Covalent Cross-Linking of Glutathione and Carnosine to Proteins by 4-Oxo-2-nonenal[§]

Xiaochun Zhu[†], Molly M. Gallogly[+], John J. Mieyal[+], Vernon E. Anderson*[,‡], and Lawrence M. Sayre[¶,†]

[†] Department of Chemistry, Case Western Reserve University

[+] Department of Pharmacology, Case Western Reserve University

[‡] Department of Biochemistry, Case Western Reserve University

Cleveland, OH 44106

Supporting Information

Table S1. GSH–ONE-modified β-LG peptides detected by HPLC-ESI-MS/MS in the chymotryptic digest

Peptide sequence	Position	Mass of GSH–ONE-modified peptide		Modified residue
		Theoretical	Observed	
LIVTQ	1–5	998.52/1007.58	998.59/1007.60	L1
LIVTQT	1–6	1099.57/1108.63	1099.59/1108.64	L1
KGL	8–10	742.38/751.44	742.46/751.48	K8
KGLDIQ	8–13	1098.55/1107.61	1098.57/1107.58	K8
KGLDIQKVAGTW	8–19	1740.89/1749.95	1740.81/1749.89	K8
KGLDIQKVAGTW	8–19	1740.89/1749.95	1740.86/1749.88	K14
DIQKVAGTW	11–19	1442.71/1451.75	1442.61/1451.64	K14
KVAGTW	14–19	1086.53/1095.59	1086.50/1095.60	K14
VEELKPTPEGDLEIL	43–57	2107.05/2116.11	1054.36/1058.90[#]	K47
LQKW	58–61	999.50/1008.55	999.52/1008.57	K60
QKW	59–61	886.41/895.47	886.35/895.43	K60
TKIPAVF	76–82	1200.63/1209.69	1200.67/1209.66	K77
KIDAL	83–87	984.51/993.56	984.50/993.56	K83
KIDALN	83–88	1098.55/1107.61	1098.54/1107.51	K83
KIDALNENKVL	83–93	1681.88/1690.94	1681.76/1690.74	K83
KIDALNENKVL	83–93	1681.88/1690.94	1681.82/1690.91	K91
NENKVL	88–93	1141.56/1150.61	1141.55/1150.57	K91
KVL	91–93	784.43/793.48	784.44/793.48	K91
KKY	100–102	863.43/872.49	863.49/872.54	K100

KKY	100–102	863.43/872.49	863.51/872.56	K101
VRTPEVDDEALEKF	123–136	2072.99/2082.04	1037.39/1041.85[#]	K135
DKAL	137–140	871.42/880.48	871.42/880.53	K138
KALPM	141–145	984.49/993.55	984.53/993.58	K141

#: double charged peak

Table S2. Carnosine–ONE-modified *β*-LG peptides detected by HPLC-ESI-MS/MS in the chymotryptic digest

Peptide sequence	Position	Mass of Carnosine–ONE-modified peptide		Modified residue
		Theoretical	Observed	
KGL	8–10	661.40/670.43	661.47/670.57	K8
VEELKPTPEGDLEIL	43–57	2026.07/2035.10	1013.80/1018.74[#]	K47
LQKW	58–61	918.52/927.55	918.56/927.57	K60
KIDAL	83–87	903.53/912.57	903.59/912.58	K83
KIDALNENKVL	83–93	1600.91/1609.93	1600.80/1609.90	K91
KKY	100–102	782.46/791.48	782.44/791.58	K100 or K101
VRTPEVDDEALEKF	123–136	1992.01/2001.03	996.85/1001.31[#]	K135
DKAL	137–140	790.45/799.47	790.50/799.49	K138
KALPM	141–145	903.51/912.54	903.60/912.58	K141
HIRL	146–149	882.53/891.56	882.66/891.86	H146

#: double charged peak

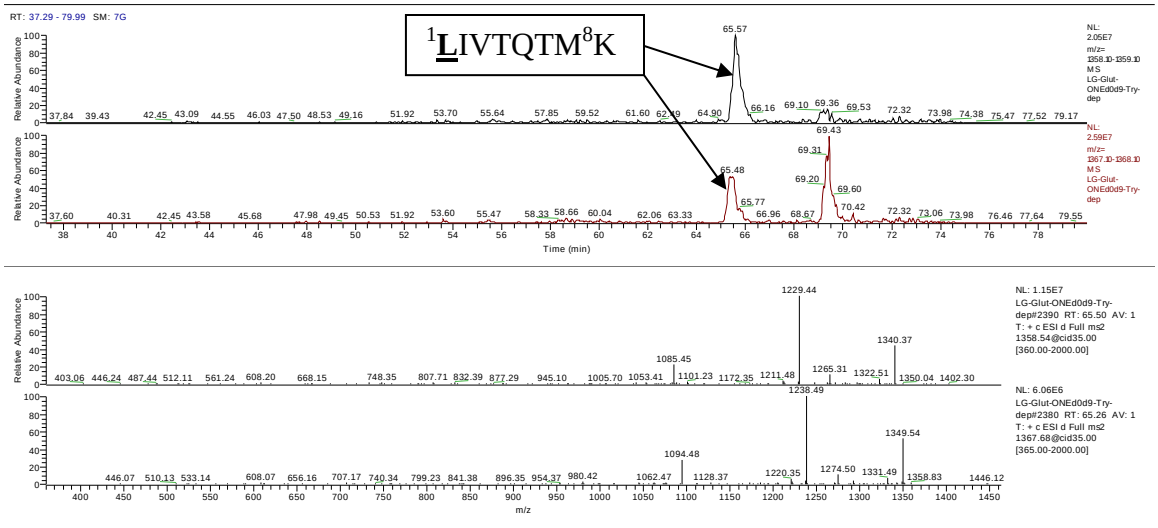

Figure S1. SIC and tandem mass spectrum of modified [1]**L**IVTQTM[8]K at L1 by GSH–d_0-ONE MA (1[nd] trace and 1[st] spectrum) or GSH–d_9-ONE MA (2[rd] trace and 2[nd] spectrum).

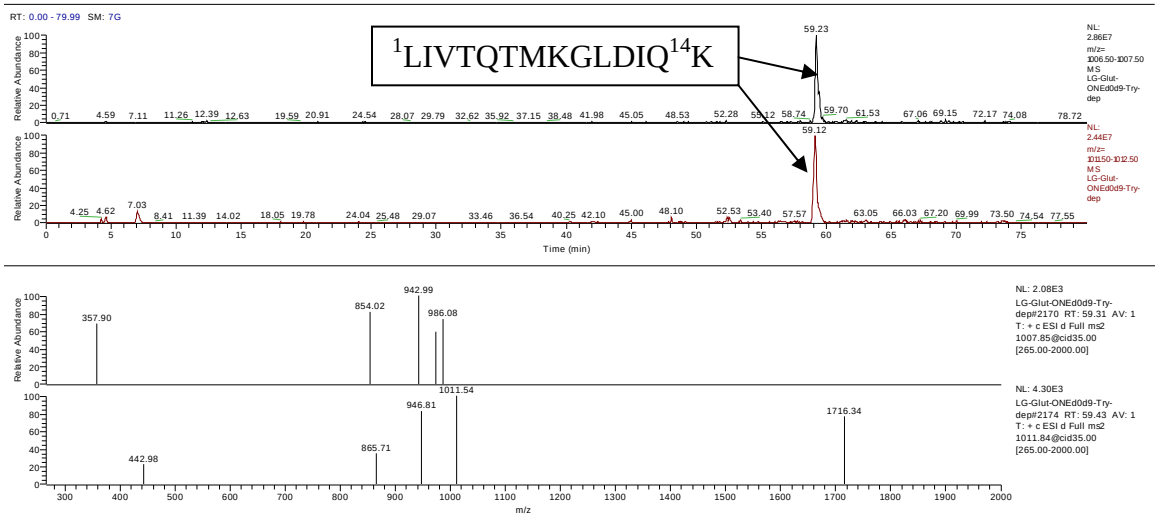

Figure S2. SIC and tandem mass spectrum of modified ^{1}LIVTQTMKGLDIQ^{14}K at L1 or K8 by GSH–d_0-ONE MA (1nd trace and 1st spectrum) or GSH–d_9-ONE MA (2rd trace and 2nd spectrum).

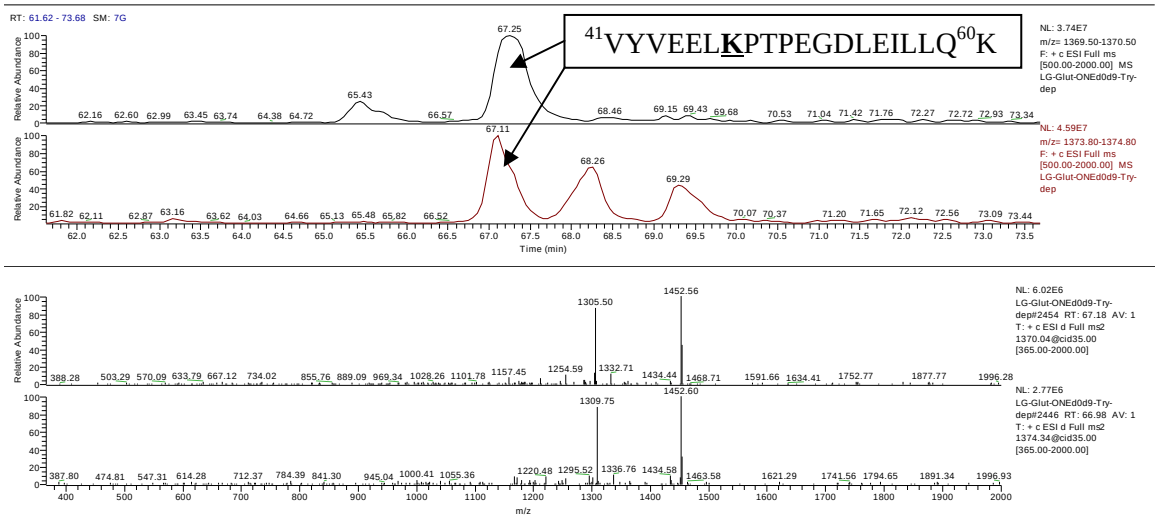

Figure S3. SIC and tandem mass spectrum of modified ^{41}VYVEEL**K**PTPEGDLEILLQ^{60}K at K47 by GSH–d_0-ONE MA (1nd trace and 1st spectrum) or GSH–d_9-ONE MA (2rd trace and 2nd spectrum).

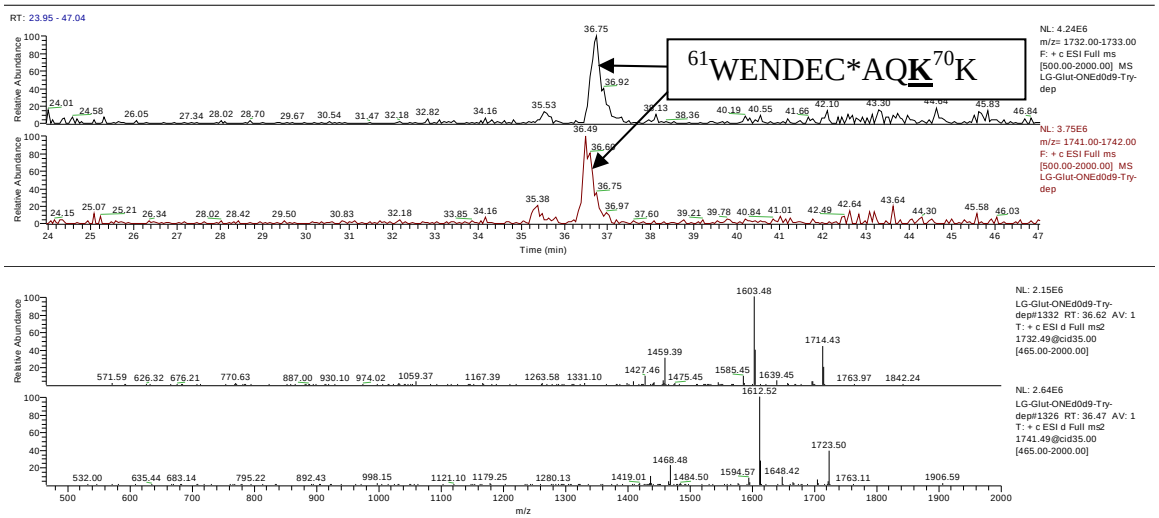

Figure S4. SIC and tandem mass spectrum of modified ^{61}WENDEC*AQK^{70}K at K69 by GSH–d_0-ONE MA (1nd trace and 1st spectrum) or GSH–d_9-ONE MA (2rd trace and 2nd spectrum).

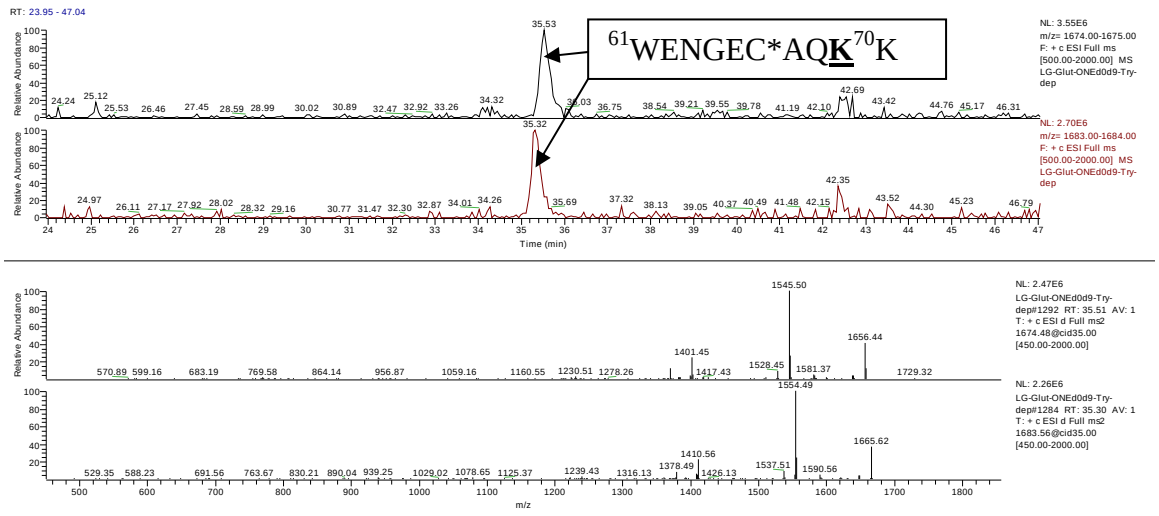

Figure S5. SIC and tandem mass spectrum of modified ^{61}WENGEC*AQ**K**^{70}K at K69 by GSH–d_0-ONE MA (1nd trace and 1st spectrum) or GSH–d_9-ONE MA (2rd trace and 2nd spectrum).

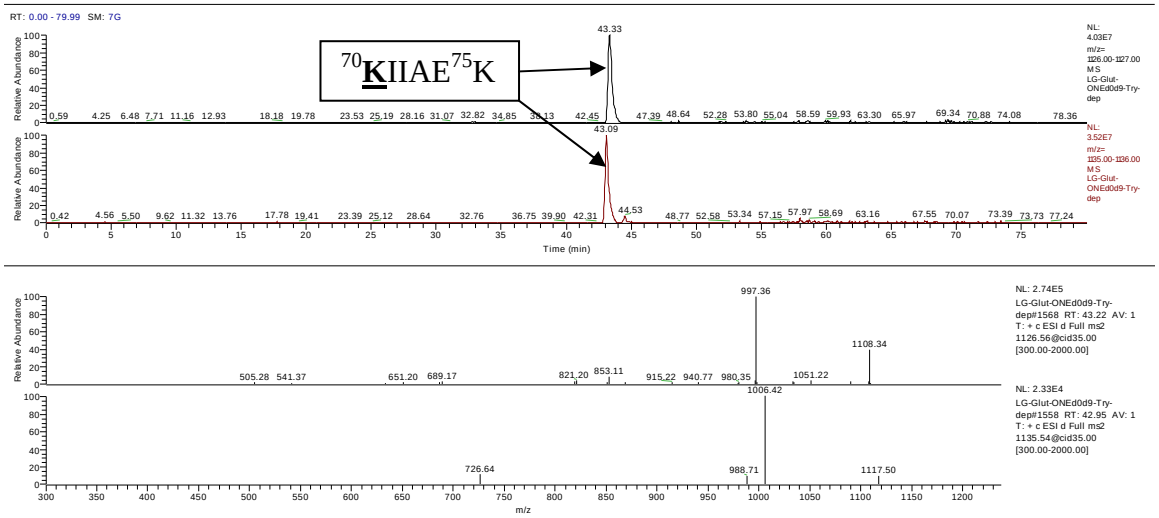

Figure S6. SIC and tandem mass spectrum of modified ^{70}**K**IIAE^{75}K at K70 by GSH–d_0-ONE MA (1nd trace and 1st spectrum) or GSH–d_9-ONE MA (2rd trace and 2nd spectrum).

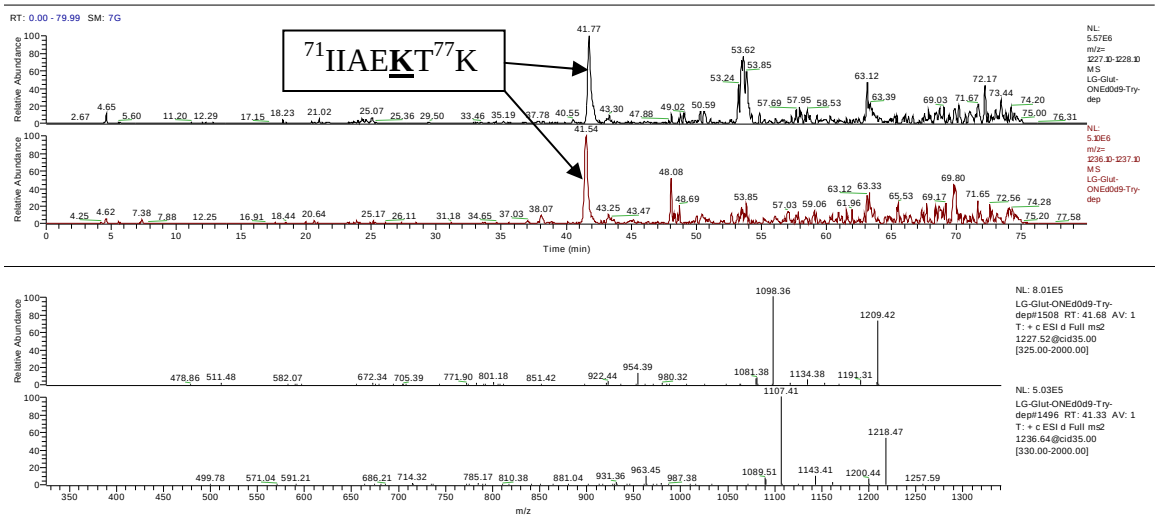

Figure S7. SIC and tandem mass spectrum of modified ^{71}IIAE**K**T^{77}K at K75 by GSH–d_0-ONE MA (1nd trace and 1st spectrum) or GSH–d_9-ONE MA (2rd trace and 2nd spectrum).

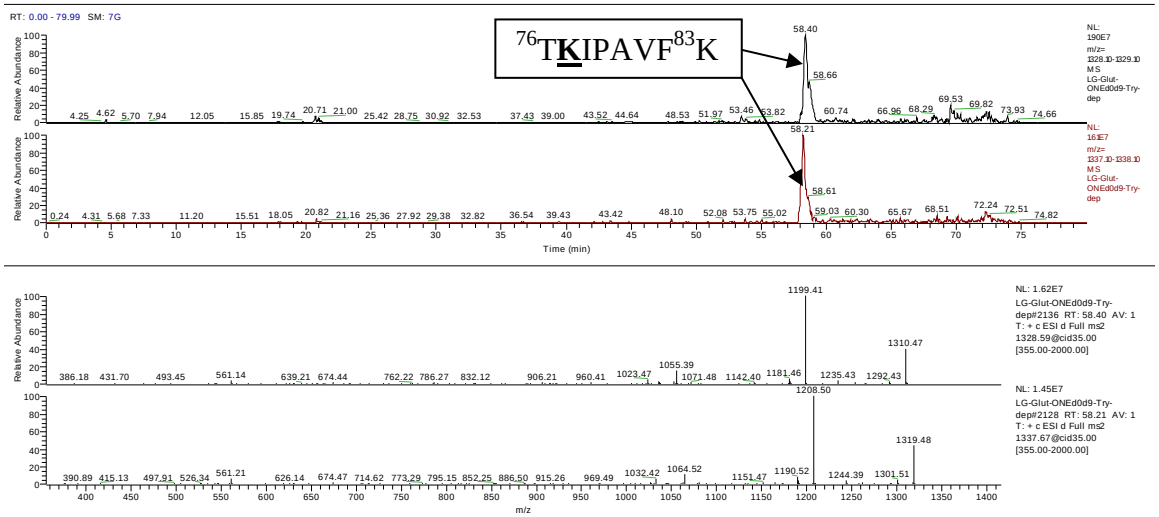

Figure S8. SIC and tandem mass spectrum of modified ^{76}T**K**IPAVF^{83}K at K77 by GSH–d_0-ONE MA (1nd trace and 1st spectrum) or GSH–d_9-ONE MA (2rd trace and 2nd spectrum).

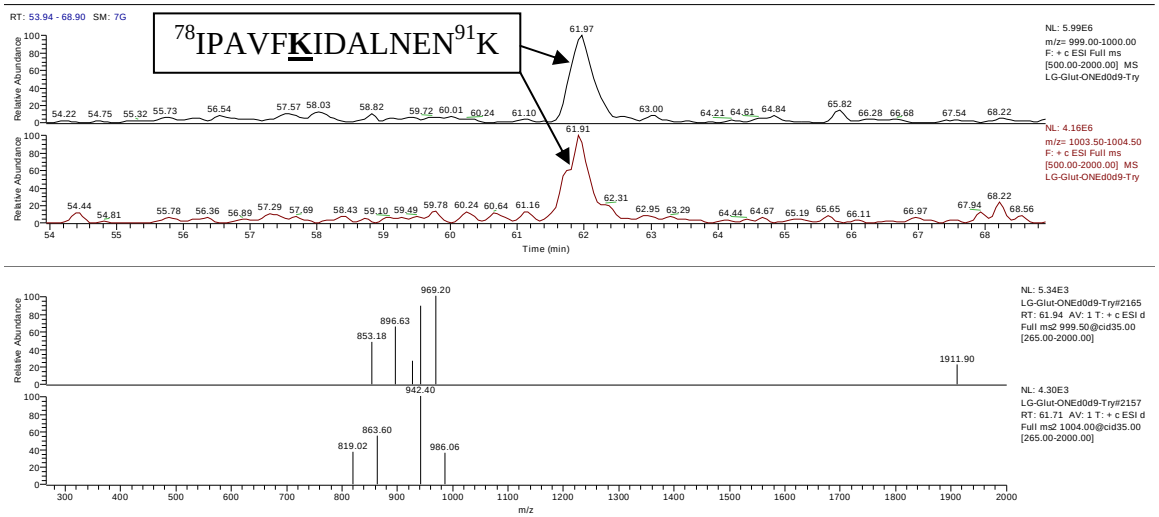

Figure S9. SIC and tandem mass spectrum of modified ^{78}IPAVF**K**IDALNEN^{91}K at K83 by GSH–d_0-ONE MA (1nd trace and 1st spectrum) or GSH–d_9-ONE MA (2rd trace and 2nd spectrum).

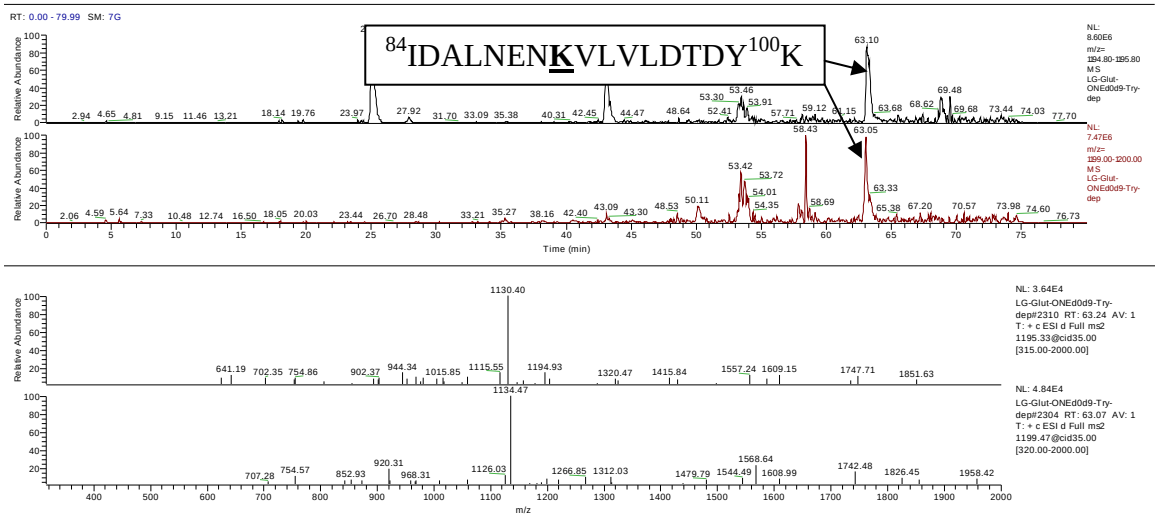

Figure S10. SIC and tandem mass spectrum of modified ^{84}IDALNEN**K**VLVLDTDY^{100}K at K91 by GSH–d_0-ONE MA (1nd trace and 1st spectrum) or GSH–d_9-ONE MA (2rd trace and 2nd spectrum).

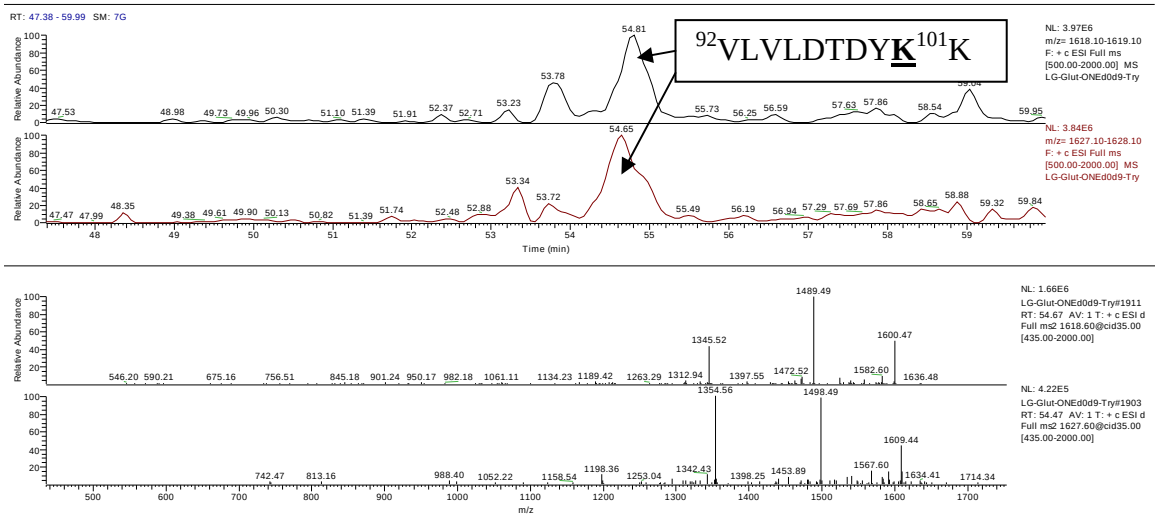

Figure S11. SIC and tandem mass spectrum of modified ^{92}VLVLDTDY**K**^{101}K at K100 by GSH–d_0-ONE MA (1nd trace and 1st spectrum) or GSH–d_9-ONE MA (2rd trace and 2nd spectrum).

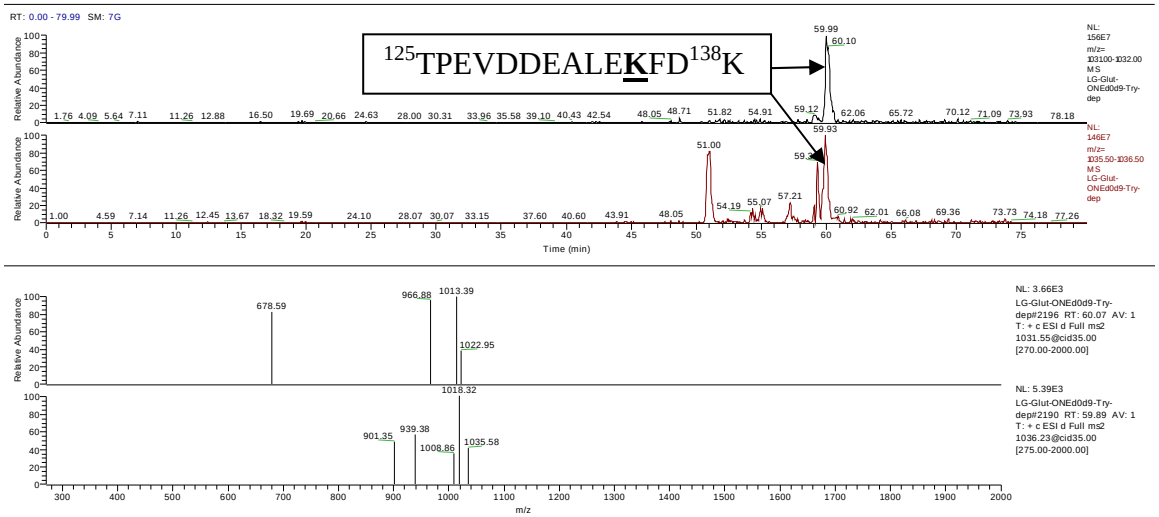

Figure S12. SIC and tandem mass spectrum of modified ^{125}TPEVDDEALE**K**FD^{138}K at K135 by GSH–d_0-ONE MA (1nd trace and 1st spectrum) or GSH–d_9-ONE MA (2rd trace and 2nd spectrum).

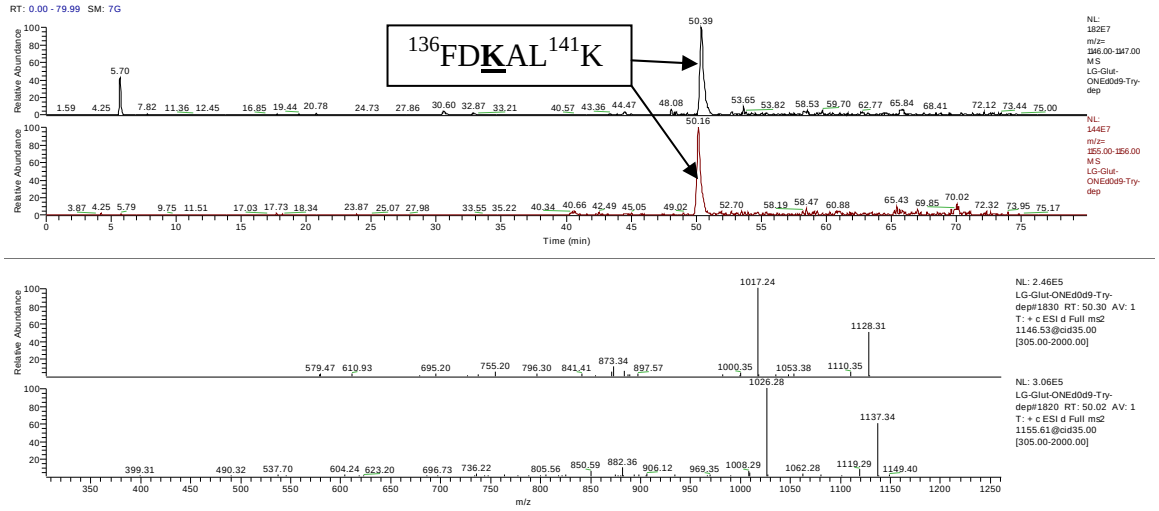

Figure S13. SIC and tandem mass spectrum of modified ^{136}FD**K**AL^{141}K at K138 by GSH–d_0-ONE MA (1nd trace and 1st spectrum) or GSH–d_9-ONE MA (2rd trace and 2nd spectrum).

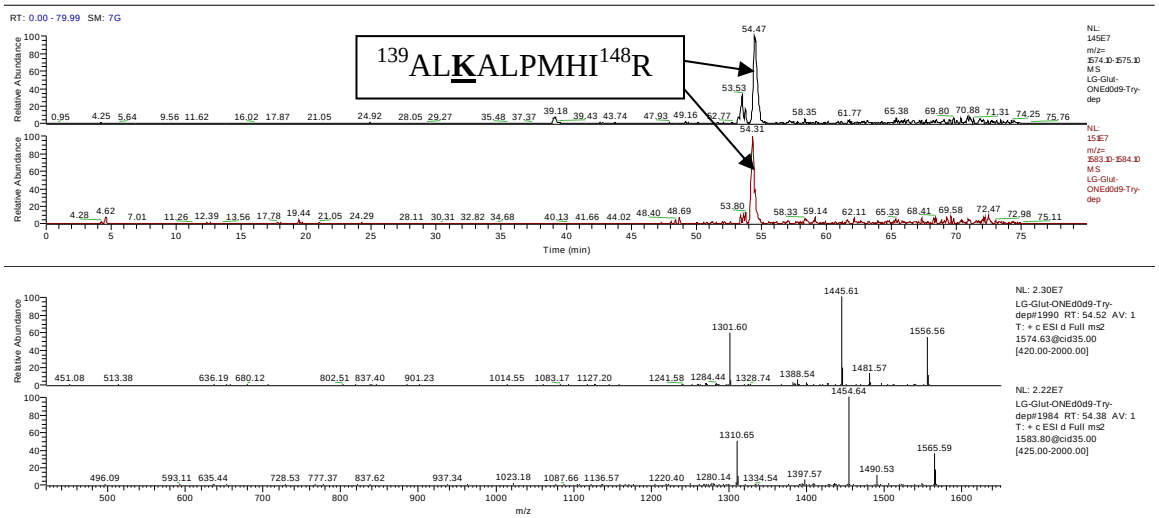

Figure S14. SIC and tandem mass spectrum of modified ^{139}AL**K**ALPMHI^{148}R at K141 by GSH–d_0-ONE MA (1nd trace and 1st spectrum) or GSH–d_9-ONE MA (2rd trace and 2nd spectrum).

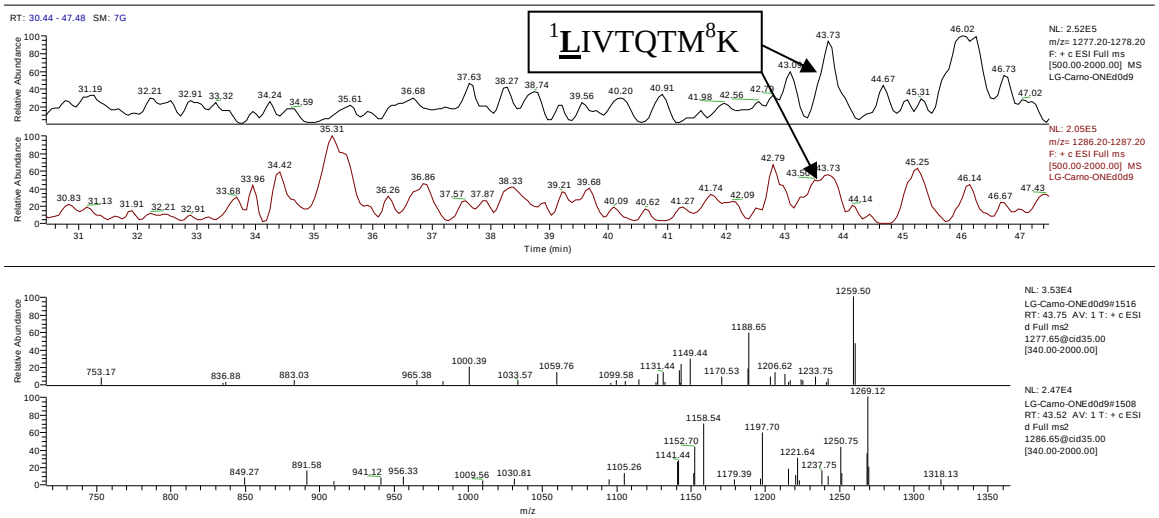

Figure S15. SIC and tandem mass spectrum of modified ^{1}**L**IVTQTM^{8}K at L1 by carnosine–d_0-ONE MA (1nd trace and 1st spectrum) or carnosine–d_9-ONE MA (2rd trace and 2nd spectrum).

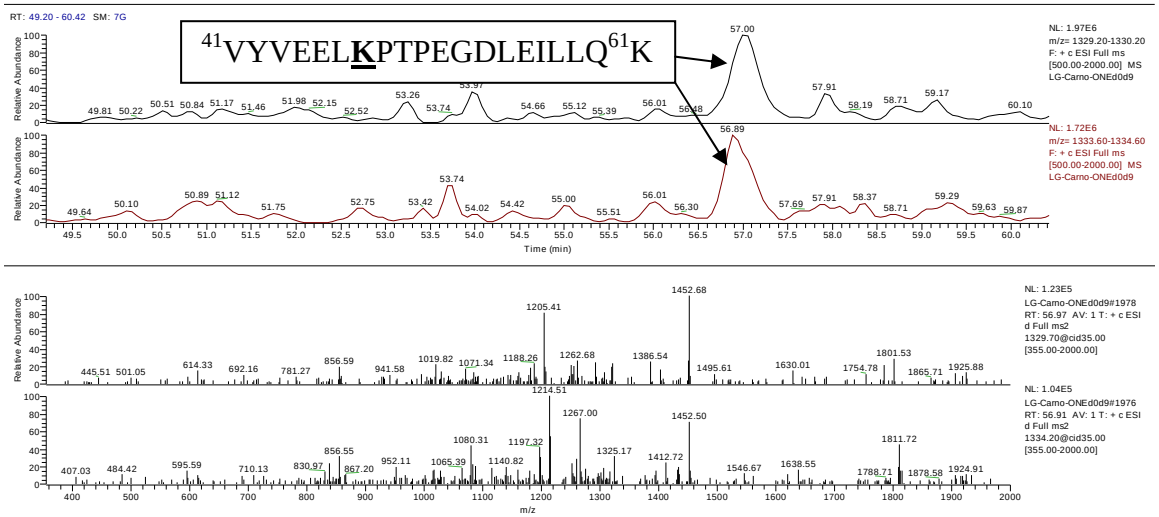

Figure S16. SIC and tandem mass spectrum of modified ^{41}VYVEEL**K**PTPEGDLEILLQ^{61}K at K47 by carnosine–d_0-ONE MA (1nd trace and 1st spectrum) or carnosine–d_9-ONE MA (2rd trace and 2nd spectrum).

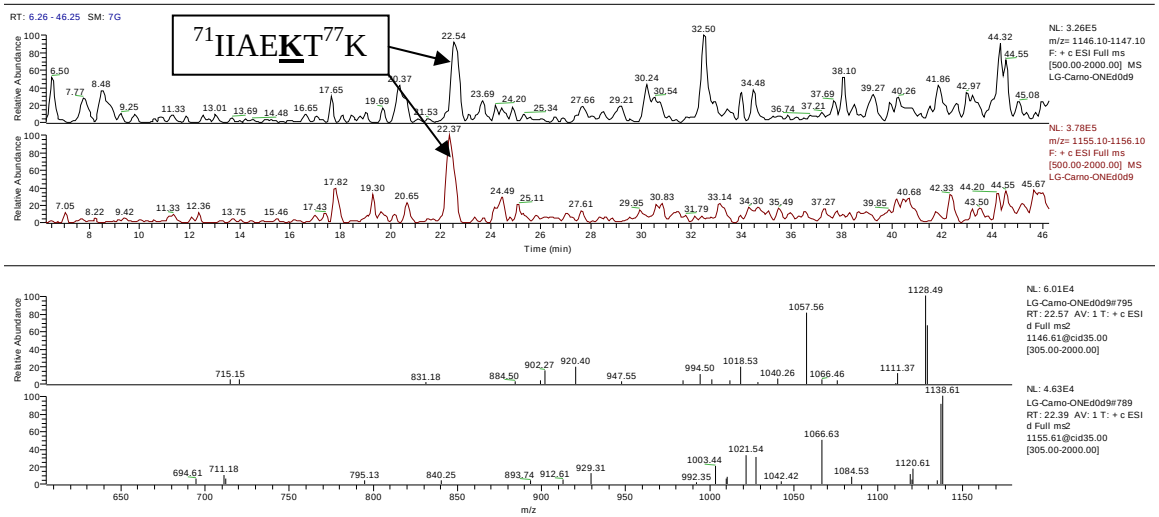

Figure S17. SIC and tandem mass spectrum of modified ^{71}IIAE**K**T^{77}K at K75 by carnosine–d_0-ONE MA (1nd trace and 1st spectrum) or carnosine–d_9-ONE MA (2rd trace and 2nd spectrum).

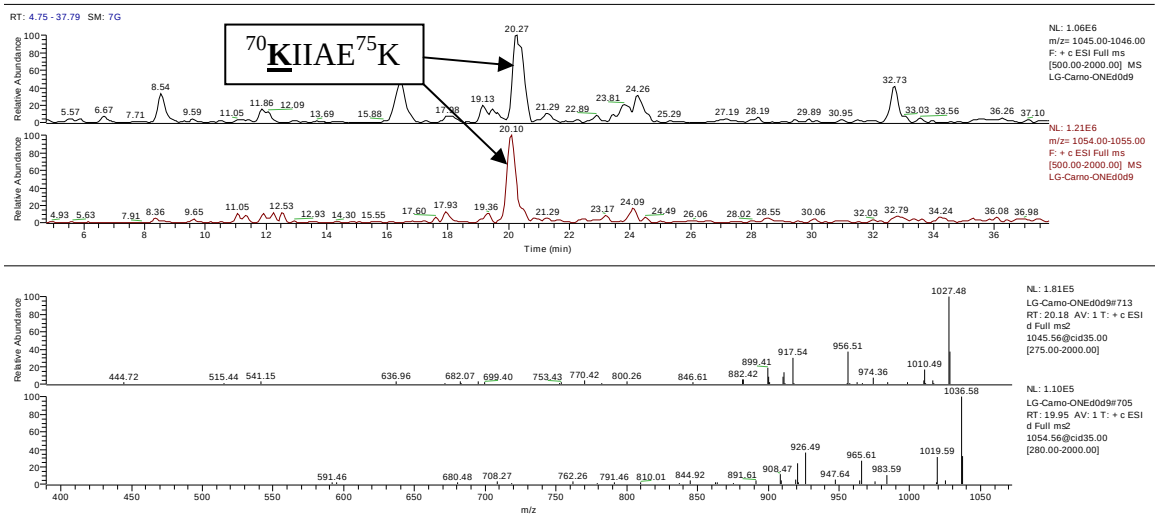

Figure S18. SIC and tandem mass spectrum of modified ^{70}**K**IIAE^{75}K at K70 by carnosine–d_0-ONE MA (1nd trace and 1st spectrum) or carnosine–d_9-ONE MA (2rd trace and 2nd spectrum).

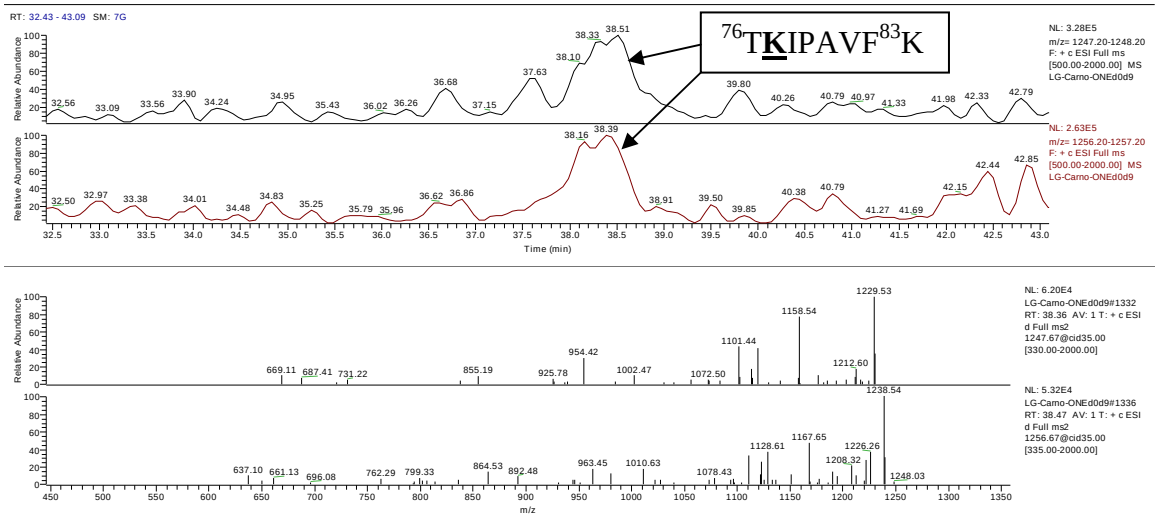

Figure S19. SIC and tandem mass spectrum of modified ^{76}T**K**IPAVF^{83}K at K77 by carnosine–d_0-ONE MA (1nd trace and 1st spectrum) or carnosine–d_9-ONE MA (2rd trace and 2nd spectrum).

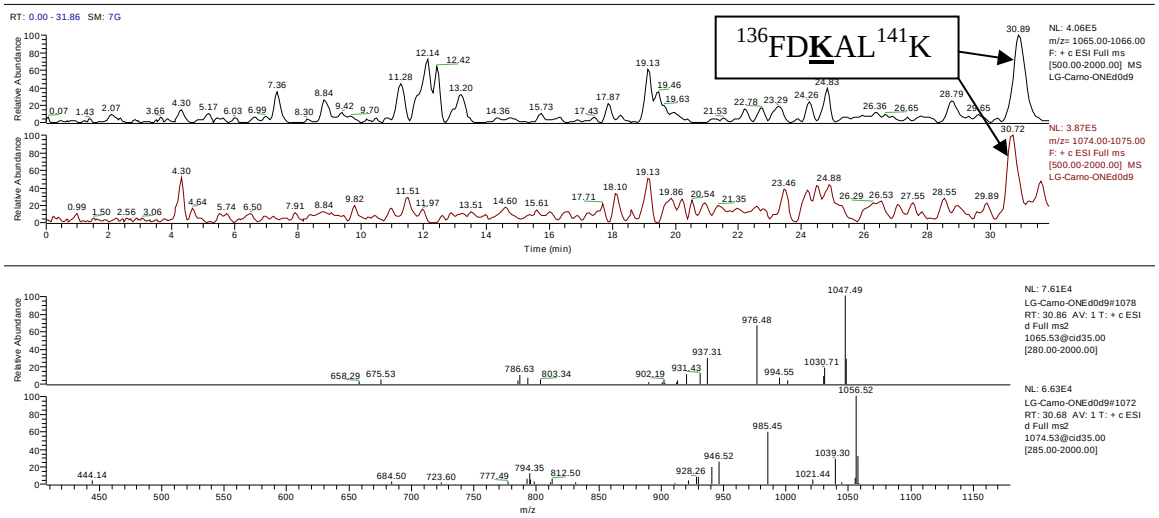

Figure S20. SIC and tandem mass spectrum of modified ^{136}FD**K**AL^{141}K at K138 by carnosine–d_0-ONE MA (1nd trace and 1st spectrum) or carnosine–d_9-ONE MA (2rd trace and 2nd spectrum).

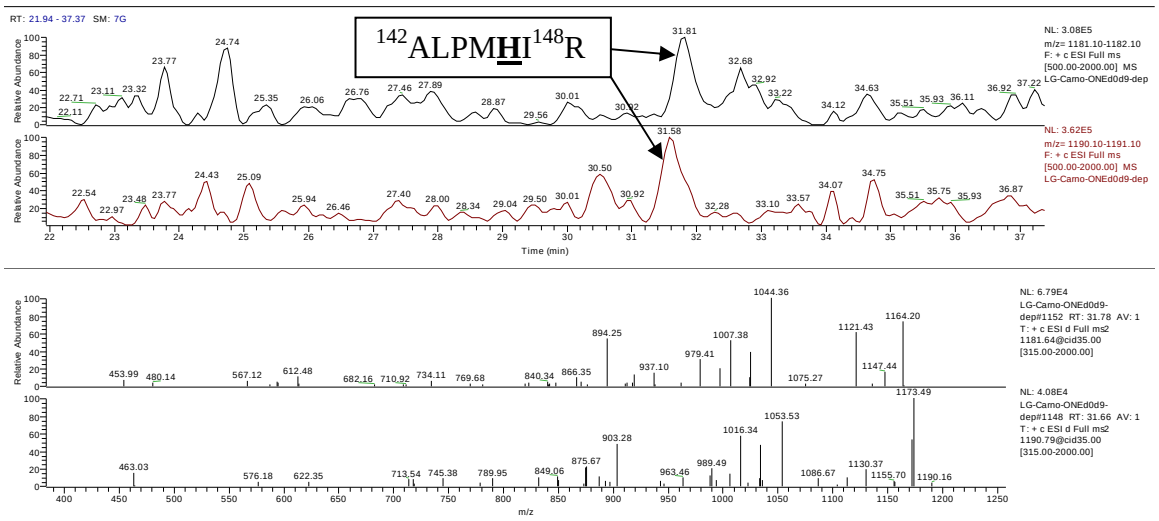

Figure S21. SIC and tandem mass spectrum of modified ^{142}ALPM**H**I^{148}R at H146 by carnosine–d_0-ONE MA (1nd trace and 1st spectrum) or carnosine–d_9-ONE MA (2rd trace and 2nd spectrum).