Supporting Information

A Water-Soluble Ruthenium Glycosylated Porphyrin Catalyst for Carbenoid Transfer Reactions in Aqueous Media with Applications in Bioconjugation Reactions

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Experimental Section

General. Myoglobin (M1882) from horse heart, ubiquitin (U6253) from bovine erythrocytes, insulin (I5500) from bovine pancreas, and RNase A (R5500) from bovine pancreas were purchased from Sigma and used without further purification. Peptides PPGFSPFR, GGG, ALILTLVS, HDMNKVLDL, and DRVYIHPFHL were also obtained from commerical sources, and were used without further purification. Water (ddH_2O) used in biological procedures or as reaction solvent was deionized using a NANOpure^{TM} purification system (Barnstead, USA). H_2(4-(Ac_4Glc)-TPP) was synthesized according to the published procedure.[1] [Ru^{II}(4-Cl-TPP)(CO)]-PEG (**2**, ruthenium porphyrin loading: 0.143 mmol/g) was prepared as described in our previous work.[2] Peptides YTSSSKNVVR, YLSGANLNL, TYGPVFMSL, AYEMWCFHQK, and SCSSCPLSSK were synthesized according to the standard procedure of solid phase Fmoc-peptide synthesis using Wang resin as solid support, and were purified by preparative reversed-phase HPLC equipped with C18 column using CH_3CN/H_2O/TFA as the solvent system. The amino acid sequence of the peptides was confirmed by tandem mass spectrometric (MS/MS) analysis. Flash chromatography was performed using silica gel 60 (230–400 mesh ASTM) with EtOAc/hexane as eluent. GC analysis was conducted by using an HP-5890 series II gas chromatograph equipped with a flame ionization detector and an HP-17 capillary column using N_2 as carrier gas. ^1H and ^{13}C NMR spectra were recorded on a Bruker DPX-300 or DPX-400 spectrometer. Chemical shifts (ppm) were referenced to TMS. IR spectra were recorded on a Bio-Rad FT-IR spectrometer (KBr pellet). UV-vis spectra were obtained on an HP 8453 diode array spectrophotometer. Mass spectra were measured on a Finnigan MAT 95 or Finnigan LCQ mass spectrometer.

The organic products in the catalytic inter- and intramolecular cyclopropanation, intramolecular ammonium/sulfonium ylide formation/[2,3]-sigmatropic rearrangement, and intermolecular carbenoid N–H insertion reactions were identified by comparing their spectral and physical data with the reported ones.[3]

Preparation of Diazo Compound 15. 2-Aminoethanol (0.2 g, 3.3 mmol) was added to a solution of dansyl chloride (0.3 g, 1.1 mmol) and Et_3N (0.22 g, 2.2 mmol) in CH_2Cl_2 at 0 ^oC for 1 h. The reaction

mixture was allowed to warm to RT and stirred for an additional 1 h. The reaction was quenched by adding water (5 mL), and extracted with CH_2Cl_2 (5 mL × 2). The combined organic layer was washed with brine (10 mL) and dried over anhydrous Na_2SO_4. Intermediate **A** (0.26 g, 85%; Scheme S1) was collected and purified by a silica gel column with hexane/EtOAc (2:1 v/v) as eluent.

Freshly distilled diketene (0.1 g, 1.4 mmol) in THF was added dropwise to a solution of intermediate **A** (0.2 g, 0.71 mmol) and Et_3N in THF (10 mL) over 10 min at RT. After 4 h, the reaction was quenched by water (5 mL) and extracted with EtOAc (10 mL × 2). The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. Intermediate **B** (0.22 g, 82%; Scheme S1) was collected and purified by a silica gel column using hexane/EtOAc (2:1 v/v) as eluent.

MsN_3 (0.1 g, 0.8 mmol) in dried CH_3CN (3 mL) was added to a solution containing intermediate **B** (0.2 g, 0.53 mmol) and Et_3N (0.1 g, 1.06 mmol) in CH_3CN (5 mL) over 20 min. The reaction mixture was allowed to stir at RT for 5 h. An aqueous solution of LiOH was added to the reaction mixture and stirred for an additional 6 h. After the solvent was removed, the residue was redissolved in EtOAc (15 mL) and water (5 mL). The mixture was extracted with EtOAc (10 mL × 2). The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. Purification by a silica gel column with hexane/EtOAc (2:1 v/v) as eluent gave **15** as a yellow solid (130 mg, 68%). R_f = 0.25 (hexane/EtOAc 2:1 v/v); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.55 (d, J = 7.3 Hz, 1H), 8.25 (m, 2H), 7.53 (m, 2H), 7.16 (d, J = 7.6 Hz, 1H), 5.42 (t, J = 6.0 Hz, 1H), 4.55 (s, 1H), 4.07 (m, 2H), 3.19 (m, 2H), 2.87 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 M Hz): δ 42.95, 45.89, 46.72, 60.92, 63.52, 115.71, 119.10, 123.65, 128.92, 130.01, 130.07, 130.42, 131.15, 135.09, 152.55; EIMS: *m/z* (relative intensity) 362 (21), 336 (11), 334 (12), 276 (5), 184 (7), 171 (100), 149 (31); HRMS (EI): *m/z* for $C_{16}H_{18}N_4O_4S^+$: calcd 362.1049, found 362.1048.

Preparation of *N*-Hydroxysuccinimide Ester 19. (4-Vinylphenyl)acetic acid (50 mg) was allowed to react with *N*-hydroxysuccimde (58 mg) in dry CH_2Cl_2 (10 mL) containing 1.5 equivalents of 1,3-dicyclohexylcarbodiimide (DCC) at RT for 12 h.[4] The product was purified by a silica gel column using hexane/EtOAc as eluent, giving **19** as a colorless white solid (yield: 65%). R_f = 0.5 (hexane/EtOAc 1:1 v/v); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25–7.7.39 (m, 4H), 6.70 (dd, J = 10.9, 17.6 Hz, 1H), 5.73 (dd, J

= 0.9, 17.6 Hz, 1H), 5.23 (dd, *J* = 0.8, 10.9 Hz, 1H), 3.63 (s, 2H), 2.70 (s, 4H); EIMS: *m/z* (relative intensity) 259 (14), 144 (30), 118 (8), 117 (100); HRMS (EI): *m/z* for $C_{14}H_{13}NO_4^+$: calcd 259.0845, found 259.0844.

Preparation of Alkene-Tethered Lys^6 Ubiquitin (23). Ubiquitin (10 mg) was first dissolved in 1 mL of PBS at pH 7.4. Two equivalents of **19** (100 mM in DMSO) were added to ubiquitin on ice for 2 h. The modified ubiquitin mixture was separated by a reversed-phase HPLC (Waters 2690 system) on a Waters Xterra RP C18 column (4.6 mm × 150 mm, 5 μm particle size) using a water/MeCN linear gradient containing 0.05% TFA in both mobile phases. The elution gradient started at 20% MeCN and increased to 70% MeCN in a 40 min period. Elution was monitored with an inline diode array detector (DAD). The fraction that was collected at 20 min showed a mass of 8710 a.m.u., which corresponds to mono-alkene tethered ubiquitin. The eluted protein band was digested with trypsin and the resulting tryptic digested peptides were analysed by LC-MS/MS. LC-MS gave a new doubly charged ion peak at *m/z* 705.4, which corresponds to Met^1-Lys^{11} peptide with the incorporation of alkenic group (*m/z* 144). MS/MS analysis further confirmed that alkene was site-specifically incorporated at Lys^6.

Typical Procedure for 1- or 2-Catalyzed Intramolecular Cyclopropanation of Allylic Diazoacetates. A solution of allylic diazoacetate (1 mmol) in a water/dioxane (9:1 mL) mixture was added dropwise to a solution of **1** or **2** (0.01 mmol) in water (10 mL) via a syringe pump over 24 h at RT. The reaction mixture was stirred until all the starting material was consumed, as monitored by TLC. The aqueous phase was extracted with Et_2O (15 mL × 3), and the combined organic extracts were dried over anhydrous Na_2SO_4. Pure cyclopropanation products were obtained by flash chromatography.

Typical Procedure for 1- or 2-Catalyzed Intramolecular Ammonium/Sulfonium Ylide Formation/[2,3]-Sigmatropic Rearrangement Reactions. A solution of diazo compound (0.5 mmol) in a water/dioxane (4.5:0.5 mL) mixture was added dropwise to a solution of **1** or **2** (0.005 mmol) in water (5 mL) via a syringe pump over 2 h at 50 ^oC. The reaction mixture was stirred until all the substrate was consumed, as monitored by TLC. The aqueous phase was extracted with Et_2O (15 mL × 3)

and the combined organic extracts were dried over anhydrous Na_2SO_4. Pure cyclic products were obtained by flash chromatography.

Typical Procedure for Recycling of Catalyst 2. After completion of the reaction, the organic products were separated from the reaction mixture by extracting with hexane-diethyl ether (1:1 v/v). The catalyst retained in the aqueous solution was reused in a next cycle. The catalyst loss in each recycle was determined through analysis of the Ru content by inductive coupling plasma-mass spectrometry (ICP-MS).

Biological Mass Spectrometry. Protein samples were co-crystallized using a sinapinic acid (SA) solution (10 mg/mL in MeCN/ddH_2O 1:1 with 0.1% TFA). MALDI-TOF mass spectra of protein molecules were recorded in linear mode using a Voyager-DE STR^{TM} system (Applied Biosystems, USA). Electrospary LC/MS and LC-MS/MS analyses were performed using a hybrid QqTOF mass spectrometer (QSTAR-XL^{TM} system, Applied Biosystems, USA) equipped with an Ionspray source and an Agilent 1100 series cap-LC pump (Agilent Technologies, USA). Protein mass reconstructuion was performed with AnalystQS software (Applied Biosystem). The tryptic digested peptide fragments chromatography was performed using an Agilent ZORBAX® 300SB-C18 reversed phase column (0.3 mm × 150 mm) with a MeCN:ddH_2O gradient mobile phase containing 0.1% formic acid (5 μL/min).

SDS-PAGE Analysis. SDS-PAGE analysis was accomplished on a Mini-Protean apparatus (Bio-Rad, USA) with a 18% polyacrylamide gel, following the general protocol of Laemmli.[5] Commercially available markers (Invitrogen, Carlsbad) were applied for calculation of apparent molecular weights. After electrophoresis, fluorescent bands were visualized and recorded by UV transillamination (Alphaimager 2200, Alpha Innotech Corp.). The gel was stained with Colloidal Coomassie Brilliant Blue G for visualization of proteins.

General Procedure for Trypsin Digestion of Modified Proteins. 50 μL of purified protein was diluted with 400 μL of 50 mM NH_4HCO_3 buffer, and then treated with 20 μL of a sequencing grade modified trypsin (Promega, 20 μg reconstituted wiht 150 μL of 50 mM acetic acid). The mixture was

incubated at 37 °C for 12 h. The resulting peptides were separated and analyzed by LC-MS/MS (QSTAR system, Applied Biosystems, USA).

Calculation of Solvent-Accessible Area of Proteins. The nitrogen atom solvent accessibility calculations were performed by an internet accessible program GETAREA 1.4[6] using the X-ray crystal structure data of Wild-type bovine pancreatic ribonuclease A (1FS3), horse heart myoglobin (1YMB), di-ubiquitin (1AAR), and insulin before high dose X-ray burn (2BN3). The default parameters for radius of the water probe and default atomic radii and atomic solvent parameters were used for all calculations. Solvent accessiblities for proteins were calculated on an area-per-atom basis.

Comparison of Catalytic Properties

From Tables S1–S5 (see below), it is evident that catalysts **1** and **2** are more active than **3** and **4**, and than $[Ru^{II}(D_4\text{-PorS*})(CO)]^{4-}$ as well (the last complex catalyzed the reaction of **5a** with EDA to give **6a** in 52% yield,[7] though with a higher *trans/cis* ratio due to the sterically-encumbered D_4-PorS* ligand). For the transformation of **11a**,**b** to **12a**,**b**, catalyst **1** is slightly superior to catalyst **2**, but for the other transformations included in Tables S1–S5, **2** is the best catalyst among **1**–**4**.

The carbenoid transfer reactions catalyzed by **2** have the following features:

(i) The cyclopropanation of styrenes **5a**–**e** and allylic diazoacetates **7a**–**d** and the carbenoid N–H insertion of primary arylamines **13a**–**e** afforded **6a**–**e**, **8a**–**d**, **14a**–**e**, respectively, in 80–94% yields (substrate conversions: 80–100%, *trans*/*cis* ratios for **6a**–**e**: (5–11):1). For the cyclopropanation of 1-octene and ethoxyethene (entries 16, 17 in Table S1), the resulting cyclopropanes were formed in 78% yield (*trans*/*cis* 8:1) and 86% yield (*trans*/*cis* 5:1), respectively, with 100% substrate conversion. Product yields of 77–91% were obtained for the carbenoid N–H insertion of *N*-methyl aniline and *N*-methyl butylamine (entries 10, 11 in Table S5).

(ii) The transformation of **9d–f** to **10d–f** (yields: 84–88%) showed a considerable *anti* selectivity with *anti*/*syn* ratios of (2.1–3.2):1 (entries 20–22 in Table S3), unlike the *anti*-**10d**/*syn*-**10d** ratio of 1:1

exclusively obtained using [Ru^{II}(Por)(CO)] catalysts in toluene[3c] (such *anti*-selectivity should be attributed to the effect of water solvent, as the reaction of **9d** catalyzed by **2** in toluene at 50 °C afforded *anti*- and *syn*-**10d** in 1:1.1 ratio with 86% yield, and heating a 1:1 mixture of *anti*- and *syn*-**10d** in an aqueous solution at 50 °C for 2 h did not change the *anti*/*syn* ratio of **10d**).

(iii) Recycling the catalyst four and nine times for the transformations of **5a** → **6a** (entries 3–6 in Table S1) and **9a** → **10a** (entries 3–11 in Table S3), respectively, had no significantly deleterious effect on the catalytic efficiency (each run features a product yield of ~90% and a catalyst loss of < 0.5%), with the respective total product turnover numbers of ~430 over five runs and ~910 over ten runs (the latter is comparable to that of ~880 over 10 runs, with 0.4% to 2.3% catalyst loss in each recycle, for the [$Rh_2(OAc)_4$]-catalyzed intramolecular C–H insertion reaction of diazo compounds in water[8]). In contrast, for the transformation of **5a** → **6a**, recycling [Ru^{II}(D_4-PorS*)(CO)]$^{4-}$ two times considerably lowered the enantioselectivity,[7] and recycling a water-soluble chiral ruthenium 2,6-bis(oxazolinyl)pyridine (pybox) catalyst three times decreased the product yield from 62% to 13% (ee: from 97% to 42%, total product turnover: ~70 over the four runs)[9] (no catalyst recycling was reported for asymmetric cyclopropanation of styrenes catalyzed by a chiral hydrophobic Ru-pybox complex in water, which afforded cyclopropanes in 31–46% yields[10]).

References

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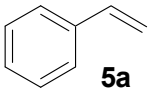
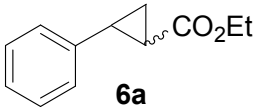
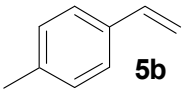
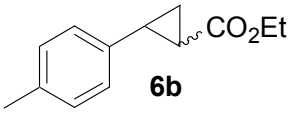
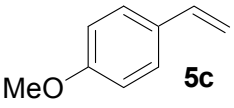
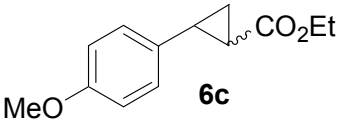
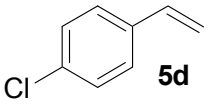
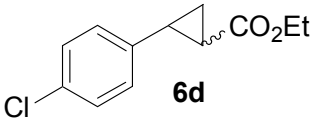
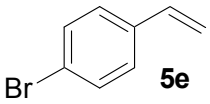
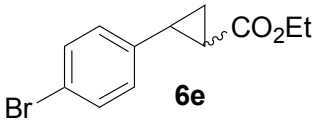
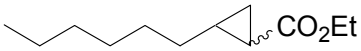
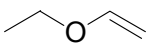
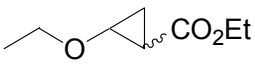
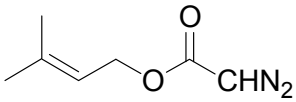
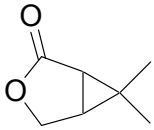
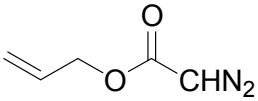
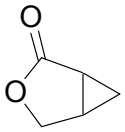
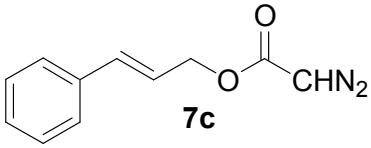
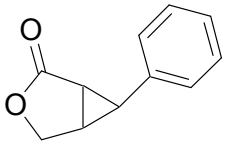
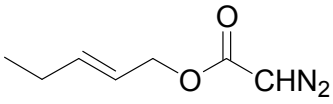
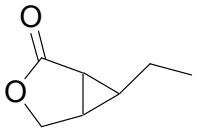
Table S1. Intermolecular Cyclopropanation of Alkenes with EDA Catalyzed by **1** in Aqueous Media.[a] Results Obtained Using Catalysts **2**–**4** Are Also Included.

entry	catalyst	substrate	product	conv (%)	yield (%)[b]	*trans*/*cis* ratio
1	**1**	 **5a**	 **6a**	92	75	5:1
2	**2**			100	90	10:1
3	**2**[c]			95	94	9.5:1
4	**2**[d]			94	92	9.5:1
5	**2**[e]			90	90	9:1
6	**2**[f]			91	88	9:1
7	**2**[g]	catalyst recycling		43	91	10:1
8	**3**			62	58	3.4:1
9	**4**			79	85	7:1
10	**1**	 **5b**	 **6b**	92	76	5:1
11	**2**			100	94	10:1
12	**2**	 **5c**	 **6c**	100	92	11:1
13	**1**	 **5d**	 **6d**	85	70	4:1
14	**2**			85	90	8:1
15	**2**	 **5e**	 **6e**	80	88	7:1
16	**2**			100	78	8:1
17	**2**			100	86	5:1

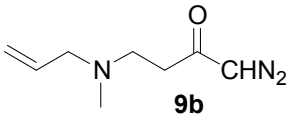
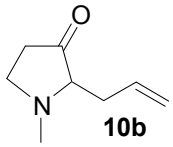
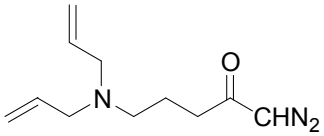
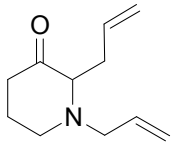
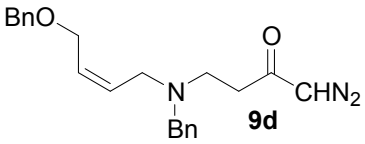
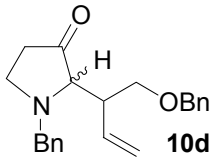
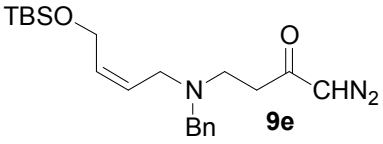
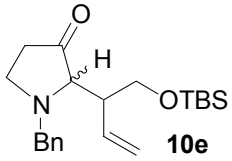
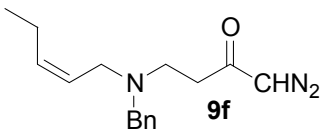
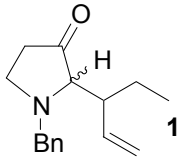
[a] Reaction conditions: substrate (1 mmol), EDA (1.2 mmol), catalyst (1 mol%), H_2O (20 mL), RT, 24 h. Conversion, yield, and *trans*/*cis* ratio were determined by GC. [b] Based on consumed substrate. [c–f] The 1st–4th recycling of catalyst. [g] Catalyst loading: 0.1 mol%.

Table S2. Intramolecular Cyclopropanation of Allylic Diazoacetate Catalyzed by **1** in Aqueous Media.[a] Results Obtained Using Catalyst **2** Are Also Included.

entry	catalyst	substrate	product	yield (%)[b]
1	**1**	 **7a**	 **8a**	68
2	**2**			84
3	**2**	 **7b**	 **8b**	82
4	**2**	 **7c**	 **8c**	86
5	**2**	 **7d**	 **8d**	85

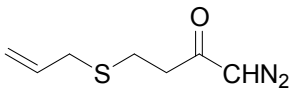
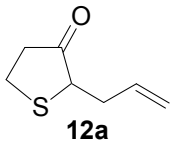
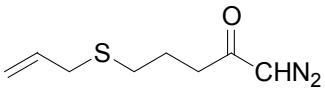
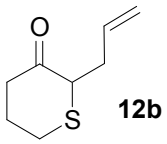
[a] Reaction conditions: substrate (1 mmol) in H_2O/dioxane (9:1 mL) was added over 24 h to the catalyst (1 mol%) in H_2O (10 mL) at RT. Conversion: 100%. [b] Isolated yield.

Table S3. Intramolecular Ammonium Ylide Formation/[2,3]-Sigmatropic Rearrangement Reaction of Diazoketones Catalyzed by **1** in Aqueous Media.[a] Results Obtained Using Catalysts **2**–**4** Are Also Included.

entry	catalyst	substrate	product	conv (%)	yield (%)[b]
1	**1**			100	89
2	**2**			100	94
3	**2**[c]			100	93
4	**2**[d]			100	93
5	**2**[e]			100	92
6	**2**[f]			100	91
7	**2**[g]			100	91
8	**2**[h]			100	90
9	**2**[i]			100	90
10	**2**[j]	catalyst recycling		100	88
11	**2**[k]			100	87
12	**3**			100	81
13	**4**			90	80
14	$[Rh_2(OAc)_4]$			100	21[l]
15	$[Cu(acac)_2]$			100	31
16	**1**	 **9b**	 **10b**	100	83
17	**2**			100	90
18	**1**	 **9c**	 **10c**	100	86
19	**2**			100	92
20	**2**	 **9d**	 **10d**	100	87 (3.2:1[m])
21	**2**	 **9e**	 **10e**	100	84 (2.1:1[m])
22	**2**	 **9f**	 **10f**	100	88 (2.5:1[m])

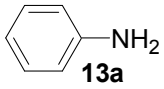
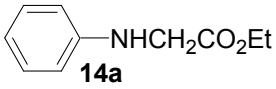
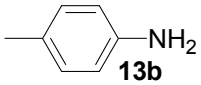
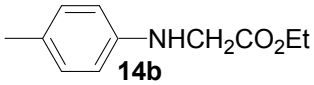
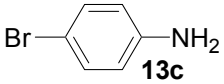
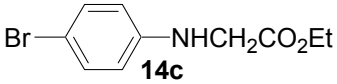
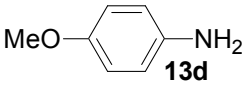
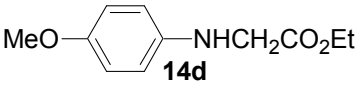
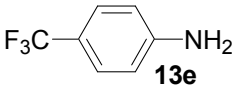
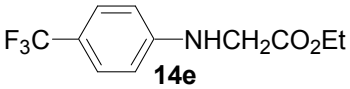
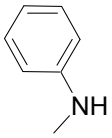
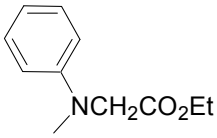
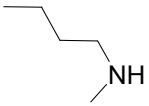
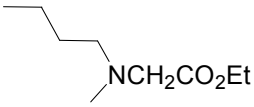
[a] Reaction conditions: substrate (0.5 mmol) in H_2O/dioxane (4.5:0.5 mL), catalyst (1 mol%) in H_2O (5 mL), 50 °C, 2 h. [b] Isolated yield based on consumed substrate. [c–k] 1st–9th recycling of catalyst, respectively. [l] O–H insertion product was isolated in 62% yield. [m] *anti*/*syn* ratio determined by 'H NMR analysis of the crude reaction mixture.

Table S4. Intramolecular Sulfonium Ylide Formation/[2,3]-Sigmatropic Rearrangement Reaction of Diazoketones Catalyzed by **1** in Aqueous Media.[a] Results Obtained Using Catalyst **2** Are Also Included.

entry	catalyst	substrate	product	conv (%)	yield (%)[b]
1	**1**	 **11a**	 **12a**	100	91
2	**2**			100	86
3	**1**	 **11b**	 **12b**	100	90
4	**2**			100	82

[a] Reaction conditions: substrate (0.5 mmol) in H_2O/dioxane (4.5:0.5 mL), catalyst (1 mol%) in H_2O (5 mL), 50 ^oC, 2 h. [b] Isolated yield.

Table S5. Intermolecular N–H Insertion of Amines with EDA Catalyzed by **1** in Aqueous Media.[a] Results Obtained Using Catalyst **2** Are Also Included.

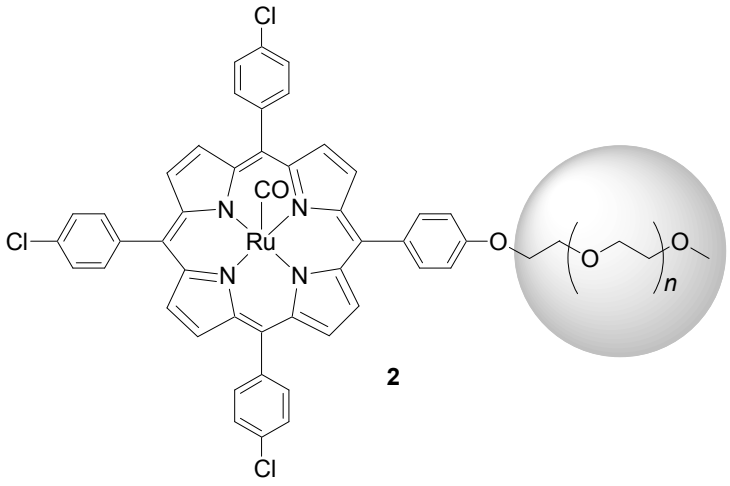
entry	catalyst	substrate	product	conv (%)	yield (%)[b]
1	**1**	 **13a**	 **14a**	100	81
2	**2**			100	87
3	**1**	 **13b**	 **14b**	100	76
4	**2**			100	88
5	**1**	 **13c**	 **14c**	100	82
6	**2**			100	83
7	**1**	 **13d**	 **14d**	100	83
8	**2**			100	90
9	**2**	 **13e**	 **14e**	100	80
10	**2**			100	77
11	**2**			100	91[c]

[a] Reaction conditions: substrate (1 mmol), EDA (0.5 mmol) in H_2O (5 mL), catalyst (1 mol %) in H_2O (5 mL), RT, 10 h. [b] Isolated yield. [c] Determined by 'H NMR spectroscopy of the crude reaction mixture.

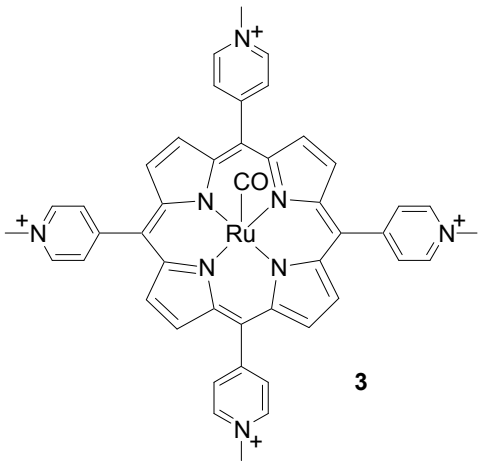
Table S6. Peptide Mass Mapping of Ubiquitin and Alkene-Tethered Ubiquitin (**23**) after Trypsin Digestion.

residues		peptide sequence	*m/z*	
start	end		ubiquitin	**23**
1	11	MQIFVKTLTGK	1265.7	1409.7
12	27	TITLEVEPSDTIENVK	1787.9	1787.9
34	42	EGIPPDQQR	1039.5	1039.5
43	48	LIFAGR	648.4	648.4
49	54	QLEDGR	717.4	717.4
55	63	TLSDYNIQK	1081.6	1081.6
64	72	ESTLHLVLR	1067.6	1067.6

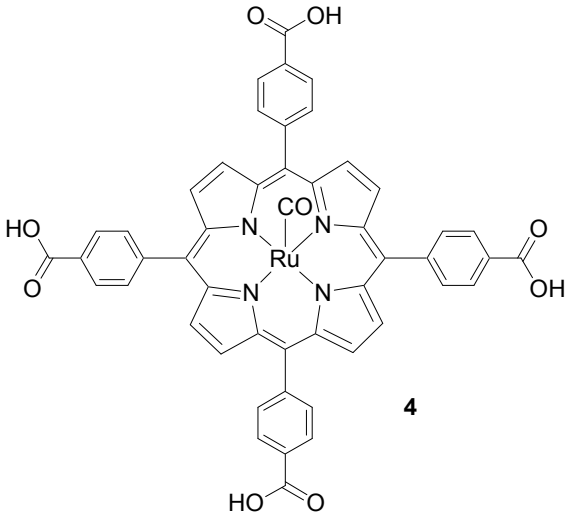
Chart S1. Water-soluble ruthenium porphyrins previously reported in literature.



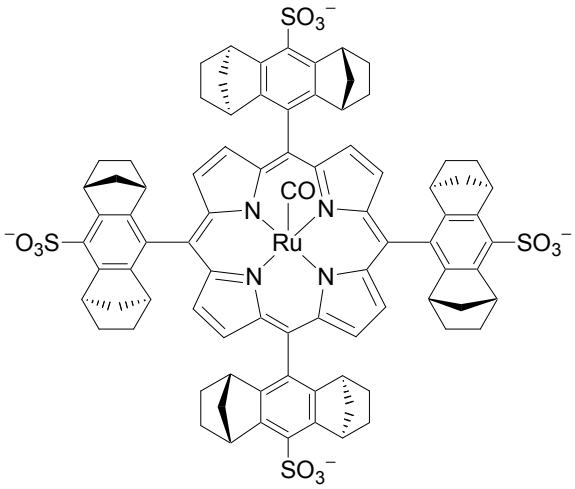
2



3

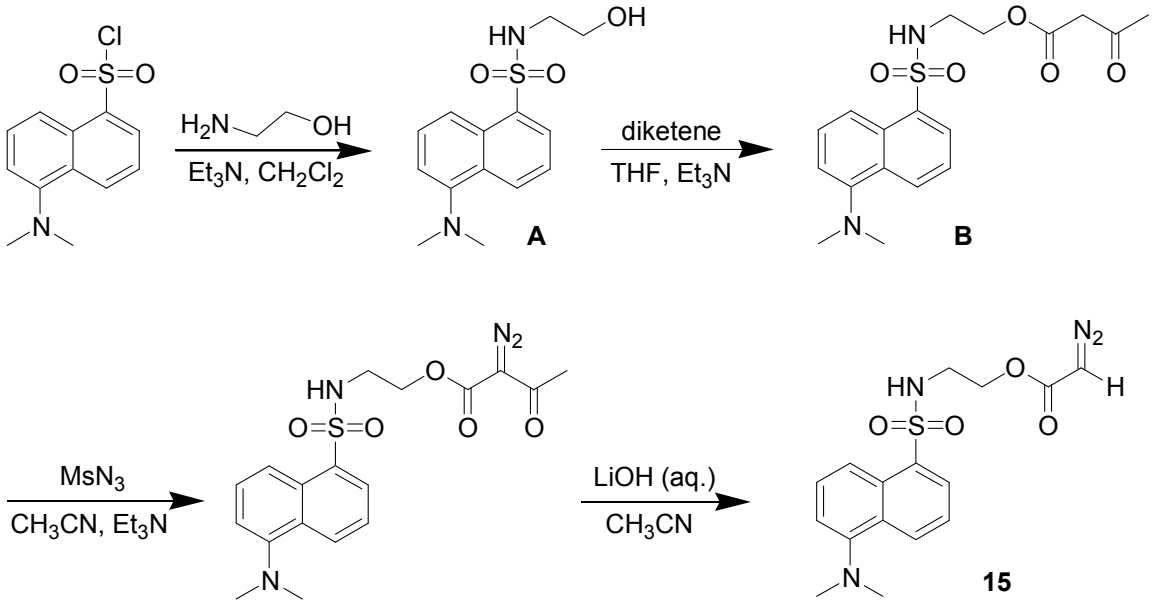


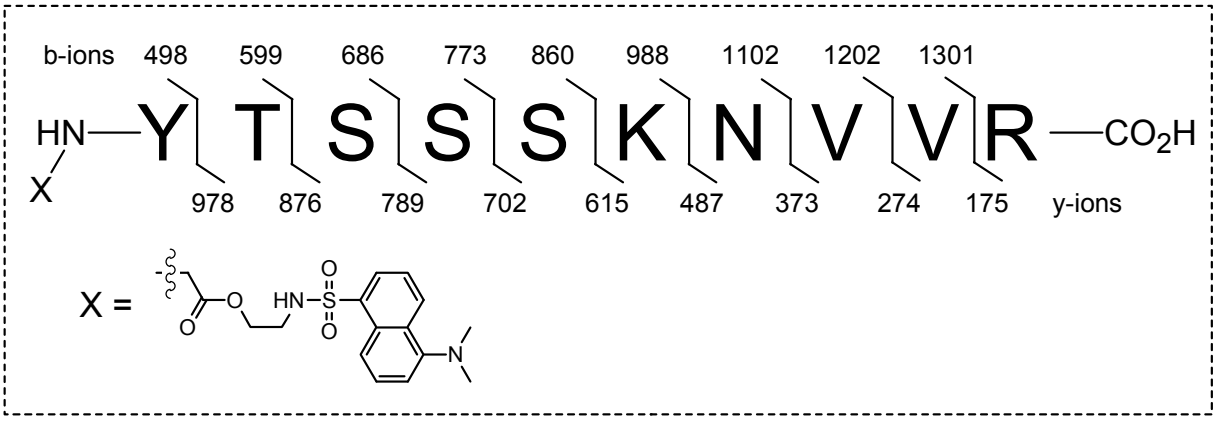
4



$[Ru^{II}(D_4\text{-PorS*})(CO)]^{4-}$

Scheme S1


Cl
O=S=O
N
H2N
OH
Et3N, CH2Cl2
HN
OH
A
diketene
THF, Et3N
O
B
MsN3
CH3CN, Et3N
N2
LiOH (aq.)
CH3CN
H
15



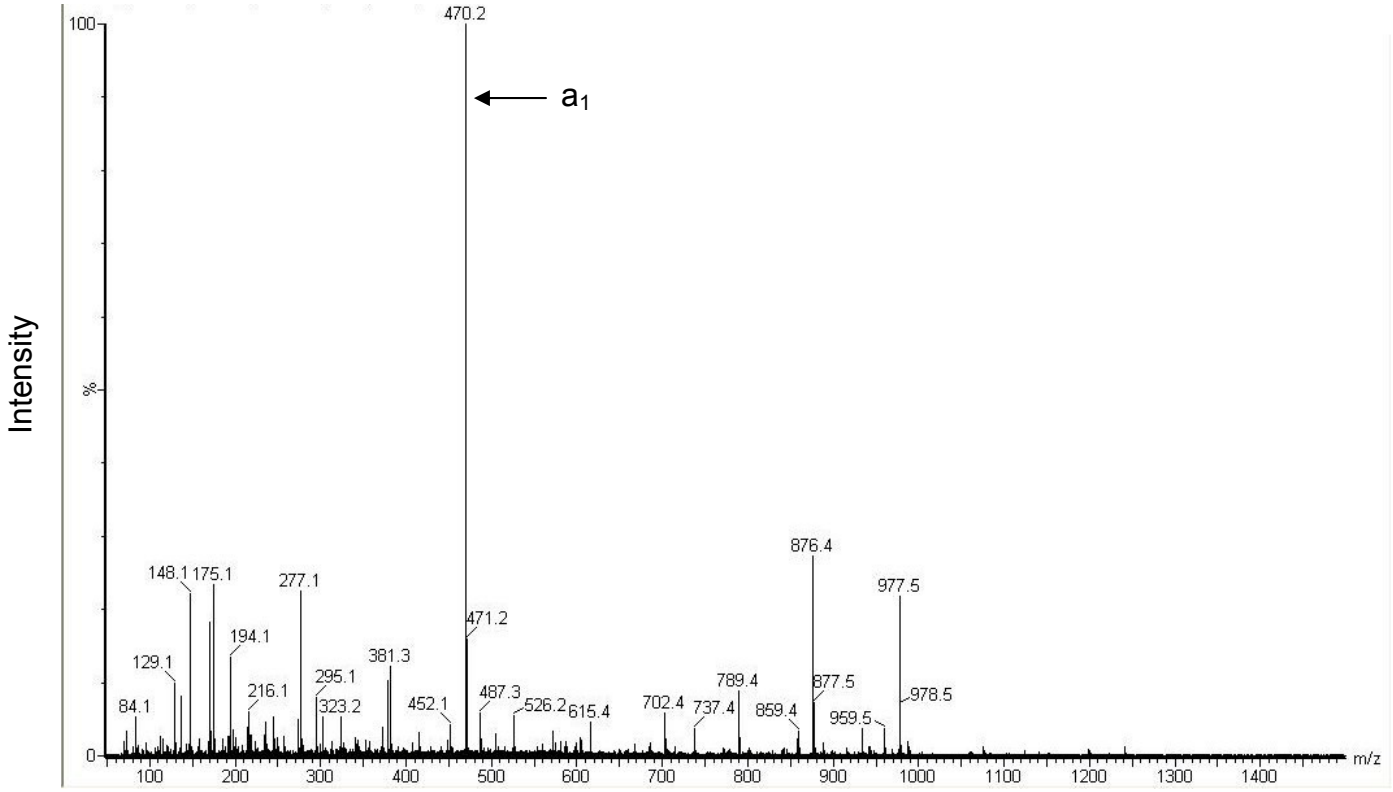


Figure S1. Q-TOF MS/MS spectrum of the N-terminal acylated peptide YTSSSKNVVR (ESI source, doubly charged ion of m/z = 737.8).

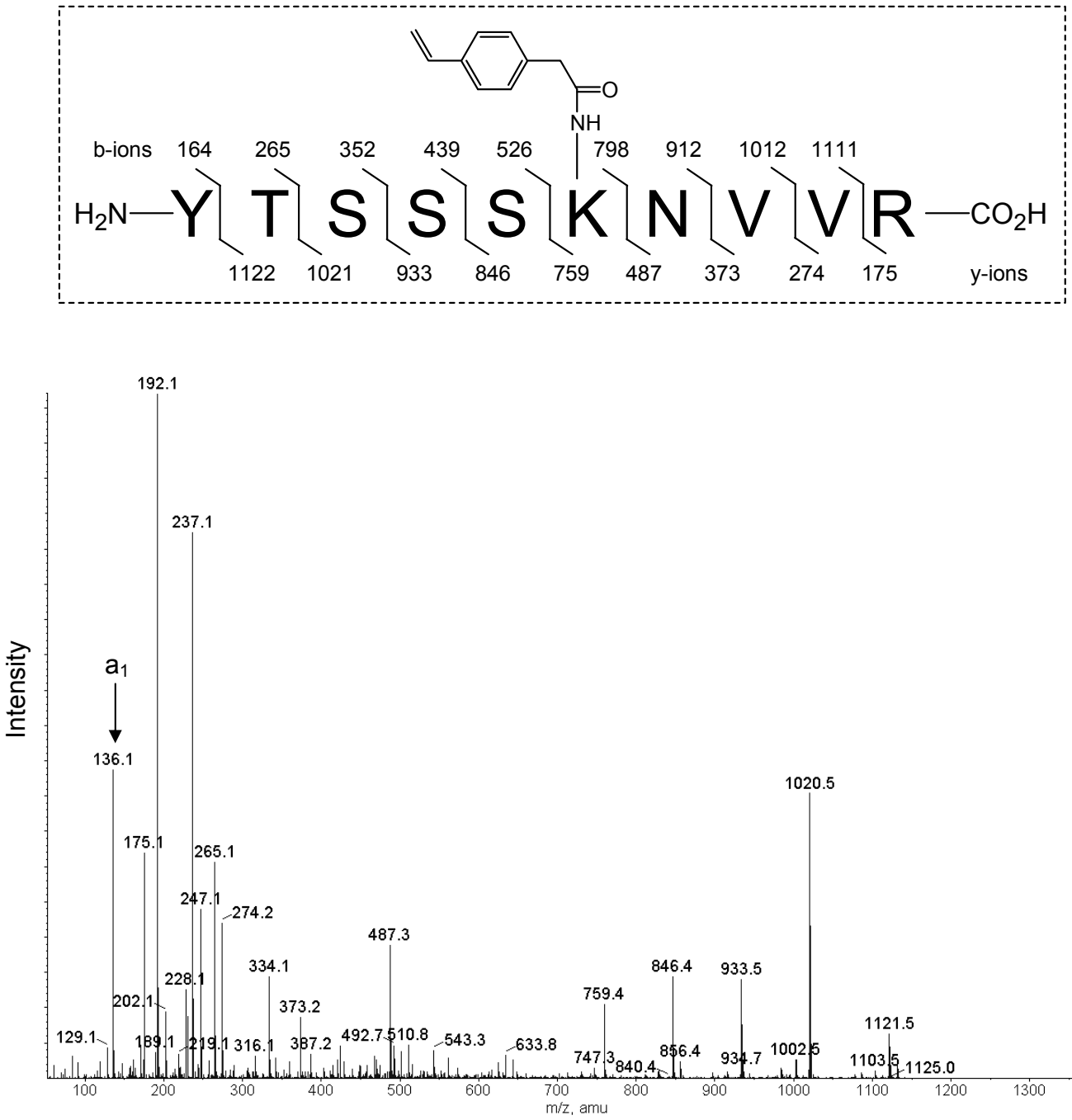


Figure S2. Q-TOF MS/MS spectrum of the lysine acylated peptide YTSSSKNVVR (ESI source, doubly charged ion of m/z = 642.8).

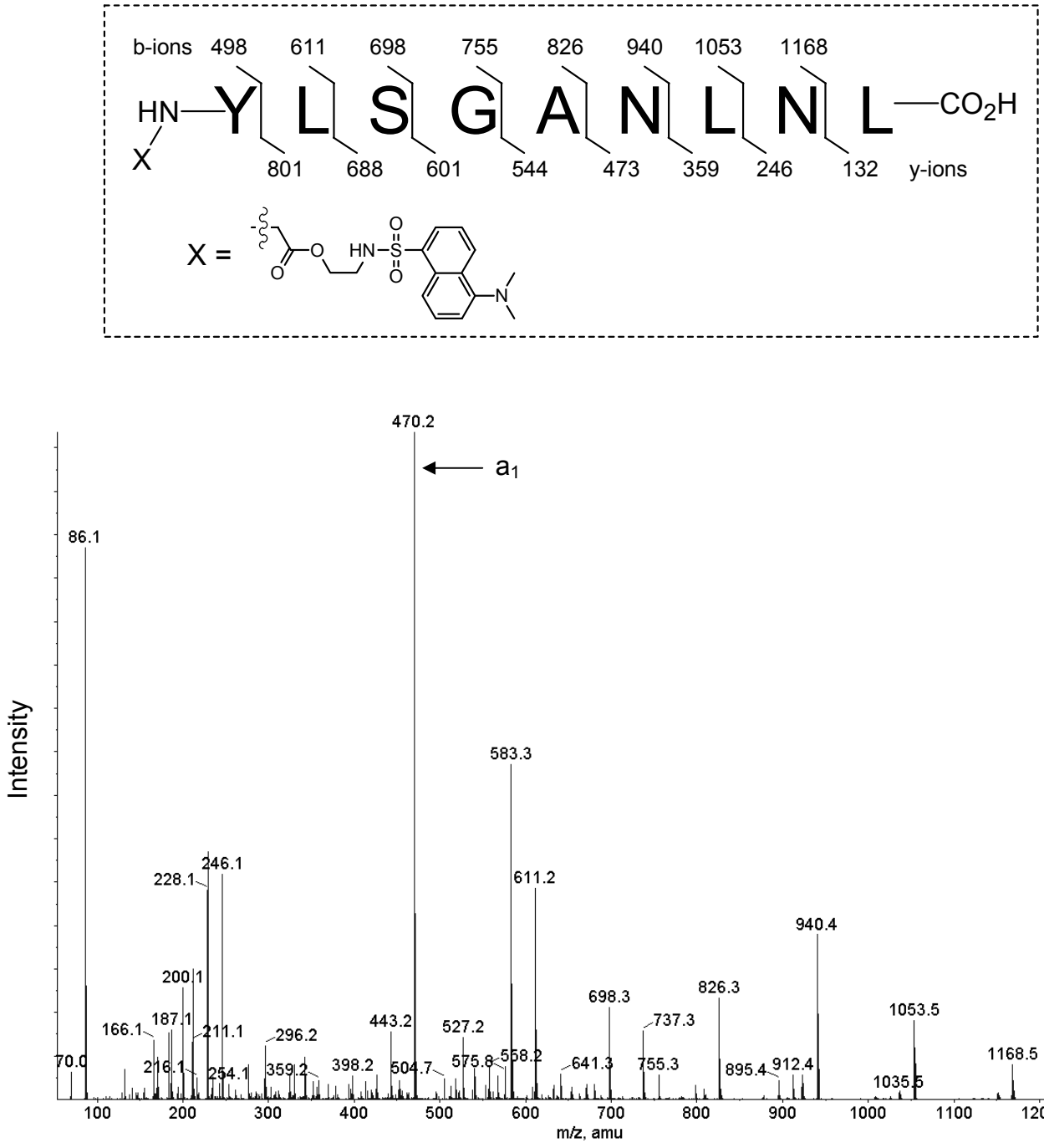
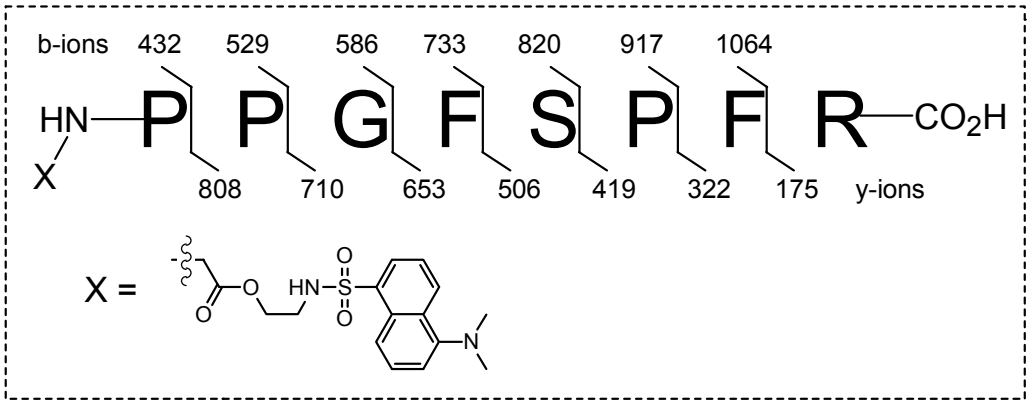


Figure S3. Q-TOF MS/MS spectrum of the N-terminal acylated peptide YLSGANLNL (ESI source, doubly charged ion of m/z = 649.8).



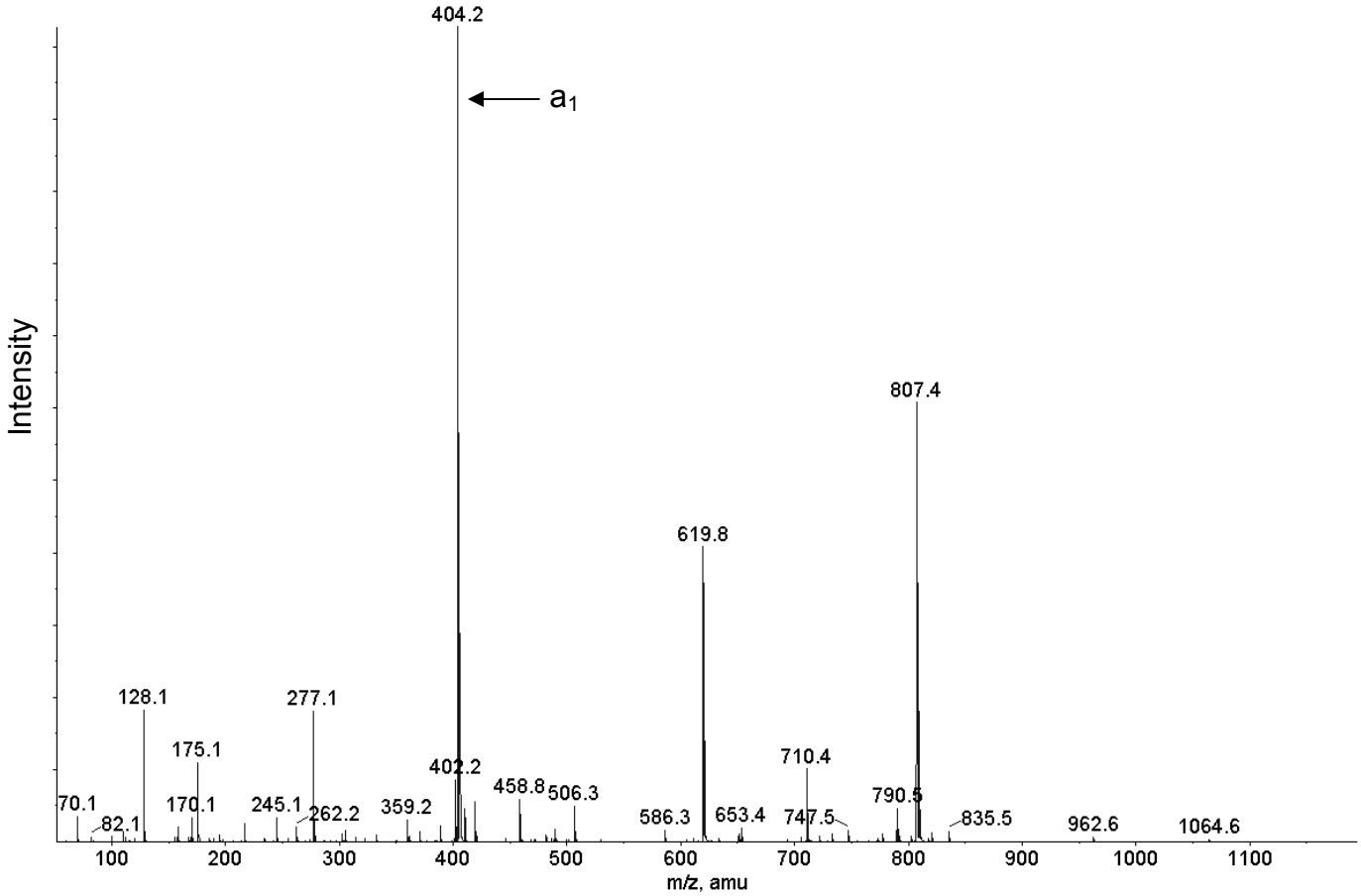


Figure S4. Q-TOF MS/MS spectrum of the N-terminal acylated peptide PPGFSPFR (ESI source, doubly charged ion of m/z = 619.8).

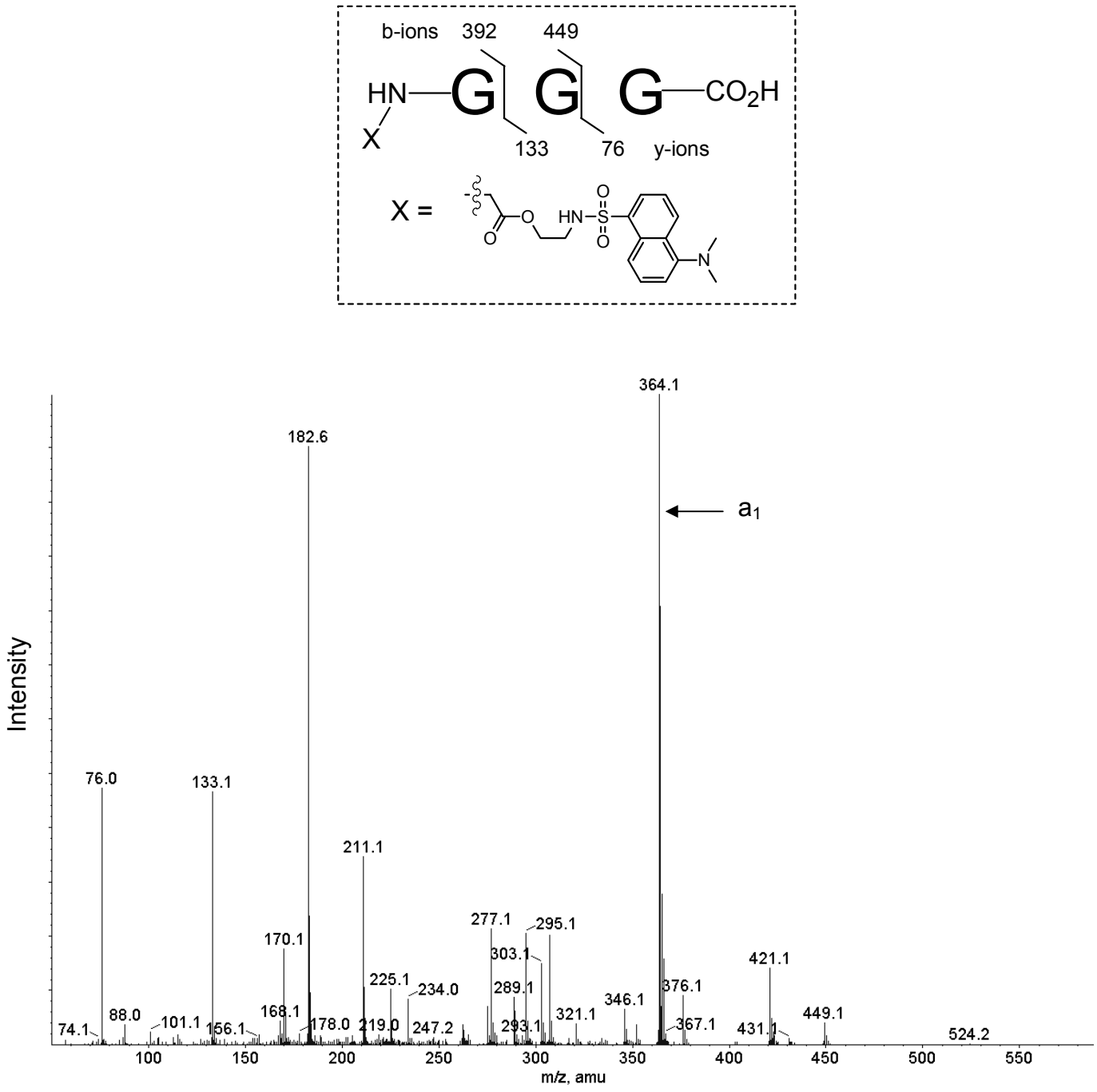
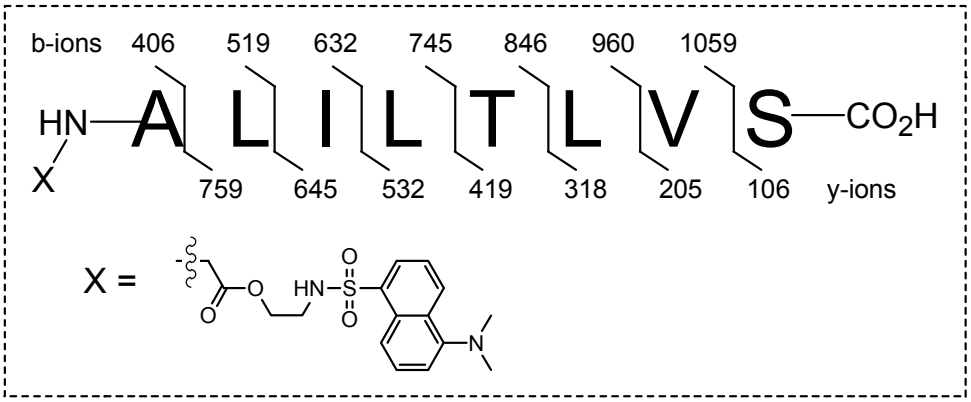


Figure S5. Q-TOF MS/MS spectrum of the N-terminal acylated peptide GGG (ESI source, doubly charged ion of $m/z = 262.5$).



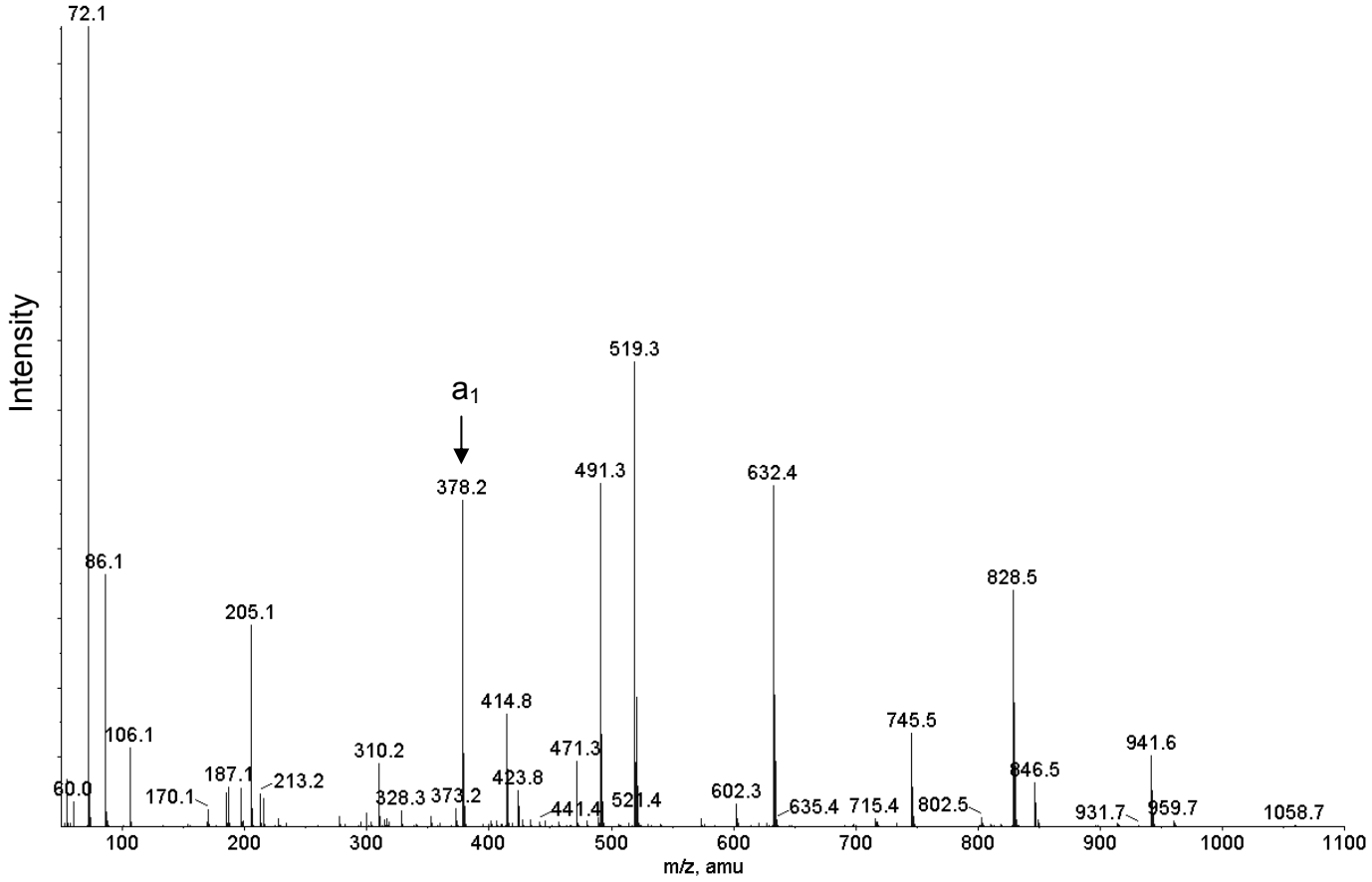
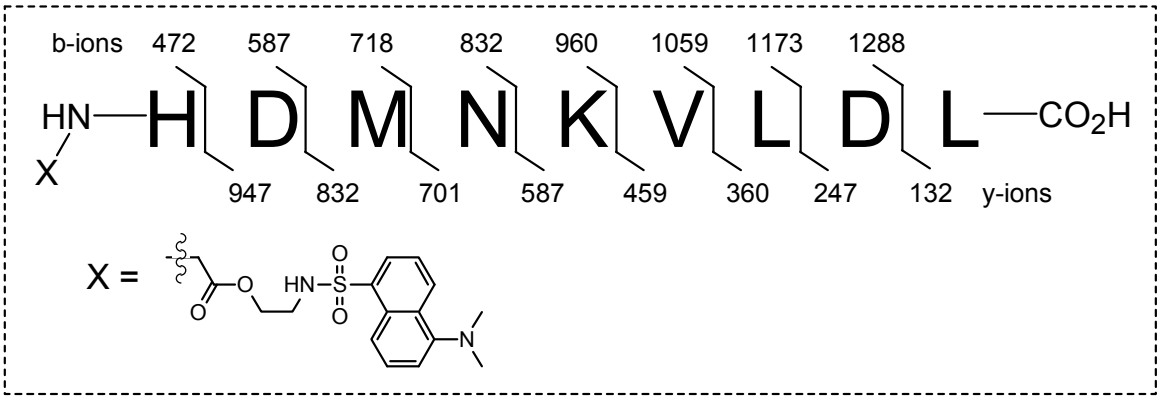


Figure S6. Q-TOF MS/MS spectrum of the N-terminal acylated peptide ALILTLVS (ESI source, doubly charged ion of m/z = 582.4).



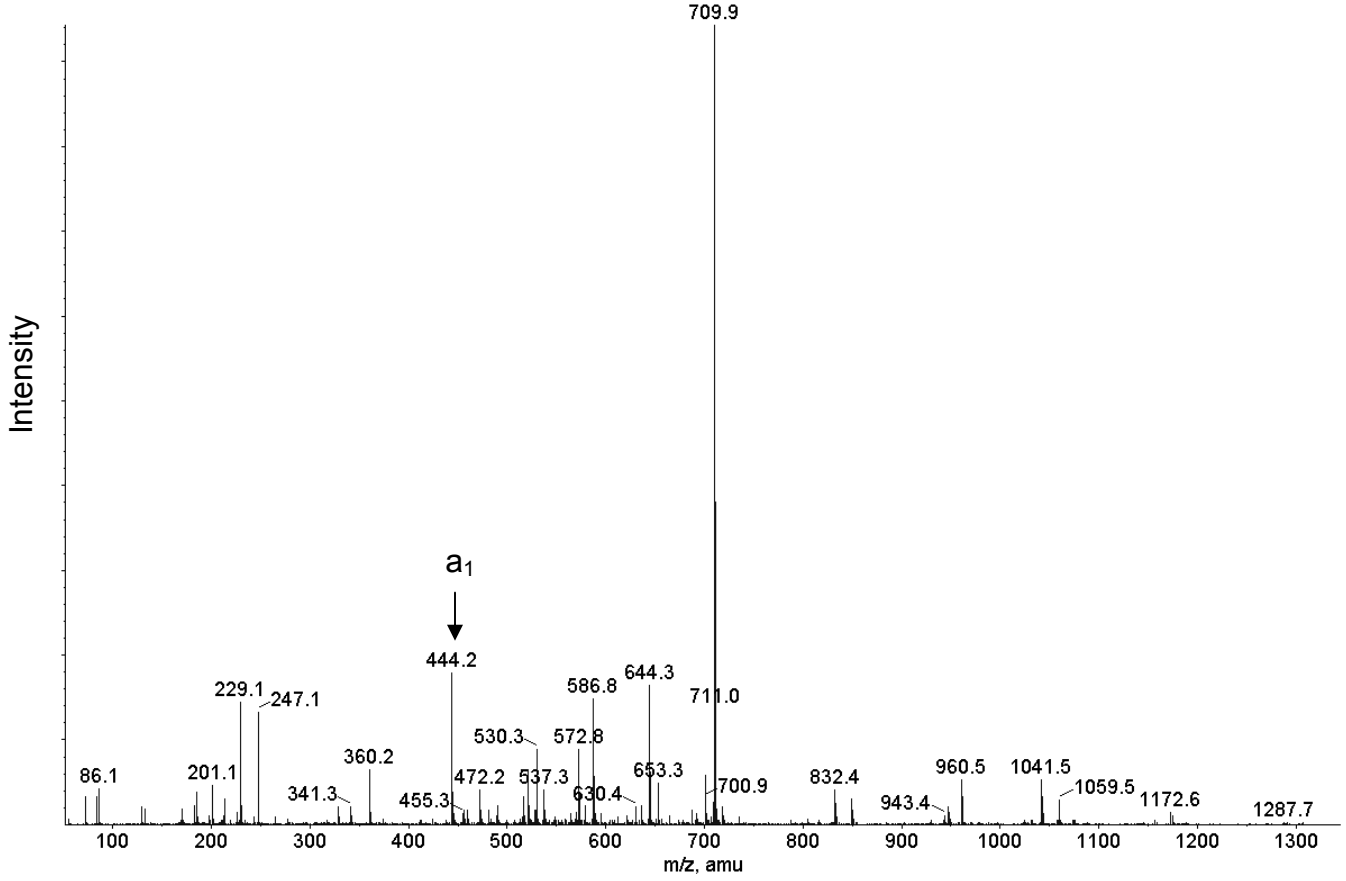
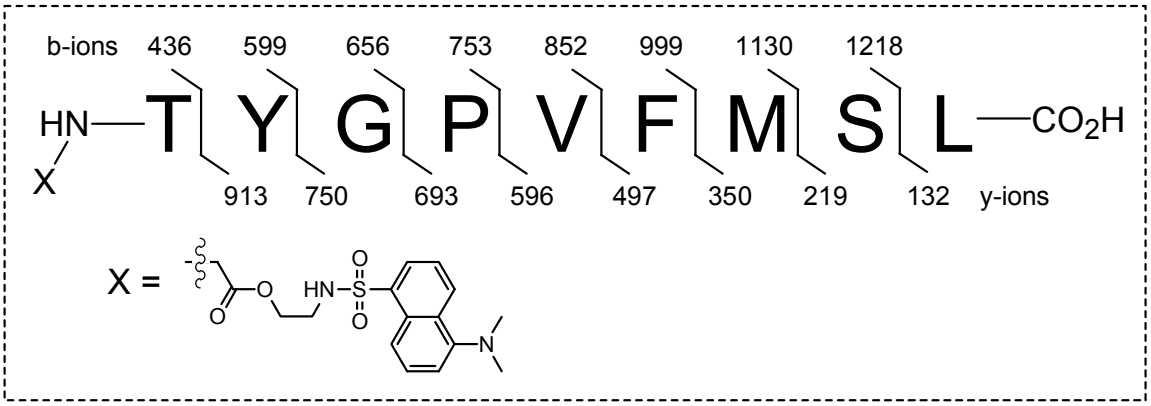


Figure S7. Q-TOF MS/MS spectrum of the N-terminal acylated peptide HDMNKVLDL (ESI source, doubly charged ion of m/z = 709.9).



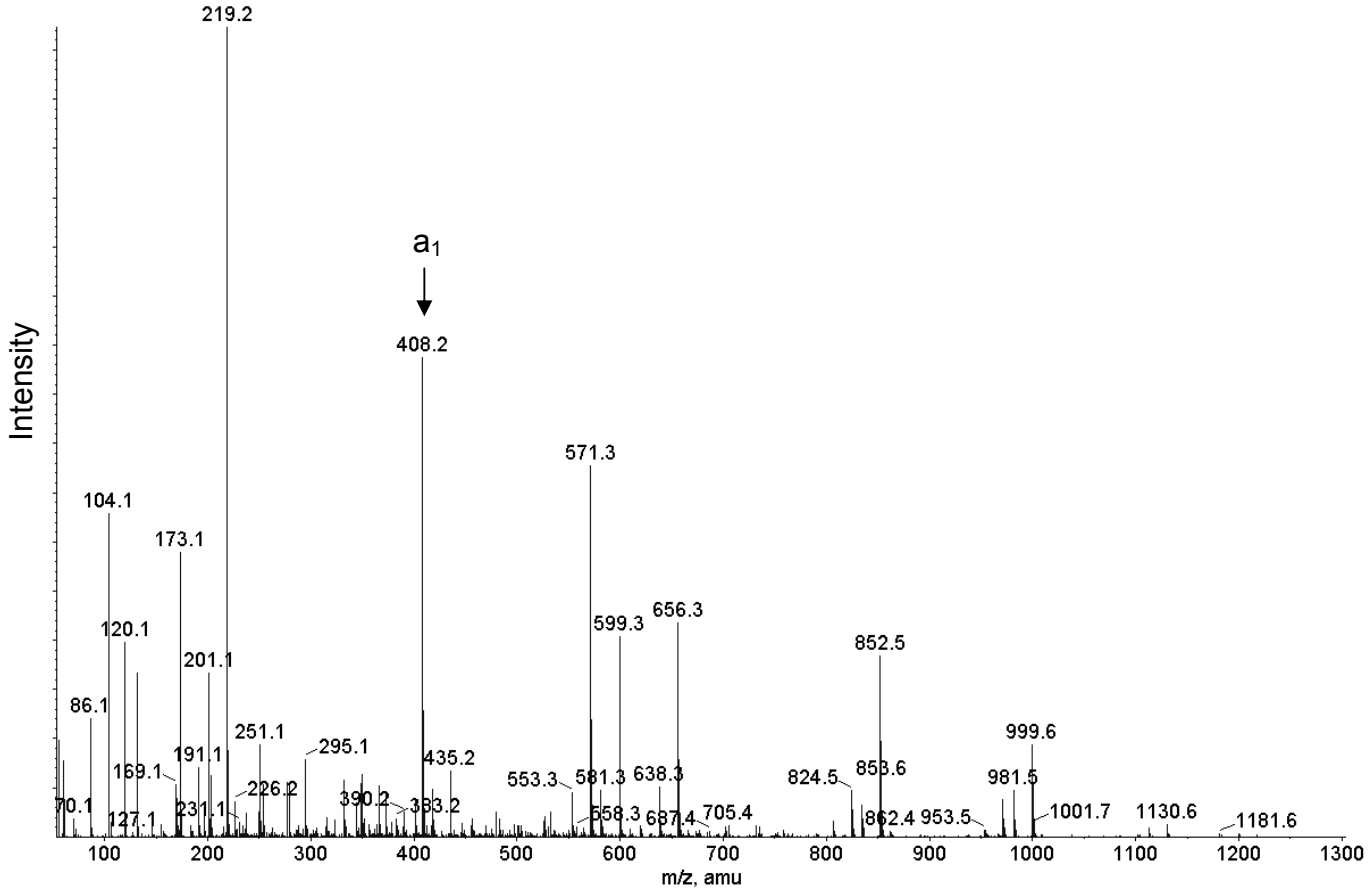
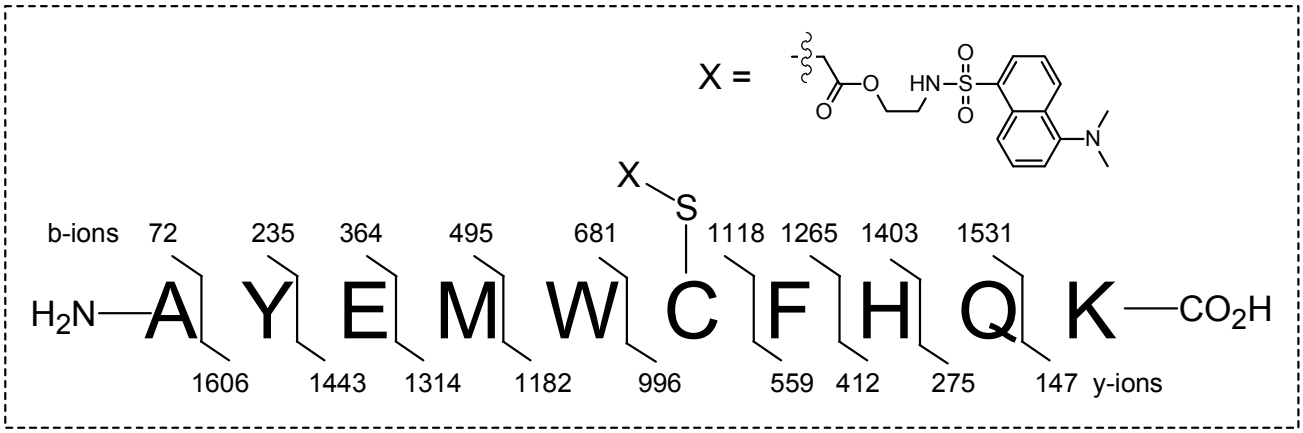


Figure S8. Q-TOF MS/MS spectrum of the N-terminal acylated peptide TYGPVFMSL (ESI source, doubly charged ion of m/z = 674.9)



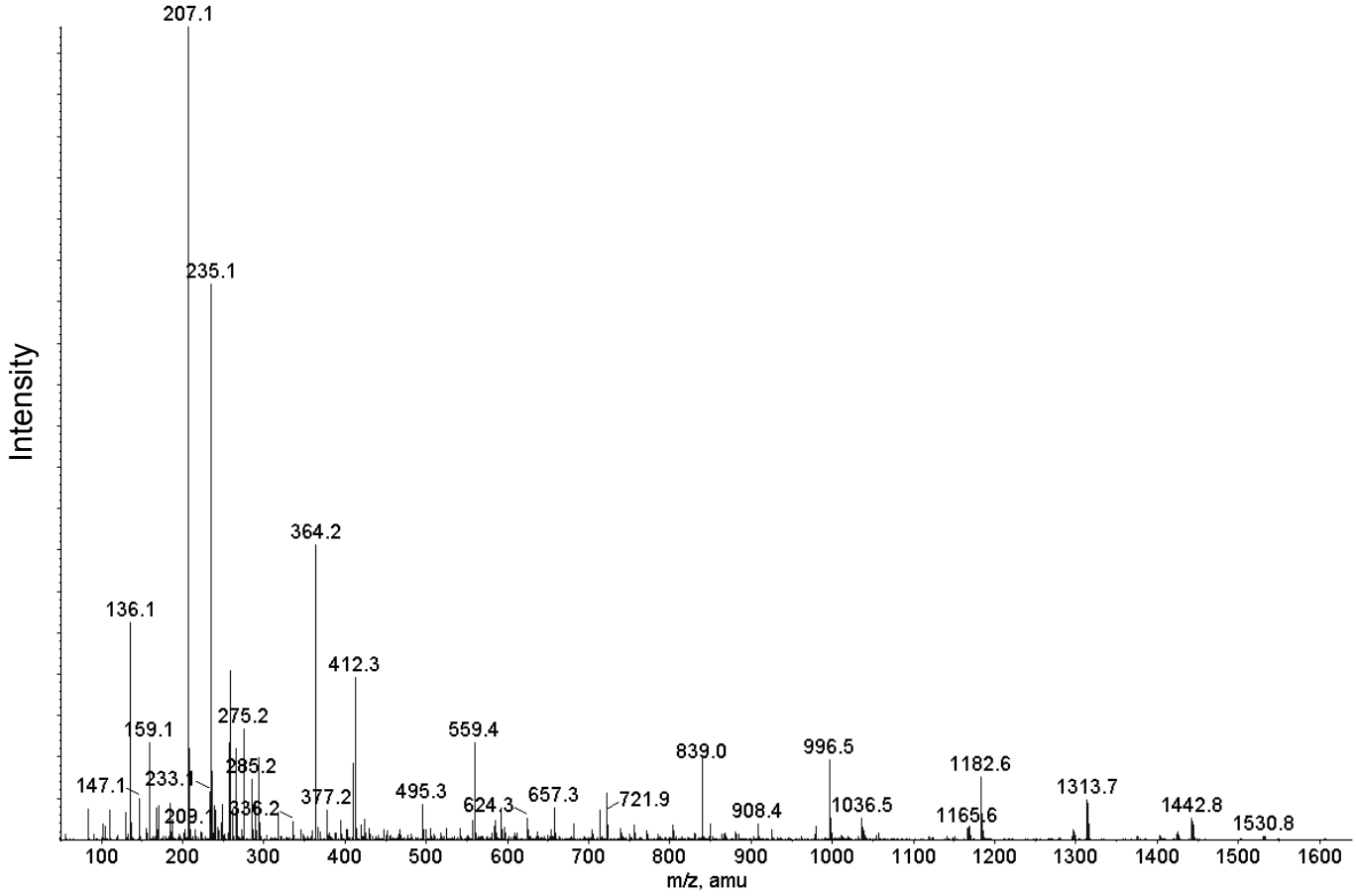
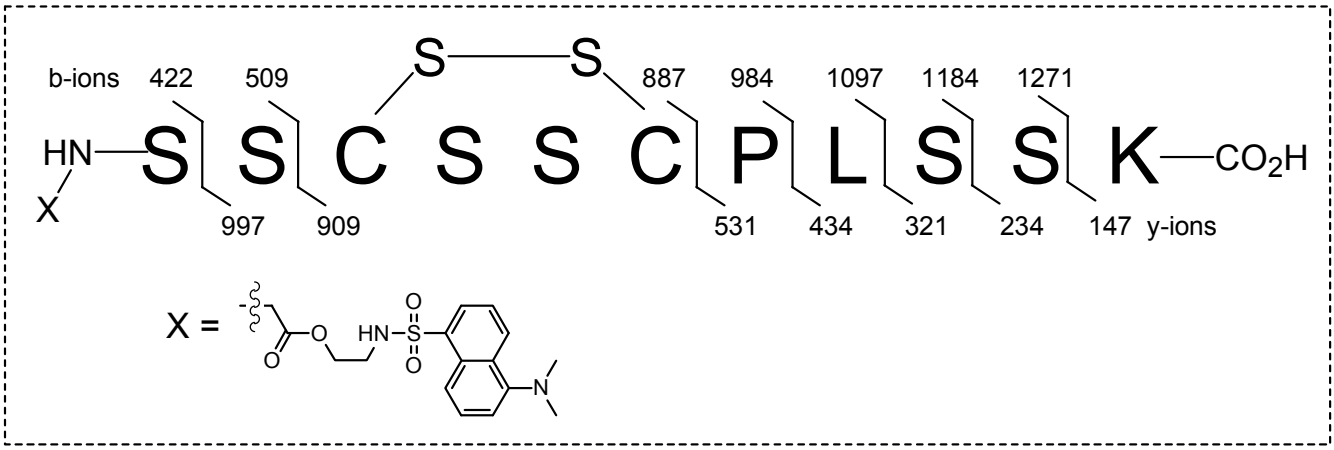


Figure S9. Q-TOF MS/MS spectrum of the N-terminal acylated peptide AYEMWCFHQK (ESI source, doubly charged ion of m/z = 838.9).



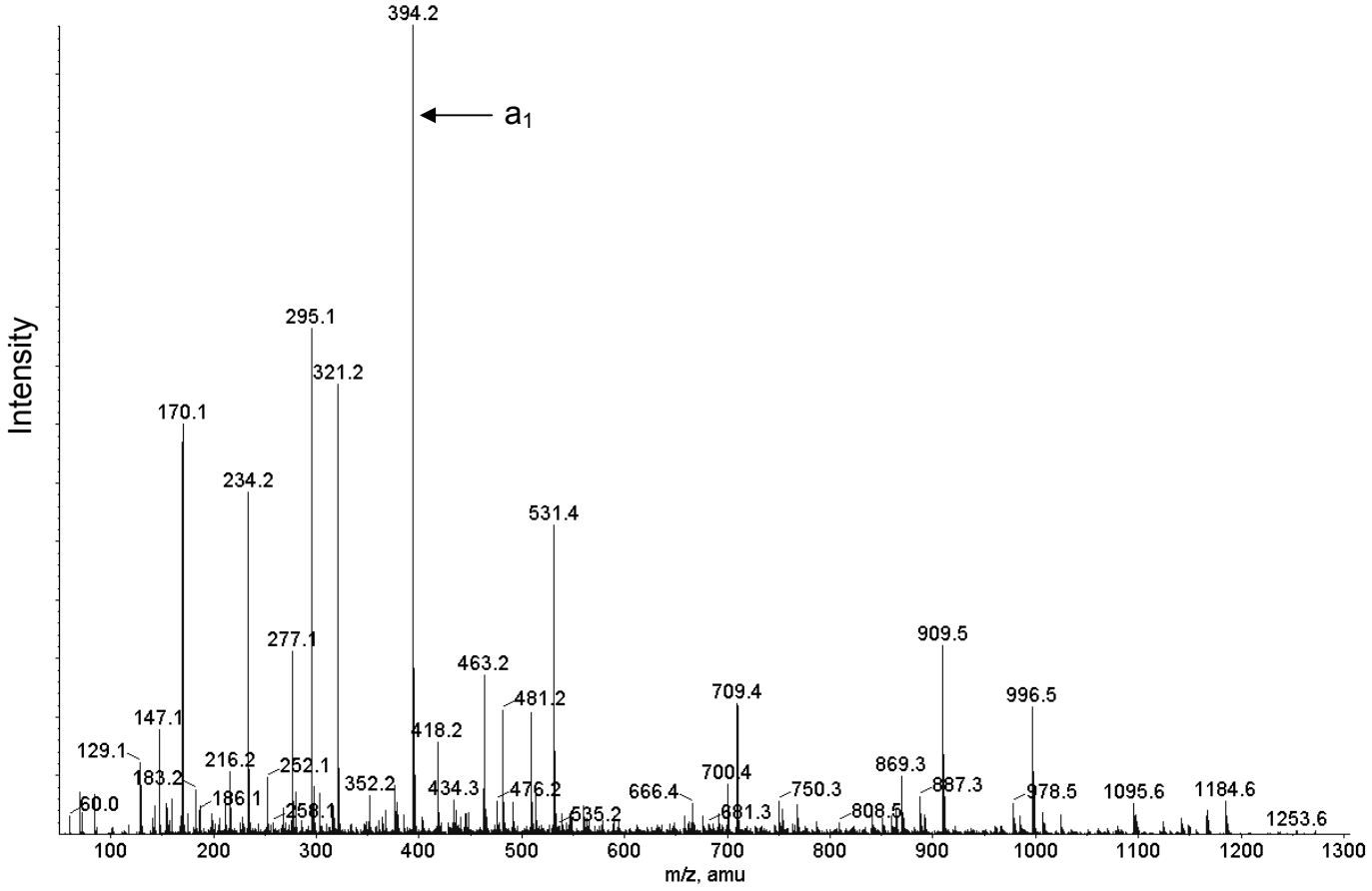


Figure S10. Q-TOF MS/MS spectrum of the N-terminal acylated peptide SSCSSCPLSSK (ESI source, doubly charged ion of m/z = 709.3).

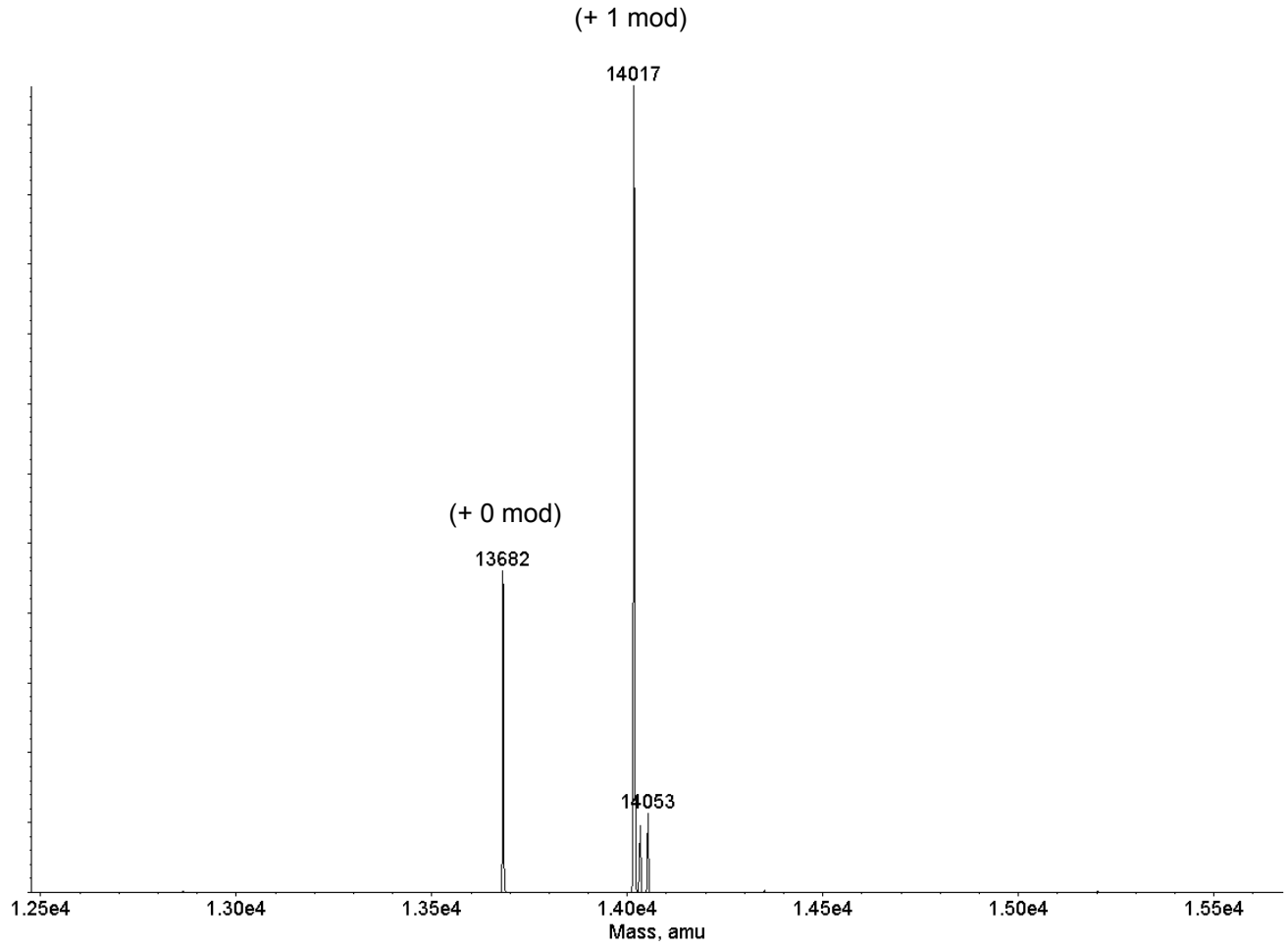


Figure S11. A representative ESI-MS spectrum of modified RNAase A. Protein mass reconstruction was performed on the charge ladder with Analyst QS software (Applied Biosystems).

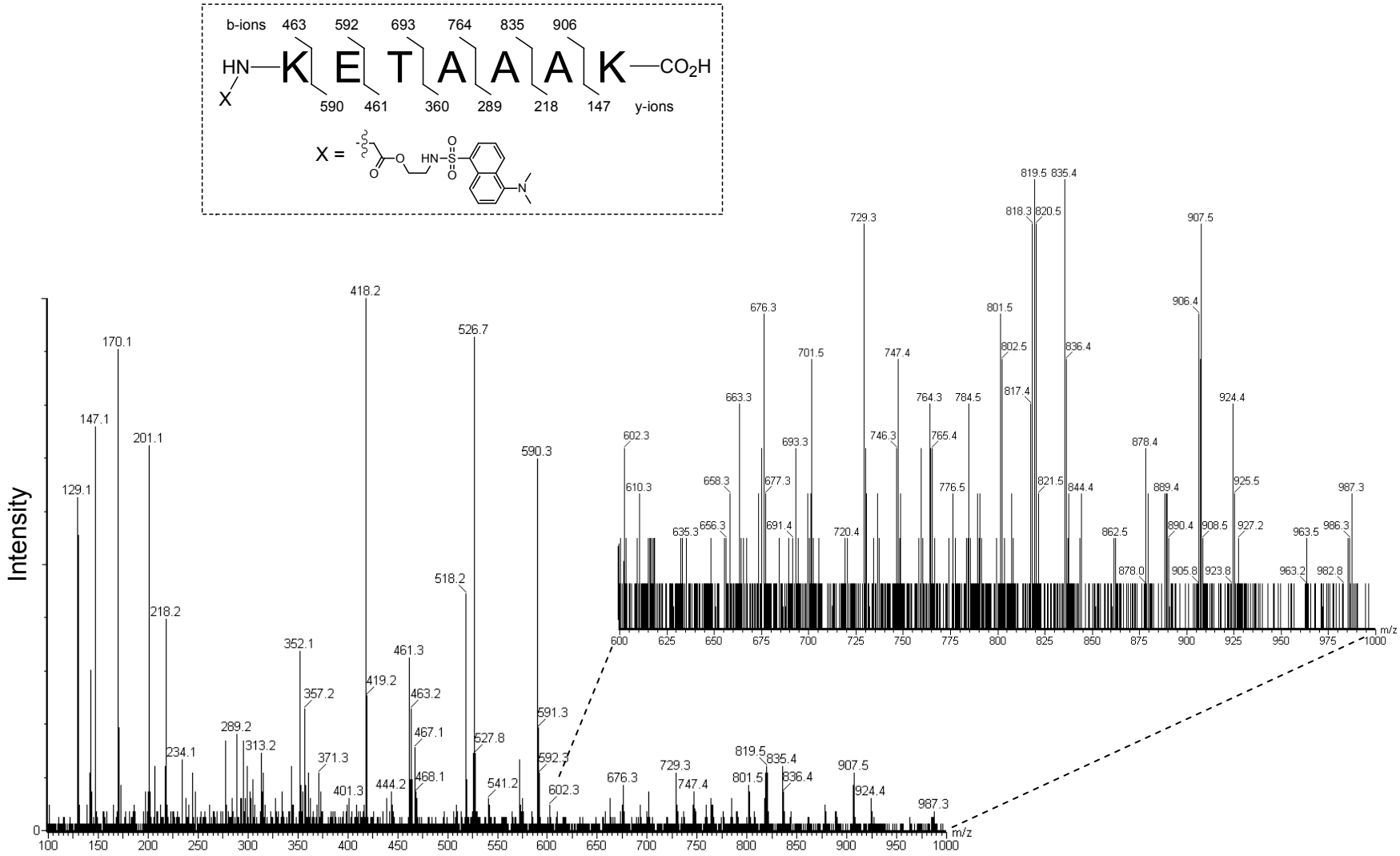


Figure S12. Q-TOF MS/MS spectrum of the N-terminal acylated peptide KETAAAK (ESI source, doubly charged ion of m/z = 526.7).

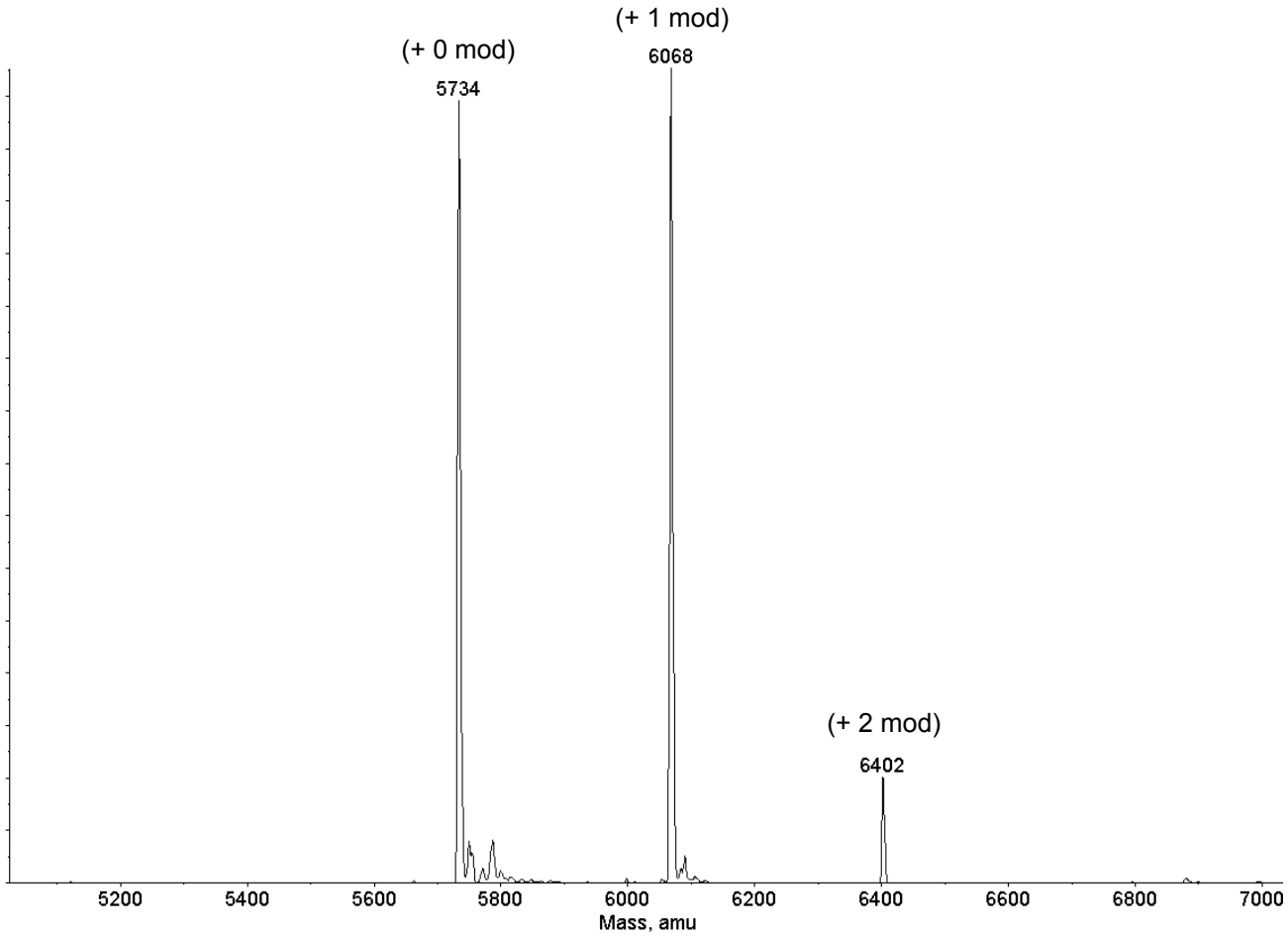


Figure S13. A representative ESI-MS spectrum of modified insulin. Protein mass reconstruction was performed on the charge ladder with Analyst QS software (Applied Biosystems).

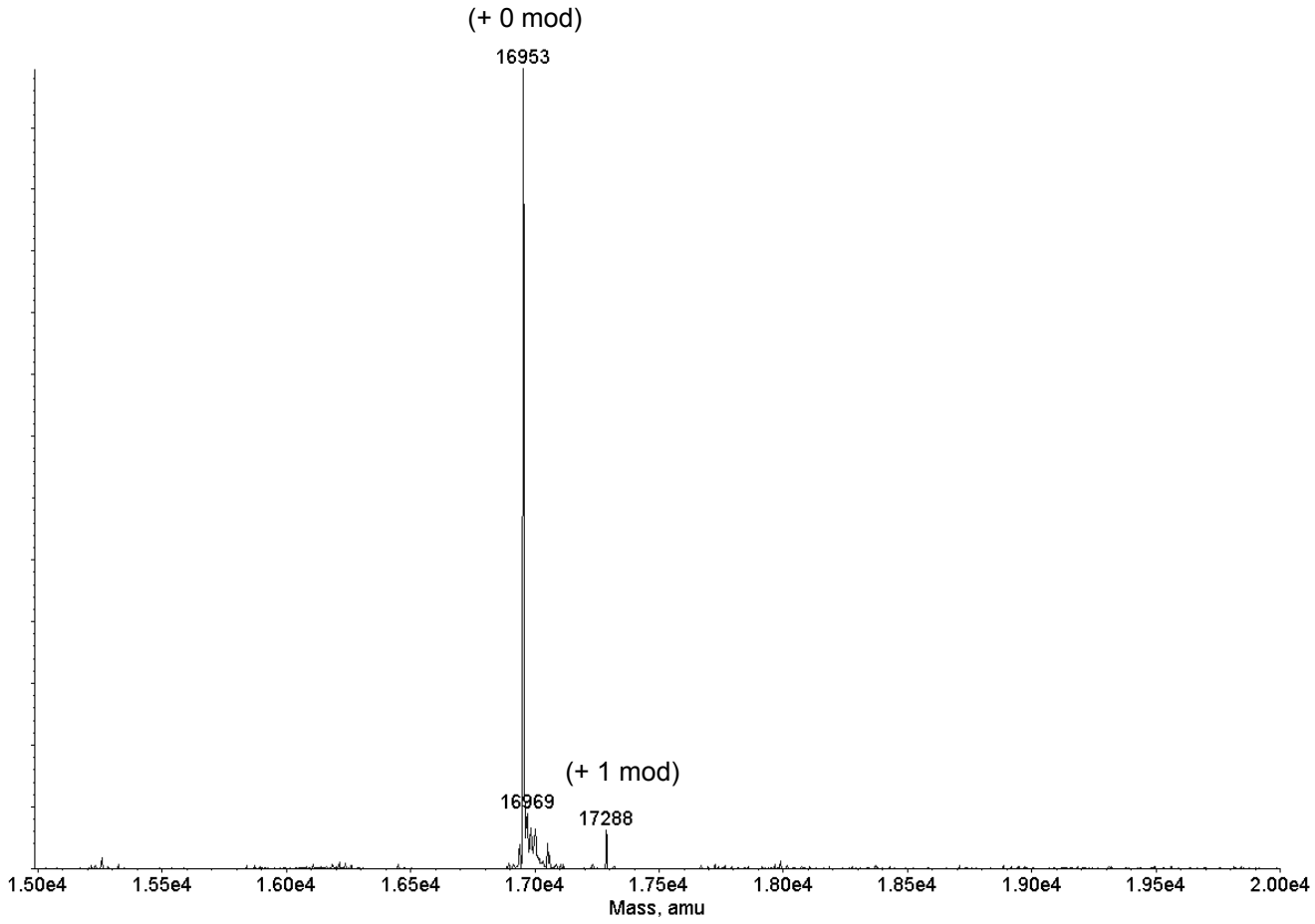


Figure S14. A representative spectrum of modified myoglobin. Protein mass reconstruction was performed on the charge ladder with Analyst QS software (Applied Biosystems).

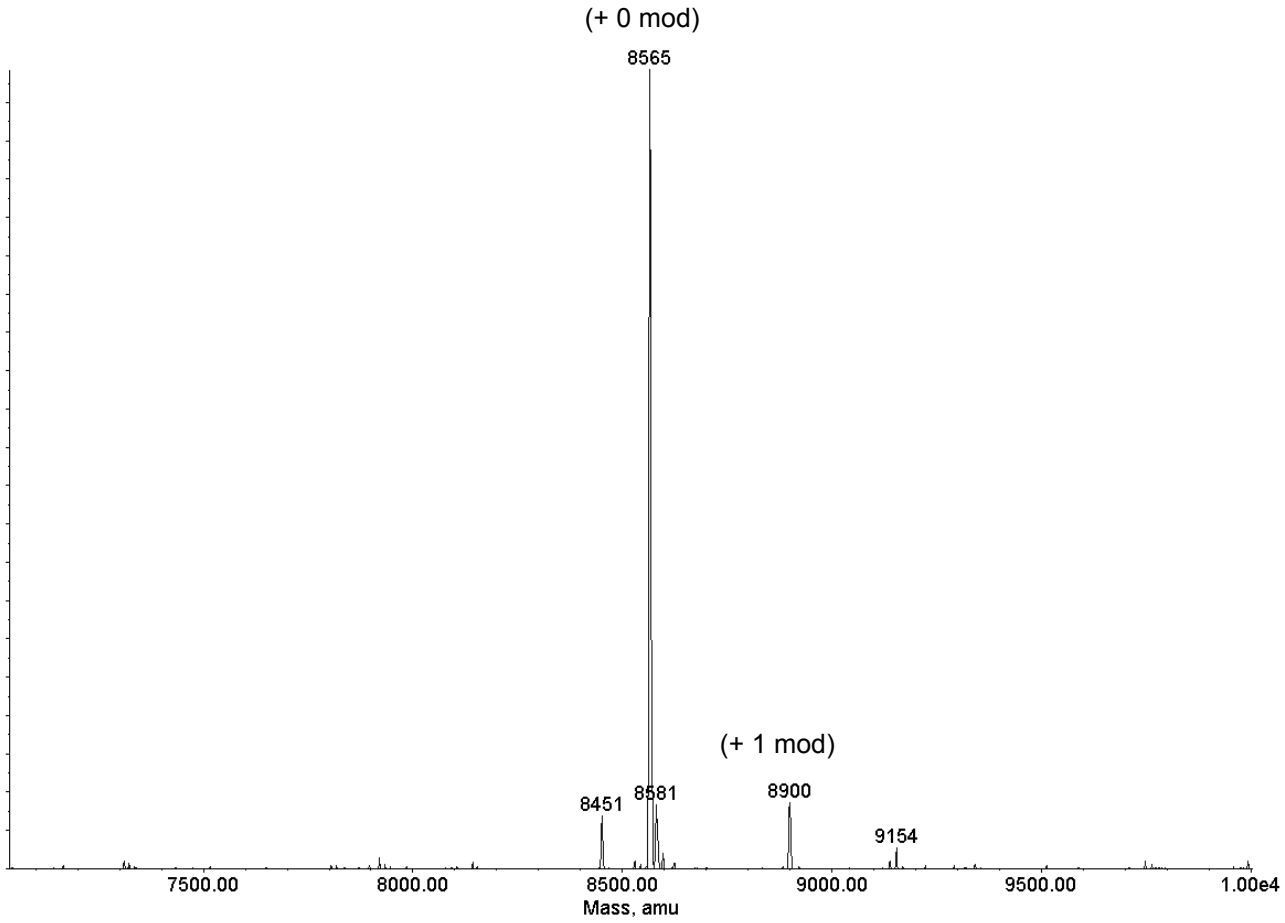


Figure S15. A representative ESI-MS spectrum of modified ubiquitin. Protein mass reconstruction was performed on the charge ladder with Analyst QS software (Applied Biosystems).

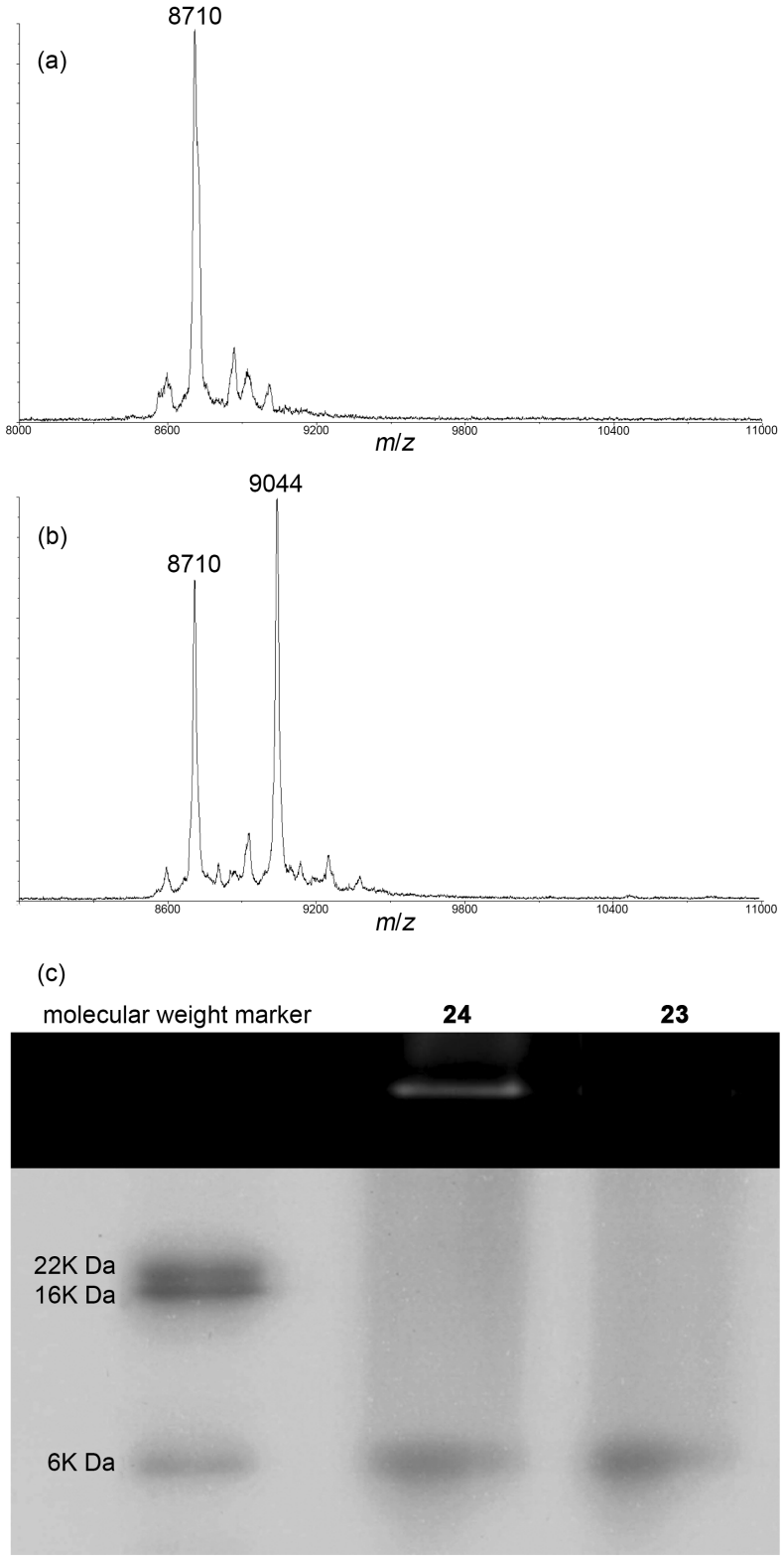


Figure S16. MALDI-TOF mass spectra of (a) **23** and (b) **24**. (c) SDS-PAGE analysis of **23** and **24**. Upper gel: record under UV irradiation; lower gel: colloidal Coomassie blue staining of the upper gel (for visualization of all the protein bands)

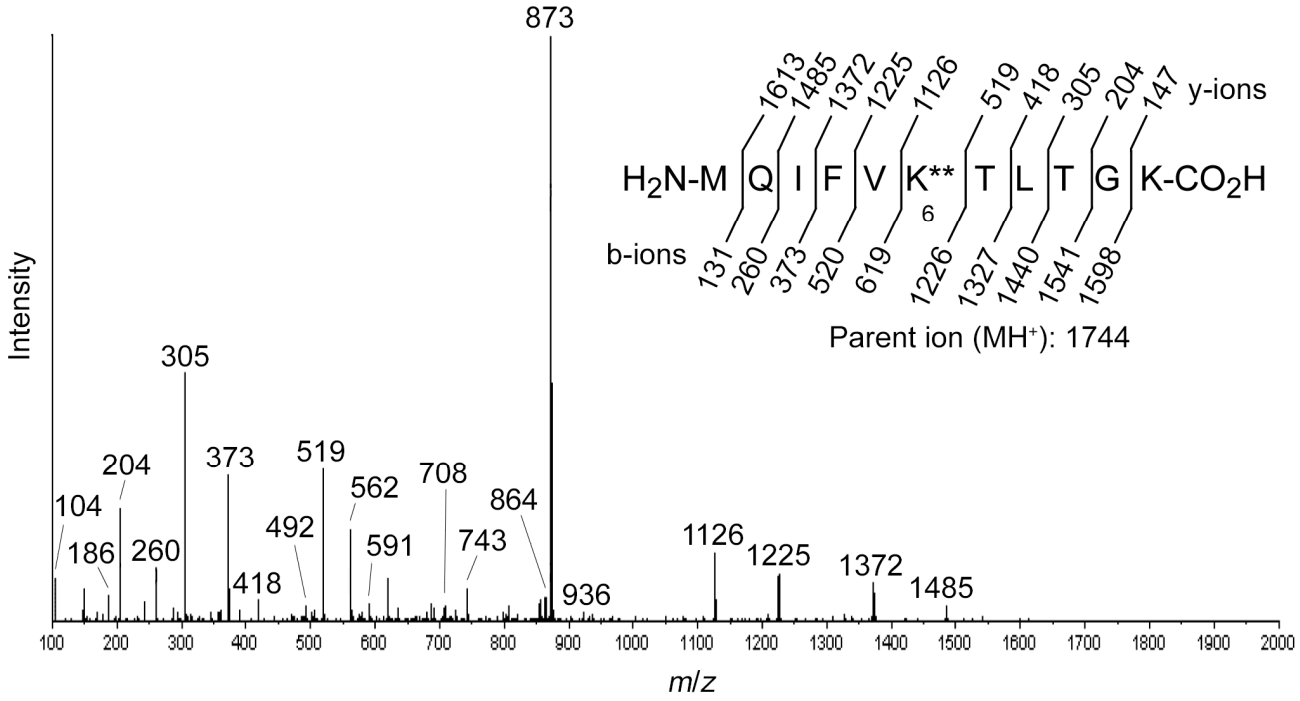


Figure 17. MS/MS spectrum of protonated peptide (*m*/*z* = 873, ESI mode) from trypsin digestion of **24**.

K** = cyclopropane modified lysine residue.