Supplementary Methods

Capture and Release of Alkyne-Derivatized Glycerophospholipids Using Cobalt Chemistry

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Materials

¹H, ¹³C, and ³¹P NMR spectra were collected on a 300 MHz NMR. All reactions were carried out under an atmosphere of argon. THF and CH₂Cl₂ were dried using a solvent purification system. Commercial anhydrous DMF, CHCl₃, Et₃N, and pyridine were used as received. Phospholipids were dried by azeotroping from benzene and dried under vacuum prior to coupling reactions. Purification by column chromatography was carried out on silica gel and TLC plates were visualized with phosphomolybdic acid. Odd-carbon glycerophospholipid internal standards (25:0, 31:1, 37:4, and 43:6 PA, PC, PE, PG, PI, and PS) were obtained from Avanti Polar Lipids. 32:0 Phosphatidylmethanol was obtained from Avanti Polar Lipids. 2-Diphenylphosphinoethyl functionalized silica gel was purchased from Sigma chemicals. Co₂(CO)₈ was obtained from Alfa Aesar or Strem.

Alkyne Lipid Synthesis

Preparation of ω-alkynyl polyunsaturated fatty acids aLA (1) and aAA (2) was achieved utilizing the general strategy outlined in Supplemental Scheme 1. Diols 6 and 7 were prepared from methyl linoleate and arachidonic acid according to previous reports.¹-³ Oxidative cleavage of the diols by sodium periodate led to the unstable aldehydes, which were immediately reduced to the corresponding alcohols. The alcohol was converted to the iodide and the methyl ester hydrolyzed in preparation for the Wittig reaction. Transformation of the iodide into the corresponding phosphonium iodide, followed by Wittig coupling with 5-hexynal yielded final products aLA (1) and aAA (2) with excellent diastereoselectivity (≥96% Z). The typical efficiency for the preparation of the modified fatty acids over
Supplemental Scheme 1. Synthesis of aLA (1) and aAA (2).

Reagents: a) NaIO₄, H₂O, THF; b) NaBH₄, MeOH; c) I₂, PPh₃, ImH, Et₂O, MeCN; d) LiOH, H₂O, THF; e) PPh₃, PhMe, MeCN; f) LiN(TMS)₂, 5-hexynal, HMPA, THF.

The sn-2-aPLPC (3) was synthesized by coupling of aLA (1) to commercially available lyso 16:0 PC. A DCC (N,N'-Dicyclohexylcarbodiimide) coupling was employed that involved the addition of glass and sonication to accelerate the reaction. Under these conditions, the reaction time was decreased from days to hours.

Supplemental Scheme 2. Synthesis of aDPPC (4).

Reagents: a) 15-hexadecynoic acid 11, DCC, DMAP (4-dimethylaminopyridine), CHCl₃

Synthesis of diol 6. To a solution of methyl linoleate (100g, 0.34mol) in CH₂Cl₂ (2.6L), was added
m-CPBA (75g, 0.43mol) at 5°C. After 5h, the reaction mixture was poured into saturated NaHCO₃ (1L). The phases were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried with MgSO₄, condensed, and the residue was chromatographed (1-6% EtOAc/hexanes) to yield 80g of a colorless oily mixture of epoxide and its regioisomer. To the mixture of epoxides (80g, 0.26mol) in THF (1.8L) at 5°C was added dropwise a cold 5% HClO₄ solution (650mL). The mixture was stirred overnight at 5°C, and neutralized with 10% NaHCO₃ solution. The majority of the organic solvent was evaporated, and the residual material was extracted with ethyl acetate. The combined organic layers were condensed to ca. 1L, washed with brine, and dried over MgSO₄. Evaporation of solvents gave a yellowish oil, which was chromatographed (5-30% EtOAc/hexanes) to yield 42g of the colorless oily mixture of diol 6 and its regioisomer. NMR analysis was consistent with the literature.⁵

Synthesis of 8a. To the mixture of diols (42g, 0.13mol) in THF (420mL) and water (160mL) at 0 °C, solid NaIO₄ (42g, 0.20mmol) was added, and the resulting suspension was vigorously stirred for 1 h. The mixture was then partitioned between water and EtOAc. The combined organic layers were washed with brine, dried over Na₂SO₄ and condensed. The residual scented oil was dissolved in MeOH (400mL), and it was treated with solid NaBH₄ (16g, 0.42mol) at room temperature. After 45 min, water was added (800mL) and the mixture was stirred for an additional 5 min, followed by an extraction with EtOAc. The combined organic layers were washed with brine, dried over MgSO₄ and condensed. The residue was chromatographed (10-22% EtOAc/hexanes) to yield compound 8a as a colorless oil (13.6g, 18% over 3 steps). ¹H NMR (300MHz) δ 5.55 (m, 1H), 5.37 (m, 1H), 3.67 (s, 3H), 3.65 (t, 2H, J = 6.6Hz), 2.32 (m, 4H), 2.06 (q, 2H, J = 6.6), 1.62 (m, 2H), 1.31 (m, 8H); ¹³C NMR (300MHz) δ 174.4, 133.3, 125.0, 62.3, 51.4, 34.0, 30.8, 29.5, 29.0, 28.9, 27.2, 24.9; HRMS Calcd. for C₁₃H₂₄LiO₃⁺ (M+Li)⁺ 235.1885, found 235.1890.

Synthesis of 8b. To a solution of compound 8a (13.6g, 60mmol) in Et₂O (220mL) and MeCN
(80mL) were added imidazole (5.73, 85mmol), PPh₃ (23.6g, 90mmol) and iodine (21.5g, 85mmol) at 0°C. After 30 min, mixture was brought to room temperature and stirred for additional 1.5h. The resulting suspension was washed with 10% Na₂SO₃ solution (350mL), organic layer was subsequently dried over MgSO₄ and condensed. The residual material was chromatographed (1-5% EtOAc/hexanes) to yield 18g of a mixture of methyl (Z)-12-iodododec-9-enoate and PPh₃ (ca. 20:1 mol/mol). The mixture was dissolved in THF (850mL) and water (160mL), and the solution was degassed by argon bubbling for 30min before and 30min after addition of 1M LiOH solution (120mL) at 5°C. The mixture was reacted overnight at 5°C, and the solution was adjusted to pH of 3 with 1M HCl. THF was evaporated, and the residual material was extracted with EtOAc. The combined organic layers were washed with brine, dried over MgSO₄ and condensed. The residue was chromatographed (6-40% EtOAc/hexanes) to yield 15.4g of (Z)-12iodododec-9-enoic acid as a colorless oil. ¹H NMR (300MHz) δ 5.52 (m, 1H), 5.32 (m, 1H), 3.14 (t, 2H, J = 7.2Hz), 2.63 (q, 2H, J = 7.2Hz), 2.35 (t, 2H, J = 7.5Hz), 2.02 (m, 2H), 1.62 (m, 2H), 1.32 (m, 8H); ¹³C NMR (300MHz) δ 180.1, 132.5, 127.8, 34.0, 31.4, 29.3, 29.1, 29.0, 28.9, 27.3, 24.6; HRMS Calcd. for C₁₂H₂₁IClO₂⁻ (M+Cl)⁻ 359.0275, found 359.0282.

**Synthesis of 8c.** To a solution of the above acid (15.4g, 48mmol) in toluene (105mL) was added PPh₃ (18.1g, 69mmol) in MeCN (37mL). The mixture was refluxed for 96h, solvent was removed and the residual thick yellow oil was chromatographed (1-17% MeOH/EtOAc). The filtrate was condensed to give the product as a yellow semi-solid (21.6g, 62% over 3 steps). ¹H NMR (300MHz) δ 7.77 (m, 15H), 5.53 (m, 1H), 5.49 (m, 1H), 3.73 (m, 2H), 2.44 (m, 2H), 2.33 (t, 2H, J = 7.2Hz), 1.78 (m, 2H), 1.58 (m, 2H), 1.21 (m, 8H); ¹³C NMR (300MHz) δ 177.7, 135.2, 135.1, 133.8, 133.6, 132.7, 130.6, 130.5, 125.9, 125.7, 118.6, 117.5, 33.9, 29.0, 28.8, 28.7, 27.1, 24.6, 23.7, 23.1, 20.6; HRMS Calcd. for C₃₀H₃₆O₂⁺ (M-I)⁺ 459.2453, found 459.2446.

**Synthesis of aLA (1).** To a solution of phosphonium iodide 8c (10.8g, 18.5mmol) in THF (225mL)
and HMPA (55mL), was added over 10 min a LiNH(TMS)\(_2\) solution (1M in THF, 38.6mL) at -78 °C. After addition of the base, the reaction mixture was stirred at -40 °C for 1h, and then it was brought back to -78 °C and 5-hexynal (1.74g, 18.1mmol) in THF (60mL) was added. The mixture was then allowed to gradually warm up to room temperature over 1.5 h. The reaction was quenched with water, and the pH of the mixture was brought to ca. 3 with 1M HCl. The mixture was partitioned between water (250mL) and EtOAc (4 x 200mL), and organic layers were combined and condensed. The residual liquid was dissolved in Et\(_2\)O (150mL) and it was washed with brine (5 x 150mL), the ethereal layer was then dried MgSO\(_4\) and condensed. The residue was chromatographed (1-30% EtOAc/hexanes) to yield the product as a pale yellow oil (1.89g, 37%). \(^1\)H NMR \(\delta\) 5.34 (m, 4H), 2.77 (t, 2H, \(J = 6.0\)Hz), 2.33 (t, 2H, \(J = 7.5\)Hz), 2.17 (m, 4H), 2.01 (m,2H), 1.94 (s, 1H), 1.60 (m, 4H), 1.30 (m, 8H); \(^{13}\)C NMR (300MHz) \(\delta\) 179.5, 130.1, 129.1, 128.6, 127.8, 80.5, 68.3, 33.9, 29.5, 29.1, 29.0, 28.9, 28.3, 27.1, 26.1, 25.6, 24.6, 17.8; HRMS Calcd. for C\(_{18}\)H\(_{27}\)O\(_2\) \(- (M-H)\) 275.2011, found 275.2023.

**Synthesis of diol 7.** To a solution of arachidonic acid (5.0g, 16.4mmol) in CH\(_2\)Cl\(_2\) (50mL) was added 1,1'-carbonyldiimidazole (2.8g, 17.3mmol). After 40 min, the resulting arachidonyl imidazolide solution was added over 5 min to a cold (ca. 5°C) 3.6 M ethereal solution of hydrogen peroxide (120mL) containing lithium imidazole (25mg, 0.34mmol). After the addition, the mixture was stirred for 5min, diluted with CH\(_2\)Cl\(_2\) (125mL) and treated with powdered anhydrous KHSO\(_4\) (35g). The mixture was stirred for another 5min, solids were then removed by filtration and the resulting filtrate was kept over anhydrous Na\(_2\)SO\(_4\) (10g) overnight at room temperature, under an argon atmosphere. The drying agent was filtered off, and the filtrate was washed with brine (5 x 50mL). The organic layer was then dried over MgSO\(_4\), and solvents were removed yielding a colorless oil. The oil was dissolved in CH\(_2\)Cl\(_2\) (45mL) and it was treated with 1,1'-carbonyldiimidazole (4.2g, 26mmol). After 45 min, MeOH (8mL) was added and the mixture was stirred for an additional 2h. Solvents were evaporated, and the residue was partitioned between H\(_2\)O and EtOAc. The combined organic layers were washed with brine, dried
over MgSO₄ and condensed. The residue was chromatographed (1-7% EtOAc/hexanes) to yield the epoxide as a colorless oil (3.0g, 55%).

To a solution of the epoxide (3.0g, 9.1mmol) in THF (80mL) at 5 ºC was added dropwise a cold 10% HClO₄ solution (27mL). The mixture was stirred overnight at 5 ºC, and it was then neutralized with solid NaHCO₃. The majority of the organic solvent was evaporated, and the residual material was extracted with EtOAc. The combined organic layers were washed with brine, dried over MgSO₄ and condensed. The residue was chromatographed (10-30% EtOAc/hexanes) to yield 2.4 g of diol 7 as a colorless oil. Spectral characterization was identical with the literature.⁶-⁸

**Synthesis of 9a.** To a mixture of diol 7 (2.4g, 6.8mmol), THF (30mL) and water (12mL) at 0 ºC, solid NaIO₄ (2.18g, 10.2mmol) was added, and the resulting suspension was vigorously stirred for 1 h. The mixture was then partitioned between water (40mL) and EtOAc (4 x 20mL). The combined organic layers were washed with brine, dried over Na₂SO₄ and condensed. The residual scented oil was dissolved in MeOH (20mL), and it was treated with solid NaBH₄ (0.86g, 22.7mmol) at 0 ºC. After 35 min, water was added (40mL) and the mixture was stirred for an additional 5 min, followed by an extraction with Et₂O. The combined organic layers were washed with brine, dried over MgSO₄ and condensed. The residue was chromatographed (10-30% EtOAc/hexanes) to yield the product as a colorless oil (1.25g, 55% over 3 steps). Analytical data for compound 9a matched well with the data presented for this compound in the literature.⁶

**Synthesis of 9b.** To a solution of compound 9a (1.25g, 5.0mmol) in Et₂O (18mL) and MeCN (6mL) was added imidazole (0.47g, 7.0mmol), PPh₃ (1.97g, 7.5mmol) and iodine (1.78g, 7.0mmol) at 0 ºC. After 30 min, the mixture was brought to room temperature and stirred for an additional 1.5h. The resulting suspension was washed with 10% Na₂SO₃ (25mL), organic layer was subsequently dried over MgSO₄ and condensed. The residual material was chromatographed (1-5% EtOAc/hexanes) to yield 1.6
g of a mixture of methyl (5Z,8Z,11Z)-14-iodotetradeca-5,8,11-trienoate and PPh₃ (ca. 20:1 mol/mol). The mixture was dissolved in THF (60mL) and water (12mL), and the solution was degassed by argon bubbling for 20 min before and 20 min after addition of a cold 1 M LiOH solution (9mL) at 5 ºC. The mixture was reacted overnight at 5 ºC, and the pH of the solution was brought to 3 with 1 M HCl. THF was evaporated, and the residual material was extracted with EtOAc. The combined organic layers were washed with brine, dried over MgSO₄ and condensed. The residue was chromatographed (10-40% EtOAc/hexanes) to yield 1.4 g of 9b as a colorless oil. 

H NMR (300MHz) δ 5.52 (m, 1H), 5.37 (m, 5H), 3.15 (t, 2H, J = 7.2Hz), 2.81 (m, 4H), 2.67 (q, 2H, J = 7.2Hz), 2.38 (t, 2H, J = 7.5Hz), 2.14 (m, 2H), 1.72 (m, 2H); ¹³C NMR (300MHz) δ 179.9, 130.3, 128.9, 128.8, 128.4, 128.3, 127.7, 33.4, 31.4, 26.4, 25.8, 25.6, 24.4; HRMS Calcd. for C₁₄H₂₁IO₂Cl (M+Cl) − 383.0275, found 383.0270.

Synthesis of 9c. To a solution of 9b (1.4g, 4.0mmol) in toluene (9.6mL) was added PPh₃ (1.57g, 6.0mmol) in MeCN (3.8mL). After the mixture was refluxed for 96h, the solvent was removed and the residual thick yellow oil was chromatographed (1-23% MeOH/EtOAc). The filtrate was condensed to give salt 10c as a yellow semi-solid material (1.5g, 49%). H NMR (300MHz) δ 7.77 (m, 15H), 5.53 (m, 1H), 5.30 (m, 5H), 3.75 (m, 2H), 2.63 (m, 4H), 2.49 (m, 2H), 2.34 (t, 2H, J = 7.2Hz), 2.05 (m, 2H), 1.64 (m, 2H); ¹³C NMR (300MHz) δ 177.4, 135.2, 135.1, 133.7, 133.6, 130.7, 130.6, 130.5, 129.0, 128.7, 128.5, 127.0, 126.3, 126.1, 118.5, 117.4, 33.4, 26.4, 25.6, 24.4, 23.5, 22.9, 20.3; HRMS Calcd. for C₃₂H₃₆O₂P (M-I)⁻ 483.2453, found 483.2447.

Synthesis of aAA (2). To a solution of phosphonium iodide 9c (1.5g, 2.4mmol) in THF (30mL) and HMPA (7mL) was added dropwise a 1 M solution of LiN(TMS)₂ in THF (4.8mL, 4.8mmol) at -78 ºC. After addition of the base, the mixture was stirred at -40 ºC for 40 min, and then it was brought back to -78ºC and 5-hexynal (0.23g, 2.4mmol) in THF (9mL) was added dropwise. The mixture was then
allowed to gradually warm up to room temperature over a period of 100 min. The reaction was quenched with water, and the pH of the mixture was brought to 3 with 1 M HCl. The mixture was partitioned between water (50mL) and EtOAc (4 x 30mL), organic layers were combined and condensed. The residual liquid was dissolved in Et₂O (30mL) and it was washed with brine (5 x 30mL), the ethereal layer was then dried over MgSO₄ and condensed. The residue was chromatographed (1-28% EtOAc/hexanes) to yield compound 2 as a pale yellow oil (0.39g, 54%). ¹H NMR (300MHz) δ 5.35 (m, 8H), 2.82 (m, 6H), 2.35 (t, 2H, J = 7.5Hz), 2.18 (m, 6H), 1.94 (s, 1H), 1.70 (m, 2H), 1.60 (m, 2H); ¹³C NMR (300MHz) δ 179.5, 129.0, 128.9, 128.8, 128.7, 128.3, 128.2, 128.1, 128.0, 127.9, 84.4, 68.4, 33.2, 28.2, 26.4, 26.1, 25.6, 25.5, 24.4, 17.8; HRMS Calcd. for C₂₀H₂₇O₂ (M-H) 299.2011, found 299.2009.

**Synthesis of 34:4 PC (sn-2-aPLPC) 3.** DCC (1.1 g, 5.4 mmol) and DMAP (0.61 g, 5.0 mmol) were added to a milky solution of lyso 16:0 PC (0.68 g, 1.4 mmol) and alkynyl linoleic acid (0.29 g, 1.1 mmol) in CHCl₃ (20 mL). Crushed glass was added and the reaction mixture sonicated. After 2.5 h, the reaction mixture was filtered, washed with MeOH, and concentrated. Acidic Dowex was added to a solution of the crude product in MeOH to remove the DMAP. After 30 min, the solution was filtered and washed with additional MeOH. Column chromatography (65:30:5, CH₂Cl₂:MeOH:H₂O) yielded the product as a sticky white powder (0.51 g, 65%). ¹H NMR (MeOH-d₄) δ 5.35 (m, 4H), 5.24 (m, 1H), 4.43 (dd, 1H, J = 2.7, 12.0 Hz), 4.27 (br s, 2H), 4.17 (dd, 1H, J = 6.9, 12.0 Hz), 3.99 (t, 2H, J = 5.8 Hz), 3.66 (m, 2H), 3.24 (s, 9H), 2.80 (t, 2H, J = 5.2 Hz), 2.33 (t, 2H, J = 7.6 Hz), 2.31 (t, 2H, J = 7.6 Hz), 2.18 (m, 5H), 2.07 (q, 2H, J = 6.1 Hz), 1.58 (m, 6H), 1.28 (m, 32H), 0.89 (t, 3H, J = 6.8 Hz); ¹³C NMR (MeOH-d₄) δ 174.8, 174.4, 131.0, 130.2, 129.7, 129.0, 84.5, 71.8 (d), 69.8, 67.4, 64.9 (d), 63.6, 60.5 (d), 54.7, 35.1, 34.9, 33.1, 30.8, 30.7, 30.5, 30.4, 30.24, 30.17, 29.7, 28.2, 27.1, 26.6, 26.0, 23.8, 18.6, 14.6; HRMS (MALDI) calculated 754.5387 (M + H), observed 754.5385.

**Synthesis of 15-hexadecynol 10.** NaH was washed with hexanes several times and dried. 1,3-
Diaminopropane (150 mL) was SLOWLY added to NaH (7.9 g, 0.33 mol) and heated to 70 °C. The reaction mixture turned brown upon heating. After 1h, 7-hexadecyn-1-ol (13.8 g, 0.058 mol) was added and the temperature lowered to 55 °C. After stirring overnight, the reaction mixture was cooled, quenched with H₂O, and extracted with ether. The organic layer was washed with 10% HCl, brine, and dried over MgSO₄. The product was isolated as a white powder (11.7 g, 84%) and used without further purification. ¹H NMR (CDCl₃) δ 3.60 (t, 2H, J = 6.6 Hz), 2.15 (dt, 2H, J = 2.6, 7.0 Hz), 1.91 (t, 1H, J = 2.6 Hz), 1.50 (m, 5H), 1.23 (m, 20H); ¹³C NMR (CDCl₃) δ 84.8, 68.0, 63.0, 32.7, 29.6, 29.54, 29.45, 29.4, 29.1, 28.7, 28.4, 25.7, 18.3.

**Synthesis of 15-hexadecynoic acid 11.** PDC (29.5 g, 0.078 mol) was added to a solution of 15-hexadecyn-1-ol (6.3 g, 0.026 mol) in DMF (100 mL). After stirring overnight, the reaction mixture was diluted with 10% HCl and extracted with EtOAc. The organics were washed with brine and dried over MgSO₄. Purification by column chromatography (2:1, hexanes:EtOAc) yielded the product (4.4 g, 67%) as a white powder. ¹H NMR (CDCl₃) δ 2.32 (t, 2H, J = 7.5 Hz), 2.15 (m, 2H), 1.91 (t, 1H, J = 2.4 Hz), 1.59 (m, 2H), 1.48 (m, 2H), 1.23 (m, 18H); ¹³C NMR (CDCl₃) δ 180.3, 84.8, 68.0, 34.0, 29.5, 29.44, 29.36, 29.2, 29.1, 29.0, 28.7, 28.4, 24.6, 18.3.

**Synthesis of 32:4 PC (aDPPC) 4.** DCC (1.1 g, 5.3 mmol) and DMAP (0.70 g, 5.7 mmol) were added to a milky solution of sn-glycero-3-phosphocholine cadmium chloride (0.81 g, 1.8 mmol) and 15-hexadecynoic acid (1.4 g, 5.4 mmol) in CHCl₃ (30 mL). Crushed glass was added and the reaction mixture sonicated. After 5h, the reaction mixture was filtered, washed with MeOH, and concentrated. The crude product was taken up in MeOH/CHCl₃/H₂O (5:4:1) and treated with Rexyn I-300 (1.0 g) to remove the CdCl₂. The mixture was filtered and washed with additional solvent. Column chromatography (65:30:5, CH₂Cl₂:MeOH:H₂O) yielded the product as a white powder (0.50 g, 38%). ¹H NMR (MeOH-d₄) δ 5.24 (m, 1H), 4.43 (dd, 1H, J = 3.1, 12.0 Hz), 4.27 (m, 2H), 4.16 (dd, 1H, J =
6.9, 12.0 Hz), 3.99 (t, 2H, J = 5.6 Hz), 3.64 (m, 2H), 3.22 (s, 9H), 2.34 (t, 2H, J = 7.4 Hz), 2.31 (t, 2H, J = 7.4 Hz), 2.15 (m, 6H), 1.49 (m, 4H), 1.30 (m, 40H); $^{13}$C NMR (MeOH-$d_4$) δ 174.9, 174.6, 85.0, 71.8 (d), 69.4, 67.5, 64.9 (d), 63.6, 60.5 (d), 54.7, 35.1, 34.9, 34.7, 30.8, 30.7, 30.5, 30.3, 29.8, 29.7, 26.7, 26.0, 19.0; HRMS (MALDI) calculated 726.5074 (M + H), observed 726.5071.

Lipid Extraction and Mass Spectral Analysis

Extraction of Macrophage Lipids. Phospholipids were extracted using a modified Bligh and Dyer procedure. The method is suitable for extraction from cell culture plates after aspirating the medium and washing the adhered cells twice with 5 mL ice cold 1X PBS. Cells are then scraped in 1mL of 1X PBS, and centrifuged (600g, 4°C, 5 min). PBS is aspirated and the cell pellet is extracted with 800 μL of cold 0.1 N HCl: MeOH (1:1) and 400 μL of cold CHCl$_3$ with vortexing (1 min) followed by centrifugation (5 min, 4°C, 18,000g). The lower organic phase is then isolated, odd-carbon internal standards are added, and solvent evaporated (Labconco Centrivap Concentrator, Kansas City, MO).

Liquid Chromatography/ Mass Spectrometry. Class separation of glycerophospholipids was achieved by the use of a previously published LC/MS technique. After extraction and solvent evaporation (as described above) the resulting lipid film is dissolved in 100 μL of IPA:Hexane:100 mM NH$_4$CO$_2$H$_{(aq)}$ 58:40:2 (mobile phase A). For our lipid screens, we utilized an Applied Biosystems/ MDS SCIEX 4000 Q TRAP hybrid triple quadrupole/ linear ion trap mass spectrometer (Applied Biosystems, Foster City, CA). Coupled to this instrument were a Shimadzu (Shimadzu Scientific Instruments, Inc., Columbia, MD) HPLC system consisting of a SCL 10 AVP controller, two LC 10 ADVP pumps and a CTC HTC PAL autosampler (Leap Technologies, Carrboro, NC). All samples shown were separated on a Phenomenex (Phenomenex, Torrance, CA) Luna Silica column (2 x 250 mm, 5 micron particle size) using a 20 μL sample injection. Lipids were separated using a binary
gradient program consisting of IPA: Hexane: 100 mM NH₄CO₂H (aq) 58:40:2 (mobile phase A), and IPA: Hexane: 100 mM NH₄CO₂H (aq) 50:40:10 (mobile phase B). The following LC gradient was used: 0-5 min, B=50%; 5-30 min, B=50%-100%; 30-40 min, B=100%; 40-41 min, B=100%-50%; 41-50 min, B=50%. The mobile phase was infused at a flow rate of 0.3mL/min. The MS spectra were acquired in negative ionization mode using a turbo spray source operated at 450°C with an ion voltage of – 3500V, and nitrogen as curtain and nebulizer gas. The curtain gas (CUR) was 30 l/h, and ion source gas 1 and 2 were both 50 l/h. The declustering potential (DP) was -110 V and the collision energy (CE) was -5 V. Scan type: EMS, unit resolution for Q1; Scan rate: 1000 amu/s; Scan range from m/z 350-1200, with the ion trap set for dynamic fill time.

**Direct Infusion Mass Spectrometry.** Additional mass spectral analysis was performed on a Finnigan TSQ Quantum triple quadrupole mass spectrometer (ThermoFinnigan, San Jose, CA) equipped with a Harvard Apparatus syringe pump (Harvard Apparatus, Holliston, MA) and an electrospray source. Samples were analyzed at an infusion rate of 10 μL/min in both positive and negative ionization modes over the range of m/z 350 to 1200.

**Cell Culture**

**RAW 264.7 Cell Cultures.** RAW 264.7 cells were obtained from the American Type Culture Collection. The cells were maintained in Dulbecco’s Modified Eagle Medium supplemented with GlutaMax, high glucose, sodium pyruvate, and pyridoxine-HCl (Gibco, Grand Island, NY) containing 10% heat inactivated fetal calf serum (Atlas Biologicals, Norcross, GA) (DMEM/FCS). Cells were plated onto 60 mm dishes at 1.5 x 10⁶ cells/dish on the day prior to experiments.

**Enrichment of RAW 264.7 Cells with Alkyne-Derivatized Linoleic (1) or Arachidonic (2) Acids.** A solution (0.25 ml) of 20 mg/mL fatty acid in chloroform was placed in an acid-washed glass tube and
the solvent was evaporated to dryness under argon. 1.25 mL of 0.015 M KOH was added to the resulting film. The tube was sealed under argon and incubated at 37°C for 1h. Fatty acid- and endotoxin-free bovine serum albumin (BSA, 1.25 g, Sigma) was dissolved in 40 mL of calcium- and magnesium-free phosphate-buffered saline (PBS), and added to the tube, which was sealed under argon and incubated at 37°C with agitation for 24h. The pH of the solution was corrected to 7.4, and PBS was added to a final volume of 50 mL. The resulting solution was stored under argon at -20°C. On the day of the experiment, the solution was thawed and diluted 1:10 in Macrophage Serum-Free Medium (Gibco) to give a final BSA concentration of 2.5 mg/mL and a final fatty acid concentration of 10 μg/mL (SFM-AA). Media from the tissue culture plates was removed and replaced with the enriched media. Each plate was incubated for 20 h.

**Enrichment of RAW 264.7 Cells with PC (3).** Alkyne-derivatized 34:4 PC (3) (41.2 mg) was dissolved in 500 μL CHCl₃, transferred to a Pyrex tube and dried under nitrogen. While the lipid was drying, 20 mL of serum free DMEM + 0.25% fatty acid free BSA was prepared. One mL of the serum free DMEM / BSA solution was added to the dried lipid in the Pyrex tube and this was sonicated for 5 min, vortexed and then sonicated an additional 5 min. The 1 mL of sonicated lipid was added back to the 19 mL of serum free DMEM / BSA and mixed well. Media from six 60 mm RAW 264.7 cell plates was removed and replaced with the enriched media. Each plate was treated with 3 mL of this media (for a total of 6.18 mg lipid/ dish) for four hours. Three plates were used for lipid quantitation while the remaining three plates were reserved for cobalt derivatization. Lipid incorporation (as determined by LC/MS quantitation): 1.2 μg (1.5 nmol)/ 3x10⁶ cells.

**Formation of Cobalt Complex (3a).** Co₂(CO)₈ (8.4 mg) was dissolved under an atmosphere of N₂ in 100 μL of IPA:Hexane:100 mM NH₄CO₂H (aq) 58:40:2. Compound 3 (3.4 mg) was resuspended in the dicobalt octacarbonyl solution under an atmosphere of N₂ and analyzed by direct infusion MS.
Treatment of PC (3)-Enriched RAW 264.7 Cells with Dicobalt Octacarbonyl. \( \text{Co}_2(\text{CO})_8 \) (8.4 mg) was dissolved in 100 µL of IPA:Hexane:100 mM NH\(_4\)CO\(_2\)H\(_{aq}\) 58:40:2 under an atmosphere of \( \text{N}_2 \). Compound 3 enriched RAW 264.7 whole cell extracts combined from three 60 mm plates were resuspended in the dicobalt octacarbonyl solution and analyzed by LC/MS.

Enrichment of RAW 264.7 Cells with PC. (4) Alkyne-derivatized 32:4 PC (4) (23.0 mg) was dissolved in 500 µL CHCl\(_3\), transferred to a Pyrex tube, and dried under nitrogen. Ten mL of serum free DMEM + 0.25% fatty acid free BSA was prepared as described above. Media from three 60 mm RAW 264.7 cell plates was removed and replaced with the enriched media. Each plate was treated with 3 mL of media (for a total of 6.90 mg lipid/dish) for four hours. Lipid incorporation (as determined by LC/MS quantitation): 1.1 µg (1.4 nmol)/ 3x10\(^6\) cells.

Capture and Release of Alkyne 5 from RAW 264.7 Cell Extracts Following PLD Activation. Alkyne-derivatized 34:4 PC (4) (25 mg) was dissolved as described above and added to media containing 20 ng/mL GM-CSF + 100 ng/mL LPS + 10 ng/mL IFN\(\gamma\). After 5 hrs of incubation, triplicate 60 mm plates of primed RAW 264.7 cells (approximately 2.5 x 10\(^6\) cells each) were stimulated with 1 µM PMA + 0.3% n-butanol for 30 minutes. Cell pellets were extracted as described previously. Samples were combined and one third was used for LC/MS analysis (initial sample) while the remaining two thirds was evaporated and used for alkyne capture and release. To the cell extract film was added \( \text{Co}_2(\text{CO})_8 \) (4.5 mg, 13.5 µmol in 200 uL 9:1 MeOH/CH\(_2\)Cl\(_2\)) and the solution stirred at room temperature for 60 min. 2-Diphenylphosphinoethyl derivatized silica gel (50 mg) was added to the mixture. The sample was heated at 50 °C for 2 h with gentle stirring. The silica gel was washed with 9:1 MeOH/CH\(_2\)Cl\(_2\) (3 x 200 µL) and centrifuged to remove unbound phospholipids. Washings were pooled and analyzed by LC/MS (post-capture wash). The rinsed gel was then treated with
Fe(NO$_3$)$_3$ (8 mg, 20 µmol) in MeOH (600 uL), and the suspension stirred at room temperature for 30 min. The silica gel was washed with 9:1 MeOH/CH$_2$Cl$_2$ (4 x 200µL), centrifuged, and the washings pooled and concentrated. The sample was resuspended in LC solvent (IPA/hexane/ammonium formate) (100 µL) for LC/MS analysis (release sample). Native lipids and alkyne PC 4 were quantitated using the 4 odd carbon PC internal standards used in all experiments. Alkyne phosphatidylbutanol 5 was quantitated with the aid of a 32:0 phosphatidylmethanol internal standard. Please note that all transfers involving cobalt were done under an atmosphere of N$_2$ (glove bag) to minimize oxidation. Only the final washing step was performed with exposure to air.

Supplemental Figures

**Supplemental Figure 1.** Intra-class glycerophospholipid distributions for four representative lipid classes. Individual lipids are displayed as percentages of the total lipid class. (A) PE lipid distributions
in control RAW 264.7 (blue bars), AA-enriched (yellow bars), alkyne-derivatized AA (2) (red bars), and alkyne-derivatized LA (1) (green bars). Green asterisk tags refer to phospholipids in compound (1) enriched cells that were found by MS/MS analysis to contain either fatty acid (1) and/or the two carbon elongation product of fatty acid (1). Red asterisk tags are used to identify phospholipids from compound (2) enriched cells containing fatty acid (2) and/or its two carbon elongated analog. (B) Distribution of PG phospholipids, (C) Distribution of PI lipids, (D) Distribution of PS phospholipids.

**Supplemental Figure 2.** MS/MS spectra of pure PC (3). MS/MS analysis of the alkyne analog 3 was performed by direct infusion MS. Compound 3 was detected as a chloride adduct in negative ionization mode. The major fragments detected were 788 m/z (chloride adduct), 738 m/z (M-15), 275
m/z (18:4 fatty acid), and 255 m/z (16:0 fatty acid). Similar MS/MS experiments were conducted for comparisons between cell extract lipids and a pure standard of compound 4.

Supplemental Figure 3. MS/MS of PC Cobalt complex 3a. Analysis of the 1038 m/z peak was consistent with a compound containing six carbonyl moieties. The mass of the parent peak matched the expected value for [3a-H].
Supplemental Figure 4. LC/MS results from PMA treatment of RAW 264.7 cells enriched with PC (4). (a) Example of a basal RAW 264.7 cell extract glycerophospholipid MS spectra. Analysis of RAW cell extracts are extremely complicated due to the presence of over 1000 glycerophospholipid species within a narrow range of spectra. Most alkyne-tagged lipids coelute and are isobaric with naturally occurring phospholipids. (b) RAW cell extract 695 m/z extracted ion chromatogram, which corresponds to the 32:4 alkynyl PtdBuOH - PLD reaction product 5. (c) Typical RAW cell extract showing extracted ion chromatograms for 4 major lipids (38:4p PE, 38:4 PI, 36:1 PS, and 34:1 PA). (d) Extracted ion chromatograms for 38:4p PE, 38:4 PI, 36:1 PS, and 34:1 PA following alkyne release from solid support using Fe(NO₃)₃. It is noteworthy that lipids not bearing an alkyne moiety were not bound to the gel. (e) Extracted ion chromatograms of alkynes 4 (red trace) and 5 (blue trace) following lipid release from functionalized silica gel. Cleavage of the silica gel/cobalt/alkyne complex with Fe(NO₃)₃ releases bound alkyne lipids.

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