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Facile Synthesis of Lysophospholipids Containing Unsaturated Fatty Acid Chains

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Abstract: The efficient synthesis of polyunsaturated phospholipids is challenging due to the sensitivity of the unsaturated moiety to the conditions employed in phosphate ester deprotection. We discuss here three independent methods that resolve this issue and enable the synthesis of a series of unsaturated lysophosphatidic acid mimics for the development of a more comprehensive understanding of the structure-activity relationship in this series. Copyright © 1996 Elsevier Science Ltd

Lysophosphatidic acid (LPA, 1-O-acyl-sn-glycerol-3-phosphate) (Figure 1) is a minor membrane constituent and is an ubiquitous intermediate in phospholipid synthesis. It has received increased attention due to its action as a pleiotropic extracellular signaling molecule. LPA evokes platelet aggregation,[1] smooth-muscle contraction,[2] cell morphology changes,[3] intracellular calcium mobilization,[4] mitogenic effects,[5] and inhibition of forskolin-driven rises in cAMP.[4] Available evidence suggests that LPA acts through one or more G-protein coupled receptors. The ability to obtain reliable data on the physiological activities of LPA is hampered by its low water solubility and metabolic lability. To address these problems, LPA mimics need to be synthesized which resolve these impediments. In addition, a more comprehensive understanding of the structure-activity relationships for this family of lipid signaling molecules needs to be generated.[4,6,7]

A variety of synthetic[8,9] and enzymatic[5,9] routes have been reported for the synthesis of phospholipids with saturated fatty acid chains. However, the availability of phospholipids with unsaturated acyl chains has lagged behind.[10] The difficulty in incorporating unsaturated fatty acid chains is the selective deprotection of the phosphate, without reducing or isomerizing the olefins present in the LPA fatty acid chain. Herein, we

report three methods that enable the incorporation of mono and poly-unsaturated fatty acid chains into a variety of LPA agonists.

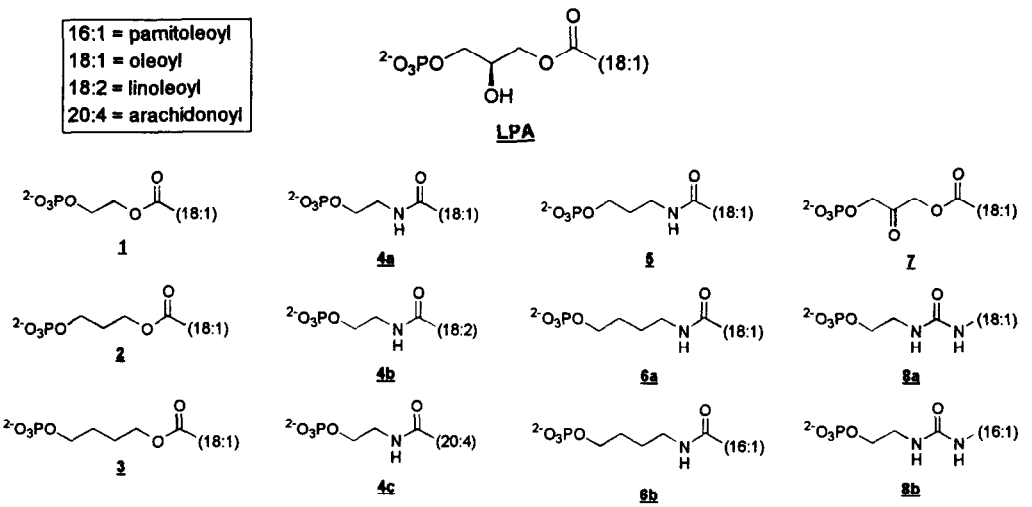
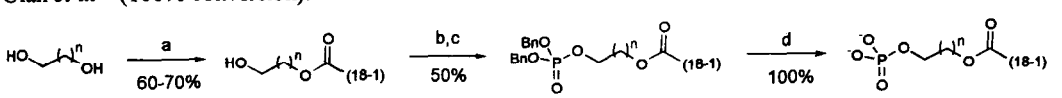


Figure 1

In the present study three classes of LPA agonists were synthesized (Figure 1). The first set of LPA agonists (**1-3**) was synthesized by Method 1 (Scheme 1). The initial acylation of the appropriate diol with oleoyl chloride proceeded smoothly in each case with typical yields of 60-70%. The remaining hydroxyl was phosphorylated with bis(benzyloxy)(diisopropylamino)phosphine[11] and oxidized to the phosphate with hydrogen peroxide (typical yields of 50%). The phosphoalkenyl esters **1-3** were prepared by hydrogenolysis of the phosphate benzyl esters *via* hydrogen transfer by modification of the conditions of Olah *et al*[12] (100% conversion).

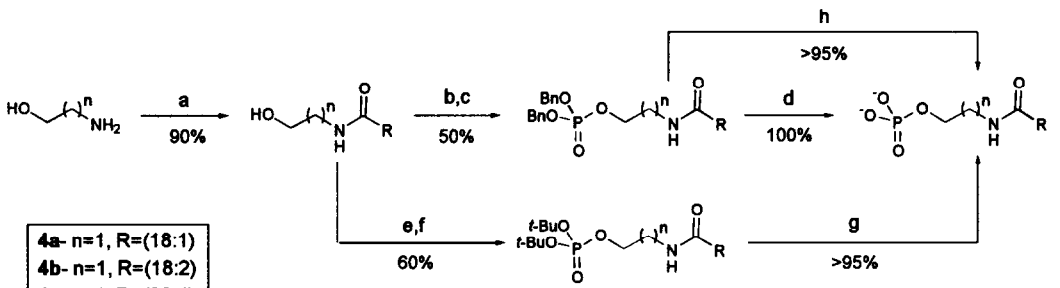


1- n=1
2- n=2
3- n=3

Scheme 1: a) pyr, oleoyl chloride; b) bis(benzyloxy)(diisopropylamino)phosphine, tetrazole; c) HOOH; d) cyclohexene, 10% Pd/C(cat).

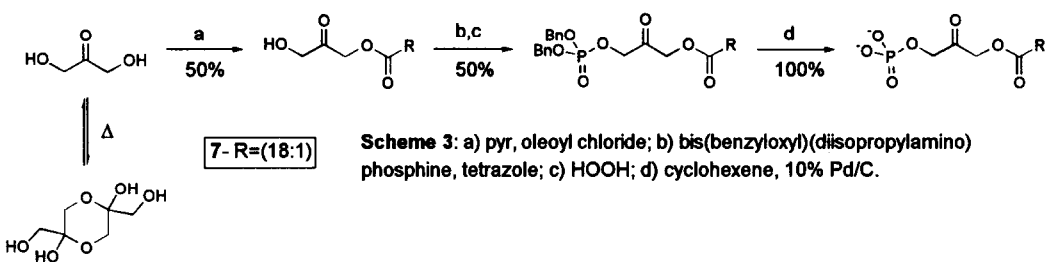
A second family of LPA agonists ($\mathbf{4}_{a-c}$, **5**, and $\mathbf{6}_{a,b}$) was synthesized using Method 2 (Scheme 2). selective amino acylation (typical yield of 90%) was followed by phosphorylation of the hydroxyl analogous to the methodology described for the O-acyl analogs above. The N-acyl analogs with a mono-unsaturated

fatty acid chain were converted to the phosphate mono-ester through the conditions described above. However, these conditions did not allow selective reduction of the phosphate benzyl esters for molecules containing poly-unsaturated fatty acid chains with *bis*-allylic protons, typically reducing these to a mixture of the corresponding mono-unsaturated derivatives. However, *t*-butyl phosphodiesters could be employed in the phosphorylation sequence and subsequently removed under acidic conditions to yield the phosphate mono ester in nearly 100% (Scheme 2).



4a- n=1, R=(18:1)
4b- n=1, R=(18:2)
4c- n=1, R=(20:4)
5 - n=2, R=(18:1)
6a- n=3, R=(18:1)
6b- n=3, R=(16:1)

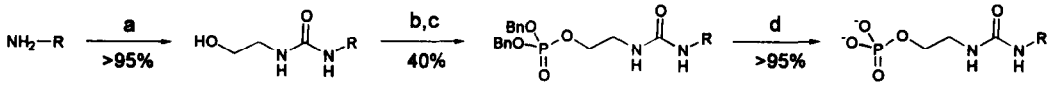
Scheme 2: a) pyr, fatty acid chloride; b) bis(benzyloxy)(diisopropylamino) phosphine,tetrazole; c) HOOH; d) cyclohexene, 10% Pd/C; e) bis(*t*-butoxy)-(diisoproylamino)phosphine, tetrazole; f) HOOH; g) HCl; h) NH_3, Li^o, dioxane.



7- R=(18:1)

Scheme 3: a) pyr, oleoyl chloride; b) bis(benzyloxyl)(diisopropylamino) phosphine, tetrazole; c) HOOH; d) cyclohexene, 10% Pd/C.

The third set of agonists (**7** & $\mathbf{8}_{a-d}$) was realized through two independent pathways. The synthesis of the 2-keto LPA analog **7** was not accessible through Method 1. The difficulty in synthesizing **7** *via* this method was due to the insolubility of dihydroxyacetone, which exists as a dimer. To alleviate this problem the initial acylating conditions required reflux (Scheme 3). The urea containing lipids $\mathbf{8}_{a-d}$ were synthesized by Method 3 (Scheme 4). A variety of long chain amines were treated with triphosgene and ethanolamine to obtain the urea functionality.[13] The hydroxyl was then phosphorylated with bis(benzyloxy) (di-isopropylamino) phosphine and converted to the phosphate mono-ester by Birch reduction.



8a- R=(18:1)
8b- R=(16:1)

Scheme 4: a) triphosgene, ethanolamine; b) bis(bezyloxy)(diisopropyl-amino)phosphine, tetrazole; c) HOOH; d) NH_3, Li^0, dioxane.

In summary, we have explored three independent routes to realize the synthesis of LPA agonists with unsaturated fatty acid chains. These methods allow the deprotection of the phosphate under neutral, acidic and basic conditions without altering the unsaturated fatty acid chains. With these methods the synthesis of different families of phospholipids containing unsaturated fatty acid chains and a variety of other functional groups has become routinely accessible. Our ongoing studies are exploring the synthesis of a variety of LPA mimetics that possess chain lengths with differing degrees of unsaturation and other functionalities.

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References:

1. Watson, S.P.; McConnell, R.T.; Lapetina, E.G. *Biochem. J.* **1985**, *232*, 61.
2. Tokumura, A.; Yube, N.; Fujimoto, H.; Tsukatane, H. *J. Pharm. Pharmocol.* **1991**, *43*, 774.
3. Ridley, A.J.; Hall, A. *Cell* **1992**, *70*, 389.
4. Moolenaar, W.H. *J. Biological Chem.* **1995**, *270*, 12949.
5. Van Corven, E.J.; Van Rijswijk, A.; Van Der Bend, R.L.; Van Blitterswijk, W.J.; Moolenaar, W.H. *Biochem. J.* **1992**, *281*, 163.
6. Durieux, M.E.; Lynch, K.R. *TiPS* **1993**, *14*, 249.
7. Moolenaar, W.H.; *Current Opinion in Cell Biology* **1995**, *7*, 203.
8. Bittman, R.; Swords, B.; Liliom, K.; Tigyi, G. *J. Lipid Res.* **1996**, *37*, 391.
9. Sugiura, T.; Tokumura, A.; Gregory, L.; Nouchi, T.; Weintraub, S.T.; Hanahan, D.J. *Arch. Biochem. Biophys.* **1994**, *311*, 358.
10. Murakami-Murofushe, K.; Kobayashi, S.; Onimura, K.; Matsumoto, M.; Shioda, M.; Yoshida, S.; Shoji, M.; Murofushi, H. *Biochimica et Biophysica Acta* **1995**, *1258*, 57.
11. Bannwarth, W.; Trzeciak, A. *Helvetica Chimia Acta* **1987**, *70*, 175.
12. Olah, G.A.; Surya Prakash, G.K.; Narang, S.C. *Synthesis* **1978**, 825.
13. Majer, P.; Randad, R.S. *J. Org. Chem.* **1994**, *59*, 1937.

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