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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<sub>C2</sub> and PIP3<sub>C4</sub>, respectively. © 1998 Elsevier Science Ltd. All rights reserved.

Phosphatidylinositol 3-kinase (PI3-kinase)<sup>1</sup>, 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)<sup>2</sup>. PIP3 and PI 3,4-P2 are thought to act as second messengers, e. g., PIP3 activates PIP3-dependent kinase 1<sup>3</sup> and aPKC<sup>4</sup>, while PI 3,4-P2 may stimulate the activity of Akt<sup>5</sup>. These polyphosphoinositides may also be involved in vesicle transport and rearrangement of the cytoskeleton<sup>6</sup>.

PIP3 analogs with saturated diacylglycerol substructure, such as distearoyl (C18)<sup>7,8</sup> and dioctanoyl (C8)<sup>9</sup> glycerol, have been synthesized by us and other groups. Recently, synthesis of natural PIP3 with unsaturated arachidonic acid has been reported.<sup>10</sup> During evaluation of the enzymatic reaction of synthetic phosphatidylinositols, we unexpectedly found that distearoyl PIP3 (PIP3<sub>C18</sub>) 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.<sup>11</sup> 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



Scheme 1







The diacylglycerol analogs were synthesized as shown in Schemes 1 and 2.<sup>8</sup> 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*,*N*-tetraisopropylphosphoramidite, which were prepared from (*S*)-(+)-2,2-dimethyldioxolane-4-methanol and phosphorus trichloride, respectively.<sup>12,13</sup> The appropriately protected homochiral key intermediate **8** was synthesized according to Estevez and Prestwich's method<sup>12</sup> from methyl  $\alpha$ -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**.<sup>11</sup> Removal of the *p*-methoxybenzyl (PMB) groups with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) in wet CH<sub>2</sub>Cl<sub>2</sub> 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<sub>4</sub>.<sup>8,9</sup>

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<sub>C2</sub>, PIP3<sub>C4</sub>, PIP3<sub>C8</sub> and PIP3<sub>C18</sub>.<sup>8</sup> The reaction mixture was developed on TLC<sup>14</sup> and radioactivity of the resulting PI 3,4P2 was visualized by autoradiography (Figure 3). In the presence of PIP3<sub>C2</sub> and PIP3<sub>C4</sub>, dephosphorylation of native [ ${}^{32}P$ ]PIP3 was evidently inhibited. On the other hand, PIP3<sub>C8</sub> and PIP3<sub>C18</sub> did not affect the dephosphorylation even at higher concentrations. Next, direct dephosphorylation of PIP3<sub>C4</sub> by PIP3 5-phosphatase was also examined. Production of PI 3,4-P2<sub>C4</sub> was detected as an apparent spot on TLC stained by CuSO<sub>4</sub>-phosphoric acid. Therefore, it appears that PIP3<sub>C2</sub> and PIP3<sub>C4</sub> 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<sub>C2</sub>, PIP3<sub>C4</sub>, PIP3<sub>C8</sub>, and PIP3C<sub>18</sub>

Lane 1: native [ ${}^{32}$ P]PIP3, lane 2: native [ ${}^{32}$ P]PIP3 + PIP3 5-phosphatase, lanes 3-5: [ ${}^{32}$ P]PIP3 +PIP3<sub>C2</sub> (×10, ×10<sup>2</sup>, ×10<sup>3</sup> from left) + PIP3 5-phosphatase, lanes 6-8: [ ${}^{32}$ P]PIP3 +PIP3<sub>C4</sub> (×10, ×10<sup>2</sup>, ×10<sup>3</sup>) + PIP3 5-phosphatase, lanes 9-11: [ ${}^{32}$ P]PIP3 +PIP3<sub>C8</sub> (×10, ×10<sup>2</sup>, ×10<sup>3</sup>) + PIP3 5-phosphatase, lanes 12-14: [ ${}^{32}$ P]PIP3 +PIP3<sub>C18</sub> (×10, ×10<sup>2</sup>, ×10<sup>3</sup>) + 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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## **References and notes**

- Auger, K. R.; Serunian, L. A.; Soltoff, S. P.; Libby, P.; Cantley, L. C. Cell, 1989, 57, 167.; Ruderman, N. B.; Kapeller, R.; White, M. F.; Cantley, L. C. Proc. Natl. Acad. Sci. USA, 1990, 87, 1411.; Stephens, L. R.; Hughes, K. T.; Irvine, R. F. Nature, 1991, 351, 33.; Carter, A. N.; Downes, C. P. J. Biol. Chem., 1992, 267, 14563.
- Whitman, M.; Downes, C. P.; Keeler, M.; Keller, T.; Cantley, L. C. Nature, 1988, 332, 644.; Shibasaki, F.; Homma, Y.; Takenawa, T. J. Biol. Chem., 1991, 266, 8108.; Hawkins, T. T.; Jackson, T. R.; Stephens, L. R. Nature, 1992, 358, 157.; Kabuyama, Y.; Nakatsu, N.; Homma, Y.; Fukui, Y. Eur. J. Biochem., 1996, 238, 350.; Guilherme, A.; Klarlund, J. K.; Krystal, G.; Czech, M. P. J. Biol. Chem., 1996, 271, 29533.; Liu, L.; Jefferson, A. B.; Zhang, X.; Norris, F. A.; Majerus, P. W.; Krysrtal, G. J. Biol. Chem., 1996, 271, 29729.
- Alessi, D. R.; James, S. R.; Downes, C. P.; Holmes, A. B.; Gaffney, P. R.; Reese, C. B.; Cohen, P. Current Biology, 1997, 8, 69.; Alessi, D. R.; Kozlowski, M. T.; Weng, Q. P.; Morrice, N.; Avruch, J. Current Biology, 1998, 8, 69.
- Nakanishi, H.; Brewer, K. A.; Exton, J. H. J. Biol. Chem., 1993, 268, 13-16.; Akimoto, K.; Takahashi, R.; Moriya, S.; Nishioka, N.; Takayanagi, J.; Kimura, K.; Fukui, Y.; Osada, S.; Mizuno, K.; Hirai, S.; Kazlauskas, A.; Ohno, S. EMBO J., 1996, 15, 788.
- 5. Franke, T. F.; Kaplan, D. R.; Cantley, L. C.; Toker, A. Science, 1997, 275, 665.
- Cockcroft, S. Curr. Opin. in Hematology, 1996, 3, 48.; Rodriguez-Viciana, P.; Warne, P. H.; Khwaja, A.; Marte, B. M.; Pappin, D.; Das, P.; Waterfield, M. D.; Ridley, A.; Downward, J. Cell, 1997, 89, 457.; Carpenter, C. L.; Cantley, L. C. Curr. Opin. Cell Biol., 1996, 8, 153.
- Gou, D. M.; Chen, C. S. J. Chem. Soc., Chem. Commun., 1994, 2125.; Watanabe, Y.; Tomioka, M.; Ozaki,
  S. Tetrahedron, 1995, 51, 8969.; Wang, D. S.; Chen, C. S. J. Org. Chem., 1996, 61, 5905., Aneja, S. G.;
  Parra, A.; Stoenescu, C.; Xia, W. Y.; Aneja, R. Tetrahedron Lett., 1997, 38, 803.
- 8. Sawada, T.; Shirai, R.; Iwasaki, S. Chem. Pharm. Bull., 1997, 45, 1521.
- 9. Reddy, K. K.; Saddy, M.; Falck, J. R. J. Org. Chem., 1995, 60, 3385.
- Sawada, T.; Shirai, R.; Matsuo, Y.; Kabuyama, Y.; Kimura, K.; Fukui, Y.; Hashimoto, Y.; Iwasaki, S. Bioorg. Med. Chem. Lett., 1995, 5, 2263.; Tanaka, K.; Imajoh-Ohmi, S.; Sawada, T.; Shirai, R.; Hashimoto, Y.; Iwasaki, S.; Kaibuchi, K.; Kanaho, Y.; Shirai, T.; Terada, Y.; Kimura, K.; Nagata, S.; Fukui, Y. Eur. J. Biochem., 1997, 245, 512.; Shirai, T.; Tanaka, K.; Terada, Y.; Sawada, T.; Shirai, R.; Hashimoto, Y.; Nagata, S.; Iwamatsu, A.; Okawa, K.; Li, S.; Hattori, S.; Mano, H.; Fukui, Y. Biochim. Biophys. Acta, 1998, 1402, 292.
- 11. Gaffney, P. R. J.; Reese, C. B. Bioorg. Med. Chem. Lett., 1997, 7, 3171.; Watanabe, Y.; Nakatomi, M. Tetrahedron Lett., 1998, 39, 1583.
- 12. Estevez V. A.; Prestwich G. D. J. Am. Chem. Soc., 1991, 113, 9885. See also, Bender S. L.; Budhu R. J. J. Am. Chem. Soc., 1991, 113, 9883.
- 13. Bannwarth W.; Trzeciak A. Helv. Chim. Acta, 70, 175 (1987).
- 14. TLC was developed with a solvent system of chloroform : acetone : methanol : acetic acid :  $H_2O = 40 : 15 : 13 : 12 : 7$  using Silica Gel 60 plates (Merck) pretreated with potassium oxalate.