Electronic Supporting information for

Analysis of Ether Glycerophosphocholines at the Level of C=C Locations from Human Plasma

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1. Protocols for lipid extraction from human plasma

Briefly, 50 μL human plasma was diluted with 950 μL water, 1 mL methanol and 2 mL chloroform containing 0.015% BHT in a 10 mL centrifuge tube. The mixture was vortex-mixed for 10 min and then was centrifuged at 10,000 rpm for 20 min to obtain layer separation. The bottom chloroform layer was collected and the extraction procedure was repeated twice by adding 2 mL chloroform for every time. Finally, the chloroform layers were combined and evaporated under a stream of nitrogen. The extract was re-dissolved in 1 mL methanol and stored at -20 ℃ prior to MS analysis.

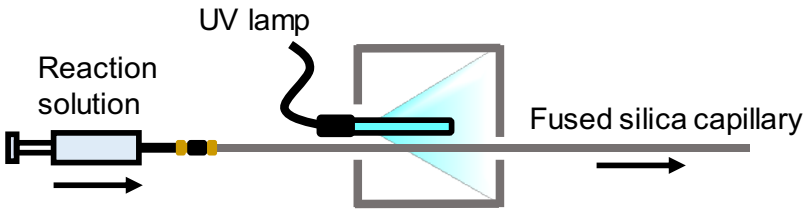
2. Supplemental methods for mass spectrometry analysis

NanoESI. Positive-ion nanoESI experiments were performed on a QTRAP 4500 mass spectrometer (Sciex, Toronto, CA) equipped with a home-built nanoESI source. NanoESI capillary was made from borosilicate glass capillary (1.5 mm o.d., 0.86 mm i.d.) by a P-1000 Flaming/Brown micropipette puller (Sutter Instrument, Novato, CA, USA). A stainless-steel wire was inserted into the nanoESI capillary to provide electric contact to the solution for nanoESI. Data collection modes include enhanced MS (EMS) and enhanced product ion (EPI). The instrument parameters were as follows: nanoESI voltage, 1500 V; curtain gas, 10 psi; declustering potential, 100V.

LC-MS. LC-MS experiments were performed on a QTRAP 4500 mass spectrometer coupled with an ExionLC AC system (Sciex, Toronto, CA) or a X500R QTOF mass spectrometer (Sciex, Toronto, CA) coupled with a 20AD HPLC system (Shimadzu, Tokyo, Japan). LC separation was performed on a hydrophilic interaction chromatography (HILIC) column (150 mm × 2.1 mm, 2.7 μm; Sigma-Aldrich, St. Louis, MO, USA) using the following parameters: flow rate 0.2 mL/min, oven temperature 30 ℃, mobile phase gradient: 90-85 % B in 0-5 min, 85 % B in 5-8 min, 85-80% B in 8-15 min, 80% B in 15-18 min, 80-70% B in 18-19 min, 70% B in 19-22 min, 70%-90% B in 22-23 min and 90% B in 23-25 min, where A was 10 mM aqueous ammonium acetate and B was acetonitrile. Injection volume was 2 μL for QTRAP and 2-10 μL for QTOF. The parameters

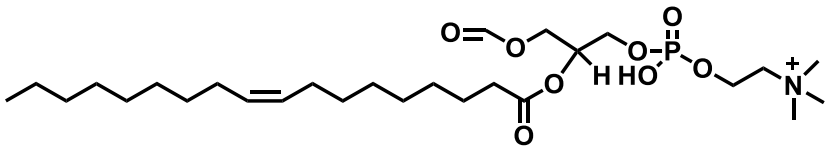
on QTRAP 4500 were as follows: ESI voltage, ±4500 V; curtain gas, 35 psi; interface heater temperature, 400°C; nebulizing gas 1 and gas 2, 30 psi; declustering potential, ±100V. The parameters on X500R QTOF were as follows: ESI voltage, 5500 V; curtain gas, 25 psi; CAD gas, 8; interface heater temperature, 500°C; nebulizing gas 1 and gas 2, 55 psi; declustering potential, 80V.

3. Setup for offline PB reaction

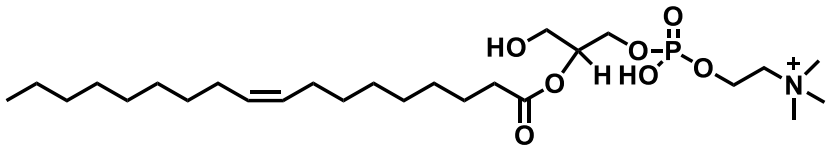


Scheme S1 A schematic representation of flow microreactor for implementing offline PB reaction. The UV lamp was parallelly positioned 1 cm away from the fused silica capillary. The PB reaction time was calculated by the total volume of the fused silica capillary exposed to UV irradiation divided by the solution flow rate.

4. Optimization of the PB reaction conditions for PC-P

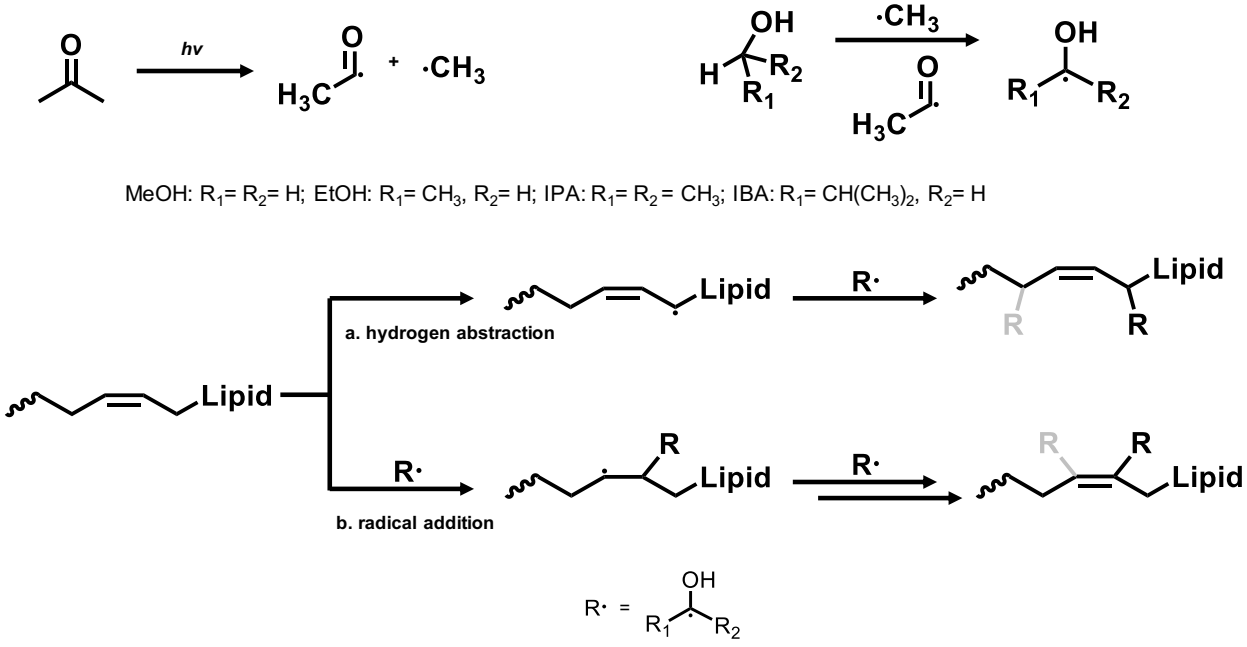


Oxidation products, *m/z* 550.4



Hydrolysis products, *m/z* 522.5

Scheme S2 The structures of oxidation products and hydrolysis products.



Scheme S3 Possible mechanism for the formation of alcohol substitution side products.

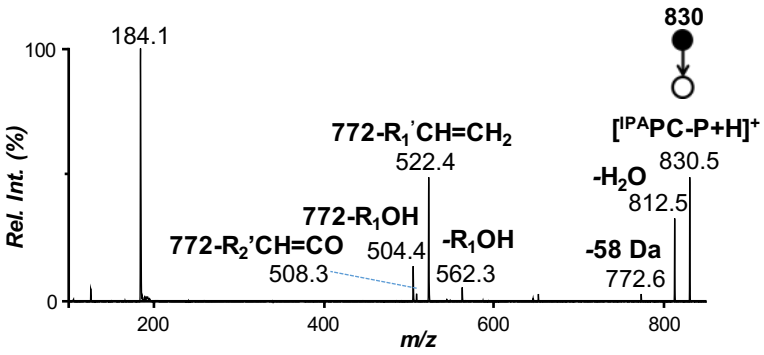


Fig. S1 Positive-ion NanoESI MS^2 CID of IPA substitution products at *m/z* 830.5.

5. Structural identification of PC-P via PB-MS/MS

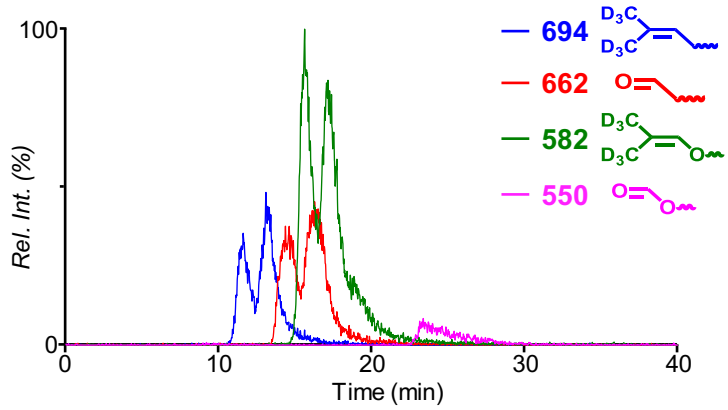


Fig. S2 Extracted ion chromatograms of *m/z* 694, 662, 582, and 550 on positive-ion RPLC-ESI-MS/MS of PB products of PC P-18:0/18:1(9Z) at *m/z* 836.5. RPLC conditions: flow rate 0.4 mL/min, oven temperature 30 ℃, isocratic gradient of 80% B over 40 min. Mobile phase A, 10 mM aqueous ammonium acetate; mobile phase B, acetone/acetonitrile/IPA, 50/25/25.

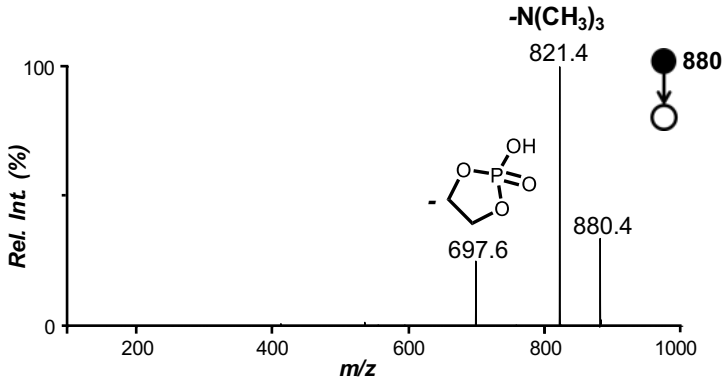


Fig. S3 Positive-ion nanoESI ion trap MS^2 CID of sodium adduct of the PB products of PC P-18:0/20:4(5Z, 8Z, 11Z, 14Z) at *m/z* 880.5. PB reaction solvent: d_6-acetone/water (20 mM NH_4OAc)/IPA (50/40/10, v/v/v).

6. PB reaction phenomena of PC-O

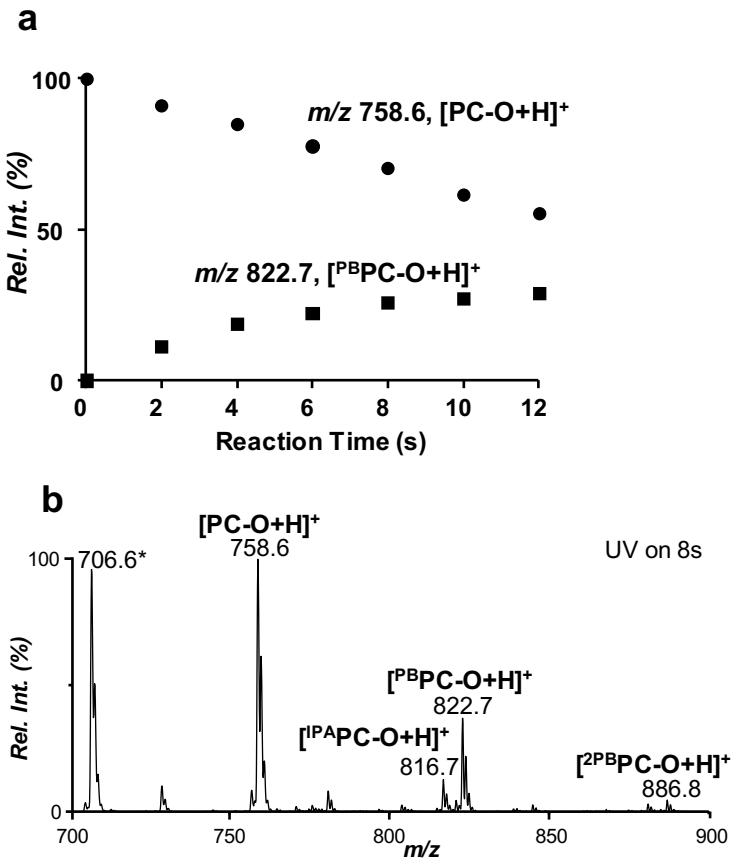


Fig. S4 (a) Kinetic curves of PB reaction of PC O-18:1(9Z)/O-18:1(9Z). (b) Positive-ion nanoESI MS^1 spectra of 2μM PC O-18:1(9Z)/O-18:1(9Z) in d_6-acetone/water/IPA/formic acid (68.6/29.4/1/1, v/v/v/v) after 8 s UV exposure. "*" denotes internal standard (IS) PC 15:0/15:0.

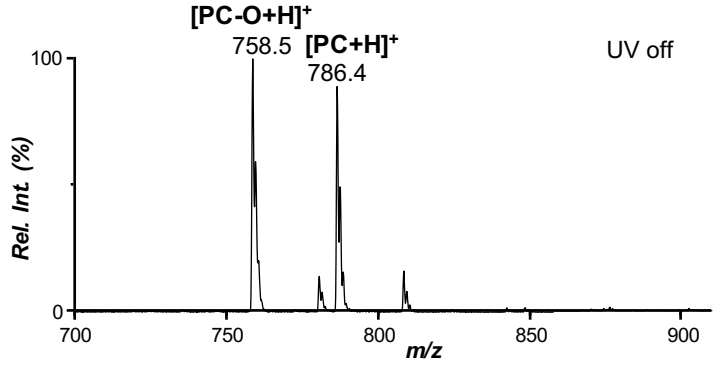


Fig. S5 Positive-ion nanoESI MS^1 spectra of an equimolar mixture (2μM each) of PC O-18:1(9Z)/O-18:1(9Z) and PC 18:1(9Z)/18:1(9Z) in acetone/water/IPA/formic acid (68.6/29.4/1/1, v/v/v/v) before UV exposure.

7. Analysis of ether PCs from human plasma

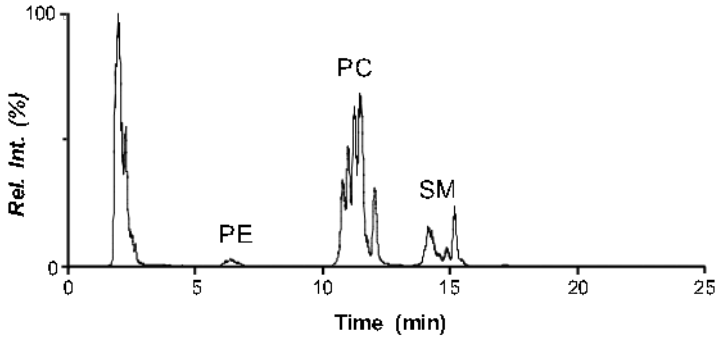
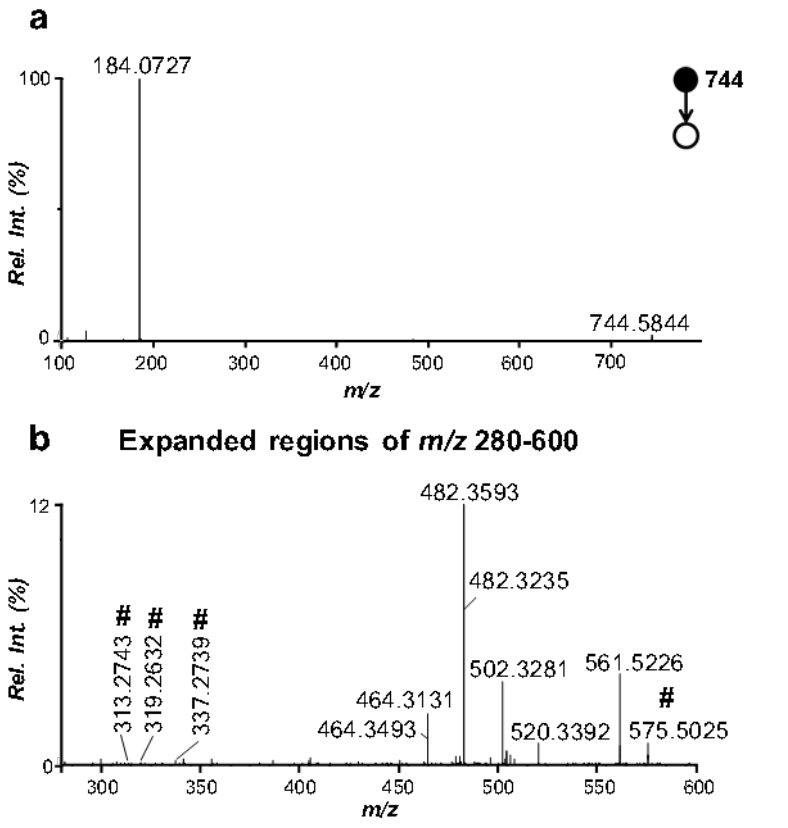


Fig. S6 Positive-ion HILIC-ESI-QTOF-MS chromatogram of total lipid extract from human plasma.



575.5025: loss of head group (169 Da) from DMPE 16:0_18:2
319.2632: loss of 16:0 fatty acid from 575
337.2739: loss of 16:0 ketene from 575
313.2743: loss of 18:2 ketene from 575
464.3131: loss of 18:2 fatty acid from PC 15:0_18:2 or DMPE 16:0_18:2
464.3493: loss of 18:2 fatty acid from PC O-16:0/18:2
482.3235: loss of 18:2 ketene from PC 15:0_18:2 or DMPE 16:0_18:2
482.3593: loss of 18:2 ketene from PC O-16:0/18:2
502.3281: loss of 15:0 fatty acid from PC 15:0_18:2
520.3392: loss of 15:0 ketene from PC 15:0_18:2

Fig. S7 (a) Positive-ion HILIC-ESI-QTOF-MS/MS of *m/z* 744.5. (b) Expanded regions of *m/z* 280-600 from (a) and the identities of fragment ions.

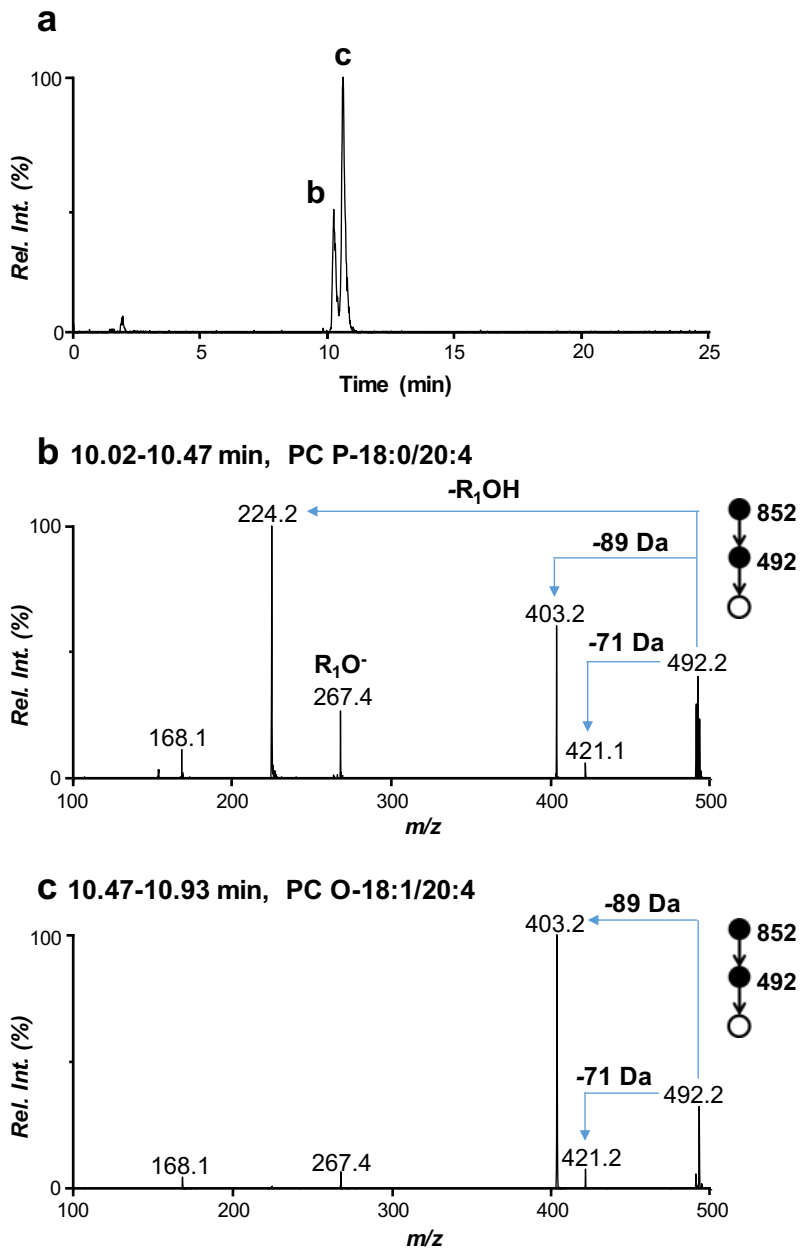


Fig. S8 (a) Negative-ion HILIC-ESI-QTRAP-MS^3 chromatogram of [M-15-20:4 ketene]$^-$ ion at *m/z* 492.2 produced from MS^2 of [M+OAc]$^-$ (*m/z* 852.6). Averaged mass spectra from (b) 10.02-10.47 and (c) 10.47-10.93 min.

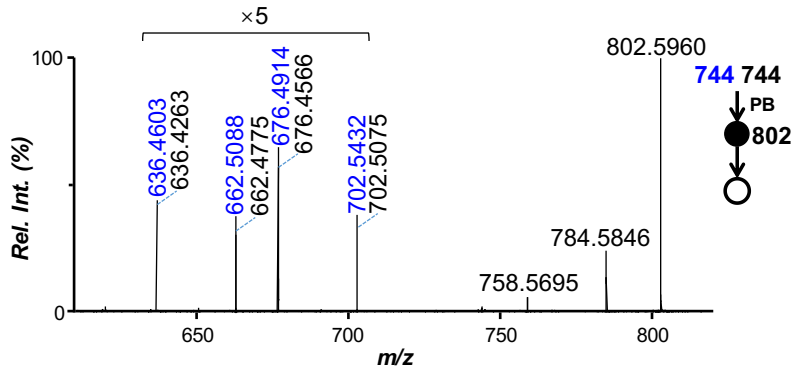


Fig. S9 Positive-ion HILIC-ESI-QTOF-MS/MS of PB products at *m/z* 802.6.

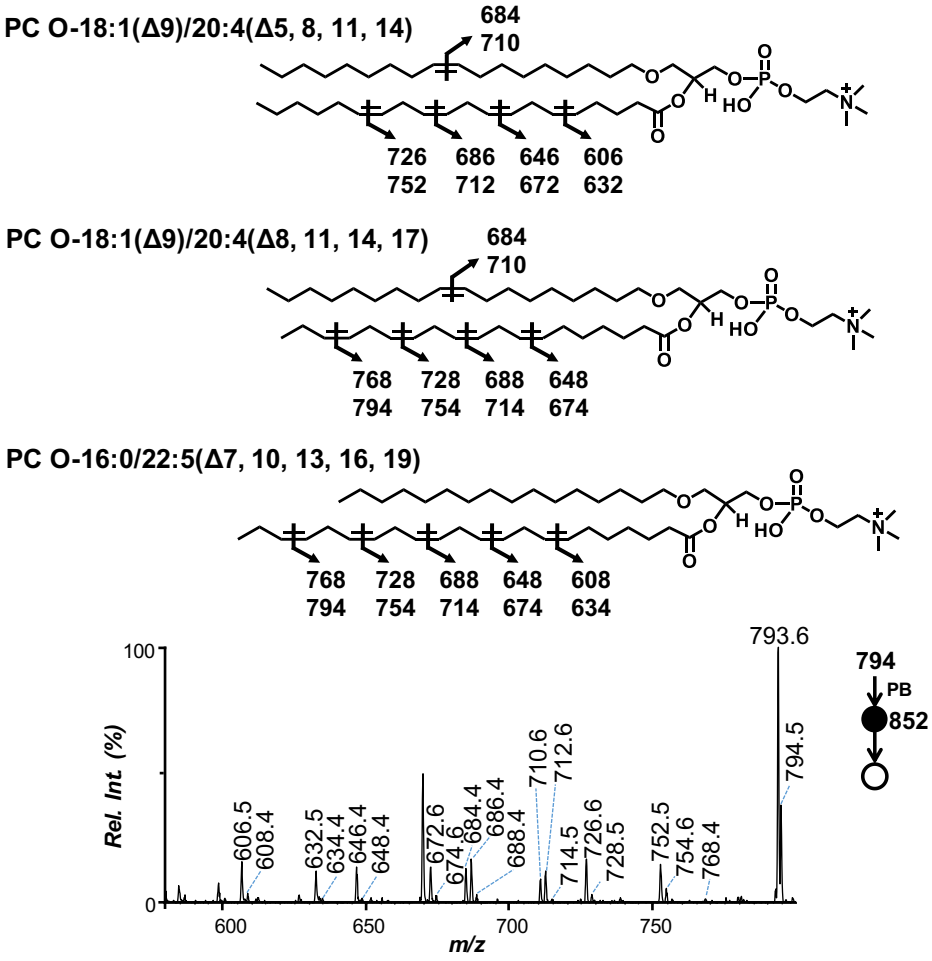


Fig. S10 Positive-ion HILIC-ESI-QTRAP-MS/MS of PB products at *m/z* 852.6.

Table S1 High resolution mass measurements of $[M+H]^+$ ions of ether PCs from human plasma and their assignments based on negative-ion MS^3 CID.

Experimental *m/z*	Theoretical *m/z*	Lipid	Relative Abundance, %	Structures
716.5612	716.5589	PC O-32:2/P-32:1	0.04	PC P-16:0/16:1
718.5408	718.5381	PC 31:1	0.06	PC 15:0_16:1
718.5759	718.5745	PC O-32:1/P-32:0	0.31	PC P-16:0/16:0 PC O-16:0/16:1
720.5554	720.5538	PC 31:0	0.16	PC 15:0_16:0
720.5905	720.5902	PC O-32:0	0.49	PC O-16:0/16:0
742.5758	742.5745	PC O-34:3/P-34:2	1.28	PC P-16:0/18:2
744.5571	744.5538	PC 33:2	0.78	PC 15:0_18:2
744.5899	744.5902	PC O-34:2/P-34:1	1.58	PC P-16:0/18:1 PC O-16:0/18:2
746.5710	746.5694	PC 33:1	0.54	PC 16:0_17:1 PC 15:0_18:1
746.6044	746.6058	PC O-34:1/P-34:0	0.92	PC O-18:1/16:0 PC O-16:0/18:1
764.5607	764.5589	PC O-36:6/P-36:5	0.13	PC P-16:0/20:5
766.5765	766.5745	PC O-36:5/P-36:4	2.17	PC P-16:0/20:4
768.5905	768.5902	PC O-36:4/P-36:3	2.94	PC P-18:1/18:2 PC P-16:0/20:3 PC O-16:0/20:4
770.5701	770.5694	PC 35:3	0.30	PC 17:1_18:2 PC 15:0_20:3
770.6038	770.6058	PC O-36:3/P-36:2	1.08	PC P-18:0/18:2 PC O-18:1/18:2 PC O-16:0/20:3
772.5873	772.5851	PC 35:2	1.40	PC 17:1_18:1 PC 17:0_18:2
772.6191	772.6215	PC O-36:2/P-36:1	0.50	PC O-18:1/18:1 PC P-18:0/18:1 PC O-18:0/18:2
790.5766	790.5745	PC O-38:7/P-38:6	0.30	PC P-16:0/22:6

792.5916	792.5902	PC O-38:6/P-38:5	0.88	PC P-18:1/20:4 PC O-18:2/20:4 PC O-18:1/20:5 PC P-16:0/22:5 PC O-16:0/22:6
794.6069	794.6058	PC O-38:5/P-38:4	2.80	PC P-18:0/20:4 PC O-18:1/20:4 PC P-16:0/22:4 PC O-16:0/22:5
796.5881	796.5851	PC 37:4	0.72	PC 17:1_20:3 PC 17:0_20:4
796.6191	796.6215	PC O-38:4/P-38:3	1.69	PC P-18:0/20:3 PC O-18:1/20:3 PC O-18:0/20:4 PC O-16:0/22:4
816.5904	816.5902	PC O-40:8/P-40:7	0.26	PC P-18:1/22:6
818.6072	818.6058	PC O-40:7/P-40:6	0.32	PC O-18:1/22:6
820.5880	820.5851	PC 39:6	0.16	PC 17:0_22:6
820.6219	820.6215	PC O-40:6/P-40:5	0.36	PC O-20:2/20:4 PC O-18:1/22:5 PC O-18:0/22:6
822.6048	822.6008	PC 39:5	0.16	PC 19:1_20:4 PC 17:0_22:5
822.6373	822.6371	PC O-40:5/P-40:4	0.38	PC O-20:1/20:4 PC O-18:1/22:4 PC O-18:0/22:5
824.6186	824.6164	PC 39:4	0.16	17:0_22:4 19:0_20:4
824.6517	824.6528	PC O-40:4/P-40:3	0.26	PC O-20:0/20:4 PC O-18:0/22:4
850.6693	850.6684	PC O-42:5/P-42:4	0.20	PC O-22:1/20:4

Table S2 Identified unsaturated ether PCs from human plasma based on PB-MS/MS (Some unconfident peaks are labelled in parentheses due to their poor signal-to-noise ratios or *m/z* errors over 5 ppm).

$[M+H]^+$ *m/z*	$[^{PB}M+H]^+$ *m/z*	Diagnostic Ions	Molecular Lipid with Defined C=C Location
718.5759	776.6	550.3860	PC P-16:0/16:0
		636.4597, (662)	PC O-16:0/16:1(Δ9)
742.5758	800.6	574; 634, 660; 674, 700	PC P-16:0/18:2(Δ9, 12)
744.5899	802.6	576.3999; 634.4454, (660)	PC P-16:0/18:1(Δ9)
		636.4603, 662.5088; 676.4914, 702.5432	PC O-16:0/18:2(Δ9,12)
746.6044	804.6	636.4594, 662.5112	PC O-18:1(Δ9)/16:0
		(664), 690.5432	PC O-16:0/18:1(Δ11)
		636.4594, 662.5112	PC O-16:0/18:1(Δ9)
		(664), 690.5432	PC O-18:1(Δ11)/16:0
766.5764	824.6	598; 578, 604; 618, 644; 658, 684; 698, 724	PC P-16:0/20:4(Δ5, 8, 11, 14)
768.5909	826.6	574; 658, 684; 660, 686; 700, 726	PC P-18:1(Δ9)/18:2(Δ9, 12)
		600; 620, 646; 660, 686; 700, 726	PC P-16:0/20:3(Δ8, 11 ,14)
		580, 606; 620, 646; 660, 686; 700, 726	PC O-16:0/20:4(Δ5, 8, 11, 14)
770.6038	828.6	574.3846; 662.4759, 688.5246; 702.5078, (728)	PC P-18:0/18:2(Δ9, 12)
		660.4608, 686.5152; 662.4756, 688.5280; 702.5078, 728.5559	PC O-18:1(Δ9)/18:2(Δ9, 12)

		622.4424, 648.4943; 662.4756, 688.5280; 702.5078, 728.5559	PC O-16:0/20:3(Δ8, 11 ,14)
772.6191	830.6	576.4013; 662.4760, 688.5257	PC P-18:0/18:1(Δ9)
		664.4924, 690.5405; 704.5195, 730.5777	PC O-18:0/18:2(Δ9, 12)
		662.4768, 688.5303	PC O-18:1(Δ9)/18:1(Δ9)
794.6069	852.6	598; 606, 632; 646, 672; 686, 712; 726, 752	PC P-18:0/20:4(Δ5, 8, 11, 14)
		626; 606, 632; 646, 672; 686, 712; 726, 752	PC P-16:0/22:4(Δ7, 10, 13, 16)
		684, 710; 606, 632; 646, 672; 686, 712; 726, 752	PC O-18:1(Δ9)/20:4(Δ5, 8, 11, 14)
		684, 710; 648, 674; 688, 714; 728, 754; 768, 794	PC O-18:1(Δ9)/20:4(Δ8, 11, 14, 17)
		608, 634; 648,674; 688, 714; 728, 754; 768, 794	PC O-16:0/22:5(Δ7, 10, 13, 16, 19)
796.6191	854.6	686.4762, 712.5286; 648.4604, 674.5132; 688.4919, 714.5434; 728.5215, 754.5731	PC O-18:1(Δ9)/20:3(Δ8, 11,14)
		608.4282, 634.4818; 648.4604, 674.5132; 688.4919, 714.5434; 728.5215, 754.5731	PC O-18:0/20:4(Δ5, 8, 11,14)
		608.4282, 634.4818; 648.4604, 674.5132; 688.4919, 714.5434; 728.5215, 754.5731	PC O-16:0/22:4(Δ7, 10, 13, 16)
822.6373	880.6	684.4612, (710); 634.4443, 660.4972; 674.4739, 700.5265; 714.5067, 740.5552; (754), (780)	PC O-20:1(Δ9)/20:4(Δ5, 8, 11, 14)