Published in final edited form as:

Bioorg Med Chem. 2011 January 1; 19(1): 580–587. doi:10.1016/j.bmc.2010.10.058.

An Efficient Synthesis of γ -Hydroxy- α,β -unsaturated Aldehydic Esters of 2-Lysophosphatidylcholine

Jaewoo Choi, James M. Laird, and Robert G. Salomon^{*}

Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106-7078

Abstract

The diverse biological activities of γ -hydroxyalkenal phospholipids and their involvement in disease are the subject of intense study. Phospholipid aldehydes, such as the 4-hydroxy-7-oxohept-5-enoic acid ester of 2-lyso-phosphatidylcholine (HOHA-PC), the 5-hydroxy-8-oxo-6-octenoic acid ester of 2-lyso-PC (HOOA-PC), and the 9-hydroxy-12-oxododec-10-enoic acid ester of 2-lyso-PC (HODA-PC), are generated by oxidative cleavage of polyunsaturated fatty acyl phospholipids. To facilitate investigations of their chemistry and biology, we now report efficient total syntheses of HOOA, HODA, and HOHA phospholipids. Because the target γ -hydroxyalkenals readily decompose through oxidation of the aldehyde group to a carboxylic acid or through cyclization to furans, these syntheses generate the sensitive functional array of the target phospholipids under mild conditions from acetal derivatives that are suitable for long-term storage.

Keywords

γ -Hydroxy- α,β -unsaturated aldehyde; oxidized phospholipid; HOHA-PC; HOOA-PC; HODA-PC; organic synthesis; cardiovascular disease; atherosclerosis

1. Introduction

Free radical-induced oxidative cleavage of phospholipids containing docosahexaenoate (C_{22}), arachidonate (C_{20}), or linoleate (C_{18}) generates truncated, biologically active aldehydes including γ -hydroxyalkenal phospholipids, i.e., the 4-hydroxy-7-oxohept-5-enoic acid ester of 2-lysophosphatidylcholine (HOHA-PC), the 5-hydroxy-8-oxo-6-octenoic acid ester of 2-lyso-PC (HOOA-PC), and the 9-hydroxy-12-oxododec-10-enoic acid ester of 2-lyso-PC (HODA-PC) respectively (Scheme 1). These γ -hydroxyalkenals react with protein lysyl ϵ -amino residues to generate biologically active carboxyalkylpyrrole derivatives (Scheme 1).^{1,2} HOHA-PC, HOOA-PC and HODA-PC and the derived protein adducts 2-(ω -carboxyethyl)pyrroles (CEPs), 2-(ω -carboxypropyl)pyrroles (CPPs) and 2-(ω -carboxyheptyl)pyrroles (CHPs), are present in human atherosclerotic lesions.^{3–5} The γ -hydroxyalkenal phospholipids contribute to the generation of atherosclerotic plaques. Thus,

© 2010 Elsevier Ltd. All rights reserved

^{*}To whom correspondence should be addressed at Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106-7078; rgs@case.edu, Phone: 216-368-2592. FAX: 216-368-3006.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Supplementary data Supplementary data associated with this article can be found, in the online version, at?

they promote the conversion of monocyte macrophages into foam cells by serving as ligands for the scavenger receptor CD36 that mediates endocytosis of oxidized low-density lipoprotein particles by macrophage cells.^{6,7} Binding of these phospholipids, and their more oxidized derivatives, to platelet CD36 receptors induces aggregation leading to thrombosis.⁸ Their binding to the macrophage scavenger receptor class B, type I (SR-BI) prevents binding of its physiological ligand, high-density lipoprotein, to SR-BI and consequently interferes with SR-BI-mediated selective uptake of cholesteryl esters in hepatocytes.⁹ Thus, oxidative stress resulting in the accumulation of specific oxidized phospholipids in plasma may have an inhibitory effect on reverse cholesterol transport. HOOA-PC induces monocyte binding to endothelial cells^{10–12} and this may promote infiltration of monocyte macrophages into the subendothelial space where they become foam cells through unregulated endocytosis of oxidized low-density lipoprotein. Also, HOOA-PC inhibits lipopolysaccharide-induced expression of E-selectin a major endothelial cell adhesion molecule involved in neutrophil binding.¹⁰ Specifically, HOOA-PC induces expression of chemokines that promote interaction with monocytes.¹³ Thus, HOOA-PC is important as a putative proinflammatory molecule regulating leukocyte endothelial interactions.^{11,14} Furthermore, covalent modification of proteins by HOOA-PC and HODA-PC results, *inter alia*, in impaired proteolytic degradation of internalized macromolecules by mouse peritoneal macrophages and inhibition of cathepsin B, a lysosomal protease. HOHA-PC also inhibits posttranslational processing of the nascent 25 kDa membrane fusion protein Rab5a to produce the active 23 kDa “mature” Rab5a in phagosomal membranes.¹⁵ Most recently, proteins containing CEPs, that are generated *in vivo* from HOHA-PC, were shown to initiate destruction of the retina in age-related macular degeneration.¹⁶ We previously reported total syntheses of HOHA-, HOOA- and HODA-PC.^{1,3} However, those syntheses were lengthy and involved reactions that are difficult to perform. Herein, we report superior syntheses of HOOA, HODA, and HOHA derivatives that will facilitate investigation of their biological involvements.

2. Results and Discussion

2.1. Synthetic Design

γ -Hydroxyalkenals readily decompose through oxidation of the aldehyde group to a carboxylic acid or cyclization to furans. Therefore, a synthetic strategy was designed that generates the sensitive functional array of the target phospholipids **1** under mild conditions from stable acetal precursors **2** that are suitable for long-term storage, and that might be readily converted to the target γ -hydroxyalkenal phospholipids under mild conditions (Scheme 2). Masking of the aldehyde carbonyl as an acetal also allows selective reduction of the ketone carbonyl in the ketoalkenal derivatives **3**. The precursors **3** might be assembled through cross-coupling of the known vinyl tin **4** and acid chlorides **5**. Alternatively, ketoalkenal precursors **6** are readily available via oxidation of furans **7**.

2.2. HOHA Ethyl Ester

To test the feasibility of generating the requisite γ -hydroxyalkenal functional array in 2 byselective reduction of a ketone from an ester of **3** ($n = 2$, $R = \text{Me}$), we synthesized (*E*)-ethyl-4-hydroxy-7-oxohept-5-enoate (**11**) (Scheme 3). The furyl ester **7a** was oxidatively ring opened with *N*-bromosuccinimide (NBS).¹⁷ The resulting keto aldehyde **8** was selectively protected using trimethyl orthoformate and Montmorillonite K10¹⁸ to give **9**. Reduction of the ketone carbonyl in **9** was readily accomplished in excellent yield to provide the masked γ -hydroxyalkenal **10**. The target γ -hydroxyalkenal **11** could be generated by hydrolysis of the dimethyl acetal in **10** promoted by the weak acid, pyridinium *p*-toluenesulfonic acid (PPTs). However, concomitant transesterification delivered the lactone

12 in excellent yield when the hydrolysis was performed in the presence of stronger acid catalysts, such as Amberlyst-15 or TFA.

2.3. HOOA Methyl Ester

In an alternative approach (Scheme 4), the requisite carbon skeleton was assembled by regio- and stereoselective tributylstannylcupration of 3,3-diethoxyprop-1-yne (**13**) with the Lipshutz reagent¹⁹, $\text{Bu}_3\text{SnCu}(\text{Bu})\text{CNLi}_2$, followed by $\text{PdCl}_2(\text{CH}_3\text{CN})_2$ catalyzed acylation of the resulting vinyltin **4**²⁰ with glutaric acid monomethyl ester chloride (**5b**).²¹ Reduction of the resulting ketone **14b** with sodium borohydride gave the stable masked γ -hydroxyalkenal **15b** in excellent yield. The target γ -hydroxyalkenal **16** could be generated by hydrolysis of **15b** promoted by a weak acid. However, as for the **10** $\rightarrow$ **11** conversion above, concomitant transesterification delivered the lactone **17** when the hydrolysis was performed in the presence of stronger acid catalysts.

2.4. HOHA-PC

Enzymatic hydrolysis of ethyl ester **9** with porcine pancreatic lipase (PPL) in phosphate buffer saline (PBS) buffer afforded key intermediate **2** (Scheme 5), that is precursor for many oxidized phospholipids.²² When lithium hydroxide was applied to hydrolyze methyl ester group, the diethyl acetal moiety was also deprotected. Therefore, selective enzymatic hydrolysis of the ester at pH 7 was considered. Synthesis of PC-ester **18** was efficiently accomplished using the coupling reagents 2,6-dichlorobenzoyl chloride and 1-methylimidazole,^{23,24} resulting in 50% shorter reaction time and improved yield of the ester coupling product, as compared to using general coupling reagents such as dicyclohexylcarbodiimide and 4-*N,N*-dimethylaminopyridine, which adhere to silica gel resulting in laborious separation via chromatography. Selective reduction of ketone **18a** using NaBH_4 in methanol and subsequent mild deprotection of the dimethylacetal **2a** gave HOHA-PC **1a** in excellent yield.

2.5. HOOA and HODA-PCs

HOOA-PC (**1b**) and HODA-PC (**1c**) were assembled by $\text{PdCl}_2(\text{CH}_3\text{CN})_2$ -catalyzed acylation of vinylstanane **4**²⁰ with glutaric acid monomethyl ester chloride (**5b**) or azelaic acid monomethyl ester chloride (**5c**)^{25–27} to generate ketones **14b** or **14c**, respectively (Scheme 6). Acylation of L- α -lysophosphatidylcholine (HO-PC) with the carboxylic acids produced by hydrolysis of the methyl esters **3** followed by selective reduction of the ketone carbonyl delivered stable precursors **2** of the γ -hydroxyalkenal phospholipids **1**.

3. Conclusion

New total syntheses readily deliver the γ -hydroxy- α,β -unsaturated aldehydic esters HOHA-PC (**1a**), HOOA-PC (**1b**), and HODA-PC (**1c**) of 2-lysophosphatidylcholine from the corresponding acetals **2**. These relatively stable precursors are protected against oxidative degradation of the aldehyde and cyclization to furans. However, our model studies with **11** and **16** revealed a potential pitfall, acid-catalyzed lactonization to **12** or **17**, respectively (Scheme 7). Since L- α -lysophosphatidylcholine (HO-PC) – that would be released by such lactonization of HOHA-PC (**1a**) or HOOA-PC (**1b**) – is biologically active, it is important to avoid strongly acidic conditions during the generation of these oxidatively truncated phospholipids from the stable acetal precursors.

Experimental Section

4.1. Ethyl (*E*)-4, 7-dioxohept-5-enoate (**8**)

N-Bromosuccinimide (NBS, 1.59 g, 8.9 mmol) was dissolved in tetrahydrofuran-acetone-H₂O (5:4:1, 12 mL) and slowly added to a solution of ethyl-(3-(2-furyl)propanoate (**7a**, 1 g, 5.9 mmol) and pyridine (962 μ L, 11.9 mmol) in tetrahydrofuran-acetone-H₂O (5:4:1, 8 mL) at -20°C . The solution was stirred for 1 h at -20°C and 3 h at room temperature. The solvents were then evaporated under reduced pressure and the residue purified by flash chromatography on a silica gel column (30% ethyl acetate/hexanes, TLC: R_f = 0.3) to give **8** (653 mg, 60%). ¹H NMR (400 MHz, CDCl₃) δ 9.76 (d, J = 7.1 Hz, 1H), 6.89 (d, J = 16.2 Hz, 1H), 6.79 (dd, J = 16.9, 7.0 Hz, 1H), 4.11 (q, J = 7.2 Hz, 2H), 2.99 (t, J = 6.2 Hz, 2H), 2.65 (t, J = 6.1 Hz, 2H), 1.22 (t, J = 6.2 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) 198.2, 193.4, 172.3, 144.5, 137.7, 60.9, 35.8, 27.9, 14.2. HRMS (FAB): m/z calcd for C₉H₁₃O₄ (MH⁺), 185.0814; found, 185.0818.

4.2. Ethyl (*E*)-7, 7-dimethoxy-4-oxohept-5-enoate (**9**)

Aldehyde **8** (299 mg, 1.6 mmol) was stirred with suspension of the Montmorillonite K-10 (500 mg) and trimethyl orthoformate (500 μ L) in dry dichloromethane (3 mL) at room temperature for 1 h. The mixture was then filtered through a pad of Celite 521, followed by washing the residue with dichloromethane (10 mL). The solvents were evaporated under reduced pressure and the residue purified by flash chromatography on a silica gel column (10% ethyl acetate/hexanes) to give **9** (280 mg, 75%). TLC (30% ethyl acetate/hexanes, R_f = 0.32): ¹H NMR (400 MHz, CDCl₃) δ 6.62 (dd, J = 19.5, 3.9 Hz, 1H), 6.37 (dd, J = 16.2, 1.1 Hz, 1H), 4.93 (m, 1H), 4.11 (q, J = 7.2 Hz, 2H), 3.32 (s, 6H), 2.89 (t, J = 6.6 Hz, 2H), 2.60 (t, J = 6.6 Hz, 2H), 1.23 (t, J = 7.2 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) 198.1, 172.8, 140.8, 131.9, 101.1, 60.8, 53.1, 35.3, 28.1, 14.4. HRMS (FAB): m/z calcd for C₁₁H₁₉O₅ (MH⁺), 231.1232; found, 231.1235.

4.3. Ethyl (*E*)-4-hydroxy-7, 7-dimethoxyhept-5-enoate (**10**)

Sodium borohydride (9.4 mg, 0.25 mmol) was added in small portions over 5 min at 0°C to a stirred solution of ester **9** (47.7 mg, 0.2 mmol) in ethanol (1.5 mL). The reaction mixture was stirred for 4 h and then quenched by addition of methanol to destroy excess sodium borohydride. The mixture was neutralized to pH 7 by addition of saturated sodium bicarbonate solution, followed by addition of brine. The aqueous layer was extracted with ethyl acetate. Solvents were evaporated from the combined organic extracts under reduced pressure, and the residue purified by flash chromatography on a silica gel column (20% ethyl acetate/hexanes) to give **10** (45.7 mg, 95%). TLC (45% ethyl acetate/hexanes, R_f = 0.21): ¹H NMR (400 MHz, CDCl₃) δ 5.88 (ddd, J = 15.8, 5.7, 1.0 Hz, 1H), 5.69 (ddd, J = 15.8, 4.7, 1.3 Hz, 1H), 4.79 (d, J = 4.8 Hz, 1H), 4.22 (m, 1H), 4.12 (q, J = 7.2 Hz, 2H), 3.31 (s, 6H), 2.45 (t, J = 6.6 Hz, 2H), 2.06 (s, -OH), 2.00–1.74 (m, 2H), 1.23 (t, J = 7.2 Hz, 3H). HRMS (FAB): m/z calcd for C₁₁H₂₀NaO₅ (MNa⁺), 255.1209; found, 255.1206.

4.4. Ethyl (*E*)-4-hydroxy-7-oxohept-5-enoate (**11**)

Acetal **10** (5 mg, 0.02 mmol) was dissolved in acetone/water = 2:1 (0.5 mL) and pyridinium *p*-toluenesulfonate (PPTS, 5.9 mg, 0.022 mmol) was added and the mixture was stirred at room temperature for 2 h. Solvents were then evaporated under reduced pressure and the residue purified by flash chromatography on a silica gel column (20% ethyl acetate/hexanes) to give **11** (3.6 mg, 90%). TLC (45% ethyl acetate/hexanes, R_f = 0.20): ¹H NMR (400 MHz, CDCl₃) δ 9.60 (d, J = 7.8 Hz, 1H), 6.80 (dd, J = 15.7, 4.4 Hz, 1H), 6.35 (ddd, J = 15.7, 7.8, 1.7 Hz, 1H), 4.54 (m, 1H), 4.15 (q, J = 7.1 Hz, 3H), 2.59 (d, J = 4.8 Hz, 1H), 2.50 (ddd, J = 7.1, 10.4, 7.6 Hz, 3H), 2.14–1.98 (m, 1H), 1.89 (dt, J = 14.4, 6.6 Hz, 2H), 1.27 (t,

$J = 7.1$ Hz, 6H). HRMS (FAB): m/z calcd for $C_9H_{13}O_3$ ($MH^+ - H_2O$), 169.0865; found, 169.0867.

4.5. (E)-3-(5-Oxotetrahydrofuran-2-yl)acrylaldehyde (12)

Method (A)—Amberlyst-15 (15 mg) was added in small portions to a stirred solution of acetal **10** (15 mg, 0.06 mmol) in acetone/water = 3:1 (1.5 mL) and the mixture was stirred at room temperature for 2 h. Solvents were then evaporated under reduced pressure and the residue purified by flash chromatography on a silica gel column (40% ethyl acetate/hexanes) to give lactone **12** (6 mg, 67%). TLC (50% ethyl acetate/hexanes, $R_f = 0.15$).

Method (B)—Acetal **10** was dissolved in TFA/ H_2O = 95:5 (1 mL) and stirred for 1.5 h. Solvents were then evaporated under reduced pressure and the residue purified by flash chromatography on a silica gel column (40% ethyl acetate/hexanes) to give lactone **12** (5.5 mg, 61%). 1H NMR (400 MHz, $CDCl_3$) δ 9.60 (d, $J = 7.6$ Hz, 1H), 6.80 (dd, $J = 15.8, 4.7$ Hz, 1H), 6.32 (ddd, $J = 15.8, 7.6, 1.6$ Hz, 1H), 5.31–5.10 (m, 1H), 2.69–2.43 (3H), 2.20–1.97 (m, 1H). HRMS (FAB): m/z calcd for $C_7H_9O_3$ (MH^+), 141.0551; found, 141.0473. The 1H NMR spectral data for **12** is in agreement with that reported previously.^{28,29}

4.6. (E)-7, 7-Dimethoxy-4-oxohept-5-enoic acid (3a)

To increase solubility at room temperature ester **9** (205 mg, 0.89 mmol) was dissolved in PBS (pH 7.4, 50 mM, 7 mL) and methanol (1 mL). To this rapidly stirred solution, was then added porcine pancreatic lipase (type II, crude, 50 mg), sodium chloride (5 mg), and calcium chloride (10 mg). The pH of the reaction mixture was maintained at 7.0–7.2 by addition of sodium hydroxide solution (0.1 N). Upon completion, the solution was evaporated under reduced pressure, the residue extracted with chloroform/methanol (2:1, v/v), filtered, and dried with anhydrous magnesium sulfate. Solvents were then evaporated from the combined organic extracts under reduced pressure and the residue purified by flash chromatography on a silica gel column (chloroform/methanol = 20:1) to give **3a** (90 mg, 50%). TLC (chloroform/methanol/ H_2O = 80:19:1, $R_f = 0.44$): 1H NMR (400 MHz, $CDCl_3$) δ 6.63 (dd, $J = 16.4, 4$ Hz, 1H), 6.38 (dd, $J = 16.4, 1.2$ Hz, 1H), 4.95 (dd, $J = 4.0, 1.6$ Hz, 1H), 3.33 (s, 6H), 2.90 (t, $J = 6.4$ Hz, 2H), 2.61 (t, $J = 6.4$ Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) 198.2, 178.4, 140.1, 131.8, 101.1, 53.2, 35.3, 28.1. HRMS (FAB): m/z calcd for $C_9H_{14}NaO_5$ (MNa^+), 225.0740; found, 225.0746.

4.7. (E)-(7, 7-Dimethoxy-4-oxohept-5-enoyl)-1-palmitoyl-*sn*-glycero-3-phosphatidylcholine (18a)

1-Methylimidazole (18.7 μ L, 0.23 mmol) and 2, 6-dichlorobenzoyl chloride (37 μ L, 0.26 mmol) were added to a solution of acid **3a** (31.7 mg, 0.16 mmol) and L- α -lysophosphatidylcholine (38.8 mg, 78 μ mol) in dry methylene chloride (4 mL). The resulting mixture was stirred for 20 h at room temperature and monitored by TLC for disappearance of starting material. Solvents were then evaporated under reduced pressure and the residue purified by flash chromatography on a silica gel column (chloroform/methanol/ H_2O = 16:9:1, TLC: $R_f = 0.23$) to give **18a** (41.5 mg, 78%). 1H NMR (400 MHz, $CD_3OD:CDCl_3$ = 1:2) δ 6.61 (dd, $J = 16.2, 3.9$ Hz, 1H), 6.36 (d, $J = 16.2$ Hz, 1H), 5.19 (m, 1H), 4.93 (m, 1H), 4.37 (m, 1H), 4.34 (m, 1H), 4.14 (dd, $J = 11.9, 6.4$ Hz, 2H), 3.98 (m, 2H), 3.83 (m, 2H), 3.37 (bs, 9H), 3.34 (s, 6H), 2.89 (m, 2H), 2.62 (m, 2H), 2.27 (t, $J = 7.6$ Hz, 2H), 1.56 (m, 2H), 1.24 (24H), 0.87 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR (100 MHz, $CD_3OD:CDCl_3$ = 1:2) 198.1, 173.8, 172.3, 141.0, 131.8, 101.1, 76.9, 71.7, 71.6, 66.7, 63.7, 62.9, 59.5, 54.7, 53.3, 35.1, 34.3, 32.2, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 28.1, 25.1, 22.3, 14.4. HRMS (FAB): m/z calcd for $C_{33}H_{63}NO_{11}$ (M^+), 680.4139; found, 680.4131.

4.8. (*E*)-2-(4-Hydroxy-7, 7-dimethoxyhept-5-enoyl)-1-palmitoyl-*sn*-glycero-3-phosphatidylcholine (**2a**)

Sodium borohydride (1.7 mg, 45.8 μ mol) was slowly added at 4 °C to a stirred solution of ester **18a** (26 mg, 38 μ mol) in dry methylene chloride (0.5 mL) and methanol (1 mL). Once complete, the reaction mixture was neutralized with acetic acid, solvents were evaporated under reduced pressure, and the residue purified by flash chromatography on a silica gel column (chloroform/methanol/H₂O = 11:9:1, TLC: R_f = 0.27) to give **2a** (22.1 mg, 85%). ¹H NMR (400 MHz, CD₃OD:CDCl₃ = 1:2) δ 5.99 (dd, J = 16, 6 Hz, 1H), 5.76 (dd, J = 16.4, 4.8 Hz, 1H), 5.34 (m, 1H), 4.89 (d, 1H), 4.51 (m, 1H), 4.42 (m, 2H), 4.27 (dd, J = 12, 6.8 Hz, 2H), 4.12 (m, 2H), 3.82 (m, 2H), 3.48 (s, 1H), 3.44 (bs, 9H), 3.37 (s, 6H), 2.56 (m, 2H), 2.43 (m, 2H), 1.93 (m, 2H), 1.70 (m, 2H), 1.41 (24H), 0.98 (t, J = 6.8 Hz, 3H). ¹³C NMR (100 MHz, CD₃OD:CDCl₃ = 1:2) 173.8, 173.0, 137.8, 128.6, 101.0, 76.9, 71.8, 71.6, 70.2, 66.4, 63.6, 62.9, 59.4, 54.7, 53.4, 35.1, 34.3, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 28.3, 25.2, 22.3, 14.4. HRMS (FAB): m/z calcd for C₃₃H₆₃NNaO₁₁P ((M-H)Na⁺), 704.4115; found, 704.4119.

4.9. (*E*)-2-(4-Hydroxy-7-oxohept-5-enoyl)-1-palmitoyl-*sn*-glycero-3-phosphatidylcholine (HOHA-PC, **1a**)

PPTS (1.3 mg, 5.1 μ mol) was added to a stirred solution of ester **2a** (3.2 mg, 4.6 μ mol) in tetrahydrofuran-acetone-H₂O = 5:4:1 (1 mL). The resulting mixture was stirred for 2 h at room temperature and monitored by TLC for disappearance of starting material. Upon completion, the solvents were evaporated under reduced pressure and the residue purified by flash chromatography on a silica gel column (chloroform/methanol/H₂O = 11:9:1, TLC: R_f = 0.2) to give **1a** (2.9 mg, 97%). ¹H NMR (400 MHz, CD₃OD:CDCl₃ = 1:2) δ 9.54 (d, J = 8 Hz, 1H), 6.92 (dd, J = 15.6, 4.4 Hz, 1H), 6.29 (dd, J = 16, 8 Hz, 1H), 5.21 (m, 1H), 4.35 (m, 1H), 4.28 (m, 2H), 4.13 (m, 2H), 3.98 (m, 2H), 3.65 (m, 2H), 3.30 (s, 9H), 2.43–2.62 (m, 2H), 2.27–2.34 (m, 2H), 1.80–1.97 (m, 2H), 1.57 (m, 2H), 1.23 (24H), 0.85 (t, J = 6.8 Hz, 3H). HRMS (FAB): m/z calcd for C₃₁H₅₉NNaO₁₀P (M+H⁺), 636.3876; found, 636.3850

4.10. 1-Tributylstannyl-3, 3-diethoxy-prop-1-ene (**4**)

According to the published procedure,²¹ 3, 3-diethoxy-1-propyne (0.97 mL, 6.8 mmol) was added dropwise via syringe to a THF solution of Bu₃SnCu(Bu)CNLi₂, and the reaction mixture was stirred for 2 h under –78 °C before quenching with water. The dark solution was extracted with ether (3 $\times$ 20 mL), followed by drying of the combined extracts over anhydrous sodium sulfate. The solvents were evaporated under reduced pressure and the residue purified by flash chromatography on a silica gel column (ethyl acetate/hexanes = 3:97, TLC: R_f = 0.28) to give **4** (2.56 g, 81%). ¹H NMR (400 MHz, CDCl₃) δ 6.40–6.26 (m, 1H), 6.03–5.88 (m, 1H), 4.89–4.72 (m, 1H), 3.63 (dq, J = 9.5, 7.1 Hz, 3H), 3.49 (dq, J = 9.5, 7.1 Hz, 3H), 1.57–1.40 (9H), 1.36–1.25 (9H), 1.24–1.14 (6H), 0.93–0.80 (9H). The ¹H NMR spectral data for stannane **4** is in agreement with that reported previously.³⁰

4.11. Methyl (*E*)-8,8-diethoxy-5-oxooct-6-enoate (**14b**)

The procedure was modified from that reported.²¹ Glutaric acid monomethyl ester chloride (371 μ L, 2.68 mmol) was added dropwise via syringe to a solution of stannane **4** (1.12 g, 2.68 mmol) and PdCl₂(MeCN)₂ (6.9 mg, 26.8 μ mol) in DMF (3 mL). The reaction mixture was then cooled in an ice bath and stirred for 1 h, and then for 4 h at room temperature. Saturated aqueous sodium fluoride and acetone were added to the brown-red reaction mixture to remove tributyltin chloride. The mixture was extracted with ethyl acetate (3 $\times$ 20 mL), followed by drying of the combined extracts over anhydrous sodium sulfate. Solvent was evaporated under reduced pressure and the residue purified by flash chromatography on a silica gel column (ethyl acetate:hexanes = 7:93) to give **14b** (450 mg, 65%). TLC: (30%

ethyl acetate/hexanes; R_f = 0.38). ^1H NMR (400 MHz, CDCl_3) δ 6.62 (dd, J = 16, 4.4 Hz, 1H), 6.32 (dd, J = 16.4, 1.2 Hz, 1H), 5.02 (m, 1H), 3.64 (s, 3H), 3.59–3.64 (m, 2H), 3.48–3.54 (m, 2H), 2.64 (t, J = 6.6 Hz, 2H), 2.34 (t, J = 7.2 Hz, 2H), 1.92 (tt, J = 7.2, 7.2 Hz, 2H), 1.20 (t, J = 7.2 Hz, 6H). ^{13}C NMR (100 MHz, CDCl_3) 199.6, 173.8, 141.5, 131.6, 99.7, 61.7, 51.8, 39.4, 33.2, 19.1, 15.4. HRMS (FAB): m/z calcd for $\text{C}_{13}\text{H}_{23}\text{O}_5$ (MH^+), 259.1545; found, 259.1504.

4.12. Methyl (*E*)-8,8-diethoxy-5-hydroxyoct-6-enoate (**15b**)

Sodium borohydride (1.5 mg, 0.03 mmol) was added in small portions over 2 min at 0 °C to a stirred solution of ester **14b** (10 mg, 0.03 mmol) in ethanol (1 mL). The reaction mixture was stirred for 4 h and then quenched by addition of methanol to destroy excess sodium borohydride. The mixture was neutralized to pH 7 by addition of saturated sodium bicarbonate solution, followed by addition of brine. The aqueous layer was extracted with ethyl acetate, solvents were evaporated from the combined organic extracts under reduced pressure, and the residue purified by flash chromatography on a silica gel column (20% ethyl acetate/hexanes) to give **15b** (9.4 mg, 94%). TLC (45% ethyl acetate/hexanes, R_f = 0.24): ^1H NMR (400 MHz, CDCl_3) δ 5.86 (dd, J = 15.7, 6.0 Hz, 1H), 5.70 (ddd, J = 15.7, 5.0, 1.2 Hz, 1H), 4.89 (d, J = 5.0 Hz, 1H), 4.12 (m, 1H), 3.68–3.58 (5H), 3.55–3.41 (m, 2H), 2.34 (m, 2H), 1.77–1.62 (3H), 1.62–1.50 (2H), 1.29–1.13 (6H). HRMS (FAB): m/z calcd for $\text{C}_{13}\text{H}_{24}\text{NaO}_5$ (MNa^+), 283.1522; found, 283.1523.

4.13. Methyl (*E*)-5-hydroxy-8-oxooct-6-enoate (**16**)

Acetal **15b** (9.4 mg, 0.03 mmol) was dissolved in acetone/water = 2:1 (1 mL), PPTS (10 mg, 0.033 mmol) was added, and the mixture stirred at room temperature for 2 h. After completion, solvents were evaporated under reduced pressure and the residue purified by flash chromatography on a silica gel column (20% ethyl acetate/hexanes) to give **16** (5.9 mg, 88%). TLC (45% ethyl acetate/hexanes, R_f = 0.20): ^1H NMR (400 MHz, CDCl_3) δ 9.58 (d, J = 7.8 Hz, 1H), 6.81 (dd, J = 15.7, 4.5 Hz, 1H), 6.32 (ddd, J = 15.7, 7.8, 1.6 Hz, 1H), 4.45 (bs, -OH), 4.13 (q, J = 7.1 Hz, 1H), 3.67 (s, 3H), 2.47–2.26 (2H), 1.86–1.51 (2H), 1.35–1.08 (2H). HRMS (FAB): m/z calcd for $\text{C}_9\text{H}_{13}\text{O}_3$ ($\text{MH}^+ - \text{H}_2\text{O}$), 167.0709; found, 167.0722.

4.14. (*E*)-3-(6-Oxotetrahydro-2H-pyran-2-yl)acrylaldehyde (**17**)

Amberlyst-15 (10 mg) was added in small portions to a stirred solution of acetal **15b** (16 mg, 0.06 mmol) in TFA/water = 95:5 (1.5 mL) and the mixture was stirred at room temperature for 1.5 h. Solvents were then evaporated under reduced pressure and the residue purified by flash chromatography on a silica gel column (45% ethyl acetate/hexanes) to give lactone **17** (8.2 mg, 87%). TLC (50% ethyl acetate/hexanes, R_f = 0.1): ^1H NMR (400 MHz, CDCl_3) δ 9.61 (d, J = 7.5 Hz, 1H), 6.76 (dd, J = 15.8, 4.3 Hz, 1H), 6.37 (ddd, J = 15.8, 7.6, 1.7 Hz, 1H), 5.19–5.04 (m, 1H), 2.76–2.62 (m, 1H), 2.55 (m, 1H), 2.18–2.05 (m, 1H), 2.06–1.87 (m, 2H), 1.83–1.64 (m, 1H). HRMS (TOF-MS/ESI): m/z calcd for $\text{C}_8\text{H}_{10}\text{NaO}_3$ (MNa^+), 177.0528; found, 177.0529. ^1H NMR spectral data for **17** is in agreement with that reported previously.²⁸

4.15. (*E*)-8,8-Diethoxy-5-oxooct-6-enoic acid (**3b**)

The acid **3b** was prepared using a solution of ester **14b** (118.5 mg, 0.45 mmol) in PBS (pH 7.4, 50 mM, 8 mL) and methanol (1 mL) to increase solubility at room temperature. Porcine pancreatic lipase (PPL, type II, crude, 100 mg), then sodium chloride (20 mg) and calcium chloride (40 mg) were added. The pH of the reaction mixture was maintained at 7.0–7.2 by addition of sodium hydroxide solution (0.1 *N*). After completion, solvents were evaporated under reduced pressure, the residue extracted with chloroform:methanol (2:1, v/v), filtered, and dried using anhydrous magnesium sulfate. Solvents were evaporated from the combined

organic extracts under reduced pressure and the residue purified by flash chromatography on a silica gel column (chloroform/methanol = 20:1) to give **3b** (65 mg, 58%). TLC (chloroform/methanol/H₂O = 80:19:1, *R_f* = 0.51): ¹H NMR (400 MHz, CDCl₃) δ 6.65 (dd, *J* = 16.2, 4 Hz, 1H), 6.34 (dd, *J* = 16, 1.2 Hz, 1H), 5.05 (dd, *J* = 4.2, 1.6 Hz, 1H), 3.63–3.69 (m, 2H), 3.50–3.56 (m, 2H), 2.69 (t, *J* = 7.2 Hz, 2H), 2.43 (t, *J* = 6.8 Hz, 2H), 1.95 (tt, *J* = 7.2, 7.2 Hz, 2H), 1.24 (t, *J* = 7.2 Hz, 6H). ¹³C NMR (100 MHz, CDCl₃) 199.7, 179.2, 141.6, 131.6, 99.7, 61.8, 39.3, 33.2, 18.8, 15.4. HRMS (FAB): *m/z* calcd for C₁₂H₂₁O₅ (MH⁺), 245.1389; found, 245.1382.

4.16. (*E*)-2-(8,8-Diethoxy-5-oxooct-6-enoyl)-1-palmitoyl-*sn*-glycero-3-phosphatidylcholine (**18b**)

1-Methylimidazole (11.5 μL, 0.14 mmol) and 2,6-dichlorobenzoyl chloride (22.7 μL, 0.16 mmol) were added to a solution of acid **3b** (31.7 mg, 0.16 mmol) and L-2-lysophosphatidylcholine (23.5 mg, 96 μmol) in dry methylene chloride (4 mL). The resulting mixture was stirred for 17 h at room temperature and monitored by TLC for disappearance of the starting material. Solvents were then evaporated under reduced pressure and the residue purified by flash chromatography on a silica gel column (chloroform/methanol/H₂O = 16:9:1, TLC: *R_f* = 0.34) to give **18b** (25.6 mg, 74%). ¹H NMR (400 MHz, CD₃OD:CDCl₃ = 1:2) δ 6.64 (dd, *J* = 16.2, 4.0 Hz, 1H), 6.33 (dd, *J* = 16.0, 1.2, 1H), 5.21 (m, 1H), 5.04 (m, 1H), 4.39 (m, 1H), 4.25 (m, 2H), 4.13 (dd, *J* = 12.0, 5.2 Hz, 2H), 4.00 (m, 2H), 3.61–3.69 (4H), 3.49–3.57 (m, 2H), 3.30 (bs, 9H), 2.70 (t, *J* = 6.8 Hz, 2H), 2.37 (t, *J* = 6.8 Hz, 2H), 2.29 (t, *J* = 7.2 Hz, 2H), 1.89 (m, 2H), 1.57 (m, 2H), 1.22 (30H), 0.85 (t, *J* = 6.8 Hz, 3H). ¹³C NMR (100 MHz, CD₃OD:CDCl₃ = 1:2) 200.5, 174.1, 172.9, 141.8, 131.3, 99.8, 70.8, 66.5, 63.9, 62.6, 61.9, 59.3, 54.0, 39.2, 34.1, 33.2, 32.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 24.9, 22.7, 18.9, 14.9, 13.8. HRMS (FAB): *m/z* calcd for C₃₃H₆₉NO₁₁P (MH⁺), 722.4608; found, 722.4589.

4.17. (*E*)-2-(5-Hydroxy-8,8-diethoxyoct-6-enoyl)-1-palmitoyl-*sn*-glycero-3-phosphatidylcholine (**2b**)

Sodium borohydride (1.9 mg, 50 μmol) was slowly added at 4 °C to a stirred solution of ester **18b** (33.4 mg, 46 μmol) in dry methylene chloride (1 mL) and methanol (2 mL). Once complete, the reaction mixture was neutralized with acetic acid, solvents were evaporated under reduced pressure, and the residue purified by flash chromatography on a silica gel column (chloroform/methanol/H₂O = 11:9:1, TLC: *R_f* = 0.22) to give **2b** (32.8 mg, 98%). ¹H NMR (400 MHz, CD₃OD:CDCl₃ = 1:2) δ 5.81 (dd, *J* = 15.6, 6 Hz, 1H), 5.62 (dd, *J* = 15.6, 6 Hz, 1H), 5.21 (m, 1H), 4.86 (d, 1H), 4.37 (m, 1H), 4.22 (m, 2H), 4.13 (dd, *J* = 12.4, 7.2 Hz, 2H), 4.07 (m, 1H), 3.64 (m, 2H), 3.47–3.59 (4H), 3.16 (bs, 9H), 2.27–2.36 (4H), 1.68 (m, 2H), 1.52–1.57 (4H), 1.16–1.26 (30H), 0.85 (t, *J* = 6.8 Hz, 3H). ¹³C NMR (100 MHz, CD₃OD:CDCl₃ = 1:2) 174.1, 173.4, 137.1, 127.3, 101.6, 70.8, 70.6, 66.5, 63.8, 62.6, 61.5, 59.2, 54.0, 36.3, 36.2, 34.0, 33.9, 32.0, 29.7, 29.5, 29.4, 29.3, 29.2, 24.9, 22.7, 20.9, 14.9, 13.9. HRMS (FAB): *m/z* calcd for C₃₆H₇₀NNaO₁₁P (MNa⁺), 746.4584; found, 746.4584.

4.18. (*E*)-2-(5-Hydroxy-8-oxooct-6-enoyl)-1-palmitoyl-*sn*-glycero-3-phosphatidylcholine (HOOA-PC, **1b**)

PPTS (4.6 mg, 18.4 μmol) was added to a stirred solution of ester **2b** (11.1 mg, 15.3 μmol) in tetrahydrofuran/acetone/H₂O = 5:4:1 (2 mL), the resulting mixture was stirred for 4 h at room temperature, and monitored by TLC for disappearance of starting material. Upon completion, solvents were evaporated under reduced pressure and the residue purified by flash chromatography on a silica gel column (chloroform/methanol/H₂O = 11:9:1, TLC: *R_f* = 0.18) to give **1b** (8.4 mg, 85%). ¹H NMR (400 MHz, CD₃OD:CDCl₃ = 1:2) δ 9.53 (d, *J* = 8 Hz, 1H), δ 6.93 (dd, *J* = 15.6, 4.4 Hz, 1H), 6.29 (ddd, *J* = 15.6, 8, 1.6 Hz, 1H), 5.21 (m,

1H), 4.36 (m, 2H), 4.11–4.26 (4H), 3.99 (m, 2H), 3.58 (m, 2H), 3.30 (s, 9H), 2.39 (m, 2H), 2.31 (m, 2H), 1.57–1.77 (6H), 1.57 (m, 2H), 1.26 (24H), 0.85 (t, $J = 6.8$ Hz, 3H). HRMS (FAB): m/z calcd for $C_{32}H_{61}NO_{10}P$ (MH^+), 650.4033; found, 650.4030.

4.19. Methyl 9-(chlorocarbonyl)octanoate (5c)

Monomethyl azelate (500 mg, 2.4 mmol) was added dropwise via syringe to a solution of oxalyl chloride (300 mg, 3.5 mmol, 1.5 eq) in benzene (3 mL). The reaction mixture was stirred for 30 min at room temperature and then refluxed for 2 h, after which time no acid starting material was detected by IR. Upon completion, the solvent was evaporated under reduced pressure and the residue purified by vacuum distillation to give **5c** (491 mg, 90%). IR (film, cm^{-1}) 2943, 2861, 1811, 1733, 1462, 1434, 1251, 1205. 1H NMR (400 MHz, $CDCl_3$) δ 3.66 (s, 3H), δ 2.87 (m, 2H), 2.30 (m, 2H), 1.65 (m, 2H), 1.60 (m, 2H), 1.35 (6H).

4.20. Methyl (E)-12,12-diethoxy-9-oxododec-10-enoate (14c)

The procedure was modified from that reported.²¹ 1-Tributylstannyl-3,3-diethoxy-prop-1-ene (**4**, 380 mg, 0.9 mmol) was added dropwise via syringe to a solution of acyl chloride **5c** (200 mg, 0.9 mmol) and $PdCl_2(MeCN)_2$ (2.3 mg, 9 μ mol) in DMF (1.5 mL) and the reaction mixture stirred first for 1 h in an ice bath and then 3 h at room temperature. Saturated aqueous sodium fluoride and acetone were added to the brown-red reaction mixture to remove tributyltin chloride. The mixture was extracted with ethyl acetate (3×20 mL), followed by drying of the combined extracts over anhydrous sodium sulfate. Solvents were evaporated from the organic extracts under reduced pressure and the residue purified by flash chromatography on a silica gel column (ethyl acetate/hexanes = 7:93) to give **14c** (171 mg, 60%). TLC (30% ethyl acetate/hexanes; R_f = 0.51). 1H NMR (400 MHz, $CDCl_3$) δ 6.62 (dd, $J = 16.1, 4.3$ Hz, 1H), 6.33 (dd, $J = 16.1, 1.3$ Hz, 1H), 5.04 (dd, $J = 4.3, 1.3$ Hz, 1H), 3.76–3.59 (m, 5H), 3.59–3.43 (m, 2H), 2.64 (t, $J = 6.6$ Hz, 2H), 2.34 (t, $J = 7.2$ Hz, 2H), 1.92 (p, $J = 7.2$ Hz, 2H), 1.20 (t, $J = 7.2$ Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) 200.8, 174.5, 141.1, 131.8, 99.8, 61.7, 51.7, 40.6, 34.3, 29.2, 25.09, 24.06, 15.54, 15.43. HRMS (FAB): m/z calcd for $C_{17}H_{31}O_5$ (MH^+), 315.2171; found, 315.2175.

4.21. (E)-12,12-Diethoxy-9-oxododec-10-enoic acid (3c)

The acid was prepared using a solution of ester **14c** (88.7 mg, 0.28 mmol) in PBS (pH 7.4, 50 mM, 8 mL) and methanol (1 mL) to increase solubility at room temperature. Porcine pancreatic lipase (type II, crude, 100 mg), sodium chloride (20 mg), and calcium chloride (40 mg) were added. The pH of the reaction mixture was maintained at 7.0–7.2 by addition of sodium hydroxide solution (0.1 *N*). Upon completion, solvents were evaporated under reduced pressure, the residue extracted with chloroform/methanol (2:1, v/v), filtered, and dried with anhydrous magnesium sulfate. Solvents were evaporated from the combined organic extracts under reduced pressure and the residue purified by flash chromatography on a silica gel column (chloroform/methanol = 15:1) to give **3c** (56 mg, 67%). TLC (chloroform/methanol/ H_2O = 80:19:1, R_f = 0.70): 1H NMR (400 MHz, $CDCl_3$) δ 6.61 (dd, $J = 16, 4.4$ Hz, 1H), 6.33 (dd, $J = 16, 1.2$ Hz, 1H), 5.04 (dd, $J = 4.4, 1.2$ Hz, 1H), 3.65–3.66 (m, 2H), 3.50–3.56 (m, 2H), 2.56 (t, $J = 7.6$ Hz, 2H), 2.33 (t, $J = 7.2$ Hz, 2H), 1.60 (m, 2H), 1.30 (6H), 1.22 (6H). ^{13}C NMR (100 MHz, $CDCl_3$) 200.9, 179.8, 141.2, 131.8, 99.8, 61.7, 40.5, 34.2, 29.2, 29.1, 29.0, 24.8, 24.1, 15.4. HRMS (FAB): m/z calcd for $C_{16}H_{28}O_5$ (M^+), 300.1937; found, 300.1930.

4.22. (E)-2-(12,12-Diethoxy-9-oxododec-10-enoyl)-1-palmitoyl-*sn*-glycero-3-phosphatidylcholine (18c)

1-Methylimidazole (10.6 μ L, 0.13 mmol) and 2,6-dichlorobenzoyl chloride (20.9 μ L, 0.14 mmol) were added to a solution of acid **3c** (20 mg, 0.06 mmol) and L- α -

lysophosphatidylcholine (22 mg, 0.04 mmol) in dry methylene chloride (4 mL). The resulting mixture was stirred for 24 h at room temperature and monitored by TLC for disappearance of starting material. The mixture was evaporated under reduced pressure and the residue purified by flash chromatography on a silica gel column (chloroform/methanol/H₂O = 16:9:1, TLC: R_f = 0.34) to give **18c** (24.5 mg, 71%). ¹H NMR (400 MHz, CD₃OD:CDCl₃ = 1:2) δ 6.61 (dd, J = 16.1, 4.4 Hz, 1H), 6.32 (dd, J = 16.1, 1.2 Hz, 1H), 5.20 (m, 1H), 5.04 (m, 1H), 4.39 (m, 1H), 4.23 (dt, J = 11.8, 6.0 Hz, 2H), 4.13 (dd, J = 11.9, 6.8 Hz, 1H), 3.95 (m, 2H), 3.72–3.46 (6H), 3.26 (bs, 9H), 2.59 (t, J = 7.4 Hz, 2H), 2.30 (4H), 1.58 (6H), 1.33–1.09 (36H), 0.85 (t, J = 6.8 Hz, 3H). ¹³C NMR (100 MHz, CD₃OD:CDCl₃ = 1:2) 200.9, 174.1, 173.6, 141.5, 141.1, 131.9, 99.8, 68.3, 67.0, 62.6, 62.1, 61.9, 57.5, 54.0, 40.5, 35.6, 34.2, 34.1, 32.0, 29.7, 29.6, 29.4, 29.3, 29.1, 29.0, 28.9, 24.9, 24.8, 23.9, 22.7, 17.7, 14.9, 13.9. HRMS (FAB): m/z calcd for C₄₀H₇₇NO₁₁P(M⁺), 778.5229; found, 778.5124.

4.23. (E)-2-(9-Hydroxy-12,12-diethoxydodec-10-enoyl)-1-palmitoyl-*sn*-glycero-3-phosphatidylcholine (**2c**)

Sodium borohydride (1.4 mg, 37 μ mol) was slowly added at 4 °C to a stirred solution of ester **18c** (24.5 mg, 31.4 μ mol) in dry methylene chloride (1 mL) and methanol (2 mL). Upon completion, the reaction mixture was neutralized with glacial acetic acid. Solvents were evaporated under reduced pressure, and residue purified by flash chromatography on a silica gel column (chloroform/methanol/H₂O = 11:9:1, TLC: R_f = 0.30) to give **2c** (20.5 mg, 87%). ¹H NMR (400 MHz, CD₃OD:CDCl₃ = 1:2) δ 5.80 (dd, J = 15.6, 6.4 Hz, 1H), 5.60 (dd, J = 15.6, 6.4 Hz, 1H), 5.21 (m, 1H), 4.86 (m, 1H), 4.41 (m, 1H), 4.26 (m, 2H), 4.13 (dd, J = 12, 6.8 Hz, 1H), 4.04 (m, 1H), 3.96 (m, 2H), 3.71–3.41 (6H), 3.18 (bs, 9H), 2.27–2.33 (4H), 1.58 (8H), 1.16–1.29 (36H), 0.85 (t, J = 6.8 Hz, 3H). ¹³C NMR (100 MHz, CD₃OD:CDCl₃ = 1:2) 174.1, 173.6, 137.6, 127.0, 101.7, 71.4, 70.5, 66.5, 63.8, 62.7, 61.4, 59.2, 54.0, 40.3, 37.1, 34.2, 34.1, 32.0, 29.7, 29.6, 29.4, 29.3, 29.1, 29.0, 28.9, 24.9, 24.8, 22.7, 15.0, 14.9, 13.9. HRMS (FAB): m/z calcd for C₄₀H₇₈NNaO₁₁P(MNa⁺), 802.5210; found, 802.5217.

4.24. (E)-(9-Hydroxy-12-oxododec-10-enoyl)-1-palmitoyl-*sn*-glycero-3-phosphatidylcholine (HODA-PC, **1c**)

PPTS (5.6 mg, 22.2 μ mol) was added to a stirred solution of ester **2c** (14.5 mg, 18.6 μ mol) in tetrahydrofuran/acetone/H₂O = 5:4:1 (2 mL). The resulting mixture was stirred for 5 h at room temperature and monitored by TLC for disappearance of starting material. Upon completion, solvents were evaporated under reduced pressure and the residue purified by flash chromatography on a silica gel column (chloroform/methanol/H₂O = 11:9:1, TLC: R_f = 0.4) to give **1c** (11.8 mg, 90%). ¹H NMR (400 MHz, CD₃OD + CDCl₃) δ 9.52 (d, J = 8 Hz, 1H), 6.91 (dd, J = 15.6, 4.8 Hz, 1H), 6.26 (ddd, J = 15.6, 8, 1.6 Hz, 1H), 5.20 (m, 1H), 4.38 (m, 1H), 4.31 (m, 1H), 4.22 (m, 2H), 4.14 (m, 1H), 3.97 (m, 2H), 3.58 (m, 2H), 3.30 (s, 9H), 2.27–2.33 (4H), 1.57 (7H), 1.23–1.31 (31H), 0.85 (t, J = 6.8 Hz, 3H). HRMS (FAB): m/z calcd for C₃₆H₆₇NO₉P(MH⁺ - H₂O), 688.4548; found, 688.4583.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful for support of this work by National Institutes of Health Grants RO1-GM021249, RO1-EY016813, and RO1-HL053315.

References

- (1). Gu X, Sun M, Gugiu B, Hazen S, Crabb JW, Salomon RG. *J Org Chem*. 2003; 68:3749–61. [PubMed: 12737551]
- (2). Sun M, Deng Y, Batyreva E, Sha W, Salomon RG. *J Org Chem*. 2002; 67:3575–84. [PubMed: 12027667]
- (3). Deng Y, Salomon RG. *J Org Chem*. 1998; 63:7789–94.
- (4). Kaur K, Salomon RG, O'Neil J, Hoff HF. *Chem Res Toxicol*. 1997; 10:1387–96. [PubMed: 9437530]
- (5). Sayre LM, Sha W, Xu G, Kaur K, Nadkarni D, Subbanagounder G, Salomon RG. *Chem Res Toxicol*. 1996; 9:1194–201. [PubMed: 8902276]
- (6). Podrez EA, Poliakov E, Shen Z, Zhang R, Deng Y, Sun M, Finton PJ, Shan L, Febbraio M, Hajjar DP, Silverstein RL, Hoff HF, Salomon RG, Hazen SL. *J Biol Chem*. 2002; 277:38517–23. [PubMed: 12145296]
- (7). Podrez EA, Poliakov E, Shen Z, Zhang R, Deng Y, Sun M, Finton PJ, Shan L, Gugiu B, Fox PL, Hoff HF, Salomon RG, Hazen SL. *J Biol Chem*. 2002; 277:38503–16. [PubMed: 12105195]
- (8). Podrez EA, Byzova TV, Febbraio M, Salomon RG, Ma Y, Valiyaveetil M, Poliakov E, Sun M, Finton PJ, Curtis BR, Chen J, Zhang R, Silverstein RL, Hazen SL. *Nat Med*. 2007; 13:1086–95. [PubMed: 17721545]
- (9). Ashraf MZ, Kar NS, Chen X, Choi J, Salomon RG, Febbraio M, Podrez EA. *J Biol Chem*. 2008; 283:10408–14. [PubMed: 18285332]
- (10). Subbanagounder G, Deng Y, Borromeo C, Dooley AN, Berliner JA, Salomon RG. *Vascul Pharmacol*. 2002; 38:201–9. [PubMed: 12449016]
- (11). Watson AD, Leitinger N, Navab M, Faull KF, Horkko S, Witztum JL, Palinski W, Schwenke D, Salomon RG, Sha W, Subbanagounder G, Fogelman AM, Berliner JA. *J Biol Chem*. 1997; 272:13597–607. [PubMed: 9153208]
- (12). Leitinger N, Tyner TR, Oslund L, Rizza C, Subbanagounder G, Lee H, Shih PT, Mackman N, Tigyi G, Territo MC, Berliner JA, Vora DK. *Proc Natl Acad Sci U S A*. 1999; 96:12010–5. [PubMed: 10518567]
- (13). Lee H, Shi W, Tontonoz P, Wang S, Subbanagounder G, Hedrick CC, Hama S, Borromeo C, Evans RM, Berliner JA, Nagy L. *Circ Res*. 2000; 87:516–21. [PubMed: 10988245]
- (14). Subbanagounder G, Watson AD, Berliner JA. *Free Radic Biol Med*. 2000; 28:1751–61. [PubMed: 10946217]
- (15). Hoff HF, O'Neil J, Wu Z, Hoppe G, Salomon RL. *Arterioscler Thromb Vasc Biol*. 2003; 23:275–82. [PubMed: 12588771]
- (16). Hollyfield JG, Bonilha VL, Rayborn ME, Yang X, Shadrach KG, Lu L, Ufret RL, Salomon RG, Perez VL. *Nat Med*. 2008; 14:194–8. [PubMed: 18223656]
- (17). Kobayashi Y, Kishihara K, Watatani K. *Tetrahedron Lett*. 1996; 37:4385–4388.
- (18). Taylor EC, Chiang C-S. *Synthesis*. 1977:467.
- (19). Beaudet I, Parrain J, Quintard J. *Tetrahedron Lett*. 1991; 32:6333–6336.
- (20). Lipshutz BH, Ellsworth EL, Dimock SH, Reuter DC. *Tetrahedron Lett*. 1989; 30:2065–2068.
- (21). Parrain J, Beaudet I, Duchene A, Watrelot S, Quintard J. *Tetrahedron Lett*. 1993; 34:5445–48.
- (22). Rodriguez A, Nomen M, Spur B, Godfroid J. *Eur. J. Org. Chem*. 1999:2655–62.
- (23). Acharya HP, Kobayashi Y. *Synlett*. 2005; 13:2015–18.
- (24). Gaffney PR, Reese CB. *J. Chem. Soc., Perkin Trans*. 2001; 1:192–205.
- (25). Abell AD, Morris KB, Litten JC. *J. Org. Chem*. 1990; 55:5217–21.
- (26). Micovic IV, Ivanovic MD, Piatak DM. *J. Serb. Chem. Soc*. 1988; 53:419–26.
- (27). Uno T, Ku J, Prudent JR, Huang A, Schultz PG. *J. Am. Chem. Soc*. 1996; 118:3811–17.
- (28). Yeh MP, Chuang L, Hsieh Y, Tsai M. *Chem. Commun*. 1999:805–06.
- (29). Gu X, Zhang W, Salomon RG. *J. Am. Chem. Soc*. 2007; 129:6088–89. [PubMed: 17441719]
- (30). Parrain J, Duchene A, Quintard J. *J. Chem. Soc., Perkin Trans*. 1990; 1:187–89.

Scheme 1.

Oxidation of polyunsaturated fatty acids generates γ -hydroxy- α,β -unsaturated aldehydes, that react with proteins and form 2-(ω -carboxylalkyl)pyrroles.

Scheme 2.
Retrosyntheses of γ -hydroxyalkenal phospholipids.

Scheme 3.

Synthesis of HOHA ethyl ester. Reagents and conditions: (a) NBS, THF/acetone/ H_2O (5:4:1), pyridine; (b) K 10 / $\text{CH}(\text{OCH}_3)_3$; (c) NaBH_4 , MeOH; (d) PPTS, acetone/ H_2O (2:1); (e) Amberlyst-15, acetone/ H_2O (2:1), 67% or TFA/ H_2O (95:5), rt, 61%

Scheme 4.

Synthesis of HOOA methyl ester. Reagents and conditions: (a) $\text{Bu}_3\text{SnCu}(\text{Bu})\text{CNLi}_2$, THF, $-78\text{ }^\circ\text{C}$, then H_2O ; (b) $\text{PdCl}_2(\text{MeCN})_2$ (1 mol%), DMF, $0\text{ }^\circ\text{C}$; (c) NaBH_4 , EtOH, $0\text{ }^\circ\text{C}$; (d) PPTS, acetone/ H_2O (2:1), rt; (e) Amberlyst 15, TFA / H_2O (95:5), rt.

Scheme 5. Synthesis of HOHA-PC

Reagents and conditions: (a) porcine pancreatic lipase, phosphate buffer; (b) L- α -lysophosphatidylcholine (HO-PC), 2,6-dichlorobenzoyl chloride, 1-methylimidazole, CH_2Cl_2 ; (c) NaBH_4 , MeOH, (d) PPTS, THF/acetone/ H_2O (5:4:1).

Scheme 6. Syntheses of HOOA and HODA-PCs

Reagents and conditions: (a) oxalyl chloride, benzene; (b) $\text{PdCl}_2(\text{MeCN})_2$ (1 mol%), DMF, 0 °C; (c) porcine pancreatin lipase, PBS; (d) L- α -lysophosphatidylcholine (HO-PC), 2,6-dichlorobenzoyl chloride, 1-methylimidazole, CH_2Cl_2 ; (e) NaBH_4 , MeOH; (f) PPTS, THF/acetone/ H_2O (5:4:1)

Scheme 7.

Lactone formation is promoted by strong acid.