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Total Syntheses of Bioactive Oxidized Ethanolamine Phospholipids

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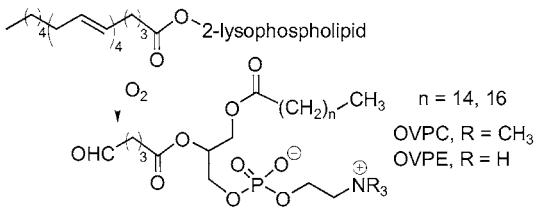
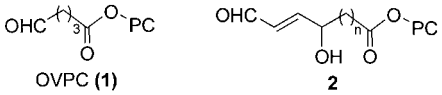
ABSTRACT

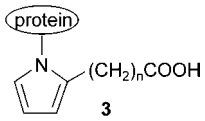


Truncated ethanolamine phospholipids containing aldehyde functionality, e.g. OVPE, and the corresponding acids, are generated by oxidative cleavage of polyunsaturated phospholipids. To confirm their identities and facilitate studies of the chemistry and biological actions of these analogues of biologically active phosphatidylcholines, e.g. OVPC, total syntheses were developed. An efficient general strategy was used that features selective *N*-protection of 2-lysophosphatidylethanolamine, and generation of the target compounds by mild deprotection of stable precursors.

Because they exhibit biological activities with apparent pathological significance, interest in the chemistry and biology of oxidized phospholipids (oxPL) is growing. Oxidative cleavage of the arachidonic acid ester of 2-lysophosphatidylcholine (PC) generates the oxovaleryl ester OVPC (**1**).[1] This aldehydic PC and the corresponding carboxylic acid inhibit lipopolysaccharide-induced E-selectin expression by human aortic endothelial cells and activate endothelial cells to bind monocytes.[2] Truncated γ-oxygenated α,β-unsaturated aldehydic **2** and carboxylic oxPLs are ligands for the macrophage scavenger receptor CD36 that mediates endocytosis of oxidized low-density lipoprotein (LDL) by macrophage cells.[3]



The aldehydes **1** and **2** also covalently modify proteins by reactions with primary amino groups of lysyl residues, e.g., generating Schiff bases and carboxyalkylpyrroles **3**[4] whose levels are elevated in cardiovascular[5] and retinal[6] diseases.



Primarily owing to the availability of authentic samples by total syntheses,[7,8] studies on oxPL have focused on phosphatidylcholines.[1,3] Phosphatidylethanolamines (PEs)

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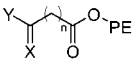
constitute an important fraction of LDL phospholipids and are major components of certain brain and retinal membranes.[9] To facilitate studies of the generation, chemistry, and biology of oxidized PEs we now report efficient methods for their preparation. The reactivity of the primary amino group of PEs toward aldehyde functionality[10] prompted us to design syntheses that avoided aldehydic intermediates or generated them in the final step from stable precursors suitable for storage.

PE analogues **4a**−**c** of OVPC (**1**) and homologues are expected to be generated by oxidative cleavage of docosahexaenoic, arachidonic, and linoleic acid esters of 2-lysophosphatidylethanolamine. Syntheses were devised for **4a**−**c** as well as the corresponding carboxylic acids **5a**−**c** and the oxPE analogues **6a**−**c** of the most avid oxPC ligands for the CD36 receptor.[3]

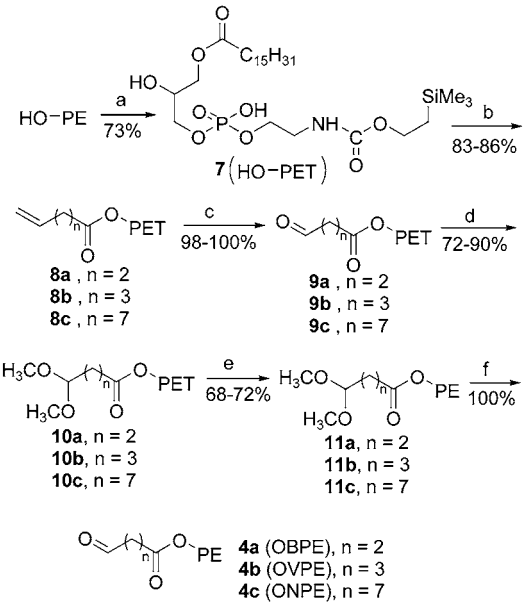


4a-c: X = O, Y = H, n = 2, 3, 7
5a-c: X = O, Y = OH, n = 2, 3, 7
6a-c: X =O, Y = CH=CHCO$_2$H, n = 2, 3, 7

Our strategy was to protect the amine moiety of the commercially available 2-lysoPE and couple the latter with the appropriate acids. Initially we examined the use of *t*-BOC.[11] This was satisfactory for preparing the acids **5a**−**c**. However, 2-trimethylsilanylethoxycarbonyl (Teoc), used with success for amino acids,[12,13] is a more suitable protecting group because deprotection can be accomplished under mild conditions with tetrabutylammonium fluoride. The present report documents the first application of the Teoc group to protect the primary amino group in 2-lyso-phosphatidylethanolamines.

Of reagents available for the protection of amines with Teoc,[13] we found *p*-nitrophenyl trimethylsilanylethyl carbonate (*p*-NPTeoc) to be most effective. With this reagent[14] we were able to introduce the Teoc protecting group selectively on the amine moiety under mild conditions. As shown in Scheme 1, protection of 2-lysoPE (HO-PE) with *p*-NPTeoc followed by coupling with the appropriate commercially available acids gave the Teoc *N*-protected alkenoylphospholipids 4-pentenoyl- (**8a**), 5-hexenoyl- (**8b**), and 9-decenoyl-

Scheme 1[a]



[a] Reagents and conditions: (a) *p*-NPTeoc, Na_2CO_3 1 M, 48 h. (b) DCC, DMAP $CHCl_3$, $CH_2{=}CH(CH_2)_nCO_2H$, 72 h, $n = 2, 3, 7$. (c) O_3, MeOH, −60 °C, then Me_2S, MeOH −10 °C to room temperature. (d) $CH(OCH_3)_3$, Montmorillonite K10, rt. (e) 1 M TBAF/ THF, 72 h, rt. (f) TFA 50%:$CHCl_3$ (1:2), 0 °C, 90 min.

(**8c**) phosphatidylethanolamine. Ozonolysis at −60 °C followed by reduction in methanol with Me_2S of the intermediate ozonides[15] produced the Teoc *N*-protected aldehydes 4-oxobutyroyl- (**9a**), 5-oxovaleroyl- (**9b**), and 9-oxononanoyl- (**9c**) phosphatidylethanolamine.

In an analogous fashion (Scheme 2) the *t*-BOC *N*-protected alkenoylphospholipids (**13a**−**c**) were prepared, as well as the corresponding aldehydes (**14a**−**c**).

Attempts to directly deprotect[12,16] the Teoc derivatives **9a**−**c** or the *t*-BOC derivatives **14a**−**c**, using a variety of conditions summarized in Scheme 1 and Table 1 in the Supporting Information, failed to provide the desired phosphatidylethanolamine aldehydes **4a**−**c**. Masking of the Teoc *N*-protected aldehyde-phospholipids **9a**−**c** (Scheme 1) as dimethylacetals, using Montmorillonite K10 and trimethylorthoformate, and deprotection of the ethanolamine moiety in **10a**−**c**, using tetrabutylammonium fluoride (TBAF) in THF at room temperature, delivers the stable precursors **11a**−**c**. The corresponding oxidized lipids (OBPE (**4a**), OVPE (**4b**) and ONPE (**4c**)) can be conveniently generated from **11a**−**c**, as needed, under mild conditions by stirring in a heterogeneous mixture of chloroform and 50% TFA (2:1) at 0 °C.[17]

The acids **15a**−**c** could be obtained from the *t*-BOC protected alkenoylphospholipids **13a**−**c** by ozonolysis to give

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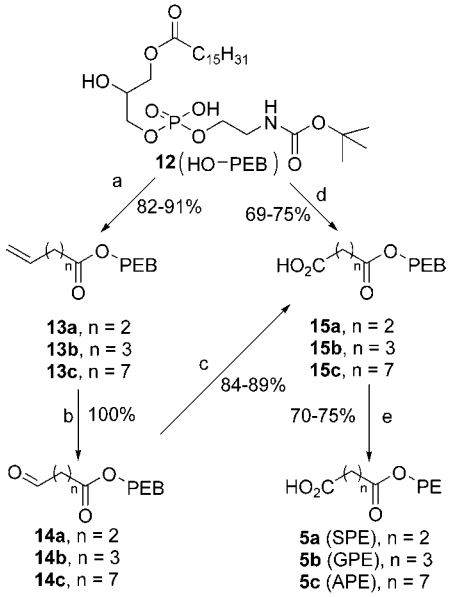
Scheme 2[a]



[a] Reagents and conditions: (a) $CH_2{=}CH(CH_2)_nCO_2H$, DCC, DMAP, $CHCl_3$, 72 h. (b) O_3, MeOH, −60 °C, then Me_2S, MeOH, −10 °C to room temperature. (c) $NaClO_2/NaH_2PO_4$, 2-methyl-2-butene, *t*-BuOH−H_2O (5:1), 2 h, rt. (d) (OC-$(CH_2)_{n-2}$-CO)O, DCC, DMAP, $CHCl_3$, 72 h. (e) TFA, 4 °C, 30 min.

aldehydes **14a**−**c** followed by $NaClO_2$ oxidation (see steps a to c in Scheme 2).[9] However, a more practical route to **15a**−**c** is available (Scheme 2, step d). The succinic, glutaric, and azelaic acid monoesters of 2-lysoPE are best obtained from the appropriate anhydride. Succinic ($n = 4$) and glutaric ($n = 5$) anhydrides are commercially available while azelaic anhydride ($n = 9$) is readily available from azelaic acid.[18] Coupling with *t*-BOC *N*-protected 2-lysoPE (HO-PEB) in the presence of DMAP in anhydrous chloroform produces the succinic, glutaric, and azelaic acid monoesters **15a**−**c** of *t*-BOC *N*-protected 2-lysoPE. Deprotection of **15a**−**c** with TFA at 0 °C delivers the corresponding monoesters of 2-lysoPE, **5a**−**c**.

The ketoacids **6a**−**c**, which are expected to be strong ligands for CD36 due to their structural similarity with the corresponding active phosphatidylcholines,[3] were readily available from the furyl acids **16a**−**c.** Furylpropionic acid **16a** is commercially available while **16b** and **16c** were prepared according to Sun et al.[8] Coupling of HO-PEB with **16a**−**c** to give the furylalkanoyl monoesters of *t*-BOC *N*-protected 2-lysoPE (**17a**−**c**), followed by a two-step oxidation and deprotection delivers **6a**−**c** (Scheme 3).

The compounds and methods reported in this letter will be used to confirm the identities of biologically active oxPEs present in oxidized LDL. Recent mass spectroscopic studies indicate that oxPEs derived from 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylethanolamine (SAPE) are present in bovine LDL, and that 1-stearoyl-2-(5-oxovaleroyl)-PE and 1-stearoyl-2-glutaroyl-PE are produced upon autoxidation[1]

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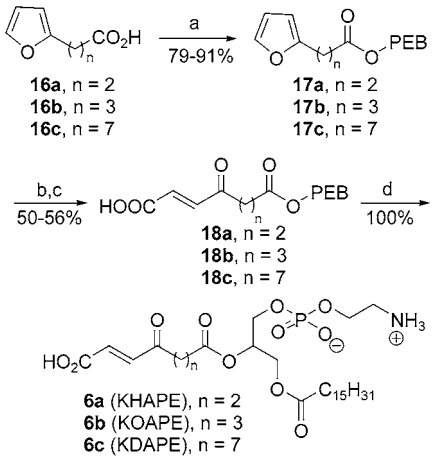
Scheme 3[a]



[a] Reagents and conditions: (a) HO-PEB, DCC, DMAP, $CHCl_3$, 72 h. (b) NBS, 1 h, −20 °C, then Py, 6 h, rt. (c) $NaClO_2/NaH_2PO_4$, 2-methyl-2-butene, *t*-BuOH−H_2O (5:1), 2 h, rt. (d) TFA 50%−$CHCl_3$ (1:2), 4 °C, 24 h.

of isolated SAPE. These oxPEs exhibited bioactivities similar to those of the corresponding oxPCs.[2] Other recent studies suggest that some oxPEs may have biological activities not shared with oxPCs. Thus, the ability of oxidized LDL to promote platelet prothrombinase activity appears to be mediated primarily by the oxPE component of the lipoprotein.[19]

Ongoing studies, to be reported elsewhere, confirm that the oxidatively truncated ethanolamine phospholipids, identical with those prepared by the total syntheses detailed herein, are formed by the oxidation of docosahexaenoic, arachidonic, and linoleic acid esters of 2-lysoPE, and that **4b**−**c** and **5b**−**c** induce monocyte binding to endothelial cells. As anticipated, the aldehydic PE **4b** is chemically unstable. Preliminary results showed that 50% of **4b** is lost within 1 h upon standing in aqueous solution at room temperature in pH 7.4 phosphate buffered saline containing 5% methanol. The ketoacids **6** are expected to be strong ligands for CD36 owing to their structural similarity with the corresponding biologically active oxPCs.[3] The affinity of the CD36 receptor for structurally defined pure oxPL as well as their ability to cause platelet activation are under investigation, and a study of the chemistry of these oxPEs is in progress.

Acknowledgment. This work was supported in part by NIH grant GM21249.

Supporting Information Available: Complete experimental details including ^1H and ^{13}C (or ^1H, HMQC, and HMBC) NMR spectra for compounds **5a**−**c** to **11a**−**c** and **13a**−**c** to **18a**−**c**; for the unstable aldehydes **4a**−**c**, ^1H, but not ^{13}C, NMR spectra were recorded. This material is available free of charge via the Internet at http://pubs.acs.org.

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