Journal of Lipid Research

Characterization and Relative Quantification of Phospholipids based on Methylation and Stable Isotopic Labeling

Supplementary Figure Legends

Supplementary Figure 1. Scheme of the derivatization procedure.

Supplementary Figure 2. The derivatization efficiency of PC species by diazomethane generated in situ.

A: PIS of m/z of 184 for the identification and quantification of PC species before derivatization. B: PIS of m/z of 184 for the identification and quantification of underivatized PC species after derivatization. C: PIS of m/z of 184 for the identification and quantification of derivatized PC species after derivatization. The drivatization efficiency is independent of the chemical properties of individual PC species including FA chain length or double bond (DB) numbers.

Supplementary Figure 3. The derivatization efficiency of SM species by diazomethane.

A: NLS of 213 Da, corresponding to phosphocholine plus methyl aldehyde from lithiated SMs, was used to characterize the individual sphingomyelin species from liver before derivatization by addition of approximately 25 pmol LiOH/ μL to the

lipid solution. B: NLS of 213 Da for the identification and quantification of underivatized SM species from liver after derivatization. C: PIS of m/z of 198 for the identification and quantification of derivatized SM species after derivatization, as the derivatized SMs tend to be ionized as proton adducts ([M+H]+) rather than lithium adducts even in the presence of high concentration of LiOH in the analyzing solution. The drivatization efficiency is independent of the chemical properties of individual PC species including FA chain length or double bond (DB) numbers.

Supplementary Figure 4. MS/MS spectra of protonated or ammonium adducted methylated PLs.

A: Collision-induced dissociation (CID) spectrum of the methylated PA 17:0/14:1 (m/z of 678, ammonium adducted). B: CID spectrum of the methylated PC 17:0/14:1 (m/z of 718, protonated). C: CID spectrum of the methylated PG 17:0/14:1 (m/z of 738, ammonium adducted). D: CID spectrum of the methylated PS 17:0/14:1 (m/z of 748, protonated).

Supplementary Figure 5. MS/MS spectra of protonated methylated lysoPLs.

A: CID spectrum of the methylated LPE 17:1. B: CID spectrum of the methylated LPA 17:1. C: CID spectrum of the methylated LPS 17:1. D: CID spectrum of the methylated LPI 17:1.

Supplementary Figure 6. Profiling of the six major LPLs by PIS or NLS under positive ionization mode.

A: PIS of m/z of 198 for the identification and quantification of lysoPC species after derivatization. B-F: NLS of 143Da, 155Da, 203Da, 213Da and 274Da were chosen for identification and quantification of LPA, LPE, LPG, LPS and LPI species after derivatization.

Supplementary Figure 7. Scheme for isotopic labeling of phospholipids by phosphate methylation based on acid catalyzed H/D exchange and methanolysis of TMS-diazomethane.

Supplementary Figure 8. Simultaneous fractionation of phospholipid species from two different samples based on isotopic labeling strategy to reduce sample complexity.

A: Full scan for detection of PLs from the lipid droplet by direct infusion ESI-MS before fractionation of phospholipid by HILIC-based chromatography. B: Full scan for detection of PLs from the lipid droplet by direct infusion ESI-MS before after fractionation of phospholipid by HILIC-based chromatography. C: The isotopic pairs of PC species were co-eluted from the column. D: The profiles of unlabeled or labeled PC species were not affected by the pre-fractionation.

Supplementary Figure 9. NLS or PIS based ESI-MS/MS analysis for the mixture of ^{1}H and labeled phospholipids at 1:3 ratios.

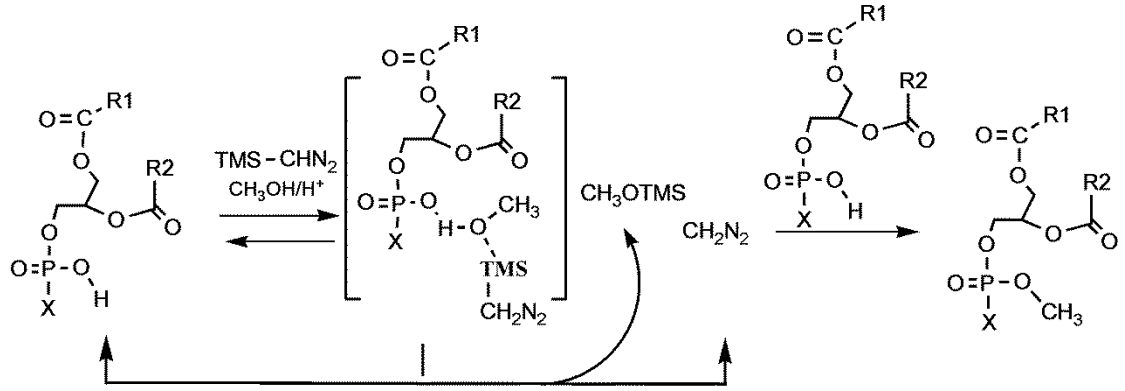
A: NLS of 155 Da for identification and quantification of ^{1}H labeled PE species from the mixture of ^{1}H and D-labeled phospholipids at 1:3 ratio. B: NLS of 157 Da for

identification and quantification of ^{1}D labeled PE species from the mixture of ^{1}H and D-labeled phospholipids at 1:3 ratio. C: PIS of m/z 603 for identification and quantification of ^{1}H labeled and D-labeled PE 34:1 from the mixture of ^{1}H and D-labeled phospholipids at 1:3 ratio. D: NLS of 213 Da for identification and quantification of ^{1}H labeled PS species from the mixture of ^{1}H and D-labeled phospholipids at 1:3 ratio. B: NLS of 217 Da for identification and quantification of ^{1}D labeled PS species from the mixture of ^{1}H and D-labeled phospholipids at 1:3 ratio. C: PIS of m/z 603 for identification and quantification of ^{1}H labeled and D-labeled PS 34:1 from the mixture of ^{1}H and D-labeled phospholipids at 1:3 ratio. The expected 1:3 intensity ratio and the 2 or 4Da shift for PE and PS, respectively, are also evident. The monoisotopic peak was employed for quantification.

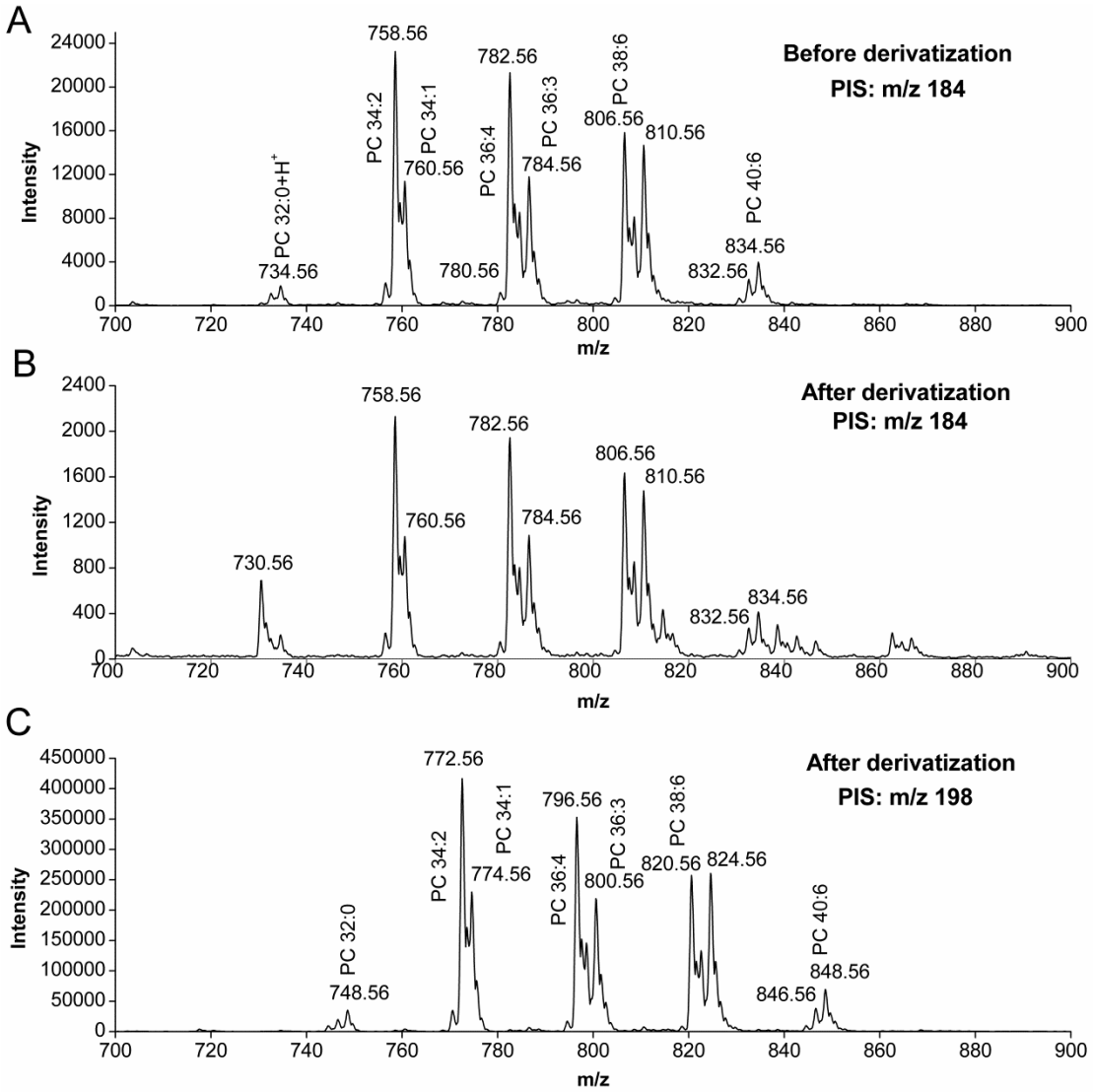
Supplementary Figure 10. The effect of the derivatization conditions on the stability of plasmalogens.

A: PIS of m/z of 196 for the identification and quantification of plasmalogens from lipid solution extracted from brain tissue, which are believed to be rich of plasmalogens, before went through our methylation procedures. B: PIS of m/z of 196 for the identification and quantification of plasmalogens from lipid lipid solution extracted from brain tissue after went through our derivatization procedures except for addition of the TMS-dizomethane.

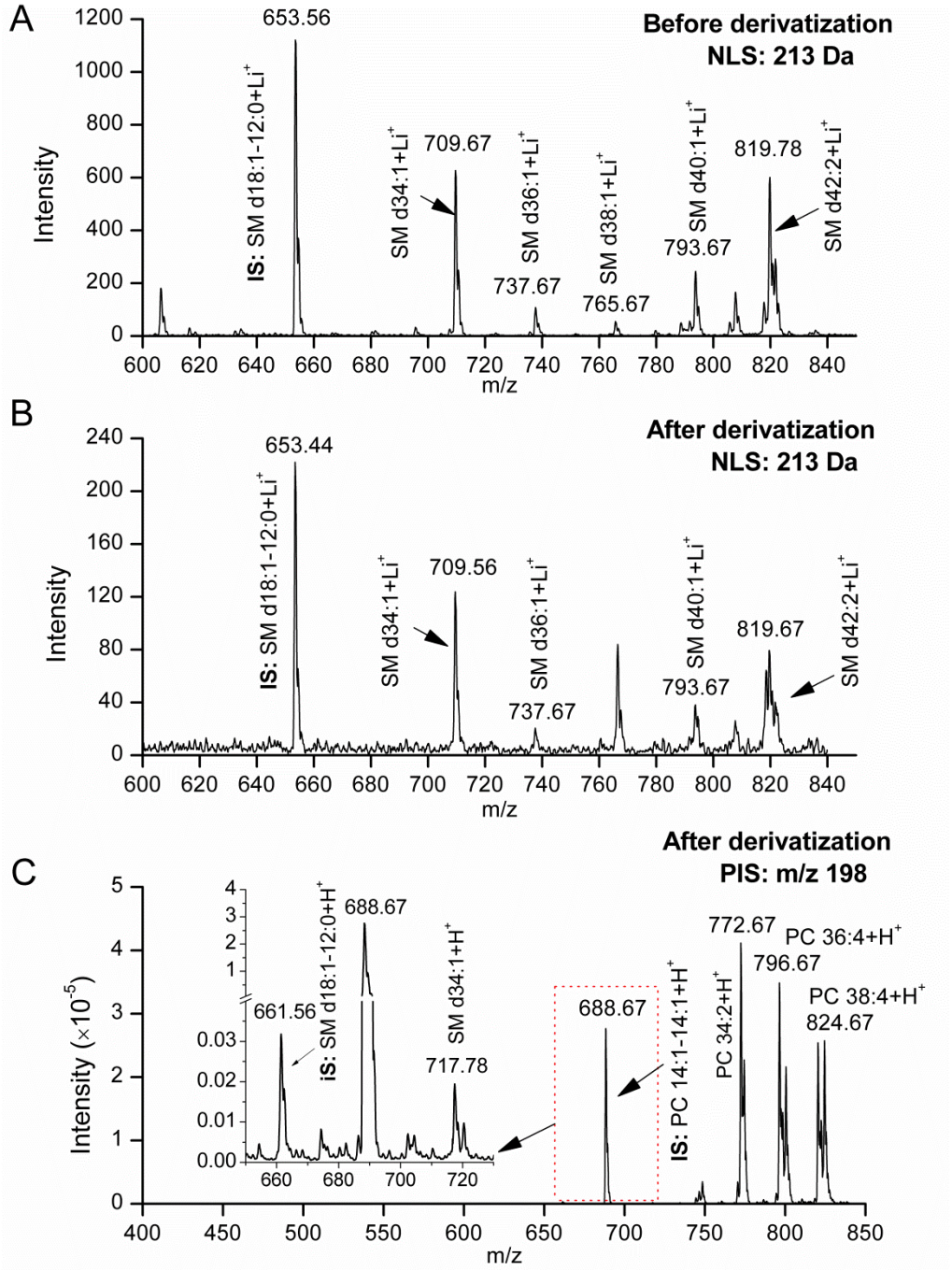
Supplementary Figure 1



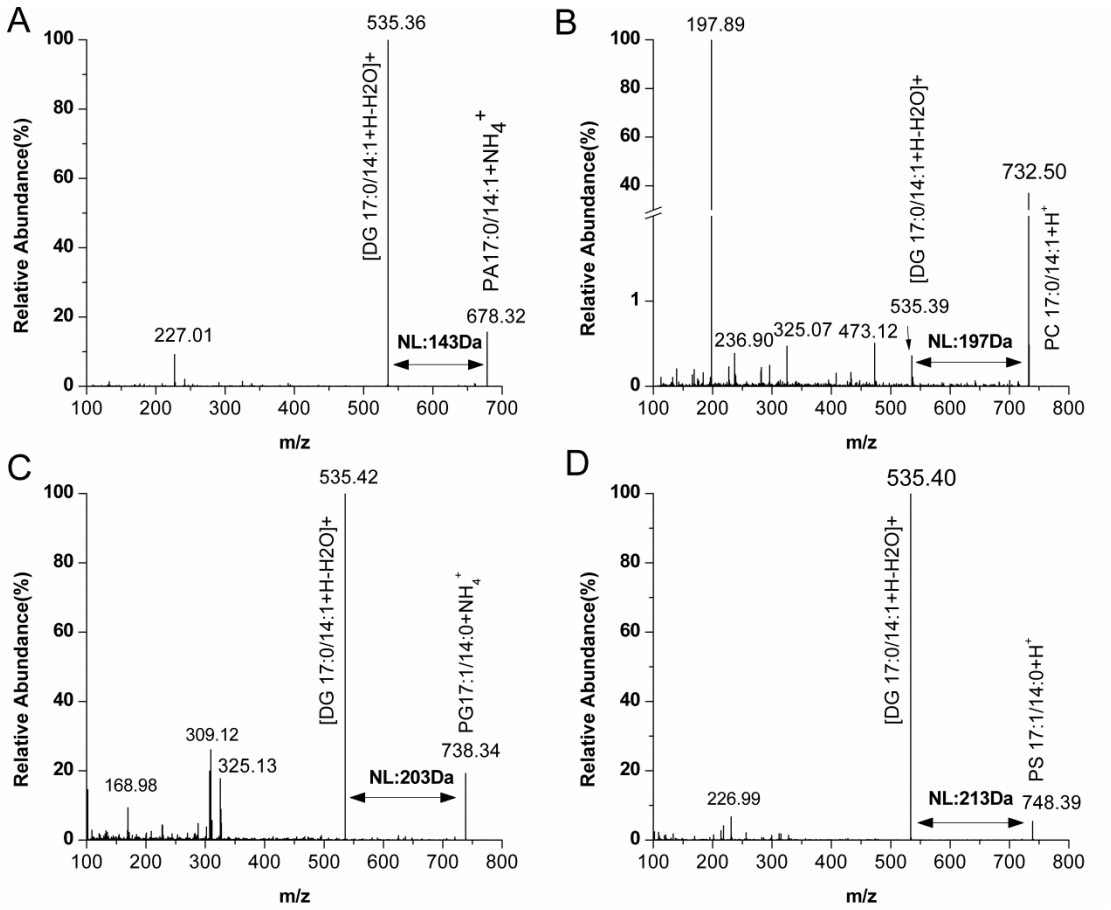
Supplementary Figure 2



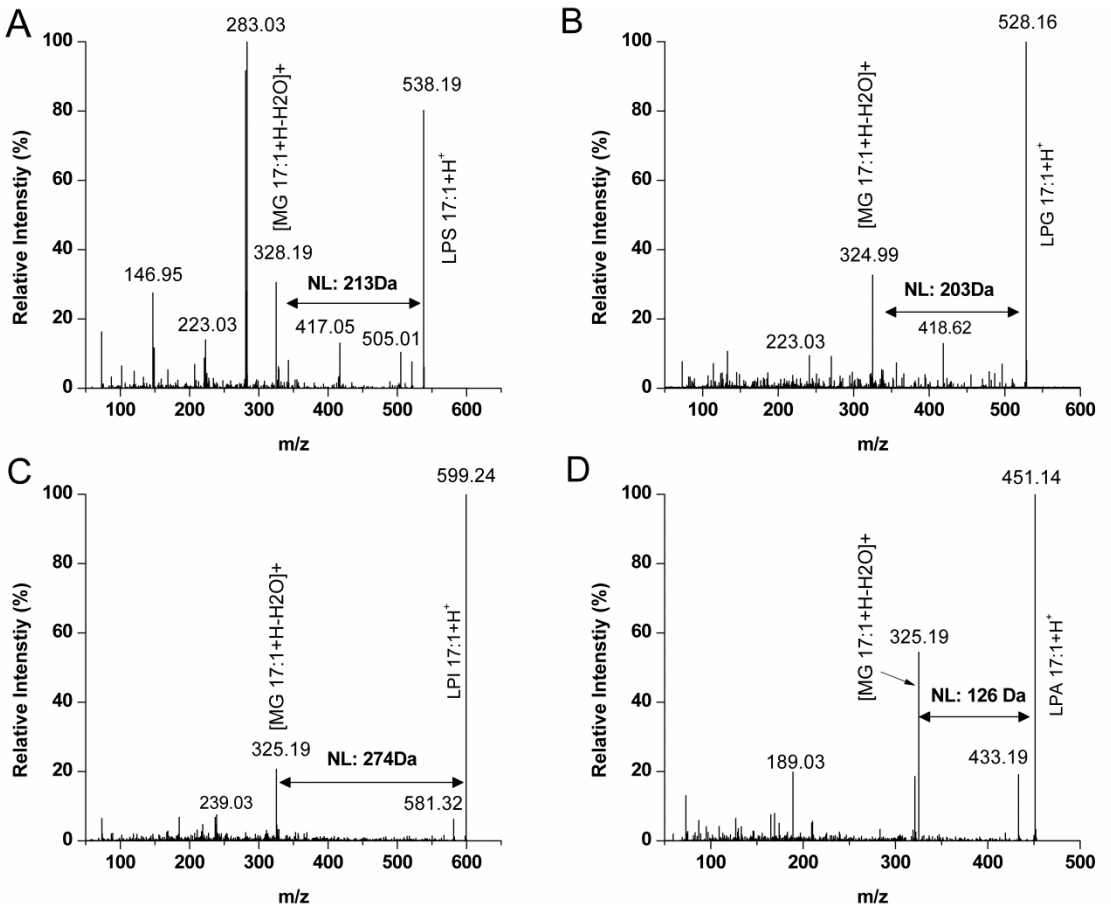
Supplementary Figure 3



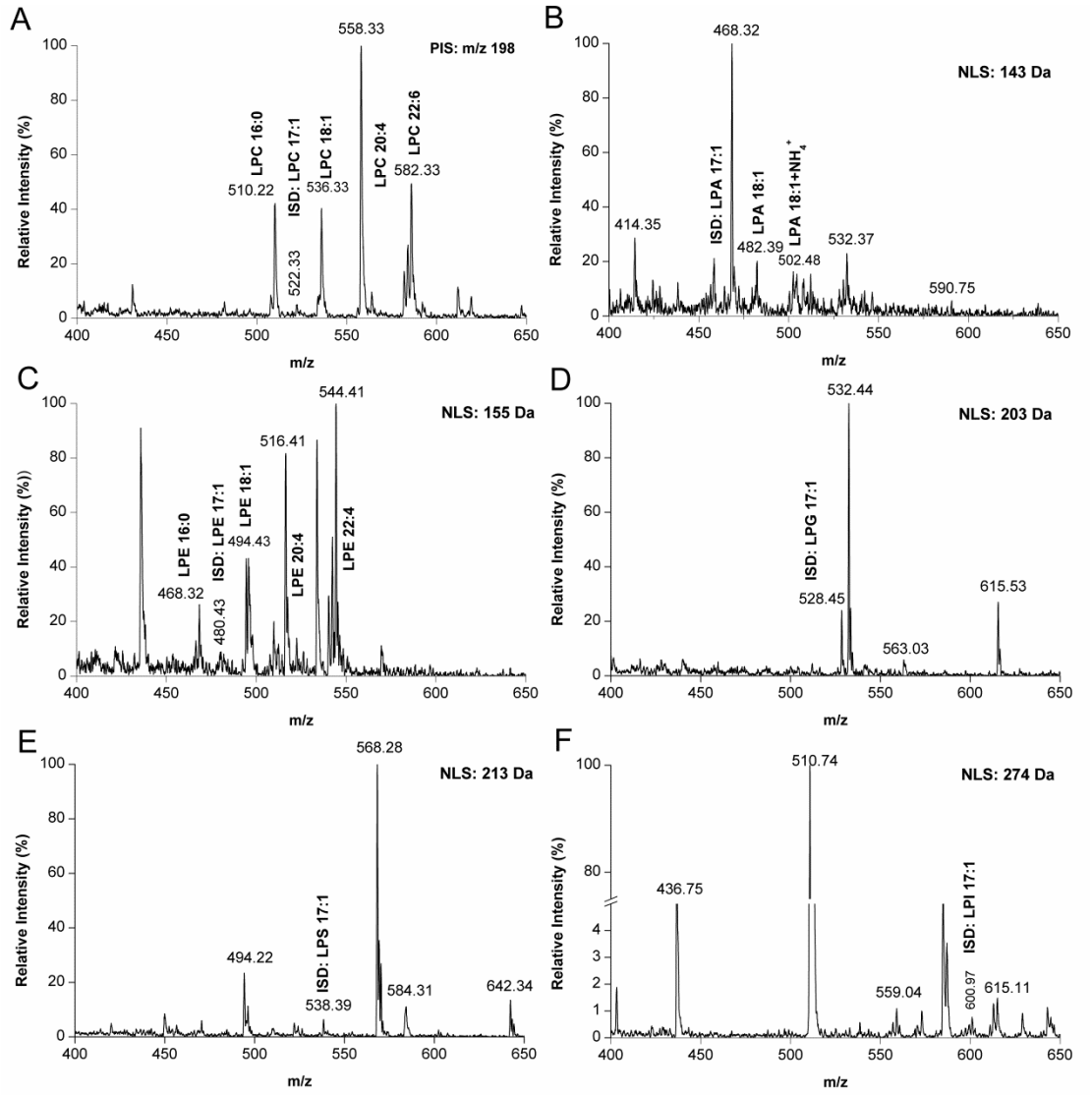
Supplementary Figure 4



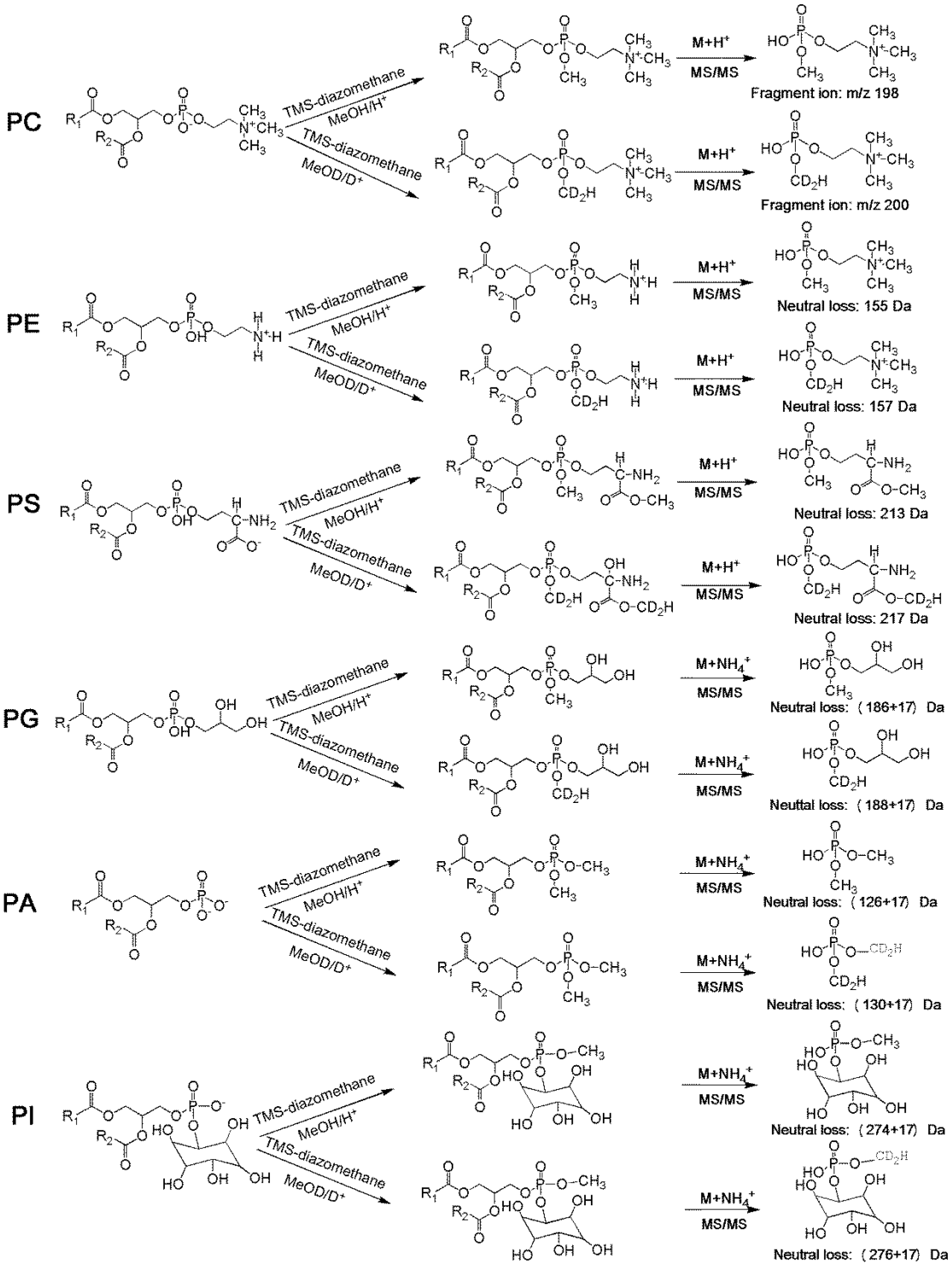
Supplementary Figure 5



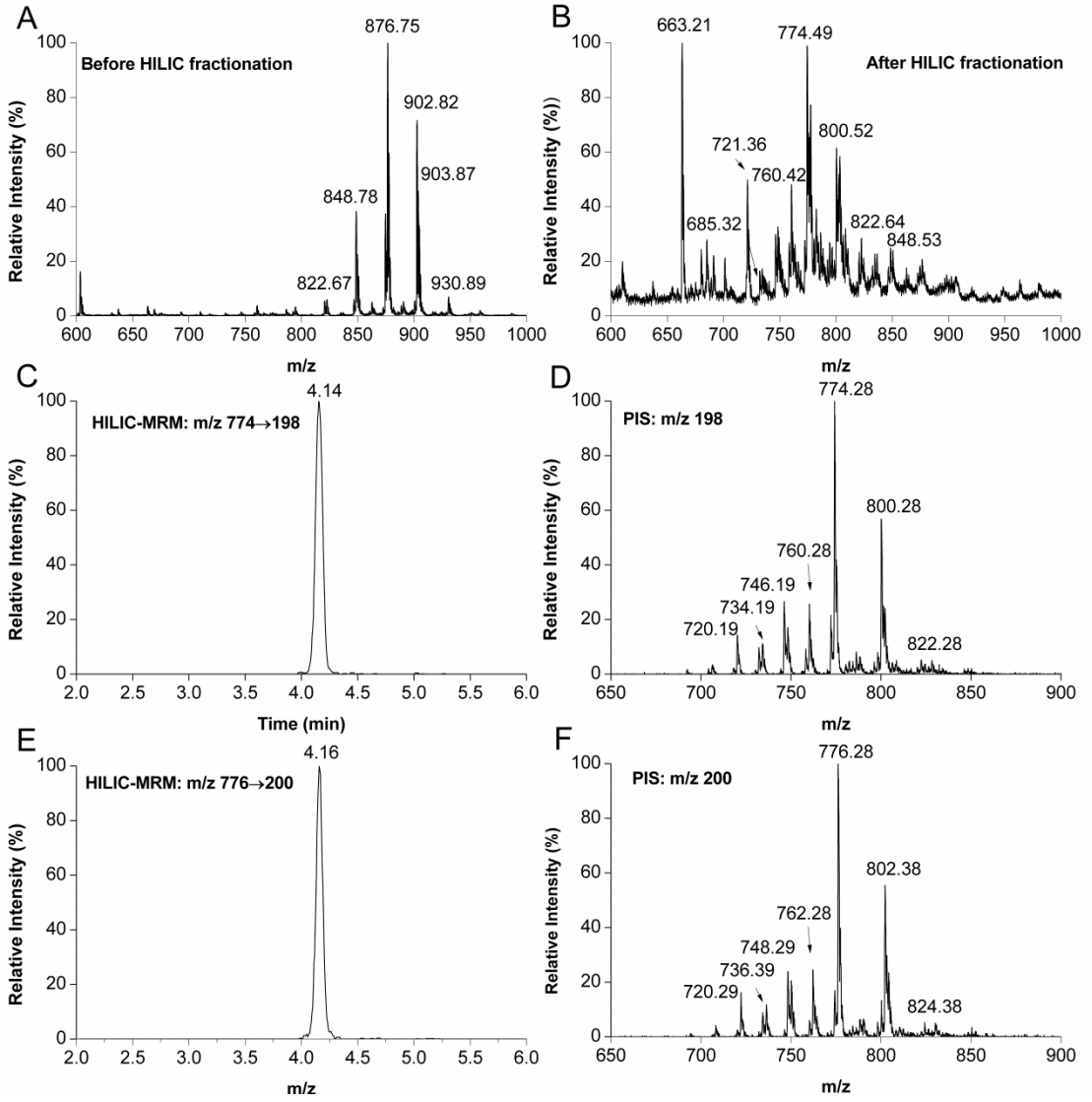
Supplementary Figure 6



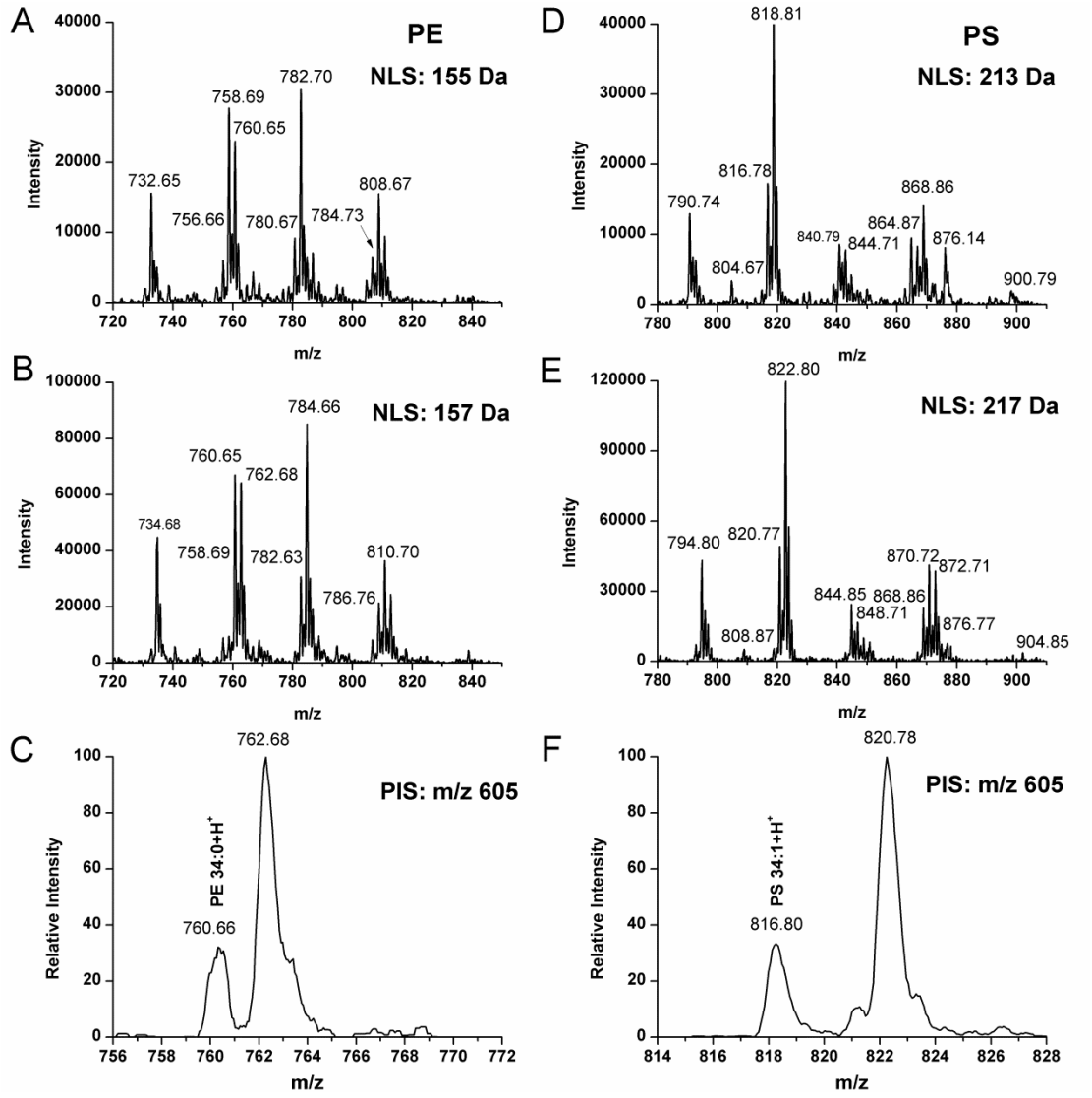
Supplementary Figure 7



Supplementary Figure 8



Supplementary Figure 9



Supplementary Figure 10