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Synthesis of Isoprostanyl Phosphatidylcholine and Isoprostanyl Phosphatidylethanolamine

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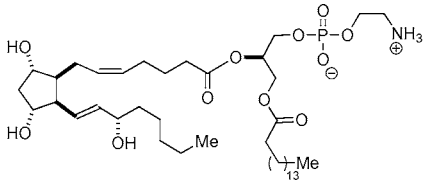
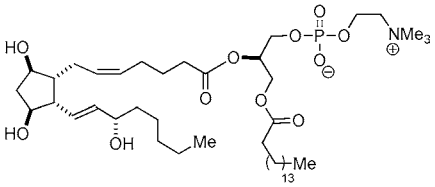


15-F_{2t}-isoprostanyl phosphatidylethanolamine (**1**)



ent-15-epi-F_{2t}-isoprostanyl phosphatidylcholine (**2**)

The syntheses of two isoprostanyl phospholipids are described. A newly established route to 15-F_{2t}-isoprostane and *ent*-15-*epi*-F_{2t}-isoprostane has allowed for the selective preparation of 15-F_{2t}-isoprostanyl phosphatidylethanolamine and *ent*-15-*epi*-F_{2t}-isoprostanyl phosphatidylcholine. The nature of the headgroups dictates the coupling strategy used to attach the appropriately protected isoprostanes to the corresponding lysophospholipids. Preliminary ^{1}H NMR and ^{31}P NMR studies indicate that these isoprostanyl phospholipids aggregate in apolar solvents.

Isoprostanyl esters are generated through oxidation of phosphatidyl arachidonates (Figure 1).[1] Phospholipase-mediated hydrolysis of the resulting isoprostanyl phospholipids then releases the isoprostanes from their lipid surroundings.[2] The biological activities of these lipid metabolites typically involve inflammatory responses, as well as activities related to smooth muscle growth and platelet aggregation factors.[3] The isoprostanes are also recognized as important indicators of oxidative stress,[4] particularly in human maladies such as Alzheimer's disease,[5] diabetes,[6] and cancer.[7] Although progress has been made in understanding the generation and distribution of the isoprostanes, the biological activities and interactions of their isoprostanyl phospholipid precursors are far less clear.

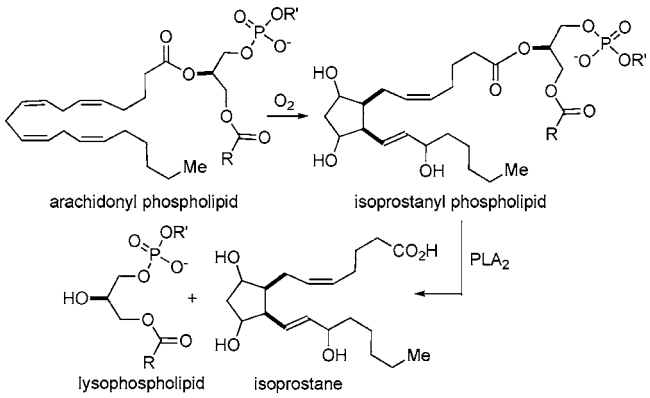


FIGURE 1. Formation of isoprostanes and isoprostanyl phospholipids from arachidonic esters.

To begin addressing questions regarding the function of the lipid-bound oxidative metabolites, we sought access to several

(1) For free radical peroxidation of arachidonic acid, see: (a) Morrow, J. D.; Hill, K. E.; Burk, R. F.; Nammour, T. M.; Badr, K. F.; Roberts, L. J., II. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9383. (b) Morrow, J. D.; Harris, T. M.; Roberts, L. J., II. *Anal. Biochem.* **1990**, *184*, 1. (c) Morrow, J. D.; Awad, J. A.; Boss, H. J.; Blair, I. A.; Roberts, L. J., II. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 10721. (d) Morrow, J. D.; Minton, T. A.; Mukundan, C. R.; Campbell, M. D.; Zackert, W. E.; Daniel, V. C.; Badr, K. F.; Blair, I. A.; Roberts, L. J., II. *J. Biol. Chem.* **1994**, *269*, 4317. (e) Harrison, K. A.; Murphy, R. C. *J. Biol. Chem.* **1995**, *270*, 17273. (f) Morrow, J. D.; Awad, J. A.; Wu, A.; Zackert, W. E.; Daniel, V. C.; Roberts, L. J., II. *J. Biol. Chem.* **1996**, *271*, 23185. (g) Basu, S. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **1998**, *58*, 319. (h) Roberts, L. J.; Fessel, J. P. *Chem. Phys. Lipids* **2004**, *128*, 173. (i) Wang, D.; DuBois, R N. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 415. For review, see: (j) Fam, S. S.; Morrow, J. D. *Curr. Med. Chem.* **2003**, *10*, 1723.

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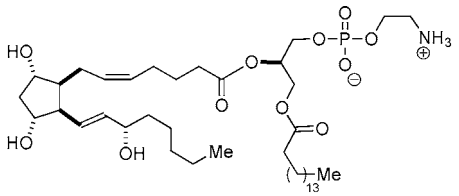
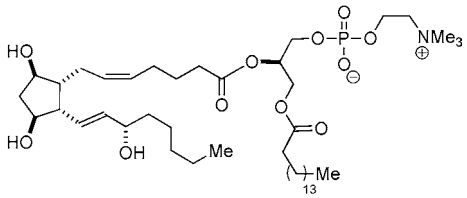
isoprostanyl phospholipids, as well as structural variants.[8] These compounds should allow for the identification of relevant interactions of the oxidized phospholipids within the membrane bilayer environment. The isoprostanyl phospholipids should also provide valuable tools for studying phospholipase processing of the oxidized phospholipids. *As a preliminary step toward these objectives, we report the synthesis of 15-F_{2t}-isoprostanyl phosphatidylethanolamine* ***1*** *and ent-15-epi-F_{2t}-isoprostanyl phosphatidylcholine* ***2****.*

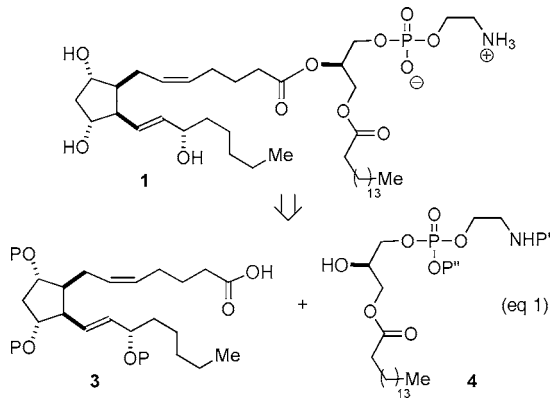


15-F_{2t}-isoprostanyl phosphatidylethanolamine (**1**)



ent-15-epi-F_{2t}-isoprostanyl phosphatidylcholine (**2**)

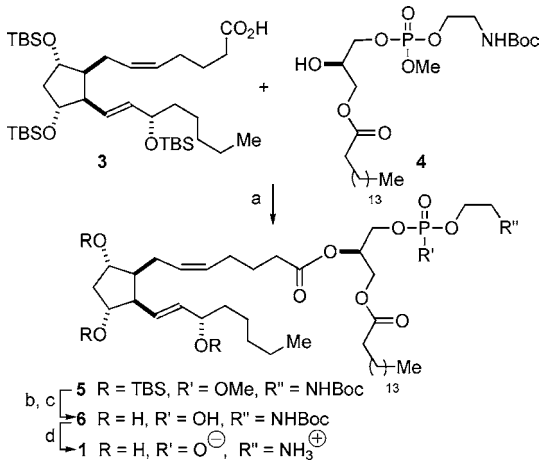
15-F_{2t}-Isoprostanyl Phosphatidylethanolamine (1). On the basis of 15-F_{2t}-isoprostane's well-established formation under oxidative conditions,[9] 15-F_{2t}-isoprostanyl phosphatidylethanolamine **1** was selected as an initial target. As illustrated in eq 1, a convergent approach was envisioned for the preparation of the 15-F_{2t}-isoprostanyl phospholipid. Phospholipid **1** could be



accessed readily by coupling the protected lysophosphatidylethanolamine derivative **4** with a tris-protected isoprostane **3**. Subsequent steps would then involve removal of the protecting groups.

The known lysophosphatidylethanolamine derivative **4** was prepared using procedures reported by Schreiber/Richards[10] and Martin.[11] Coupling of tris-TBS-protected isoprostane **3**, an intermediate generated in our stereodivergent route to all the isoprostane isomers,[12] to the protected lysophosphatidylethanolamine fragment **4** was accomplished using DCC and DMAP to give the protected phospholipid **5** in 74% yield (Scheme 1). The synthesis of **1** was completed by selective hydrolysis of the phosphate methyl ester using Finkelstein conditions to give the phosphate in 93% yield. An acid-mediated deprotection of the silyl ethers and the Boc-protected amine gave the desired 15-F_{2t}-isoprostanyl phosphatidylethanolamine **1** in 26% yield along with fractions containing Boc-protected compound **6** after silica gel chromatography. The mixture containing **6** was treated with TFA in CH_2Cl_2 to deliver an additional 5% yield of the desired phospholipid **1**.

SCHEME 1. Synthesis of 15-F_{2t}-Isoprostanyl Phosphatidylethanolamine 1[a]



[a] (a) DCC, DMAP, CH_2Cl_2; 74%. (b) NaI, 2-butanone, 80 °C; 93%. (c) 1 M HCl, THF/H_2O; mixture of **6** and **1**. (d) TFA, CH_2Cl_2; 32% from **5**.

Preliminary studies indicate that 15-F_{2t}-isoprostanyl phosphatidylethanolamine **1** can serve as a substrate for secretory phospholipase A_2 (bee venom *s*-PLA_2).[13] Specifically, HPLC analysis indicated that 15-F_{2t}-isoprostane is liberated when **1** is treated with *s*-PLA_2. Although an interesting observation, additional studies with more relevant phospholipases may be necessary before any conclusions of biological significance can be made.

***ent*-15-*epi*-F_{2t}-Isoprostanyl Phosphatidylcholine (2).** A preliminary screen of the 15-F_2-isoprostanes indicated that *ent*-15-*epi*-F_{2t}-isoprostane is more active in a whole blood platelet aggregation assay than the known 15-F_{2t}-isoprostane.[14] This new isoprostanyl activity inspired us to examine *ent*-15-*epi*-F_{2t}-isoprostanyl phosphatidylcholine **2**, a likely cellular precursor to *ent*-15-*epi*-F_{2t}-isoprostane. With access to all the isoprostanes in hand,[12] a convergent esterification of the protected isoprostane

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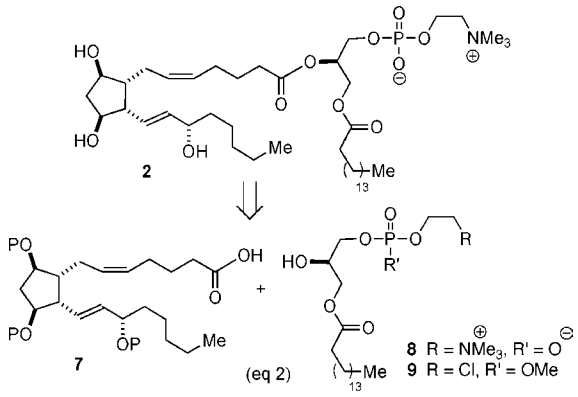
(13) *s*-PLA_2 Assay Kit from Cayman Chemical, Ann Arbor, MI (Catalog No. 765001). For related work from our laboratory, see: (a) Cheung, A. K.; Murelli, R.; Snapper, M. L. *J. Org. Chem.* **2004**, *69*, 5712. (b) Cheung, A. K.; Snapper, M. L. *J. Am. Chem. Soc.* **2002**, *124*, 11584.

(14) Schrader, T. O. Ph.D. Thesis, Boston College, Chestnut Hill, MA, 2002.

acid **7** with commercially available phosphatidylcholine alcohol **8** (eq 2) was envisioned as our preferred route to this likely lipid metabolite. Unfortunately, no desired product was obtained



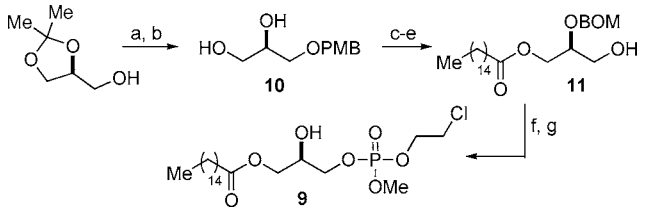
using a variety of coupling protocols,[15] and only starting material was usually observed. The problem was thought to arise from the zwitterionic nature of the lipid fragment **8**. We decided, therefore, to use the neutral lipid precursor **9** in coupling with a protected form of **7**, which can then be converted to phospholipid **2** with the desired choline headgroup.

The initial steps toward **9** were performed under modified Martin conditions from an optically pure, protected glycerol (Scheme 2). PMB protection of the primary alcohol and deprotection of the acetonide gave **10**. DCC coupling of **10** with palmitic acid, BOM protection of the secondary alcohol, and oxidative removal of the PMB group with DDQ in CH_2Cl_2/H_2O yielded alcohol **11**. The subsequent phosphorylation was problematic with Martin's procedure; instead, treating **11** with 2-chloroethyl phosphorodichloridate and Et_3N in CH_2Cl_2, followed by addition of methanol, and removal of the BOM group by hydrogenolysis yielded the desired lipid fragment **9**.

Esterification of the protected isoprostane **12** with **9** was now successful using EDC/DMAP to obtain **13** in 55% yield (Scheme 3). Global deprotection of the TBS groups with 1 M HCl yielded triol **14**, which was then treated with excess anhydrous trimethylamine to give the desired *ent*-15-*epi*-F_{2t}-isoprostanyl phosphatidylcholine **2** in 80% yield.

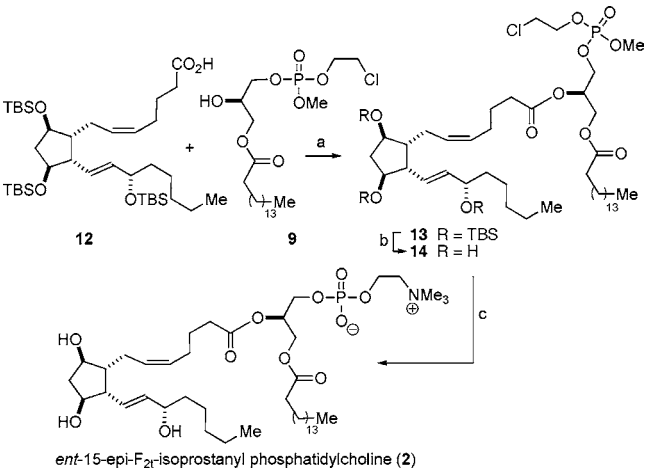
NMR analyses of phospholipids **1** and **2** revealed interesting solvation effects. Although the ^{1}H NMR spectra of both lipids taken in CD_3OD showed relatively sharp resonances, the ^{1}H NMR spectra taken in $CDCl_3$ displayed broadened peaks.[16] A similar phenomenon was observed in the ^{31}P NMR spectra. Single resonances were observed when the ^{31}P NMR spectra were taken in CD_3OD. However, no phosphorus resonance was observed in $CDCl_3$, even after several thousand scans. Kai et al. also observed similar solvent-dependent behavior of phospholipid bolaamphiphiles:[17] Increased peak sharpening was observed for the polar headgroup ($R-NMe_3^+$) with increasing polarity of the solvent (9:1, 3:1, 1:1, 1:3, $CDCl_3$/CD_3OD), and the reverse trend was observed for the terminal methyl groups on the fatty acid chains. Their data suggested that micelle-like structures are formed in polar solvents, whereas reverse micelle

SCHEME 2. Synthesis of Lipid Fragment 9[a]



[a] (a) PMBCl, NaH, DMF, 0 °C to room temperature; 93%. (b) AcOH, MeOH; 99%. (c) palmitic acid, DCC, DMAP, CH_2Cl_2; 78%. (d) BOMCl, *i*-Pr_2NEt, CH_2Cl_2; 90%. (e) DDQ, H_2O/CH_2Cl_2; 90%. (f) (1) $Cl_2(O{=})PO(CH_2)_2Cl$, Et_3N, CH_2Cl_2, room temperature; (2) MeOH, Et_3N, room temperature; 63%. (g) Pd/C, H_2, THF/H_2O; 99%.

SCHEME 3. Completion of *ent*-15-*epi*-F_{2t}-Isoprostanyl Phosphatidylcholine 2[a]



[a] (a) EDC, DMAP, CH_2Cl_2; 55%. (b) 1 M HCl, THF/H_2O; 92%. (c) Me_3N (excess), $CHCl_3$/EtOH, 62 °C, 60 h; 80%.

structures are generated in apolar solvents. In contrast, isoprostanyl phospholipids (**1** and **2**) showed line broadening of all signals in the ^{1}H NMR spectra, suggesting that aggregates are forming in $CDCl_3$, whereas in more polar CD_3OD, the molecule appears to be monomeric.

In summary, the total synthesis of 15-F_{2t}-isoprostanyl phosphatidylethanolamine **1** and *ent*-15-*epi*-F_{2t}-isoprostanyl phosphatidylcholine **2** was accomplished via a convergent strategy by an esterification of a protected isoprostane and a phosphatidyl lipid fragment. ^{1}H NMR spectroscopy indicated that aggregates are forming in apolar solvents, whereas in more polar solvents, **1** and **2** appear to be monomeric. The synthetic phospholipids will aid us to further understand mechanisms associated with oxidative stress. Furthermore, our synthesis will allow us to prepare photoaffinity probe reagents to assess the lipid interactions of these cellular metabolites.

Experimental Section

Tris-TBS-Protected Isoprostane (3). To a solution of bis-TBS isoprostane **3b** (41 mg, 0.070 mmol, 1 equiv) and Et_3N (100 μL, 0.72 mmol, 10 equiv) in CH_2Cl_2 (700 μL, 0.10 M) was added TBSCl (48 mg, 0.32 mmol, 4.6 equiv) followed by DMAP (8 mg, 0.07 mmol, 1 equiv). After stirring at 23 °C for 12 h, Et_2O (15 mL) was added and the reaction mixture was washed sequentially with HCl (1 M, 15 mL) and brine (15 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was then filtered through a 1 cm plug of silica gel (15:1, pentane/Et_2O as

(15) The following coupling conditions were attempted: DCC/DMAP, EDC/DMAP, CDI, and Yamaguchi esterification.

(16) The full ^{1}H NMR spectral data are shown in the Supporting Information.

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elutant) and concentrated. The resulting residue was dissolved in THF (1 mL) and H_2O (1 mL), and LiOH (10 mg, 0.41 mmol) was added. The reaction was stirred at 23 °C for 2 h (judged complete by TLC) at which time the reaction mixture was diluted with Et_2O and (15 mL) and HCl (0.5 M, 15 mL). The organic layer was separated and washed with brine (15 mL). The organics were dried over $MgSO_4$, filtered, and concentrated to a crude oil that was purified by silica gel chromatography (pentane/Et_2O/AcOH; gradient 6:1:0.1 to 1:1:0.1; R_f = 0.25, pentane/Et_2O/AcOH, 6:1:0.1) to deliver **3** as a colorless oil (18 mg, 0.026 mmol, 42% yield based on recovered **3b**), followed by recovered **3b** (R_f = 0.0, pentane/Et_2O/AcOH, 6:1:0.1; 5 mg, 0.009 mmol, 12% recovery). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.46 (1H, dd, J = 15.2, 6.0 Hz), 5.42 (1H, m), 5.32 (1H, m), 5.29 (1H, dd, J = 15.2, 10.4 Hz), 4.05 (1H, q, J = 6.4 Hz), 3.92 (1H, m), 3.77 (1H, q, J = 6.8 Hz), 2.56 (1H, m), 2.34 (3H, m), 2.08 (4H, m), 1.82 (1H, m), 1.69 (2H, m), 1.56−1.39 (3H, m), 1.36−1.21 (6H, m), 0.90−0.85 (30H, m), 0.04 (3H, s), 0.03 (9H, s), 0.01 (6H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 178.9, 135.8, 129.9, 128.7, 127.6, 76.4, 76.2, 73.5, 52.8, 50.2, 44.6, 38.9, 33.6, 32.2, 27.0, 26.6, 26.4, 26.2, 25.3, 24.9, 23.0, 18.6, 18.4, 18.3, 14.4, −3.9, −4.0, −4.1, −4.20, −4.25, −4.33. IR (thin film, NaCl): 2961 (s), 2936 (m), 2860 (m), 1721 (s), 1482 (m), 1262 (m), 1086 (m), 834 (w) cm^{-1}. HRMS (ESI$^+$): calcd for $C_{38}H_{76}O_5Si_3Na$ (M + Na) 719.4898, found 719.4904.

Tris-TBS−Isoprostanyl Phospholipid (5). To a solution of acid **3** (18 mg, 0.026 mmol, 1 equiv), alcohol **4** (15 mg, 0.026 mmol, 1 equiv), and DMAP (1.4 mg, 0.011 mmol, 0.4 equiv) in CH_2Cl_2 (370 μL, 0.07 M) was added a solution of DCC (8 mg, 0.04 mmol, 1.5 equiv) in CH_2Cl_2 (150 μL) over 30 min at 23 °C under N_2. After stirring for a total of 5.5 h (judged complete by TLC), the reaction was filtered through a 1 cm layer of Celite and concentrated. The resulting material was dissolved in pentane (1 mL) and filtered through cotton to remove insoluble impurities. The solution was concentrated and purified by silica gel chromatography (pentane/EtOAc; gradient 4:1, 1:1) to give recovered **3** (R_f = 0.4, pentane/EtOAc, 4:1; 3.5 mg, 0.0050 mmol, 19% recovery), followed by the protected lipid **5** (R_f = 0.15, pentane/EtOAc, 4:1; 19.3 mg, 0.0155 mmol, 74% yield based on recovered **3**) isolated as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.45 (1H, dd, J = 15.6, 6.0 Hz), 5.42 (1H, m), 5.34−5.27 (2H, m), 5.24 (1H, m), 5.08 (1H, br s), 4.32 (1H, dd, J = 11.6, 4.4 Hz), 4.22−4.04 (6H, m), 3.92 (1H, m), 3.77 (3H, overlapping d, J = 4.0 Hz), 3.76 (1H, m), 3.41 (2H, m), 2.54 (1H, m), 2.32 (5H, m), 2.18−2.00 (4H, m), 1.82 (1H, m), 1.72−1.56 (3H, m), 1.55−1.34 (3H, m), 1.43 (9H, s), 1.32−1.21 (31H, m), 0.90−0.84 (33H, m), 0.04 (3H, s), 0.02 (9H, s), 0.01 (6H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.2, 172.6, 172.5, 155.8, 135.9, 129.8, 128.6, 127.6, 79.8, 76.4, 76.2, 73.5, 69.6, 67.5, 65.7, 61.8, 54.9, 52.8, 50.2, 44.6, 41.2, 38.9, 34.3, 34.0, 32.3, 32.2, 30.0, 29.9, 29.8, 29.7, 29.6, 29.5, 28.7, 27.0, 26.6, 26.24, 26.20, 25.3, 25.2, 23.1, 23.0, 18.6, 18.4, 18.3, 14.5, 14.4, −3.88, −3.94, −4.1, −4.20, −4.24, −4.3. ^{31}P NMR ($CDCl_3$, 162 MHz): δ 1.23, 1.19. IR (thin film, NaCl): 3345 (b), 2967 (s), 2923 (s), 2849 (s), 1747 (s), 1734 (s), 1514 (m), 1469 (m), 1375 (m), 1262 (s), 1174 (s), 840 (s) cm^{-1}. HRMS (ESI$^+$): calcd for $C_{65}H_{128}NO_{13}PSi_3Na$ (M + Na) 1268.8303, found 1268.8329.

15-F_{2t}-Isoprostanyl Phosphatidylethanolamine (1). To a stirred solution of **5** (11.7 mg, 9.38 μmol, 1 equiv) in 2-butanone (freshly distilled from P_2O_5, 1.9 mL, 0.005 M) was added NaI (7.0 mg, 0.047 mmol, 5 equiv). The solution was refluxed (80 °C) for 2 h (judged complete by TLC). After evaporation of the solvent, the residue was suspended in $CHCl_3$ (15 mL) and the solution was washed with 5% aqueous HCl (2 × 15 mL) followed by H_2O (15 mL). The aqueous layer was back-extracted with $CHCl_3$ (25 mL), and the combined organics were dried over Na_2SO_4, filtered, and concentrated to give **6** as a pale yellow oil (10.8 mg, 8.76 μmol, 93% yield).

The protected lipid **6** (6.60 mg, 5.36 μmol, 1 equiv) was stirred in a solution of HCl (1 M, 750 μL, 140 equiv) and THF (750 μL, 0.007 M) for 75 h at 23 °C (monitored by TLC). The volatiles were evaporated, and the material was purified by silica gel chromatography (*i*-PrOH/H_2O; 25:1, R_f = 0.15) to give impure fractions containing the Boc-protected compound **6** (multiple spots by TLC, 3.5 mg) followed by pure **1** (1.1 mg, 1.4 μmol, 26% yield).

The material containing **6** was dissolved in TFA (250 μL) and CH_2Cl_2 (250 μL) and stirred at 23 °C for 1 h. The volatiles were evaporated (25 °C, 0.1 mmHg), and the material was purified by silica gel chromatography (*i*-PrOH/H_2O; 25:1) to give additional **1** (0.2 mg, 0.3 μmol, 32% total yield from **6**). ^{1}H NMR (CD_3OD, 400 MHz): the proton NMR spectra for compound **1** was difficult to interpret, so selected peaks are shown; δ 5.49 (1H, m), 5.48−5.26 (2H, m), 5.22 (1H, m), 4.41 (1H, m), 4.21 (1H, dd, J = 7.6, 4.0 Hz), 4.17 (1H, m), 4.12−3.83 (6H, m), 3.70−3.40 (2H, m), 3.61 (br s, impurity from silica gel), 3.14 (2H, m), 2.60 (4H, m), 2.42 (4H, t, J = 7.6 Hz), 1.80−1.50 (6H, m), 1.48−1.22 (34H, m), 0.90 (6H, t, J = 6.8 Hz). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.15 (2H, br s), 5.60−5.10 (4H, m), 4.40 (1H, m), 4.45−3.80 (7H, m), 3.80−3.30 (2H, m), 3.62 (br s, impurity from silica gel), 3.20 (2H, br s), 2.40−2.20 (4H, m), 2.10−1.90 (4H, m), 1.80−1.48 (14H, m), 1.38−1.18 (26H, m), 0.87 (6H, t, J = 6.8 Hz). ^{31}P NMR (CD_3OD, 162 MHz): δ 4.30. IR (thin film, NaCl): 3415 (b), 2936 (s), 2867 (m), 1741 (m), 1470 (m), 1369 (w), 1224 (w), 1086 (m) cm^{-1}. MS (ESI$^-$): 788.42 (M − H), 770.42 (M − H_3O). HRMS (ESI$^+$): calcd for $C_{41}H_{76}NO_{11}PNa$ (M + Na) 812.5054, found 812.5020. HRMS (ESI$^-$): calcd for $C_{38}H_{75}NO_{11}P$ (M − H) 788.5078, found 788.5080.

BOM-Protected Phospholipid (11b). In a flame-dried 10 mL Schlenk flask, alcohol **11** (265 mg, 0.59 mmol, 1 equiv) and CH_2Cl_2 (2.7 mL, 0.2 M) were added under N_2. Et_3N (164 μL, 1.18 mmol, 2 equiv) was added to the mixture, and the mixture was stirred at 23 °C for 5 min. Reagent $Cl_2(O{=})PO(CH_2)_2Cl$ (75 μL, 0.59 mmol, 1 equiv) was added dropwise, and the reaction was stirred at 23 °C under N_2 for 12 h. Et_3N (82 μL, 0.59 mmol, 1 equiv) and methanol (36 μL, 0.88 mmol, 1.5 equiv) were added to the reaction mixture, and the mixture was stirred for another 2 h at 23 °C. The reaction was opened to air, and the solution was filtered through a cotton-plugged pipet, flushed with CH_2Cl_2 (2.0 mL), and concentrated. The residue was purified by silica gel chromatography (hexanes/EtOAc; gradient 2:1, 1:1, 1:10; R_f = 0.3, hexane/EtOAc, 1:1) to give intermediate **11b** as a pale yellow oil (225 mg, 0.37 mmol, 63% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.38−7.27 (5H, m), 4.86 (2H, s), 4.66 (2H, s), 4.30−4.24 (3H, m), 4.21−4.14 (3H, m), 4.10 (1H, q, J = 5.1 Hz), 3.79 (3H, d, J_{H-P1} = 11.3 Hz, d, J_{H-P2} = 11.3 Hz), 3.70−3.67 (2H, m), 2.30 (2H, t, J = 7.4 Hz), 1.64−1.56 (2H, m), 1.28−1.24 (24H, m), 0.88 (3H, t, J = 6.6 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.4, 137.5, 128.5, 127.9, 94.1, 77.5, 77.2, 76.9, 73.5, 73.4, 69.9, 67.4, 67.3, 67.0, 66.9, 62.9, 42.7, 34.4, 32.2, 29.9, 29.8, 29.6, 29.5, 29.4, 25.2, 23.0, 14.5. ^{31}P NMR ($CDCl_3$, 162 MHz): δ 0.93. IR (neat, NaCl): 2891 (s), 2859 (s), 1739 (s), 1463 (m), 1281 (s), 1174 (w), 1114 (m), 1040 (s), 1023 (s), 866 (w), 821 (w), 738 (w). HRMS (ESI$^+$): calcd for $C_{30}H_{53}ClO_8P$ (M + H) 607.3161, found 607.3167.

Alcohol Phospholipid (9). Pd/C (10% by wt., dry, 13 mg) was added to a stirred solution of BOM−phosphate **11b** (100 mg, 0.16 mmol, 1 equiv) in THF (129 μL) and H_2O (14.0 μL). Hydrogen gas was bubbled into the reaction mixture for 5 min. The reaction was stirred under H_2 (1 atm) at 23 °C for 3 h (judged complete by TLC). The solution was filtered through a pad of Celite and was washed with EtOAc (5 mL). The mixture was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel chromatography (hexanes/EtOAc; 1:1; R_f = 0.15) to give **9** as a colorless oil (78 mg, 0.16 mmol, quantitative yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.27 (1H, td, J = 5.5, 1.0 Hz), 4.25 (1H, td, J = 5.8, 1.0 Hz), 4.22−4.16 (3H, m), 4.16−4.12 (1H, m), 4.12−4.08 (1H, m), 3.83 (3H, d, J_{H-P1} = 11.3 Hz, d, J_{H-P2} = 11.2 Hz), 3.72 (2H, t, J = 5.6 Hz), 2.34 (2H, t, J = 7.4 Hz), 1.66−1.59 (2H, m), 1.29−1.25 (24H, m), 0.88 (3H, t, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.8, 69.3, 69.2, 69.1, 69.0, 67.6, 64.5, 55.1, 42.8, 42.7, 34.4, 32.3, 30.0, 29.91, 29.89, 29.8, 29.7, 29.6,

29.5, 25.2, 23.0, 14.5. ^{31}P NMR ($CDCl_3$, 162 MHz): δ 1.89. IR (neat, NaCl): 3388 (b), 2957 (s), 2891 (s), 2860 (s), 1739 (s), 1466 (s), 1377 (m), 1254 (s), 1176 (s), 1038 (s), 877 (m), 822 (m), 739 (m), 699 (m), 670 (m). HRMS (ESI$^+$): calcd for $C_{22}H_{45}ClO_7P$ (M + H) 487.2591, found 487.2591.

Tris-TBS−Isoprostanyl Phospholipid (13). Acid **12** (22.0 mg, 0.032 mmol, 1 equiv) was dissolved in CH_2Cl_2 (800 μL, 0.04 M). EDC (11.0 mg, 0.057 mmol, 1.8 equiv) and DMAP (2.0 mg, 0.016 mmol, 0.5 equiv) were added to the solution followed by alcohol **9** (23.0 mg, 0.047 mmol, 1.5 equiv). The reaction mixture was stirred for 12 h at 23 °C. The solution was diluted with CH_2Cl_2 (3.0 mL) and was washed with saturated NH_4Cl (aq) (1 × 3 mL) and brine (1 × 3 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by silica gel chromatography (hexanes/EtOAc 5:1; R_f = 0.15, 0.1) to obtain **13** as a colorless oil (20.0 mg, 0.017 mmol, 54% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.46 (1H, dd, J = 15.2, 6.0 Hz), 5.44 (1H, m), 5.37−5.31 (2H, m), 5.27−5.21 (1H, m), 4.35−4.09 (6H, m), 4.04 (1H, q, J = 6.2 Hz), 3.89 (1H, dt, J = 6.8, 3.9 Hz), 3.80 (3H, d, J_{H-P1} = 11.3 Hz, d, J_{H-P2} = 11.3 Hz), 3.70 (2H, t, J = 5.7 Hz), 2.62−2.57 (1H, m), 2.36−2.29 (4H, m), 2.12−2.01 (4H, m), 1.88 (1H, q, J = 7.1 Hz), 1.72−1.59 (3H, m), 1.54−1.40 (2H, m), 1.25 (34H, m), 0.88−0.86 (33H, m), 0.04 (3H, s), 0.03−0.01 (15H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.2, 172.5, 136.0, 129.9, 128.7, 128.1, 76.4, 76.3, 73.7, 69.7, 69.6, 67.51, 67.46, 65.9, 61.8, 55.0, 54.9, 52.6, 50.3, 44.6, 42.7, 42.6, 38.8, 34.3, 34.0, 32.27, 32.2, 30.04, 30.00, 29.97, 29.8, 29.7, 29.6, 29.5, 27.0, 26.6, 26.3, 26.22, 26.20, 25.3, 25.2, 23.1, 23.0, 18.6, 18.42, 18.37, 14.5, 14.4, −3.87, −3.94, −4.2, −4.3. ^{31}P NMR ($CDCl_3$, 162 MHz): 0.77, 0.74. IR (thin film, NaCl): 3059 (m), 3026 (s), 2950 (s), 2924 (s), 2859 (s), 1745 (s), 1462 (m), 1254 (s), 1144 (m), 1045 (s), 972 (w), 836 (s), 227 (m). HRMS (ESI$^+$): calcd for $C_{60}H_{118}ClO_{11}Si_3Na$ (M + Na) 1187.7302, found 1187.7306. $[\alpha]^{20}_D$ −14° (c = 1.0, $CHCl_3$).

***ent*-15-*epi*-F_{2t}-Isoprostanyl Phosphatidylcholine (2).** Aqueous HCl (163 μL, 0.16 mmol, 10 equiv, 1 M) was added to a stirred solution of **13** (19.0 mg, 0.016 mmol, 1 equiv) in THF (500 μL, 0.3 M), and the mixture was stirred at 23 °C for 10 h (judged complete by TLC). The reaction mixture was concentrated, and the residue was purified by silica gel chromatography (CH_2Cl_2/MeOH; 9:1; R_f = 0.4, 0.38) to obtain the triol isoprostanyl lipid **13b** as a colorless oil (12.0 mg, 0.014 mmol, 92% yield).

In a heavy-walled sealed tube, **13b** (2.0 mg, 2.4 μmol) and $CHCl_3$/EtOH (100 μL/100 μL) were added and a rubber septum was placed on top. Anhydrous trimethylamine (approximately 200 μL) was condensed to the solution at −78 °C until the volume approximately doubled. The reaction mixture was allowed to warm to 23 °C, and the rubber septum was replaced with a Teflon screw cap. The reaction mixture was heated to 62 °C and was stirred at this temperature for 60 h. The solution was allowed to cool to 23 °C and was concentrated. The crude residue was purified by silica gel chromatography (CH_3OH/H_2O; gradient 100:0, 50:1, 10:1; R_f = 0.25, CH_3OH/H_2O, 10:1) to obtain **2** as an off-white solid (1.3 mg, 1.6 μmol, 65% yield). ^{1}H NMR (CD_3OD, 400 MHz): δ 5.52−5.49 (2H, m), 5.44 (1H, dd, J = 11.8, 3.3 Hz), 5.39−5.33 (1H, m), 5.24−5.19 (1H, m), 4.40 (1H, dd, J = 12.0, 3.3 Hz), 4.30−4.23 (3H, m), 4.17 (2H, m), 4.01−3.92 (3H, m), 3.84 (1H, dt, J = 7.6, 5.4 Hz), 3.63−3.61 (2H, m), 2.66 (1H, m), 2.46 (1H, p, J = 7.4 Hz), 2.32 (4H, m), 2.13−1.99 (5H, m), 1.65 (2H, m), 1.61−1.39 (12H, m), 1.27 (34H, m), 0.92 (3H, t, J = 7.5 Hz), 0.89 (3H, t, J = 7.1 Hz). ^{31}P NMR (CD_3OD, 162 MHz): δ 0.47. IR (thin film, NaCl): 3355 (b), 3059 (m), 3027 (m), 2954 (s), 2892 (m), 2856 (s), 1737 (s), 1661 (w), 1465 (w), 1234 (m), 1085 (s), 971 (m). HRMS (ESI$^+$): calcd for $C_{44}H_{83}NO_{11}P$ (M + H) 832.5701, found 832.5704. $[\alpha]^{20}_D$ +46.3° (c = 0.05, $CHCl_3$).

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Supporting Information Available: Experimental details and NMR spectra are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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