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Oxidatively Truncated Docosahexaenoate Phospholipids: Total Synthesis, Generation, and Peptide Adduction Chemistry

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The recent immunological detection of extraordinarily high levels of carboxyethylpyrrole (CEP) modifications of proteins from the retinas of individuals with age-related macular degeneration provided presumptive evidence for the involvement of docosahexaenoate-derived oxidatively truncated phospholipids in retinal pathology. To facilitate the in vivo detection and characterization of the chemistry and biological activities of these postulated naturally occurring molecules, a family of oxidatively truncated phospholipids was prepared by total syntheses. Their formation in oxidation reactions of a docosahexaenoate ester of 2-lysophosphatidylcholine (DHA-PC) was also demonstrated. Free radical-induced oxidative cleavage of DHA-PC promoted by myeloperoxidase or copper ions generates similar mixtures of these phospholipids. The most abundant products were 1-palmitoyl-2-succinoyl-*sn*-glycero-3-phosphatidylcholine (4.7%) and 2-(6-carboxy-4-oxohex-5-enoyl)-1-palmitoyl-*sn*-glycero-3-phosphatidylcholine (1.7%). Both of these oxidatively truncated phospholipids are homologues of biologically active arachidonate-derived phospholipids. A minor product from DHA-PC, 2-(4-hydroxy-7-oxohept-5-enoyl)-1-palmitoyl-*sn*-glycero-3-phosphatidylcholine (0.4% yield), reacted with the ϵ-amino group of a peptide lysyl residue to produce a CEP derivative in 0.7% yield. These observations support the previous conclusion, based on immunological evidence, that CEPs are generated by the reaction of an oxidatively truncated phospholipid with proteins in the retina and further indicate that CEP protein modifications probably represent only a tiny fraction of the products generated upon oxidative damage of DHA-PC in photoreceptor disk membranes.

Introduction

Retinal membrane disks, stacked like coins in photoreceptor rod outer segments (ROS), are rich in phospholipids containing docosahexaenoic acid (DHA). Approximately 80 mol % of the polyunsaturated fatty acids in mammalian ROS are DHA.[1] The ROS tips, containing the oldest disks, are clipped off and digested in lysosomes of the adjacent retinal pigmented epithelial cells, while newly minted disks are added to the other end of the stack. The entire stack of disks is replaced every 10 days[2−4] probably, in part, because of the high oxidizability of DHA and the oxygen-rich environment of the retina, in a process that combats photoinitiated[5−8] free-radical-induced lipid oxidation. We are examining the hypothesis that retinal diseases, such as age-related macular degeneration, may be promoted by the failure of cellular systems which prevent or repair oxidative damage to photoreceptor membrane lipids. Oxidative modifications of proteins caused by the adduction of electrophilic oxidatively truncated phospholipids could exacerbate cellular dysfunction, leading to retinal pathologies. Previous studies demonstrated that 2-substituted pyrrole modifications of proteins can be formed by reactions of γ-hydroxy-α,β-unsaturated aldehydes.[9,10] This led us to postulate that a 2-(ω-carboxyethyl)pyrrole (CEP) derivative of proteins might originate from carboxylic phospholipid esters containing γ-hydroxy-α,β-unsaturated aldehyde functionality that are generated by oxidative cleavage of DHA phospholipids (Scheme 1).

To facilitate investigations of the possible role of oxidatively truncated docosahexaenoate phospholipids in disease, we now report total syntheses of DHA phospho-

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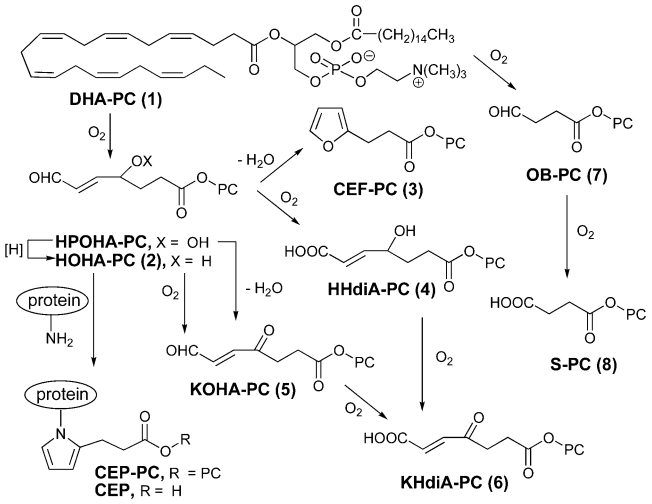
SCHEME 1

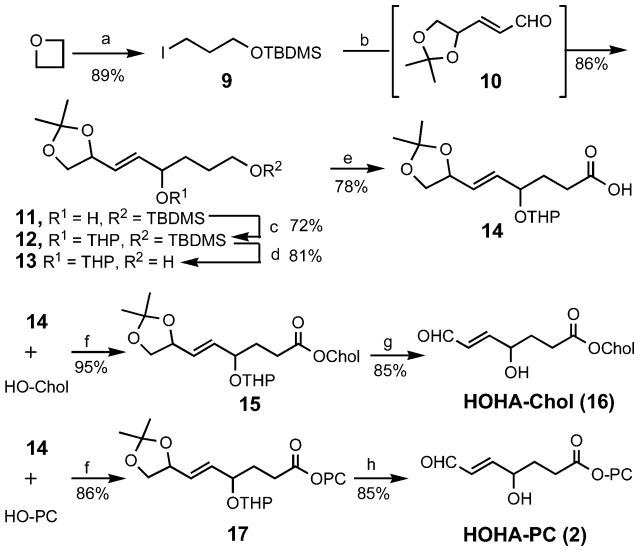


lipid oxidation products **2**−**8** (Scheme 1). Analogy with the chemistry of arachidonate and linoleate phospholipids suggests the likely formation of **2**−**8** from DHA-PC (**1**), as well as their potential for modifying proteins through covalent adduction[10] and for affecting cell-signaling pathways through receptor recognition.[11−14] We also now show that oxidatively truncated phospholipids are generated during free-radical-induced oxidation of liposomes containing the DHA ester of 2-lysophosphatidylcholine (DHA-PC, **1**). Similar product evolution profiles were found for two different free-radical-initiating systems, myeloperoxidase (MPO)[13]/H_2O_2/NO_2^- and copper(II). Finally, as a prelude to exploring in vivo protein adduction, we demonstrate that the DHA-PC oxidation product 2-(4-hydroxy-7-oxohept-5-enoyl)-1-palmitoyl-*sn*-glycero-3-phosphatidylcholine (HOHA-PC, **2**) reacts with the ϵ-amino group of a peptide lysyl residue to form various adducts, including a CEP derivative.

Results and Discussion

Syntheses of Oxidatively Truncated DHA-PC Lipids. Synthesis of HOHA Esters. Esters of 4-hydroxy-7-oxohept-5-enoic acid (HOHA) with 2-lysophosphatidylcholine (HO-PC) and cholesterol (HO-Chol) were prepared (Scheme 2). An intermediate **11**, which contains the carbon skeleton of HOHA with a 3,3-dimethyl-2,4-dioxolanyl moiety as a latent aldehyde,[15] was generated by 1,2-addition of a Grignard reagent, prepared from a TBDMS ether **9** of 3-iodopropanol, with aldehyde **10**[15] (Scheme 2). The starting 3-iodopropanol TBDMS ether **9** is readily available in a single step from trimethylene oxide by treatment with NaI and TBDMSCl. The secondary allylic alcohol **11** was protected as tetrahydropyranyl (THP) ether **12**. After desilyation, the primary alcohol **13** was obtained. Oxidation of the alcohol with pyridinium dichromate (PDC) delivered latent carboxyaldehydic acid **14** in 78% yield.

SCHEME 2[a]



[a] Key: (a) NaI, TBDMSCl, $CaCO_3$, THF; (b) Mg, Et_2O; (c) DHP, PPTS, CH_2Cl_2; (d) Bu_4NF, THF; (e) PDC, DMF; (f) DCC, DMAP, $CHCl_3$; (g) $NaIO_4$, AcOH, H_2O; (h) AcOH/H_2O, 40 °C then $Pb(OAc)_4$, −78 °C.

Esterification of acid **14** with HO-Chol or HO-PC provided air-stable precursors **15** or **17**, respectively, from which the unstable (vide infra) target aldehydes HOHA-Chol (**16**) or HOHA-PC (**2**) can be freshly generated as needed. Exposure of the ester **15** to aqueous acetic acid and periodate generated the desired HOHA-Chol (**16**) in one step. An alternative method was employed to prepare HOHA-PC (**2**). The acetonide **17** was hydrolyzed in aqueous acetic acid. After evaporation of the aqueous acetic acid, oxidative cleavage of the resulting vicinal diol intermediate with lead tetraacetate at −78 °C delivered HOHA-PC (**2**) in 85% yield.

Syntheses of Oxidatively Truncated Phospholipids 3−**8.** Keto aldehyde KOHA-PC (**5**) was prepared in 65% yield by oxidative ring opening of carboxyethylfuryl PC (CEF-PC, **3**), which was obtained by esterification of HO-PC with 3-furan-2-ylpropionic acid (Scheme 3). Hydroxy acid HHdiA-PC (**4**) and keto acid KHdiA-PC (**6**) were prepared from the corresponding HOHA-PC (**2**) and KOHA-PC (**5**), respectively, by selective oxidation of the aldehyde functional groups.

The monoester of succinic acid S-PC (**8**) was obtained by esterification of HO-PC with succinic anhydride. 1-Palmitoyl-2-(4-oxobutyryl)-*sn*-glycero-3-phosphatidylcholine (**7**, OB-PC) was obtained by ozonolysis of 1-palmitoyl-2-(4-pentenoyl)-*sn*-glycero-3-phosphatidylcholine, which was produced by coupling of 2-lysophosphatidylcholine with 4-pentenoic acid (Scheme 3).

Generation of Oxidatively Truncated Phospholipids from DHA-PC. LC−**MS Detection of Products from DHA-PC Oxidation.** To demonstrate generation of oxidatively truncated phospholipids during the oxidation of DHA-PC, DHA-PC liposomes were exposed to air

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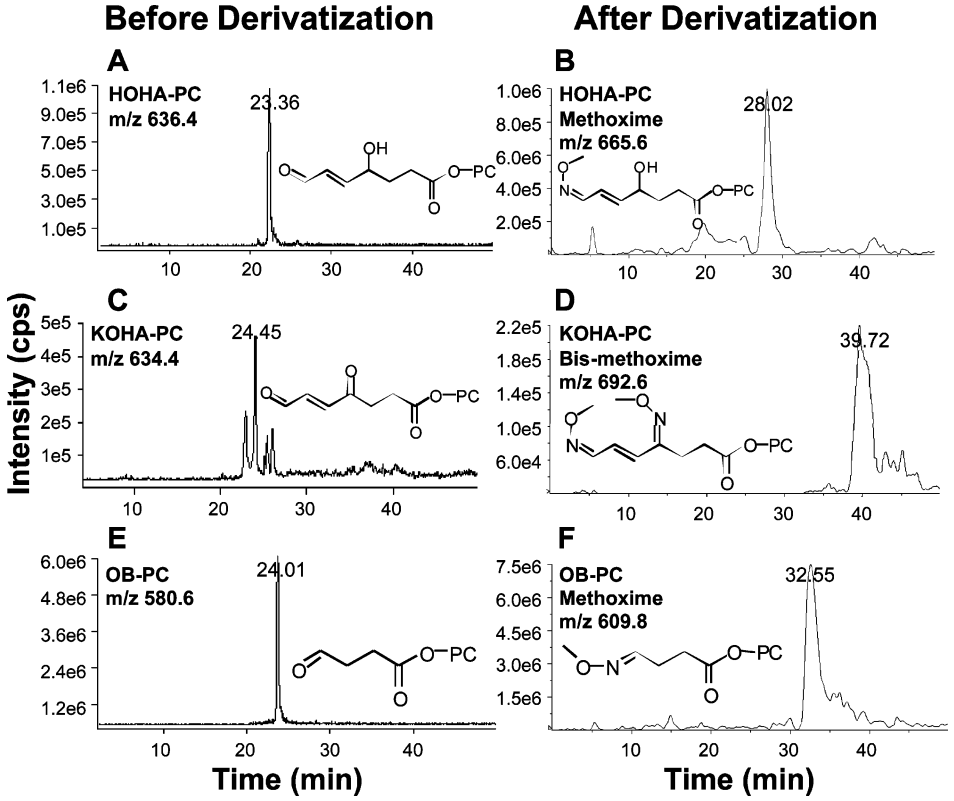
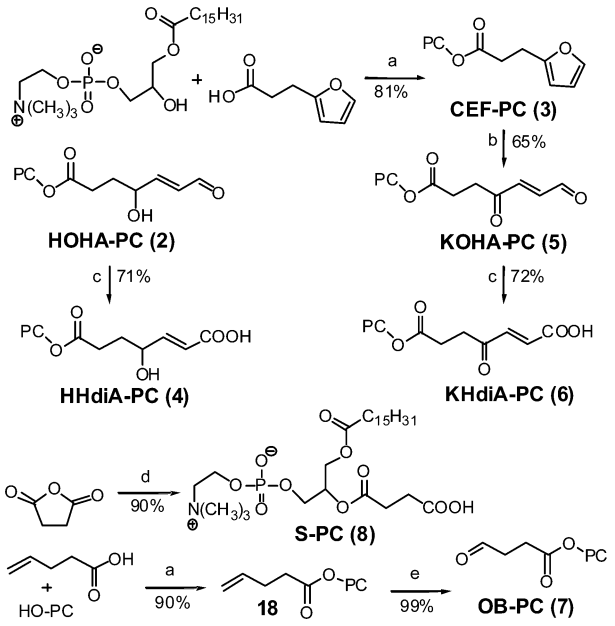


FIGURE 1. LC−MS analysis of lipid products from DHA-PC oxidation in the presence of the MPO/H_2O_2/NO_2^- system for 12 h: (A) HOHA-PC, *m*/*z* 636.4 ion extract chromatogram; (B) HOHA-PC methoxime, *m*/*z* 665.6 ion extract chromatogram; (C) KOHA-PC, *m*/*z* 634.4 ion extract chromatogram; (D) KOHA-PC methoxime, *m*/*z* 692.6 ion extract chromatogram; (E) OB-PC, *m*/*z* 580.4 ion extract chromatogram; (F) OB-PC methoxime, *m*/*z* 609.8 ion extract chromatogram.

SCHEME 3[a]



[a] Key: (a) DCC, DMAP, $CHCl_3$; (b) NBS, pyridine, acetone/THF/H_2O; (c) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH/H_2O; (d) HO-PC, DMAP, $CHCl_3$; (e) O_3/MeOH −60 °C then Me_2S/MeOH.

and either copper ions or myeloperoxidase (MPO). Lipid products were extracted into chloroform/methanol[16] from aliquots collected at various times. Reaction product mixtures were analyzed by LC−MS. Preliminary identification of each truncated phospholipid was provided by a peak in the ion extraction chromatogram with a specific mass of interest and a retention time comparable to the corresponding standard that was available from unambiguous total syntheses (Figures 1−3).

MPO-promoted oxidation of DHA-PC liposomes produced the anticipated oxidatively truncated lipids. A single peak with a retention time that is identical to standard is present in the ion extract chromatogram of HOHA-PC (*m*/*z* 636.4, Figure 1A), OB-PC (*m*/*z* 580.4, Figure 1E), and S-PC (*m*/*z* 596.4, Figure 2A). Although multiple peaks are present in the ion extract chromatogram of KOHA-PC (*m*/*z* 634.4, Figure 1C), KHdiA-PC (*m*/*z* 650.4, Figure 2C), and CEF-PC (*m*/*z* 618.4, Figure 3A), only the predominant peak has a retention time that is the same as the standard. The other peaks correspond to other, as yet unidentified, products with the same molecular weight as the compounds identified by HPLC comparison with authentic standards.

Derivatization of Oxidation Products. Additional analyses confirmed the identities of the oxidatively truncated phospholipids. The oxidation product mixtures from DHA-PC were treated with methoxylamine hydrochloride or a mixture of pentafluorobenzyl bromide (PFB-Br) and diisopropylethylamine to derivatize aldehyde, ketone, or carboxylic acid functionality. Methoxime derivatization of a single aldehyde or a ketone results in a net increase of 29 Da, while pentafluorobenzyl esterifi-

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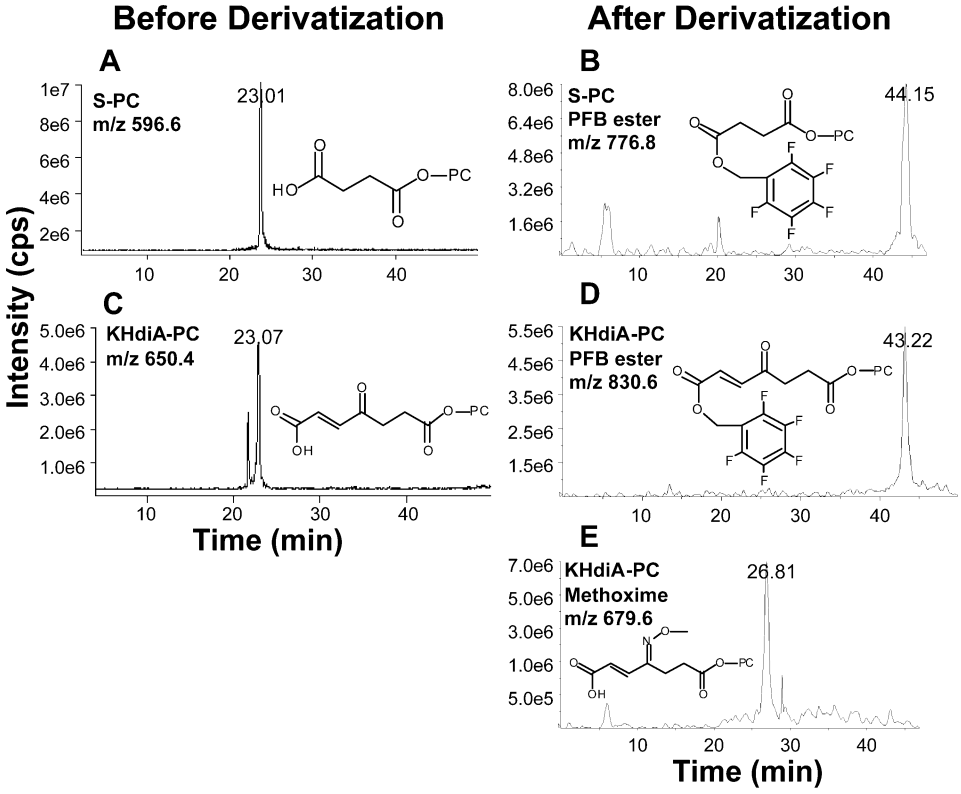


FIGURE 2. LC−MS analysis of lipid products from DHA-PC oxidation in the presence of the MPO/H_2O_2/NO_2^- system for 12 h: (A) S-PC, *m*/*z* 596.4 ion extract chromatogram; (B) S-PC PFB ester, *m*/*z* 776.8 ion extract chromatogram; (C) KHdiA-PC, *m*/*z* 650.4 ion extract chromatogram; (D) KHdiA-PC PFB ester, *m*/*z* 830.6 ion extract chromatogram; (E) KHdiA-PC methoxime, *m*/*z* 679.6 ion extract chromatogram.

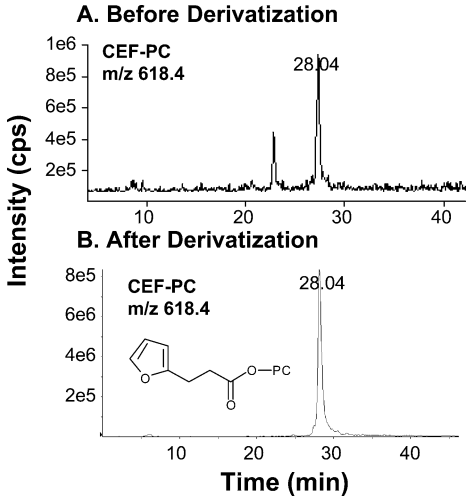


FIGURE 3. LC−MS analysis of lipid products from DHA-PC oxidation in the presence of MPO for 12 h: (A) CEF-PC, *m*/*z* 618.4 ion extract chromatogram from oxidation mixture before derivatization; (B) CEF-PC, *m*/*z* 618.4 ion extract chromatogram from oxidation mixture after derivatization.

cation of a carboxylic acid introduces a net increase of 180 Da. Methoxime derivatives of pure HOHA-PC, KOHA-PC, KHdiA-PC, and OB-PC and pentafluorobenzyl esters of pure KHdiA-PC and S-PC were also prepared to provide authentic samples for analyzing product mixtures from DHA-PC oxidation. Each derivative was purified using a C18 minicolumn and then subjected to LC−ES/MS analyses in the positive mode.

Representative ion extract chromatographs are shown in Figures 1−3. The predicted ions for methoxime derivatives of HOHA-PC and OB-PC are *m*/*z* 665.6, 609.6, which are consistent with the addition of a methoxime group (29 Da) to HOHA-PC (*m*/*z* 636.4) and OB-PC (*m*/*z* 580.4). The derivatization of KOHA-PC (*m*/*z* 634.4) was expected to introduce two methoxime groups (58 Da) to the lipid because of the presence of two carbonyl groups. Before the treatment with methoxylamine hydrochloride, no peaks were present at the same retention time as their corresponding derivatized standards in the *m*/*z* 665.6, 609.6, and 692.6 ion extract chromatographs (data not shown). However, after derivatization, intense new peaks appeared at the appropriate retention time in the *m*/*z* 665.6, 609.6, and 692.6 ion extract chromatographs (Figure 1B,D,F). In contrast, peaks representing underivatized lipids were lost (data not shown). Analyses following treatment with PFB-Br are shown in Figure 2B,D. Prior to derivatization, there were no peaks present at *m*/*z* 776.8 and 830.6, which are 180.2 Da greater than peaks corresponding to the putative KHdiA-PC and SA-PC (data not shown). However, after the reaction, intense peaks appeared at *m*/*z* 776.6 and 830.6 in the ion extract chromatographs (Figure 2B,D) with a concomitant loss of the peaks in the *m*/*z* 650.4 and 596.4 ion extract chromatographs (data not shown). These newly emerged peaks have the same retention times as their corresponding derivatized standards. In addition to being derivatized as a PFB ester, the generation of an ion with *m*/*z* 679.6 indicates that KHdiA-PC can also react with one molecule of methoxylamine (Figure 2E). This observation

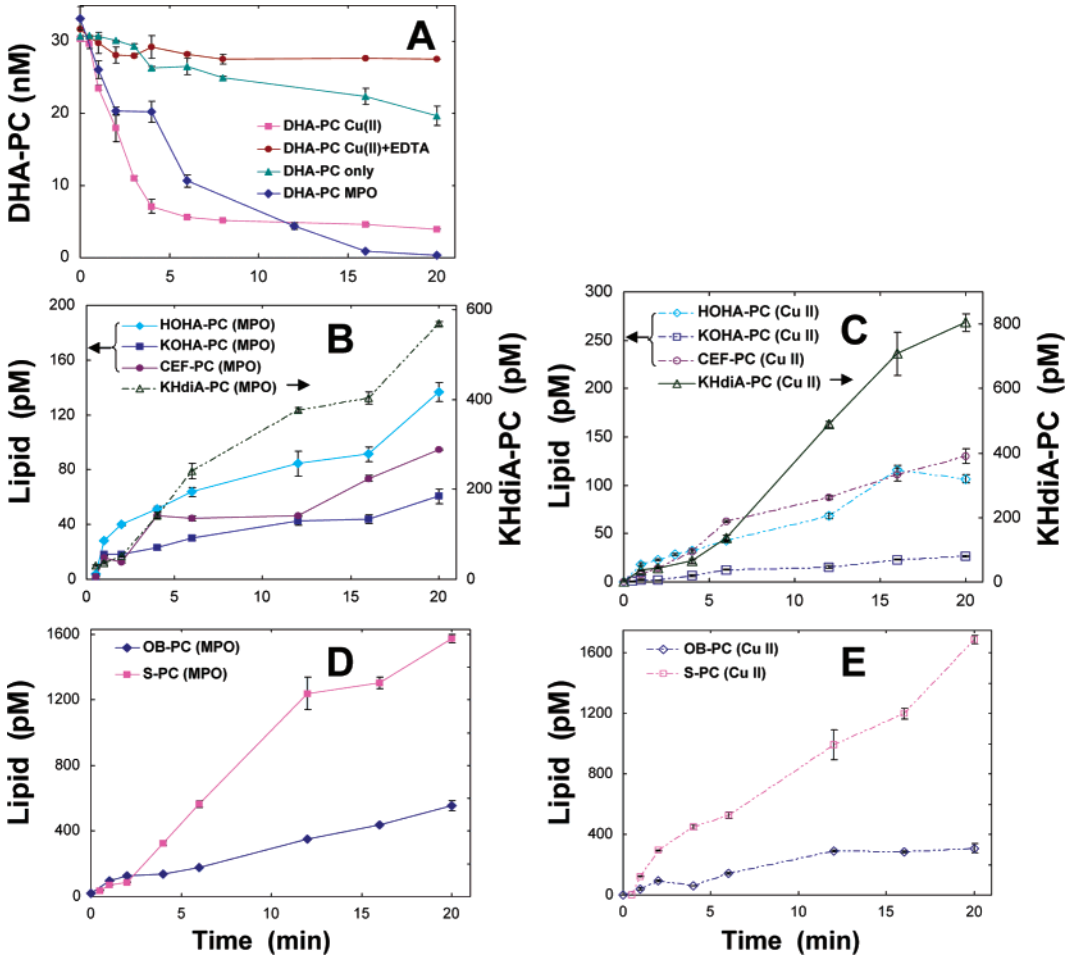


FIGURE 4. Evolution profiles of (A) DHA-PC oxidation at various conditions; (B) and (D) lipid fragmentations generated from MPO-initiated oxidation of DHA-PC; (C) and (E) lipid fragmentations generated from copper (II) initiated oxidation of DHA-PC.

is consistent with the fact that KHdiA-PC contains a carboxyl group as well as a ketone carbonyl. Because CEF-PC has no carboxylic acid, aldehyde, or ketone functional groups, peaks corresponding to CEF-PC in the *m*/*z* 618.4 ion extract chromatograph remained unchanged upon treatment with either methoxylamine or PFB-Br. Figure 3B shows the *m*/*z* 618.4 ion extract chromatograph of the reaction mixture after treatment with methoxylamine hydrochloride.

Oxidation Product Evolution Profiles. Quantification was achieved using the multiple reaction monitoring (MRM) mode for maximum sensitivity, by monitoring the precursor ions of interest (ions with *m*/*z* 636.4, 634.4, 650.4, 618.4, 580.4, and 596.4) in the first quadrupole and the common product ion with *m*/*z* 184.0 at the second analyzer (the third quadrupole). As internal standard, 1,2-dimyristoyl-*sn*-glycerol-3-phosphocholine was added prior to the reaction. The absolute amount of each compound was determined by integration of the peak area and comparison with that of the corresponding authentic standard of known concentration. Results are presented in Table 1 and Figure 4.

Oxidation of DHA-PC by copper or MPO gave rise to similar product evolution profiles. After 20 h of incubation, 99% of DHA-PC was consumed in the MPO-promoted oxidation while 87% of DHA-PC was consumed in the copper-promoted oxidation. Without MPO or Cu(II), the consumption of DHA-PC is much slower, 36% after 20 h. Oxidation promoted by copper can be inhibited by a metal chelator, EDTA. The evolution profiles for oxidized lipids are shown in Figure 4B−E. S-PC is the most abundant compound formed under both reaction conditions. The amount of KOHA-PC after 20 h is low but detectable. Further oxidation of HOHA-PC produces a more stable product, KHdiA-PC. Therefore, it is reasonable that the amount of KHdiA-PC is much higher than HOHA-PC. The content of HOHA-PC reached 106 ± 4 pM in the Cu(II)-promoted oxidation and 136 ± 7 pM in the MPO-promoted oxidation, corresponding to 0.35% and 0.41% yields, respectively, based on DHA-PC. The total yield of the oxidatively truncated phospholipids HOHA-PC, KOHA-PC, KHdiA-PC, CEF-PC, OB-PC, and

TABLE 1. Yields (%) of Oxidatively Truncated Phospholipids[a]

	HOHA-PC	KOHA-PC	KHdiA-PC	CEF-PC	OB-PC	S-PC
Cu(II)	0.35	0.09	2.6	0.43	1.0	5.6
MPO	0.41	0.18	1.7	0.28	1.7	4.7

[a] Each value is the average of three independent studies (n = 3) after 20 h of oxidation, starting with DHA-PC (30 nM).

TABLE 2. Comparison of Ion Intensity[a] of Michael and Schiff Base Adducts for ESI Analysis of Figure 5

	0.5 h	1 h	2 h	8 h	12 h	24 h
Michael adducts (*m*/*z* 895.6)	3.5e6	1.3e6	5.4e5	3.6e5	2.2e5	1.3e5
Schiff base adducts (*m*/*z* 877.6)	3e5	2.1e5	1.2e5	1.4e5	4.3e5	7.7e5
ratio (Michael/Schiff base)	11.6	6.2	4.3	2.6	0.57	0.17

[a] cps: count per second.

S-PC accounted for 10% of DHA-PC in the Cu(II)-initiated oxidation and 9% in MPO-initiated oxidation.

It is especially noteworthy that KHdiA-PC (**6**) and S-PC (**8**) are major products of DHA-PC oxidation. A homologue of KHdiA-PC, that is produced during oxidation of arachidonyl phosphatidylcholine, was recently shown to trigger CD36 receptor-mediated endocytosis of oxidatively modified low-density lipoprotein particles by macrophage cells.[12,13] Analogy suggests that KHdiA-PC plays a similar role in the retina where retinal pigmented epithelial (RPE) cells also have CD36 receptors.[17] Thus, KHdiA-PC (**6**) may trigger endocytosis of oxidatively damaged photoreceptor rod outer segments (ROS). Endocytosis of ROS by RPE cells also involves membrane to membrane adhesion. A homologue of S-PC that is produced by oxidation of arachidonyl phosphatidylcholine promotes adhesion of monocyte macrophage cells to endothelial cells.[11] This suggests that S-PC (**8**) may foster adhesion of ROS tips to RPE cells.

Covalent Adduction of HOHA-PC with Ac-Gly-Lys-OMe. To characterize HOHA-derived lysine modifications, a 2-fold excess of the dipeptide, Ac-Gly-Lys-OMe (0.33 mM), was incubated with HOHA-PC (0.16 mM) in methanol at room temperature. The products in aliquots withdrawn after various reaction times were analyzed by ESI-MS. Two series of products were observed. One series corresponds to products from the reaction of HOHA-PC with the dipeptide Ac-Gly-Lys-OMe (Figure 5), and the other corresponds to products from decomposition reactions of HOHA-PC.

Identification of Peptide Adducts. The positive-ion ESI mass spectrum of the reaction mixture from HOHA-PC with Ac-Gly-Lys-OMe demonstrated the formation of products with mass-to-charge ratios (*m*/*z*) from 850 to 950, and no peak could be detected in this mass range before incubation (0 h) (Figure 5A). Three peaks, at *m*/*z* 895.8, 877.8, 859.8, were assigned to the protonated Michael adduct, Schiff base adduct, and pyrrole adduct, respectively. On the basis of the ion intensity, a relatively large amount of Michael adduct is already present after only 30 min of incubation. After 12 h of incubation, the level of Michael adduct had decreased while the level of Schiff Base adduct had increased, and detectable levels of pyrrole adduct had appeared (Table 2).

These observations show that pyrrole adduct formation is a very slow process, resulting in irreversible modification of lysine ϵ-amino groups. Under the conditions of this experiment, Schiff base and Michael adducts are trapped as methyl aminal and acetal derivatives, respectively. Thus, a peak with *m*/*z* 909.8 increased with incubation time. This peak corresponds to the molecular weight of methyl aminal derivative **19** of the Schiff base and methyl acetal derivative **20** of the Michael adduct that are

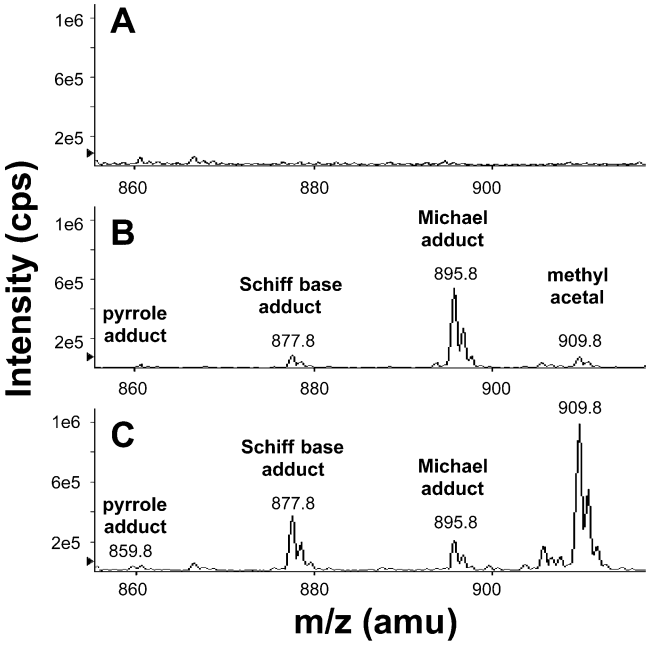
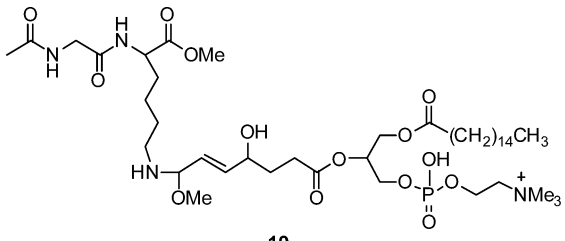
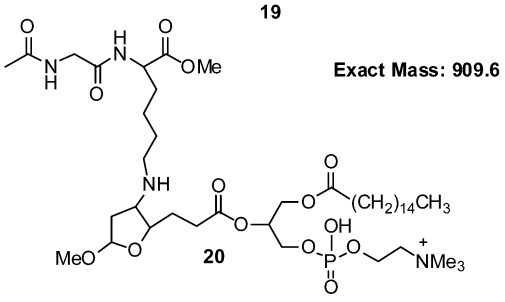


FIGURE 5. Representative positive ion ESI mass spectrometric analysis of mixtures from reaction of HOHA-PC with Ac-Gly-Lys-OMe after (A) 0 h incubation, (B) 0.5 h incubation, (C) 12 h incubation.

expected to arise from reactions of methanol with the imine moiety of the Schiff base and the hemiacetal moiety of the Michael adduct.





Representative product ion spectra of precursor ions with *m*/*z* 895.8, 877.8, and 859.8 are shown in Figure 6 to exhibit a series of fragment ions that allow confirmation of Michael, Schiff base, and pyrrole modification on the peptide. Collisional induced decomposition (CID) of

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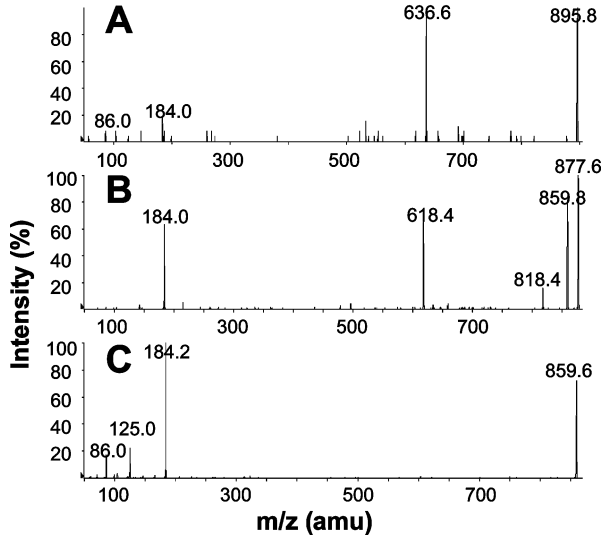


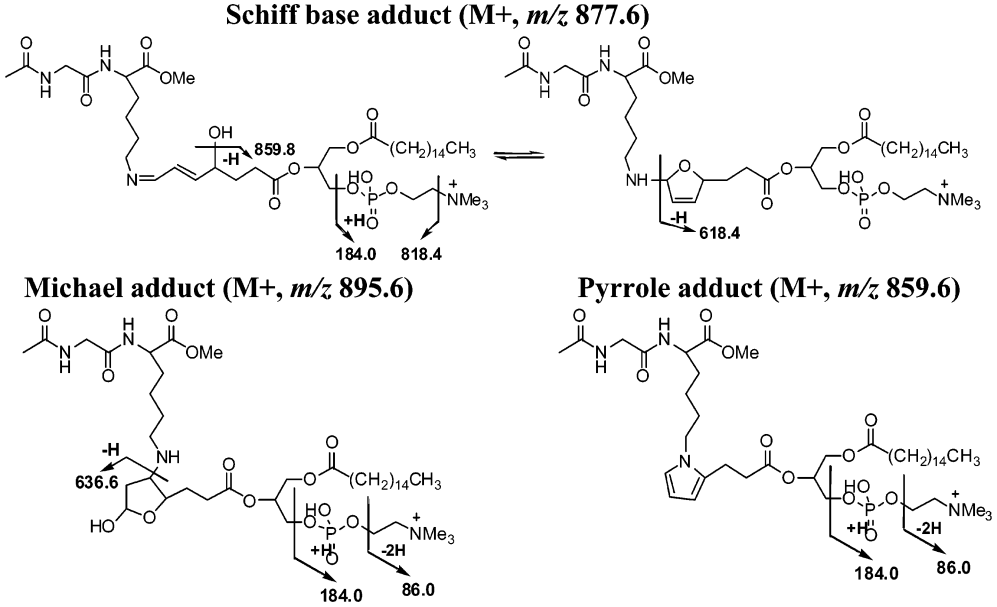
FIGURE 6. Product ion spectra of (A) Michael adduct, (B) Schiff base adduct, and (C) pyrrole derivatives generated from HOHA-PC and Ac-Gly-Lys-OMe.

$[M + H]^+$ of all three parent ions yielded an ion at *m*/*z* 184 corresponding to the mass of the phosphocholine group present at the *sn*-3 position. The formation of this ion is useful for specifically identifying glycerophosphocholine molecular species. For the Michael adduct, the ion with *m*/*z* 636.6 was produced by a fragmentation that occurred adjacent to the nitrogen atom. The Schiff base adduct may be in equilibrium with a dihydrofuran that fragments to release an aromatic furan with *m*/*z* 618.4. In contrast, no fragmentation adjacent to the nitrogen atom was observed for the pyrrole adduct because the nitrogen atom was incorporated into an aromatic moiety. Decomposition of the Schiff base adduct resulted in the abundant formation of an ion with *m*/*z* 859.8, corresponding to the loss of water, and an ion with *m*/*z* 818.4 induced by the neutral loss of trimethylamine (59 amu) or methoxycarbonyl (59 amu) from the polar phosphatidylcholine headgroup. Fragmentations of modified peptides are summarized in Chart 1.

LC−MS Analyses. To further characterize the specific HOHA-PC-derived lysine modifications, reaction mixtures were subjected to analysis by reversed-phase HPLC interfaced with a quadrupole mass spectrometer. Typical ion chromatograms showing starting materials HOHA-PC and the dipeptide Ac-Gly-Lys-OMe are shown in Figure 7. The dipeptide eluted from the column as a broad peak with a retention time of about 5 min. Two predominant peaks corresponding to HOHA-PC were observed. The major peak in the *m*/*z* 636.4 ion current chromatogram with a retention time of 23.3 min represents the HOHA-PC with a palmitic acid (C16:0) at the *sn*-1 position, while the one in the *m*/*z* 664.4 ion current chromatogram with a retention time of 27.5 min represents the HOHA-PC homologue with a stearic acid (C18:0) at the *sn*-1 position. To simplify, only products from the HOHA-PC homologue containing palmitic acid will be discussed.

After 24 h of incubation, more complex chromatograms were observed (Figure 8). The products derived from the reaction of HOHA-PC and the dipeptide, Ac-Gly-Lys-OMe, were remarkably reproducible. Only slight variations were observed between preparations. Although numerous products were present in the reaction mixture, we concentrated on three ions corresponding to protonated Michael, Schiff base, and pyrrole adducts whose identities were well characterized by ESI-MS/MS analysis (Figure 6). In the *m*/*z* 859.6 ion extract chromatogram, a single peak eluting at 24.4 min represents the isomerically homogeneous pyrrole adduct. In the *m*/*z* 877.6 ion extract chromatogram, a series of peaks eluted over a 2 min interval. These peaks represent compounds possessing a molecular mass expected for Schiff base adducts which are expected to be a mixture of cis and trans geometric isomers as well as various diastereomers and enamine tautomers. Similarly, numerous peaks were detected in ion extract chromatogram for *m*/*z* 895.6, the expected ion mass for Michael adducts. Numerous peaks

CHART 1. Fragmentation of Schiff Base Adduct, Michael Adduct, and Pyrrole Adduct 22



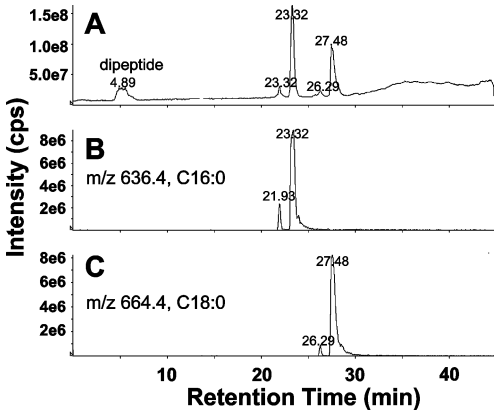


FIGURE 7. LC−MS analysis of the reaction product mixture from HOHA-PC and Ac-Gly-Lys-OMe at the beginning of reaction (0 h of incubation): (A) total ion current chromatogram for the mixture; (B) extracted ion chromatogram of *m*/*z* 636.4 from (A), which corresponds to the molecular weight of HOHA-PC with a palmitic acid (C16:0) at the *sn*-1 position; (C) extracted ion chromatogram of *m*/*z* 664.4 from (A), which corresponds to the molecular weight of HOHA-PC with a stearic acid (C18:0) at the *sn*-1 position.

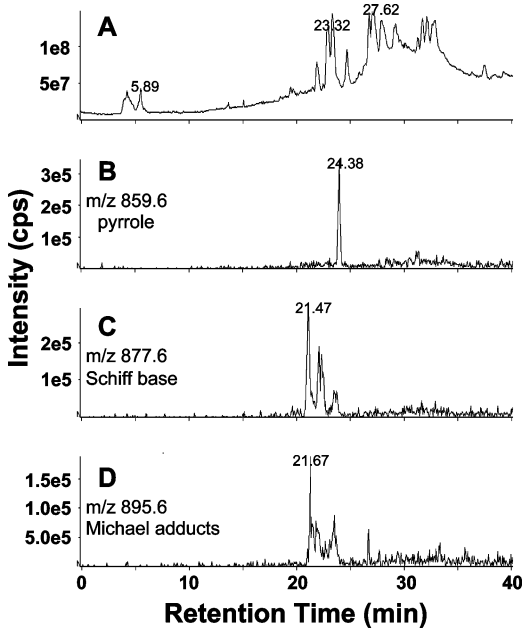


FIGURE 8. LC−MS analysis of diluted reaction mixture products from HOHA-PC and Ac-Gly-Lys-OMe (20 μL), containing 26 μM lipid and 42 μM peptide, after incubation at room temperature for 24 h: (A) total ion current chromatogram for the mixture; (B) extracted ion chromatogram of *m*/*z* 859.6 from (A), which corresponds to the molecular weight of pyrrole adduct; (C) extracted ion chromatogram of *m*/*z* 877.6 from (A), which corresponds to the molecular weight of Schiff base adducts; (D) extracted ion chromatogram of *m*/*z* 859.6 from (A), which corresponds to the molecular weight of Michael adducts.

are expected since the Michael adducts are comprised of a mixture of aldehyde and hemiacetal diastereomers.

The identity of the carboxyethylpyrrole (CEP) derivative **22** was confirmed by LC-MS comparisons with an authentic sample prepared by unambiguous total synthesis. Paal-Knorr reaction of the 4,7-dioxoheptanoic acid ester **21** of 2-lysophosphatidylcholine with the dipeptide Ac-Gly-Lys-OMe gave dipeptide-derived CEP-PC ester **22**.

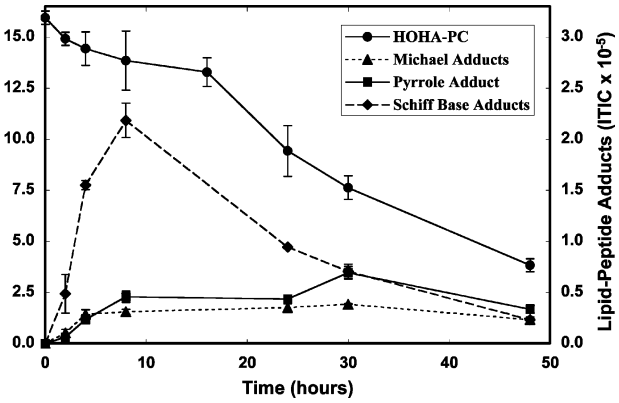
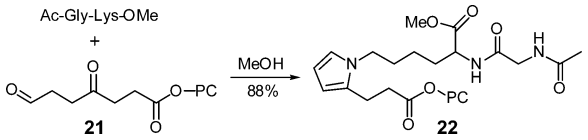


FIGURE 9. Time course for generation of peptide adducts from HOHA-PC. Relative amounts of each compound were determined by LC−ESI/MS in units of integrated total ion count (ITIC).



Peptide Adduction Product Evolution Profiles. We examined the time course for the formation of Michael, Schiff base, and pyrrole adducts. For these studies, product mixtures from reaction of HOHA-PC with dipeptide were subjected to LC−ESI/MS using the multiple reaction monitoring mode (MRM). In the MRM mode, both the precursor ions of interest (ions with *m*/*z* 859.6, 877.6, 895.6) and the product ion at *m*/*z* 184 were monitored in order to provide sensitive and specific quantitative analysis of these three adducts. The integrated total ion current (ITIC) of each compound from different runs was normalized by comparing with the ITIC of an internal standard, 1,2-dimyristoyl-*sn*-glycerol-3-phosphocholine that was added prior to the reaction.

As shown in Figure 9, HOHA-PC was constantly consumed, and the levels of three adducts changed dramatically in a time-dependent manner. Especially noteworthy is the rapid generation of a relatively large amount of Schiff base that is subsequently consumed. This is understandable because Schiff base adducts are unstable and are transformed into other stable products, such as pyrrole or methyl aminal **19**.

The ITIC value for the Schiff base is far larger than that of Michael adducts through the period from 2 to 24 h of incubation. In contrast to the results obtained by infusion ESI/MS, the ratio of ion intensity (cps) of Michael adducts to Schiff base adducts is above 1 before 12 h of incubation, indicating the ion intensity of Michael adducts was greater than that of Schiff base adducts. Neither method is expected to be quantitative. Direct infusion of sample into the mass spectrometer can result in ion suppression with the high (26 μM) total lipid concentration used in our study.[18] LC−MS analysis, that

(18) DeLong, C. J.; Baker, P. R.; Samuel, M.; Cui, Z.; Thomas, M. J. *J. Lipid Res.* **2001**, *42*, 1959−68.

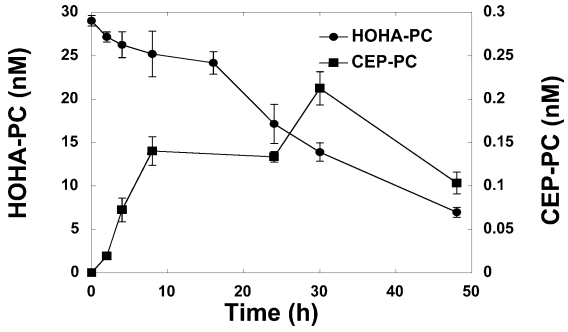
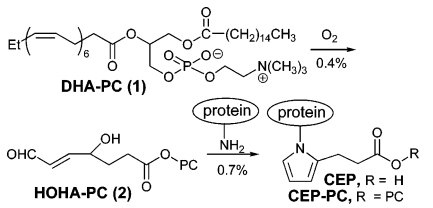


FIGURE 10. Absolute amounts of HOHA-PC (●) and Ac-Gly-Lys-OMe-derived CEP-PC-dipeptide (■) in the reaction of HOHA-PC with Ac-Gly-Lys-OMe as a function of time.

features the prior separation of compounds with HPLC columns, can bypass the problem caused by ion suppression. However, this method can cause selective loss of certain molecular species due to the use of a column. Considering the instability of Michael adducts, it is possible that this compound is decomposing during the process of separation, thus the ITIC values only represent the amount that eluted from the column. Calibration was not possible because pure standards of the Michael and Schiff base adducts were unavailable owing to chemical instability. Nevertheless, one important conclusion can be drawn from these experiments. Based on both ESI/MS and LC−ESI/MS analyses, the generation of pyrrole adduct is relatively slower than formation of the Schiff base or Michael adducts. The absolute amounts of HOHA-PC and pyrrole adduct (CEP-PC-dipeptide, **19**) were determined by calibration with respective standard curves and are shown in Figure 10. After 30 h, CEP-PC-dipeptide (**19**) was generated in 0.7% yield from HOHA-PC. This result is consistent with immunoassay studies that showed that CEP modification of human serum albumin reached 0.5% yield based on HOHA-PC after 24 h of incubation (details will be reported elsewhere).

Conclusions. The present LC−ESI/MS studies showed that free-radical-induced cleavage of DHA-PC produces a family of oxidatively truncated phospholipids that include HOHA-PC (0.4%), KOHA-PC (0.2%), KHdiA-PC (1.7%), CEF-PC (0.3%), OB-PC (1.7%), and S-PC (4.7%). To provide authentic standards for detection and quantification, these truncated phospholipids, and the HOHA-derived peptide adduct CEP-PC-dipeptide (**19**), were prepared by unambiguous total syntheses. These syntheses will facilitate ongoing studies of retinal physiology and age-related retinal dystrophies. The availability of these and other phospholipid oxidation products by total syntheses developed in our laboratories[11,15,19−26] will make possible the identification of lipoxidation products with a potential pathogenic role in age-related macular degeneration (AMD). Patients with this eye disease accumulate extracellular debris on a membrane beneath the retina. Our recent report[27] that this debris and the membrane contain proteins with a variety of oxidative modifications, including CEP adducts and cross-links, has kindled hope that an understanding of the molecular links between oxidative damage and AMD will "open up avenues for therapeutic research"[28] and "pave the way" for developing a hard scientific basis for understanding the etiology of AMD.[29] Identification of the major lipid oxidation products causing oxidative damage in AMD will provide insights toward the development of methods to prevent or limit the pathogenesis of this debilitating disease.

SCHEME 4



The oxidation of DHA-PC promoted by MPO[13] or by copper ions generated similar product evolution profiles. The γ-hydroxy-α, β-unsaturated aldehyde moiety of HOHA-PC (**2**) reacted with the lysyl residue of a dipeptide to form Michael and Schiff base adducts as well as a low yield (0.7 %) of pyrrole (CEP) adduct. These observations support the previous conclusion, based on immunological evidence, that CEPs are generated by the reaction of an oxidatively truncated phospholipid **2** with proteins in the retina (Scheme 4), and further indicate that CEP protein modifications probably represent only a tiny fraction of the products generated upon oxidative damage of DHA-PC in photoreceptor disk membranes.

Experimental Procedures

General Methods. ^{1}H NMR spectra were recorded on a Varian Gemini spectrometer operating at 200, 300, or 600 MHz using $CHCl_3$ (δ 7.24) or CD_3OH (δ 3.30) as internal standard. All chemical shifts are reported in parts per million (ppm) on the δ scale relative to the internal standard used. ^{1}H NMR spectral data are tabulated in terms of multiplicity of proton absorption (s, singlet; d, doublet; dd, doublet of doublets; t triplet; m multiplet; br broad), coupling constants (Hz), number of protons. ^{13}C NMR spectra were recorded on a Varian Gemini spectrometer operating at 75 MHz using $CHCl_3$ (δ 77.0), or CD_3OH (δ 49.0) as internal standard. Signal multiplicities were established by DEPT experiments. HMQC and HMBC spectra were recorded on a Varian INOVA 600. High-resolution mass spectra were recorded on a Kratos AEI MS25 RFA high-resolution mass spectrometer at 20 eV. All solvents were distilled under nitrogen atmosphere prior to use. Tetrahydrofuran (THF) was distilled over potassium and benzophenone. Methylene chloride, diethyl ether, and *N*,*N*-dimethylformamide (DMF) were distilled over calcium hydride. Chloroform was distilled over P_2O_5. Chromatography was performed

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with ACS grade solvents (ethyl acetate, hexane). Thin-layer chromatography (TLC) was performed on glass plates precoated with silica gel (Kieselgel 60 F_{254}, E. Merck, Darmstadt, West Germany). R_f values are quoted for plates of thickness 0.25 mm. The plates were visualized by viewing the developed plates under short-wavelength UV light, iodine, or a phospholipid spray.[30] Flash column chromatography was performed on 230−400 mesh silica gel supplied by E. Merck. For all reactions performed in an inert atmosphere, argon was used unless otherwise specified.

Purification of phospholipids by solid-phase extraction (SPE) was performed with Sep-Pak cartridges (Waters, Milford, MA) or Supeclean LC-18 C18 minicolumns (Supelco Inc., Bellefonte, PA) to remove small polar organic molecules (i.e., DMAP) and inorganic compounds from phospholipids. The maximum capacity for a 6 mL SPE cartridge is about 30 mg of phospholipids. Both 3 mL and 6 mL cartridges were used according to the amount of phospholipids applied to the cartridges. Vacuum or pressure was applied as the driving force for solvent. Typically, for 3 mL cartridges, methanol (20 mL) was passed through the cartridge over 2 min. Then water (20 mL) was passed through the cartridge over 2 min. Water (2 mL) was loaded onto the cartridge followed by phospholipids in methanol (0.5 mL), and the water and methanol in the cartridge were mixed with a pipet. Then solvent was sucked through the cartridge at a rate of 1 mL/min with a vacuum. The cartridge was eluted with 50%, 70%, 90% methanol in water (20 mL of each) at a rate of 5 mL/min. Finally, the cartridge was eluted with methanol (20 mL). The fractions were monitored with TLC. Phospholipids generally eluted in the 90% methanol or pure methanol fractions.

Total Syntheses of Oxidatively Truncated Docosahexaenoate Lipids. 3-Iodo-1-(1,1,2,2,-tetramethyl-1-silapropoxy)propane (9). To a stirred suspension of $CaCO_3$ (2.6 g, 26 mmol) and NaI (3.9 mg, 26 mmol) in methylene chloride (10 mL) were added trimethylene oxide (1.0 g, 17.2 mmol), and *tert*-butyldimethylsilyl chloride (TBDMSCl) (3.9 g, 26 mmol).[31] The resulting mixture was boiled under reflux for 48 h and then quenched with 6% aqueous $Na_2S_2O_3$ at room temperature. The whole mixture was extracted with diethyl ether (2 × 30 mL) and washed with brine. The combined organic phase was dried (Na_2SO_4) and concentrated by rotary evaporation to give a residue that was purified by flash chromatography on a silica gel column with 1.5% ethyl acetate in hexanes to afford compound **9** (4.6 g, 89%): TLC (ethyl acetate/hexanes, 1:49) $R_f = 0.25$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.64 (t, $J = 5.7$, 2H), 3.26 (t, $J = 5.9$, 2H), 1.91−2.06 (m, 2H), 0.87 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (APT, $CDCl_3$) δ 61.87 (CH_2), 33.53 (CH_2), 26.06 (CH_3), 14.78 (CH_2), 7.18 (C), −5.28 (CH_3); HRMS calcd for $C_5H_{12}IOSi$ ($M^+ - CMe_3$) 242.9501, found 242.9506.

1-(3,3-Dimethyl-2,4-dioxolanyl)-6-(1,1,2,2-tetramethyl-1-silapropoxy)hex-1-en-3-ol (11). Turnings of Mg (1.5 g, 61.7 mmol) and 20 mL of dried diethyl ether were placed in a 250 mL, flame-dried, three necked flask with a mechanical stirrer and condenser under argon atmosphere at room temperature. A few drops of iodide **9** (4.0 g, 13.3 mmol) in 4 mL of dry diethyl ether were added. After the formation of Grignard reagent, another 160 mL of dry diethyl ether was added, and the remaining iodide was added at a rate sufficient to maintain gentle boiling. The reaction mixture was stirred for another 1 h and then chilled at 0 °C followed by slow addition of 4,5-*O*-isopropylidene-2-pentenal (**10**)[32−34] (462.0 mg, 3.0 mmol). After being stirred for another 30 min, the reaction was quenched by the addition of saturated aqueous NH_4Cl, extracted with diethyl ether, and dried with magnesium sulfate. The solvent was removed by rotary evaporation. The residue was flash chromatographed on a silica gel column (20% ethyl acetate in hexanes) to give **11** (862 mg, 86%, based on **10**); TLC (ethyl acetate/hexanes, 3:7) $R_f = 0.25$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.85 (dd, $J = 15.38$, 5.6 Hz, 1H), 5.68 (dd, $J = 15.4$, 7.1 Hz, 1H), 4.52 (q, $J = 6.9$ Hz, 1H), 4.05−4.22 (m, 1H), 4.09 (dd, $J = 7.8$, 1.6 Hz, 1H), 3.56−3.69 (3H), 1.52−1.75 (4H), 1.43 (s, 3H), 1.40 (s, 3H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (APT, $CDCl_3$, 2*S* + 2*R*) 137.55, 137.34 (CH), 127.45, 127.34 (CH), 109.30 (−C-), 76.66, 76.48 (CH), 71.44, 71.36 (CH), 69.48 (CH_2), 63.42 (CH_2), 34.75, 34.43 (CH_2), 28.84, 26.71 (CH_2), 25.96 (CH_3), 18.35 (−C−), −5.35 (CH_3); HRMS (EI) (*m*/*z*) calcd for $C_{13}H_{25}O_4Si$ ($M^+ - CMe_3$) 273.1522, found 273.1525.

1-(4-(2-Oxanyloxy)-6-(3,3-dimethyl-2,4-dioxolanyl)hex-5-enyloxy)-1,1,2,2-tetramethyl-1-silapropane (12). A small amount of pyridinium *p*-toluenesulfonate (PPTS, 50.0 mg, 0.2 mmol) was added to the solution of compound **11** (1.4 g, 4.2 mmol) and dihydropyran (DHP, 790 mg, 9.2 mmol, freshly distilled) in 60 mL of dry methylene chloride.[35] The resulting solution was stirred overnight at room temperature, diluted and extracted with diethyl ether, and washed with brine. The solvent was removed by rotary evaporation. The crude product was purified by chromatography on a silica gel flash column (8% EtOAc in hexanes) to afford **12** (1.3 g, 72%): TLC (ethyl acetate/hexanes, 4:46) $R_f = 0.2$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.60−5.90 (m, 2H), 4.65−4.70 (m, 1H), 4.49−4.59 (m, 1H), 4.0−4.12 (m, 2H), 3.78−3.90 (m, 1H), 3.53−3.62 (3H), 3.47−3.53 (m, 1H), 1.5−1.8 (br, 10H), 1.43 (s, 3H), 1.40 (s, 3H), 0.89 (s, 9H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz, APT) δ 135.69 (CH), 135.23 (CH), 134.29 (CH), 130.82 (CH), 130.51 (CH), 128.32 (CH), 127.96 (CH), 109.47 (C), 109.43 (C), 109.31 (C), 97.68 (CH), 97.55 (CH), 95.11 (CH), 94.95 (CH), 76.88 (CH), 76.72 (CH), 76.56 (CH), 76.07 (CH), 74.96 (CH), 74.77 (CH), 69.62 (CH_2), 69.53 (CH_2), 63.13 (CH_2), 63.09 (CH_2), 63.02 (CH_2), 62.57 (CH_2), 62.29 (CH_2), 62.13 (CH_2), 32.12 (CH_2), 32.08 (CH_2), 30.89 (CH_2), 30.87 (CH_2), 30.81 (CH_2), 30.74 (CH_2), 30.69 (CH_2), 28.94 (CH_2), 28.90 (CH_2), 28.31 (CH_2), 28.24 (CH_2), 26.76 (CH_3), 26.74 (CH_3), 26.72 (CH_3), 26.71 (CH_3), 26.03 (CH_3), 25.99 (CH_3), 25.61 (CH_2), 25.45 (CH_2), 19.72 (CH_2), 19.67 (CH_2), 19.55 (CH_2), 19.43 (CH_2) 18.42 (C), −5.21 (CH_3); HRMS (EI) (*m*/*z*) calcd for $C_{21}H_{39}O_5$ Si ($M^+ - CH_3$) 399.2550, found 399.2551.

4-(2-Oxanyloxy)-6-(3,3-dimethyl-2,4-dioxolanyl)hex-5-en-1-ol (13). *n*-Bu_4NF (10 mL, 1 M in THF) was added dropwise to a stirred solution of the silyl ether **12** (1.3 g, 3.1 mmol) in THF at room temperature.[36] The resulting mixture was stirred 12 h, and then 10 mL of water was added. The whole mixture was extracted with diethyl ether (3 × 30 mL), washed with brine, dried ($MgSO_4$), and concentrated via rotary evaporator. The resulting residue was flashed chromatographed on a silica gel column (30% ethyl acetate in hexanes) to afford **13** (750 mg, 81%): TLC (ethyl acetate/hexanes, 4:6) $R_f = 0.15$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.62−5.89 (2H), 4.48−4.71 (2H), 4.06−4.15 (2H), 3.78−3.91 (m, 1H), 3.56−3.64 (3H), 3.40−3.50 (m, 1H), 1.53−1.74 (br, 10H), 1.42 (s, 3H), 1.38 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 135.32 (CH), 134.79 (CH), 133.85 (CH), 133.81 (CH), 130.78 (CH), 130.55 (CH), 128.45 (CH), 128.11 (CH), 109.37 (C), 109.25 (C), 97.73 (CH), 97.60 (CH), 95.49 (CH), 95.36 (CH), 76.70 (CH), 76.49 (CH), 76.19 (CH), 74.92 (CH), 74.78 (CH), 69.47 (CH_2), 69.38 (CH_2), 62.71 (CH_2), 62.49 (CH_2), 32.28 (CH_2), 30.96 (CH_2), 30.85 (CH_2), 30.72 (CH_2), 30.65 (CH_2), 28.59 (CH_2), 28.10 (CH_2), 26.61 (CH_3), 25.84 (CH_3), 25.38 (CH_2), 25.32 (CH_2), 19.67 (CH_2), 19.55 (CH_2); HRMS (EI) (*m*/*z*) calcd for $C_{16}H_{28}O_5$ (M^+) 301.2015, found 301.2017, calcd for $C_{15}H_{25}O_5$ ($M^+ - CH_3$) 285.1702, found 285.1708.

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4-(2-Oxanyloxy)-6-(3,3-dimethyl-2,4-dioxolanyl)hex-5-enoic Acid (14). A solution of compound **13** (750 mg, 2.5 mmol) and pyridinium dichromate (PDC) (5.7 g, 15 mmol) in anhydrous DMF (9 mL) was stirred at room temperature for 20 h, diluted with aqueous acetic acid to pH 4, and extracted with ethyl acetate (3 × 30 mL). The combined organic phase was washed with brine, dried over $MgSO_4$, and concentrated by rotary evaperation. Flash chromatography on a silica gel column (45% ethyl acetate in hexanes) afforded **14** (615 mg, 78.2%): TLC (ethyl acetate/hexanes, 11:9) $R_f = 0.25$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.60−5.86 (2H), 4.47−4.68 (2H), 4.05−4.20 (2H), 3.78−3.88 (m, 1H), 3.53−3.60 (m, 1H), 3.38−3.52 (m, 1H), 2.35−2.55 (dt, J = 7.1, 7.5 Hz, 2H), 1.52−1.94 (br, 8H), 1.42 (s, 3H), 1.38 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz, APT) δ 178.54 (CO), 178.51 (CO), 178.39 (CO), 178.35 (CO), 134.63 (CH), 134.19 (CH), 133.20 (CH), 131.45 (CH), 131.19 (CH), 129.07 (CH), 128.76 (CH), 109.57 (C), 109.45 (C), 97.91 (CH), 97.81 (CH), 95.39 (CH), 95.37 (CH), 95.27 (CH), 76.67 (Ch), 75.66 (CH), 75.21 (CH), 74.06 (CH), 73.89 (CH), 69.54 (CH_2), 69.46 (CH_2), 62.62 (CH_2), 62.55 (CH_2), 62.47 (CH_2), 62.34 (CH_2), 30.70 (CH_2), 30.63 (CH_2), 30.51 (CH_2), 30.46 (CH_2), 30.16 (CH_2), 30.11 (CH_2), 29.35 (CH_2), 29.20 (CH_2), 29.09 (CH_2), 26.70 (CH_3), 25.94 (CH_3), 25.48 (CH_2), 19.55 (CH_2), 19.52 (CH_2), 19.41 (CH_2); HRMS (EI) (m/z) calcd for $C_{16}H_{27}O_6$ (MH^+) 315.1808, found 315.1851, calcd for $C_{15}H_{23}O_6$ ($M^+ - CH_3$) 299.1495, found 299.1504.

Cholesteryl 4-(2-Oxanyloxy)-6-(3,3-dimethyl-2,4-dioxolanyl)hex-5-enoate (15). Dicyclohexylcarbodiimide (DCC) (80.0 mg, 0.38 mmol) and *N*,*N*-dimethylaminopyridine (DMAP, 20 mg, 0.16 mmol) were added to the solution of the acid **14** (60 mg, 0.19 mmol) and cholesterol (50 mg, 0.13 mmol) in dry ethanol free $CHCl_3$ (2 mL). The resulting mixture was stirred at room temprature for 48 h. The solution was filtered, and solvent was removed. The residue was purified on a silica gel flash column (10% ethyl acetate in hexanes) to afford **15** (84.4 mg, 95%): TLC (ethyl acetate/hexanes, 3:22) $R_f = 0.22$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.53−5.89 (2H), 5.32 (d, J = 4.0, 1H), 4.53−4.68 (2H), 4.53 (m, 1H), 4.06 (m, 2 H), 3.85 (m, 1 H), 3.53 (m, 1H), 3.46 (m, 1H), 2.23−2.48 (4H), 0.8−1.98 (41H), 1.41 (s, 3H), 1.37 (s, 3H), 0.99 (s, 3H), 0.68 (s, 3H).

Cholesteryl 4-Hydroxy-7-oxohept-5-enoate (16). A solution of **15** (20 mg, 0.03 mmol) in acetic acid/water (2:1, v/v, 1.2 mL) was stirred for 4 h at 40 °C. Then sodium metaperidate (9.7 mg, 0.045 mmol) was added. After another 1.5 h of stirring at room temperature, the solution was neutralized by the portionwise addition of saturated aqueous sodium bicarbonate and then extracted with diethyl ether. The ether phase was dried over $MgSO_4$ and evaporated. The residue was flash chromatographed (25% ethyl acetate in hexanes) to provide **16** (14.3 mg, 85%): TLC (ethyl acetate/hexanes, 2:3) $R_f = 0.26$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.53−5.89 (m, 2H), 5.32 (bd, J = 4.0, 1H), 4.53−4.68 (m, 2H), 4.50 (m, 1H), 4.06 (m, 2 H), 3.85 (m, 1 H), 3.53 (m, 1H), 3.46 (m, 1H), 2.23 (t, J = 7.7 Hz, 2 H), 2.25 (d, J = 7.7 Hz, 2H), 0.8−2.2 (41H), 1.41 (s, 3H), 1.37 (s, 3H), 0.99 (s, 3H), 0.88 (d, J = 6.5 Hz, 3H), 0.84 (d, J = 6.7 Hz, 3H), 0.836 (d, J = 6.7 Hz, 3H), 0.65 (s, 3H); ^{13}C NMR (APT, $CDCl_3$) 197.68 (CHO), 173.29 (CO), 158.79 (CH), 139.65 (C), 130.76 (CH), 123.01 (CH), 73.78 (CH), 71.08 (CH), 56.68 (−), 56.17 (−), 50.05 (+), 42.34 (+), 39.77 (−), 39.56 (+), 38.22 (+), 37.03(+), 36.66 (+), 36.51 (+), 36.19(+), 35.87 (−), 31.96 (+), 31.89 (−), 29.18 (+), 29.11 (+), 28.96 (+), 28.26 (+), 28.07 (−), 27.86 (+), 25.12 (+), 24.96 (−), 24.33(+), 23.81 (+), 22.88 (−), 22.60 (−), 21.09 (+), 18.75 (−), 11.98 (−).

Phospholipid 17. Dicyclohexylcarbodiimide (DCC) (57.7 mg, 0.28 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) (15 mg, 0.12 mmol) were added to a solution of the acid **14** (55.1 mg, 0.12 mmol) and L-α-lysophosphatidylcholine (75 mg, 0.15 mmol) in dry $CHCl_3$ (5 mL). The resulting mixture was stirred for 48 h at room temperature. The solution was then filtered, and the solvent was removed. Flash chromatography of the residue ($CHCl_3$/MeOH/H_2O, 60:36:4) gave **17** (102 mg, 86%): TLC ($CHCl_3$/MeOH/H_2O, 30:19:1) $R_f = 0.16$; ^{1}H NMR (300 MHz, $CDCl_3$), 5.50−5.88 (2H), 5.12−5.22 (1H), 4.43−4.68 (m, 1H), 4.18−4.42 (3H), 4.02−4.18 (3H), 3.86−3.98 (3H), 3.68−3.95 (3H), 3.50−3.60 (m, 1H), 3.38−3.50 (m, 1H), 3.35 (s, 9H), 2.20−2.50 (4H), 1.43−1.90 (11H), 1.41 (s, 3H), 1.38 (s, 3H), 1.22 (apparent s, 33H), 0.85 (t, J = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz, APT) δ 173.6 (CO), 172.7 (CO), 134.65 (CH), 134.21 (CH), 133.24 (CH),131.41 (CH), 131.23 (CH), 129.09 (CH), 128.74 (CH), 109.50 (C), 109.48 (C), 109.37 (C), 97.96 (CH), 97.83 (CH), 95.32 (CH), 95.23 (CH), 76.64 (CH), 76.33 (CH), 75.70 (CH), 75.17 (CH), 73.96 (CH), 73.82 (CH), 71 (CH), 69.55 (CH_2), 69.46 (CH_2), 66.47 (CH_2), 66.35 (CH_2), 66.29 (CH_2, d, $^3J_{CP}$ = 6.7 Hz), 63.01 (CH_2), 62.62 (CH_2), 62.46 (CH_2, d, $^2J_{CP}$ = 6.7 Hz), 59.29 (CH_2, d, $^2J_{CP}$ = 7.1 Hz), 54.47 (CH_3), 34.12 (CH_2), 31.95 (CH_2), 31.9 (CH_2), 29.74 (CH_2), 29.69 (CH_2), 29.56 (CH_2), 29.39 (CH_2), 29.35 (CH_2), 29.19 (CH_2), 26.72 (CH_3), 25.92 (CH_3), 25.50 (CH_2), 25.40 (CH_2), 24.90 (CH_2), 22.72 (CH_2), 19.63 (CH_2), 19.54 (CH_2), 14.16 (CH_3); HRMS (FAB, CsI/NaI/glycerol) (m/z) calcd for $C_{40}H_{74}NNaO_{12}P$ (MNa^+) 814.4846, found 814.4867.

2-(4-Hydroxy-7-oxohept-5-enoyl)-1-palmitoyl-*sn*-glycero-3-phosphatidylcholine (HOHA-PC, 2). A solution of the compound **17** (20 mg, 0.025 mmol) in acetic acid/water (2:1, v/v, 1.2 mL) was stirred magnetically for 4 h at 40 °C, and then the solvent was removed by rotary evaporation under reduced pressure. The last traces of HOAc were removed by azeotropic distillation with *n*-heptane (3 × 1 mL) under high vacuum. Dry methylene chloride (2 mL) and Na_2CO_3 (5.7 mg, 0.054 mmol) were added to the residue. The solution was stirred magnetically at −78 °C under argon atmosphere, and $Pb(OAc)_4$ (12.4 mg, 0.03 mmol) was added. The resulting solution was stirred for 30 min, the solvent was removed, and the residue was flashed chromatographed on a silica gel column ($CHCl_3$/MeOH/H_2O, 60:36:4) to give **2** (13.0 mg, 83%): TLC ($CHCl_3$/MeOH/H_2O, 15:9:1) $R_f = 0.1$; ^{1}H NMR (300 Hz, $CDCl_3$), δ 9.50 (d, J = 8.1), 6.88 (dd, J = 15.2 Hz, 3.8 Hz, 1H), 6.30 (ddd, J = 15.2 Hz, 8.0 Hz, 1.4 Hz), 5.18−5.28 (1H), 4.10−4.43 (5H), 3.85−4.08 (2H), 3.71−3.82 (2H), 3.32 (s, 9H), 2.32−2.51 (2H), 2.25 (t, J = 7.5 Hz, 2H), 1.5−1.70 (4H), 1.22 (apparent s, 31H), 1.85 (t, J = 6.6 Hz, 3H); HRMS (FAB, CsI/NaI/glycerol) (m/z) calcd for $C_{31}H_{58}NNaO_{10}P$ (MNa^+) 658.3696, found 658.3678.

2-(3-(2-Furyl)propanoyl)-1-palmitoyl-*sn*-glycero-3-phosphatidylcholine (CEF-PC, 3). A mixture of 3-furan-2-ylpropionic acid (56 mg, 0.4 mmol) and 1-palmitoyl-2-lyso-*sn*-glycero-3-phosphatidylcholine (100 mg, 0.2 mmol), which were dried on a vacuum pump (0.1 mmHg) equipped with a dry ice−acetone trap for 10 h at room temperature, was dissolved in dry $CHCl_3$ (5 mL, shaken with P_2O_5 for 0.5 h and distilled). Dicyclohexylcarbodiimide (DCC, 240 mg, 1.2 mmol) and *N*,*N*-dimethylaminopyridine (DMAP, 24 mg, 0.2 mmol) were added. The mixture was stirred for 48 h under nitrogen. The mixture was then concentrated, and the residue was purified by flash chromatography on silica with $CHCl_3$/MeOH/H_2O (16/9/1) to produce the furyl phospholipid CEF-PC (**3**, 100 mg, 81%): TLC ($CHCl_3$/MeOH/H_2O, 15:9:1) $R_f = 0.26$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.28 (d, J = 1.2 Hz, 1H), 6.25 (d, J_1 = 3.2 Hz, J_2 = 1.2 Hz, 1H), 6.00 (d, J = 3.1 Hz, 1H), 5.21 (m, 1H), 4.36 (dd, J = 12.4, 2.8 Hz, 1H), 4.26 (bm, 2H), 4.11 (dd, J_1 = 12.2 Hz, J_3 = 7.1 Hz, 1H), 3.93 (m, 2H), 3.76 (bm, 2H), 3.33 (bs, 9H), 2.92 (t, J = 7.2 Hz, 2H), 2.65 (t, J = 7.6 Hz, 2H), 2.24 (t, J = 7.4 Hz, 2H), 1.5−1.7 (m, 2H), 1.24 (24H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz, APT) δ 173.6 (CO), 172.0 (CO), 154.0 (C), 141.3 (CH), 110.3 (CH), 105.4 (CH), 71.1 (CH, d, $^3J_{CP}$ = 10.9 Hz), 66.4 (CH_2, d, $^3J_{CP}$ = 8.1 Hz), 63.4 (CH_2, d, $^2J_{CP}$ = 7.1 Hz), 62.9 (CH_2), 59.3 (CH_2, d, $^2J_{CP}$ = 7.6 Hz), 54.4 (CH_3), 34.1 (CH_2), 32.7 (CH_2), 31.9 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 24.9 (CH_2), 23.4 (CH_2), 22.7 (CH_2), 14.2 (CH_3); HRMS (MALDI-TOF) m/z 618.3693 (MH^+) calcd for $C_{31}H_{56}NO_9P$, found 618.3698.

2-(4-Oxo-7-oxohept-6-enoyl)-1-palmitoyl-*sn*-glycero-3-phosphatidylcholine (KOHA-PC, 5). NBS (9.15 mg, 0.051 mmol) and pyridine (5.75 μL, 0.068 mmol) were sequentially

added to the solution of furyl phosphatidyl choline **3** (21 mg, 0.034 mmol) in THF/acetone/water (5/4/2) at −20 °C. The resulting mixture was stirred for 1 h at this temperature, and then kept at room temperature for 6 h. The solvent was then removed quickly, and the residue was purified on a silica gel column ($CHCl_3$/MeOH/H_2O = 16/9/1) affording KOHA-PC (15 mg, 65%): TLC ($CHCl_3$/MeOH/H_2O, 15:9:1) R_f = 0.15. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.79 (d, J = 7.8 Hz, 1H), 6.95 (d, J = 16.8 Hz, 1H), 6.80 (dd, J = 16.8, 7.8 Hz, 1H), 5.17 (m, 1H), δ 4.30 (3H), 4.12 (dd, J = 11.4, 7.2 Hz, 1H), 3.94 (m, 2H), 3.79 (m, 2H), 3.34 (bs, 9H), 3.0−3.1 (2H), 2.65−2.75 (2H), 2.26 (t, J = 7.2 Hz, 2H), 1.54 (2H), 1.09 (24H), 0.83 (t, J = 7.2 Hz, 3H); HRMS (MALDI-TOF) m/z (MH^+) 634.3642 calcd for $C_{31}H_{56}NO_{10}P$, found 634.3661.

2-(6-Carboxy-4-oxohex-5-enoyl)-1-palmitoyl-*sn*-glycero-3-phosphatidylcholine (KHdiA-PC, 6). To a magnetically stirred solution of KOHA-PC (**5**) (20 mg, 0.032 mmol) in *t*-BuOH−H_2O (5:1, v/v, 0.5 mL) were added NaH_2PO_4 (6 mg, 0.045 mmol), 2-methyl-2-butene (171 μL, 0.35 mmol, 2 M solution in THF), and $NaClO_2$ (8.1 mg, 0.09 mmol). The resulting mixture was stirred for 2 h at room temperature under Ar. The solvent was removed. The residue was purified by the general SPE procedure to give KHdiA-PC (15 mg, 72%): TLC ($CHCl_3$/MeOH/H_2O, 15:9:1) R_f = 0.12. ^{1}H NMR (CD_3OD, 300 MHz) δ 6.82 (d, J = 16.1 Hz, 1H), 6.71 (d, J = 16.1 Hz, 1H), 5.21 (m, 1H), 4.36 (dd, J_1 =12.0 Hz, J_2 = 3.6 Hz, 1H), 4.28 (m, 2H), 4.19 (dd, J_1 = 12.1 Hz, J_2 = 6.7 Hz, 1H), 4.00 (m, 2H), 3.88 (m, 1H), 3.65 (m, 2H), 3.22 (s, 9H), 2.95−3.05 (m, 2H), 2.5−2.7 (m, 2H), 2.35 (t, J = 7.5 Hz, 2H), 1.5−1.7 (m, 2H), 1.28 (24 H), 0.87 (t, J = 6.6 Hz, 3H); ^{13}C NMR from HMQC, HMBC (150 MHz, CD_3OD) δ 200.1 (CO), 173.9 (CO), 172.9 (CO), 172.4 (CO), 139.7 (CH), 134.1 (CH), 71.1 (CH), 66.3 (CH_2), 63.6 (CH_2), 62.1 ($2CH_2$), 59.3 (CH_2), 53.5 ($3CH_3$), 34.7 (CH_2), 27.4 (CH_2), 33.5 (CH_2), 29.1 ($11CH_2$), 24.6 (CH_2), 13.1 (CH_3); HRMS (MALDI-TOF) m/z calcd for $C_{31}H_{56}NO_{11}P^+$ (MH^+) 650.3591, found 650.3689.

(4-Hydroxy-6-carboxyhex-5-enoyl)-1-palmitoyl-2-*sn*-glycero-3-phosphatidylcholine (HHdiA-PC, 4). To a magnetically stirred solution of HOHA-PC (**2**) (23 mg, 0.036 mmol) in *t*-BuOH−H_2O (5:1, v/v, 0.3 mL) was added a solution containing NaH_2PO_4 (7.2 mg, 0.054 mmol), 2-methyl-2-butene (205 μL, 0.4 mmol, 2M solution in THF) and $NaClO_2$ (9.8 mg, 0.108 mmol) in *t*-BuOH−H_2O (5:1, v/v, 0.5 mL). The resulting mixture was stirred for 2 h at room temperature under Argon. The solvent was removed by vacuum.The residue was purified by the general SPE procedure to give HHdiA-PC (**4**, 16 mg, 71%): TLC ($CHCl_3$/MeOH/H_2O, 15:9:1) R_f = 0.12; ^{1}H NMR (CD_3OD, 200 MHz) δ 6.60 (d, J_1 = 15.6 Hz, J_2 = 6 Hz, 1H), 5.98 (d, J = 15.6 Hz, 1H), 5.24 (m, 1H), 4.40 (dd, J_1 = 12 Hz, J_2 = 3.6 Hz, 1H), 4.21−4.29 (m, 2H), 4.14 (dd, J_1 = 12 Hz, J_2 = 6.6 Hz, 1H), 4.02 (m, 2H), 3.63−3.67 (2H), 3.19 (s, 9H), 2.47 (t, J = 7.5 Hz, 2H), 2.34 (t, J = 7.5 Hz, 2H), 1.86−1.77 (m, 2H), 1.50−1.65 (m, 2H), 1.25 (24 H), 0.86 (t, J = 6 Hz, 3H); ^{13}C NMR from HMQC, HMBC (CD_3OD, 150 MHz) δ 173.6 (CO), 173.1 (CO), 172.9 (CO), 144.9 (CH), 126.4 (CH), 70.8 (CH), 62.3 (CH_2), 62.1 (CH), 59.3 (CH_2), 63.8 (CH_2), 66.1 (CH_2), 53.5 ($3CH_3$), 29.7 (CH_2), 33.5 (CH_2), 31.5 (CH_2), 31.4 (CH_2), 24.6 (CH_2), 29.2 ($11CH_2$), 13.1 (CH_3); HRMS (FAB) m/z calcd for $C_{32}H_{61}NO_{11}P^+$ (MH^+) 652.3748, found 652.3525.

1-Palmitoyl-2-succinoyl-*sn*-glycero-3-phosphatidylcholine (S-PC, 8). Succinic anhydride (100 mg, 1 mmol) and DMAP (12 mg, 0.1 mmol) were added to a solution of 2-lyso-PC (50 mg, 0.1 mmol) in dry $CHCl_3$ under argon. The mixture was stirred for 2 days, and the solvent was removed under reduced pressure. The residue was purified on a silica gel flash column ($CHCl_3$/MeOH/H_2O = 14/9/2), producing S-PC (**8**, 50 mg, 76%): TLC ($CHCl_3$/MeOH/H_2O, 15:9:1) R_f = 0.1; ^{1}H NMR (CD_3OD, 300 MHz) δ 5.23 (m, 1H), 4.37 (dd, J_1 = 12.0 Hz, J_2 = 3.6 Hz 1H), 4.27 (m, 2H), 4.11 (dd, J_1 = 12.0 Hz, J_2 = 6.6 Hz, 1H), 4.01 (m, 2H), 3.63 (bm, 2H); 3.21 (bs, 9H), 2.5−2.7 0 (4H), 2.32 (t, J = 7.4 Hz, 2H), 1.50−1.65 (m, 2H), 1.23 (24H), δ 0.89 (t, J = 6.9 Hz, 3H); ^{13}C NMR from HMQC, HMBC (CD_3OD, 150 MHz): δ 175.2 (CO), 173.9 (CO), 172.4 (CO), 70.9 (CH), 66.3 (CH_2), 63.6 (CH_2), 62.1 (CH_2), 59.2 (CH_2), 53.3 ($3CH_3$), 28.9 (CH_2), 33.5 (CH_2), 24.7 (CH_2), 28.9 ($12CH_2$), 13.1 (CH_3); HRMS (MALDI-TOF) m/z 596.3564 (MH^+) calcd for $C_{28}H_{54}NO_{10}P$, found 596.3748.

1-Palmitoyl-2-(4-pentenoyl)-*sn*-glycero-3-phosphatidylcholine (18). The esterification of 2-lysophosphatidylcholine with 4-pentenoic acid was accomplished by a general method. Traces of moisture were removed from 1-palmitoyl-2-lyso-*sn*-glycero-3-phosphatidylcholine (50 mg, 0.1 mmol, 1.0 equiv) by azeotropic distillation with benzene (3 × 5 mL) by rotary evaporation under reduced pressure at room temperature and then on a high vacuum pump (1 mmHg) for 1 h. Freshly distilled anhydrous $CHCl_3$ (2 mL), dicyclohexylcarbodiimide (62.4 mg, 0.3 mmol, 3 equiv), *N*,*N*-dimethylaminopyridine (34 mg, 0.3 mmol, 3 equiv), and 4-pentenoic acid (30.3 mg, 0.3 mmol, 3 equiv) were added. The flask was flushed with nitrogen and sealed. The reaction was stirred at room temperature for 72 h monitoring by TLC for the disappearance of lysophosphatidylcholine using a phospholipid detection spray.[30] The solvent was removed completely under reduced pressure and the resulting solid was flash chromatographed on silica gel with $CHCl_3$/MeOH/H_2O (30/19/1) as eluant to afford the title compound (53 mg, 90% based on lyso-PC): TLC ($CHCl_3$/MeOH/H_2O, 30/19/1) R_f = 0.22; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.78 (m,1H), 5.19 (m, 1H), 4.96−5.06 (2H), 4.32−4.39 (m, 2H), 4.06−4.15 (m, 2H), 3.95 (t, J = 5.6 Hz, 2H), 3.81 (m, 2H), 3.36 (s, 9H), 2.23−2.40 (6H), 1.55 (t, 2H, J = 7.0 Hz), 1.23 (24H), 0.86 (t, 3H, J = 6.3 Hz); ^{13}C NMR from HMQC, HMBC ($CDCl_3$, 150 MHz,) δ 173.5 (CO), 172.4 (CO), 136.4 (CH), 115.6 (CH), 70.8 (CH), 66.2 (CH_2), 63.3 (CH_2), 62.9 (CH_2), 59.2 (CH_2), 54.3 ($3CH_3$), 34.1 (CH_2), 33.4 (CH_2), 31.9 (CH_2), 29.6 ($10CH_2$), 28.7 (CH_2), 24.8 (CH_2), 22.6 (CH_2), 14.1 (CH_3); MALDI-MS m/z calcd for $C_{29}H_{57}NO_8P^+$ (MH^+) 578.3822, found 578.3950.

2-(4-Oxobutyryl)-1-palmitoyl-*sn*-glycero-3-phosphatidylcholine (OB-PC, 7). The ozonolysis of 1-palmitoyl-2-(4-pentenoyl)-*sn*-glycero-3-phosphatidylcholine was done at −60 °C in methanol. Thus, PPC (22 mg, 0.038 mmol) was dissolved in methanol (10 mL) and cooled with magnetic stirring to −60 °C. Ozone was bubbled through the stirred solution until a blue color appeared (2−5 min). The excess of ozone was flushed with nitrogen. While still at −10 °C, Me_2S (1 mL) was added and the solution was allowed to come to −60 °C. The solution was stirred 1 h at −10 °C, 1 h at 0 °C, and finally, at room temperature 1 h. After evaporation of the solvent under reduced pressure, the aldehyde phospholipid **7** was obtained in quantitative yield: TLC (CH_3CN/MeOH/H_2O, 15:9:1) R_f = 0.16; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 9.76 (s, 1H), 5.18 (m, 1H), 4.09−4.35 (4H), 3.95 (t, J = 5.4 Hz, 2H), 3.79 (m, 2H), 3.34 (s, 9H), 2.56−2.81 (4H), 2.26 (t, J = 7.6 Hz, 2H), 1.55 (t, J = 7.2 Hz, 2H), 1.23 (24H), 0.85 (t, J = 6.7 Hz, 3H); ^{13}C NMR from HMQC, HMBC ($CDCl_3$, 150 MHz): δ 200.3 (CO), 173.6 (CO), 171.9 (CO), 71.6 (CH), 66.6 (CH_2), 63.8 (CH_2), 62.7 (CH_2), 54.6 ($3CH_3$), 38.5 (CH_2), 34.2 (CH_2), 29.55 ($12CH_2$), 24.99 (CH_2), 14.24 (CH_3); MALDI-MS m/z calcd for $C_{28}H_{55}NO_9P^+$ (MH^+) 580.3614, found 580.3597.

Generation of Oxidatively Truncated Docosahexaenoate Lipids from DHA-PC. Oxidation of DHA-PC Promoted by MPO/H_2O_2/$NaNO_2$. A mixture of the dry lipids, DHA-PC and DM-PC, was hydrated at 37 °C for 1 h in phosphate buffer.[37] Then the mixture of hydrated lipids was made into unilamellar vesicles by extrusion using an Avanti Mini-Extruder (Avanti Polar Lipids, Inc.). The vesicles (DHA-PC, 200 ug/mL, dimyrisitoyl-PC, 50 μg/mL) were incubated in the presence of MPO (5 nM), glucose oxidase (100 ng/mL), $NaNO_2$ (500 μM), and glucose (100 μg/mL) in sodium phosphate buffer (50 mM) with DTPA (200 μM) at 37 °C. The reaction was stopped by adding BHT (40 μM) and catalase (150

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μM). The lipids were extracted by the method of Bligh and Dyer.[16] The solvent was evaporated under a stream of dry nitrogen from the extract in a vial. Samples were stored in vials sealed under argon at −80 °C.

LC−MS Analysis of DHA-PC Oxidation Products. The oxidized DHA-PC was analyzed by LC−MS. Oxidized DHA-PC (100 μg) was dissolved in 1000 μL of MeOH. Ten microliters of this solution was then diluted with 30% acetonitrile in water containing 0.02% trifluoroacetic acid (90 μL). Twenty microliters of this solution (containing about 0.2 μg of lipids) was chromatograghed on a 1.0 × 250 mm Vydac C18 column (particle size 5 μm, pore size 300 A, Hesperia, CA) using a linear gradient of acetonitrile from 40% to 98% in water in the presence of 0.02% trifluoroacetic acid over 30 min. Then 98% acetonitrile with 2% water was eluted for another 50 min at a flow rate of 40 μL/min. Ions of interest were monitored by a PE Sciex API 3000 triple quadrupole mass spectrometer. The total ion current was obtained in the mass range of *m*/*z* 200−1000 at 40 V of orifice voltage in the positive ion mode. Electrospray voltage was 5000 V.

Phospholipid Derivatization Experiments. Methoxylamine derivatives of lipids were prepared by resuspending dried samples (100 μg) in freshly dried pyridine containing 10% methoxylamine hydrochloride (500 μL). Reaction mixtures were incubated at room temperature overnight. Pentafluorobenzyl ester derivatives of lipids were prepared by resuspending dried samples (100 μg) in freshly dried acetonitrile containing 10% pentafluorobenzyl bromide (PFB-Br) and 20% *N*,*N*-diisopropylethylamine (500 μL). Reaction mixtures were incubated at room temperature overnight. After reaction, solutions were dried under anhydrous nitrogen and then resuspended in 50% methonol. Nonlipid components were removed by passage over a C18 minicolumn (Supeclean LC-18 SPE tubes, 3 mL, Supelco Inc., Bellefonte, PA). Lipids were dried again under anhydrous nitrogen. Approximately 10 μg of lipids was chromatographed on a Luna C18 250 × 1 mm, 5 μm column (Phenomennex, Torrance, CA) at a flow rate of 80 μL/min. A gradient was used as follows: 40% acetonitrile with 0.02% trifluoroacetic acid (TFA) in water was increased linearly to 95% acetonitrile with 0.02% trifluoroacetic acid (TFA) in water over 30 min, and isocratic elution with this gradient was continued for another 40 min. The column effluent was split that 20 μL/min was introduced to the mass spectrometer and analyzed by LC/MS in the positive mode.

Mass Spectroscopic Study of HOHA-PC Adduction with the Lysyl Residue of a Peptide. Preparation of HOHA-PC Adducts with AcGly-Lys-OMe. A 50 μL aliquot of a freshly made stock solution of HOHA-PC (1 μg/μL in methanol) and 20 μL of a freshly made stock solution of AcGly-Lys-OMe (1 μg/μL in methanol) were mixed with 430 μL of methanol to make the final concentrations, 100 ng/μL and 40 ng/μL, respectively. The reaction was allowed to proceed at room temperature. Aliquots (50 μL) were taken at various times, and solvent was evaporated under argon. The solvent-free samples were stored at −80 °C before analysis.

CEP-PC-dipeptide (22). The 4,7-dioxoheptanoic acid ester of 2-lysoPC (**21**, 5 mg, 8 μmol) and Ac-Gly-Lys-OMe (5 mg, 0.02 mmol) were dissolved in MeOH (1.5 mL), and stirred for 8 h under nitrogen at room temperature. Solvents were then removed via rotary evaporator under reduced vacuum. The crude product was purified by silica gel chromatography with $CHCl_3$/MeOH (9:1, v/v) to deliver **22** (6 mg, 88%): TLC ($CHCl_3$/MeOH, 9:1): $R_f = 0.2$; ^{1}H NMR $CDCl_3$ (200 Hz) δ 6.52 (m, 1H), 5.98 (m, 1H), 5.82 (m, 1H), 5.13−5.22 (1H), 4.2−4.5 (4H), 4.03−4.16 (m, 1H), 3.86−3.99 (3H), 3.60−3.80 (9H), 3.30 (s, 9H), 2.82−2.93 (m, 2H), 2.60−2.70 (m, 2H), 2.20−2.50 (5H), 1.97 (s, 3H), 1.23 (apparent s, 26H), 0.85 (t, J = 6.6 Hz, 3H); ^{13}C NMR from HMQC, HMBC ($CDCl_3$, 150 MHz) δ 173.6 (CO), 173.1 (CO), 170.8 (CO), 170.1 (CO), 131.0 (C), 120.3 (CH), 107.1 (CH), 105.3 (CH), 71.1 (CH), 66.9 (CH_2), 64.1 (CH_2), 62.8 (CH_2), 59.1 (CH), 55.0 ($3CH_3$), 52.6 (CH_2), 52.3 (CH_2), 46.3 (CH_2), 43.0 (CH_2), 34.2 ($2CH_2$), 30.9 (CH_2), 29.7 ($14CH_2$), 25.0 (CH_2), 23.1 (CH_2), 21.9 (CH_2), 14.3 (CH_3); HRMS calcd $C_{42}H_{76}N_4O_{12}P^+$ (M^+) 859.5197, found 859.5203.

Characterization of Lipid Adducts. Different lipid adducts were analyzed by tandem mass spectrometric analysis in the positive-ion mode. Samples in aqueous solutions of 70% acetonitrile containing 0.02% trifluoroacetic acid, were introduced directly into the mass spectrometer by infusion at a flow rate of 5 μL/min. Precursor ions were selected by using the first quadrupole (Q1), and collision induced dissociation (CID) was effected by collisions with nitrogen gas in the second (RF-only) quadrupole (Q2). Product ion spectra were obtained by scanning the third quadrupole (Q3) from *m*/*z* 50 up to the molecular mass of interest in the positive-ion mode.

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Supporting Information Available: ^{1}H, ^{13}C, DEPT, HMQC, and HMBC NMR spectra of new compounds; details of LC/MS quantification of lipid fragments and analysis and quantification of lipid-peptide adducts. This material is available free of charge via the Internet at http://pubs.acs.org.

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