Tetrahedron Letters, Vol. 34, No. 35, pp. 5579-5582, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

Synthesis of phosphatidyl-2-O-alkylinositols as potential inhibitors for PI specific PLC

Venkata R. Garigapati and Mary F. Roberts
Department of Chemistry, Boston College, Chestnut Hill, MA 02154

Abstract: (±)-Racemic phosphatidyl-2-O-methylinositol and phosphatidyl-2-O-heptylinositol were synthesized and tested as mechanism-based inhibitors of bacterial PI-PLC activity.

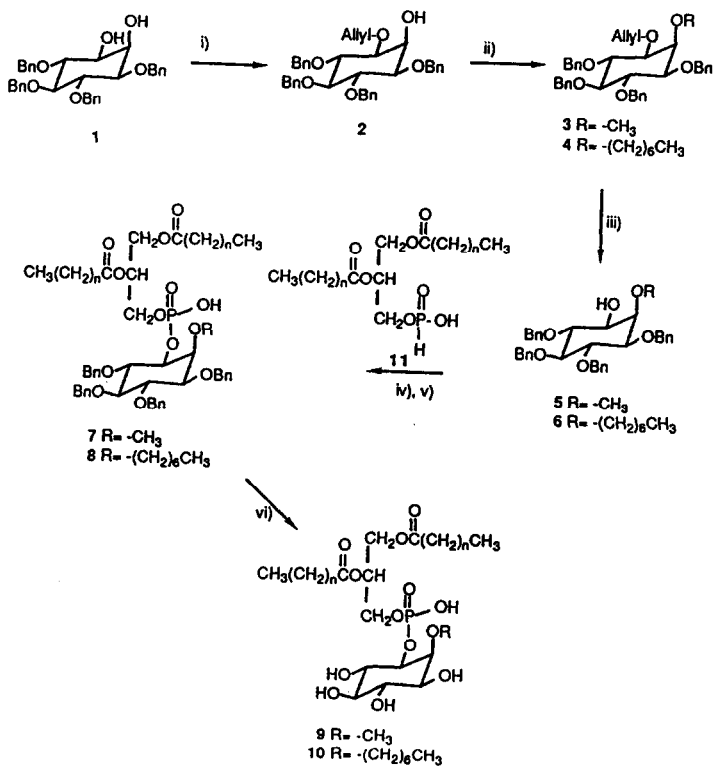
Phosphatidylinositol (PI) is an important phospholipid in the cell membranes which participates in signal transduction and in variety of physiological functions.[1] PI undergoes two phosphorylation steps: PI-kinases first yield phosphatidylinositol-4-phosphate (PIP) which is further phosphorylated to phosphatidyl-inositol-4,5-bisphosphate (PIP_2). The latter, as a substrate for PI-specific phospholipase C (PI-PLC), is converted to diacylglycerol and inositol-1,4,5-trisphosphate, both secondary messenger products.[1,2] The key event of the transduction mechanism is the hydrolysis of PIP_2. There has been limited development of specific inhibitors of PI-PLC action. Two which have appeared include tetravanadate[3] and a fluorophopshonate analog of myo-inositol-1,2-cyclic phosphate.[3] Both of these water-soluble inhibitors might also function to modify enzymes which process inositol phosphates.[4] This paper deals with the design and synthesis of phosphatidyl-2-O-alkyl-inositols as amphipathic inhibitors of PI-PLC. These membrane-active compounds should specifically block early steps of the phosphoinositide cycle and as such may be therapeutically useful.

The bacterial PI-PLC enzyme posesses both phosphotranseferase and phosphodiesterase activities. In the hydrolysis of PI by PI-PLC, from either *Bacillus cereus* [4] or *Bacillus thuringiensis* [5], D-myo-inositol-1,2-cyclic phosphate is initially formed via the phosphotransferase activity of the enzyme. The same enzyme then converts the cyclic phosphate to D-myo-inositol-1-phosphate. The formation of the cyclic phosphodiester has been postulated to require the axial hydroxyl at the second position. Also consistent with the observation is that PIs having a palmitoyl group at the 2nd position of the inositol ring were highly resistant to the PI-PLC.[6] Based on this mechanism for PI-PLC action, we designed compounds **9** and **10** (see scheme) as potential inhibitors for PI-PLC. Compound **9** has a methoxy group at the 2nd position while compound **10** has the more hydrophobic O-heptyl group at the inositol C-2 position. These groups will prevent the formation of the cyclic phosphodiester which is an essential intermediate in the hydrolysis process. These compounds will also provide information on how the size of the alkyl group is related to inhibitory activity. Heptanoyl chains were chosen as the fatty acyl linkages to generate a micellar rather than bilayer-forming PI. Synthetic short chain PI's have been shown to be excellent substrates for bacterial PI-PLC.[5] They are potentially deliverable to cells when packaged in short-chain phospholipid/long-chain phospholipid bilayer disks.[7]

Short chain PIs were successfully synthesized by the H-phosphonate approach using NPCl as a condensing agent.[5] The same phosphonate chemistry has been coupled with suitably protected inositol derivatives prepared as shown in scheme 1 to synthesize **9** and **10**. 3,4,5,6-tetrabenzylinositol, **1**, was readily obtained from myo-inositol in three steps (74% yield).[8] Selective O-allylation at C-1 of **1** was carried out using dibutyltin oxide in dry benzene with tetrabutylammonium bromide and allylbromide to yield 1-O-allyl-3,4,5,6-tetrabenzylinositol, **2**, which was purified on a silica gel colum as a colorless gum in 98% yield. Compound **2** was initially treated with diazomethane in ether to form the methoxy derivative, but this was not successful.

Hence, NaH and CH_3I or $CH_3(CH_2)_6I$ in anhydrous DMF were used to give 1-O-allyl-2-O-methyl 3,4,5,6-tetrabenzylinositol 3 or 1-O-allyl-2-O-heptyl 3,4,5,6-tetrabenzylinositol 4 in 97% yield.[9]

Scheme 1



i) dibutyl tin oxide, dry benzene 24h, tetrabutylammonium bromide, allylbromide; ii) NaH, DMF, methyl iodide; iii) Wilkinson's catalyst, DABCO, 90% ethanol, 3h then acetic acid, water, THF, 4h; iv) **11**, NPCl, pyridine; v) I_2-pyridine-water; vi) 10% Pd-C, ethanol, hydrogen.

Compounds **3** and **4** were readily deallylated using tris(triphenylphosphine)-rhodium(I) chloride and DABCO in 90% ethanol followed by boiling in acetic acid-water-tetrahydrofuran to form 2-O-methyl-3,4,5,6-tetrabenzylinositol, **5**, and 2-O-heptyl-3,4,5,6-tetrabenzylinositol, **6**. Both **5** and **6** were purified on silica gel in 95% yield then recrystallized in alcohol to give white crystals. [8,9] Compounds **5** and **6** were subsequently used to couple with the triethylammonium salt of 1,2-diheptanoyl-sn-glycero-3-H-phosphonate **11**.[5] **5** and **6** were condensed with the H-phosphonate using 5,5-dimethyl-2-oxo-2-chloro-1,3,2-dioxaphosphorinan (NPCl) as the condensing agent in pyridine in the ratio of 1:2:4 eq (protected inositol: H-phosphonate: NPCl) to give H-phosphonate diesters. These were subjected to *in situ* oxidation with I_2 in 98:2 pyridine/water to give triethylammonium salts of phosphate diester **7** and **8**.[9,10] The latter were purified on a silica gel column and

obtained as colorless gums in 78% yield. **7** and **8** were subjected to hydrogenolysis in ethanol with 10% Pd-C under 50 psi for 3h to yield diC$_7$-2-O-methyl-PI, **9**, and diC$_7$-2-O-heptyl-PI, **10**. These compounds were chromatographed on silica gel: **9** eluted with 25% methanol-chloroform, while **10** eluted with 5 to 8% methanol in chloroform. Fractions were pooled, evaporated under reduced pressure, and lyophilized to give white powders. The diC$_7$-2-O-heptyl-PI, **10**, insoluble in water, was characterized by NMR in $CDCl_3$: CD_3OD (1:1).[9]

When assayed by ^{31}P NMR spectroscopy neither O-alkylated PI derivative was a substrate for PI-PLC from *Bacillus thuringiensis*.[5] Furthermore, **9** was a more potent inhibitor of D-diC$_7$PI hydrolysis than **10** as monitored by ^{31}P integrated intensity (at 202.3 MHz using instrumentation and parameters described previously[5]) (Figure 1). The enzyme specific activities were 514, 520, and 275 μmol min^{-1} mg^{-1} for D-diC$_7$PI (4.5 mM) alone, mixed with 0.75 mM **10**, and mixed with 0.75 mM **9**, respectively. The low concentrations of inhibitors were chosen so that the heptyl derivative would form mixed micelles in the D-diC$_7$PI (rather than phase separate). Under these conditions the inhibitory potency of both O-alkylated species could be compared directly. In conclusion, the modification of the hydroxyl functionality at the inositol C-2 is clearly important in the development of inhibitors for PI-PLC. Furthermore, size of the modification at C-2 is also critical for fine-tuning amphipathic PI-PLC inhibitors.

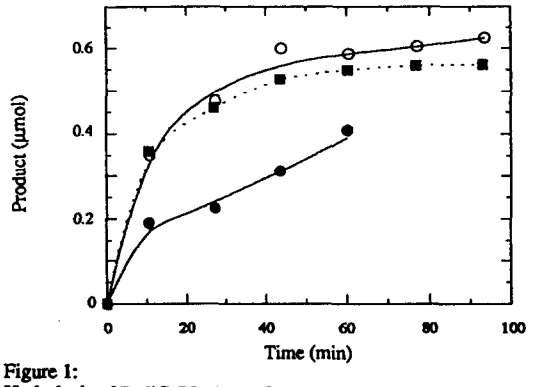


Figure 1:
Hydrolysis of D-diC$_7$PI alone (○) and with **9** (●) and **10** (■).

Acknowledgments: This work was supported by N.I.H. GM 26762. We would like to thank Ms. Chun Zhou for measuring the enzyme assays toward these modified PI substrates.

References and notes:

1. Berridge, M. J.; Irvine, R. F. *Nature* **1984**, *312*, 315; Nishizuka, Y. *Nature* **1984**, *308*, 693.
2. Chung, D.-M. *Ann. Rev. Pharmacol. Toxicol.* **1989**, *29*, 71.
3. Campbell, A. S.; Thatcher, G. R. J. *Bioorg. Med. Chem. Lett.* **1992**, *12*, 655; *Tet. Lett* **1991**, *32*, 2207.
4. Lin, G.; Bennet, C. F.; Tsai, M. D. *Biochemistry* **1990**, 29, 2747.
5. Garigapati, V. R.; Roberts, M. F. *Tetrahedron Lett.* **1993**, 34, 769; Lewis, K.; Garigapati, V. R.; Zhou, C.; Roberts, M. F. *Biochemistry* **1993**, *32*, in press.
6. Roberts, W. L.; Myher, J. J.; Kuksis, A.; Low, M. G.; Rosenberry, T. L. *J. Biol. Chem.* **1988**, 263, 18766; Roberts, W. L.; Santikarn, S.; Reinhold, V. N.; Rosenberry, T. L. *J. Biol. Chem.* **1988**, *263*, 18776.
7. Gabriel, N.E.; Roberts, M. F. *Biochemistry* **1987**, *26*, 2432.
8. Baker, G. R.; Billington, D. C.; Gani, D. *Tetrahedron* **1991**, *47*, 3895.
9. **2** was purified by elution from silica gel with 20-35% ethylacetate in petroleum ether. Its ^1H NMR data corresponded well with the literature data.[8] **3**, a colorless syrup, was purified by elution from silica gel with

0-15% ethyl acetate in petroleum ether: δ_H (300 MHz: $CDCl_3$, unless otherwise noted) 3.20-3.22 (dd, 1H, Ins-H); 3.36-3.39 (dd, 1H, Ins-H); 3.41-3.43 (m, 1H, Ins-H); 3.59-3.61 (m, 1H, Ins-H); 3.96-3.98 (m, 1H, Ins-H); 4.15-4.18 (dd, 1H, Ins-H); 3.62 (s, 3H, $-OCH_3$); 4.7-4.90 (m, 8H, benzyl CH_2 groups); 5.18-5.20 dd, 5.22-5.40 dd, 5.60-5.80 m (5H, -O-allyl protons); 7.2-7.45 (m, 20H, aromatic benzyl protons). **4** was purified on a silica gel column to a colorless viscous syrup: δ_H resonances at 0.78-0.82 (3H, t, heptyl ω-CH_3); 1.18-1.38 (8H, broad m, heptyl $(CH_2)_n$); 1.40-1.50 (2H, broad m, heptyl β-CH_2); 3.18-3.20 (dd, 1H, Ins-H); 3.30-3.32 (dd, 1H, Ins); 3.90-4.1 (m, 1H, Ins-H); 3.74-3.76 (t, 2H, heptyl $-OCH_2$); 3. 81-3.82 (t, 1H, Ins-H); 3.90-3.94 (m, 1H, Ins-H); 4.08-4.12 (dd, 1H, Ins-H); 4.65-4.95 (m, 8H, benzyl CH_2); 5.16-5.19 dd, 5.24-5.28, dd and 5.85-5.97 (5H, -O-allyl group); 7.2-7.4 (m, 20H, aromatic H). **5**, eluted from silica gel with 15-30% ethyl acetate in petroleum ether, was recrystallized in absolute ethanol to yield white fluffy crystals, m.p.146° C. ^1H resonances included: 2.35 (bs, inositol-1-OH, exchangeable with D_2O), 3.40-3.42 (dd, 1H, Ins-H); 3.44-3.46 (dd, 1H, Ins-H); 3.48-3.50 (t, 1H, Ins-H); 3.60-3.61(m, 1H, Ins-H); 3.63 (s, 3H, $-OCH_3$); 3.78-3.80 (t, 1H, Ins-H); 3.96-3.99 (m, 1H, Ins-H); 4.65-4.95 (m, 8H, benzyl CH_2); 7.2-7.40 (m, 20H, aromatic protons). **6** was eluted from the silica gel column with 5-10% ethyl acetate in petroleum ether; it was recrystallized in absolute ethanol, m.p.44-46° C. ^1H resonances at 0.82-0.88 (t, 3H, heptyl ω-CH_3); 1.2-1.38 (m, 8H, heptyl $(CH_2)_n$); 1.46-1.51 (m, 2H, heptyl β-CH_2); 2.28-2.30 (bs, 1H, Ins-OH, exchangeable with D_2O); 3.39-3.41 (dd, 1H, Ins-H); 3.42-3.44 (t, 2H, heptyl $-OCH_2$); 3.58-3.61 (m, 1H, Ins-H); 3.66-3.68 (t, 1H, Ins-H); 3.82-3.84 (m, 1H, Ins-H); 3.86-3.88 (dd, 1H, Ins-H); 3. 88-3.90 (t, 1H, Ins-H); 4.64-4.93 (m, 8H, benzyl CH_2); 7.20-7.41 (m, 20H, aromatic H). **7** was eluted with 5-15% methanol in chloroform. ^1H resonances included: 0.65-0.90 (t, 6H, ω-CH_3); 1.20-1.30 (m, 16H, acyl chain $(CH_2)_n$); 1.38-1.52 (m, 4H, β-CH_2); 2.12-2.25 (m, 4H, α-CH_2); 3.24-3.27 (m, 1H, Ins-H); 3.40-3.42 (dd, 1H, Ins-H); 3.48 (s, 3H, $-OCH_3$); 3.82-3.86 (m, 3H, Ins-H and *sn*-1-CH_2); 4.05-4.11 (m, 1H, Ins-H); 4.15-4.19 (m, 1H, Ins-H); 4.22-4.27 (m, 1H, Ins-H); 4.45-4.95 (m, 10H, benzyl CH_2 and *sn*-3 CH_2); 5.20-5.25 (m, 1H, *sn*-2 CH); 7.2-7.4 (m, 20H, aromatic H). **8** was eluted with 5% methanol in chloroform; δ_H resonances at 0.75-0.85 (m, 9H, acyl chain and -O-heptyl ω-CH_3); 1.12-1.38 (m, 24H, acyl and heptyl $(CH_2)_n$); 1.42-1.56 (m, 6H, acyl and heptyl β-CH_2); 2.18-2.28 (m, 4H, acyl chain α-CH_2); 3.25-3.4 (m, 2H, Ins-H); 3.7-3.78 (m, 3H, heptyl $-OCH_2$ and Ins-H); 3.85-4.12 (m, *sn*-1 CH_2, Ins-H); 4.18-4.22 (m, 1H, Ins-H); 4.55-4.98 (m, 10H, benzyl CH_2 and *sn*-3 CH_2); 5.2-5.25 (m, 1H, *sn*-2 CH); 7.1-7.42 (m, 20H, aromatic H). ^1H chemical shifts for **9** (in D_2O) included: 0.75-0.82 (t, 6H, ω-CH_3); 1.0-1.2 (m, 16H, (acyl chain $(CH_2)_n$); 1.5-1.7 (m, 4H, β-CH_2); 2.38-2.45 (m, 4H, α-CH_2); 3.28-3.33 (m, 1H, Ins-H); 3.5-3.75 (m, $-OCH_3$ appears as singlet overlapping *sn*-1 CH_2 and Ins-H); 3.95-3.98 (m, 3H, Ins-H and *sn*-3 CH_2); 4.05-4.11 (m, 1H, Ins-H); 4.25-4.32 (m, 1H, Ins-H); 4.41-4.48 (m, 1H, Ins-H); 5.30-5.35 (m, 1H, *sn*-2 CH); ^{31}P chemical shift in D_2O is -1.45 ppm with respect to external H_3PO_4. ^1H chemical shifts for **10** in $CDCl_3$:CD_3OD (1:1) were 0. 8-0.91 (m, 9H, acyl and O-heptyl ω-CH_3); 1.40-1.80 (m, 24H, $(CH_2)_n$); 1.55-1.22 (m, 6H, β-CH_2); 2.42-2.50 (m, 4H, α-CH_2); 3.05-3.09 (dd, 1H, Ins-H); 3.12-3.15 (t, 2H, heptyl $-OCH_2$); 3.41-3.44 (m, 1H, Ins-H); 3.58-3.62 (m, 1H, Ins-H); 3.75-3.80 (m, 1H, Ins-H); 4.01-4.08 (m, 3H, *sn*-1-CH_2 and Ins-H); 4.15-4.20 (m, 1H, Ins-H); 4.35-4.44 (m, 2H, *sn*-3 CH_2); 5.22-5.27 (m, 1H, *sn*-2 CH).

10. Lindh, I.; Stawinski, J. *J. Org. Chem.* **1989**, *54*, 1338.

(Received in USA 10 March 1993; revised 10 June 1993; accepted 9 July 1993)