Supporting Information for:
Di-Lysine-Methylene Diphenyl Diisocyanate (MDI), a Urine Biomarker of MDI Exposure?

Adam V. Wisnewski[†*], *Ala F. Nassar*[†], *Jian Liu*[†] *and Dhimiter Bello*[‡]
[†]Department of Internal Medicine, Yale University, New Haven, CT, USA
[‡]Department of Public Health, University of Massachusetts, Lowell, MA, USA

*Corresponding Author: Adam V. Wisnewski, PhD, D(ABMLI); Yale University / PO Box 208057; 300 Cedar Street / TACS-415; New Haven, CT 06524 / USA, Email: Adam.Wisnewski@Yale.edu, Phone: (203)-737-4054, Fax: (203)-785-3826

Figure # Page#

Figure S1. LC-UV and MS characterization of reaction mixtures to which mice were exposed. S2

Figure S2. MS/MS characterization of the major MDI-GSH reaction product, an 865 *m/z* $[M+H]^+$ parent ion. S3

Figure S3. Partial ^1H NMR spectra for MDI-GSH reaction products. S4

Figure S4. Expected MS/MS CID-fragmentation for di-lysine-MDI. S5

Figure S5. General schematic of di-lysine-MDI chemical synthesis. S6

Figure S6. Expected isotope distribution for urine molecules from mice exposed to MDI-GSH. S7

Figure S7. MS/MS of urinary 558.17 *m/z* $[M+H]^+$ parent ion. S8

Figure S8. MS/MS of urinary 863.23 *m/z* $[M+H]^+$ parent ion. S9

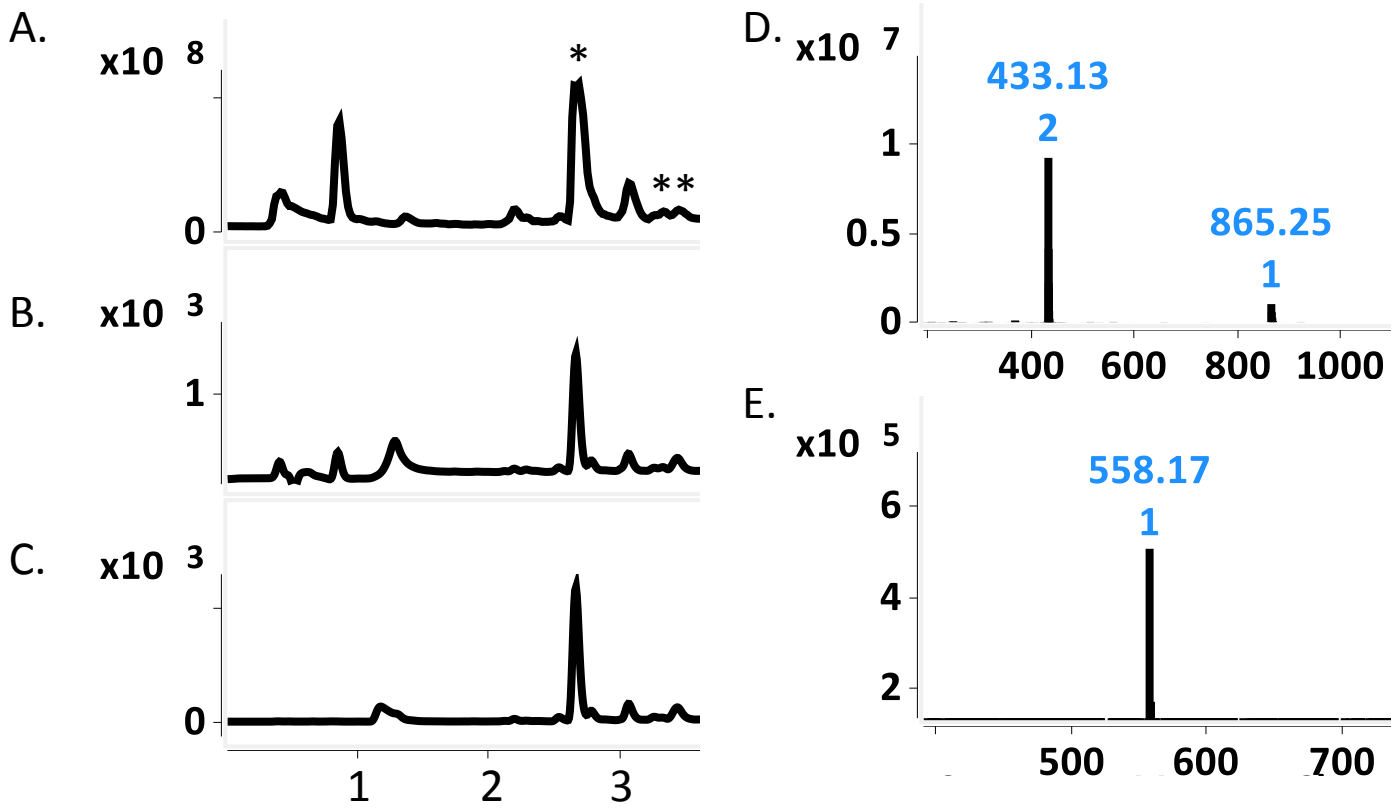

Figure S1. LC-MS and UV characterization of MDI-GSH reaction mixtures to which mice were exposed. The LC-MS TIC (A) LC-UV-A_{210} (B) and the LC-UV-A_{245} (C) are shown; Y-axis is intensity and X-axis reflects retention time in minutes. The MS for the (peak) reaction products with a retention time ~2.8 minutes (*) is shown in Panel D, while a minor peak (**) with retention time > 3.5 min is shown in Panel E. For D and E the X-axis reflects m/z, and the peak mass and charge are labeled.

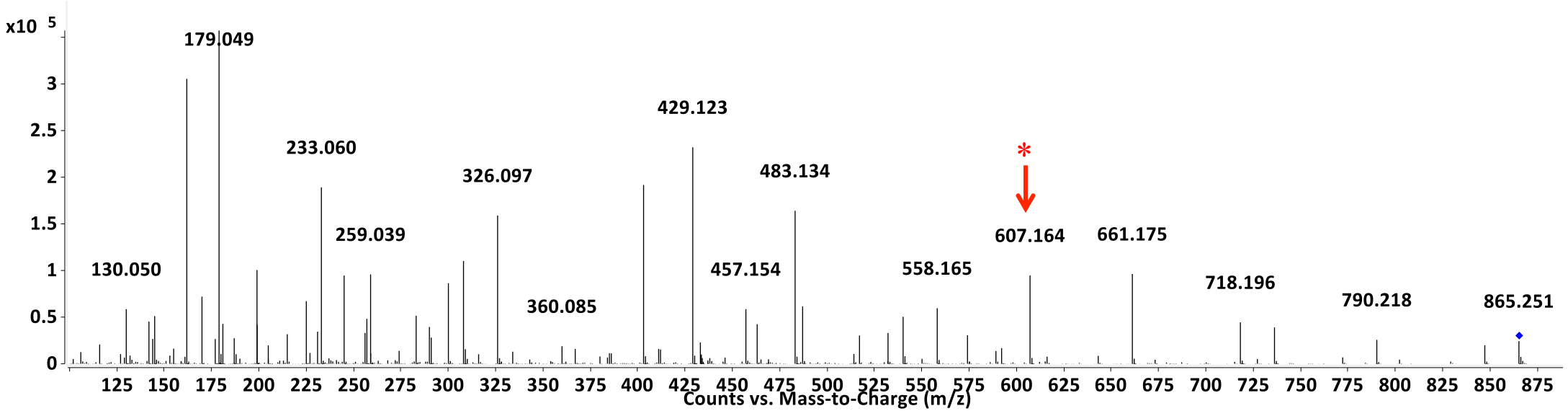

Figure S2. MS/MS characterization of the major MDI-GSH reaction product, an 865 *m/z* $[M+H]^+$ parent ion, to which mice are exposed via the respiratory tract. Note the 607.164 m/z daughter ion corresponding to bis(cys-gly)-MDI as we previously published (reference # 48 in manuscript), as evidence for *S,S*'-linked bis(GSH)-MDI as the parent ion.

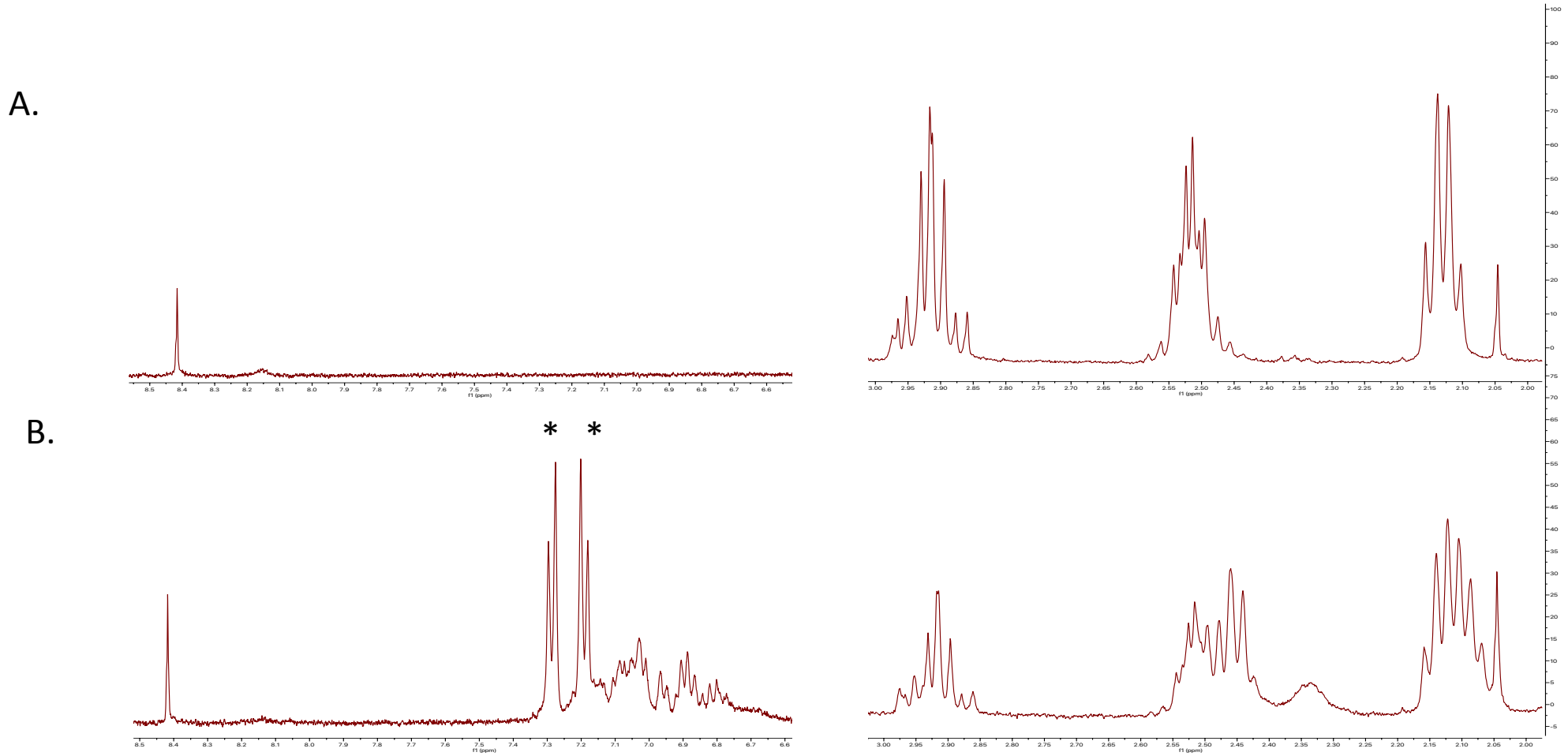

Figure S3. Partial ^{1}H NMR spectra for MDI-GSH reaction products. Partial ^{1}H-NMR spectra are show for mock reacted GSH (A) and MDI-reacted GSH (B) in D_2O containing phosphate buffer. Regions of the spectra not obscured by water and buffer peaks are shown and highlight (*) doublets ~7ppm that likely correspond to hydrogens from MDI's aromatic rings among the reaction products.

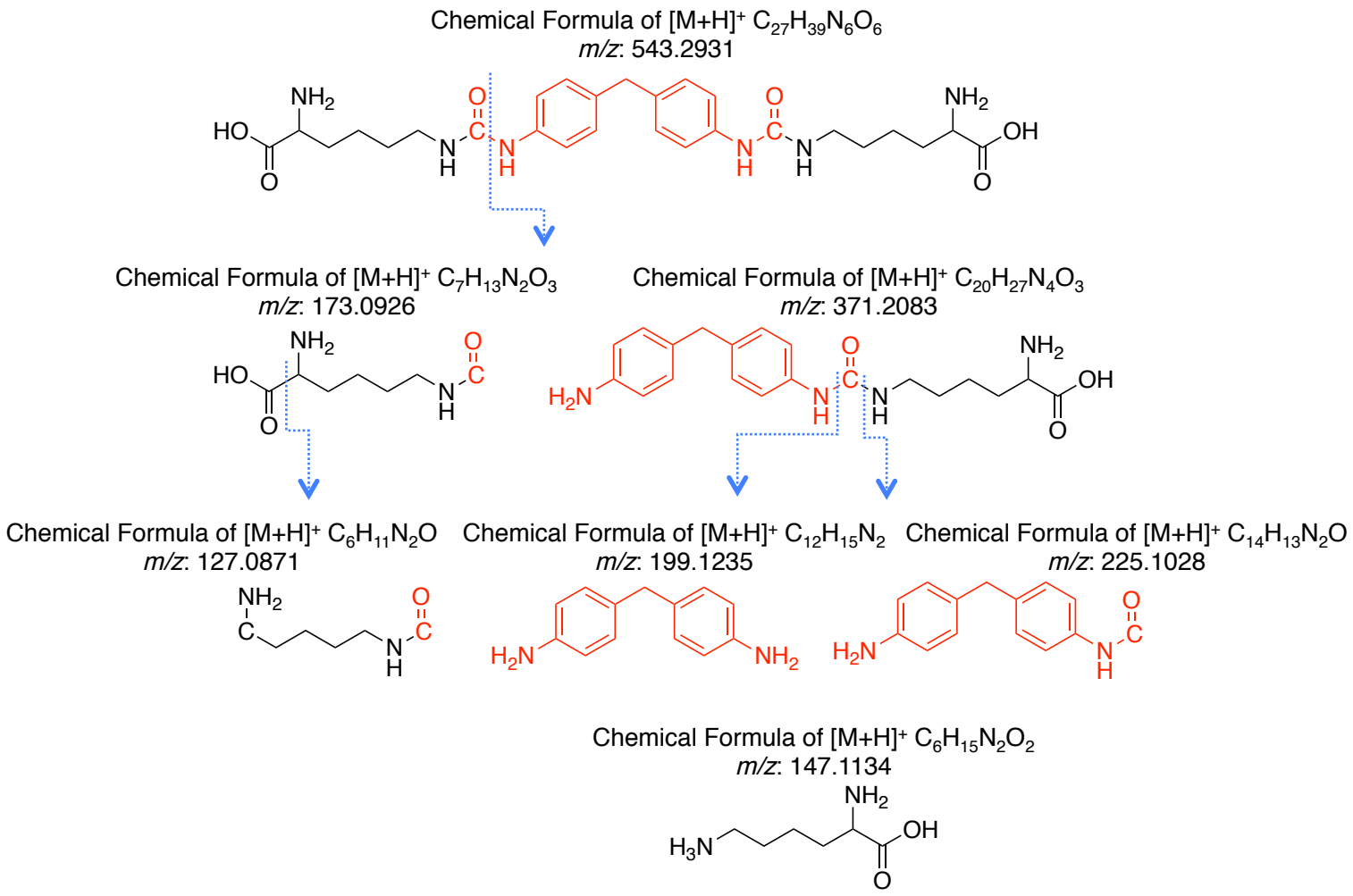

Figure S4. Expected fragmentation pattern of di-lysine-MDI. The predicted ions expected to result upon CID during MS/MS of the parent 543.29 *m/z* $[M+H]^+$ ion are shown as mentioned in the Results Section.

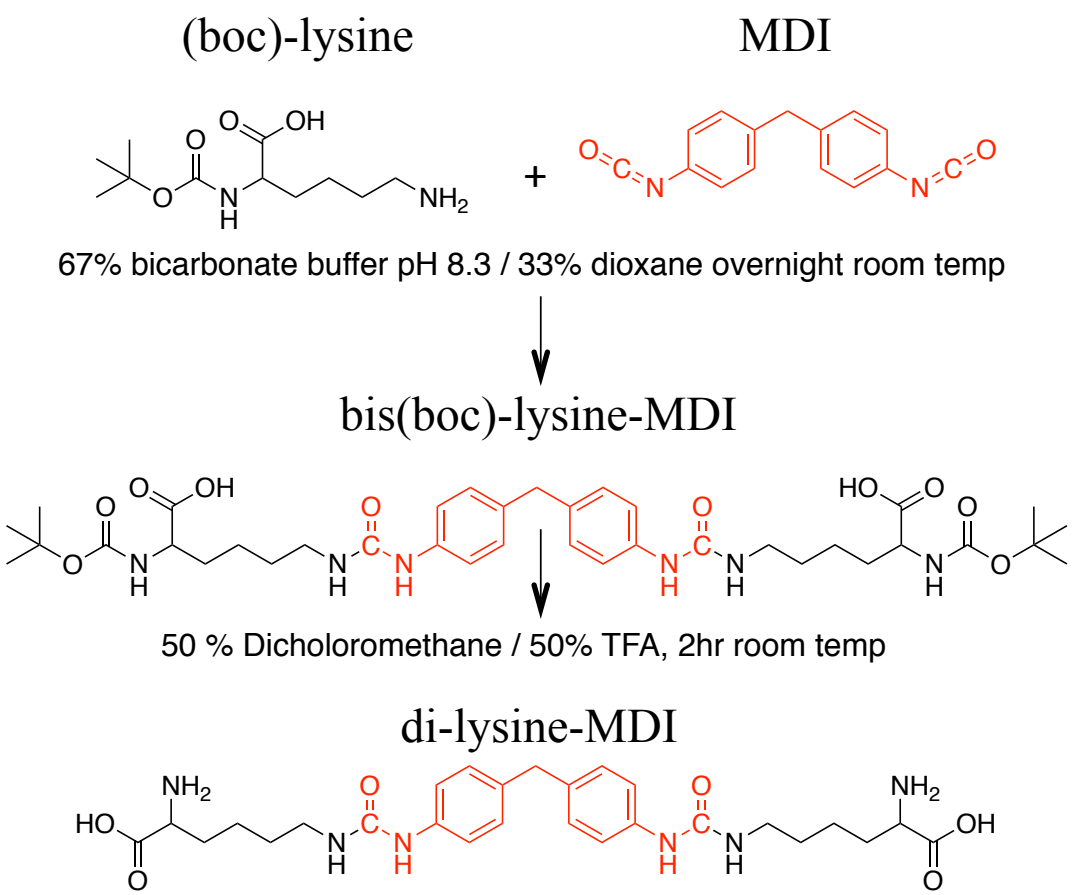

Figure S5. General schematic of di-lysine-MDI chemical synthesis, with MDI cross linking the epsilon amine groups of lysine.

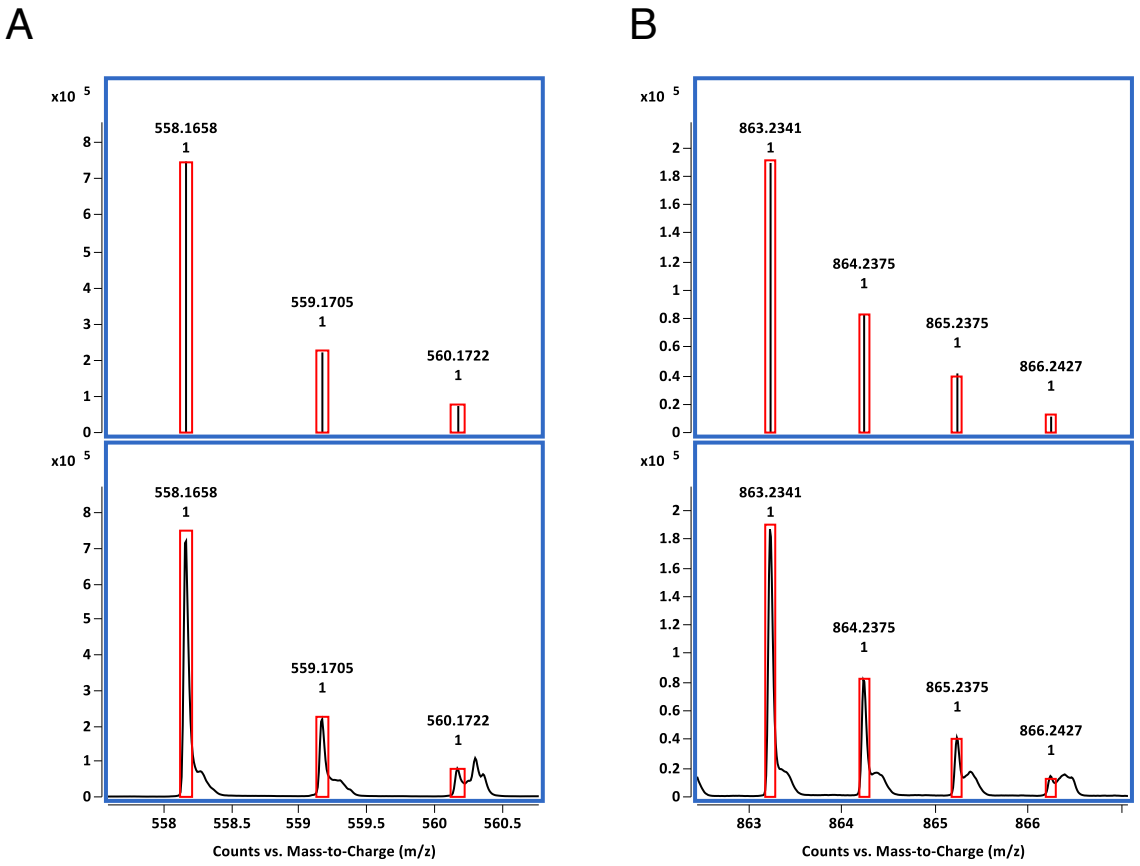

Figure S6. Expected isotope distribution for urine molecules from mice exposed to MDI-GSH. Mass chromatograms with the background subtracted (top row) and raw data (bottom row) are show for the the 558.17 *m/z* $[M+H]^+$ ion (A) and the 863.23 *m/z* $[M+H]^+$ ion (B). Red boxes depict the signal expected for molecules with the formulas $C_{25}H_{27}N_5O_8S$ or $C_{35}H_{42}N_8O_{14}S_2$ in A and B respectively. Y-axis is intensity and X-axis depicts *m/z*.

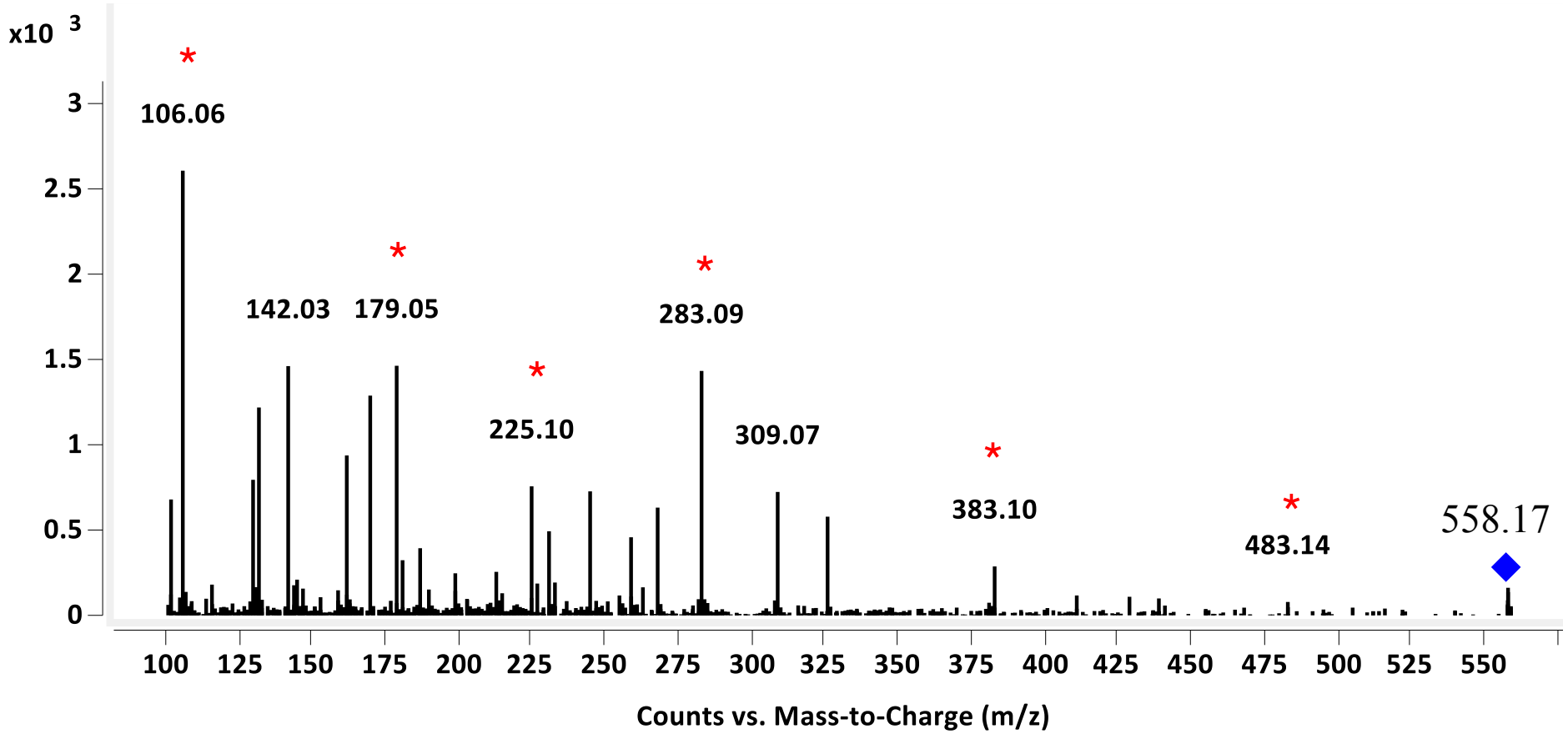

Figure S7. MS/MS of the 558.17 *m/z* $[M+H]^+$ parent ion (blue diamond) uniquely found in urine from mice exposed via the respiratory tract to MDI-GSH reaction products. Ions with * are described in the text.

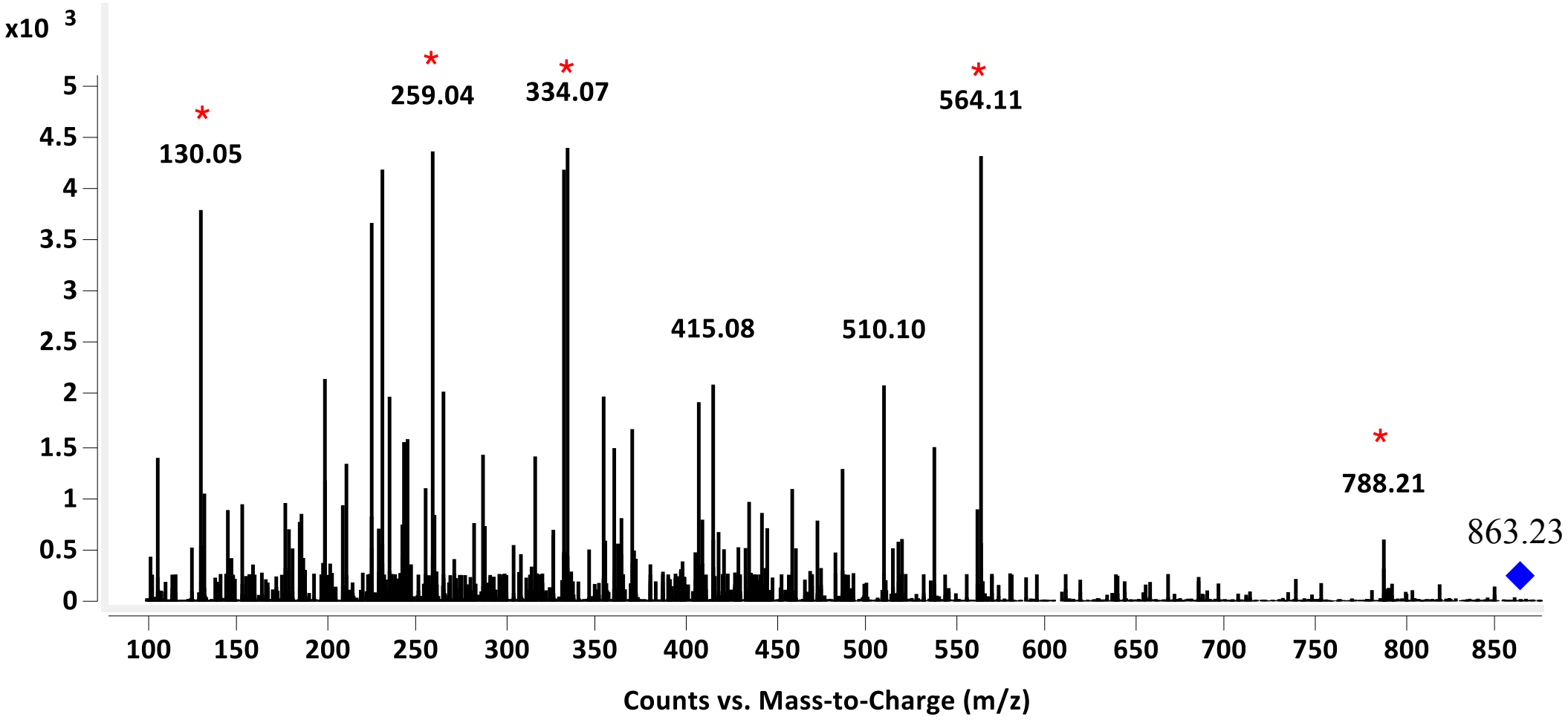

Figure S8. MS/MS of the 863.23 *m/z* $[M+H]^+$ parent ion uniquely found in urine from mice exposed via the respiratory tract to MDI-GSH reaction products. Ions with * are described in the text.