# Supporting Information

## Tertiary Amine Coupling by Oxidation for Selective Labeling of Dimethyl Lysine Posttranslational Modifications

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**I. General.** All commercial materials (Sigma-Aldrich, Fluka and Novabiochem) were used without further purification. All solvents were reagent or HPLC (Fisher) grade. All reactions were performed under air in glass vials. Yields refer to chromatographically pure compounds; % yields were obtained by comparing HPLC peak areas of products and starting materials. HPLC and MS were used to monitor reaction progress, and product elucidation was done using MS and NMR.

**II. Materials.** Fmoc-amino acids, Rink amide resin, 3-[bis(dimethylamino)methyliumyl]-3H-benzotriazol-1-oxide hexafluorophosphate (HBTU), 1-hydroxy-7-azabenzotriazole (HOAt), N,N′-iisopropylcarbodiimide(DIC), and N,N-diisopropylethylamine (DIEA) were obtained from CreoSalus (Louisville, Kentucky). Piperidine, trifluoroacetic acid (TFA), were obtained from Alfa Aesar (Ward Hill, Massachusetts). N,Ndimethylformamide (DMF), dichloromethane (DCM), methanol (MeOH), acetonitrile (ACN), were obtained from VWR (100 Matsonford Road Radnor, Pennsylvania). Selectfluor (1-Chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate)), 4-Dimethylaminopyridine and pyridine were obtained from Sigma.

**III. Purification. HPLC:** Purification of peptide starting materials was performed using high performance liquid chromatography (HPLC) on an Agilent 1100 series HPLC equipped with a C-18 reverse phase column with a particle size of 5 µm. All separations involved a mobile phase of water (solvent A) and acetonitrile (solvent B). The HPLC method used a linear gradient of 0-80% solvent B over 30 minutes at ambient temperature with a flow rate of 1 mL $min^{-1}$. The eluent was monitored by absorbance at 220 nm.

**IV. Instrumentation and sample analysis. NMR.** $^{1}H$ and $^{13}C$ spectra were acquired at 25 °C in DMSO-$d_6$, $CDCl_3$ using an Agilent DD2 (600 MHz) spectrometer with a 3-mm He triple resonance (HCN) cryoprobe. All $^{1}H$ NMR chemical shifts (δ) were referenced relative to the residual DMSO-$d_6$ peak at 2.50 ppm, $CDCl_3$ peak at 7.26 ppm or internal tetramethylsilane (TMS) at 0.00 ppm. $^{13}C$ NMR chemical shifts were referenced to DMSO-$d_6$ at 39.52 ppm and $CDCl_3$ at 77.2 ppm. $^{13}C$ NMR spectra were proton decoupled. NMR spectral data are reported as chemical shift (multiplicity, coupling constants (*J*), integration). Multiplicity is reported as follows: singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), doublet of triplets (td), triplet (t) and multiplet (m). Coupling constant (J) in hertz (Hz).

**Analytical HPLC.** Analytical HPLC chromatography (HPLC) was performed on an Agilent 1200 series HPLC equipped with a 5 mm C-18 reversed-phase column. The reaction was monitored by analytical reverse phase HPLC using a gradient of water versus acetonitrile. All separations involved mobile phase with 0.1 % formic acid in water (solvent A) and 0.1 % formic acid in acetonitrile (solvent B). Analytical HPLC method used for purification of peptides a linear gradient of 0-80% solvent B over 30 min at room temperature with a flow rate of 1.0 mL $min^{-1}$. The oxidative tertiary amine reactions were analyzed by HPLC, and MS. HPLC was carried out with 0.1% formic acid: water (solvent A): acetonitrile (solvent B) at detection wavelength 220 nm. HPLC **METHOD A:** Gradient: 0 to 80 % **B** (0.1% formic acid in ACN) in 30 min; 80-100 % B in 31-35 min at a flow rate of 1 mL/min.
**HPLC METHOD B:** Gradient: 0 to 50 % **B** (0.1% formic acid in ACN) in 30 min; 50-100 % B in 31-35 min at a flow rate of 0.5 mL/min.

**LC/MS.** High resolution LC-MS conditions for all purified peptides: Analyses were performed on an ultraperformance LC system (ACQUITY, Waters Corp., USA) coupled with a quadrupole

time-of-flight mass spectrometer (Q-ToF Premier, Waters) with electrospray ionization (ESI) in positive mode using Mass lynx software (V4.1) or high-performance LC system (Agilent, 1100 series) coupled with triple quadrupole.

LC-MS (Agilent technologies 6460) with electrospray ionization (ESI) in positive mode using Agilent mass hunter (10.0). Unless otherwise mentioned a sample was injected either onto a C4 column (Phenomenex Aeris™ 3.6 µm WIDEPORE C4 200 Å, LC Column 50 x 2.1 mm) with a 400 µL/min flow rate of mobile phase of solution A (90 % $H_2O$, 10 % acetonitrile and 0.1 % formic acid (FA)) and solution B (95 % acetonitrile, 5 % $H_2O$, and 0.1 % formic acid) beginning gradient- Time- 0 min 10 % B; 5 min 28 % B; 20 min 38 % B; 22 min 90 % B; C18 column (ACQUITY UPLC BEH 1.7 µm 1x 50 mm) with a 200 µL/min flow rate of mobile phase of solution A (90 % $H_2O$, 10 % acetonitrile and 0.1 % formic acid) and solution B (90 % acetonitrile, 10 % $H_2O$, and 0.1 % formic acid) beginning gradient- Time- 1 min 0% B; 1-10 min 100% B for chromatography analysis (or) directly injected with mobile phase 90 % $H_2O$: 10 % ACN, 0.1% formic acid at 400 µL/min flow rate in ESI positive mode. HPLC and MS of the cyclized products were performed without the addition of formic acid to the eluting solvent because the triazene compound is acid labile.

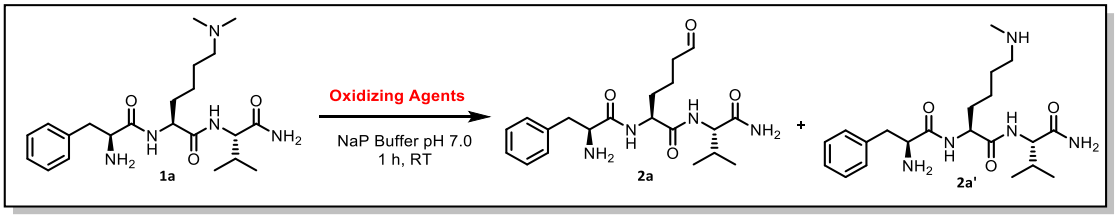
**HRMS.** High resolution MS data were acquired on Thermo Exactive Plus using a heated electrospray source. The solution was infused at a rate of 10-25 µL/min/electrospray using 3.3 KV. The typical settings were Capillary temp 320 $^{o}$C. S-lens RF level was between 30-80 with an AGC setting of 1 E6. The maximum injection time was set to 50 ms. Spectra were taken at 140,000 resolutions at m/z 200 using Tune software and analyze with Thermo's Freestyle software.

**V. Fmoc Solid-Phase Peptide Synthesis (Fmoc-SPPS).**[1] Peptides were synthesized manually on a 0.25 mm scale using Rink amide resin. Resin was swollen with DCM for 1 h at room temperature. Fmoc was deprotected using 20% piperidine–DMF for 5 min to obtain a deprotected peptide-resin. First, Fmoc protected amino acid (1.25 mm/5 equiv.) was coupled using HOAt (1.25 mm/5 equiv.) and DIC (1.25 mm/5 equiv.) in DMF for 15 min at room temperature. Fmoc-protected amino acids (0.75 mm/3 equiv.) were sequentially coupled on the resin using HBTU (0.75 mm/3 equiv.) and DIEA (1.5 mm/6 equiv.) in DMF for 5 min at room temperature. Peptides were synthesized using standard protocols. Peptides were cleaved from the resin using a cocktail of 95:5, trifluoroacetic acid: water for 2 h. The resin was removed by filtration and the resulting solution was concentrated. The residue was diluted with ACN/water mixture. The resulting solution was purified by HPLC.

**VI. General procedure 1: Tertiary amine coupling by Oxidation (TACO) of dimethyllysine peptides to generate aldehyde-peptide products.**

To 1 mg (6-10mM) of unprotected dimethyllysine peptide dissolved in 300 µL of 10 mM sodium phosphate buffer (NaP, pH 7.0), was added pyridine (5 eq) and selectfluor (2 eq). The reaction mixture was stirred for 1 h and subsequently injected into the HPLC for determining the % conversion of dimethyllysine peptides to the aldehyde peptide product and the mass confirmed with LC-MS. HPLC was carried out with 0.1% formic acid: water (solvent A): acetonitrile (solvent B) at detection wavelength 220 nm.

**HPLC METHOD A: HPLC METHOD A:** Gradient: 0 to 80 % **B** (0.1% formic acid in ACN) in 30 min; 80-100 % B in 31-35 min at a flow rate of 1 mL/min.
**HPLC METHOD B:** Gradient: 0 to 50 % **B** (0.1% formic acid in ACN) in 30 min; 50-100 % B in 31-35 min at a flow rate of 0.5 mL/min.
**VII. Supplementary Figure 1:** Evaluation of oxidizing reagents for conversion of $Kme_2$ to aldehyde

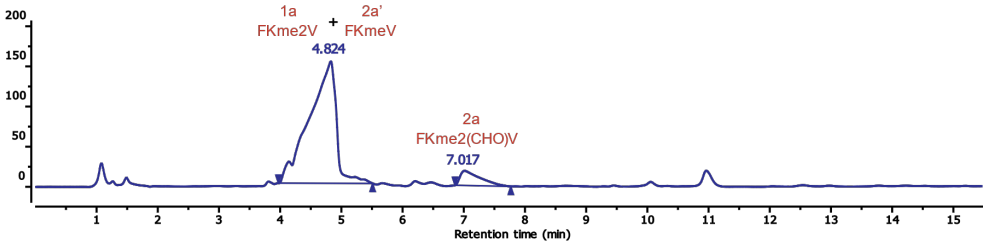



To 1.0 mg (8mM) of $FKme_2V$ peptide **1a** in 300 µL of 10 mM sodium phosphate buffer (NaP, pH 7.0) was added different oxidizing reagents such selectfluor, tropylium, NBS, DEAD, and $FeCl_3$/t-BuOOH (2 eq). The reaction mixture was stirred for 1 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the generation of aldehyde product $FKme_2(CHO)V$ **2a**. The reaction mixture was analyzed by HPLC using method A and the % conversion to aldehyde-peptide product **2a** was determined and compiled in the table below.

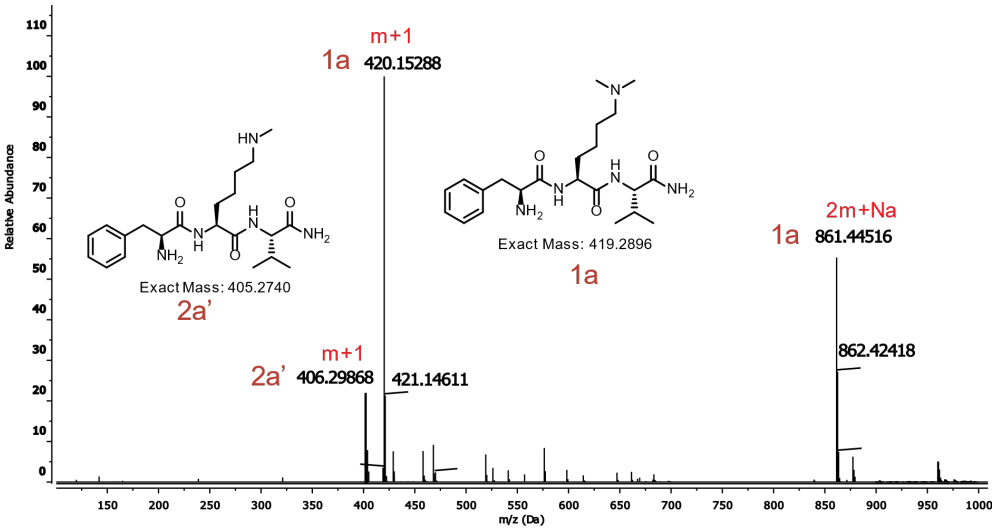
**$FKme_2V$ linear peptide 1a.** LCMS: *m/z* 420.29315 (calcd [M+H]+ = 420.2896), (HPLC analysis at 220 nm). Retention time in HPLC: 4.824

**$FKme_2(CHO)V$ peptide aldehyde product 2a.** LCMS: *m/z* 391.23049 (calcd [M+H]+ = 391.2340), (HPLC analysis at 220 nm). Retention time in HPLC: 7.017

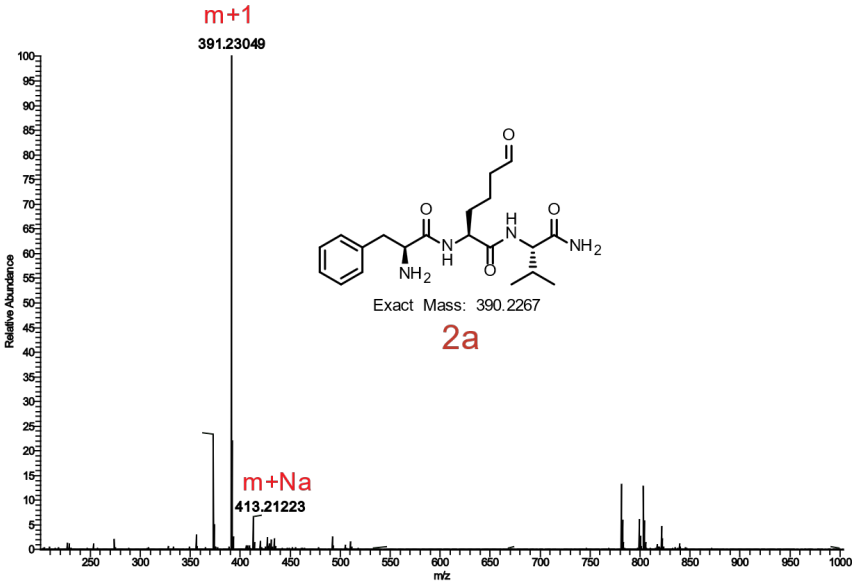
**HPLC Trace of aldehyde generation reaction from 1a using selectfluor**

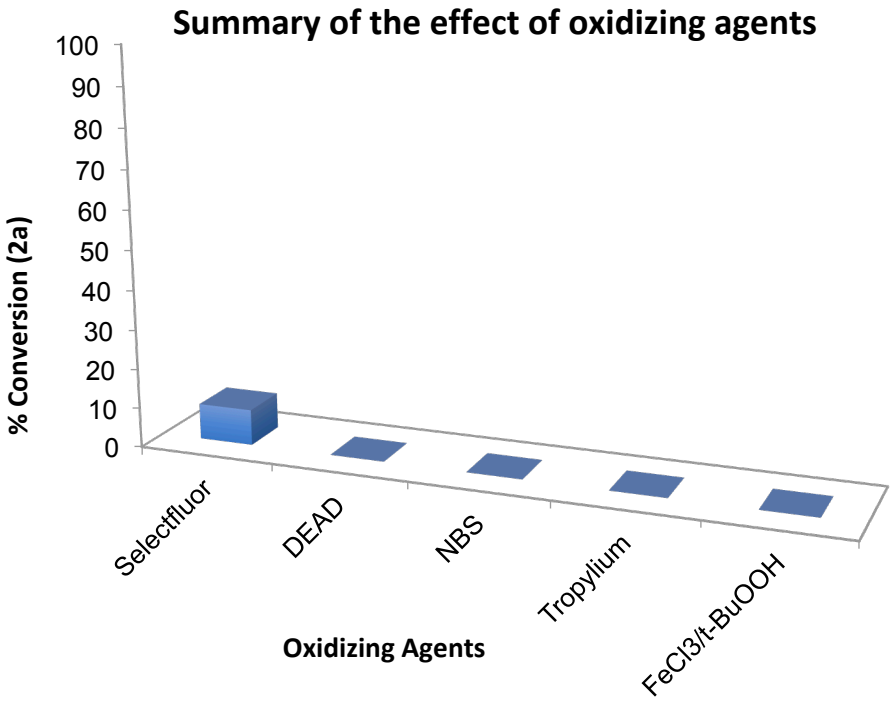


## MS-Trace of peak 4.824



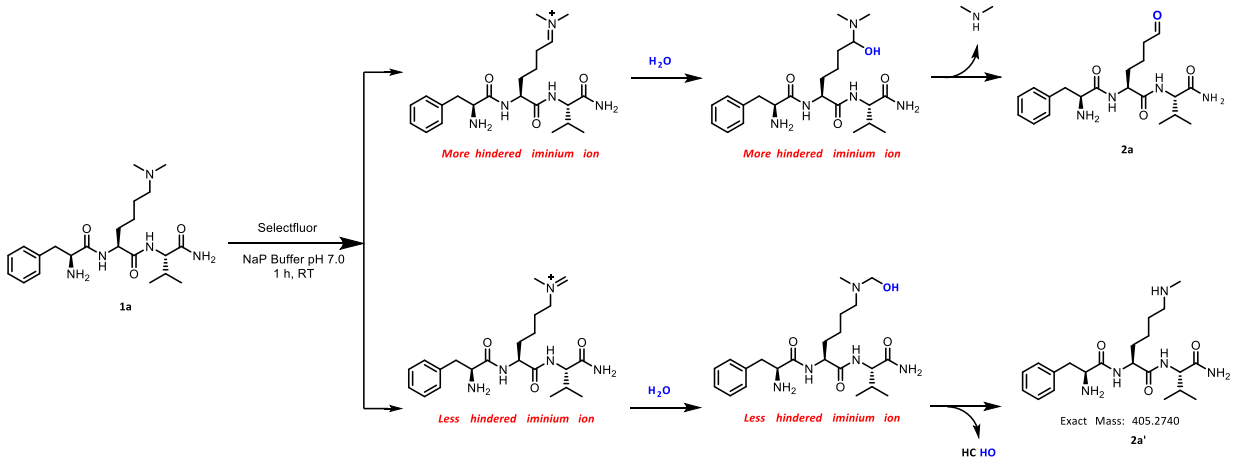

## MS-Trace of peak 7.017



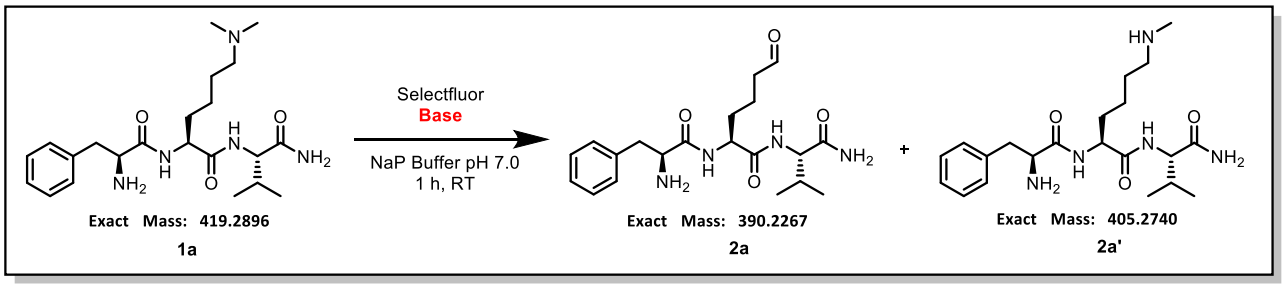



| Oxidizing agents | Selectfluor | DEAD | NBS | Tropylium | $FeCl_3$/t-BuOOH |
|---|---|---|---|---|---|
| % Conversion (2a) | 9 | 0 | 0 | 0 | 0 |

**VIII. Supplementary Figure 2:** Plausible mechanism for the formation of peptide aldehyde and monomethyllysine peptides.



**IX. Supplementary Figure 3:** Evaluation of various bases for the oxidative tertiary amine transformation of dimethyllysine peptide **1a** to peptide aldehyde **2a**.

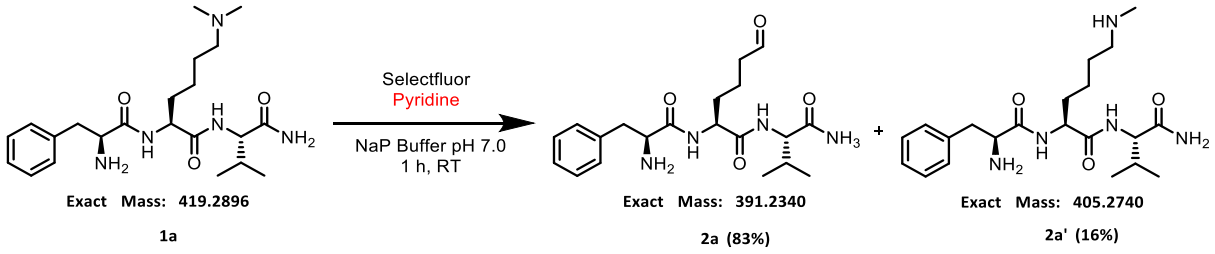



To 1.0 mg of FKme$_2$V **1a** dissolved in 300 µL of 10 mM sodium phosphate buffer (NaP, pH 7.0), was added varying **base** such as pyridine, piperidine, DMAP and proton-sponge (1-5 eq) and selectfluor (2 eq). The reaction mixtures were stirred for 1 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the generation of peptide aldehyde **2a**. The reaction mixtures were analyzed by HPLC using method A to determine the % conversion to peptide aldehyde **2a** and monomethyllysine peptide **2a'**.

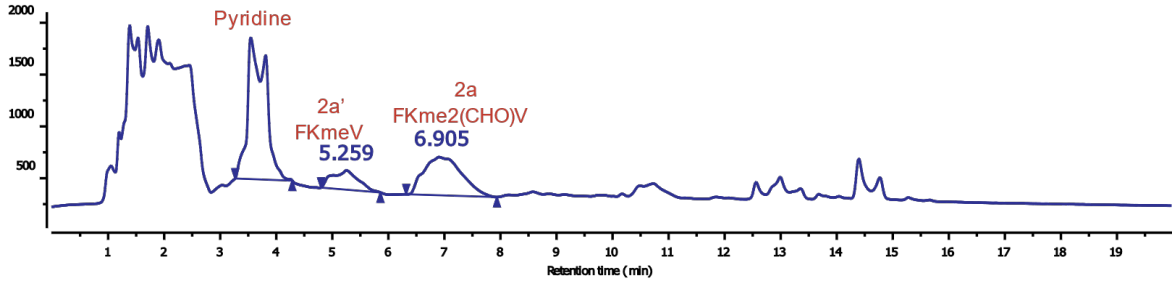
**FKme$_2$(CHO)V peptide-aldehyde product 2a.** LCMS: *m/z* 391.23049 (calcd $[M+H]^+$ = 391.2340), m/z 413.21223 (calcd [M+Na]+ =413.21), (HPLC analysis at 220 nm). Retention time in HPLC: 6.905

**FKmeV monomethyllysine peptide product 2a'.** LCMS: *m/z* 406.18372 (calcd $[M+H]^+$ = 391.23049), m/z 811.59185 (calcd [2M+1]+ = 811.15), (HPLC analysis at 220 nm). Retention time in HPLC: 5.259

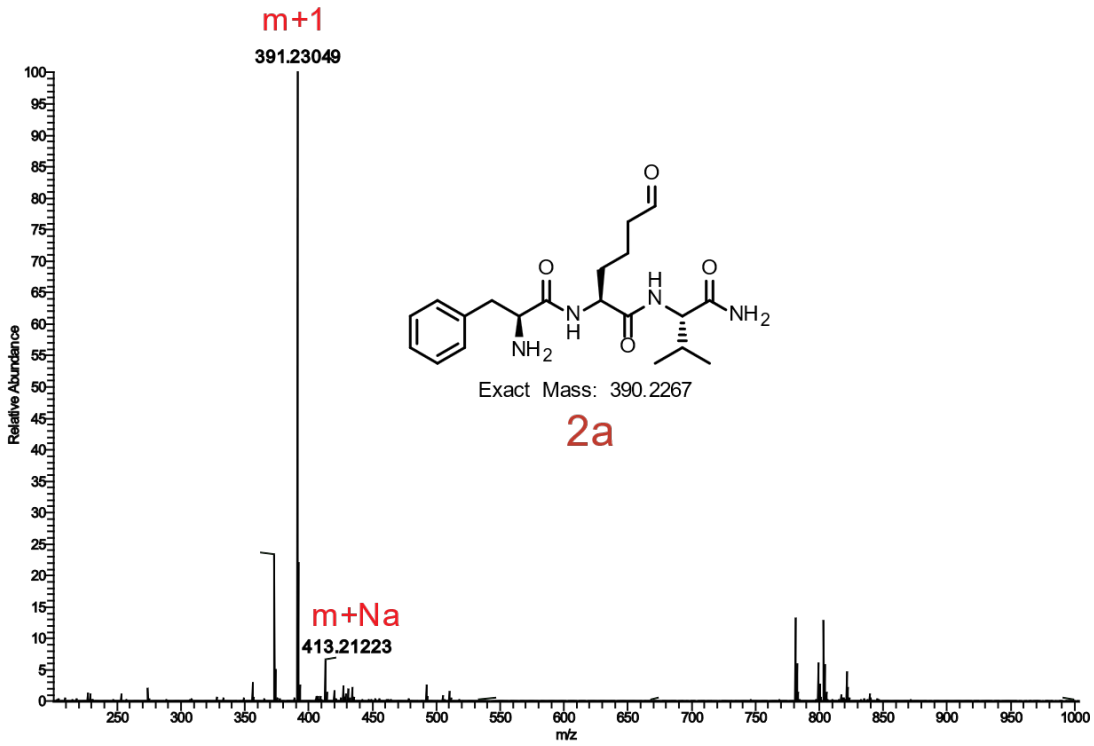
**Evaluation of pyridine:**



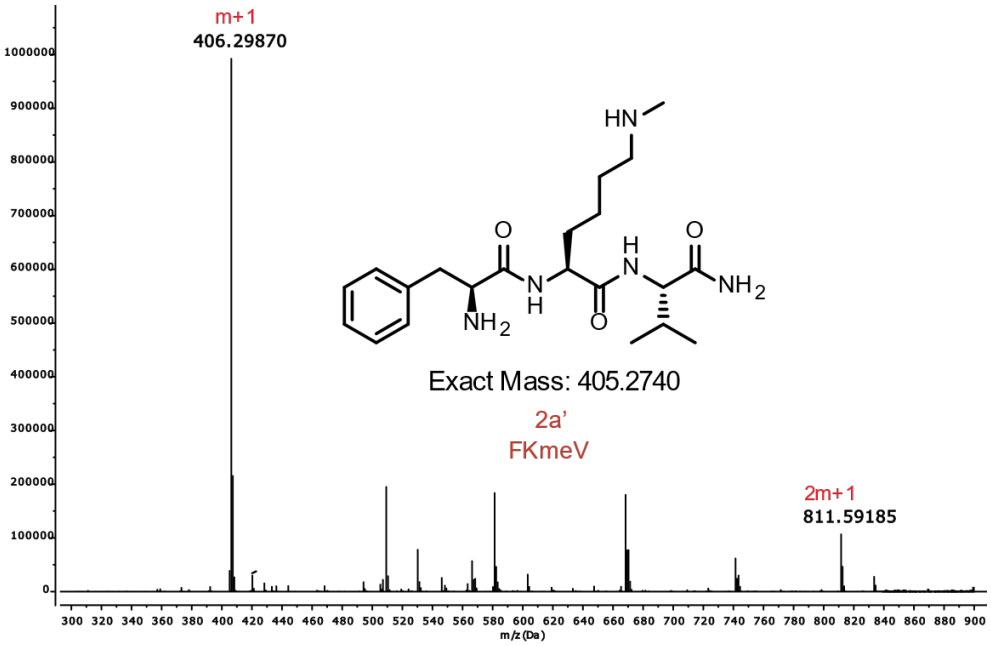
**HPLC Trace of aldehyde generation reaction from 1a using pyridine**



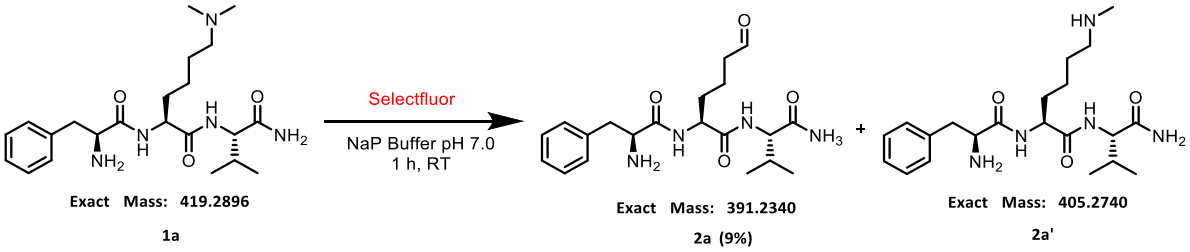

**MS-Trace of 2a (Peak 6.905)**



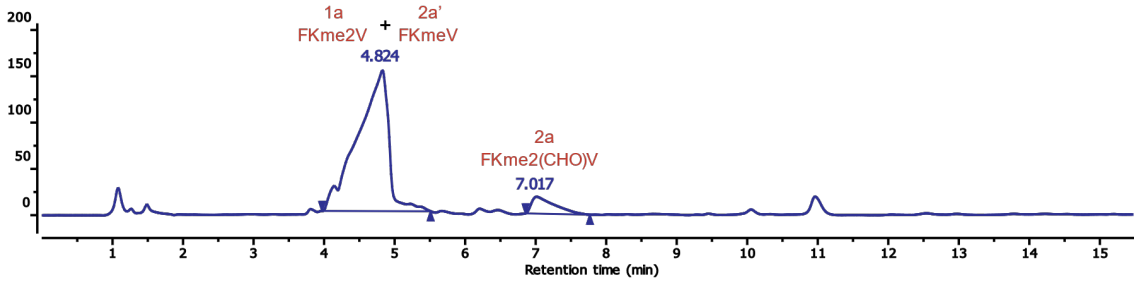
## MS-Trace of 2a' (peak 5.259)



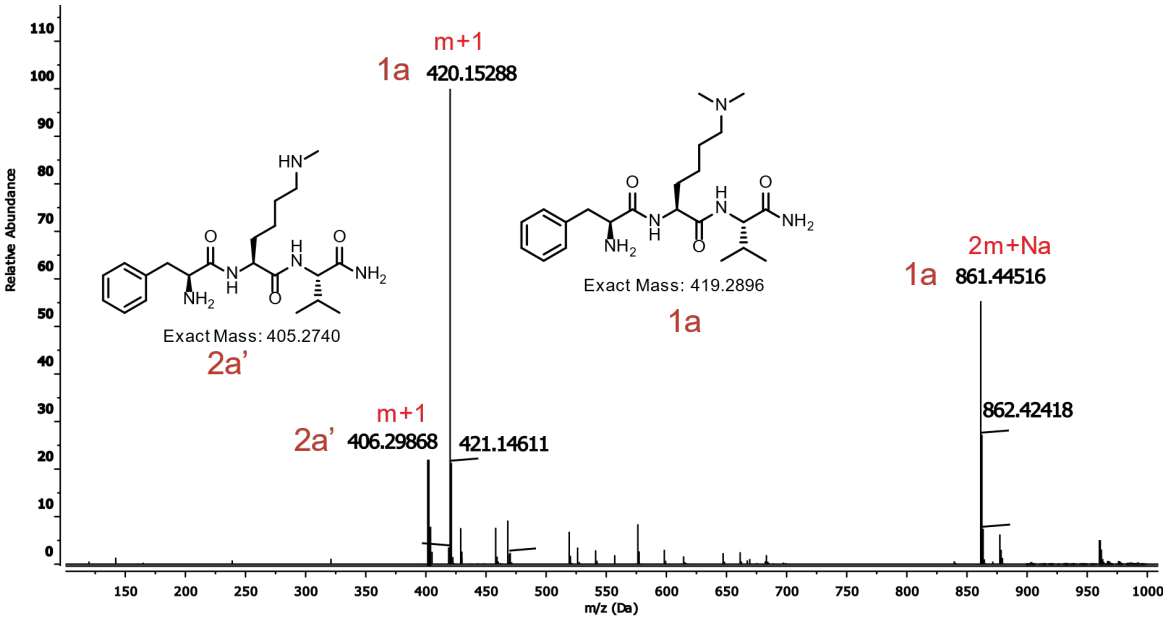

## Evaluation of in-situ base generated from selectfluor without any external base.



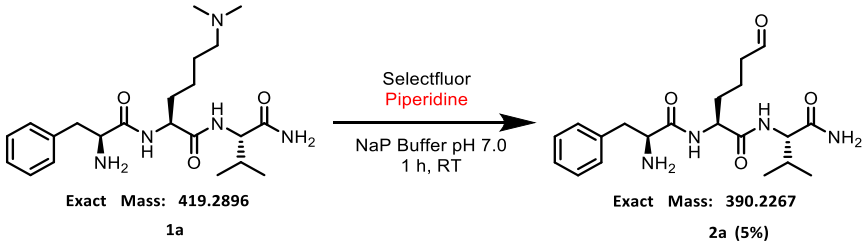

## HPLC Trace of aldehyde generation reaction from 1a using Dabco-like base



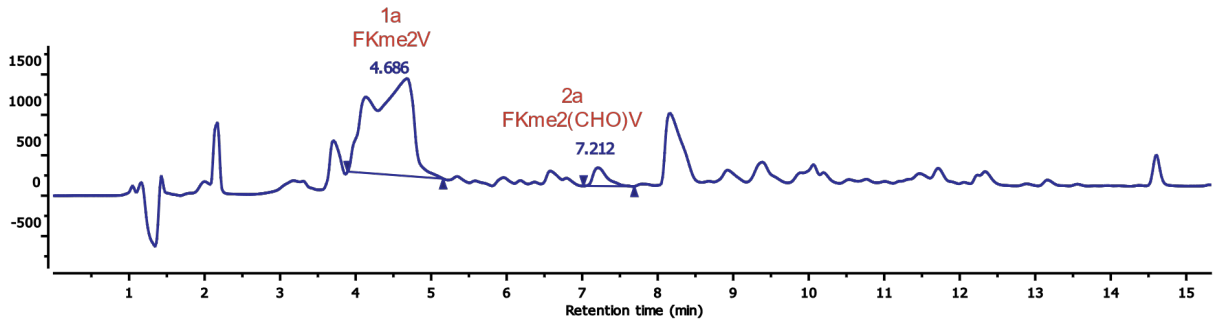
## MS-Trace of peak 4.824



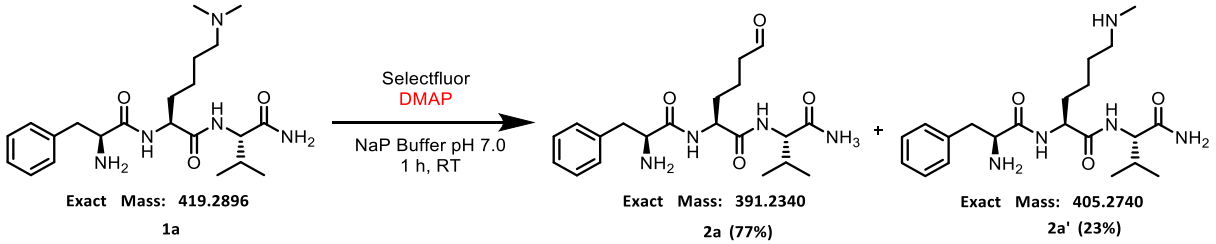

## Evaluation of piperidine:



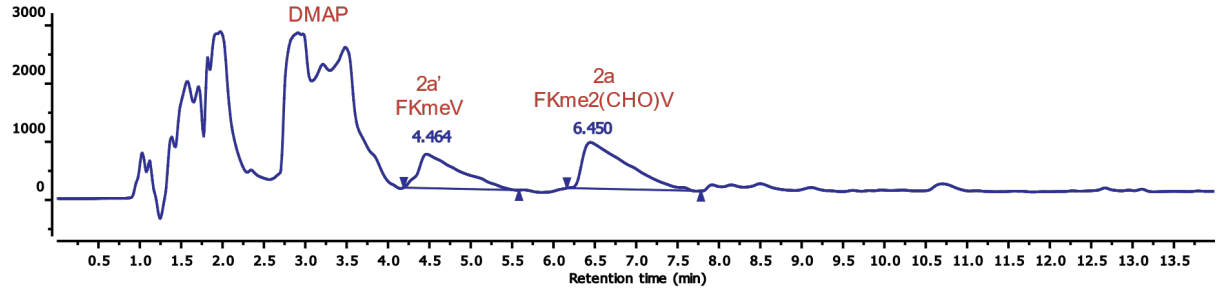

## HPLC Trace of aldehyde generation reaction from 1a using piperidine



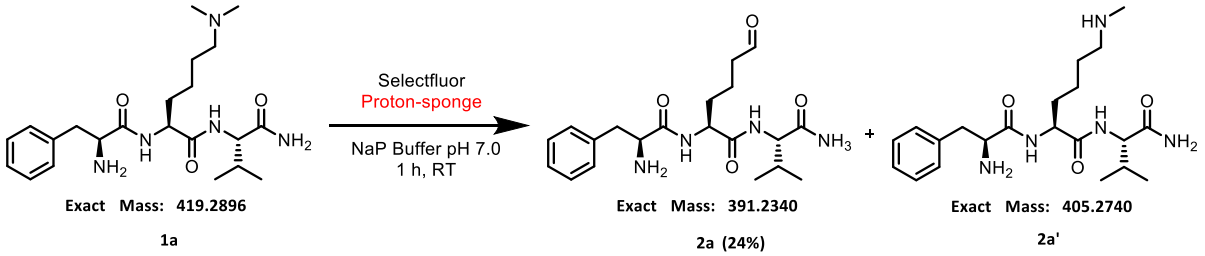
## Evaluation of DMAP:



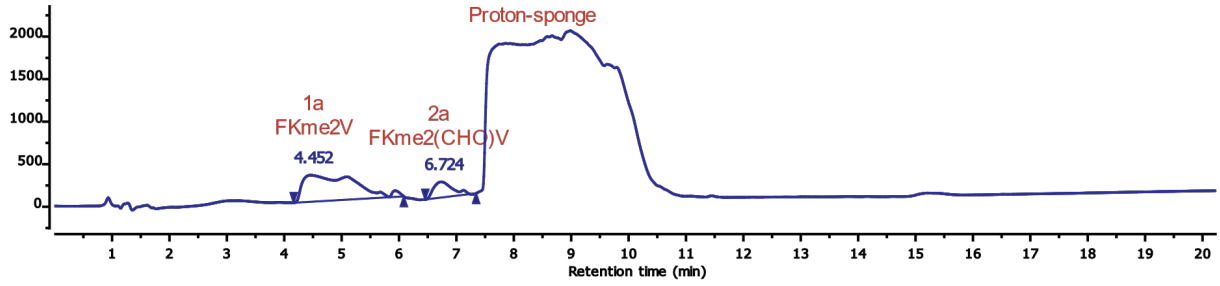

### HPLC Trace of aldehyde generation reaction from 1a using DMAP

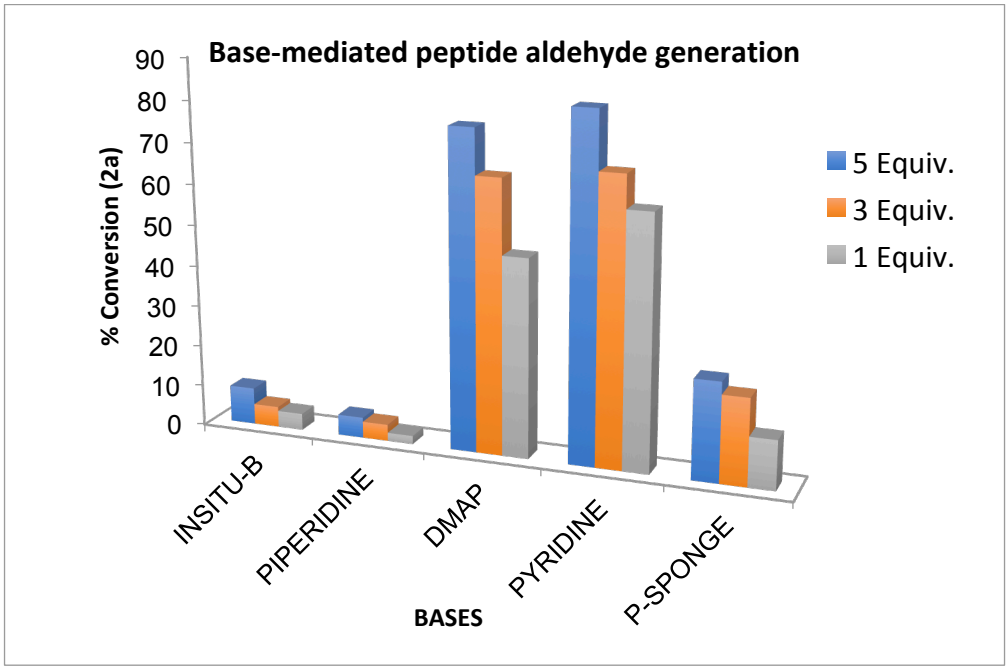



## Evaluation of proton-sponge:



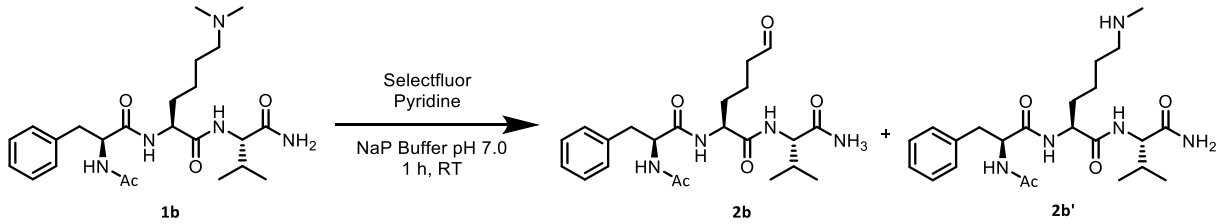

### HPLC Trace of aldehyde generation reaction from 1a using proton-sponge






| Bases | Equivalence | % Conversion (2a) |
|---|---|---|
| **No base (Insitu-Base)** | 1 | 4 |
| | 3 | 5 |
| | 5 | 9 |
| **Piperidine** | 1 | 5 |
| | 3 | 4 |
| | 5 | 2 |
| **DMAP** | 1 | 48 |
| | 3 | 66 |
| | 5 | **77** |
| **Pyridine** | 1 | 61 |
| | 3 | 69 |
| | 5 | **83** |
| **Proton-sponge** | 1 | 12 |
| | 3 | 21 |
| | 5 | 24 |

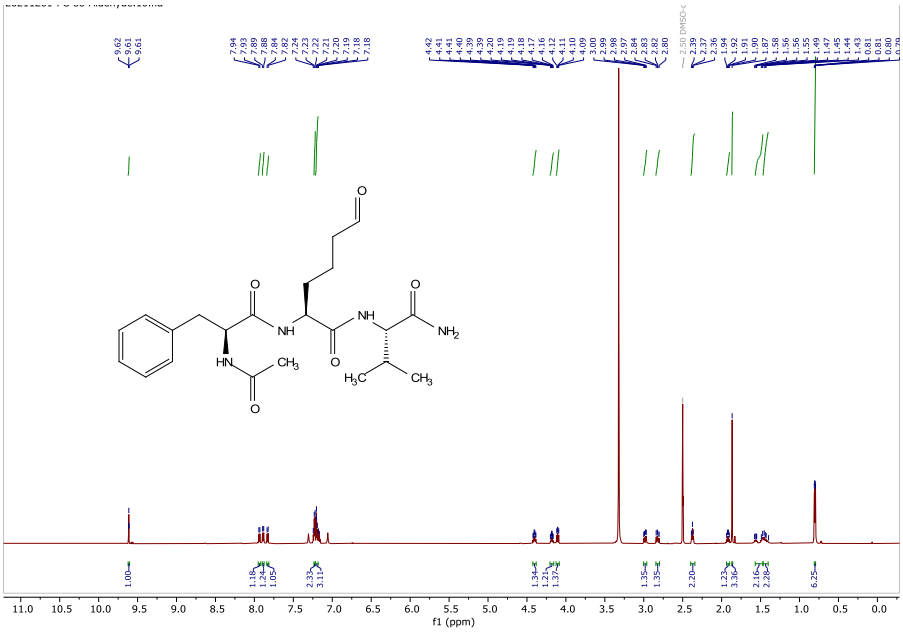
**X. Supplementary Figure 4:** Characterization of peptide-aldehyde product **2b** and monomethyllysine peptide **2b'**.



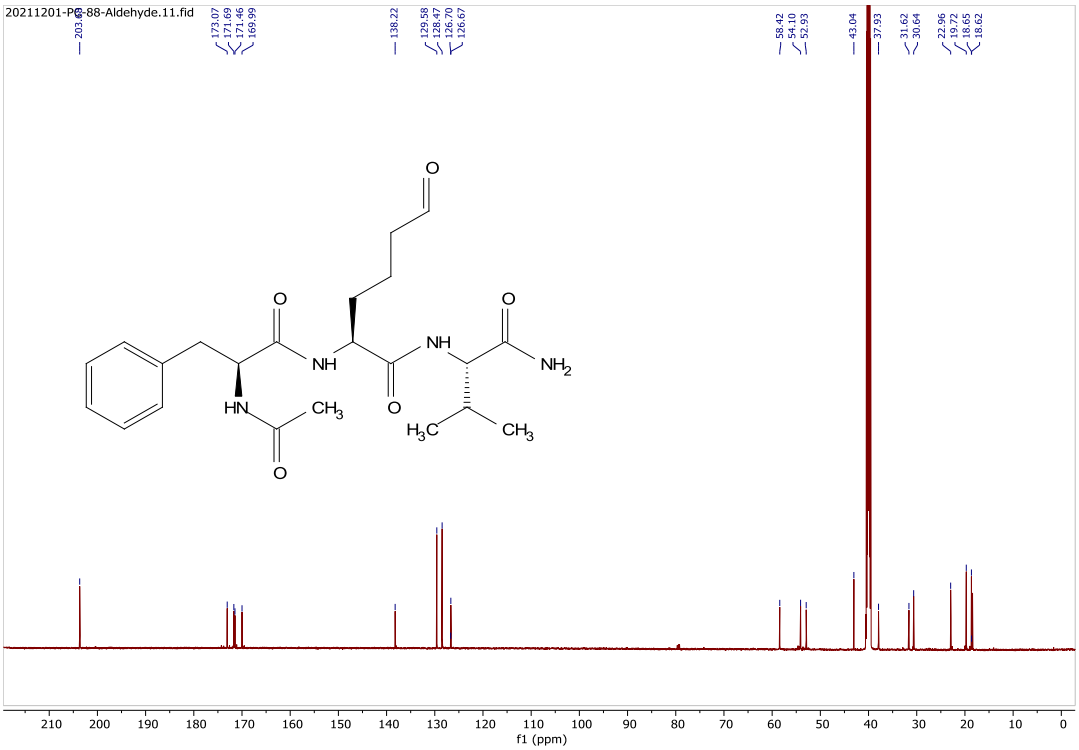

**(OAc)FKme$_2$(CHO)V 2b: $^1$H NMR** (600 MHz, DMSO-d$_6$) δ 9.61 (t, *J* = 1.6 Hz, 1H), 7.94 (d, *J* = 7.8 Hz, 1H), 7.89 (d, *J* = 8.4 Hz, 1H), 7.83 (d, *J* = 8.3 Hz, 1H), 7.23 (d, *J* = 7.1 Hz, 3H), 7.21 – 7.18 (m, 3H), 4.40 (td, *J* = 8.6, 5.1 Hz, 1H), 4.18 (td, *J* = 8.0, 5.3 Hz, 1H), 4.10 (dd, *J* = 8.4, 6.7 Hz, 1H), 2.98 (dd, *J* = 13.8, 5.1 Hz, 1H), 2.82 (dd, *J* = 13.9, 8.8 Hz, 1H), 2.40 – 2.34 (m, 2H), 1.92 (q, *J* = 6.8 Hz, 1H), 1.87 (s, 3H), 1.57 – 1.40 (m, 5H), 0.80 (dd, *J* = 6.8, 2.4 Hz, 6H). **$^{13}$C NMR** (151 MHz, DMSO) δ 203.68, 173.07, 171.69, 171.46, 169.99, 138.22, 129.58, 128.47, 126.67, 58.42, 54.10, 52.93, 43.04, 37.93, 31.62, 30.64, 22.96, 19.72, 18.65.

**(OAc)FKmeV 2b': $^1$H NMR** (600 MHz, DMSO-d$_6$) δ 8.02 (d, *J* = 7.7 Hz, 1H), 7.99 (d, *J* = 8.3 Hz, 1H), 7.86 (d, *J* = 8.2 Hz, 1H), 7.26 – 7.22 (m, 2H), 7.22 – 7.17 (m, 3H), 4.40 (td, *J* = 8.6, 5.0 Hz, 1H), 4.16 (td, *J* = 8.3, 5.3 Hz, 1H), 4.09 (dd, *J* = 8.3, 6.7 Hz, 1H), 3.00 (dd, *J* = 13.9, 5.0 Hz, 1H), 2.86 – 2.82 (m, 1H), 2.61 (d, *J* = 1.9 Hz, 2H), 2.61 (t, *J* = 7.4 Hz, 2H), 2.40 (s, 3H), 1.93 (h, *J* = 6.7 Hz, 2H), 1.88 (s, 3H), 1.59 – 1.48 (m, 2H), 1.47 – 1.41 (m, 2H), 1.25 – 1.16 (m, 2H), 0.81 (dd, *J* = 6.9, 1.2 Hz, 6H). **$^{13}$C NMR** (151 MHz, DMSO) δ 173.19, 171.76, 171.63, 170.09, 138.27, 129.59, 128.47, 126.67, 58.58, 54.16, 53.14, 49.75, 37.90, 34.39, 31.72, 30.60, 22.94 (d, *J* = 7.5 Hz), 19.74, 18.70.

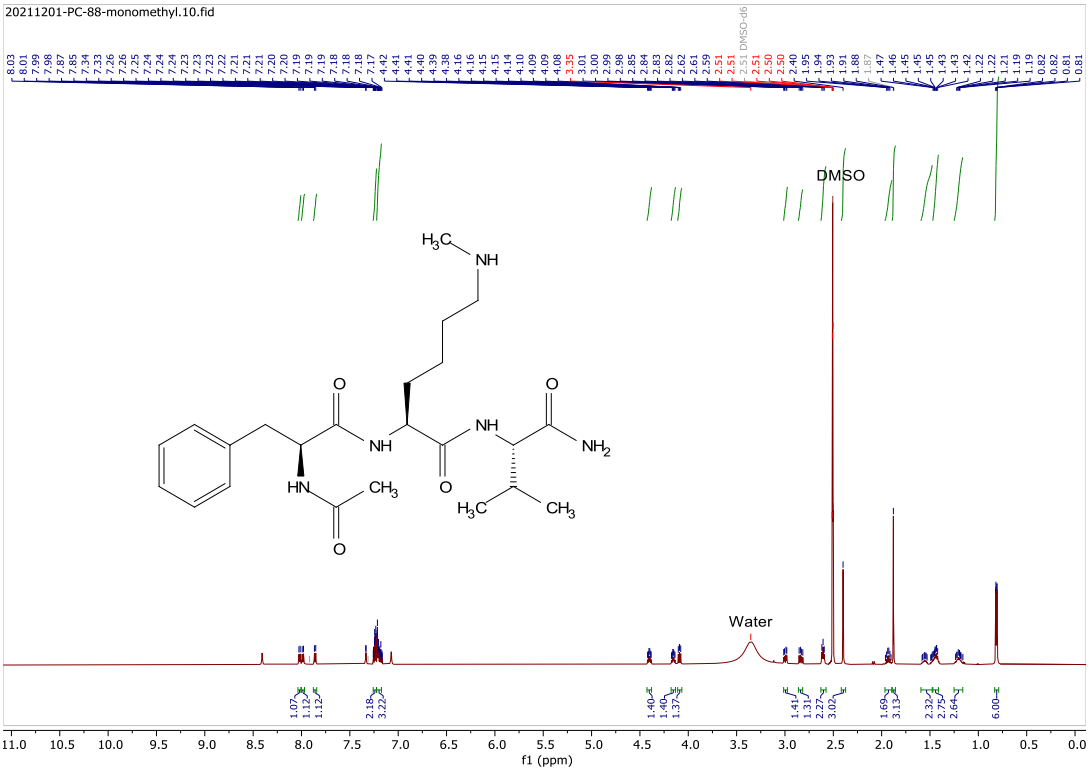
**$^1$H NMR (600 MHz, DMSO-d$_6$) of aldehyde-peptide 2b**



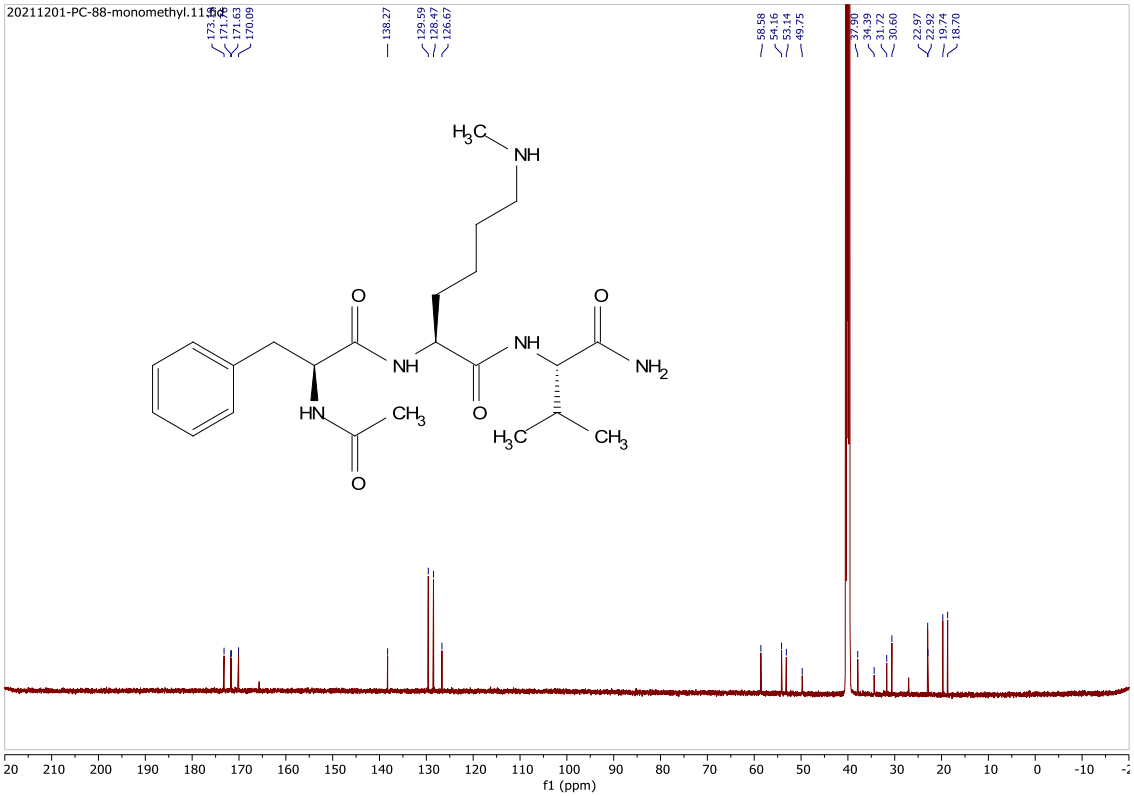
## $^{13}$C NMR (151 MHz, DMSO-d6) of aldehyde-peptide 2b



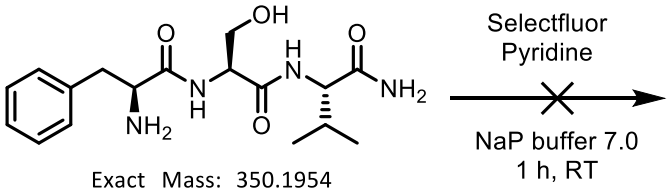

## $^{1}$H NMR (600 MHz, DMSO-$d_6$) of monomethyl lysine peptide (OAc)FKmeV 2b'



**$^{13}$C NMR (151 MHz, DMSO-$d_6$) of of monomethyl lysine peptide (OAc)FKmeV 2b'**



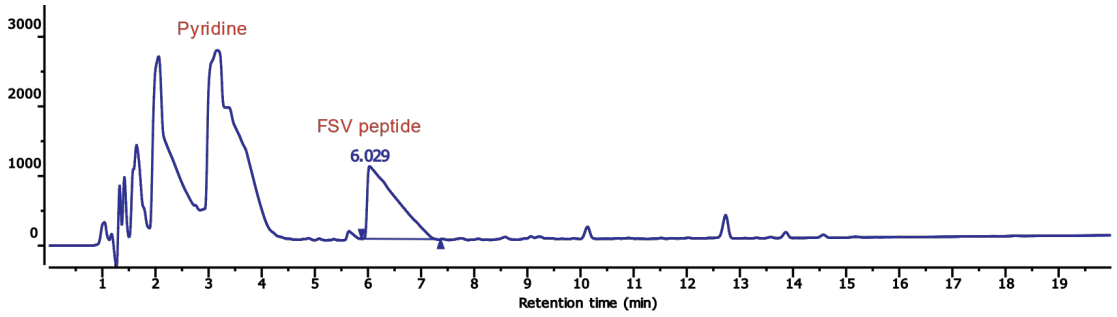

**XI. Supplementary Figure 5:** Chemoselectivity studies for aldehyde-peptide formation**.**



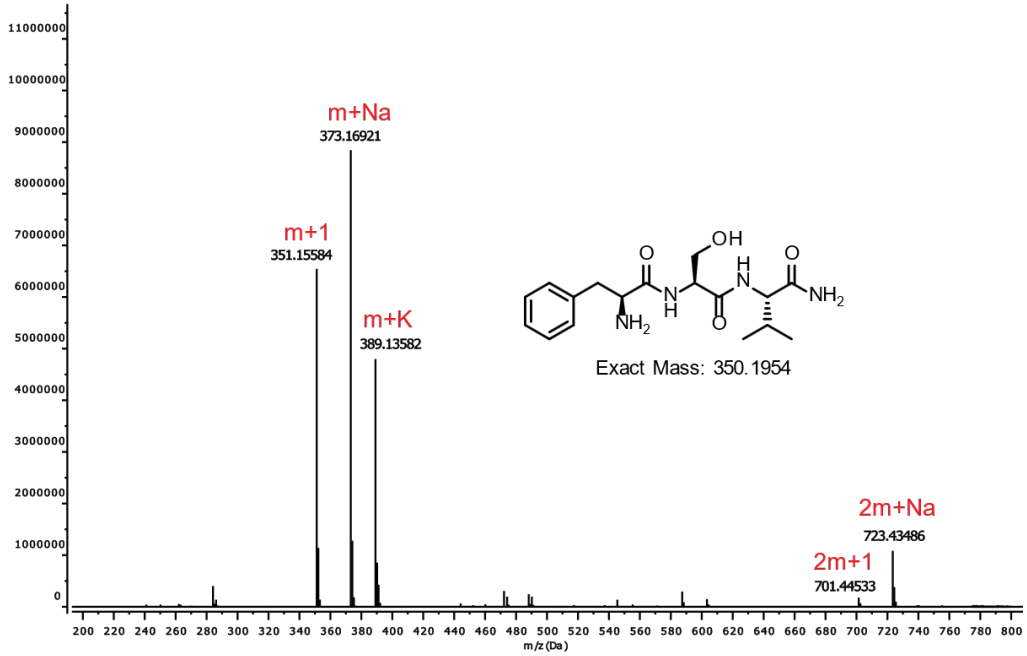

1.0 mg of serine containing tripeptide FSV was modified using **general procedure 1.** The reaction mixture was analyzed by HPLC using method A to determine the % conversion. No modification of the starting peptide was observed.

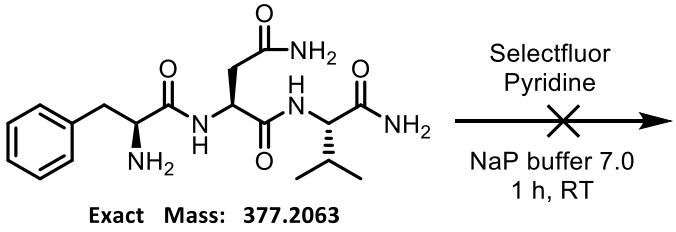
FSV linear peptide. LCMS: *m/z* 351.15584 (calcd [M+H]+ = 351.2027), *m/z* 373.16921 (calcd [M+Na] = 373.16), *m/z* 389.13582 (calcd [M+K] = 389.1591), *m/z* 701.44533 (calcd [2M+H]+ = 701.3981), *m/z* 723.43486 (calcd [2M+Na] = 723.2800), (HPLC analysis at 220 nm). Retention time in HPLC: 6.029

**HPLC Trace of chemoselectivity evaluation of serine**




**MS-Trace of peak 6.029**



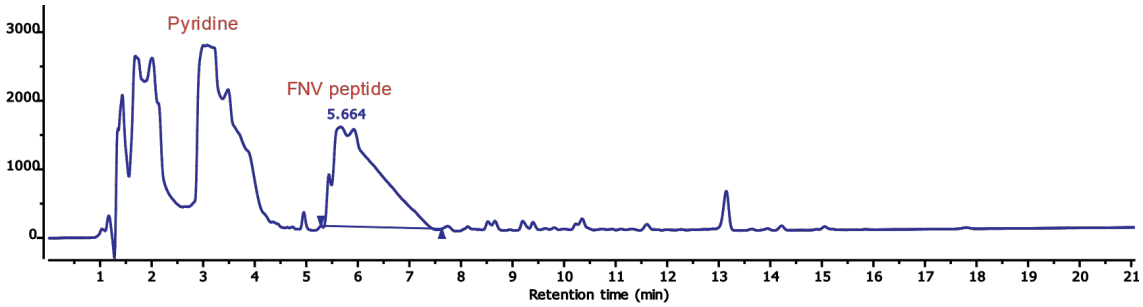




**Exact Mass: 377.2063**

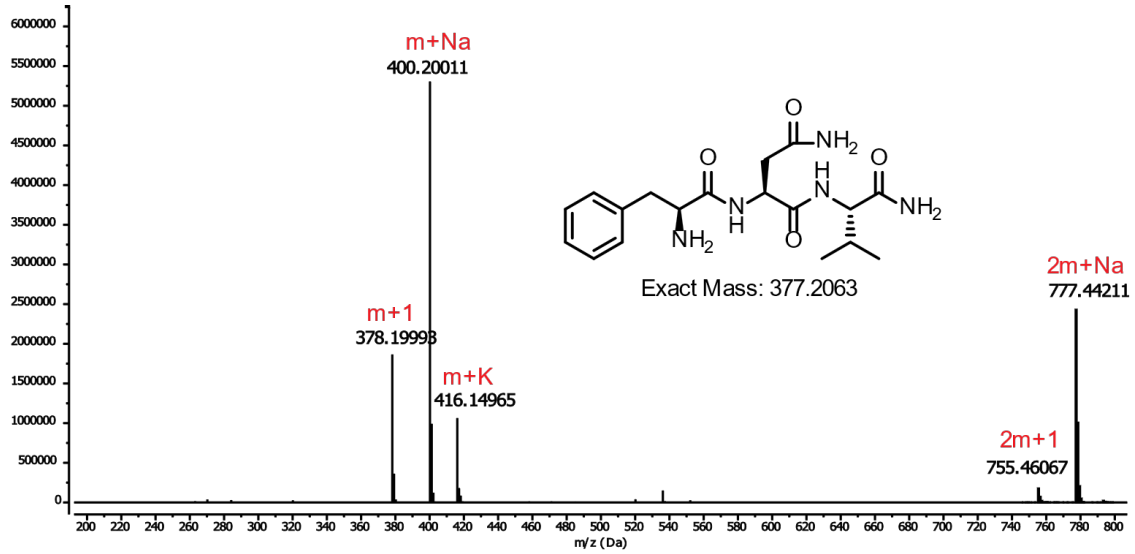
1.0 mg of asparagine containing tripeptide FNV was modified using **general procedure 1.** The reaction mixture was analyzed by HPLC using method A to determine the % conversion. No modification of the starting peptide was observed.

FNV linear peptide. LCMS: *m/z* 378.19993 (calcd [M+H]+ = 378.2136), *m/z* 400.20011 (calcd [M+Na]+ = 400.1955), *m/z* 416.14965 (calcd [M+K]+ = 416.1695), *m/z* 755.46067 (calcd [2M+H]+ = 755.4199), *m/z* 777.44211 (calcd [2M+Na]+ = 777.4018), (HPLC analysis at 220 nm). Retention time in HPLC: 5.664

**HPLC Trace of chemoselectivity evaluation of asparagine**

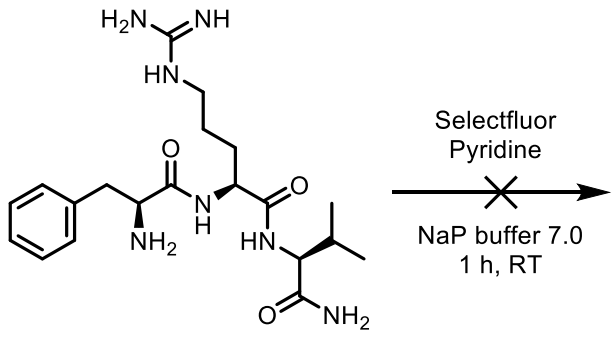



**MS-Trace of peak 5.664**






Exact Mass: 377.2063

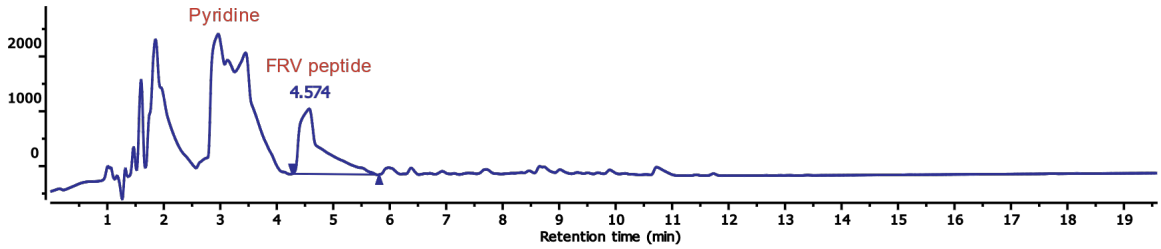



Exact Mass: 419.2645

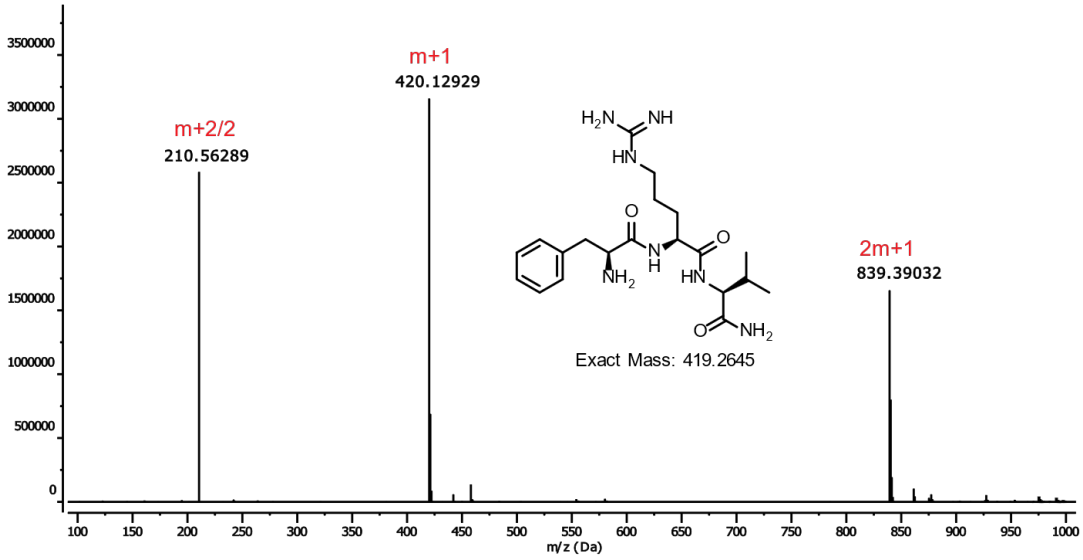
1.0 mg of arginine containing peptide FRV was modified using **general procedure 1.** The reaction mixture was analyzed by HPLC using method A to determine the % conversion. No modification of the starting peptide was observed.

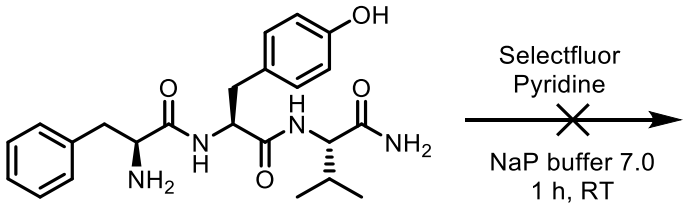
FRV linear peptide. LCMS: *m/z* 420.12929 (calcd $[M+H]^+$ = 420.2718), *m/z* 210.56289 (calcd $[M+2/2]^+$ = 210.5659), *m/z* 839.39032 (calcd $[2M+H]^+$ = 839.5363), (HPLC analysis at 220 nm). Retention time in HPLC: 4.574

**HPLC Trace of chemoselectivity evaluation of arginine**




**MS-Trace of peak 4.574**



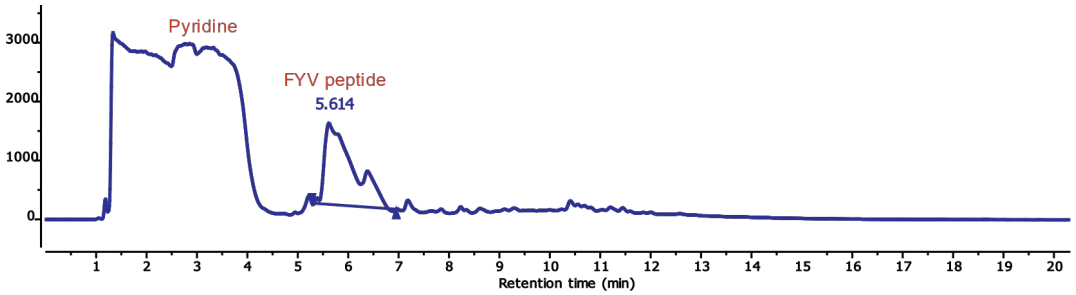




Exact Mass: 426.2267

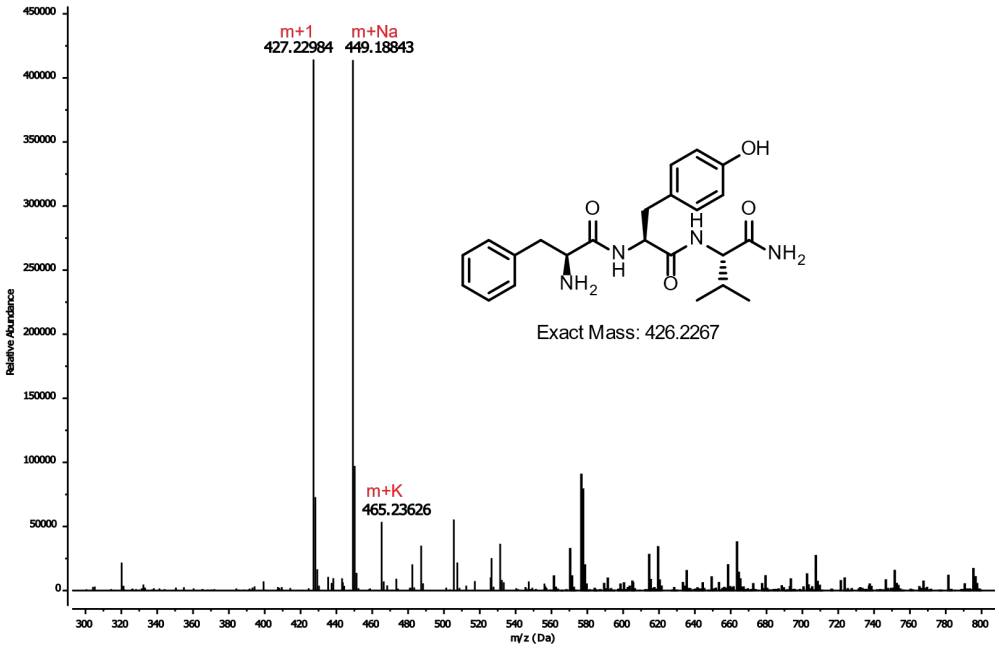
1.0 mg of tyrosine containing tripeptide FYV was modified using **general procedure 1.** The reaction mixture was analyzed by HPLC using method A to determine the % conversion. No modification of the starting peptide was observed.

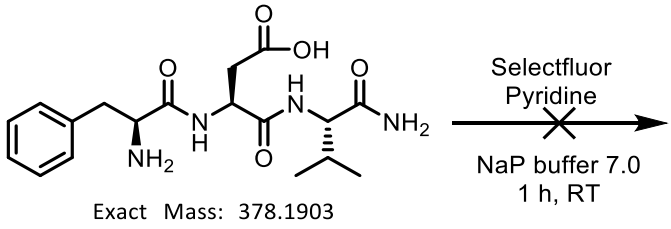
FYV linear peptide. LCMS: *m/z* 427.22984 (calcd [M+H]+ =427.2340), *m/z* 449.18843 (calcd [M+Na]+ = 449.2159), *m/z* 465.23626 (calcd [M+K]+ = 465.1899), (HPLC analysis at 220 nm). Retention time in HPLC: 5.614

**HPLC Trace of chemoselectivity evaluation of tyrosine**




**MS-Trace of peak 5.614**

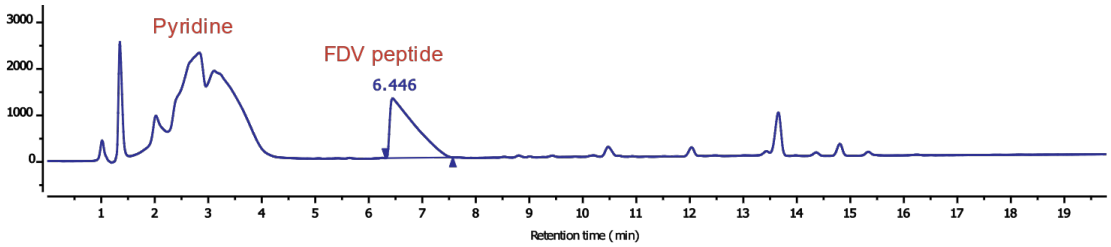


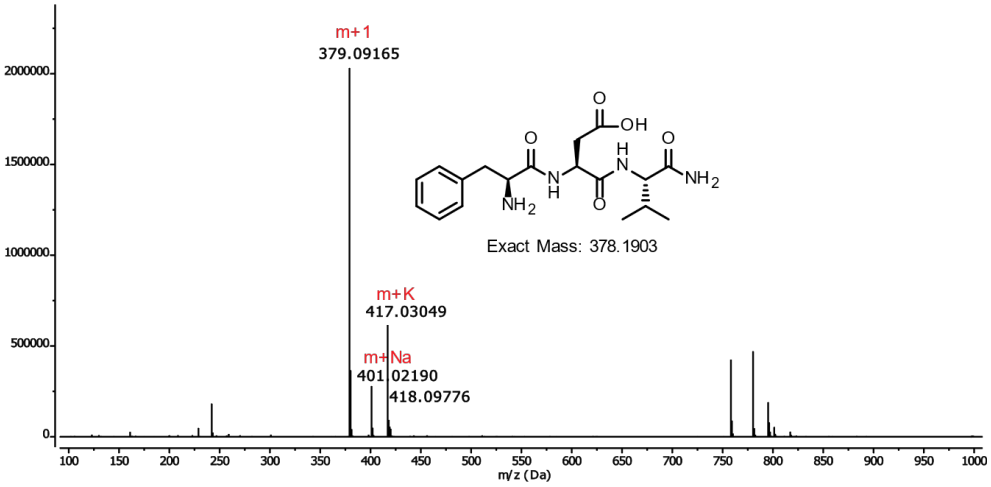

1.0 mg of aspartic acid containing tripeptide FDV was modified using **general procedure 1.** The reaction mixture was analyzed by HPLC using method A to determine the % conversion. No modification of the starting peptide was observed

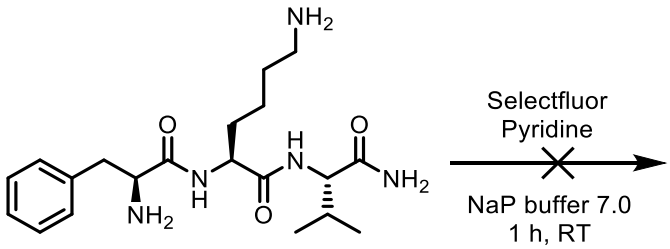
FDV linear peptide. LCMS: m/z 379.09165 (calcd [M+H]+ = 379.1976), m/z 401.02190 (calcd [M+Na]+ = 401.1795), m/z 417.03049 (calcd [M+K]+ = 417.1535), (HPLC analysis at 220 nm). Retention time in HPLC: 6.446

**HPLC Trace of chemoselectivity evaluation of aspartic acid**




**MS-Trace of peak 6.446**



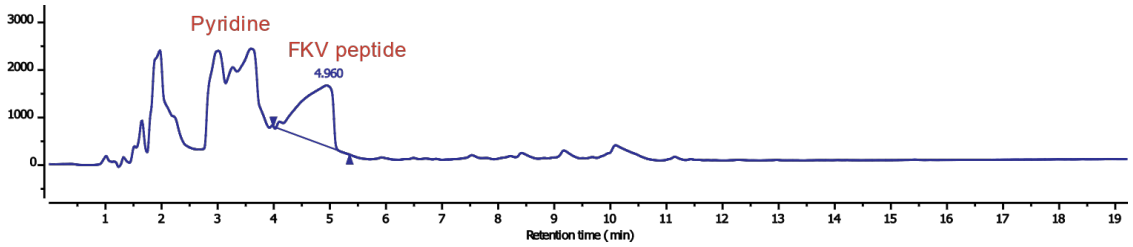




Exact Mass: 391.2583

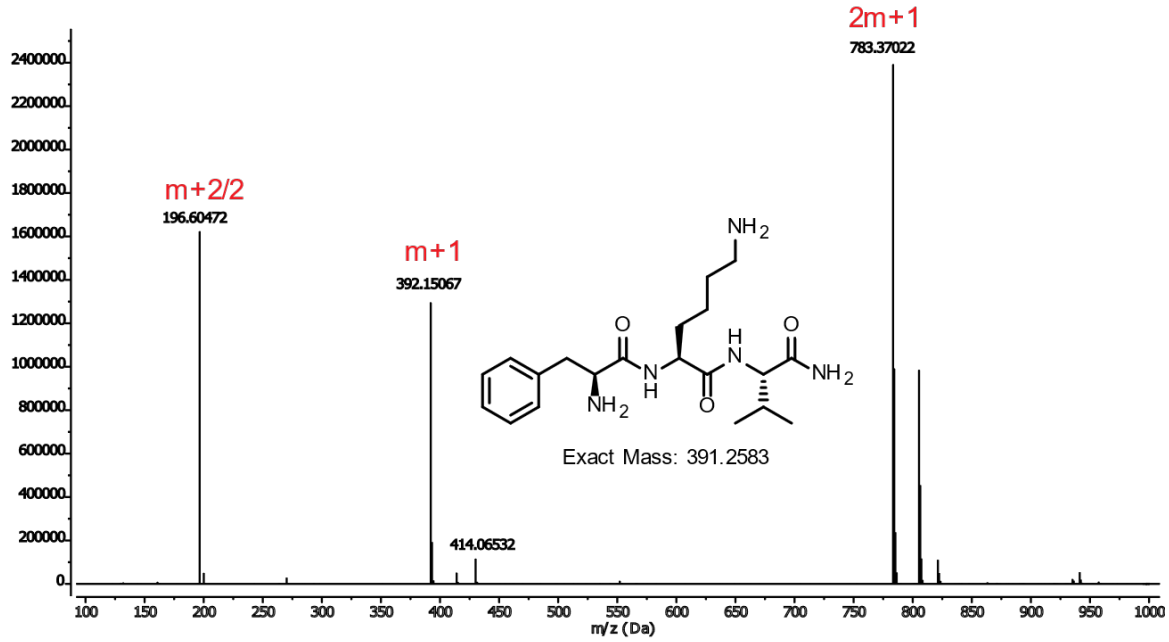
1.0 mg of lysine containing tripeptide FKV was modified using **general procedure 1.** The reaction mixture was analyzed by HPLC using method A to determine the % conversion. No modification of the starting peptide was observed.

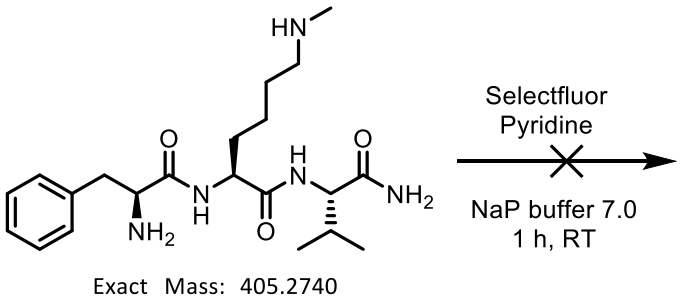
FKV linear peptide. LCMS: m/z 392.15067 (calcd [M+H]+ = 392.2656), m/z 392.15067 (calcd [M+2/2]+ = 196.6291), m/z 783.37022 (calcd [2M+H]+ = 783.5240), (HPLC analysis at 220 nm). Retention time in HPLC: 4.960

**HPLC Trace of chemoselectivity evaluation of lysine**




**MS-Trace of peak 4.960**

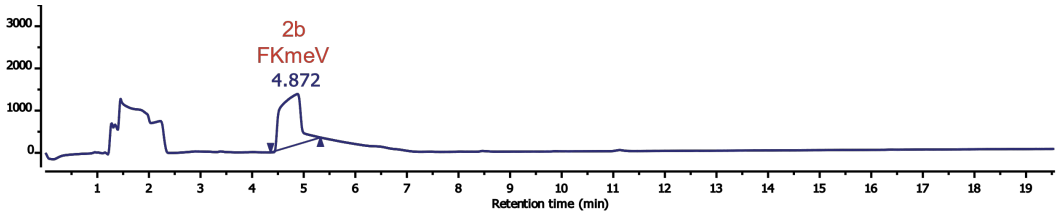


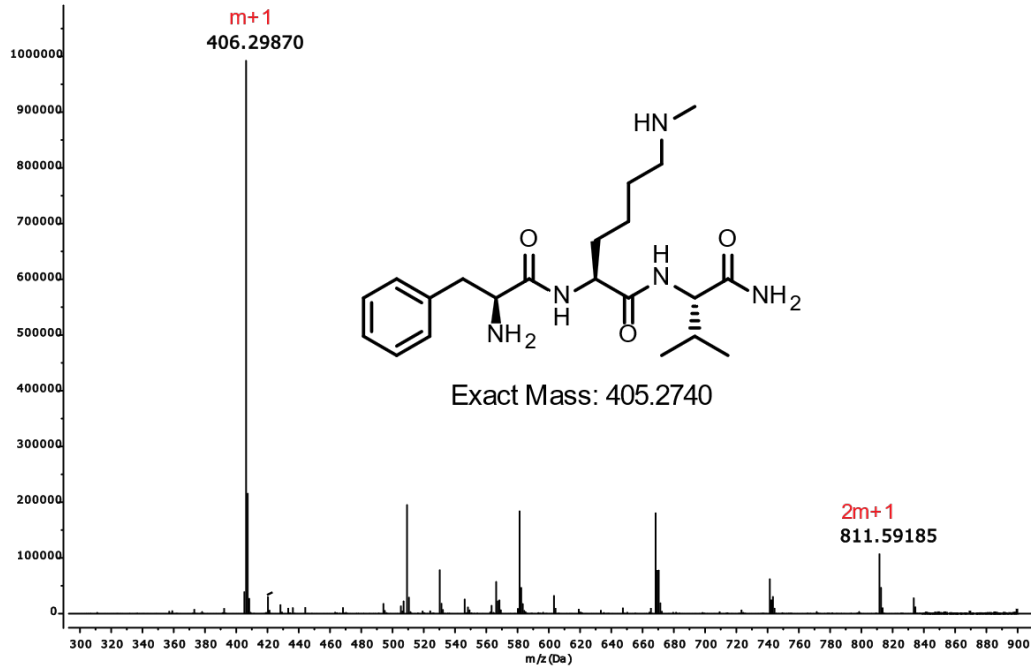

1.0 mg of monomethyllysine containing tripeptide FKmeV was modified using **general procedure 1.** The reaction mixture was analyzed by HPLC using method A to determine the % conversion. No modification of the starting peptide was observed.

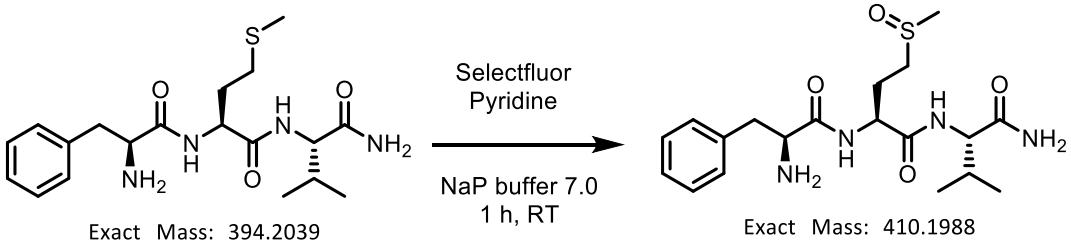
FKmeV linear peptide **2b**. LCMS: m/z 406.29870 (calcd [M+H]+ = 406.54), m/z 811.59185 (calcd [2M+H]+ = 811.59), (HPLC analysis at 220 nm). Retention time in HPLC: 4.872

**HPLC Trace of chemoselectivity evaluation of monomethyllysine**




**MS-Trace of peak 4.872**



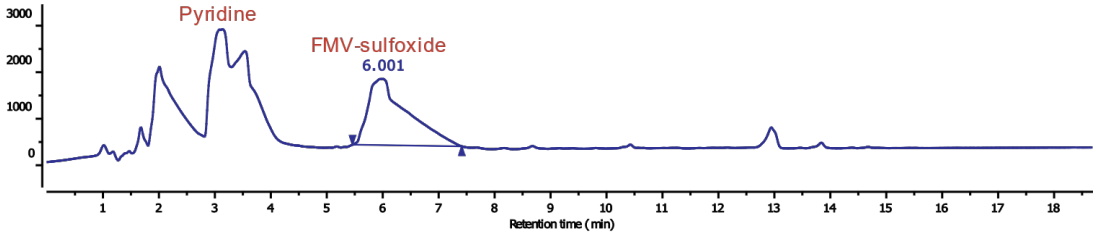




1.0 mg of methionine containing tripeptide FMV was modified using **general procedure 1.** The reaction mixture was analyzed by HPLC using method A to determine the % conversion. The conversion of the sulfoxide product was determined to be (97%).

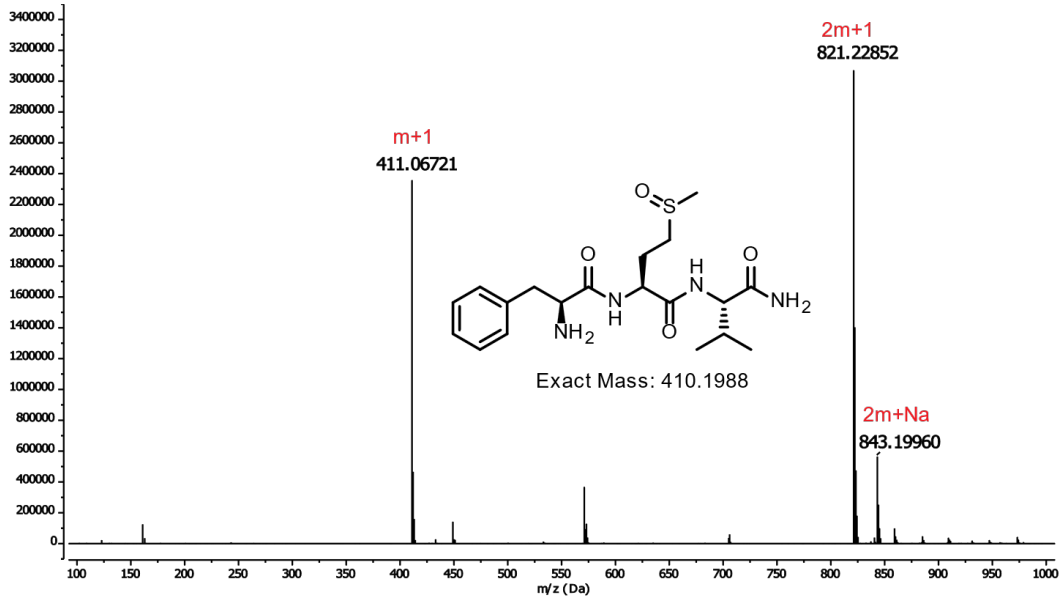
FMV linear peptide. LCMS: *m/z* 395.06721 (calcd [M+H]+ = 395.2111), (HPLC analysis at 220 nm). Retention time in HPLC: 6.839

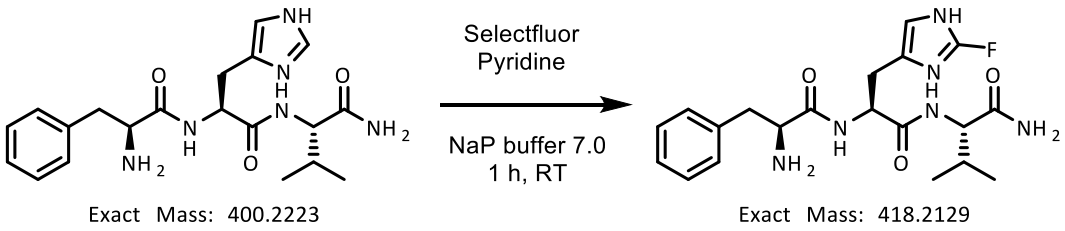
FMV sulfoxide peptide product. LCMS: *m/z* 411.06721 (calcd [M+H]+ = 411.2061), *m/z* 821.22852 (calcd [2M+H]+ = 821.4048), *m/z* 843.19960 (calcd [2M+Na]+ = 843.3868), (HPLC analysis at 220 nm). Retention time in HPLC: 6.001

**HPLC Trace of chemoselectivity evaluation of methionine**




**MS-Trace of peak 6.001**



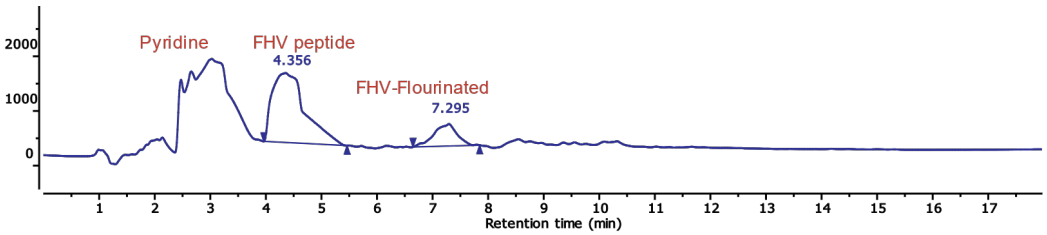

Exact Mass: 400.2223 Exact Mass: 418.2129

1 mg of histidine containing tripeptide FHV was modified using **general procedure 1.** The reaction mixture was analyzed by HPLC using method A to determine the % conversion. The conversion of the fluorinated product was determined to be (14%).

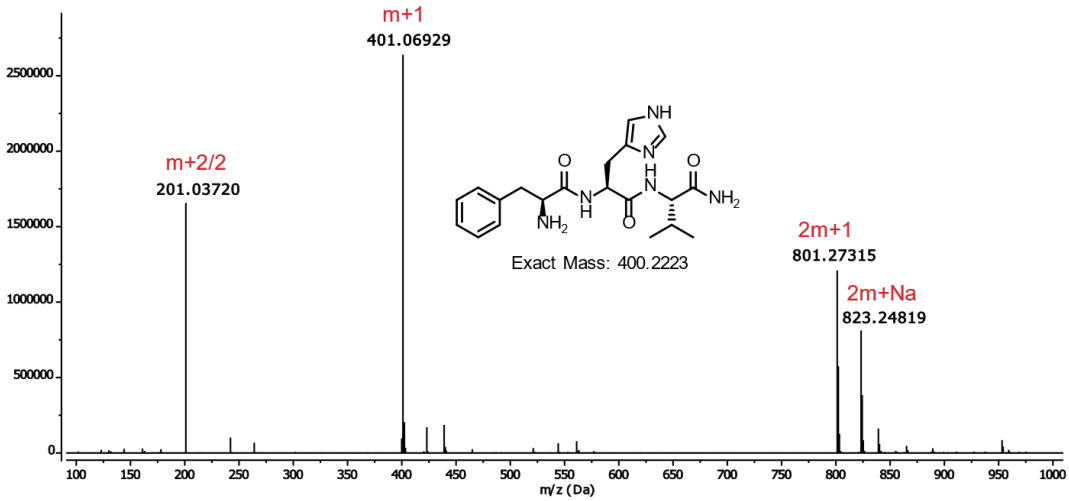
FHV linear peptide. LCMS: *m/z* 401.06929 (calcd [M+H]+ = 401.2296), *m/z* 201.03720 (calcd [M+2/2]+ = 201.1148), *m/z* 801.27315 (calcd [2M+H]+ = 801.4519), *m/z* 823.24819 (calcd [2M+Na]+ = 823.4338), (HPLC analysis at 220 nm). Retention time in HPLC: 4.356

FHV flourination peptide product. LCMS: *m/z* 419.06721 (calcd [M+H]+ = 419.2201), *m/z* 210.03720 (calcd [M+2/2]+ = 210.1100), (HPLC analysis at 220 nm). Retention time in HPLC: 7.295

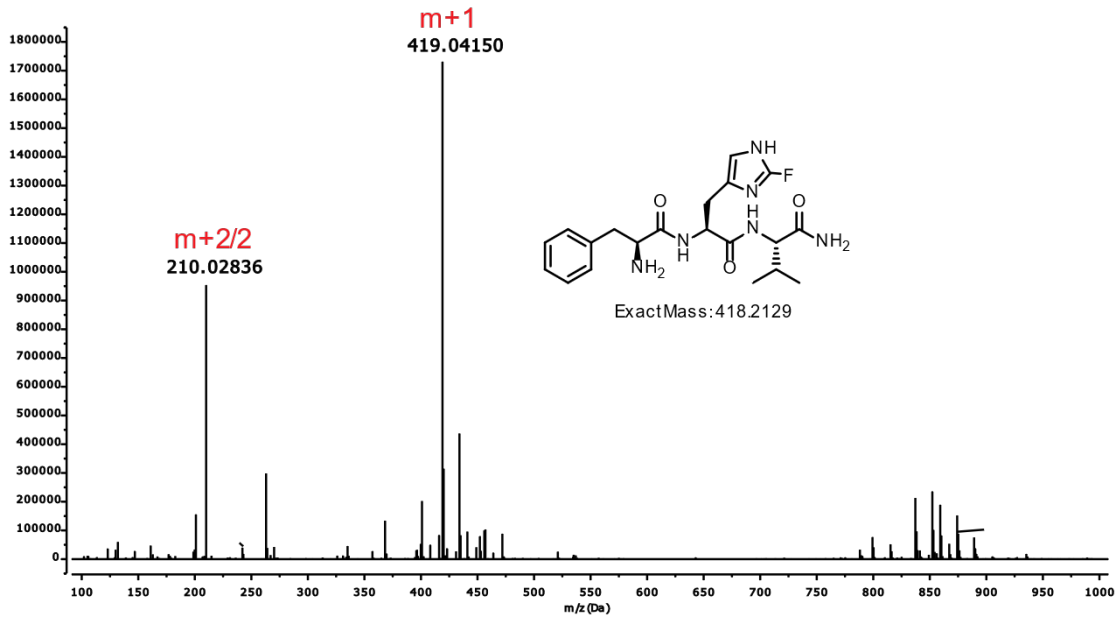
**HPLC Trace of chemoselectivity evaluation of histidine**

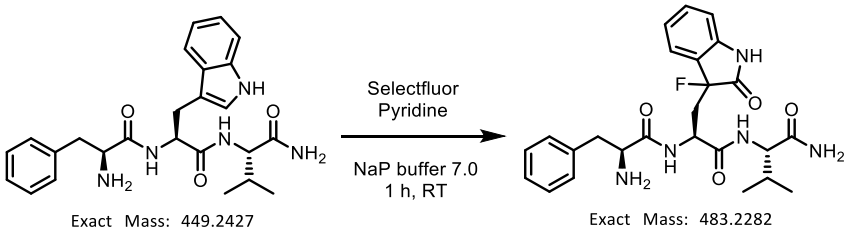



**MS-Trace of peak 4.356**



**MS-Trace of peak 7.295**

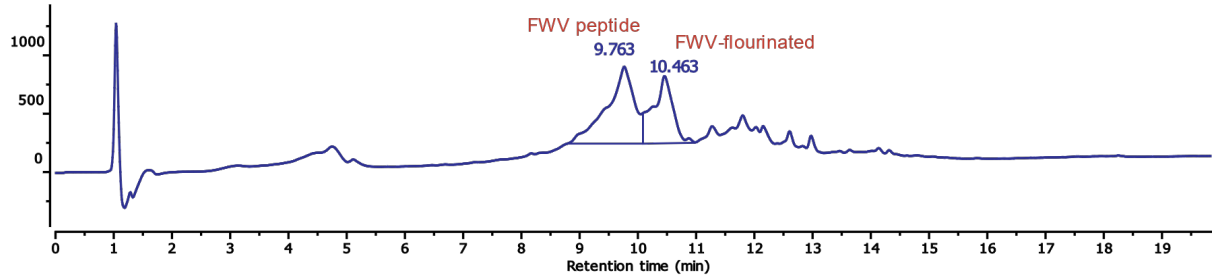


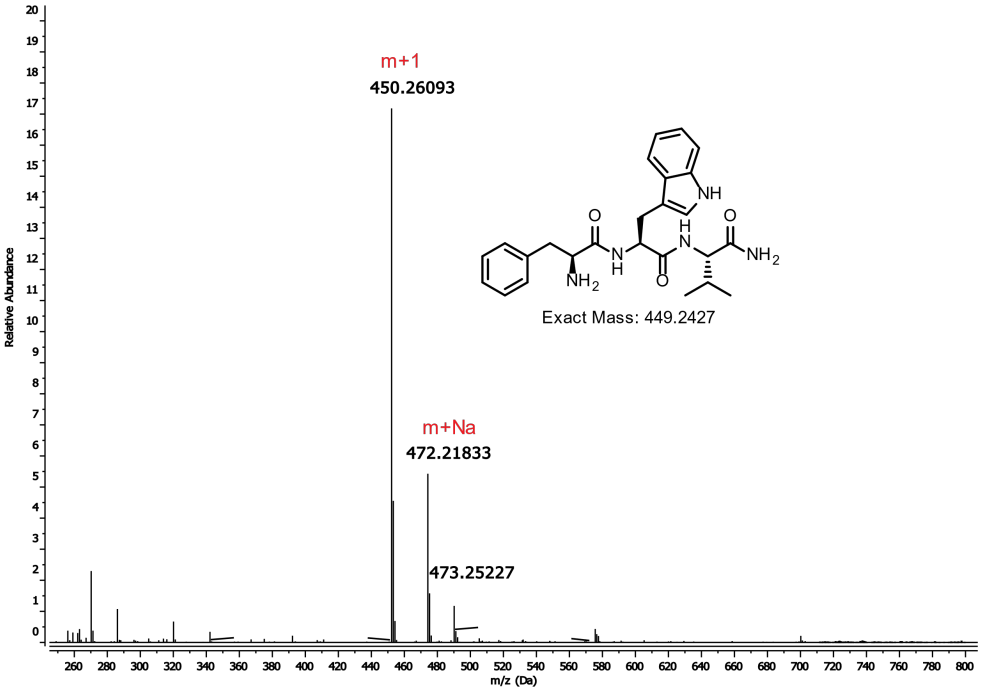

1.0 mg of tryptophan containing tripeptide FWV was modified using **general procedure 1.** The reaction mixture was analyzed by HPLC using method A to determine the % conversion. The conversion of the fluorinated product was determined to be (57%).

FWV linear peptide. LCMS: m/z 450.26093 (calcd [M+H]+ = 450.2500), LCMS: m/z 472.21833 (calcd [M+Na]+ = 472.2319), (HPLC analysis at 220 nm). Retention time in HPLC: 9.763

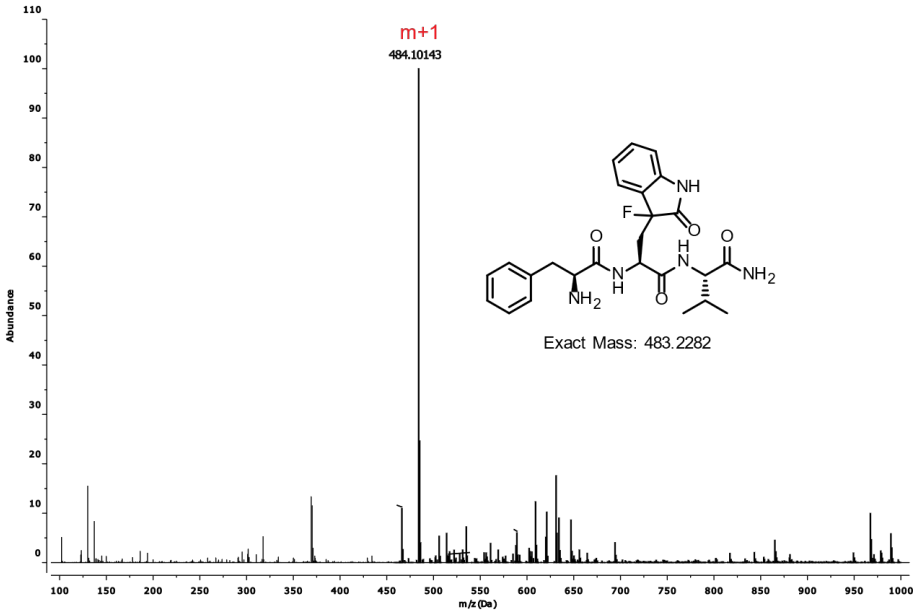
FWV flourinated peptide product. LCMS: m/z 484.10143 (calcd [M+H]+ = 484.2355), (HPLC analysis at 220 nm). Retention time in HPLC: 10.463

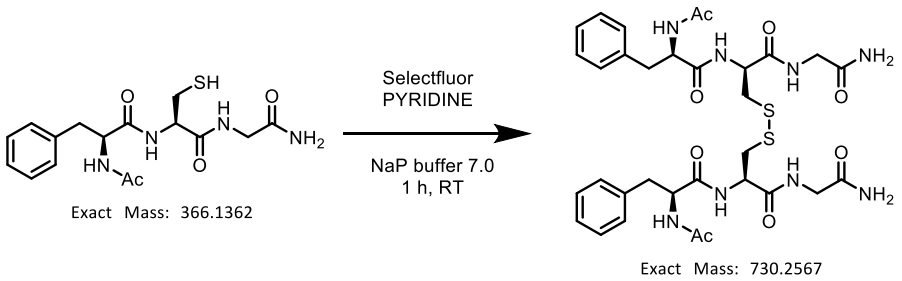


## MS-Trace of peak 9.763




## MS-Trace of peak 10.463



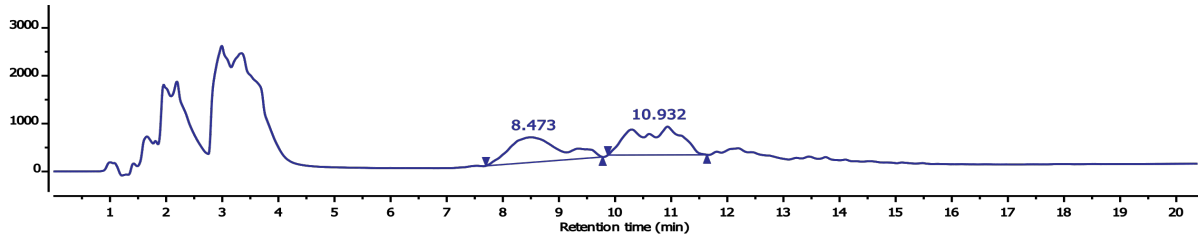



1.0 mg of cysteine containing tripeptide FCV was modified using **general procedure 1.** The reaction mixture was analyzed by HPLC using method A to determine the % conversion. The disulfide product was observed to be (54%).

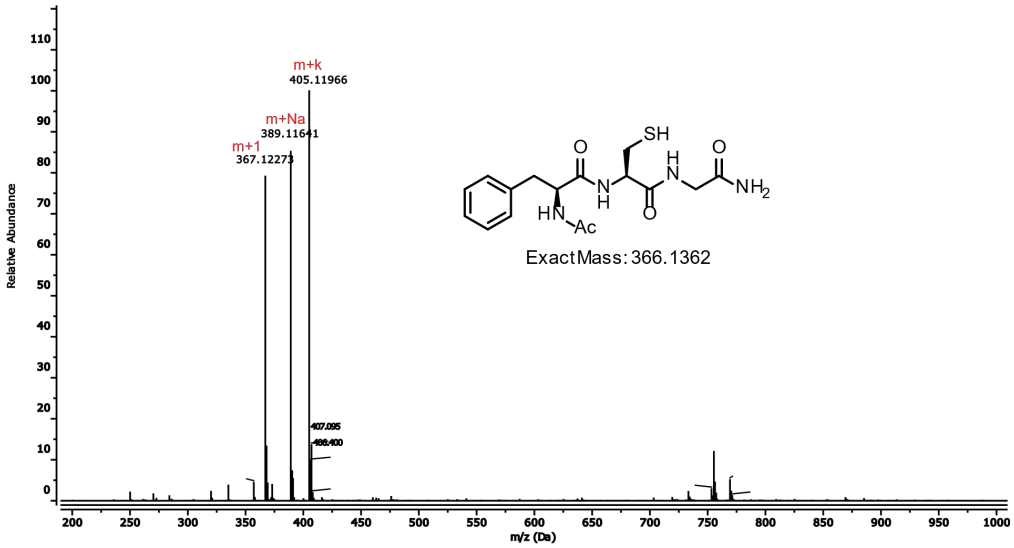
FCV linear peptide. LCMS: m/z 367.12273 (calcd [M+H]+ = 367.1435), m/z 389.11641 (calcd [M+H]+ = 389.1254), m/z 405.11966 (calcd [M+H]+ = 405.0993), (HPLC analysis at 220 nm). Retention time in HPLC: 8.473

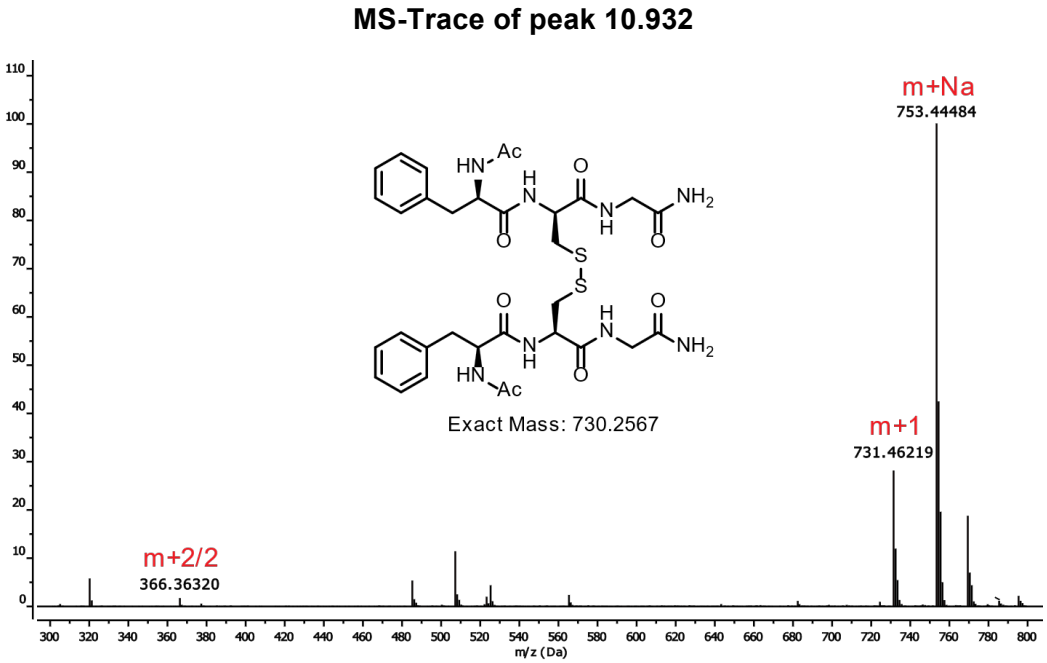
FCV disulfide peptide product. LCMS: *m/z* 731.46219 (calcd [M+H]+ = 731.2640), m/z 366.36320 (calcd [M+2/2]+ = 366.1327), *m/z* 753.44483 (calcd [M+Na]+ = 753.2459), (HPLC analysis at 220 nm). Retention time in HPLC: 10.932

**HPLC Trace of chemoselectivity evaluation of cysteine**

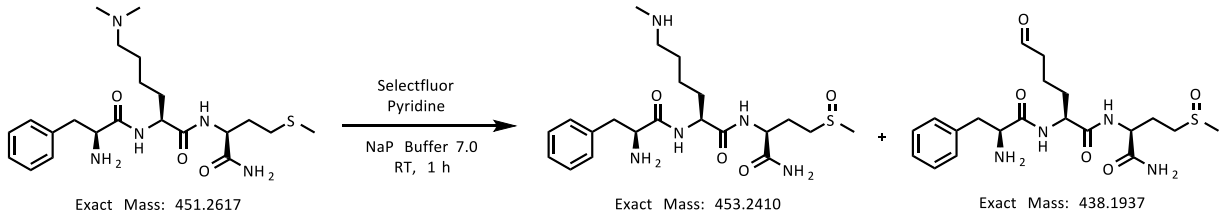



**MS-Trace of peak 8.473**






**XII. Supplementary Figure 6.** Evaluation of effect of adducts on enrichment of $Kme_2$-generated aldehyde.

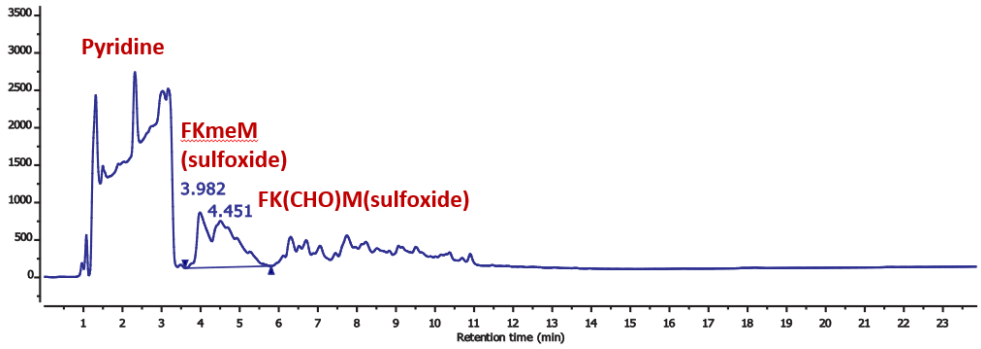



To 1.0 mg of $FKme_2M$ peptide dissolved in 300 µL of 10 mM sodium phosphate buffer (NaP, pH 7.0), was added pyridine (5 eq) and selectfluor (2 eq). The reaction mixture was stirred for 1 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the generation of peptide aldehyde and sulfoxide product. The reaction mixture was analyzed by HPLC using method A and product peak purified by preparative HPLC.

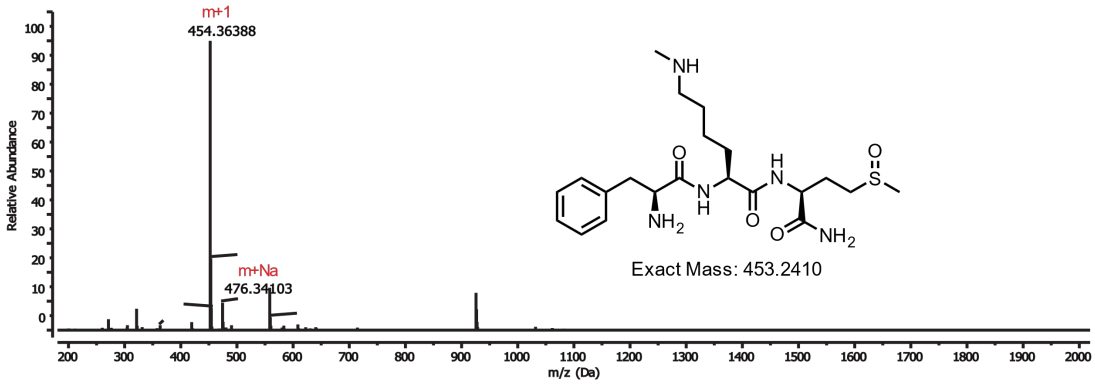
**FKmeM(sulfoxide).** LCMS: *m/z* 454.36388 (calcd [M+H]+ = 454.2483), *m/z* 476.34103 (calcd [M+Na]+ = 476.2302), (HPLC analysis at 220 nm, **method A**). Retention time in HPLC: 3.982

**FK(CHO)M(sulfoxide).** LCMS: *m/z* 439.05783 (calcd [M+H]+ = 439.2010), *m/z* 877.11566 (calcd [2M+1]+ = 877.3947), (HPLC analysis at 220 nm, **method A**). Retention time in HPLC: 4.451

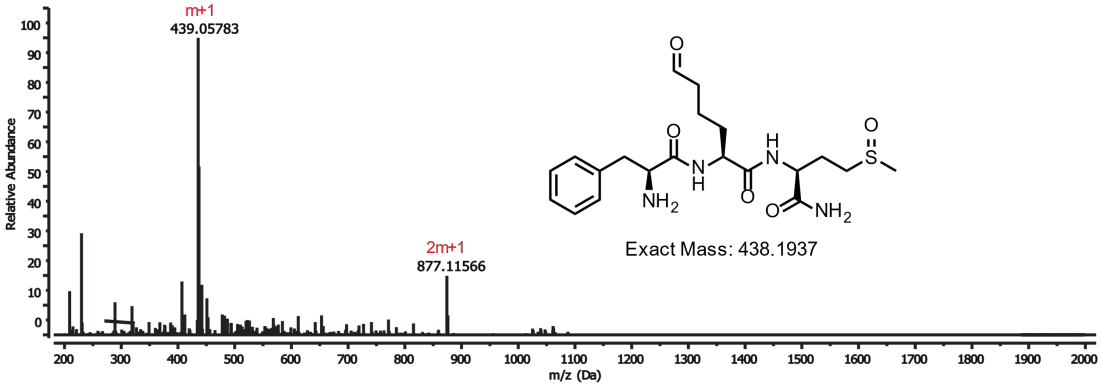
## HPLC Trace of reaction

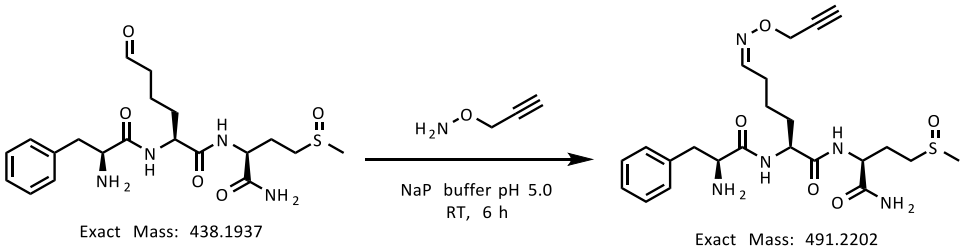



## MS-Trace of peak 3.982 (FKmeM(sulfoxide))




## MS-Trace of peak 4.451 (FK(CHO)M(sulfoxide))

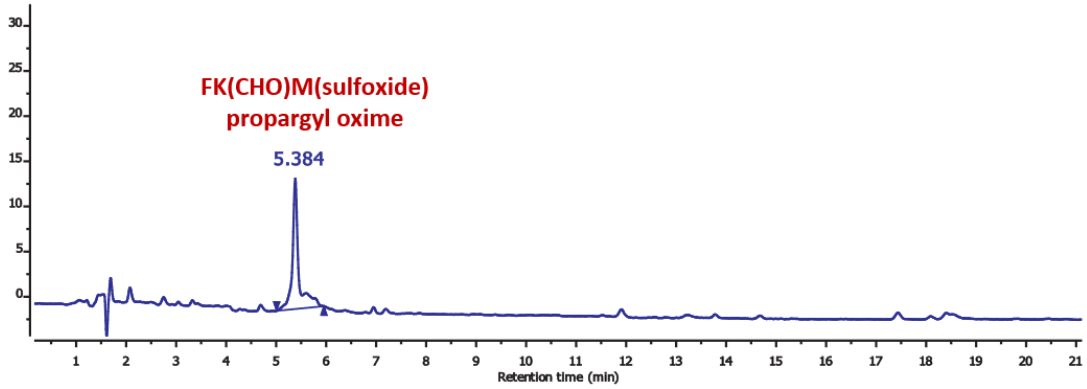




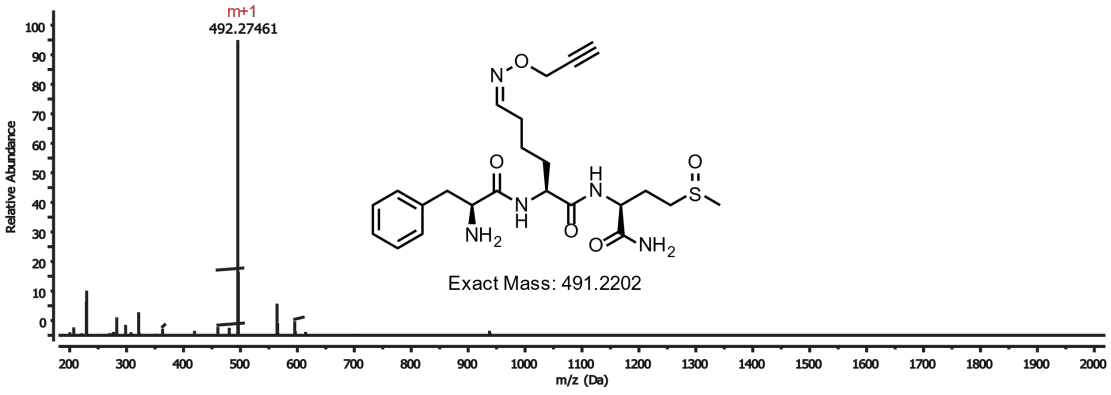

To 1 mg of aldehyde-sulfoxide peptide **FK(CHO)M(sulfoxide)** in 300 µL of NaP buffer was added 4 equivalences of O-2-Propynylhydroxylamine hydrochloride. The reaction was stirred for 6 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the modification of the peptide **FK(CHO)M(sulfoxide)** to the oxime propargyl peptide product. The reaction mixture was analyzed by HPLC using method B and the % conversion to the oxime propargyl peptide product (>98%).

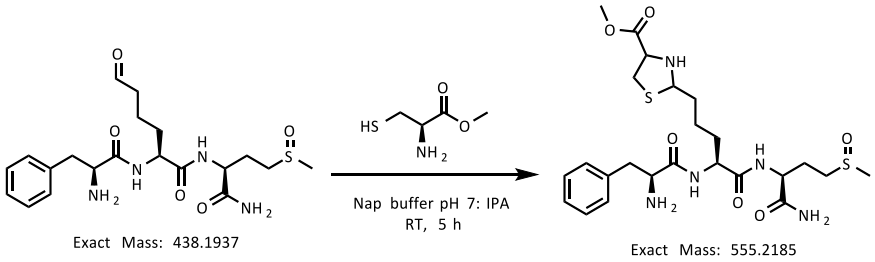
**FK(CHO)M(sulfoxide) propargyl oxime.** LCMS: *m/z* 492.27461 (calcd [M+H]+ = 492.2275), (HPLC analysis at 220 nm, **method A**). Retention time in HPLC: 5.384

**HPLC Trace of reaction**




**MS-Trace of peak 5.384 (FK(CHO)M(sulfoxide) propargyl oxime)**

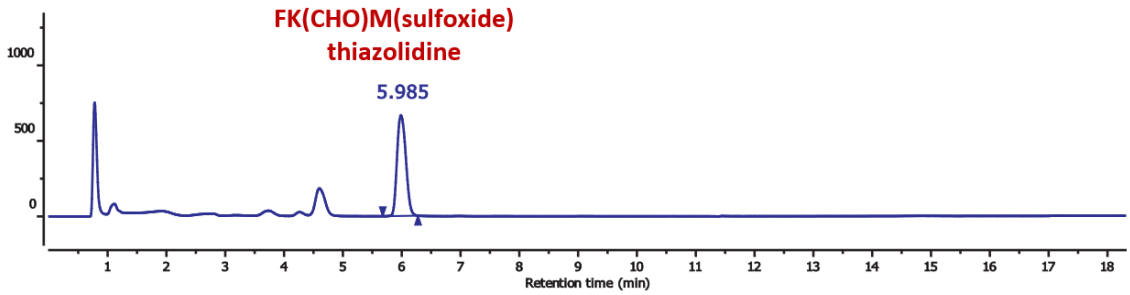




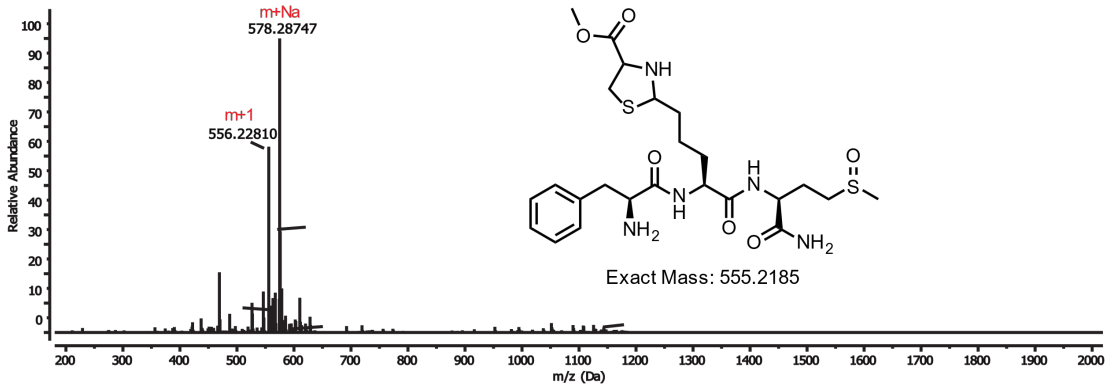

To 1 mg of aldehyde-sulfoxide peptide **FK(CHO)M(sulfoxide)** in 300 µL of NaP buffer and isopropyl alcohol (IPA) was added 5 equivalences of cysteine methylester. Reaction was stirred at room temperature for 5 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the formation of thiazolidine. The reaction mixture was analyzed by HPLC using method A and % conversion to thiazolidine product was >99%.

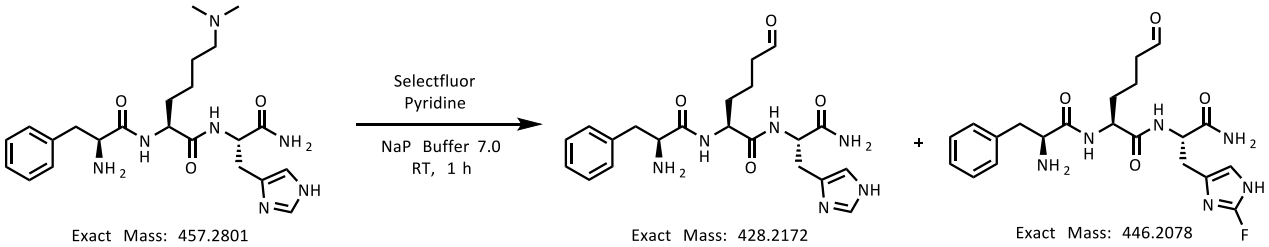
**FK(CHO)M(sulfoxide) thiazolidine.** LCMS: *m/z* 556.22810 (calcd [M+H]+ = 556.2258), *m/z* 578.28747 (calcd [M+Na]+ = 578.2077), (HPLC analysis at 220 nm, **method A**). Retention time in HPLC: 5.985

**HPLC Trace of reaction**




**MS-Trace of peak 5.985 (FK(CHO)M(sulfoxide) thiazolidine)**



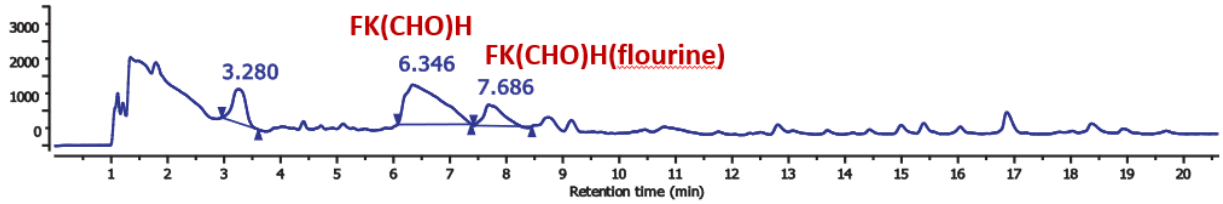



To 1.0 mg of FK$me_2$H peptide dissolved in 300 µL of 10 mM sodium phosphate buffer (NaP, pH 7.0), was added pyridine (5 eq) and selectfluor (2 eq). The reaction mixture was stirred for 1 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the generation of fluorinated peptide aldehyde product. The reaction mixture was analyzed by HPLC using method A and product peak purified by preparative HPLC.

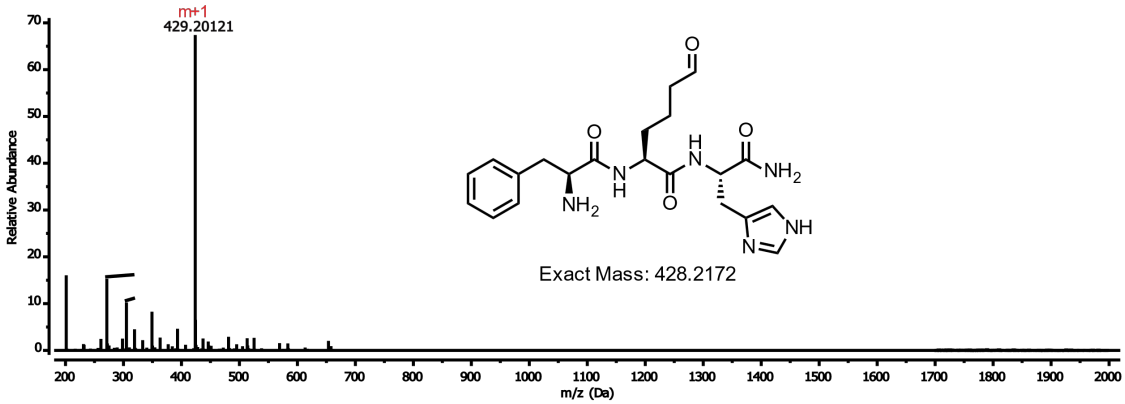
**FK(CHO)H.** LCMS: *m/z* 429.20121 (calcd [M+H]+ = 429.2245), (HPLC analysis at 220 nm, **method A**). Retention time in HPLC: 6.346

**FK(CHO)H(flourine).** LCMS: *m/z* 447.26419 (calcd [M+H]+ = 447.2151), (HPLC analysis at 220 nm, **method A**). Retention time in HPLC: 7.686

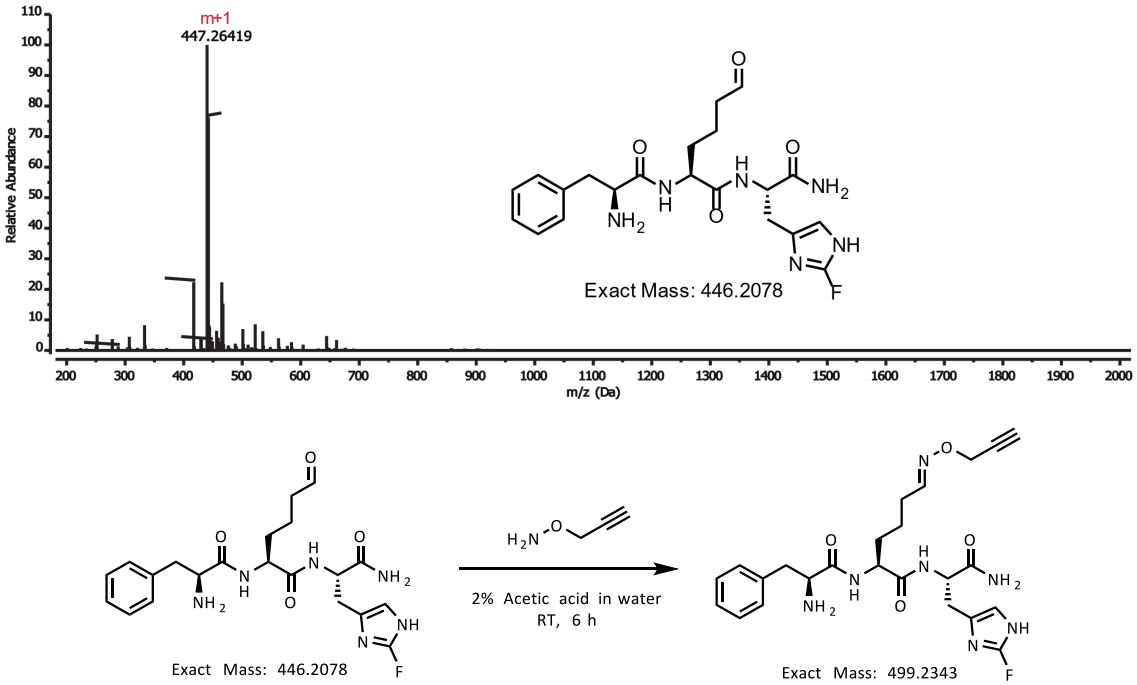
**HPLC Trace of reaction**




**MS-Trace of peak 6.346 (FK(CHO)H)**



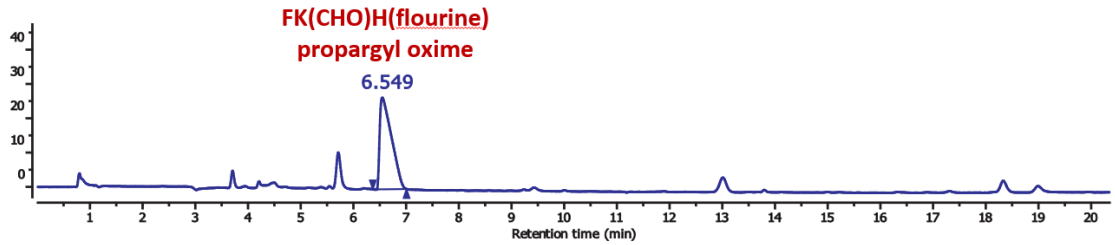
**MS-Trace of peak 7.686 (FK(CHO)H(flourine))**



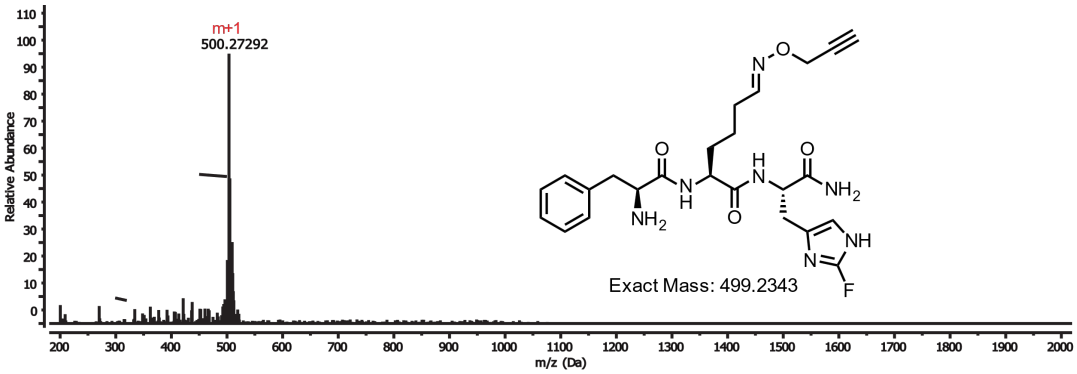

To 1 mg of aldehyde-flourinated peptide **FK(CHO)H(flourine)** in 300 µL of NaP buffer was added 4 equivalences of O-2-Propynylhydroxylamine hydrochloride. The reaction was stirred for 6 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the modification of the peptide **FK(CHO)H(flourine)** to the oxime propargyl peptide product. The reaction mixture was analyzed by HPLC using method B and the % conversion to the oxime propargyl peptide product (>98%).

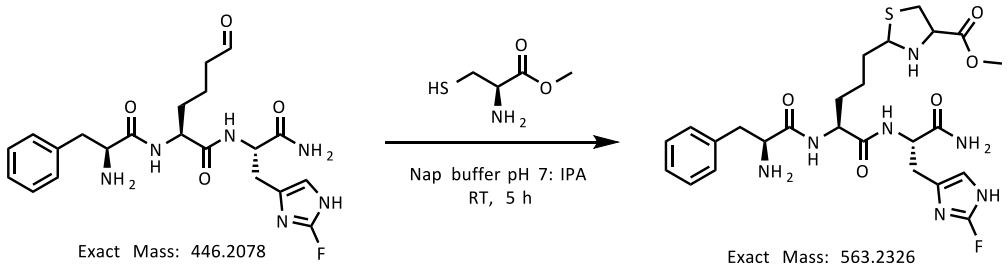
**FK(CHO)H(flourine) propargyl oxime.** LCMS: *m/z* 500.27292 (calcd [M+H]+ = 500.2416), (HPLC analysis at 220 nm, **method A**). Retention time in HPLC: 6.549

**HPLC Trace of reaction**



**MS-Trace of peak 6.549 (FK(CHO)H(flourine) propargyl oxime)**

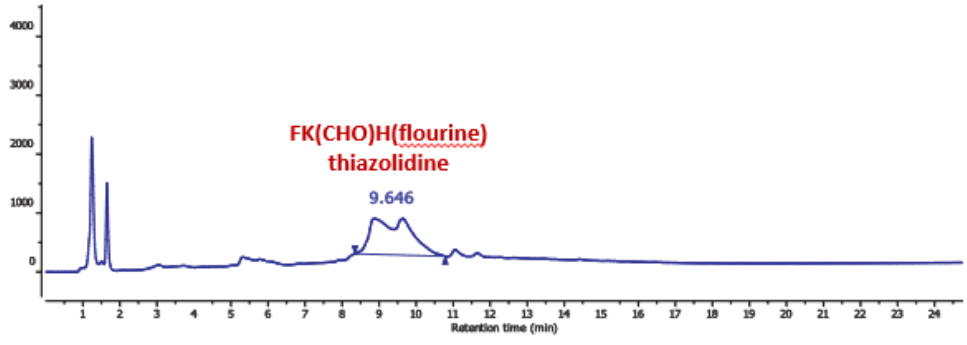


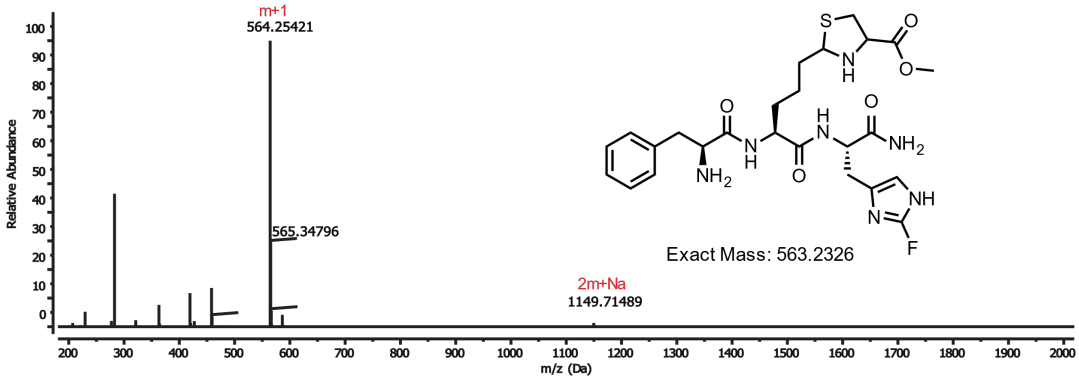

To 1 mg of aldehyde-flourinated peptide **FK(CHO)H(flourine)** in 300 µL of NaP buffer and isopropyl alcohol (IPA) was added 5 equivalences of cysteine methylester. Reaction was stirred at room temperature for 5 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the formation of thiazolidine. The reaction mixture was analyzed by HPLC using method A and % conversion to thiazolidine product was >99%.

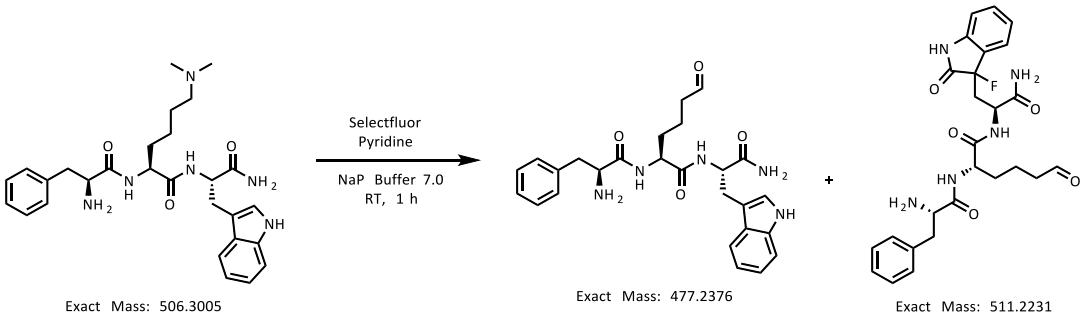
**FK(CHO)H(flourine) thiazolidine.** LCMS: *m/z* 564.25421 (calcd [M+H]+ = 564.2399), *m/z* 1149.71489 (calcd [2M+H]+ = 1149.4545), (HPLC analysis at 220 nm, **method A**). Retention time in HPLC: 9.646

**HPLC Trace of reaction**



**MS-Trace of peak 9.646 (FK(CHO)H(flourine) thiazolidine)**



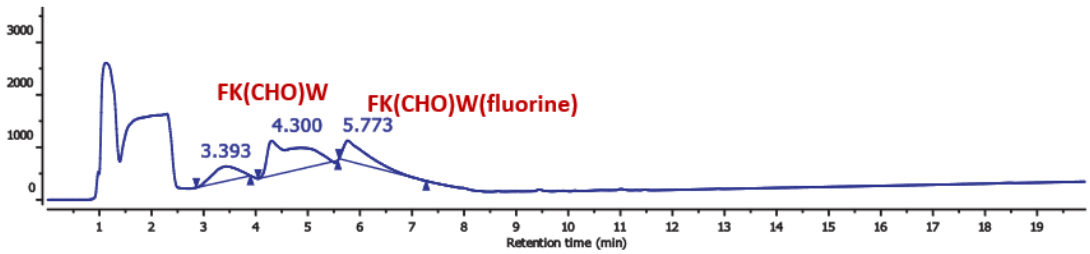




To 1.0 mg of FKme$_2$W peptide dissolved in 300 µL of 10 mM sodium phosphate buffer (NaP, pH 7.0), was added pyridine (5 eq) and selectfluor (2 eq). The reaction mixture was stirred for 1 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the generation of fluorinated peptide aldehyde product. The reaction mixture was analyzed by HPLC using method A and product peak purified by preparative HPLC.

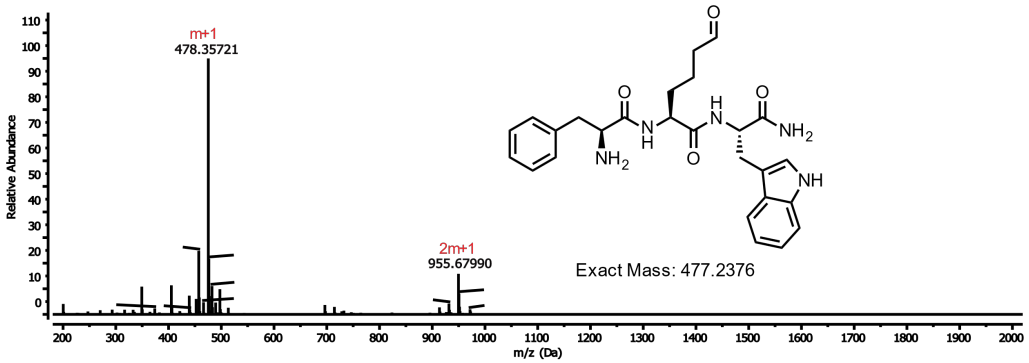
**FK(CHO)W.** LCMS: *m/z* 478.35721 (calcd [M+H]+ = 478.2449), *m/z* 955.67990 (calcd [2M+H]+ = 955.4825), (HPLC analysis at 220 nm, **method A**). Retention time in HPLC: 4.300

**FK(CHO)W(fluorine).** LCMS: *m/z* 512.22616 (calcd [M+H]+ = 512.2304), *m/z* 256.53616 (calcd [M+2/2]+ = 256.6115), (HPLC analysis at 220 nm, **method A**). Retention time in HPLC: 5.773

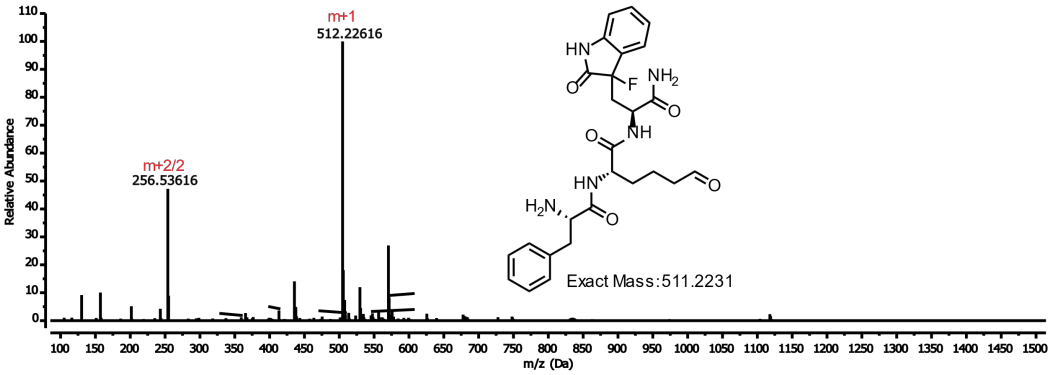
**HPLC Trace of reaction**

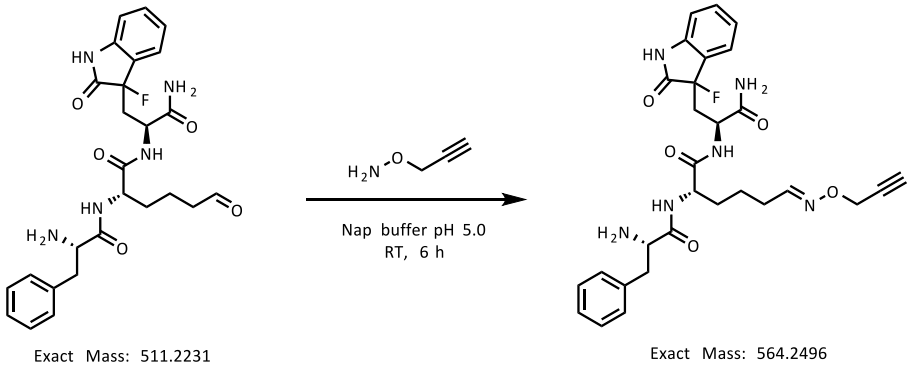



**MS-Trace of peak 4.300 (FK(CHO)W)**




**MS-Trace of peak 5.773 (FK(CHO)W(fluorine))**

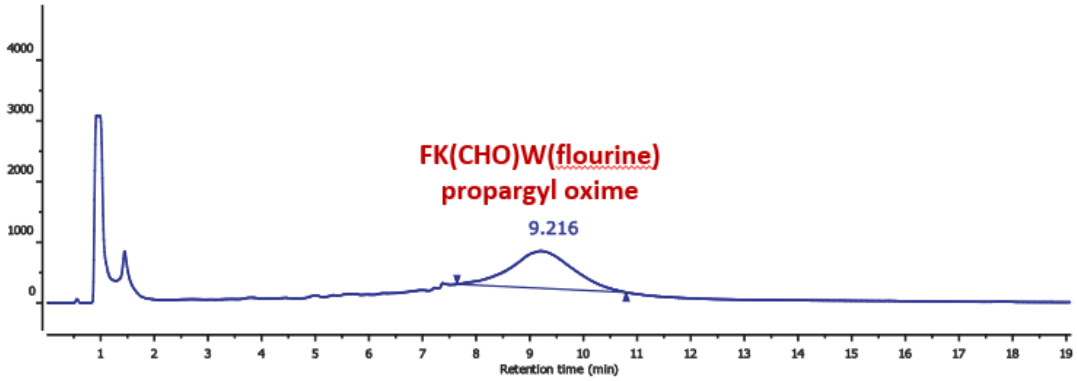


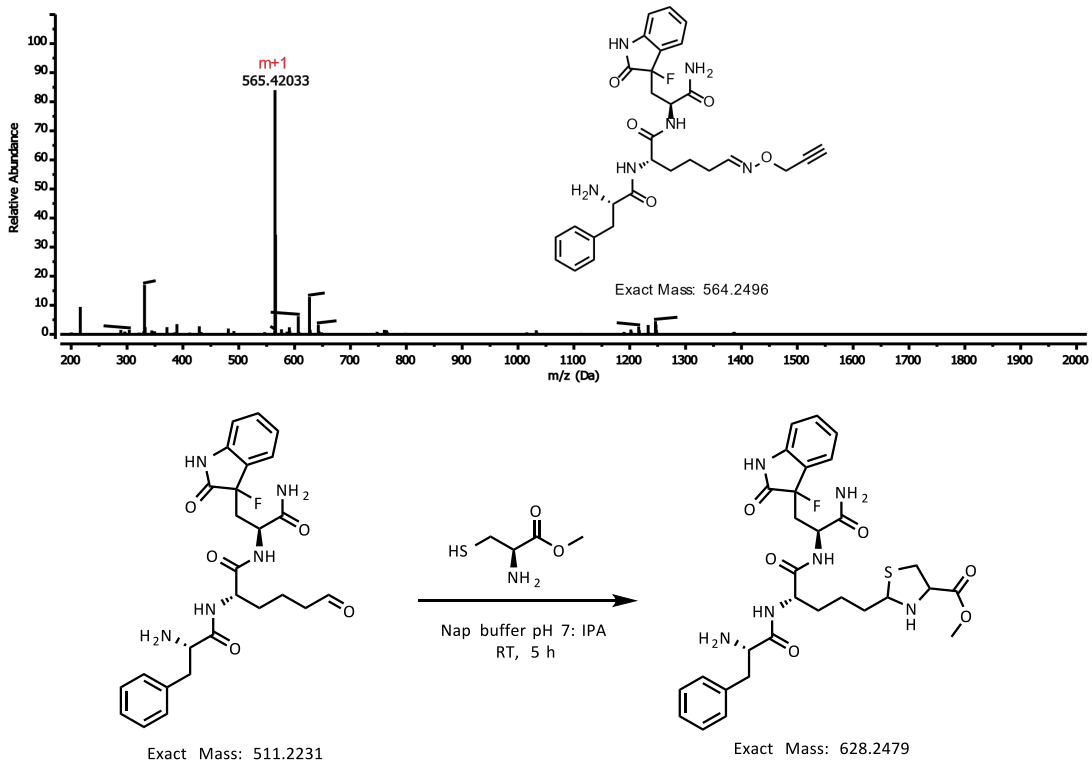

To 1 mg of aldehyde-flourinated peptide **FK(CHO)W(flourine)** in 300 µL of NaP buffer was added 4 equivalences of O-2-Propynylhydroxylamine hydrochloride. The reaction was stirred for 6 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the modification of the peptide **FK(CHO)W(flourine)** to the oxime propargyl peptide product. The reaction mixture was analyzed by HPLC using method B and the % conversion to the oxime propargyl peptide product (>98%).

**FK(CHO)W(flourine) propargyl oxime.** LCMS: *m/z* 565.42033 (calcd [M+H]+ = 565.2569), (HPLC analysis at 220 nm, **method A**). Retention time in HPLC: 9.216

**HPLC Trace of reaction**



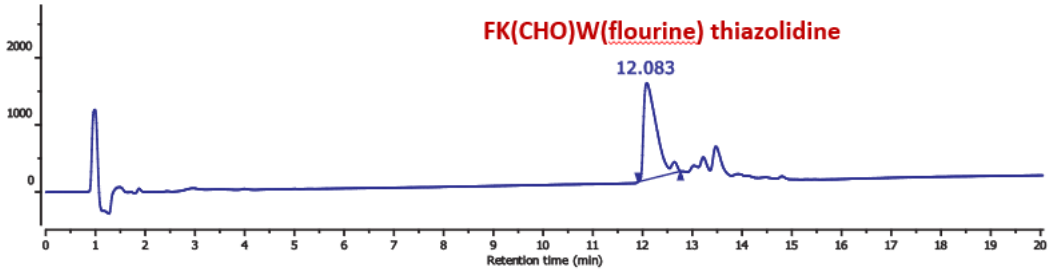

**MS-Trace of peak 9.216 (FK(CHO)W(flourine) propargyl oxime)**



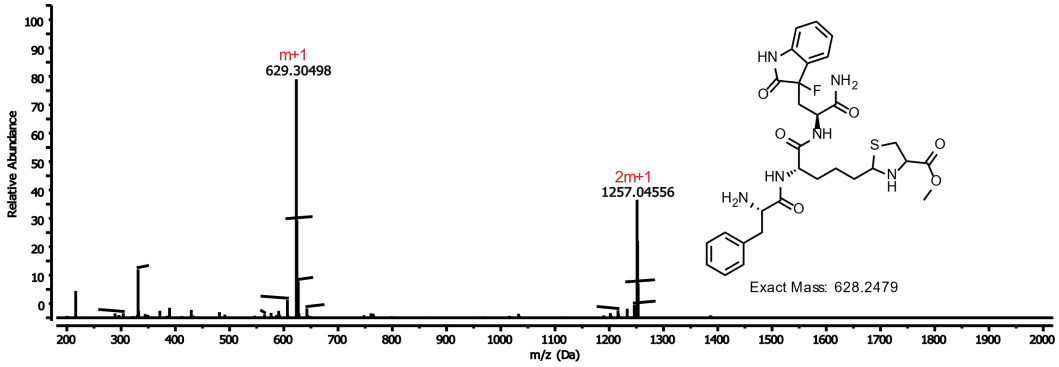

To 1 mg of aldehyde-flourinated peptide **FK(CHO)W(flourine)** in 300 µL of NaP buffer and isopropyl alcohol (IPA) was added 5 equivalences of cysteine methylester. Reaction was stirred at room temperature for 5 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the formation of thiazolidine. The reaction mixture was analyzed by HPLC using method A and % conversion to thiazolidine product was >99%.

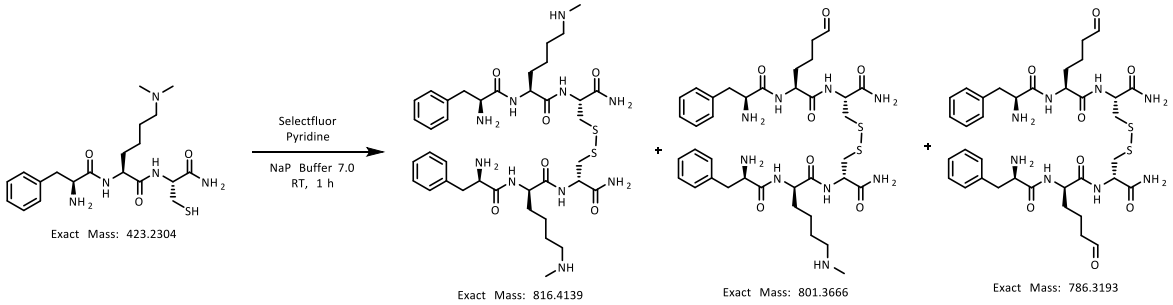
**FK(CHO)W(flourine) thiazolidine.** LCMS: *m/z* 629.30498 (calcd [M+H]+ = 629.2552), *m/z* 1257.04556 (calcd [2M+H]+ = 1257.5031), *m/z* 1149.71489 (calcd [2M+H]+ = 1149.4545), (HPLC analysis at 220 nm, **method A**). Retention time in HPLC: 12.083

**HPLC Trace of reaction**




**MS-Trace of peak 12.083 (FK(CHO)W(flourine) thiazolidine)**



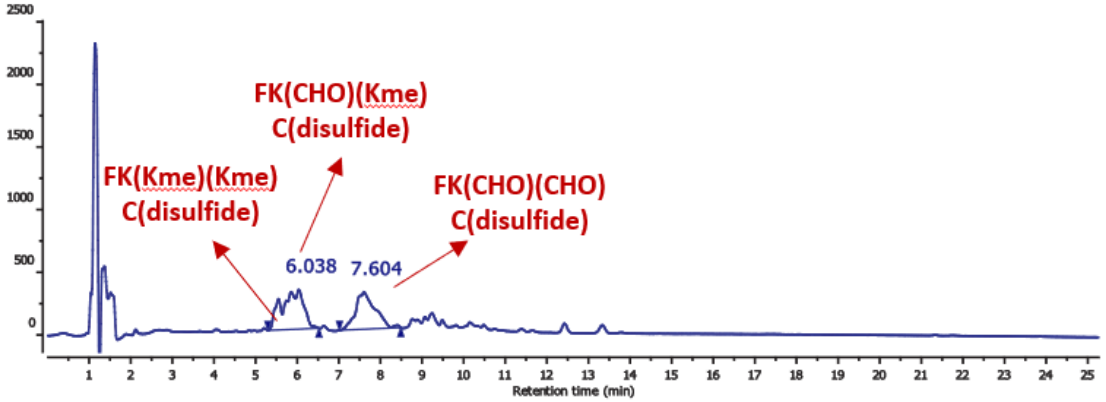

To 1.0 mg of FK$me_2$C peptide dissolved in 300 µL of 10 mM sodium phosphate buffer (NaP, pH 7.0), was added pyridine (5 eq) and selectfluor (2 eq). The reaction mixture was stirred for 1 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the generation of peptide aldehyde disulfide product. The reaction mixture was analyzed by HPLC using method A and product peak purified by preparative HPLC.

**FK(Kme)(Kme)C(disulfide).** LCMS: *m/z* 817.39377 (calcd [M+H]+ = 817.4211), *m/z* 839.96313 (calcd [M+Na]+ = 839.4031), (HPLC analysis at 220 nm, **method A**). Retention time in HPLC: 5.491

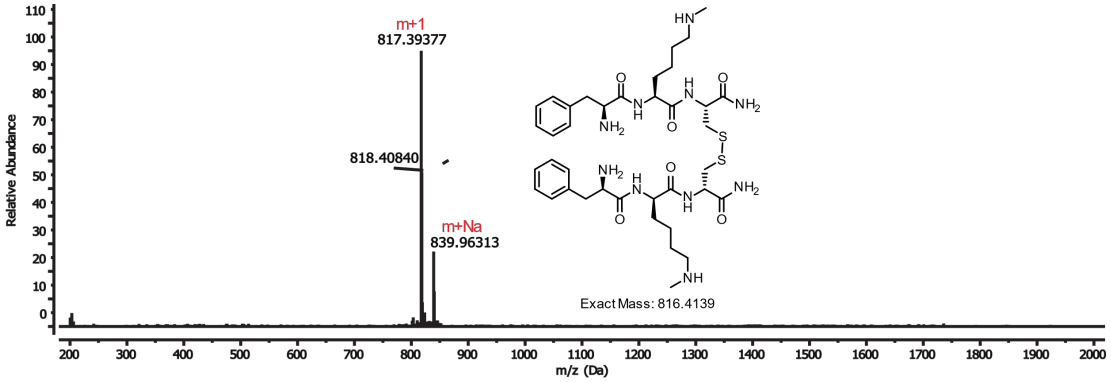
**FK(CHO)(Kme)C(disulfide).** LCMS: *m/z* 802.15983 (calcd [M+H]+ = 802.3739), *m/z* 1063.22263 (calcd [2M+H]+ = 1603.3404), (HPLC analysis at 220 nm, **method A**). Retention time in HPLC: 6.038

**FK(CHO)(CHO)C(disulfide).** LCMS: *m/z* 787.34160 (calcd [M+H]+ = 787.3266), *m/z* 809.30939 (calcd [M+Na]+ = 809.3085), *m/z* 394.10974 (calcd [M+2/2]+ = 394.1596), (HPLC analysis at 220 nm, **method A**). Retention time in HPLC: 7.604

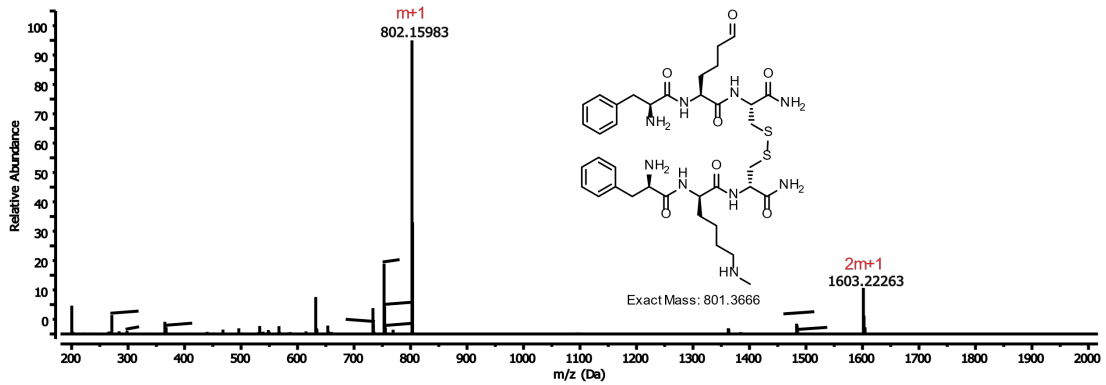
**HPLC Trace of reaction**



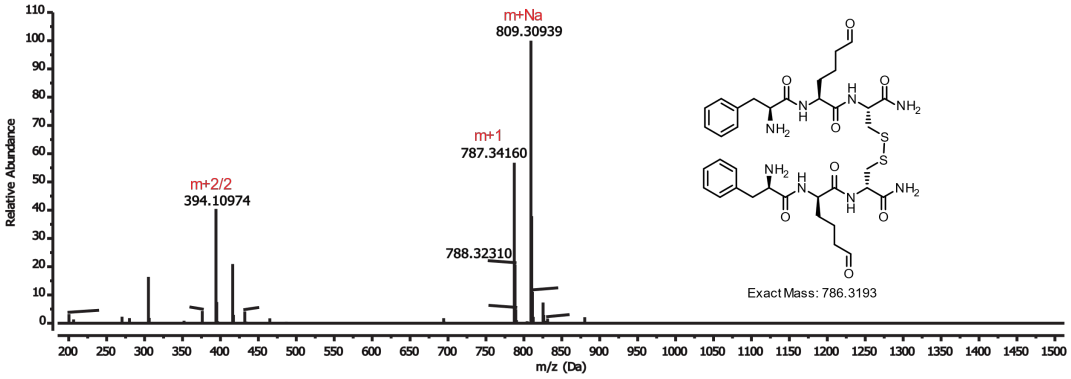

**MS-Trace of peak 5.491**

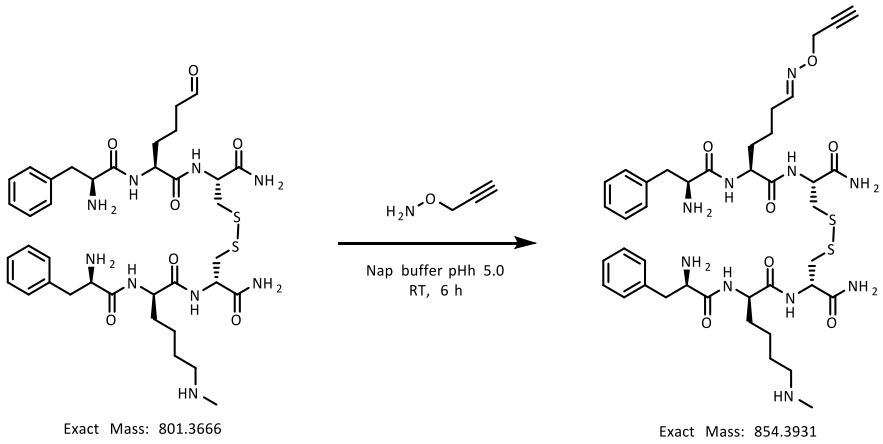



**MS-Trace of peak 6.038**



**MS-Trace of peak 7.604**

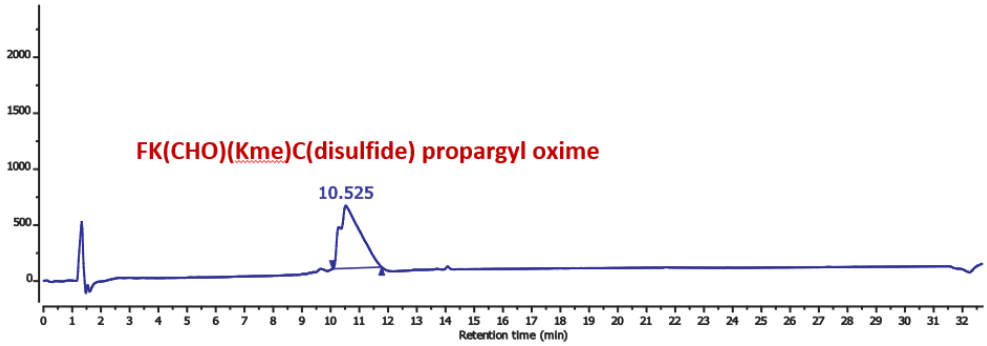


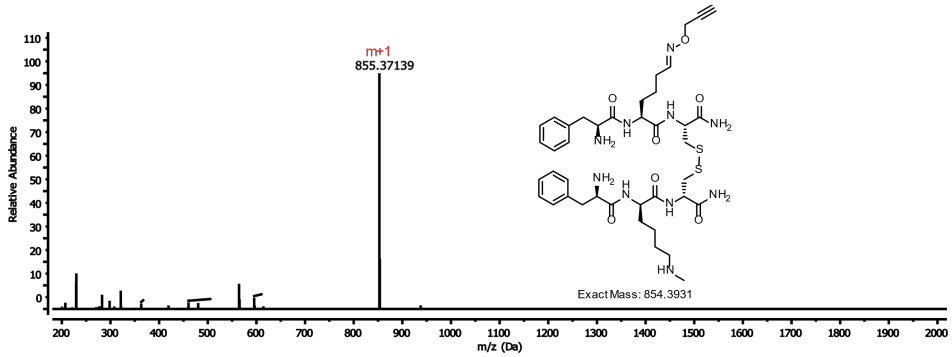

To 1 mg of aldehyde-disulfide peptide **FK(CHO)(Kme)C(disulfide)** in 300 µL of NaP buffer was added 4 equivalences of O-2-Propynylhydroxylamine hydrochloride. The reaction was stirred for 6 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the modification of the peptide **FK(CHO)(Kme)C(disulfide)** to the oxime propargyl peptide product. The reaction mixture was analyzed by HPLC using method B and the % conversion to the oxime propargyl peptide product (>98%).

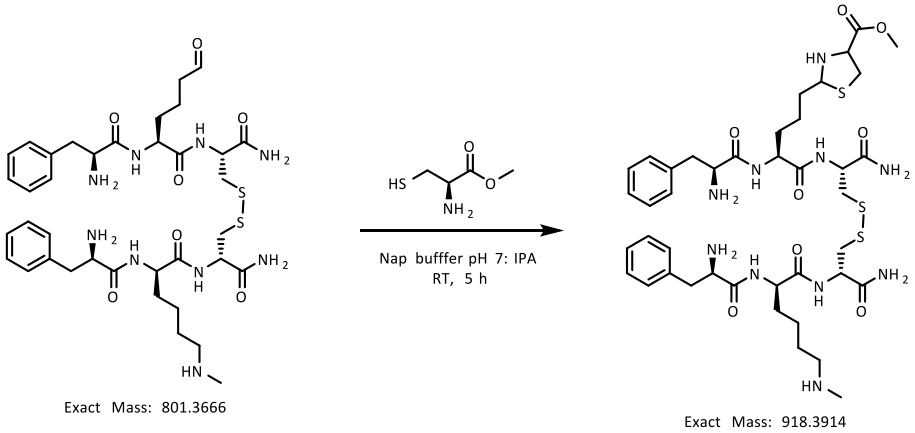
**FK(CHO)(Kme)C(disulfide) propargyl oxime.** LCMS: *m/z* 855.37139 (calcd [M+H]+ = 855.4004), (HPLC analysis at 220 nm, **method A**). Retention time in HPLC: 10.525

**HPLC Trace of reaction**



**MS-Trace of peak 10.525**

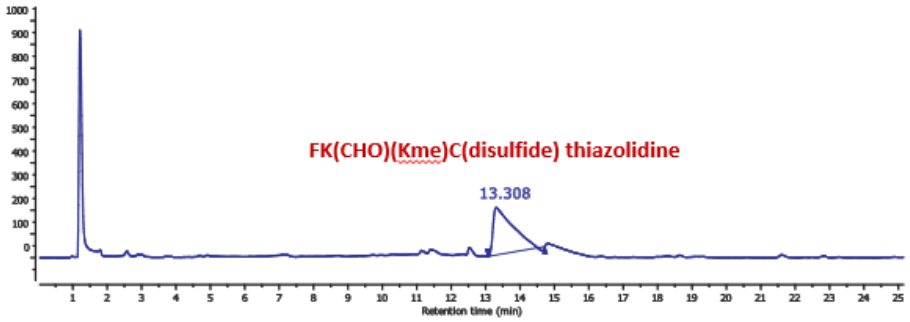


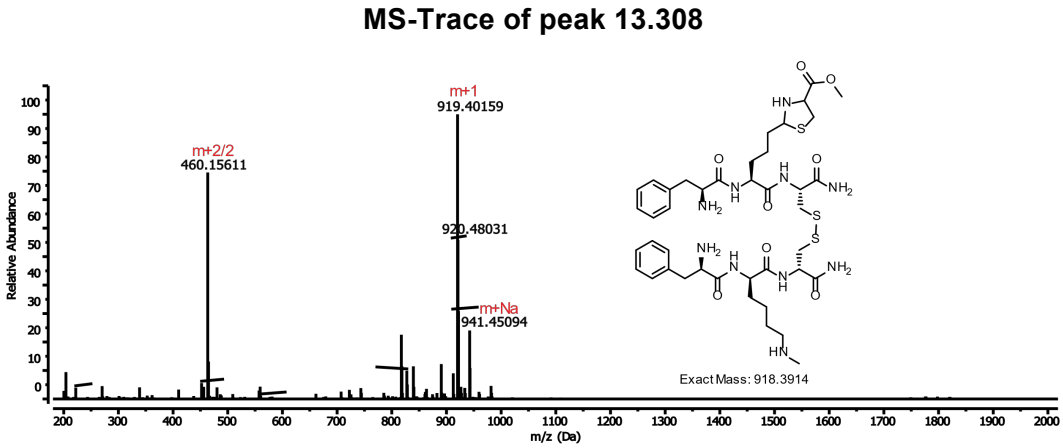




To 1 mg of aldehyde-disulfide peptide **FK(CHO)(Kme)C(disulfide)** in 300 µL of NaP buffer and isopropyl alcohol (IPA) was added 5 equivalences of cysteine methylester. Reaction was stirred at room temperature for 5 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the formation of thiazolidine. The reaction mixture was analyzed by HPLC using method A and % conversion to thiazolidine product was >99%.

**FK(CHO)(Kme)C(disulfide) thiazolidine.** LCMS: *m/z* 919.40159 (calcd [M+H]+ = 919.3987), *m/z* 941.45094 (calcd [M+Na]+ = 941.3804), *m/z* 460.15611 (calcd [M+2/2]+ = 460.2030), (HPLC analysis at 220 nm, **method A**). Retention time in HPLC: 13.308

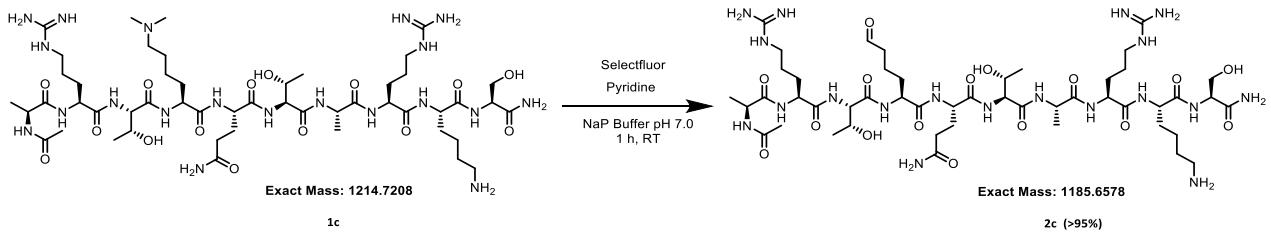
**HPLC Trace of reaction**






**XIII. Supplementary Figure 7:** Pan-specificity evaluation of dimethyllysine containing histone peptide fragments to generate histone peptide aldehydes.

**Kme$_2$4K9 (ARTKme$_2$QTARKS) 1c:**

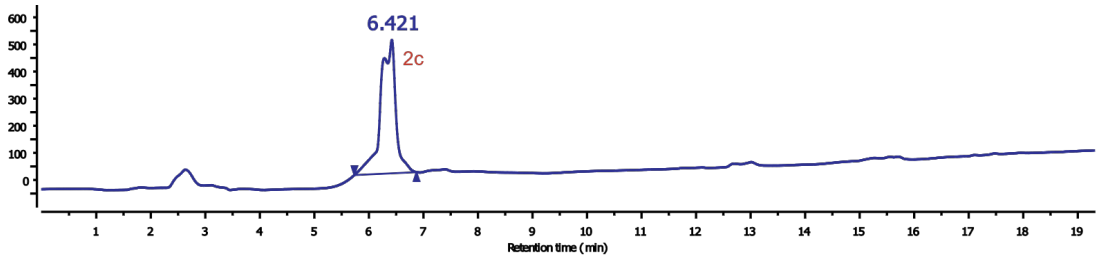



To 1.0 mg of histone peptide fragment Kme$_2$4K9 (ARTKme$_2$QTARKS) **1c** dissolved in 300 µL of 10 mM sodium phosphate buffer (NaP, pH 7.0), was added pyridine (5 eq) and selectfluor (2 eq). The reaction mixture was stirred for 1 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the generation of histone peptide aldehyde product **2c**. The reaction mixture was analyzed by HPLC using method B and % conversion to peptide aldehyde **2c** was >95%.

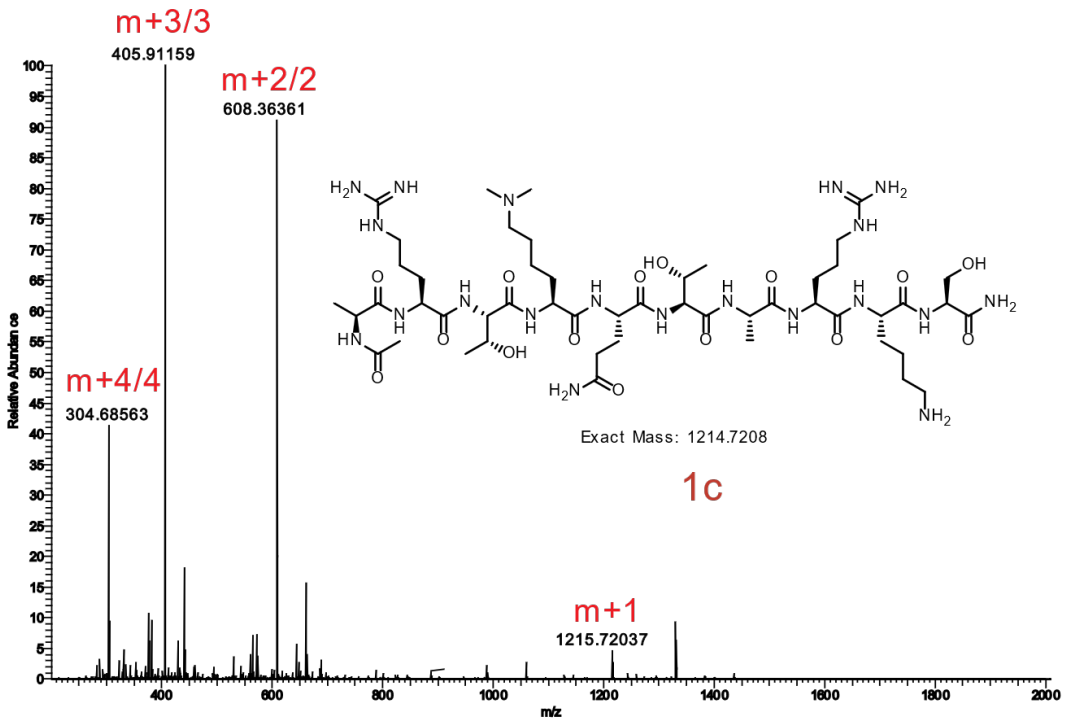
**Kme$_2$4K9 (ARTKme$_2$QTARKS) 1c.** LCMS: *m/z* 1215.72037 (calcd [M+H]+ = 1215.7280), *m/z* 608.36361 (calcd [M+2/2]+ = 608.3641), *m/z* 405.91159 (calcd [M+3/3]+ = 405.9093), *m/z* 304.68563 (calcd [M+4/4]+ = 304.6826).

**Kme$_2$(CHO)4K9 (ARTKme$_2$(CHO)QTARKS) 2c.** LCMS: *m/z* 1186.65866 (calcd [M+H]+ = 1186.6651), *m/z* 593.83341 (calcd [M+2/2]+ = 593.8326), *m/z* 396.21955 (calcd [M+3/3]+ = 396.2217), (HPLC analysis at 220 nm, **method B**). Retention time in HPLC: 6.421

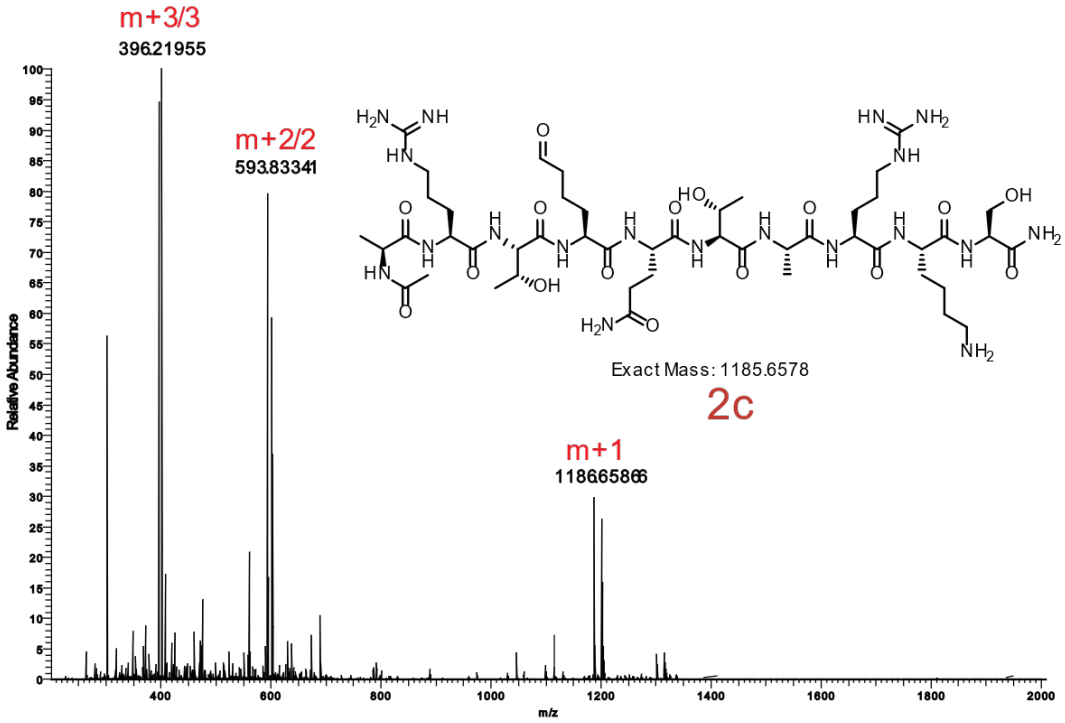
**HPLC Trace of reaction**



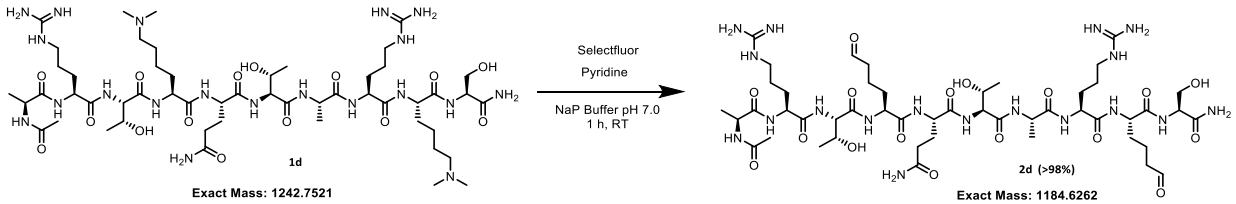

**MS-Trace of starting histone peptide fragment 1c**



**MS-Trace of histone aldehyde peptide product 2c**




**Modification of histone peptide fragment 1d:**

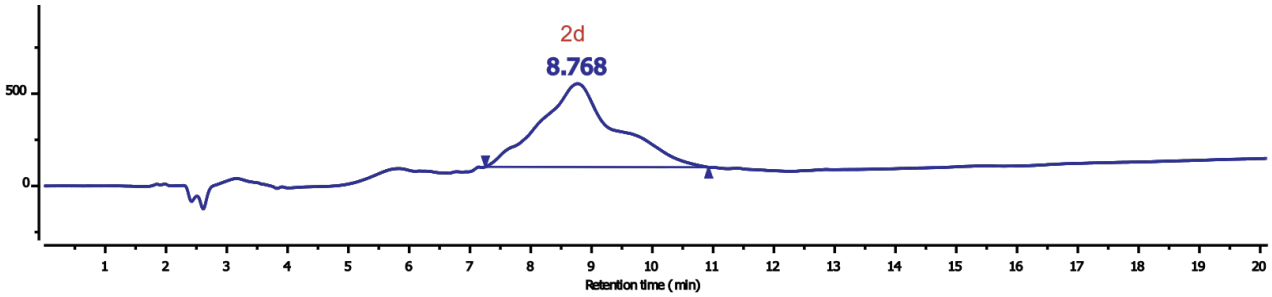



To 1.0 mg of histone peptide fragment $Kme_2$4$Kme_2$9 (ARTK$me_2$QTARK$me_2$S) **1d** dissolved in 300 µL of 10 mM sodium phosphate buffer (NaP, pH 7.0), was added pyridine (5 eq) and selectfluor (2 eq). The reaction mixture was stirred for 1 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the generation of histone peptide aldehyde product **2d**. The reaction mixture was analyzed by HPLC using method B and % conversion to peptide aldehyde **2d** was >98%.

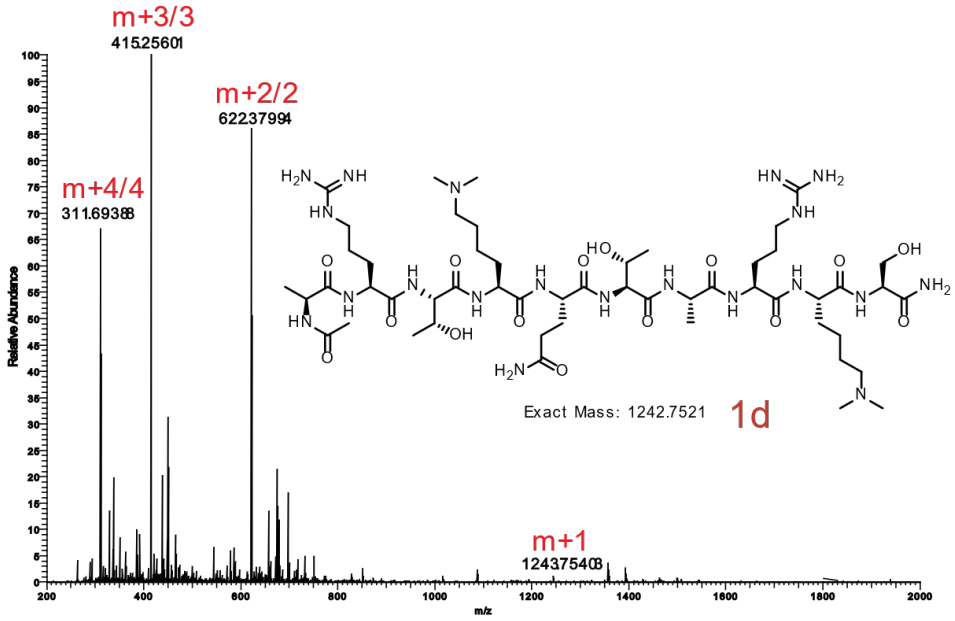
**$Kme_2$4$Kme_2$9 (ARTK$me_2$QTARK$me_2$S) 1d.** LCMS: *m/z* 1243.75401 (calcd [M+H]+ = 1243.7593), *m/z* 622.37994 (calcd [M+2/2]+ = 622.3796), *m/z* 415.25601 (calcd [M+3/3]+ = 415.2531), *m/z* 311.69388 (calcd [M+4/4]+ = 311.6898).

**$Kme_2$(CHO)4$Kme_2$(CHO)9 (ARTK$me_2$(CHO)QTARK$me_2$(CHO)S) 2d.** LCMS: *m/z* 1185.62600 (calcd [M+H]+ = 1185.6335), *m/z* 593.31680 (calcd [M+2/2]+ = 593.3167), (HPLC analysis at 220 nm, **method B**). Retention time in HPLC: 8.768

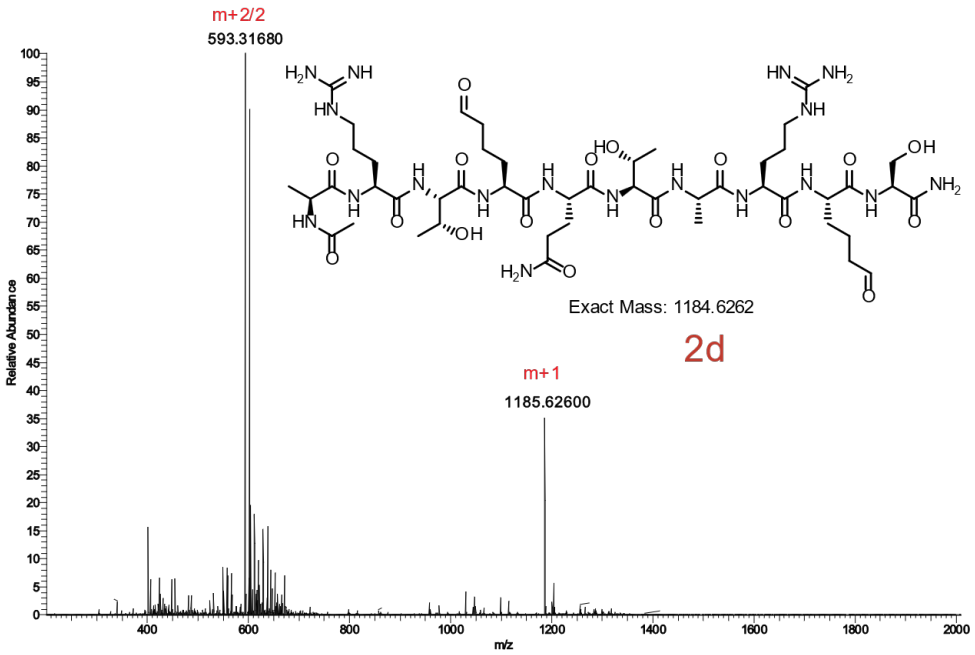
## HPLC Trace of reaction



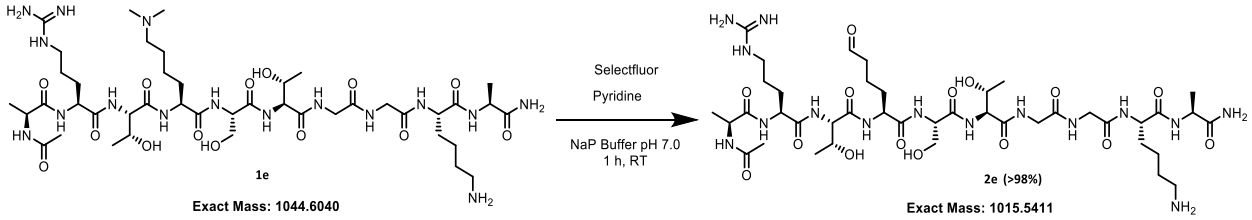

## MS-Trace of 1d



**MS-Trace of product 2d**




**Modification of histone peptide fragment 1e:**

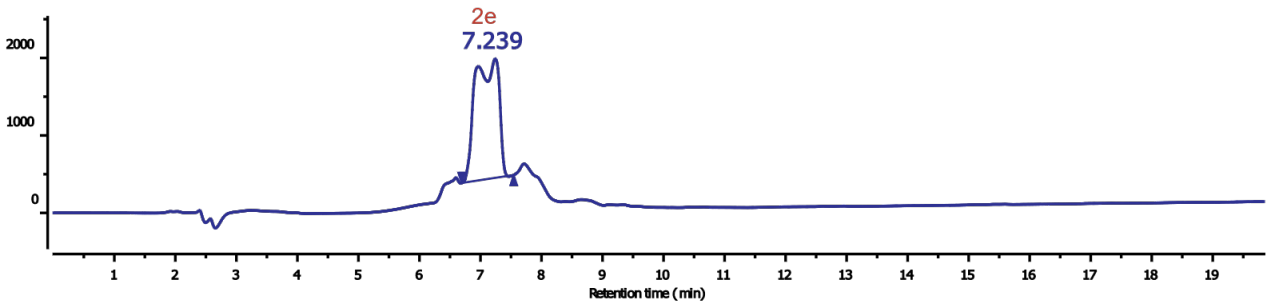



To 1.0 mg of $Kme_2$9K14 (ART$Kme_2$STGGKA) **1e** dissolved in 300 µL of 10 mM sodium phosphate buffer (NaP, pH 7.0), was added pyridine (5 eq) and selectfluor (2 eq). The reaction mixture was stirred for 1 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the generation of histone peptide aldehyde product $Kme_2$(CHO)9K14 (ART$Kme_2$(CHO)STGGKA) **2e**. The reaction mixture was analyzed by HPLC using method B and % conversion to peptide aldehyde **2e** was >98%.

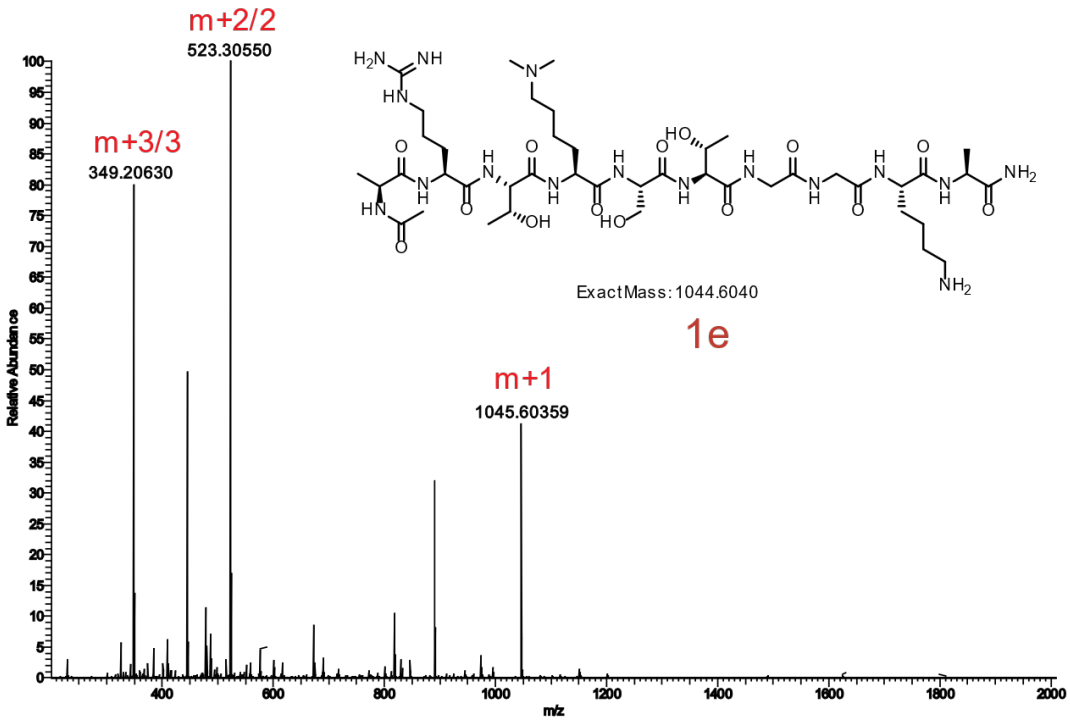
**Linear peptide of histone peptide fragment 1e.** LCMS: *m/z* 1045.60359 (calcd [M+H]+ = 1045.6113), *m/z* 523.30550 (calcd [M+2/2]+ = 523.30565), *m/z* 349.20630 (calcd [M+3/3]+ = 349.2037).

**Histone aldehyde peptide product 2e.** LCMS: *m/z* 1016.54144 (calcd [M+H]+ = 1016.5483), *m/z* 508.77432 (calcd [M+2/2]+ = 508.7741), (HPLC analysis at 220 nm, **method B**). Retention time in HPLC: 7.239

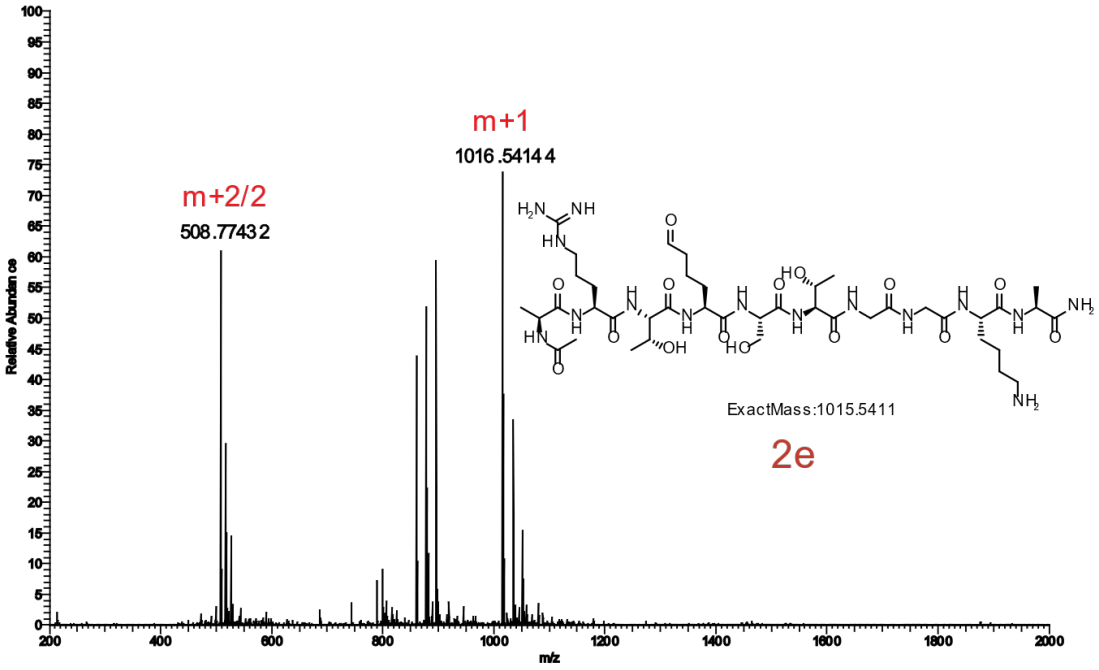
## HPLC Trace of reaction



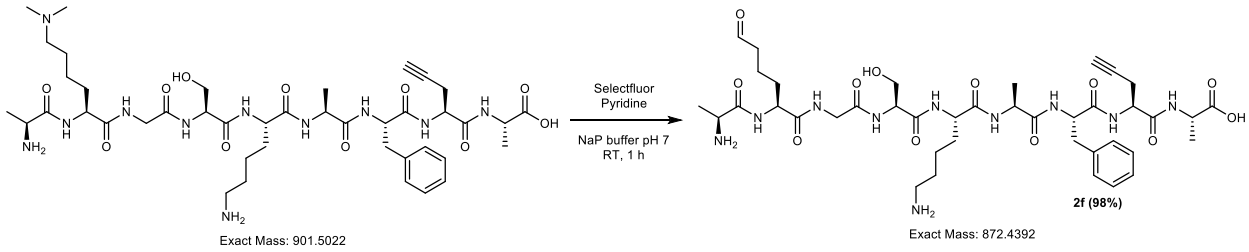

## MS-Trace of 1e



**MS-Trace of product 2e**



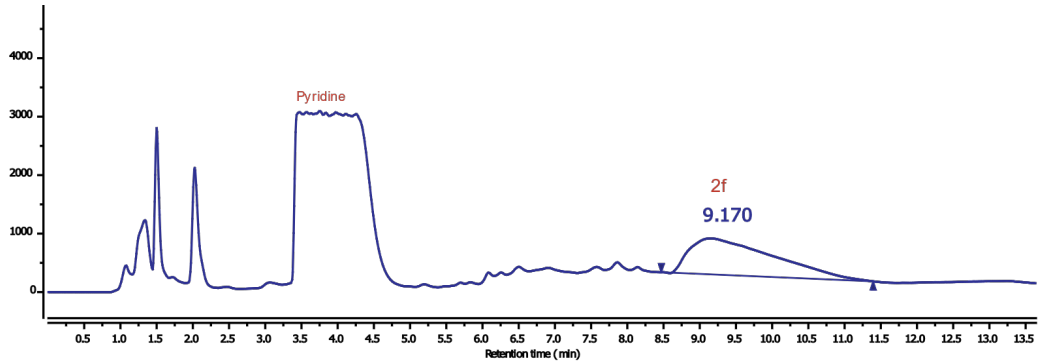

**Modification of peptide fragment 1f:**



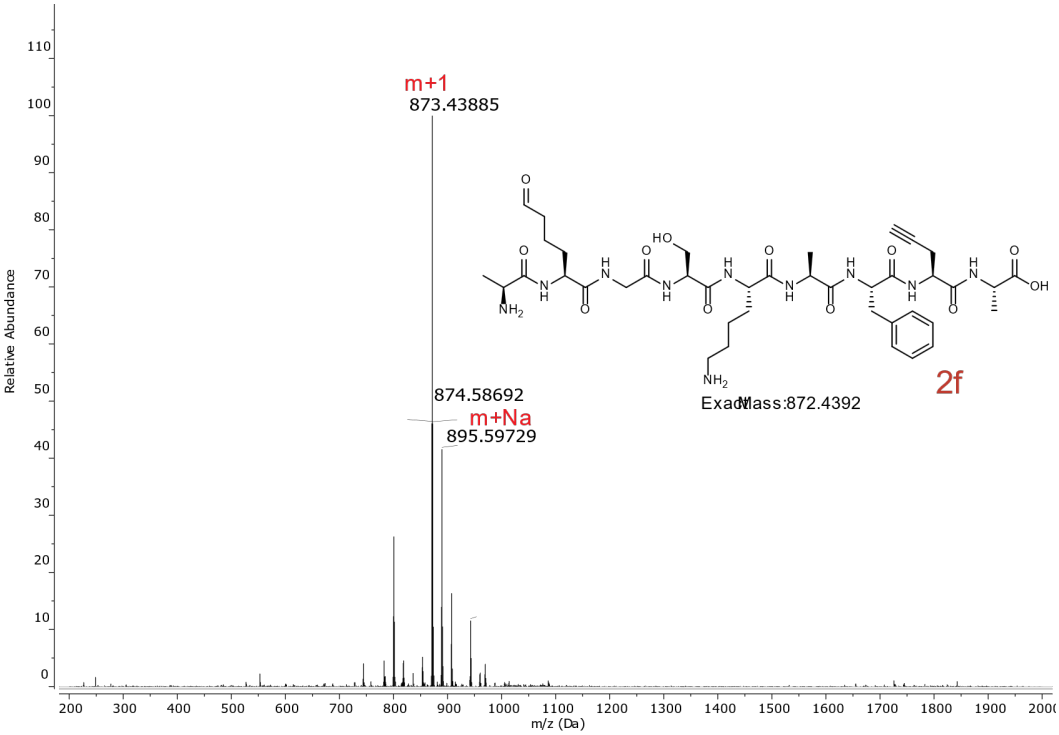

**Linear peptide 1f**: To 1.0 mg of a peptide fragment AKme$_2$GSKAF(PrG)A (where PrG = propargyl glycine) dissolved in 300 µL of 10 mM sodium phosphate buffer (NaP, pH 7.0), was added pyridine (5 eq) and selectfluor (2 eq). The reaction mixture was stirred for 1 h. The reaction mixture was analyzed by HPLC using method A and % conversion to aldehyde product **2f** (>98%).

**Peptide aldehyde product 2f.** LCMS: *m/z* 873.43885 (calcd [M+H]+ = 873.4392), *m/z* 895.59729 (calcd [M+Na]+ = 895.4284), (HPLC analysis at 220 nm). Retention time in HPLC: 9.170

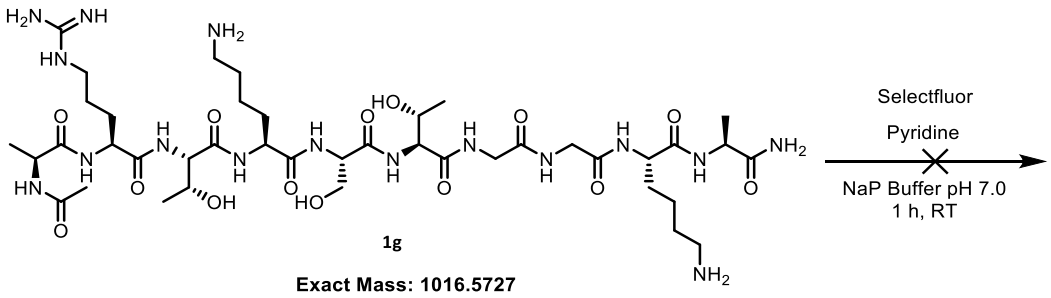
## HPLC Trace of peptide aldehyde product 2f




## MS-Trace of peak 9.170



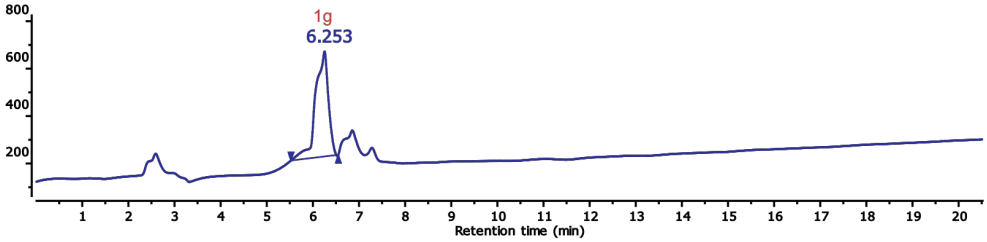

## Modification of histone negative control peptide fragment 1g without $Kme_2$:



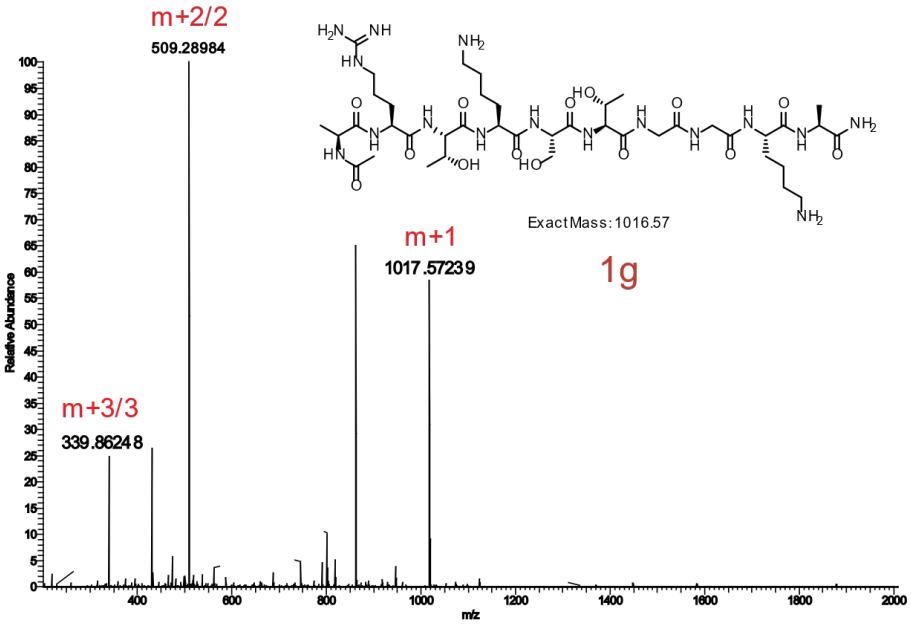
To 1.0 mg of histone peptide fragment, ARTKQTARKS **1g** dissolved in 300 µL of 10 mM sodium phosphate buffer (NaP, pH 7.0), was added pyridine (5 eq) and selectfluor (2 eq). The reaction mixture was stirred for 1 h. The reaction mixture was analyzed by HPLC using method B. No modification of peptide **1g** was observed.

**K4K9 (ARTKQTARKS) 1g.** LCMS: *m/z* 1017.57249 (calcd [M+H]+ = 1017.5800), *m/z* 509.28979 (calcd [M+2/2]+ = 509.2900), *m/z* 339.86256 (calcd [M+3/3]+ = 339.8600), (HPLC analysis at 220 nm, **method B**). Retention time in HPLC: 6.253

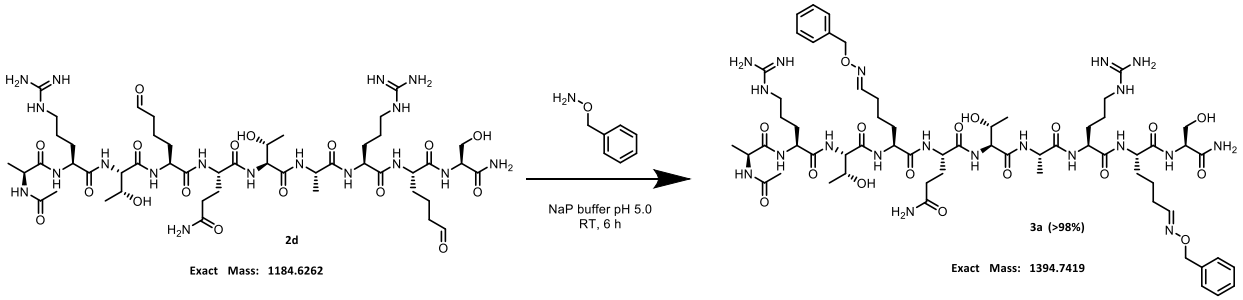
**HPLC Trace of reaction**




**MS-Trace of peak 6.253: unreacted control peptide 1g**



**XIV. Supplementary Figure 8:** Derivatization of di-aldehyde ARTK$me_2$(CHO)QTARK$me_2$(CHO)S **2d** with benzyl hydroxylamine to generate oxime adducts.



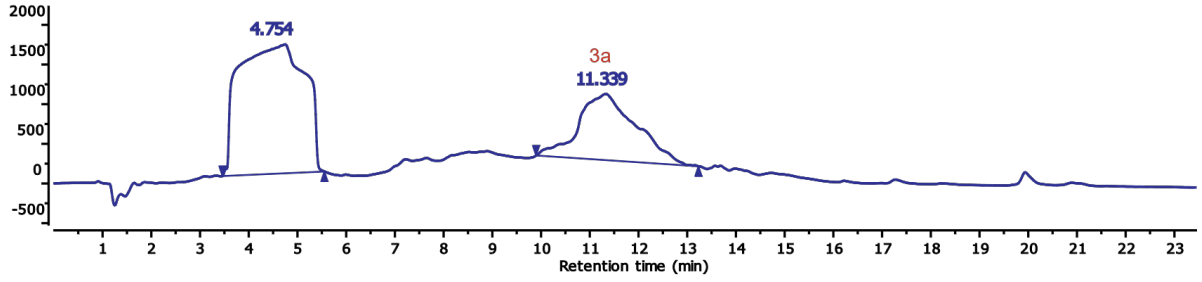

To 1 mg of aldehyde peptide **2d** in 300 µL of NaP buffer was added 4 equivalences of o-benzylhydroxylamine. The reaction was stirred for 6 h. The samples were taken from the reaction mixture, injected into LC-MS to monitor the modification of the peptide **2d** to the oxime peptide product **3a**. The reaction mixture was analyzed by HPLC using method B and the % conversion to the double oxime peptide product **3a** (>98%).

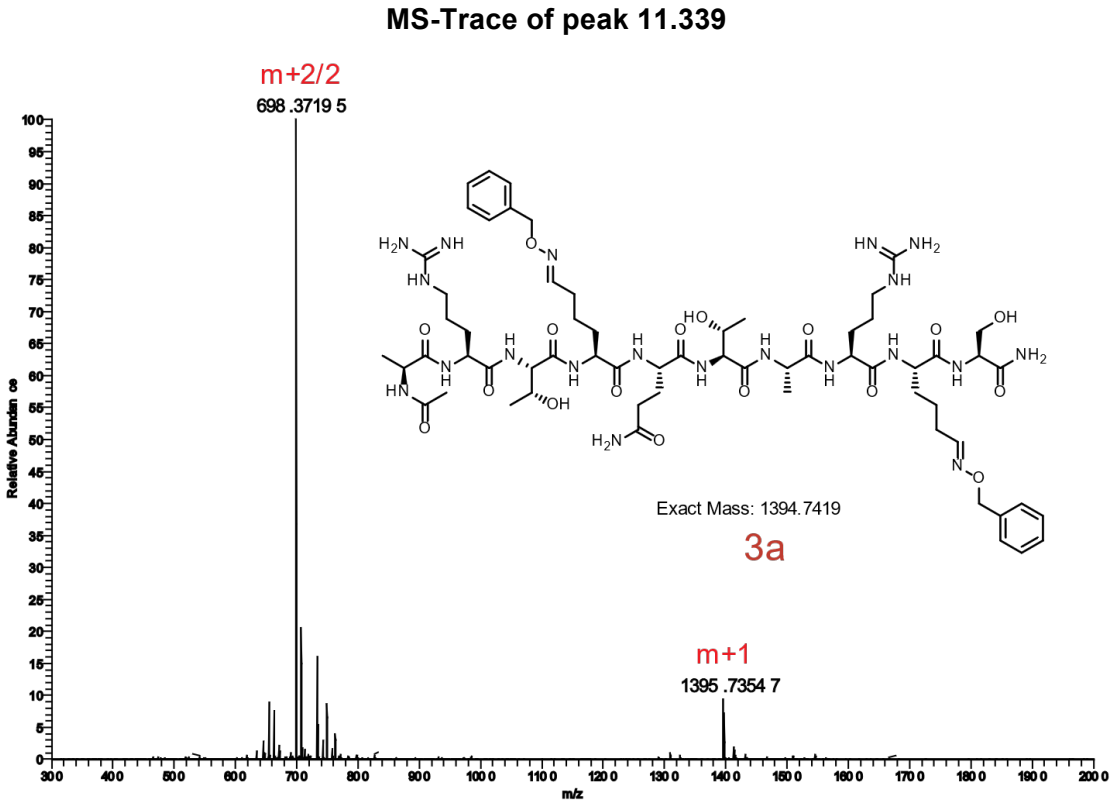
**K$me_2$(CHO)4K$me_2$(CHO)9 (ARTK$me_2$(CHO)QTARK$me_2$(CHO)S) 2d.** LCMS: *m/z* 1185.62600 (calcd [M+H]+ = 1185.6335), *m/z* 593.31680 (calcd [M+2/2]+ = 593.3167), (HPLC analysis at 220 nm). Retention time in HPLC: 8.768

**Doubly modified oxime histone peptide product 3a.** LCMS: *m/z* 1395.73547 (calcd [M+H]+ = 1395.7592), *m/z* 698.37195 (calcd [M+2/2]+ = 698.3796), (HPLC analysis at 220 nm). Retention time in HPLC: 11.339

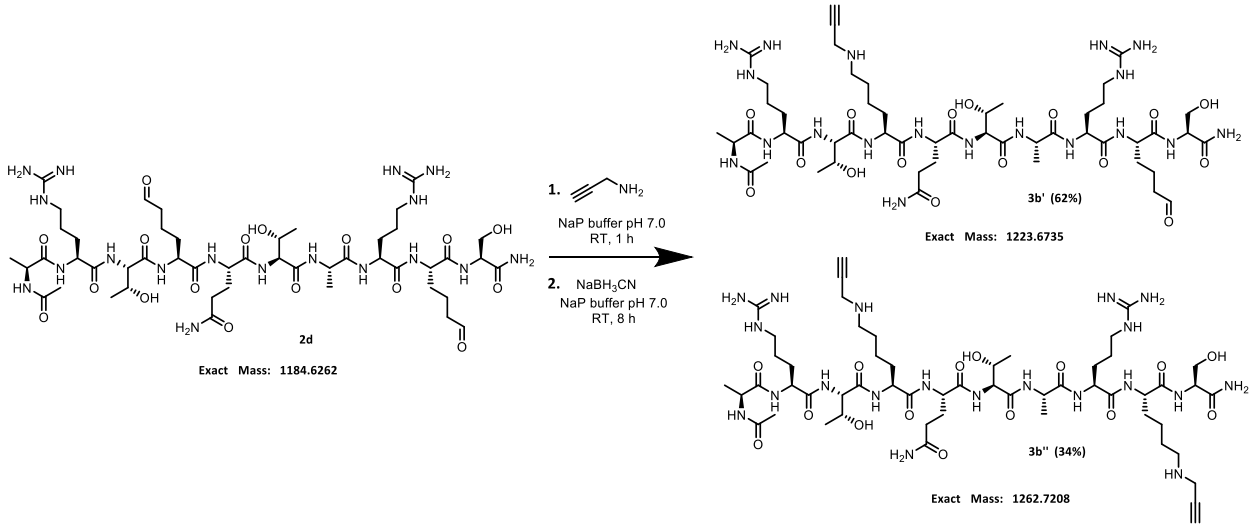
**Benzylhydroxylamine.** (HPLC analysis at 220 nm). Retention time in HPLC: 4.754

**HPLC Trace of oxime formation reaction**






**XV. Supplementary Figure 9:** Reductive amination of dialdehyde peptide ARTK$me_2$(CHO)QTARK$me_2$(CHO)S **2d** with propargylamine and sodium cyanoborohydride.




To 1 mg of aldehyde peptide **2d** in 300 µL of NaP buffer was added 5 equivalences of propargylamine. The reaction was stirred for 1 h, followed by the addition of 10 equivalence of sodium cyanoborohydride. Reaction was stirred for 8 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the modification of dialdehyde peptide ARTK$me_2$(CHO)QTARK$me_2$(CHO)S **2d**. The reaction mixture was analyzed by HPLC using

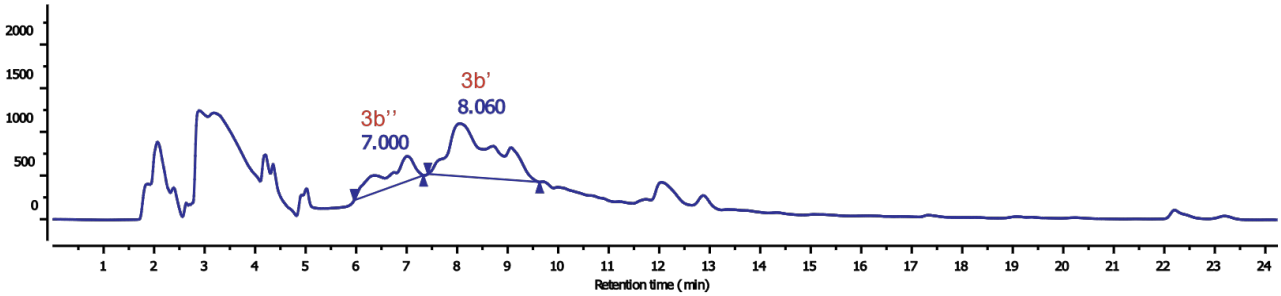
method B and % conversion to single alkynylated peptide product **3b'** was 62% and double alkynylated **3b''** was 34%.

**$Kme_2(CHO)4Kme_2(CHO)9$ ($ARTKme_2(CHO)QTARKme_2(CHO)S$) 2d.** LCMS: *m/z* 1185.62600 (calcd [M+H]+ = 1185.6335), *m/z* 593.31680 (calcd [M+2/2]+ = 593.3167), (HPLC analysis at 220 nm). Retention time in HPLC: 8.768

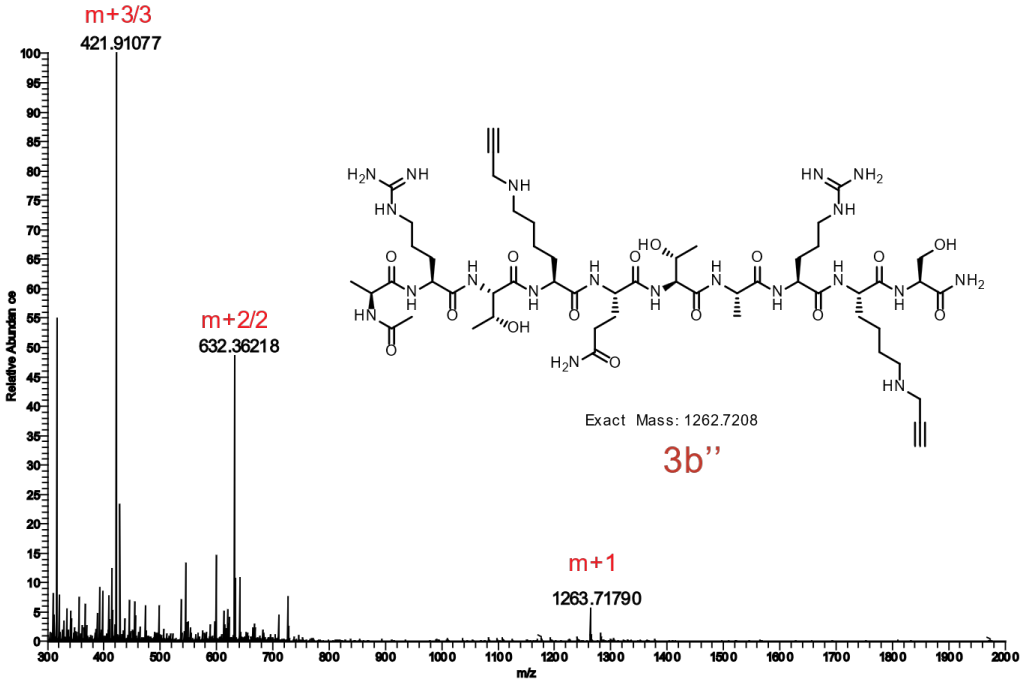
**Single-modified propargylamine histone peptide 3b'.** LCMS: *m/z* 1224.67163 (calcd [M+H]+ = 1224.6807), (HPLC analysis at 220 nm). Retention time in HPLC: 8.060

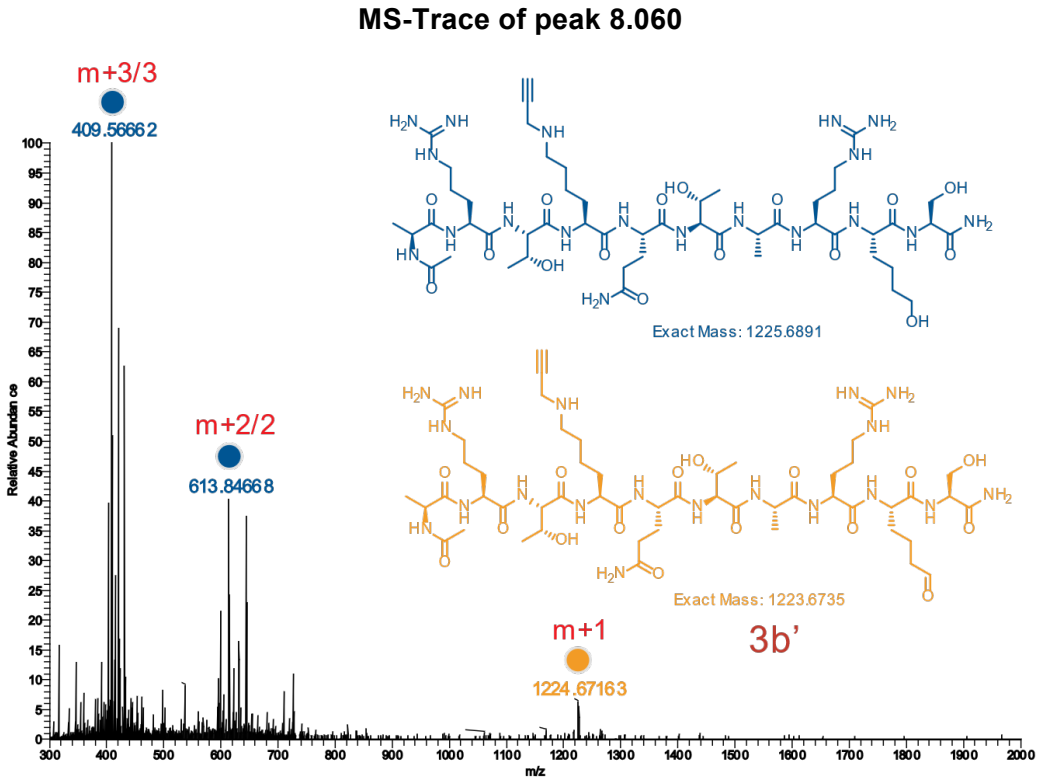
**Double-modified propargylamine histone peptide fragment 3b''.** LCMS: *m/z* 1263.71790 (calcd [M+H]+ = 1263.7280), *m/z* 632.36218 (calcd [M+2/2]+ = 632.3640), *m/z* 421.91077 (calcd [M+3/3]+ = 421.9093), (HPLC analysis at 220 nm). Retention time in HPLC: 7.000

**HPLC Trace of reductive amination reaction of** $ARTKme_2(CHO)QTARKme_2(CHO)S$ **2d**

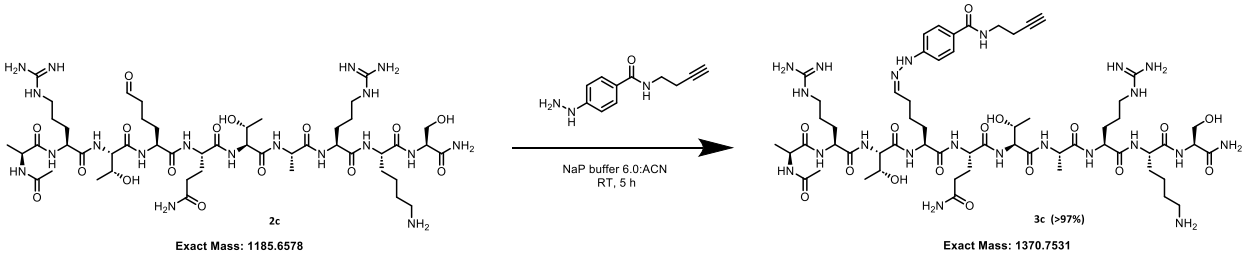



**MS-Trace of peak 7.000**






**XVI. Supplementary Figure 10.** Derivatization of $Kme_2$4K9-aldehyde (ARTK$me_2$(CHO)QTARKS) **2c** peptide aldehyde with hydrazone.



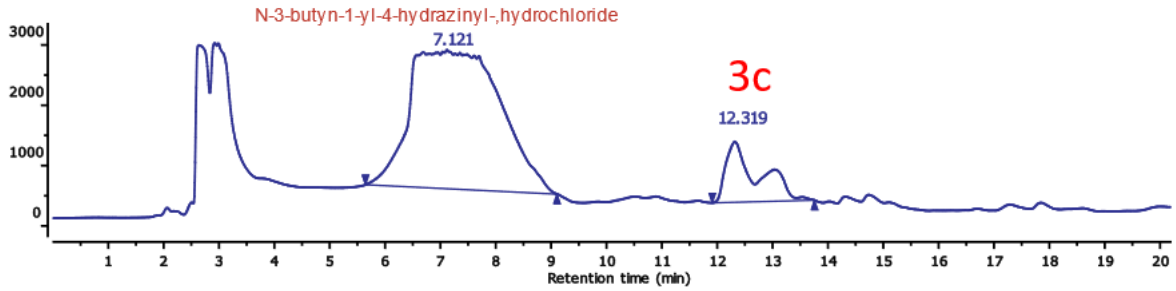

To 1 mg of aldehyde peptide **2c** in 300 µL of NaP buffer and acetonitrile was added 5 equivalences of N-3-butyn-1-yl-4-hydrazinyl-, hydrochloride. The reaction was stirred at room temperature for 5 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the modification of aldehyde peptide $Kme_2$4K9-aldehyde (ARTK$me_2$(CHO)QTARKS) **2c**. The reaction mixture was analyzed by HPLC using method B and % conversion to alkynylated peptide product **3c** was >97%.

**$Kme_2$(CHO)4K9 (ARTK$me_2$(CHO)QTARKS) 2c.** LCMS: *m/z* 1186.65866 (calcd [M+H]+ = 1186.6651), *m/z* 593.83341 (calcd [M+2/2]+ = 593.8225), *m/z* 396.21955 (calcd [M+3/3]+ = 396.2217), (HPLC analysis at 220 nm). Retention time in HPLC: 6.421

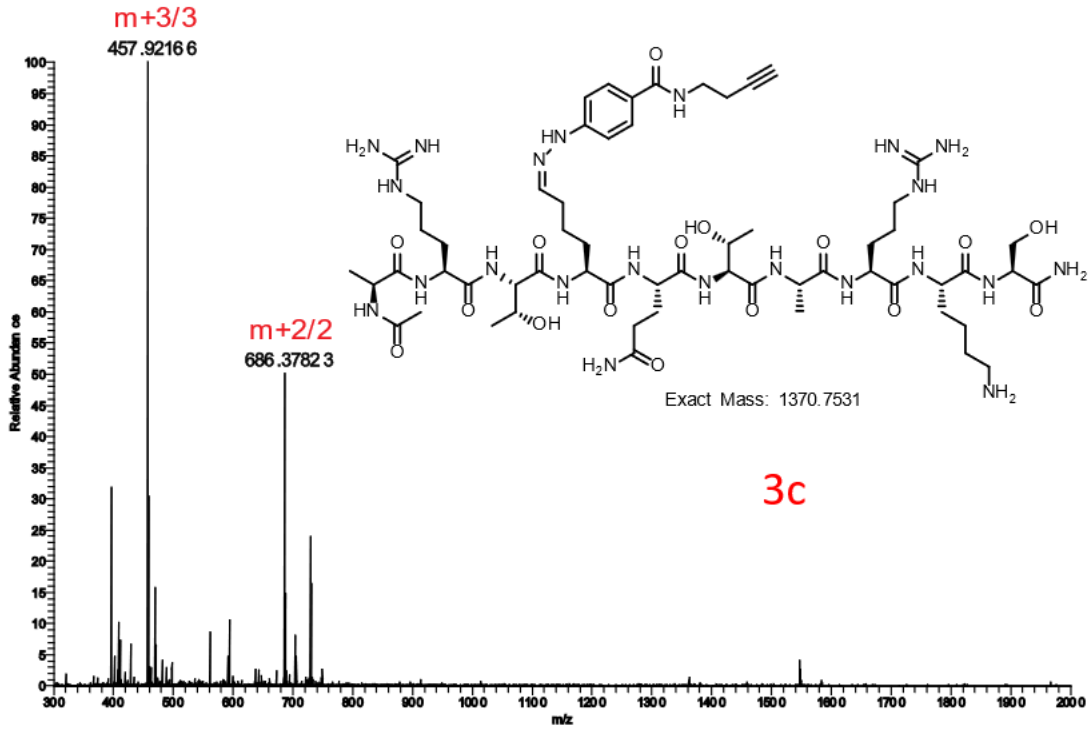
**Alkyne-modified hydrazone peptide 3c.** LCMS: *m/z* 686.37823 (calcd [M+2/2]+ = 686.3802), *m/z* 457.91166 (calcd [M+3/3]+ = 457.9201), (HPLC analysis at 220 nm). Retention time in HPLC: 12.319

**N-3-butyn-1-yl-4-hydrazinyl-hydrochloride.** (HPLC analysis at 220 nm). Retention time in HPLC: 7.121.

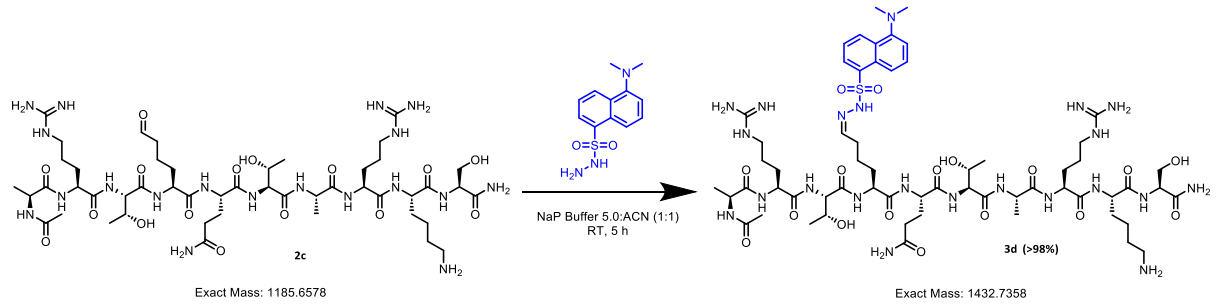
**HPLC Trace of reaction of hydrzone modification of 2c with alkyne hydrazine**




**MS-Trace of peak 12.319**



**XVII. Supplementary Figure 11:** Derivatization of $Kme_2$4K9-aldehyde (ARTK$me_2$(CHO)QTARKS) **2c** with dansyl sulfonylhydrazine.

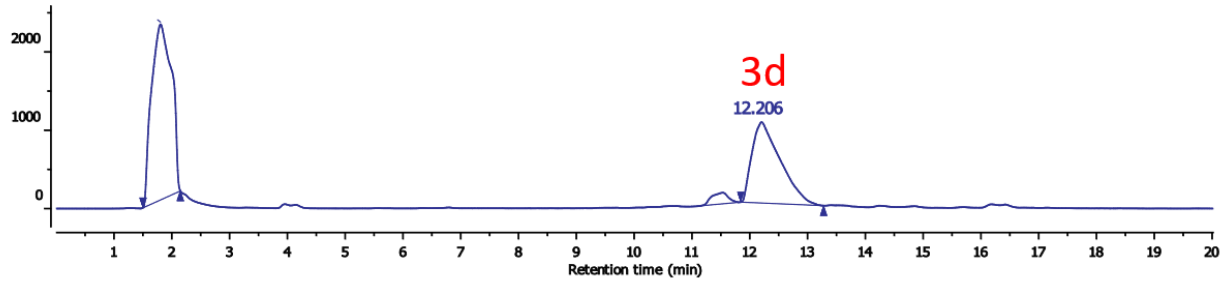



To 1 mg of $Kme_2$4K9-aldehyde (ARTK$me_2$(CHO)QTARKS) aldehyde **2c** in 150 µL of NaP buffer and acetonitrile was added 5 equivalences of dansyl sulfonylhydrazine. Reaction was stirred at room temperature for 5 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the modification of the histone peptide aldehyde **2c.** The reaction mixture was analyzed by HPLC using method B and % conversion to dansyl hydrazone peptide product **3d** was >98%.

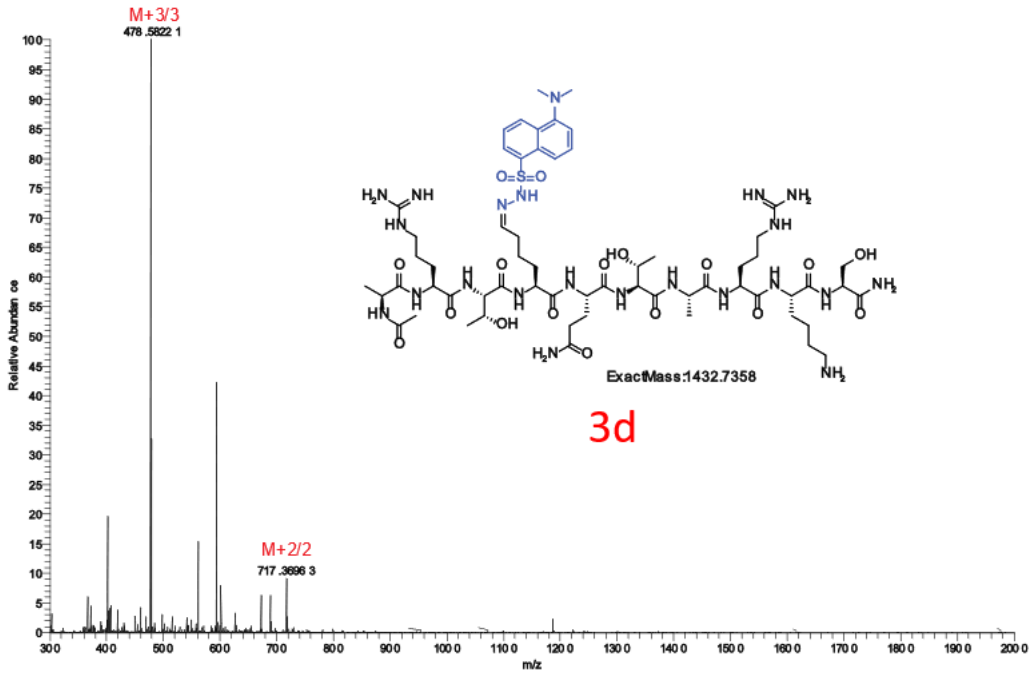
**$Kme_2$(CHO)4K9 (ARTK$me_2$(CHO)QTARKS) 2c.** LCMS: *m/z* 1186.65866 (calcd [M+H]+ = 1186.6651), *m/z* 593.83341 (calcd [M+2/2]+ = 593.8325), *m/z* 396.21955 (calcd [M+3/3]+ = 396.2217), (HPLC analysis at 220 nm). Retention time in HPLC: 6.421

**Dansyl sulfonylhydrazine-modified histone peptide 3d.** LCMS: *m/z* 717.36963 (calcd [M+2/2]+ =717.3715), *m/z* 478.58221 (calcd [M+3/3]+ =478.5810), (HPLC analysis at 220 nm). Retention time in HPLC: 12.206

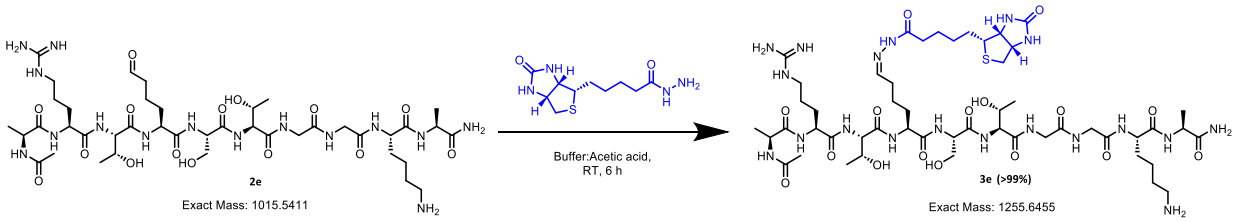
**HPLC Trace of reaction**



**MS-Trace of peak 12.206**




**XVIII. Supplementary Figure 12:** Derivatization of (ARTKme$_2$(CHO)STGGKA) **2e** with biotin-hydrazide.

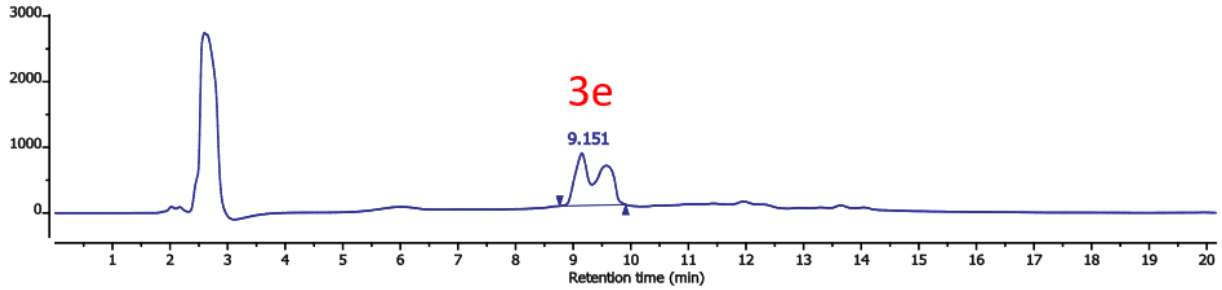


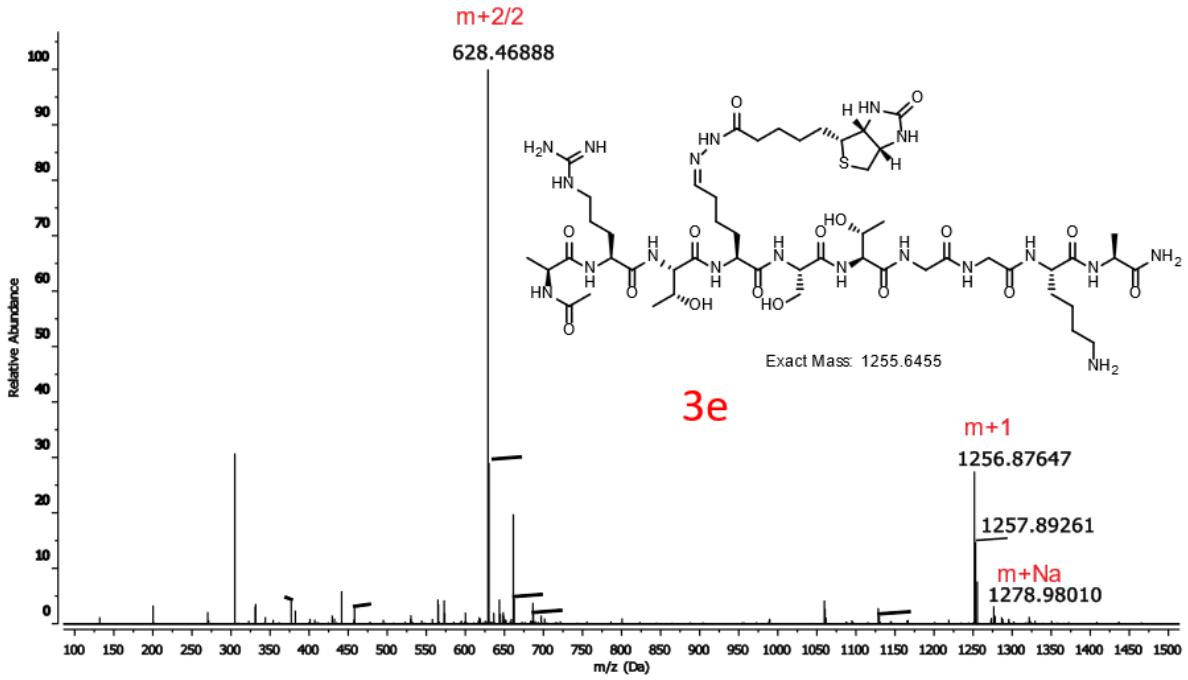

To 1 mg of aldehyde peptide (ARTKme$_2$(CHO)STGGKA) **2e** in 100 µL of NaP buffer and 200 µL acetic acid was added 10 equivalences of biotinhydrazine. The reaction was stirred at room temperature for 6 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the modification of the histone peptide aldehyde ARTKme$_2$(CHO)STGGKA) **2e.** The reaction mixture was analyzed by HPLC using method B and % conversion to biotin-hydrazone peptide product **3e** was >99%.

**Histone aldehyde peptide product 2e.** LCMS: *m/z* 1016.54144 (calcd [M+H]+ = 1016.5483), *m/z* 508.77432 (calcd [M+2/2]+ = 508.7741), (HPLC analysis at 220 nm). Retention time in HPLC: 7.239

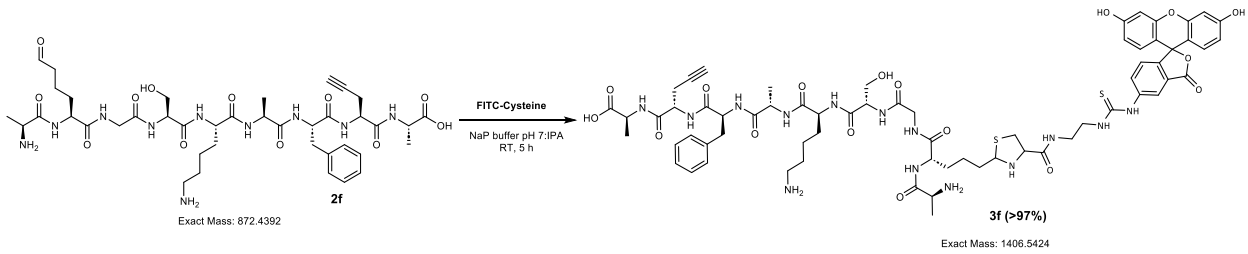
**Biotin-hydrazide modified histone peptide product 3e.** LCMS: *m/z* 1256.87647 (calcd [M+H]+ = 1256.6528), *m/z* 1278.98010 (calcd [M+Na]+ = 1278.6348), *m/z* 628.46888 (calcd [M+2/2]+ = 628.8227), (HPLC analysis at 220 nm). Retention time in HPLC: 9.151

**HPLC Trace of reaction**




**XIX. Supplementary Figure 13:** Modification of peptide aldehydes with cysteine-derivatives to thiazolidine products.

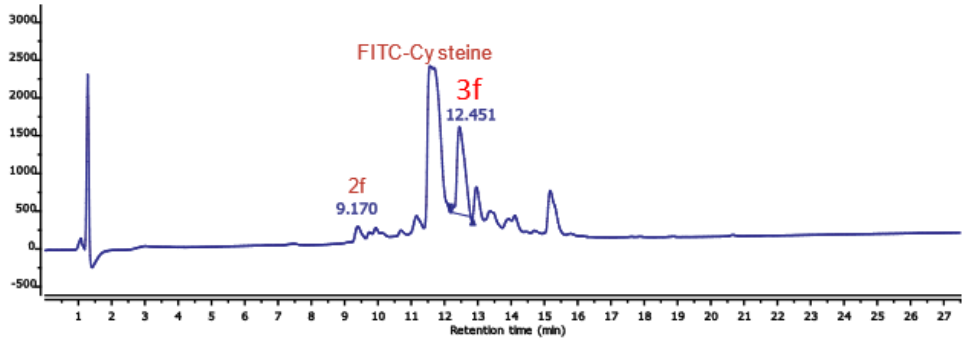


**Labeling of peptide aldehyde 2f by FITC-cysteine:** To 1 mg of peptide aldehyde AKme$_2$(CHO)GSKAF(PrG)A (where PrG = propargyl glycine) **2f** in 300 µL of NaP buffer and isopropyl alcohol (IPA) was added 3 equivalences of FITC-Cysteine. Reaction was stirred at room temperature for 5 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the formation of thiazolidine **3f**. The reaction mixture was analyzed by HPLC using method A and % conversion to thiazolidine product **3f** was >97%.

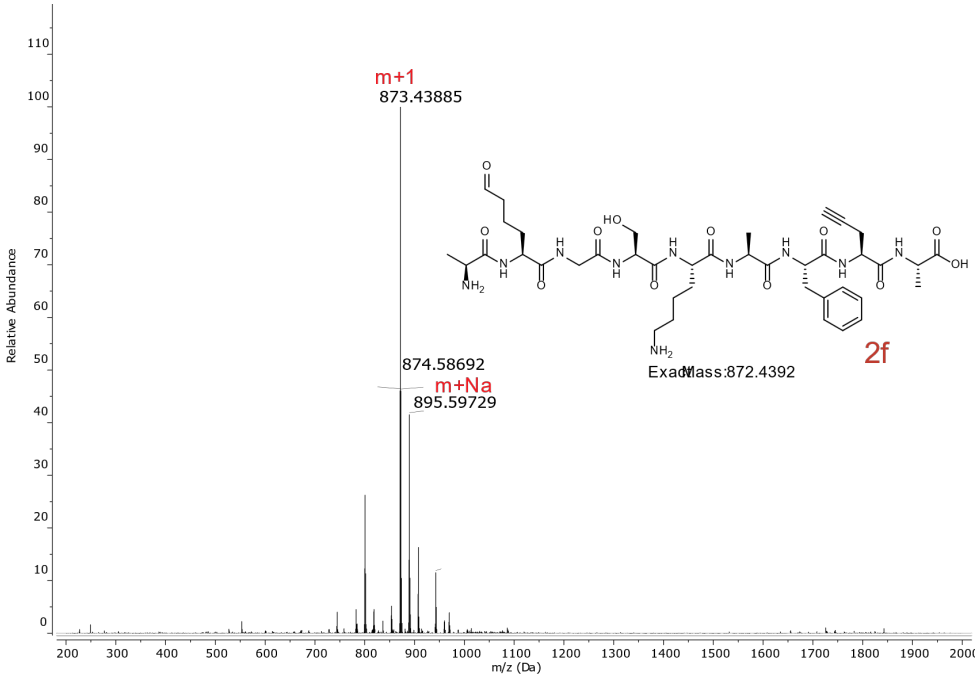
**Peptide aldehyde product 2f.** LCMS: *m/z* 873.43885 (calcd [M+H]+ = 873.4284), *m/z* 895.59729 (calcd [M+Na]+ = 895.4284), (HPLC analysis at 220 nm). Retention time in HPLC: 9.170

**FITC-Thiazolidine peptide product 3f.** LCMS: *m/z* 1407.54264 (calcd [M+H]+ = 1407.5497), *m/z* 704.31022 (calcd [M+2/2]+ = 704.7748), (HPLC analysis at 220 nm). Retention time in HPLC: 12.451

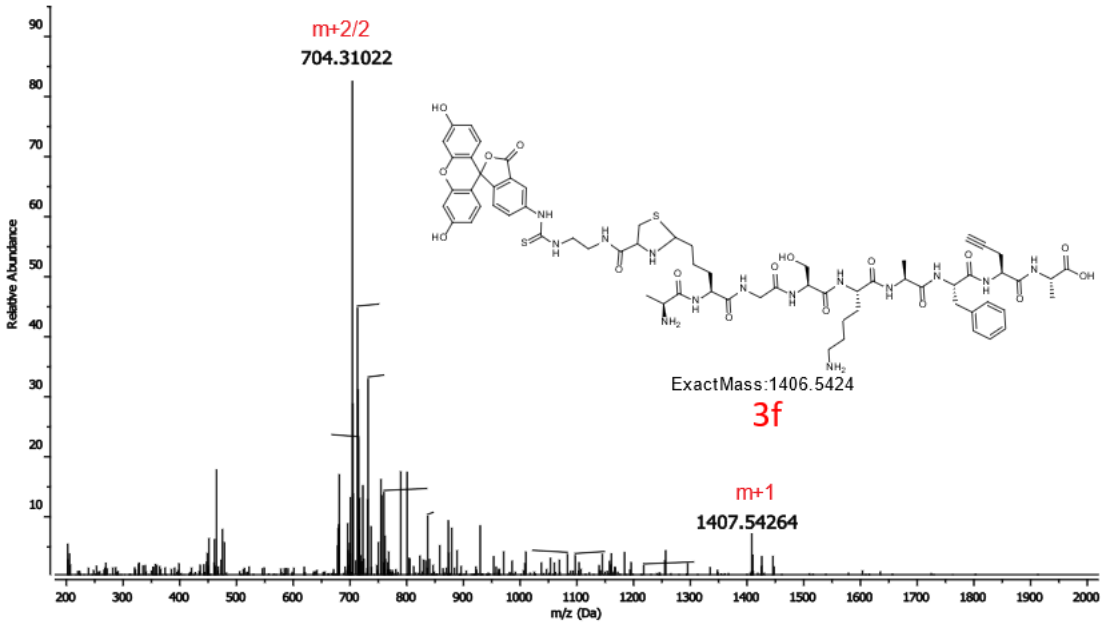
**HPLC Trace of thiazolidine product 3f**

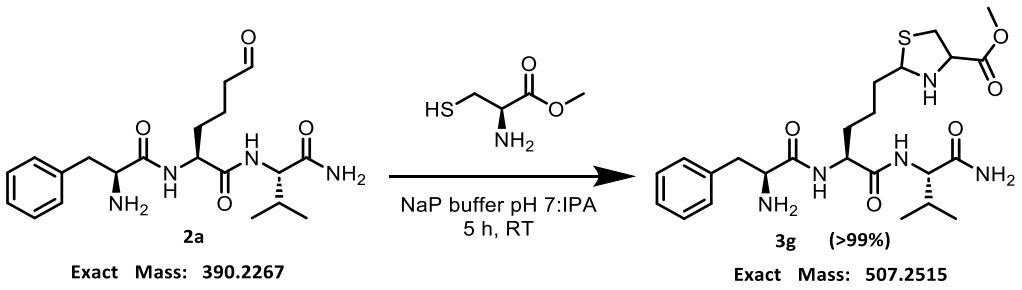



**MS-Trace of peak 9.170**



**MS-Trace of peak 12.451**




**2a**

**Exact Mass: 390.2267**

**3g (>99%)**

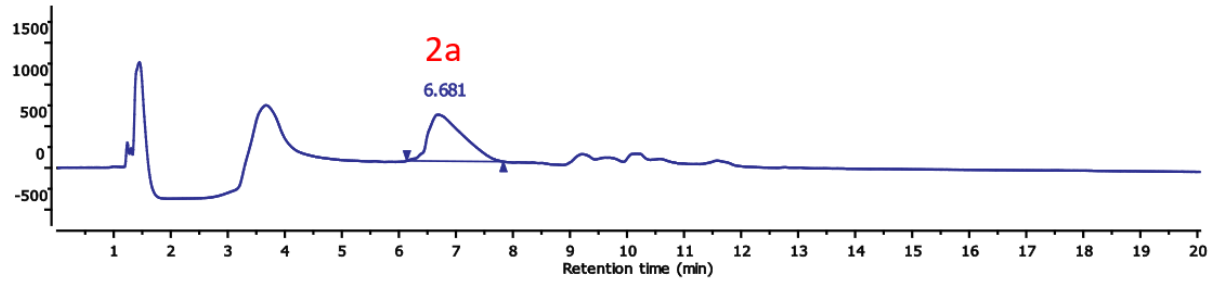
**Exact Mass: 507.2515**

To 1 mg of peptide aldehyde Fkme$_2$(CHO)V **2a** in 300 µL of NaP buffer and isopropyl alcohol (IPA) was added 5 equivalences of cysteine methylester. Reaction was stirred at room temperature for 5 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the formation of thiazolidine. The reaction mixture was analyzed by HPLC using method A and % conversion to thiazolidine product was >99%.

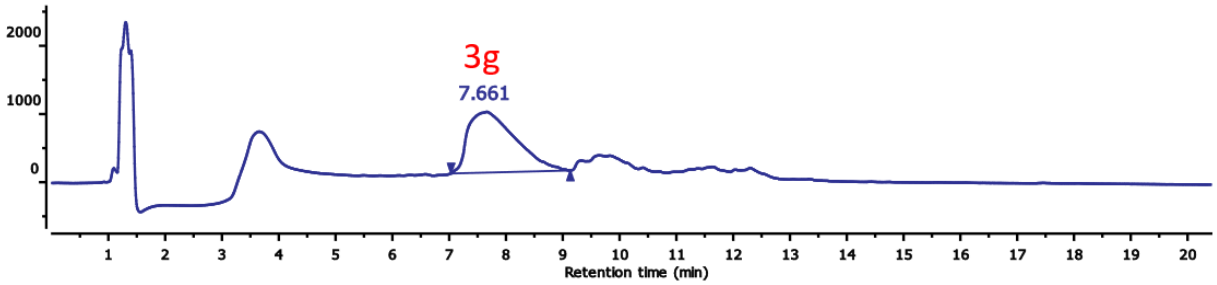
**FKme$_2$(CHO)V 2a.** LCMS: *m/z* 391.23049 (calcd [M+H]+ = 391.2340), (HPLC analysis at 220 nm). Retention time in HPLC: 6.681

**Thiazolidine peptide product of aldehyde 3g.** LCMS: *m/z* 508.25449 (calcd [M+H]+ = 508.2588), *m/z* 254.63107 (calcd [M+2/2]+ = 254.6294), *m/z* 530.23617 (calcd [M+Na]+ = 530.2408), (HPLC analysis at 220 nm). Retention time in HPLC: 7.661

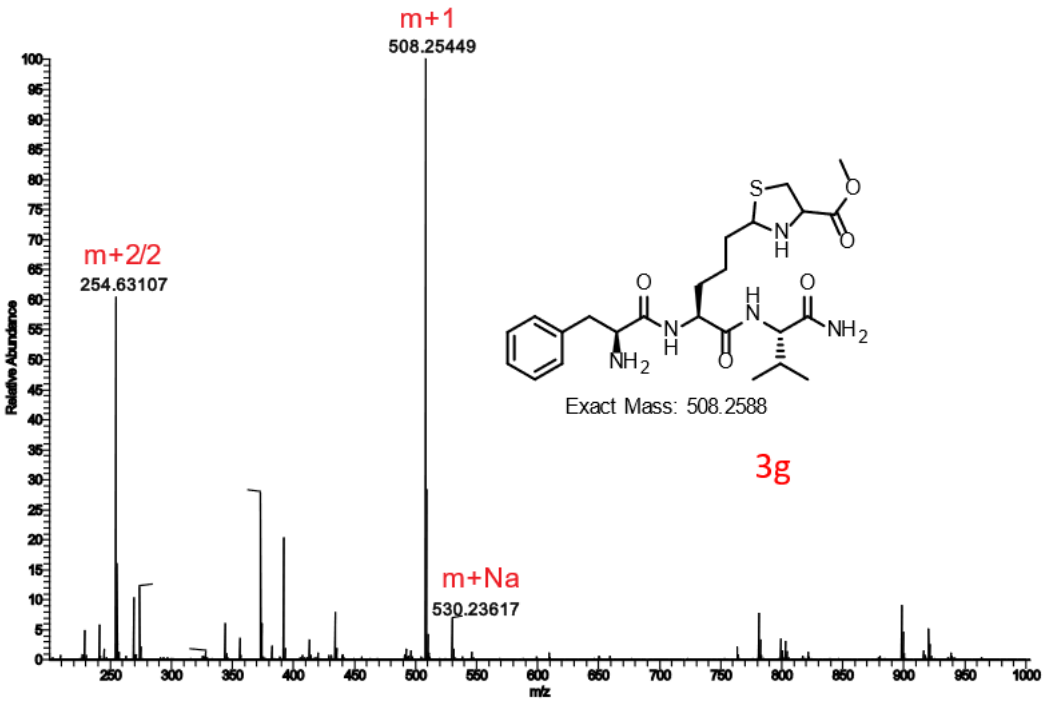
**HPLC Trace of FKme$_2$(CHO)V 2a**



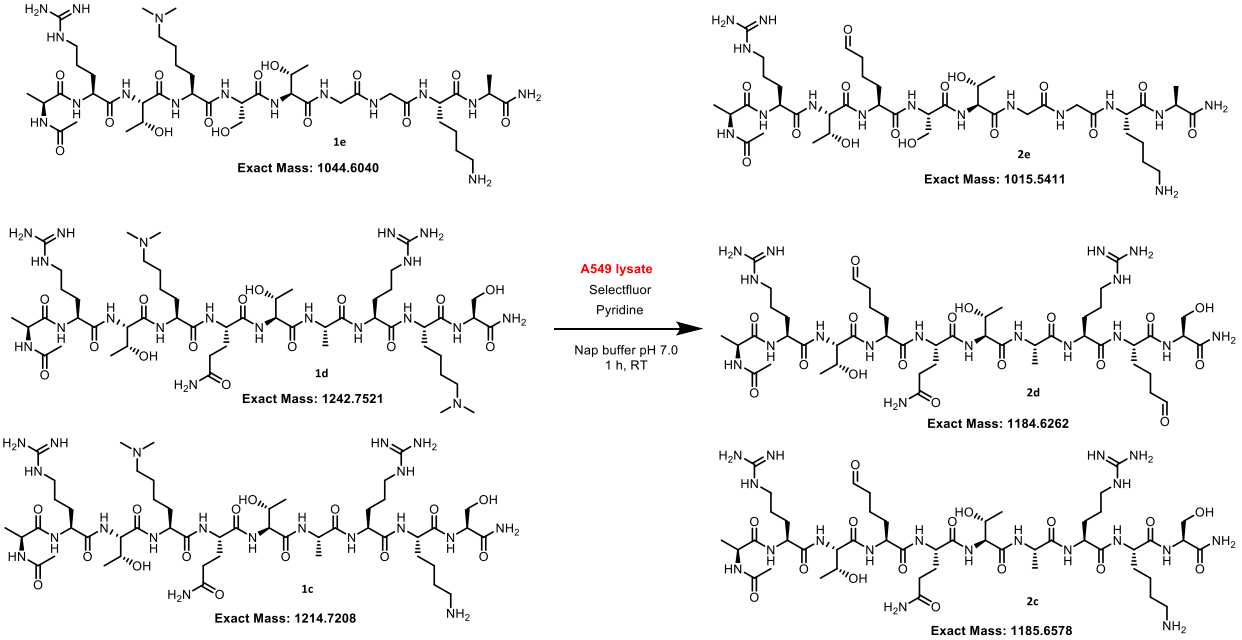

**HPLC Trace of thiazolidine product**




**MS-Trace of peak 7.661**

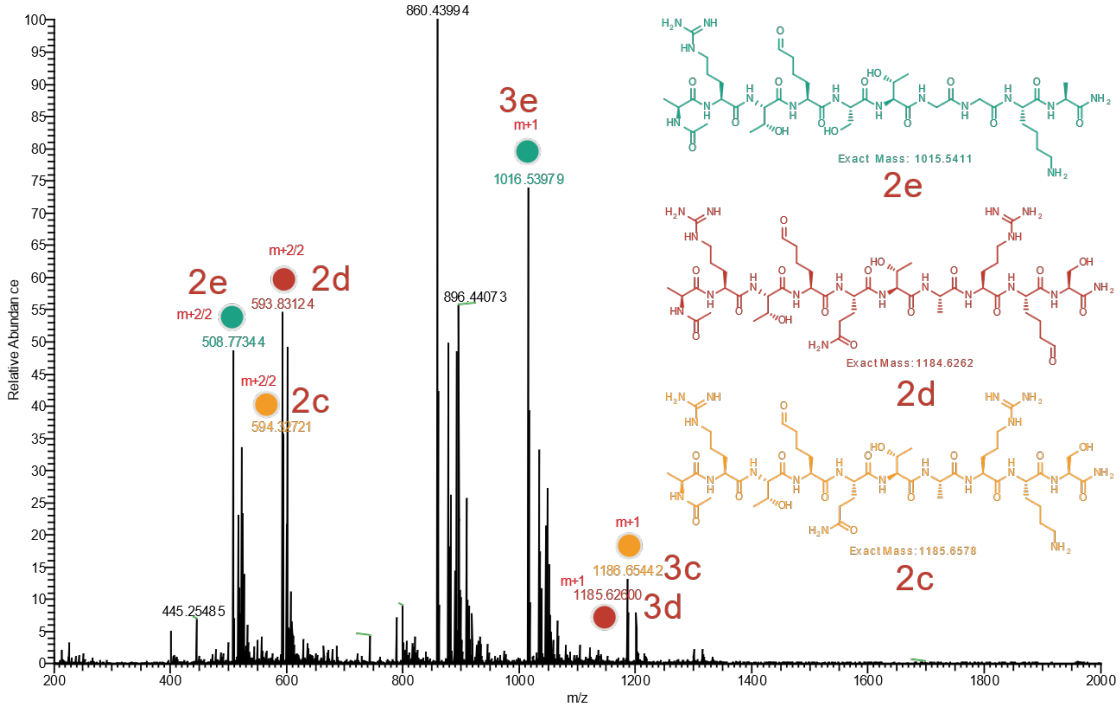


**XX. Supplementary Figure 14:** Oxidative tertiary amine transformation of dimethyllysine containing histone peptides fragments (**1c-1e**) spiked into a complex mixture of epithelial carcinoma A549 whole cell lysate.

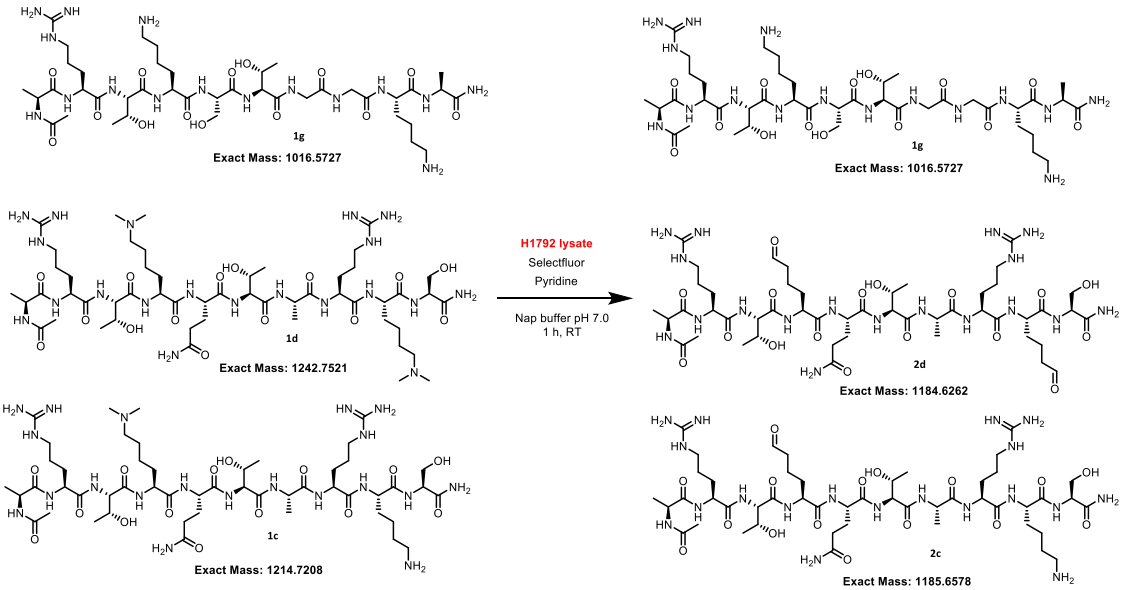



To 100 µg of A549 whole cell lysate in 200 µL of NaP buffer pH 7.0 was added 0.1 mg of histone peptide (ARTK$me_2$QTARKS) **1c**, 0.1 mg of histone peptide K$me_2$4K$me_2$9 (ARTK$me_2$QTARK$me_2$S) **1d**, and 0.1 mg of histone peptide K$me_2$9K14 (ARTK$me_2$STGGKA**) 1e**. To this mixture of lysate and histone peptides was added pyridine (30 eq with respect to peptides) and selectfluor (10 eq. with respect to peptides). The reaction mixture was stirred for 1 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the modification of dimethyllysine containing histone peptides into aldehyde products. The reaction mixture was analyzed by HPLC using method B.

**MS-Trace of the Cell lysate after the reaction**

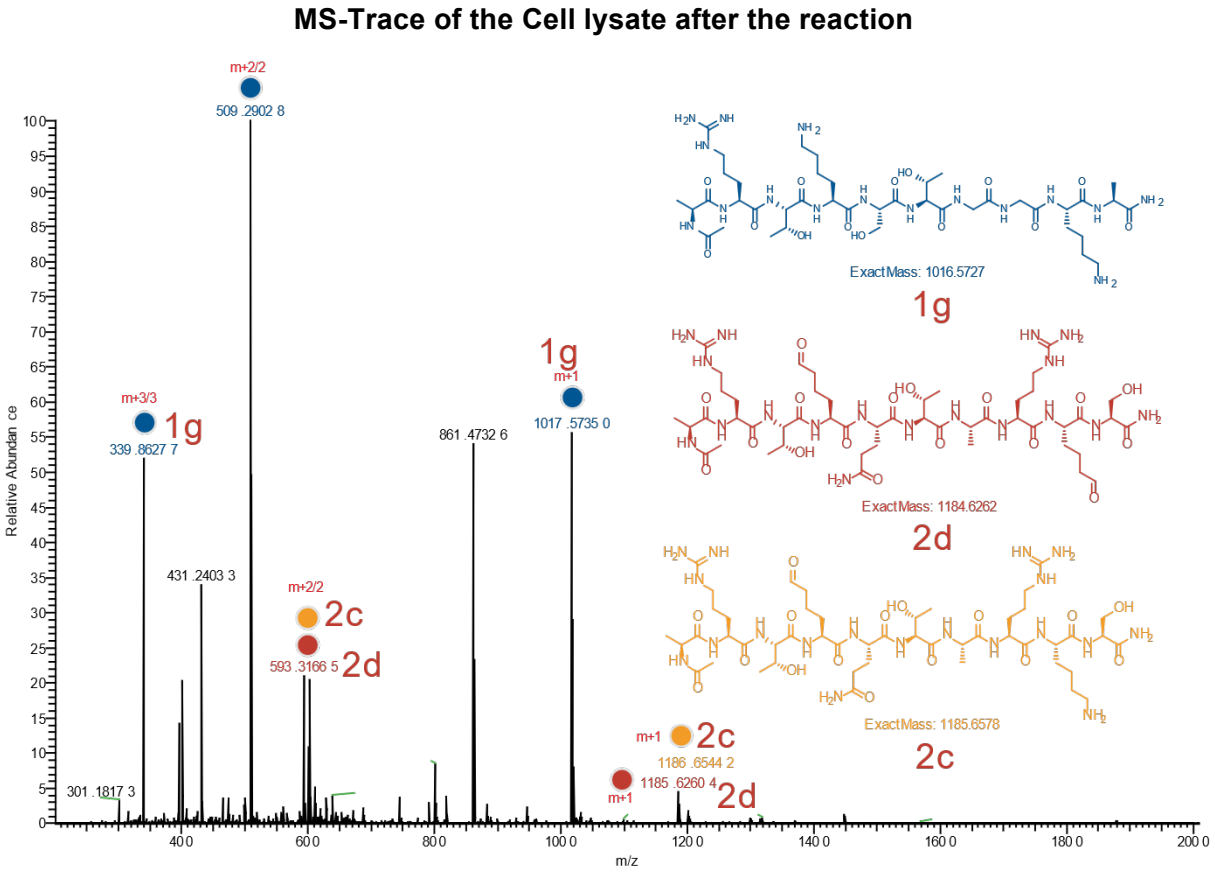



**XXI. Supplementary Fig. 15:** Oxidative tertiary amine transformation of dimethyllysine containing histone peptides fragments (**1c, 1d and 1g**) spiked into a complex mixture of H1792-lung cancer cell lysate.




To 100 µg of H1792 whole cell lysate in 200 µL of NaP buffer pH 7.0 was added 0.1 mg of histone peptide (ARTK$me_2$QTARKS) **1c**, 0.1 mg of histone peptide $Kme_2$4$Kme_2$9

(ARTKme$_2$QTARKme$_2$S) **1d**, and 0.1 mg of histone peptide Kme$_2$9K14 (ARTKme$_2$STGGKA) **1g**. To this mixture of lysate and histone peptides was added pyridine (30 eq with respect to peptides) and selectfluor (10 eq. with respect to peptides). The reaction mixture was stirred for 1 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the modification of dimethyllysine containing histone peptides into aldehyde products. The reaction mixture was analyzed by HPLC using method B.

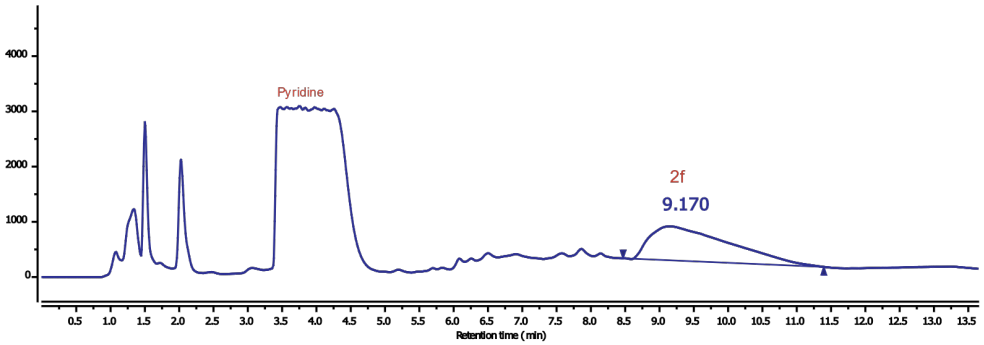



**XXII. Supplementary Figure 16.** Single-molecule sequencing for identification of Kme$_2$ sites by TACO

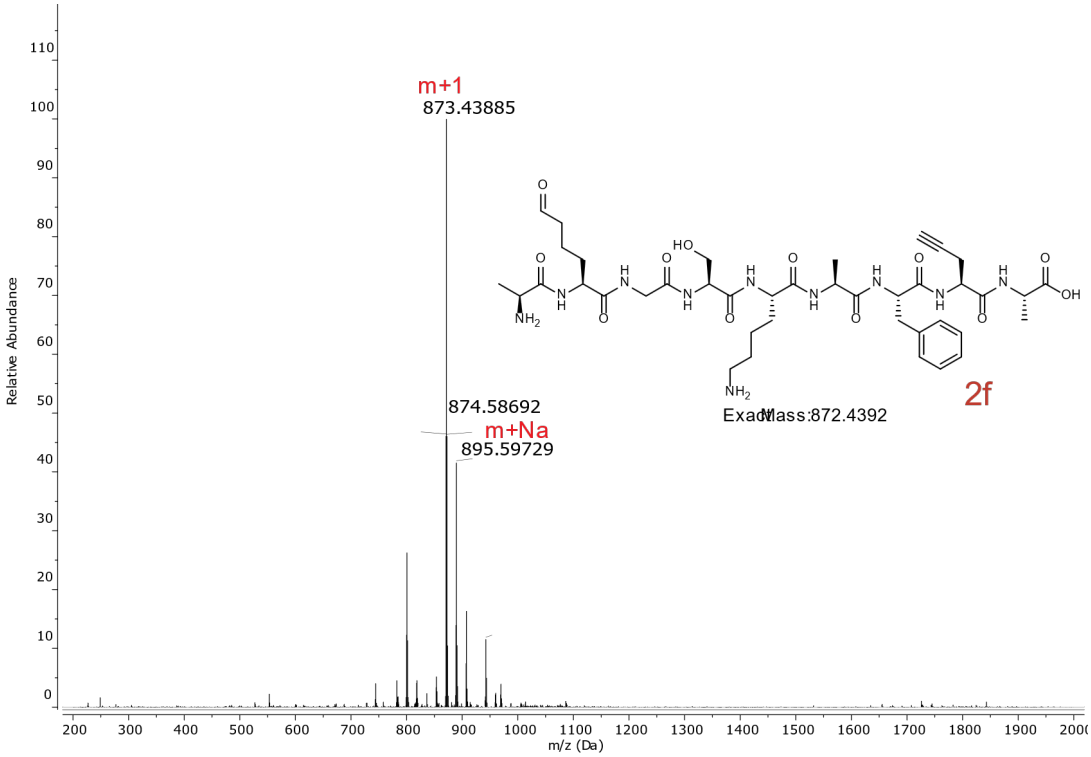
**Aldehyde generation**: To 1.0 mg of fluorosequencing peptide fragment AKme$_2$GSKAF(PrG)A (where PrG = propargyl glycine) dissolved in 300 µL of 10 mM sodium phosphate buffer (NaP, pH 7.0), was added pyridine (5 eq) and selectfluor (2 eq). The reaction mixture was stirred for 1 h. Samples were taken from the reaction mixture, injected into LC-MS to monitor the generation of peptide aldehyde product AKme$_2$(CHO)GSKAF(PrG)A **2f** (>98%).

AKme$_2$(CHO)GSKAF(PrG)A **2f.** LCMS: *m/z* 873.43885 (calcd [M+H]+ = 508.4465), *m/z* 895.59729 (calcd [M+Na]+ = 895.4284), (HPLC analysis at 220 nm). Retention time in HPLC: 9.17

**HPLC trace of reaction**

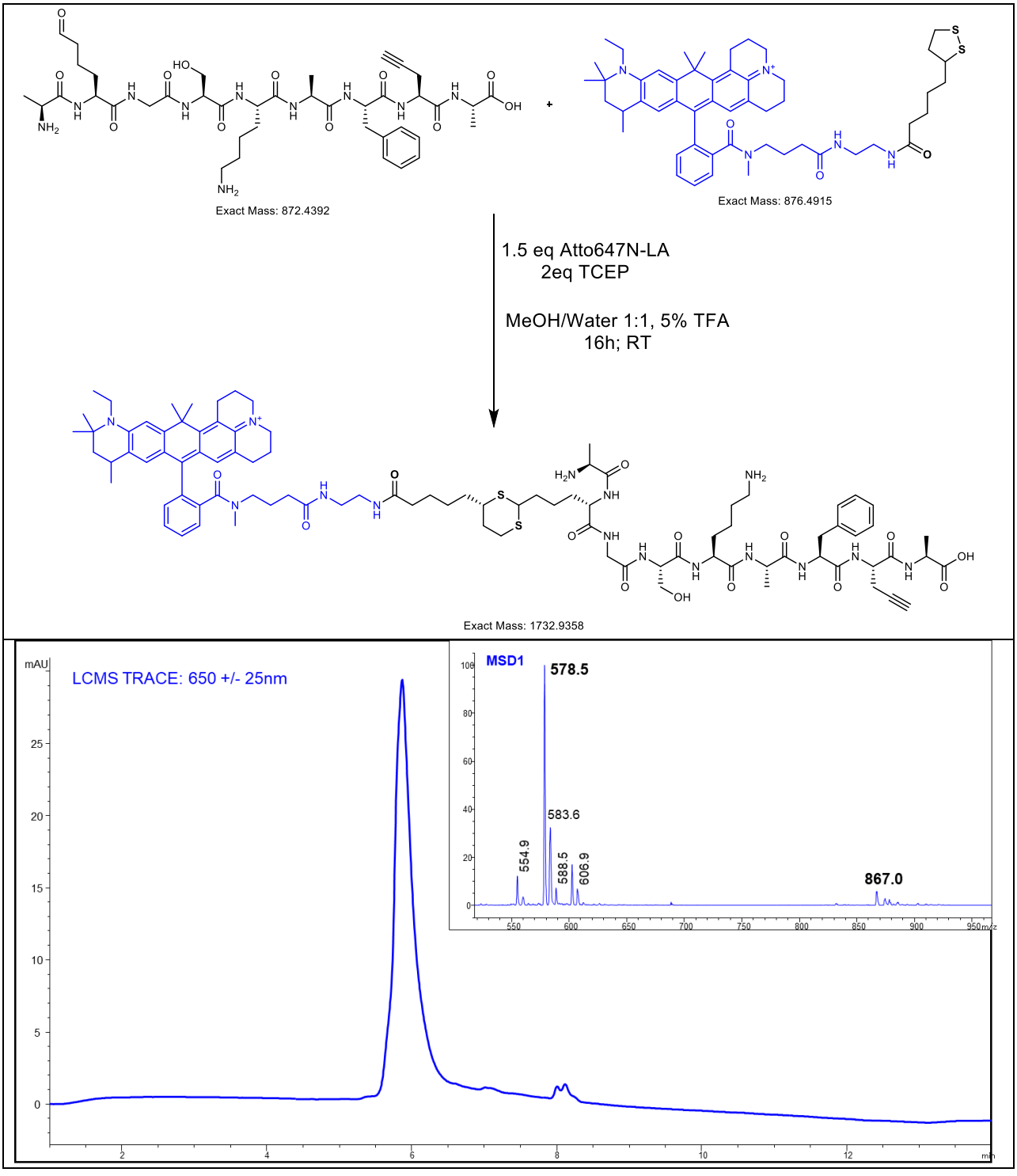



**MS-Trace of peak 9.170**




**Fluorophore labeling of $Kme_2$ peptide:** Atto647N-LA (Lipoic acid) was custom synthesized by Atto-tec (Germany), by reacting 1 mg of Atto647N-Amine (Cat# AD 647N-91) with 2 eq. lipoic acid (Cas # 1077-28-7; Sigma (Cat # T5625-1G)) and 2 eq. of triethylamine in dimethylformamide for 2 h at RT. Standard HPLC purification was performed to isolate the Atto647N-LA compound and lyophilized. Atto647N-LA was coupled to modified $Kme_2$ peptide (AK$me_2$(CHO)GSKAF(PrG)A) **2f** by reacting with 1.5 eq of Atto647N-LA, 2 eq. TCEP (tris(2-carboxyethyl)phosphine) in solution of 5% TFA in methanol/water (1:1 vv) solution for 16 h. The labeled peptide was purified using standard HPLC method and lyophilized.

**Atto647N-peptide product.** LCMS: *m/z* 876.0 (calcd [M+2/2]+ = 867.4679), *m/z* 578.5 (calcd [M+3/3]+ =578.6452), (HPLC analysis at 220 nm). Retention time in HPLC: 6.0



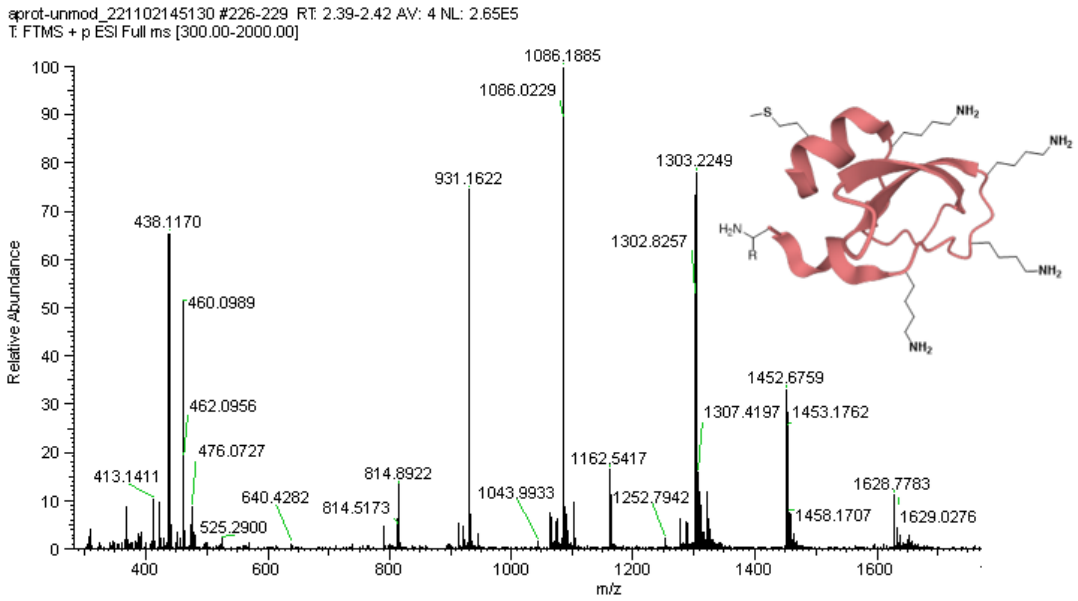
**Surface immobilization of peptide**. For single molecule peptide sequencing, a 40 mm German Desag 263 borosilicate glass coverslip (Bioptechs) surface was first cleaned by UV/ozone (Jelight Company) and then functionalized by chemical vapor deposition of 3-azidopropyltriethoxysilane (Gelest, Cat # SIA0770.0) under vacuum at 70 $^{o}$C for 1 h. Atto647N labeled $Kme_2$ peptides were covalently coupled to the coverslip surface *via* copper catalyzed click chemistry between the propargyl amino acid on peptide and the azido silane. A fresh solution of of 2 mM copper sulfate, 1 mM Tris(3-hydroxypropyltriazolylmethyl)amine (Sigma, Cat # 762342), 100 mM HEPES (pH 8.5), 5mM sodium ascorbate with Atto647N labeled $Kme_2$ peptides was incubated for 10 minutes at room temperature on the azido silane functionalized coverslip, washed with water to remove unbound peptides, and dried under a nitrogen gas stream.

**Fluorosequencing.**[2] Single molecule sequencing was performed as described with minor modifications in the solvents use, incubation times and use of a different camera (Orca Fusion BT, Hamamatsu). The wash solvent ethyl acetate was substituted with acetonitrile and the phenyl isothiocyanate solution is now 20% vv in pyridine. Incubation time for cleavage step (use of trifluoroacetic acid) is reduced to 5mins. Fluorosequencing datasets were analyzed using the *SigProc* software tool, available as part of the Plaster package at https://github.com/erisyon/plaster_v1. Further details of the experimental methods and analysis are available on request.

## XXIII. Supplementary Figure 17. Modification of $Kme_2$ proteins using TACO

**General procedure for dimethylation of proteins:** To 2 mg of protein in 1 mL of NaP buffer (pH 7), was added 500 µL of 10 % formaldehyde solution in water. The reaction was vortexed for 2 min, followed by the addition of 500 µL of 600 mM $NaBH_3CN$ solution in water, and vortexed for additional 2 min. The reaction was incubated at room temperature for 6 min, filtered through a molecular weight cut off (3 kDa) to remove small molecules and the resulting purified protein was analyzed by MS.

**Mass spectrum of unmodified aprotinin protein**
**Molecular weight: 6511 (4 lysines, 1 N-terminus)**
**Sequence:**
RPDFCLEPPYTGPCKARIIRYFYNAKAGLCQTFVYGGCRAKRNNFKSAEDCMRTCGGA

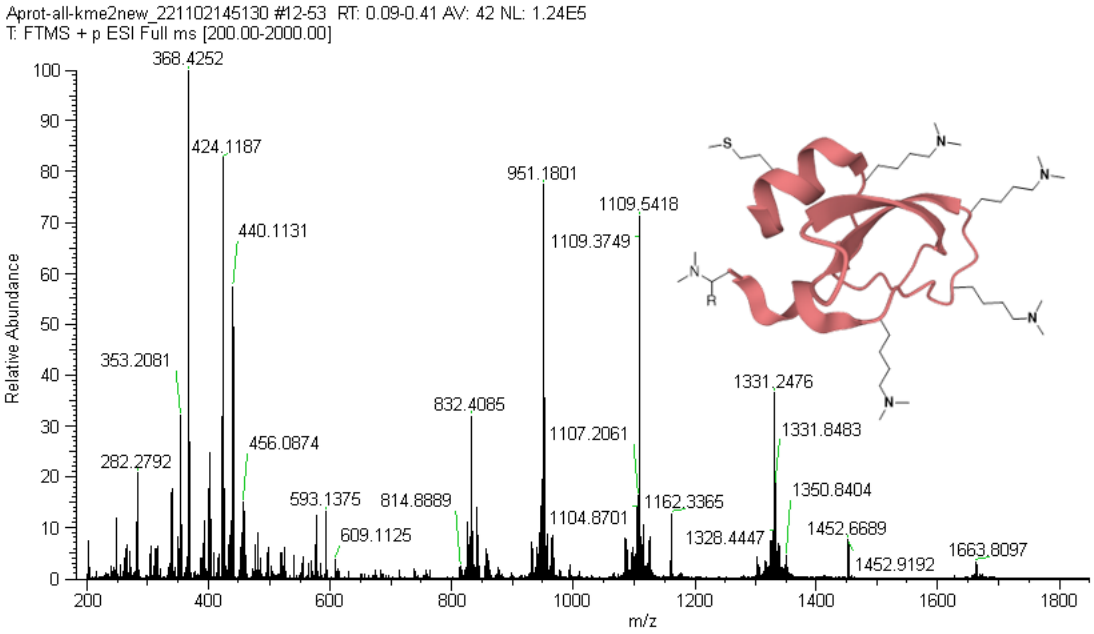



**Mass spectrum of dimethylated lysine aprotinin protein**

**Molecular weight: 6511 (4 lysines, 1 N-terminus)**

**Sequence:**

**(me2)**RPDFCLEPPYTGPCKme$_2$ARIIRYFYNAKme$_2$AGLCQTFVYGGCRAKme$_2$RNNFKme$_2$SAEDCMRTCGGA

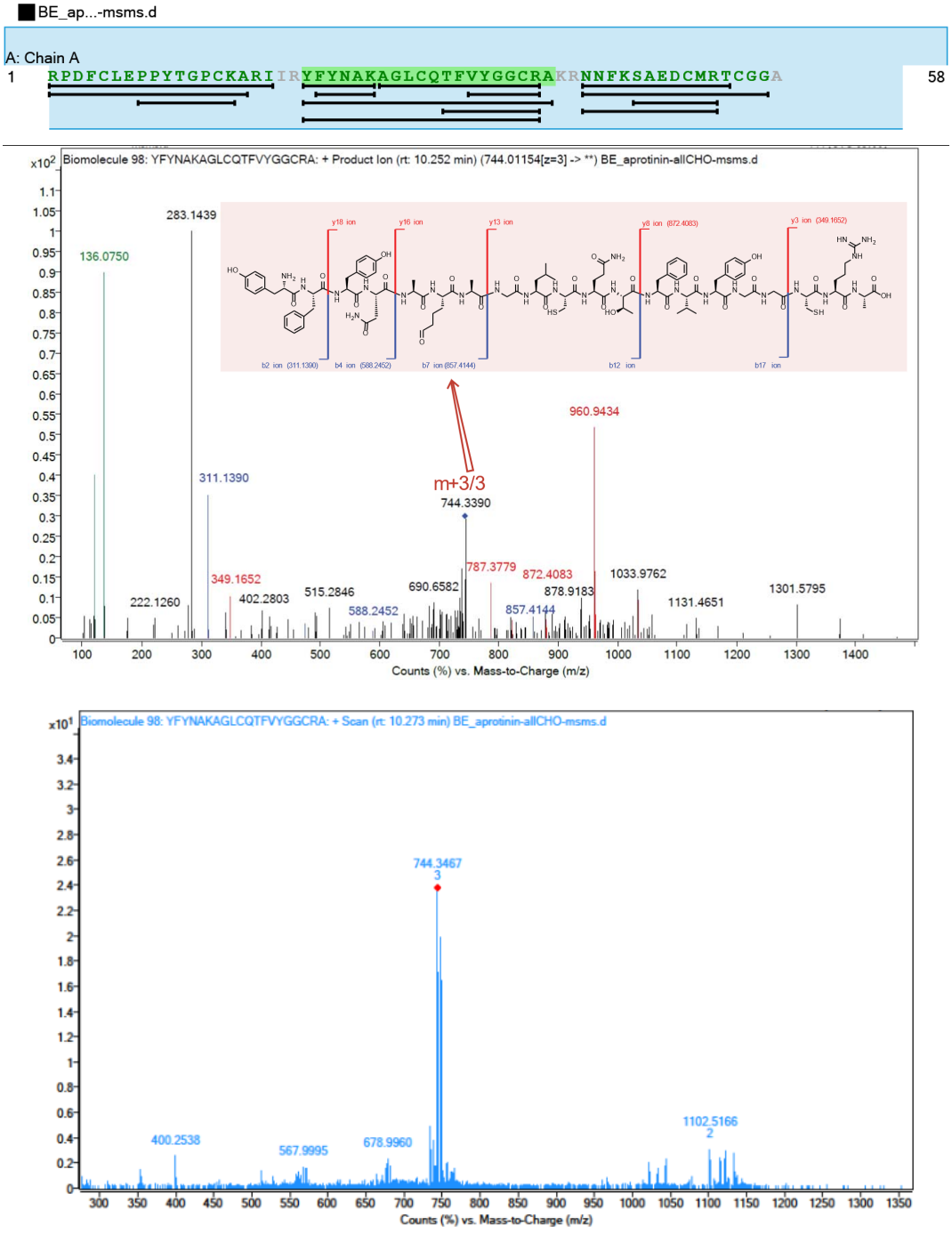


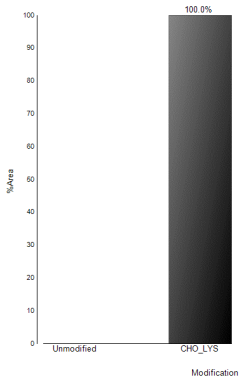

**TACO modification of Kme$_2$ containing proteins:** To 2 mg Kme$_2$ proteins in 1 mL of NaP buffer pH 7, was added 10 mg of selectfluor and 50 µL of pyridine. The reaction was stirred at room temperature for 30 min and filtered through a molecular weight cutoff to remove chemical

reagents at the end of reaction. Protein samples were digested using the thermofischer scientific lysine/Lys C protein digestion kit. Digested samples were analyzed using LC-MS/MS.

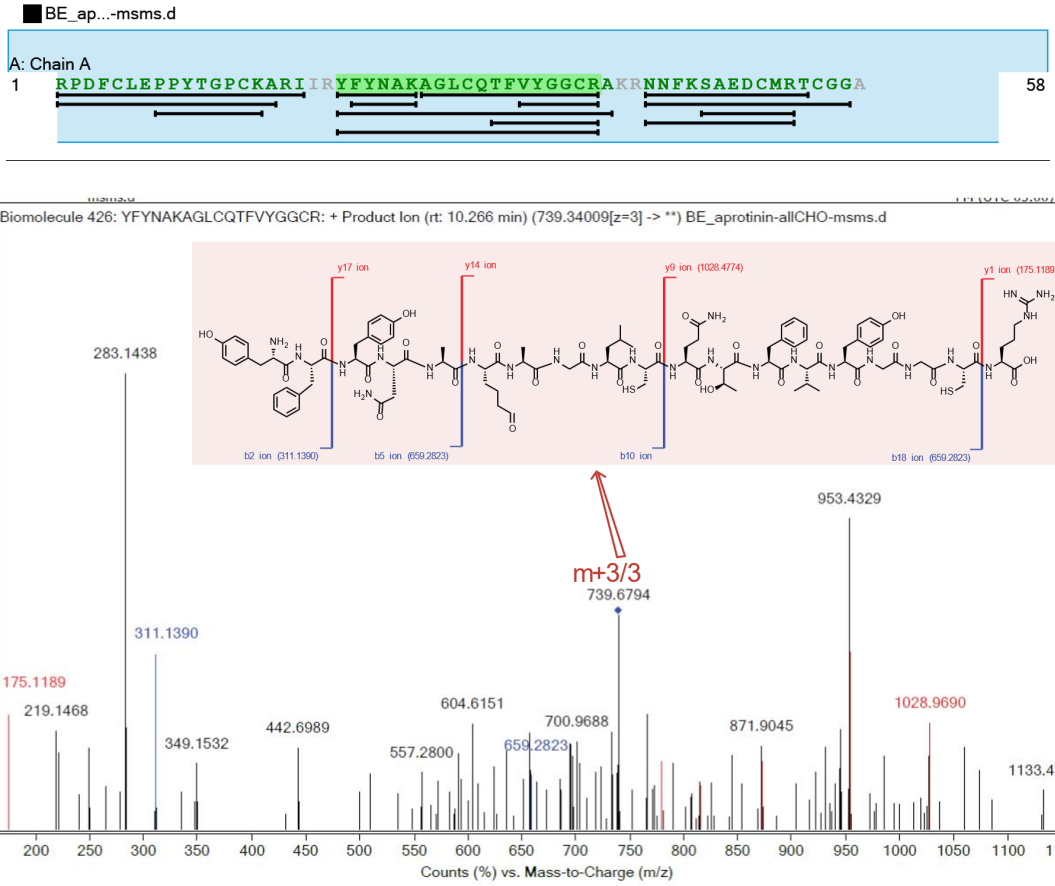
## APROTININ

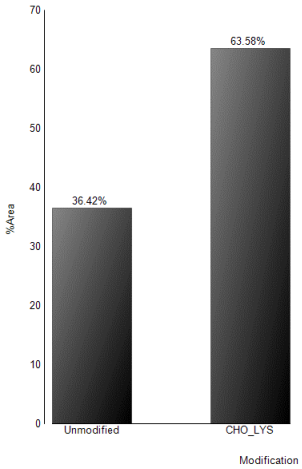
### Fragment 1 mass spectrum data for aprotinin





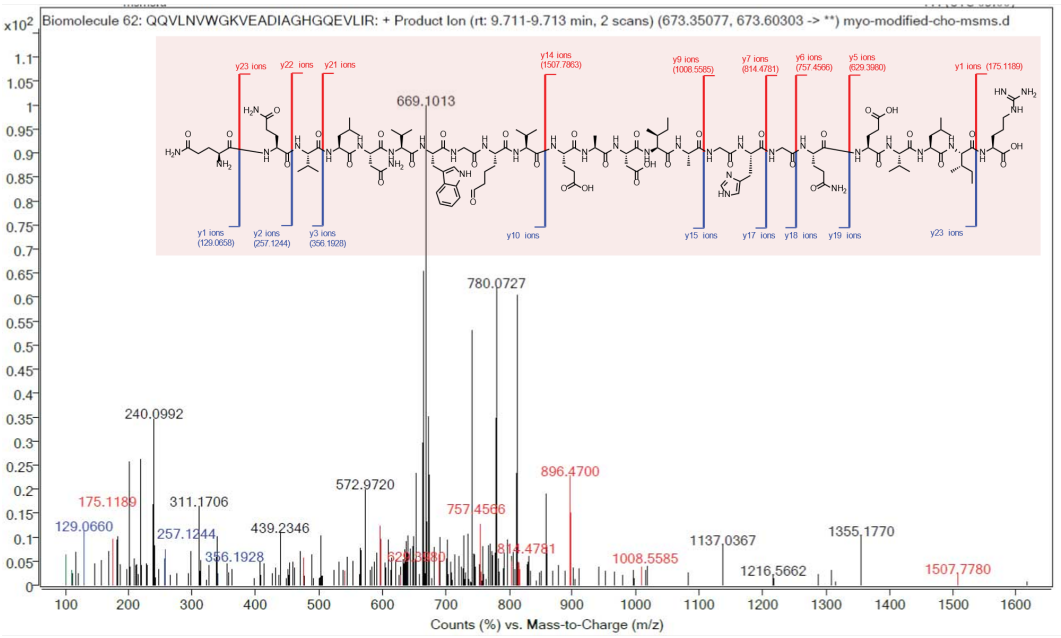

## Fragment 2 mass spectrum data for aprotinin

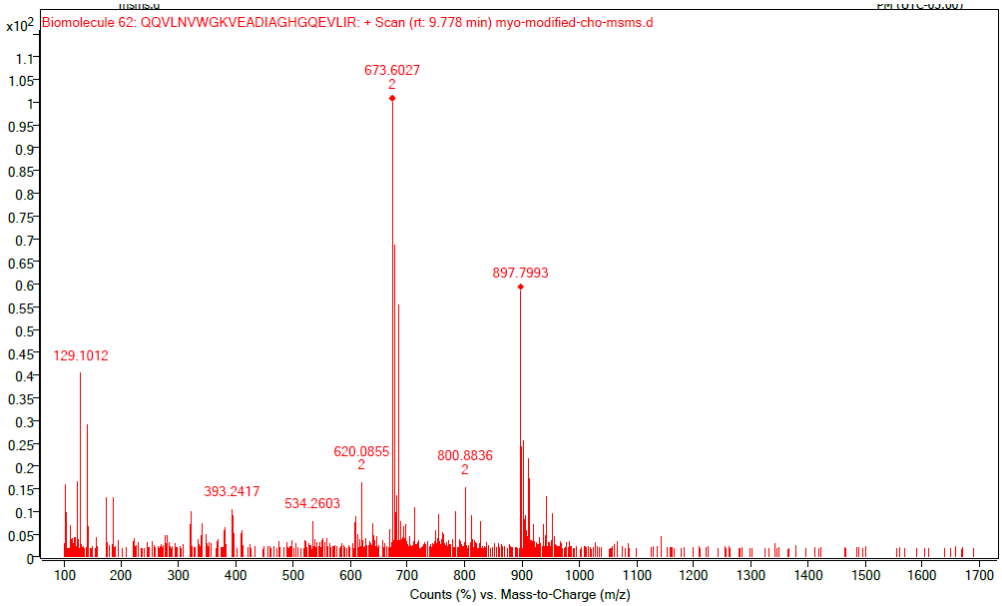


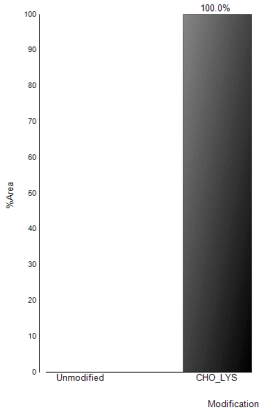



**MYOGLOBIN**

## Fragment mass spectrum data for myoglobin

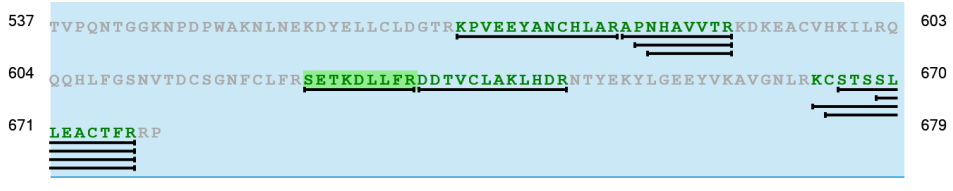



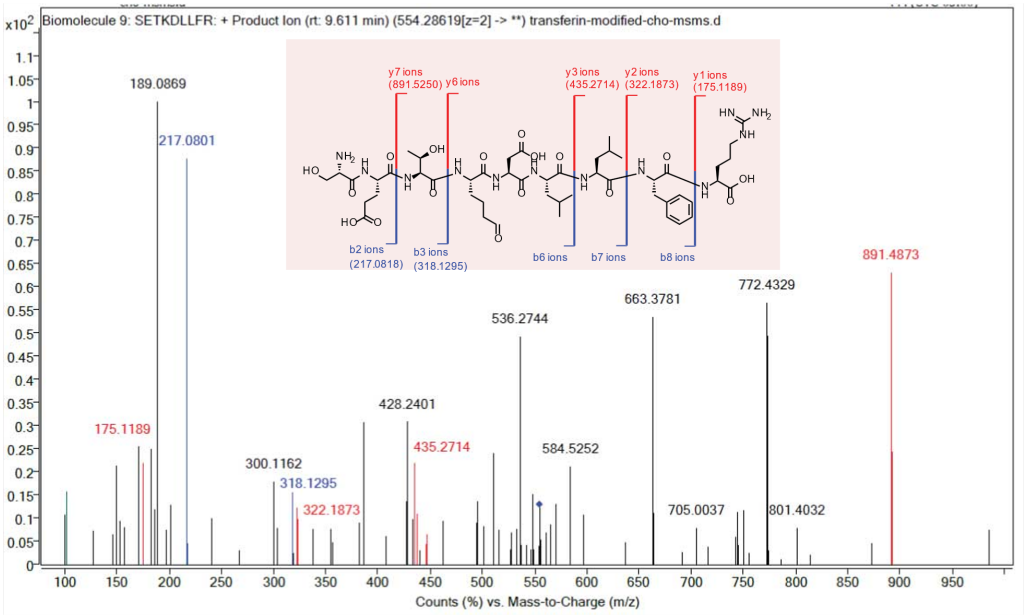


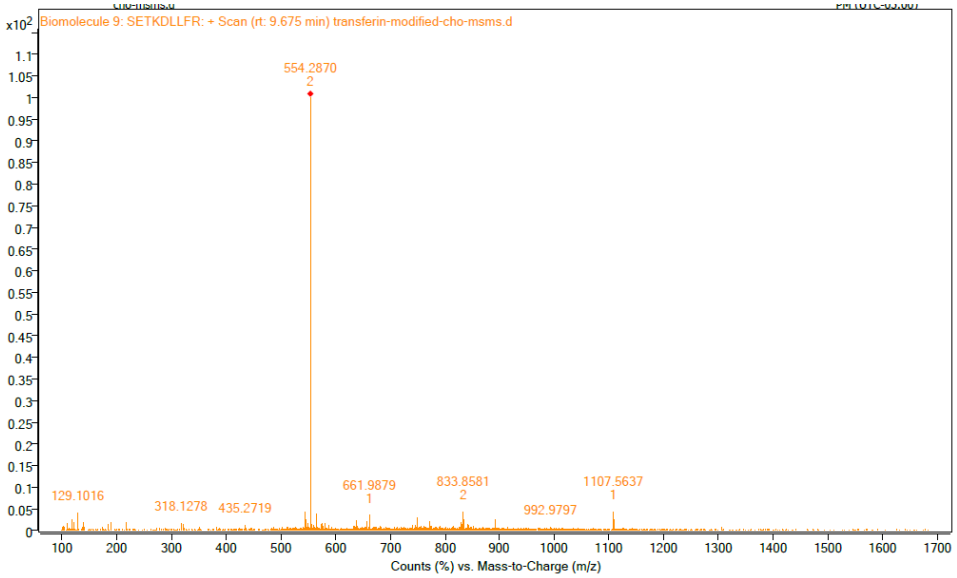



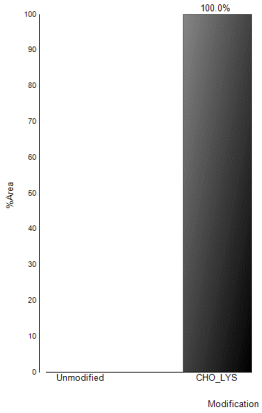

## TRANSFERRIN

**Fragment 1 mass spectrum data for transferrin**

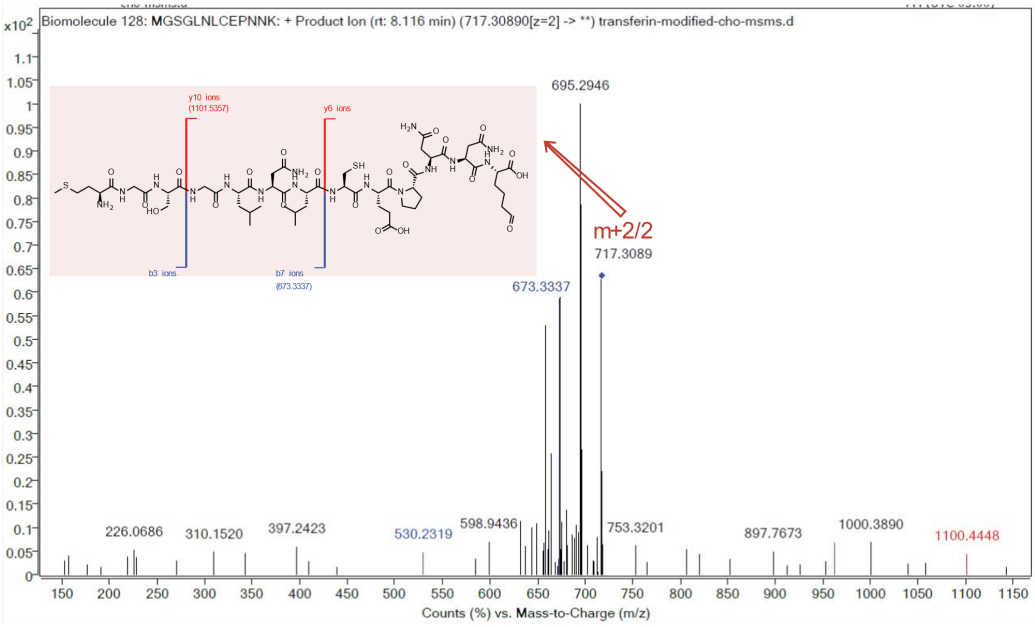


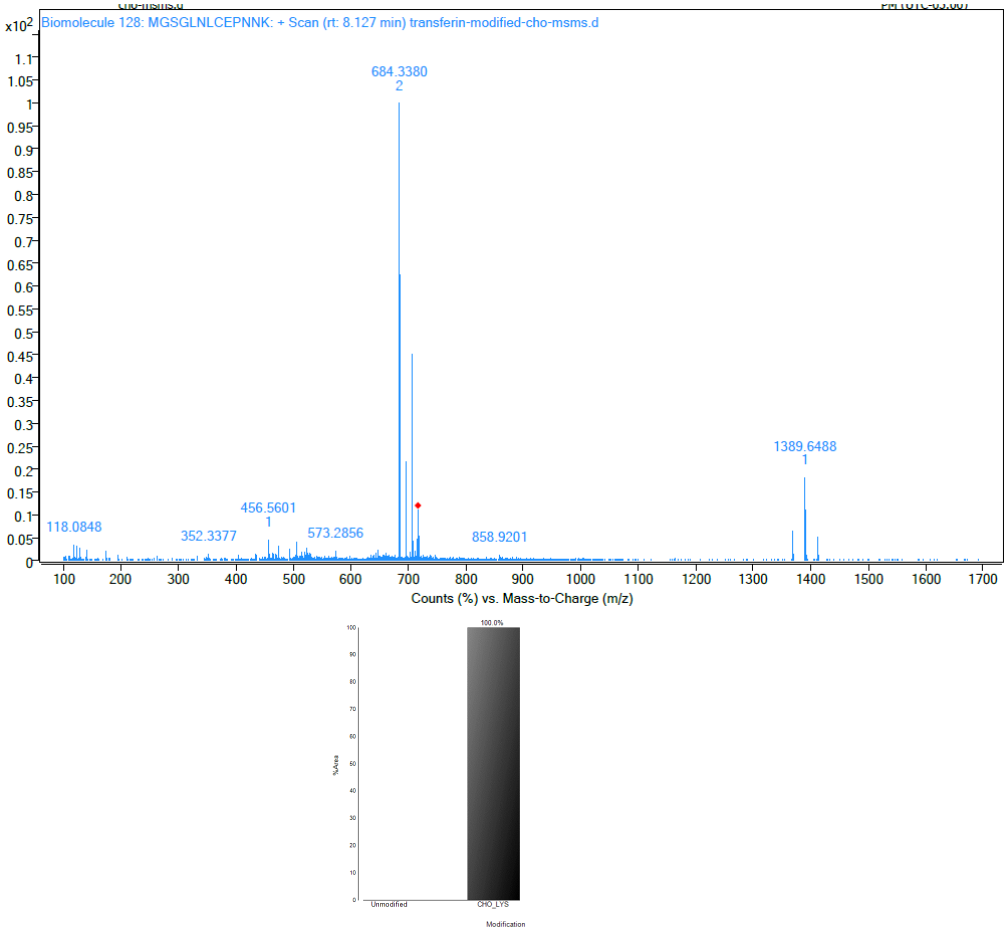

Biomolecule 9: SETKDLLFR: + Product Ion (rt: 9.611 min) (554.28619[z=2] -> **) transferin-modified-cho-msms.d
x10^2
189.0869
217.0801
175.1189
300.1162
318.1295
322.1873
428.2401
435.2714
536.2744
584.5252
663.3781
705.0037
772.4329
801.4032
891.4873
y7 ions (891.5250)
y6 ions
y3 ions (435.2714)
y2 ions (322.1873)
y1 ions (175.1189)
b2 ions (217.0818)
b3 ions (318.1295)
b6 ions
b7 ions
b8 ions
Counts (%) vs. Mass-to-Charge (m/z)


Biomolecule 9: SETKDLLFR: + Scan (rt: 9.675 min) transferin-modified-cho-msms.d
x10^2
554.2870
2
129.1016
318.1278
435.2719
661.9879
1
833.8581
2
992.9797
1107.5637
1
Counts (%) vs. Mass-to-Charge (m/z)


100.0%
%Area
Unmodified
CHO_LYS
Modification

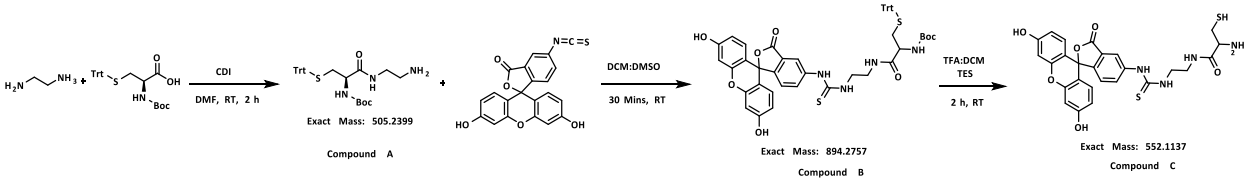
## Fragment 2 mass spectrum data for transferrin




**XXIV. Supplementary Figure 18. Modification of $Kme_2$ proteins with FITC-cysteine fluorophore.**

**Synthetic scheme for FITC-Cysteine probe synthesis.**

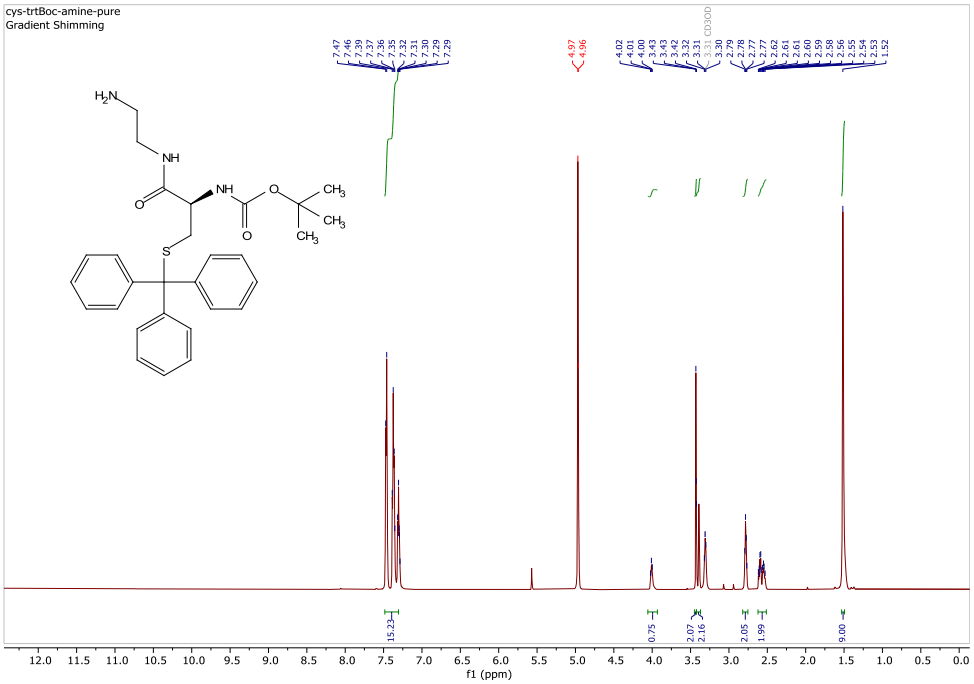



**Synthesis of tert-butyl (R)-(1-((2-aminoethyl)amino)-1-oxo-3-(tritylthio)propan-2-yl)carbamate (A).** To 1 g of N-Boc-S-trityl-L-cysteine in 10 mL of DMF was added 420 mg (2.6 mmol) of carbonyl diimidazole and reaction stirred at room temperature for 30 min. After 30 min, ethylenediamine (1.4 mL, 21.6 mmol) was added in a single portion to the reaction mixture and stirred for an additional 2 h at room temperature. Upon completion, DMF was removed in vacuo, and residue was dissolved in 50 mL of dichloromethane and washed with water (3 x 50 mL), and saturated brine (50 mL). The resulting extracted solution was dried with anhydrous magnesium sulfate, and solvent was reduced to 1 mL in vacuo and solid was collected by filtration and dried in vacuo.

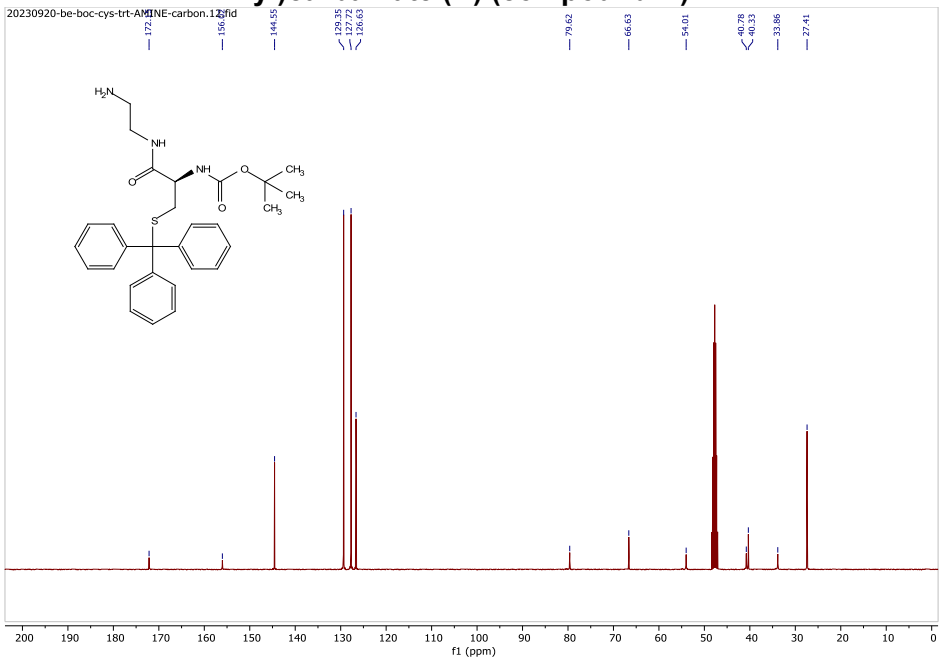
**$^1$H NMR** (600 MHz, $CD_3OD$) δ 7.48 – 7.31 (m, 15H), 4.01 (t, 1H), 3.43 (d, *J* = 2.7 Hz, 2H), 3.40

(d, *J* = 22.7 Hz, 2H), 2.78 (q, J = 5.3, 4.6 Hz, 2H), 2.62 – 2.51 (m, 2H), 1.51 (s, 9H).
**$^{13}$C NMR** (151 MHz, $CD_3OD$) δ 172.15, 156.02, 144.55, 129.35, 127.72, 126.63, 79.62, 66.63, 54.01, 40.78, 40.33, 33.86, 27.41.

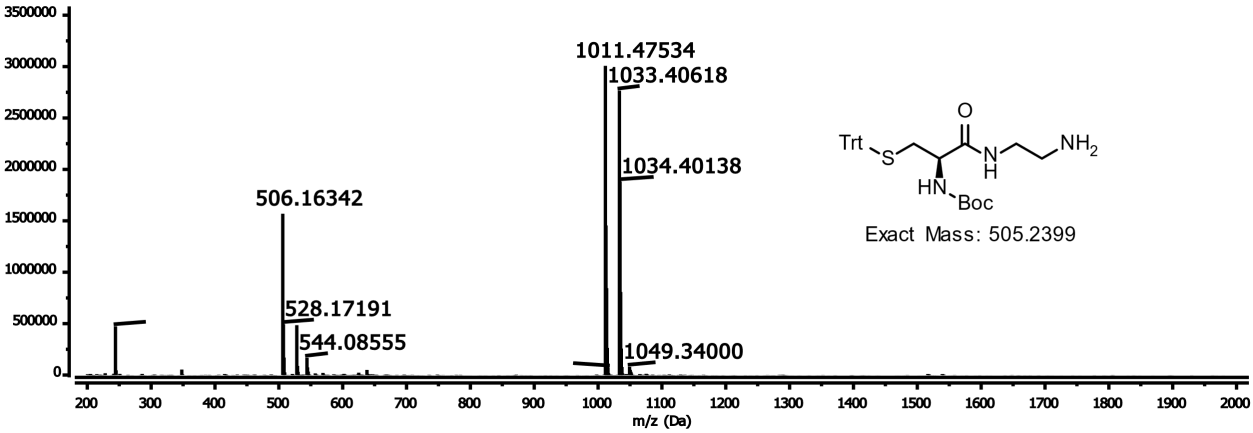
**Proton NMR of tert-butyl (R)-(1-((2-aminoethyl)amino)-1-oxo-3-(tritylthio)propan-2-yl)carbamate (A) (compound A)**




**Carbon NMR of tert-butyl (R)-(1-((2-aminoethyl)amino)-1-oxo-3-(tritylthio)propan-2-yl)carbamate (A) (compound A)**



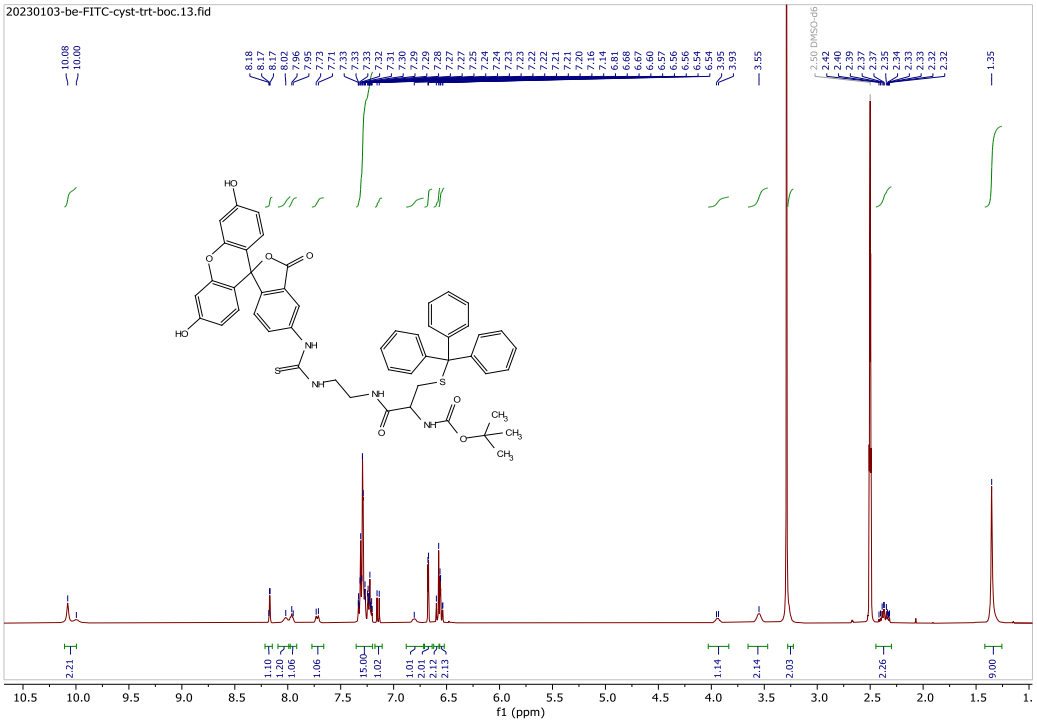
**MS-Trace of tert-butyl (R)-(1-((2-aminoethyl)amino)-1-oxo-3-(tritylthio)propan-2-yl)carbamate (A) (compound A)**



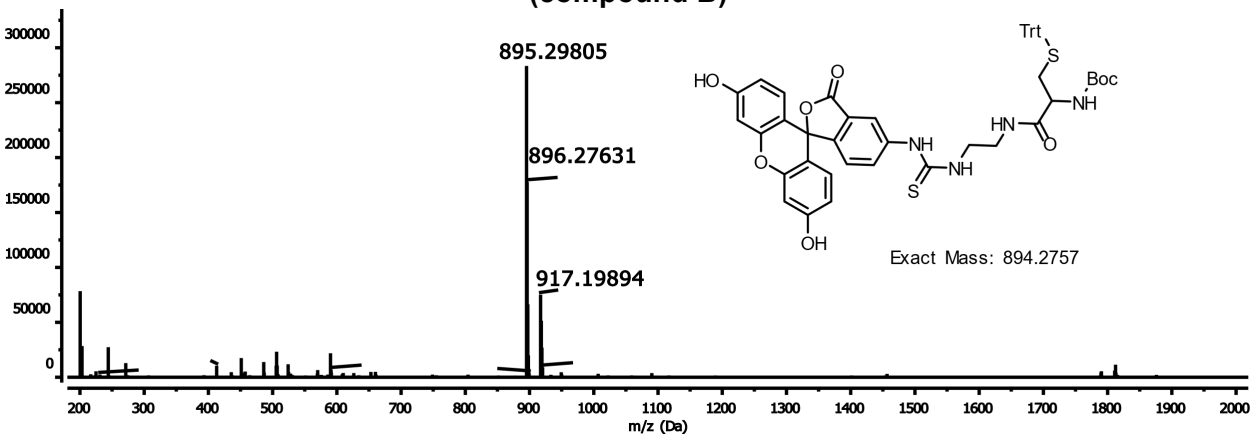

**Synthesis of tert-butyl (1-((2-(3-(3',6'-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-5-yl)thioureido)ethyl)amino)-1-oxo-3-(tritylthio)propan-2-yl)carbamate (B).** To 100 mg of tert-butyl (R)-(1-((2-aminoethyl)amino)-1-oxo-3-(tritylthio)propan-2-yl)carbamate in 10 mL of dichloromethane was added 77 mg (1.98 mmol) of fluorescein isothiocyanate dissolved in 1 mL of DMSO. Reaction was stirred at room temperature for 30 min. After 30 min, dichloromethane was removed in vacuo and acetonitrile was added to the residue and purified by preparative HPLC.

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 10.05 (d, *J* = 32.7 Hz, 2H), 8.18 (d, *J* = 2.0 Hz, 1H), 8.03 (s, 1H), 7.96 (d, *J* = 5.4 Hz, 1H), 7.73 (d, *J* = 8.4 Hz, 1H), 7.36 – 7.21 (m, 15H), 7.15 (d, *J* = 8.3 Hz, 1H), 6.81 (s, 1H), 6.68 (d, *J* = 2.2 Hz, 2H), 6.59 (d, *J* = 8.6 Hz, 2H), 6.56 (dd, *J* = 8.6, 2.2 Hz, 2H), 3.95 (d, *J* = 7.6 Hz, 1H), 3.56 (d, 2H), 2.45 – 2.31 (m, 2H), 1.36 (s, 9H).

**$^1$H NMR of tert-butyl (1-((2-(3-(3',6'-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-5-yl)thioureido)ethyl)amino)-1-oxo-3-(tritylthio)propan-2-yl)carbamate (compound B)**



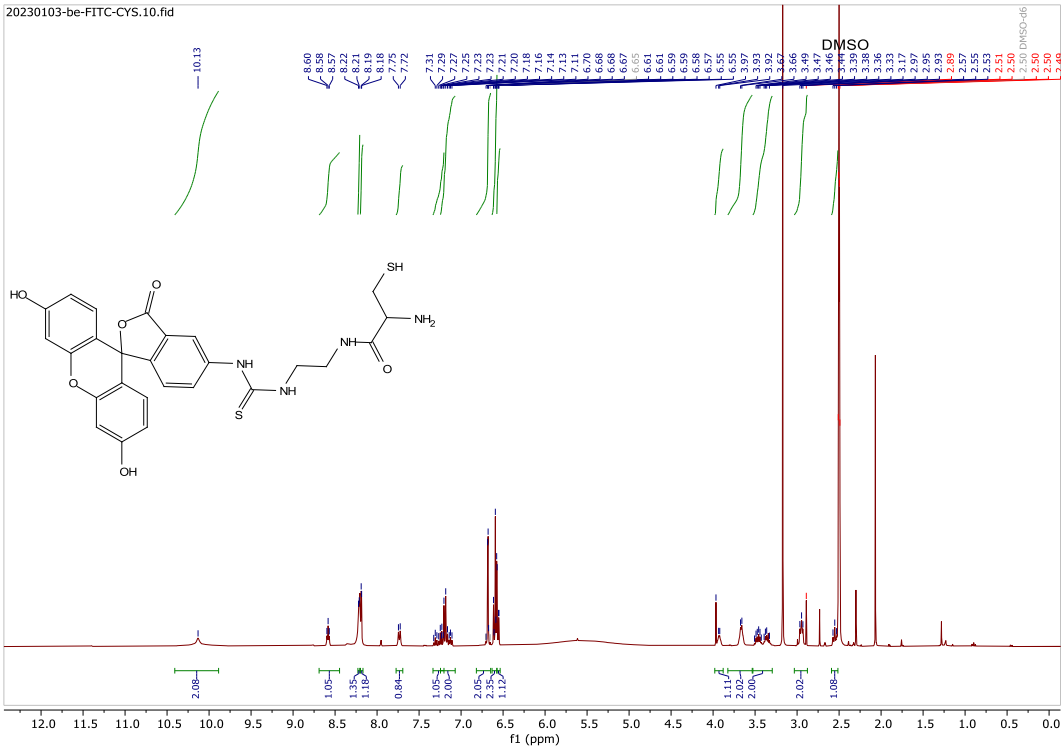

**MS-Trace of tert-butyl (1-((2-(3-(3',6'-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-5-yl)thioureido)ethyl)amino)-1-oxo-3-(tritylthio)propan-2-yl)carbamate (compound B)**



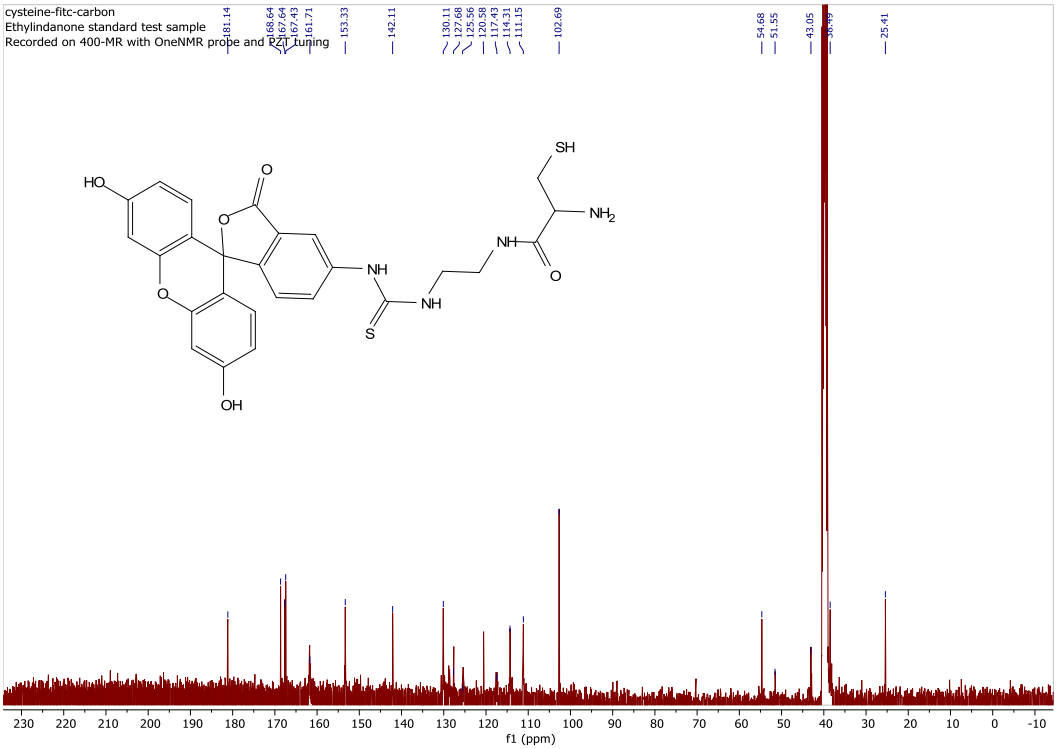

**Synthesis of 2-amino-N-(2-(3-(3',6'-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-5-yl)thioureido)ethyl)-3-mercaptopropanamide (compound C) (FITC-Cysteine).** To 100 mg of tert-butyl (1-((2-(3-(3',6'-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-5-yl)thioureido)ethyl)amino)-1-oxo-3-(tritylthio)propan-2-yl)carbamate in 5 mL of dichloromethane was added 5 mL of trifluoroacetic acid, 300µL of triethyl silane, and 100 µL of water. Reaction was stirred at room temperature for 2 h. After 2 h, the reaction was dried in vacuo and residue

washed with hexane (3x). Solid was dried in vacuo to obtain the title compound C, **FITC-Cysteine** as yellow oil.
**$^{1}$H NMR** (400 MHz, DMSO-$d_6$) δ 10.13 (s, 2H), 8.58 (t, *J* = 5.7 Hz, 1H), 8.21 (d, 1H), 8.19 (t, *J* = 2.1 Hz, 1H), 7.74 (d, *J* = 8.4 Hz, 1H), 7.33 – 7.20 (m, 1H), 7.24 – 7.07 (m, 2H), 6.68 (d, *J* = 2.2 Hz, 2H), 6.63 – 6.57 (m, 2H), 6.57 – 6.53 (m, 1H), 3.95 (t, *J* = 12.7 Hz, 1H), 3.67 (t, *J* = 6.4 Hz, 2H), 3.53 – 3.30 (m, 2H), 3.03 – 2.88 (m, 2H), 2.56 (t, 1H).
**$^{13}$C NMR** (151 MHz, DMSO-$d_6$) δ 181.14, 168.64, 167.64, 167.43, 161.71, 153.33, 142.11, 130.11, 127.68, 125.56, 120.58, 117.43, 114.31, 111.15, 102.69, 54.68, 51.55, 43.05, 38.49, 25.41.

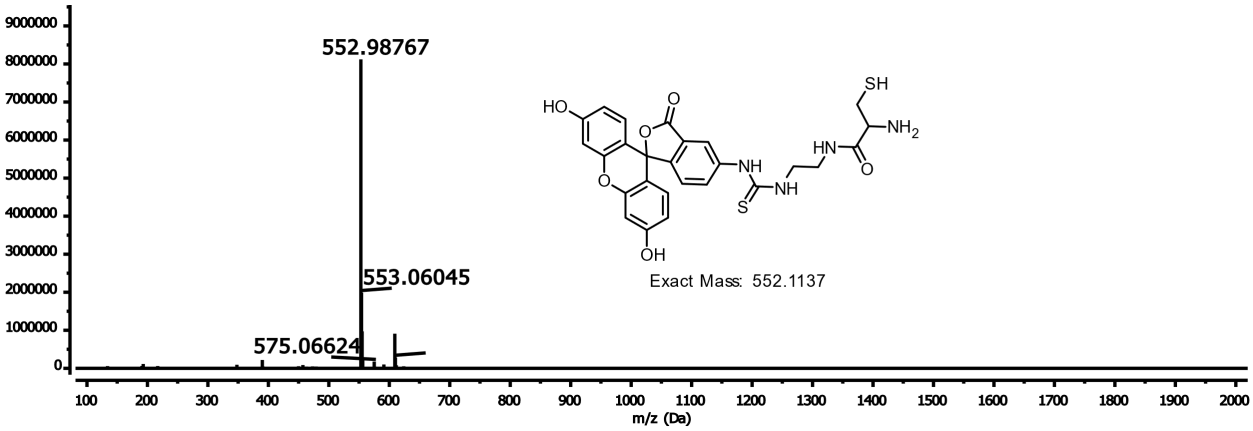
**$^{1}$H of 2-amino-N-(2-(3-(3',6'-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-5-yl)thioureido)ethyl)-3-mercaptopropanamide (compound C, FITC-Cysteine)**



**$^{13}C$ of 2-amino-N-(2-(3-(3',6'-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-5-yl)thioureido)ethyl)-3-mercaptopropanamide (compound C, FITC-Cysteine)**

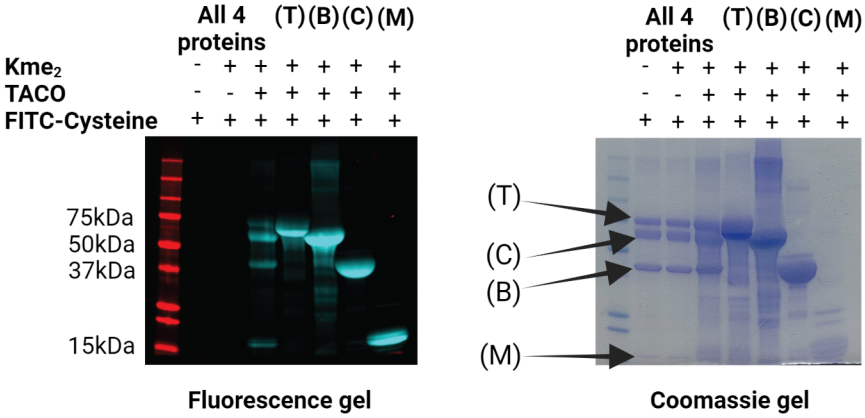



**Mass spectrum of 2-amino-N-(2-(3-(3',6'-dihydroxy-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthen]-5-yl)thioureido)ethyl)-3-mercaptopropanamide (compound C, FITC-Cysteine)**

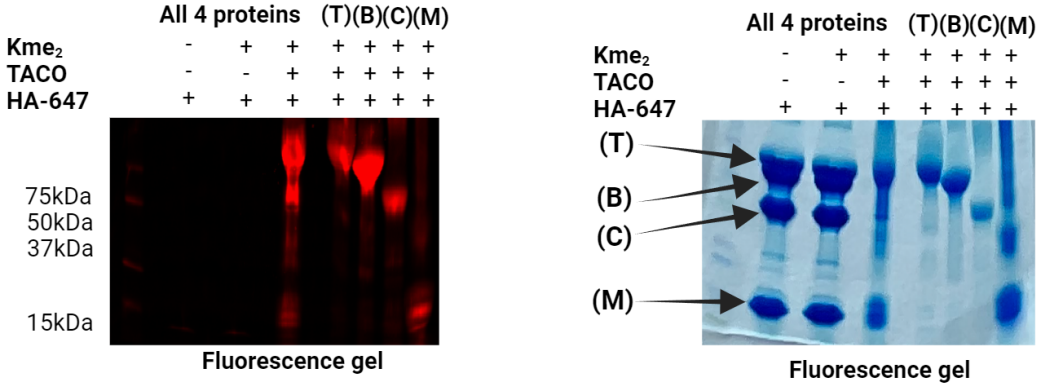



**TACO modification of $Kme_2$ proteins and conjugation with FITC-cysteine:** To 2 mg $Kme_2$ proteins transferrin (T), BSA (B), creatine kinase (C) and myoglobin (M) in 1 mL of NaP buffer pH 7, was added 10 mg of selectfluor and 50 µL of pyridine. The reaction was stirred at room temperature for 30 min and filtered through a molecular weight cutoff to remove small molecules to obtain pure protein. Reaction was filtered with a 3 kDa molecular weight cutoff, and proteins resuspended in 300 µL of PBS buffer. To the sample was added 200µL of 100 µM solution of

FITC-cysteine and incubated for 2 h. Excess fluorophores were washed off through the molecular weight cuttoff. Labelled proteins were then analyzed through in gel fluorescence imaging and coomassie blue staining.

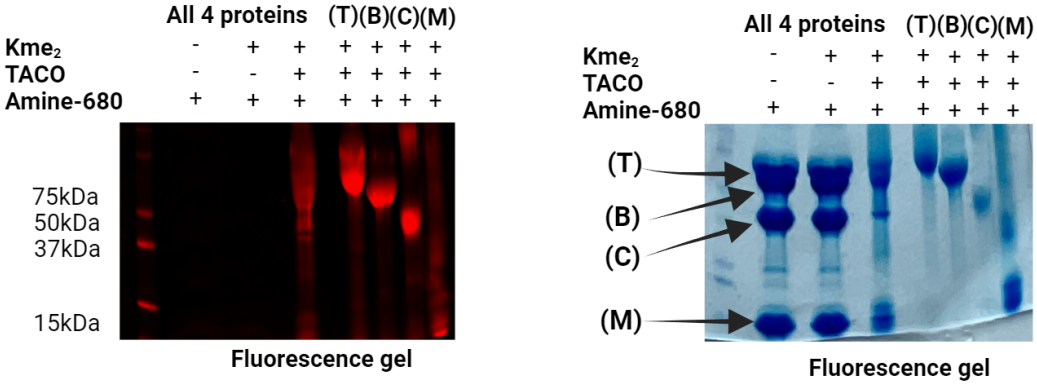



**TACO modification of $Kme_2$ proteins and conjugation with Hydroxylamine-647 dye:** To 2 mg $Kme_2$ proteins transferrin (T), BSA (B), creatine kinase (C) and myoglobin (M) in 1 mL of NaP buffer pH 7, was added 10 mg of selectfluor and 50 µL of pyridine. The reaction was stirred at room temperature for 30 min and filtered through a molecular weight cutoff to remove small molecules to obtain pure protein. Reaction was filtered with a 3 kDa molecular weight cutoff, and proteins resuspended in 300 µL of PBS buffer. To the sample was added 200µL of 100 µM solution of hydroxylamine-647 and incubated for 2 h. Excess fluorophores were washed off through the molecular weight cuttoff. Labelled proteins were then analyzed through in gel fluorescence imaging and coomassie blue staining.




**TACO modification of $Kme_2$ proteins and conjugation with Amine-680 dye:** To 2 mg $Kme_2$ proteins transferrin (T), BSA (B), creatine kinase (C) and myoglobin (M) in 1 mL of NaP buffer pH 7, was added 10 mg of selectfluor and 50 µL of pyridine. The reaction was stirred at room temperature for 30 min and filtered through a molecular weight cutoff to remove small molecules to obtain pure protein. Reaction was filtered with a 3 kDa molecular weight cutoff, and proteins resuspended in 300 µL of PBS buffer. To the sample was added 200µL of 100 µM solution of Amine-680 dye and incubated for 2 h. Excess fluorophores were washed off through the

molecular weight cuttoff. Labelled proteins were then analyzed through in gel fluorescence imaging and coomassie blue staining.




**XXV. Supplementary Figure 19. TACO modification of $Kme_2$ proteins in cell lysates**

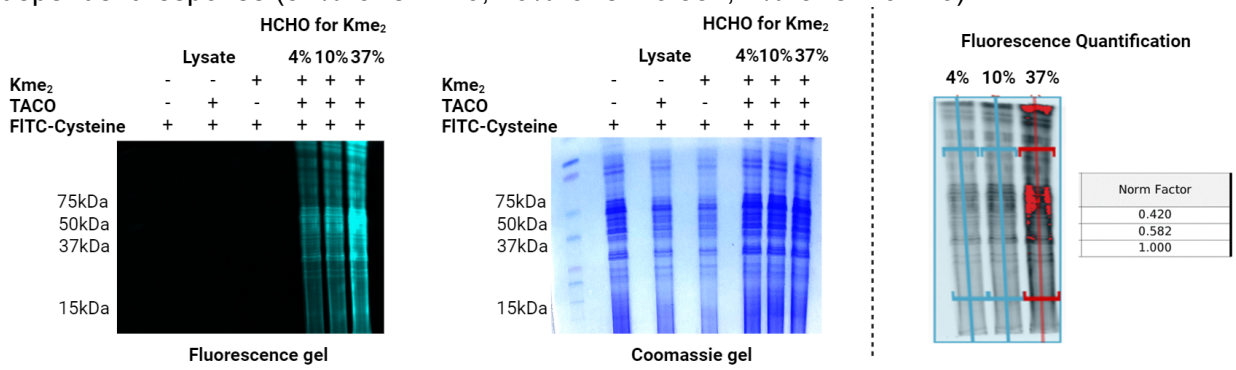
**Dose Dependent labeling of cell lysate with $Kme_2$ (4%):** To 300 µg Lncap cell lysate in 200 µL of NaP buffer (pH 7), was added 50 µL of 4% formaldehyde solution in water. The reaction was vortexed for 2 min, followed by the addition of 50 µL of 600 mM $NaBH_3CN$ solution in water, and vortexed for additional 2 min. The reaction was incubated at room temperature for 16 min, and subjected to acetone precipitated to obtain $Kme_2$ containing proteins. Lysates were resuspended in 500 µL NaP buffer pH 7.

**Dose Dependent labeling of cell lysate with $Kme_2$ (10%):** To 300 µg Lncap cell lysate in 200 µL of NaP buffer (pH 7), was added 50 µL of 10% formaldehyde solution in water. The reaction was vortexed for 2 min, followed by the addition of 50 µL of 600 mM $NaBH_3CN$ solution in water, and vortexed for additional 2 min. The reaction was incubated at room temperature for 16 min, and subjected to acetone precipitated to obtain $Kme_2$ containing proteins. Lysates were resuspended in 500 µL NaP buffer pH 7.

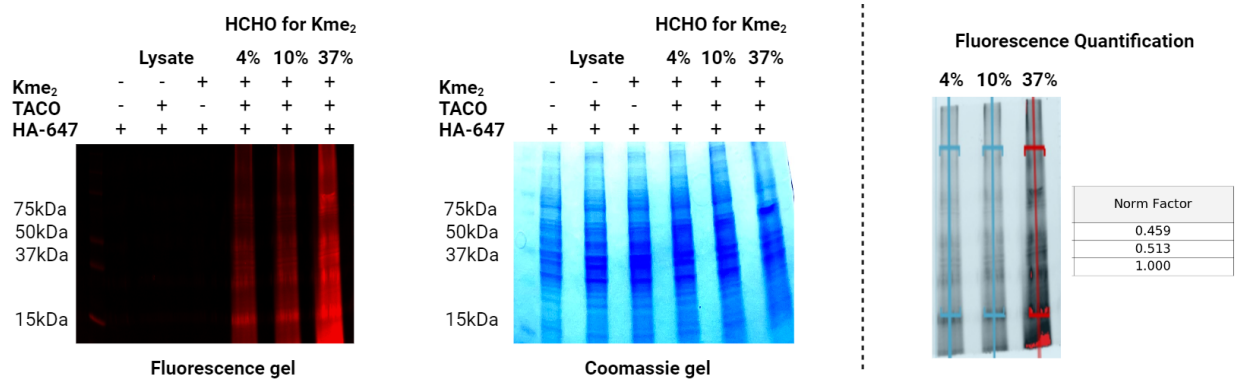
**Dose Dependent labeling of cell lysate with $Kme_2$ (37%):** To 300 µg Lncap cell lysate in 200 µL of NaP buffer (pH 7), was added 50 µL of 37% formaldehyde solution in water. The reaction was vortexed for 2 min, followed by the addition of 50 µL of 600 mM $NaBH_3CN$ solution in water, and vortexed for additional 2 min. The reaction was incubated at room temperature for 16 min, and subjected to acetone precipitated to obtain $Kme_2$ containing proteins. Lysates were resuspended in 500 µL NaP buffer pH 7.

**TACO modification of $Kme_2$ containing lysates and conjugation of FITC-cysteine:** To 300 µg of $Kme_2$-lysate in 500 µL of NaP buffer was added 2 mg of selectfluor and 10 µL of pyridine. The reaction was stirred at room temperature for 30 min. Samples were subjected to acetone precipitation to remove small molecules and obtain pure proteins, dissolved in 200 µL of PBS buffer pH 7.5 and incubated with 200 µL of 15 mM of iodoacetamide in the dark for 30 min. Proteins in cell lysate were acetone precipitated, resuspended in 100 µL PBS buffer. 200 µL of 100 µM solution of FITC-cysteine (compound C) was added and incubated for 2 h. Excess fluorophores were removed by another round of acetone precipitation, followed by analysis of proteins through in gel fluorescence imaging and Coomassie blue staining. Quantification of gel lanes using Invitrogen ibright FL-1500 image processing software clearly showed a dose-

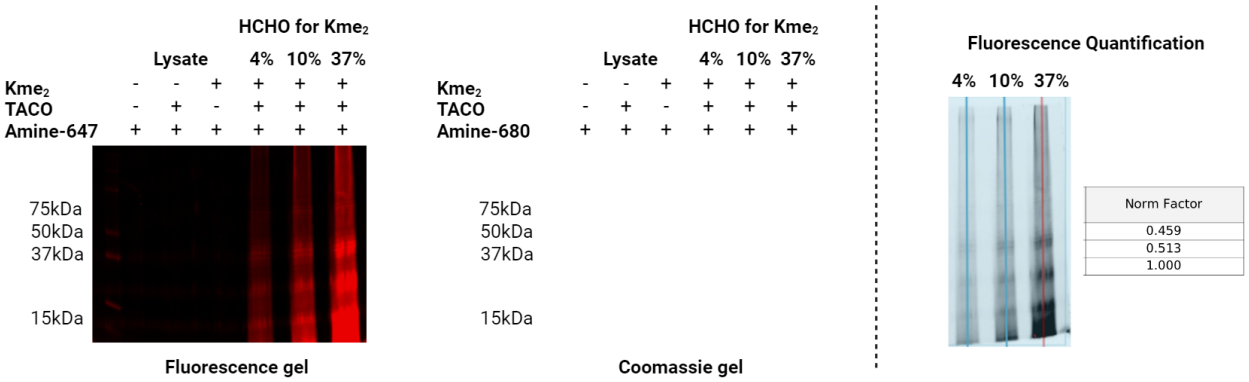
dependent response (37% lane = 1.0, 10% lane = 0.582, 4% lane = 0.420).



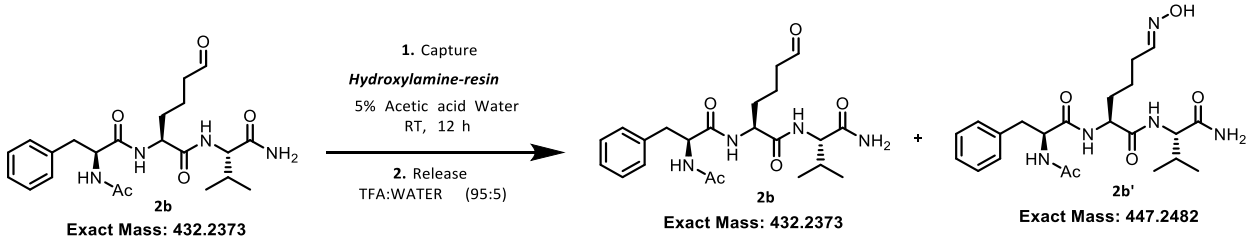

**TACO modification of $Kme_2$ containing lysates and conjugation of Hydroxylamine-647 dye:** To 300 µg of $Kme_2$-lysate in 500 µL of NaP buffer was added 2 mg of selectfluor and 10 µL of pyridine. The reaction was stirred at room temperature for 30 min. Samples were subjected to acetone precipitation to remove small molecules and obtain pure proteins, dissolved in 200 µL of PBS buffer pH 7.5 and incubated with 200 µL of 15 mM of iodoacetamide in the dark for 30 min. Proteins in cell lysate were acetone precipitated, resuspended in 100 µL PBS buffer. 200 µL of 100 µM solution of hydroxylamine-647 dye was added and incubated for 2 h. Excess fluorophores were removed by another round of acetone precipitation, followed by analysis of proteins through in gel fluorescence imaging and Coomassie blue staining. Quantification of gel lanes using Invitrogen ibright FL-1500 image processing software clearly showed a dose-dependent response (37% lane = 1.0, 10% lane = 0.513, 4% lane = 0.459).




**TACO modification of $Kme_2$ containing lysates and conjugation of Amine-680:** To 300 µg of $Kme_2$-lysate in 500 µL of NaP buffer was added 2 mg of selectfluor and 10 µL of pyridine. The reaction was stirred at room temperature for 30 min. Samples were subjected to acetone precipitation to remove small molecules and obtain pure proteins, dissolved in 200 µL of PBS buffer pH 7.5 and incubated with 200 µL of 15 mM of iodoacetamide in the dark for 30 min. Proteins in cell lysate were acetone precipitated, resuspended in 100 µL PBS buffer. 200 µL of 100 µM solution of amine-680 dye was added and incubated for 2 h. Excess fluorophores were removed by another round of acetone precipitation, followed by analysis of proteins through in gel fluorescence imaging and Coomassie blue staining. Quantification of gel lanes using Invitrogen ibright FL-1500 image processing software clearly showed a dose-dependent response (37% lane = 1.0, 10% lane = 0.672, 4% lane = 0.373).

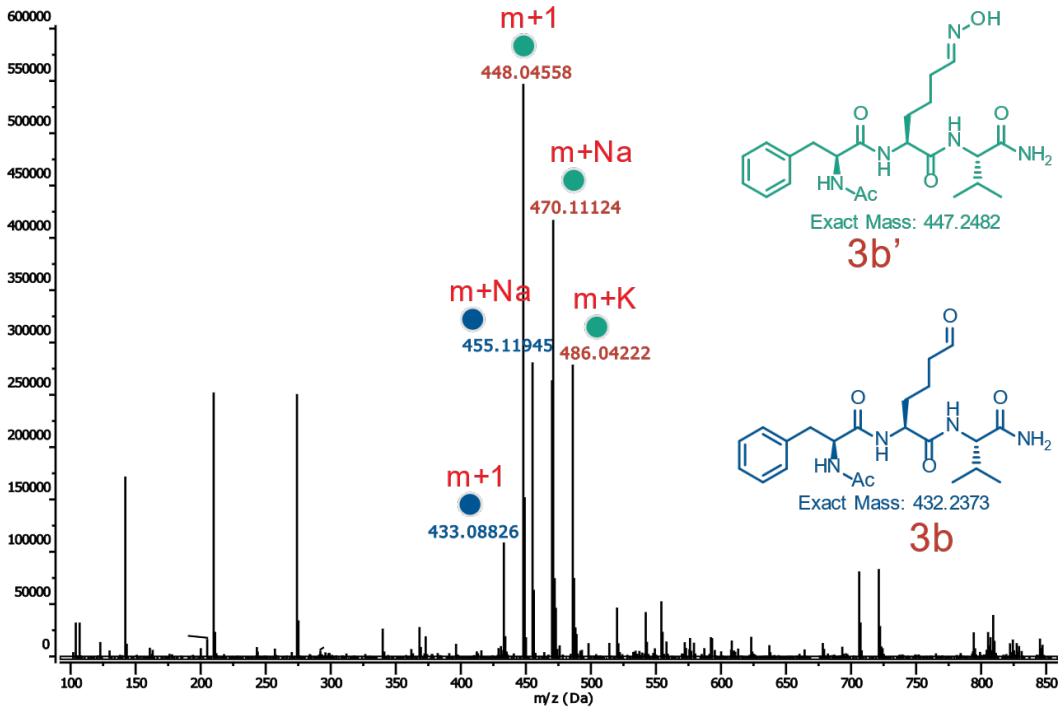



**XXVI. Supplementary Figure 20.** Solid support mediated enrichment of dimethyllysine containing peptide aldehyde OAcFKme$_2$(CHO)V **2b** by using hydroxylamine solid-support.




For optimizing the enrichment of aldehyde peptides, we carried out enrichment with a model peptide. To 5 mg of peptide aldehyde OAcFKme$_2$(CHO)V **2b** in 200 µL of NaP buffer pH 7.0 was added hydroxylamine resin. 5% acetic acid in water (500 µL) was added to the resin and reaction was incubated for 12 h. The resin was filtered and washed with methanol (3x), and DMF (3x). Resin was dried and cleaved using 95:5 (TFA:Water) for 2 h at room temperature to release the enriched peptides **2b** and oxime-modified **2b'**. Eluate was concentrated and analyzed using LC-MS.

**MS-Trace after the release of peptide aldehyde 2b from resin**




**XXVII. Supplementary Figure 21.** Solid support mediated enrichment of dimethyllysine containing proteins from nucleosomes obtained from prostate cancer cell lysate.

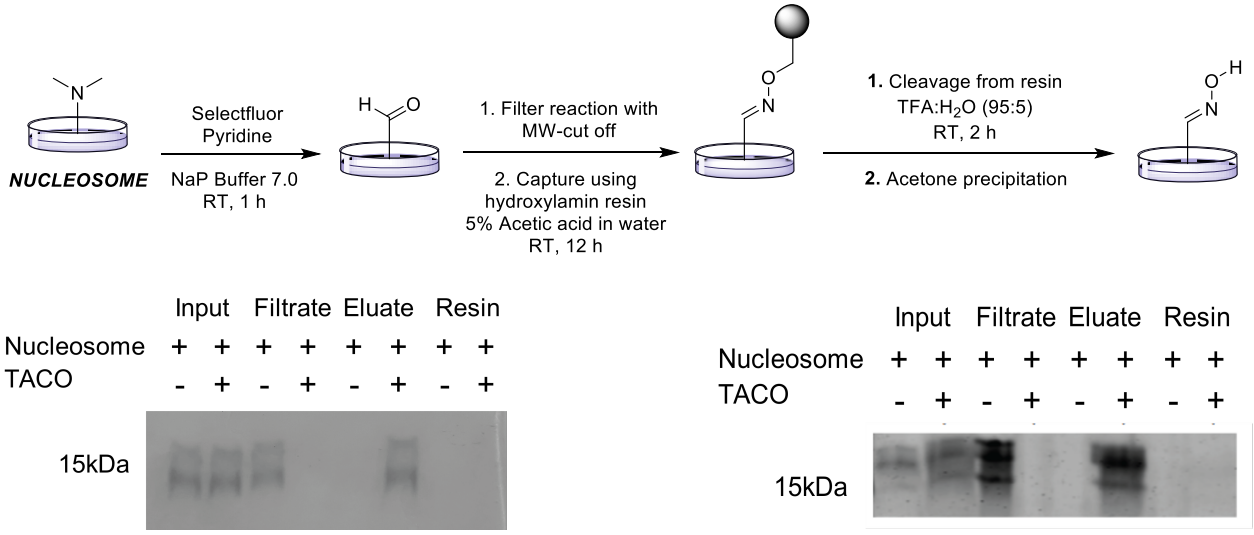
**Cell culture and drugs.** Cells were maintained at 37 °C and 5% $CO_2$. LNCaP cells were cultured in RPMI supplemented with 10% (V/V) fetal bovine serum (FBS) and 1% (V/V) penicillin/streptomycin (100 µg/mL).

**Cell lysis and western blotting.** Whole cell lysate was generated by lysing cells on ice in RIPA buffer (50 mM TrisHCl [pH 8], 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with protease and phosphatase inhibitors. Lysates were centrifuged 6,500 x g, 10 m at 4°C, and soluble lysate was collected. Whole cell lysate proteins were separated using 16% SDS-PAGE. SDS-PAGE gels were stained with coomassie brilliant blue dye and visualized (Odyssey, Li-Cor). Primary antibodies used were as follows: H3 (Abcam).

**Nucleosome isolation.** Purified nucleosomes were generated by resuspending pelleted cells in lysis buffer (20 mM HEPES [pH 7.5], 0.25 M sucrose, 3 mM $MgCl_2$, 0.5% V/V NP-40, supplemented with protease inhibitors) and Dounce homogenization prior to centrifugation at 3,000 x g, 15 m. Nuclei were subsequently washed twice with lysis buffer, once with Buffer B (20 mM HEPES [pH 7.5], 3 mM $MgCl_2$, 0.2 mM EDTA), once with 0.6 M KCl + 10% glycerol in Buffer B, and pelleted 5,000 x g. Nuclei were then washed in MSB (20 mM HEPES [pH 7.5], 0.4 M NaCl, 1 mM EDTA, 5% V/V glycerol), centrifuged 10,000 x g, 10 m, and resuspended in HSB (20 mM HEPES [pH 7.5], 0.65 M NaCl, 1 mM EDTA, 0.34 M sucrose, supplemented with protease inhibitors) and Dounce homogenized to release oligonucleosome fragments.

Oligonucleosomes were then centrifuged 10,000 x g, 20 m and supernatant dialyzed o/n against LSB (20 mM HEPES [pH 7.5], 0.1 M NaCl, 1 mM EDTA,) with 6-8 kDa MWCO membranes. The next day, dialysate was collected and $CaCl_2$ was added to a final concentration of 3 mM and incubated at 37 $^{o}$C for 5 m, after which micrococcal nuclease (Sigma) was added to a final concentration of 10 U/mL and incubated for 10 m at 37 $^{o}$C. Digestion was stopped with 0.1 volumes of 0.5 M EDTA on ice, and NaCl was added to a final concentration of 0.6 M. Mononucleosomes were separated via ultracentrifugation by layering 1 mL of material onto equilibrated linear gradients in 1 x 3.5-inch polyallomer centrifuge tubes (Beckman) with 1 mL each of HSB + 10% V/V glycerol, HSB + 20% V/V glycerol, HSB + 30% V/V glycerol, HSB + 40% V/V glycerol, from top to bottom. Samples were centrifuged 100,000 x g, 16 h, in an ultracentrifuge equipped with a SW-55Ti rotor (Beckman). 500 µL fractions were harvested from top to bottom and nucleosomes were assessed via 16% SDS PAGE followed by coomassie stain. Fractions containing nucleosomes were pooled for subsequent experimentation.

**TACO modification and enrichment of modified nucleosome.** To 100 µg of prostate cancer cell nucleosome extract in 200 µL NaP buffer (pH 7), was added 2 mg of selectfluor and 10 µL of pyridine. The reaction mixture was stirred for 1 h. Reaction mixture was filtered using a 3 kDa molecular weight cut off to remove small molecules. To rest of the solution was added hydroxylamine solid support resin, 5% acetic acid in water (500 µL) and reaction incubated for 12 h. The resin was filtered and washed with methanol (3x), and DMF (3x). Resin was dried and cleaved using 95:5 (TFA: Water) to release the enriched nucleosomes. Eluate was concentrated followed by acetone precipitation. The eluate and filtrate were analyzed using SDS PAGE. This reaction was performed in duplicates.




**XXVIII. Supplementary Figure 22.** TACO modification of nuclear extract and proteomics analysis.

**Nuclear extract isolation.** Nuclear extracts were generated using a modified protocol of Active Motif Nuclear Extract Kit. Pelleted cells were resuspended in hypotonic lysis buffer (20 mM TrisHCl [pH 7.5], 150 mM NaCl, 5 mM $MgCl_2$, 1% V/V NP-40, supplemented with protease inhibitors) and incubated on ice for 15 min. 50 µL of detergent was added and vortexed for 10 seconds. Samples were dounce homogenized prior to centrifugation at 14,000 x g (5415 rotor)

for 1 m. Cytoplasmic extracts (supernatant) were transferred and nuclear extract (pellet) resuspended in complete lysis buffer and 15 µL of detergent. Sample was dounce homogenized and vortexed for 10 seconds and incubated on ice for 30 m, followed by centrifugation at 14,000 x g (12200 rpm for 5415 rotor) for 10 m to obtain the nuclear extract (supernatant).

**TACO modification and propargylation of nuclear extract:** To 500 µg of nuclear extract in 500 µL of NaP buffer pH 7 was added 5 mg of selectfluor and 20 µL of pyridine. The reaction was stirred at room temperature for 1 h. Samples were subjected to acetone precipitation to remove small molecules to obtain pure proteins. To proteins resuspended in 500 µL of NaP buffer pH 5.0 was added 5 µL of propargylamine and stirred for 1 h, followed by the addition of 5 mg sodium cyanoborohydride. Reaction was stirred for 5 h followed by acetone precipitation.

**Proteomics analysis of proteins:** Proteins were digested using Thermo scientific EasyPep Mini MS sample Prep Kit. Digested peptides were analyzed by LC/MS. Samples were analyzed by LC-MS using a Q-Exactive Plus orbitrap mass spectrometer equipped with Dionex UltiMate 3000 LC system (Thermo). Briefly, lyophilized digested peptides were resuspended in 0.1% formic acid in 10% acetonitrile and loaded onto a trap column (PepMap™ NEO 5 µm C18 300 µm X 5 mm Trap Cartridge) and resolved through a custom analytical column packed with ReproSil-Pur 120 C18-AQ 3 µm beads (Dr. Maisch GmbH) at a flow rate of 0.3 µL/min with a gradient solvent A (0.1% formic acid in 2% acetonitrile) and a gradient solvent B (0.1% formic acid in 80% acetonitrile) for 150 minutes. MS analysis was conducted in a data-dependent manner with full scans in the range from 400 to 1800 m/z using an Orbitrap mass analyzer set as follows: MS1: resolution = 70,000, AGC target = 3e6, Max IT = 100ms; MS2: resolution = 17,500, AGC target 1e5, Max IT = 50ms. The top fifteen most intense precursor ions were selected for MS2 with an isolation window of 4 m/z. Isolated precursors were fragmented by high energy collisional dissociation (HCD) with normalized collision energy (NCE) of 27. LC-MS RAW files were searched against the human proteome sequence database from UniProt using the Sequest HT search engine embedded in Proteome Discoverer 3.0 (Thermo) with 10 ppm MS1 precursor mass tolerance, 0.02 Da MS2 fragment mass tolerance, 0.01 false discovery rate. Search included the following modifications: Methionine oxidation (+15.99492 Da), asaparagine and glutamine deamidation (+0.98402 Da) and protein N-terminal acetylation (+42.03670), histidine fluorination (+17.9906), tryptophan fluorination (+33.9855), lysine propargylation (+38.0157), and lysine-to-aldehyde (-1.0316) were variable modifications (up to 3 allowed per peptide); cysteine was assigned a fixed carbamidomethyl modification (+57.021465 Da).

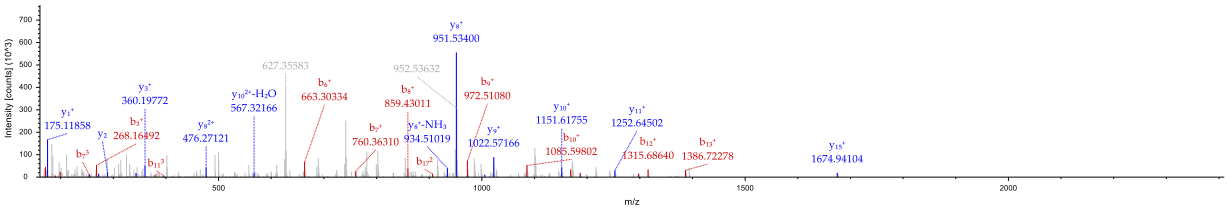
**Representative peptide spectra of modified peptides (propargyl and aldehyde sites, +38 on lysine)**

**+TACO modified peptides: <u>All these peptides were identified after the TACO chemistry on the nuclear extract obtained from LnCap</u>.**

A5A3E0 (POTE ankyrin domain family member F)

Sequence: VAPEEHPVLLTEATLNPKANR, K18-Ben_K1 (38.01570 Da) (position: lysine 813)

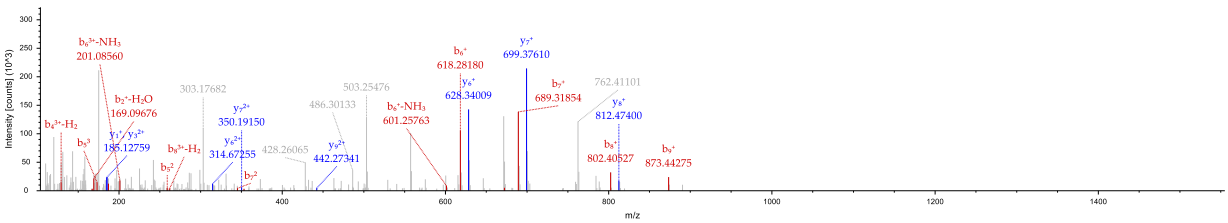
| #1 | b+ | b2+ | b3+ | Seq. | y+ | y2+ | y3+ | #2 |
|---|---|---|---|---|---|---|---|---|
| 1 | 100.07569 | 50.54148 | 34.03008 | V | | | | 21 |
| 2 | 171.11280 | 86.06004 | 57.70912 | A | 2238.187... | 1119.597... | 746.73410 | 20 |
| 3 | 268.16557 | 134.58642 | 90.06004 | P | 2167.150... | 1084.078... | 723.05507 | 19 |
| 4 | 397.20816 | 199.10772 | 133.07424 | E | 2070.097... | 1035.552... | 690.70414 | 18 |
| 5 | 526.25075 | 263.62902 | 176.08844 | E | 1941.055... | 971.03128 | 647.68995 | 17 |
| 6 | 663.30967 | 332.15847 | 221.77474 | H | 1812.012... | 906.50998 | 604.67575 | 16 |
| 7 | 760.36243 | 380.68485 | 254.12566 | P | 1674.953... | 837.98053 | 558.98944 | 15 |
| 8 | 859.43084 | 430.21906 | 287.14847 | V | 1577.901... | 789.45415 | 526.63852 | 14 |
| 9 | 972.51491 | 486.76109 | 324.84315 | L | 1478.832... | 739.91994 | 493.61572 | 13 |
| 10 | 1085.598... | 543.30312 | 362.53784 | L | 1365.748... | 683.37791 | 455.92103 | 12 |
| 11 | 1186.646... | 593.82696 | 396.22040 | T | 1252.664... | 626.83588 | 418.22634 | 11 |
| 12 | 1315.689... | 658.34826 | 439.23460 | E | 1151.616... | 576.31204 | 384.54378 | 10 |
| 13 | 1386.726... | 693.86682 | 462.91364 | A | 1022.574... | 511.79074 | 341.52959 | 9 |
| 14 | 1487.774... | 744.39066 | 496.59620 | T | 951.53709 | 476.27218 | 317.85055 | 8 |
| 15 | 1600.858... | 800.93269 | 534.29088 | L | 850.48941 | 425.74834 | 284.16799 | 7 |
| 16 | 1714.901... | 857.95415 | 572.30519 | N | 737.40535 | 369.20631 | 246.47330 | 6 |
| 17 | 1811.953... | 906.48053 | 604.65611 | P | 623.36242 | 312.18485 | 208.45899 | 5 |
| 18 | 1978.064... | 989.53586 | 660.02634 | K-Ben... | 526.30966 | 263.65847 | 176.10807 | 4 |
| 19 | 2049.101... | 1025.054... | 683.70537 | A | 360.19899 | 180.60314 | 120.73785 | 3 |
| 20 | 2163.144... | 1082.075... | 721.71968 | N | 289.16188 | 145.08458 | 97.05881 | 2 |
| 21 | | | | R | 175.11895 | 88.06311 | 59.04450 | 1 |




A0A1W2PQ09 (TATA-box-binding protein-associated factor 11-like protein 11)

Sequence: SVSENTAIAMAGIAK, K15-Ben_K1 (38.01570 Da) (position: lysine 147)

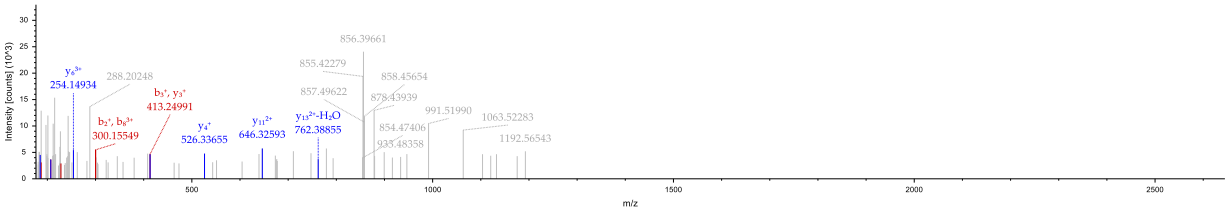
| #1 | b+ | b2+ | b3+ | Seq. | y+ | y2+ | y3+ | #2 |
|---|---|---|---|---|---|---|---|---|
| 1 | 88.03930 | 44.52329 | 30.01795 | S | | | | 15 |
| 2 | 187.10772 | 94.05750 | 63.04076 | V | 1413.740... | 707.37398 | 471.91841 | 14 |
| 3 | 274.13975 | 137.57351 | 92.05143 | S | 1314.672... | 657.83977 | 438.89561 | 13 |
| 4 | 403.18234 | 202.09481 | 135.06563 | E | 1227.640... | 614.32376 | 409.88493 | 12 |
| 5 | 517.22527 | 259.11627 | 173.07994 | N | 1098.597... | 549.80246 | 366.87073 | 11 |
| 6 | 618.27295 | 309.64011 | 206.76250 | T | 984.55471 | 492.78100 | 328.85642 | 10 |
| 7 | 689.31006 | 345.15867 | 230.44154 | A | 883.50704 | 442.25716 | 295.17386 | 9 |
| 8 | 802.39412 | 401.70070 | 268.13623 | I | 812.46992 | 406.73860 | 271.49482 | 8 |
| 9 | 873.43124 | 437.21926 | 291.81526 | A | 699.38586 | 350.19657 | 233.80014 | 7 |
| 10 | 1004.471... | 502.73950 | 335.49543 | M | 628.34874 | 314.67801 | 210.12110 | 6 |
| 11 | 1075.508... | 538.25806 | 359.17446 | A | 497.30826 | 249.15777 | 166.44094 | 5 |
| 12 | 1132.530... | 566.76879 | 378.18162 | G | 426.27115 | 213.63921 | 142.76190 | 4 |
| 13 | 1245.614... | 623.31082 | 415.87631 | I | 369.24968 | 185.12848 | 123.75475 | 3 |
| 14 | 1316.651... | 658.82938 | 439.55534 | A | 256.16562 | 128.58645 | 86.06006 | 2 |
| 15 | | | | K-Ben... | 185.12850 | 93.06789 | 62.38102 | 1 |



P12259 (Coagulation factor V)

Sequence: WIISSLTPKHLQAGMQAYIDIK, M15-Ben_Met2 (15.99490 Da), K22-Ben_K1 (38.01570 Da) (position: lysine 327)

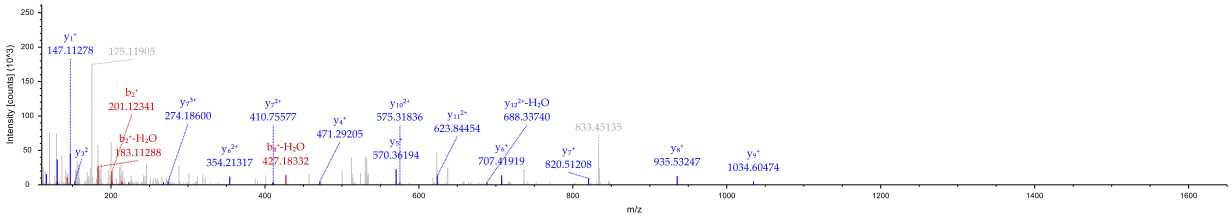
| #1 | b+ | b2+ | b3+ | Seq. | y+ | y2+ | y3+ | #2 |
|---|---|---|---|---|---|---|---|---|
| 1 | 187.08659 | 94.04693 | 63.03371 | W | | | | 22 |
| 2 | 300.17065 | 150.58897 | 100.72840 | I | 2381.289... | 1191.148... | 794.43477 | 21 |
| 3 | 413.25472 | 207.13100 | 138.42309 | I | 2268.205... | 1134.606... | 756.74008 | 20 |
| 4 | 500.28675 | 250.64701 | 167.43377 | S | 2155.121... | 1078.064... | 719.04540 | 19 |
| 5 | 587.31877 | 294.16303 | 196.44444 | S | 2068.089... | 1034.548... | 690.03472 | 18 |
| 6 | 700.40284 | 350.70506 | 234.13913 | L | 1981.057... | 991.03243 | 661.02404 | 17 |
| 7 | 801.45052 | 401.22890 | 267.82169 | T | 1867.973... | 934.49040 | 623.32936 | 16 |
| 8 | 898.50328 | 449.75528 | 300.17261 | P | 1766.925... | 883.96656 | 589.64680 | 15 |
| 9 | 1026.598... | 513.80276 | 342.87093 | K | 1669.873... | 835.44017 | 557.29588 | 14 |
| 10 | 1163.657... | 582.33222 | 388.55724 | H | 1541.778... | 771.39269 | 514.59755 | 13 |
| 11 | 1276.741... | 638.87425 | 426.25192 | L | 1404.719... | 702.86324 | 468.91125 | 12 |
| 12 | 1404.799... | 702.90354 | 468.93812 | Q | 1291.635... | 646.32121 | 431.21656 | 11 |
| 13 | 1475.836... | 738.42209 | 492.61715 | A | 1163.576... | 582.29192 | 388.53037 | 10 |
| 14 | 1532.858... | 766.93283 | 511.62431 | G | 1092.539... | 546.77336 | 364.85133 | 9 |
| 15 | 1679.893... | 840.45052 | 560.63610 | M-Ben... | 1035.517... | 518.26263 | 345.84418 | 8 |
| 16 | 1807.952... | 904.47981 | 603.32230 | Q | 888.48259 | 444.74494 | 296.83238 | 7 |
| 17 | 1878.989... | 939.99836 | 627.00133 | A | 760.42402 | 380.71565 | 254.14619 | 6 |
| 18 | 2042.052... | 1021.530... | 681.35578 | Y | 689.38690 | 345.19709 | 230.46715 | 5 |
| 19 | 2155.136... | 1078.072... | 719.05047 | I | 526.32358 | 263.66543 | 176.11271 | 4 |
| 20 | 2270.163... | 1135.585... | 757.39278 | D | 413.23951 | 207.12339 | 138.41802 | 3 |
| 21 | 2383.247... | 1192.127... | 795.08747 | I | 298.21257 | 149.60992 | 100.07571 | 2 |
| 22 | | | | K-Ben... | 185.12850 | 93.06789 | 62.38102 | 1 |




Q09666 (Neuroblast differentiation-associated protein AHNAK)

Sequence: ISMPDVDLHVKGTK, M3-Ben_Met2 (15.99490 Da), K11-Ben_K1 (38.01570 Da) (position: lysine 707)

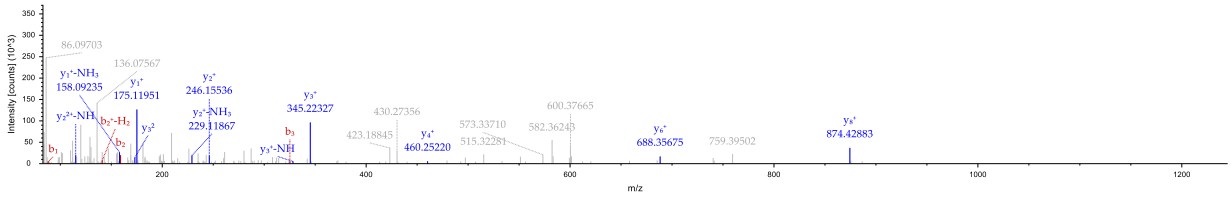
| #1 | b+ | b2+ | b3+ | Seq. | y+ | y2+ | y3+ | #2 |
|---|---|---|---|---|---|---|---|---|
| 1 | 114.09134 | 57.54931 | 38.70196 | I | | | | 14 |
| 2 | 201.12337 | 101.06532 | 67.71264 | S | 1480.746... | 740.87688 | 494.25368 | 13 |
| 3 | 348.15875 | 174.58302 | 116.72444 | M-Ben... | 1393.714... | 697.36086 | 465.24300 | 12 |
| 4 | 445.21152 | 223.10940 | 149.07536 | P | 1246.679... | 623.84317 | 416.23121 | 11 |
| 5 | 560.23846 | 280.62287 | 187.41767 | D | 1149.626... | 575.31679 | 383.88028 | 10 |
| 6 | 659.30687 | 330.15708 | 220.44048 | V | 1034.599... | 517.80332 | 345.53797 | 9 |
| 7 | 774.33382 | 387.67055 | 258.78279 | D | 935.53094 | 468.26911 | 312.51517 | 8 |
| 8 | 887.41788 | 444.21258 | 296.47748 | L | 820.50400 | 410.75564 | 274.17285 | 7 |
| 9 | 1024.476... | 512.74203 | 342.16378 | H | 707.41993 | 354.21361 | 236.47816 | 6 |
| 10 | 1123.545... | 562.27624 | 375.18659 | V | 570.36102 | 285.68415 | 190.79186 | 5 |
| 11 | 1289.655... | 645.33157 | 430.55681 | K-Ben... | 471.29261 | 236.14994 | 157.76905 | 4 |
| 12 | 1346.677... | 673.84230 | 449.56396 | G | 305.18195 | 153.09461 | 102.39883 | 3 |
| 13 | 1447.725... | 724.36614 | 483.24652 | T | 248.16048 | 124.58388 | 83.39168 | 2 |
| 14 | | | | K | 147.11280 | 74.06004 | 49.70912 | 1 |




O94760 (N(G),N(G)-dimethylarginine dimethylaminohydrolase 1)

Sequence: SAKGEEVDVAR, K3-Ben_K1 (38.01570 Da) (position: lysine 34)

| #1 | b+ | b2+ | Seq. | y+ | y2+ | #2 |
|---|---|---|---|---|---|---|
| 1 | 88.03930 | 44.52329 | S | | | 11 |
| 2 | 159.07642 | 80.04185 | A | 1111.574... | 556.29077 | 10 |
| 3 | 325.18708 | 163.09718 | K-Ben... | 1040.537... | 520.77221 | 9 |
| 4 | 382.20855 | 191.60791 | G | 874.42649 | 437.71688 | 8 |
| 5 | 511.25114 | 256.12921 | E | 817.40502 | 409.20615 | 7 |
| 6 | 640.29373 | 320.65050 | E | 688.36243 | 344.68485 | 6 |
| 7 | 739.36215 | 370.18471 | V | 559.31984 | 280.16356 | 5 |
| 8 | 854.38909 | 427.69818 | D | 460.25142 | 230.62935 | 4 |
| 9 | 953.45750 | 477.23239 | V | 345.22448 | 173.11588 | 3 |
| 10 | 1024.494... | 512.75095 | A | 246.15607 | 123.58167 | 2 |
| 11 | | | R | 175.11895 | 88.06311 | 1 |

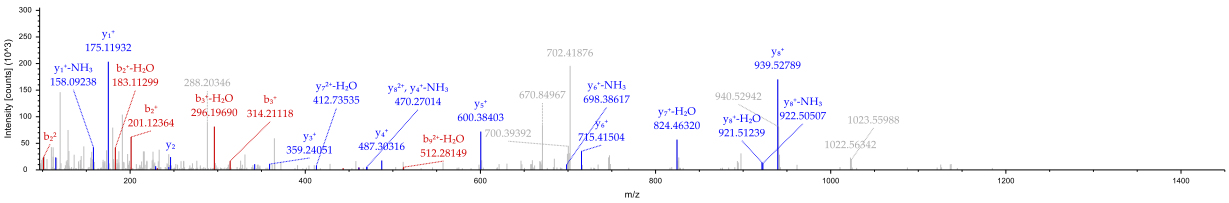


**Representative peptide spectra of modified peptides (aldehydes on lysine, -1 on lysine) +TACO modified peptides:**

**P68431** (Histone H3.1)

Sequence: VTIMPKDIQLAR, M4-Ben_Met2 (15.99490 Da), K6-Ben_K2 (-1.03160 Da) (position: lysine 123)

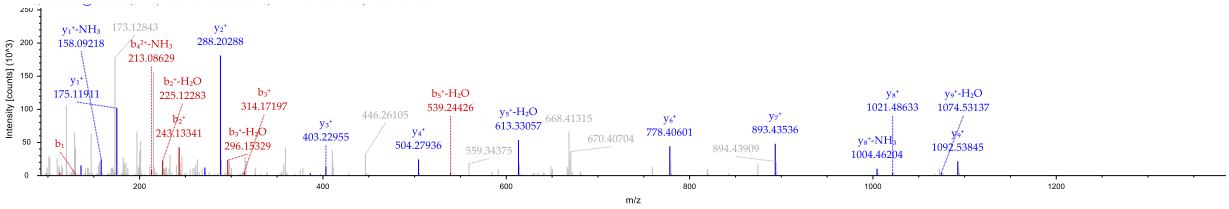
| #1 | b+ | b2+ | Seq. | y+ | y2+ | #2 |
|---|---|---|---|---|---|---|
| 1 | 100.07569 | 50.54148 | V | | | 12 |
| 2 | 201.12337 | 101.06532 | T | 1300.692... | 650.85012 | 11 |
| 3 | 314.20743 | 157.60735 | I | 1199.645... | 600.32628 | 10 |
| 4 | 461.24282 | 231.12505 | M-Ben... | 1086.561... | 543.78425 | 9 |
| 5 | 558.29558 | 279.65143 | P | 939.52584 | 470.26656 | 8 |
| 6 | 685.35894 | 343.18311 | K-Ben... | 842.47308 | 421.74018 | 7 |
| 7 | 800.38589 | 400.69658 | D | 715.40971 | 358.20850 | 6 |
| 8 | 913.46995 | 457.23861 | I | 600.38277 | 300.69502 | 5 |
| 9 | 1041.528... | 521.26790 | Q | 487.29871 | 244.15299 | 4 |
| 10 | 1154.612... | 577.80993 | L | 359.24013 | 180.12370 | 3 |
| 11 | 1225.649... | 613.32849 | A | 246.15607 | 123.58167 | 2 |
| 12 | | | R | 175.11895 | 88.06311 | 1 |




**P68431** (Histone H3.1)

Sequence: EIAQDFKTDLR, K7-Ben_K2 (-1.03160 Da) (position: lysine 80)

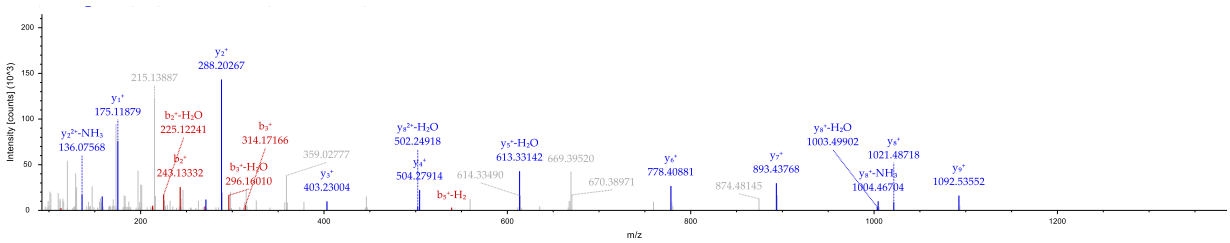
| #1 | b+ | b2+ | Seq. | y+ | y2+ | #2 |
|---|---|---|---|---|---|---|
| 1 | 130.04987 | 65.52857 | E | | | 11 |
| 2 | 243.13393 | 122.07061 | I | 1205.616... | 603.31169 | 10 |
| 3 | 314.17105 | 157.58916 | A | 1092.532... | 546.76966 | 9 |
| 4 | 442.22962 | 221.61845 | Q | 1021.494... | 511.25111 | 8 |
| 5 | 557.25657 | 279.13192 | D | 893.43636 | 447.22182 | 7 |
| 6 | 704.32498 | 352.66613 | F | 778.40941 | 389.70835 | 6 |
| 7 | 831.38834 | 416.19781 | K-Ben... | 631.34100 | 316.17414 | 5 |
| 8 | 932.43602 | 466.72165 | T | 504.27764 | 252.64246 | 4 |
| 9 | 1047.462... | 524.23512 | D | 403.22996 | 202.11862 | 3 |
| 10 | 1160.547... | 580.77715 | L | 288.20302 | 144.60515 | 2 |
| 11 | | | R | 175.11895 | 88.06311 | 1 |



**Q71DI3** (Histone 3.2)

Sequence: EIAQDFKTDLR, K7-Ben_K2 (-1.03160 Da) (position: lysine 80)

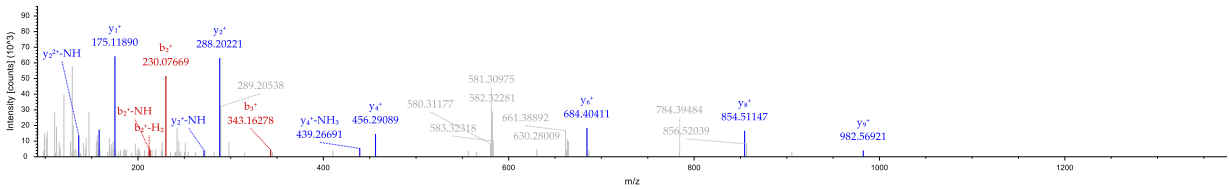
| #1 | b+ | b2+ | Seq. | y+ | y2+ | #2 |
|---|---|---|---|---|---|---|
| 1 | 130.04987 | 65.52857 | E | | | 11 |
| 2 | 243.13393 | 122.07061 | I | 1205.616... | 603.31169 | 10 |
| 3 | 314.17105 | 157.58916 | A | 1092.532... | 546.76966 | 9 |
| 4 | 442.22962 | 221.61845 | Q | 1021.494... | 511.25111 | 8 |
| 5 | 557.25657 | 279.13192 | D | 893.43636 | 447.22182 | 7 |
| 6 | 704.32498 | 352.66613 | F | 778.40941 | 389.70835 | 6 |
| 7 | 831.38834 | 416.19781 | K-Ben... | 631.34100 | 316.17414 | 5 |
| 8 | 932.43602 | 466.72165 | T | 504.27764 | 252.64246 | 4 |
| 9 | 1047.462... | 524.23512 | D | 403.22996 | 202.11862 | 3 |
| 10 | 1160.547... | 580.77715 | L | 288.20302 | 144.60515 | 2 |
| 11 | | | R | 175.11895 | 88.06311 | 1 |



**P62805** (Histone H4)

Sequence: DNIQGITKPAIR, K8-Ben_K2 (-1.03160 Da) (position: lysine 32)

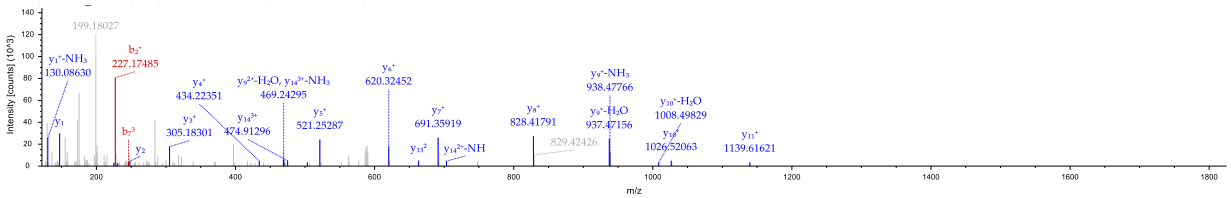
| #1 | b+ | b2+ | Seq. | y+ | y2+ | #2 |
|---|---|---|---|---|---|---|
| 1 | 116.03422 | 58.52075 | D | | | 12 |
| 2 | 230.07715 | 115.54221 | N | 1209.695... | 605.35115 | 11 |
| 3 | 343.16121 | 172.08424 | I | 1095.652... | 548.32969 | 10 |
| 4 | 471.21979 | 236.11353 | Q | 982.56804 | 491.78766 | 9 |
| 5 | 528.24125 | 264.62426 | G | 854.50946 | 427.75837 | 8 |
| 6 | 641.32532 | 321.16630 | I | 797.48800 | 399.24764 | 7 |
| 7 | 742.37299 | 371.69014 | T | 684.40394 | 342.70561 | 6 |
| 8 | 869.43636 | 435.22182 | K-Ben... | 583.35626 | 292.18177 | 5 |
| 9 | 966.48912 | 483.74820 | P | 456.29289 | 228.65009 | 4 |
| 10 | 1037.526... | 519.26676 | A | 359.24013 | 180.12370 | 3 |
| 11 | 1150.610... | 575.80879 | I | 288.20302 | 144.60515 | 2 |
| 12 | | | R | 175.11895 | 88.06311 | 1 |



**P68431** (Histone H2B type 1-K)

Sequence: LLLPGELAKHAVSEGTK, K9-Ben_K2 (-1.03160 Da) (position: lysine 109)

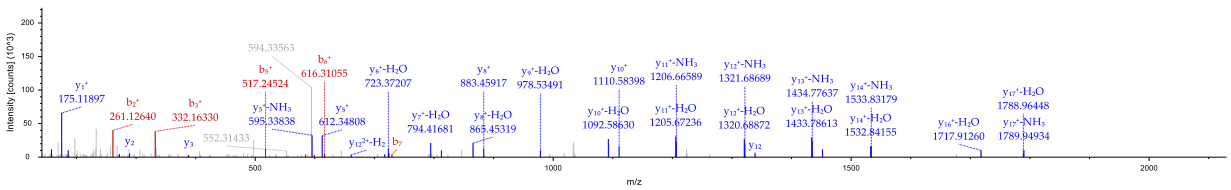
| #1 | b+ | b2+ | b3+ | Seq. | y+ | y2+ | y3+ | #2 |
|---|---|---|---|---|---|---|---|---|
| 1 | 114.09134 | 57.54931 | 38.70196 | L | | | | 17 |
| 2 | 227.17540 | 114.09134 | 76.39665 | L | 1648.890... | 824.94889 | 550.30168 | 16 |
| 3 | 340.25947 | 170.63337 | 114.09134 | L | 1535.806... | 768.40685 | 512.60700 | 15 |
| 4 | 437.31223 | 219.15975 | 146.44226 | P | 1422.722... | 711.86482 | 474.91231 | 14 |
| 5 | 494.33370 | 247.67049 | 165.44942 | G | 1325.669... | 663.33844 | 442.56139 | 13 |
| 6 | 623.37629 | 312.19178 | 208.46361 | E | 1268.648... | 634.82771 | 423.55423 | 12 |
| 7 | 736.46035 | 368.73381 | 246.15830 | L | 1139.605... | 570.30641 | 380.54003 | 11 |
| 8 | 807.49747 | 404.25237 | 269.83734 | A | 1026.521... | 513.76438 | 342.84535 | 10 |
| 9 | 934.56083 | 467.78405 | 312.19179 | K-Ben... | 955.48437 | 478.24582 | 319.16631 | 9 |
| 10 | 1071.619... | 536.31351 | 357.87810 | H | 828.42101 | 414.71414 | 276.81185 | 8 |
| 11 | 1142.656... | 571.83207 | 381.55714 | A | 691.36210 | 346.18469 | 231.12555 | 7 |
| 12 | 1241.725... | 621.36627 | 414.57994 | V | 620.32498 | 310.66613 | 207.44651 | 6 |
| 13 | 1328.757... | 664.88229 | 443.59062 | S | 521.25657 | 261.13192 | 174.42371 | 5 |
| 14 | 1457.799... | 729.40358 | 486.60481 | E | 434.22454 | 217.61591 | 145.41303 | 4 |
| 15 | 1514.821... | 757.91432 | 505.61197 | G | 305.18195 | 153.09461 | 102.39883 | 3 |
| 16 | 1615.869... | 808.43815 | 539.29453 | T | 248.16048 | 124.58388 | 83.39168 | 2 |
| 17 | | | | K | 147.11280 | 74.06004 | 49.70912 | 1 |




**P07910** (Heterogeneous nuclear ribonucleoproteins C1/C2)

Sequence: MIAGQVLDINLAAEPKVNR, M1-Ben_Met2 (15.99490 Da), K16-Ben_K2 (-1.03160 Da) (position: lysine 89)

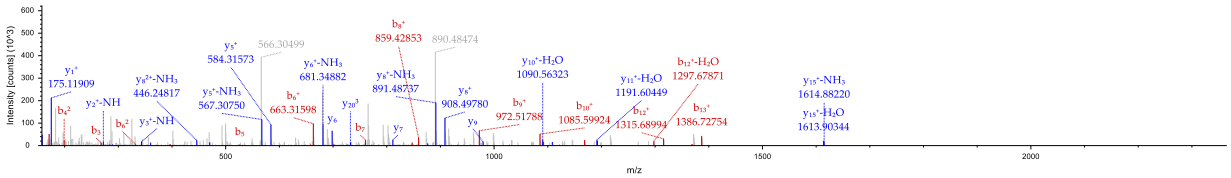
| #1 | b+ | b2+ | Seq. | y+ | y2+ | #2 |
|---|---|---|---|---|---|---|
| 1 | 148.04266 | 74.52497 | M-Ben... | | | 19 |
| 2 | 261.12673 | 131.06700 | I | 1920.054... | 960.53111 | 18 |
| 3 | 332.16384 | 166.58556 | A | 1806.970... | 903.98907 | 17 |
| 4 | 389.18530 | 195.09629 | G | 1735.933... | 868.47052 | 16 |
| 5 | 517.24388 | 259.12558 | Q | 1678.912... | 839.95979 | 15 |
| 6 | 616.31229 | 308.65979 | V | 1550.853... | 775.93050 | 14 |
| 7 | 729.39636 | 365.20182 | L | 1451.785... | 726.39629 | 13 |
| 8 | 844.42330 | 422.71529 | D | 1338.701... | 669.85426 | 12 |
| 9 | 957.50736 | 479.25732 | I | 1223.674... | 612.34079 | 11 |
| 10 | 1071.550... | 536.27878 | N | 1110.590... | 555.79875 | 10 |
| 11 | 1184.634... | 592.82082 | L | 996.54730 | 498.77729 | 9 |
| 12 | 1255.671... | 628.33937 | A | 883.46324 | 442.23526 | 8 |
| 13 | 1326.708... | 663.85793 | A | 812.42613 | 406.71670 | 7 |
| 14 | 1455.751... | 728.37923 | E | 741.38901 | 371.19814 | 6 |
| 15 | 1552.803... | 776.90561 | P | 612.34642 | 306.67685 | 5 |
| 16 | 1679.867... | 840.43729 | K-Ben... | 515.29366 | 258.15047 | 4 |
| 17 | 1778.935... | 889.97150 | V | 388.23029 | 194.61879 | 3 |
| 18 | 1892.978... | 946.99296 | N | 289.16188 | 145.08458 | 2 |
| 19 | | | R | 175.11895 | 88.06311 | 1 |



**P63261** (Actin cytoplasmic 2)

Sequence: VAPEEHPVLLTEAPLNPKANR, K18-Ben_K2 (-1.03160 Da) (position: lysine 113)

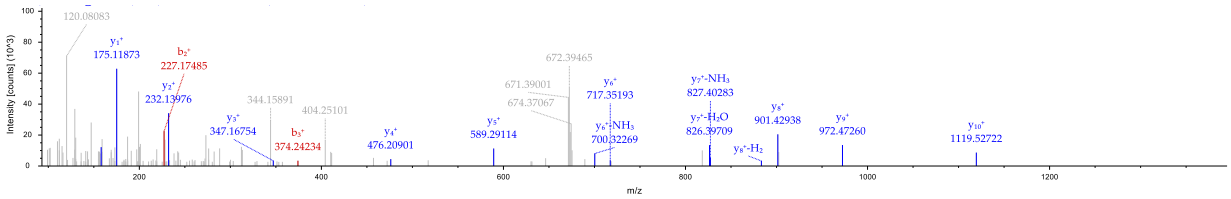
| #1 | b⁺ | b²⁺ | b³⁺ | Seq. | y⁺ | y²⁺ | y³⁺ | #2 |
|---|---|---|---|---|---|---|---|---|
| 1 | 100.07569 | 50.54148 | 34.03008 | V | | | | 21 |
| 2 | 171.11280 | 86.06004 | 57.70912 | A | 2195.145... | 1098.076... | 732.38670 | 20 |
| 3 | 268.16557 | 134.58642 | 90.06004 | P | 2124.108... | 1062.557... | 708.70766 | 19 |
| 4 | 397.20816 | 199.10772 | 133.07424 | E | 2027.055... | 1014.031... | 676.35674 | 18 |
| 5 | 526.25075 | 263.62902 | 176.08844 | E | 1898.013... | 949.51017 | 633.34254 | 17 |
| 6 | 663.30967 | 332.15847 | 221.77474 | H | 1768.970... | 884.98888 | 590.32834 | 16 |
| 7 | 760.36243 | 380.68485 | 254.12566 | P | 1631.911... | 816.45942 | 544.64204 | 15 |
| 8 | 859.43084 | 430.21906 | 287.14847 | V | 1534.858... | 767.93304 | 512.29112 | 14 |
| 9 | 972.51491 | 486.76109 | 324.84315 | L | 1435.790... | 718.39883 | 479.26831 | 13 |
| 10 | 1085.598... | 543.30312 | 362.53784 | L | 1322.706... | 661.85680 | 441.57363 | 12 |
| 11 | 1186.646... | 593.82696 | 396.22040 | T | 1209.622... | 605.31477 | 403.87894 | 11 |
| 12 | 1315.689... | 658.34826 | 439.23460 | E | 1108.574... | 554.79093 | 370.19638 | 10 |
| 13 | 1386.726... | 693.86682 | 462.91364 | A | 979.53199 | 490.26963 | 327.18218 | 9 |
| 14 | 1483.779... | 742.39320 | 495.26456 | P | 908.49488 | 454.75108 | 303.50314 | 8 |
| 15 | 1596.863... | 798.93523 | 532.95925 | L | 811.44211 | 406.22469 | 271.15222 | 7 |
| 16 | 1710.906... | 855.95669 | 570.97355 | N | 698.35805 | 349.68266 | 233.45753 | 6 |
| 17 | 1807.958... | 904.48308 | 603.32448 | P | 584.31512 | 292.66120 | 195.44322 | 5 |
| 18 | 1935.022... | 968.01476 | 645.67893 | K-Ben... | 487.26236 | 244.13482 | 163.09230 | 4 |
| 19 | 2006.059... | 1003.533... | 669.35797 | A | 360.19899 | 180.60314 | 120.73785 | 3 |
| 20 | 2120.102... | 1060.554... | 707.37228 | N | 289.16188 | 145.08458 | 97.05881 | 2 |
| 21 | | | | R | 175.11895 | 88.06311 | 59.04450 | 1 |




**P62979** (Ubiquitin-ribosomal protein eS31 fusion protein)

Sequence: LIFAGKQLEDGR, K6-Ben_K2 (-1.03160 Da) (position: lysine 48)

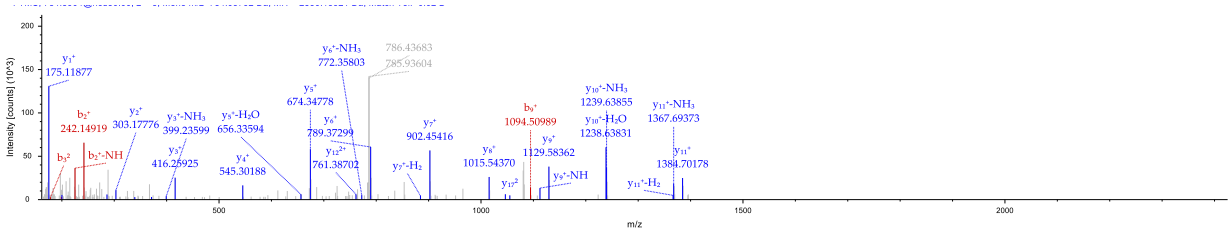
| #1 | b⁺ | b²⁺ | Seq. | y⁺ | y²⁺ | #2 |
|---|---|---|---|---|---|---|
| 1 | 114.09134 | 57.54931 | L | | | 12 |
| 2 | 227.17540 | 114.09134 | I | 1232.627... | 616.81714 | 11 |
| 3 | 374.24382 | 187.62555 | F | 1119.542... | 560.27511 | 10 |
| 4 | 445.28093 | 223.14410 | A | 972.47453 | 486.74091 | 9 |
| 5 | 502.30240 | 251.65484 | G | 901.43742 | 451.22235 | 8 |
| 6 | 629.36576 | 315.18652 | K-Ben... | 844.41596 | 422.71162 | 7 |
| 7 | 757.42434 | 379.21581 | Q | 717.35259 | 359.17993 | 6 |
| 8 | 870.50840 | 435.75784 | L | 589.29402 | 295.15065 | 5 |
| 9 | 999.55099 | 500.27913 | E | 476.20995 | 238.60861 | 4 |
| 10 | 1114.577... | 557.79261 | D | 347.16736 | 174.08732 | 3 |
| 11 | 1171.599... | 586.30334 | G | 232.14042 | 116.57385 | 2 |
| 12 | | | R | 175.11895 | 88.06311 | 1 |



**Q15149** (Plectin)

Sequence: LQLEETDHQKNLLDEELQR, K10-Ben_K2 (-1.03160 Da) (position: lysine 2339)

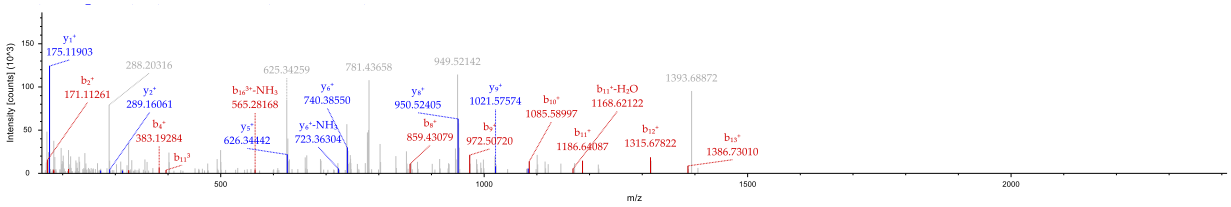
| #1 | b⁺ | b²⁺ | b³⁺ | Seq. | y⁺ | y²⁺ | y³⁺ | #2 |
|---|---|---|---|---|---|---|---|---|
| 1 | 114.09134 | 57.54931 | 38.70196 | L | | | | 19 |
| 2 | 242.14992 | 121.57860 | 81.38816 | Q | 2237.068... | 1119.037... | 746.36088 | 18 |
| 3 | 355.23398 | 178.12063 | 119.08285 | L | 2109.009... | 1055.008... | 703.67468 | 17 |
| 4 | 484.27657 | 242.64193 | 162.09704 | E | 1995.925... | 998.46636 | 665.98000 | 16 |
| 5 | 613.31917 | 307.16322 | 205.11124 | E | 1866.882... | 933.94506 | 622.96580 | 15 |
| 6 | 714.36685 | 357.68706 | 238.79380 | T | 1737.840... | 869.42376 | 579.95160 | 14 |
| 7 | 829.39379 | 415.20053 | 277.13611 | D | 1636.792... | 818.89992 | 546.26904 | 13 |
| 8 | 966.45270 | 483.72999 | 322.82242 | H | 1521.765... | 761.38645 | 507.92673 | 12 |
| 9 | 1094.511... | 547.75928 | 365.50861 | Q | 1384.706... | 692.85700 | 462.24042 | 11 |
| 10 | 1221.574... | 611.29096 | 407.86306 | K-Ben... | 1256.648... | 628.82771 | 419.55423 | 10 |
| 11 | 1335.617... | 668.31242 | 445.87737 | N | 1129.584... | 565.29603 | 377.19978 | 9 |
| 12 | 1448.701... | 724.85445 | 483.57206 | L | 1015.541... | 508.27456 | 339.18547 | 8 |
| 13 | 1561.785... | 781.39649 | 521.26675 | L | 902.45779 | 451.73253 | 301.49078 | 7 |
| 14 | 1676.812... | 838.90996 | 559.60906 | D | 789.37372 | 395.19050 | 263.79609 | 6 |
| 15 | 1805.855... | 903.43125 | 602.62326 | E | 674.34678 | 337.67703 | 225.45378 | 5 |
| 16 | 1934.897... | 967.95255 | 645.63746 | E | 545.30419 | 273.15573 | 182.43958 | 4 |
| 17 | 2047.981... | 1024.494... | 683.33215 | L | 416.26159 | 208.63444 | 139.42538 | 3 |
| 18 | 2176.040... | 1088.523... | 726.01834 | Q | 303.17753 | 152.09240 | 101.73069 | 2 |
| 19 | | | | R | 175.11895 | 88.06311 | 59.04450 | 1 |




**Q562R1** (Beta-actin-like protein 2)

Sequence: VAPDEHPILLTEAPLNPKINR, K18-Ben_K2 (-1.03160 Da) (position: lysine 114)

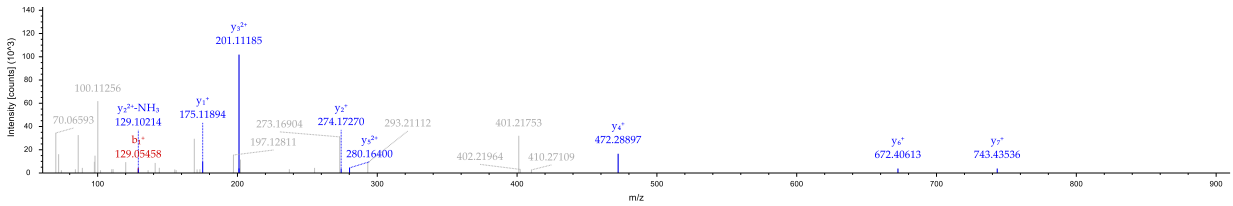
| #1 | b⁺ | b²⁺ | b³⁺ | Seq. | y⁺ | y²⁺ | y³⁺ | #2 |
|---|---|---|---|---|---|---|---|---|
| 1 | 100.07569 | 50.54148 | 34.03008 | V | | | | 21 |
| 2 | 171.11280 | 86.06004 | 57.70912 | A | 2237.192... | 1119.099... | 746.40235 | 20 |
| 3 | 268.16557 | 134.58642 | 90.06004 | P | 2166.155... | 1083.581... | 722.72331 | 19 |
| 4 | 383.19251 | 192.09989 | 128.40235 | D | 2069.102... | 1035.054... | 690.37239 | 18 |
| 5 | 512.23510 | 256.62119 | 171.41655 | E | 1954.075... | 977.54147 | 652.03007 | 17 |
| 6 | 649.29402 | 325.15065 | 217.10286 | H | 1825.033... | 913.02018 | 609.01588 | 16 |
| 7 | 746.34678 | 373.67703 | 249.45378 | P | 1687.974... | 844.49072 | 563.32957 | 15 |
| 8 | 859.43084 | 430.21906 | 287.14847 | I | 1590.921... | 795.96434 | 530.97865 | 14 |
| 9 | 972.51491 | 486.76109 | 324.84315 | L | 1477.837... | 739.42231 | 493.28396 | 13 |
| 10 | 1085.598... | 543.30312 | 362.53784 | L | 1364.753... | 682.88028 | 455.58928 | 12 |
| 11 | 1186.646... | 593.82696 | 396.22040 | T | 1251.669... | 626.33824 | 417.89459 | 11 |
| 12 | 1315.689... | 658.34826 | 439.23460 | E | 1150.621... | 575.81440 | 384.21203 | 10 |
| 13 | 1386.726... | 693.86682 | 462.91364 | A | 1021.578... | 511.29311 | 341.19783 | 9 |
| 14 | 1483.779... | 742.39320 | 495.26456 | P | 950.54183 | 475.77455 | 317.51879 | 8 |
| 15 | 1596.863... | 798.93523 | 532.95925 | L | 853.48906 | 427.24817 | 285.16787 | 7 |
| 16 | 1710.906... | 855.95669 | 570.97355 | N | 740.40500 | 370.70614 | 247.47318 | 6 |
| 17 | 1807.958... | 904.48308 | 603.32448 | P | 626.36207 | 313.68467 | 209.45887 | 5 |
| 18 | 1935.022... | 968.01476 | 645.67893 | K-Ben... | 529.30931 | 265.15829 | 177.10795 | 4 |
| 19 | 2048.106... | 1024.556... | 683.37362 | I | 402.24594 | 201.62661 | 134.75350 | 3 |
| 20 | 2162.149... | 1081.578... | 721.38793 | N | 289.16188 | 145.08458 | 97.05881 | 2 |
| 21 | | | | R | 175.11895 | 88.06311 | 59.04450 | 1 |




**Q9BPW5** (Ras-like protein family member 11B)

Sequence: QALSAKVR, K6-Ben_K2 (-1.03160 Da) (position: lysine 241)

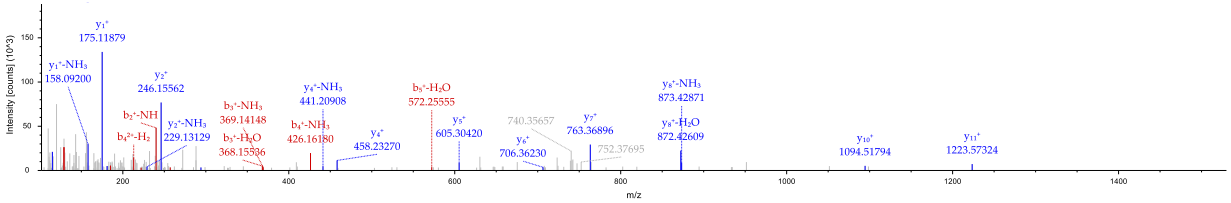
| #1 | b⁺ | b²⁺ | Seq. | y⁺ | y²⁺ | #2 |
|---|---|---|---|---|---|---|
| 1 | 129.06585 | 65.03657 | Q | | | 8 |
| 2 | 200.10297 | 100.55512 | A | 743.44105 | 372.22416 | 7 |
| 3 | 313.18703 | 157.09715 | L | 672.40394 | 336.70561 | 6 |
| 4 | 400.21906 | 200.61317 | S | 559.31987 | 280.16357 | 5 |
| 5 | 471.25617 | 236.13173 | A | 472.28784 | 236.64756 | 4 |
| 6 | 598.31954 | 299.66341 | K-Ben... | 401.25073 | 201.12900 | 3 |
| 7 | 697.38795 | 349.19761 | V | 274.18737 | 137.59732 | 2 |
| 8 | | | R | 175.11895 | 88.06311 | 1 |




**Q15233** (Non-POU domain-containing octamer-binding protein)

Sequence: QQEGFKGTFPDAR, K6-Ben_K2 (-1.03160 Da) (position: lysine 371)

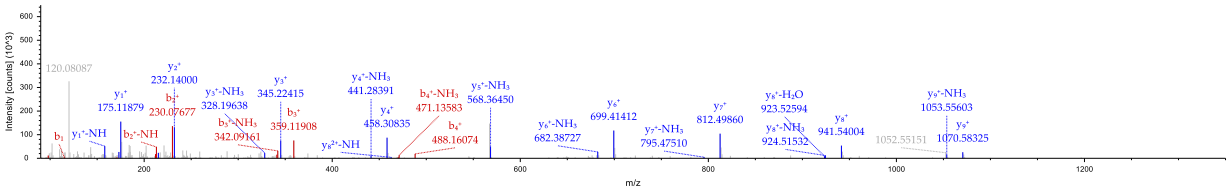
| #1 | b⁺ | b²⁺ | Seq. | y⁺ | y²⁺ | #2 |
|---|---|---|---|---|---|---|
| 1 | 129.06585 | 65.03657 | Q | | | 13 |
| 2 | 257.12443 | 129.06585 | Q | 1351.627... | 676.31751 | 12 |
| 3 | 386.16702 | 193.58715 | E | 1223.569... | 612.28822 | 11 |
| 4 | 443.18849 | 222.09788 | G | 1094.526... | 547.76692 | 10 |
| 5 | 590.25690 | 295.63209 | F | 1037.505... | 519.25619 | 9 |
| 6 | 717.32027 | 359.16377 | K-Ben... | 890.43669 | 445.72198 | 8 |
| 7 | 774.34173 | 387.67450 | G | 763.37333 | 382.19030 | 7 |
| 8 | 875.38941 | 438.19834 | T | 706.35187 | 353.67957 | 6 |
| 9 | 1022.457... | 511.73255 | F | 605.30419 | 303.15573 | 5 |
| 10 | 1119.510... | 560.25893 | P | 458.23577 | 229.62152 | 4 |
| 11 | 1234.537... | 617.77240 | D | 361.18301 | 181.09514 | 3 |
| 12 | 1305.574... | 653.29096 | A | 246.15607 | 123.58167 | 2 |
| 13 | | | R | 175.11895 | 88.06311 | 1 |



**P04908** (Histone H2A type 1-B/E)

Sequence: NDEELNKLLGR, K7-Ben_K2 (-1.03160 Da) (position: lysine 96)

| #1 | b⁺ | b²⁺ | Seq. | y⁺ | y²⁺ | #2 |
|---|---|---|---|---|---|---|
| 1 | 115.05020 | 58.02874 | N | | | 11 |
| 2 | 230.07715 | 115.54221 | D | 1185.611... | 593.30915 | 10 |
| 3 | 359.11974 | 180.06351 | E | 1070.584... | 535.79568 | 9 |
| 4 | 488.16233 | 244.58480 | E | 941.54149 | 471.27438 | 8 |
| 5 | 601.24640 | 301.12684 | L | 812.49890 | 406.75309 | 7 |
| 6 | 715.28932 | 358.14830 | N | 699.41483 | 350.21106 | 6 |
| 7 | 842.35269 | 421.67998 | K-Ben... | 585.37191 | 293.18959 | 5 |
| 8 | 955.43675 | 478.22201 | L | 458.30854 | 229.65791 | 4 |
| 9 | 1068.520... | 534.76405 | L | 345.22448 | 173.11588 | 3 |
| 10 | 1125.542... | 563.27478 | G | 232.14042 | 116.57385 | 2 |
| 11 | | | R | 175.11895 | 88.06311 | 1 |

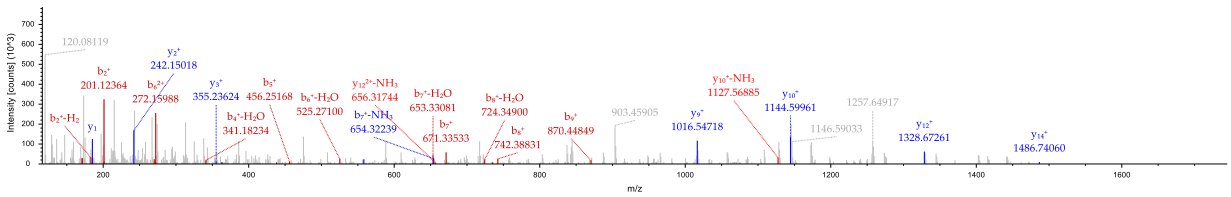



**Representative peptide spectra of modified peptides (propargyl sites, +38 on lysine)**
***-TACO modified peptides: All these peptides are identified from nuclear extract of LnCap without treatment with TACO chemistry.***

Q6ZMV9 (Kinesin-like protein KIF6)

Sequence: LSSAPSQAQDFSILGK, K16-Ben_K1 (38.01570 Da) (position: lysine 538)

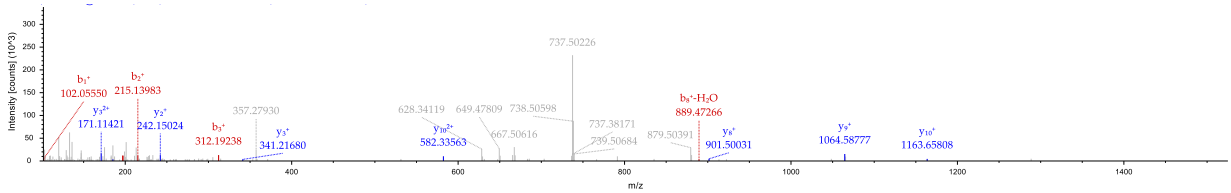
| #1 | b⁺ | b²⁺ | Seq. | y⁺ | y²⁺ | #2 |
|---|---|---|---|---|---|---|
| 1 | 114.09134 | 57.54931 | L | | | 16 |
| 2 | 201.12337 | 101.06532 | S | 1573.785... | 787.39649 | 15 |
| 3 | 288.15540 | 144.58134 | S | 1486.753... | 743.88048 | 14 |
| 4 | 359.19251 | 180.09989 | A | 1399.721... | 700.36447 | 13 |
| 5 | 456.24527 | 228.62628 | P | 1328.684... | 664.84591 | 12 |
| 6 | 543.27730 | 272.14229 | S | 1231.631... | 616.31953 | 11 |
| 7 | 671.33588 | 336.17158 | Q | 1144.599... | 572.80351 | 10 |
| 8 | 742.37299 | 371.69014 | A | 1016.541... | 508.77422 | 9 |
| 9 | 870.43157 | 435.71942 | Q | 945.50406 | 473.25567 | 8 |
| 10 | 985.45851 | 493.23290 | D | 817.44548 | 409.22638 | 7 |
| 11 | 1132.526... | 566.76710 | F | 702.41854 | 351.71291 | 6 |
| 12 | 1219.558... | 610.28312 | S | 555.35012 | 278.17870 | 5 |
| 13 | 1332.643... | 666.82515 | I | 468.31810 | 234.66269 | 4 |
| 14 | 1445.727... | 723.36718 | L | 355.23403 | 178.12065 | 3 |
| 15 | 1502.748... | 751.87791 | G | 242.14997 | 121.57862 | 2 |
| 16 | | | K-Ben... | 185.12850 | 93.06789 | 1 |




Q12955 (Ankyrin-3)

Sequence: TLPVYVSFVQVGK, K13-Ben_K1 (38.01570 Da) (position: lysine 3102)

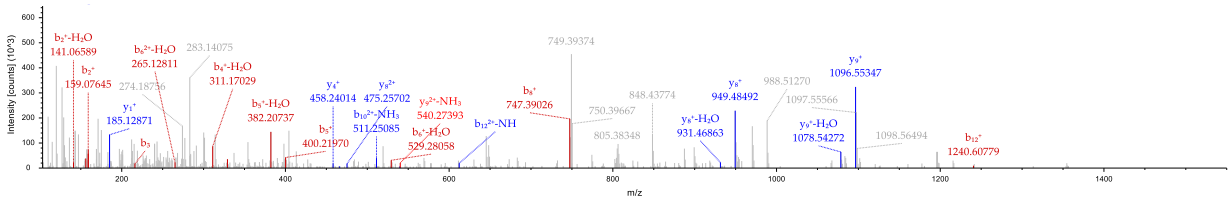
| #1 | b+ | b2+ | Seq. | y+ | y2+ | #2 |
|---|---|---|---|---|---|---|
| 1 | 102.05496 | 51.53112 | T | | | 13 |
| 2 | 215.13902 | 108.07315 | L | 1373.782... | 687.39504 | 12 |
| 3 | 312.19178 | 156.59953 | P | 1260.698... | 630.85301 | 11 |
| 4 | 411.26020 | 206.13374 | V | 1163.645... | 582.32662 | 10 |
| 5 | 574.32353 | 287.66540 | Y | 1064.577... | 532.79242 | 9 |
| 6 | 673.39194 | 337.19961 | V | 901.51423 | 451.26075 | 8 |
| 7 | 760.42397 | 380.71562 | S | 802.44582 | 401.72655 | 7 |
| 8 | 907.49238 | 454.24983 | F | 715.41379 | 358.21053 | 6 |
| 9 | 1006.560... | 503.78404 | V | 568.34537 | 284.67632 | 5 |
| 10 | 1134.619... | 567.81332 | Q | 469.27696 | 235.14212 | 4 |
| 11 | 1233.687... | 617.34753 | V | 341.21838 | 171.11283 | 3 |
| 12 | 1290.709... | 645.85826 | G | 242.14997 | 121.57862 | 2 |
| 13 | | | K-Ben... | 185.12850 | 93.06789 | 1 |




P50454 (Serpin H1)

Sequence: SAGLAFSLYQAMAK, K14-Ben_K1 (38.01570 Da) (position: lysine 60)

| #1 | b+ | b2+ | Seq. | y+ | y2+ | #2 |
|---|---|---|---|---|---|---|
| 1 | 88.03930 | 44.52329 | S | | | 14 |
| 2 | 159.07642 | 80.04185 | A | 1408.729... | 704.86833 | 13 |
| 3 | 216.09788 | 108.55258 | G | 1337.692... | 669.34977 | 12 |
| 4 | 329.18195 | 165.09461 | L | 1280.670... | 640.83904 | 11 |
| 5 | 400.21906 | 200.61317 | A | 1167.586... | 584.29701 | 10 |
| 6 | 547.28747 | 274.14738 | F | 1096.549... | 548.77845 | 9 |
| 7 | 634.31950 | 317.66339 | S | 949.48121 | 475.24425 | 8 |
| 8 | 747.40357 | 374.20542 | L | 862.44919 | 431.72823 | 7 |
| 9 | 910.46689 | 455.73709 | Y | 749.36512 | 375.18620 | 6 |
| 10 | 1038.525... | 519.76637 | Q | 586.30179 | 293.65454 | 5 |
| 11 | 1109.562... | 555.28493 | A | 458.24322 | 229.62525 | 4 |
| 12 | 1240.603... | 620.80517 | M | 387.20610 | 194.10669 | 3 |
| 13 | 1311.640... | 656.32373 | A | 256.16562 | 128.58645 | 2 |
| 14 | | | K-Ben... | 185.12850 | 93.06789 | 1 |

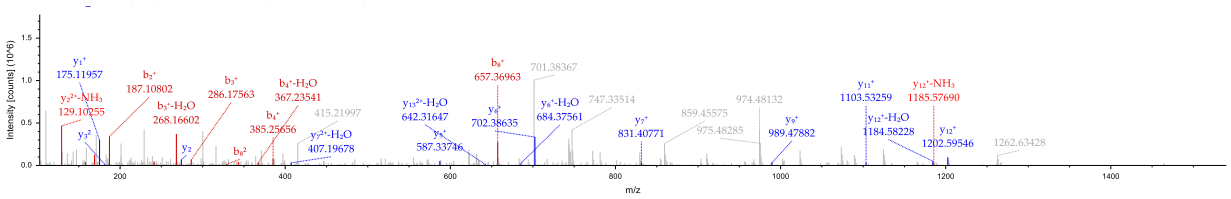



**Representative peptide spectra of modified peptides (aldehydes on lysine, -1 on lysine)**
***-TACO modified peptides: All these peptides are identified from nuclear extract of LnCap without treatment with TACO chemistry.***

**Q9NYQ6** (Cadherin EGF LAG seven-pass G-type receptor 1)

Sequence: SVVVGGASEDKVSVR, K11-Ben_K2 (-1.03160 Da) (position: lysine 1830)

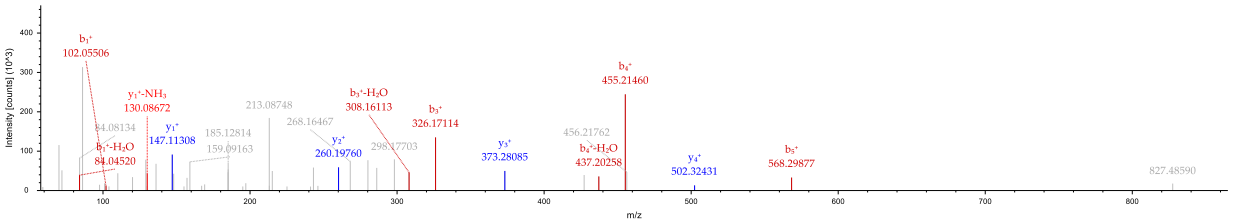
| #1 | b+ | b2+ | Seq. | y+ | y2+ | #2 |
|---|---|---|---|---|---|---|
| 1 | 88.03930 | 44.52329 | S | | | 15 |
| 2 | 187.10772 | 94.05750 | V | 1400.738... | 700.87265 | 14 |
| 3 | 286.17613 | 143.59170 | V | 1301.669... | 651.33844 | 13 |
| 4 | 385.24455 | 193.12591 | V | 1202.601... | 601.80423 | 12 |
| 5 | 442.26601 | 221.63664 | G | 1103.532... | 552.27003 | 11 |
| 6 | 499.28747 | 250.14738 | G | 1046.511... | 523.75929 | 10 |
| 7 | 570.32459 | 285.66593 | A | 989.48985 | 495.24856 | 9 |
| 8 | 657.35662 | 329.18195 | S | 918.45274 | 459.73001 | 8 |
| 9 | 786.39921 | 393.70324 | E | 831.42071 | 416.21399 | 7 |
| 10 | 901.42615 | 451.21671 | D | 702.37811 | 351.69270 | 6 |
| 11 | 1028.489... | 514.74840 | K-Ben... | 587.35117 | 294.17922 | 5 |
| 12 | 1127.557... | 564.28260 | V | 460.28781 | 230.64754 | 4 |
| 13 | 1214.589... | 607.79862 | S | 361.21939 | 181.11334 | 3 |
| 14 | 1313.658... | 657.33282 | V | 274.18737 | 137.59732 | 2 |
| 15 | | | R | 175.11895 | 88.06311 | 1 |



**Q9C0D3** (Protein zyg-11 homolog B)

Sequence: TKPEILK, K2-Ben_K2 (-1.03160 Da) (position: lysine 360)

| | | | | |
|---|---|---|---|---|
| 1 | 102.05496 | T | | 7 |
| 2 | 229.11832 | K-Ben... | 726.43965 | 6 |
| 3 | 326.17108 | P | 599.37629 | 5 |
| 4 | 455.21367 | E | 502.32353 | 4 |
| 5 | 568.29774 | I | 373.28093 | 3 |
| 6 | 681.38180 | L | 260.19687 | 2 |
| 7 | | K | 147.11280 | 1 |



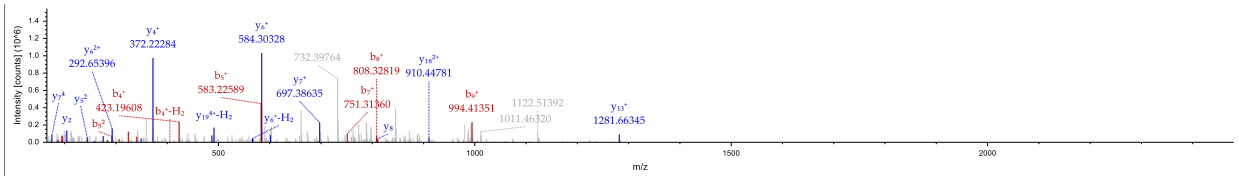

**List of MCF-10A Kme$_2$ modification sites and proteins**

**Representative peptide spectra of modified peptides (propargyl and aldehydes on lysine, +38, -1 on lysine). *All these peptides are identified from cell lysate of MCF-10A after treatment with TACO chemistry.***

**Q13162** (Peroxiredoxin-4)

Sequence: VCPAGWkPGSETI, K10-Ben_K2 (-1.03160 Da) (position: lysine 250)

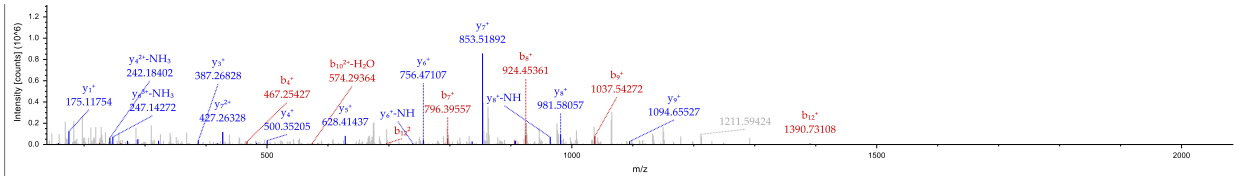
| #1 | b+ | b2+ | b3+ | b4+ | Seq. | y+ | y2+ | y3+ | y4+ | #2 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 138.06619 | 69.53673 | 46.69358 | 35.27200 | H | | | | | 23 |
| 2 | 195.08765 | 98.04746 | 65.70074 | 49.52737 | G | 2265.085... | 1133.046... | 755.70007 | 567.02687 | 22 |
| 3 | 324.13025 | 162.56876 | 108.71493 | 81.78802 | E | 2208.064... | 1104.535... | 736.69291 | 552.77150 | 21 |
| 4 | 423.19866 | 212.10297 | 141.73774 | 106.55512 | V | 2079.021... | 1040.014... | 693.67871 | 520.51085 | 20 |
| 5 | 583.22931 | 292.11829 | 195.08129 | 146.56278 | C-Car... | 1979.953... | 990.48022 | 660.65591 | 495.74375 | 19 |
| 6 | 680.28207 | 340.64467 | 227.43221 | 170.82598 | P | 1819.922... | 910.46490 | 607.31236 | 455.73609 | 18 |
| 7 | 751.31919 | 376.16323 | 251.11125 | 188.58525 | A | 1722.869... | 861.93852 | 574.96144 | 431.47290 | 17 |
| 8 | 808.34065 | 404.67396 | 270.11840 | 202.84062 | G | 1651.832... | 826.41996 | 551.28240 | 413.71362 | 16 |
| 9 | 994.41996 | 497.71362 | 332.14484 | 249.36045 | W | 1594.811... | 797.90923 | 532.27525 | 399.45825 | 15 |
| 10 | 1121.483... | 561.24530 | 374.49929 | 281.12629 | K-Ben... | 1408.731... | 704.86957 | 470.24881 | 352.93843 | 14 |
| 11 | 1218.536... | 609.77168 | 406.85021 | 305.38948 | P | 1281.668... | 641.33789 | 427.89435 | 321.17258 | 13 |
| 12 | 1275.557... | 638.28241 | 425.85737 | 319.64485 | G | 1184.615... | 592.81151 | 395.54343 | 296.90939 | 12 |
| 13 | 1362.589... | 681.79843 | 454.86804 | 341.40285 | S | 1127.594... | 564.30078 | 376.53628 | 282.65403 | 11 |
| 14 | 1491.632... | 746.31973 | 497.88224 | 373.66350 | E | 1040.562... | 520.78476 | 347.52560 | 260.89602 | 10 |
| 15 | 1592.679... | 796.84356 | 531.56480 | 398.92542 | T | 911.51966 | 456.26347 | 304.51140 | 228.63537 | 9 |
| 16 | 1705.763... | 853.38560 | 569.25949 | 427.19644 | I | 810.47198 | 405.73963 | 270.82884 | 203.37345 | 8 |
| 17 | 1818.847... | 909.92763 | 606.95418 | 455.46745 | I | 697.38792 | 349.19760 | 233.13416 | 175.10244 | 7 |
| 18 | 1915.900... | 958.45401 | 639.30510 | 479.73064 | P | 584.30385 | 292.65556 | 195.43947 | 146.83142 | 6 |
| 19 | 2030.927... | 1015.967... | 677.64741 | 508.48738 | D | 487.25109 | 244.12918 | 163.08855 | 122.56823 | 5 |
| 20 | 2127.980... | 1064.493... | 709.99833 | 532.75057 | P | 372.22415 | 186.61571 | 124.74623 | 93.81149 | 4 |
| 21 | 2199.017... | 1100.012... | 733.67737 | 550.50985 | A | 275.17138 | 138.08933 | 92.39531 | 69.54830 | 3 |
| 22 | 2256.039... | 1128.523... | 752.68453 | 564.76521 | G | 204.13427 | 102.57077 | 68.71627 | 51.78902 | 2 |
| 23 | | | | | K | 147.11280 | 74.06004 | 49.70912 | 37.53366 | 1 |



**P05556** (Integrin beta-1)

Sequence: GTAEKLkPEDITQ, K2-Ben_K2 (-1.03160 Da) (position: lysine 107)

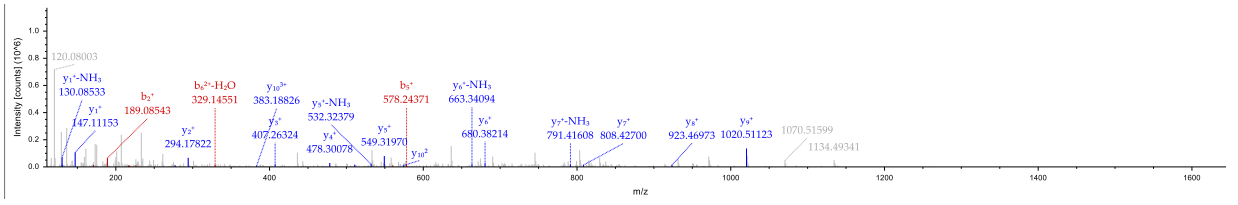
| #1 | b⁺ | b²⁺ | b³⁺ | Seq. | y⁺ | y²⁺ | y³⁺ | #2 |
|---|---|---|---|---|---|---|---|---|
| 1 | 114.09134 | 57.54931 | 38.70196 | L | | | | 17 |
| 2 | 241.15470 | 121.08099 | 81.05642 | K-Ben... | 1905.044... | 953.02566 | 635.68620 | 16 |
| 3 | 338.20747 | 169.60737 | 113.40734 | P | 1777.980... | 889.49398 | 593.33174 | 15 |
| 4 | 467.25006 | 234.12867 | 156.42154 | E | 1680.927... | 840.96759 | 560.98082 | 14 |
| 5 | 582.27700 | 291.64214 | 194.76385 | D | 1551.885... | 776.44630 | 517.96662 | 13 |
| 6 | 695.36107 | 348.18417 | 232.45854 | I | 1436.858... | 718.93283 | 479.62431 | 12 |
| 7 | 796.40875 | 398.70801 | 266.14110 | T | 1323.774... | 662.39079 | 441.92962 | 11 |
| 8 | 924.46732 | 462.73730 | 308.82729 | Q | 1222.726... | 611.86695 | 408.24706 | 10 |
| 9 | 1037.551... | 519.27933 | 346.52198 | I | 1094.668... | 547.83767 | 365.56087 | 9 |
| 10 | 1165.609... | 583.30862 | 389.20817 | Q | 981.58399 | 491.29563 | 327.86618 | 8 |
| 11 | 1262.662... | 631.83500 | 421.55909 | P | 853.52541 | 427.26634 | 285.17999 | 7 |
| 12 | 1390.721... | 695.86429 | 464.24529 | Q | 756.47265 | 378.73996 | 252.82907 | 6 |
| 13 | 1518.779... | 759.89358 | 506.93148 | Q | 628.41407 | 314.71067 | 210.14287 | 5 |
| 14 | 1631.863... | 816.43561 | 544.62617 | L | 500.35549 | 250.68139 | 167.45668 | 4 |
| 15 | 1730.932... | 865.96982 | 577.64897 | V | 387.27143 | 194.13935 | 129.76199 | 3 |
| 16 | 1844.016... | 922.51185 | 615.34366 | L | 288.20302 | 144.60515 | 96.73919 | 2 |
| 17 | | | | R | 175.11895 | 88.06311 | 59.04450 | 1 |



**Q9Y2A7** (Nck-associated protein 1)

Sequence: MRTSFDkPDQMAA, K5-Ben_K2 (-1.03160 Da) (position: lysine 886)

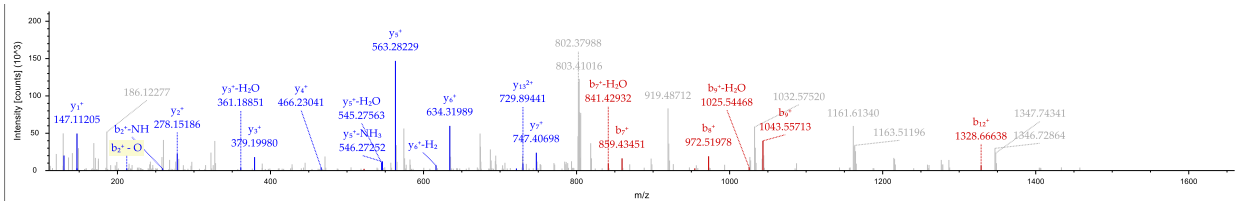
| #1 | b⁺ | b²⁺ | b³⁺ | Seq. | y⁺ | y²⁺ | y³⁺ | #2 |
|---|---|---|---|---|---|---|---|---|
| 1 | 102.05496 | 51.53112 | 34.68984 | T | | | | 14 |
| 2 | 189.08698 | 95.04713 | 63.70051 | S | 1496.709... | 748.85815 | 499.57453 | 13 |
| 3 | 336.15540 | 168.58134 | 112.72332 | F | 1409.677... | 705.34214 | 470.56385 | 12 |
| 4 | 451.18234 | 226.09481 | 151.06563 | D | 1262.608... | 631.80793 | 421.54105 | 11 |
| 5 | 578.24570 | 289.62649 | 193.42009 | K-Ben... | 1147.581... | 574.29446 | 383.19873 | 10 |
| 6 | 675.29847 | 338.15287 | 225.77101 | P | 1020.518... | 510.76278 | 340.84428 | 9 |
| 7 | 790.32541 | 395.66634 | 264.11332 | D | 923.46551 | 462.23640 | 308.49336 | 8 |
| 8 | 918.38399 | 459.69563 | 306.79951 | Q | 808.43857 | 404.72292 | 270.15104 | 7 |
| 9 | 1049.424... | 525.21587 | 350.47968 | M | 680.37999 | 340.69364 | 227.46485 | 6 |
| 10 | 1120.461... | 560.73443 | 374.15871 | A | 549.33951 | 275.17339 | 183.78469 | 5 |
| 11 | 1191.498... | 596.25299 | 397.83775 | A | 478.30240 | 239.65484 | 160.10565 | 4 |
| 12 | 1304.582... | 652.79502 | 435.53244 | L | 407.26528 | 204.13628 | 136.42661 | 3 |
| 13 | 1451.651... | 726.32923 | 484.55524 | F | 294.18122 | 147.59425 | 98.73192 | 2 |
| 14 | | | | K | 147.11280 | 74.06004 | 49.70912 | 1 |



**Q562R1** (Beta-actin-like protein 2)

Sequence: IADRMQkEIITLA, K3-Ben_K2 (-1.03160 Da) (position: lysine 316)

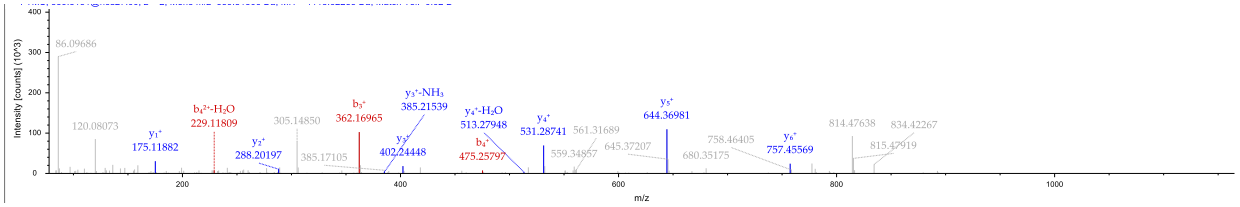
| #1 | b+ | b2+ | Seq. | y+ | y2+ | #2 |
|---|---|---|---|---|---|---|
| 1 | 148.04268 | 74.52498 | M-Oxi... | | | 14 |
| 2 | 276.10125 | 138.55427 | Q | 1458.787... | 729.89728 | 13 |
| 3 | 403.16462 | 202.08595 | K-Ben... | 1330.728... | 665.86799 | 12 |
| 4 | 532.20721 | 266.60724 | E | 1203.665... | 602.33631 | 11 |
| 5 | 645.29127 | 323.14928 | I | 1074.622... | 537.81501 | 10 |
| 6 | 758.37534 | 379.69131 | I | 961.53868 | 481.27298 | 9 |
| 7 | 859.42302 | 430.21515 | T | 848.45462 | 424.73095 | 8 |
| 8 | 972.50708 | 486.75718 | L | 747.40694 | 374.20711 | 7 |
| 9 | 1043.544... | 522.27574 | A | 634.32287 | 317.66507 | 6 |
| 10 | 1140.596... | 570.80212 | P | 563.28576 | 282.14652 | 5 |
| 11 | 1227.628... | 614.31813 | S | 466.23300 | 233.62014 | 4 |
| 12 | 1328.676... | 664.84197 | T | 379.20097 | 190.10412 | 3 |
| 13 | 1459.717... | 730.36221 | M | 278.15329 | 139.58028 | 2 |
| 14 | | | K | 147.11280 | 74.06004 | 1 |




**P17540** (Creatine kinase S-type, mitochondrial)

Sequence: KDPRFSkILENLR, K3-Ben_K2 (-1.03160 Da) (position: lysine 344)

| #1 | b+ | b2+ | Seq. | y+ | y2+ | #2 |
|---|---|---|---|---|---|---|
| 1 | 148.07569 | 74.54148 | F | | | 9 |
| 2 | 235.10772 | 118.05750 | S | 971.55206 | 486.27967 | 8 |
| 3 | 362.17108 | 181.58918 | K-Ben... | 884.52003 | 442.76365 | 7 |
| 4 | 475.25515 | 238.13121 | I | 757.45666 | 379.23197 | 6 |
| 5 | 588.33921 | 294.67324 | L | 644.37260 | 322.68994 | 5 |
| 6 | 717.38180 | 359.19454 | E | 531.28854 | 266.14791 | 4 |
| 7 | 831.42473 | 416.21600 | N | 402.24594 | 201.62661 | 3 |
| 8 | 944.50879 | 472.75804 | L | 288.20302 | 144.60515 | 2 |
| 9 | | | R | 175.11895 | 88.06311 | 1 |



**Q15154** (Pericentriolar material 1 protein)

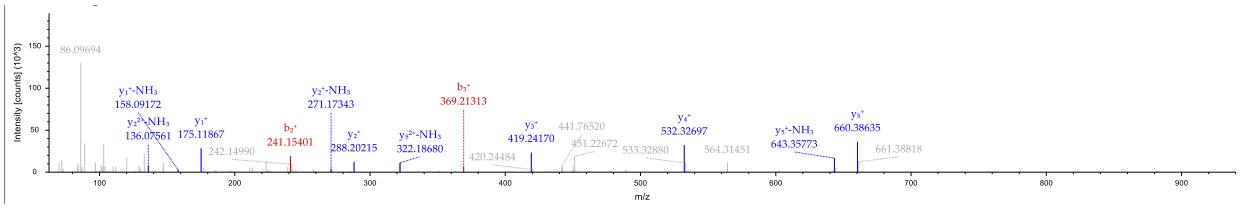
Sequence: QLSENRkPFNFLP, K1-Ben_K2 (-1.03160 Da) (position: lysine 139)

| #1 | b⁺ | b²⁺ | Seq. | y⁺ | y²⁺ | #2 |
|---|---|---|---|---|---|---|
| 1 | 128.07064 | 64.53896 | K-Ben... | | | 14 |
| 2 | 225.12340 | 113.06534 | P | 1579.793... | 790.40050 | 13 |
| 3 | 372.19182 | 186.59955 | F | 1482.740... | 741.87412 | 12 |
| 4 | 486.23474 | 243.62101 | N | 1335.672... | 668.33991 | 11 |
| 5 | 633.30316 | 317.15522 | F | 1221.629... | 611.31845 | 10 |
| 6 | 746.38722 | 373.69725 | L | 1074.561... | 537.78424 | 9 |
| 7 | 843.43999 | 422.22363 | P | 961.47714 | 481.24221 | 8 |
| 8 | 990.47539 | 495.74133 | M-Oxi... | 864.42438 | 432.71583 | 7 |
| 9 | 1118.533... | 559.77062 | Q | 717.38898 | 359.19813 | 6 |
| 10 | 1231.618... | 616.31265 | I | 589.33040 | 295.16884 | 5 |
| 11 | 1345.660... | 673.33412 | N | 476.24634 | 238.62681 | 4 |
| 12 | 1446.708... | 723.85795 | T | 362.20341 | 181.60534 | 3 |
| 13 | 1560.751... | 780.87942 | N | 261.15573 | 131.08150 | 2 |
| 14 | | | K | 147.11280 | 74.06004 | 1 |

**Q15276** (Rab GTPase-binding effector protein 1)

Sequence: TRDQVKkLQLMLR, K1-Ben_K2 (-1.03160 Da) (position: lysine 559)

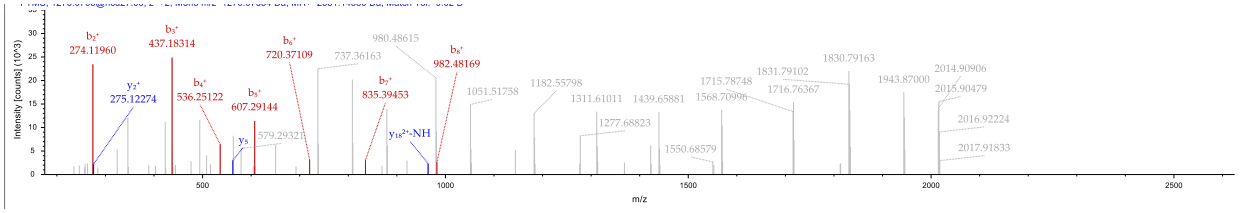
| #1 | b⁺ | b²⁺ | Seq. | y⁺ | y²⁺ | #2 |
|---|---|---|---|---|---|---|
| 1 | 128.07064 | 64.53896 | K-Ben... | | | 7 |
| 2 | 241.15470 | 121.08099 | L | 773.47021 | 387.23874 | 6 |
| 3 | 369.21328 | 185.11028 | Q | 660.38614 | 330.69671 | 5 |
| 4 | 482.29734 | 241.65231 | L | 532.32756 | 266.66742 | 4 |
| 5 | 613.33783 | 307.17255 | M | 419.24350 | 210.12539 | 3 |
| 6 | 726.42189 | 363.71459 | L | 288.20302 | 144.60515 | 2 |
| 7 | | | R | 175.11895 | 88.06311 | 1 |



**P68032** (Actin, alpha cardiac muscle 1)

Sequence: LCYVALDFENEMATAASSSSLEK, K23-Ben_K2 (-1.03160 Da) (position: lysine 240)

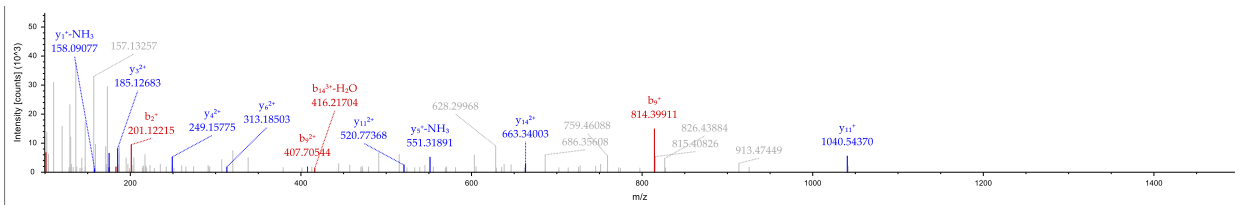
| #1 | b+ | b2+ | Seq. | y+ | y2+ | #2 |
|---|---|---|---|---|---|---|
| 1 | 114.09134 | 57.54931 | L | | | 23 |
| 2 | 274.12199 | 137.56463 | C-Car... | 2438.037... | 1219.522... | 22 |
| 3 | 437.18532 | 219.09630 | Y | 2278.006... | 1139.507... | 21 |
| 4 | 536.25373 | 268.63050 | V | 2114.943... | 1057.975... | 20 |
| 5 | 607.29085 | 304.14906 | A | 2015.875... | 1008.441... | 19 |
| 6 | 720.37491 | 360.69109 | L | 1944.837... | 972.92260 | 18 |
| 7 | 835.40185 | 418.20456 | D | 1831.753... | 916.38057 | 17 |
| 8 | 982.47027 | 491.73877 | F | 1716.726... | 858.86710 | 16 |
| 9 | 1111.512... | 556.26007 | E | 1569.658... | 785.33289 | 15 |
| 10 | 1225.555... | 613.28153 | N | 1440.615... | 720.81160 | 14 |
| 11 | 1354.598... | 677.80283 | E | 1326.572... | 663.79013 | 13 |
| 12 | 1501.633... | 751.32053 | M-Oxi... | 1197.530... | 599.26884 | 12 |
| 13 | 1572.670... | 786.83908 | A | 1050.494... | 525.75114 | 11 |
| 14 | 1673.718... | 837.36292 | T | 979.45788 | 490.23258 | 10 |
| 15 | 1744.755... | 872.88148 | A | 878.41020 | 439.70874 | 9 |
| 16 | 1815.792... | 908.40004 | A | 807.37309 | 404.19018 | 8 |
| 17 | 1902.824... | 951.91605 | S | 736.33597 | 368.67163 | 7 |
| 18 | 1989.856... | 995.43207 | S | 649.30395 | 325.15561 | 6 |
| 19 | 2076.888... | 1038.948... | S | 562.27192 | 281.63960 | 5 |
| 20 | 2163.920... | 1082.464... | S | 475.23989 | 238.12358 | 4 |
| 21 | 2277.004... | 1139.006... | L | 388.20786 | 194.60757 | 3 |
| 22 | 2406.047... | 1203.527... | E | 275.12380 | 138.06554 | 2 |
| 23 | | | K-Ben... | 146.08120 | 73.54424 | 1 |




**Q9H165** (B-cell lymphoma/leukemia 11A)

Sequence: LSAKGATDAGAKPPR, K4-Ben_K2 (+38.0157 Da) (position: lysine 559)

| #1 | b+ | b2+ | b3+ | Seq. | y+ | y2+ | y3+ | #2 |
|---|---|---|---|---|---|---|---|---|
| 1 | 114.09134 | 57.54931 | 38.70196 | L | | | | 15 |
| 2 | 201.12337 | 101.06532 | 67.71264 | S | 1325.680... | 663.34406 | 442.56513 | 14 |
| 3 | 272.16048 | 136.58388 | 91.39168 | A | 1238.648... | 619.82804 | 413.55445 | 13 |
| 4 | 399.22385 | 200.11556 | 133.74613 | K-Ben... | 1167.611... | 584.30949 | 389.87542 | 12 |
| 5 | 456.24531 | 228.62629 | 152.75329 | G | 1040.548... | 520.77780 | 347.52096 | 11 |
| 6 | 527.28242 | 264.14485 | 176.43233 | A | 983.52687 | 492.26707 | 328.51381 | 10 |
| 7 | 628.33010 | 314.66869 | 210.11488 | T | 912.48976 | 456.74852 | 304.83477 | 9 |
| 8 | 743.35704 | 372.18216 | 248.45720 | D | 811.44208 | 406.22468 | 271.15221 | 8 |
| 9 | 814.39416 | 407.70072 | 272.13624 | A | 696.41513 | 348.71121 | 232.80990 | 7 |
| 10 | 871.41562 | 436.21145 | 291.14339 | G | 625.37802 | 313.19265 | 209.13086 | 6 |
| 11 | 942.45274 | 471.73001 | 314.82243 | A | 568.35656 | 284.68192 | 190.12370 | 5 |
| 12 | 1070.547... | 535.77749 | 357.52075 | K | 497.31944 | 249.16336 | 166.44467 | 4 |
| 13 | 1167.600... | 584.30387 | 389.87167 | P | 369.22448 | 185.11588 | 123.74634 | 3 |
| 14 | 1264.653... | 632.83025 | 422.22259 | P | 272.17172 | 136.58950 | 91.39542 | 2 |
| 15 | | | | R | 175.11895 | 88.06311 | 59.04450 | 1 |



**XXIX. Supplementary Figure 23.** TACO modification of nuclear extract, biotin enrichment of modified $Kme_2$ proteins from nuclear extract and identification of $Kme_2$ proteins and $Kme_2$ sites by proteomic analysis.

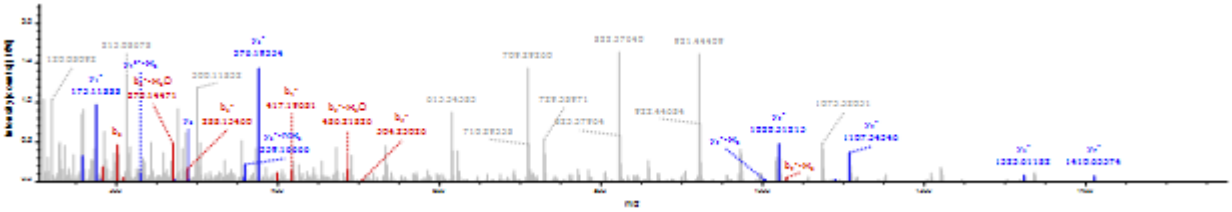
**TACO modification and enrichment of $Kme_2$ proteins from nuclear extract:** To 2 mg of nuclear extract in 2 mL of NaP buffer pH 7 was added 20 mg of selectfluor and 80 µL of pyridine. The reaction was stirred at room temperature for 1 h. Samples were subjected to acetone precipitation to remove small molecules. To proteins resuspended in 2 mL of NaP buffer pH 5.0 was added a solution of 0.5 mg of Biotin-dPEG®3-oxyamine hydrochloride in DMSO and stirred for 6 h, followed by acetone precipitation. TACO modified nuclear lysate was resuspended in 2 mL binding buffer (Tris-buffered saline, pH 7.5). 100 µL of prepped magnetic streptavidin beads were added to biotinylated nuclear lysate and incubated at 4 $^{O}$C for 1 h. Beads were washed 3x with washing buffer (TBS, 2M urea, pH 7.5) and finally with PBS pH 7. After washing, captured proteins were eluted by adding 1X SDS sample buffer (200 uL) and heated at 98 $^{O}$C for 10 m. Eluted proteins were passed through a 3 kDa MW-cut off filter, followed by digestion with Thermo scientific EasyPep Mini MS sample Prep Kit.

**Proteomics analysis of enriched proteins:** Proteins were digested using Thermo scientific EasyPep Mini MS sample Prep Kit. Digested peptides were analyzed by LC/MS. Samples were analyzed by LC-MS using a Q-Exactive Plus orbitrap mass spectrometer equipped with Dionex UltiMate 3000 LC system (Thermo). Briefly, lyophilized digested peptides were resuspended in 0.1% formic acid in 10% acetonitrile and loaded onto a trap column (PepMap™ NEO 5 µm C18 300 µm X 5 mm Trap Cartridge) and resolved through a custom analytical column packed with ReproSil-Pur 120 C18-AQ 3 µm beads (Dr. Maisch GmbH) at a flow rate of 0.3 µL/min with a gradient solvent A (0.1% formic acid in 2% acetonitrile) and a gradient solvent B (0.1% formic acid in 80% acetonitrile) for 150 minutes. MS analysis was conducted in a data-dependent manner with full scans in the range from 400 to 1800 m/z using an Orbitrap mass analyzer set as follows: MS1: resolution = 70,000, AGC target = 3e6, Max IT = 100ms; MS2: resolution = 17,500, AGC target 1e5, Max IT = 50ms. The top fifteen most intense precursor ions were selected for MS2 with an isolation window of 4 m/z. Isolated precursors were fragmented by high energy collisional dissociation (HCD) with normalized collision energy (NCE) of 27. LC-MS RAW files were searched against the human proteome sequence database from UniProt using the Sequest HT search engine embedded in Proteome Discoverer 3.0 (Thermo) with 10 ppm MS1 precursor mass tolerance, 0.02 Da MS2 fragment mass tolerance, 0.01 false discovery rate. Search included the following modifications: Methionine oxidation (+15.99492 Da), asaparagine and glutamine deamidation (+0.98402 Da) and protein N-terminal acetylation (+42.03670), histidine fluorination (+17.9906), tryptophan fluorination (+33.9855), and lysine biotin-oxime (+415.1777) were variable modifications (up to 3 allowed per peptide); cysteine was assigned a fixed carbamidomethyl modification (+57.021465 Da).

**Representative peptide spectra of modified peptides (Biotin-oxime sites, +415 on lysine)**
***All these peptides are identified from nuclear extract of LnCap after treatment with TACO chemistry.***

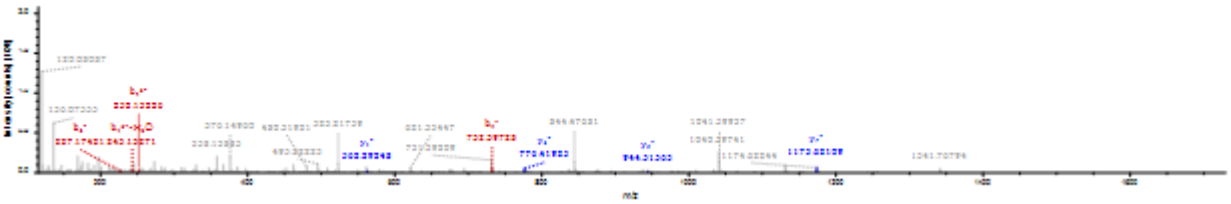
**Q6R327** (Rapamycin-insensitive companion of mTOR)
Sequence: LSSESKTSNR, K6-BenKPlus415 (415.17770 Da) (position: lysine 1125)

| #1 | b+ | b2+ | Seq. | y+ | y2+ | #2 |
|---|---|---|---|---|---|---|
| 1 | 114.09134 | 57.54931 | L | | | 10 |
| 2 | 201.12337 | 101.06532 | S | 1410.652... | 705.83010 | 9 |
| 3 | 288.15540 | 144.58134 | S | 1323.620... | 662.31409 | 8 |
| 4 | 417.19799 | 209.10263 | E | 1236.588... | 618.79807 | 7 |
| 5 | 504.23002 | 252.61865 | S | 1107.546... | 554.27678 | 6 |
| 6 | 1047.502... | 524.25498 | K-Ben... | 1020.514... | 510.76076 | 5 |
| 7 | 1148.550... | 574.77882 | T | 477.24159 | 239.12443 | 4 |
| 8 | 1235.582... | 618.29483 | S | 376.19391 | 188.60059 | 3 |
| 9 | 1349.625... | 675.31630 | N | 289.16188 | 145.08458 | 2 |
| 10 | | | R | 175.11895 | 88.06311 | 1 |



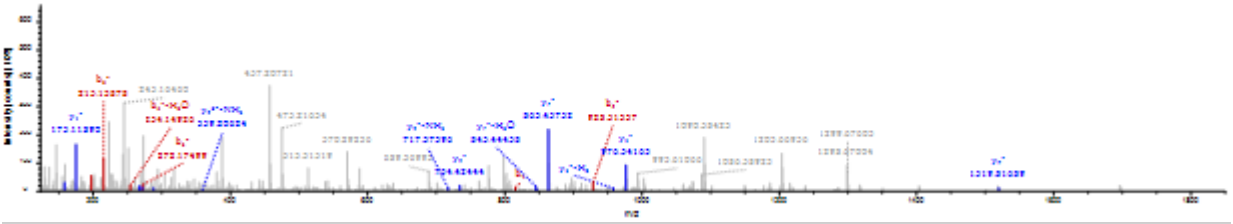
**P17098** (Zinc finger protein 8)
Sequence: LIFEQTPALTK, K11-BenKPlus415 (415.17770 Da) (position: lysine 441)

| #1 | b+ | b2+ | Seq. | y+ | y2+ | #2 |
|---|---|---|---|---|---|---|
| 1 | 114.09134 | 57.54931 | L | | | 11 |
| 2 | 227.17540 | 114.09134 | I | 1562.813... | 781.91036 | 10 |
| 3 | 374.24382 | 187.62555 | F | 1449.729... | 725.36833 | 9 |
| 4 | 503.28641 | 252.14684 | E | 1302.660... | 651.83412 | 8 |
| 5 | 631.34499 | 316.17613 | Q | 1173.618... | 587.31283 | 7 |
| 6 | 732.39267 | 366.69997 | T | 1045.559... | 523.28354 | 6 |
| 7 | 829.44543 | 415.22635 | P | 944.51212 | 472.75970 | 5 |
| 8 | 900.48254 | 450.74491 | A | 847.45936 | 424.23332 | 4 |
| 9 | 1013.566... | 507.28694 | L | 776.42225 | 388.71476 | 3 |
| 10 | 1114.614... | 557.81078 | T | 663.33818 | 332.17273 | 2 |
| 11 | | | K-Ben... | 562.29050 | 281.64889 | 1 |



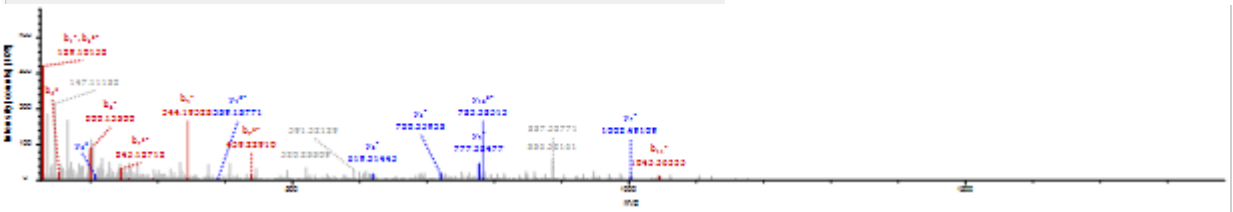
**Q9H270** (Vacuolar protein sorting-associated protein 11 homolog)
Sequence: TIGKLEPSYVIR, K4-BenKPlus415 (415.17770 Da) (position: lysine 417)

| #1 | b+ | b2+ | Seq. | y+ | y2+ | #2 |
|---|---|---|---|---|---|---|
| 1 | 102.05496 | 51.53112 | T | | | 12 |
| 2 | 215.13902 | 108.07315 | I | 1689.924... | 845.46584 | 11 |
| 3 | 272.16048 | 136.58388 | G | 1576.840... | 788.92381 | 10 |
| 4 | 815.43315 | 408.22021 | K-Ben... | 1519.818... | 760.41307 | 9 |
| 5 | 928.51721 | 464.76224 | L | 976.54621 | 488.77674 | 8 |
| 6 | 1057.559... | 529.28354 | E | 863.46214 | 432.23471 | 7 |
| 7 | 1154.612... | 577.80992 | P | 734.41955 | 367.71341 | 6 |
| 8 | 1241.644... | 621.32594 | S | 637.36679 | 319.18703 | 5 |
| 9 | 1404.707... | 702.85760 | Y | 550.33476 | 275.67102 | 4 |
| 10 | 1503.776... | 752.39181 | V | 387.27143 | 194.13935 | 3 |
| 11 | 1616.860... | 808.93384 | I | 288.20302 | 144.60515 | 2 |
| 12 | | | R | 175.11895 | 88.06311 | 1 |



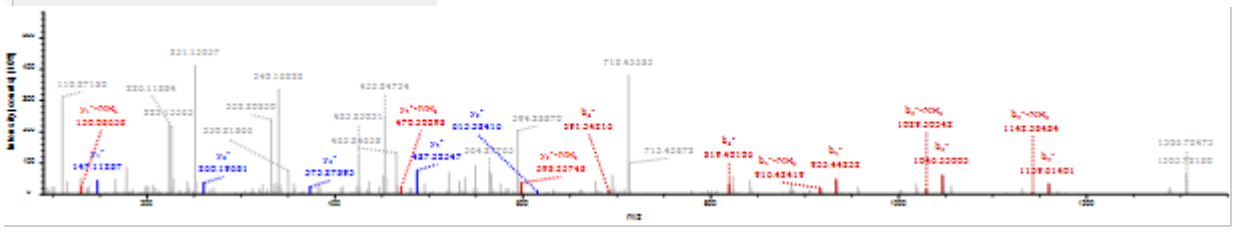
**Q99741** (Cell division control protein 6 homolog)
Sequence: KAGSLYLSGAPGTGK, K15-BenKPlus415 (415.17770 Da) (position: lysine 208)

| #1 | b+ | b2+ | b3+ | Seq. | y+ | y2+ | y3+ | #2 |
|---|---|---|---|---|---|---|---|---|
| 1 | 129.10224 | 65.05476 | 43.70560 | K | | | | 15 |
| 2 | 200.13935 | 100.57331 | 67.38464 | A | 1693.846... | 847.42691 | 565.28703 | 14 |
| 3 | 257.16082 | 129.08405 | 86.39179 | G | 1622.809... | 811.90835 | 541.60799 | 13 |
| 4 | 344.19285 | 172.60006 | 115.40247 | S | 1565.787... | 783.39762 | 522.60084 | 12 |
| 5 | 457.27691 | 229.14209 | 153.09715 | L | 1478.755... | 739.88161 | 493.59016 | 11 |
| 6 | 620.34024 | 310.67376 | 207.45160 | Y | 1365.671... | 683.33957 | 455.89548 | 10 |
| 7 | 733.42430 | 367.21579 | 245.14628 | L | 1202.608... | 601.80791 | 401.54103 | 9 |
| 8 | 820.45633 | 410.73180 | 274.15696 | S | 1089.524... | 545.26588 | 363.84634 | 8 |
| 9 | 877.47779 | 439.24254 | 293.16412 | G | 1002.492... | 501.74986 | 334.83567 | 7 |
| 10 | 948.51491 | 474.76109 | 316.84315 | A | 945.47099 | 473.23913 | 315.82851 | 6 |
| 11 | 1045.567... | 523.28747 | 349.19407 | P | 874.43387 | 437.72058 | 292.14948 | 5 |
| 12 | 1102.589... | 551.79821 | 368.20123 | G | 777.38111 | 389.19419 | 259.79855 | 4 |
| 13 | 1203.636... | 602.32205 | 401.88379 | T | 720.35965 | 360.68346 | 240.79140 | 3 |
| 14 | 1260.658... | 630.83278 | 420.89094 | G | 619.31197 | 310.15962 | 207.10884 | 2 |
| 15 | | | | K-Ben... | 562.29050 | 281.64889 | 188.10169 | 1 |



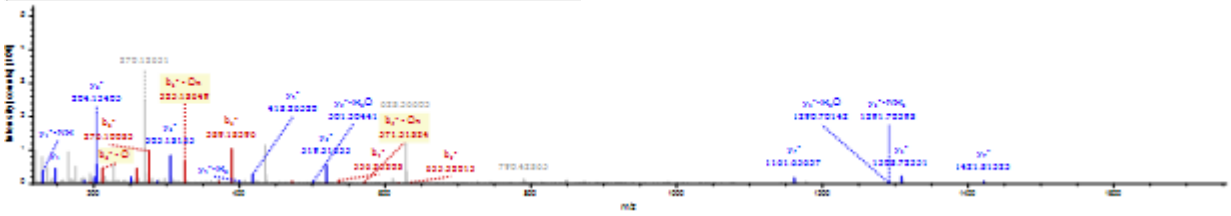
**Q6ZSZ5** (Rho guanine nucleotide exchange factor 18)
Sequence: KFQNLIK, K1-BenKPlus415 (415.17770 Da) (position: lysine 573)

| #1 | b+ | Seq. | y+ | #2 |
|---|---|---|---|---|
| 1 | 544.27994 | K-Ben... | | 7 |
| 2 | 691.34835 | F | 762.45085 | 6 |
| 3 | 819.40693 | Q | 615.38244 | 5 |
| 4 | 933.44986 | N | 487.32386 | 4 |
| 5 | 1046.533... | L | 373.28093 | 3 |
| 6 | 1159.617... | I | 260.19687 | 2 |
| 7 | | K | 147.11280 | 1 |



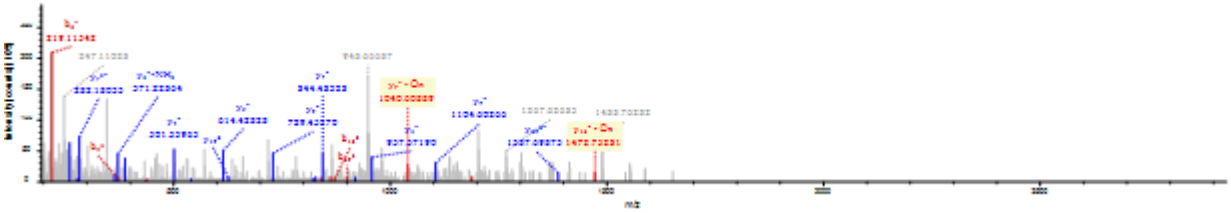
**P62979** (Ubiquitin-ribosomal protein eS31 fusion protein)
Sequence: MQIFVKTLTGK, M1-Oxidation (15.99492 Da), K6-BenKPlus415 (415.17770 Da) (position: lysine 6)

| #1 | b+ | b2+ | Seq. | y+ | y2+ | #2 |
|---|---|---|---|---|---|---|
| 1 | 148.04268 | 74.52498 | M-Oxi... | | | 11 |
| 2 | 276.10125 | 138.55427 | Q | 1549.865... | 775.43655 | 10 |
| 3 | 389.18532 | 195.09630 | I | 1421.807... | 711.40726 | 9 |
| 4 | 536.25373 | 268.63050 | F | 1308.723... | 654.86523 | 8 |
| 5 | 635.32215 | 318.16471 | V | 1161.654... | 581.33102 | 7 |
| 6 | 1178.594... | 589.80104 | K-Ben... | 1062.586... | 531.79681 | 6 |
| 7 | 1279.642... | 640.32488 | T | 519.31369 | 260.16048 | 5 |
| 8 | 1392.726... | 696.86691 | L | 418.26601 | 209.63664 | 4 |
| 9 | 1493.774... | 747.39075 | T | 305.18195 | 153.09461 | 3 |
| 10 | 1550.795... | 775.90148 | G | 204.13427 | 102.57077 | 2 |
| 11 | | | K | 147.11280 | 74.06004 | 1 |



**Q99832** (T-complex protein 1 subunit)
Sequence: AFFAKMVVDAVMMLDDLLQLK, M12-Oxidation (15.99492 Da), M13-Oxidation (15.99492 Da), K5-BenKPlus415 (415.17770 Da) (position: lysine 177)

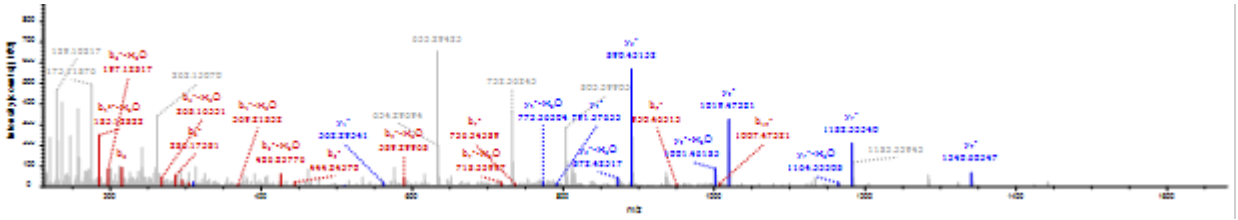
| #1 | b+ | b2+ | b3+ | Seq. | y+ | y2+ | y3+ | #2 |
|---|---|---|---|---|---|---|---|---|
| 1 | 72.04439 | 36.52583 | 24.68631 | A | | | | 21 |
| 2 | 219.11280 | 110.06004 | 73.70912 | F | 2774.388... | 1387.697... | 925.46775 | 20 |
| 3 | 366.18122 | 183.59425 | 122.73192 | F | 2627.320... | 1314.163... | 876.44495 | 19 |
| 4 | 437.21833 | 219.11280 | 146.41096 | A | 2480.251... | 1240.629... | 827.42215 | 18 |
| 5 | 980.49099 | 490.74914 | 327.50185 | K-Ben... | 2409.214... | 1205.111... | 803.74311 | 17 |
| 6 | 1111.531... | 556.26938 | 371.18201 | M | 1865.942... | 933.47469 | 622.65222 | 16 |
| 7 | 1210.599... | 605.80358 | 404.20482 | V | 1734.901... | 867.95445 | 578.97206 | 15 |
| 8 | 1309.668... | 655.33779 | 437.22762 | V | 1635.833... | 818.42024 | 545.94925 | 14 |
| 9 | 1424.695... | 712.85126 | 475.56993 | D | 1536.764... | 768.88603 | 512.92645 | 13 |
| 10 | 1495.732... | 748.36982 | 499.24897 | A | 1421.737... | 711.37256 | 474.58413 | 12 |
| 11 | 1594.800... | 797.90403 | 532.27178 | V | 1350.700... | 675.85401 | 450.90510 | 11 |
| 12 | 1741.836... | 871.42173 | 581.28358 | M-Oxi... | 1251.632... | 626.31980 | 417.88229 | 10 |
| 13 | 1888.871... | 944.93943 | 630.29538 | M-Oxi... | 1104.596... | 552.80210 | 368.87049 | 9 |
| 14 | 2001.955... | 1001.481... | 667.99006 | L | 957.56152 | 479.28440 | 319.85869 | 8 |
| 15 | 2116.982... | 1058.994... | 706.33238 | D | 844.47746 | 422.74237 | 282.16400 | 7 |
| 16 | 2232.009... | 1116.508... | 744.67469 | D | 729.45052 | 365.22890 | 243.82169 | 6 |
| 17 | 2345.093... | 1173.050... | 782.36938 | L | 614.42357 | 307.71543 | 205.47938 | 5 |
| 18 | 2458.177... | 1229.592... | 820.06407 | L | 501.33951 | 251.17339 | 167.78469 | 4 |
| 19 | 2586.236... | 1293.621... | 862.75026 | Q | 388.25545 | 194.63136 | 130.09000 | 3 |
| 20 | 2699.320... | 1350.163... | 900.44495 | L | 260.19687 | 130.60207 | 87.40381 | 2 |
| 21 | | | | K | 147.11280 | 74.06004 | 49.70912 | 1 |



**Q8TDJ6** (DmX-like protein 2)

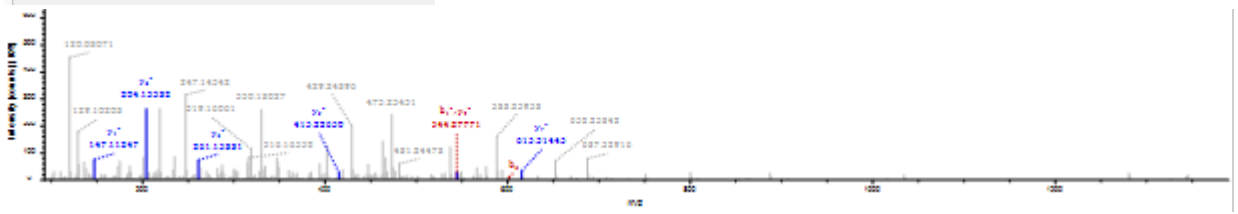
Sequence: TLATGYEVDGGK, K12-BenKPlus415 (415.17770 Da) (position: lysine 2068)

| #1 | b+ | b2+ | Seq. | y+ | y2+ | #2 |
|---|---|---|---|---|---|---|
| 1 | 102.05496 | 51.53112 | T | | | 12 |
| 2 | 215.13902 | 108.07315 | L | 1524.725... | 762.86615 | 11 |
| 3 | 286.17613 | 143.59170 | A | 1411.640... | 706.32412 | 10 |
| 4 | 387.22381 | 194.11554 | T | 1340.603... | 670.80556 | 9 |
| 5 | 444.24527 | 222.62628 | G | 1239.556... | 620.28173 | 8 |
| 6 | 607.30860 | 304.15794 | Y | 1182.534... | 591.77099 | 7 |
| 7 | 736.35120 | 368.67924 | E | 1019.471... | 510.23933 | 6 |
| 8 | 835.41961 | 418.21344 | V | 890.42879 | 445.71803 | 5 |
| 9 | 950.44655 | 475.72691 | D | 791.36037 | 396.18383 | 4 |
| 10 | 1007.468... | 504.23765 | G | 676.33343 | 338.67035 | 3 |
| 11 | 1064.489... | 532.74838 | G | 619.31197 | 310.15962 | 2 |
| 12 | | | K-Ben... | 562.29050 | 281.64889 | 1 |



**O14686** (Histone-lysine N-methyltransferase 2D)
Sequence: KGEAEGPGGK, K1-BenKPlus415 (415.17770 Da) (position: lysine 4832)

| #1 | b+ | Seq. | y+ | #2 |
|---|---|---|---|---|
| 1 | 544.27994 | K-Ben... | | 10 |
| 2 | 601.30140 | G | 801.37372 | 9 |
| 3 | 730.34400 | E | 744.35226 | 8 |
| 4 | 801.38111 | A | 615.30967 | 7 |
| 5 | 930.42370 | E | 544.27255 | 6 |
| 6 | 987.44517 | G | 415.22996 | 5 |
| 7 | 1084.497... | P | 358.20850 | 4 |
| 8 | 1141.519... | G | 261.15573 | 3 |
| 9 | 1198.540... | G | 204.13427 | 2 |
| 10 | | K | 147.11280 | 1 |



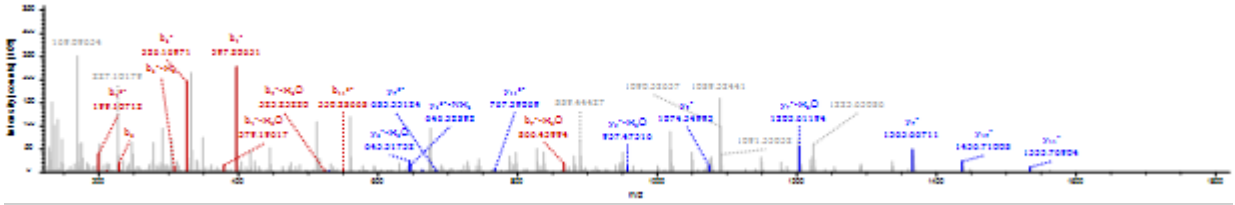
**Representative peptide spectra of modified peptides (Biotin-oxime sites, +415 on lysine)**
***All these peptides are identified from nuclear extract of LnCap without treatment with TACO chemistry.***

**-TACO modified peptides:**

**Q86YW9** (Mediator of RNA polymerase II transcription subunit 12-like protein)

Sequence: LDPAGSFVPTNTK, K13-BenKPlus415 (415.17770 Da) (position: lysine 1851)

| #1 | $b^+$ | $b^{2+}$ | Seq. | $y^+$ | $y^{2+}$ | #2 |
|---|---|---|---|---|---|---|
| 1 | 114.09134 | 57.54931 | L | | | 13 |
| 2 | 229.11828 | 115.06278 | D | 1648.788... | 824.89798 | 12 |
| 3 | 326.17105 | 163.58916 | P | 1533.761... | 767.38451 | 11 |
| 4 | 397.20816 | 199.10772 | A | 1436.708... | 718.85813 | 10 |
| 5 | 454.22962 | 227.61845 | G | 1365.671... | 683.33957 | 9 |
| 6 | 541.26165 | 271.13446 | S | 1308.650... | 654.82884 | 8 |
| 7 | 688.33007 | 344.66867 | F | 1221.618... | 611.31283 | 7 |
| 8 | 787.39848 | 394.20288 | V | 1074.549... | 537.77862 | 6 |
| 9 | 884.45124 | 442.72926 | P | 975.48155 | 488.24441 | 5 |
| 10 | 985.49892 | 493.25310 | T | 878.42879 | 439.71803 | 4 |
| 11 | 1099.541... | 550.27456 | N | 777.38111 | 389.19419 | 3 |
| 12 | 1200.589... | 600.79840 | T | 663.33818 | 332.17273 | 2 |
| 13 | | | K-Ben... | 562.29050 | 281.64889 | 1 |



## References


1. Chan, W. C.; White, P. D. Fmoc solid phase peptide synthesis: A practical approach (Oxford Univ. Press, New York, 2000).
2. Swaminathan, J.; Boulgakov, A.A.; Hernandez, E. T.; Bardo, A. M.; Bachman, J. L.; Marotta, J.; *et al.* Highly parallel single-molecule identification of proteins in zeptomole-scale mixtures. *Nat. Biotechnol.* **36**, 1076–1082 (2018).