SUPPORTING INFORMATION

Glutathione Adduct Patterns of Michael-Acceptor Carbonyls

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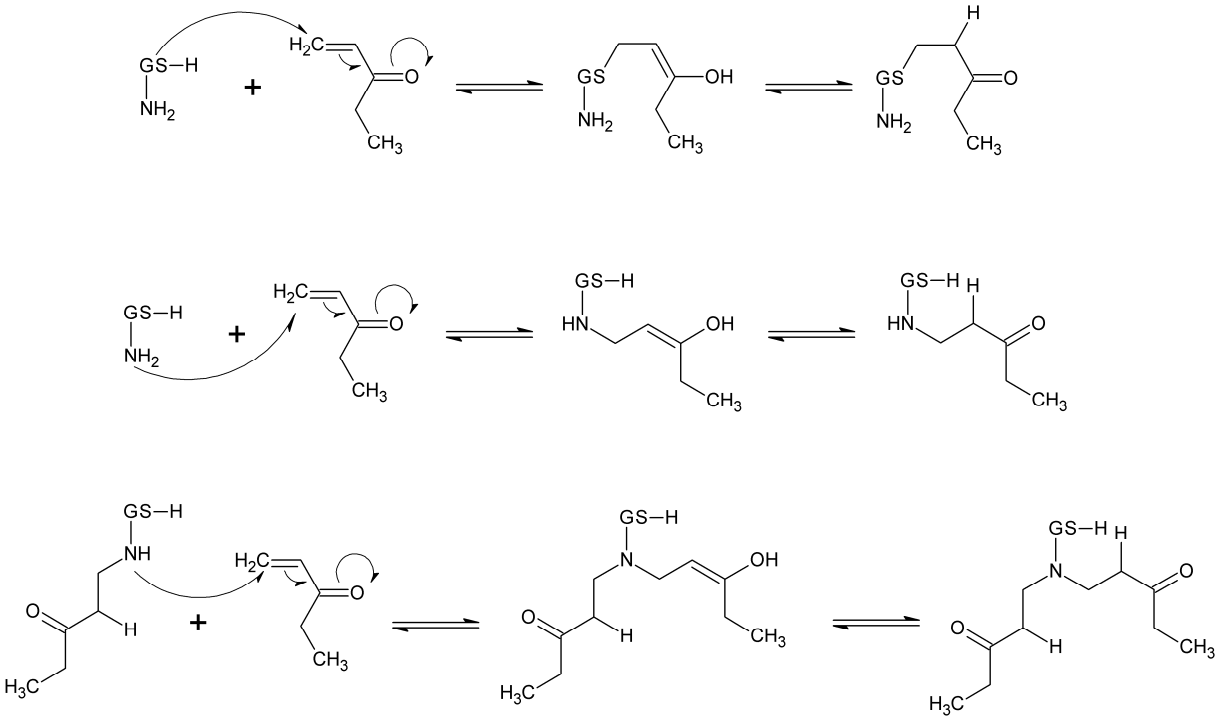
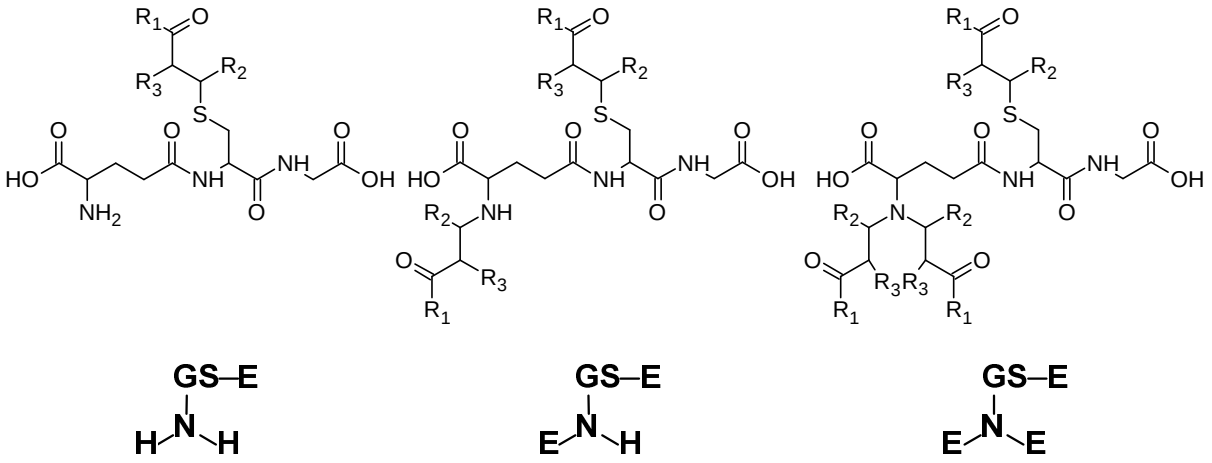
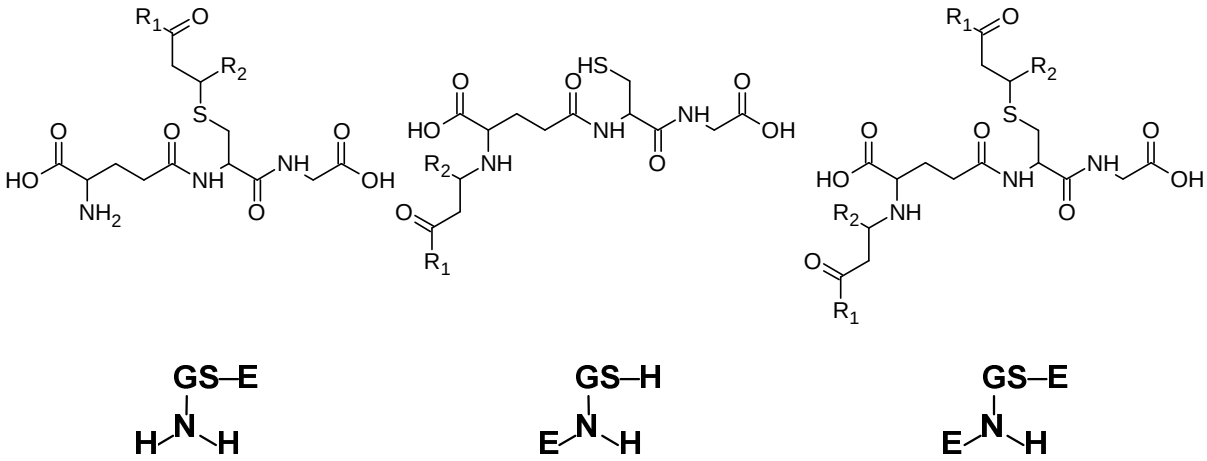
Chemicals and reagents. 1-Hexen-3-one (purity 90%), 1-octen-3-one (purity 97%), 3-penten-2-one (purity 85%), 2-octen-4-one (purity 97%), and ethyl acrolein (purity 90%) were provided by Alfa Aesar (Karlsruhe, Germany). 1-Penten-3-one (Fluka, purity 97%), 4-hexen-3-one (purity 92%), and ethyl crotonate (Fluka, purity 95%) were obtained from Sigma Aldrich (Missouri, USA). Reduced glutathione (GSH), ethyl acrylate (purity 99%), disodium hydrogen phosphate (anhydrous), potassium dihydrogen phosphate, dimethyl sulfoxide, acetonitrile (HPLC-grade), and formic acid were purchased from Merck (Darmstadt, Germany). Doubly distilled water was freshly prepared using a GFL 2102 distillation apparatus (Burgwedel, Germany).



Scheme S1. Adduct formation of GSH with α,β-unsaturated carbonyls. The reactions with the thiol group and the N-terminus of GSH are shown for 1-hexen-3-one (A2) and represent Michael additions as known organic reaction mechanism.



Scheme S2. General structures of GSH adducts formed by the reaction with α,β-unsaturated carbonyls in excess concentration. R_1 = H (aldehydes), alkyl (ketones), O-alkyl (ester), R_2 = H (terminal), CH_3 (non-terminal), R_3 = H, C_2H_5 (2-ethyl acrolein, C1), E = electrophile.



Scheme S3. Structures of the GSH adducts formed by the reaction with 1-hexen-3-one (A2) and 4-hexen-3-one (B2) with GSH being in excess. Under these conditions, the GSH-S single adduct, the GSH-N single adduct, a double adduct featuring S and N alkylation were observed, whereas no GSH triple adduct (see Scheme S1) has been detected. $R_1 = C_3H_7$, $R_2 = H$ (1-hexen-3-one, A2); $R_1 = C_2H_5$, $R_2 = CH_3$ (4-hexen-3-one, B2), E = electrophile.

Characterization of GSH adducts. Mass spectrometric analysis of GSH adducts has been done based on literature.[1-3] Figures S1-S4 and Tables S1-S4 summarize respective results for adducts formed by the reaction of GSH with 1-hexen-3-one. By analogy to the experiments with 1-hexene-3-one (main text p.10 ff), analysis of adduct formation was performed for all investigated compounds, with the electrophile being in excess to GSH (see Table S5). In addition, for 1-hexen-3-one measurements with GSH in excess has been done. The product ion mass spectra of the adduct masses ($[M+H]^+$) are shown in the figures S1-S4 with some main fragments and the important fragmentation pathway to prove adduct formation at the SH group and/or N terminus. Moreover, a more detailed description of the fragments is presented in the Tables S1-S4. The fragments given in theses tables refer to respective masses shown in Figures S1-S4.

Due to the fact, that a neutral loss from a precursor ion is the most likely fragmentation after collision induced dissociation (CID) and aiming for traceability of our results, Tables S1-S4 list possible precursor ions, neutral losses, and structures of the fragments derived from the masses shown in Figures S1-S4. Additionally, the information given by Tables S1-S4 is divided into three parts: First, fragments proving adduct formation at the SH group and/or the N terminus are described. Secondly, further fragments are given to explain all masses shown in Figures S1-S4. Finally, GSH related fragments are given to clarify that all other fragments in the table result from adducts with GSH (see Schemes S2 and S3).

Tables S7-S9 summarize masses of the fragments that have been used to confirm single, double or triple adduct formation for the reaction of GSH and the other eight Michael-acceptor carbonyls. The fragment numbers given therein shall support the understanding of adduct confirmations by referring to the fragments found for the reaction of 1-hexen-3-one and GSH, for which fragment analysis is presented exemplarily for all investigated compounds in very much detail.

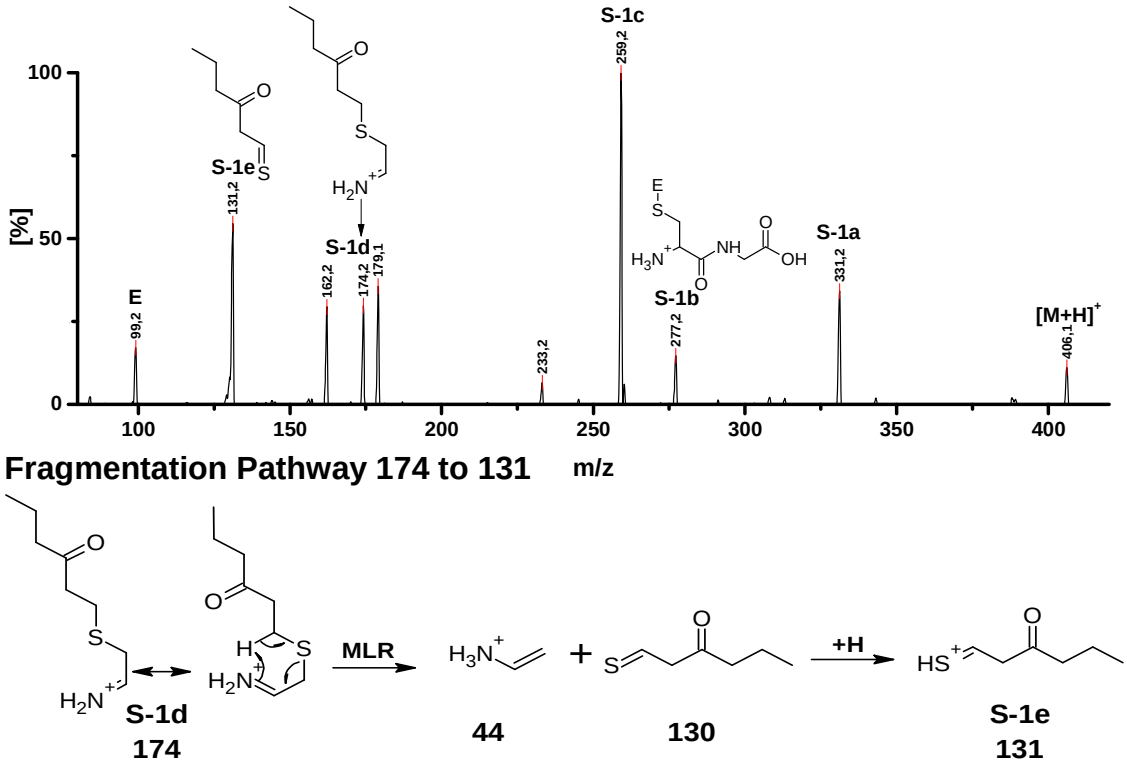
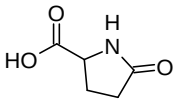
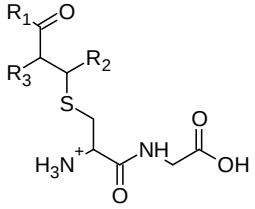
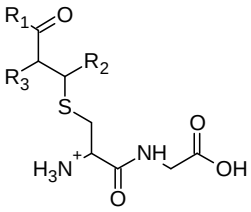
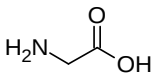
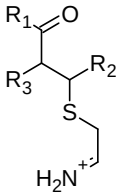
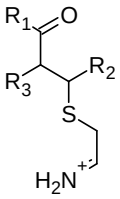
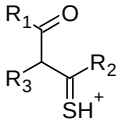
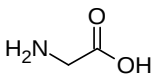
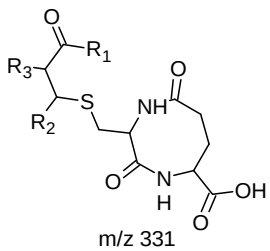
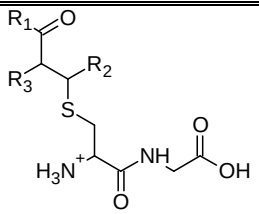
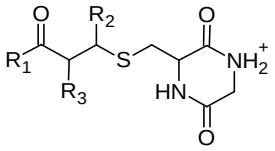
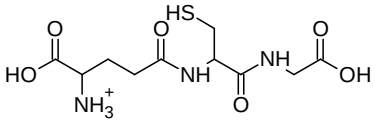
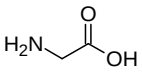
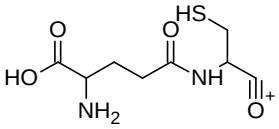
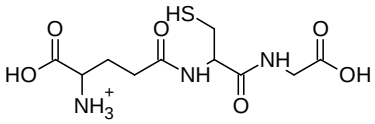
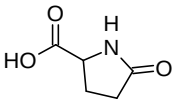
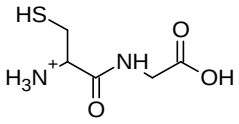
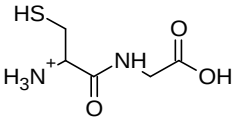
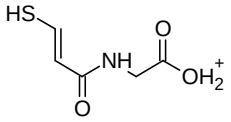
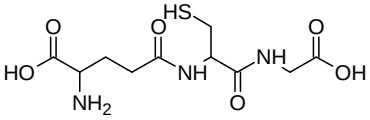
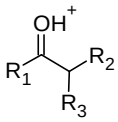


Figure S1. Product ion spectrum and three typical fragments for the single GSH-S-adduct (S-1) formed by the reaction between GSH and 1-hexen-3-one (**A2**, R_1=C_3H_7, $R_{2,3}$= H). This adduct was found in both investigated reaction mixtures (1. electrophile in excess, 2. GSH in excess). The shown fragmentation ($[M+H]^+$ 174 to 131) confirms the 1,4-addition of the GSH thiol group to the electrophile A2. E = electrophile A2 (the structure adduct is presented in Scheme S2 and S3), MLR = McLafferty rearrangement.

Table S1. Fragment structures taken from the product ion spectrum of the 1-hexen-3-one (**A2**, R_1=C_3H_7, $R_{2,3}$= H) single GSH-S-adduct (S-1, m/z 406). The table comprises the proposed precursor ions, the neutral loss, the fragment structures and the masses for the main peaks of the mass spectra (Figure S1). GSH related fragments are given to clarify that all other fragments in the table result from adducts with GSH (see Scheme S2). No. Frag. = Number of Fragment.

No. Frag.	Precursor Ion	Neutral Loss	Fragment Structure
Fragments proving adduct formation with the SH group GSH			
S-1b	$[M+H]^+$ m/z 406	 anhydroglutamic acid 129 Da	 m/z 277
S-1d	 m/z 277	 Glycin 75 Da  CO-Lost 28 Da	 m/z 174
S-1e	 m/z 174	 43 Da	 m/z 131
Further fragments shown in Figure S1			
S-1a	[M+H]+ m/z 406	 Glycin 75 Da	 m/z 331

No. Frag.	Precursor Ion	Neutral Loss	Fragment Structure
S-1c	 m/z 277	H_2O Water 18 Da	 m/z 259
	GSH-related fragments		
GSH-I	 GSH $[M+H]^+$ m/z 308	 Glycin 75 Da	 m/z 233
GSH-II	 GSH $[M+H]^+$ m/z 308	 anhydroglutamic acid 129 Da	 m/z 179
GSH-III	 m/z 179	NH_3 Ammonia 17 Da	 m/z 162
E	[M+H]+ m/z 406	 GSH $[M+H]^+$ m/z 308	 m/z 99

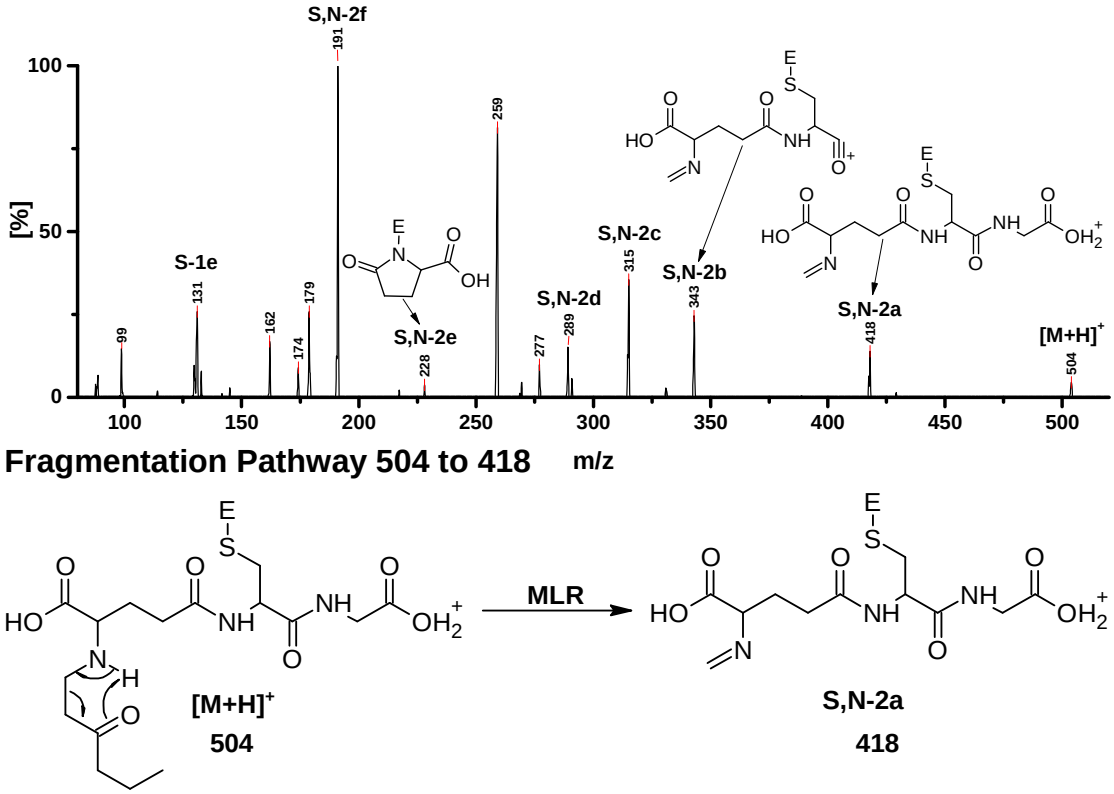
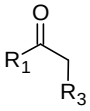
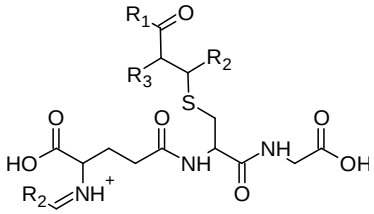
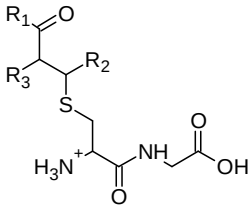
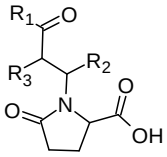
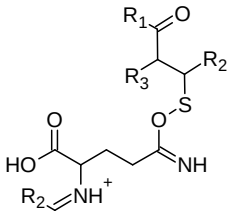
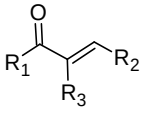
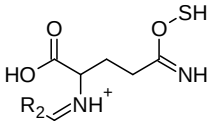
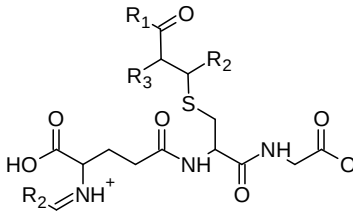
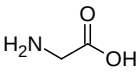
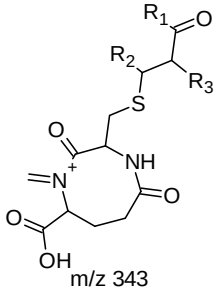
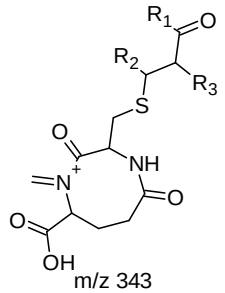
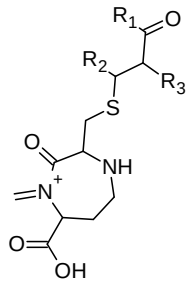
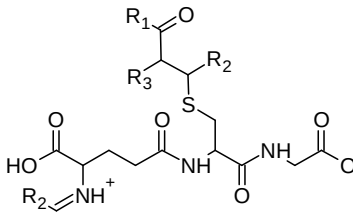
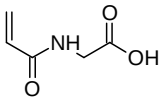
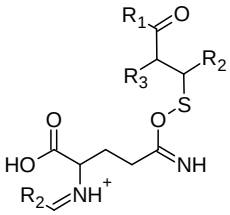
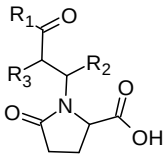
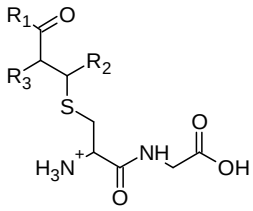
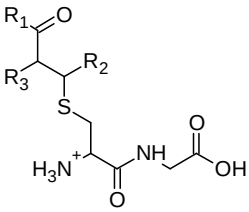
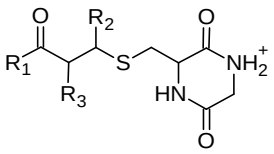
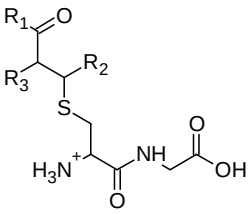
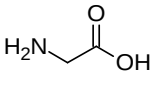
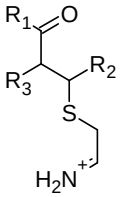
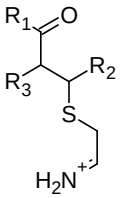
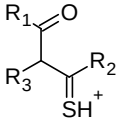
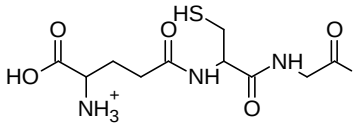
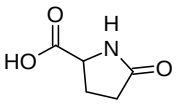
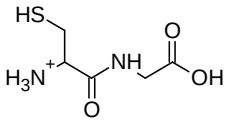
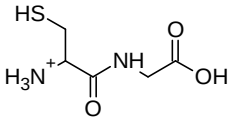
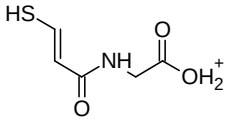
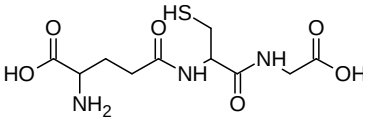
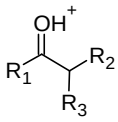


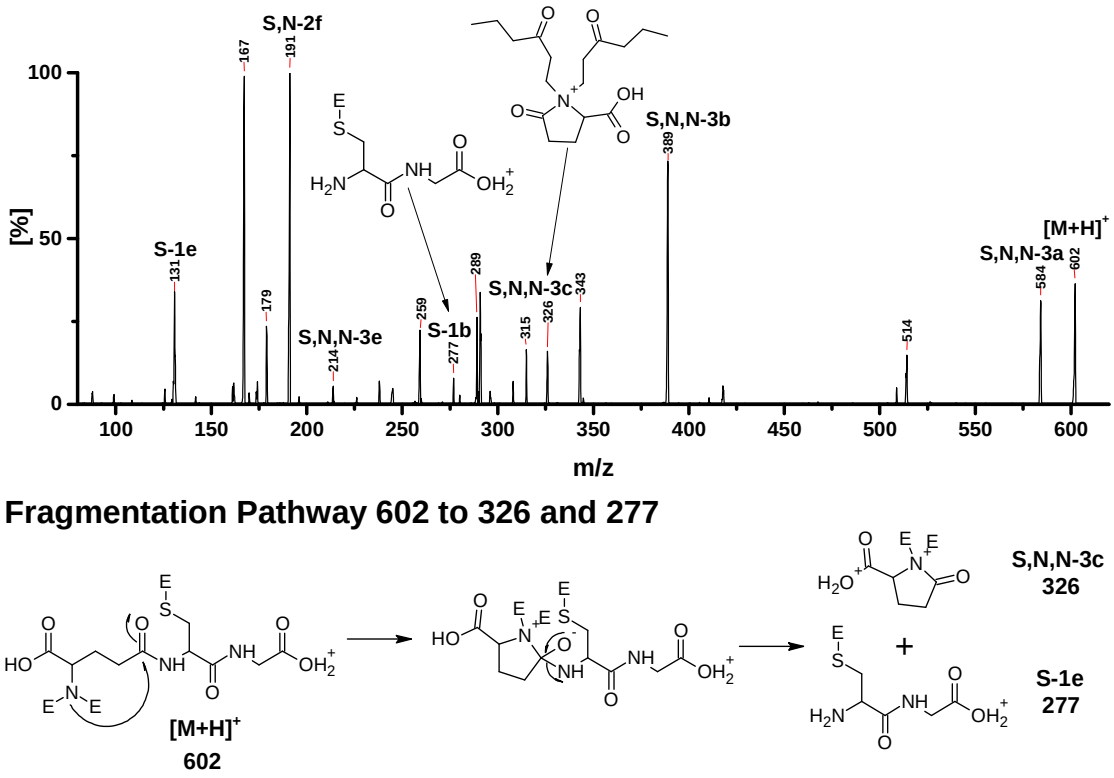
Figure S2. Product ion spectrum and three typical fragments for the double GSH adduct (single S and single N terminus adduct, S,N-2) formed by the reaction between GSH and 1-hexen-3-one (**A2**, R_1=C_3H_7, $R_{2,3}$= H). The shown fragmentation ($[M+H]^+$ 504 to 413) confirms the 1,4-addition of the free amino group of the single GSH-S-adduct to electrophile A2. E = electrophile A2 (the structure of this GSH adduct is presented in Schemes S2 and S3), MLR = McLafferty rearrangement.

Table S2. Fragment structures taken from the product ion spectrum of the 1-hexen-3-one (**A2**, R_1=C_3H_7, $R_{2,3}$= H) double GSH adduct (S,N-2, m/z 504). The table comprises the proposed precursor ions, the neutral loss, the fragment structures and the masses for the main peaks of the mass spectra (Figure S3). GSH related fragments are given to clarify that all other fragments in the table result from adducts with GSH (see Schemes S2 and S3). No. Frag. = Number of Fragment.

No. Frag.	Precursor Ion	Neutral Loss	Fragment Structure
Fragments proving adduct formation with the SH group and the N terminus of GSH			
S,N-2a	 $[M+H]^+$ m/z 504	 86 Da	 m/z 418
S,N-2e	 $[M+H]^+$ m/z 504	 276 Da	 m/z 228
S,N-2f	 m/z 289	 1-hexen-3-one 98 Da	 m/z 191
Further fragments shown in Figure S3			
S,N-2b	 m/z 418	 Glycin 75 Da	 m/z 343

No. Frag.	Precursor Ion	Neutral Loss	Fragment Structure
S,N-2c	 m/z 343	 CO 28 Da	 m/z 315
S,N-2d	 m/z 418	 129 Da	 m/z 289
S-1b	$[M+H]^+$ m/z 504	 227 Da	 m/z 277
S-1c	 m/z 277	H_2O Water 18 Da	 m/z 259
S-1d	 m/z 277	 Glycin 75 Da  CO-Lost 28 Da	 m/z 174
S-1e	 m/z 174	 43 Da	 m/z 131

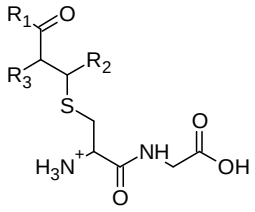
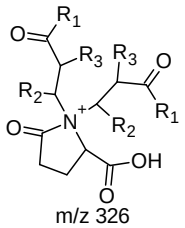
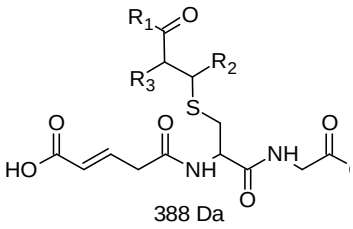
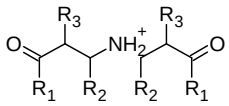
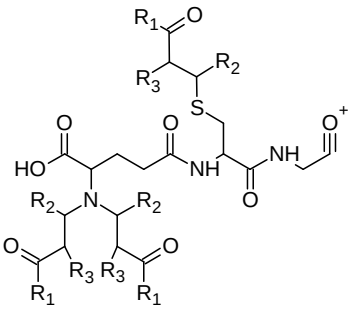
No. Frag.	Precursor Ion	Neutral Loss	Fragment Structure
	GSH-related fragments		
GSH-II	 GSH $[M+H]^+$ m/z 308	 anhydroglutamic acid 129 Da	 m/z 179
GSH-III	 m/z 179	NH_3 Ammonia 17 Da	 m/z 162
E	[M+H]+ m/z 504	 GSH $[M+H]^+$ m/z 308	 m/z 99

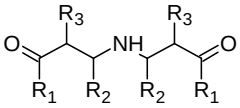
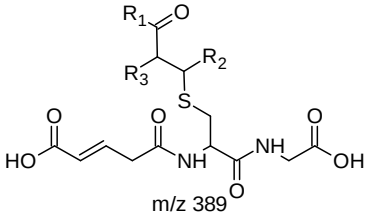
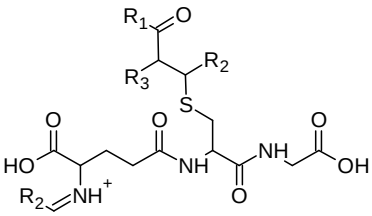
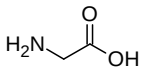
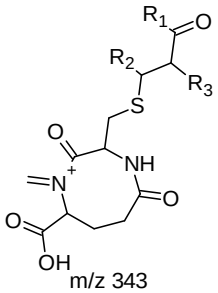
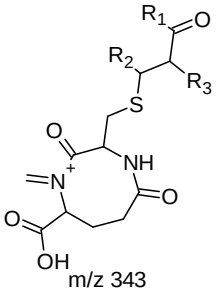
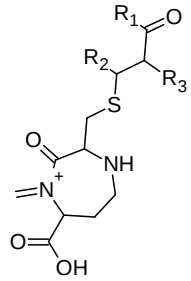
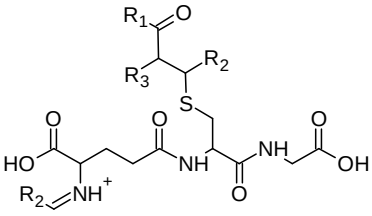
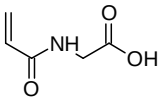
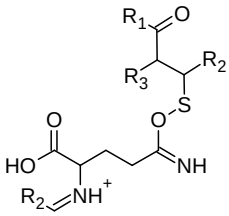


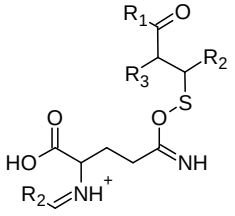
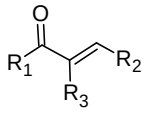
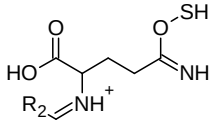
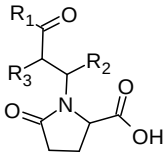
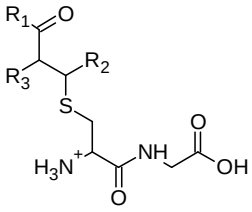
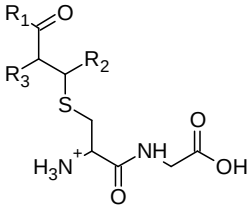
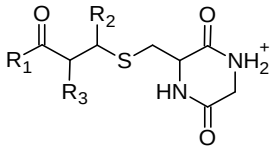
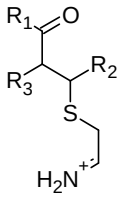
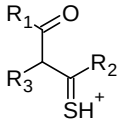
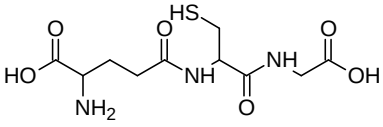
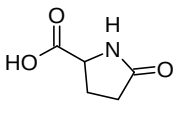
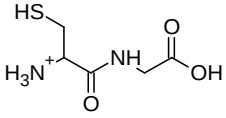
Fragmentation Pathway 602 to 326 and 277

Figure S3. Product ion spectrum and two typical fragments for the triple GSH adduct (Single S, double N terminus adduct, S,N,N-3) formed by the reaction between GSH and 1-hexen-3-one (**A2**, R_1=C_3H_7, $R_{2,3}$= H). The shown fragment 326 confirms further addition of electrophile A2 to the secondary amino group of the GSH double-adduct. E = electrophile A2 (the structure of this GSH adduct is shown in Scheme S2).

Table S3. Fragment structures taken from the product ion spectrum of the 1-hexen-3-one (**A2**, R_1=C_3H_7, $R_{2,3}$= H) triple GSH adduct (single S, double N terminus; S,N,N-3, m/z 602). The table comprises the proposed precursor ions, the neutral loss, the fragment structures and the masses for the main peaks of the mass spectra (Figure S4). GSH related fragments are given to clarify that all other fragments in the table result from adducts with GSH (see Scheme S2). No. Frag. = Number of Fragment.

No. Frag.	Precursor Ion	Neutral Loss	Fragment Structure
Fragments proving triple adduct formation with GSH (single S & double N terminus)			
S,N,N -3c	$[M+H]^+$ m/z 602	 276 Da	 m/z 326
S,N,N -3d	$[M+H]^+$ m/z 602	 388 Da	 m/z 214
Further fragments shown in Figure S4			
S,N,N -3a	$[M+H]^+$ m/z 602	H_2O Water 18 Da	 m/z 584

No. Frag.	Precursor Ion	Neutral Loss	Fragment Structure
S,N,N -3b	$[M+H]^+$ m/z 602	 213 Da	 m/z 389
S,N- 2b	 m/z 418	 Glycin 75 Da	 m/z 343
S,N- 2c	 m/z 343	 CO 28 Da	 m/z 315
S,N- 2d	 m/z 418	 129 Da	 m/z 289

No. Frag.	Precursor Ion	Neutral Loss	Fragment Structure
S,N-2f	 m/z 289	 1-hexen-3-one 98 Da	 m/z 191
S-1b	$[M+H]^+$ m/z 504	 227 Da	 m/z 277
S-1c	 m/z 277	H_2O Water 18 Da	 m/z 259
S-1e	 m/z 174	 43 Da	 m/z 131
GSH-related fragments			
GSH-II	 GSH $[M+H]^+$ m/z 308	 anhydroglutamic acid 129 Da	 m/z 179

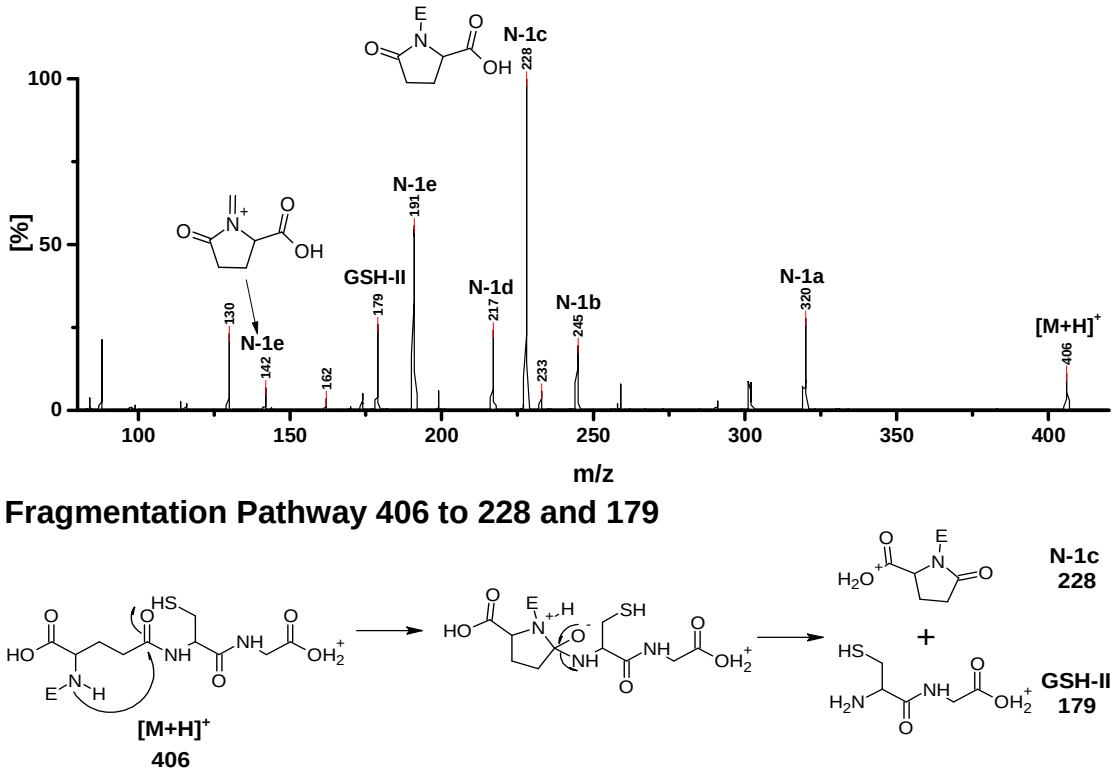
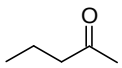
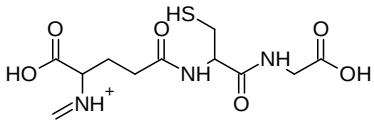
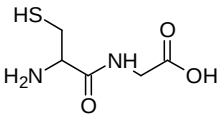
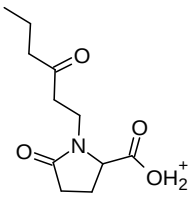
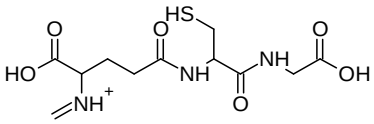
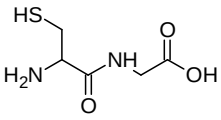
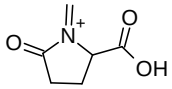
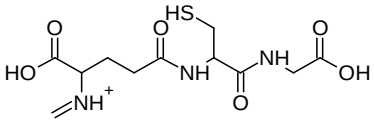
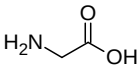
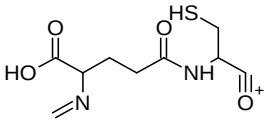
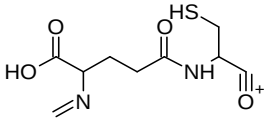
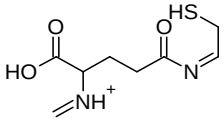
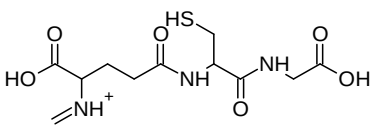
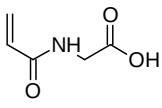
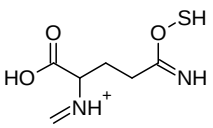
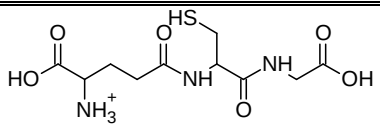
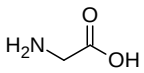
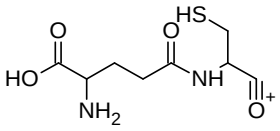
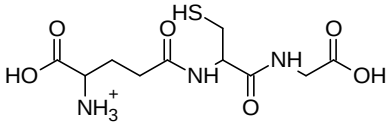
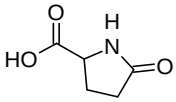
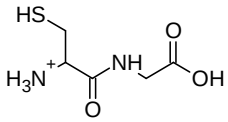
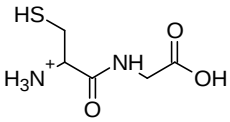
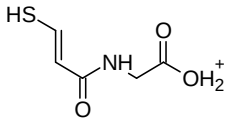
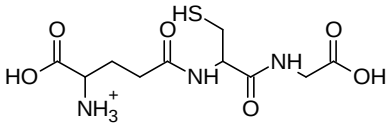
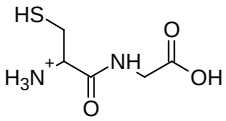
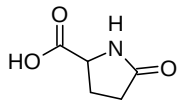


Figure S4. Product ion spectrum and five typical fragments for the single GSH-N-adduct (N-1) formed by the reaction between GSH and 1-hexen-3-one (**A2**, R_1=C_3H_7, $R_{2,3}$= H) with GSH being in excess. The shown fragmentation ([M+H]+ 406 to 228) confirms the 1,4-addition of the GSH N terminus to the electrophile A2 (see Scheme S3). E = electrophile A2.

Table S4. Fragment structures taken from the product ion spectrum of the 1-hexen-3-one (**A2**, $R_1=C_3H_7$, $R_{2,3}=H$) GSH-N-terminus single adduct (N-1, m/z 406). The table comprises the proposed precursor ions, the neutral loss, the fragment structures and the masses for the main peaks of the mass spectra (Figure S2). GSH related fragments are given to clarify that all other fragments in the table result from adducts with GSH (see Scheme S3). No. Frag. = Number of Fragment.

No. Frag.	Precursor Ion	Neutral Loss	Fragment Structure
Fragments proving adduct formation with the N terminus of GSH			
N-1a	$[M+H]^+$ m/z 406	 86 Da	 m/z 320
N-1c	$[M+H]^+$ m/z 406	 178 Da	 m/z 228
N-1f	 m/z 320	 178 Da	 m/z 142
Further fragments shown in Figure S2			
N-1b	 m/z 320	 Glycin 75 Da	 m/z 245
N-1d	 m/z 245	 CO 28 Da	 m/z 217
N-1e	 m/z 320	 129 Da	 m/z 191
GSH-related fragments			

No. Frag.	Precursor Ion	Neutral Loss	Fragment Structure
GSH-I	 GSH $[M+H]^+$ m/z 308	 Glycin 75 Da	 m/z 233
GSH-II	 GSH $[M+H]^+$ m/z 308	 anhydroglutamic acid 129 Da	 m/z 179
GSH-III	 m/z 179	NH_3 Ammonia 17 Da	 m/z 162
GSH-II	 GSH $[M+H]^+$ m/z 308	 178 Da	 anhydroglutamic acid m/z 130

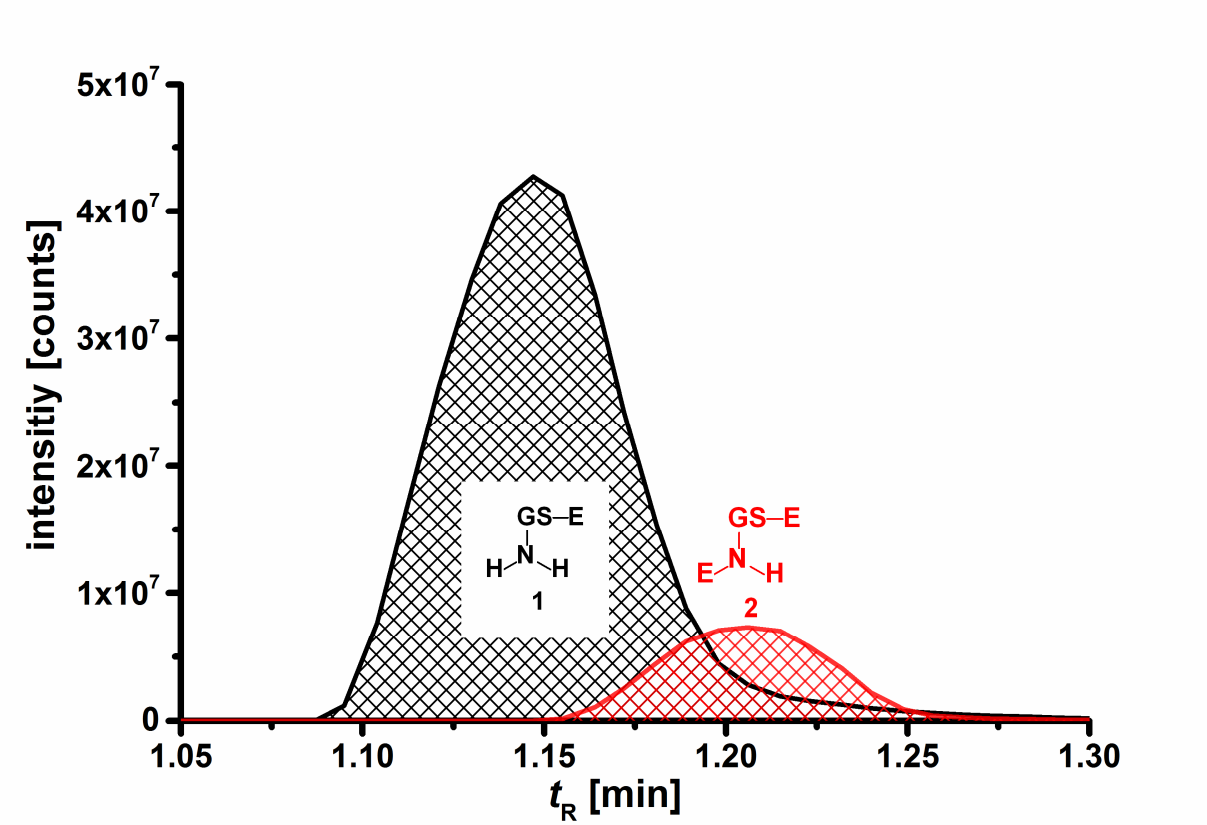


Figure S5. Extracted and overlapped ion chromatograms of two GSH adducts formed by the reaction between GSH and 4-hexen-3-one (B2). In contrast to its structural isomer 1-hexen-3-one (A2) no triple adduct was detected. The data were obtained after 24 h with an electrophile-to-GSH concentration ratio of 100:1. Peak 1 (black) = single adduct, m/z 406, Peak 2 (red) = double adduct, m/z 504, E = electrophile B2 (the general structure of the GSH adducts is shown in Scheme S2).

Table S5. GSH adducts formed by the reaction of GSH with the investigated α,β-unsaturated carbonyls in excess (electrophile/nucleophile concentration ratio $R_{E/Nu}$ = 100). m/z = [M+H]+ of adducts, t_R = retention times of adducts, n.d. = not detectable, n.a. = not available.

Electrophile	m/z $_S$	m/z $_{S,N}$	m/z $_{S,N,N}$	$t_{R,S}$ [min]	$t_{R,S,N}$ [min]	$t_{R,S,N,N}$ [min]
1-penten-3-one (A1)	392	476	560	1.10	1.15	1.20
1-hexen-3-one (A2)	406	504	602	1.16	1.25	1.32
1-octen-3-one (A3)	434	560	686	1.26	1.39	1.54
3-penten-2-one (B1)	392	476	n.d.	1.09	1.13	n.a.
4-hexen-3-one (B2)	406	504	n.d.	1.15	1.21	n.a.
2-octen-4-one (B3)	434	560	n.d.	1.25	1.37	n.a.
2-ethyl acrolein (C1)	392	476	560	1.19	1.25	1.28
ethyl acrylate (C2)	408	508	608	1.14	1.21	1.29
ethyl crotonate (C3)	422	536	n.d.	1.18	1.24	n.a.

Table S6. GSH adducts formed by the reactions of 1-hexen-3-one (A2) and 4-hexen-3-one (B2) with GSH in excess (electrophile/nucleophile concentration ratio $R_{E/Nu}$ = 0.01). m/z = [M+H]+ of adducts, t_R = retention times of adducts.

Electrophile	m/z $_N$	m/z $_S$	m/z $_{S,N}$	$t_{R,N}$ [min]	$t_{R,S}$ [min]	$t_{R,S,N}$ [min]
1-hexen-3-one (A2)	406	406	504	1.35	1.48	1.74
4-hexen-3-one (B2)	406	406	504	1.27	1.43	1.63

Table S7. Summary of the structure analysis of single GSH-S-adducts formed by the reaction of GSH with the investigated α,β-unsaturated carbonyls in excess (electrophile/nucleophile concentration ratio RE/Nu = 100). The fragments proving adduct formation with the SH group GSH (see Table S1) are used to confirm the proposed adduct structure for all other electrophiles. $[M+H]^+$ = mass of protonated adduct, R_{1-3} = specific structure elements of electrophiles.

Electrophile	R_1	R_2	R_3	$[M+H]^+$	Fragment S-1b	Fragment S-1d	Fragment S-1e
1-penten-3-one (A1)	C_2H_5	H	H	392	263	160	117
1-hexen-3-one (A2)	C_3H_7	H	H	406	277	174	131
1-octen-3-one (A3)	C_5H_{11}	H	H	434	305	202	159
3-penten-2-one (B1)	CH_3	CH_3	H	392	263	160	117
4-hexen-3-one (B2)	C_2H_5	CH_3	H	406	277	174	131
2-octen-4-one (B3)	C_4H_9	CH_3	H	434	305	202	159
2-ethyl acrolein (C1)	H	H	C_2H_5	392	263	160	117
ethyl acrylate (C2)	OC_2H_5	H	H	408	279	176	133
ethyl crotonate (C3)	OC_2H_5	CH_3	H	422	293	190	147

Table S8. Summary of the structure analysis of double GSH-S,N-adducts formed by the reaction of GSH with the investigated α,β-unsaturated carbonyls in excess (electrophile/nucleophile concentration ratio RE/Nu = 100). The fragments proving adduct formation with the SH group and the N terminus of GSH (see Table S2) are used to confirm the proposed adduct structure. $[M+H]^+$ = mass of protonated adduct, R_{1-3} = specific structure elements of electrophiles.

Electrophile	R_1	R_2	R_3	$[M+H]^+$	Fragment S,N-2a	Fragment S,N-2e	Fragment S,N-2f
1-penten-3-one (A1)	C_2H_5	H	H	476	404	214	191
1-hexen-3-one (A2)	C_3H_7	H	H	504	418	228	191
1-octen-3-one (A3)	C_5H_{11}	H	H	560	446	256	191
3-penten-2-one (B1)	CH_3	CH_3	H	476	418	214	205
4-hexen-3-one (B2)	C_2H_5	CH_3	H	504	432	228	205
2-octen-4-one (B3)	C_4H_9	CH_3	H	560	460	256	205
2-ethyl acrolein (C1)	H	H	C_2H_5	476	-	214	191
ethyl acrylate (C2)	OC_2H_5	H	H	508	420	230	-
ethyl crotonate (C3)	OC_2H_5	CH_3	H	536	448	244	-

Table S9. Summary of the structure analysis of triple GSH-S,N,N-adducts formed by the reaction of GSH with the investigated α,β-unsaturated carbonyls in excess (electrophile/nucleophile concentration ratio RE/Nu = 100). The fragments proving triple adduct formation with GSH (single S & double N terminus; see Table S3) are used to confirm the proposed adduct structure. $[M+H]^+$ = mass of protonated adduct, R_{1-3} = specific structure elements of electrophiles.

Electrophile	R_1	R_2	R_3	$[M+H]^+$	Fragment S,N,N-3c	Fragment S,N,N-3d
1-penten-3-one (A1)	C_2H_5	H	H	560	298	186
1-hexen-3-one (A2)	C_3H_7	H	H	602	326	214
1-octen-3-one (A3)	C_5H_{11}	H	H	686	382	270
2-ethyl acrolein (C1)	H	H	C_2H_5	560	298	186
ethyl acrylate (C2)	OC_2H_5	H	H	608	320	218

Table S10. Summary of the structure analysis of single GSH-N-adducts formed by the reactions of 1-hexen-3-one (A2) and 4-hexen-3-one (B2) with GSH in excess (electrophile/nucleophile concentration ratio RE/Nu = 0.01). The fragments proving adduct formation with the N terminus of GSH (see Table S4) are used to validate the proposed adduct structure. $[M+H]^+$= mass of protonated adduct, R_{1-3} = specific structure elements of electrophiles.

Electrophile	R_1	R_2	R_3	$[M+H]^+$	Fragment N-1a	Fragment N-1b	Fragment N-1f
1-hexen-3-one (A2)	C_3H_7	H	H	406	320	228	142
4-hexen-3-one (B2)	C_2H_5	CH_3	H	406	320	228	142

Table S11. Calculated molecular geometries in terms of Cartesian coordinates (x, y, z) in Å of the cyclic model products **P1**-**P7** with reactants BDA (2-butene-1,4-dial), methyl sulfide (CH_3SH), and methylamine (CH_3NH_2) (Scheme 2), and of the non-cyclic model products **P8**-**P14** with reactants acrolein (CH_2=CHCHO) and H_2O (Scheme 3) used for the quantum chemical thermodynamic analysis, employing B3LYP/def2-tzvpp as implemented in Orca for the ground-state geometry optimizations.

P1

C	-1.76514968778138	1.21863181202543	0.43764864584022
C	-0.49597389835174	1.85088686716664	0.22962129349076
C	-1.56983259967952	-0.14581003462634	0.30247642034168
C	0.43072424136671	0.84388874724568	-0.02591775618813
N	-0.23806433843183	-0.37250022358403	0.02137036588606
C	0.38064657517121	-1.67400794808671	-0.19362107605817
H	-0.38277457114129	-2.45047996172675	-0.09656910167288
H	1.16717947293756	-1.86061027423944	0.54883243145645
H	0.81974299839054	-1.73728461245845	-1.19755303221342
S	-0.24913069281285	3.59909711678453	0.30395322327215
C	1.53921958243349	3.71384171468993	-0.04601022832509
H	1.79049717351731	4.77803072286637	-0.02150608111268
H	1.77025362109331	3.31054926588690	-1.03799825007879
H	2.12187149493485	3.18714269968036	0.71763995273646
H	1.48967671543201	0.88560991219738	-0.23371080845730
H	-2.70210300604031	1.71254061963593	0.65895452317826
H	-2.26648308103806	-0.96956642345742	0.38295947790442

P2

C	-1.97273418258591	1.07463888634187	0.47266611725903
C	-0.72672814333858	1.74450033265835	0.26997192250336
C	-1.74963880429567	-0.28185355699790	0.33155804116083
C	0.22788651131753	0.76445498588309	0.00873610326061
N	-0.40213968890593	-0.47135758140105	0.04755441615650
C	0.24829345227368	-1.75650281001513	-0.17403772345739
H	-0.49871806105276	-2.54844270800951	-0.08021271903057
H	1.04041683020928	-1.92610459289514	0.56627675975416
H	0.69044047120341	-1.80263541964662	-1.17737064079542
S	1.96075531420067	0.84557956766972	-0.33353103344028
H	-2.92340844422358	1.54113203821228	0.69670146180261
H	-2.41676428396558	-1.12968256046960	0.40531929570773
H	-0.55284743963655	2.81037342493705	0.31063101835813
C	2.20119488203474	2.65296267252111	-0.24933371191798
H	3.26206668231033	2.82664832548827	-0.44946632725464
H	1.94860305244176	3.02946976394049	0.74657690291024
H	1.59651185201315	3.15815923178272	-1.00862988297695

P3

C	-1.76905367738368	1.93877893998624	1.53627020369470
C	-0.63847514499723	2.74931354353831	0.94601201913998
C	-1.49520653808060	0.50686439772324	1.03028656117034
C	0.14349062230516	1.92190864489836	0.22841478834257
N	-0.33570675861397	0.59324737327610	0.25900993548028
C	0.25966676448441	-0.56023239341099	-0.40409327828438
H	-0.44991458349028	-1.00544715680819	-1.11173111639156
H	0.53346281518288	-1.32696259988686	0.33050321279164
H	1.15518852423495	-0.24031602071066	-0.94338230226574
O	-2.12986893620413	-0.52111414711320	1.23376231016201
H	-2.76529601415725	2.25988043468602	1.20262202783757
H	-1.78869579043405	1.94058369776669	2.63469558162354
H	-0.48550728796651	3.81234045115403	1.07864439890197
H	1.04276600512030	2.14401483490089	-0.33527434220291

P4

C	-1.70282568948686	1.94455427257093	1.49857833992621
C	-0.73715848166928	2.73990967691381	1.01456404637368
C	-1.47924668283818	0.53880865505332	1.03115147935384
C	0.22357164511929	1.93176034962755	0.17544637343525
N	-0.32962775237525	0.58772573057410	0.25400032598412
C	0.23507820346489	-0.58604859274683	-0.39515368061358
H	-0.48505049085753	-1.03047934872259	-1.09416535931641
H	0.50202181544946	-1.35150674724686	0.34481618448826
H	1.13368761218858	-0.29421667399319	-0.94646377635990
H	1.25080008002471	1.97648568238866	0.57559018776146
H	0.26405365066193	2.29847782157199	-0.86435617060342
O	-2.14351877699270	-0.47112577107641	1.26068175931606
H	-2.53927000047903	2.21541552360280	2.13237905154232
H	-0.61668513221003	3.80712942148273	1.17056123871212

P5

C	-1.92755142073762	1.83309381532201	1.55978559013274
C	-0.67814208702292	2.72052429591307	1.40655027646783
C	-1.48539423544261	0.45526485221468	1.04130474293074
C	0.12901482306067	2.03037694392965	0.27432292811521
N	-0.29478112806986	0.63326525056209	0.36727197982140
C	0.35520374820666	-0.42561792802919	-0.38672106940444
H	0.30188133765090	-0.23202142204429	-1.46852230559914
H	-0.16268477747102	-1.36258795158896	-0.16414344247994
H	1.41179109561479	-0.51304545926027	-0.10001384321401
S	-1.13085250395504	4.46603972889044	1.08699216777350
C	0.53259596293195	5.23574456080638	1.08895710223541
H	1.14261291021537	4.87964178155242	0.25260967209014
H	1.04755105136137	5.04365844879035	2.03680343871448
H	0.37921494050514	6.31355399420920	0.98062436061953
H	1.21333853189611	2.11758380670630	0.42077284644436
H	-0.12132420449532	2.46080677503507	-0.70898046818755

O	-2.08201996356598	-0.60748024365585	1.17865314319142
H	-2.75460451453689	2.18103917283524	0.92468535550169
H	-2.29915275673986	1.76634701691334	2.58501083956120
H	-0.09140680940584	2.68770256089833	2.33261668528542

P6

C	-1.51254518946843	-0.49594212563161	2.30420114855502
C	-0.82478125990684	0.83646431581346	2.29208297883102
C	-1.49982280668800	-0.99353316180435	1.04722914194090
C	-0.09252093571551	0.82064438998537	0.92736650198681
N	-0.82167550593088	-0.18601828519014	0.11266741058361
C	-0.01975828251313	-0.81282238382966	-0.93966399266607
H	-0.61338553759901	-1.53844655085961	-1.50673262193982
H	0.87292798554530	-1.32950489545393	-0.53946083350819
H	0.30796721593178	-0.04036953838808	-1.64332079650678
S	-2.00972072632553	2.30559572845479	2.31131482456247
C	-2.73237383272902	2.06983438287156	3.97474757610609
H	-3.41598208796727	2.90658469519830	4.14743596913625
H	-1.95360660639991	2.08272999886786	4.74639686185993
H	-3.29714429598904	1.13340623724419	4.03108434780529
H	0.94780960503039	0.48251540102282	1.07656696517653
H	-0.07507650045888	1.79320114932316	0.42445342111498
O	-2.10377791230969	-2.14762040631790	0.63445326235374
H	-2.24694975607657	-2.10883335133581	-0.32662375960239
H	-0.13387211942661	1.01033588745075	3.12348913116355
H	-2.09515145100316	-0.89512148742119	3.12311246304707

P7

C	-2.09943019187892	2.82264712615538	0.61296276028632
C	-0.69465180322981	2.75533533794829	0.32926559723842
C	-2.58175483997587	1.52139343020040	0.61698174691015
C	-0.37003641767693	1.40624976743753	0.16963398688565
N	-1.54010818652853	0.66482995711234	0.34972512401995
C	-1.62326785227006	-0.78341223236650	0.26417740783216
H	-2.65600328810647	-1.09277886410162	0.44787729788027
H	-0.97631467536572	-1.25993707836910	1.01348245308046
H	-1.32361184615119	-1.13694859726549	-0.73196769028214
N	0.17312870427890	3.83112187838139	0.23291986883277
C	1.57907164888259	3.66156318982103	-0.05799477953899
H	2.06656029595552	4.64086012923369	-0.08511766063448
H	1.73966766483291	3.17122844794600	-1.03434416819753
H	2.08509959122006	3.04749028047655	0.70771916149413
H	0.56923178568655	0.92086182897557	-0.05105995260422
H	-2.67974861316715	3.71944131498670	0.79147365776553
H	-3.58061992084405	1.14285148955086	0.78867022187827
H	-0.19443205566181	4.75852259387698	0.37145496715326

H_2N-CH_2-CH_2-SH

C	-4.29555118156633	-1.35290952858595	0.27766153037306

C	-4.06899246167907	0.16273274101042	0.26765278635393
H	-4.94743947491027	-1.61391872803205	1.12051672454026
N	-3.00112068877350	-2.03892779666382	0.47356508285147
H	-4.82546031311559	-1.64853143880906	-0.64581432826686
H	-3.65083370561184	0.48176200370364	1.22631512275014
S	-5.69109345365952	1.01318329791768	-0.02565693715085
H	-3.37291389294629	0.43707123560515	-0.53351474496519
H	-5.23042876263439	2.27221460534220	0.13970589132189
H	-3.15047924158802	-3.02962826452577	0.66547489765906
H	-2.44108682351518	-1.98944812696244	-0.37881602546690

P8

C	-4.45136677633196	-1.07173767820770	0.04473086550268
C	-3.79397562070916	0.30960633376667	-0.08047696406705
H	-5.29736748775256	-1.01229045248703	0.74062498942091
N	-3.47885536554802	-2.03093047675827	0.61063525237499
H	-4.86171391207292	-1.36069199927340	-0.93801947641600
H	-3.39842626376325	0.63356104815761	0.88936365616988
S	-4.89917986273507	1.61070722103521	-0.75636845909959
H	-2.95173976556325	0.26789932009060	-0.78317645989573
C	-6.02208024279843	1.87825506816249	0.68102125523865
H	-3.93910115091711	-2.91196389567072	0.84043021840597
H	-2.75717667232620	-2.25172958451631	-0.07744722439058
C	-6.81371434998117	3.17072952106433	0.45159054602232
H	-6.69776947821217	1.02607498723138	0.80366067456669
H	-5.40599999073543	1.96345737575906	1.58627254702701
H	-7.39501085662416	3.14219556606393	-0.47786969640680
C	-5.92505128906524	4.40760796316285	0.44370003859503
H	-7.53342081901289	3.30191534156244	1.27710938604531
O	-6.15098436979858	5.41424723365434	-0.19626558530115
H	-5.03349572605243	4.34359710720251	1.11708443620747

P9

C	-4.27614525154199	-0.70857461959772	-0.09435297636770
C	-3.67038889210222	0.69935365784598	-0.11518187182471
H	-5.19184037297157	-0.70002072493821	0.50993954990030
N	-3.33219776769974	-1.65689059126784	0.51752391819581
H	-4.57567639905433	-0.99492220635760	-1.12068221384273
H	-3.37729139424458	1.00495032568093	0.89619329283847
S	-4.76769258473111	1.97794278562007	-0.84287219863875
H	-2.76848448593029	0.71851066542265	-0.74070772479373
C	-6.01950420616745	2.15032637737678	0.49931270497110
C	-3.82504002751752	-3.04488552207391	0.58800463532768
H	-2.46057514994766	-1.64426545348091	-0.01265271391971
C	-6.84555088361785	3.41567021773328	0.24081816710600
H	-6.66522886180301	1.26734315167630	0.53675328708166
H	-5.48765666682163	2.23075271548268	1.45697135695480
H	-7.34140660366340	3.39562976475808	-0.73704464841917
C	-6.01622472688322	4.68853483699644	0.35113610266450

H	-7.63959206727642	3.48658908564412	1.00306313956726
O	-6.22731191967793	5.70468127335646	-0.27834154545837
C	-4.82018943896155	-3.22911551395773	1.73957754806992
H	-2.96513052780044	-3.70162939967258	0.75947439821382
H	-4.29443268734533	-3.37929232955159	-0.35576654212482
H	-4.40096080561601	-2.81803316486866	2.67330061211324
C	-5.21311587056960	-4.66556482143988	2.00553533323407
H	-5.75308388787781	-2.66524245580426	1.57608490942395
O	-4.78894301699255	-5.63044304397180	1.39794464660706
H	-5.94724283028032	-4.79951237634025	2.83893579700553
H	-5.18826267290445	4.64024736572920	1.10258303611451

P10

C	-4.27610564242125	-0.62821314529230	-0.23342892364244
C	-3.76366859318786	0.77342739946162	0.13008571822040
H	-5.29343590442269	-0.74092290209728	0.15444563856696
N	-3.43481618046520	-1.68377218518942	0.36308006574986
H	-4.34821731938929	-0.72636101289216	-1.33261088622447
H	-3.73838360928448	0.89556903720683	1.21935255503888
S	-4.73897412264707	2.13733128864301	-0.61731207846167
H	-2.74477958753208	0.92994341321095	-0.24139962771087
C	-6.29993863108046	2.01209086878613	0.35612374420057
C	-4.19351019034568	-2.91793687826243	0.64478800621251
C	-2.23281134698824	-1.94182337691246	-0.44931939999186
C	-7.11732576437521	3.29075175542199	0.13872281543064
H	-6.87305084978781	1.13056965810223	0.05175585412436
H	-6.03534781483668	1.90479152515325	1.41693782889366
H	-7.34528373264778	3.46236967348898	-0.92005284392055
C	-6.43082330354827	4.52688406835038	0.70593290091516
H	-8.08230723005462	3.19290666221905	0.66375319195278
O	-6.54304317792653	5.64634669693946	0.25119414227067
C	-5.05605105216259	-2.78512206035627	1.90381889456479
H	-3.48489604503455	-3.73564557777571	0.80351483656434
H	-4.82028085653618	-3.21988422772680	-0.21507029906257
H	-4.44187124414335	-2.43081965721098	2.74817967822691
C	-5.73178611716801	-4.06995748759703	2.33386652648950
H	-5.84652377640543	-2.02522264882184	1.79383964950027
O	-5.61369859229037	-5.14138165124604	1.77089009319938
H	-1.88000546734706	-0.98035132247909	-0.84183269520143
H	-2.46508122353265	-2.57470478640210	-1.32821031080883
C	-1.08942112857190	-2.58435427232520	0.36523638906270
H	-0.94354256468621	-1.97573396192715	1.27128860606307
C	0.20726107505831	-2.57993769939803	-0.41864466664240
H	-1.31658567675495	-3.61248255938147	0.66389467319676
O	0.84535730306946	-3.56885473472873	-0.71969855973298
H	-6.37090157092430	-3.97561541798176	3.24677302097828
H	0.55992819493937	-1.56270484027651	-0.72890766796622
H	-5.81935825656839	4.33868035929687	1.62441312994384

P11

C	-4.56133344245279	-0.68057426972315	-0.21770929909834
C	-3.95238878524447	0.72233335694063	-0.29424077872711
H	-5.51025610757679	-0.62625445985968	0.33182325966584
N	-3.65603828197531	-1.58832188608851	0.50596636401108
H	-4.80649059714345	-1.03399462912579	-1.23740597919729
H	-3.72338191059280	1.09938638703462	0.70696748587310
S	-5.00686828031057	1.93755436416866	-1.19712393829010
H	-3.01410554166033	0.70452936440905	-0.86340821123750
C	-4.16284247421691	-2.96622636282260	0.64547117035764
C	-5.21751366437011	-3.06705376408327	1.75343456589691
H	-3.31826488749992	-3.61357943927583	0.90598387626350
H	-4.58563713615549	-3.36246119751980	-0.29654540407151
H	-4.84578320926319	-2.58950082253593	2.67569407124812
C	-5.62856865978003	-4.48030043892089	2.10270510020971
H	-6.13809489244375	-2.51588688821271	1.50162830607212
O	-5.17421654240492	-5.48744823989949	1.59335348101421
H	-6.00741853229412	1.97678073238039	-0.28832216008823
H	-2.75955530776496	-1.61721883989719	0.01961982689570
H	-6.40822174685009	-4.55206296696851	2.90183826320216

P12

C	-4.40281842160884	-0.78625698639184	-0.41255412252498
C	-4.05275052073917	0.67164229257573	-0.08497982854389
H	-5.43399524835615	-0.97239462514485	-0.09281430929480
N	-3.51343935570449	-1.72794080504208	0.29556626458163
H	-4.38392796418599	-0.94095897994684	-1.50716047093884
H	-4.12358621345924	0.84750284314769	0.99253734080805
S	-5.09181501365608	1.89417471732163	-0.99652146378842
H	-3.02831588901304	0.91194865944305	-0.39008961385246
C	-4.17391153193198	-3.01337647164956	0.59502070704762
C	-2.23936667569862	-1.91037523529501	-0.42165072900991
C	-5.13678429525249	-2.90130121526098	1.78105409795197
H	-3.40604384293422	-3.74923345038410	0.84974110361162
H	-4.70419168756710	-3.41803208576707	-0.28733230338998
H	-4.62521394750759	-2.44090821714597	2.64252527176077
C	-5.71253587556988	-4.22337898217675	2.24198450666538
H	-5.98787778326081	-2.23372606285624	1.57086174350078
O	-5.44399718508464	-5.30691555089938	1.75921481866717
H	-1.94795961770591	-0.93904898212309	-0.83971721161712
H	-2.35140229639152	-2.60185568986958	-1.27982207295575
C	-1.10226130438721	-2.40524988311857	0.49811337481504
H	-1.08107198224921	-1.74927459825776	1.38247334512467
C	0.24169435405548	-2.30386464913541	-0.19361310783938
H	-1.25145277617174	-3.43786715558385	0.82862298812079
O	0.99415402218504	-3.23488480300655	-0.40093248802293
H	-6.26614343406927	1.56360109226438	-0.41261939721029
H	-6.42787440825056	-4.14504095269698	3.09804041678897
H	0.51546889451524	-1.27005422300004	-0.52812886045573

P13

C	-6.54807423178730	-0.99195586683792	-1.70349186146817
C	-5.49897263970632	0.07404700513840	-1.33204602815187
N	-6.92611495342534	-0.87462379486928	-3.10289363976828
H	-5.04715532901675	-0.15437517922918	-0.36181791977675
S	-6.14812745128832	1.80227667576652	-1.30063048598526
H	-4.70423471726399	0.08589923103238	-2.08517133175392
C	-8.10282728223154	-0.44113153051298	-3.37132641405994
C	-8.55393011925552	-0.24300487668018	-4.74468878053934
C	-9.78081721441058	0.21003151120441	-5.05220598521411
H	-7.41106803002974	-0.91676882985659	-1.01613302459038
H	-6.08400084210725	-1.97953632576942	-1.55950038162140
H	-8.83038403221225	-0.19985878736480	-2.57491951665383
H	-7.82902312486642	-0.48245577374130	-5.52242709012932
H	-10.09699033002944	0.35466599466412	-6.08193726939940
H	-10.50473838847035	0.45294137225267	-4.27513567276225
H	-6.86487886389890	1.67047242480318	-0.16145403812579

P14

C	-6.89047468796316	-0.58986382290299	-1.18923452781704
C	-5.36887367066240	-0.33997090539232	-1.19277798709352
N	-7.38552863119491	-0.69530075936257	-2.55390902682869
H	-4.95607049799293	-0.44838252765733	-0.18319121398632
S	-4.85944637222001	1.30641580128734	-1.84687524002235
H	-4.88671891228510	-1.08128066779712	-1.83831237646095
C	-4.66349509939049	2.27012245748510	-0.29466080870295
C	-5.97838401365615	2.62429281220906	0.43747679219754
H	-3.98838213229460	1.72620232769079	0.37667188514760
H	-4.14523361608867	3.18445829791822	-0.61016445806752
H	-6.65482394557753	3.11873326079444	-0.27438782202753
C	-5.69811888807206	3.57320667392846	1.58665277541116
H	-6.46691732240664	1.72737236645668	0.83009959211783
O	-5.81956940808765	3.29389469718014	2.76355462057783
H	-5.32826080020840	4.58437290527040	1.27900189528152
C	-8.16475188811285	0.22947660778737	-2.98083516889035
C	-8.67733306858872	0.23398240302688	-4.34679073370165
C	-9.49497633741542	1.19101054679404	-4.81651475050755
H	-7.40499139320776	0.19542675712478	-0.60921925980175
H	-7.06811143907271	-1.55465870591751	-0.68985868186060
H	-8.48605708777217	1.06529380396203	-2.33238776776524
H	-8.35389363280884	-0.59274821640915	-4.97888917059409
H	-9.86399048147527	1.17874094533462	-5.83866313921635
H	-9.81711970344553	2.01885698118861	-4.18577950738903

Acrolein

C	-6.14440228606092	1.88559546718268	0.56956826325326
C	-6.66560433987157	3.11906156644210	0.48015050556886
C	-5.80062627888658	4.31536703221629	0.45684569753738

O	-6.21144964477355	5.46305208103379	0.37720273711514
H	-4.70539174472828	4.09826155916593	0.51897354489589
H	-7.73960162141642	3.29393991897702	0.42122353011539
H	-5.06586611253514	1.73791241199032	0.62728756900768
H	-6.76455797172755	0.99286996299186	0.58814815250639

BDA (2-butene-1,4-dial)

C	-4.63031619882585	0.56853743749434	0.04509390540076
C	-3.40928529189036	1.30248794040258	-0.35520653089981
C	-2.17629334478409	0.77055009766570	-0.53557813695248
C	-1.80246988712901	-0.65149528679485	-0.36867993995861
H	-3.56644724172425	2.37088372770861	-0.50805202037118
H	-1.34676146568099	1.41326662919029	-0.83277734121349
O	-5.70728315497602	1.13108618684254	0.18468882727967
O	-0.65978625005934	-1.04647954267297	-0.55325343518627
H	-2.60239838070887	-1.36052240622934	-0.06644201967127
H	-4.53894878422121	-0.52522478360689	0.21624669157269

CH_3NH_2

C	-3.95653667534821	2.52854826743533	0.00391659524595
N	-2.47870371559964	2.52890506638265	0.01030883428584
H	-4.43442209502302	2.58556438817361	0.99839245241404
H	-4.31175653003685	1.61555751835577	-0.48901120297820
H	-4.31174182527867	3.37923077128998	-0.59012034100966
H	-2.12907380196051	1.74321020786502	0.56005285345232
H	-2.12906535675309	3.37233378049763	0.46655080858971

CH_3SH

C	-4.04009576200175	2.51230713210003	-0.01222232362344
S	-2.19770319661395	2.56011528600542	-0.00410052752690
H	-4.44243745757095	2.59237004248688	1.00114791793617
H	-4.41023739135893	1.60613205877359	-0.49909991732205
H	-4.36116304338507	3.38285110070858	-0.59139177091489
H	-2.01255314906935	1.44333437992550	0.73428662145111

H_2O

O	-1.58062404034341	0.46263447770037	0.01436023090193
H	-0.61088872982627	0.49293617283923	-0.01808634472908
H	-1.86200722983031	1.09667934946040	-0.66473388617285

REFERENCES

(1) Baillie, T. A.; Davis, M. R. Mass spectrometry in the analysis of glutathione conjugates. *Biol. Mass Spectrom.* **1993**, *22*, 319–325.

(2) Harrison, A. G. To b or not to b: The ongoing saga of peptide b ions. *Mass Spectrom. Rev.* **2009**, *28*, 640–654.

(3) Johansson Mali'n, T.; Lindberg, S.; Åstot, C. Novel glutathione conjugates of phenyl isocyanate identified by ultra-performance liquid chromatography/electrospray ionization mass spectrometry and nuclear magnetic resonance. *J. Mass Spectrom.* **2014**, *49*, 68–79.