Supplementary Material (ESI) for Chemical Science

Proximity-Driven Metallopeptide Catalysis: Remarkable Side-Chain Scope Enables Modification of Fos bZip Domain

Brian V. Popp and Zachary T. Ball*

Department of Chemistry, Rice University MS 60, 6100 Main Street, Houston, Texas

Supporting Information

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General Considerations.

Solvents and reagents were purchased from Fisher Scientific and used as received. Millipore ultra-purified water (18 MΩ) was used in all cases.

Experimental protocols. The terms "aqueous buffer" and "10X aqueous buffer" refers to aqueous solutions of 0.1 M and 1M *t*-BuNHOH·HCl at the indicated pH, respectively. Stock solutions of substrate peptides and metallopeptide catalysts were prepared in water and frozen in between uses. All modification reactions were carried out in microcentrifuge tubes (600 µL capacity). Reaction temperature and mixing was maintained with the following conditions: at 25 °C—rotary mixer or bed shaker, at 4 °C—magnetic stirring in an environmental "walk-in" refrigerator, at –15 °C—magnetic stirring in a Neslab cryobath.

Peptide synthesis. All peptides were synthesized with an AAPPTEC APEX 396 Automated Multipeptide Synthesizer using standard solid-phase Fmoc protocols.[1] Peptides were prepared using Rink amide MBHA resin (AAPPTEC) to afford the C-terminal amide and were acetylated at the N-terminus prior to cleavage from the resin. The purification was accomplished by reverse-phase HPLC with gradients of water-acetonitrile containing 0.1% trifluoroacetic acid, and peptides were isolated by lyophilization. Analysis and purity assessment was attained by mass spectrometry and analytical HPLC.

HPLC analysis. Reversed-phase HPLC (rp-HPLC) was performed using a Shimadzu Prominence system with a pair of LC-6AD pumps with Phenomenex Jupiter 4µ Proteo 90A (250 × 15 mm preparative) and Phenomenex Jupiter 4µ Proteo 90A (250 × 4.6 mm analytical) columns. Flow rates of 8 mL/min and 1 mL/min were used for preparative and analytical columns, respectively. Analytical spectra reported here were obtained using a 5% MeCN gradient in water. Both solvents contained 0.1% trifluoroacetic acid (TFA).

Mass Spectrometry. MALDI-MS and MS/MS analyses were performed on a Bruker Daltonics Autoflex MALDI-TOF/TOF mass spectrometer with CHCA matrix (10 mg/mL, Thermo Scientific Pierce). ESI-MS was performed on a Bruker Daltonics micrOTOF instrument. MS/MS spectra were collected at high substrate conversion on crude reaction mixtures. Data analysis was performed with the mMass program.[2]

LCMS analysis of c-Fos modification. Reversed-phase HPLC (rp-HPLC) was performed using a Shimadzu Prominence UFLCXR system with a pair of LC-20ADXR pumps with a Shimadzu Shim-pack XR-ODS column with 2.2 µm particle size (50 × 2.0 mm) at a flow rate of 0.25 mL/min. In-line mass spectrometry was performed with Shimadzu's 3-D ion trap time-of-flight ms (IT-TOF). Extracted ion current (EIC) spectra reported here were obtained using a 2.8% MeCN gradient in water. Both solvents contained 0.1% formic acid (FA). A sample of crude reaction (100 µM) was diluted to 1 µM with H_2O (0.1% FA) and 25 µL was injected onto the column. For MS analysis, ions in the range of 750–1300 m/z were collected with event and ion accumulation time set at 200 and 20 msec, respectively. For MS/MS analysis, ions with m/z = 1038.7 $[Fos•Mod]^{4+}$ and 1115.3 $[Fos•2Mod]^{4+}$ (width 3 m/z) were analyzed over 725 msec (repeat 3 times) with a 10 msec ion accumulation time. Major MS/MS fragment ions 1131.9 and 1174.6 (width 3 m/z) were selected for MS^3 analysis (time and ion accumulation 1560 and 50 msec, respectively).

Circular Dichroism Spectroscopy. CD spectra were obtained on a Jasco-J810 spectropolarimeter. The spectra were acquired with a 0.2-nm interval in the range of 185–260 nm. The temperature was maintained by a Jacso PTC423S water bath. Job plot data were obtained by maintaining a total peptide concentration of 0.2 mM in aqueous buffer at pH 6.9 using a 0.01 cm cell. Temperature denaturation experiments (–10 – 50 °C with a gradient of 1 °C/min) were performed on solutions of 0.1 mM peptide and 0.1 mM dirhodium metallopeptide in aqueous buffer in a 0.1 cm sealed cell, and ellipticity data were acquired at 222 nm. Temperature denaturation curves were fit to a two-state unfolding model as described previously.[3] All CD data were converted to residual mean ellipticity (mdeg•cm^2•dm^{-1}•residue^{-1}) by the equation

residual mean ellipticity = $[\theta] = \theta_{obs} / (10 \times l \times C \times N)$

where θ_{obs} is the ellipticity in millidegrees of rotation, l is the optical path length of the cell in cm, C is the concentration of the peptide in mol/L, and N is the number of residues in the peptide.

Synthesis of known compounds. The dirhodium precursor *cis*-$Rh_2(tfa)_2(OAc)_2$,[4] substrates **E3$_g$X** (X = W, Y, F),[5] catalyst **K3$_{a,e}$Rh$_2$**,[5] and diazo reagent [2-(2-methoxyethoxy)ethoxy]ethyl (*E*)-4-phenyl-2-diazo-3-butenoate (**1**)[6a] were prepared and purified according to published procedures.

Synthesis of metallopeptide Jun(Rh_2) from *cis*-$Rh_2(tfa)_2(OAc)_2$. Peptide **Jun** (7.0 mg, 1.9 μmol) and *cis*-$Rh_2(tfa)_2(OAc)_2$ (1.0 mg, 1.9 μmol) were added in a 1-dram vial equipped with a stir bar. A solution of MES buffer (2-(*N*-morpholino)ethanesulfonic acid, 1.6 mL, 0.1 M aq soln, pH 4.9) was added. The reaction was heated to 50 °C for 3 h. The dirhodium-peptide complex was purified by direct injection of the reaction mixture onto a preparative HPLC column. The complex was isolated by lyophilization to afford a dense blue powder (2 mg, 26% yield).

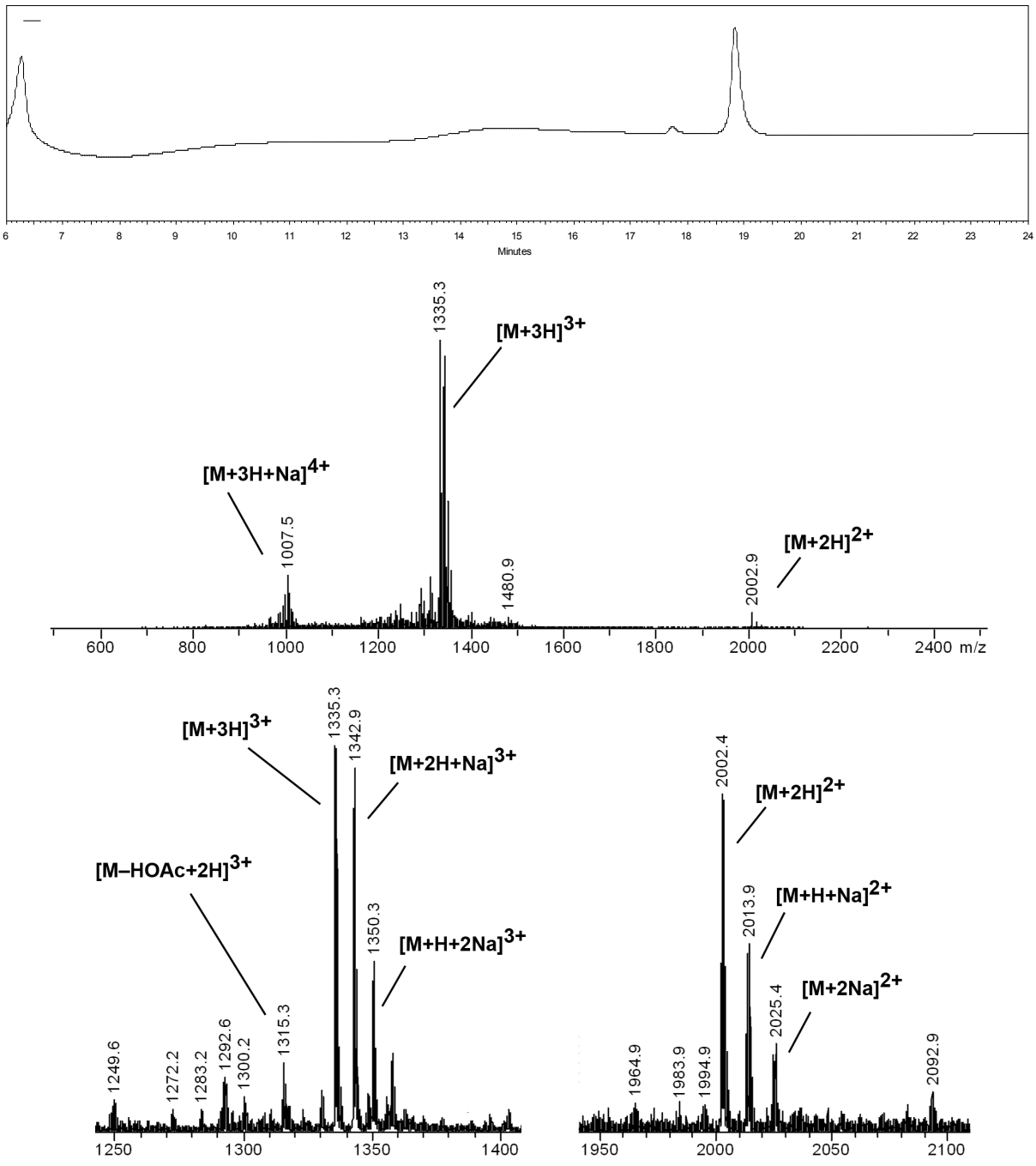


Fig. S-1 Analytical HPLC trace (300 nm) and ESI mass spectrum of isolated **Jun(Rh_2)**. Calculated mass for most abundant ion $[M+3H]^{3+}$: 1335.6; found: 1335.3.

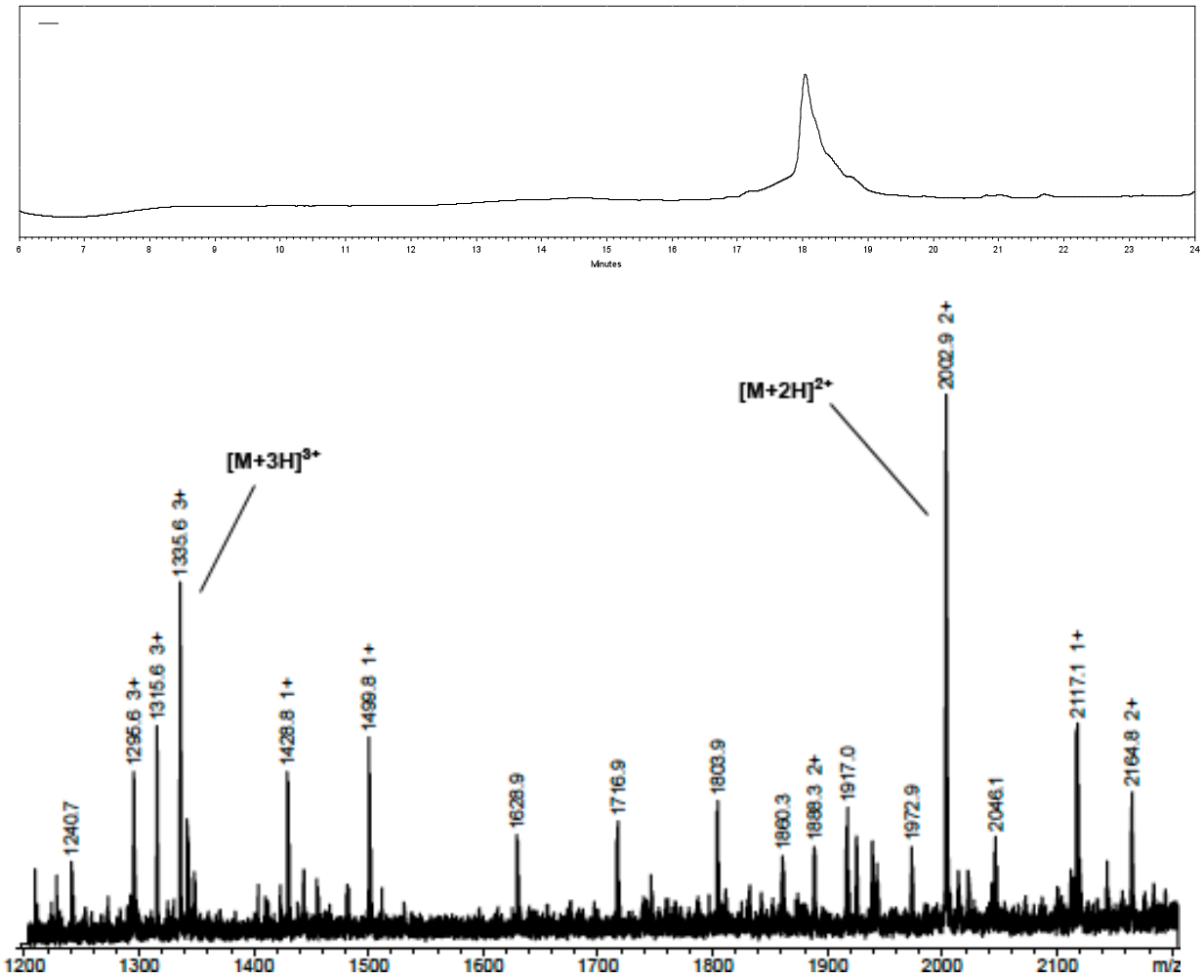


Fig. S-2 Analytical HPLC trace (300 nm) and ESI mass spectrum of crude **Jun(Rh_2)**.

Synthesis of metallopeptide Max(Rh$_2$) from *cis*-Rh$_2$(tfa)$_2$(OAc)$_2$. Following the procedure described for the synthesis of **Jun(Rh$_2$)**, **Max** peptide (2.9 mg, 0.0007 mmol) was metalated with the Rh$_2$ precursor (0.46 mg, 0.0008 mmol, 1.1 equiv) to afford **Max(Rh$_2$)** as a fluffy blue powder after HPLC purification and lyophilization (2.5 mg, 81% yield).

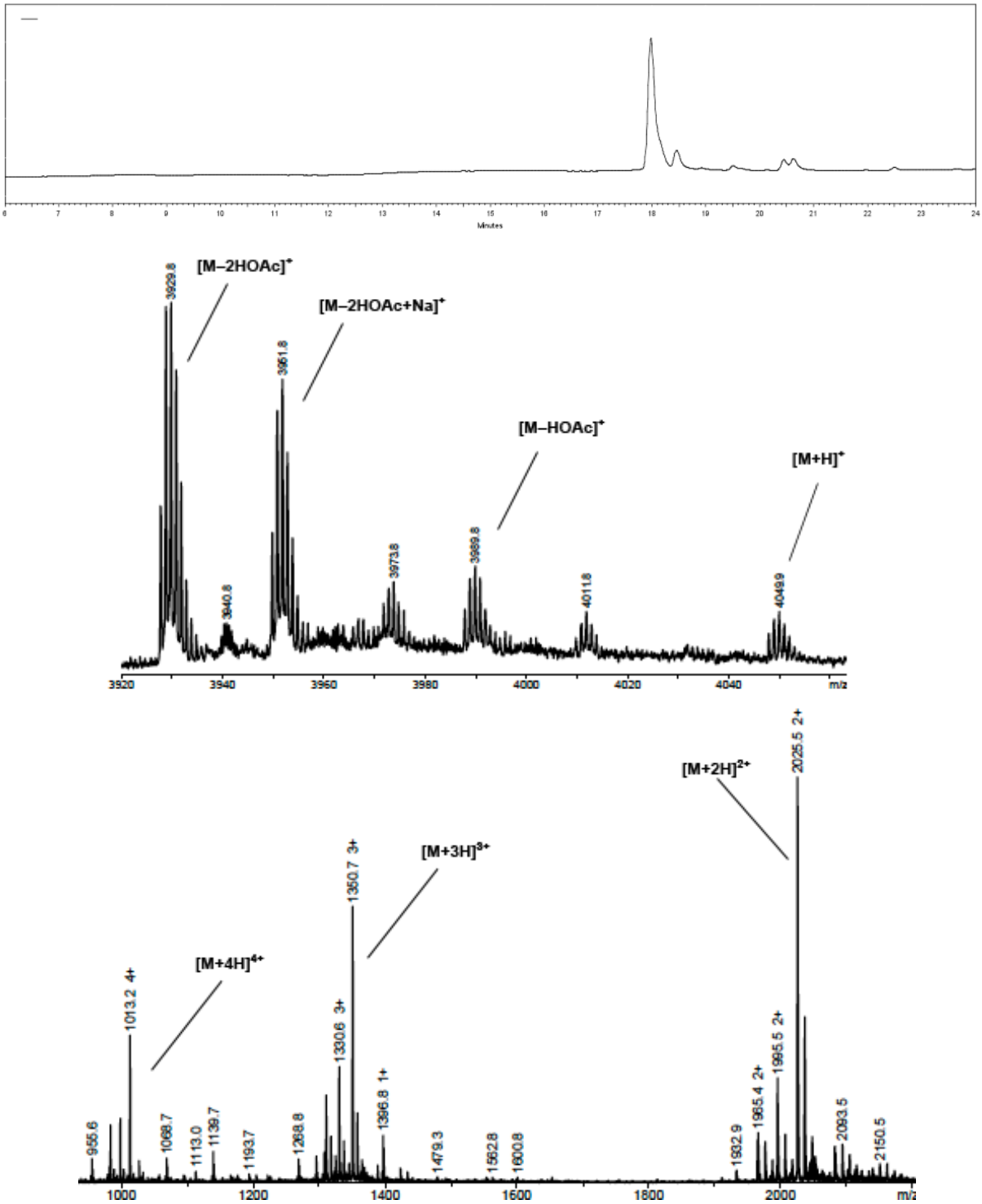


Fig. S-3 Analytical HPLC trace (300 nm) and ESI mass spectrum of isolated **Max(Rh$_2$)**. Calculated mass for monoisotopic [M+H]$^+$: 4049.8; found: 4047.9.

General Procedures:

***Procedure A* for catalytic side-chain modification: Reaction of $E3_gQ$ with diazo 1 (75 equiv) and $K3_{a,e}Rh_2$ (10 mol %).** Stock solutions of $E3_gQ$ (2.5 mM) and $K3_{a,e}Rh_2$ (0.25 mM) were prepared. A stock solution of diazo reagent **1** (23 mg in 329 μL *t*-BuOH, ~0.2 M) was also prepared. To a microcentrifuge tube, $E3_gQ$ stock (5.0 μL, 50 μM final concn) was dissolved in water (235 μL) and 10X aqueous buffer (25 μL, pH 6.2), followed by addition of $K3_{a,e}Rh_2$ stock (10 μL, 5 μM final concn). The reaction was initiated by addition of diazo stock (5 μL, 3.75 mM final concn). The total reaction volume was 250 μL with 2% *t*-BuOH co-solvent. The reaction tube was initially mixed for ca. 30 s with a bench-top vortex mixer and was then placed on either a bed shaker or a rotary mixer to react at room temperature. Aliquots (5 μL) for time-course analysis of the reaction were removed at specific times and were quenched by diluting in at least 10 μL of 70% MeCN in H_2O with 0.1% TFA.

***Procedure B* for catalytic side-chain modification: Reaction of $E3_gR$ with diazo 1 (100 equiv) and $K3_{a,e}Rh_2$ (50 mol %).** The method described in *Procedure A* was employed with the following alteration: two portions of diazo **1** (2x2 μL, ~0.6 M in *t*-BuOH) were added to initiate the reaction (t = 0 h) and at 6 h.

Procedure for $E3_gQ/W_{random}$ competitive modification experiment. Employing the metallopeptide procedure outlined in *General Procedure A* above, an additional stock of control peptide soln (5 μL of a 2.5 mM soln in water, 50 μM final concn) was added prior to addition of the metallopeptide stock solution.

General procedure for catalytic side-chain modification: Reaction of c-Fos with diazo 1 (50 equiv) and Jun(Rh_2) (200 mol % total). Stock solutions of **c-Fos** (2.5 mM) and **Jun(Rh_2)**[1] (0.25 mM) were prepared in water. A stock solution of diazo reagent **1** (10 mg in 112 μL *t*-BuOH, ~0.25 M) was also prepared. To a microcentrifuge tube equipped with a stirbar **c-Fos** stock (4.0 μL, 100 μM final concn) was dissolved in water (44 μL) and 10X aqueous buffer (10 μL, pH 6.6)[2] were mixed, followed by addition of **Jun(Rh_2)** stock (40 μL, 100 μM final concn). The reaction was initiated by addition of diazo stock (2 μL, 7.5 mM final concn). The initial reaction volume was 100 μL with 2% *t*-BuOH co-solvent. After 6h, a second bolus (2 μL) of diazo **1** stock solution was added. After 20 h, a third bolus of diazo **1** stock and a second bolus of **Jun(Rh_2)** (40 μL, 2 equiv total catalyst loading) were added**.** The reaction tube was mixed for ca. 30 s with a bench-top vortex mixer after every addition of diazo stock. Reactions were magnetically stirred at either 4 or –15 °C. In the latter case, ethylene glycol (20%) was also added to the reaction. Aliquots (2.5 μL) for time-course analysis of the reaction were removed at specific times and were quenched by diluting into a mixture of MeCN/H_2O (7:3) with 0.1% TFA.

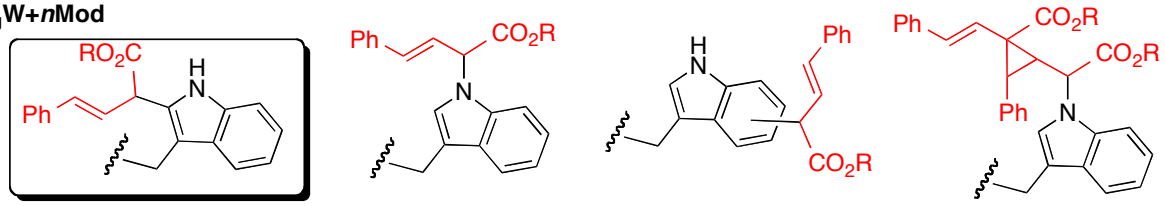
Modification Analysis: $E3_gX$ peptide conversion was determined exclusively from the ratio of modified to unmodified peptide using peak intensity from MALDI-TOF MS analysis and is uncorrected. Three spectra from different locations on the sample spot were acquired and averaged to obtain the reported conversion. This analysis was validated in our previous study in which conversion of $E3_gW$ was monitored by rp-HPLC/UV absorbance spectroscopy and MALDI-TOF MS methods as a function of time, which yielded comparable conversion data with variations ≤10%.[5]

[1] *Isolated* **Jun(Rh_2)** stock solution could be substituted with *unpurified* **Jun(Rh_2)** (Fig. S-2), in which case, the crude reaction used for metallopeptide preparation was added to the reaction directly.

[2] Optimal conversions were obtained at pH 6.5. Modest decreases in conversion were observed when the reaction was buffered bewteen pH 7-7.5.

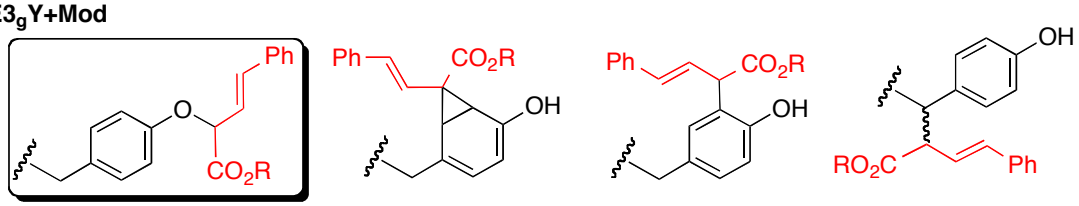
Chart S-1 Illustrations of possible side-chain bond connectivities.

E3$_g$W+*n*Mod

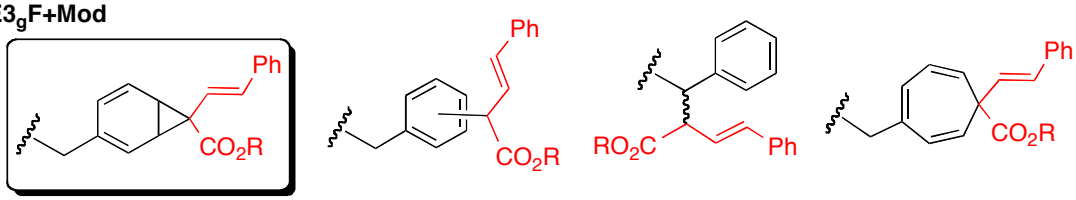


multiple modifications could arise from a combination of these mono-modied species or via cyclopropanation

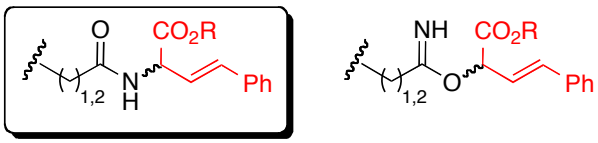
E3$_g$Y+Mod



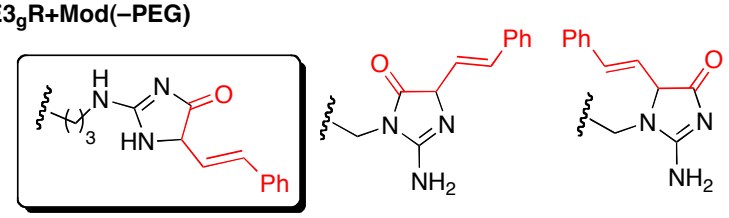
E3$_g$F+Mod



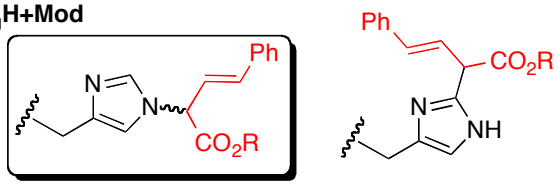
E3$_g$Q(N)+Mod



E3$_g$R+Mod(–PEG)



E3$_g$H+Mod



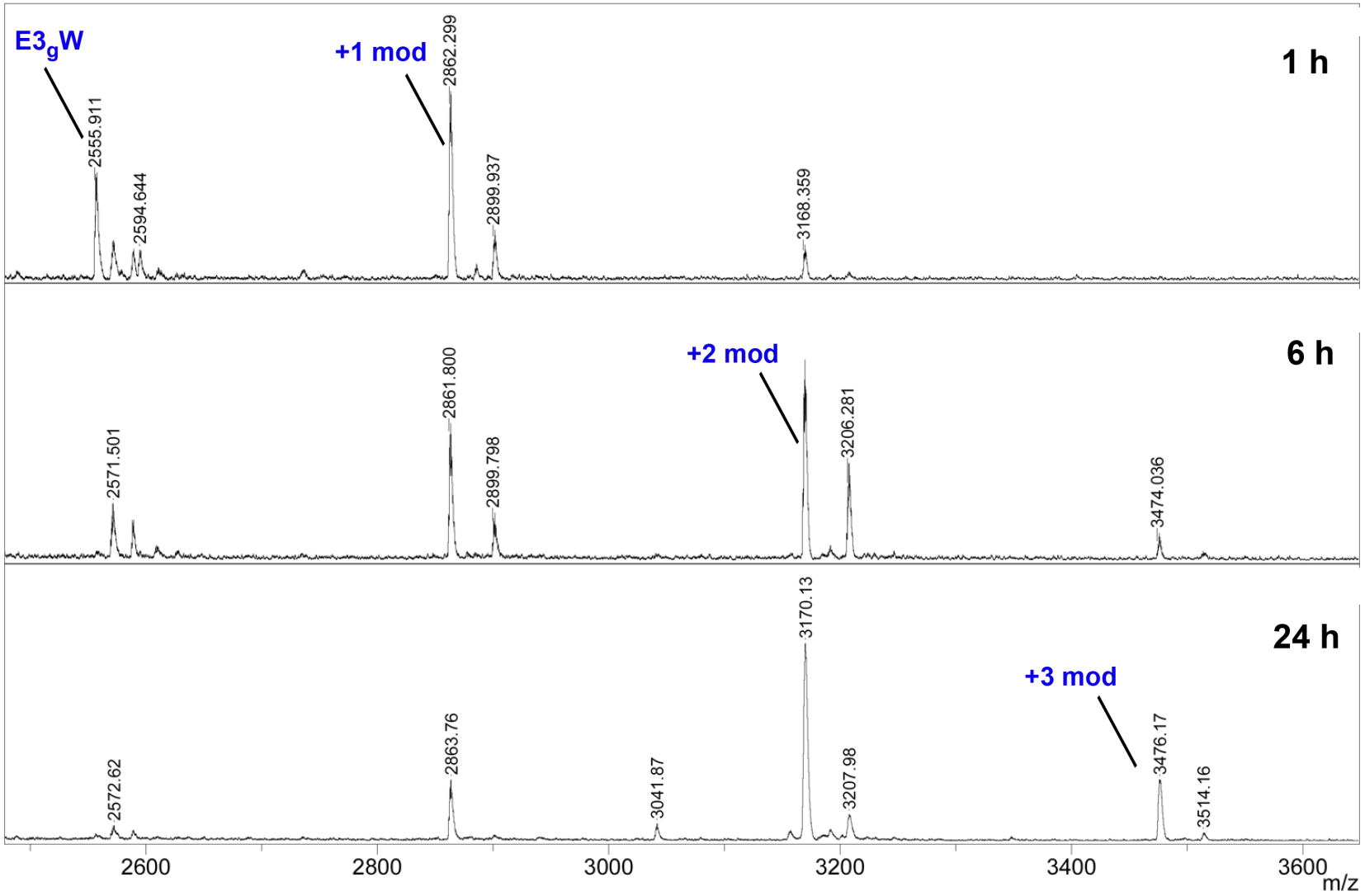


Fig. S-4 MALDI-TOF MS spectra of the modification (*Procedure A)* of peptide **E3$_g$W** with **K3$_{a,e}$Rh$_2$** (1 mol %) and 50 equiv diazo **1** . See Table 1 for conversion data.

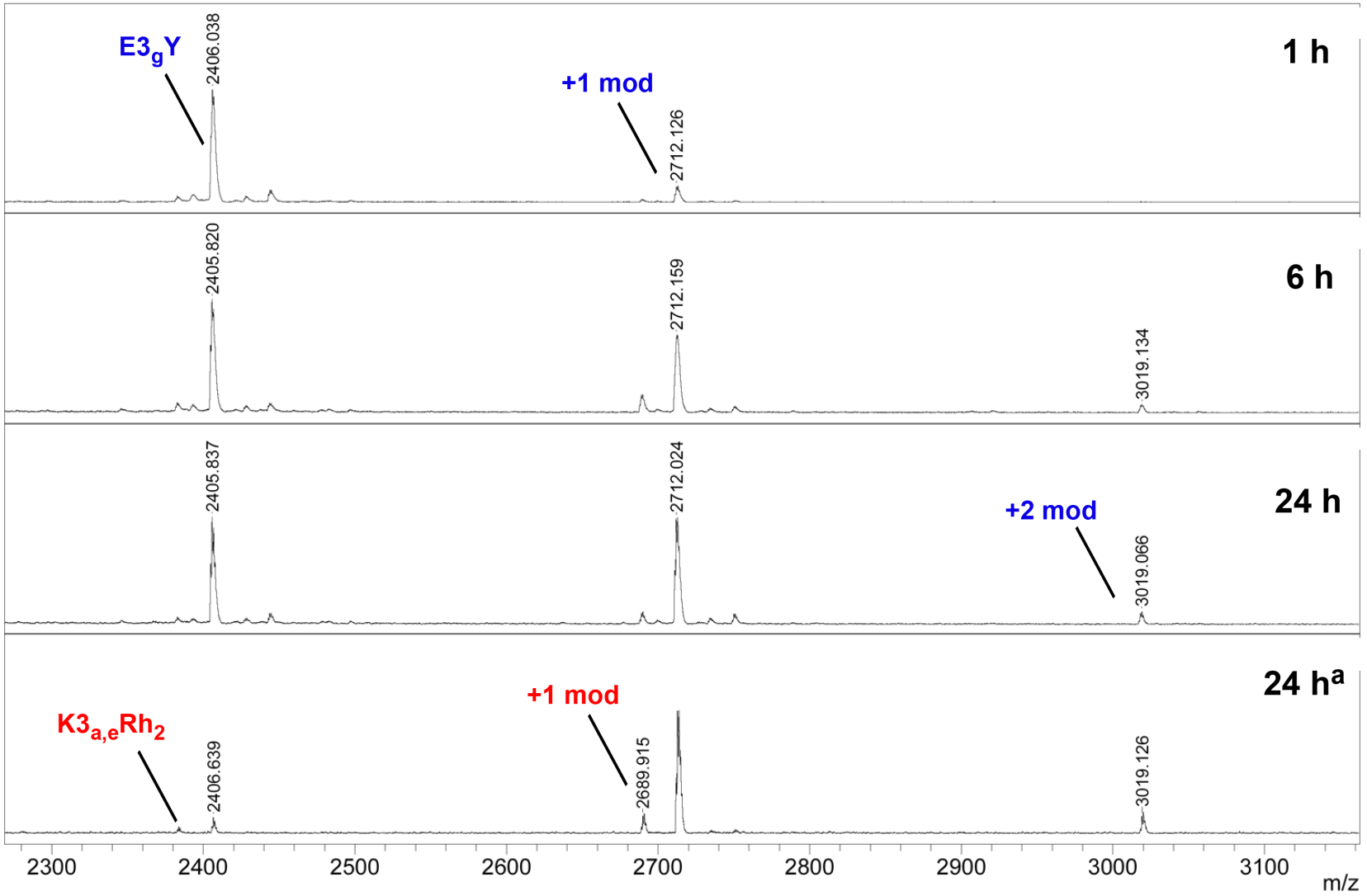


Fig. S-5 MALDI-TOF MS spectra of the modification (*Procedure A)* of peptide **E3$_g$Y** with **K3$_{a,e}$Rh$_2$** (10 mol %) and 50 equiv diazo **1**. a) Following *Procedure B* with **K3$_{a,e}$Rh$_2$** (10 mol %) and 50 equiv diazo **1**. See Table 1 for conversion data.

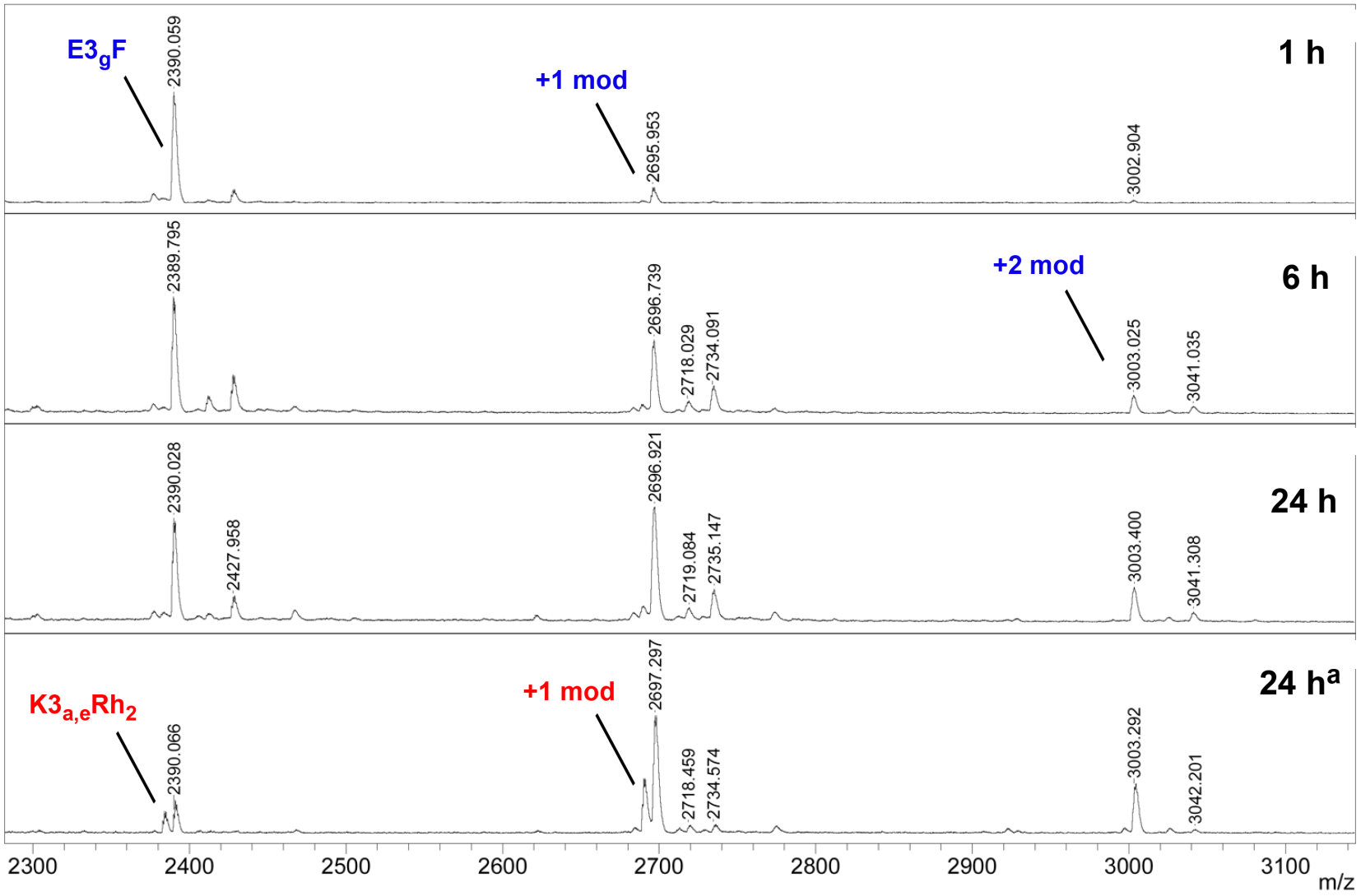


Fig. S-6 MALDI-TOF MS spectra of the modification (*Procedure A)* of peptide **E3$_g$F** with **K3$_{a,e}$Rh$_2$** (10 mol %) and 50 equiv diazo **1**. a) Following *Procedure B* with **K3$_{a,e}$Rh$_2$** (10 mol %) and 50 equiv diazo **1**. See Table 1 for conversion data.

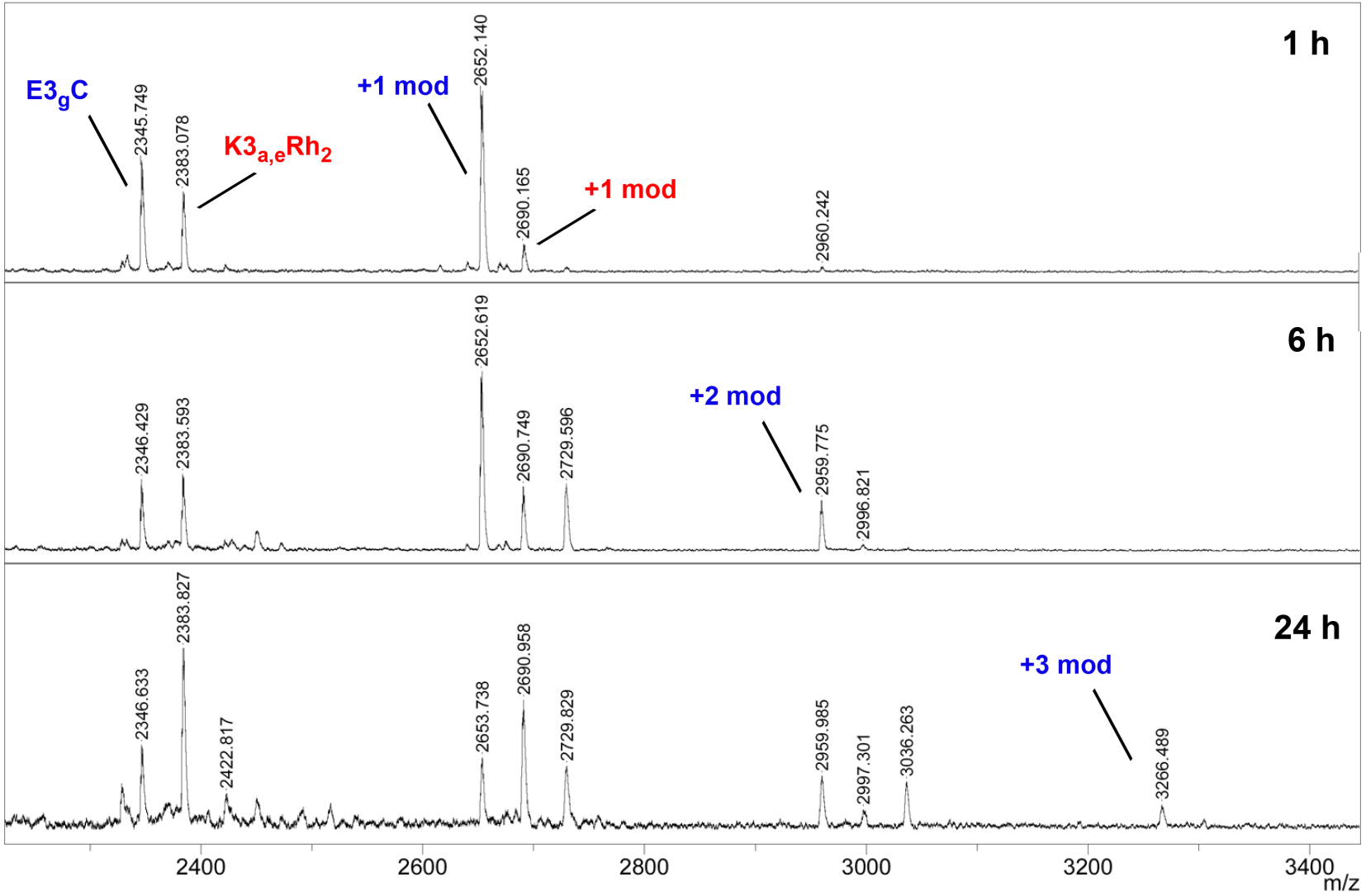


Fig. S-7 MALDI-TOF MS spectra of the modification (*Procedure A)* of peptide **E3$_g$C** with **K3$_{a,e}$Rh$_2$** (10 mol %) and 50 equiv diazo **1**. See Table 1 for conversion data.

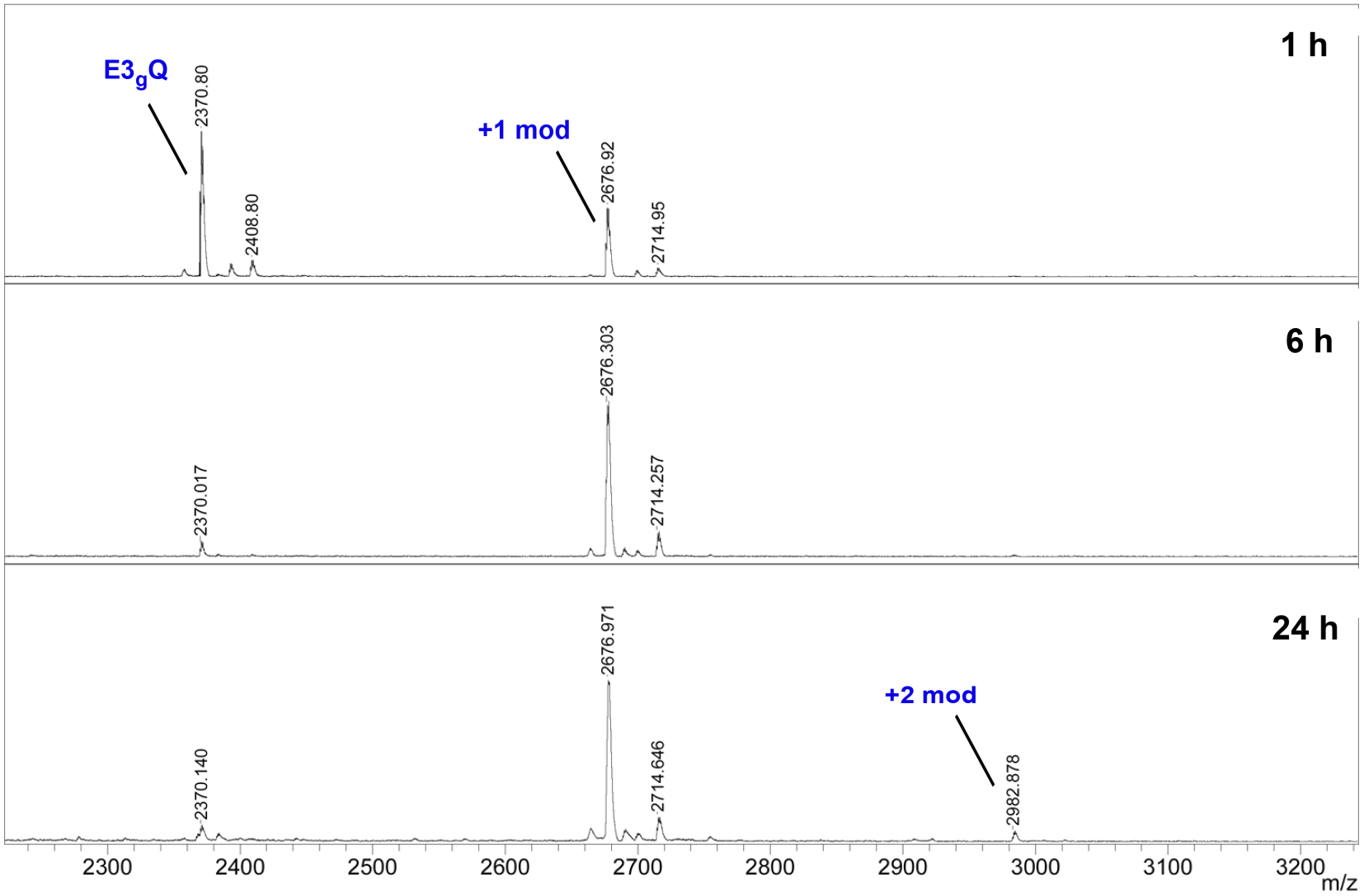


Fig. S-8 MALDI-TOF MS spectra of the modification (*Procedure A)* of peptide **E3$_g$Q** with **K3$_{a,e}$Rh$_2$** (10 mol %) and 50 equiv diazo **1**. See Table 1 for conversion data.

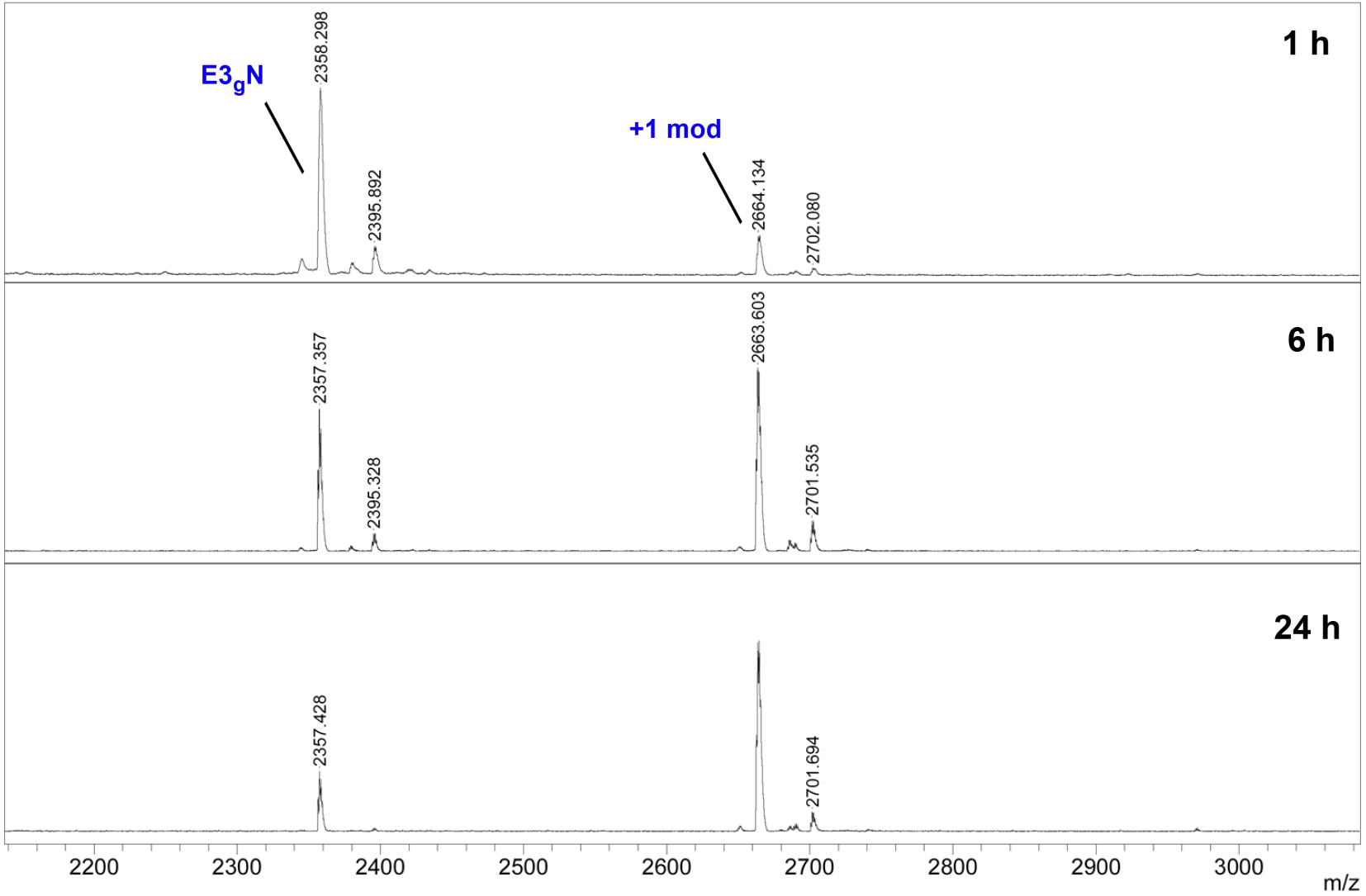


Fig. S-9 MALDI-TOF MS spectra of the modification (*Procedure B)* of peptide **E3$_g$N** with **K3$_{a,\epsilon}$Rh$_2$** (10 mol %) and 50 equiv diazo **1**. See Table 1 for conversion data.

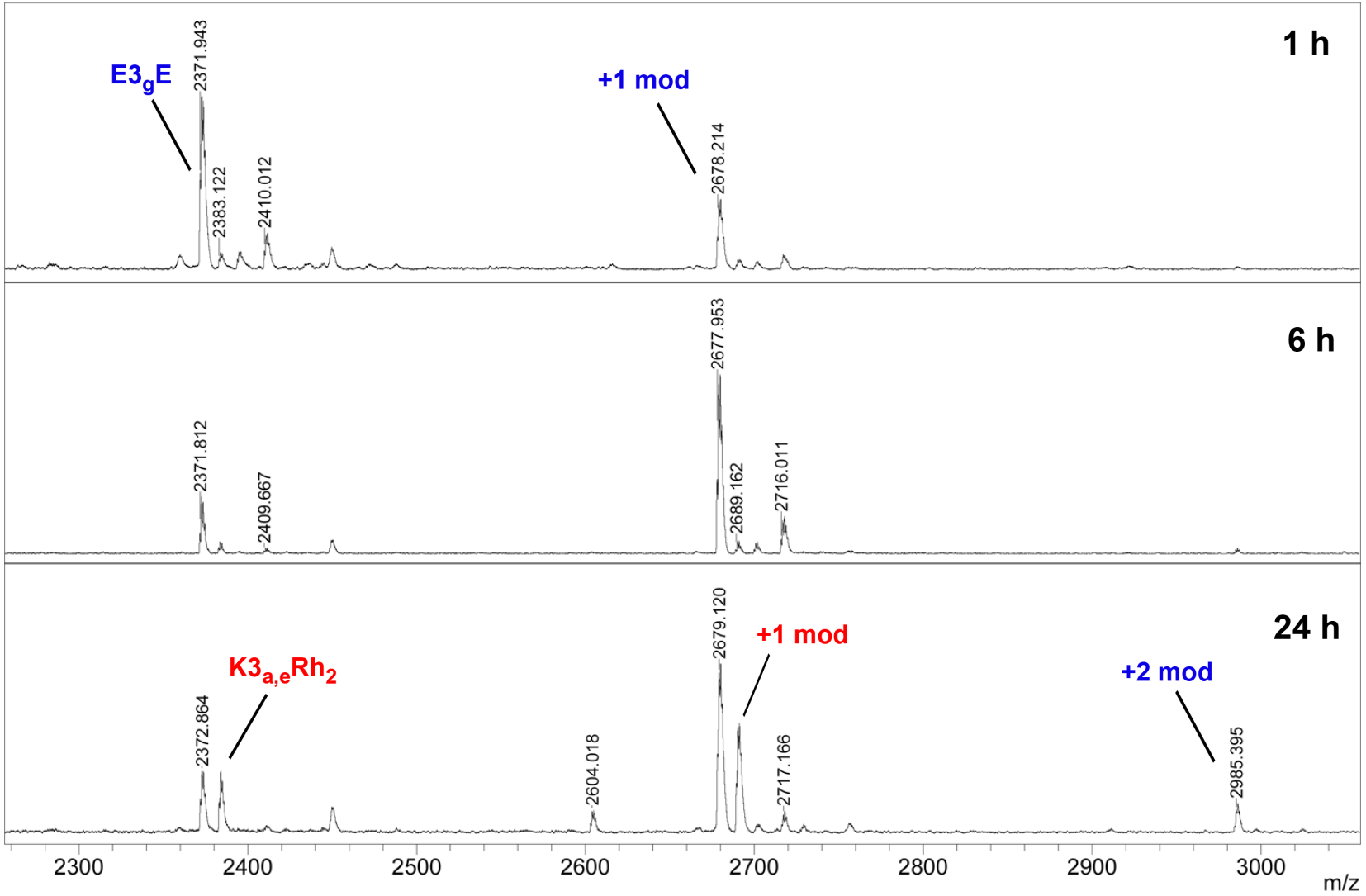


Fig. S-10 MALDI-TOF MS spectra of the modification (*Procedure B)* of peptide **E3$_g$E** with **K3$_{a,e}$Rh$_2$** (20 mol %) and 100 equiv diazo **1**. See Table 1 for conversion data.

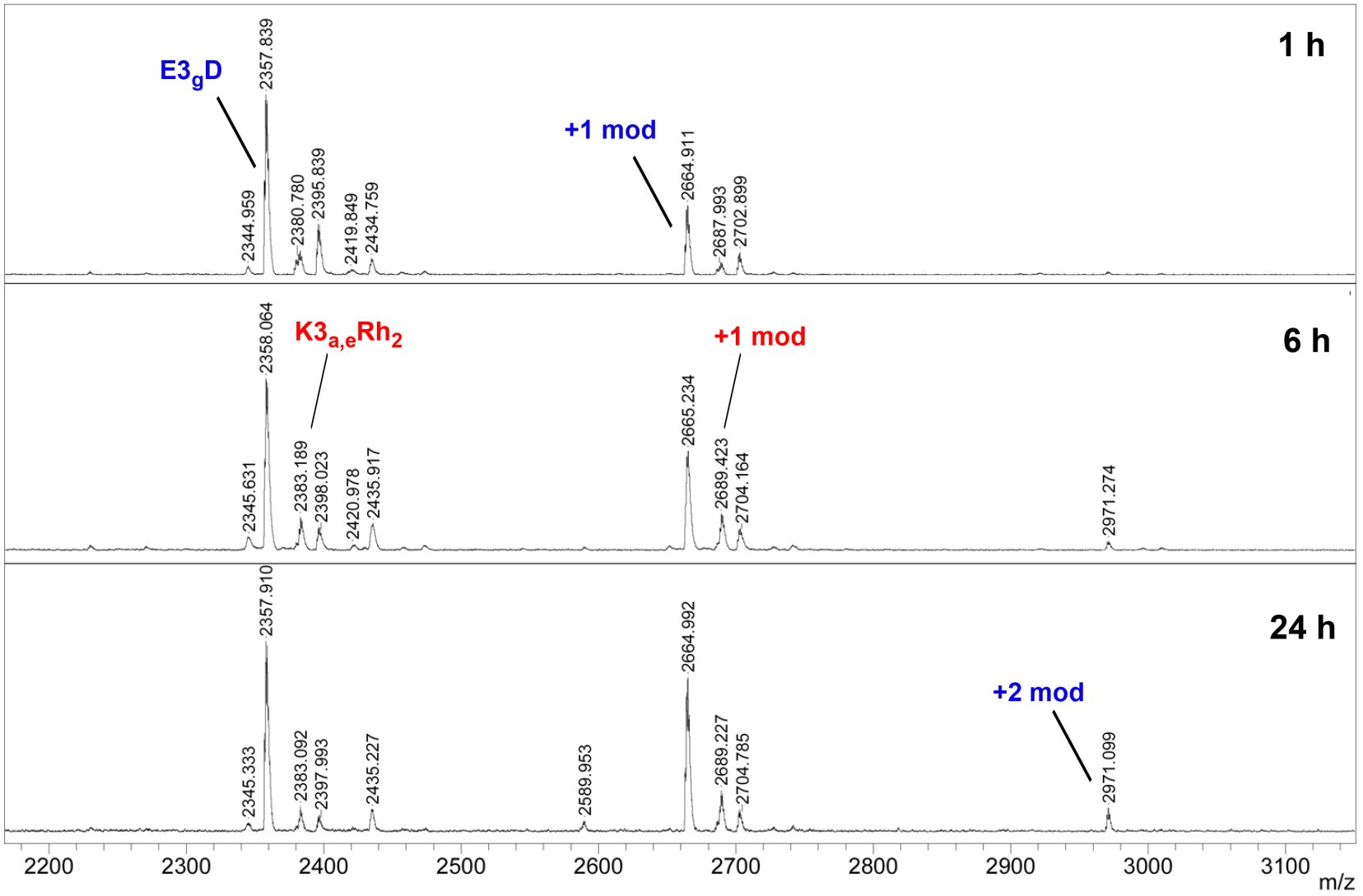


Fig. S-11 MALDI-TOF MS spectra of the modification (*Procedure B)* of peptide **E3$_g$D** with **K3$_{a,e}$Rh$_2$** (20 mol %) and 100 equiv diazo **1**. See Table 1 for conversion data.

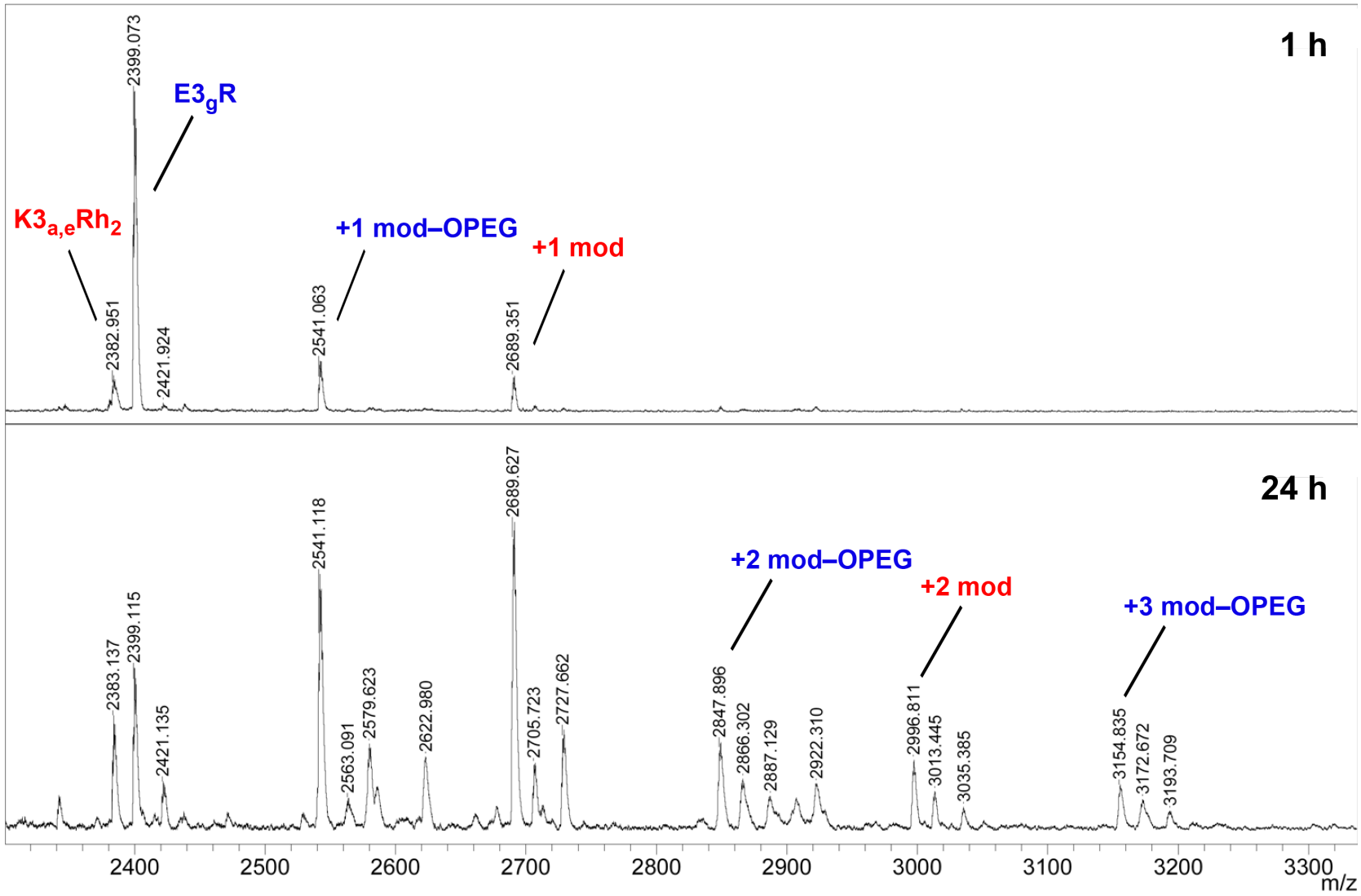


Fig. S-12 MALDI-TOF MS spectra of the modification (*Procedure B)* of peptide **E3$_g$R** with **K3$_{a,e}$Rh$_2$** (50 mol %) and 100 equiv diazo **1**. Loss of the OPEG side-chain from the ester is observed in the first modification product. See Table 1 for conversion data.

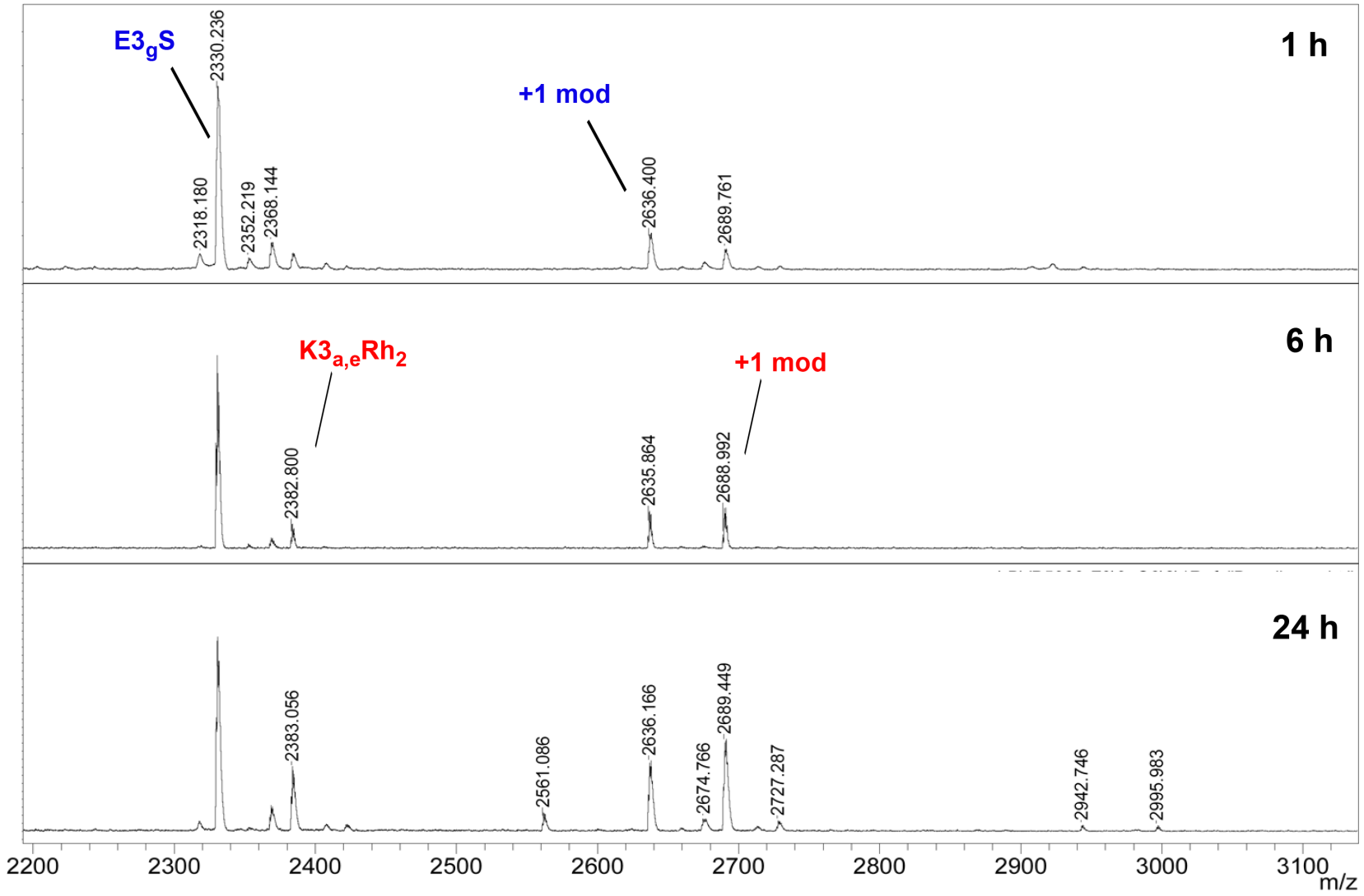


Fig. S-13 MALDI-TOF MS spectra of the modification (*Procedure B)* of peptide **E3$_g$S** with **K3$_{a,e}$Rh$_2$** (50 mol %) and 100 equiv diazo 1. See Table 1 for conversion data.

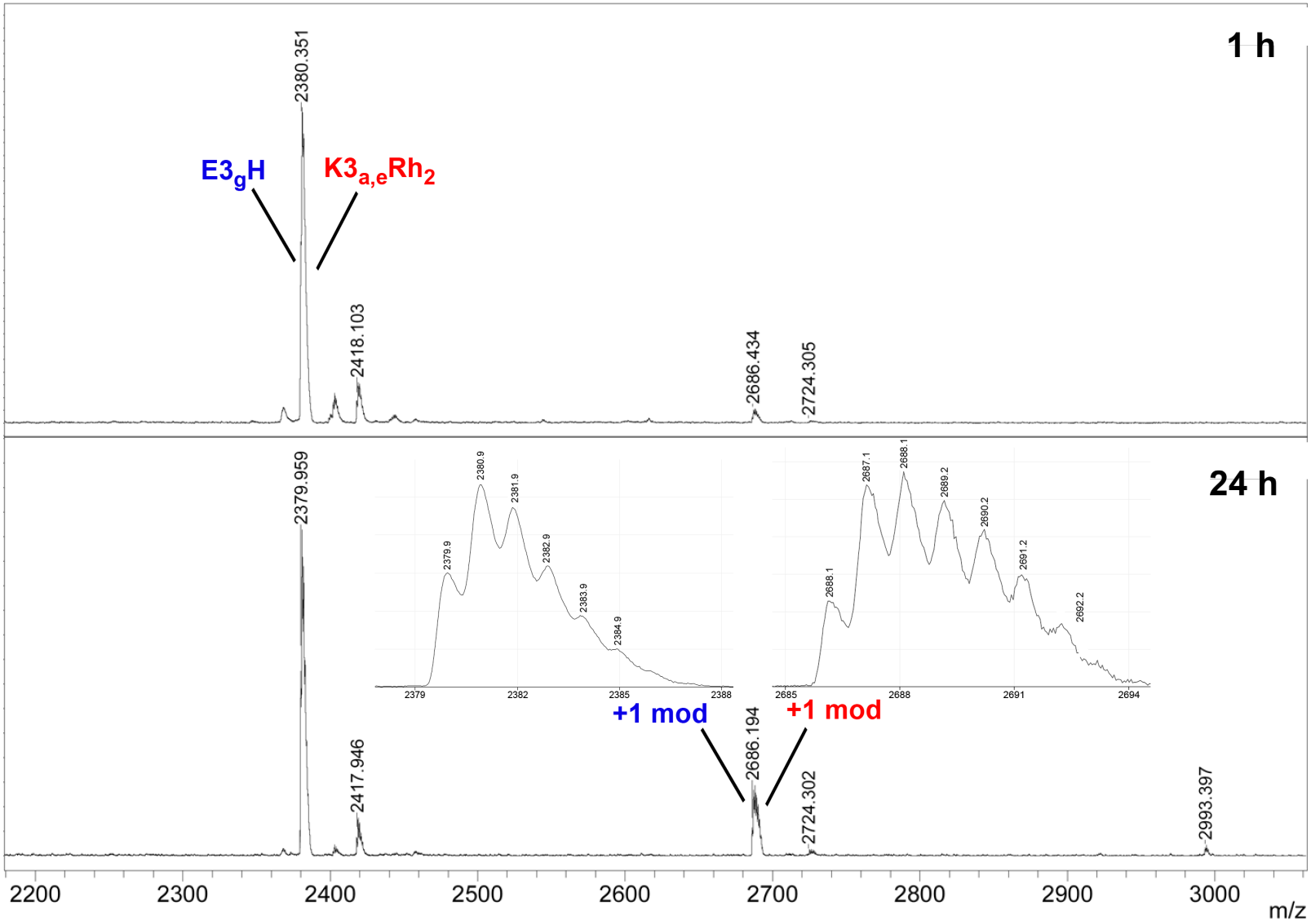


Fig. S-14 MALDI-TOF MS spectra of the modification (*Procedure B)* of peptide **E3$_g$H** with **K3$_{a,e}$Rh$_2$** (50 mol %) and 100 equiv diazo **1**. Conversion See Table 1 for conversion data.

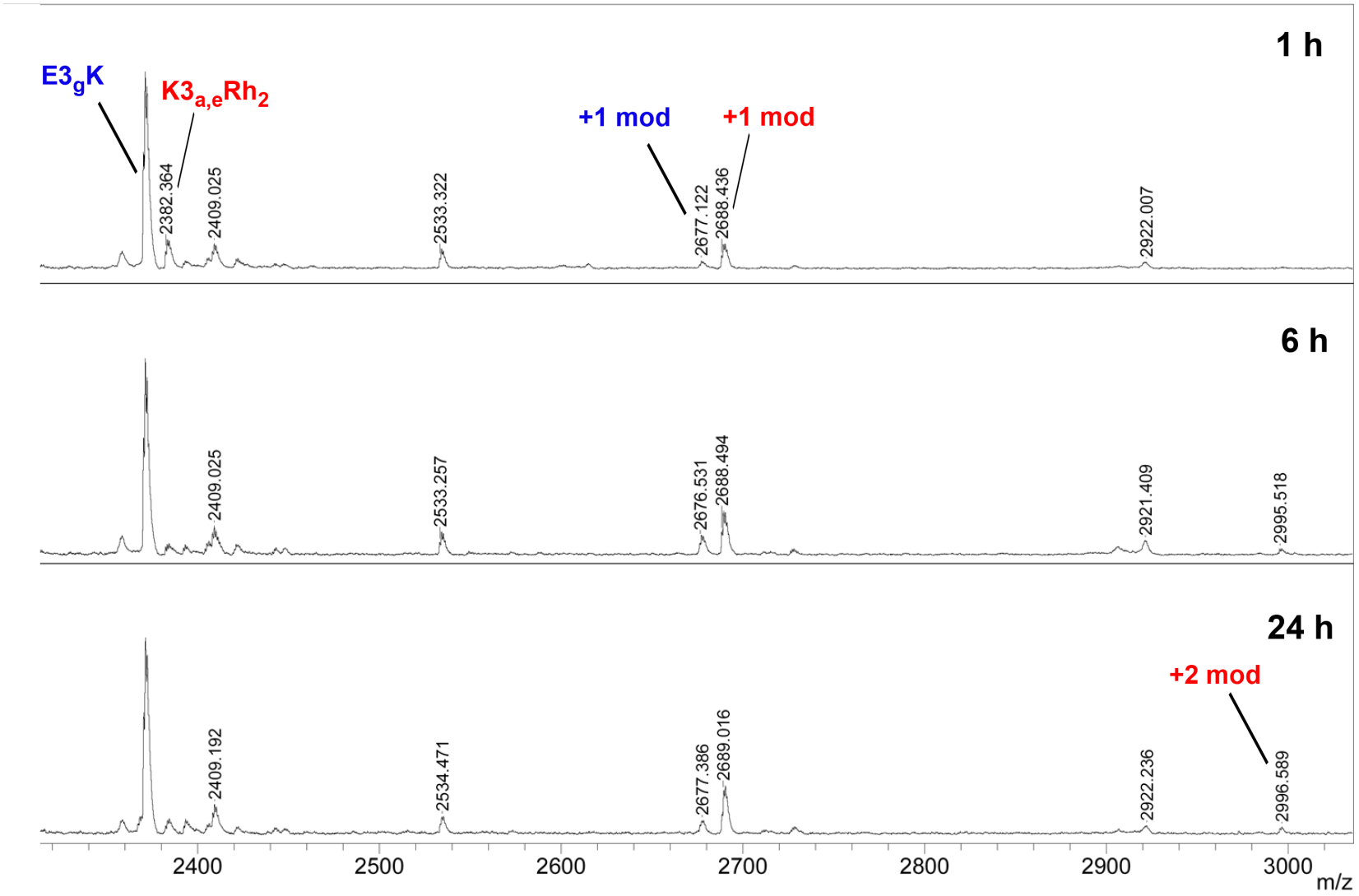


Fig. S-15 MALDI-TOF MS spectra of the modification (*Procedure B)* of peptide **E3$_g$K** with **K3$_{a,e}$Rh$_2$** (50 mol %) and 100 equiv diazo **1**. See Table 1 for conversion data.

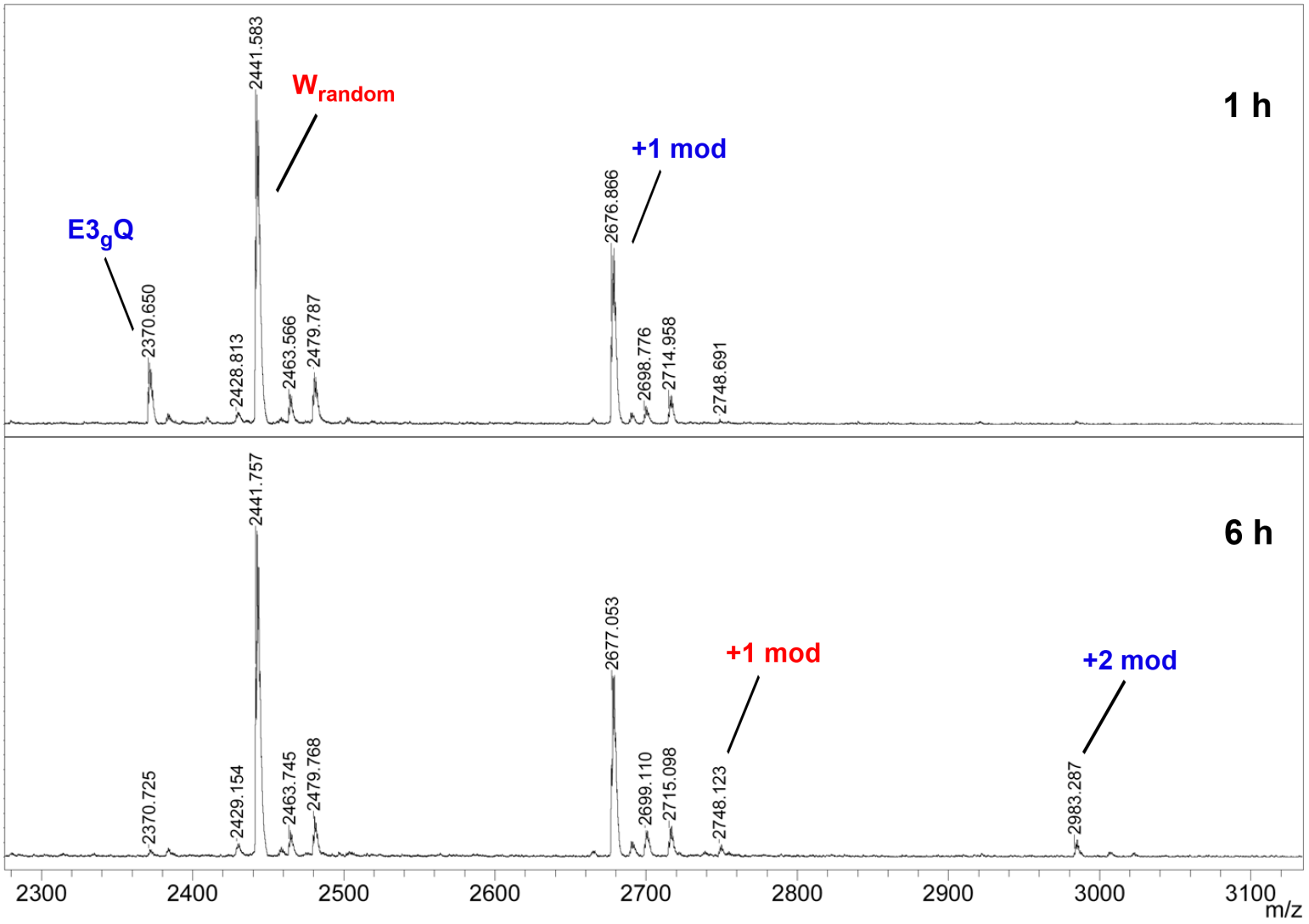


Fig. S-16 MALDI-TOF MS spectra of the competitive modification of peptide **E3$_g$Q** and **W$_{random}$** with **K3$_{a,e}$Rh$_2$** (10 mol %) and 50 equiv diazo **1**.

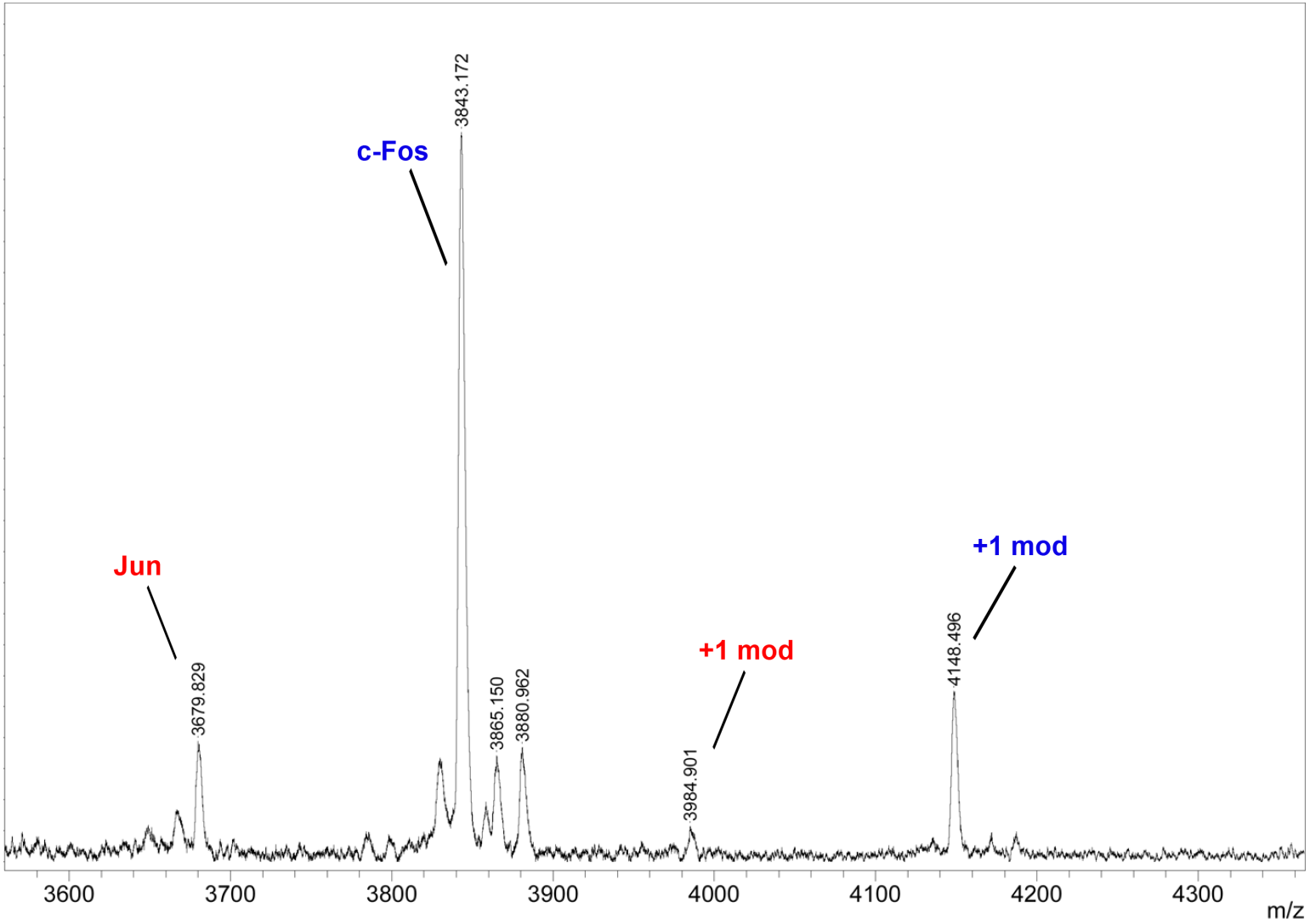


Fig. S-17 MALDI-TOF MS spectra of the modification of peptide **c-Fos** with isolated **Jun(Rh$_2$)** (100 mol %) and 100 equiv diazo **1** after 24 h at 25 °C.

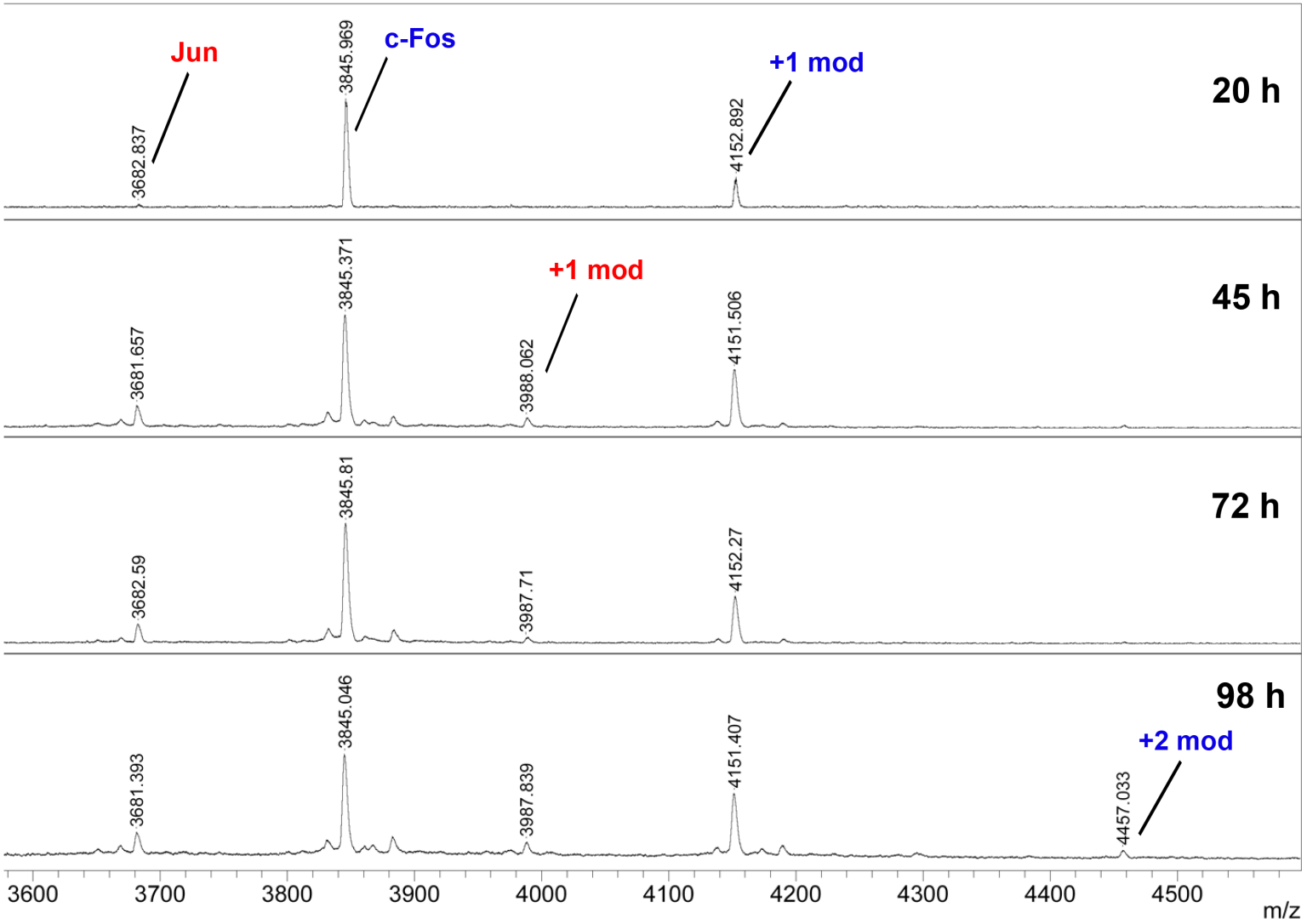


Fig. S-18 MALDI-TOF MS spectra of the modification of peptide **c-Fos** with isolated **Jun(Rh$_2$)** (100 mol% initial, 200 mol% after 20 h) and 75 equiv diazo **1** at 4 °C. See Fig. 2 for conversion data.

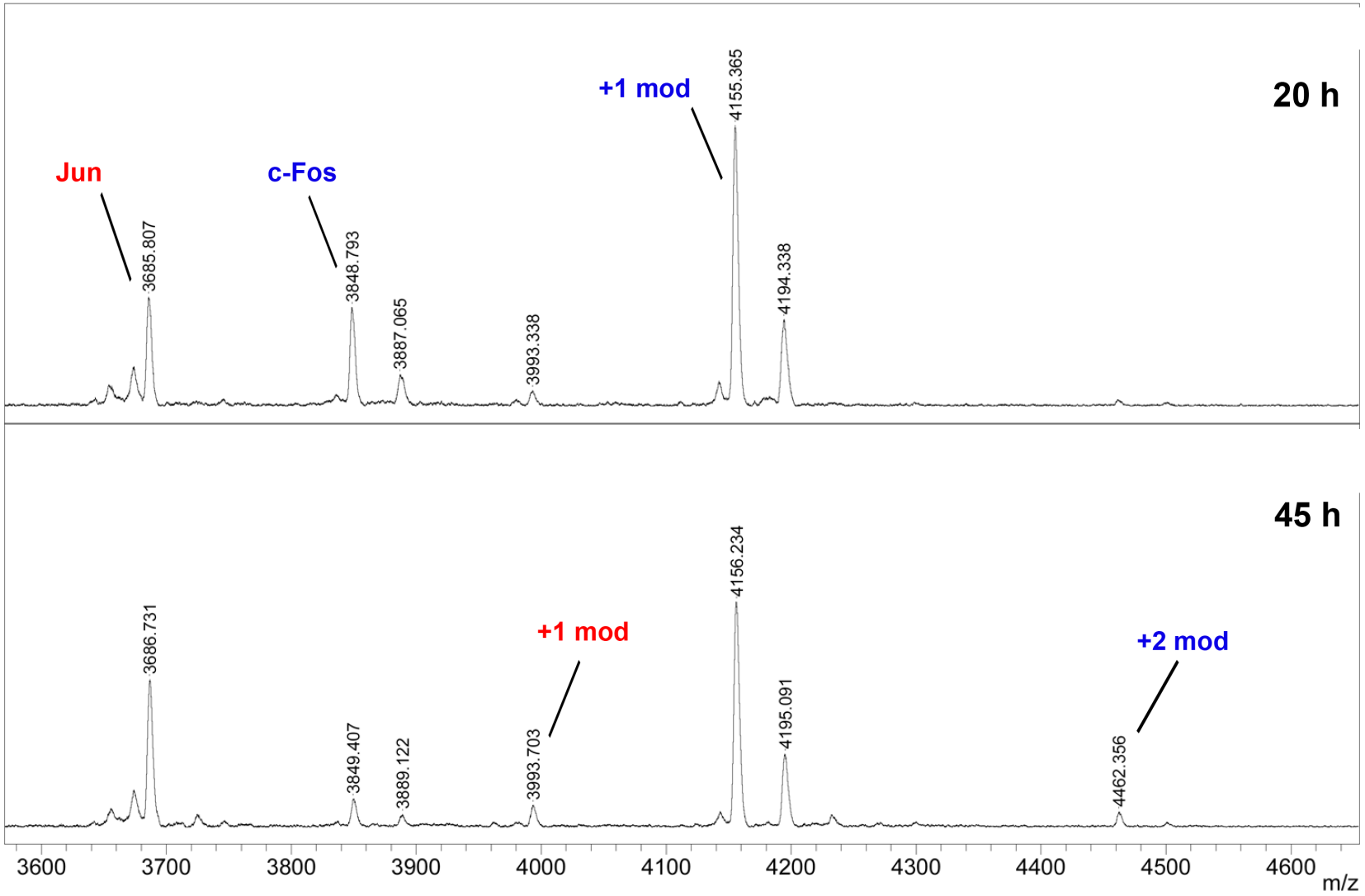


Fig. S-19 MALDI-TOF MS spectra of the modification of peptide **c-Fos** with crude **Jun(Rh$_2$)** (100 mol% initial, 200 mol% after 20 h) and 75 equiv diazo **1** at –15 °C. See Fig. 2 for conversion data.

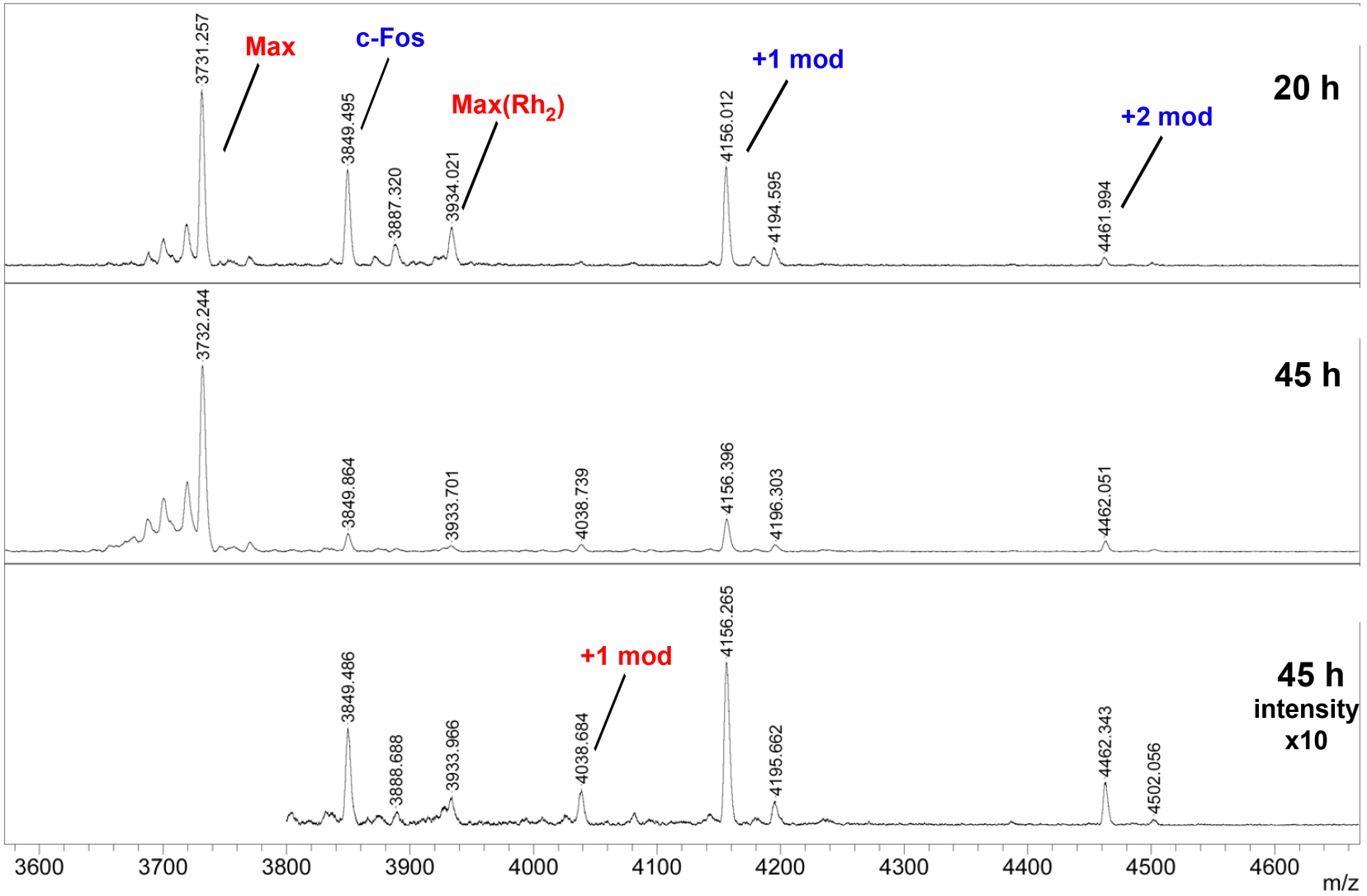


Fig. S-20 MALDI-TOF MS spectra of the modification of peptide **c-Fos** with **Max(Rh$_2$)** (100 mol% initial, 200 mol% after 20 h) and 75 equiv diazo **1** at 4 °C. See Fig. 2 for conversion data.

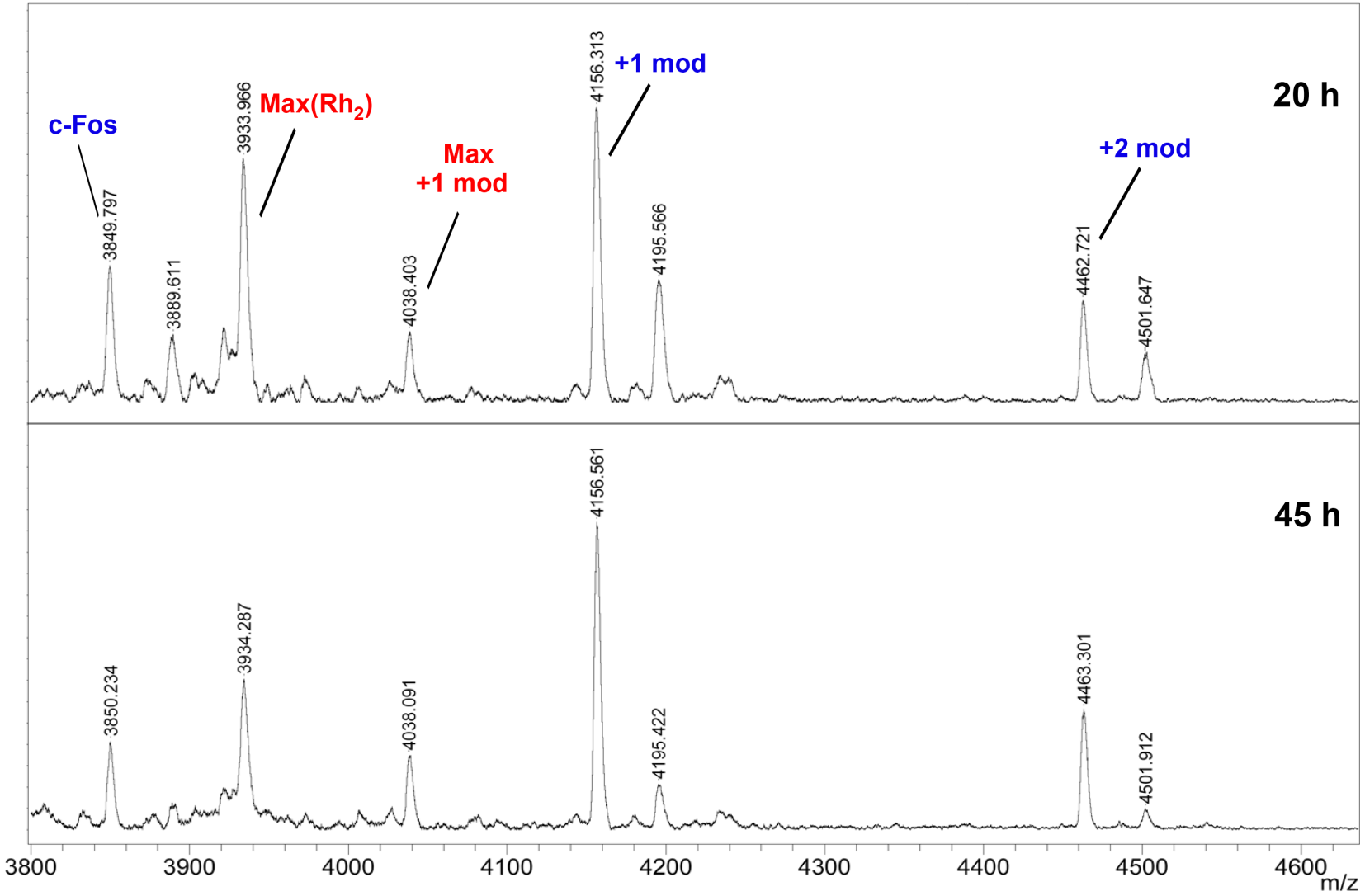


Fig. S-21 MALDI-TOF MS spectra of the modification of peptide **c-Fos** with **Max(Rh$_2$)** (100 mol% initial, 200 mol% after 20 h) and 75 equiv diazo **1** at –15 °C. See Fig. 2 for conversion data.

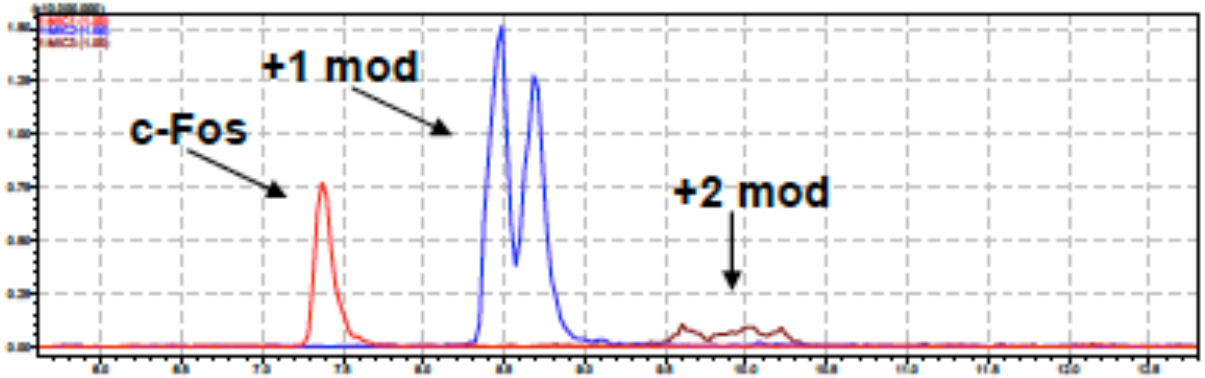


Fig. S-22 LCMS-IT-TOF EIC spectra of the crude modification reaction of peptide **c-Fos** with **Jun(Rh$_2$)** (100 mol% initial, 200 mol% after 20 h) and 75 equiv diazo **1** at –15 °C. Reaction conversion is 82 % based on peak area (uncorrected) with a 10:1 ratio of singly to doubly modified products. See Fig. 2 for comparative MALDI-TOF conversion data.

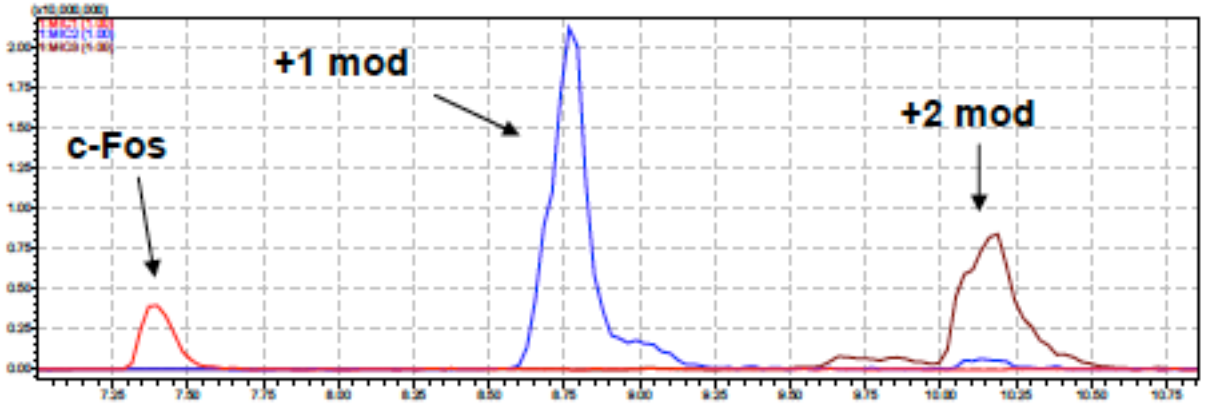


Fig. S-23 LCMS-IT-TOF EIC spectra of the crude modification reaction of peptide **c-Fos** with **Max(Rh$_2$)** (100 mol% initial, 200 mol% after 20 h) and 75 equiv diazo **1** at –15 °C. Reaction conversion is 91 % based on peak area (uncorrected) with a 1.8:1 ratio of singly to doubly modified products. See Fig. 3 for comparative MALDI-TOF conversion data.

MALDI-TOF MS/MS Modification Analysis—Jun(Rh_2)/c-Fos: c-Fos(+1mod)

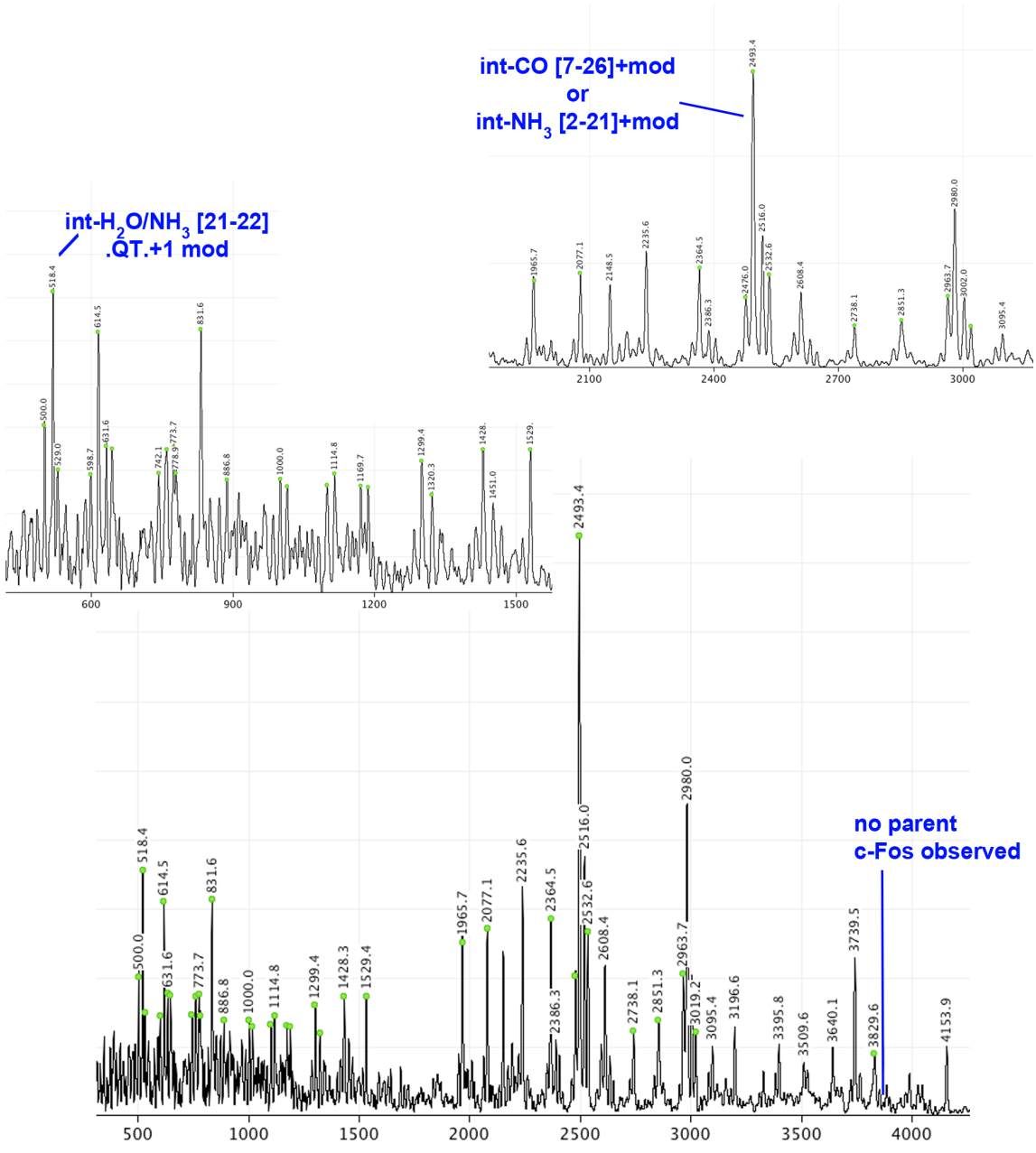


Fig. S-24 MALDI-TOF MS/MS spectrum and identified ion fragments (1.5 Da tolerance) of singly modified peptide **c-Fos**. Some diagnostic ions are labeled in the spectrum and identified ions (Table S-2) are labeled with a green dot.

Table S.1 Identified ion fragments (1.5 Da tolerance) from singly modified peptide **c-Fos**. Some diagnostic ions are in bold. Note: The sequence AS precedes the **c-Fos** sequence and is accounted for in this analysis.

assignment			mass (Da)	sequence (SDP=mod)	error (Da)
y-NH3	4	[31-34]	499.3	e.KEKL. [1xAmide]	0.7
int	-	[28-31]	499.3	l.LKEK.e	0.7
b-H2O	5	[1-5]	500.2	.ASTDT.l [1xAcetyl]	-0.2
int-H2O	-	[2-6]	500.2	a.STDTL.q	-0.2
b-NH3	5	[1-5]	501.2	.ASTDT.l [1xAcetyl]	-1.2
int-NH3	-	[2-6]	501.2	a.STDTL.q	-1.2
int-CO	-	[22-26]	501.3	q.TEIAN.l	-1.3
int-CO	-	[4-8]	501.3	t.DTLQA.e	-1.3
int-CO	-	[16-20]	501.3	d.EKSAL.q	-1.3
int-CO	-	[8-12]	517.2	q.AETDQ.l	1.2
int-CO	-	[7-11]	517.2	l.QAETD.q	1.2
b	5	[1-5]	518.2	.ASTDT.l [1xAcetyl]	0.2
int	-	[2-6]	518.2	a.STDTL.q	0.2
int-H2O	**-**	**[21-22]**	**518.2**	**l.QT.e [1xSDP]**	**0.2**
int-NH3	**-**	**[21-22]**	**519.2**	**l.QT.e [1xSDP]**	**-0.8**
int-NH3	-	[8-12]	528.2	q.AETDQ.l	0.8
int-NH3	-	[7-11]	528.2	l.QAETD.q	0.8
int	-	[22-26]	529.3	q.TEIAN.l	-0.3
int	-	[4-8]	529.3	t.DTLQA.e	-0.3
int	-	[16-20]	529.3	d.EKSAL.q	-0.3
int-H2O	**-**	**[20-21]**	**530.3**	**a.LQ.t [1xSDP]**	**-1.3**
int-H2O	-	[12-16]	597.3	d.QLEDE.k	1.4
int-H2O	-	[13-17]	597.3	q.LEDEK.s	1.4
int-NH3	-	[12-16]	598.2	d.QLEDE.k	0.5
int-NH3	-	[13-17]	598.3	q.LEDEK.s	0.4
int	-	[26-30]	598.4	a.NLLKE.k	0.3
int-NH3	-	[3-8]	613.3	s.TDTLQA.e	1.2
b-H2O	6	[1-6]	613.3	.ASTDTL.q [1xAcetyl]	1.2
b-NH3	6	[1-6]	614.3	.ASTDTL.q [1xAcetyl]	0.2
int-CO	-	[22-27]	614.4	q.TEIANL.l	0.1
int	-	[12-16]	615.3	d.QLEDE.k	-0.8
int	-	[13-17]	615.3	q.LEDEK.s	-0.8
int-CO	-	[29-33]	615.4	l.KEKEK.l	-0.9
int-CO	-	[8-13]	630.3	q.AETDQL.e	1.3
int-CO	-	[6-11]	630.3	t.LQAETD.q	1.3
int-CO	-	[4-9]	630.3	t.DTLQAE.t	1.3
int	-	[3-8]	630.3	s.TDTLQA.e	1.3
b	6	[1-6]	631.3	.ASTDTL.q [1xAcetyl]	0.3
int-H2O	**-**	**[20-22]**	**631.3**	**a.LQT.e [1xSDP]**	**0.2**
int-CO	-	[14-19]	632.3	l.EDEKSA.l	-0.7
int-NH3	**-**	**[20-22]**	**632.3**	**a.LQT.e [1xSDP]**	**-0.7**
int-H2O	-	[14-19]	642.3	l.EDEKSA.l	1.2
int	-	[22-27]	642.3	q.TEIANL.l	1.1
int-NH3	-	[14-19]	643.3	l.EDEKSA.l	0.2
int	-	[29-33]	643.4	l.KEKEK.l	0
int	-	[15-20]	644.3	e.DEKSAL.q	-0.9
int	-	[5-10]	644.3	d.TLQAET.d	-0.9

int-H2O	-		[5-11]	741.3	d.TLQAETD.q	0.8
int-H2O	-		[4-10]	741.3	t.DTLQAET.d	0.8
int-H2O	-		[3-9]	741.3	s.TDTLQAE.t	0.8
b-H2O		7	[1-7]	741.3	.ASTDTLQ.a [1xAcetyl]	0.8
int	-		[27-32]	741.5	n.LLKEKE.k	0.7
int-NH3	-		[5-11]	742.3	d.TLQAETD.q	-0.2
int-NH3	-		[4-10]	742.3	t.DTLQAET.d	-0.2
int-NH3	-		[3-9]	742.3	s.TDTLQAE.t	-0.2
b-NH3		7	[1-7]	742.3	.ASTDTLQ.a [1xAcetyl]	-0.2
int	-		[12-17]	743.4	d.QLEDEK.s	-1.3
int-CO	-		[7-13]	758.4	l.QAETDQL.e	0.8
int-CO	-		[6-12]	758.4	t.LQAETDQ.l	0.8
int-CO	-		[8-14]	759.4	q.AETDQLE.d	-0.1
int	-		[5-11]	759.4	d.TLQAETD.q	-0.1
int	-		[4-10]	759.4	t.DTLQAET.d	-0.1
int	-		[3-9]	759.4	s.TDTLQAE.t	-0.1
b		7	[1-7]	759.4	.ASTDTLQ.a [1xAcetyl]	-0.1
int-H2O	**-**		**[21-24]**	**760.4**	**l.QTEI.a [1xSDP]**	**-1.2**
int-H2O	**-**		**[20-23]**	**760.4**	**a.LQTE.i [1xSDP]**	**-1.2**
int	-		[14-20]	773.4	l.EDEKSAL.q	0.3
int	-		[13-19]	773.4	q.LEDEKSA.l	0.3
y		6	[29-34]	773.5	l.KEKEKL. [1xAmide]	0.2
int	**-**		**[21-24]**	**778.4**	**l.QTEI.a [1xSDP]**	**0.6**
int	**-**		**[20-23]**	**778.4**	**a.LQTE.i [1xSDP]**	**0.6**
int-CO	**-**		**[18-22]**	**779.4**	**k.SALQT.e [1xSDP]**	**-0.5**
int-H2O	-		[25-31]	779.5	i.ANLLKEK.e	-0.5
int	-		[12-18]	830.4	d.QLEDEKS.a	1.2
int-CO	-		[11-17]	830.4	t.DQLEDEK.s	1.2
b		8	[1-8]	830.4	.ASTDTLQA.e [1xAcetyl]	1.2
int	-		[10-16]	831.3	e.TDQLEDE.k	0.2
int	-		[9-15]	831.3	a.ETDQLED.e	0.2
int-H2O	**-**		**[21-25]**	**831.4**	**l.QTEIA.n [1xSDP]**	**0.2**
int-H2O	**-**		**[19-23]**	**831.4**	**s.ALQTE.i [1xSDP]**	**0.2**
int-NH3	**-**		**[21-25]**	**832.4**	**l.QTEIA.n [1xSDP]**	**-0.8**
int-NH3	**-**		**[19-23]**	**832.4**	**s.ALQTE.i [1xSDP]**	**-0.8**
int-CO	-		[3-10]	832.4	s.TDTLQAET.d	-0.8
int-NH3	-		[8-15]	885.3	q.AETDQLED.e	1.5
int	-		[13-20]	886.5	q.LEDEKSAL.q	0.4
y		7	[28-34]	886.6	l.LKEKEKL. [1xAmide]	0.3
int-CO	-		[7-14]	887.4	l.QAETDQLE.d	-0.6
int	-		[5-12]	887.4	d.TLQAETDQ.l	-0.6
int-NH3	-		[11-19]	999.4	t.DQLEDEKSA.l	0.6
y		8	[27-34]	999.7	n.LLKEKEKL. [1xAmide]	0.3
int-CO	-		[6-14]	1000.5	t.LQAETDQLE.d	-0.5
int	-		[5-13]	1000.5	d.TLQAETDQL.e	-0.5
int-H2O	-		[8-16]	1013.4	q.AETDQLEDE.k	1.1
int-NH3	-		[7-15]	1013.4	l.QAETDQLED.e	1.1
int-NH3	-		[8-16]	1014.4	q.AETDQLEDE.k	0.1
int	-		[12-20]	1014.5	d.QLEDEKSAL.q	0
int-H2O	**-**		**[19-25]**	**1015.5**	**s.ALQTEIA.n [1xSDP]**	**-1.1**
int-H2O	-		[4-13]	1097.5	t.DTLQAETDQL.e	0.8
int-NH3	-		[4-13]	1098.5	t.DTLQAETDQL.e	0.2

int-H2O	-	[10-31]	1099.5	e.TDQLEDEKSA.l	-0.8
y	9	[26-34]	1113.7	a.NLLKEKEKL. [1xAmide]	1.1
int-CO	-	[6-15]	1115.5	t.LQAETDQLED.e	-0.7
int	-	[4-13]	1115.5	t.DTLQAETDQL.e	-0.7
int	-	[23-32]	1168.7	t.EIANLLKEKE.k	1
y	10	[25-34]	1184.7	i.ANLLKEKEKL. [1xAmide]	0.6
int-H2O	-	[8-19]	1299.6	q.AETDQLEDEKSA.l	-0.1
int-H2O	-	[21-29]	1299.7	l.QTEIANLLK.e [1xSDP]	-0.3
int-NH3	-	[8-19]	1300.6	q.AETDQLEDEKSA.l	-1.1
int-NH3	**-**	**[21-29]**	**1300.7**	**l.QTEIANLLK.e [1xSDP]**	**-1.3**
int-CO	**-**	**[18-27]**	**1319.7**	**k.SALQTEIANL.l [1xSDP]**	**0.6**
int	**-**	**[13-21]**	**1320.7**	**q.LEDEKSALQ.t [1xSDP]**	**-0.3**
y	12	[23-34]	1426.9	t.EIANLLKEKEKL. [1xAmide]	1.5
int-H2O	-	[7-19]	1427.6	l.QAETDQLEDEKSA.l	0.7
int-NH3	-	[7-19]	1428.6	l.QAETDQLEDEKSA.l	-0.3
int-H2O	**-**	**[21-30]**	**1428.8**	**l.QTEIANLLKE.k [1xSDP]**	**-0.4**
int-NH3	**-**	**[21-30]**	**1429.7**	**l.QTEIANLLKE.k [1xSDP]**	**-1.4**
y	13	[22-34]	1527.9	q.TEIANLLKEKEKL. [1xAmide]	1.4
b-NH3	14	[1-14]	1528.7	.ASTDTLQAETDQLE.d [1xAcetyl]	0.7
int-H2O	-	[2-15]	1529.7	a.STDTLQAETDQLED.e	-0.3
int-NH3	-	[2-15]	1530.6	a.STDTLQAETDQLED.e	-1.3
int-CO	-	[7-20]	1530.7	l.QAETDQLEDEKSAL.q	-1.4
int-CO	-	[6-19]	1530.7	t.LQAETDQLEDEKSA.l	-1.4
int-CO	**-**	**[7-21]**	**1964.9**	**l.QAETDQLEDEKSALQ.t [1xSDP]**	**0.7**
int	**-**	**[8-22]**	**1965.9**	**q.AETDQLEDEKSALQT.e [1xSDP]**	**-0.3**
b	19	[1-19]	2075.9	.ASTDTLQAETDQLEDEKSA.l [1xAcetyl]	1.2
int	-	[2-20]	2076	a.STDTLQAETDQLEDEKSAL.q	1.2
int-H2O	-	[7-22]	2076	l.QAETDQLEDEKSALQT.e [1xSDP]	1.2
int-H2O	-	[8-23]	2076.9	q.AETDQLEDEKSALQTE.i [1xSDP]	0.2
int-NH3	-	[7-22]	2076.9	l.QAETDQLEDEKSALQT.e [1xSDP]	0.2
int-NH3	-	[8-23]	2077.9	q.AETDQLEDEKSALQTE.i [1xSDP]	-0.8
int-CO	-	[6-21]	2078	t.LQAETDQLEDEKSALQ.t [1xSDP]	-0.9
int-CO	-	[8-26]	2365.1	q.AETDQLEDEKSALQTEIAN.l [1xSDP]	-0.6
int	-	[15-33]	2475.3	e.DEKSALQTEIANLLKEKEK.l [1xSDP]	0.7
int	-	[14-32]	2476.3	l.EDEKSALQTEIANLLKEKE.k [1xSDP]	-0.3
int-H2O	-	[2-21]	2492.2	a.STDTLQAETDQLEDEKSALQ.t [1xSDP]	1.2
int-CO	-	[6-25]	2492.2	t.LQAETDQLEDEKSALQTEIA.n [1xSDP]	1.2
int-NH3	-	[2-21]	2493.1	a.STDTLQAETDQLEDEKSALQ.t [1xSDP]	0.3
int-CO	-	[7-26]	2493.2	l.QAETDQLEDEKSALQTEIAN.l [1xSDP]	0.2
int-NH3	-	[9-28]	2531.2	a.ETDQLEDEKSALQTEIANLL.k [1xSDP]	1.4
int-H2O	-	[5-24]	2532.2	d.TLQAETDQLEDEKSALQTEI.a [1xSDP]	0.4
int-NH3	-	[5-24]	2533.2	d.TLQAETDQLEDEKSALQTEI.a [1xSDP]	-0.6
int-CO	-	[3-24]	2738.3	s.TDTLQAETDQLEDEKSALQTEI.a [1xSDP]	-0.2
int	-	[4-26]	2850.3	t.DTLQAETDQLEDEKSALQTEIAN.l [1xSDP]	1
int	-	[4-27]	2963.4	t.DTLQAETDQLEDEKSALQTEIANL.l [1xSDP]	0.2
b-H2O	25	[1-25]	3019.4	.ASTDTLQAETDQLEDEKSALQTEIA.n [1xAcetyl; 1xSDP]	-0.2
b-NH3	25	[1-25]	3020.4	.ASTDTLQAETDQLEDEKSALQTEIA.n [1xAcetyl; 1xSDP]	-1.2
int-H2O	-	[2-26]	3020.4	a.STDTLQAETDQLEDEKSALQTEIAN.l [1xSDP]	-1.2
y-H2O	31	[4-34]	3831	t.DTLQAETDQLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]	-1.4

LCMS-IT-TOF MS/MS and MS^3 Analysis—Jun(Rh$_2$)/c-Fos: c-Fos(+1mod)

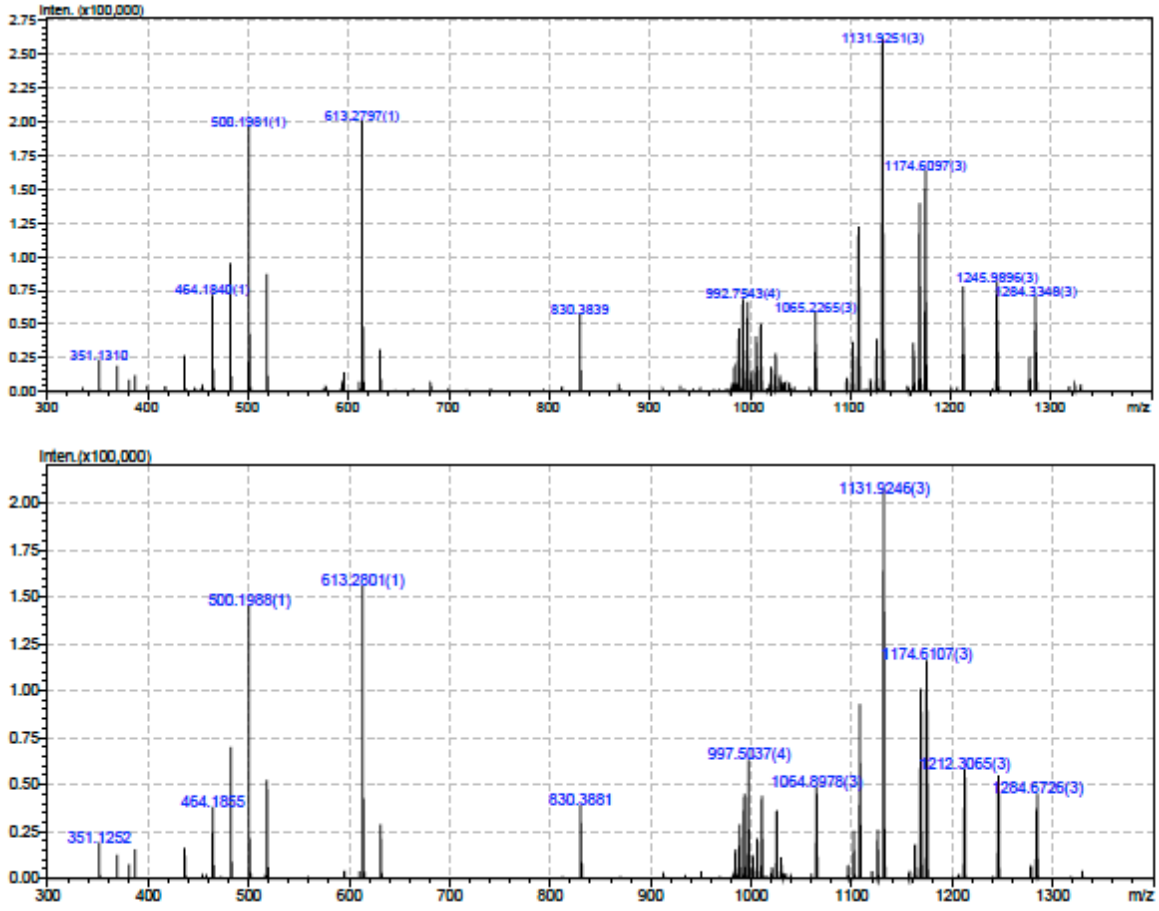


Fig. S-25 LCMS-IT-TOF MS/MS spectra of the two EIC peaks for singly modified **c-Fos** (Fig. S-22). The spectra illustrate that the two prducts have similar fragmentation patterns.

Table S-2 Identified ion fragments from LCSM-IT-TOF MS/MS spectra (Fig. S-25) of singly modified peptide **c-Fos** (Fig. 2).

m/z	*z*	*mass-actual*	*assignment*		*mass-theory*	*Ion fragment*[a]
351.1	1	351.1	int-H2O	[12-14]	353.4	d.QLE.d
436.2	1	436.2	int-CO	[15-18]	432.4	e.DEKS.a
464.2	1	464.2	int-H2O	[27-30]	466.6	n.LLKE.k
482.2	1	482.2	int-NH3	[28-31]	482.6	l.LKEK.e
500.2	1	500.2	b4-H2O	[1-5]	500.5	.ASTDT.l [1xAcetyl]
518.2	1	518.2	b5	[1-5]	518.5	.ASTDT.l [1xAcetyl]
595.3	1	595.3	int-NH3	[27-31]	595.8	n.LLKEK.e
613.3	1	613.3	b6-H2O	[1-6]	613.6	.ASTDTL.q [1xAcetyl]
631.3	1	631.3	b6	[1-6]	631.7	.ASTDTL.q [1xAcetyl]
992.8	3	2976.4	y23	[12-34]	2977.4	d.QLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]
1025.5	3	3074.5	y24-H2O	[11-34]	3074.5	t.DQLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]
1065.2	3	3193.6	y25	[10-34]	3193.6	e.TDQLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]
1108.2	3	3322.6	y26	[9-34]	3322.7	a.ETDQLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]
1131.9	3	3393.7	y27	[8-34]	3393.8	q.AETDQLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]
1174.6	3	3521.8	y28	[7-34]	3521.9	l.QAETDQLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]
1215.3	3	3643.9	y32	[3-34]	3646	s.TDTLQAETDQLEDEKSALQTEIANLLKEKEKL. [1xAmide]
1246	3	3736	y30	[5-34]	3736.2	d.TLQAETDQLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]
1284.3	3	3850.9	y31	[4-34]	3851.3	t.DTLQAETDQLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]

a) SDP = modifying carbenoid group.

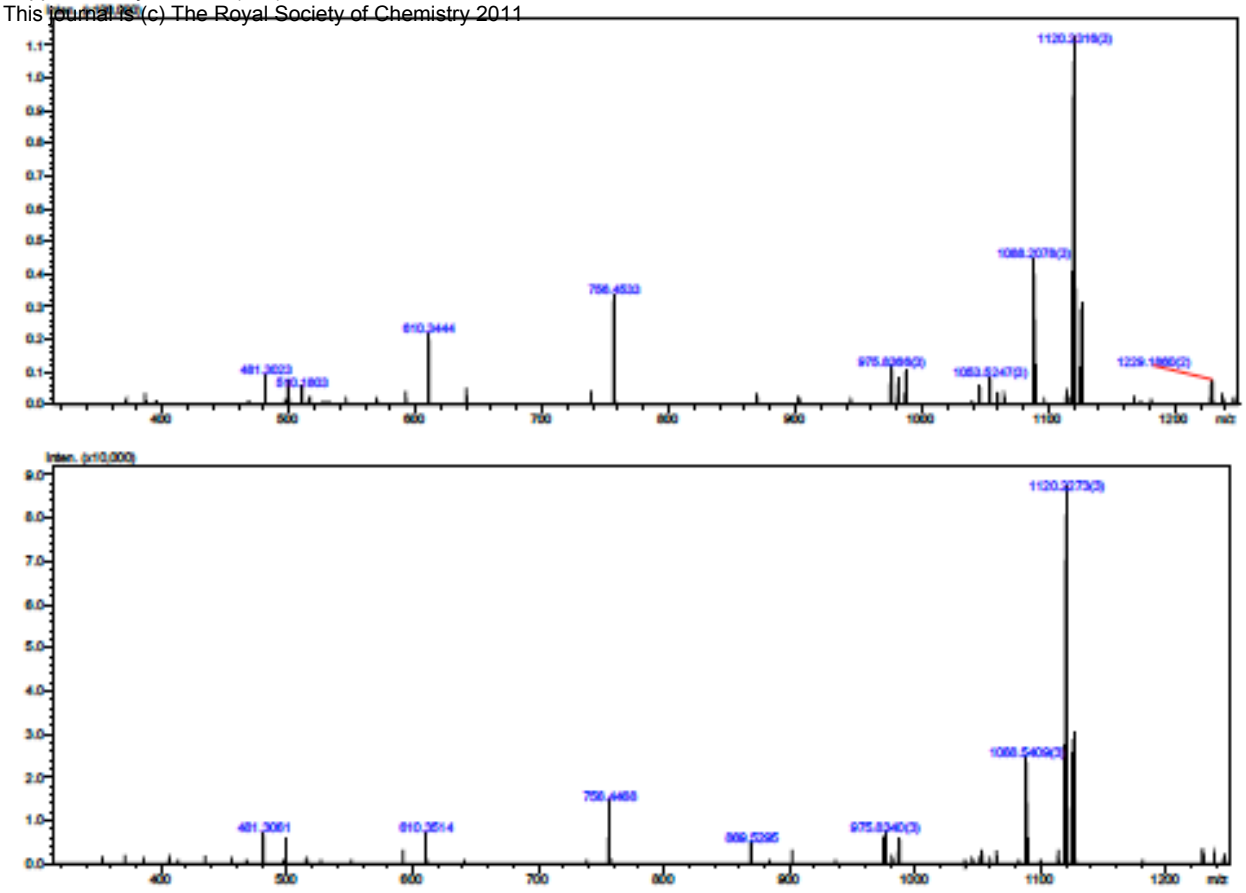


Fig. S-26 LCMS-IT-TOF MS^3 spectra from peak 1131.9 m/z (Fig. S-25) of the two EIC peaks for singly modified peptide **c-Fos** (Fig. S-22). The spectra illustrate that the two products have similar MS^3 fragmentation patterns.

Table S-3 Identified ion fragments from LCSM-IT-TOF MS^3 spectra (MS/MS peak = 1131.9 m/z, Fig. S-26) of singly modified modified **c-Fos** (Fig. 2).

m/z	*z*	*mass-actual*	*Assignment(s)*		*mass-theory*	*ion fragment*[a]
481.3	1	481.3	int-H2O	[28-31]	481.6	l.LKEK.e
488.3	1	488.3	NA[b]			
610.3	1	610.3	int-H2O	[28-32]	610.7	l.LKEKE.k
			int-NH3	[28-32]	611.7	l.LKEKE.k
756.5	1	756.5	int-NH3	[13-19]	756.8	q.LEDEKSA.l
			int-NH3	[14-20]	756.8	l.EDEKSAL.q
			int	[28-33]	756.9	l.LKEKEK.l
			y6-NH3	[29-34]	756.9	l.KEKEKL. [1xAmide]
976	3	2926	int-CO	[3-26]	2925.1	s.TDTLQAETDQLEDEKSALQTEIAN.l [1xSDP]
			int	[2-25]	2926.1	a.STDTLQAETDQLEDEKSALQTEIA.n [1xSDP]
982	3	2944	int-H2O	[11-33]	2944.3	t.DQLEDEKSALQTEIANLLKEKEK.l [1xSDP]
			int-H2O	[5-28]	2945.2	d.TLQAETDQLEDEKSALQTEIANLL.k [1xSDP]
			int-NH3	[11-33]	2945.3	t.DQLEDEKSALQTEIANLLKEKEK.l [1xSDP]
987.5	3	2960.5	y23-H2O	[12-34]	2959.4	d.QLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]
			y23-NH3	[12-34]	2960.4	d.QLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]
1088.5	3	3263.5	int	[7-32]	3263.5	l.QAETDQLEDEKSALQTEIANLLKEKE.k [1xSDP]
			int	[8-33]	3263.6	q.AETDQLEDEKSALQTEIANLLKEKEK.l [1xSDP]
1120.2	3	3358.6	int-H2O	[6-32]	3358.7	t.LQAETDQLEDEKSALQTEIANLLKEKE.k [1xSDP]
			int-NH3	[6-32]	3359.7	t.LQAETDQLEDEKSALQTEIANLLKEKE.k [1xSDP]
1125	3	3373	int-H2O	[7-33]	3373.7	l.QAETDQLEDEKSALQTEIANLLKEKEK.l [1xSDP]
			int-NH3	[7-33]	3374.7	l.QAETDQLEDEKSALQTEIANLLKEKEK.l [1xSDP]
1229.2	2	2457.4	int-H2O	[15-33]	2458.8	e.DEKSALQTEIANLLKEKEK.l [1xSDP]

a) SDP = modifying carbenoid group. b) not assigned.

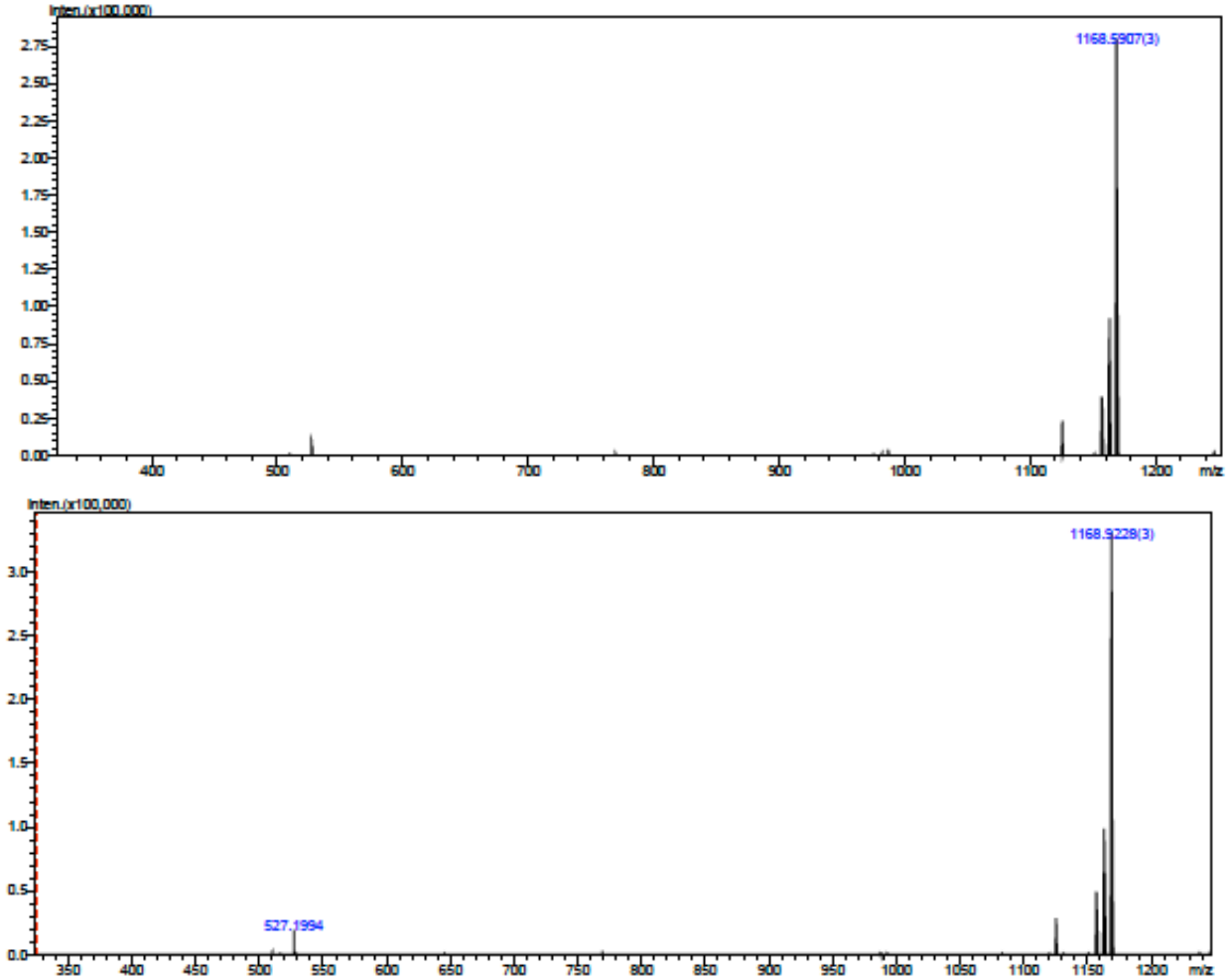


Fig. S-27. LCMS-IT-TOF MS^3 spectra from peak 1174.6 m/z (Fig. S-25) of the two EIC peaks for singly modified peptide **c-Fos (**Fig. S-22). The spectra illustrate that the two products have similar MS^3 fragmentation patterns.

Table S.4 Identified ion fragments from LCSM-IT-TOF MS^3 spectra (MS/MS peak = 1174.6 m/z, Fig. S-27) of singly modified modified **c-Fos** (Fig. 2).

m/z	*z*	*mass-actual*	*assignment(s)*		*mass-theory*	*ion fragment*[a]
527.2	1	527.2	int-H2O	[7-11]	527.5	l.QAETD.q
			int-H2O	[8-12]	527.5	q.AETDQ.l
1125.2	3	3373.6	int-H2O	[7-33]	3373.7	l.QAETDQLEDEKSALQTEIANLLKEKEK.l [1xSDP]
			int-NH3	[7-33]	3374.7	l.QAETDQLEDEKSALQTEIANLLKEKEK.l [1xSDP]
1156.9	3	3468.7	NA			
1162.9	3	3486.7	int-H2O	[6-33]	3486.9	t.LQAETDQLEDEKSALQTEIANLLKEKEK.l [1xSDP]
			int-NH3	[6-33]	3487.8	t.LQAETDQLEDEKSALQTEIANLLKEKEK.l [1xSDP]
1168.9	3	3504.7	int	[6-33]	3504.9	t.LQAETDQLEDEKSALQTEIANLLKEKEK.l [1xSDP]
			y28-NH3	[7-34]	3504.9	l.QAETDQLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]
			int-H2O	[2-30]	3505.8	a.STDTLQAETDQLEDEKSALQTEIANLLKE.k [1xSDP]
527.2	1	527.2	int-H2O	[7-11]	527.5	l.QAETD.q
			int-H2O	[8-12]	527.5	q.AETDQ.l
1125.2	3	3373.6	int-H2O	[7-33]	3373.7	l.QAETDQLEDEKSALQTEIANLLKEKEK.l [1xSDP]
			int-NH3	[7-33]	3374.7	l.QAETDQLEDEKSALQTEIANLLKEKEK.l [1xSDP]
1156.9	3	3468.7	NA			
1162.9	3	3486.7	int-H2O	[6-33]	3486.9	t.LQAETDQLEDEKSALQTEIANLLKEKEK.l [1xSDP]
			int-NH3	[6-33]	3487.8	t.LQAETDQLEDEKSALQTEIANLLKEKEK.l [1xSDP]
1168.9	3	3504.7	int	[6-33]	3504.9	t.LQAETDQLEDEKSALQTEIANLLKEKEK.l [1xSDP]
			y28-NH3	[7-34]	3504.9	l.QAETDQLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]
			int-H2O	[2-30]	3505.8	a.STDTLQAETDQLEDEKSALQTEIANLLKE.k [1xSDP]
527.2	1	527.2	int-H2O	[7-11]	527.5	l.QAETD.q
			int-H2O	[8-12]	527.5	q.AETDQ.l

a) SDP = modifying carbenoid group. b) not assigned.

MALDI-TOF MS/MS Modification Analysis—Max(Rh_2)/c-Fos: c-Fos(+1mod)

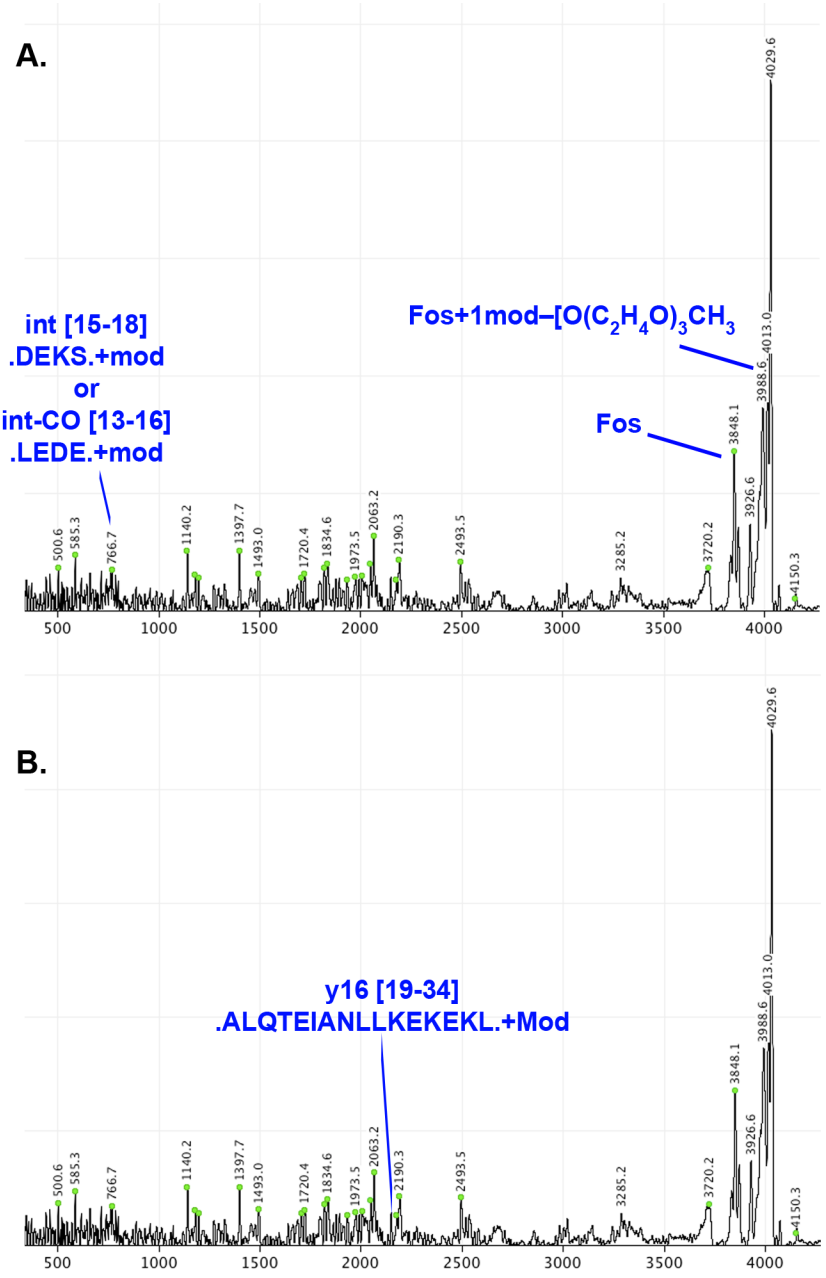


Fig. S-28. MALDI-TOF MS/MS spectra and identified ion fragments (2 Da tolerance) of singly modified peptide **c-Fos** in which analysis was performed for E14 (A) or E21 (B) modification (see Fig. 3A–C). Some diagnostic ions are labeled in the spectra and identified ions (See Tables S-5 and S-6) are labeled with a green dot.

Table S-5. Identified ion fragments (2 Da tolerance) from singly modified peptide **c-Fos** in which analysis was performed for E14 modification (see Fig 3, Fig. S-28A). Some diagnostic ions are in bold. Note: The sequence AS precedes the **c-Fos** sequence and is accounted for in this analysis.

Assignment			*mass (Da)*	*Sequence (SDP=mod)*	*error (Da)*
y-NH3	4	[31-34]	499.3	e.KEKL. [1xAmide]	1.3
int	-	[28-31]	499.3	l.LKEK.e	1.3
b-H2O	5	[1-5]	500.2	.ASTDT.l [1xAcetyl]	0.4
int-H2O	-	[2-6]	500.2	a.STDTL.q	0.4
int-CO	-	[17-21]	500.3	e.KSALQ.t	0.3
b-NH3	5	[1-5]	501.2	.ASTDT.l [1xAcetyl]	-0.6
int-NH3	-	[2-6]	501.2	a.STDTL.q	-0.6
int-CO	-	[22-26]	501.3	q.TEIAN.l	-0.7
int	-	[18-22]	501.3	k.SALQT.e	-0.7
int-CO	-	[4-8]	501.3	t.DTLQA.e	-0.7
int-CO	-	[16-20]	501.3	d.EKSAL.q	-0.7
int	-	[14-17]	502.2	l.EDEK.s	-1.6
int-NH3	-	[11-15]	584.2	t.DQLED.e	1.1
int-CO	-	[27-31]	584.4	n.LLKEK.e	0.9
int	-	[20-24]	585.3	a.LQTEI.a	0
int-CO	-	[12-16]	587.3	d.QLEDE.k	-2
int	-	[10-14]	587.3	e.TDQLE.d	-2
int	-	[9-13]	587.3	a.ETDQL.e	-2
int-CO	**-**	**[13-16]**	**765.4**	**q.LEDE.k [1xSDP]**	**1.4**
int-NH3	-	[24-30]	765.5	e.IANLLKE.k	1.3
int-NH3	-	[23-29]	765.5	t.EIANLLK.e	1.3
int	**-**	**[15-18]**	**766.4**	**e.DEKS.a [1xSDP]**	**0.4**
int-H2O	-	[7-13]	768.4	l.QAETDQL.e	-1.6
int-H2O	-	[6-12]	768.4	t.LQAETDQ.l	-1.6
int-CO	-	[24-33]	1139.7	e.IANLLKEKEK.l	0.5
int	-	[21-30]	1140.6	l.QTEIANLLKE.k	-0.4
int-CO	-	[23-32]	1140.7	t.EIANLLKEKE.k	-0.4
int	-	[22-31]	1140.7	q.TEIANLLKEK.e	-0.4
int-H2O	-	[7-16]	1141.5	l.QAETDQLEDE.k	-1.2
int-H2O	-	[8-17]	1141.5	q.AETDQLEDEK.s	-1.3
int-CO	-	[17-27]	1141.7	e.KSALQTEIANL.l	-1.4
int-NH3	**-**	**[16-23]**	**1176.6**	**d.EKSALQTE.i [1xSDP]**	**1.1**
int-H2O	-	[19-29]	1177.7	s.ALQTEIANLLK.e	-0.1
int-NH3	-	[19-29]	1178.7	s.ALQTEIANLLK.e	-1
int	**-**	**[15-22]**	**1179.6**	**e.DEKSALQT.e [1xSDP]**	**-1.9**
int-CO	**-**	**[14-21]**	**1179.6**	**l.EDEKSALQ.t [1xSDP]**	**-1.9**
int-CO	**-**	**[12-19]**	**1179.6**	**d.QLEDEKSA.l [1xSDP]**	**-1.9**
int	**-**	**[16-23]**	**1193.6**	**d.EKSALQTE.i [1xSDP]**	**1.7**
int	-	[19-29]	1195.7	s.ALQTEIANLLK.e	-0.5
int-H2O	-	[15-27]	1395.7	e.DEKSALQTEIANL.l	2
int-NH3	-	[15-27]	1396.7	e.DEKSALQTEIANL.l	1
int	-	[21-32]	1397.8	l.QTEIANLLKEKE.k	-0.1
int	-	[22-33]	1397.8	q.TEIANLLKEKEK.l	-0.1
b-H2O	13	[1-13]	1398.6	.ASTDTLQAETDQL.e [1xAcetyl]	-0.9
b-NH3	13	[1-13]	1399.6	.ASTDTLQAETDQL.e [1xAcetyl]	-1.9

int	**-**	**[16-26]**	**1491.8**	**d.EKSALQTEIAN.l [1xSDP]**	**1.3**
int	**-**	**[15-25]**	**1492.7**	**e.DEKSALQTEIA.n [1xSDP]**	**0.3**
int-H2O	-	[20-32]	1492.8	a.LQTEIANLLKEKE.k	0.2
int-NH3	-	[20-32]	1493.8	a.LQTEIANLLKEKE.k	-0.8
int	-	[10-24]	1701.8	e.TDQLEDEKSALQTEI.a	2
int-H2O	**-**	**[15-27]**	**1701.9**	**e.DEKSALQTEIANL.l [1xSDP]**	**1.9**
int-NH3	**-**	**[15-27]**	**1702.8**	**e.DEKSALQTEIANL.l [1xSDP]**	**0.9**
int	**-**	**[4-18]**	**1703.8**	**t.DTLQAETDQLEDEKS.a**	**0**
int-NH3	**-**	**[14-26]**	**1718.8**	**l.EDEKSALQTEIAN.l [1xSDP]**	**1.6**
int-H2O	**-**	**[8-20]**	**1718.8**	**q.AETDQLEDEKSAL.q [1xSDP]**	**1.6**
int-NH3	**-**	**[8-20]**	**1719.8**	**q.AETDQLEDEKSAL.q [1xSDP]**	**0.6**
int	**-**	**[15-27]**	**1719.9**	**e.DEKSALQTEIANL.l [1xSDP]**	**0.5**
int-NH3	**-**	**[15-28]**	**1815.9**	**e.DEKSALQTEIANLL.k [1xSDP]**	**2**
int-CO	**-**	**[16-29]**	**1818**	**d.EKSALQTEIANLLK.e [1xSDP]**	**-0.1**
int	**-**	**[15-28]**	**1833**	**e.DEKSALQTEIANLL.k [1xSDP]**	**1.7**
int-CO	**-**	**[12-25]**	**1834.9**	**d.QLEDEKSALQTEIA.n [1xSDP]**	**-0.3**
int-CO	-	[6-22]	1872.9	t.LQAETDQLEDEKSALQT.e	1.1
int-CO	-	[5-21]	1872.9	d.TLQAETDQLEDEKSALQ.t	1.1
int-H2O	-	[2-18]	1873.8	a.STDTLQAETDQLEDEKS.a	0.2
int-CO	-	[9-25]	1873.9	a.ETDQLEDEKSALQTEIA.n	0.1
int-CO	-	[8-24]	1873.9	q.AETDQLEDEKSALQTEI.a	0.1
int-NH3	-	[2-18]	1874.8	a.STDTLQAETDQLEDEKS.a	-0.8
int	-	[3-19]	1875.8	s.TDTLQAETDQLEDEKSA.l	-1.8
int	-	[2-18]	1891.8	a.STDTLQAETDQLEDEKS.a	0.4
int-H2O	-	[15-31]	1894	e.DEKSALQTEIANLLKEK.e	-1.8
int-CO	**-**	**[15-29]**	**1933.1**	**e.DEKSALQTEIANLLK.e [1xSDP]**	**-0.7**
int-CO	**-**	**[14-28]**	**1934**	**l.EDEKSALQTEIANLL.k [1xSDP]**	**-1.6**
int-CO	**-**	**[13-27]**	**1934**	**q.LEDEKSALQTEIANL.l [1xSDP]**	**-1.6**
int-NH3	-	[3-20]	1971.9	s.TDTLQAETDQLEDEKSAL.q	1.6
int-CO	-	[10-27]	1972	e.TDQLEDEKSALQTEIANL.l	1.5
int	-	[8-25]	1972.9	q.AETDQLEDEKSALQTEIA.n	0.6
int-CO	-	[5-22]	1974	d.TLQAETDQLEDEKSALQT.e	-0.5
int-H2O	**-**	**[7-21]**	**1974.9**	**l.QAETDQLEDEKSALQ.t [1xSDP]**	**-1.4**
int	**-**	**[16-30]**	**1975.1**	**d.EKSALQTEIANLLKE.k [1xSDP]**	**-1.6**
b	18	[1-18]	2004.9	.ASTDTLQAETDQLEDEKS.a [1xAcetyl]	1.1
int-H2O	**-**	**[9-23]**	**2005.9**	**a.ETDQLEDEKSALQTE.i [1xSDP]**	**0.1**
int-H2O	**-**	**[3-17]**	**2005.9**	**s.TDTLQAETDQLEDEK.s [1xSDP]**	**0.1**
int-NH3	**-**	**[9-23]**	**2006.9**	**a.ETDQLEDEKSALQTE.i [1xSDP]**	**-0.9**
int-NH3	**-**	**[3-17]**	**2006.9**	**s.TDTLQAETDQLEDEK.s [1xSDP]**	**-0.9**
int-H2O	-	[12-29]	2007.1	d.QLEDEKSALQTEIANLLK.e	-1.1
int	-	[10-24]	2008	e.TDQLEDEKSALQTEI.a [1xSDP]	-1.9
int-CO	-	[13-28]	2047.1	q.LEDEKSALQTEIANLL.k [1xSDP]	-0.6
int-CO	-	[2-20]	2048	a.STDTLQAETDQLEDEKSAL.q	-1.5
int-NH3	**-**	**[10-25]**	**2062**	**e.TDQLEDEKSALQTEIA.n [1xSDP]**	**1.2**
int-NH3	**-**	**[5-20]**	**2062**	**d.TLQAETDQLEDEKSAL.q [1xSDP]**	**1.2**
int-CO	**-**	**[12-27]**	**2062.1**	**d.QLEDEKSALQTEIANL.l [1xSDP]**	**1.1**
int-CO	**-**	**[15-30]**	**2062.1**	**e.DEKSALQTEIANLLKE.k [1xSDP]**	**1.1**
int-CO	**-**	**[14-29]**	**2062.1**	**l.EDEKSALQTEIANLLK.e [1xSDP]**	**1.1**
int-H2O	**-**	**[4-19]**	**2062.9**	**t.DTLQAETDQLEDEKSA.l [1xSDP]**	**0.3**
int-NH3	**-**	**[4-19]**	**2063.9**	**t.DTLQAETDQLEDEKSA.l [1xSDP]**	**-0.7**
int-CO	**-**	**[11-26]**	**2064**	**t.DQLEDEKSALQTEIAN.l [1xSDP]**	**-0.8**
int-H2O	**-**	**[10-26]**	**2175**	**e.TDQLEDEKSALQTEIAN.l [1xSDP]**	**0.3**

int-CO	**-**	**[12-26]**	**2175.1**	**d.QLEDEKSALQTEIANLL.k [1xSDP]**	**0.2**
int-CO	**-**	**[13-29]**	**2175.2**	**q.LEDEKSALQTEIANLLK.e [1xSDP]**	**0.1**
int-NH3	**-**	**[10-26]**	**2176**	**e.TDQLEDEKSALQTEIAN.l [1xSDP]**	**-0.7**
int-H2O	**-**	**[4-20]**	**2176**	**t.DTLQAETDQLEDEKSAL.q [1xSDP]**	**-0.7**
int-CO	-	[2-21]	2176	a.STDTLQAETDQLEDEKSALQ.t	-0.7
int-NH3	-	[4-20]	2177	t.DTLQAETDQLEDEKSAL.q [1xSDP]	-1.7
int-CO	-	[11-27]	2177.1	t.DQLEDEKSALQTEIANL.l [1xSDP]	-1.8
b	20	[1-20]	2189	.ASTDTLQAETDQLEDEKSAL.q [1xAcetyl]	1.3
int-H2O	-	[6-22]	2189	t.LQAETDQLEDEKSALQT.e [1xSDP]	1.2
int-H2O	-	[5-21]	2189	d.TLQAETDQLEDEKSALQ.t [1xSDP]	1.2
int-H2O	-	[9-25]	2190	a.ETDQLEDEKSALQTEIA.n [1xSDP]	0.3
int-H2O	-	[8-24]	2190	q.AETDQLEDEKSALQTEI.a [1xSDP]	0.3
int-NH3	-	[6-22]	2190	t.LQAETDQLEDEKSALQT.e [1xSDP]	0.3
int-NH3	-	[5-21]	2190	d.TLQAETDQLEDEKSALQ.t [1xSDP]	0.3
int-CO	-	[3-22]	2190	s.TDTLQAETDQLEDEKSALQT.e	0.3
int-CO	-	[15-31]	2190.2	e.DEKSALQTEIANLLKEK.e [1xSDP]	0.1
int-NH3	-	[9-25]	2191	a.ETDQLEDEKSALQTEIA.n [1xSDP]	-0.7
int-NH3	-	[8-24]	2191	q.AETDQLEDEKSALQTEI.a [1xSDP]	-0.7
int-CO	-	[14-30]	2191.1	l.EDEKSALQTEIANLLKE.k [1xSDP]	-0.8
int-H2O	-	[2-21]	2492.2	a.STDTLQAETDQLEDEKSALQ.t [1xSDP]	1.3
int-CO	-	[6-25]	2492.2	t.LQAETDQLEDEKSALQTEIA.n [1xSDP]	1.2
int-NH3	-	[2-21]	2493.1	a.STDTLQAETDQLEDEKSALQ.t [1xSDP]	0.3
int-CO	-	[7-26]	2493.2	l.QAETDQLEDEKSALQTEIAN.l [1xSDP]	0.3
b	20	[1-20]	2495.2	.ASTDTLQAETDQLEDEKSAL.q [1xAcetyl; 1xSDP]	-1.7
int	-	[4-32]	3284.6	t.DTLQAETDQLEDEKSALQTEIANLLKEKE.k	0.6
int	-	[4-33]	3718.9	t.DTLQAETDQLEDEKSALQTEIANLLKEKEK.l [1xSDP]	1.3
y	31	[4-34]	3849	t.DTLQAETDQLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]	-0.9

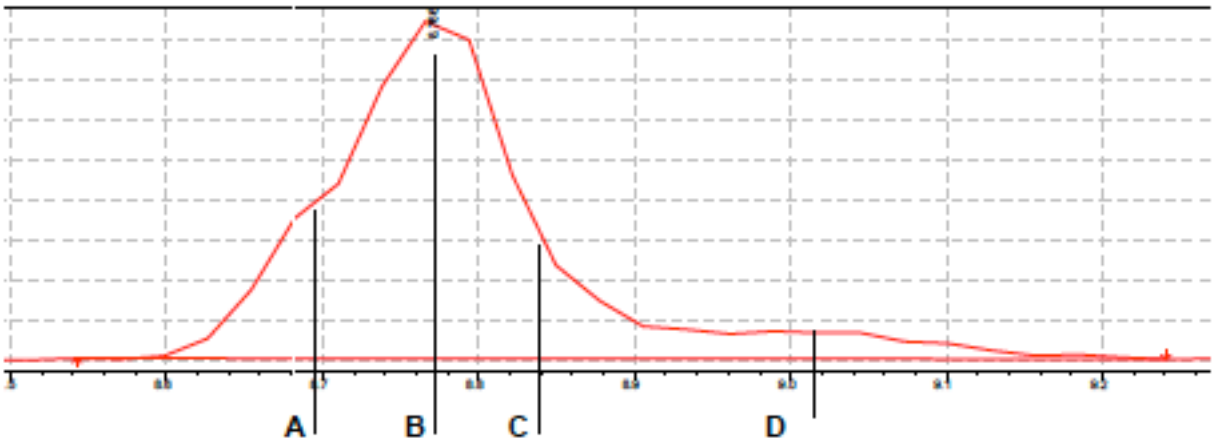
Table S-6. Identified ion fragments (2 Da tolerance) from singly modified peptide **c-Fos** in which analysis was performed for E21 modification (see Fig 3, Fig. S-28B). Some diagnostic ions are in bold. Note: The sequence AS precedes the c-Fos sequence and is accounted for in this analysis.

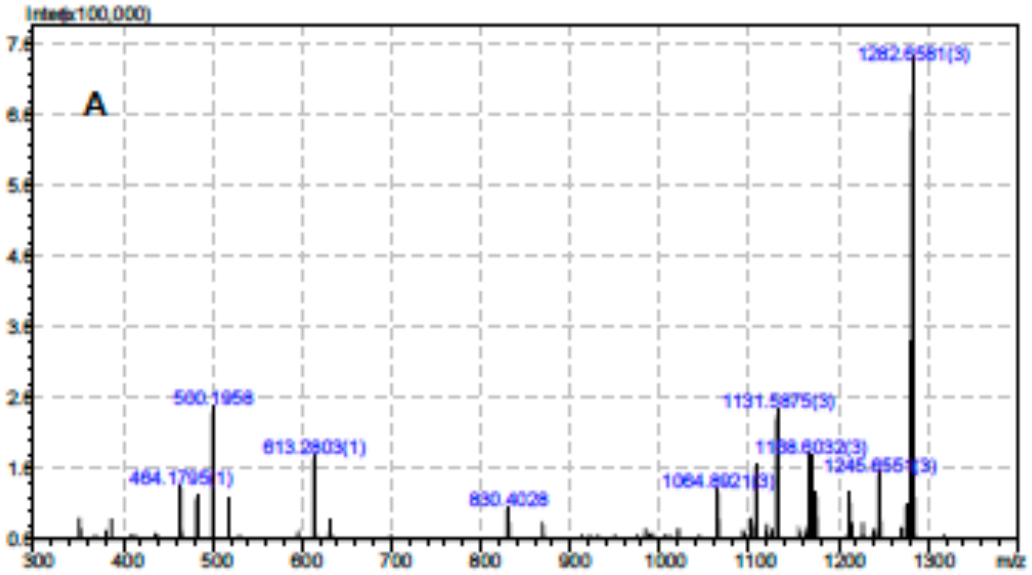
assignment			*mass (Da)*	*sequence (SDP=mod)*	*error (Da)*
y-NH3	4	[31-34]	499.3	e.KEKL. [1xAmide]	1.3
int	-	[28-31]	499.3	l.LKEK.e	1.3
b-H2O	5	[1-5]	500.2	.ASTDT.l [1xAcetyl]	0.4
int-H2O	-	[2-6]	500.2	a.STDTL.q	0.4
int-CO	-	[17-21]	500.3	e.KSALQ.t	0.3
b-NH3	5	[1-5]	501.2	.ASTDT.l [1xAcetyl]	-0.6
int-NH3	-	[2-6]	501.2	a.STDTL.q	-0.6
int-CO	-	[22-26]	501.3	q.TEIAN.l	-0.7
int	-	[18-22]	501.3	k.SALQT.e	-0.7
int-CO	-	[4-8]	501.3	t.DTLQA.e	-0.7
int-CO	-	[16-20]	501.3	d.EKSAL.q	-0.7
int	-	[14-17]	502.2	l.EDEK.s	-1.6
int-NH3	-	[11-15]	584.2	t.DQLED.e	1.1
int-CO	-	[27-31]	584.4	n.LLKEK.e	0.9
int	-	[20-24]	585.3	a.LQTEI.a	0

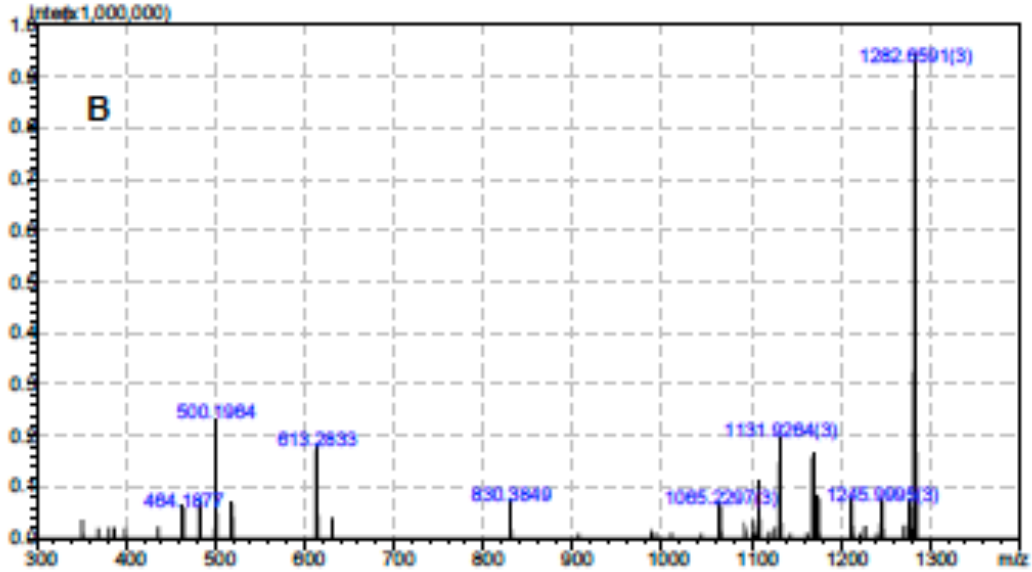
int-CO	-	[12-16]	587.3	d.QLEDE.k	-2
int	-	[10-14]	587.3	e.TDQLE.d	-2
int	-	[9-13]	587.3	a.ETDQL.e	-2
int-NH3	-	[24-30]	765.5	e.IANLLKE.k	1.3
int-NH3	-	[23-29]	765.5	t.EIANLLK.e	1.3
int-H2O	-	[7-13]	768.4	l.QAETDQL.e	-1.6
int-H2O	-	[6-12]	768.4	t.LQAETDQ.l	-1.6
int-CO	-	[24-33]	1139.7	e.IANLLKEKEK.l	0.5
int	-	[21-30]	1140.6	l.QTEIANLLKE.k	-0.4
int-CO	-	[23-32]	1140.7	t.EIANLLKEKE.k	-0.4
int	-	[22-31]	1140.7	q.TEIANLLKEK.e	-0.4
int-H2O	-	[7-16]	1141.5	l.QAETDQLEDE.k	-1.2
int-H2O	-	[8-17]	1141.5	q.AETDQLEDEK.s	-1.3
int-CO	-	[17-27]	1141.7	e.KSALQTEIANL.l	-1.4
int-NH3	**-**	**[16-23]**	**1176.6**	**d.EKSALQTE.i [1xSDP]**	**1.1**
int	**-**	**[17-24]**	**1177.6**	**e.KSALQTEI.a [1xSDP]**	**0**
int-H2O	-	[19-29]	1177.7	s.ALQTEIANLLK.e	-0.1
int-NH3	-	[19-29]	1178.7	s.ALQTEIANLLK.e	-1
int	**-**	**[16-23]**	**1193.6**	**d.EKSALQTE.i [1xSDP]**	**1.7**
int	-	[19-29]	1195.7	s.ALQTEIANLLK.e	-0.5
int-H2O	-	[15-27]	1395.7	e.DEKSALQTEIANL.l	2
int-NH3	-	[15-27]	1396.7	e.DEKSALQTEIANL.l	1
int	-	[21-32]	1397.8	l.QTEIANLLKEKE.k	-0.1
int	-	[22-33]	1397.8	q.TEIANLLKEKEK.l	-0.1
b-H2O	13	[1-13]	1398.6	.ASTDTLQAETDQL.e [1xAcetyl]	-0.9
b-NH3	13	[1-13]	1399.6	.ASTDTLQAETDQL.e [1xAcetyl]	-1.9
int	**-**	**[16-26]**	**1491.8**	**d.EKSALQTEIAN.l [1xSDP]**	**1.3**
int	**-**	**[15-25]**	**1492.7**	**e.DEKSALQTEIA.n [1xSDP]**	**0.3**
int-H2O	-	[20-32]	1492.8	a.LQTEIANLLKEKE.k	0.2
int-NH3	-	[20-32]	1493.8	a.LQTEIANLLKEKE.k	-0.8
int	-	[10-24]	1701.8	e.TDQLEDEKSALQTEI.a	2
int-H2O	**-**	**[15-27]**	**1701.9**	**e.DEKSALQTEIANL.l [1xSDP]**	**1.9**
int-NH3	**-**	**[15-27]**	**1702.8**	**e.DEKSALQTEIANL.l [1xSDP]**	**0.9**
int	-	[4-18]	1703.8	t.DTLQAETDQLEDEKS.a	0
int	**-**	**[21-32]**	**1703.9**	**l.QTEIANLLKEKE.k [1xSDP]**	**-0.1**
int	**-**	**[22-33]**	**1703.9**	**q.TEIANLLKEKEK.l [1xSDP]**	**-0.2**
int-NH3	**-**	**[14-26]**	**1718.8**	**l.EDEKSALQTEIAN.l [1xSDP]**	**1.6**
int	**-**	**[15-27]**	**1719.9**	**e.DEKSALQTEIANL.l [1xSDP]**	**0.5**
int-NH3	**-**	**[15-28]**	**1815.9**	**e.DEKSALQTEIANLL.k [1xSDP]**	**2**
y-H2O	**13**	**[22-34]**	**1816**	**q.TEIANLLKEKEKL. [1xAmide; 1xSDP]**	**1.8**
int	**-**	**[20-32]**	**1817**	**a.LQTEIANLLKEKE.k [1xSDP]**	**0.9**
y-NH3	**13**	**[22-34]**	**1817**	**q.TEIANLLKEKEKL. [1xAmide; 1xSDP]**	**0.9**
int-CO	**-**	**[18-31]**	**1818**	**k.SALQTEIANLLKEK.e [1xSDP]**	**-0.1**
int-CO	**-**	**[17-30]**	**1818**	**e.KSALQTEIANLLKE.k [1xSDP]**	**-0.1**
int-CO	**-**	**[16-29]**	**1818**	**d.EKSALQTEIANLLK.e [1xSDP]**	**-0.1**
int	-	[15-28]	1833	e.DEKSALQTEIANLL.k [1xSDP]	1.7
y	13	[22-34]	1834.1	q.TEIANLLKEKEKL. [1xAmide; 1xSDP]	0.6
int-CO	-	[12-25]	1834.9	d.QLEDEKSALQTEIA.n [1xSDP]	-0.3
int-CO	-	[6-22]	1872.9	t.LQAETDQLEDEKSALQT.e	1.1
int-CO	-	[5-21]	1872.9	d.TLQAETDQLEDEKSALQ.t	1.1

int-H2O	-	[2-18]	1873.8	a.STDTLQAETDQLEDEKS.a	0.2
int-CO	-	[9-25]	1873.9	a.ETDQLEDEKSALQTEIA.n	0.1
int-CO	-	[8-24]	1873.9	q.AETDQLEDEKSALQTEI.a	0.1
int-NH3	-	[2-18]	1874.8	a.STDTLQAETDQLEDEKS.a	-0.8
int	-	[3-19]	1875.8	s.TDTLQAETDQLEDEKSA.l	-1.8
int	-	[2-18]	1891.8	a.STDTLQAETDQLEDEKS.a	0.4
int-H2O	-	[15-31]	1894	e.DEKSALQTEIANLLKEK.e	-1.8
int-CO	**-**	**[15-29]**	**1933.1**	**e.DEKSALQTEIANLLK.e [1xSDP]**	**-0.7**
int-CO	**-**	**[14-28]**	**1934**	**l.EDEKSALQTEIANLL.k [1xSDP]**	**-1.6**
int-CO	**-**	**[13-27]**	**1934**	**q.LEDEKSALQTEIANL.l [1xSDP]**	**-1.6**
int-NH3	-	[3-20]	1971.9	s.TDTLQAETDQLEDEKSAL.q	1.6
int-CO	-	[10-27]	1972	e.TDQLEDEKSALQTEIANL.l	1.5
int	-	[8-25]	1972.9	q.AETDQLEDEKSALQTEIA.n	0.6
int-CO	-	[5-22]	1974	d.TLQAETDQLEDEKSALQT.e	-0.5
int	**-**	**[17-31]**	**1974.1**	**e.KSALQTEIANLLKEK.e [1xSDP]**	**-0.6**
int	**-**	**[18-32]**	**1975.1**	**k.SALQTEIANLLKEKE.k [1xSDP]**	**-1.6**
int	**-**	**[16-30]**	**1975.1**	**d.EKSALQTEIANLLKE.k [1xSDP]**	**-1.6**
b	18	[1-18]	2004.9	.ASTDTLQAETDQLEDEKS.a [1xAcetyl]	1.1
int-H2O	**-**	**[9-23]**	**2005.9**	**a.ETDQLEDEKSALQTE.i [1xSDP]**	**0.1**
int-NH3	**-**	**[9-23]**	**2006.9**	**a.ETDQLEDEKSALQTE.i [1xSDP]**	**-0.9**
int-H2O	-	[12-29]	2007.1	d.QLEDEKSALQTEIANLLK.e	-1.1
int	-	[10-24]	2008	e.TDQLEDEKSALQTEI.a [1xSDP]	-1.9
int-CO	-	[13-28]	2047.1	q.LEDEKSALQTEIANLL.k [1xSDP]	-0.6
int-CO	-	[2-20]	2048	a.STDTLQAETDQLEDEKSAL.q	-1.5
int-NH3	-	[10-25]	2062	e.TDQLEDEKSALQTEIA.n [1xSDP]	1.2
int-CO	-	[12-27]	2062.1	d.QLEDEKSALQTEIANL.l [1xSDP]	1.1
int-CO	-	[15-30]	2062.1	e.DEKSALQTEIANLLKE.k [1xSDP]	1.1
int-CO	-	[14-29]	2062.1	l.EDEKSALQTEIANLLK.e [1xSDP]	1.1
int-CO	-	[11-26]	2064	t.DQLEDEKSALQTEIAN.l [1xSDP]	-0.8
y	**16**	**[19-34]**	**2146.2**	**s.ALQTEIANLLKEKEKL. [1xAmide; 1xSDP]**	**0.4**
int-H2O	-	[10-26]	2175	e.TDQLEDEKSALQTEIAN.l [1xSDP]	0.3
int-CO	-	[12-28]	2175.1	d.QLEDEKSALQTEIANLL.k [1xSDP]	0.2
int-CO	-	[13-29]	2175.2	q.LEDEKSALQTEIANLLK.e [1xSDP]	0.1
int-NH3	-	[10-26]	2176	e.TDQLEDEKSALQTEIAN.l [1xSDP]	-0.7
int-CO	-	[2-21]	2176	a.STDTLQAETDQLEDEKSALQ.t	-0.7
int-CO	-	[11-27]	2177.1	t.DQLEDEKSALQTEIANL.l [1xSDP]	-1.8
b	20	[1-20]	2189	.ASTDTLQAETDQLEDEKSAL.q [1xAcetyl]	1.3
int-H2O	-	[9-25]	2190	a.ETDQLEDEKSALQTEIA.n [1xSDP]	0.3
int-H2O	-	[8-24]	2190	q.AETDQLEDEKSALQTEI.a [1xSDP]	0.3
int-CO	-	[3-22]	2190	s.TDTLQAETDQLEDEKSALQT.e	0.3
int-CO	-	[15-31]	2190.2	e.DEKSALQTEIANLLKEK.e [1xSDP]	0.1
int-NH3	-	[9-25]	2191	a.ETDQLEDEKSALQTEIA.n [1xSDP]	-0.7
int-NH3	-	[8-24]	2191	q.AETDQLEDEKSALQTEI.a [1xSDP]	-0.7
int-CO	-	[14-30]	2191.1	l.EDEKSALQTEIANLLKE.k [1xSDP]	-0.8
int-CO	-	[6-25]	2492.2	t.LQAETDQLEDEKSALQTEIA.n [1xSDP]	1.2
int-CO	-	[7-26]	2493.2	l.QAETDQLEDEKSALQTEIAN.l [1xSDP]	0.3
int	-	[4-32]	3284.6	t.DTLQAETDQLEDEKSALQTEIANLLKEKE.k	0.6
int	-	[4-33]	3718.9	t.DTLQAETDQLEDEKSALQTEIANLLKEKEK.l [1xSDP]	1.3
y	31	[4-34]	3849	t.DTLQAETDQLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]	-0.9

LCMS-IT-TOF MS/MS and MS^3 Analysis—Max(Rh$_2$)/c-Fos: c-Fos(+1mod)







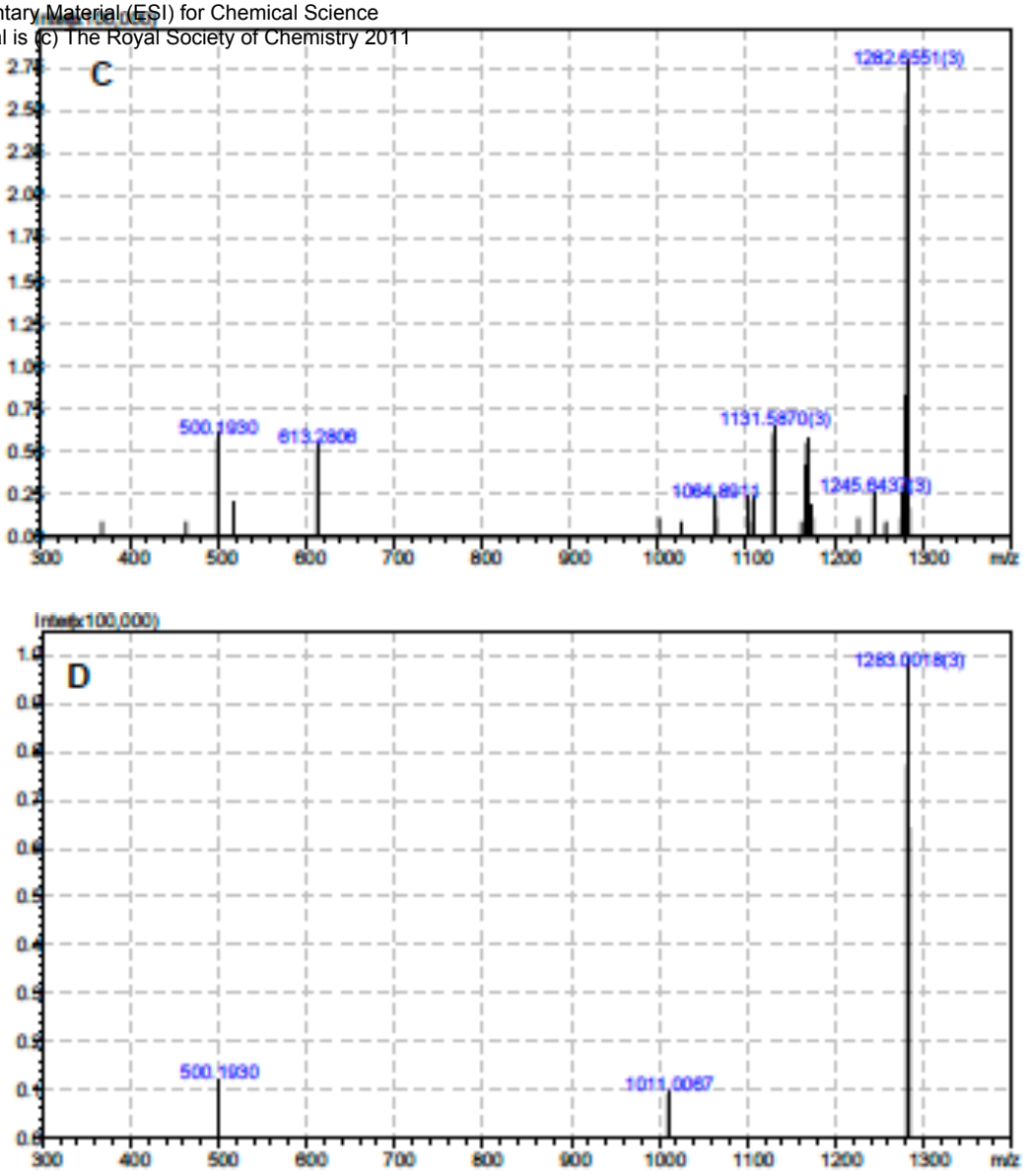
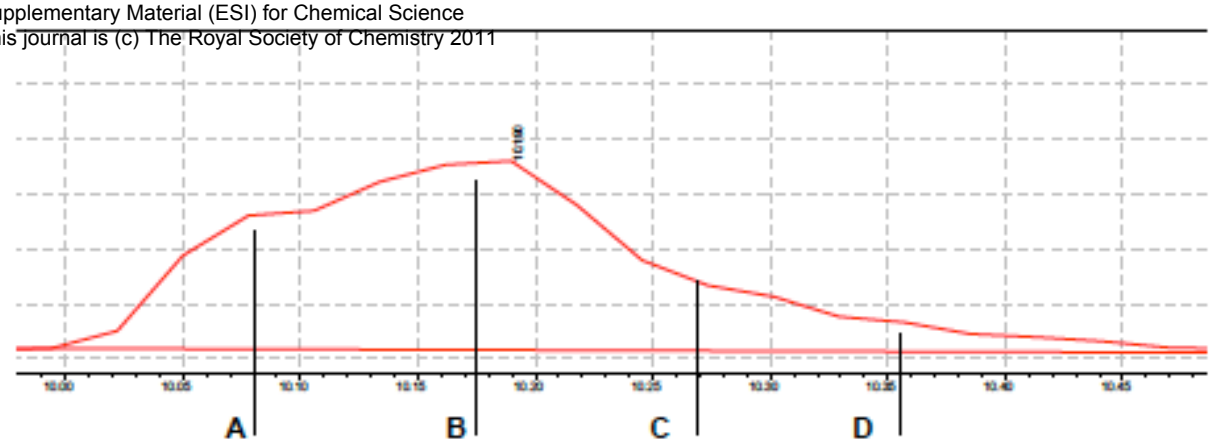


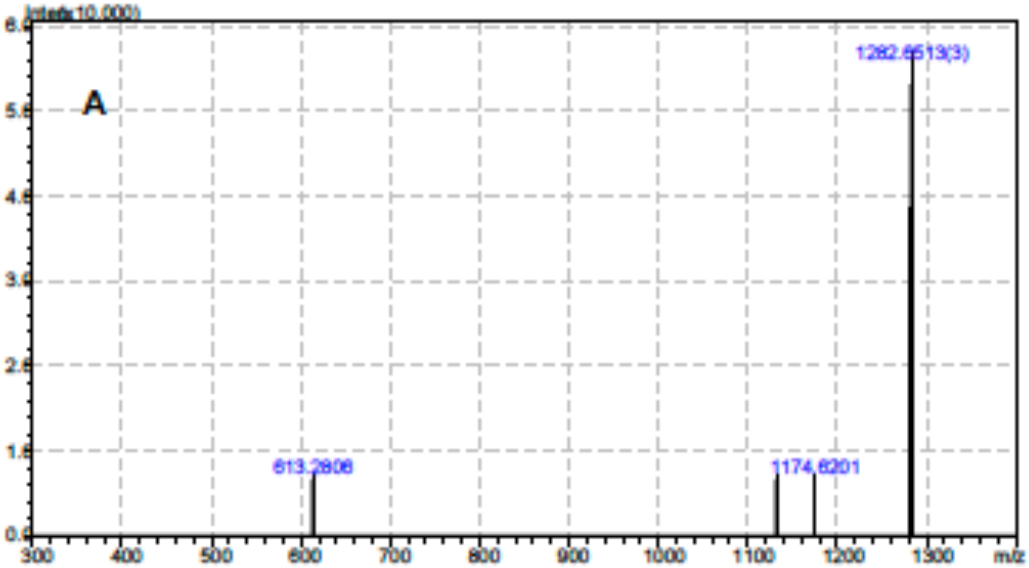
Fig. S-29 LCMS-IT-TOF MS/MS spectra of four region (A-D) of the EIC peak for singly modified **c-Fos** (Fig. 3, Fig. S-23). All spectra show similar fragments. The largest fragment 1282.6(z=3) m/z (MW_{avg}^{exp} = 3844.8 Da) corresponds to peptide **c-Fos** (MW_{avg}^{theory} = 3845.2 Da) resulting from modification cleavage.

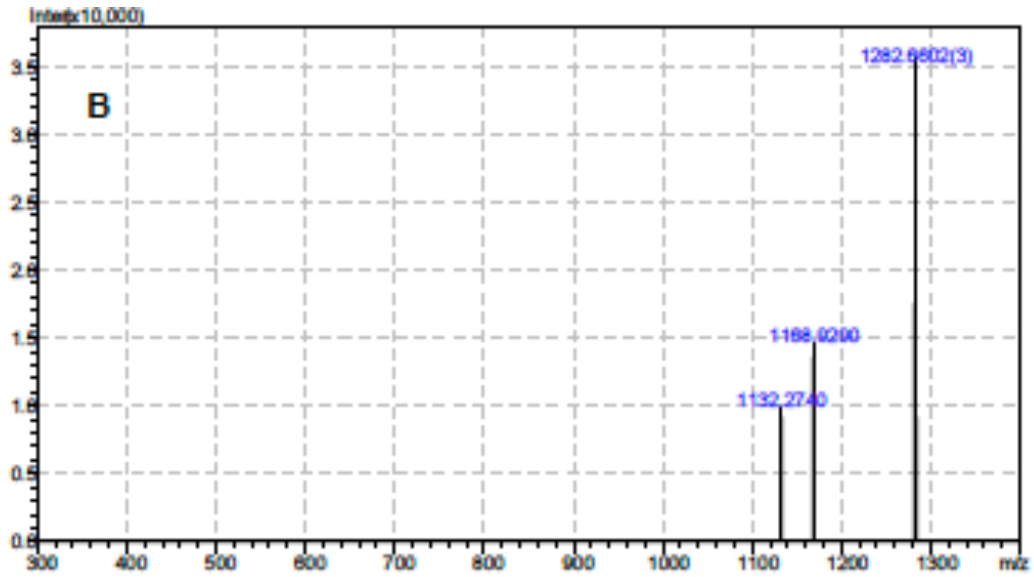
Table S-7 Identified ion fragments from LCSM-IT-TOF MS/MS spectra (Fig. S-29A-D) of singly modified peptide **c-Fos** (Fig. 3). All spectra reveal identical fragmentation patterns so only spectrum Fig. S-29B is assigned. In Fig. S-29D, a fragment ion is observed that is not present in other spectra; although we provide possible assignments below, we cannot be sure that this peak corresponds to a z=1 fragment.

m/z	*z*	*mass-actual*	*assignment*		*mass-theory*	*Ion fragment*[a]
Fig. S-29 A-C						
464.2	1	463.2	int-H2O	[27-30]	466.6	n.LLKE.k
483	1	482	int-NH3	[28-31]	482.6	l.LKEK.e
500.2	1	499.2	int	[28-31]	499.6	l.LKEK.e
613.3	1	612.3	int	[27-31]	612.8	n.LLKEK.e
830.4	1	829.4	b8	[1-8]	830.9	.ASTDTLQA.e [1xAcetyl]
1064.9	3	3193.7	y25	[10-34]	3193.6	e.TDQLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]
1105	3	3314	b30-NH3	[1-30]	3313.5	.ASTDTLQAETDQLEDEKSALQTEIANLLKE.k [1xAcetyl]
1131.6	3	3393.8	y27	[8-34]	3393.8	q.AETDQLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]
1168.6	3	3504.8	y28-NH3	[7-34]		
1215	3	3644	y32	[3-34]	3646	t.DTLQAETDQLEDEKSALQTEIANLLKEKEKL. [1xAmide]
1245.7	3	3736.1	y30	[5-34]	3736.2	.t.LQAETDQLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]
1282.7	3	3847.1	**c-Fos**	[1-34]	3848	ASDTLQAETDQLEDEKSALQTEIANLLKEKEKL. [1xAcetyl;1xAmide]
Fig. S-29D						
1011.0	1	1011.0	int-CO	[11-16]	1009	t.DQLEDE.k [1xSDP]
			int	[13-18]	1009.1	q.LEDEKS.a [1xSDP]
			int-H2O	[6-14]	1011.1	t.LQAETDQLE.d
			int-NH3	[6-14]	1012	t.LQAETDQLE.d
			int	[21-29]	1012.2	l.QTEIANLLK.e
			int-CO	[23-31]	1012.2	t.EIANLLKEK.e
			int-CO	[24-32]	1012.2	e.IANLLKEKE.k

a) SDP = modifying carbenoid group.


A
B
C
D


A
1282.6513(3)
613.2806
1174.6201
m/z


B
1282.9802(3)
1168.6200
1132.2740
m/z

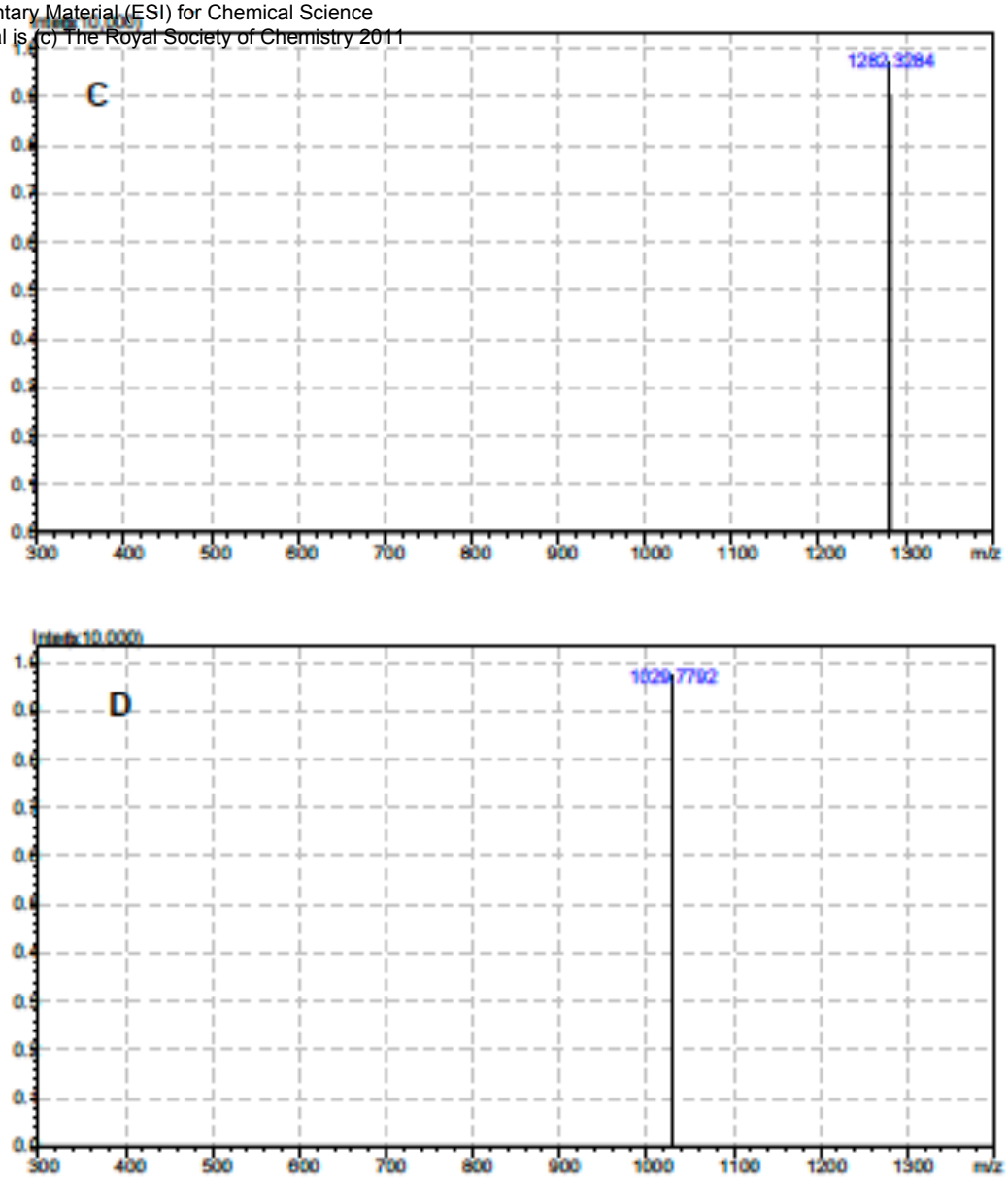


Fig. S-30 LCMS-IT-TOF MS/MS spectra of four regions (A-D) of the EIC peak for doubly modified **c-Fos** (Fig. 3, Fig. S-23). All spectra, with the exception of (D), show similar fragments. The largest fragment 1282.6(z=3) m/z (MW_{avg}^{exp} = 3844.8 Da) corresponds to peptide **c-Fos** (MW_{avg}^{theory} = 3845.2 Da), which results from modification cleavage.

Table S7 Identified ion fragments from LCSM-IT-TOF MS/MS spectra (Fig. S-29A-D) of doubly modified **c-Fos** (Fig. 3). Spectra S-29A-C reveal identical fragmentation patterns thus only spectrum Fig. S-29B is assigned. In Fig. S-29D, a fragment ion is observed that is not present in the other spectra; although we provide a possible assignment below, there is doubt as to whether this peak corresponds to a z=1 fragment due to poor signal-to-noise.

m/z	*z*	*mass-actual*	*assignment*		*mass-theory*	*Ion fragment*[a]
Fig. S-29 A-C						
464.2	1	463.2	int-H2O	[27-30]	466.6	n.LLKE.k
483	1	482	int-NH3	[28-31]	482.6	l.LKEK.e
500.2	1	499.2	int	[28-31]	499.6	l.LKEK.e
613.3	1	612.3	int	[27-31]	612.8	n.LLKEK.e
830.4	1	829.4	b8	[1-8]	830.9	.ASTDTLQA.e [1xAcetyl]
1064.9	3	3193.7	y25	[10-34]	3193.6	e.TDQLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]
1105	3	3314	b30-NH3	[1-30]	3313.5	.ASTDTLQAETDQLEDEKSALQTEIANLLKE.k [1xAcetyl]
1131.6	3	3393.8	y27	[8-34]	3393.8	q.AETDQLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]
1168.6	3	3504.8	y28-NH3	[7-34]		
1215	3	3644	y32	[3-34]	3646	t.DTLQAETDQLEDEKSALQTEIANLLKEKEKL. [1xAmide]
1245.7	3	3736.1	y30	[5-34]	3736.2	.t.LQAETDQLEDEKSALQTEIANLLKEKEKL. [1xAmide; 1xSDP]
1282.7	3	3847.1	**c-Fos**	[1-34]	3848	ASDTLQAETDQLEDEKSALQTEIANLLKEKEKL. [1xAcetyl;1xAmide]
Fig. S-29D						
1011.0	1	1011.0	int-CO	[11-16]	1009	t.DQLEDE.k [1xSDP]
			int	[13-18]	1009.1	q.LEDEKS.a [1xSDP]
			int-H2O	[6-14]	1011.1	t.LQAETDQLE.d
			int-NH3	[6-14]	1012	t.LQAETDQLE.d
			int	[21-29]	1012.2	l.QTEIANLLK.e
			int-CO	[23-31]	1012.2	t.EIANLLKEK.e
			int-CO	[24-32]	1012.2	e.IANLLKEKE.k

a) SDP = modifying carbenoid group.

MS/MS Modification Analysis: E3$_g$W(+2mod)

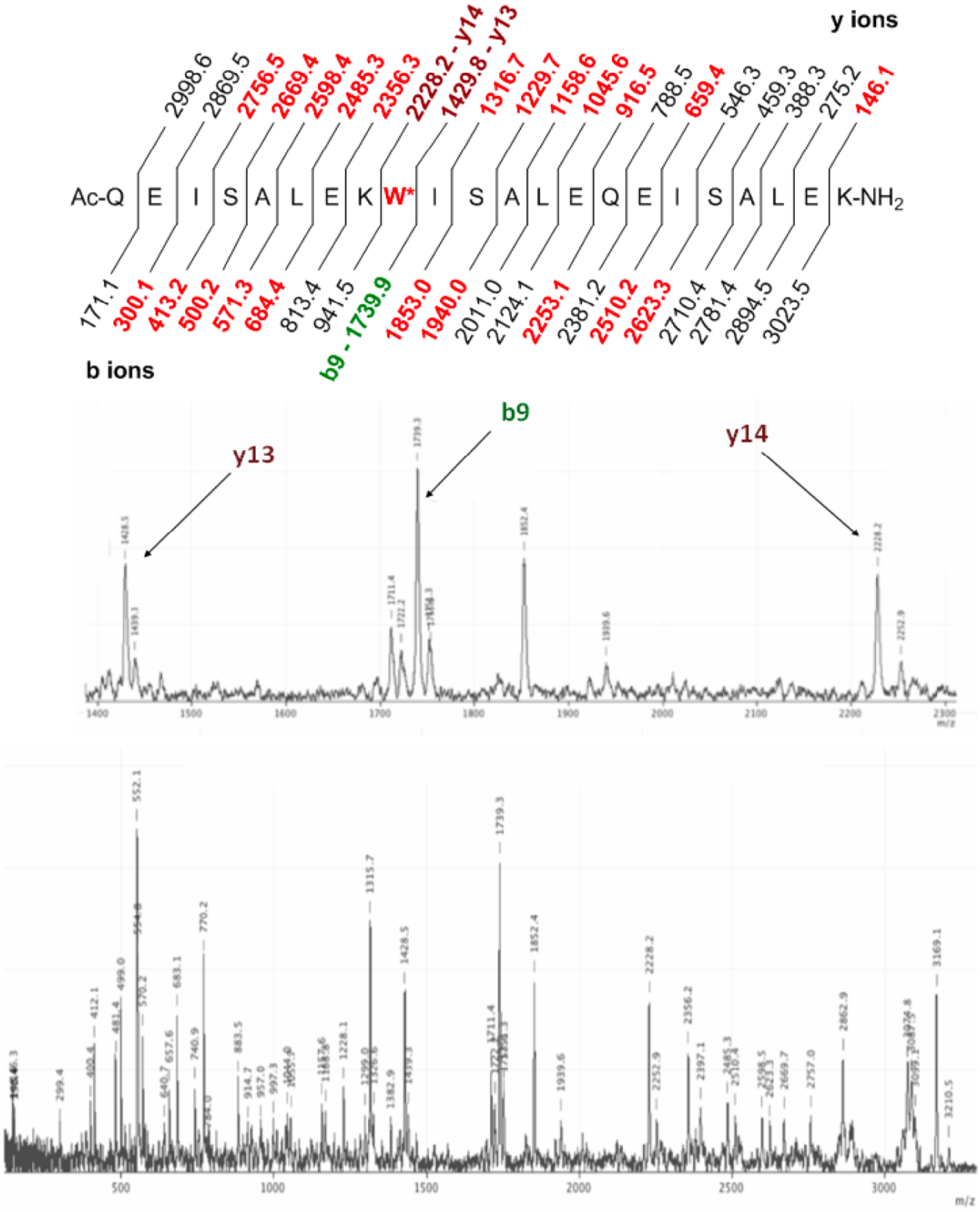


Fig. S-31. MALDI-TOF MS/MS spectrum and y/b ion fragmentation diagram of doubly modified **E3$_g$W** peptide. Located ions (2 Da tolerance) are highlighted in red. Critical ions indicating the loss of modified residue are labeled in either maroon (y ion) or green (b ion) in the fragmentation diagram. Calculated doubly modified Trp-immonium monoisotopic mass: 771.4 Da; found: 770.2 Da.

MS/MS Modification Analysis: E3$_g$W(+3mod)

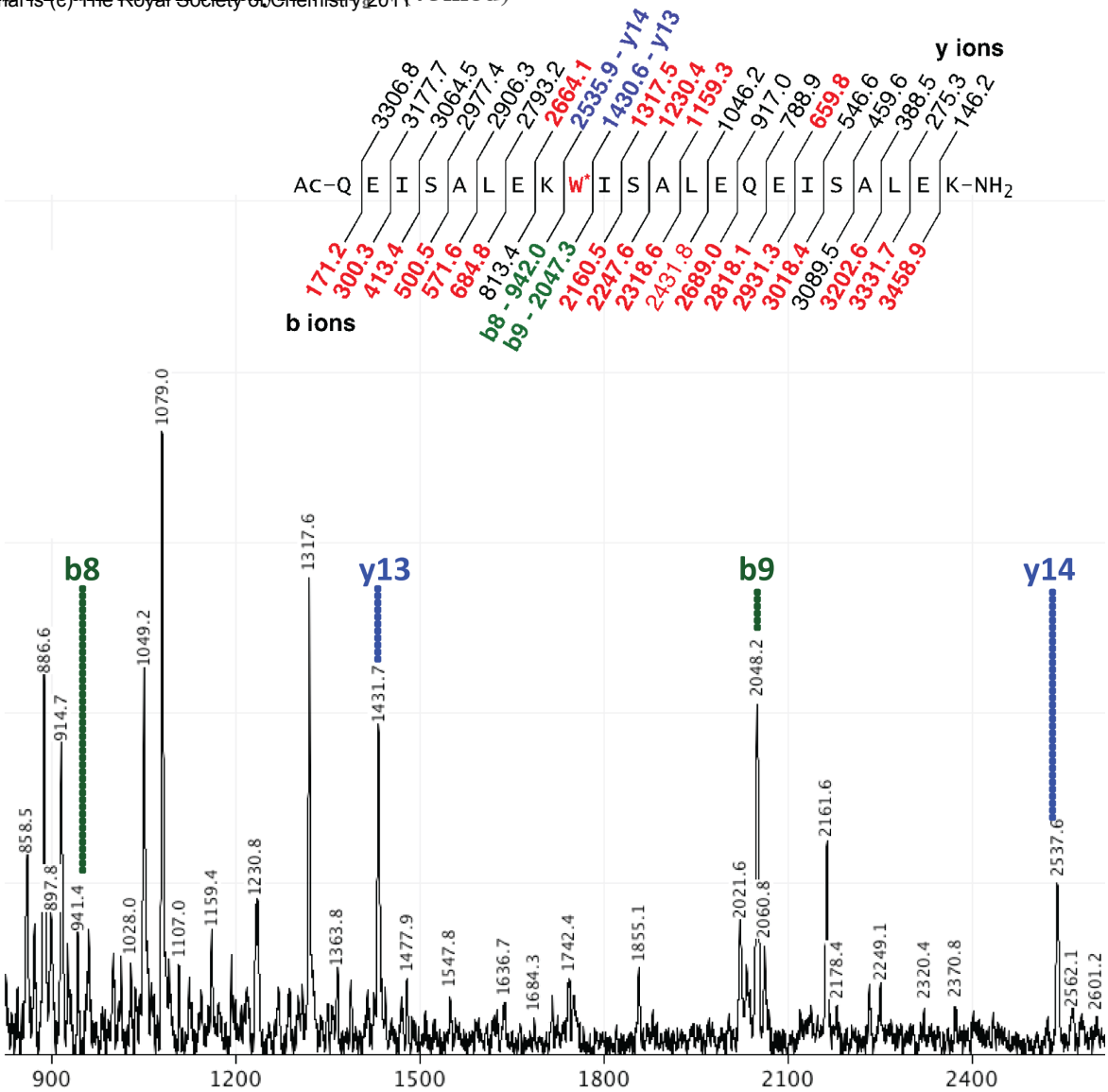


Fig. S-32. MALDI-TOF MS/MS spectrum and y/b ion fragmentation diagram of triply modified **E3$_g$W** peptide. Located ions (2 Da tolerance) are highlighted in red. Critical ions indicating the loss of modified residue are labeled in either blue (y ion) or green (b ion) in the fragmentation diagram. Calculated triply modified Trp-immonium monoisotopic mass: 1077.5 Da; found: 1079.0 Da.

MS/MS Modification Analysis: E3gQ(+1mod)

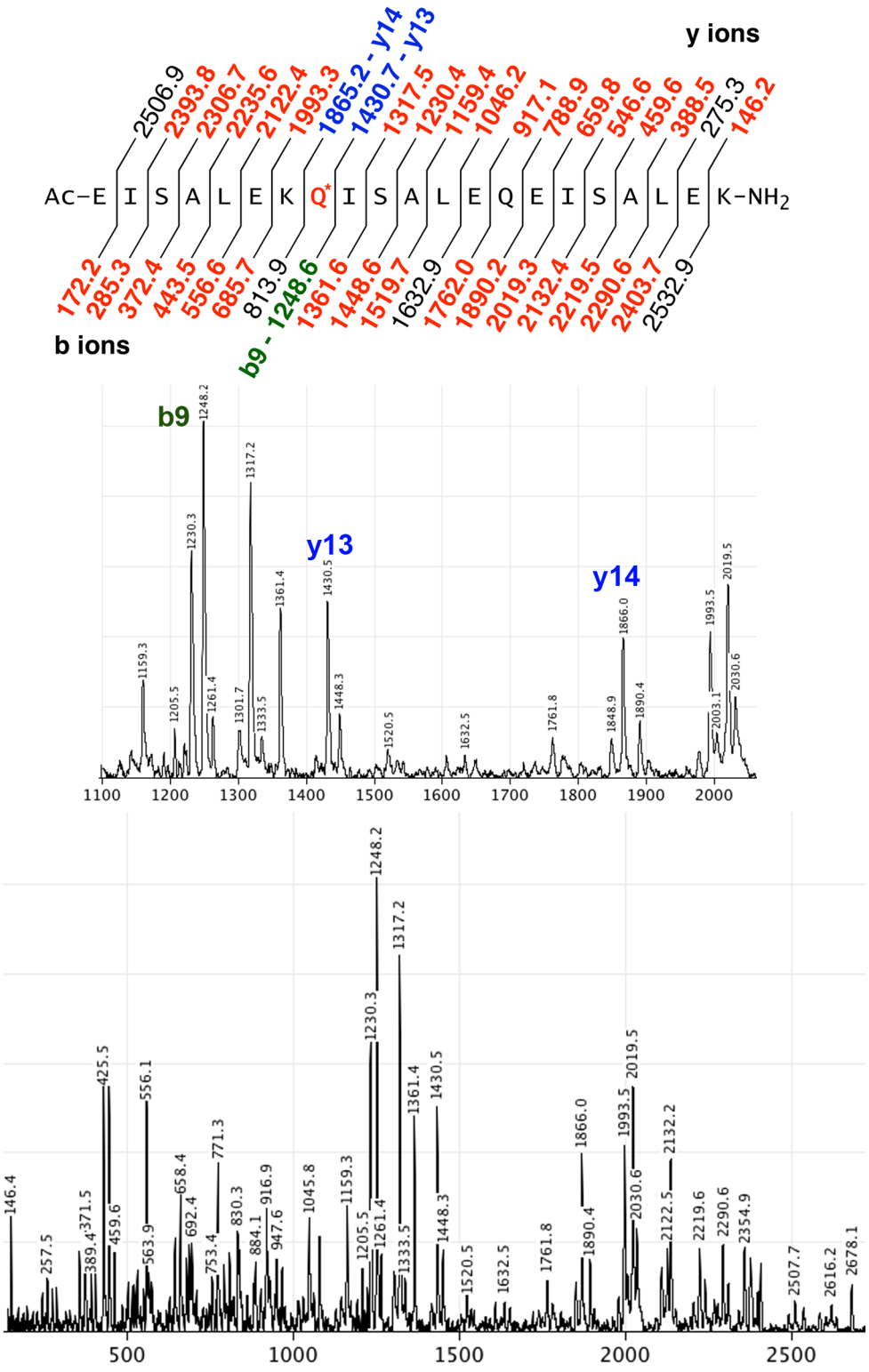


Fig. S-33. MALDI-TOF MS/MS spectrum and y/b ion fragmentation diagram of singly modified **E3$_g$Q** peptide. Located ions (2 Da tolerance) are highlighted in red. Critical ions indicating the loss of modified residue are labeled in either blue (y ion) or green (b ion) in the fragmentation diagram.

MS/MS Modification Analysis: $E3_gN$(+1mod)

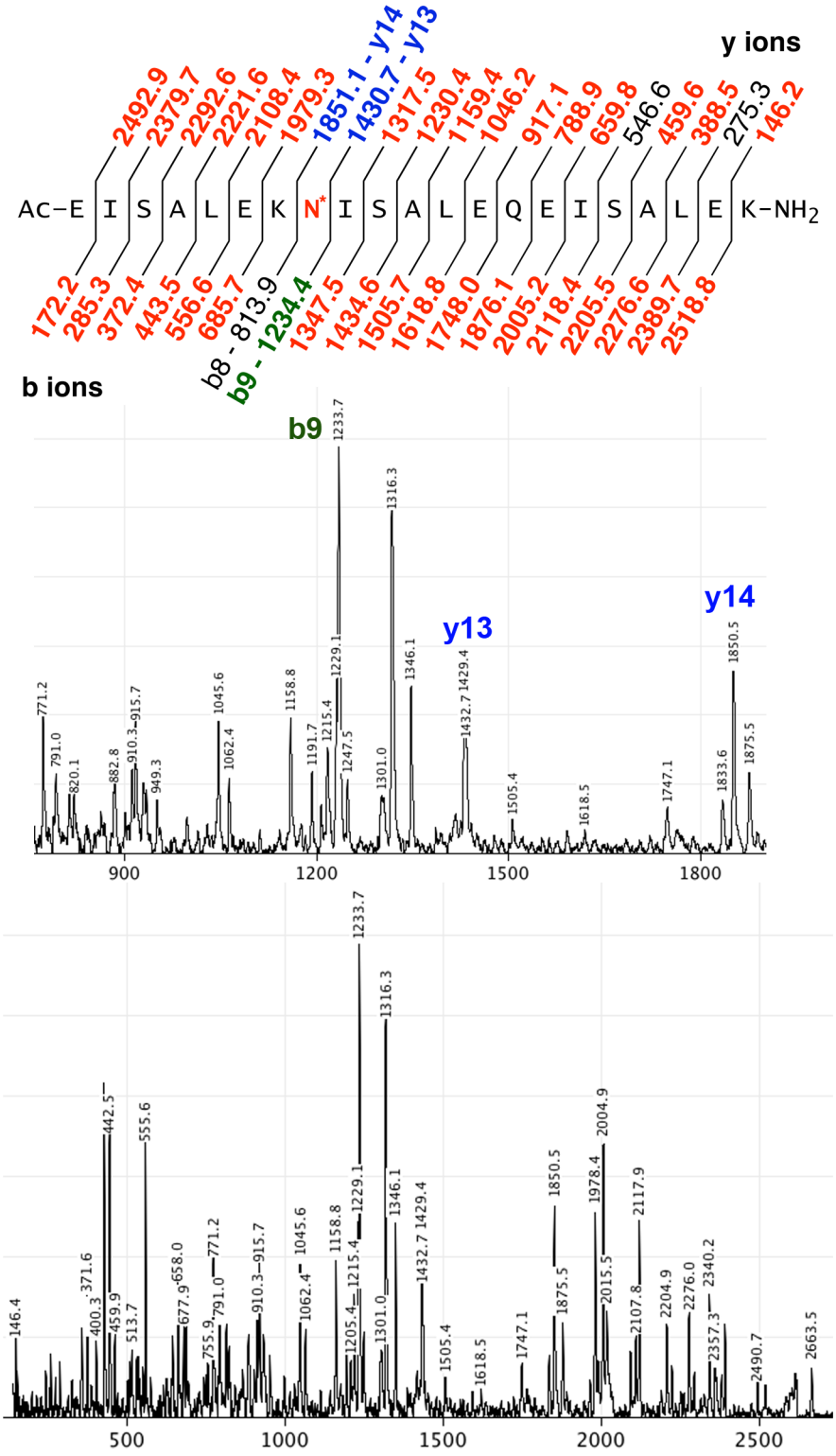


Fig. S-34. MALDI-TOF MS/MS spectrum and y/b ion fragmentation diagram of singly modified **$E3_gN$** peptide. Located ions (2 Da tolerance) are highlighted in red. Critical ions indicating the loss of modified residue are labeled in either blue (y ion) or green (b ion) in the fragmentation diagram.

MS/MS Modification Analysis: E3$_g$C(+1mod)

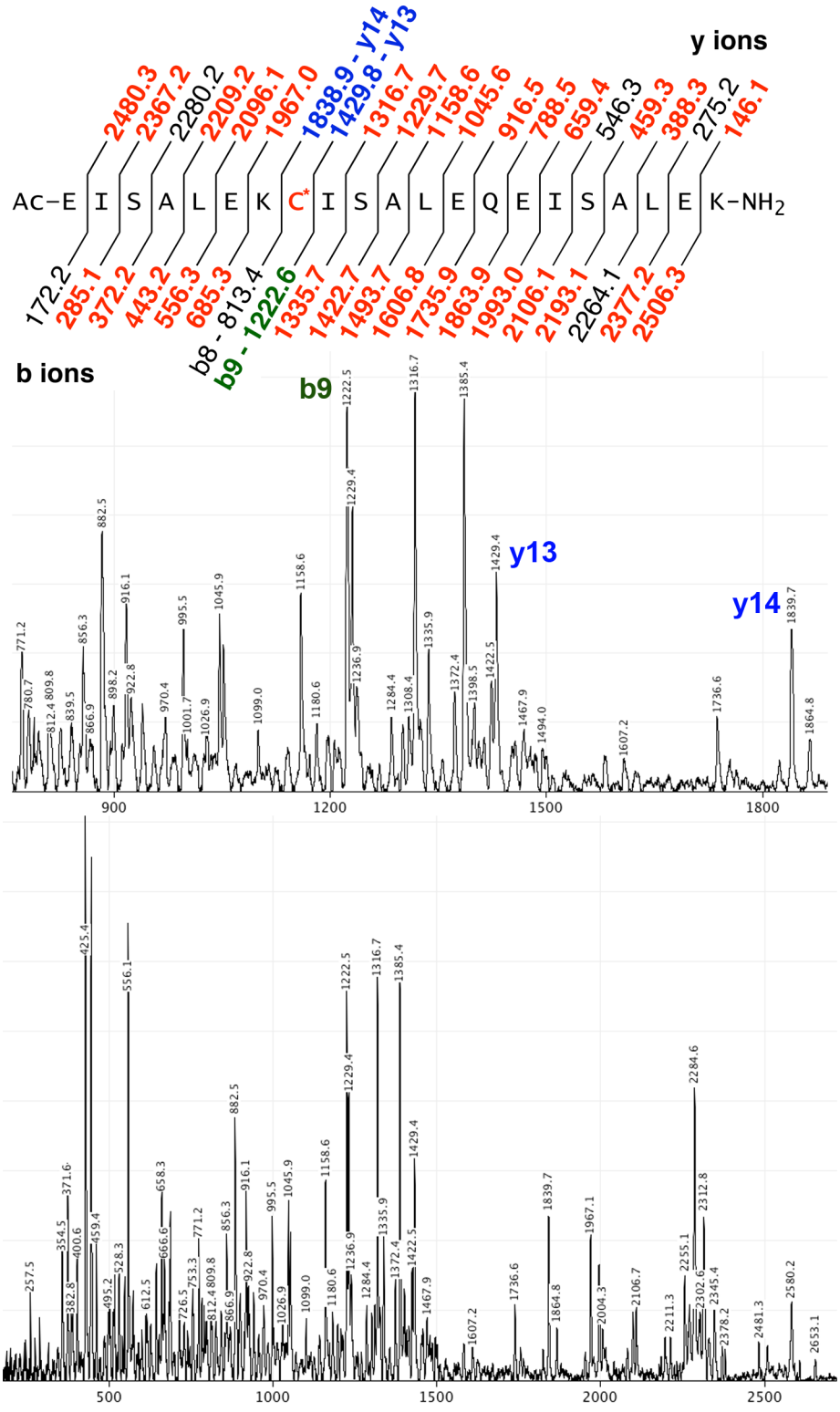


Fig. S-35. MALDI-TOF MS/MS spectrum and y/b ion fragmentation diagram of singly modified **E3$_g$C** peptide. Located ions (2 Da tolerance) are highlighted in red. Critical ions indicating the loss of modified residue are labeled in either blue (y ion) or green (b ion) in the fragmentation diagram.

MS/MS Modification Analysis: E3$_g$E(+1mod)

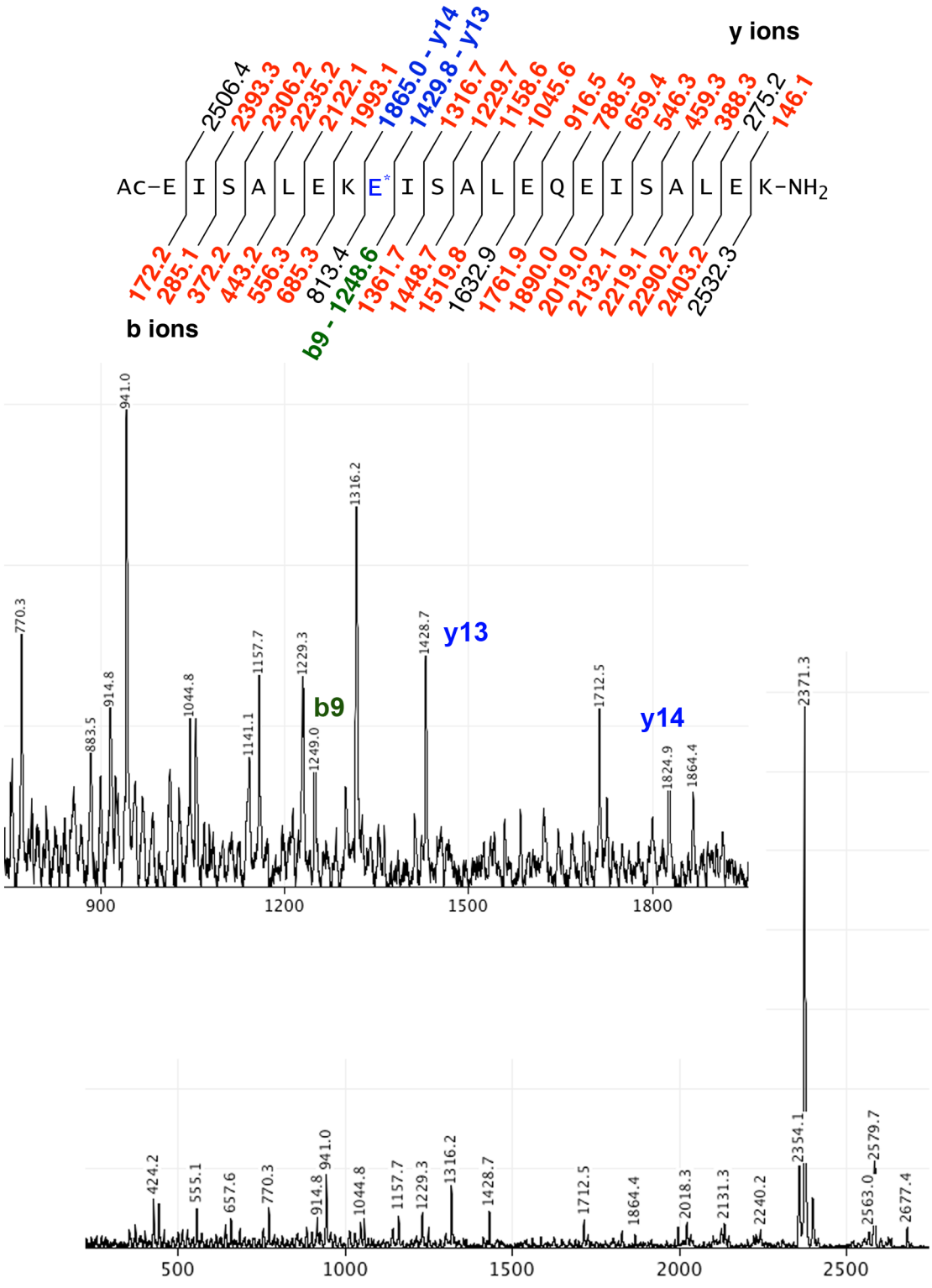


Fig. S-36. MALDI-TOF MS/MS spectrum and y/b ion fragmentation diagram of singly modified peptide **E3$_g$E**. Located ions (2 Da tolerance) are highlighted in red. Critical ions indicating the loss of modified residue are labeled in either blue or green in the fragmentation diagram. The parent peptide, **E3$_g$E**, is the most abundant ion indicating preferential fragmentation of the modifying group.

MS/MS Modification Analysis: E3$_g$D(+1mod)

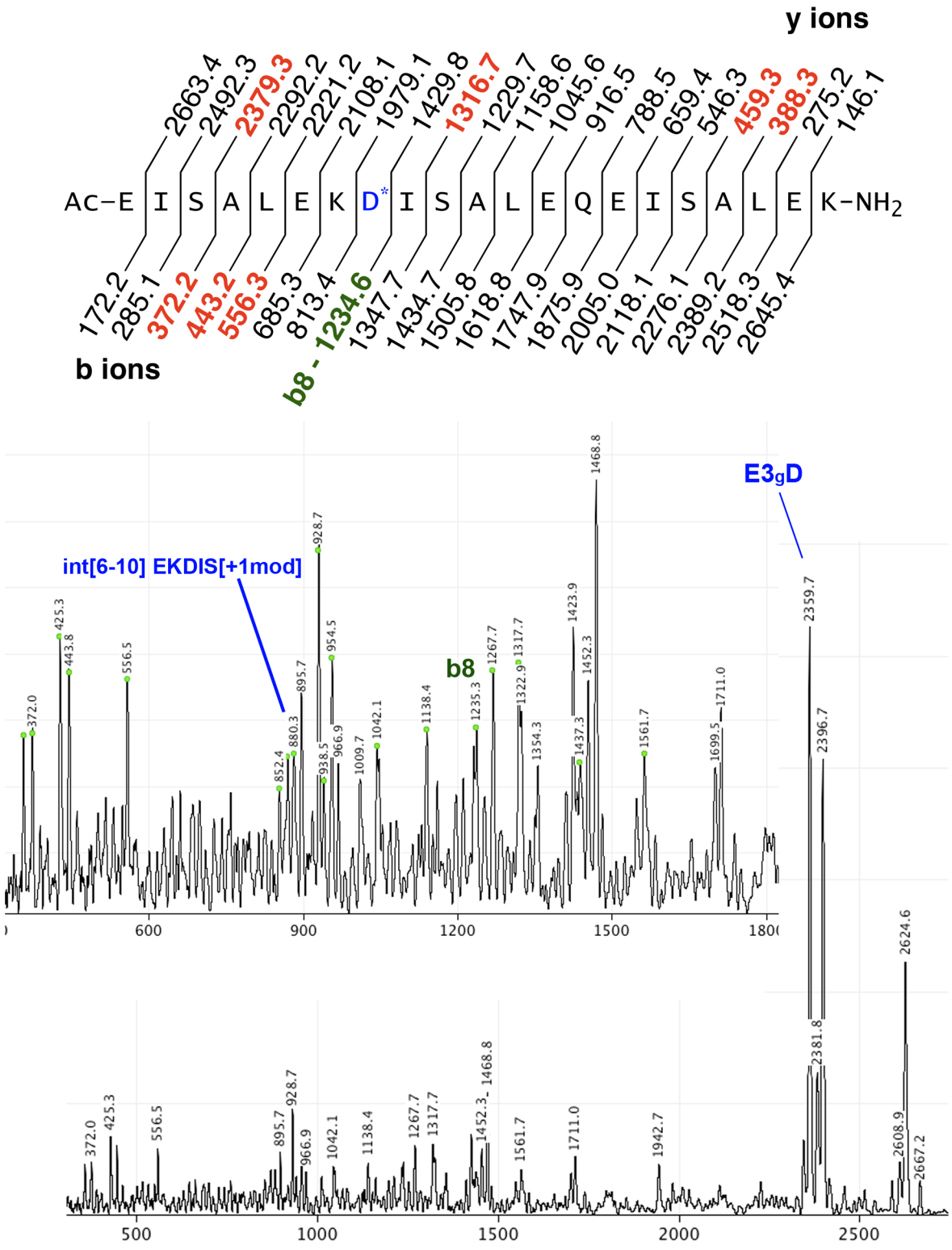


Fig. S-37. MALDI-TOF MS/MS spectrum and y/b ion fragmentation diagram of singly modified peptide **E3$_g$D**. Located ions (2 Da tolerance) are highlighted in red. Critical ions are labeled in either blue or green in the fragmentation diagram. The parent peptide, **E3$_g$D**, is the most abundant ion indicating preferential fragmentation of the modifying group.

MS/MS Modification Analysis: E3$_g$R(+1mod)

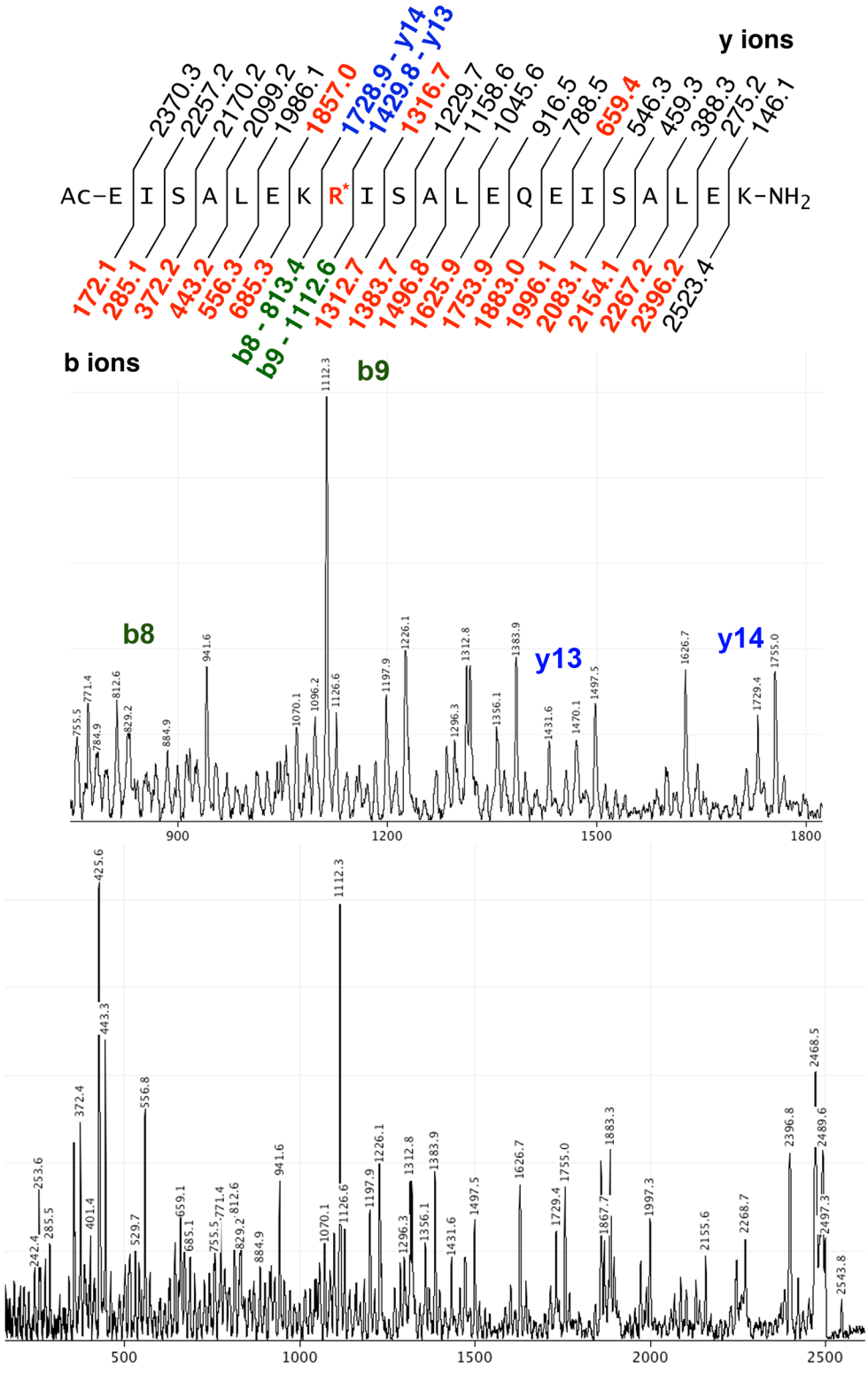


Fig. S-38. MALDI-TOF MS/MS spectrum and y/b ion fragmentation diagram of singly modified **E3$_g$R** peptide with a presumed imidazalone structure (+141 Da). Located ions (2 Da tolerance) are highlighted in red. Critical ions indicating the loss of modified residue are labeled in either blue (y ion) or green (b ion) in the fragmentation diagram.

MS/MS Modification Analysis: E3$_g$S(+1mod)

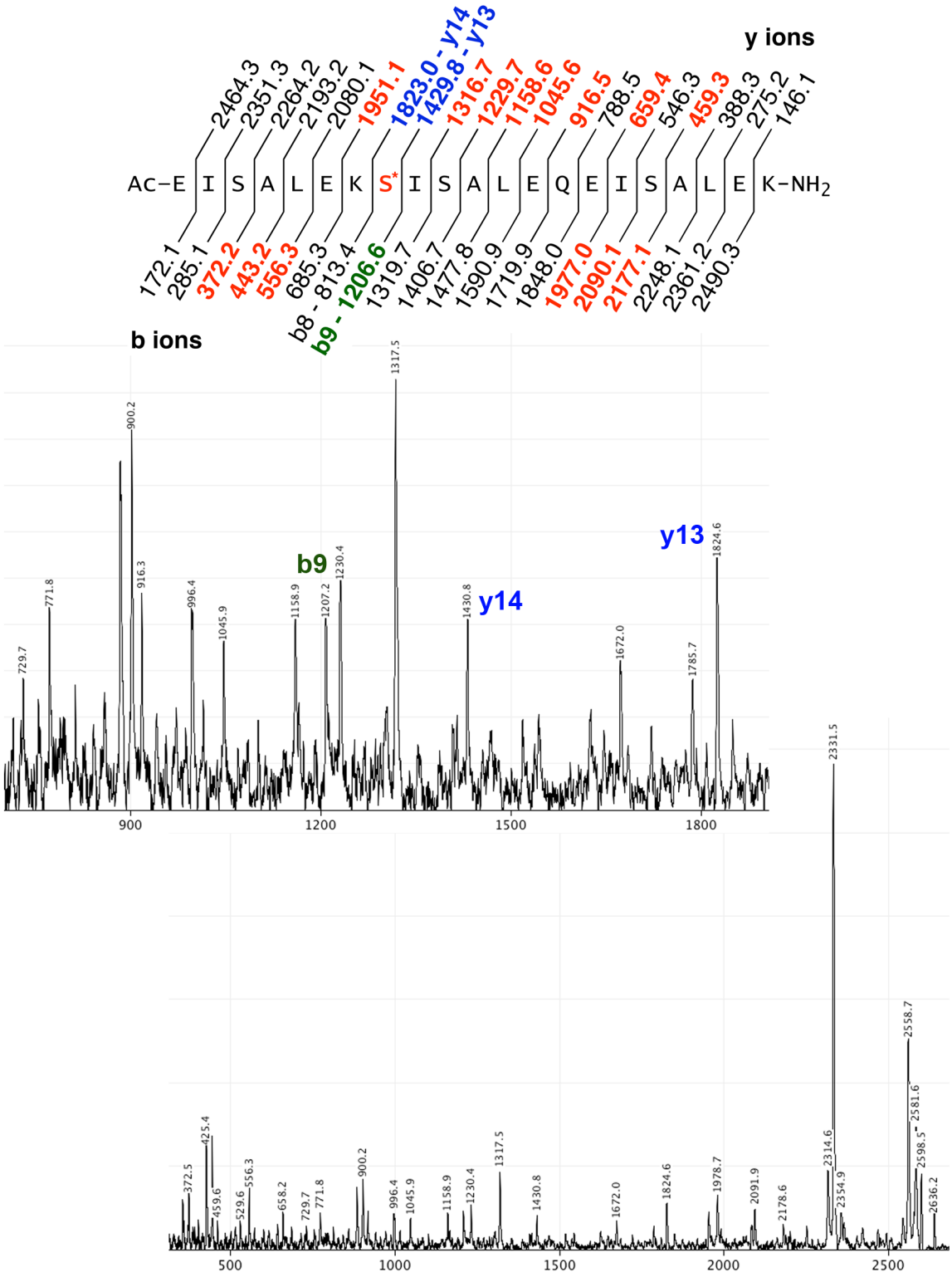


Fig. S-39. MALDI-TOF MS/MS spectrum and y/b ion fragmentation diagram of singly modified **E3$_g$R** peptide. Located ions (2 Da tolerance) are highlighted in red. Critical ions indicating the loss of modified residue are labeled in either blue (y ion) or green (b ion) in the fragmentation diagram.

MS/MS Modification Analysis: E3$_g$H(+1mod)

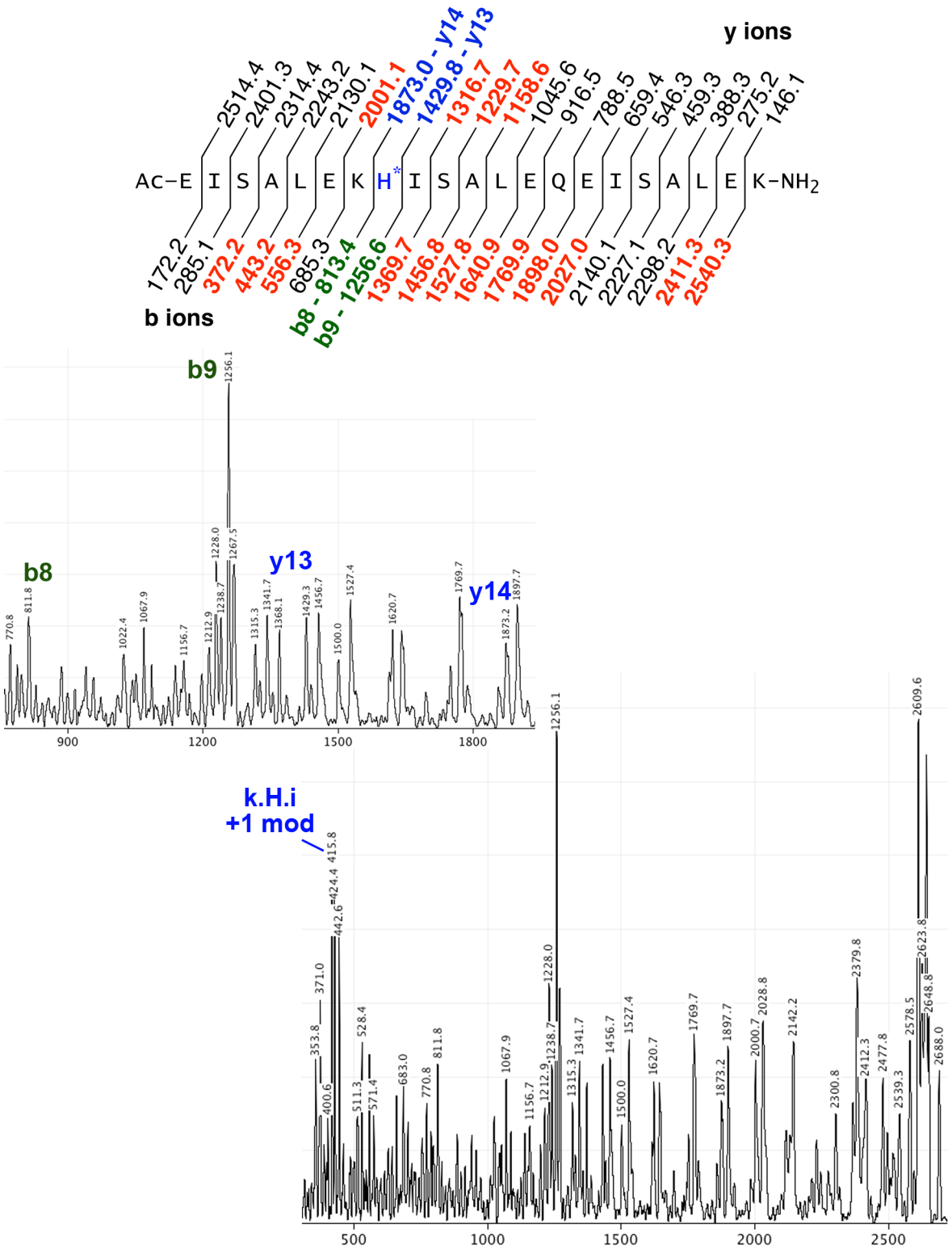


Fig. S-40. MALDI-TOF MS/MS spectrum and y/b ion fragmentation diagram of singly modified peptide **E3$_g$H**. Located ions (2 Da tolerance) are highlighted in red. Critical ions indicating the loss of modified residue are labeled in either blue or green in the fragmentation diagram.

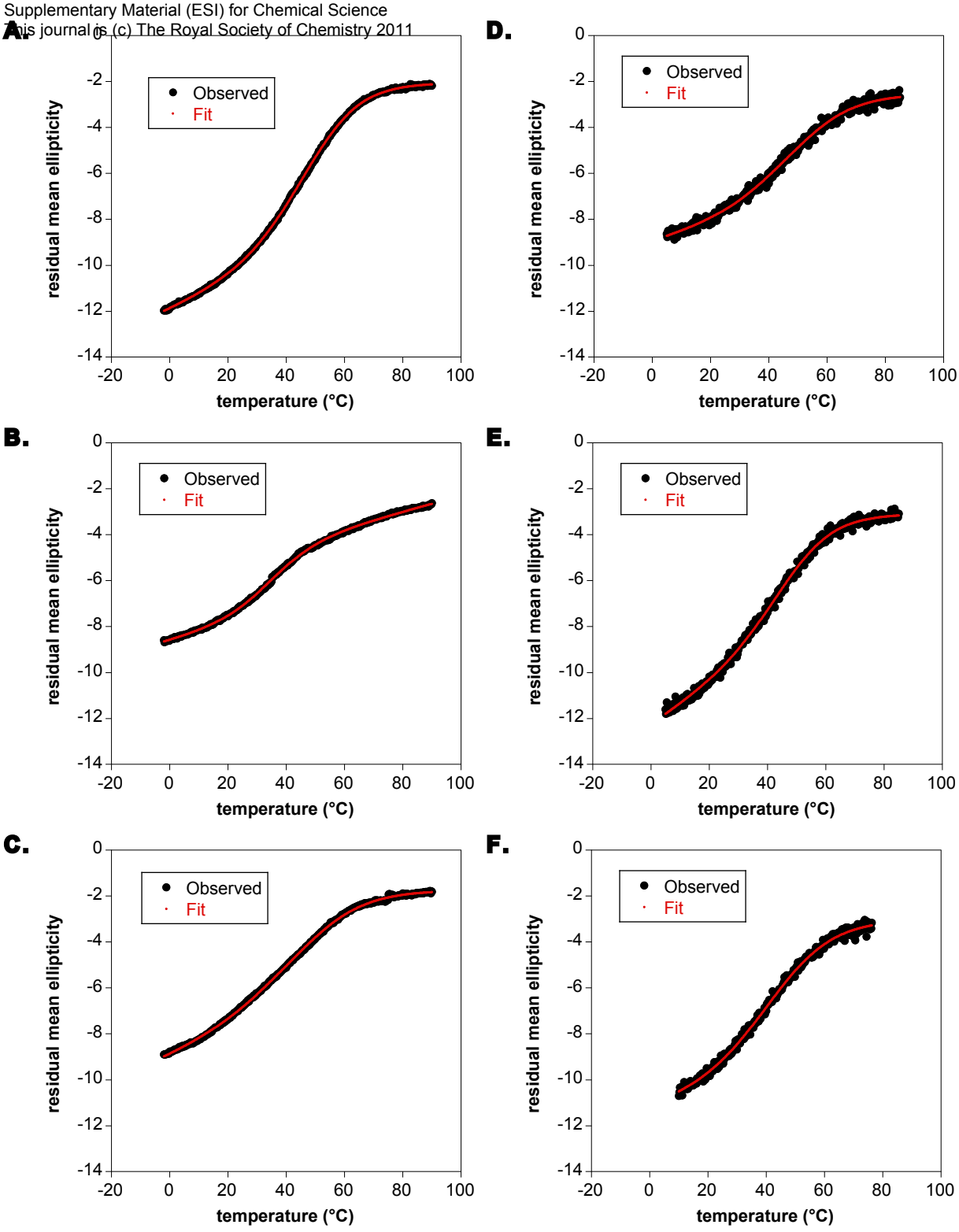


Fig. S-40. CD spectroscopic thermal profiles at 222 nm of stoichiometric mixtures of $\mathbf{K3_{a,e}Rh_2}$ (100 μM) and **E3gX** (100 μM) in aqueous buffer. X = A) **E3gQ**, B) **E3gR**, C) **E3gC**, D) **E3gW**, E) **E3gY**, F) **E3gF**. Data D-F was originally reported in ref. 5 and is reproduced here with fitting result.

Table S-8. Thermodynamic parameters melting temperature (T_m)[a] and dissociation constant (units of μM) at room temperature (K_d)[b] extracted from thermal denaturation profiles shown in Fig. S-32.

	T_m	$K_d^{(obs)}$	$K_d^{(fit)}$
E3$_g$Q	46.9	7.3	7.2
E3$_g$R	42.4	34.4	33.1
E3$_g$C	33.7	39.7	39.4
E3$_g$W	47.1	15.2	11.4
E3$_g$Y	46.7	4.7	5.7
E3$_g$F	39.9	27.3	29.8

a) In units of °C with a standard error of +/– 0.1 °C.
b) Data averaged over the range of 24.6 – 25.4 °C.

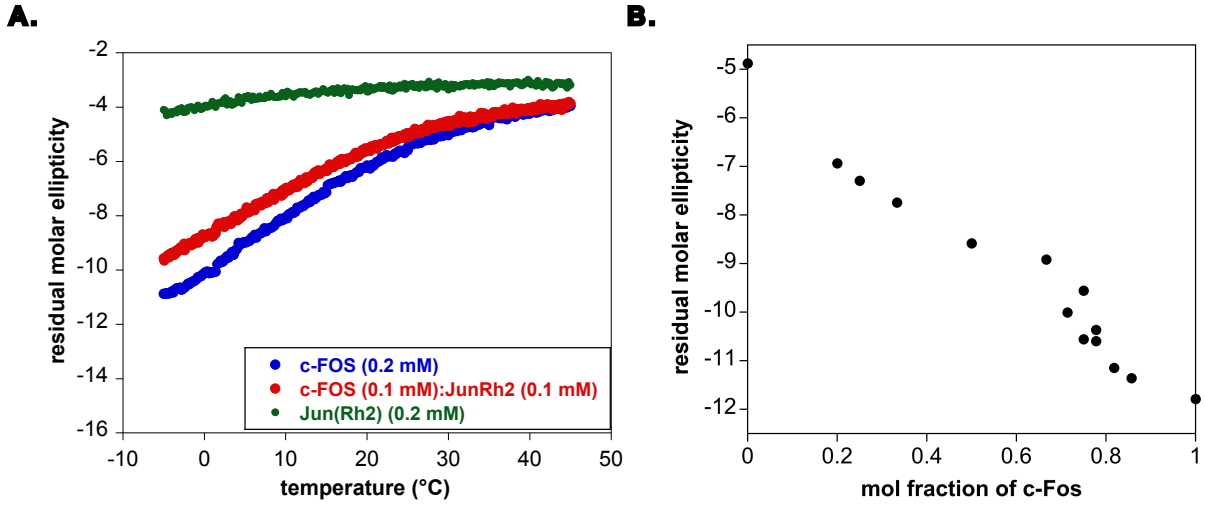


Fig. S-41. CD spectroscopy studies of **c-Fos/Jun(Rh$_2$)** in aqueous buffer at pH 6.5. A) Thermal profiles monitored at 222 nm with 100 μM **c-Fos** and 100 μM **Max(Rh$_2$)**. B) Job plot at 4 °C with $[peptide]_{total}$=200 μM.

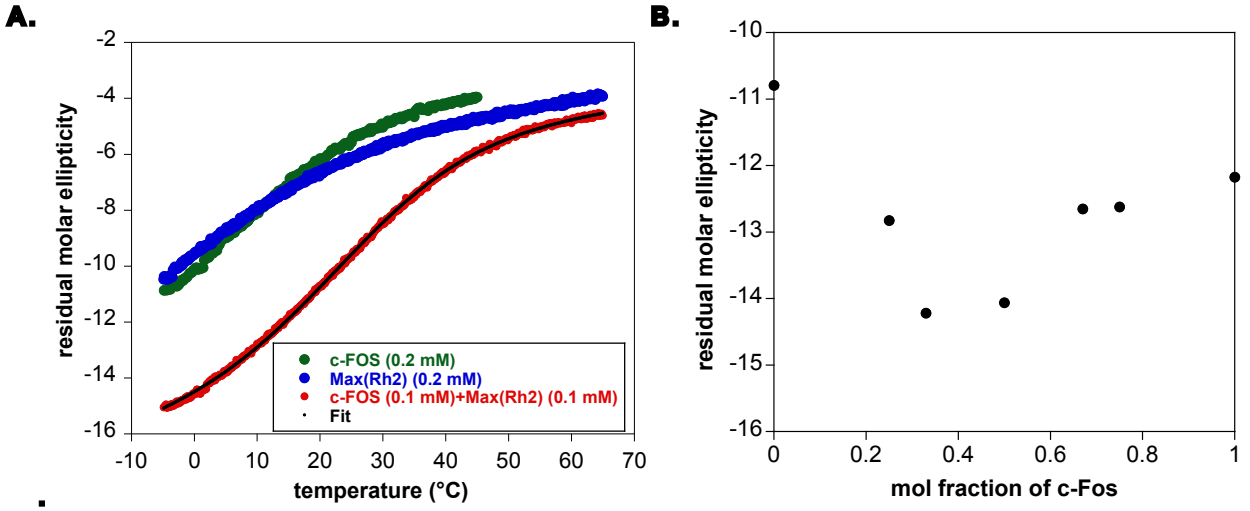


Fig. S-42. CD spectroscopy studies of **c-Fos/Max(Rh$_2$)** in aqueous buffer at pH 6.5. A) Thermal denaturation profiles monitored at 222 nm with 100 μM **c-Fos** and 100 μM **Max(Rh$_2$)**. Thermal binding parameters extracted and extrapolated from the fit curve are: T_m = 20.2 °C and K_d = 28 and 4.4 μM at 4 and –15 °C, respectively. B) Job plot at 4 °C with $[peptide]_{total}$=200 μM. Data averaged over multiple runs.

Analytical Data for Peptides.

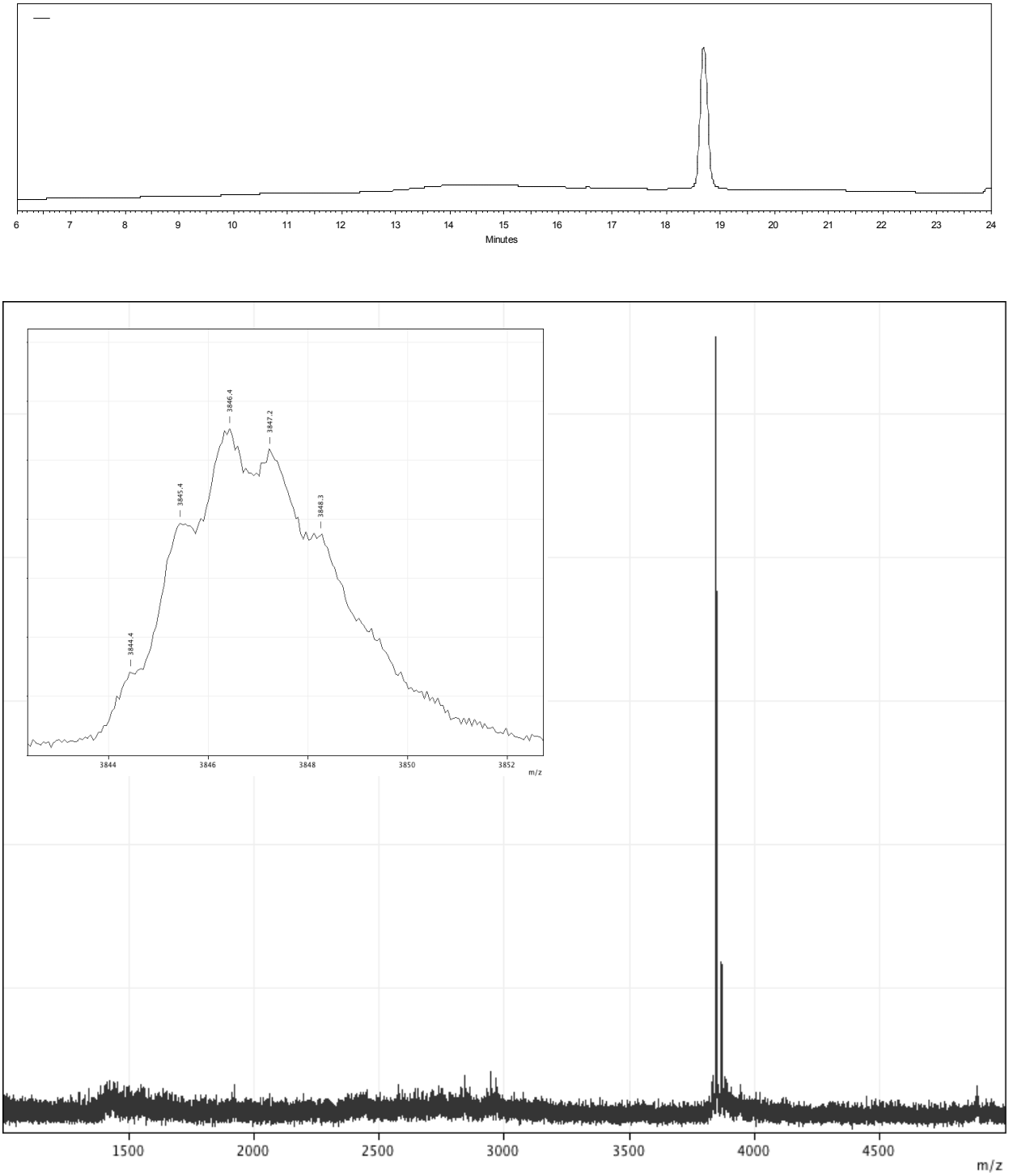


Fig. S-43. Analytical HPLC trace and MALDI-TOF spectrum of isolated peptide **c-Fos** (sequence: Ac-ASTDTLQAETDQLEDEKSALQTEIANLLKEKEKL-NH_2). Calculated monoisotopic mass $[M+H]^+$: 3844.0; found: 3844.4.

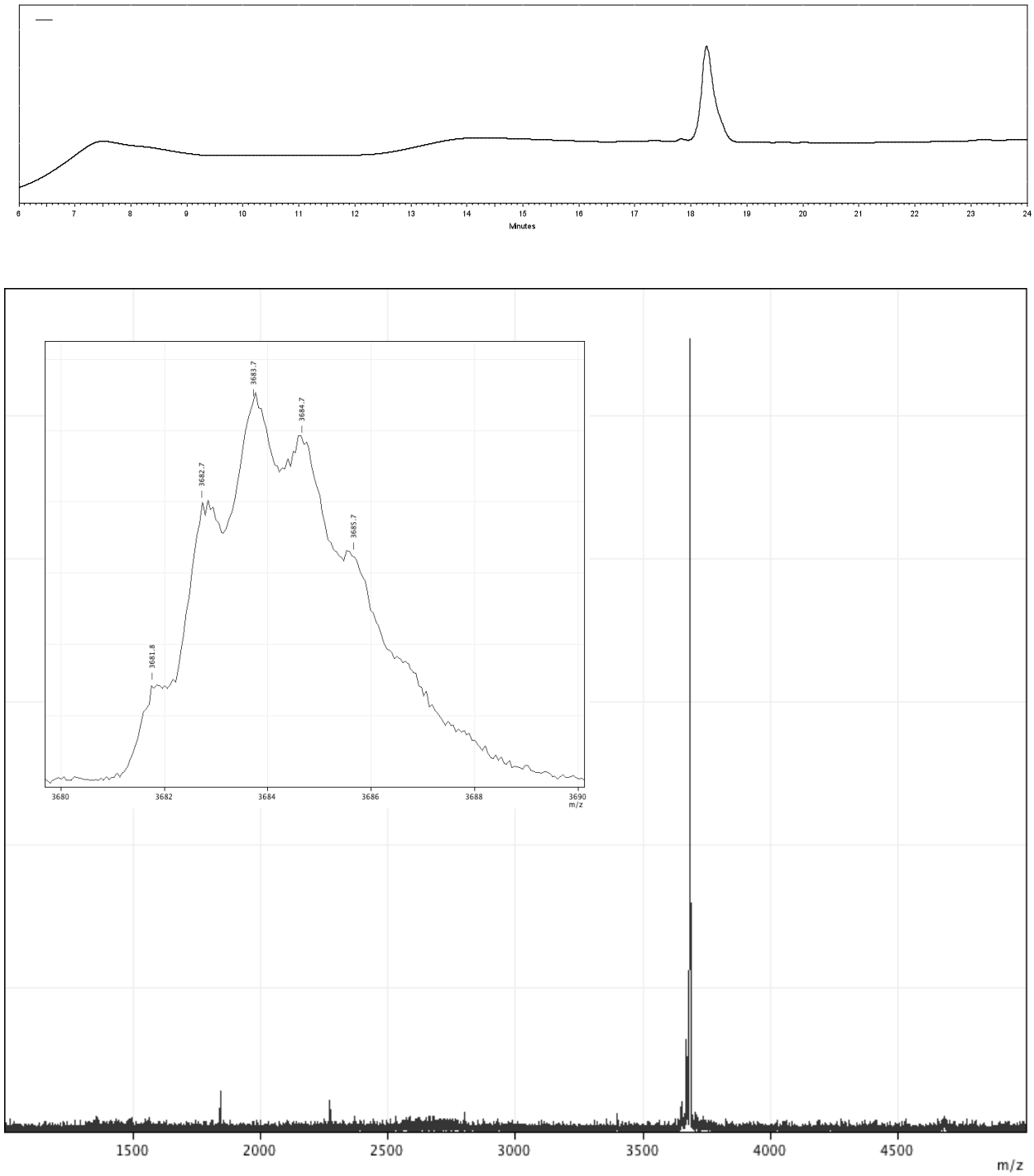


Fig. S-44. Analytical HPLC trace and MALDI-TOF spectrum of isolated peptide **Jun** (sequence: Ac-ASAAQLQQRVKTLKAEISSEASTANSLRQQIAQL-NH_2). Calculated monoisotopic mass $[M+H]^+$: 3681.0; found: 3681.8.

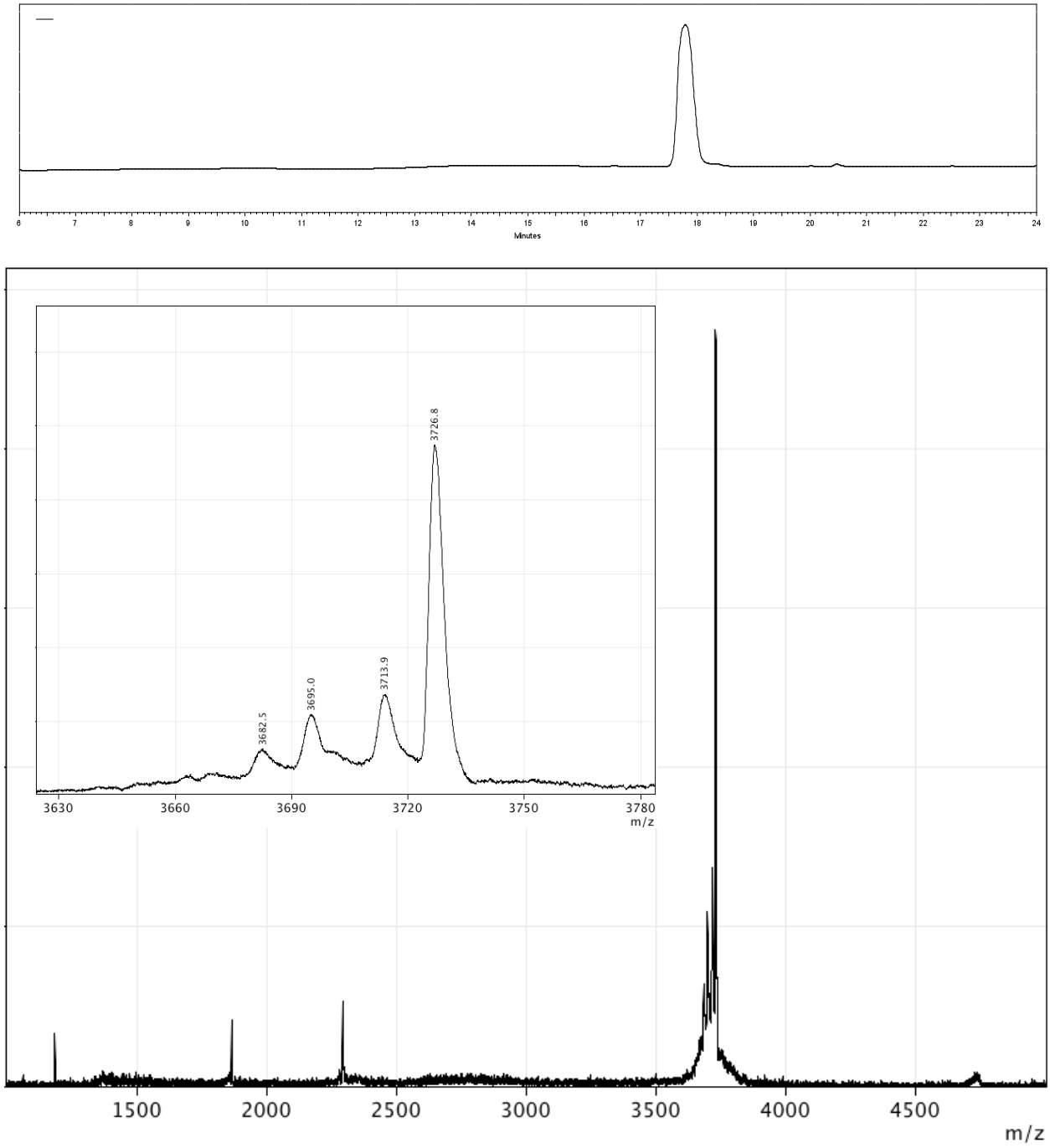


Fig. S-45. Analytical HPLC trace and MALDI-TOF spectrum of isolated peptide **Jun** (sequence: Ac-ASARRKNFTFQQNINNLKRQEALLEQQVRAL-NH_2). Calculated average mass $[M+H]^+$: 3728.2; found: 3727.8.

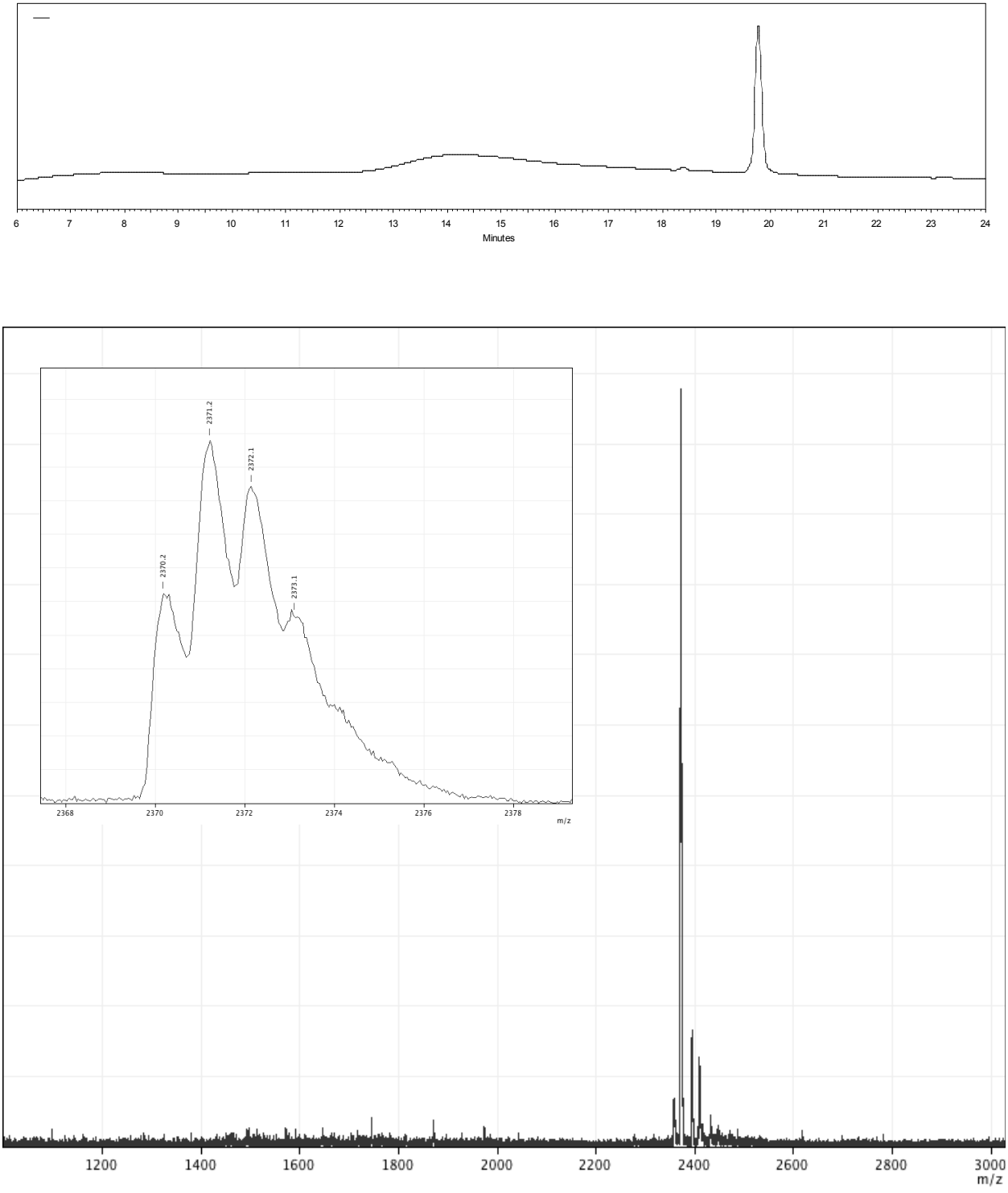


Fig. S-46. Analytical HPLC trace and MALDI-TOF spectrum of isolated peptide **E3$_g$Q** (sequence: Ac-EISALEKQISALEQEISALEK-NH_2). Calculated monoisotopic mass $[M+H]^+$: 2370.3; found: 2370.2.

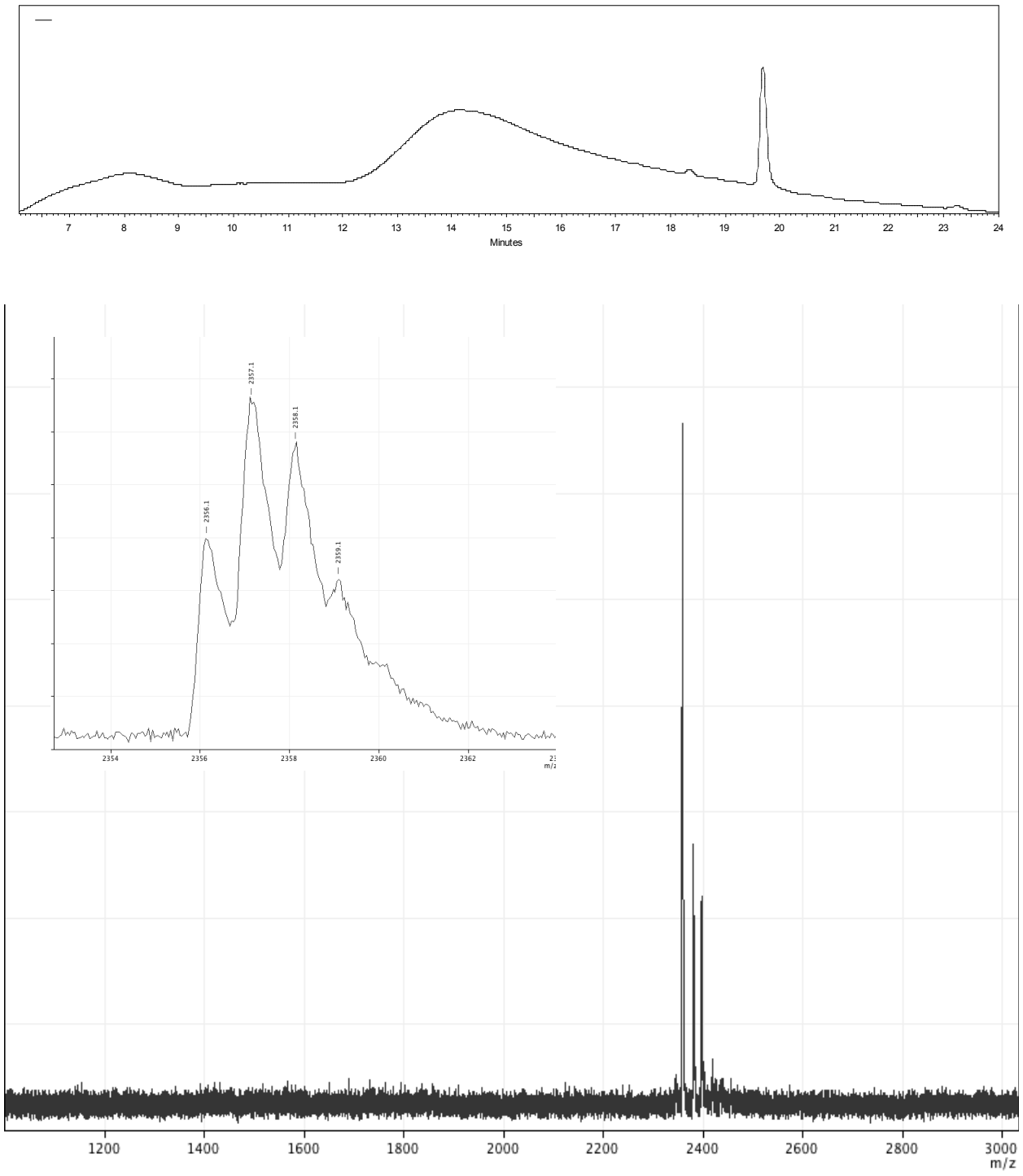


Fig. S-47. Analytical HPLC trace and MALDI-TOF spectrum of isolated peptide **E3$_g$N** (sequence: Ac-EISALEKNISALEQEISALEK-NH_2). Calculated monoisotopic mass $[M+H]^+$: 2356.3; found: 2356.1.

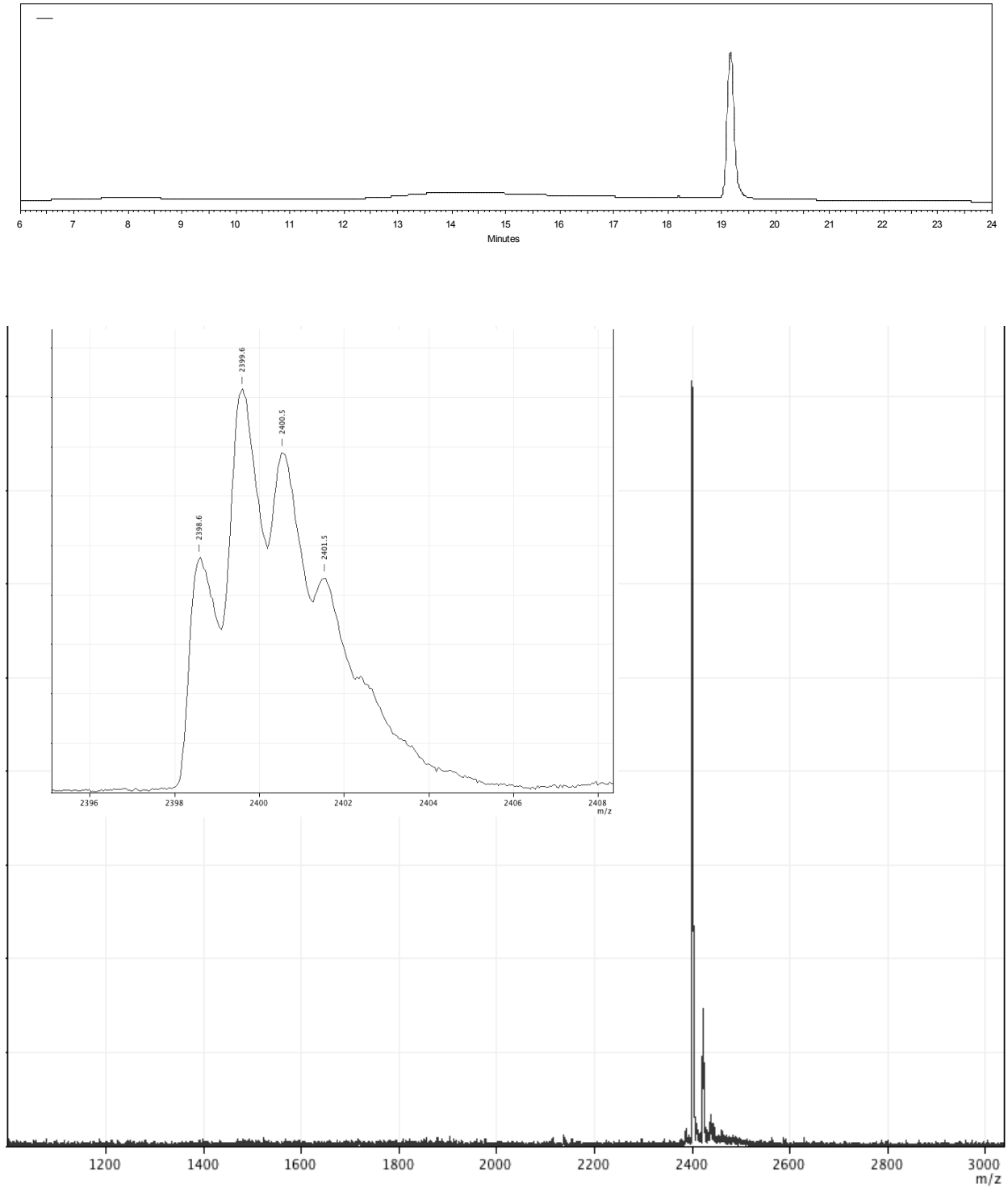


Fig. S-48. Analytical HPLC trace and MALDI-TOF spectrum of isolated peptide **E3$_g$R** (sequence: Ac-EISALEKRISALEQEISALEK-NH_2). Calculated monoisotopic mass $[M+H]^+$: 2398.3; found: 2398.6.

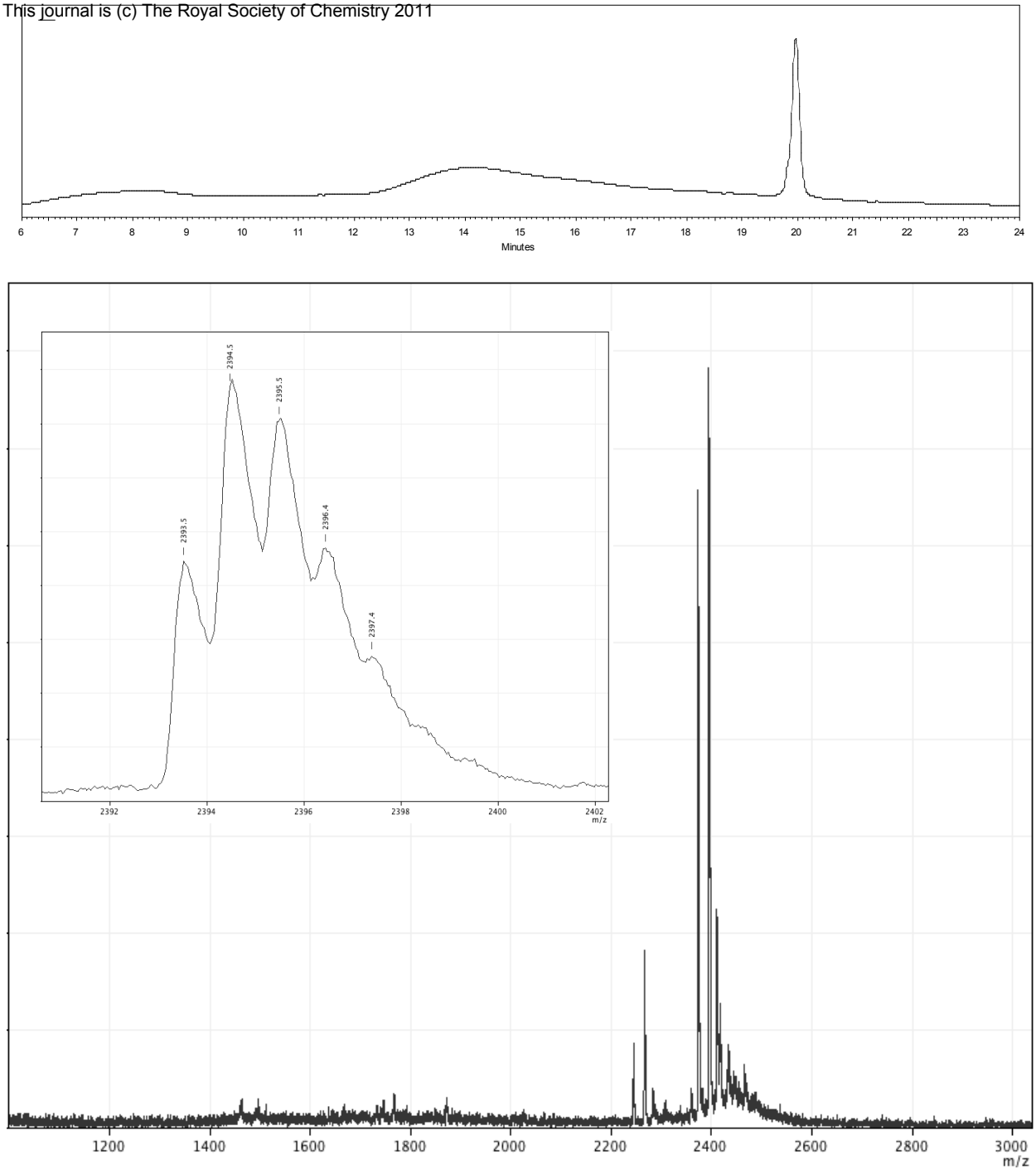


Fig. S-49. Analytical HPLC trace and MALDI-TOF spectrum of isolated peptide **E3$_g$E** (sequence: Ac-EISALEKEISALEQEISALEK-NH_2). Calculated monoisotopic mass $[M+Na]^+$: 2393.2; found: 2393.5.

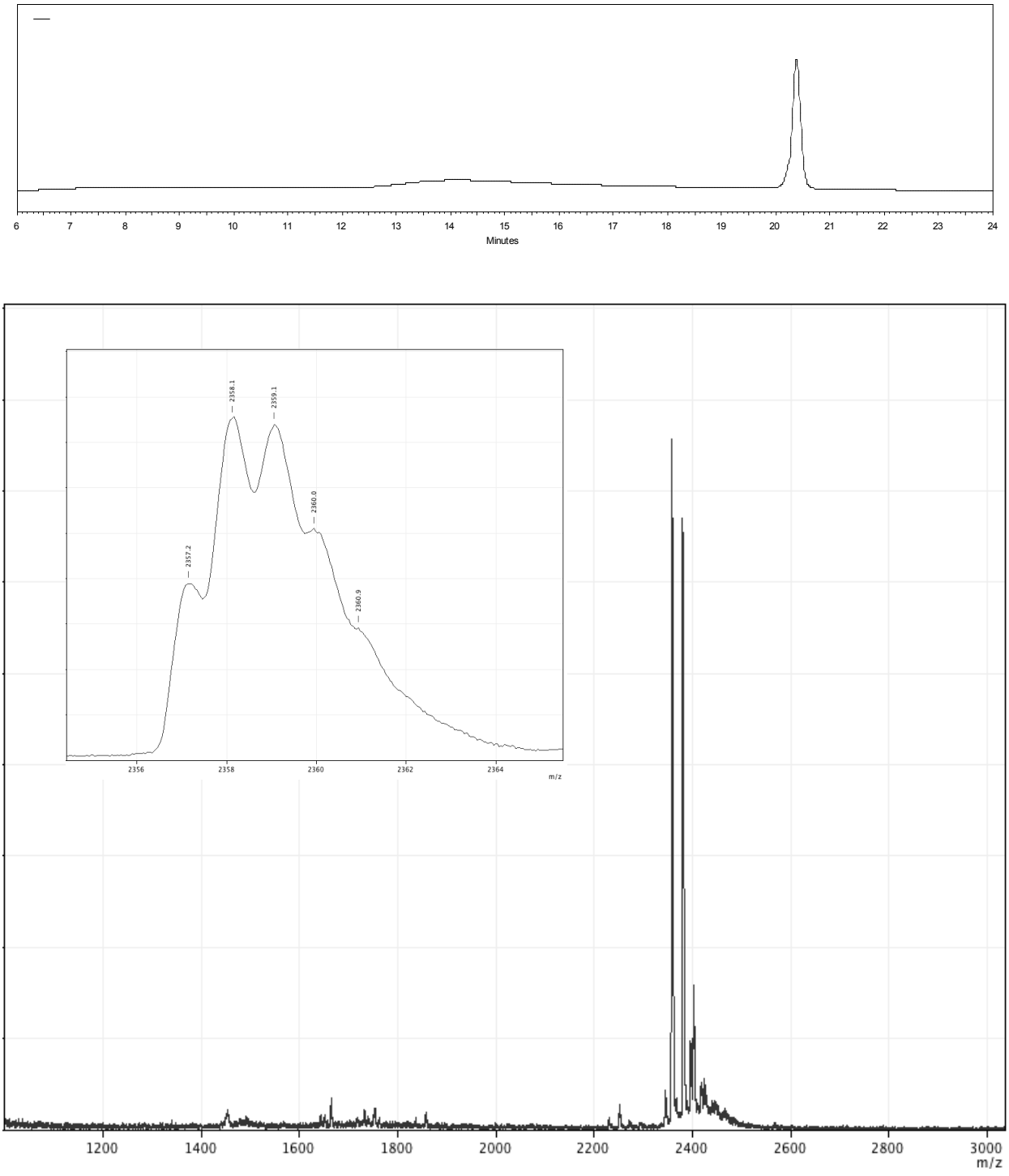


Fig. S-50. Analytical HPLC trace and MALDI-TOF spectrum of isolated peptide **E3$_g$D** (sequence: Ac-EISALEKDISALEQEISALEK-NH_2). Calculated monoisotopic mass $[M+H]^+$: 2357.3; found: 2357.2.

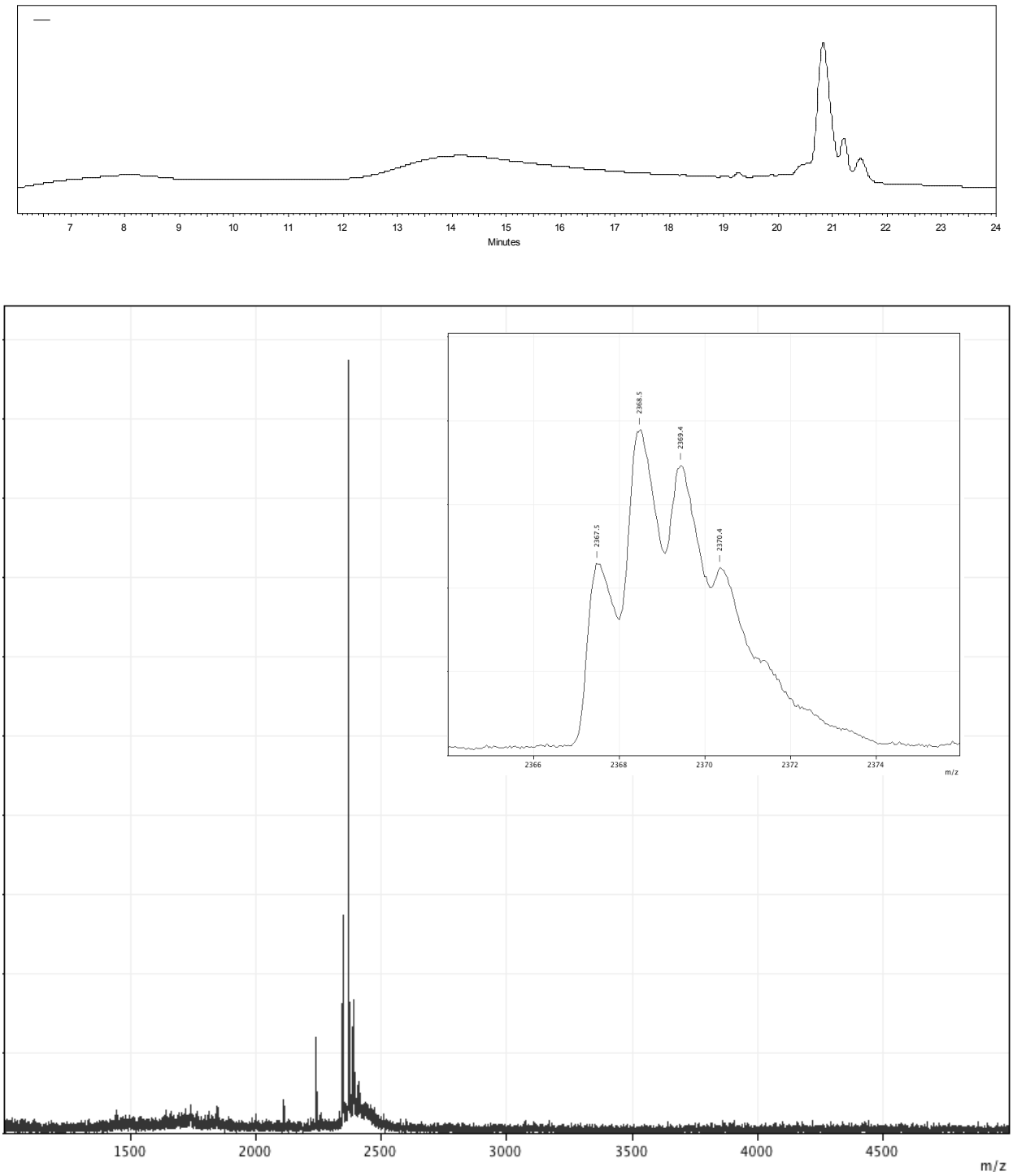


Fig. S-51. Analytical HPLC trace and MALDI-TOF spectrum of isolated peptide **E3$_g$C** (sequence: Ac-EISALEKCISALEQEISALEK-NH_2). Calculated monoisotopic mass $[M+Na]^+$: 2367.2; found: 2367.5.

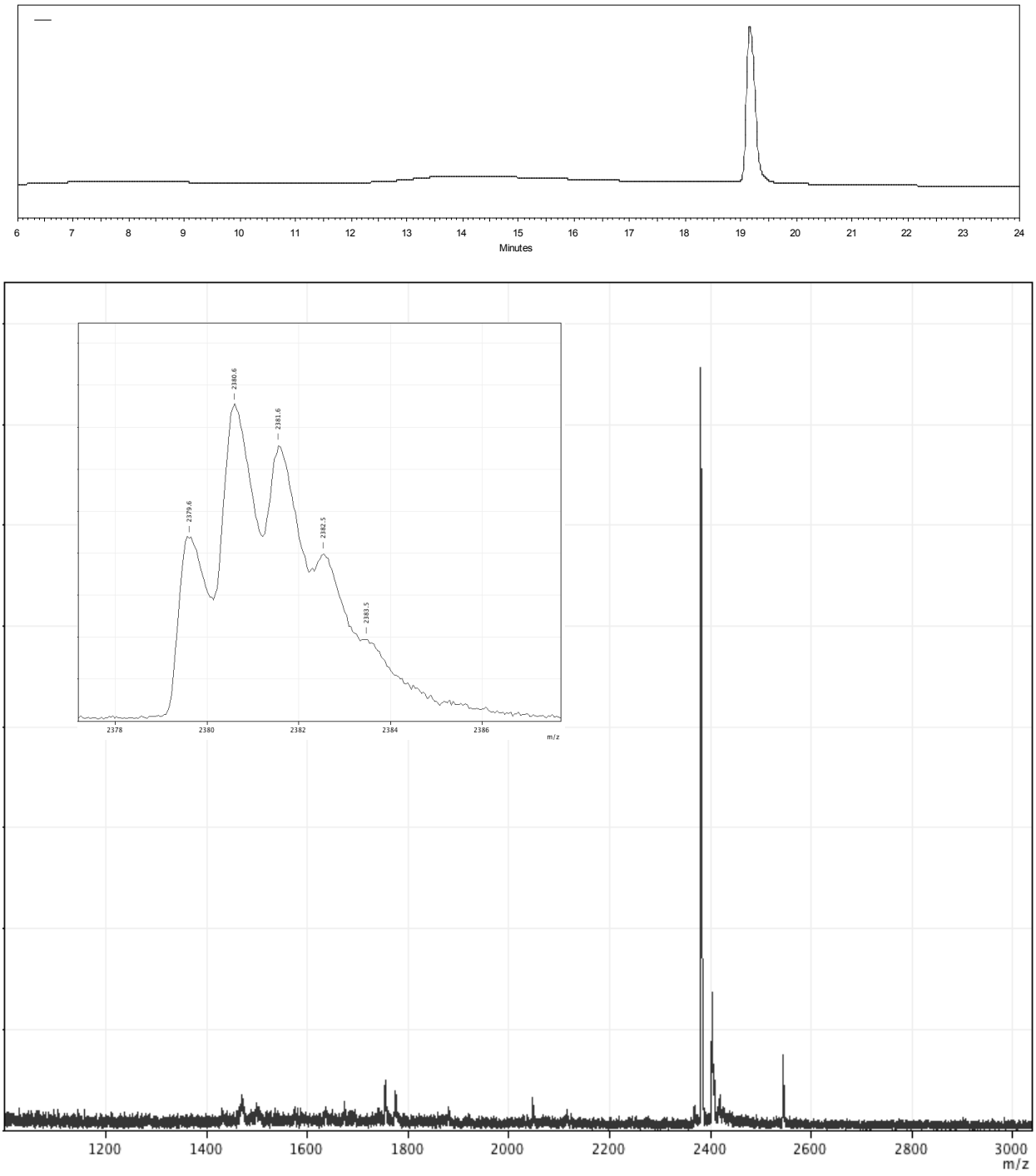


Fig. S-52. Analytical HPLC trace and MALDI-TOF spectrum of isolated peptide **E3$_g$H** (sequence: Ac-EISALEKHISALEQEISALEK-NH_2). Calculated monoisotopic mass $[M+H]^+$: 2379.3; found: 2379.6.

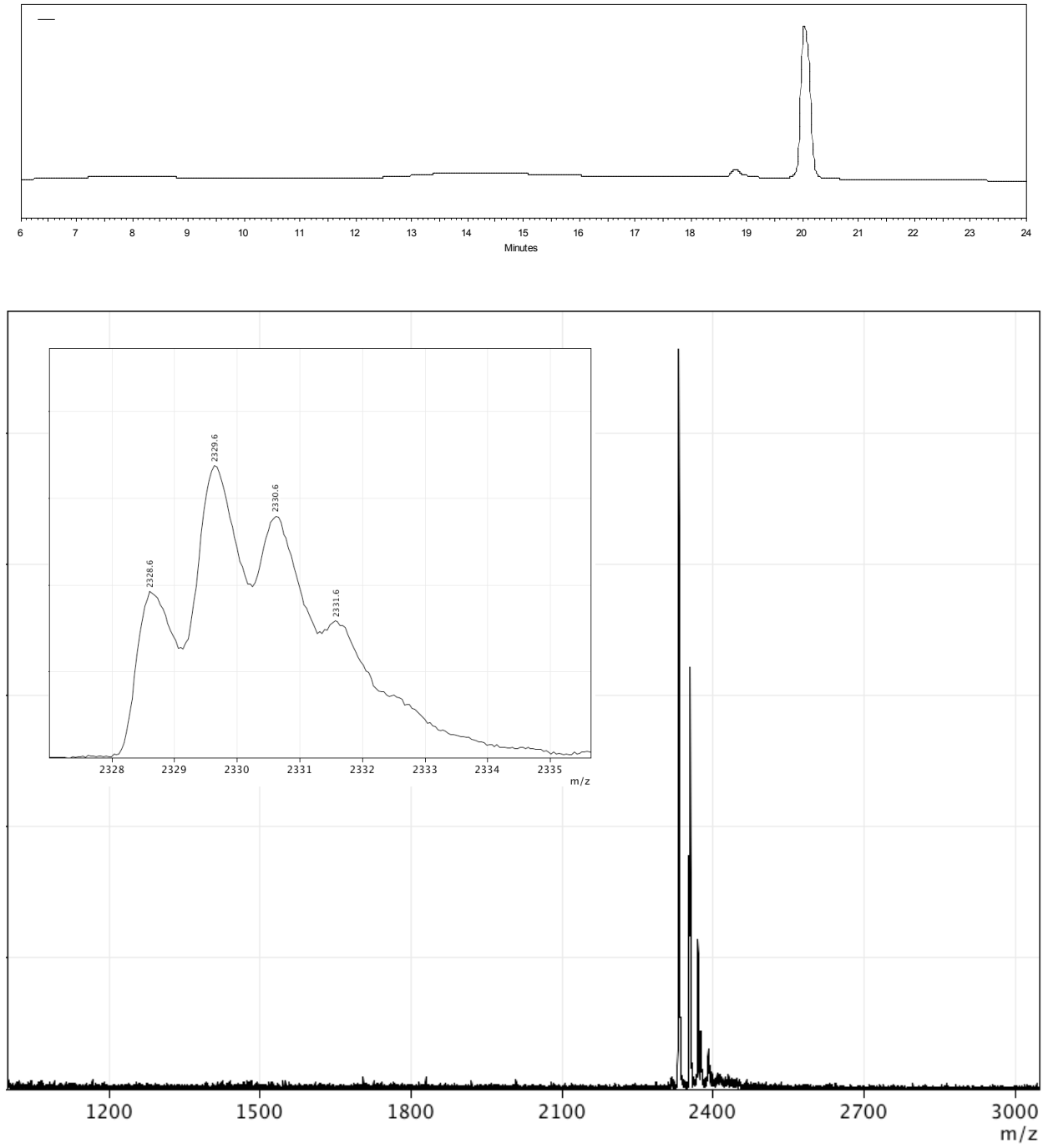


Fig. S-53. Analytical HPLC trace and MALDI-TOF spectrum of isolated peptide **E3$_g$S** (sequence: Ac-EISALEKSISALEQEISALEK-NH_2). Calculated monoisotopic mass $[M+H]^+$: 2329.2; found: 2328.6.

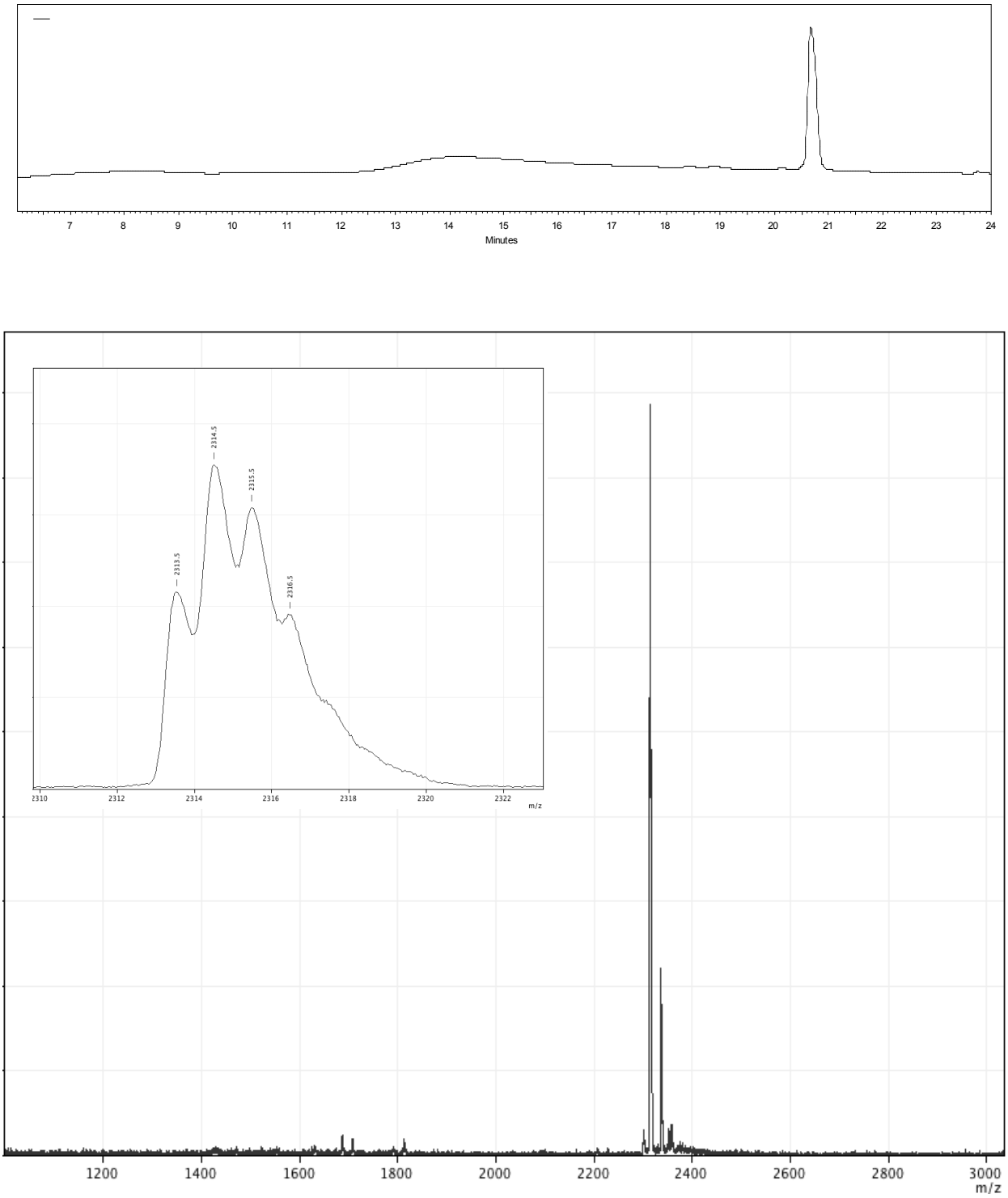


Fig. S-54. Analytical HPLC trace and MALDI-TOF spectrum of isolated peptide **E3$_g$A** (sequence: Ac-EISALEKAISALEQEISALEK-NH_2). Calculated monoisotopic mass $[M+H]^+$: 2313.2; found: 2313.5.

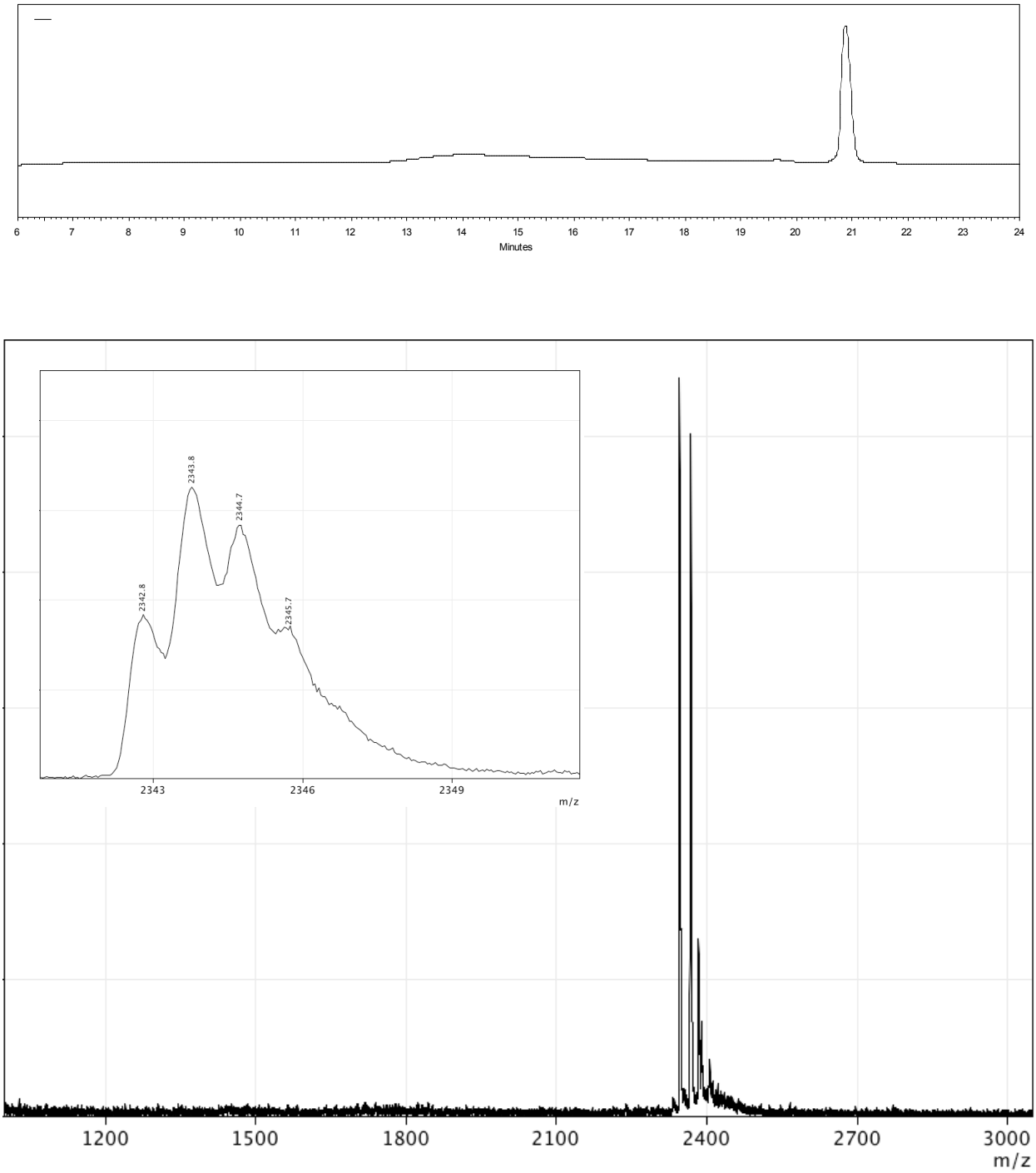


Fig. S-55. Analytical HPLC trace and MALDI-TOF spectrum of isolated peptide **E3$_g$T** (sequence: Ac-EISALEKTISALEQEISALEK-NH_2). Calculated monoisotopic mass $[M+H]^+$: 2343.2; found: 2342.8.

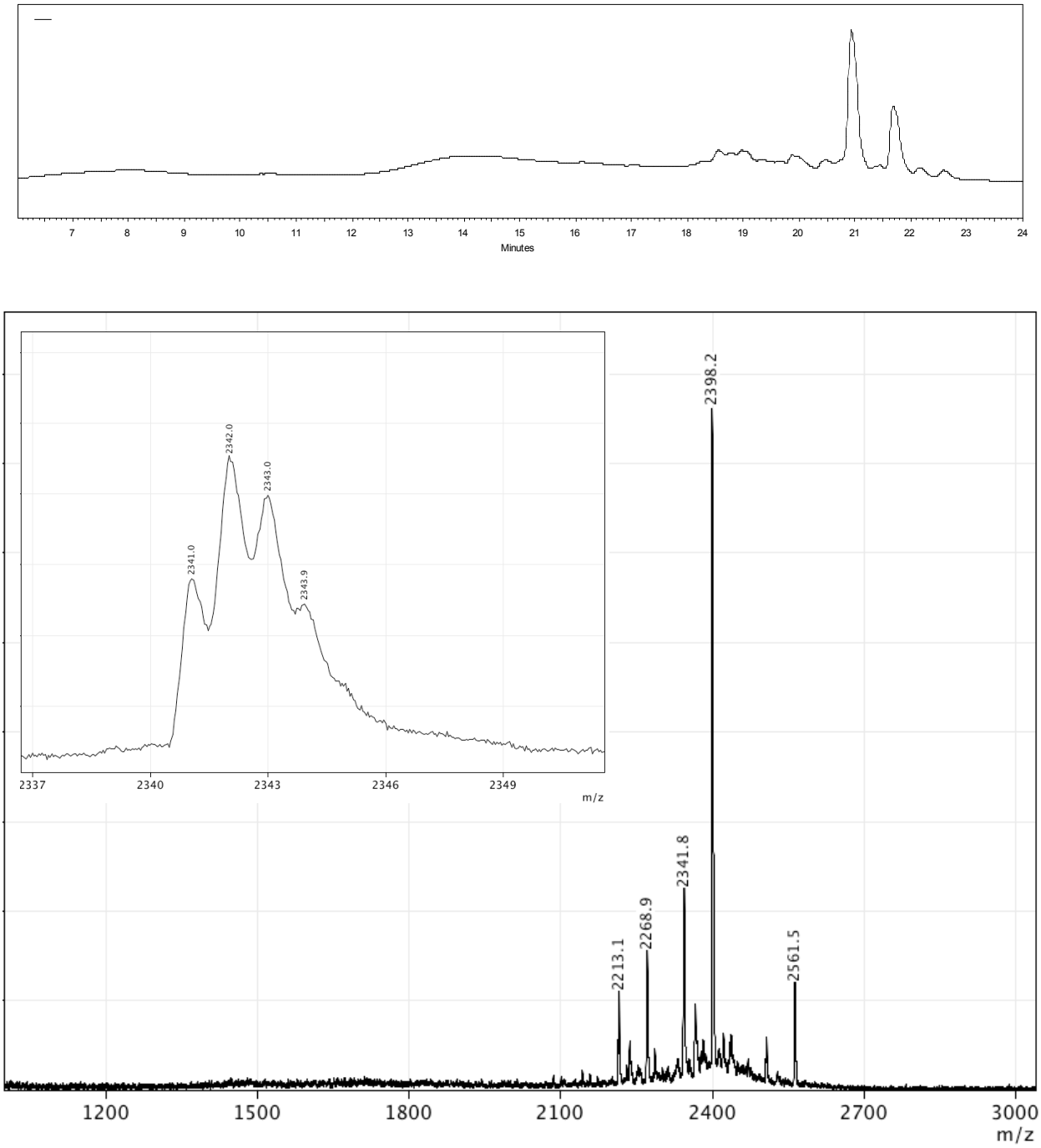


Fig. S-56. Analytical HPLC trace and MALDI-TOF spectrum of crude peptide **E3$_g$V** (sequence: Ac-EISALEKVISALEQEISALEK-NH_2). Calculated monoisotopic mass $[M+H]^+$: 2341.3; found: 2341.0.

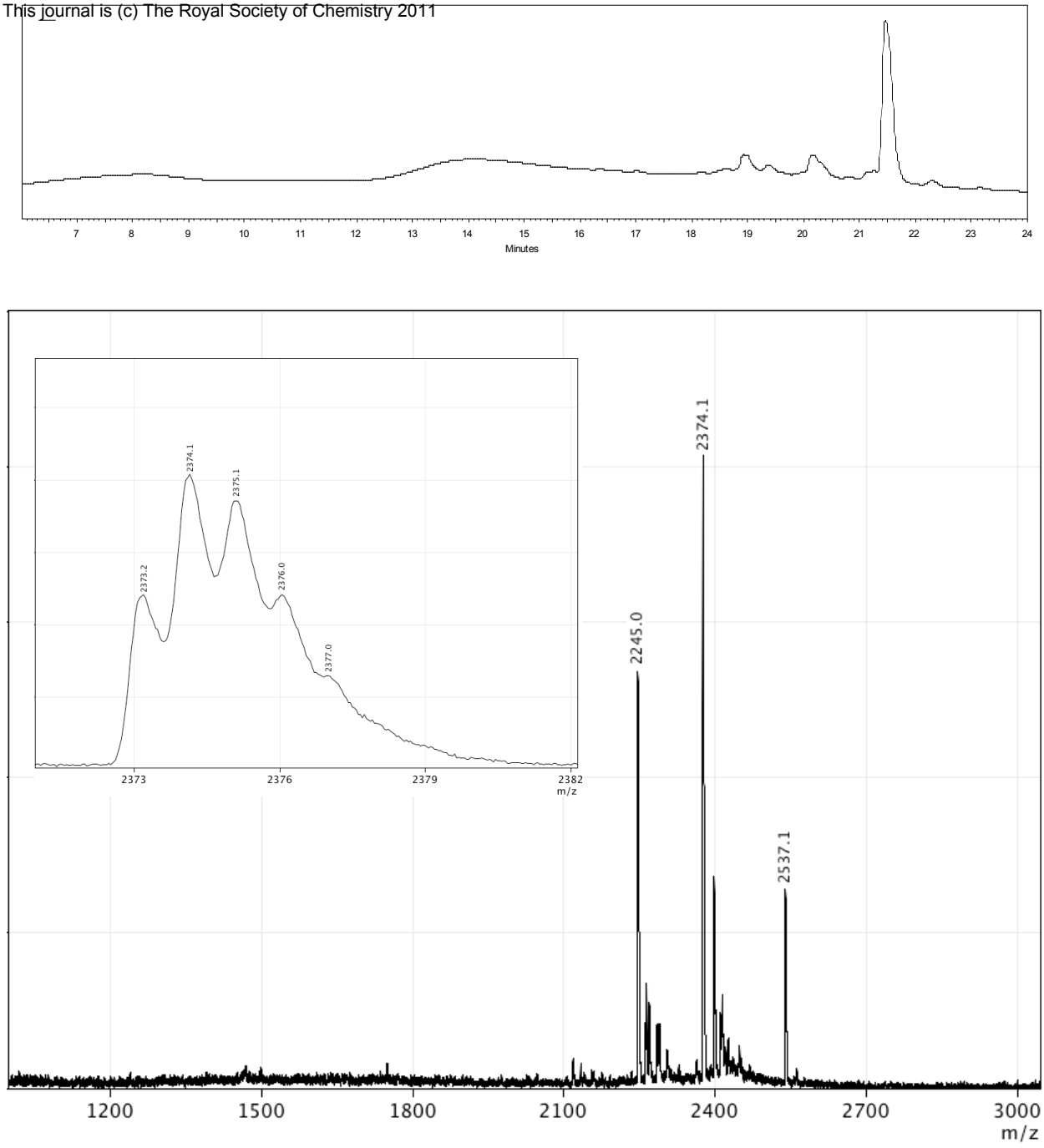


Fig. S-57. Analytical HPLC trace and MALDI-TOF spectrum of crude peptide **E3$_g$M** (sequence: Ac-EISALEKMISALEQEISALEK-NH_2). Calculated monoisotopic mass $[M+H]^+$: 2373.2; found: 2373.2.

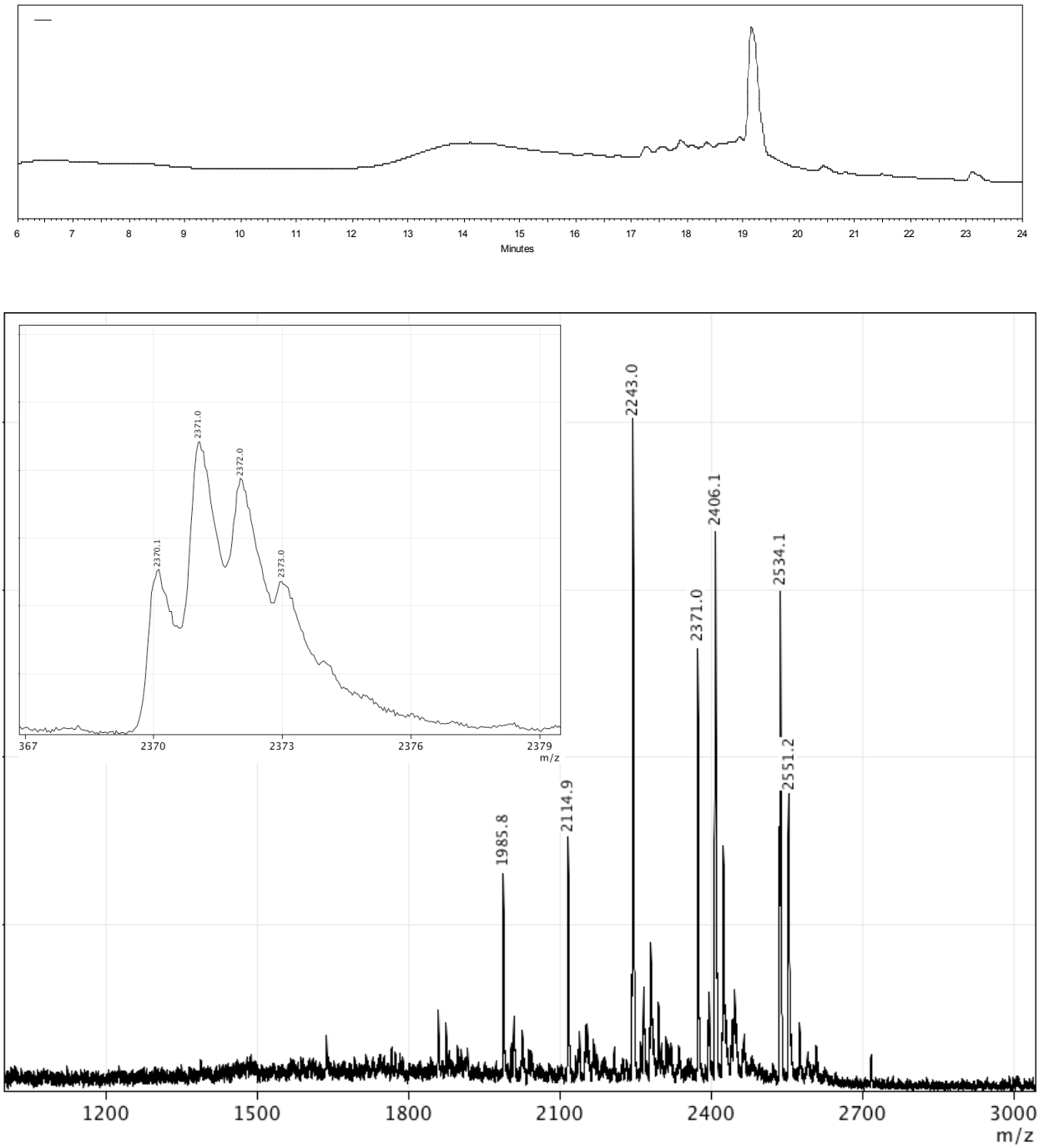


Fig. S-58. Analytical HPLC trace and MALDI-TOF spectrum of crude peptide **E3$_g$K** (sequence: Ac-EISALEKKISALEQEISALEK-NH_2). Calculated monoisotopic mass $[M+H]^+$: 2370.3; found: 2370.1.

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