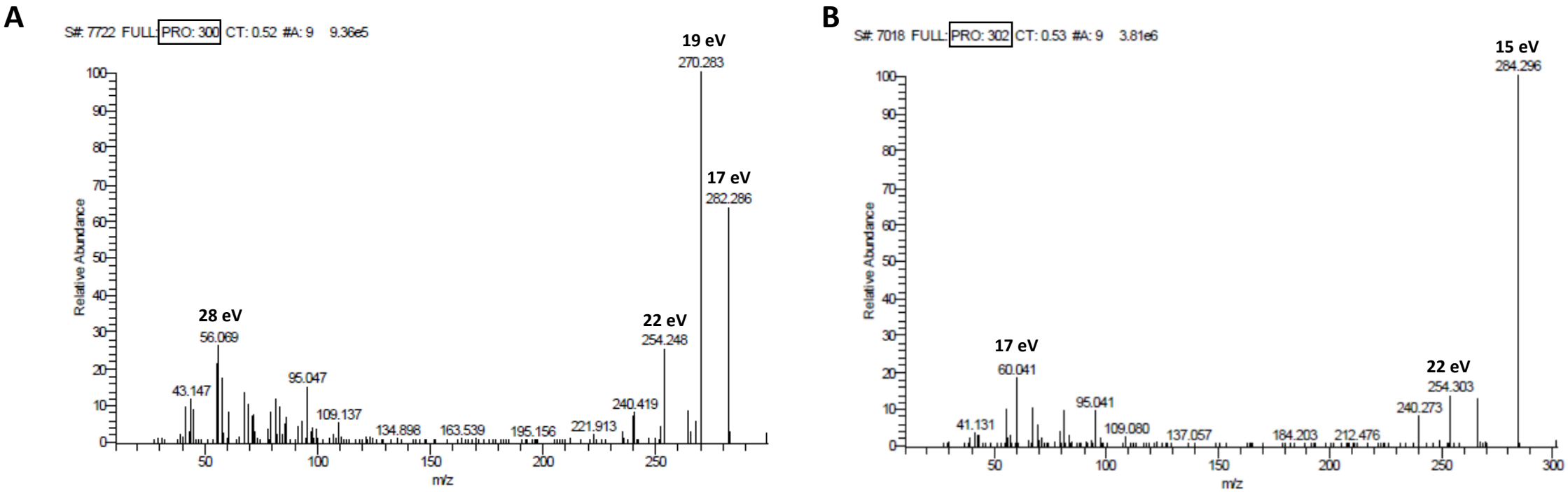
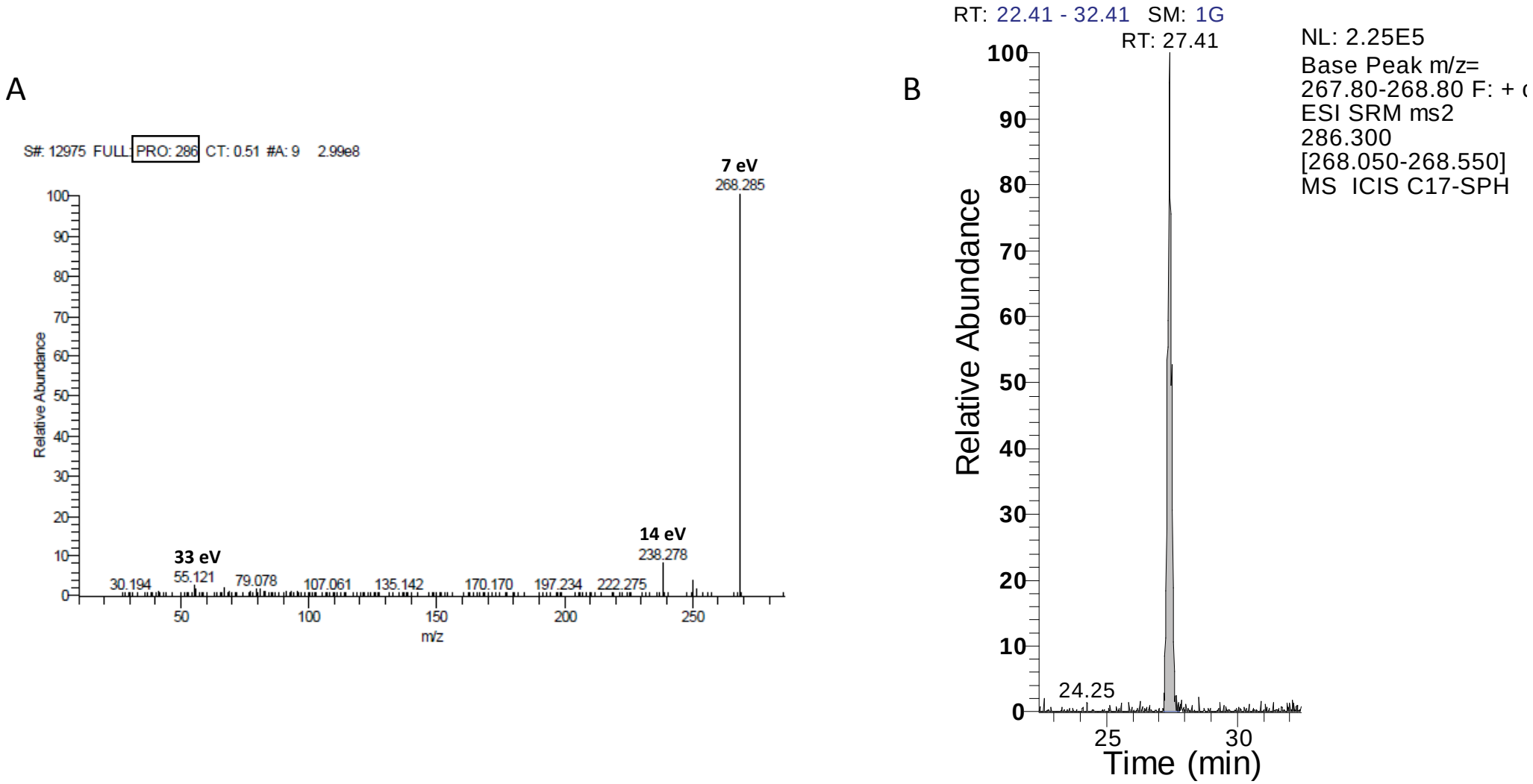
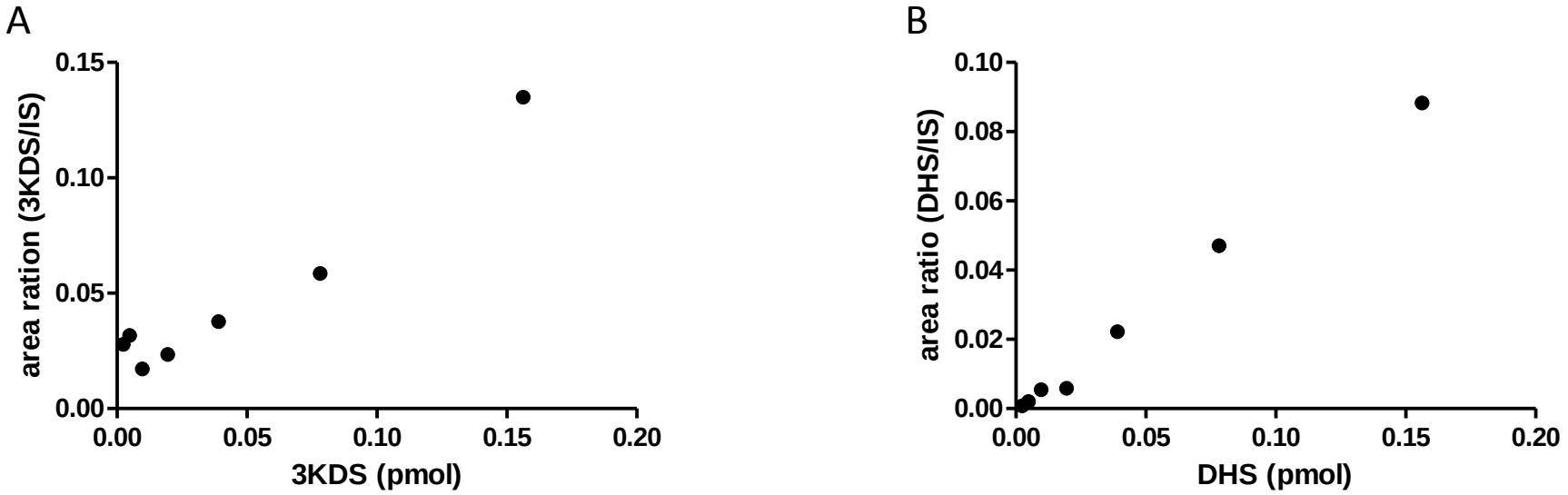


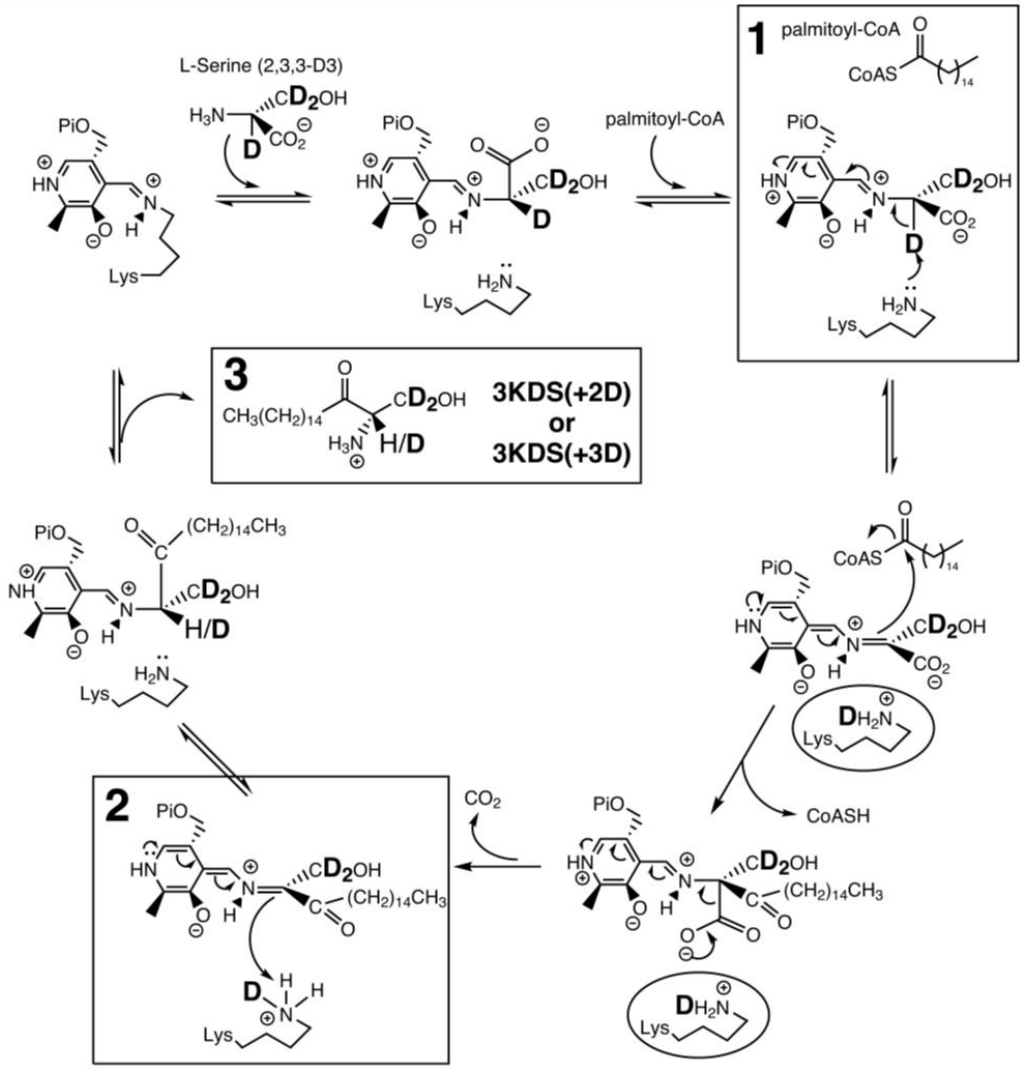
Supplemental Figure S1. Product ion spectrums of 3KDS and DHS generated from compound optimization report. Compounds were optimized using Thermo TSQ tune software for the most abundant fragment ions. 300 m/z and 302 m/z corresponding to 3KDS (A) and DHS(B) respectively, was fragmented at 1.5 mTorr and product ions were monitored with full scan between 0-298 m/z and 0-300 m/z for 3KDS and DHS respectively. Collision energy for each abundant product ion was also shown.



Supplemental Figure S2. HPLC-ESI-MS/MS characterization of internal standard C17-SPH. (A) Product ion spectrum of C17-SPH generated from Thermo TSQ tune software. 286 m/z corresponding to C17-SPH was fragmented at 1.5 mTorr and product ions were monitored with full scan between 0-284 m/z. Collision energy for each abundant fragment ion was also shown. (B) SRM peak of C17-SPH. Ion intensities, retention time and parent/product ion pairs are shown in the legends on the right.



Supplemental Figure S3. Detection of 3KDS (A) and DHS (B) that fall below the linear range of the standard curve. 0.002 – 0.156 pmol of 3KDS (A) and DHS (B) were applied to HPLC followed by ESI-MS/MS using the same transition as in Fig 1. The absolute quantity of the analyte is plotted against the peak area ratio of the analyte to the internal standard (C17-SPH).



Supplemental Figure S4. The fate of C2-deuterium from L-Ser (2,3,3-D3) predicted by the proposed PLP-dependent SPT catalytic mechanism. This figure is modified from Raman et al., 2009 (10). Box 1 shows the deprotonation of the external aldimine formed between PLP and L-Ser (2,3,3-D3) with abstraction of C2-deuterium by active Lys residue (presumably Lys_{366} on LCB2) upon binding of palmitoyl-CoA. The deuterated base is circled. The deuterium is retained on the lysine base presuming no hydrogen exchange occurs. Box 2 shows the re-protonation process of 3KDS product quinonoid by abstraction of hydrogen or deuterium from Lys366 base. Box 3 shows the production of either 3KDS (+2D) or 3KDS (+3D) depending on which hydrogen or deuterium is abstracted.