Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

Method of coupling Paternò-Büchi reaction with direct infusion ESI-MS/MS for locating C=C bond in glycerophospholipids

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Experimental procedures

Solutions. Stock solutions of lipids and yeast polar extract were transferred to glass vials with Teflon lined caps and stored at -20 ℃. 5 μL of stock solution was transferred to an Eppendorf tube containing 95 μL isopropanol using a stainless steel tip glass syringe. From this solution 1-7.5 μL was further diluted to a 1 mL working solution. Solvent ratios were reported as v/v for all working solutions. Unless otherwise specified, solutions were purged with N_2 prior to analysis to remove dissolved oxygen. For N_2 purging of solutions Eppendorf tubes containing working lipid solutions were covered with parafilm (Parafilm "M"; Bemis Company, Inc.; Oshkosh, WI, USA) and a stainless steel needle (Precision Glide Needle; 18 gauge, 1.5" length; BD; Franklin Lakes, NJ, USA) was inserted to deliver N_2 at a bubble frequency of ~ 5 Hz for 15 min.

Mass Spectrometry. All experiments were performed on a triple quadrupole / linear ion trap (LIT) hybrid mass spectrometer (4000 QTRAP; Applied Biosystems / Sciex; Toronto, Canada). Data was collected and processed with Analyst software (1.5.2; Applied Biosystems / Sciex; Toronto, Canada). Neutral loss and precursor ion scans were performed in triple quadrupole mode.[1] For all LIT scans ions were collected and scanned out of Q3 by mass selective axial ejection (MSAE).[2] MS^2 beam-type CID consisted of precursor ion selection in Q1, ion acceleration into q2 collision cell, followed by fragment ion collection and subsequent mass analysis in Q3. MS^3 CID involved precursor ion selection in Q1, ion acceleration into q2 collision cell, fragment ion collection in the LIT, fragment ion isolation, ion secular frequency resonance excitation, ion cooling step, followed by MSAE of fragment ions.[3] For low abundance ions the "Q0 trapping" function was activated to increase sensitivity.[4] Extracted ion chromatograms (XICs) were obtained by peak integration of ±0.5 Da from peak centroid. The typical instrument settings were: curtain gas, 5 psi; declustering potential, ±100 V; and 1000 Da/s scan time unless

otherwise specified. MS^1 spectra typically employed a 20 ms LIT fill time and were averaged over 30-60 spectra while tandem MS spectra were averaged for at least 120 spectra with LIT fill times of up to 2 s.

Experimental Data

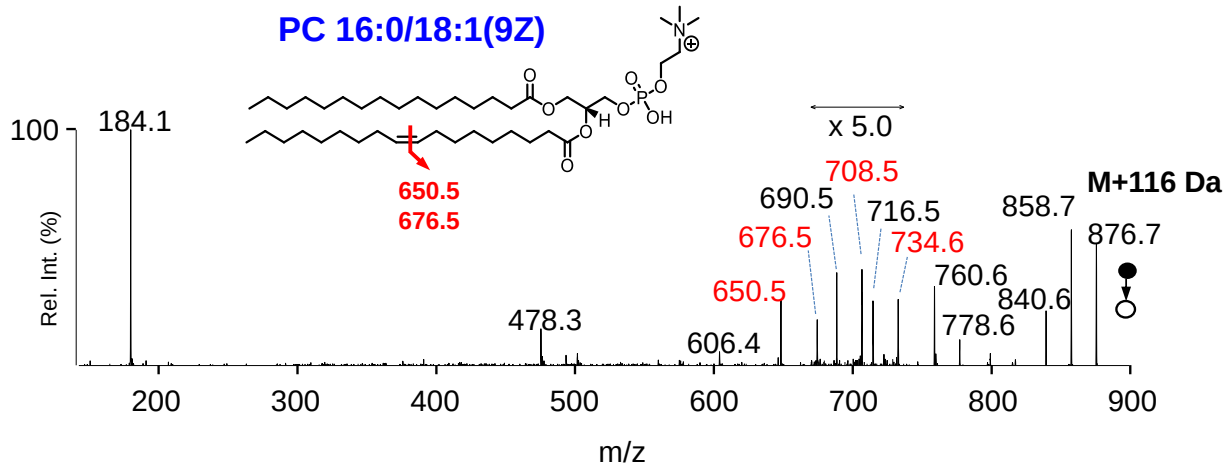

Figure S-1: MS^2 CID of a side reaction product at *m/z* 876 from UV irradiation of solution containing PC 16:0/18:1(9Z) from Figure 1b of the main text.

Continuous UV exposure micro flow reactor for PB-ESI-MS/MS.

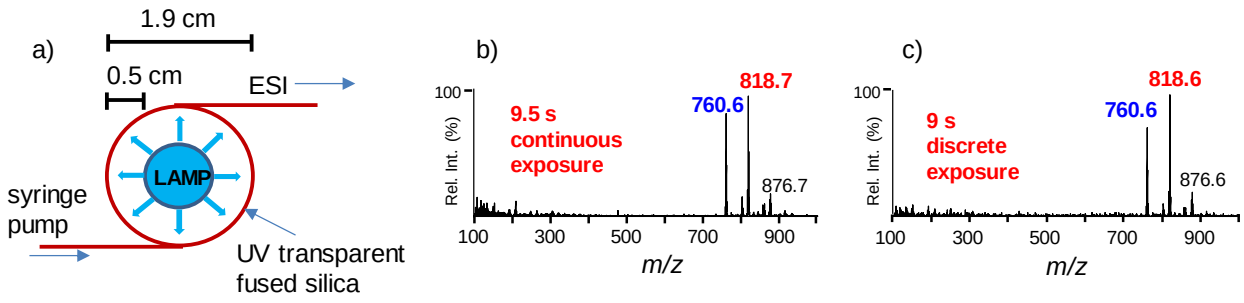

Figure S-2: (a) Drawing of a continuous UV exposure reactor used to effect the PB reaction prior to ESI MS. (b) MS^1 reaction spectra showing 9.5 s continuous UV exposure of a solution containing N_2 purged 5 µM PC 16:0/18:1(9Z) in 7:3 acetone: H_2O 1% acetic acid using the experimental setup shown in (a). The GP precursor and PB product are at *m/z* 760.6 and 818.7, respectively. (c) MS^1 reaction spectrum where the same sample was exposed to 9 s discrete UV exposure using the experimental setup as shown in Figure 1 of the main text.

Detection limit for PC 16:0/18:1 (9Z)

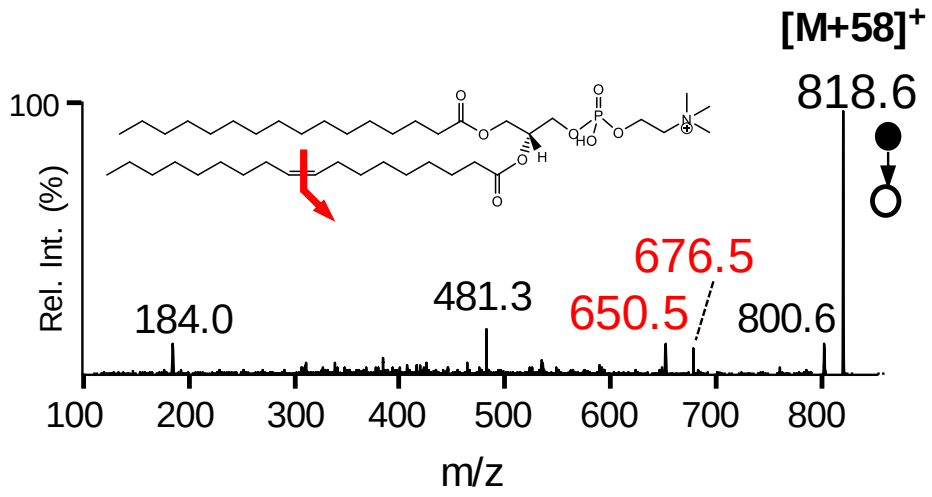

Figure S-3: MS/MS spectra of the PB reaction product (*m/z* 818.6) of 5 nM PC 16:0/18:1(9Z) dissolved in 7:3 acetone: H_2O 1% acetic acid to determine the limit of detection of C=C diagnostic ions at *m/z* 650.5 and 676.5 (S:N > 3) . UV exposure was 6 s using the continuous UV exposure reaction system from SI Figure 1a. The MS/MS spectra were averaged for 10 minutes with a 2 second LIT fill time.

Analysis of Yeast Polar Lipid Extract

Procedures. For the yeast polar extract analysis 20 μL of commercially purchased stock (25 mg/mL $CHCl_3$) was dissolved to 1 mL in 7:3 acetone: H_2O 1% acetic acid or NH_4OH for a final concentration of 0.5 mg/mL. Prior to direct injection ESI MS/MS the solution was N_2 purged as described in the Experimental Section. Triple quadrupole scans identified PL class and cumulative number of fatty acyl carbons and degree of saturation. Precursor ion scans (PIS) and neutral loss scans (NLS) were all implemented at 6 s scan speed. Negative ionization beam-type CID LIT product ion scans determined the individual fatty acyl chain lengths and degree of saturation. The PB reaction was implemented using the continuous reaction setup as shown in SI Figure S1a at 5 μL/min and UV exposure of 6-8 s. PB reaction products (+58 Da from precursor ion) for all PL classes (excluding PC) were subjected to tandem MS to detect C=C diagnostic ions. Ion trap CID was performed on beam-type CID fragment ions displaying a +58 Da shift from the fatty acyl fragment ion (i.e. *m/z* 339.3 and/or 311.3 for fatty acyl *m/z* 281.3 and 253.3, respectively). PC PB product ions were subjected to beam-type CID in positive ionization and C=C location was assigned based on fragment ions and the knowledge of fatty acyl composition from MS/MS beam-type CID of $[M-15]^-$ PC ions.

Table S-1: Complete list of unsaturated GPs analyzed in the polar yeast extract, including ionization mode and monoisotopic *m/z*. Triple quadrupole scans performed to identify GP head group include neutral loss (NLS) and precursor ion (PIS) scans.

Phospholipid	Neutral mass	Ionization Mode	Phospholipid Monoisotopic *m/z*	scan / headgroup identifier
PI 16:0_16:1(9)	808.5	(-)	807.5	PI, *m/z* 241.1
PI 16:1(9)_16:1(9)	806.5	(-)	805.5	
PI 16:0_18:1(9)	836.5	(-)	835.5	
PI 18:0_16:1(9)	836.5	(-)	835.5	
PI 16:1(9)_18:1(9)	834.5	(-)	833.5	
PI 18:0_18:1(9)	864.6	(-)	863.6	
PI 18:1(9) _18:1(9)	862.6	(-)	861.5	
LPI 18:1(9)	598.3	(-)	597.3	
LPI 16:1(9)	570.3	(-)	569.3	
PC 16:1(9)_16:1(9)	729.5	(+)	730.5	PI, *m/z* 184.1
PC 16:0_16:1(9)	731.6	(+)	732.6	
PC 16:1(9)_18:1(9)	757.6	(+)	758.6	
PC 16:0_18:1(9)	759.6	(+)	760.6	
PC 18:0_16:1(9)	759.6	(+)	760.6	
PC 18:1(9)_18:1(9)	785.6	(+)	786.6	
LPC 18:1(9)	522.4	(+)	522.4	
LPC 16:1(9)	493.3	(+)	494.3	
PE 18:0_16:1(9)	717.5	(+)	718.5	NL, 141.0 Da
PE 16:0_18:1(9)	717.5	(+)	718.5	
PE 16:1(9)_16:1(9)	715.5	(+)	716.5	
PE 16:1(9)_18:1(9)	715.5	(+)	716.5	
LPE 18:1(9)	479.3	(+)	480.3	
LPE 16:1(9)	451.3	(+)	452.3	
PS 16:1(9)_16:1(9)	703.4	(-)	702.4	NL, 87.1 Da
PS 16:0_16:1(9)	705.5	(-)	704.5	
PS 18:0_16:1(9)	733.5	(-)	732.5	
PS 16:0_18:1(9)	733.5	(-)	732.5	
PS 16:1(9)_18:1(9)	731.5	(-)	730.5	
LPS 18:1(9)	523.3	(-)	522.3	
LPS 16:1(9)	495.3	(-)	494.3	
PA 16:1(9)_16:1(9)	644.4	(-)	643.4	PI, *m/z* 153.1
PA 16:0_18:1(9)	646.5	(-)	673.5	
PA 16:0_16:1(9)	646.5	(-)	645.4	
PA 16:1(9)_18:1(9)	644.4	(-)	671.5	
LPA 18:1(9)	436.3	(-)	435.3	

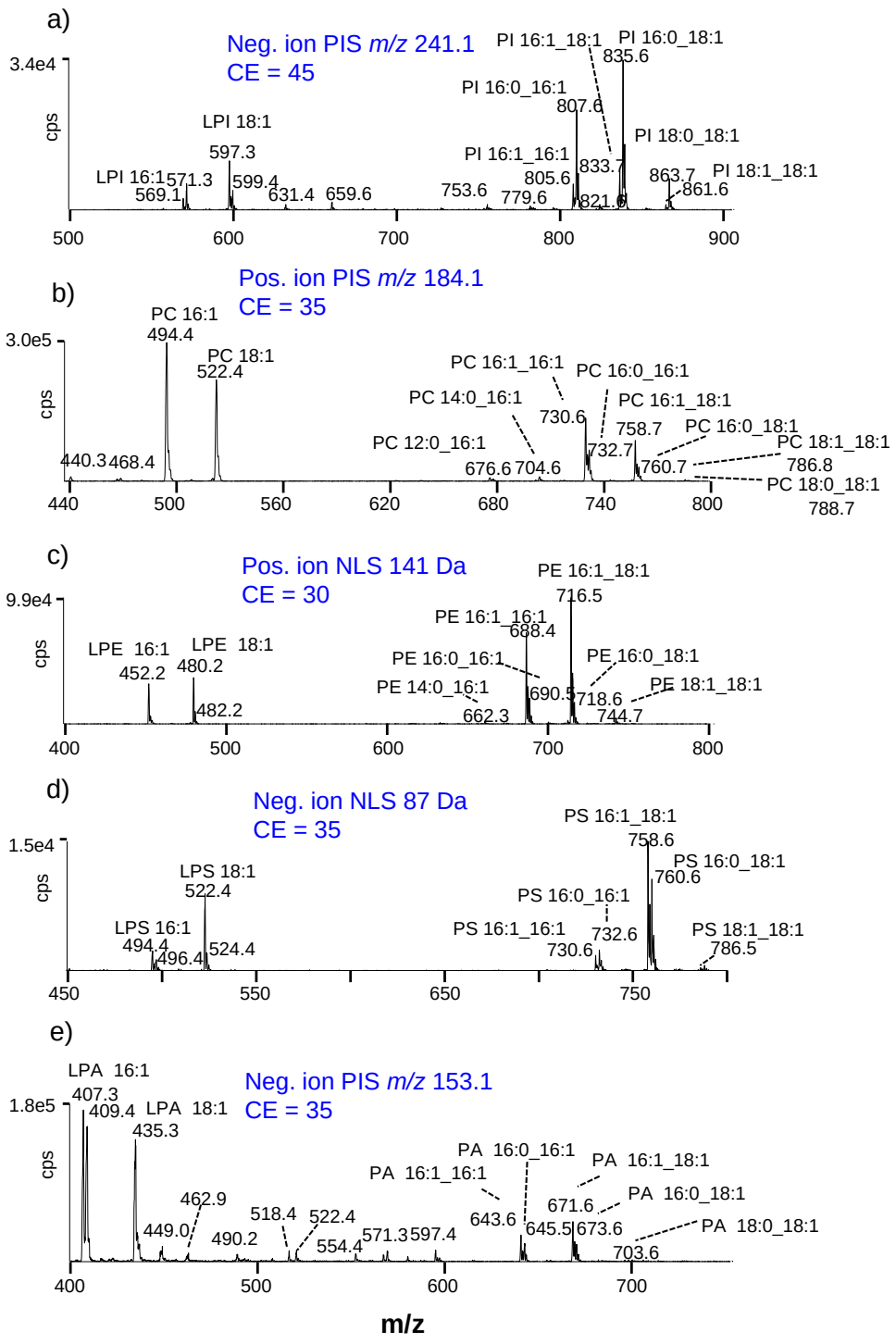

Figure S-4: Triple quadrupole neutral loss scans (NLS) and precursor ion scans (PIS) for determining GP head-group applied to direct injection of yeast polar lipid extract. a) Negative ion PIS *m/z* 241.1 for PI at CE = 45. b) Positive ion PIS *m/z* 184.1 for PC at CE = 35. c) Positive ion NLS 141 Da for PE at CE = 30. d) Negative ion NLS 87 Da for PS at CE = 35. e) Negative ion PIS *m/z* 153.1 for PA/PG at CE = 35.

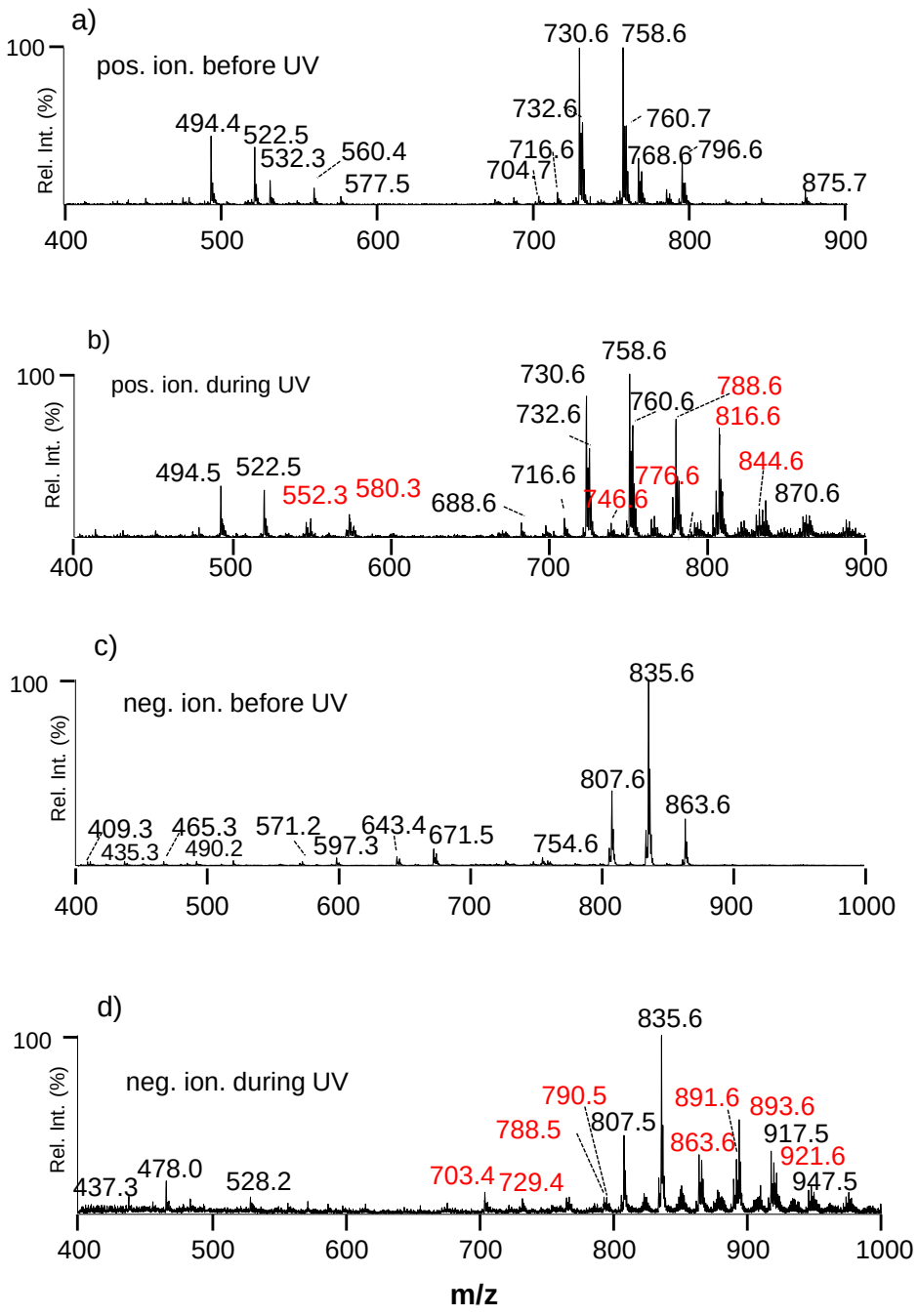

Figure S-5: Positive and negative ionization MS^1 spectra for the yeast polar lipid extract before and during application of UV using the experimental setup shown in SI Figure S1a. a) Positive ionization MS^1 spectra before UV and (b) during UV (PB product *m/z* in red). c) Negative ionization MS^1 spectra before UV and (d) during UV (PB product *m/z* in red).

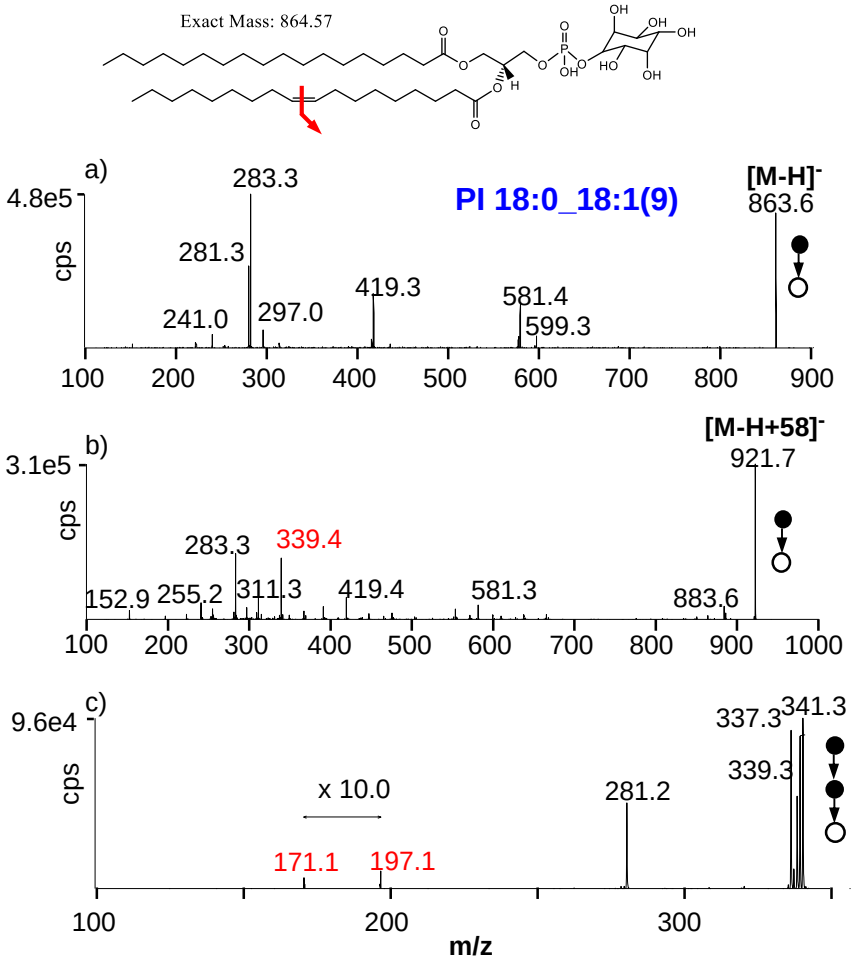

Figure S-6: Proposed structure, fatty acyl structural determination, and PB diagnostic ions for PI 18:0_18:1(9) from direct injection of yeast polar lipid extract. a) Beam-type CID of *m/z* 863.3. b) Beam-type CID of PB product *m/z* 921.7. c) MS^3 ion trap CID of *m/z* 339.3 from (b).

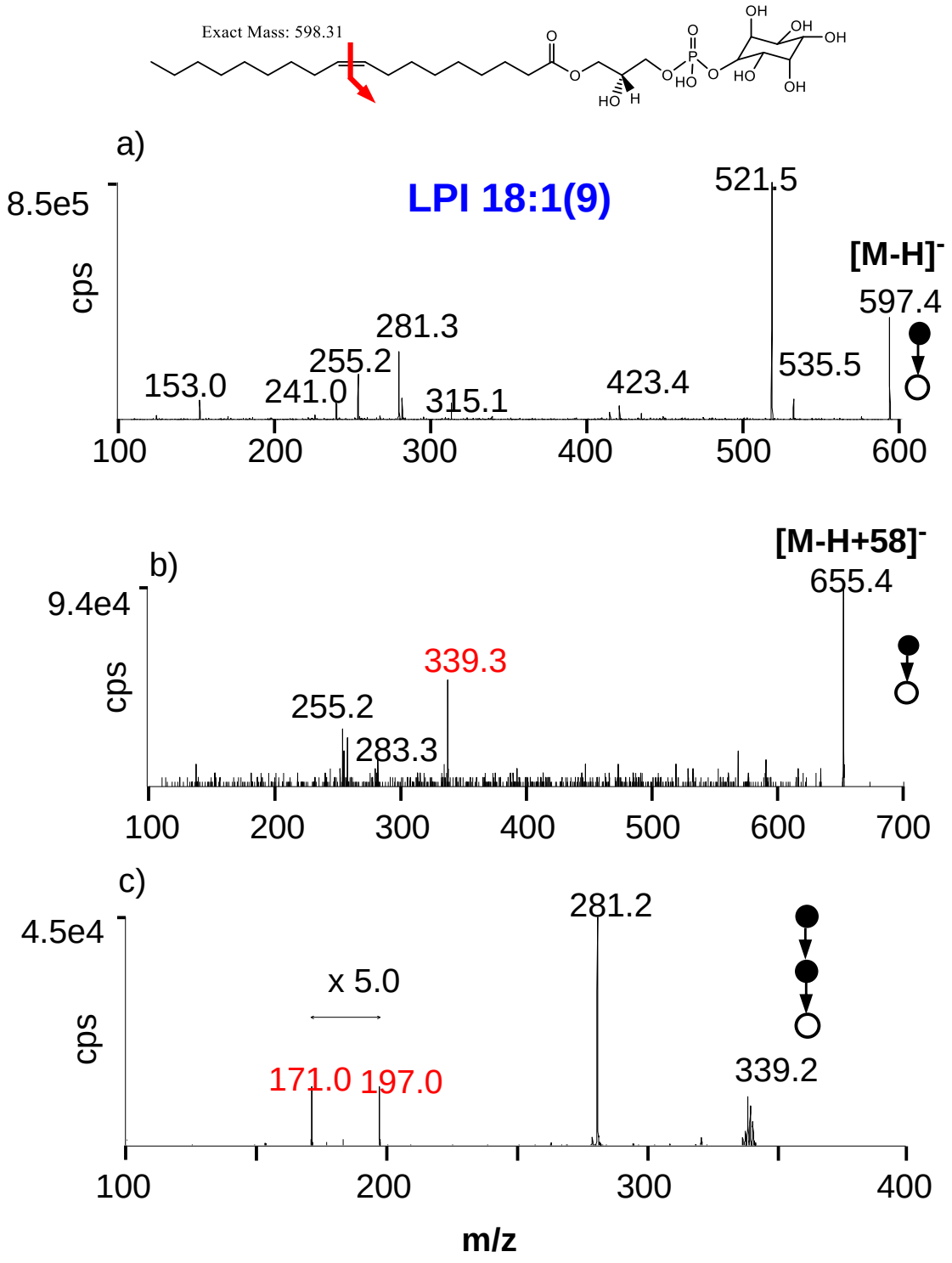

Figure S-7: Proposed structure, fatty acyl structural determination, and PB diagnostic ions for LPI 18:1(9) from direct injection of yeast polar lipid extract. a) Beam-type CID of *m/z* 597.4. b) Beam-type CID of PB product *m/z* 655.4. c) MS^3 ion trap CID of *m/z* 339.3 from (b).

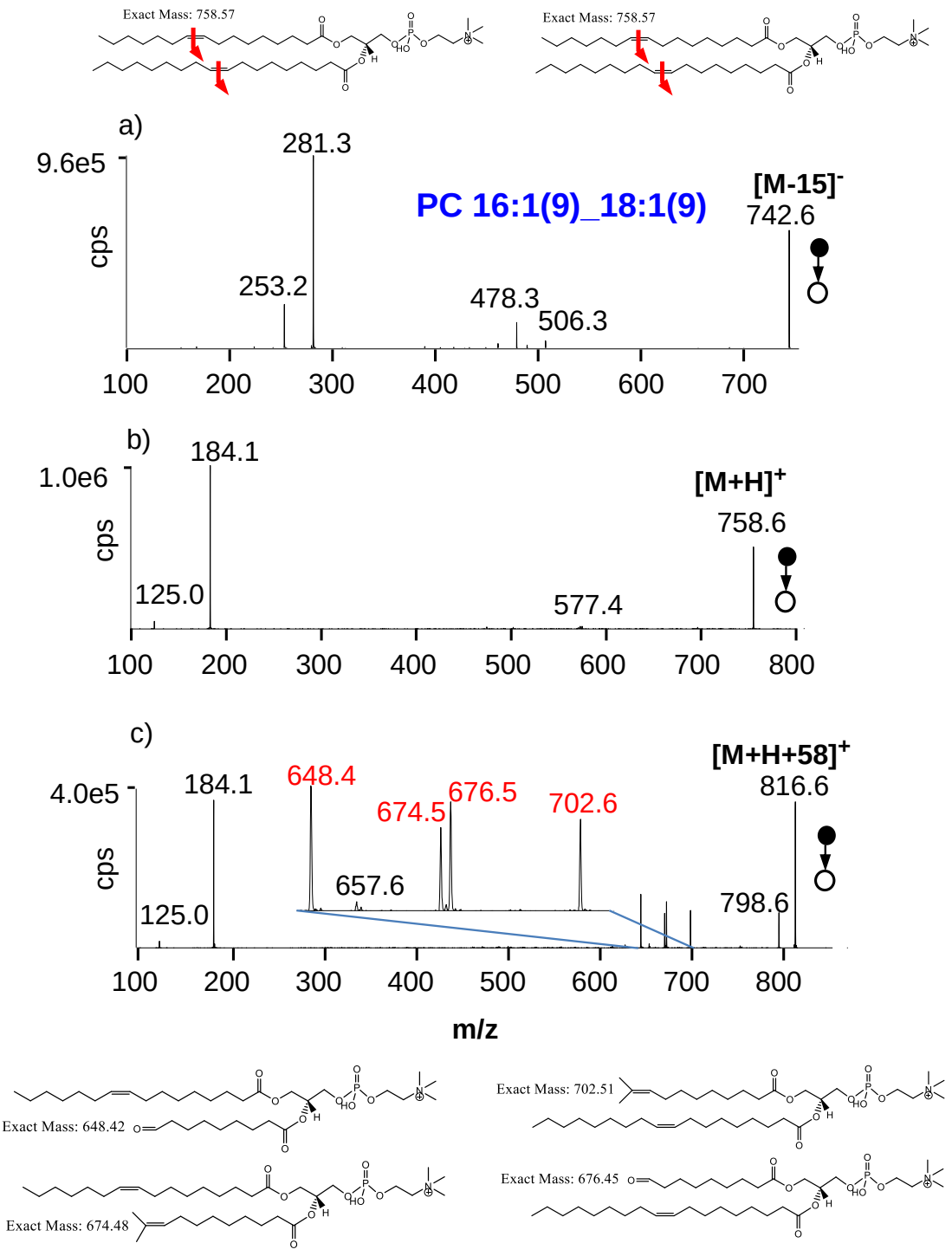

Figure S-8: Proposed structure, fatty acyl structural determination, and PB diagnostic ions for PC 16:1(9)_18:1(9) from direct injection of yeast polar lipid extract. a) Beam-type CID of *m/z* 742.6 ([M-15]$^-$). b) Beam-type CID of precursor positive ion *m/z* 758.6. c) Beam-type CID of PB product *m/z* 816.6.

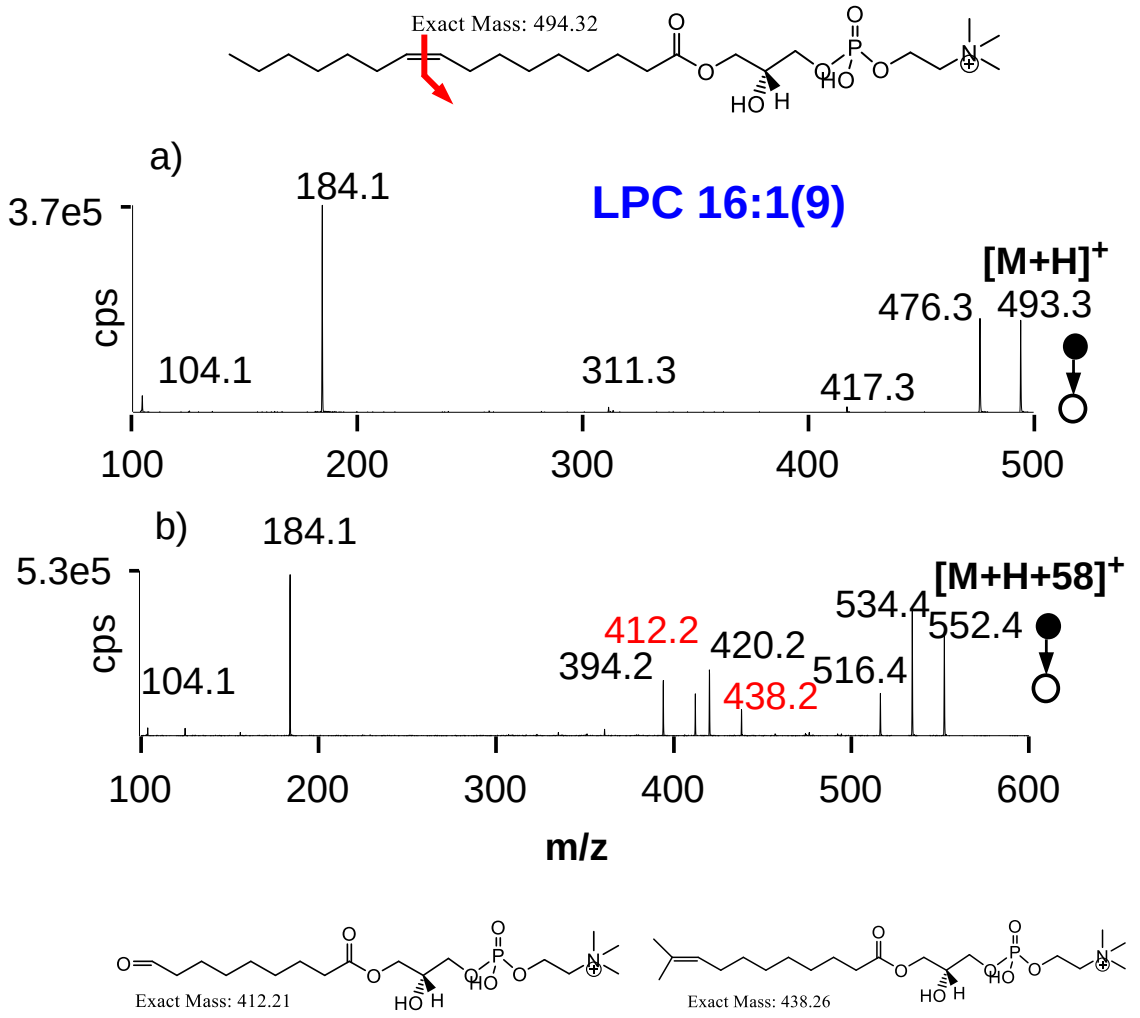

Figure S-9: Proposed structure, fatty acyl structural determination, and PB diagnostic ions for LPC 16:1(9) from direct injection of yeast polar lipid extract. a) Beam-type CID of *m/z* 493.3. b) Beam-type CID of PB product *m/z* 552.4.

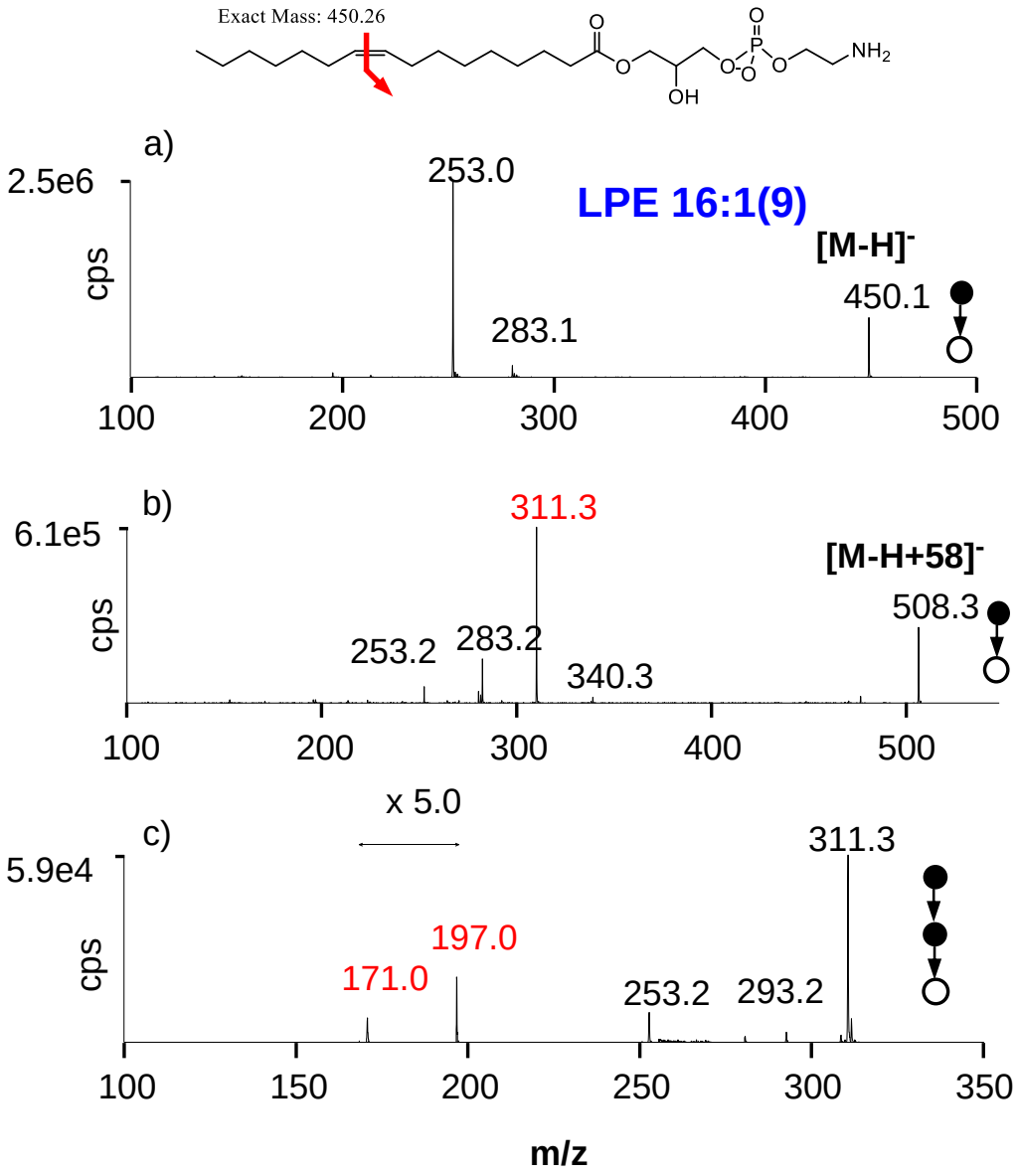

Figure S-10: Proposed structure, fatty acyl structural determination, and PB diagnostic ions for LPE 16:1(9) from direct injection of yeast polar lipid extract. a) Beam-type CID of *m/z* 450.1. b) Beam-type CID of PB product *m/z* 508.3. c) MS^3 ion trap CID of *m/z* 311.3 from (b).

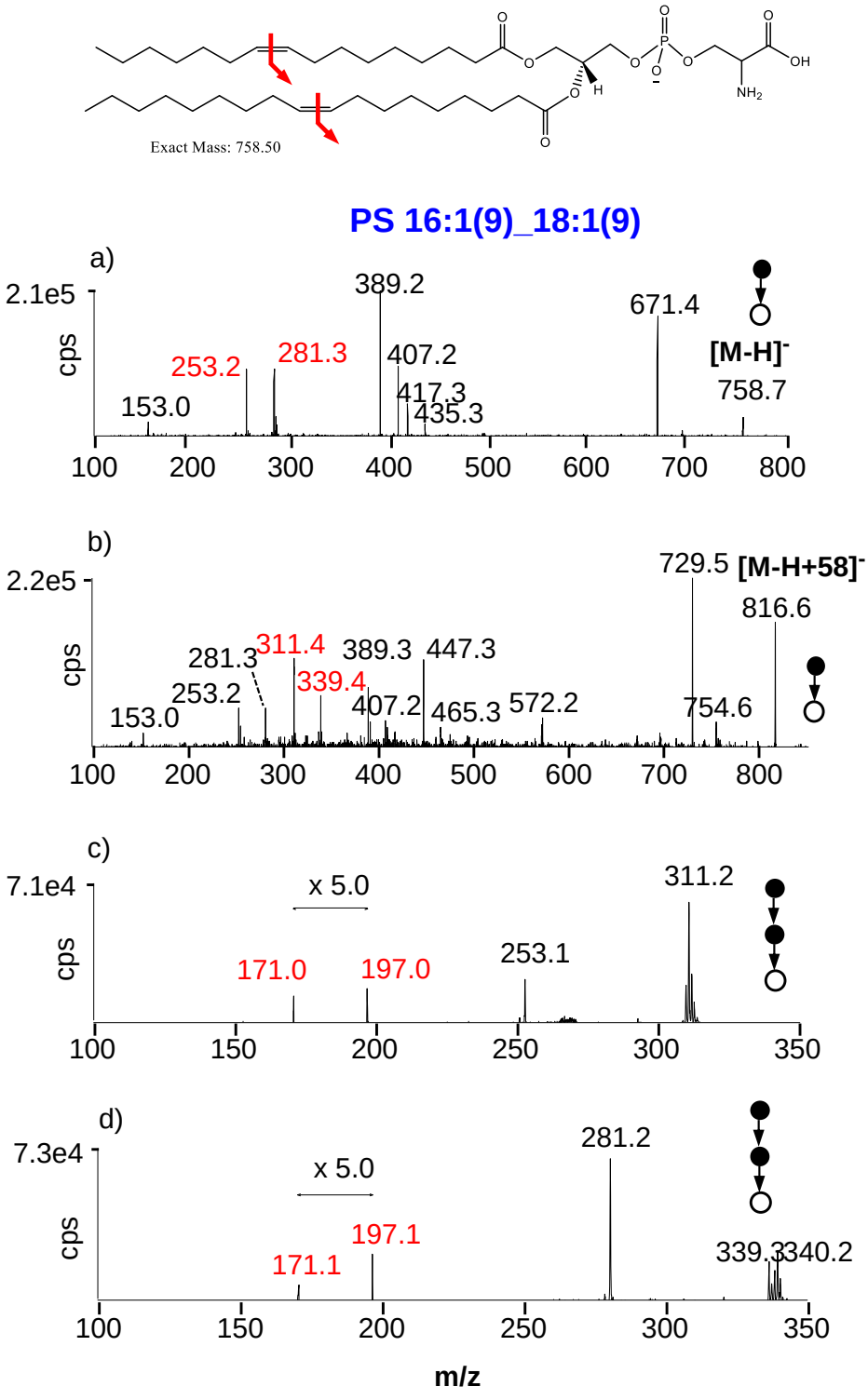

Figure S-11: Proposed structure, fatty acyl structural determination, and PB diagnostic ions for PS 16:1(9)_18:1(9) from direct injection of yeast polar lipid extract. a) Beam-type CID of *m/z* 758.7. b) Beam-type CID of PB product *m/z* 816.6. c) MS^3 ion trap CID of *m/z* 311.3 from (b). d) MS^3 ion trap CID of *m/z* 339.3 from (b).

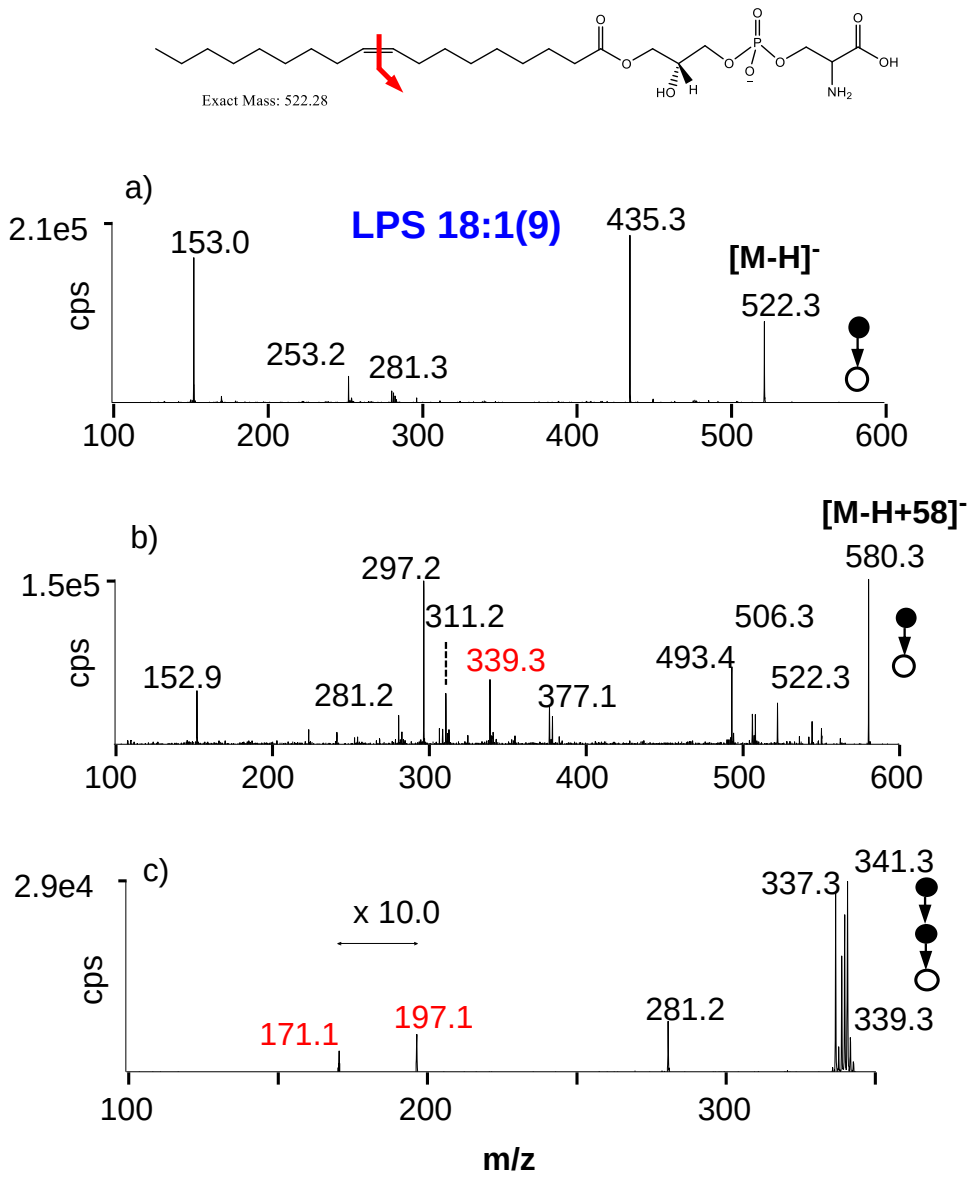

Figure S-12: Proposed structure, fatty acyl structural determination, and PB diagnostic ions for LPS 18:1(9) from direct injection of yeast polar lipid extract. a) Beam-type CID of *m/z* 522.3. b) Beam-type CID of PB product *m/z* 580.3. c) MS^3 ion trap CID of *m/z* 339.3 from (b).

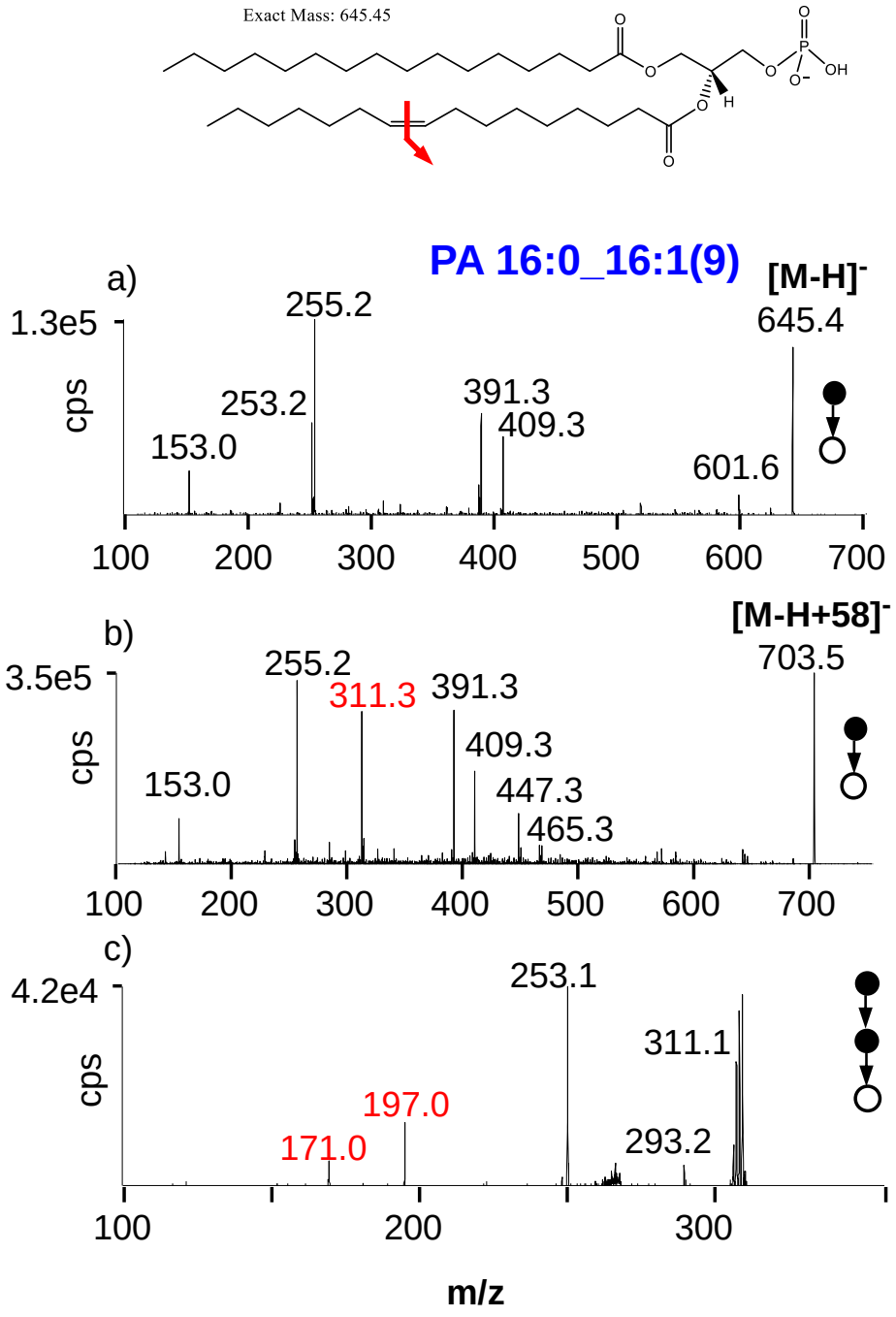

Figure S-13: Proposed structure, fatty acyl structural determination, and PB diagnostic ions for PA 16:0_16:1(9) from direct injection of yeast polar lipid extract. a) Beam-type CID of *m/z* 645.4. b) Beam-type CID of PB product *m/z* 703.5. c) MS^3 ion trap CID of *m/z* 311.3 from (b).

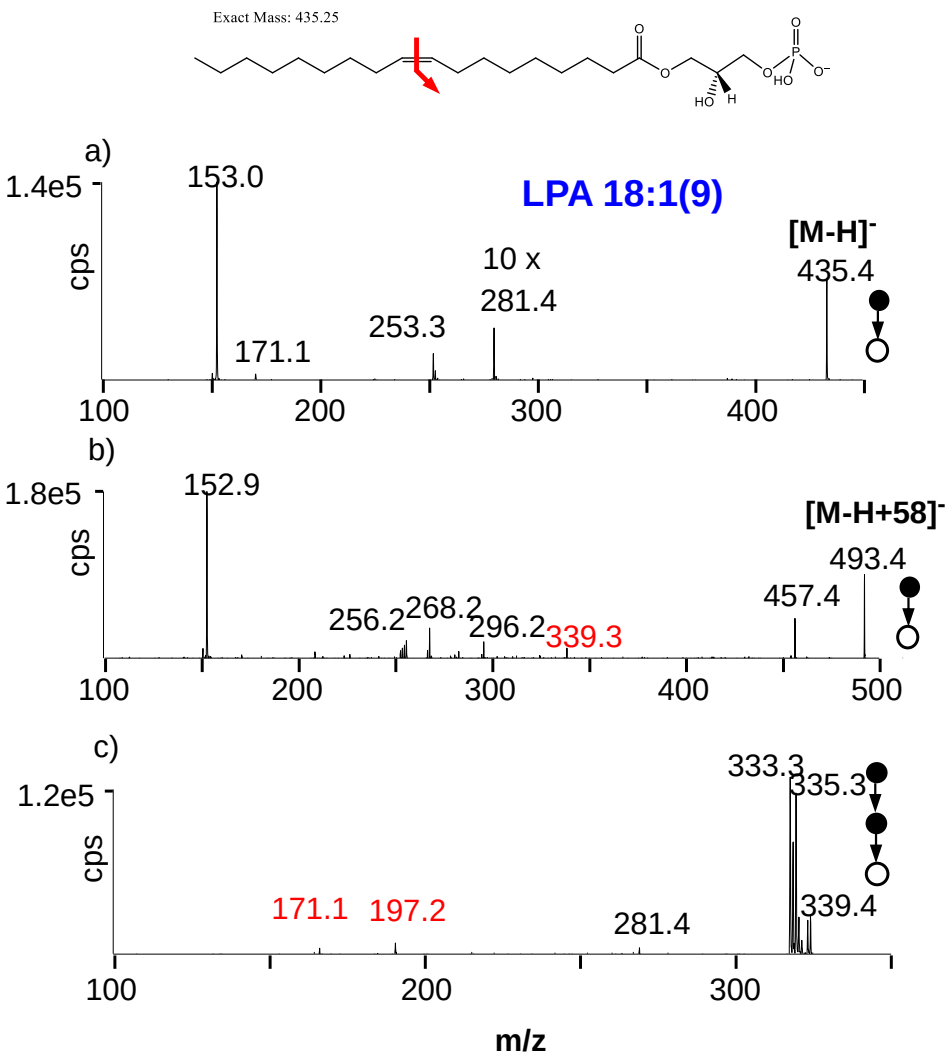

Figure S-14: Proposed structure, fatty acyl structural determination, and PB diagnostic ions for LPA 18:1(9) from direct injection of yeast polar lipid extract. a) Beam-type CID of *m/z* 435.4. b) Beam-type CID of PB product *m/z* 493.4. c) MS^3 ion trap CID of *m/z* 339.3 from (b).

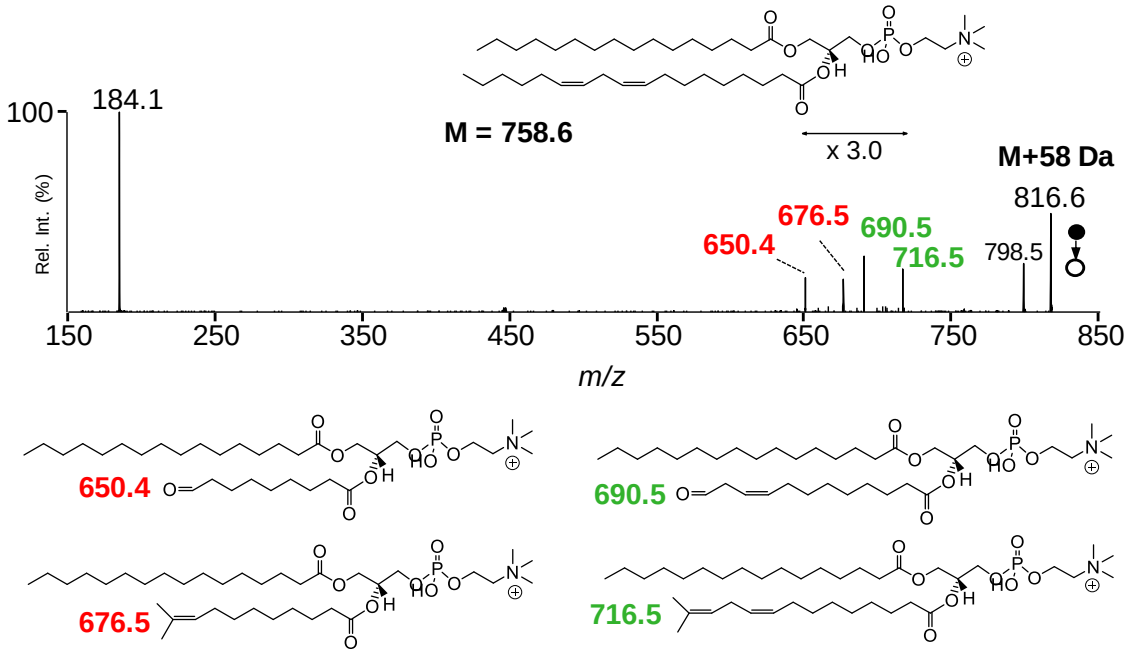

Figure S-15: Application of direct infusion ESI-PB-MS/MS on a PC standard, PC 16:0/18:2 (9Z, 12 Z). Beam-type CID of *m/z* 816.6, the first generation PB product of PC16:0/18:2, leads to the formation of two pairs of C=C diagnostic ions at *m/z* 650/676 and 690/716. The structure of diagnostic ions are shown below the spectrum.

(1) Han, X.; Yang, K.; Gross, R. W. *Mass Spectrom. Rev.* **2012**, 31, 134.
(2) Hager, J. W. *Rapid Commun. Mass Spectrom.* **2002**, 16, 512.
(3) Collings, B.; Stott, W.; Londry, F. *J. Am. Soc. Mass. Spectrom.* **2003**, 14, 622.
(4) Hager, J. W.; Yves Le Blanc, J. C. *Rapid Commun. Mass Spectrom.* **2003**, 17, 1056.