Supplementary Material

Authors: Gang Sun[1,2], Kui Yang[1,2], Zhongdan Zhao[1,2], Shaoping Guan [1,2], Xianlin Han[1,2], Richard W. Gross[1,2,3,4]

Affiliations: [1]Division of Bioorganic Chemistry and Molecular Pharmacology, Departments of [2]Medicine and [3]Molecular Biology & Pharmacology, Washington University School of Medicine, St. Louis, Missouri 63110 and [4]Department of Chemistry, Washington University, St. Louis, MO 63130

Title: Matrix-assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometric Analysis of Cellular Glycerophospholipids Enabled by Multiplexed Solvent Dependent Analyte-Matrix Interactions

Contents

Figure S1.

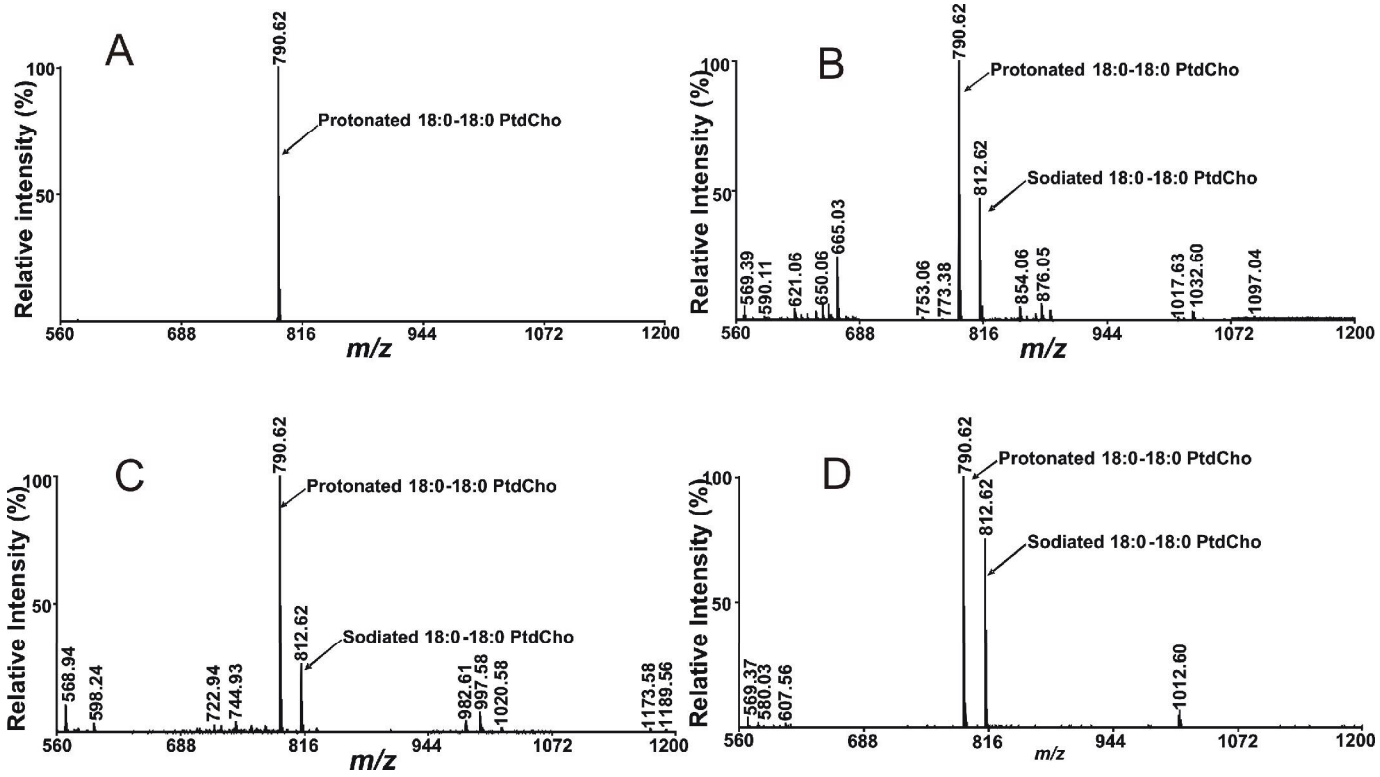

Figure S1. MALDI mass spectrum of D18:0-18:0 PtdCho acquired on a 4800 MALDI-TOF/TOF Analyzer in the positive ion mode using different matrices: A) 9-aminoacridine (10mg/mL) dissolved in isopropanol/acetonitrile (60/40, v/v); B) CHCA (10 mg/mL)

dissolved in methanol containing 0.1% TFA; C) DHB (0.5 M) dissolved in methanol containing 0.1% TFA; and D) THAP (40 mM) dissolved in methanol. The prefix ‘‘D’’ stands for diacyl (i.e., phosphatidyl-) species.

Figure S2

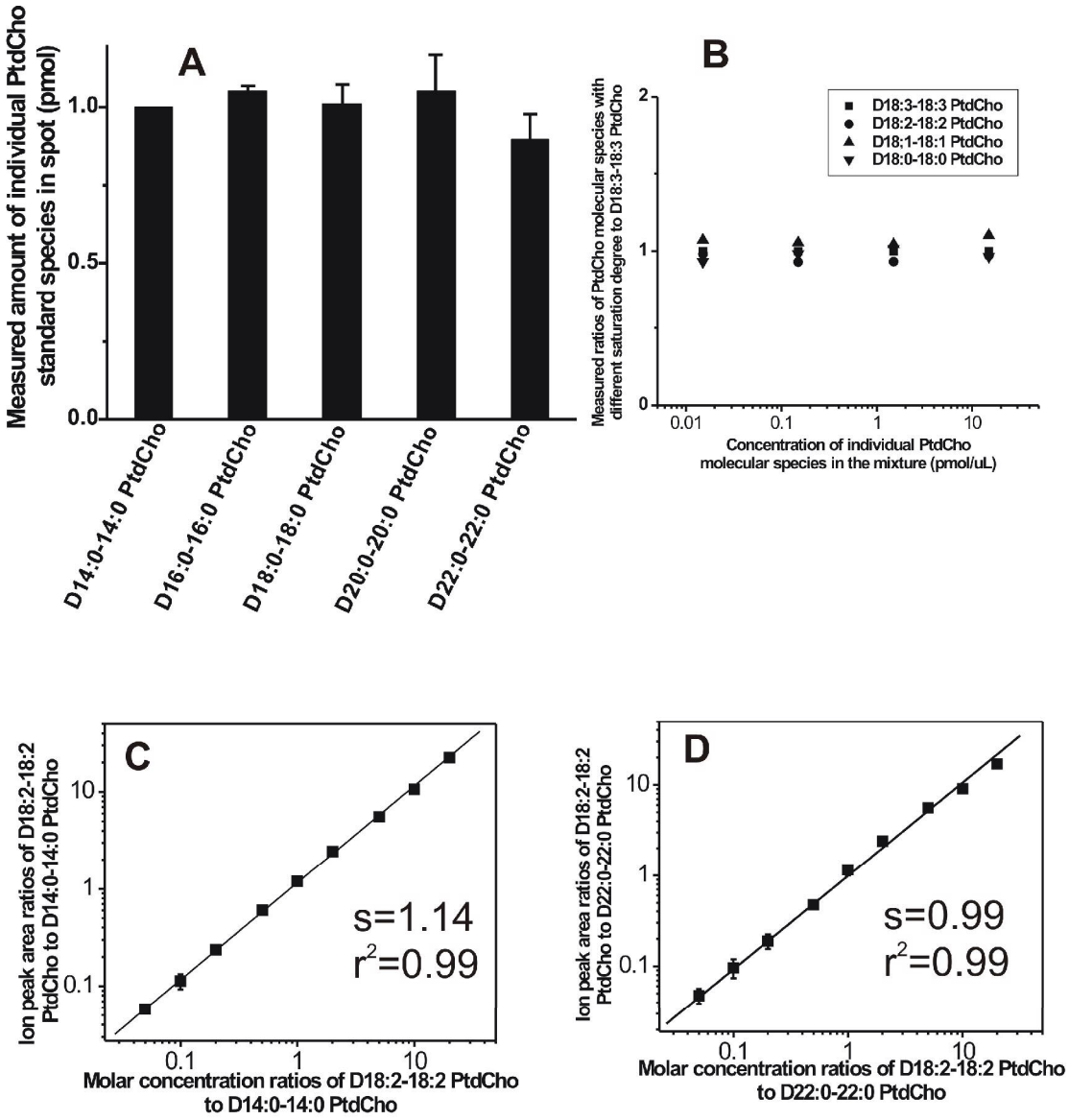

Figure S2 Quantitative analyses of standard PC molecular species using MALDI-TOF MS with 9-aminoacridine as a matrix. A) Measured amount of D14:0-14:0 PtdCho, D16:0-16:0 PtdCho, D18:0-18:0 PtdCho, D20:0-20:0 PtdCho and D22:0-22:0 PtdCho in spot resulted from Figure 1 using D14:0-14:0 as internal standard (1 pmol/spot) after correction for ^{13}C isotope effect. The error bars in A) are the result of multiple independent preparations representing the mean ±SD. B) Measured ratios of D18:2-

18:2 PtdCho, D18:1-18:1 PtdCho and D18:0-18:0 PtdCho to D18:3-18:3 PtdCho at different concentrations. Each PtdCho molecule species was present in equal amounts per spot. C) Linear correlation between ion peak area ratios and the molar ratios of D18:2-18:2 PtdCho to D14:0-14:0 PtdCho in the molar ratio range 0.05–20. D) Linear correlation between the ion peak area ratios and the molar ratios of D18:2-18:2 PtdCho and D22:0-22:0 PtdCho in the molar ratio range 0.05–20. Each of the peak area ratios was determined from 17 fmol-5 pmol phospholipid per spot. Each data point represents the mean ± SD from different concentrations of each PtdCho to another at each ratio tested. “s” and “r^2” represent the slope and correlation coefficient, respectively. The prefix ‘‘D’’ stands for diacyl (i.e., phosphatidyl-) species.

Figure S3

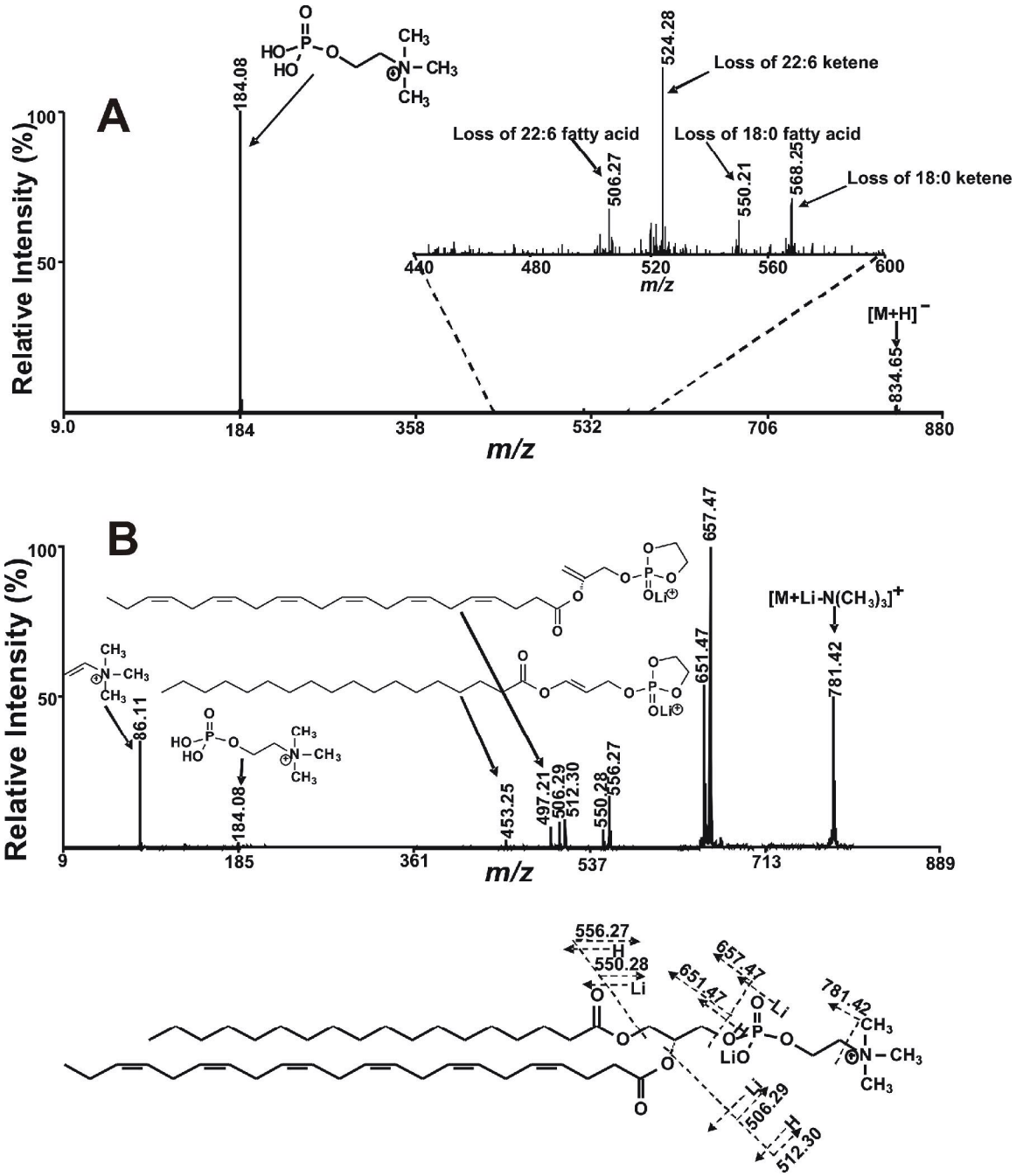

Figure S3. MALDI tandem mass spectral comparison of D18:0-22:6 PtdCho molecular species present in mouse heart lipid extracts. The tandem mass spectra were acquired on a 4800 MALDI-TOF/TOF Analyzer in the positive ion mode. A) The tandem MS spectrum of protonated D18:0-22:6 PtdCho molecular species was

recorded in the positive ion mode; B) The tandem MS spectrum of lithiated D18:0-22:6 PtdCho was recorded in the positive ion mode. Tandem mass analyses of D18:0-22:6 PtdCho was performed on a 4800 MALDI-TOF/TOF Analyzer using 9-aminoacridine as matrix with CID on, metastable suppressor on and timed ion selector enabled. The voltages of source 1, the collision cell and the collision cell offset were 8.0 kV, 7.0 kV and -0.035 kV, respectively. The tandem MS spectrum was obtained by averaging 2000 consecutive laser shots (50 shots per subspectra with 40 total subspectra). The prefix ‘‘D’’ stands for diacyl (i.e., phosphatidyl-) species.

Figure S4

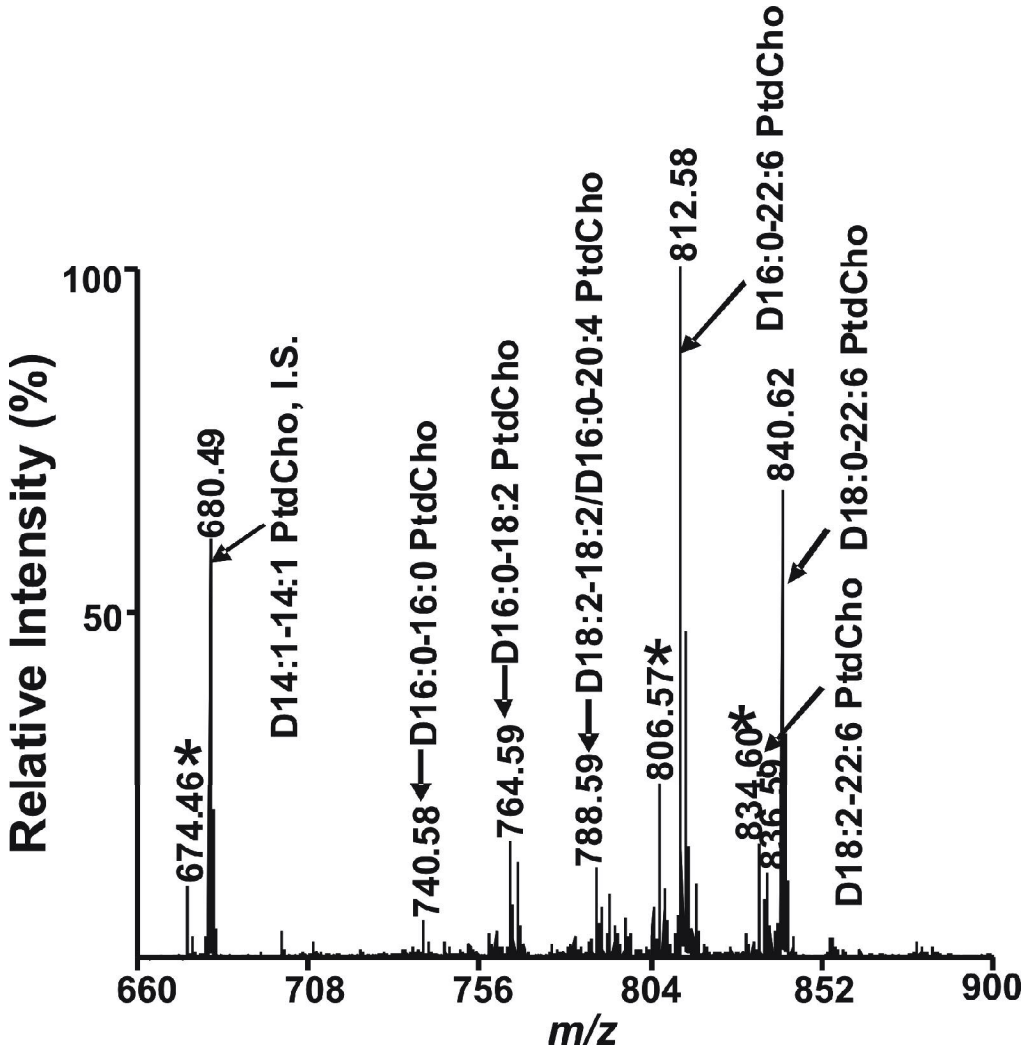

Figure S4 MALDI mass spectrum of lithiated PC molecular species present in mouse heart lipid extracts. The mass spectrum was acquired on a 4800 MALDI-TOF/TOF Analyzer in the positive ion mode as described in the Experimental Section. 9-aminoacridine matrix dissolved in isopropanol/acetonitrile (60/40, vv) containing 10 mM lithium acetate was used as matrix. The majority of PC molecular species are detected as lithium adducts. The prefix ‘‘D’’ stands for diacyl (i.e., phosphatidyl-) species. An asterisk indicates protonated PC molecular species.

Figure S5

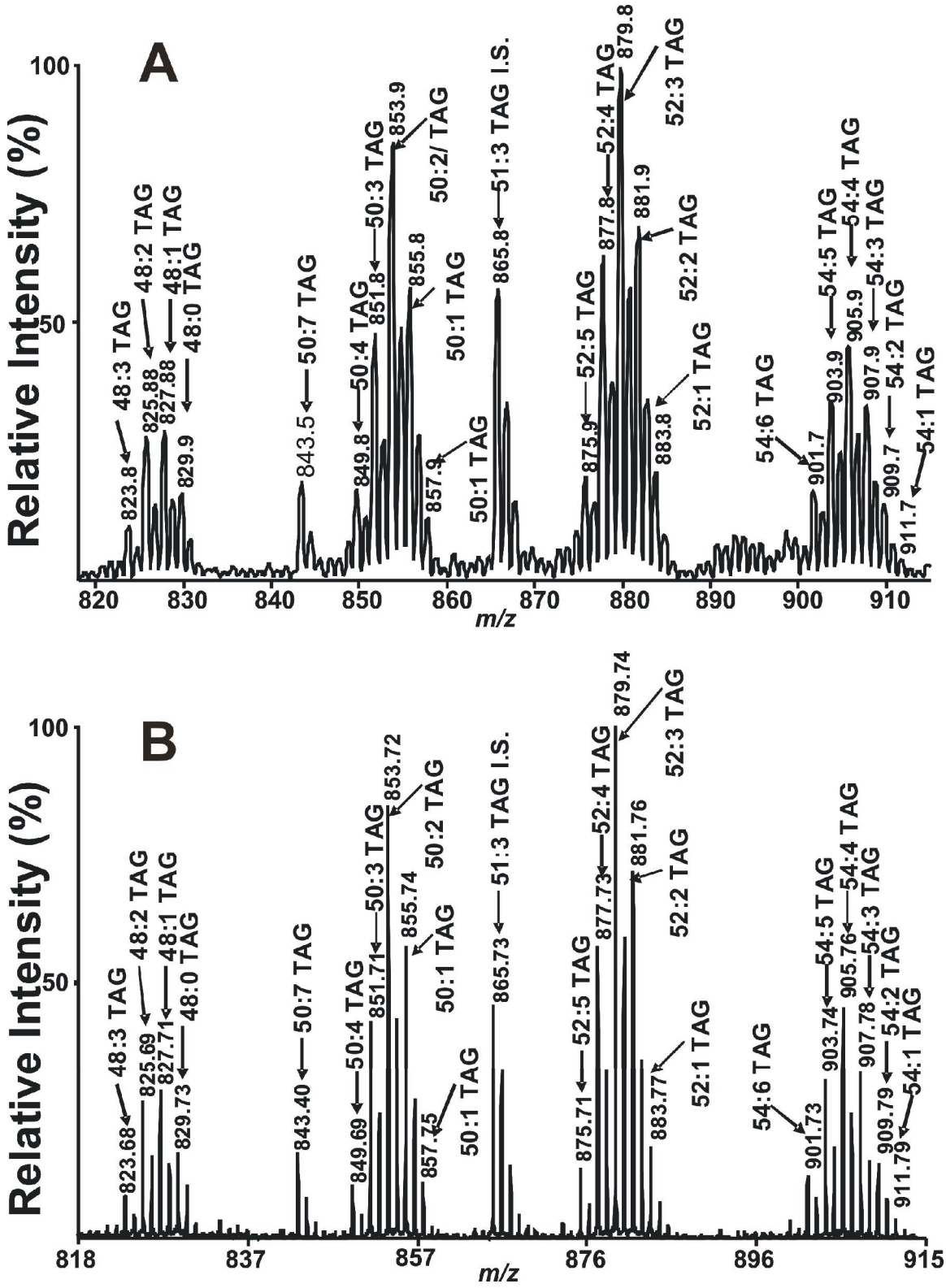
A
Relative Intensity (%)
100
50
m/z
820
830
840
850
860
870
880
890
900
910
823.8
48:3 TAG
825.88
48:2 TAG
827.88
48:1 TAG
829.9
48:0 TAG
843.5
50:7 TAG
849.8
50:4 TAG
851.8
50:3 TAG
853.9
855.8
50:2/ TAG
857.9
50:1 TAG
865.8
51:3 TAG I.S.
875.9
52:5 TAG
877.8
52:4 TAG
879.8
881.9
52:3 TAG
883.8
52:2 TAG
52:1 TAG
54:6 TAG
901.7
903.9
54:5 TAG
905.9
54:4 TAG
907.9
54:3 TAG
909.7
54:2 TAG
911.7
54:1 TAG
B
Relative Intensity (%)
100
50
m/z
818
837
857
876
896
915
823.68
48:3 TAG
825.69
48:2 TAG
827.71
48:1 TAG
829.73
48:0 TAG
843.40
50:7 TAG
849.69
50:4 TAG
851.71
50:3 TAG
853.72
855.74
50:2 TAG
857.75
50:1 TAG
865.73
51:3 TAG I.S.
875.71
52:5 TAG
877.73
52:4 TAG
879.74
881.76
52:3 TAG
883.77
52:2 TAG
52:1 TAG
54:6 TAG
901.73
903.74
54:5 TAG
905.76
54:4 TAG
907.78
54:3 TAG
909.79
54:2 TAG
911.79
54:1 TAG

Figure S5. Mass spectral comparison of sodiated TAG molecular species present in mouse adipose tissue lipid extracts acquired by: A) ESI-MS; and B) MALDI-TOF MS. Mouse fat tissue TAG extracts were prepared using hexane as described in the Experimental Section. The MALDI-TOF mass spectrum of TAG molecular species was acquired on a 4800 MALDI-TOF/TOF Analyzer in the positive ion mode as described in the Experimental Section. 9-aminoacridine matrix dissolved in isopropanol/acetonitrile (60/40, v/v) containing 15 mM sodium acetate was used as matrix. The ESI mass spectrum was recorded on a TSQ Quantum Ultra Plus triple-quadrupole mass spectrometer in the positive ion mode as described in the Experimental Section. The numbers by each peak reflect the total chain length and number of double bonds in each triglyceride molecular species

Figure S6

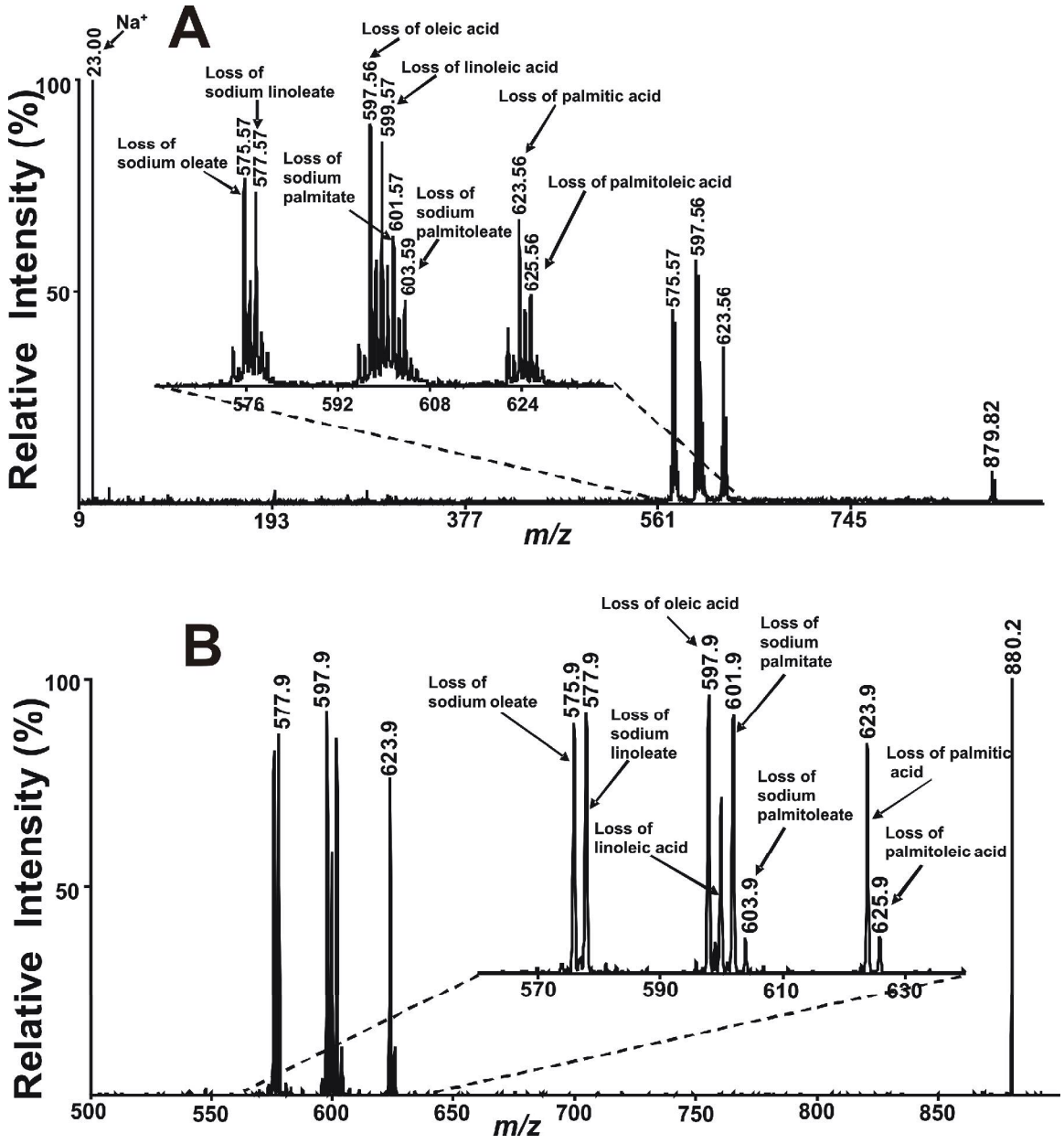

Figure S6. Tandem mass spectral comparison of sodiated18:1-18:1-16:1/18:2-18:1-16:0 TAG molecular species present in mouse adipose tissue lipid extract acquired by: A) MALDI-TOF/TOF MS; and B) ESI-MS/MS. The MALDI tandem mass spectrum was recorded on a 4800 MALDI-TOF/TOF Analyzer in the positive ion mode using 9-aminoacridine as matrix with CID on, metastable suppressor on and timed ion selector

enabled. The voltages of source 1, collision cell and collision cell offset were 8.0 kV, 7.0 kV and -0.035 kV, respectively.The tandem MS spectrum was obtained by averaging 2000 consecutive laser shots (50 shots per subspectra with 40 total subspectra). B) The ESI tandem mass spectrum was recorded on a TSQ Quantum Ultra Plus triple-quadrupole mass spectrometer in the positive ion mode. After selection for the sodiated TAG ion in the first quadrupole, collision activation was performed in the second quadrupole with collision energy of 35 V and the resultant product ions were analyzed in the third quadrupole as described in the Experimental Section.

Figure S7

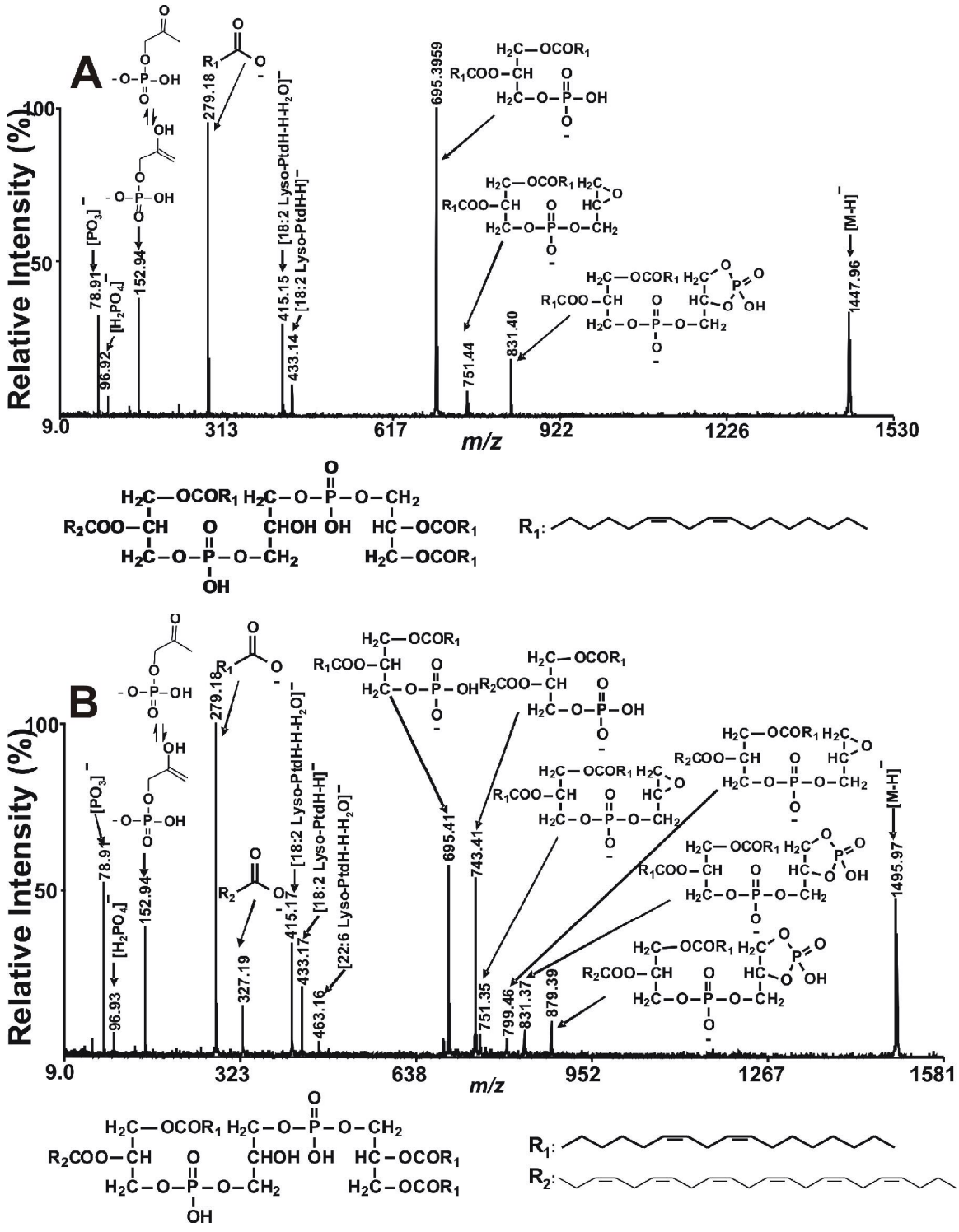
A
Relative Intensity (%)
100
50
9.0
313
617
922
1226
1530
m/z
78.91 [PO3]-
96.92
152.94 [H2PO4]-
279.18 R1COO-
415.15 [18:2 Lyso-PtdH-H-H2O]-
433.14
[18:2 Lyso-PtdH-H]-
695.3959
751.44
831.40
1447.96 [M-H]-
B
Relative Intensity (%)
100
50
9.0
323
638
952
1267
1581
m/z
78.91 [PO3]-
96.93
152.94 [H2PO4]-
279.18 R1COO-
327.19
415.17
433.17
[18:2 Lyso-PtdH-H-H2O]-
[18:2 Lyso-PtdH-H]-
463.16
[22:6 Lyso-PtdH-H-H2O]-
695.41
743.41
751.35
799.46
831.37
879.39
1495.97 [M-H]-
R1:
R2:

Figure S7 MALDI tandem mass spectra of a symmetric CL molecular species (A: T18:2 CL) and an asymmetric CL molecular species (B: 18:2-18:2-18:2-22:6 CL) present in mouse heart lipid extracts. The tandem mass spectra were recorded on a 4800 MALDI-TOF/TOF Analyzer in the negative ion mode using 9-aminoacridine as matrix with CID on, metastable suppressor on and timed ion selector enabled. The voltages of source 1, collision cell and collision cell offset were 8.0 kV, 7.0 kV and -0.035 kV, respectively. The tandem MS spectrum was obtained by averaging 2000 consecutive laser shots (50 shots per subspectra with 40 total subspectra).

Table S1 List of Detected Ion Peaks of PC

Species	Monoisotopic mass $[PC+H]^+$	
	Theoretical	Experimental
D14:1-14:1	674.48	674.47
D16:1-16:0/D14:1-18:0	732.55	732.56
D16:0-16:0	734.57	734.57
A16:0-18:3	742.58	742.58
P18:1-16:0	744.59	744.58
P16:0-18:0/P18:0-16:0/A18:1-16:0	746.61	746.59
D16:1-18:2	756.55	756.55
D16:0-18:2	758.57	758.57
D16:0-18:1	760.59	760.58
D16:0-18:0	762.60	762.60
P18:1-18:1	770.61	770.60
P18:1-18:0	772.62	772.60
A18:0-18:1/P18:0-18:0	774.64	774.61
D18:2-18:3/D16:1-20:4	780.55	780.56
D18:2-18:2/D16:0-20:4	782.57	785.57
D18:1-18:2/D16:0-20:3	784.59	784.58
D18:0-18:2/D18:1-18:1	786.60	786.59
D18:0-18:1	788.62	788.61
P18:1-20:4	792.59	792.57
P18:0-20:4	794.61	794.60
D16:0-22:6/D18:2-20:4	806.57	806.56
D18:1-20:4/D16:0-22:5	808.59	808.57
D18:2-20:2/D18:0-20:4	810.60	810.59
D18:0-20:3	812.62	812.60
D18:0-20:2/P18:2-22:6	814.63	814.62
P18:1-22:6	816.65	816.60
A18:0-22:6/P18:0-22:5	820.62	820.59
D18:2-22:6	830.57	830.56
D18:1-22:6/D18:2-22:5	832.59	832.58
D18:0-22:6/D18:1-22:5	834.60	834.60
D18:0-22:5/D20:2-20:3	836.62	836.60
D18:0-22:4/D20:0-20:4/D20:2-20:2	838.63	838.60
P20:1-22:4	848.65	848.62
P20:0-22:2	854.70	854.55
P20:0-22:1	856.72	856.57
P21:1-22:6	858.64	858.59
P21:1-22:5	860.65	860.60
P21:1-22:4	862.67	862.64
D20:0-22:5/D20:1-22:4	864.65	864.60

The prefix “D”, “P” and “A” stand for diacyl (i.e., phosphatidyl-), alkenyl-acyl (plasmenyl-) and alkyl-acyl (plasmanyl-) species