Supporting Information

AuCl-Mediated Selective Cysteine Modification of Peptides and Proteins Using Allenes

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Experimental Section

General. RNaseA was purchased from Sigma and used without further purification. Peptides STSSSCNLSK, AYEMWCFHQR, ECG, CSKFR, KSTFC and allene **1f** were obtained from commercial sources and used without further purification. Water (ddH_2O) used as reaction solvent and in peptide/ protein modifications was deionized using a NANOpure^{TM} purification system (Barnstead, USA). Chemicals purchased from commercial sources were used without further purification. Flash column chromatography was performed using silica gel 60 (230-400 mesh ASTM) with ethyl acetate/n-hexane as eluent. ^{1}H and ^{13}C-NMR spectra were recorded on a Avance DPX300, AV400, DRX500, AV600 spectrometer. Chemical shifts (ppm) were referenced to TMS. Mass spectra were measured using a DFS High Resolution Magnetic Sector MS or Finnigan LCQ Classic mass spectrometers.

Biological Mass Spectrometry. LC-MS and LC-MS/MS analysis was performed using a hybrid Q-TOF mass spectrometer (QSTAR-XL^{TM} system, ABI) equipped with an Ionspray source and a Agilent 1100 series cap-LC pump or a QqQ mass spectrometer (4000 Q-Trap system, ABI) equipped with an Ionspray source and a Agilent 1100 series cap-LC pump. The modified peptides and tryptic digested peptide fragments of modified RNaseA chromatography was performed using a Agilent ZORBAX 300SB-C18 (0.3 mm × 150 mm) reverse phase column on the Q-TOF mass spectrometer or a Agilent Eclipse XDB-C18 (4.6 mm × 150 mm) reverse phase column on the QqQ mass spectrometer with a CH_3CN/ddH_2O gradient mobile phase containing 0.1% formic acid (flow rate: 5 μL/min for the Q-TOF mass spectrometer and 1 mL/min for the QqQ mass spectrometer).

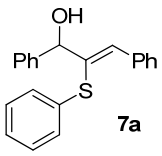
Preparation of allenes 1a–e. Allenes **1a–e** were prepared according to literature procedure (V. K.-Y. Lo, M.-K. Wong and C.-M. Che, *Org. Lett.* 2008, **10**, 517–519).

Preparation of allenes 1g. Allene **1g** was prepared by first synthesizing a propargylamine through a three-component coupling reaction of benzaldehyde, piperidine and propargyl alcohol according to literature procedure (E. Ramu, R. Varala, N. Sreelatha and S. R. Adapa, *Tetrahedron Lett.* 2007, **48**, 7184). The resulting propargylamine was then used for the preparation of allene **1g** according to literature procedure (D. A. Mundal, K. E. Lutz and R. J. Thomson, *J. Am. Soc. Chem.* 2012, **134**, 5782).

Preparation of allenes 1h. A solution of 7-(Diethylamino)-coumarin-3-carboxylic acid (0.0263 g, 0.1 mmol), allene **1g** (0.030 g, 0.2 mmol), EDC·HCl (0.029 g, 0.15 mmol) and DMAP (0.019 g, 0.15 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with 5% HCl aqueous solution (3×10 mL), $NaHCO_3$ saturated aqueous solution (3×10 mL), and brine (3×10 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (50% EtOAc in hexane) to yield allene **1h** as a yellow oil. Yield: 65%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.29–7.31 (m, 4H), 7.24 (d, J = 9.0 Hz, 1H), 7.19–7.23 (m, 1H), 6.58 (dd, J = 9.0, 1.5 Hz, 1H), 6.44 (d, J = 2.5 Hz, 1H). 6.31–7.33 (m, 1H), 5.85 (q, J = 6.5 Hz, 1H), 4.87–4.97 (m, 2H), 3.43 (q, J = 7.0 Hz, 4H), 1.22 (t, J = 7.0 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.8, 163.9, 158.7, 158.4, 153.2, 149.6, 133.7, 131.3, 128.9, 127.5, 127.2, 109.8, 108.6, 107.9, 96.9, 96.8, 91.6, 62.6, 45.3, 12.7; ESI-MS *m/z* 390 $[M+H]^+$

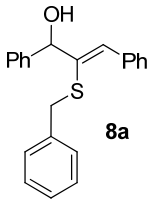
General Procedure for Modification of Peptides (2–6) using Allenes (1a–h). In a 1.0 mL eppendorf tube, allene (10 equiv, 10 µL of a 10 µmol/mL stock solution of allene in CH_3CN), AuCl (10 equiv, 10 µL of a 10 µmol/mL stock solution of AuCl in CH_3CN) and AgOTf (10 equiv, 10 µL of a 10 µmol/mL stock solution of AuCl in CH_3CN) were mixed in 60 µL of H_2O followed by the addition of a peptide solution in H_2O (1 µmol/mL, 10 µL). The reaction mixture was kept at room temperature for 1 h. The conversions of peptides **2–6** were determined from TIC (total ion count) of LC-MS (liquid chromatography-mass spectrometry) analysis of the reaction mixture. The identities of the modified residues of peptides were confirmed by LC-MS/MS (liquid chromatography-tandem mass spectrometry) analysis.

General Procedure for Reaction of Thiophenol (7) or Benzyl Thiol (8) with Allenes in the Presence of AuCl. To a 1.5 mL CH_3CN/H_2O (2:1) solution of 0.52 mmol of allene **1a** or **1g**, 10 mol% AuCl (0.052 mmol) was added. After the reaction mixture turned dark blue, 15.6 mmol of thiol thiophenol (**7**) or benzyl thiol (**8**), (30 equiv) was added and was stirred at room temperature for overnight in an open flask. After concentrated the resulting reaction mixture under reduced pressure, the desired product was purified by flash column chromatography (10 % EtOAc in hexane).



7a

7a (**Z**-isomer, 136 mg, 82 % yield): analytical TLC (silica gel 60) (20 % EtOAc in *n*-hexane), R*f* = 0.29. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 (d, *J* = 7 Hz, 2H), 7.37–7.13 (m, 14 H), 5.27 (s, 1H), 2.51 (bs, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 141.7, 135.8, 135.6, 134.9, 134.5, 129.6, 129.5, 129.1, 128.5, 128.4, 128.1, 128.08, 127.0,126.5, 76.9; EIMS *m/z* 318 [M^+]; HRMS (EI) for $C_{21}H_{18}SO$, calcd. 318.11, found 318.1072.



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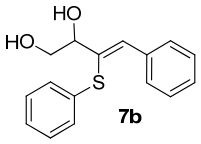
8a (**Z**-isomer, 38 mg, 22 % yield): analytical TLC (silica gel 60) (20 % EtOAc in *n*-hexane), R*f* = 0.25. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (d, *J* = 1 Hz, 2H), 7.72–7.17 (m, 11 H), 7.07-7.05 (m, 3 H), 5.28 (s, 1H), 3.61 (dd, *J* = 10 Hz, 13 Hz, 2H), 2.40 (bs, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 141.5, 137.6, 137.5, 136.0,133.3, 129.4, 128.9,128.5, 128.4, 128.3, 128.1, 127.9, 127.8, 127.3, 127.0, 126.7, 78.2, 37.6; EIMS *m/z* 332 [M^+]; HRMS (EI) for $C_{22}H_{20}SO$, calcd. 332.12, found 332.1229.



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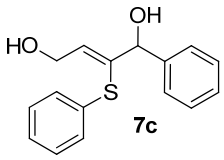
7b (**Z**-isomer, 12 mg, 6 % yield): analytical TLC (silica gel 60) (40 % EtOAc in *n*-hexane), R*f* = 0.27. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.66–7.65 (d, *J* = 7.5 Hz, 2H), 7.36–7.17 (m, 8H), 4.32 (s, 1H), 3.87–3.70 (m, 1H), 3.69–3.67 (m, 1H), 2.74 (s, 1H), 2.04 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 135.8, 135.4, 134.4, 131.9, 129.5, 129.3, 129.1, 128.2, 128.1, 126.6, 75.1, 66.1; EIMS *m/z* 272 [M^+]; HRMS (EI) for $C_{16}H_{16}SO_2$, calcd. 272.0871, found 272.0868.



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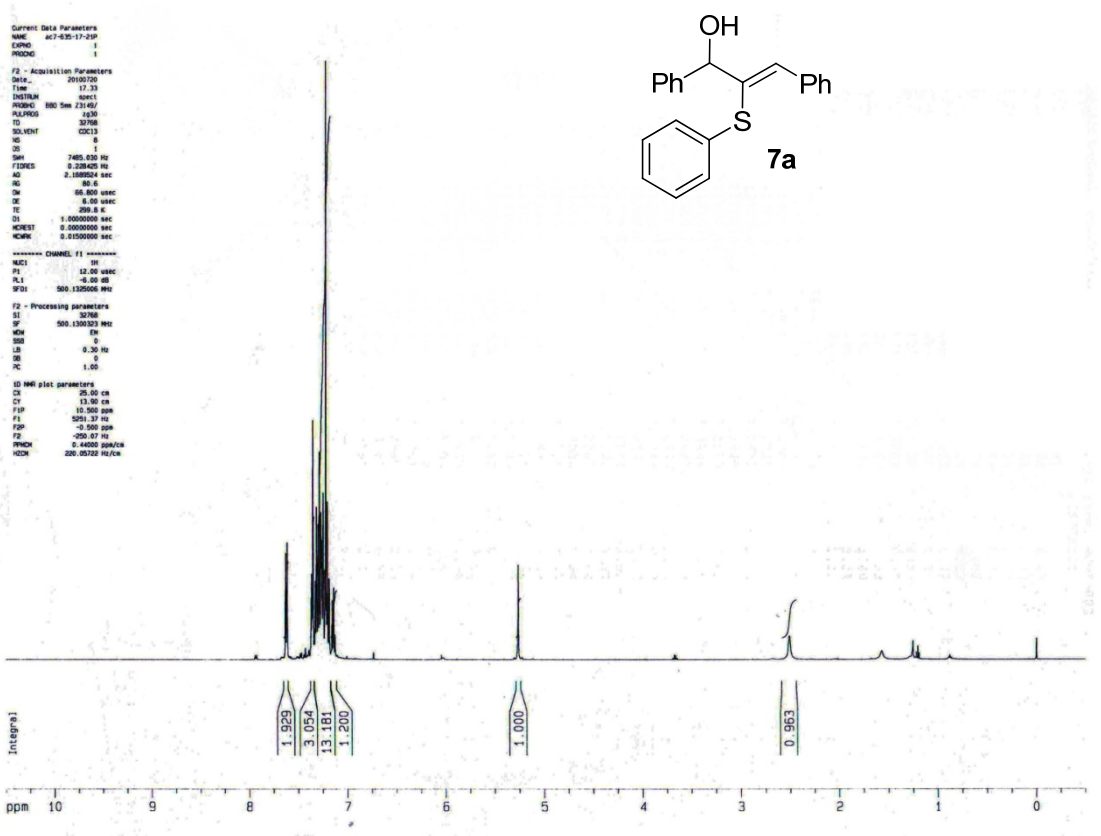
7c (**Z**-isomer, 4 mg, 2 % yield): analytical TLC (silica gel 60) (40 % EtOAc in *n*-hexane), R*f* = 0.27. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34─7.18 (m, 10H), 6.53 (t, *J* = 6 Hz, 1H), 5.2 (s, 1H), 4.48─4.36 (m, 2H), 2.40 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 141.0, 137.9, 136.3, 134.4, 129.25,129.22, 128.5, 128.1, 126.8, 126.7, 76.2, 60.8; EIMS *m/z* 272 [M^+]; HRMS (EI) for $C_{16}H_{16}SO_2$, calcd. 272.0871, found 272.0865.

Modification of RNaseA using allene 1a in the presence of AuCl and AgOTf. In a 1.0-mL eppendorf tube, RNaseA solution in H_2O (1 μmol/mL, 50 μL) was mixed with dithiothreitol (DTT) solution in H_2O (400 μmol/mL, 50 μL) at 70 ^{o}C for 10 min. The number of free cysteine residue generated through reduction of disulfide linkages of RNaseA was confirmed by LC-MS analysis (M. Scigelova, P. S. Green, A. E. Giannakopulos, A. Rodger, D. H. G. Crout and P. J. Derrick, *Eur. J. Mass Spectrom.* 2001, **7**, 29-34). The reaction mixture of DTT treated RNaseA was then centrifuged at 13.2 rpm for 10 min to remove the excess DTT. 50 μL of H_2O was added to the dried and centrifuged DTT-treated RNaseA. 10 μL of this DTT treated-RNaseA solution was added to a mixture of allene **1a** (10 equiv, 10 μL of a 10 μmol/mL stock solution of allene in CH_3CN), AuCl (10 equiv, 10 μL of a 10 μmol/mL stock solution of AuCl in CH_3CN) and AgOTf (10 equiv, 10 μL of a 10 μmol/mL stock solution of AuCl in CH_3CN) and allowed to react at r.t. for 1 h. The conversion of the DTT treated RNaseA in the cysteine modification using allene **1a** in the presence of AuCl and AgOTf was determined from TIC (total ion count) of LC-MS analysis.

Trypsin Digestion of allene 1a-modified RNaseA. In a 1.0-mL eppendorf tube, allene **1a**-modified RNaseA mixture (100 μL) was mixed with 1:1 butanol/H_2O (100 μL) at 65 °C for 15 min. The reaction mixture was diluted 4-fold by ammonium bicarbonate solution (50 mM, 600 μL). Trypsin solution [1 mg/mL, 2.74 μL; ratio of trypsin to RNaseA ~1:70 (w/w)] was added to the eppendorf tube at 0 °C. The reaction mixture was incubated at 37 °C overnight and the trypsin digested mixture was analyzed using LC-MS/MS analysis.

Reaction of thiophenol with allene 1a in the presence of AuCl and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO). To a 1.5 mL CH_3CN/H_2O (2:1) solution, allene **1a** and 10 mol% of AuCl were mixed. When the reaction mixture turned dark blue, thiophenol (3 mmol) and TEMPO (3 mmol) were added and the reaction mixture was stirred in air at room temperature for overnight in an open flask. The resulting reaction mixture was purified by flash column chromatography (10 % EtOAc in hexane) and subjected to ESI-MS analysis.

Reaction of thiophenol with allene 1a in the presence of AuCl in H_2O^{18}. The procedure is the same as the general procedure for reaction of thiophenol with allenes in the presence of AuCl, except that H_2O^{18} was used. The resulting reaction mixture was purified by flash column chromatography (10 % EtOAc in hexane) and subjected to ESI-MS analysis.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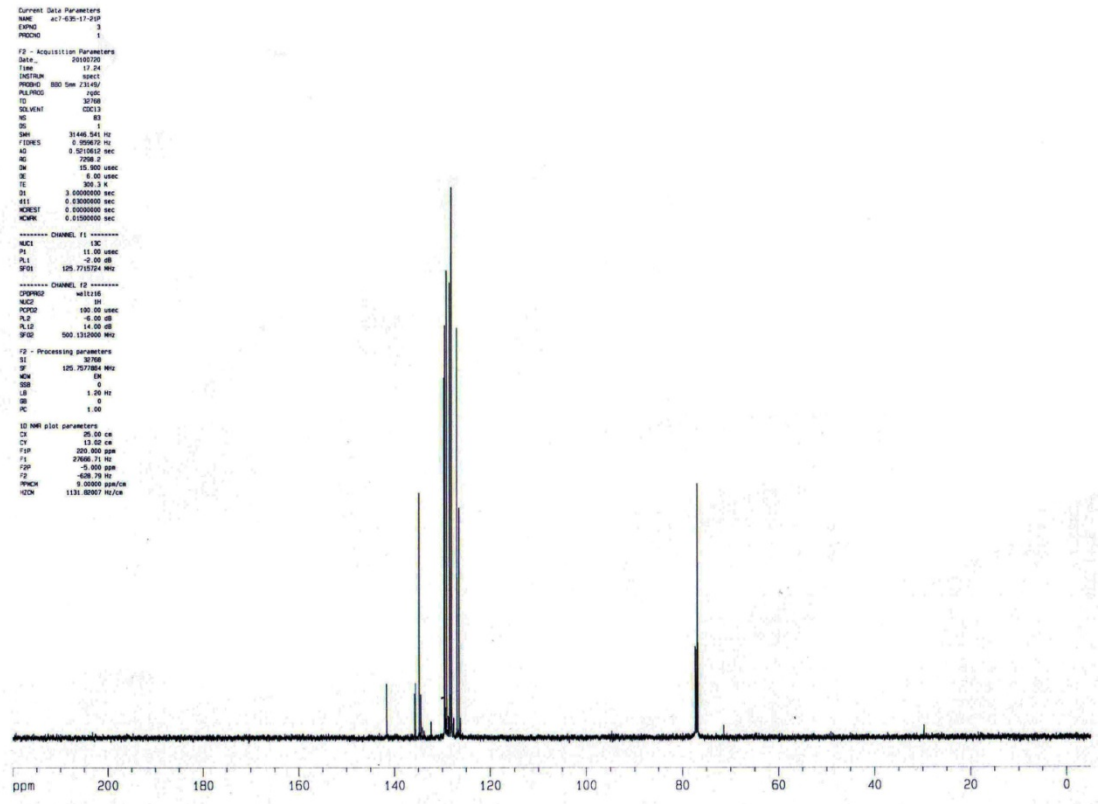
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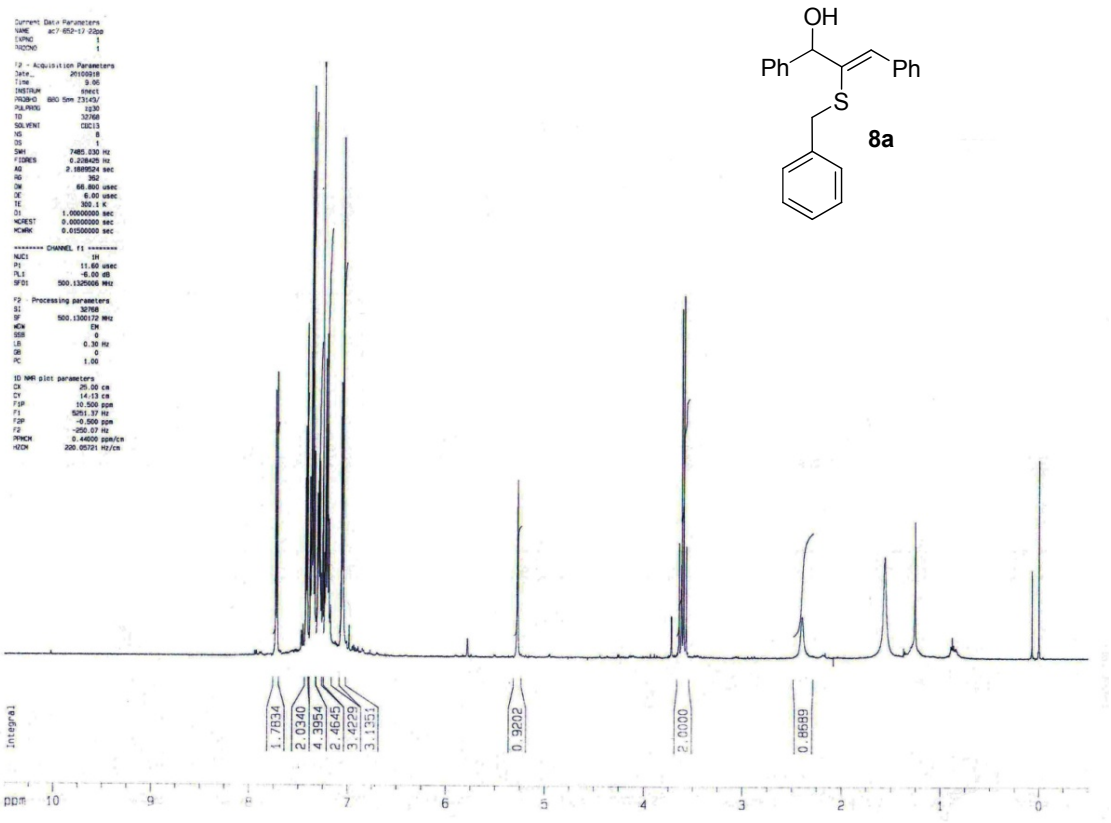
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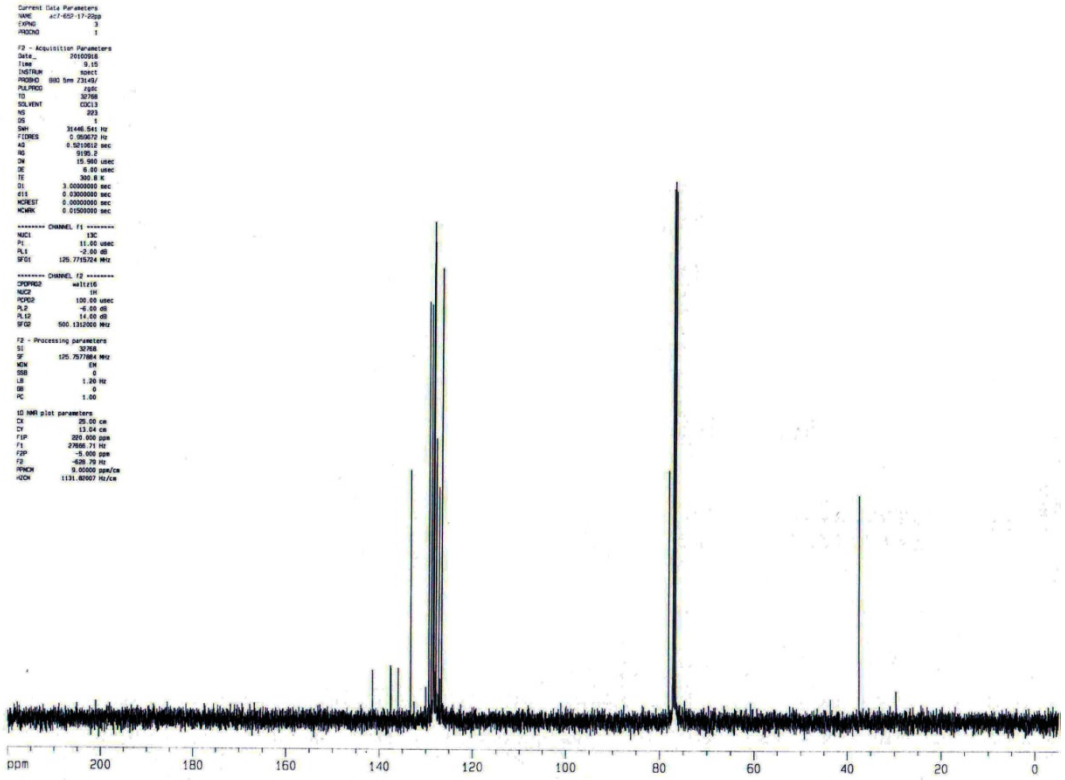
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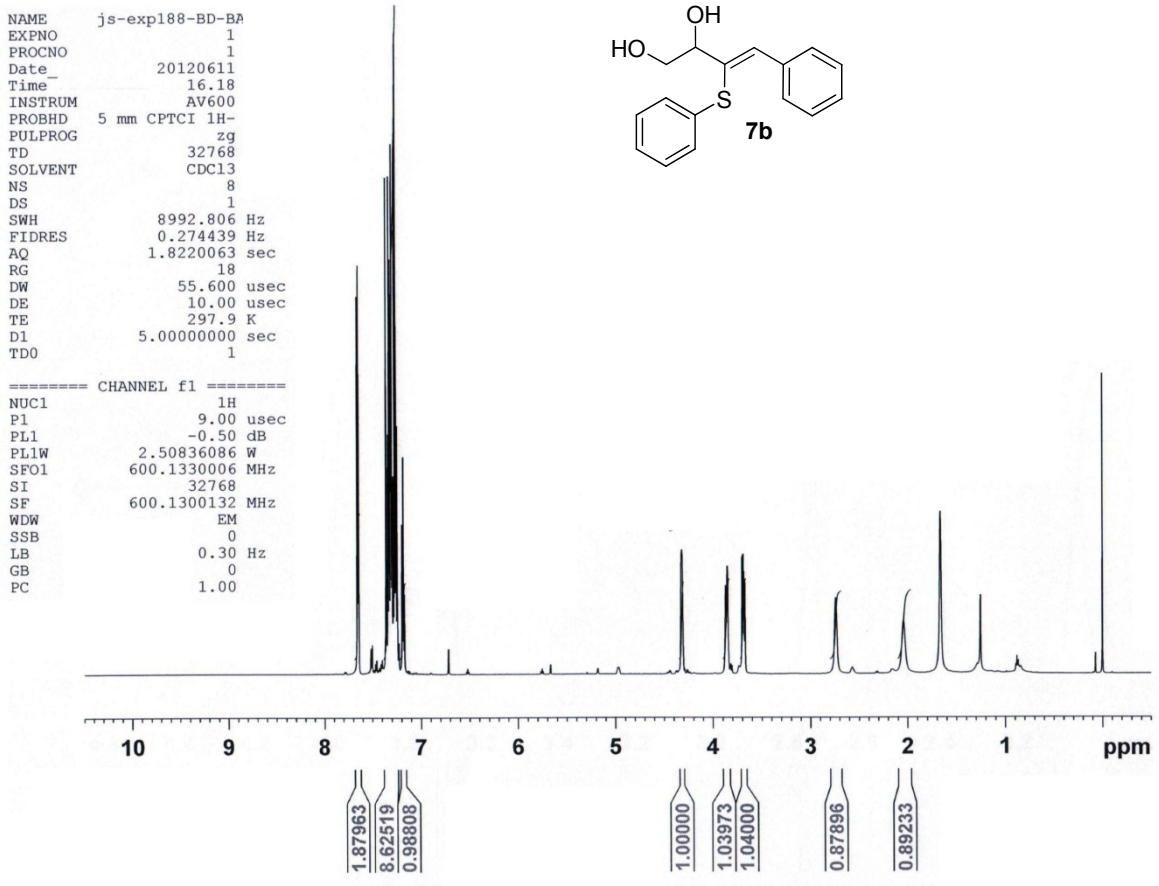
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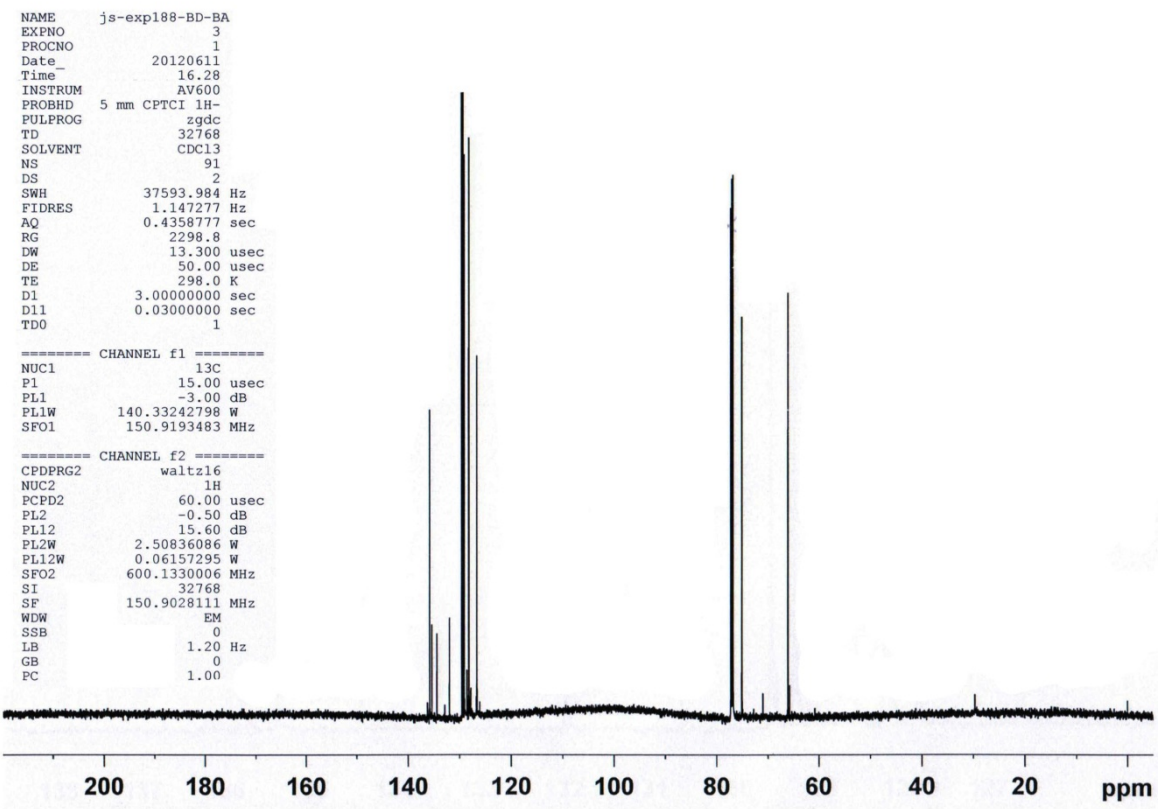
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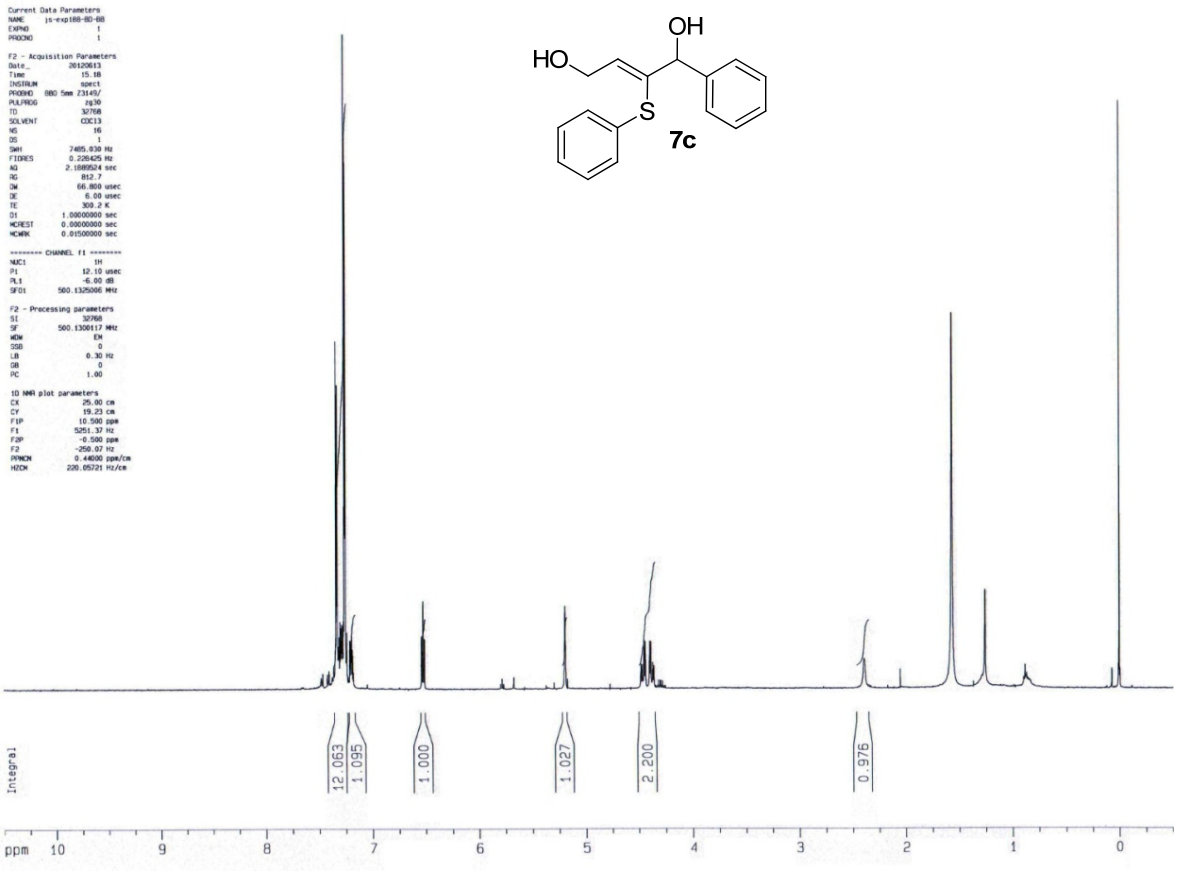
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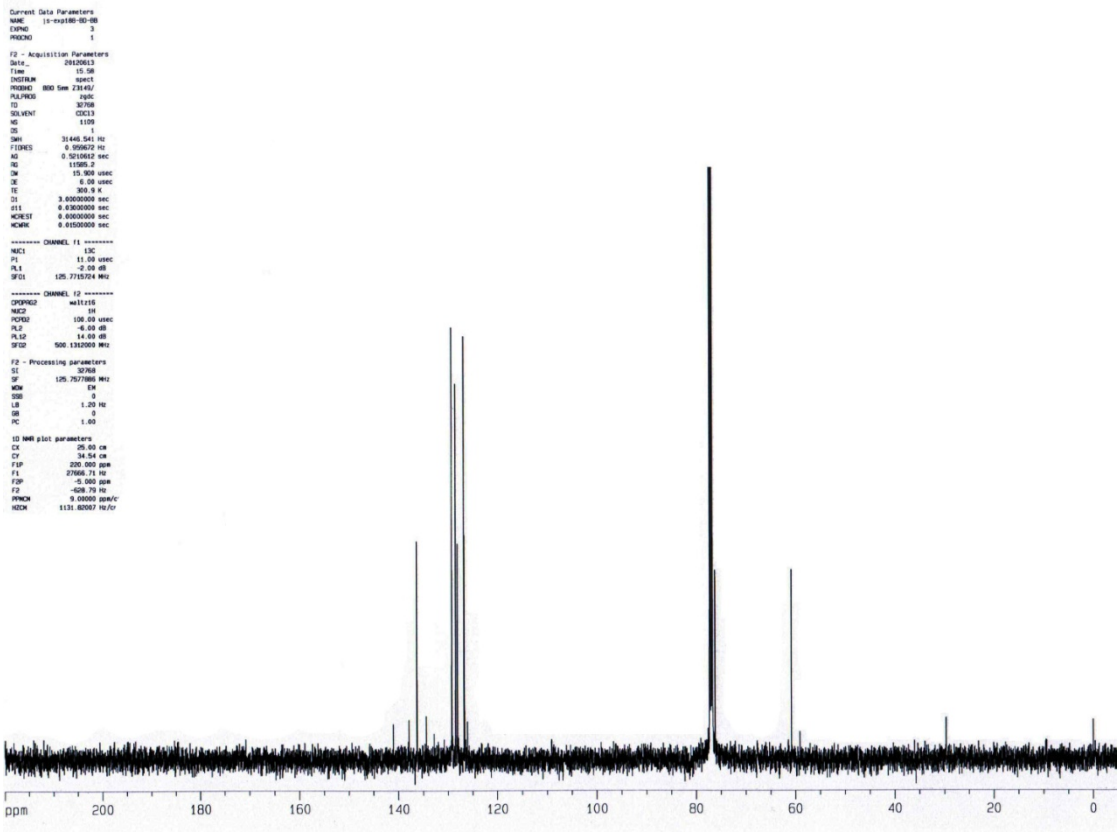
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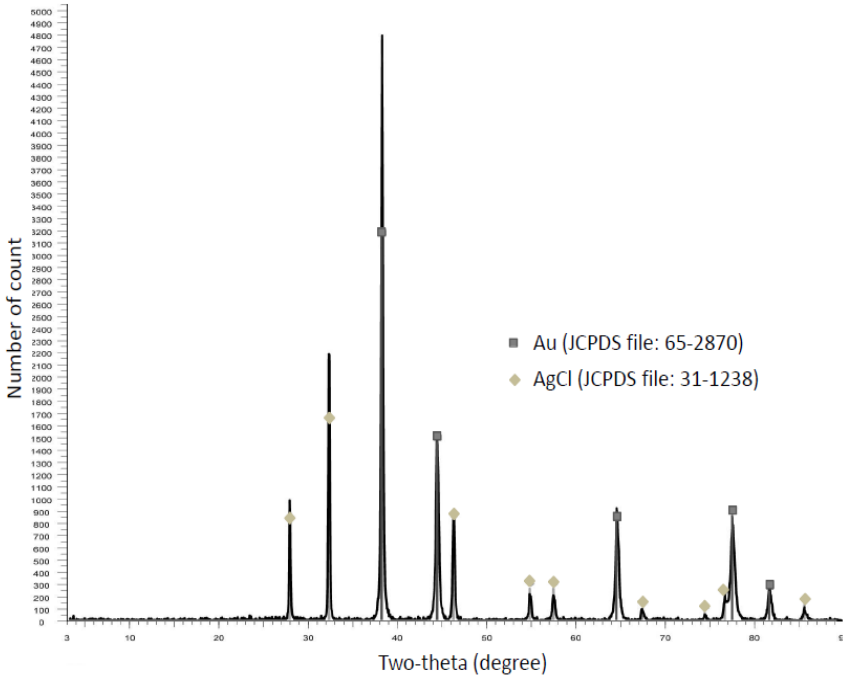

Figure S-1. XRD spectrum of the residues collected in the cysteine modification of peptide using gold mediated allene coupling reaction.

Optimization of reaction conditions of the gold compound mediated cysteine modification of peptide 2 using allene 1a.

1. Using different equivalents of AuCl and AgOTf.

Table S-1. Cysteine modification of peptide STSSSCNLSK (**2**) using allene **1a** in the presence of different equivalents of AuCl and AgOTf[a]

Entry	Metal complex	Equiv.	**2**(%)[b]	:	**2a**(%)[b]	:	**2a'**(%)[b]
1	AuCl	0.5	100	:	n.d.[c]	:	n.d.
2	AuCl	1	100	:	n.d.	:	n.d.
3	AuCl	1.5	100	:	n.d.	:	n.d.
4	AuCl	2	92	:	3	:	5
5	AuCl	5	33	:	62	:	5
6	AuCl	10	34	:	49	:	17
7	AuCl	10	22	:	77	:	1
	AgOTf	10					
8	AuCl	20	19	:	74	:	7
	AgOTf	20					
9	AuCl	30	7	:	89	:	4
	AgOTf	30					
10	AuCl	40	1	:	57	:	42
	AgOTf	40					
11	AuCl	50	10	:	15	:	75
	AgOTf	50					

[a]STSSSCNLSK (**2**) (0.01 µmol), allene **1a** (0.1 µmol) and AuCl (0.05 µmol, i.e. 0.5 equivalents) and AgOTf (0.05 µmol, i.e. 0.5 equivalents) in CH_3CN/H_2O (2:1) solution (100 µL), 1h, r.t. [b]Determined by total ion count (TIC) of LC-MS analysis.[c]n.d. = not detected in LC-MS analysis.

By LC-MS analysis, no cysteine modified peptide **2a** was observed using 0.5, 1 or 1.5 equivalents of AuCl (Table S-1, entries 1–3). Increasing the equivalent of AuCl used from 2 to 5 gave a rise in the formation of peptide **2a** (from 3% to 62%, Table S-1, entries 4–5). Using 10 equivalents of AuCl, the ratio of **2** to **2a** to **2a'** obtained in LC-MS analysis was 34:49:17, which indicated an increase in the formation of both cysteine modified peptide **2a** and peptide dimer **2a'**. The increase in the formation of peptide dimer **2a'** in entry 6 of Table S-1 is attributed to the increase in the amount of AuCl used which could lead to over oxidation of the cysteine thiol residue.

Upon the addition of 10 equivalents of AgOTf, the ratio of **2** to **2a** to **2a'** obtained in LC-MS analysis changed from 34:49:17 to 22:77:1 (Table S-1, entries 6–7). Further increasing the equivalents of AuCl and AgOTf used to 20 and 30 led to an increase in the ratio of **2** to **2a** to **2a'** which were found to be 19:74:7 to 7:89:4, respectively (Table S-1, entries 8–9). These results suggested that the addition of AgOTf could lead to an increase in the formation of cysteine modified peptide **2a**. In the presence of 40 and 50 equivalents each of AuCl and AgOTf, the ratio of **2** to **2a** to **2a'** obtained in LC-MS analysis were 1:57:42 and 10:15:75, respectively (Table S-1, entries 10–11). These results reveal a drop in the formation of cysteine modified peptide **2** and a significant increase in the amount of peptide dimer **2a'** due to over oxidation of the cysteine thiol residue in the presence of excessive AuCl and AgOTf. Since using 10 equivalents each of AuCl and AgOTf gave the highest conversion of cysteine modified peptide **2a** and the lowest conversion of peptide dimer **2a'**, this reaction condition was thus used as the optimal condition in the following cysteine modifications of peptides.

2. Using different reaction temperature.

Table S-2. Cysteine modification of peptide STSSSCNLSK (**2**) using allene **1a** in the presence of AuCl and AgOTf at different reaction temperature[a]

Entry	Temp.	**2** (%)[b]	:	**2a** (%)[b]
1	4 °C	35	:	65
2	r.t.	27	:	73[c]
3	37 °C	87	:	13[c]
4	55 °C	89	:	11[c]

[a]STSSSCNLSK (**2**) (0.01 μmol), allene **1a** (0.1 μmol) and AuCl (0.1 μmol) and AgOTf (0.1 μmol) in CH_3CN/H_2O (2:1) solution (100 μL), 1h. [b]Determined by total ion count (TIC) of LC-MS analysis. [c]Only a trace amount of peptide dimer **2a'** was observed in each case.

At low temperature, the conversion of cysteine modified peptide **2a** was compatible with that conducted at room temperature (Table S-2, entry 1). At elevated temperature, the conversions of cysteine modified peptide **2a** were 13 and 11% as confirmed by LC-MS analysis (Table S-2, entries 2–4). Only trace amount of peptide dimer **2a'** was observed. Since modification of peptide **2** conducted at room temperature gave the highest conversion of cysteine modified peptide **2a** (Table S-2, entry 2), room temperature was thus chosen as the optimal reaction temperature for the modification of peptides.

The use of CH_3CN could be reduced to 20 % in the reaction of peptide **2** (1 μmol/mL in H_2O, 10 μL) with allene **1a** (10 μmol/mL in CH_3CN, 10 μL) in the presence of AuCl (10 μmol/mL in CH_3CN, 10 μL)/AgOTf (10 μmol/mL in H_2O, 10 μL). The total volume of the reaction mixture was made up to 100 μL by water. The conversion of the cysteine-modified peptide **2** was maintained at 70 % as confirmed by LC-MS/MS analysis.

3. Using different gold compounds.

Table S-3. Cysteine modification of peptide STSSSCNLSK (**2**) using allene **1a** in the presence of different metal complexes.[a]

Entry	Metal complexes	**2** (%)[b]	:	**2a** (%)[b]
1	AuCl/AgOTf	27	:	73[c]
2	$Au(PPh_3)Cl/AgNO_3$	91	:	9[c]
3	Au(TPP)Cl/AgOTf	92	:	8[c]
4	$KAuCl_4$/AgOTf	90	:	10[c]
5	AgOTf	100	:	n.d.[d]
6	$AgNO_3$	100	:	n.d.
7	$ZnCl_2$	100	:	n.d.
8	$CuSO_4$	100	:	n.d.

[a]STSSSCNLSK (**2**) (0.01 μmol), allene **1a** (0.1 μmol) and metal complex (0.1 μmol, i.e. 10 equivalents) in CH_3CN/H_2O (2:1) solution (100 μL), 1h, r.t. [b]Determined by total ion count (TIC) of LC-MS analysis. [c]Only a trace amount of dimer **2a'** was observed. [d]n.d. = not detected.

Apart from AuCl, $Au(PPh_3)Cl$ was applied in the cysteine modification of peptide **2** using allene **1a** which gave 9% conversion of cysteine modified peptide **2a** as confirmed by LC-MS analysis (Table S-3, entry 2). Using gold(III) compounds such as Au(TTP)Cl and $KAuCl_4$, the conversion of cysteine modified peptide **2a** was 8 and 10%, respectively (Table S-3, entries 3–4). Since AuCl gave the highest conversion of peptide **2a**, it was chosen for the following modification reactions of peptides.

Control experiments conducted using either AgOTf or $AgNO_3$ gave no cysteine modified peptide **2a** as confirmed by LC-MS analysis (Table S-3, entries 5–6). These results show that silver compound alone does not lead to cysteine modification of cysteine containing-peptides using allenes. Apart from gold compounds, $ZnCl_2$ and $CuSO_4$ led to no modified peptide **2a** as revealed by LC-MS/MS analysis (Table S-3, entries 7–8).

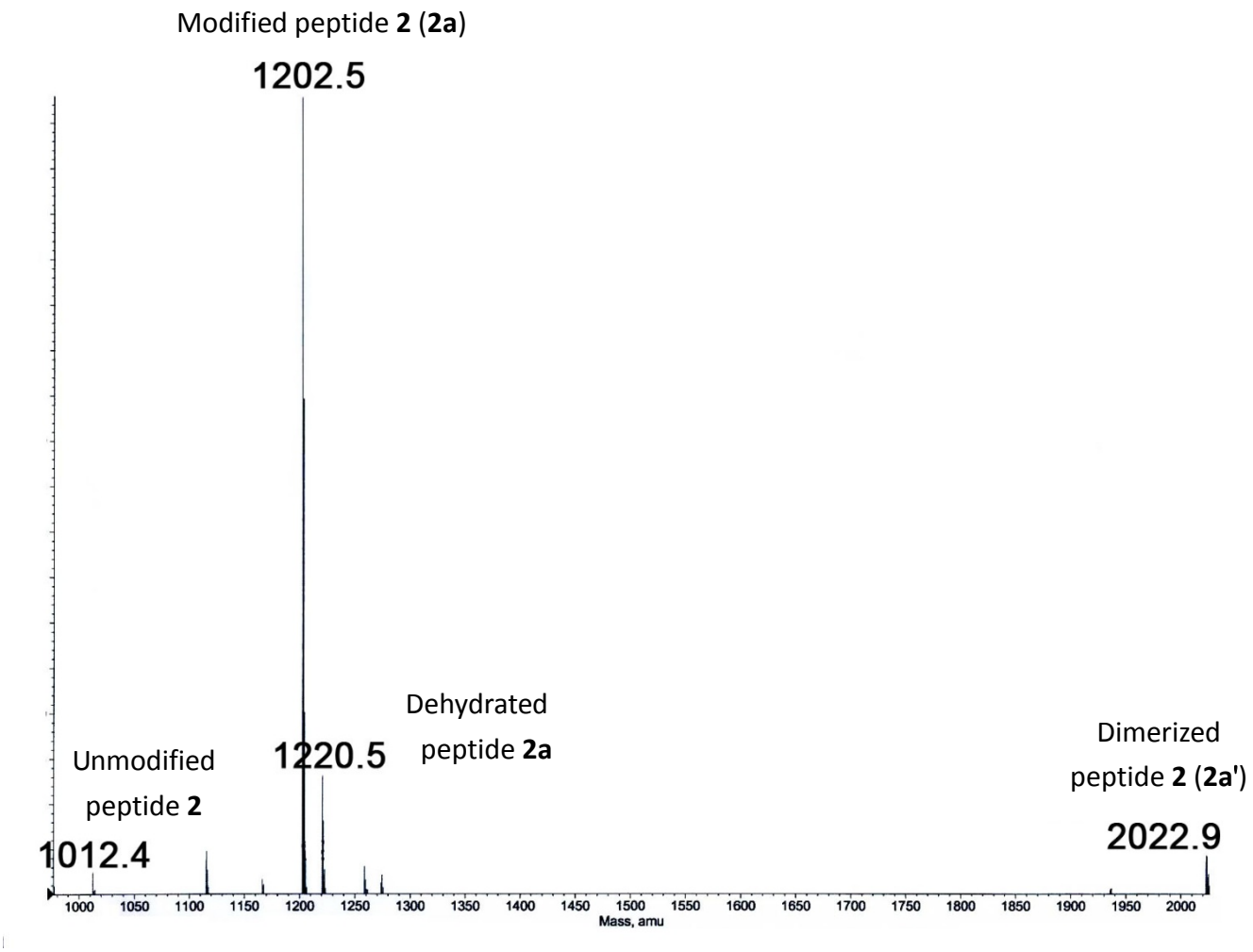

Figure S-2. Mass reconstruction spectrum in LC-MS analysis of the reaction mixture of peptide STSSSCNLSK (**2**) using allene **1a**.

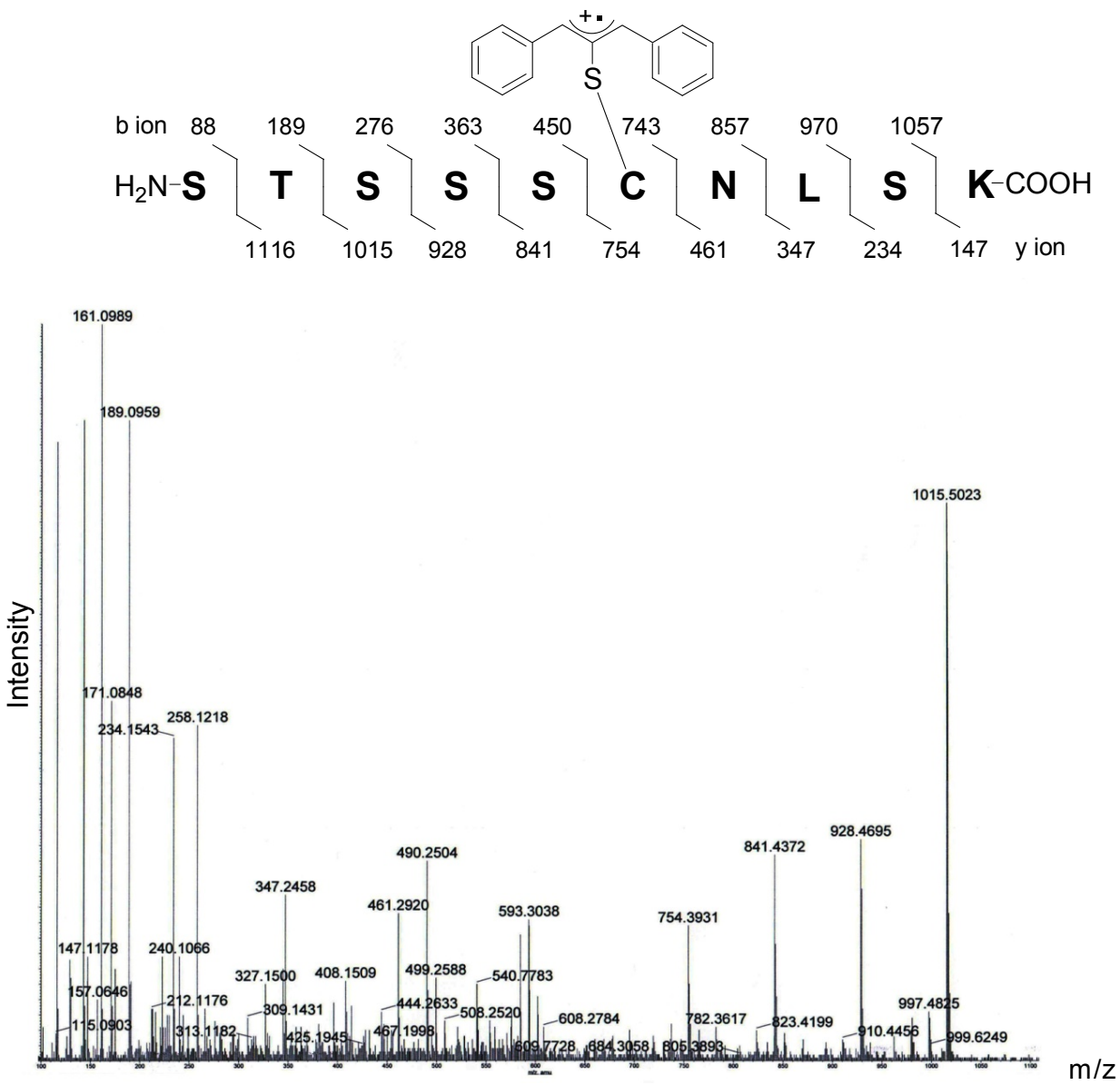

Figure S-3. Q-TOF MS/MS spectrum of the cysteine modified peptide STSSSCNLSK (**2**) using allene **1a** (ESI source, doubly charged ion of *m/z* = 611.2). The cysteine modified peptide dehydrated ([M-18] Da) during mass spectrometric analysis (ESI source, doubly charged ion of *m/z* = 602.2).

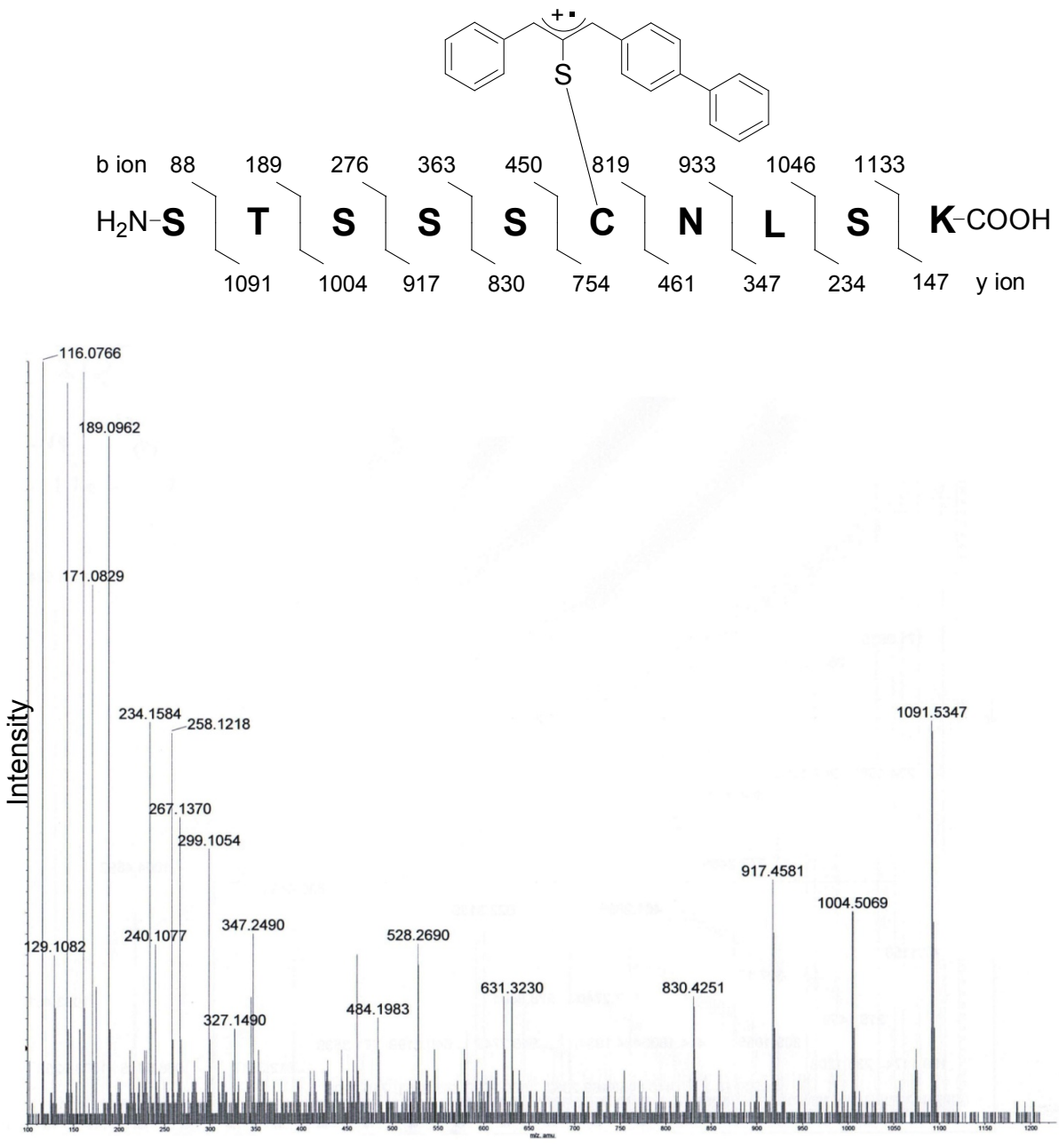

Figure S-4. Q-TOF MS/MS spectrum of the cysteine modified peptide STSSSCNLSK (**2**) using allene **1b** (ESI source, doubly charged ion of *m/z* = 649.2). The cysteine modified peptide dehydrated ([M-18] Da) during mass spectrometric analysis (ESI source, doubly charged ion of *m/z* = 640.2).

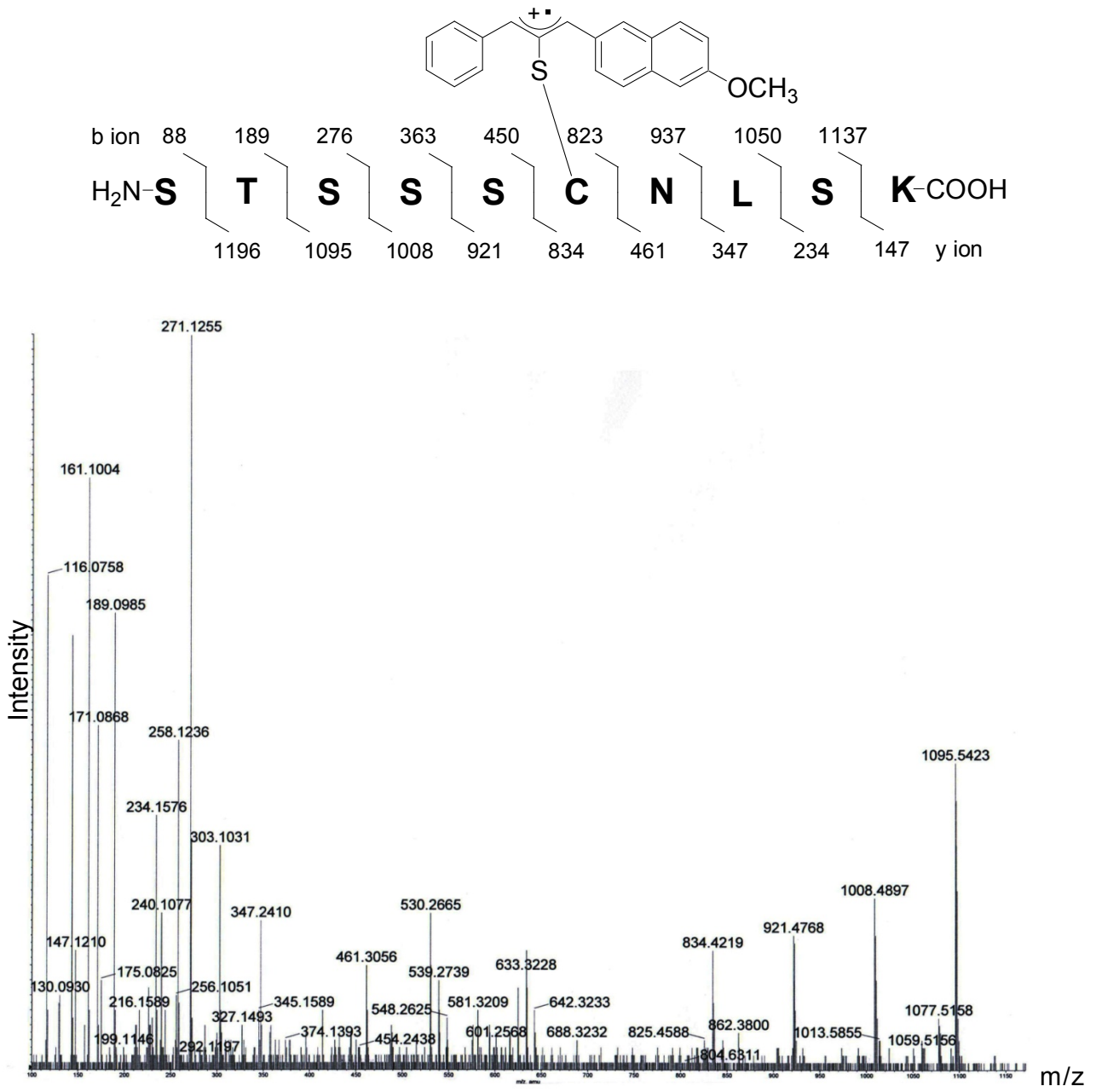

Figure S-5. Q-TOF MS/MS spectrum of the cysteine modified peptide STSSSCNLSK (**2**) using allene **1c** (ESI source, doubly charged ion of *m/z* = 651.2). The cysteine modified peptide dehydrated ([M-18] Da) during mass spectrometric analysis (ESI source, doubly charged ion of *m/z* = 642.2).

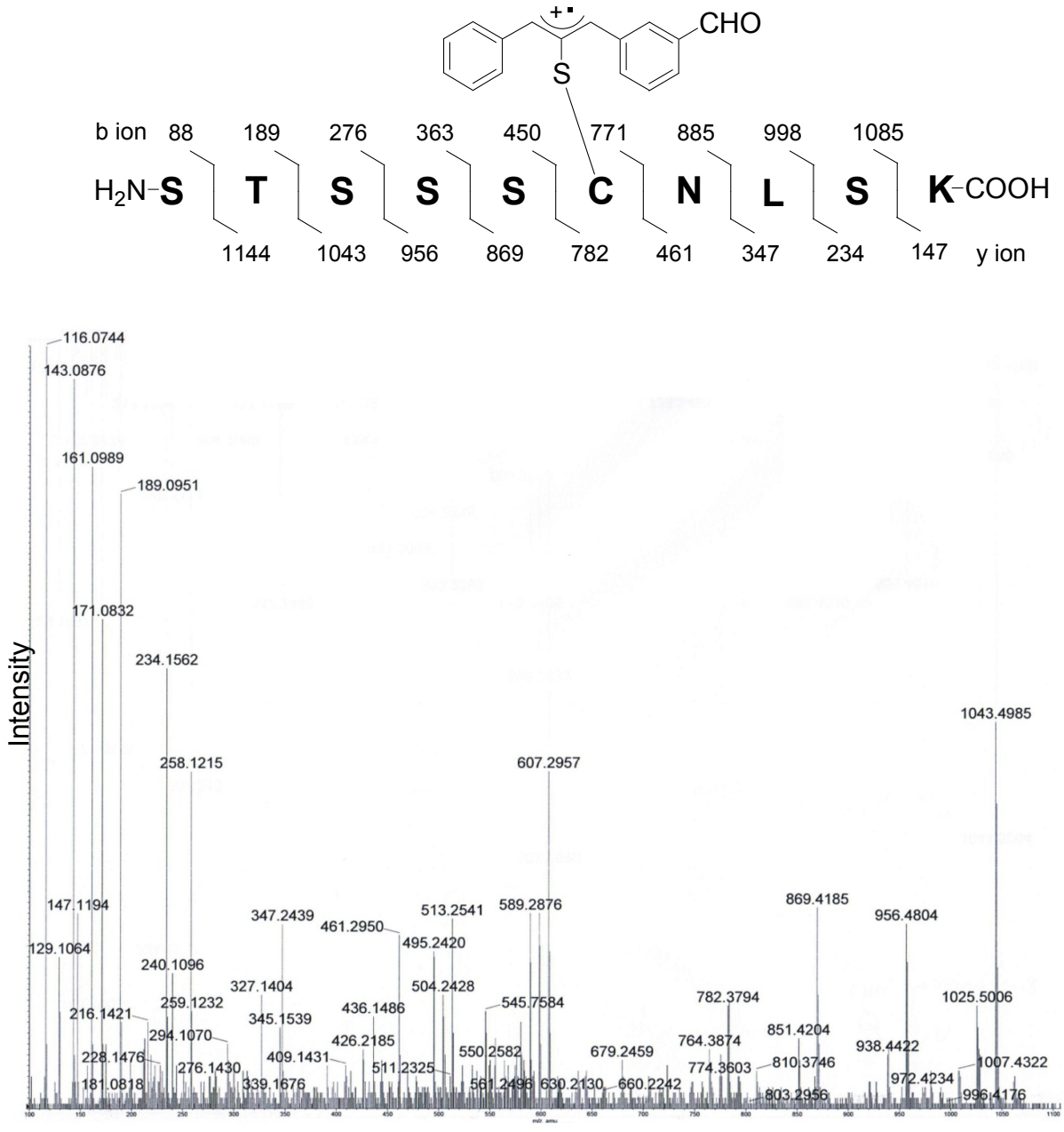

Figure S-6. Q-TOF MS/MS spectrum of the cysteine modified peptide STSSSCNLSK (**2**) using allene **1d** (ESI source, doubly charged ion of *m/z* = 625.2). The cysteine modified peptide dehydrated ([M-18] Da) during mass spectrometric analysis (ESI source, doubly charged ion of *m/z* = 616.2).

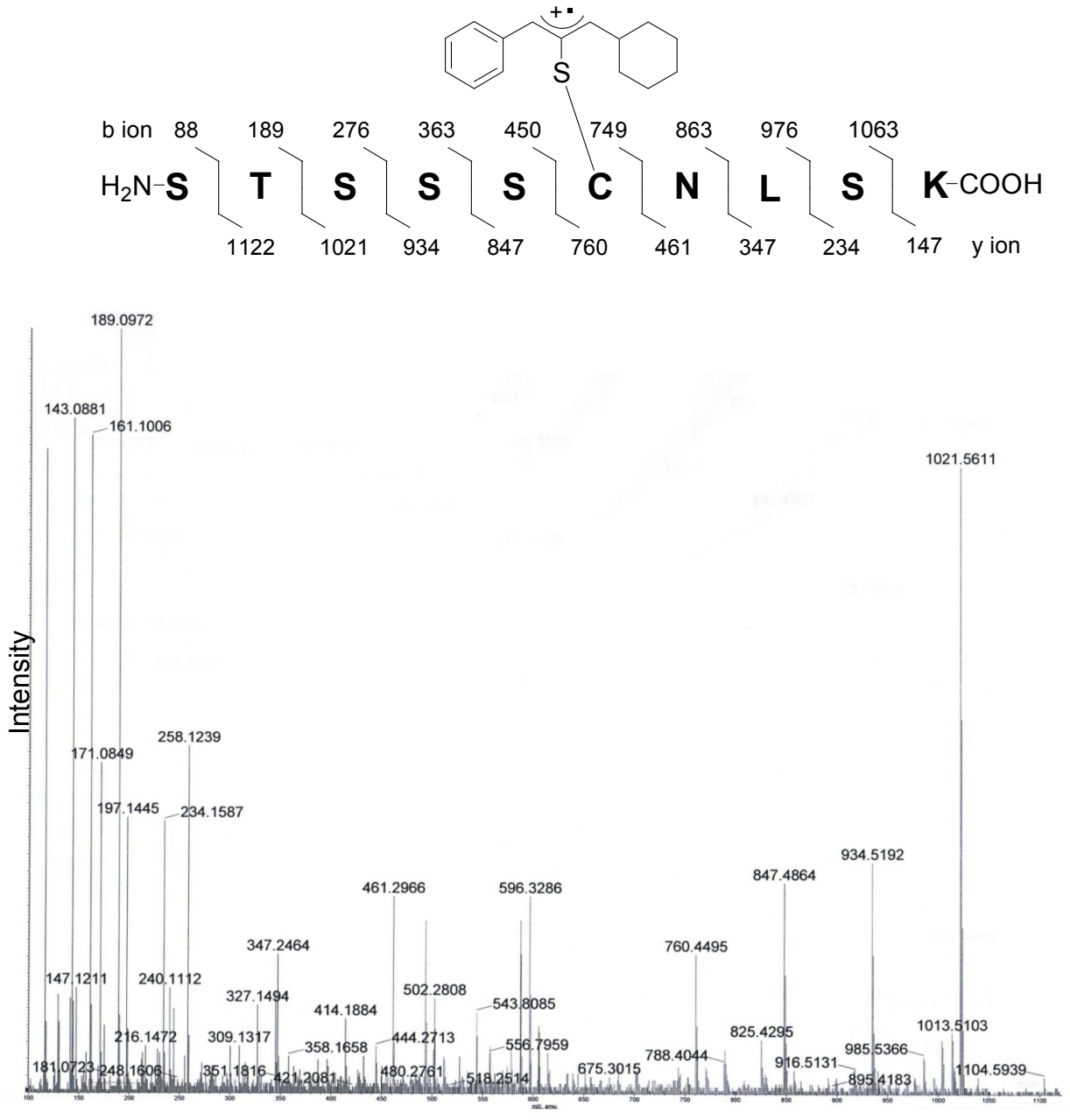

Figure S-7. Q-TOF MS/MS spectrum of the cysteine modified peptide STSSSCNLSK (**2**) using allene **1e** (ESI source, doubly charged ion of *m/z* = 614.2). The cysteine modified peptide dehydrated ([M-18] Da) during mass spectrometric analysis (ESI source, doubly charged ion of *m/z* = 605.2).

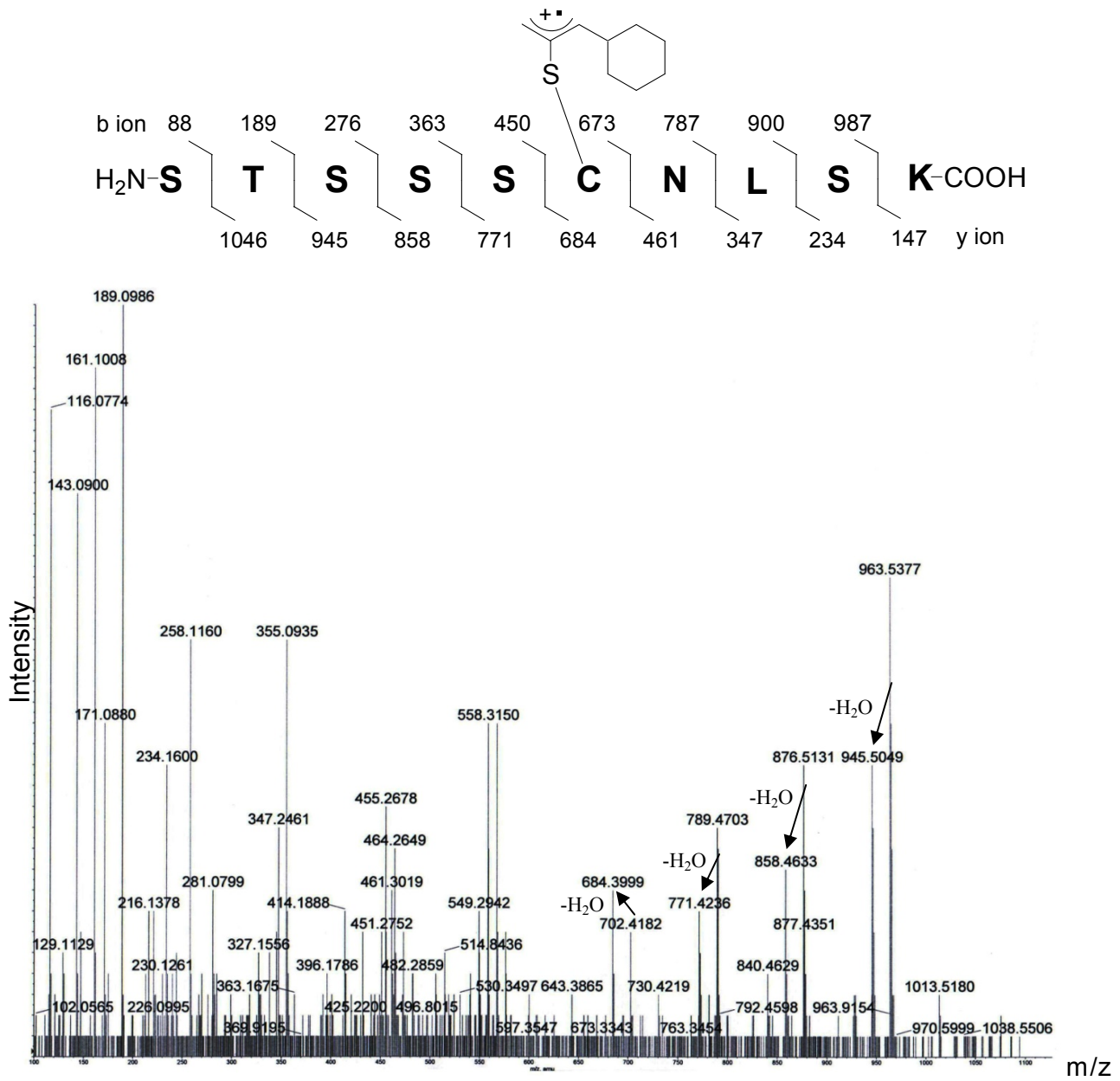

Figure S-8. Q-TOF MS/MS spectrum of the cysteine modified peptide STSSSCNLSK (**2**) using allene **1f** (ESI source, doubly charged ion of *m/z* = 576.2). The cysteine modified peptide dehydrated ([M-18] Da) during mass spectrometric analysis (ESI source, doubly charged ion of *m/z* = 567.2).

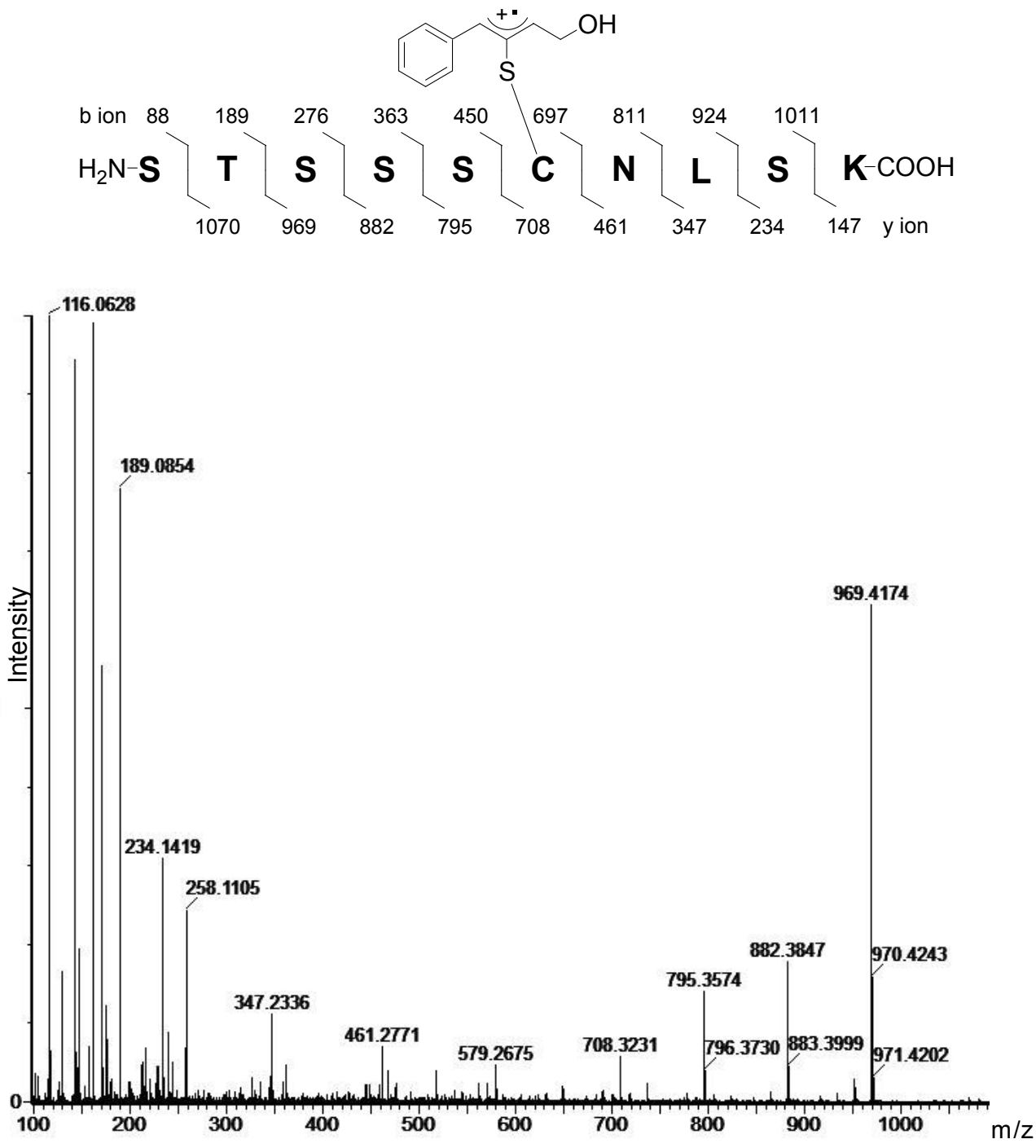

Figure S-9. Q-TOF MS/MS spectrum of the cysteine modified peptide STSSSCNLSK (**2**) using allene **1g** (ESI source, doubly charged ion of *m/z* = 588.2). The cysteine modified peptide dehydrated ([M-18] Da) during mass spectrometric analysis (ESI source, doubly charged ion of *m/z* = 579.2).

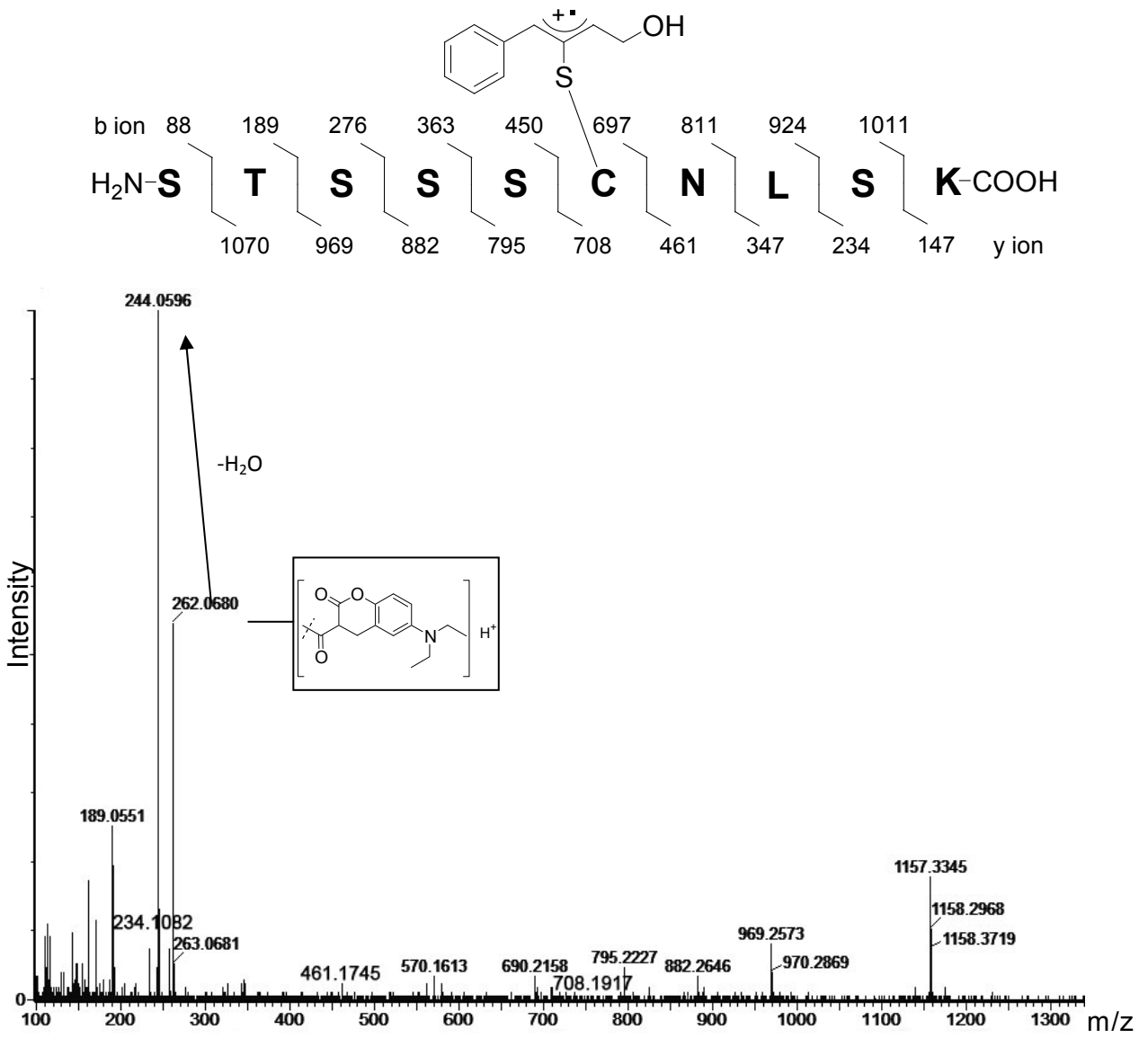

Figure S-10. Q-TOF MS/MS spectrum of the cysteine modified peptide STSSSCNLSK (**2**) using allene **1h**. The fluorescent probe on **1h** cleaved under MS analysis to yield a cysteine modified peptide **2** with doubly charged ion of *m/z* = 588.2. The cleaved fluorescent fragment with *m/z* = 262 dehydrated under MS analysis to give a fragment of *m/z* = 244.

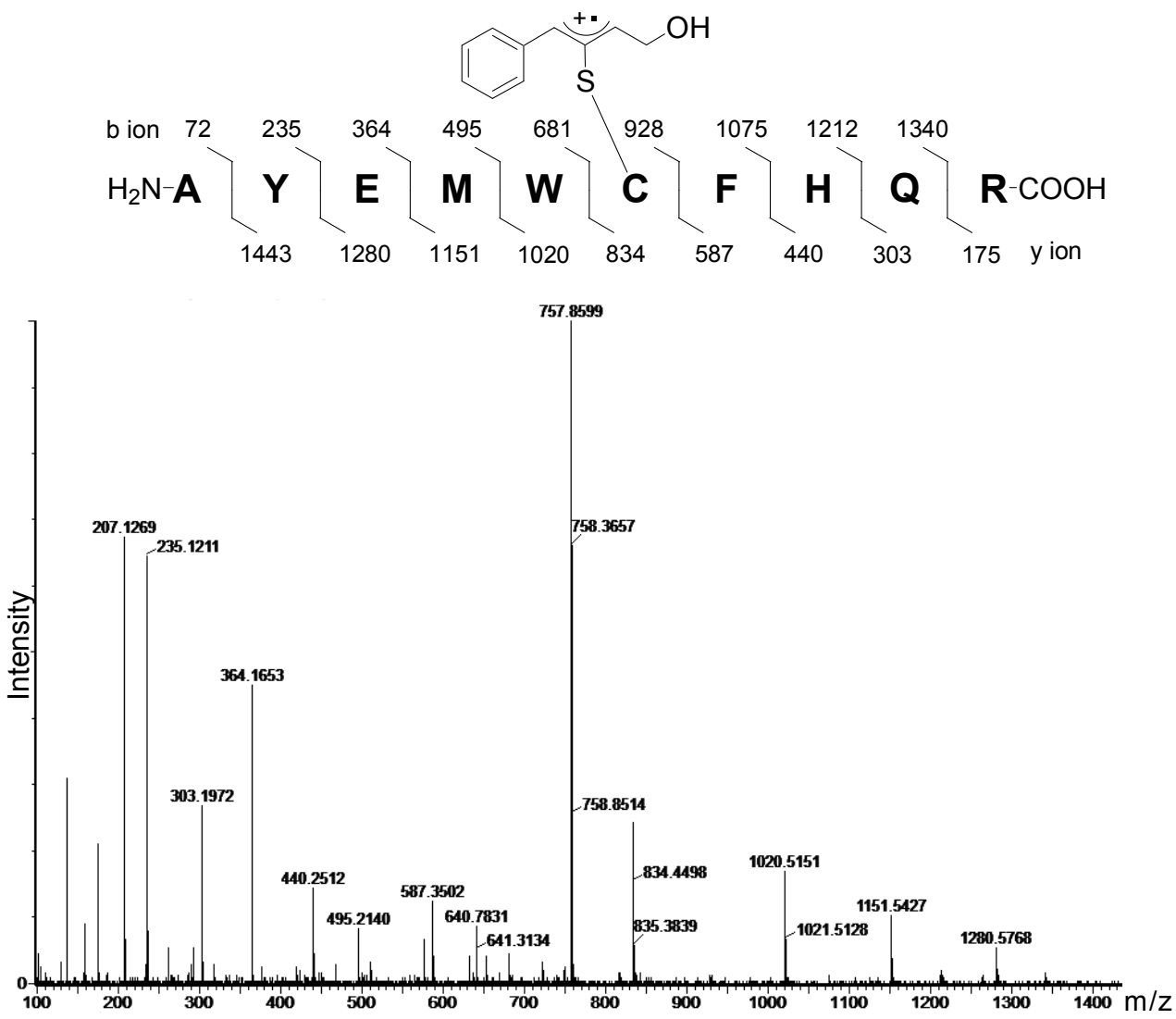

Figure S-11. Q-TOF MS/MS spectrum of the cysteine modified peptide AYEMWCFHQR (**3**) using allene **1g** (ESI source, doubly charged ion of *m/z* = 766.8). The cysteine modified peptide dehydrated ([M-18] Da) during mass spectrometric analysis (ESI source, doubly charged ion of *m/z* = 757.8).

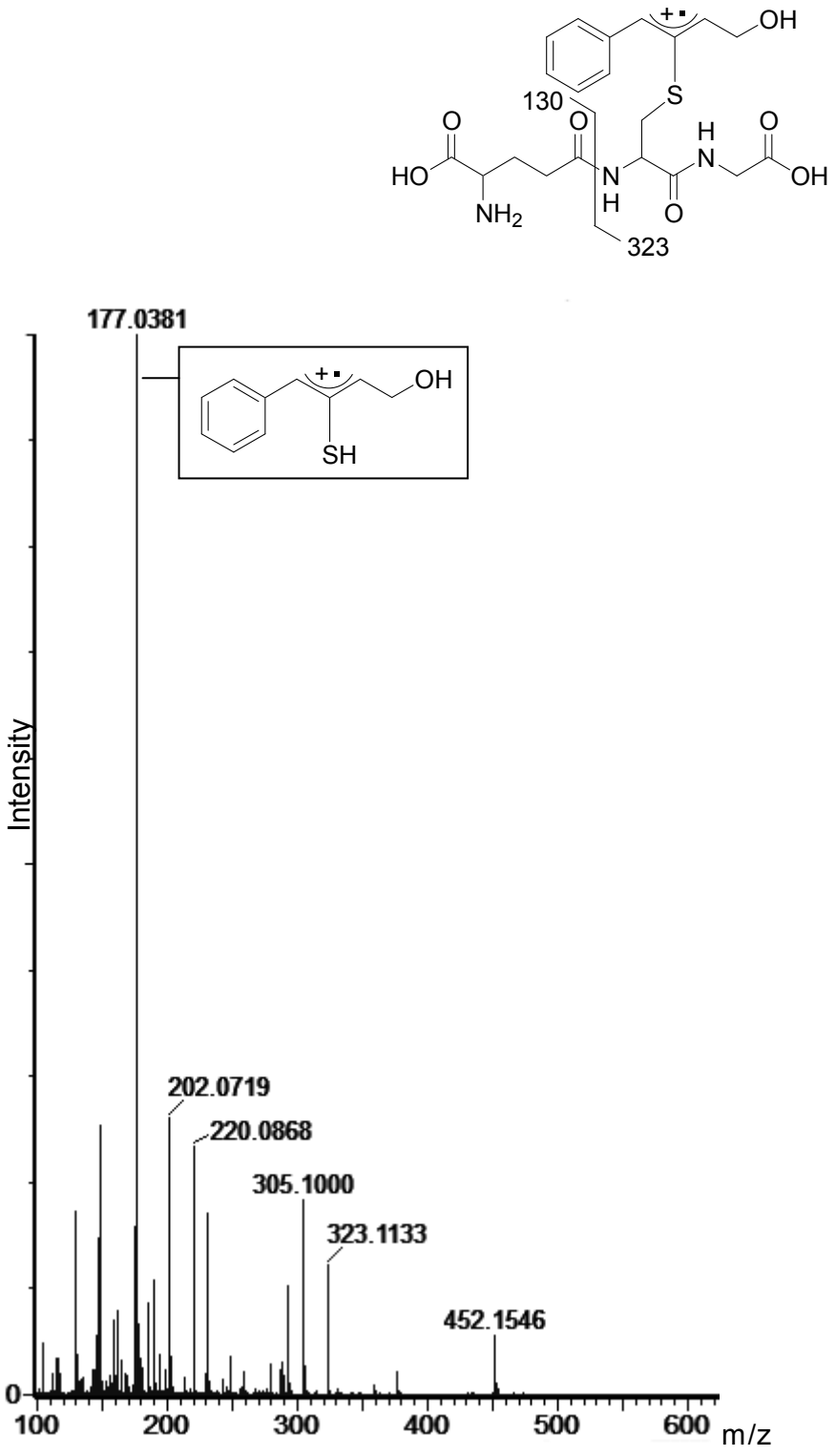

Figure S-12. Q-TOF MS/MS spectrum of the cysteine modified glutathione (GSH) (**4**) using allene **1g** (ESI source, doubly charged ion of *m/z* = 470.1). The cysteine modified peptide dehydrated ([M-18] Da) during mass spectrometric analysis (ESI source, doubly charged ion of *m/z* = 452.1).

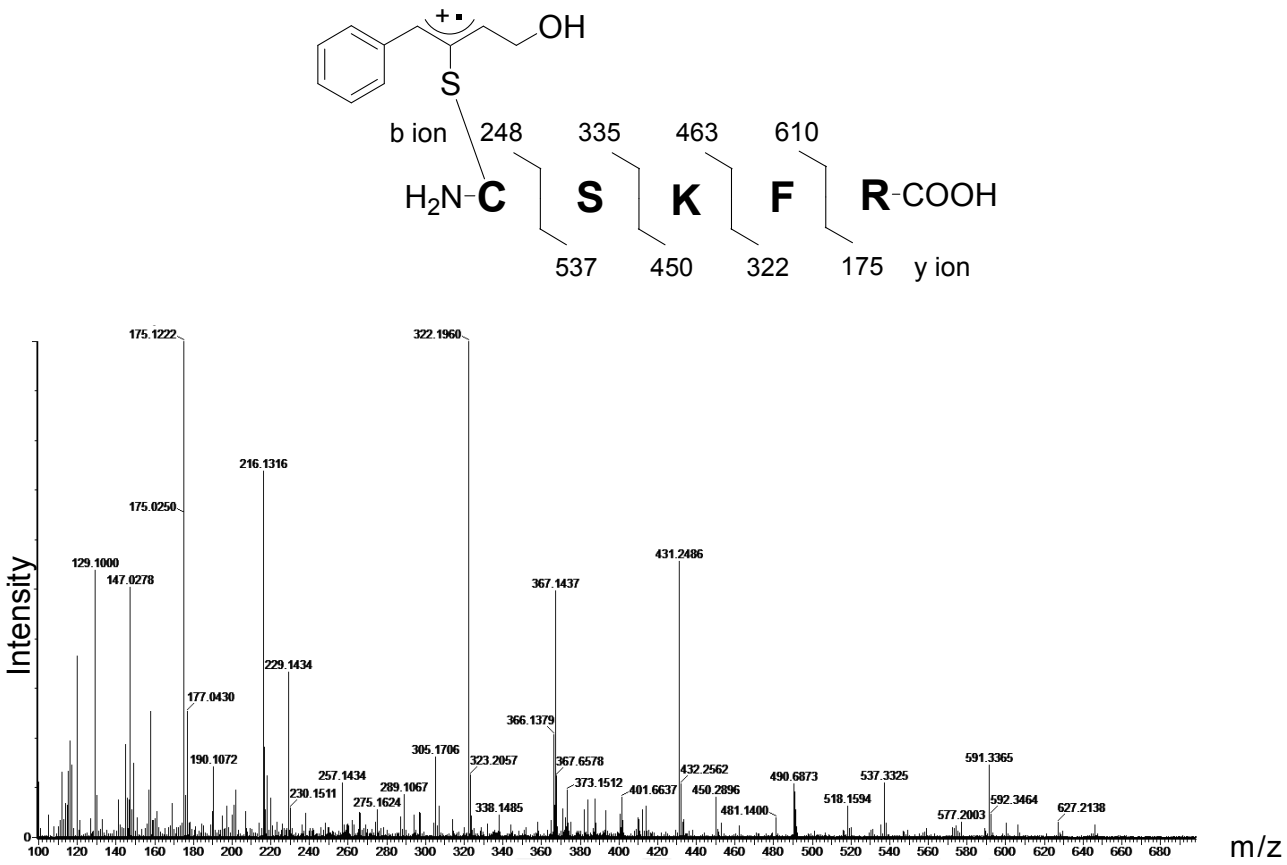

Figure S-13. Q-TOF MS/MS spectrum of the cysteine modified peptide CSKFR (**5**) using allene **1g** (ESI source, doubly charged ion of *m/z* = 401.7). The cysteine modified peptide dehydrated ([M-18] Da) during mass spectrometric analysis (ESI source, doubly charged ion of *m/z* = 392.7).

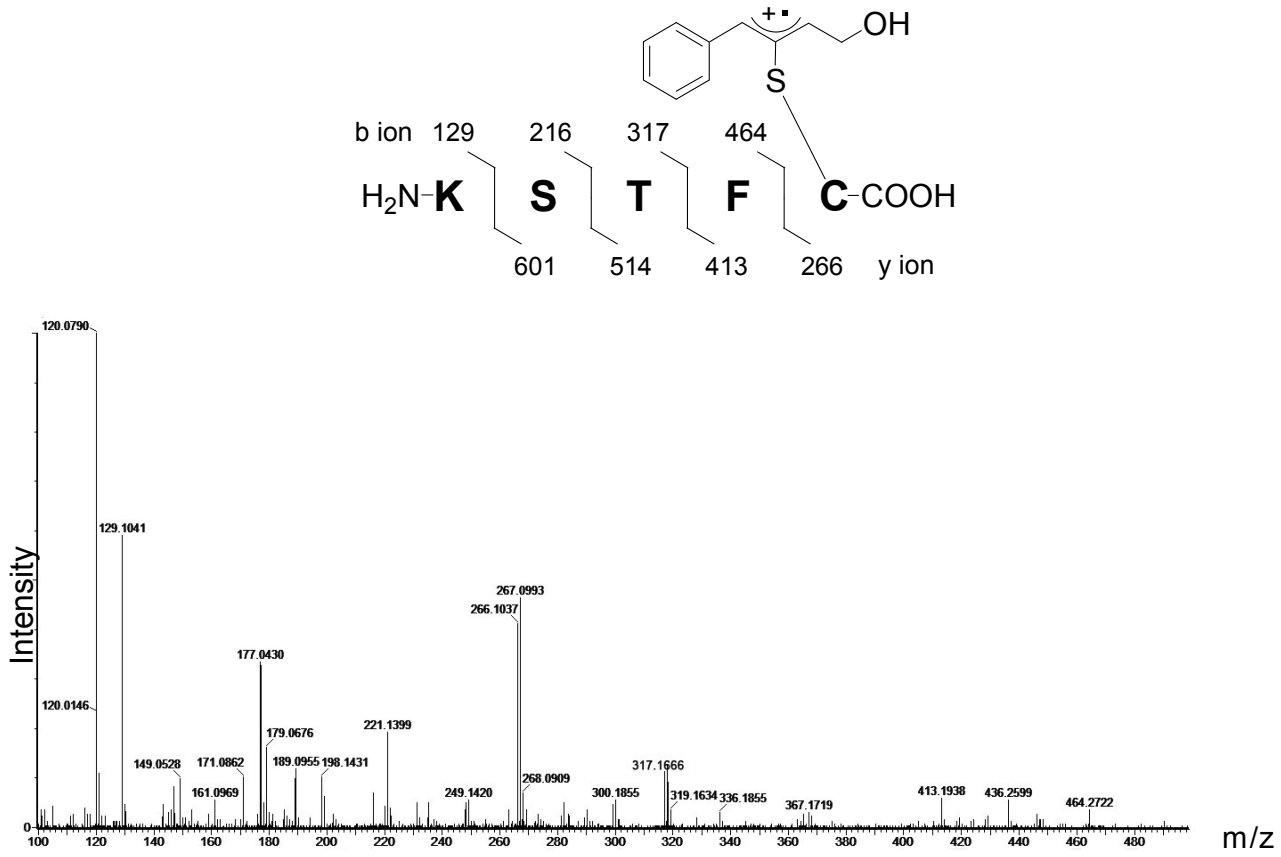

Figure S-14. Q-TOF MS/MS spectrum of the cysteine modified peptide KSTFC (**6**) using allene **1g** (ESI source, doubly charged ion of *m/z* = 374.2). The cysteine modified peptide dehydrated ([M-18] Da) during mass spectrometric analysis (ESI source, doubly charged ion of *m/z* = 365.2).

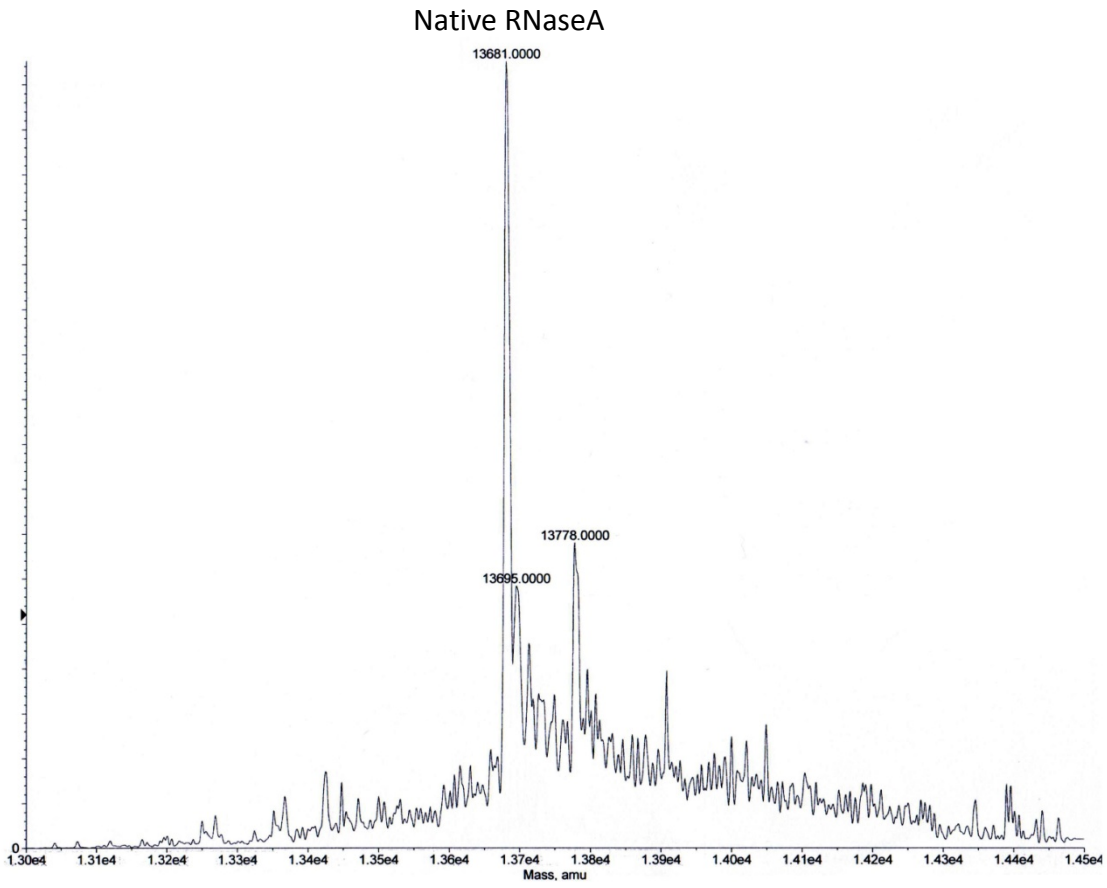

Figure S-15. Mass reconstruction spectrum in LC-MS analysis of native RNaseA.

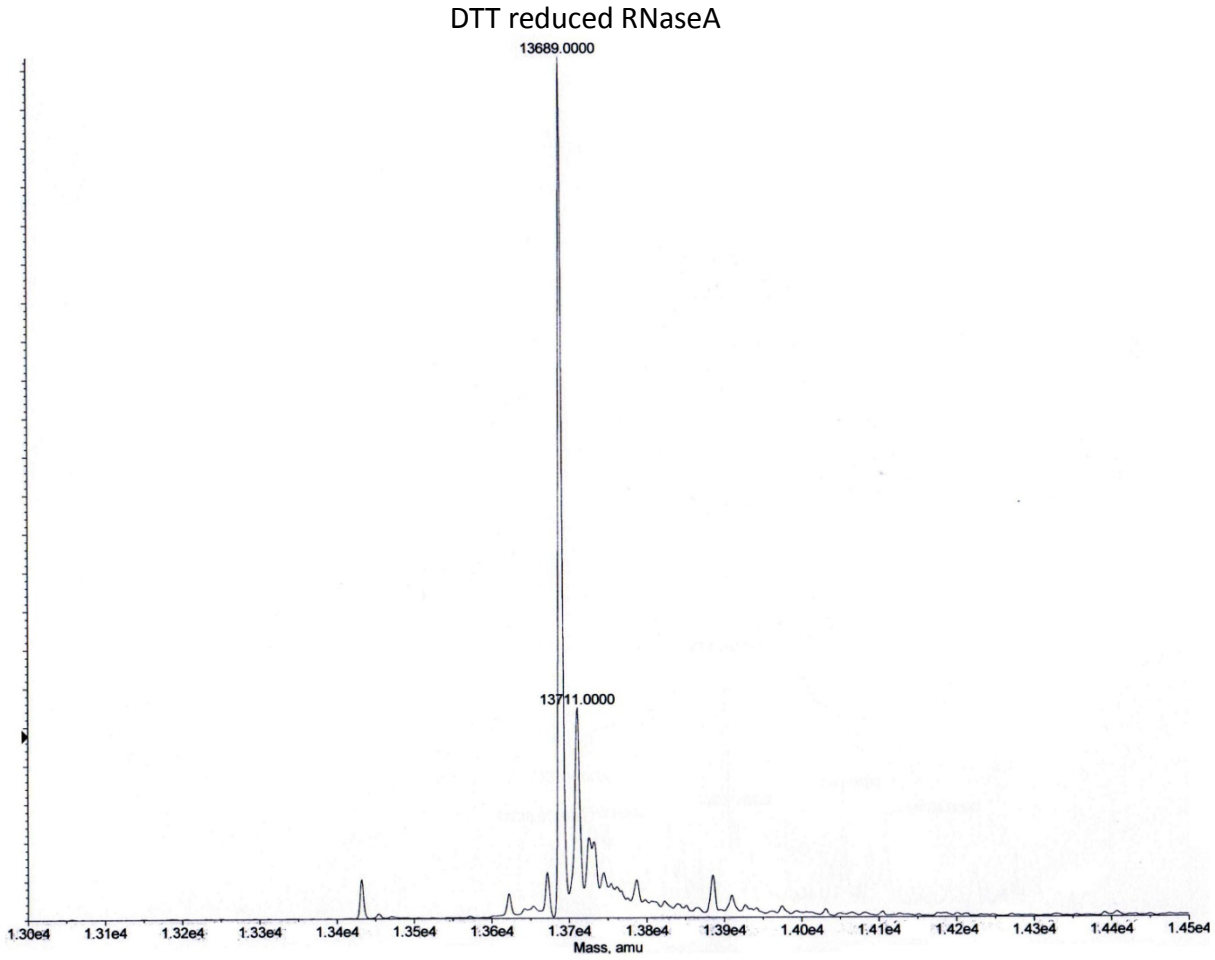

Figure S-16. Mass reconstruction spectrum in LC-MS analysis of the reaction mixture in the DTT reduction of RNaseA.

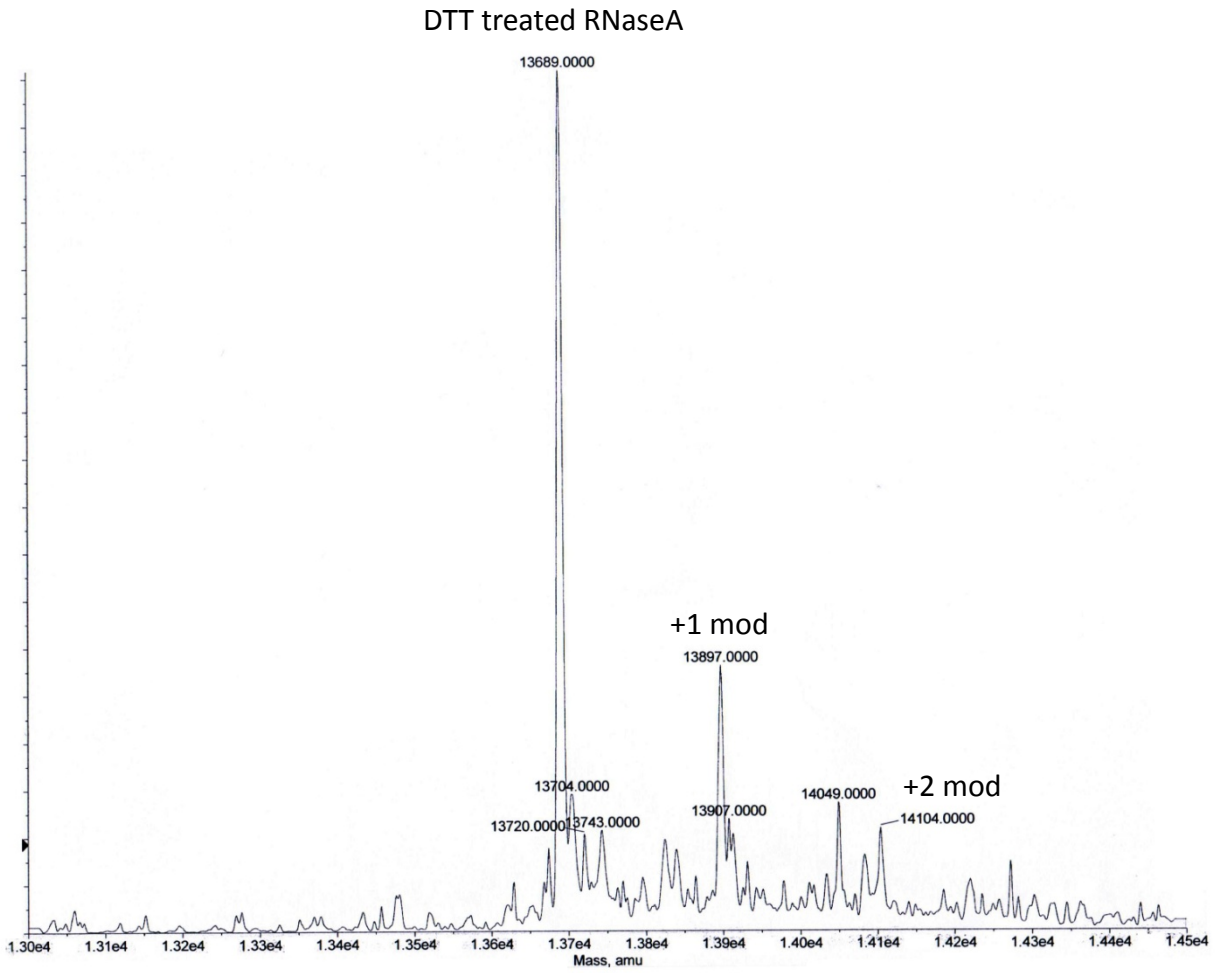

Figure S-17. Mass reconstruction spectrum in LC-MS analysis of the reaction mixture in the modification of RNaseA polypeptide.

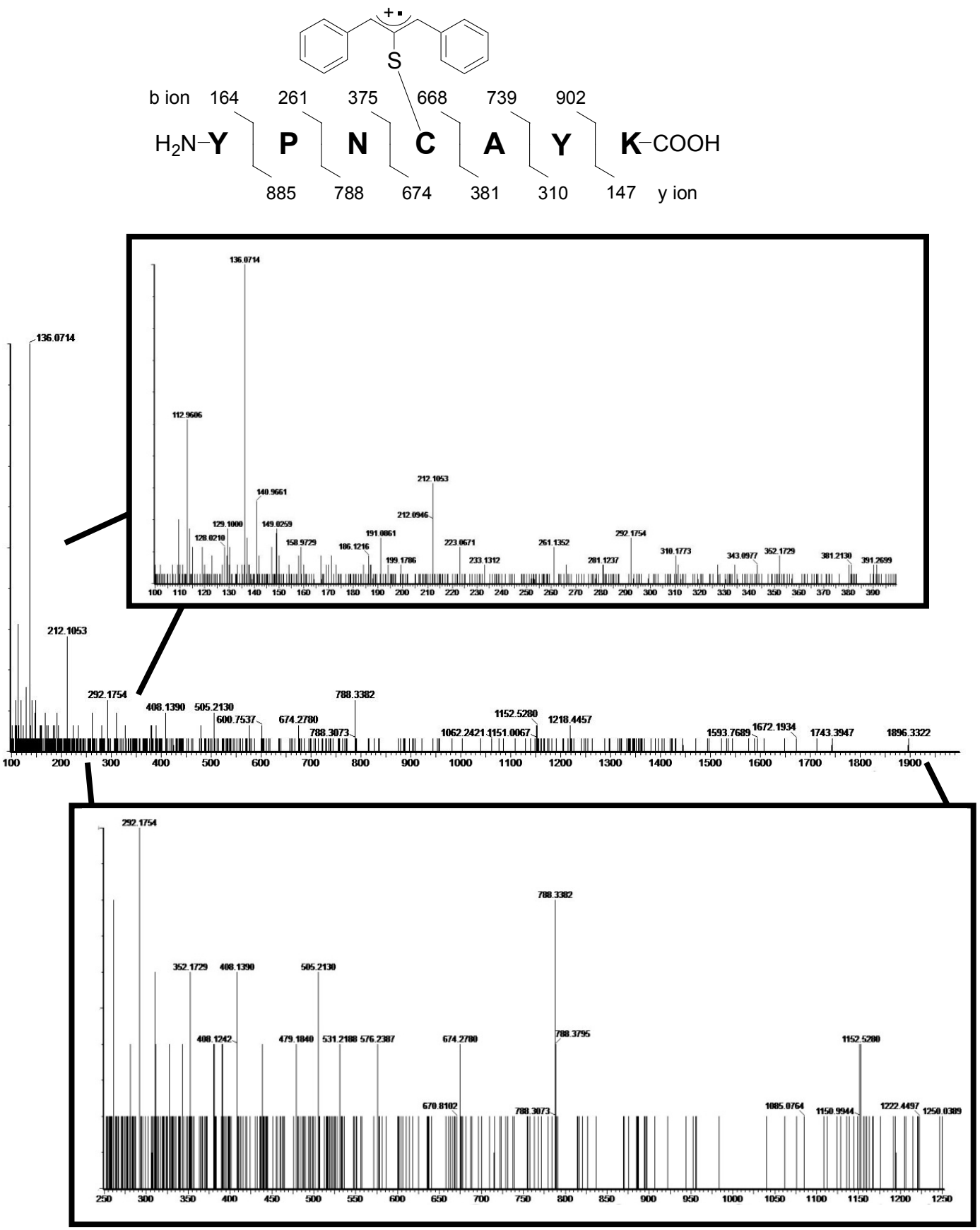

Figure S-18. MS/MS spectrum of the trypsin digested reaction mixture of modified RNaseA polypeptide.