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SYNTHESIS OF DIACYLGLYCEROL ANALOGS OF PHOSPHATIDYLINOSITOL 3,4,5-TRISPHOSPHATE

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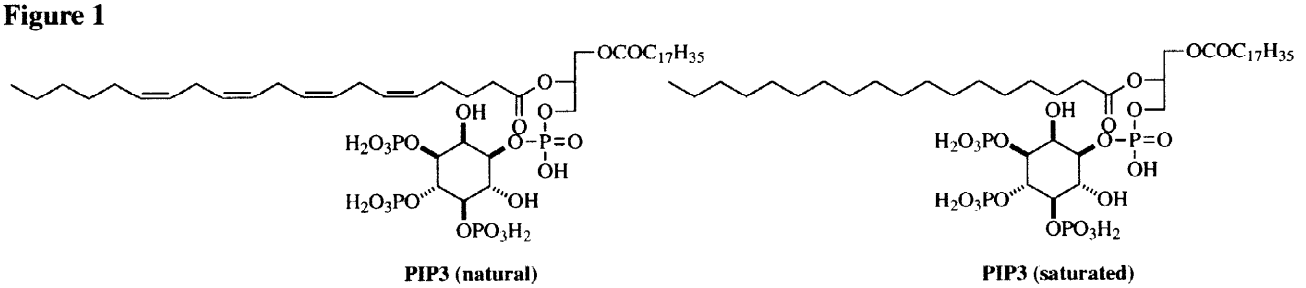
Abstract: Phosphatidylinositol 3,4,5-trisphosphate analogs with saturated diacylglycerol substructure have been designed, focusing on their reactivity with PIP3 5-phosphatase. Dephosphorylation of native PIP3 was competitively inhibited in the presence of synthetic $PIP3_{C2}$ and $PIP3_{C4}$, respectively.

Phosphatidylinositol 3-kinase (PI3-kinase)[1], when activated by stimulation with growth factors, phosphorylates phosphatidylinositol 4,5-bisphosphate (PI 4,5-P2) to generate phosphatidylinositol 3,4,5-trisphosphate (PIP3), which is subsequently dephosphorylated by PIP3 5-phosphatase to generate phosphatidylinositol 3,4-bisphosphate (PI 3,4-P2)[2]. PIP3 and PI 3,4-P2 are thought to act as second messengers, e. g., PIP3 activates PIP3-dependent kinase 1[3] and aPKC[4], while PI 3,4-P2 may stimulate the activity of Akt[5]. These polyphosphoinositides may also be involved in vesicle transport and rearrangement of the cytoskeleton[6].

PIP3 analogs with saturated diacylglycerol substructure, such as distearoyl (C18)[7,8] and dioctanoyl (C8)[9] glycerol, have been synthesized by us and other groups. Recently, synthesis of natural PIP3 with unsaturated arachidonic acid has been reported.[10] During evaluation of the enzymatic reaction of synthetic phosphatidylinositols, we unexpectedly found that distearoyl PIP3 ($PIP3_{C18}$) could not be dephosphorylated to PI 3,4-P2 by PIP3 5-phosphatase. Several reports have described the importance of unsaturated fatty acids at the sn-2 center, but the structural requirement for the sn-2 fatty acid is still unclear. In attempts to develop phosphatidylinositol analogs as biochemical probes and/or synthetic second messenger molecules, the saturated diacylglycerol substructure should provide a feasible basis for molecular design.[11] Here, we describe the design, synthesis and evaluation of PIP3 analogs with saturated diacylglycerol substructure, focusing on their reactivity with PIP3 5-phosphatase.

Figure 1



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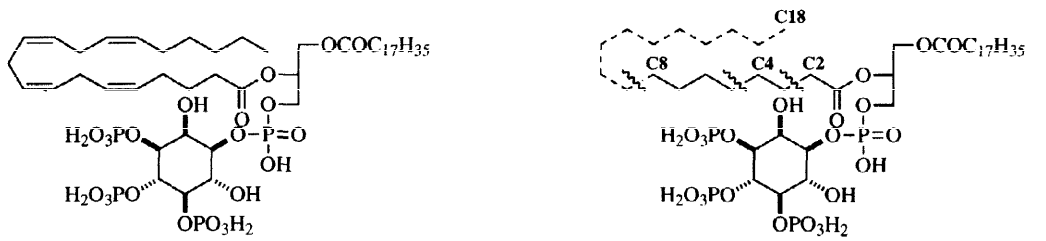
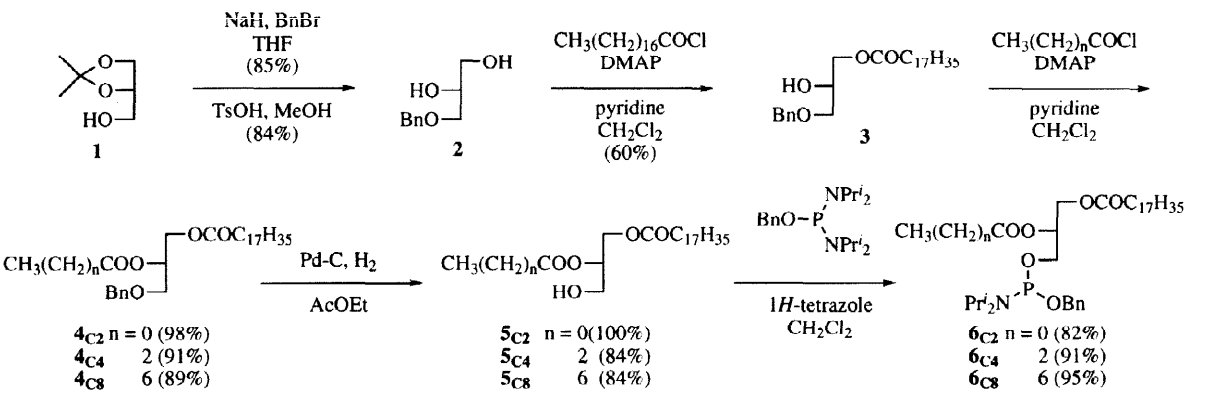
The basic design of the diacylglycerol substructure was as follows. The sn-1 fatty acid was fixed as stearic acid, which is typically found in many natural phosphatidylinositols. Since most natural phosphatidylinositols contain arachidonic acid at the sn-2 center, the spherical size of their diacylglycerol substructure might be smaller than that of distearate diacylglycerol owing to folding of the unsaturated chain of arachidonate. Therefore, we introduced a short saturated fatty acid at the sn-2 center in place of arachidonic acid (Figure 2).

Figure 2

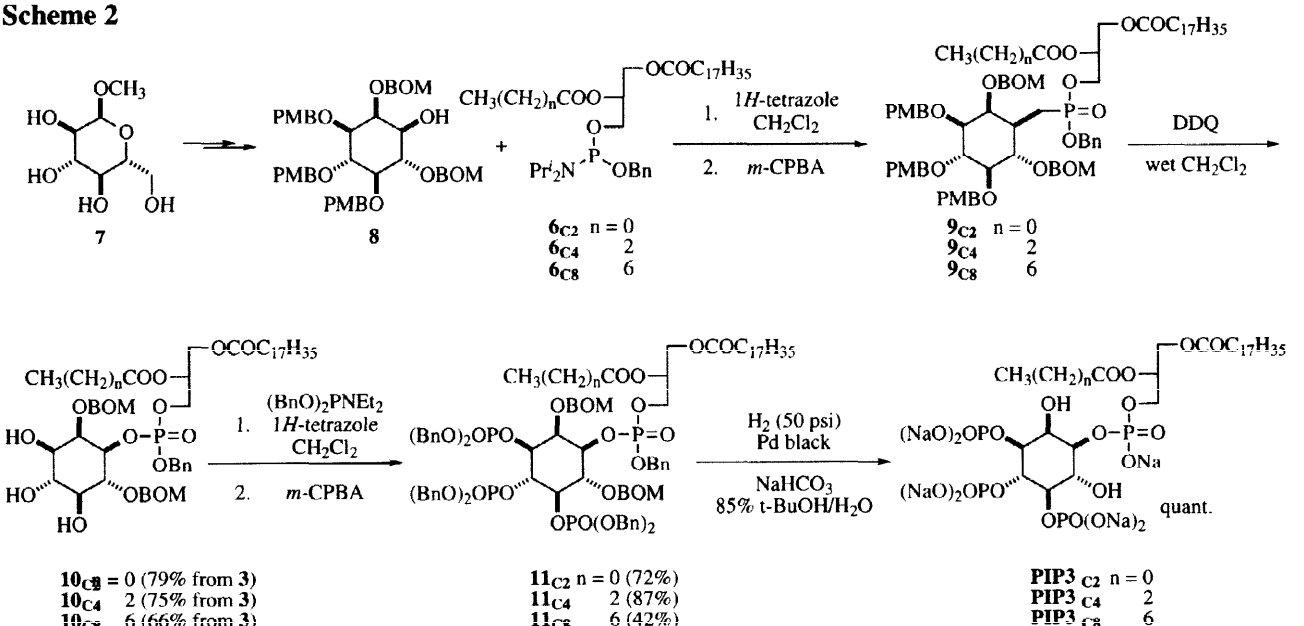


PIP3 (folded) **PIP3$_{Cn}$ (n = 2, 4, 8, 18)**

Scheme 1



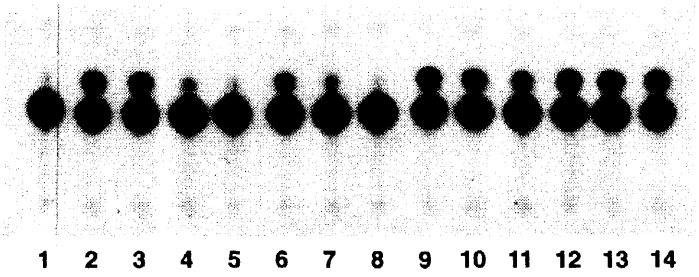
Scheme 2



The diacylglycerol analogs were synthesized as shown in Schemes 1 and 2.[8] The amidites **6** with two different acyl groups were obtained from 2-*O*-acyl-1-*O*-stearoyl-*sn*-glycerols and benzyl *N,N,N',N'*-tetraisopropylphosphoramidite, which were prepared from (*S*)-(+)-2,2-dimethyldioxolane-4-methanol and phosphorus trichloride, respectively.[12,13] The appropriately protected homochiral key intermediate **8** was synthesized according to Estevez and Prestwich's method[12] from methyl α-D-glucopyranoside (**7**) through 9 steps in 26% overall yield. The coupling of the inositol derivative **8** with the amidite **6** followed by *m*-CPBA oxidation in one pot gave an epimeric mixture of **9**.[11] Removal of the *p*-methoxybenzyl (PMB) groups with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) in wet CH_2Cl_2 gave the triol **10**. Phosphitylation of the resulting triol with dibenzyl *N,N*-diethylphosphoramidite, followed by oxidation with *m*-CPBA in one pot gave fully protected PIP3 (**11**). Deprotection of all benzyl and benzyloxymethyl (BOM) groups of **11** was carried out by hydrogenolysis over Pd black in 85% t-BuOH in the presence of $NaHCO_3$.[8,9]

The reactivity of synthetic PIP3 analogs with PIP3 5-phosphatase was evaluated by competitive dephosphorylation assay with native [^{32}P]PIP3. Native [^{32}P]PIP3 was incubated with PIP3 5-phosphatase in the presence of various concentrations of synthetic $PIP3_{C2}$, $PIP3_{C4}$, $PIP3_{C8}$ and $PIP3_{C18}$.[8] The reaction mixture was developed on TLC[14] and radioactivity of the resulting PI 3,4P2 was visualized by autoradiography (Figure 3). In the presence of $PIP3_{C2}$ and $PIP3_{C4}$, dephosphorylation of native [^{32}P]PIP3 was evidently inhibited. On the other hand, $PIP3_{C8}$ and $PIP3_{C18}$ did not affect the dephosphorylation even at higher concentrations. Next, direct dephosphorylation of $PIP3_{C4}$ by PIP3 5-phosphatase was also examined. Production of PI 3,4-$P2_{C4}$ was detected as an apparent spot on TLC stained by $CuSO_4$-phosphoric acid. Therefore, it appears that $PIP3_{C2}$ and $PIP3_{C4}$ can be substrates of PIP3 5-phosphatase.

Figure 3



Competitive dephosphorylation reaction of native [^{32}P]PIP3 by PIP3 5-phosphatase in the presence of synthetic $PIP3_{C2}$, $PIP3_{C4}$, $PIP3_{C8}$, and $PIP3C_{18}$

Lane 1: native [^{32}P]PIP3, lane 2: native [^{32}P]PIP3 + PIP3 5-phosphatase, lanes 3-5: [^{32}P]PIP3 +$PIP3_{C2}$ (×10, ×10^2, ×10^3 from left) + PIP3 5-phosphatase, lanes 6-8: [^{32}P]PIP3 +$PIP3_{C4}$ (×10, ×10^2, ×10^3) + PIP3 5-phosphatase, lanes 9-11: [^{32}P]PIP3 +$PIP3_{C8}$ (×10, ×10^2, ×10^3) + PIP3 5-phosphatase, lanes 12-14: [^{32}P]PIP3 +$PIP3_{C18}$ (×10, ×10^2, ×10^3) + PIP3 5-phosphatase

These findings indicate that synthetic PIP3 analogs with saturated diacylglycerol substructure may represent a new approach to the design of enzyme inhibitors, biochemical probes, and synthetic second messenger molecules. Further investigations are in progress.

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