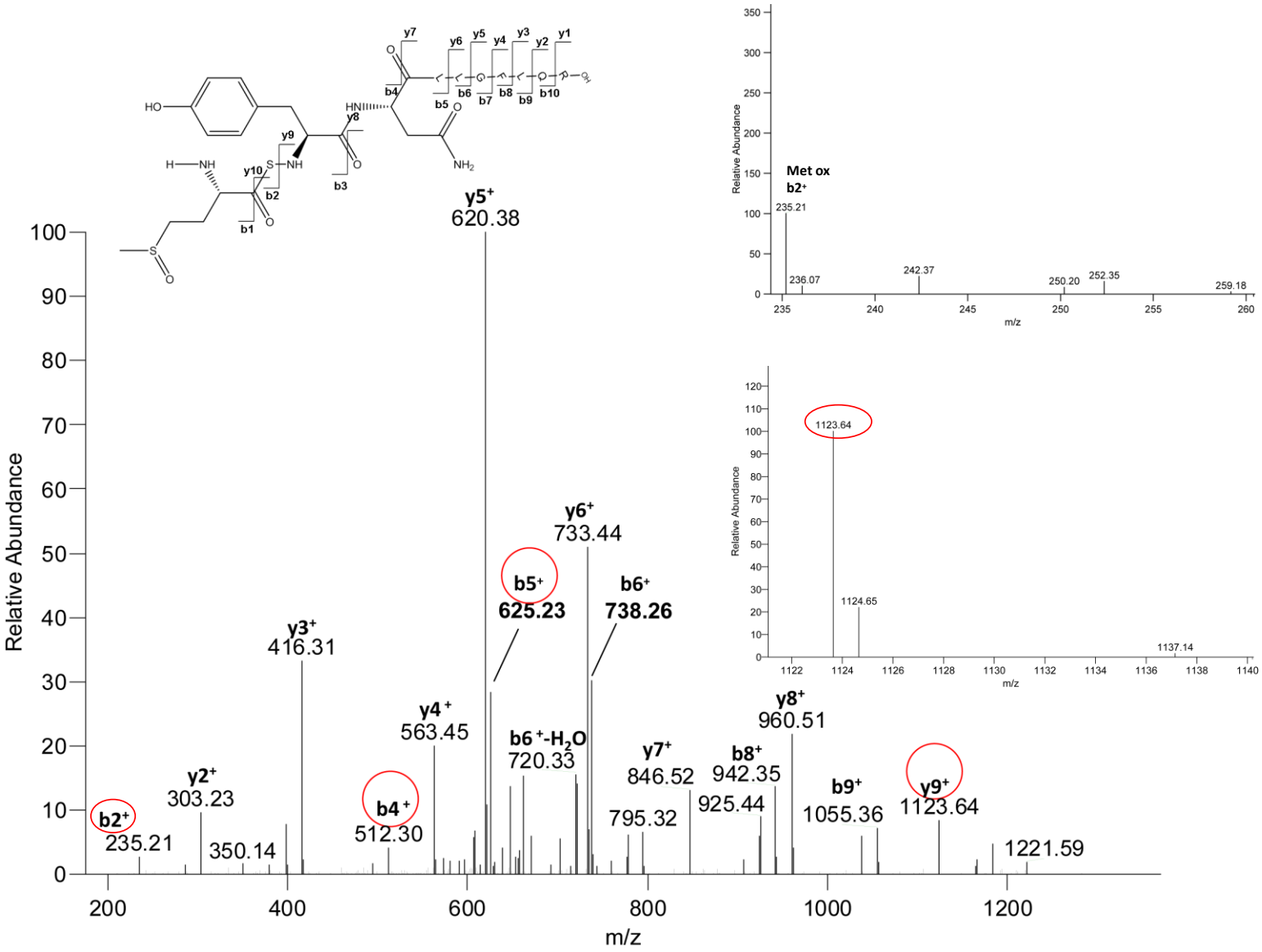


Figure S1 MS/MS spectrum and suggested chemical structure for the oxidized (+16 Da) sequence **M_1SYNLLGFLQR$_{11}$** (theoretical *mass* 1356.70, experimental *m/z* 679.35 $[M+H]^{2+}$, Δm from parent peptide 0.00).

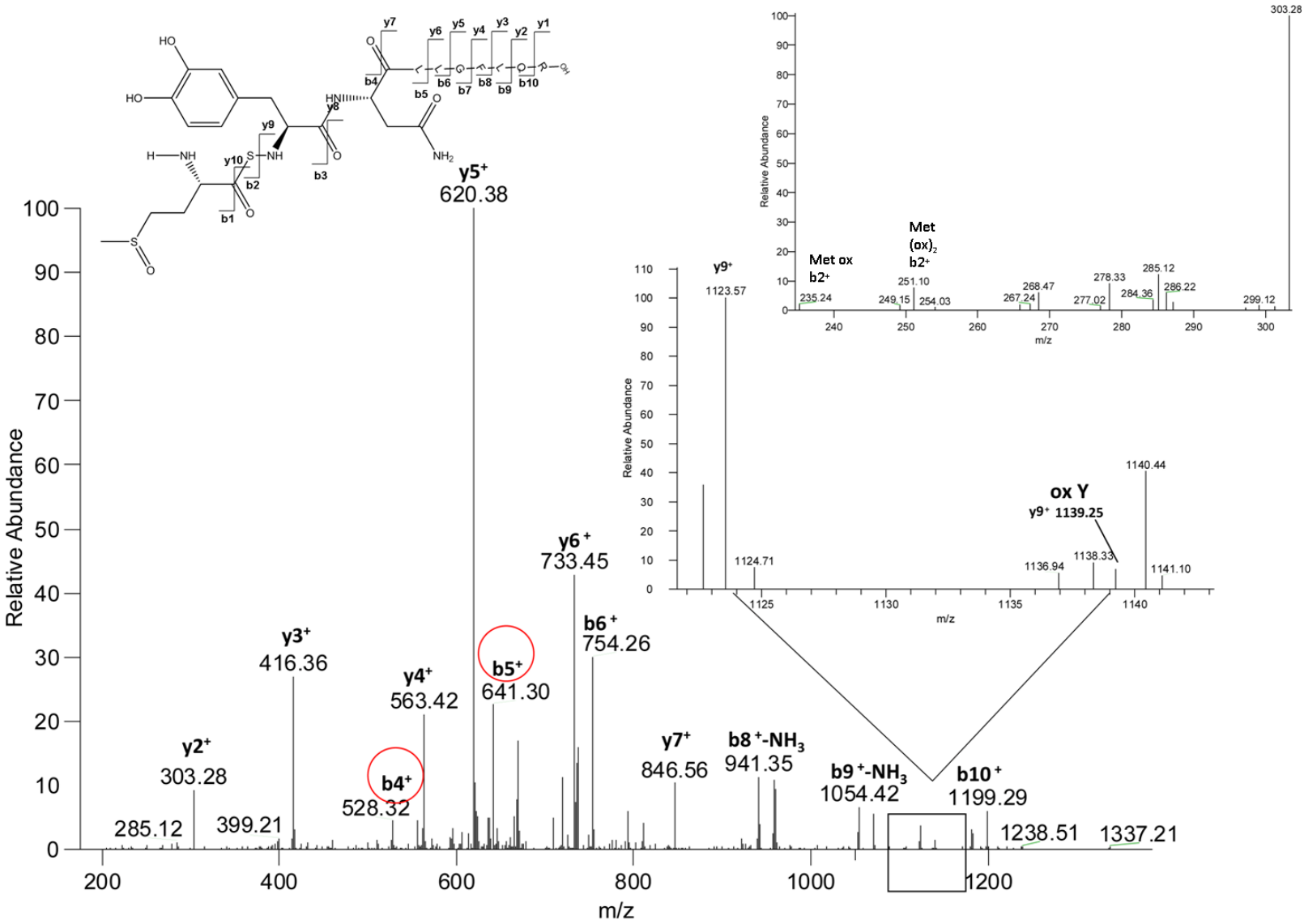


Figure S2 MS/MS spectrum and suggested chemical structure for the oxidized (+32 Da) sequence **M_1SYNLLGFLQR$_{11}$** (theoretical *mass* 1372.69, experimental *m/z* 687.35 $[M+H]^{2+}$, Δm from parent peptide +0.01).

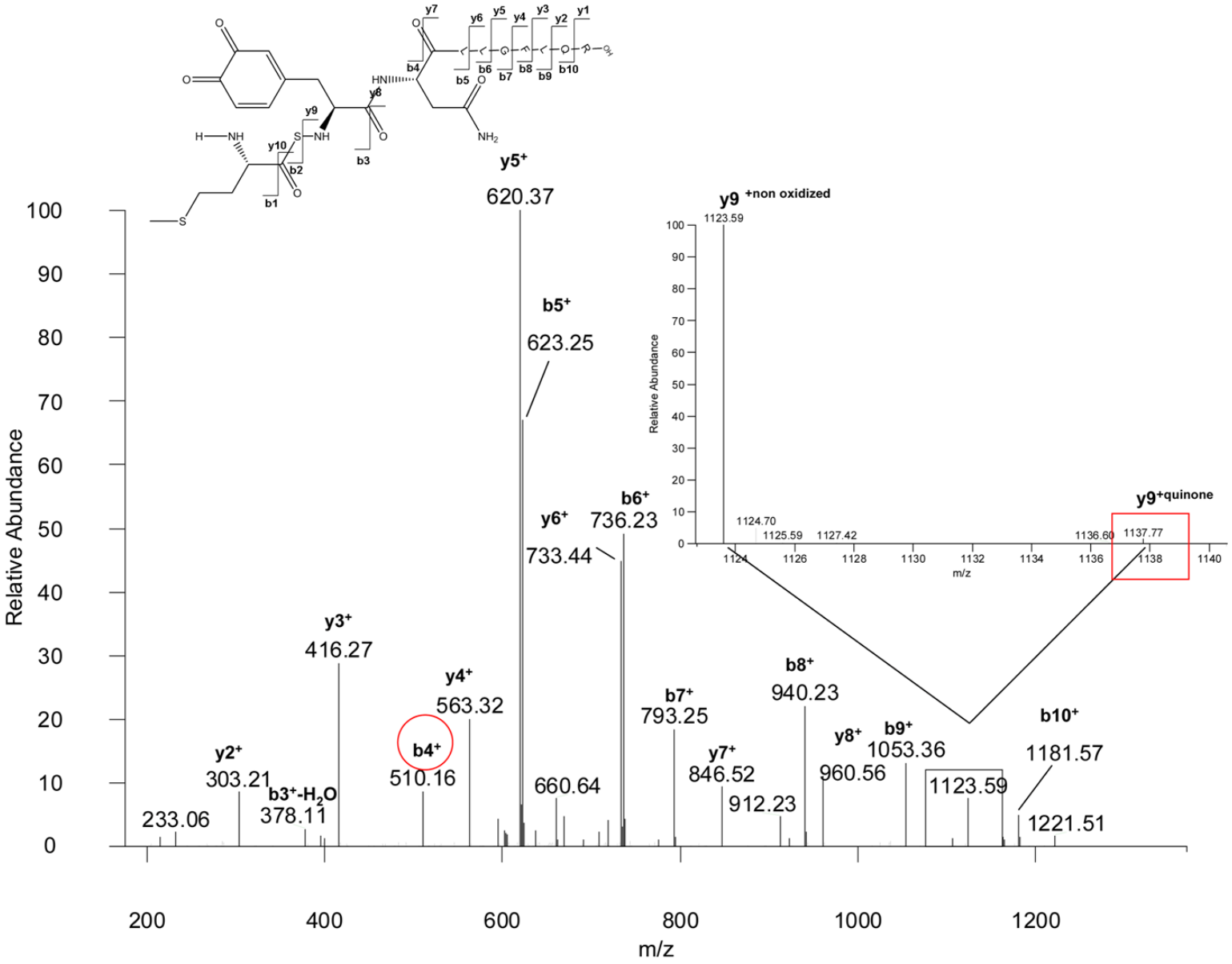


Figure S3 MS/MS spectrum and suggested chemical structure for the oxidized (+14 Da) sequence **M_1SYNLLGFLQR$_{11}$** (theoretical *mass* 1354.68, experimental *m/z* 678.34 $[M+H]^{2+}$, Δm from parent peptide 0.00).

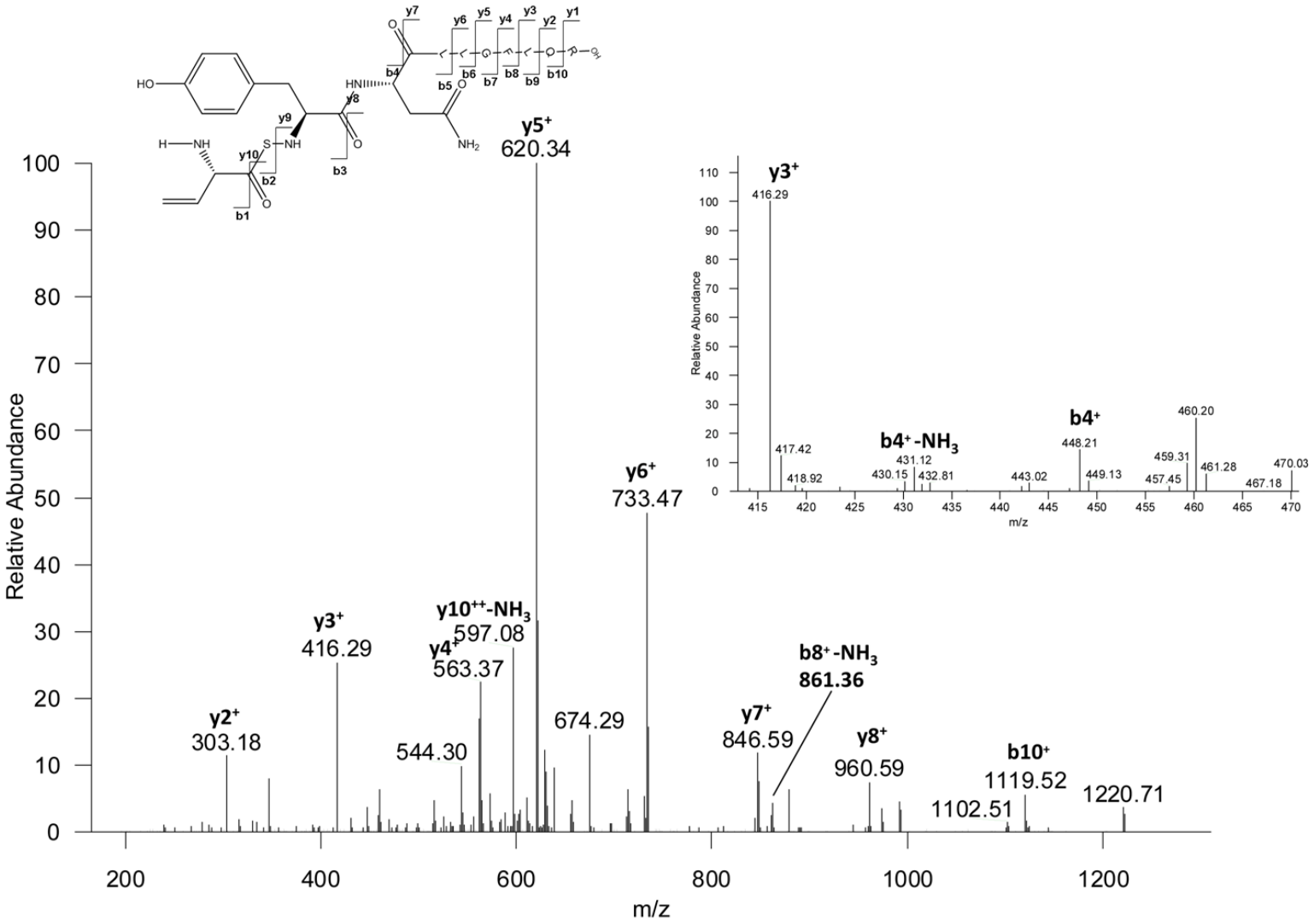


Figure S4 MS/MS spectrum and suggested chemical structure for the modify sequence **M_1SYNLLGFLQR_{11}**, where loss of methane sulfenic acid (CH3SOH, -64 Da) occurred as a consequence of Met1 oxidation. For comparison, please refer to Figure S1, where the MS/MS spectrum for the same sequence containing oxidized Met1 (+16 Da) is shown (theoretical *mass* 1292.68, experimental *m/z* 647.35 $[M+H]^{2+}$, Δm from parent peptide +0.02).

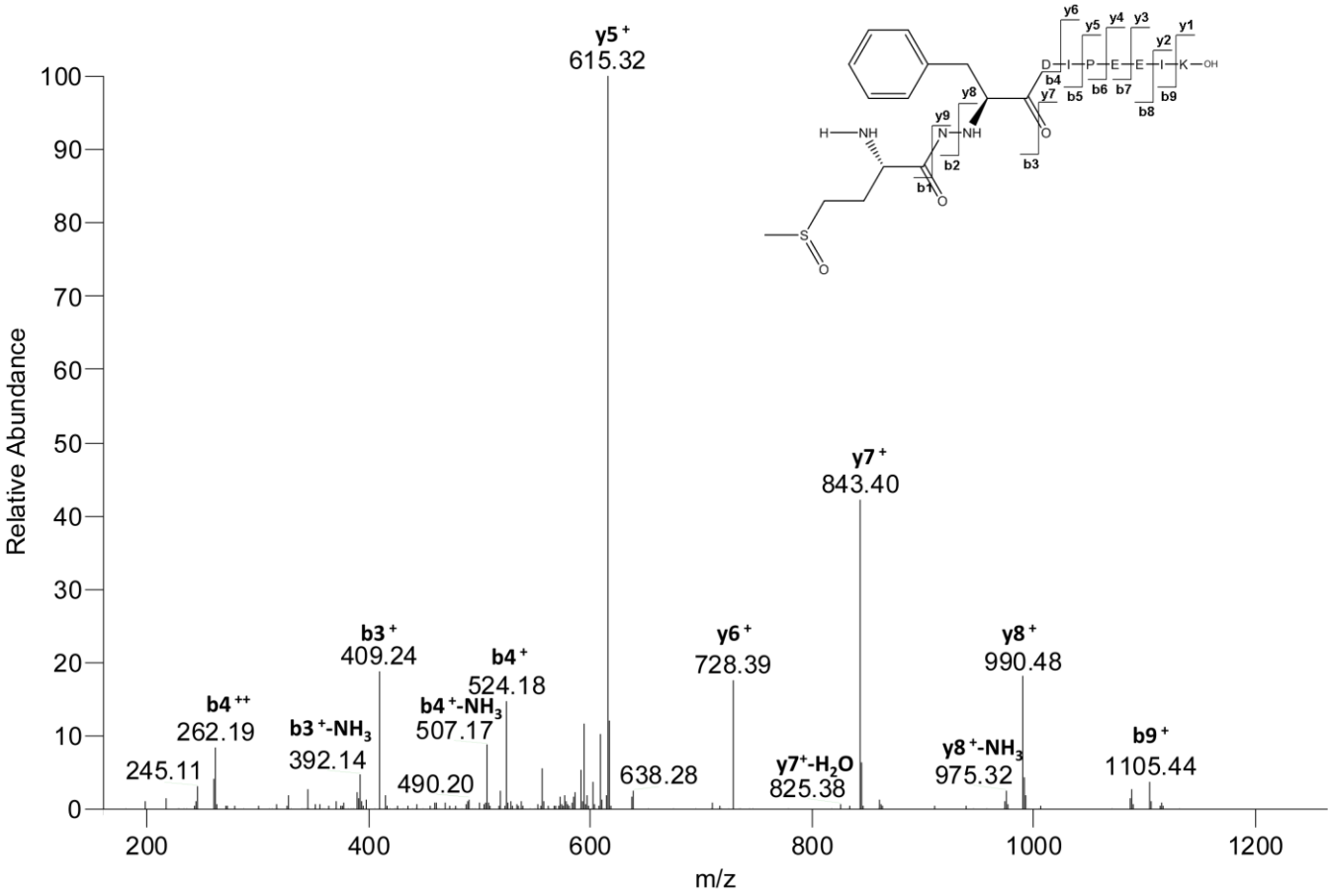


Figure S5a MS/MS spectrum and suggested chemical structure for the oxidized (+16 Da) sequence **M_{36}NFDIPEEIK$_{45}$** (theoretical *mass* 1250.59, experimental *m/z* 626.30 $[M+H]^{2+}$, Δm from native parent peptide +0.01). Note that either Met36 or Phe38 is oxidized.

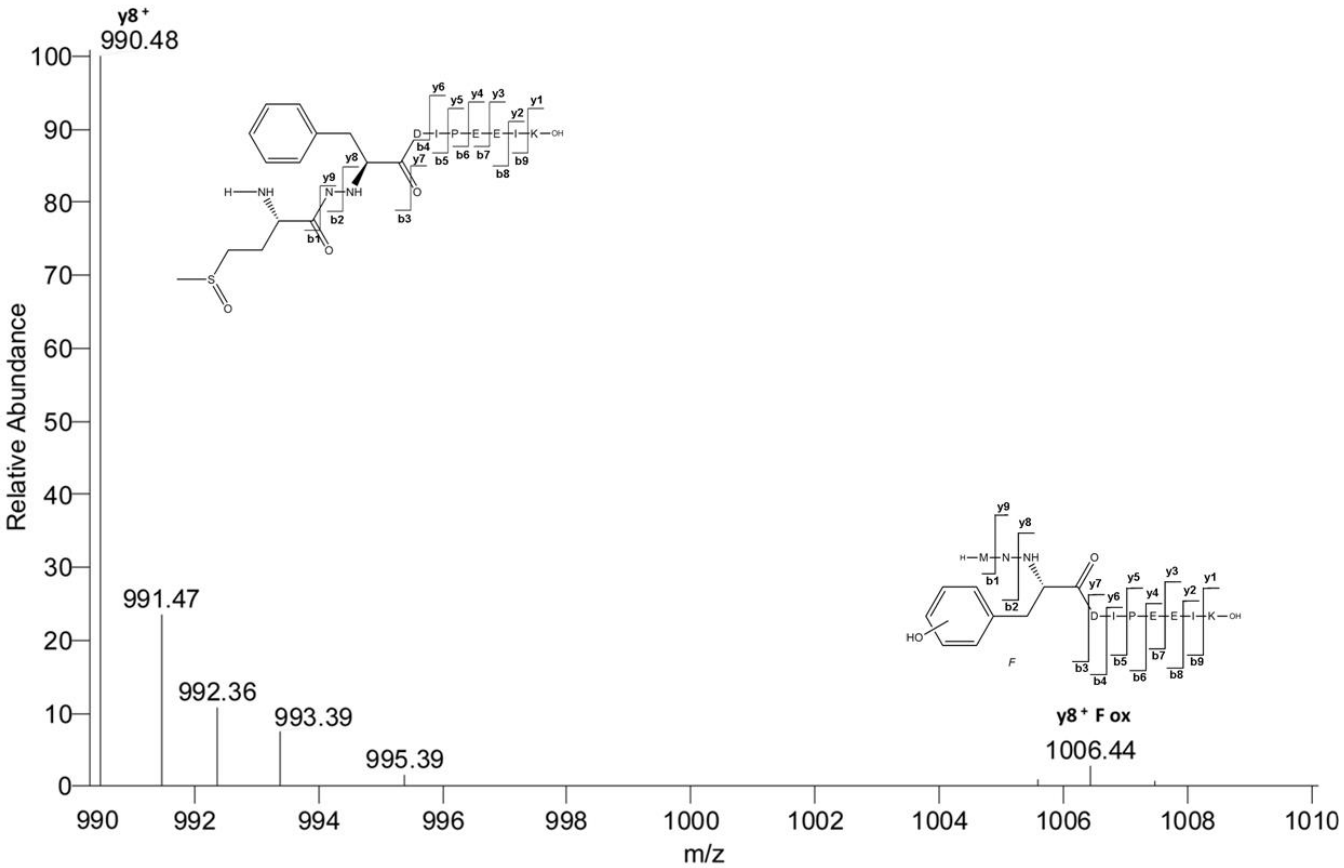


Figure S5b Zoom in (m/z range 988-1010) of the MS/MS spectrum depicted in figure S5a and suggested chemical structures for the oxidized (+16 Da) sequence **M_{36}NFDIPEEIK_{45}** in which either Met or Phe is oxidized.

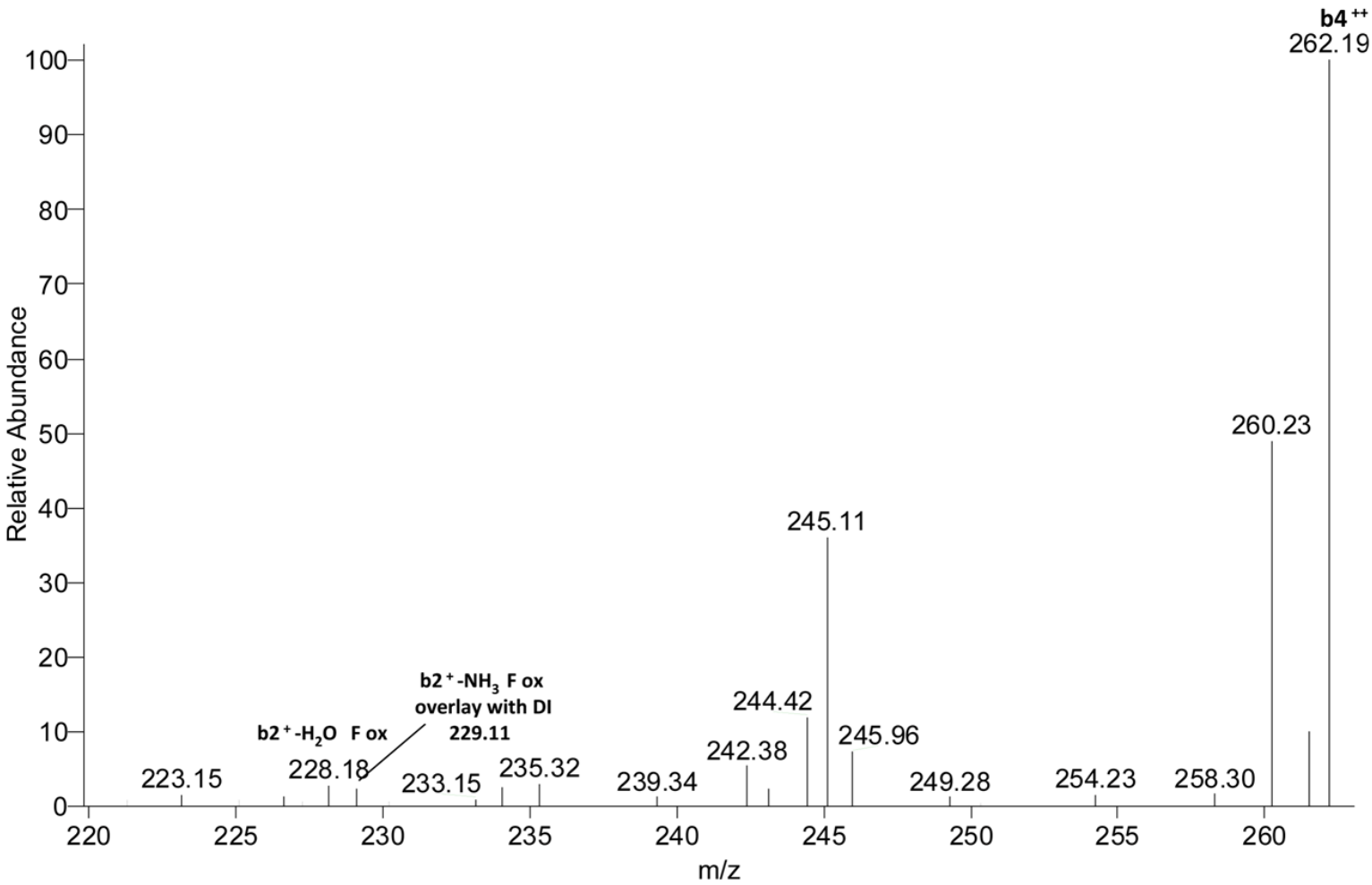


Figure S5c Zoom in (m/z range 220-265) of the MS/MS spectrum depicted in figure S5a.

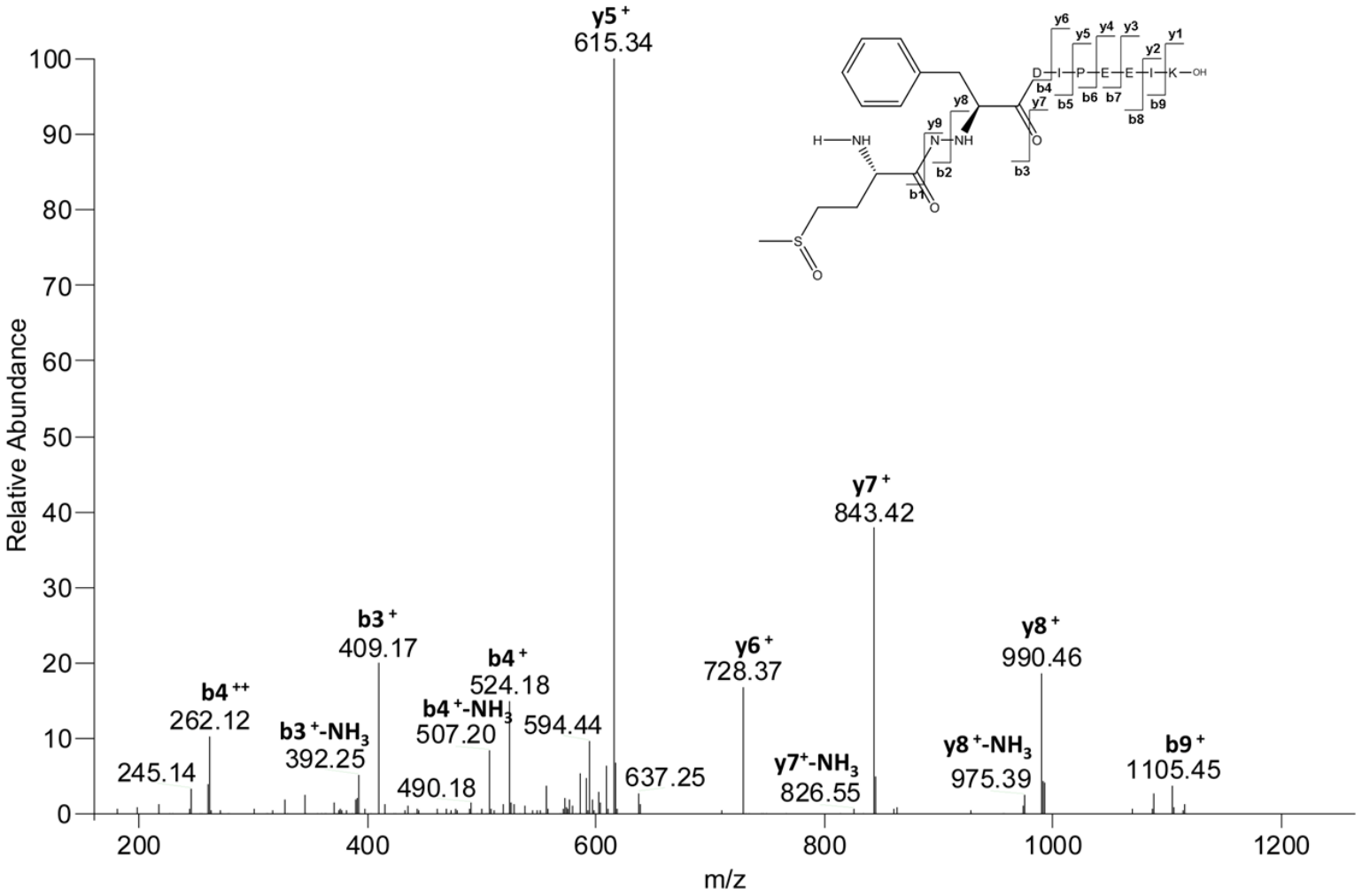


Figure S6a MS/MS spectrum and suggested chemical structure for the oxidized (+16 Da) sequence **M_{36}NFDIPEEIK$_{45}$** (theoretical *mass* 1250.59, experimental *m/z* 626.30 $[M+H]^{2+}$, Δm from native parent peptide +0.01). Note that this spectrum was obtained from non-oxidized IFNβ1a, where only Met36 is oxidized.

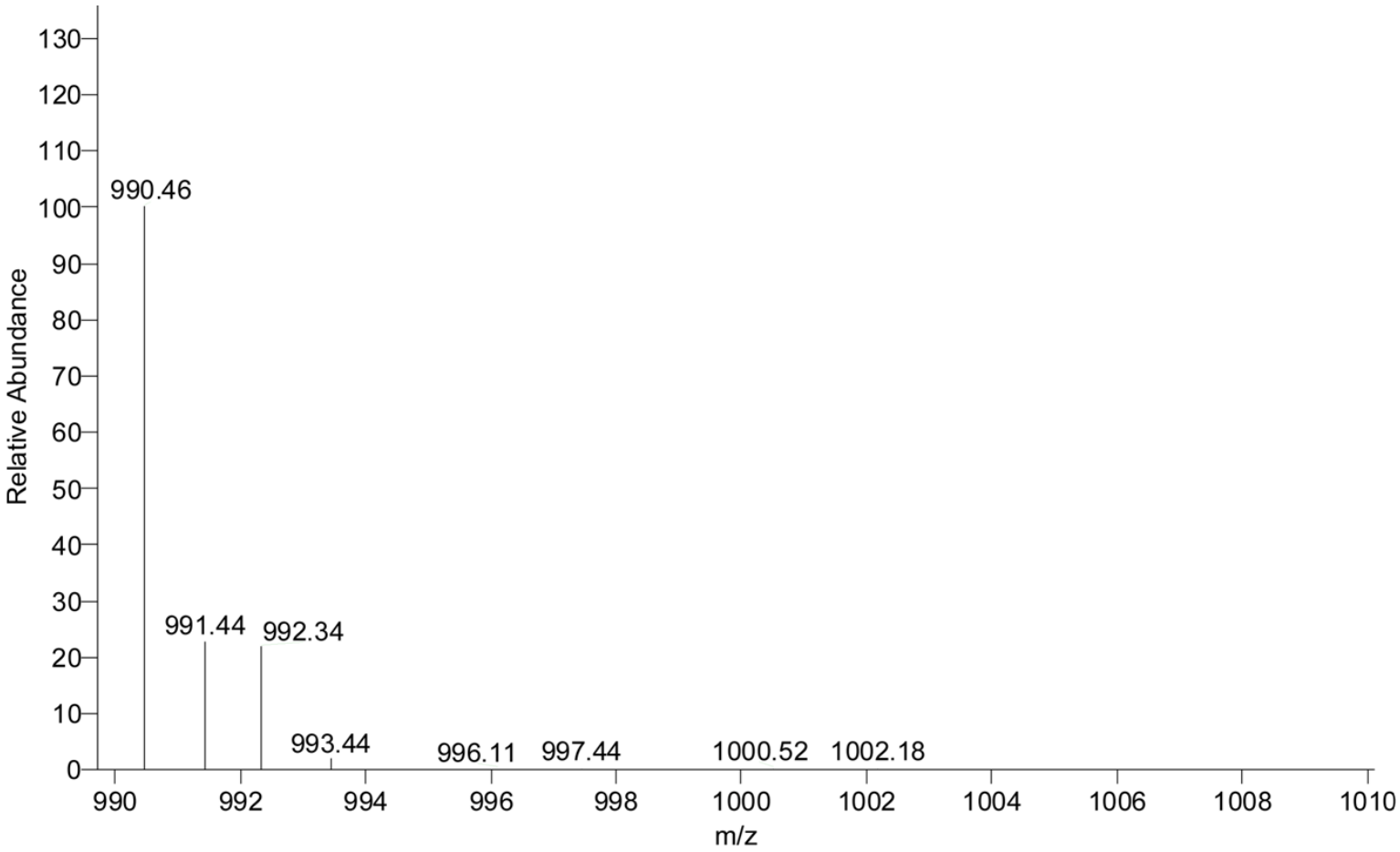


Figure S6b Zoom in (m/z range 988-1010) of the MS/MS spectrum depicted in figure S6a

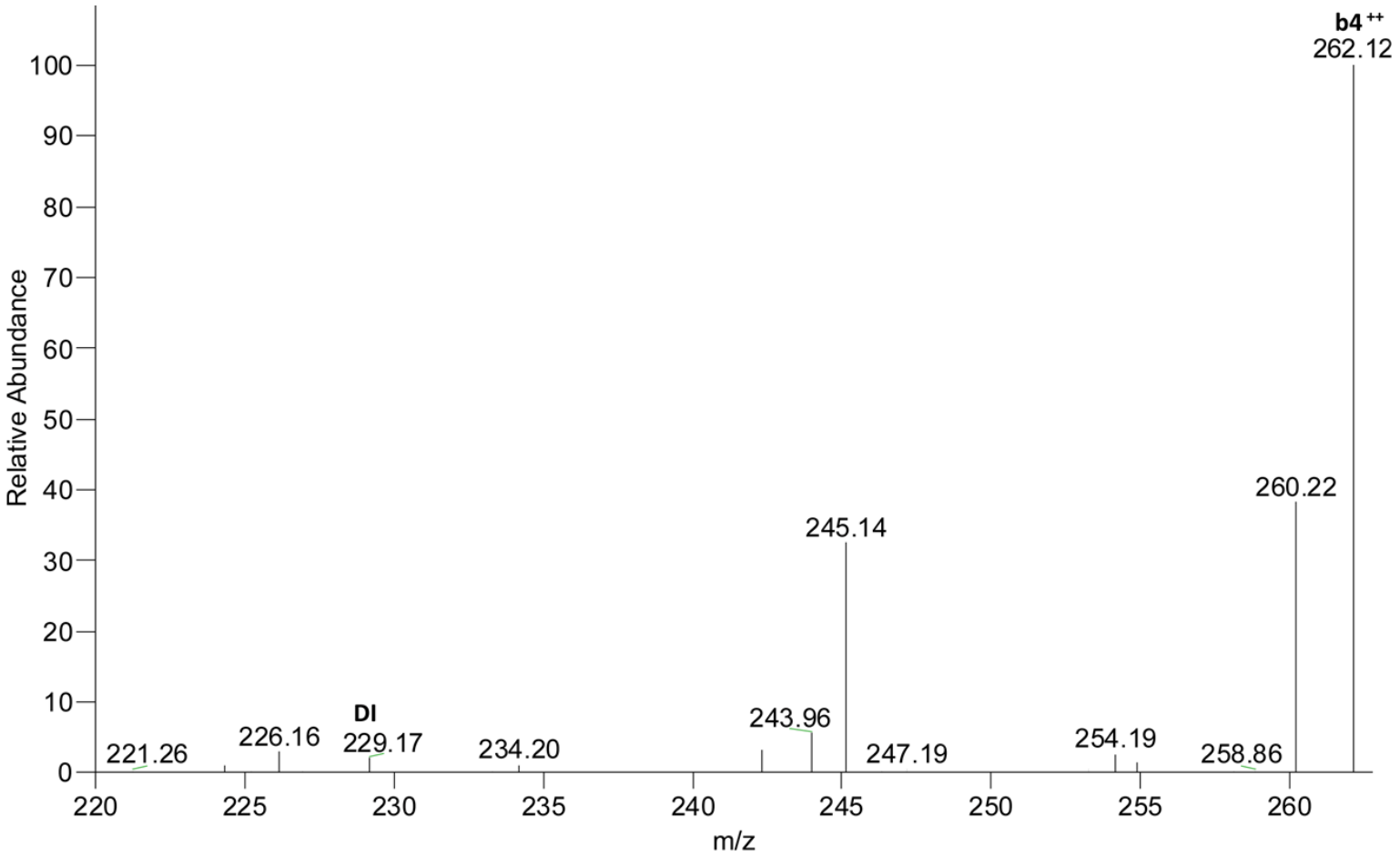
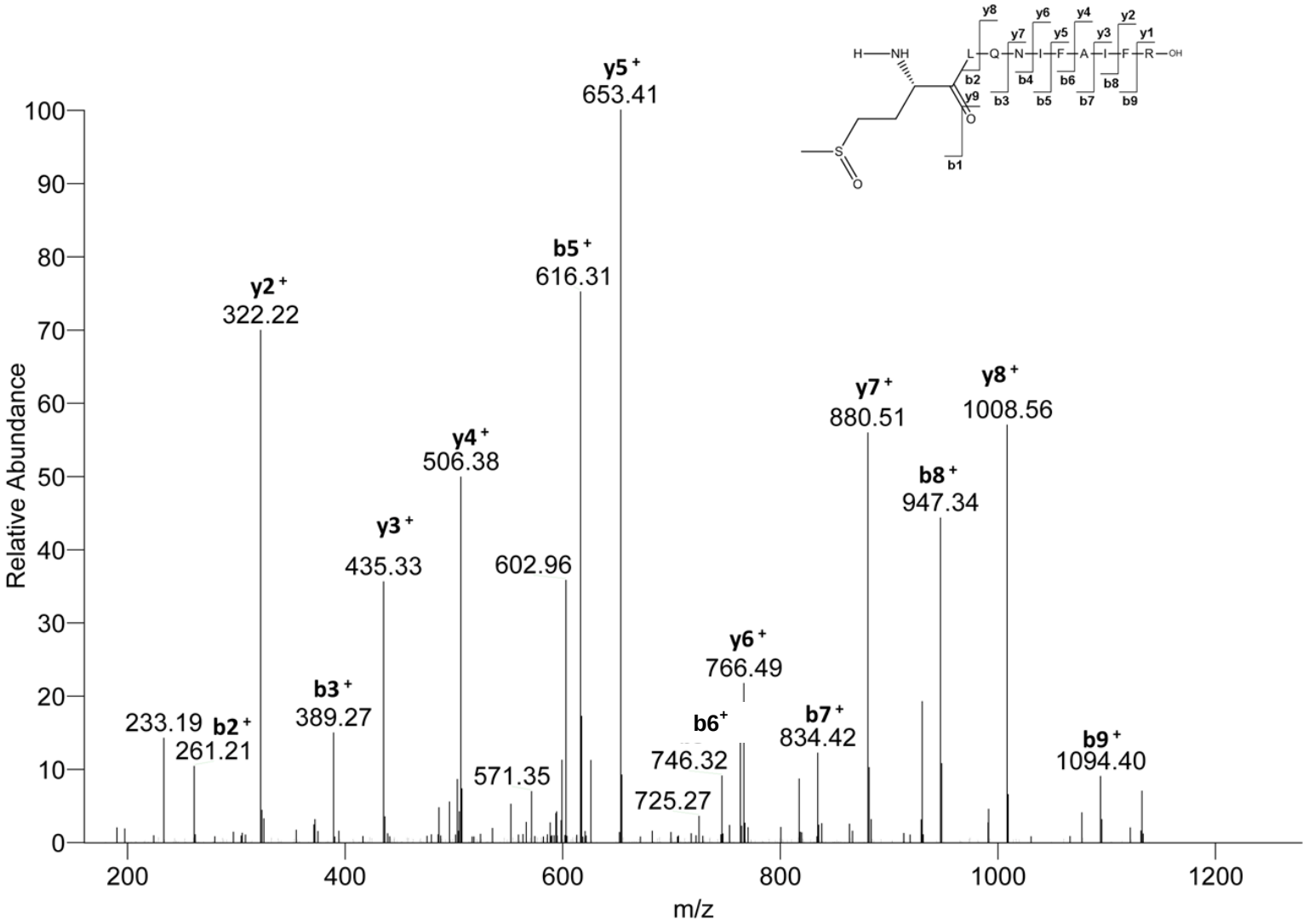


Figure S6c Zoom in (m/z range 220-265) of the MS/MS spectrum depicted in figure S6a.



FigureS7 MS/MS spectrum and suggested chemical structure for the oxidized (+16 Da) sequence **M_{62}LQNIFAIFR_{71}** (theoretical *mass* 1267.68, experimental *m/z* 634.84 $[M+H]^{2+}$, Δm from native parent peptide 0.00).

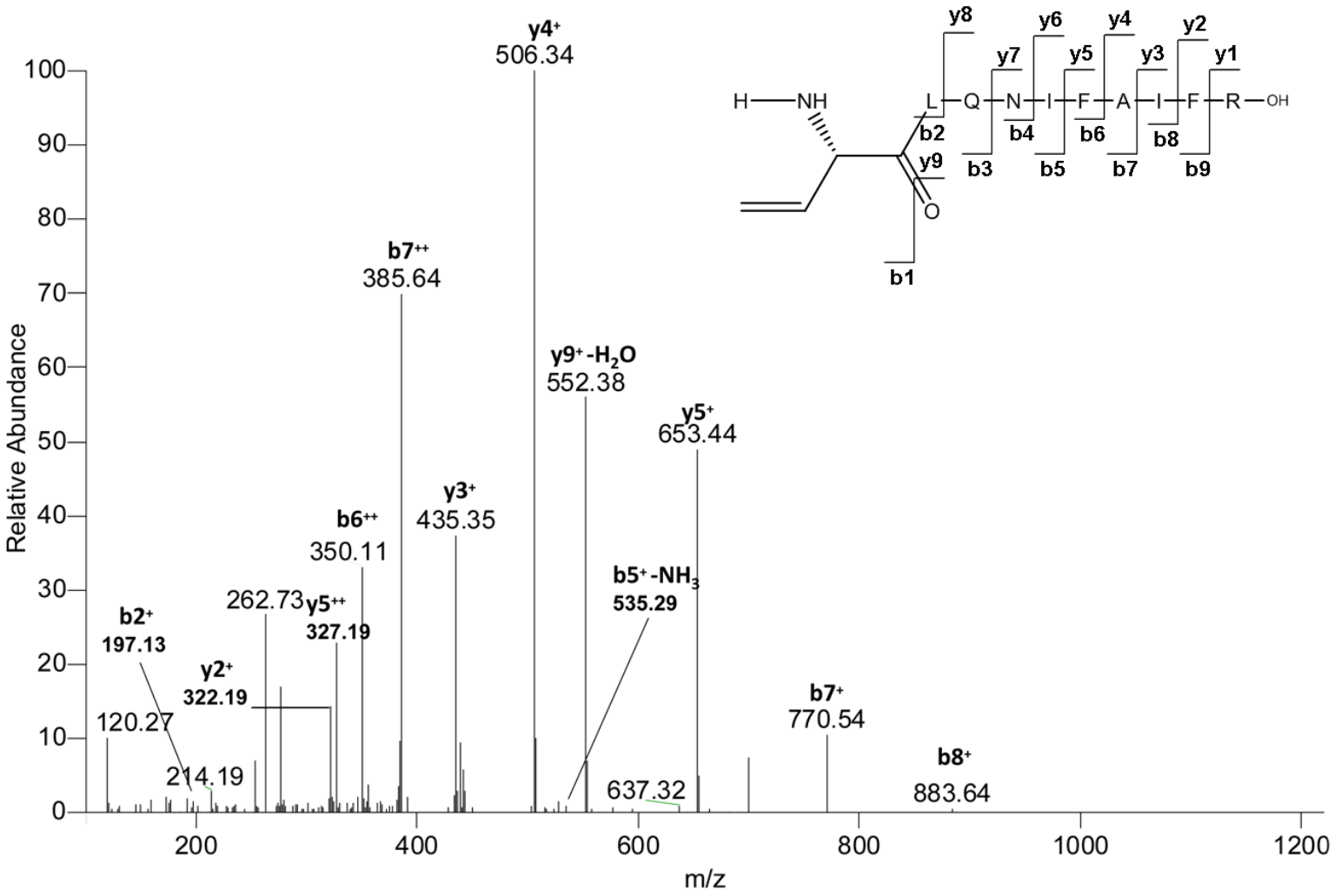


Figure S8 MS/MS spectrum and suggested chemical structure for the modify sequence **M_{62}LQNIFAIFR_{71}** where loss of methane sulfenic acid (CH3SOH, -64 Da) occurred as a consequences of Met62 oxidation. For comparison, please refer to Figure S7, where the MS/MS spectrum for the same sequence containing Met62 oxidized (+16 Da) is shown (theoretical *mass* 1203.68, experimental *m/z* 402.23 $[M+H]^{3+}$, Δm from native parent peptide +0.01).

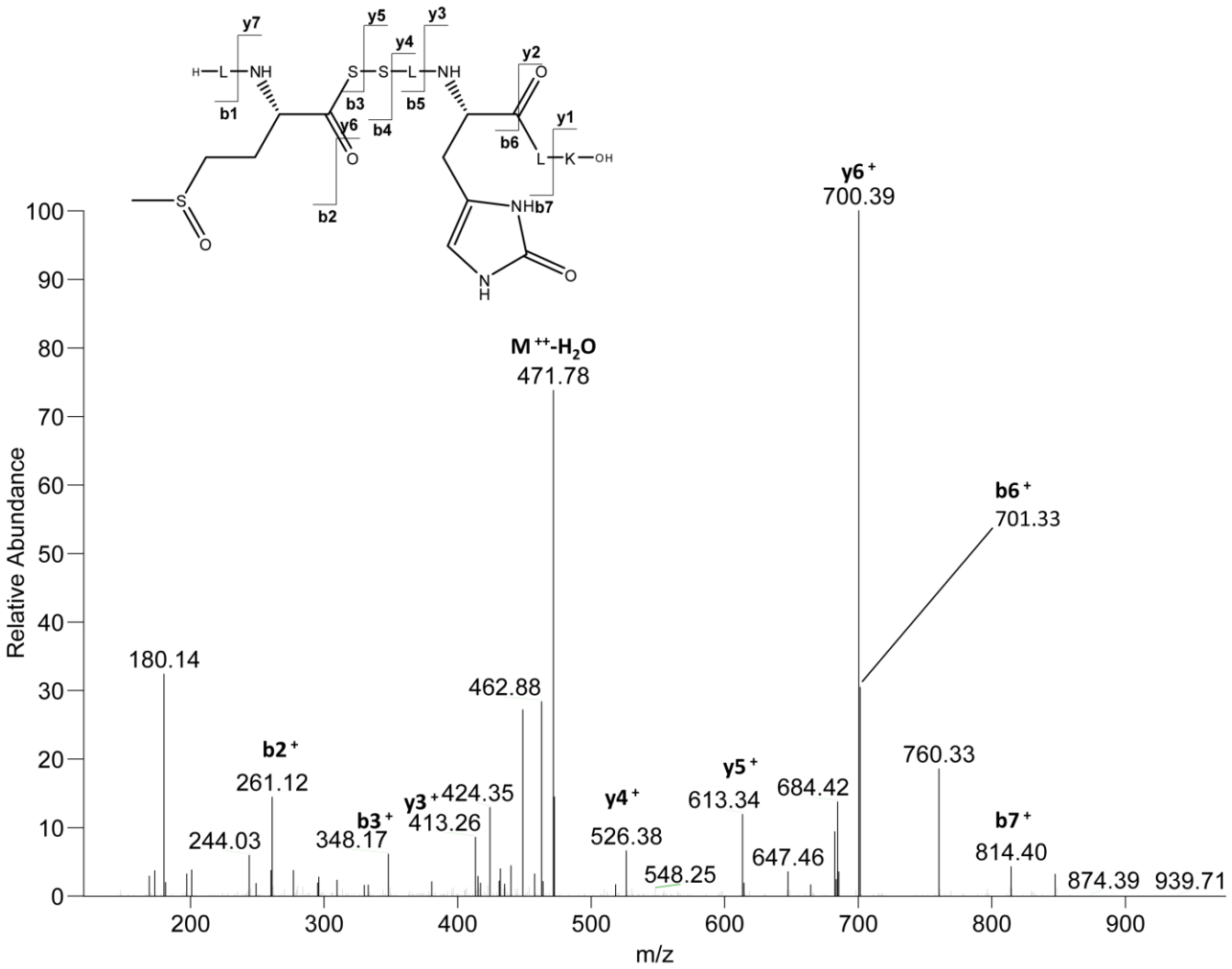


Figure S9 MS/MS spectrum and suggested chemical structure for the oxidized (+32 Da) sequence **L_{116}MSSLHLK$_{123}$** (theoretical *mass* 959.52, experimental *m/z* 480.76 $[M+H]^{2+}$, Δm from native parent peptide 0.00).

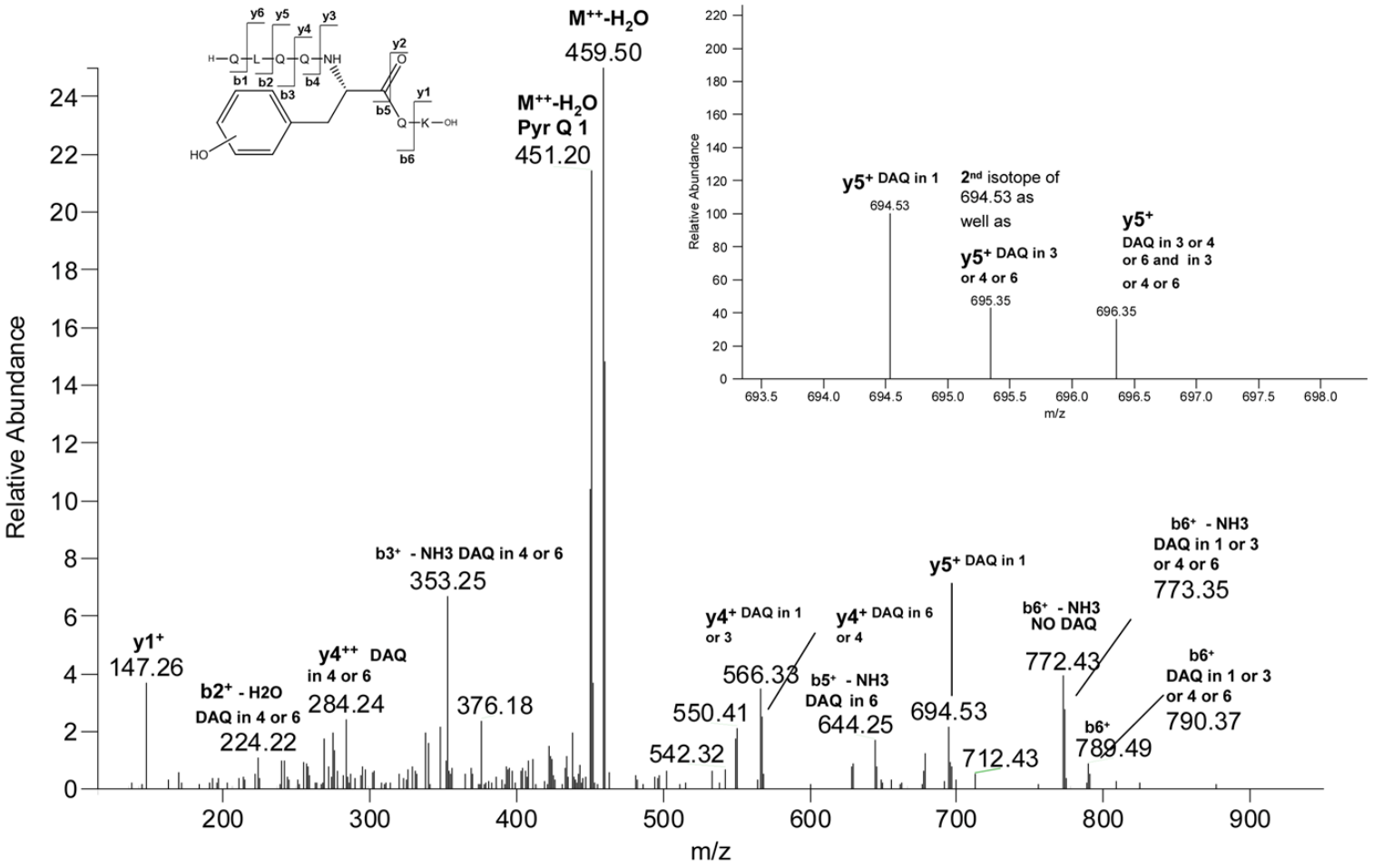


Figure S10 MS/MS spectrum and suggested chemical structure for the oxidized (+16 Da) sequence **Q_{46}LQQFQK$_{52}$** (theoretical *mass* 935.48, experimental *m/z* 468.76 $[M+H]^{2+}$, Δm from native parent peptide +0.04).

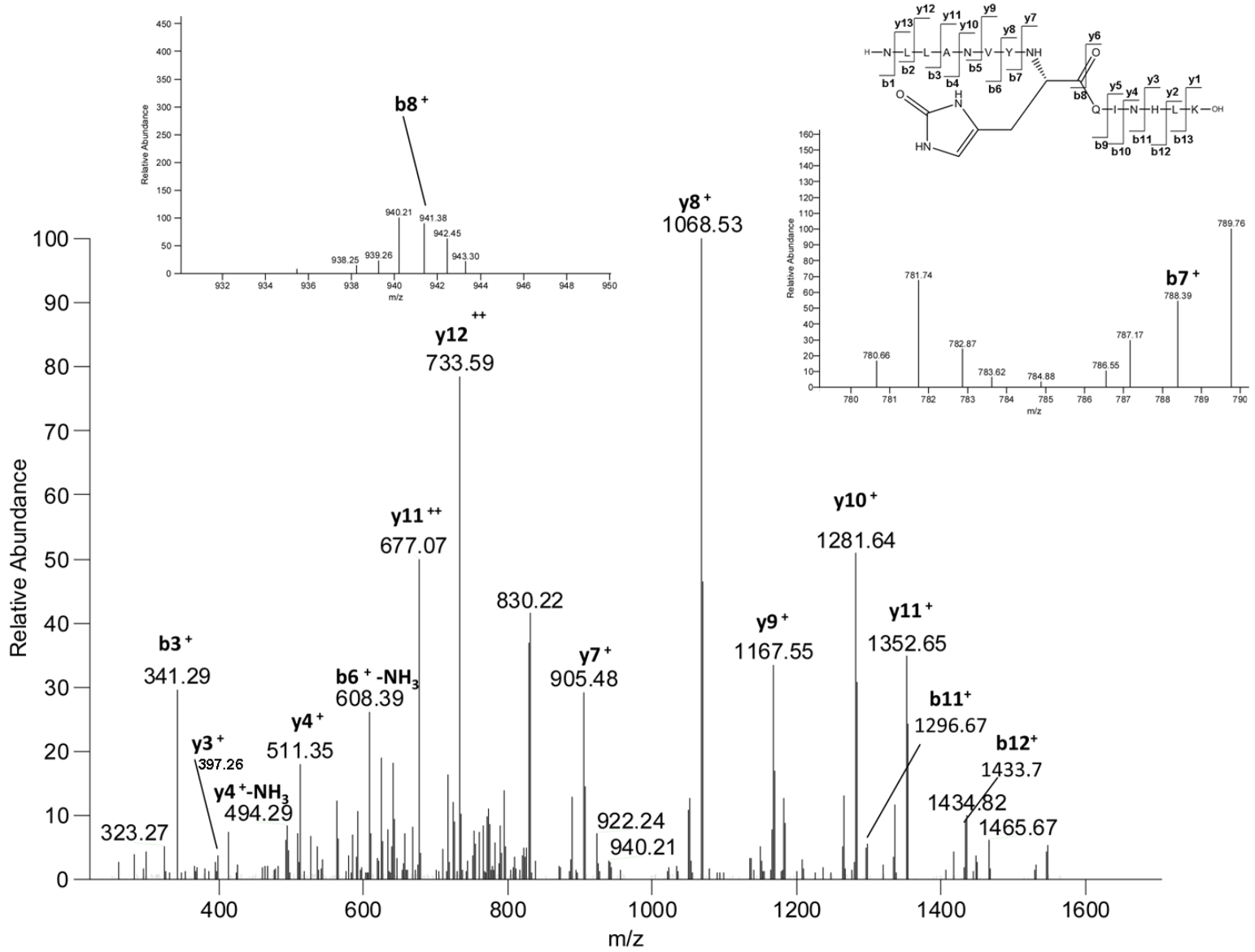


Figure S11 MS/MS spectrum and suggested chemical structure for the oxidized (+16 Da) sequence **N_{86}LLANVYHQINHLK$_{99}$** (theoretical *mass* 1691.92, experimental *m/z* 846.96 $[M+H]^{2+}$, Δm from native parent peptide 0.00).

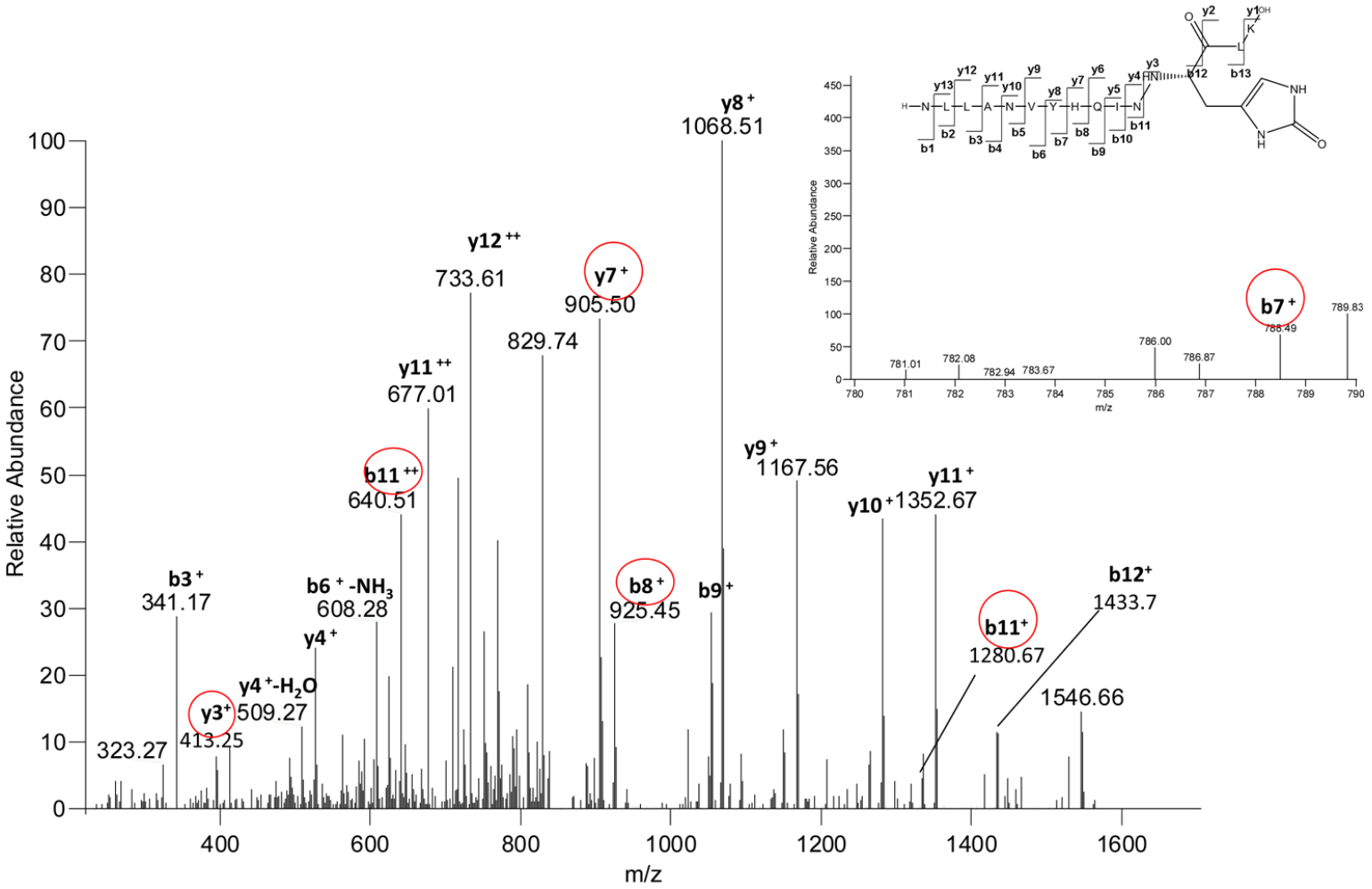


Figure S12 MS/MS spectrum and suggested chemical structure for the oxidized (+16 Da) sequence **N_{86}LLANVYHQINHLK$_{99}$** (theoretical *mass* 1691.92, experimental *m/z* 846.96 $[M+H]^{2+}$, Δm from native parent peptide 0.00).

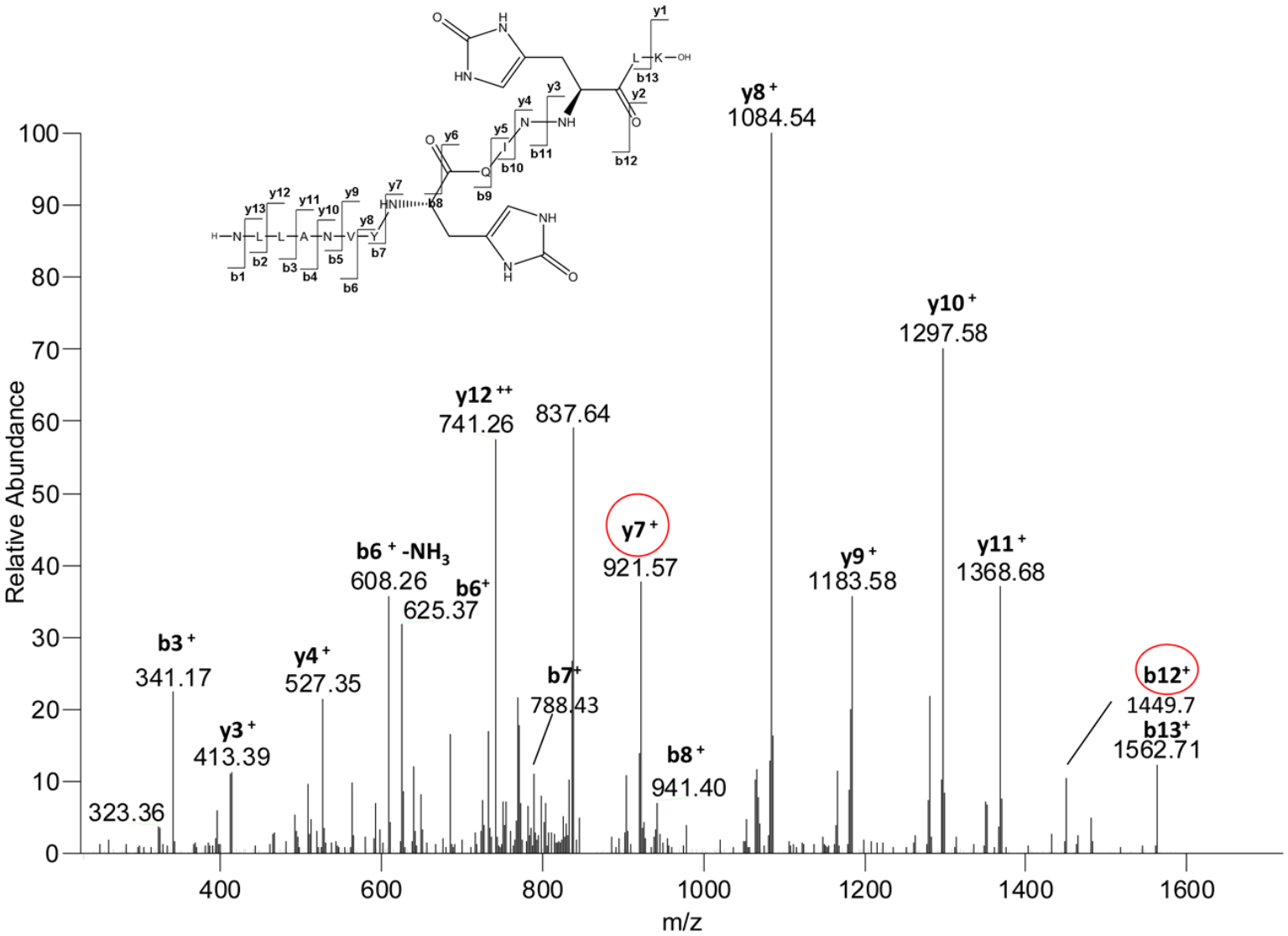


Figure S13 MS/MS spectrum and suggested chemical structure for the oxidized (+32 Da) sequence **N_{86}LLANVYHQINHLK$_{99}$** (theoretical *mass* 1707.91, experimental *m/z* 854.96 $[M+H]^{2+}$, Δm from native parent peptide +0.01).

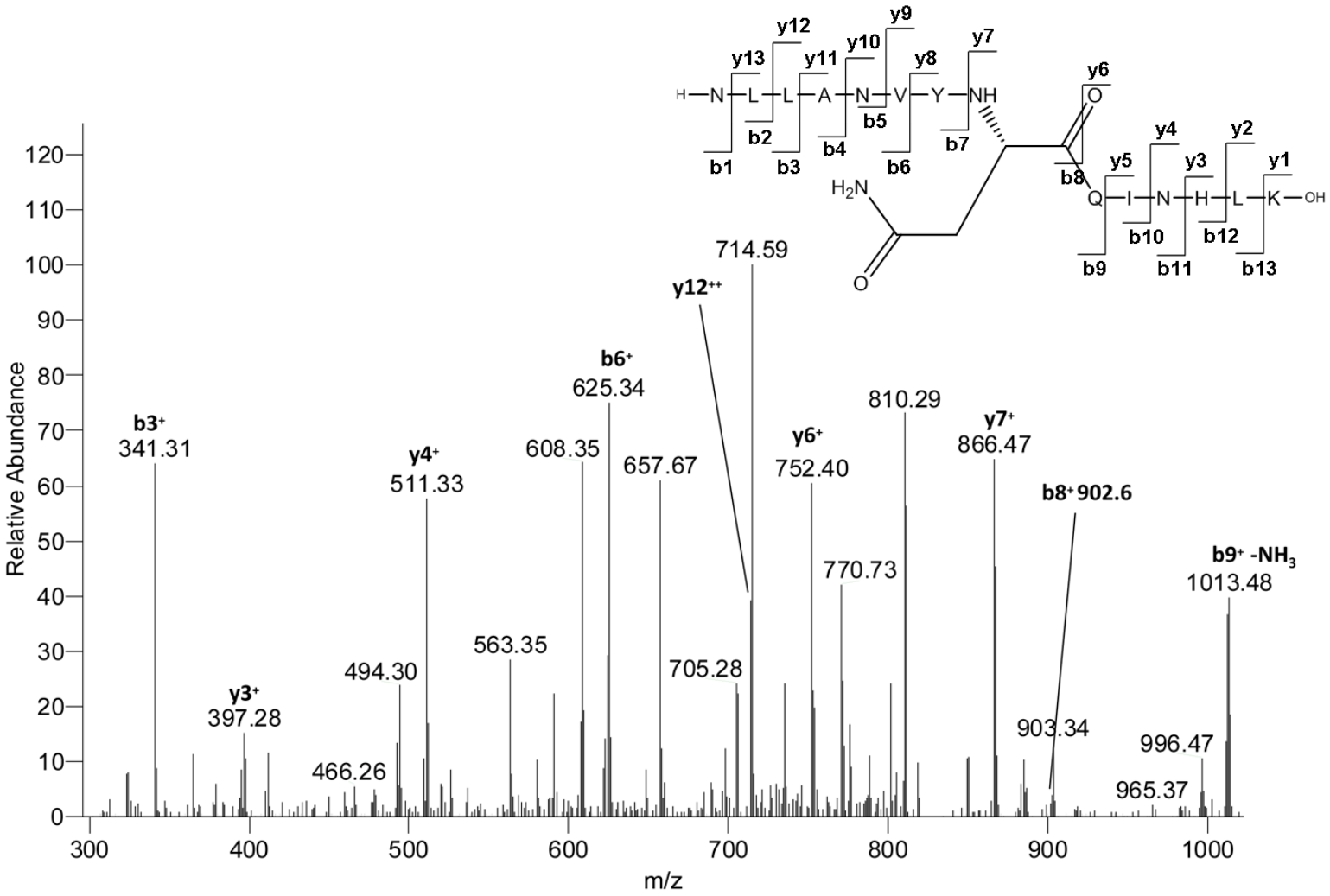


Figure S14a MS/MS spectrum and chemical structure for the oxidized (-23 Da) sequence **N_{86}LLANVYHQINHLK$_{99}$** (theoretical *mass* 1652.91, experimental *m/z* 827.46 $[M+H]^{2+}$, Δm from native parent peptide +0.01).

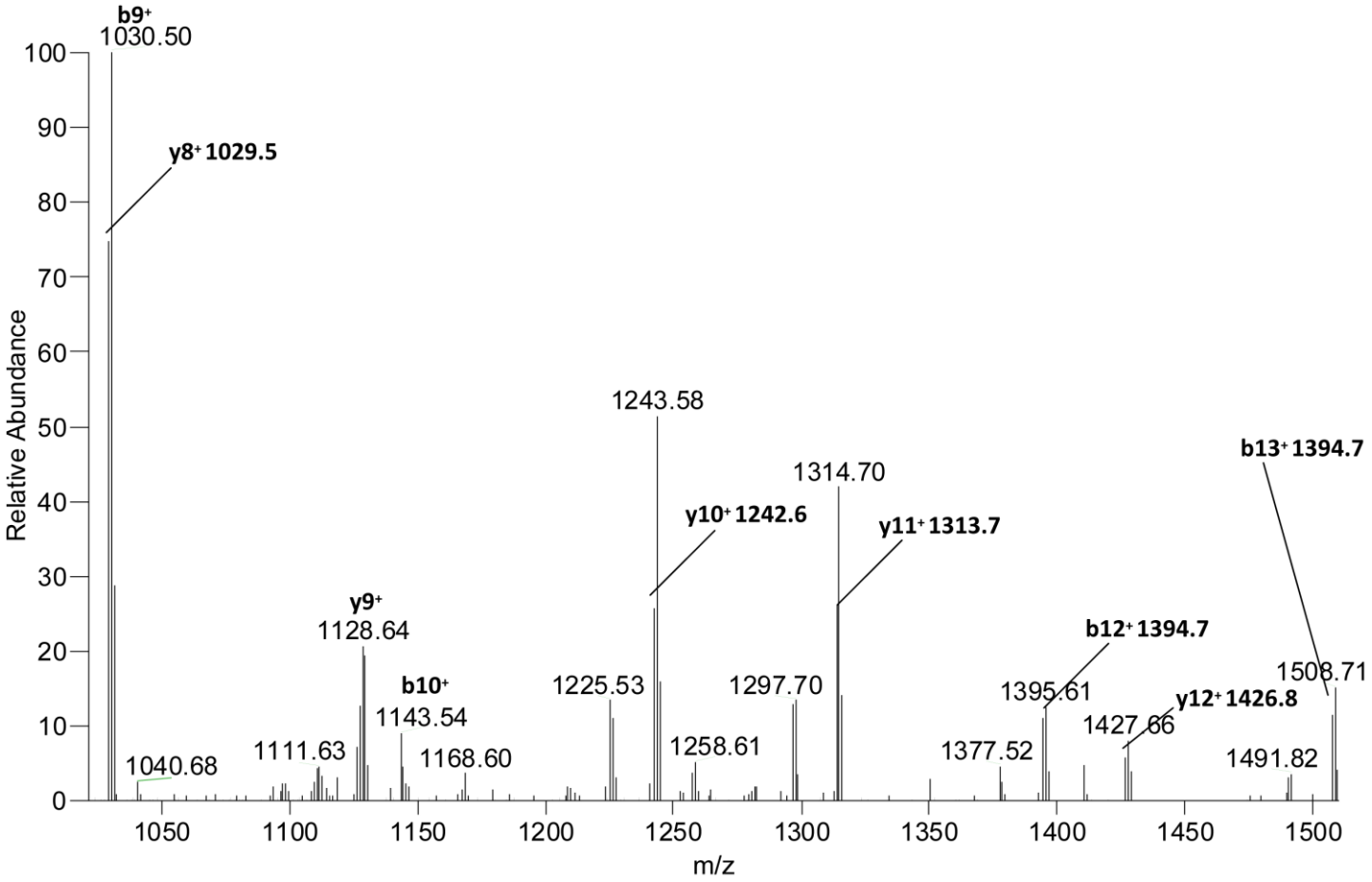


Figure S14b Zoom in (m/z range 1010-1510) of the MS/MS spectrum depicted in figure S14a.

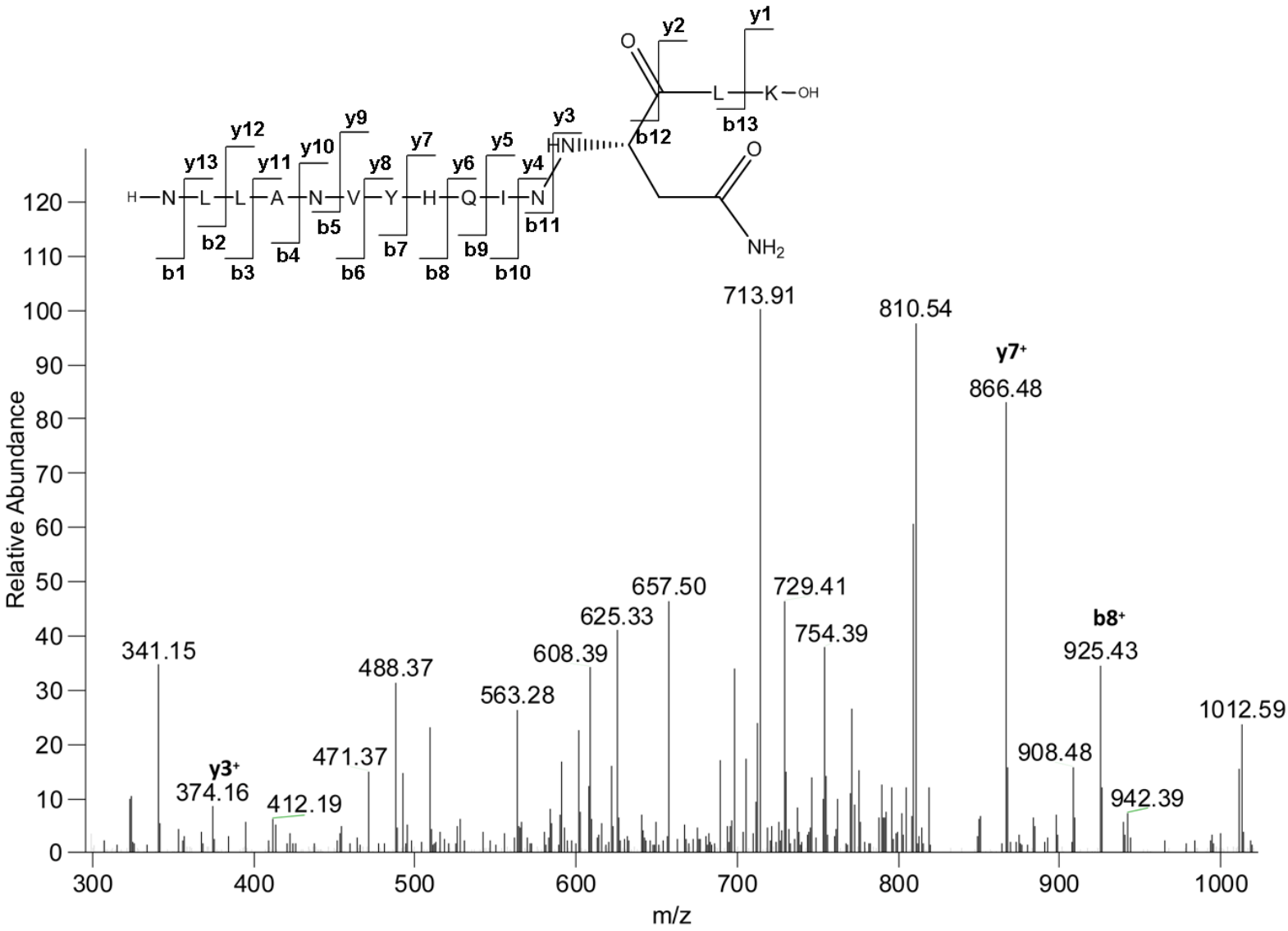


Figure S15a MS/MS spectrum and suggested chemical structure for the oxidized sequence **N_{86}LLANVYHQINHLK$_{99}$** (theoretical *mass* 1652.91, experimental *m/z* 827.46 $[M+H]^{2+}$, Δm from native parent peptide +0.01).

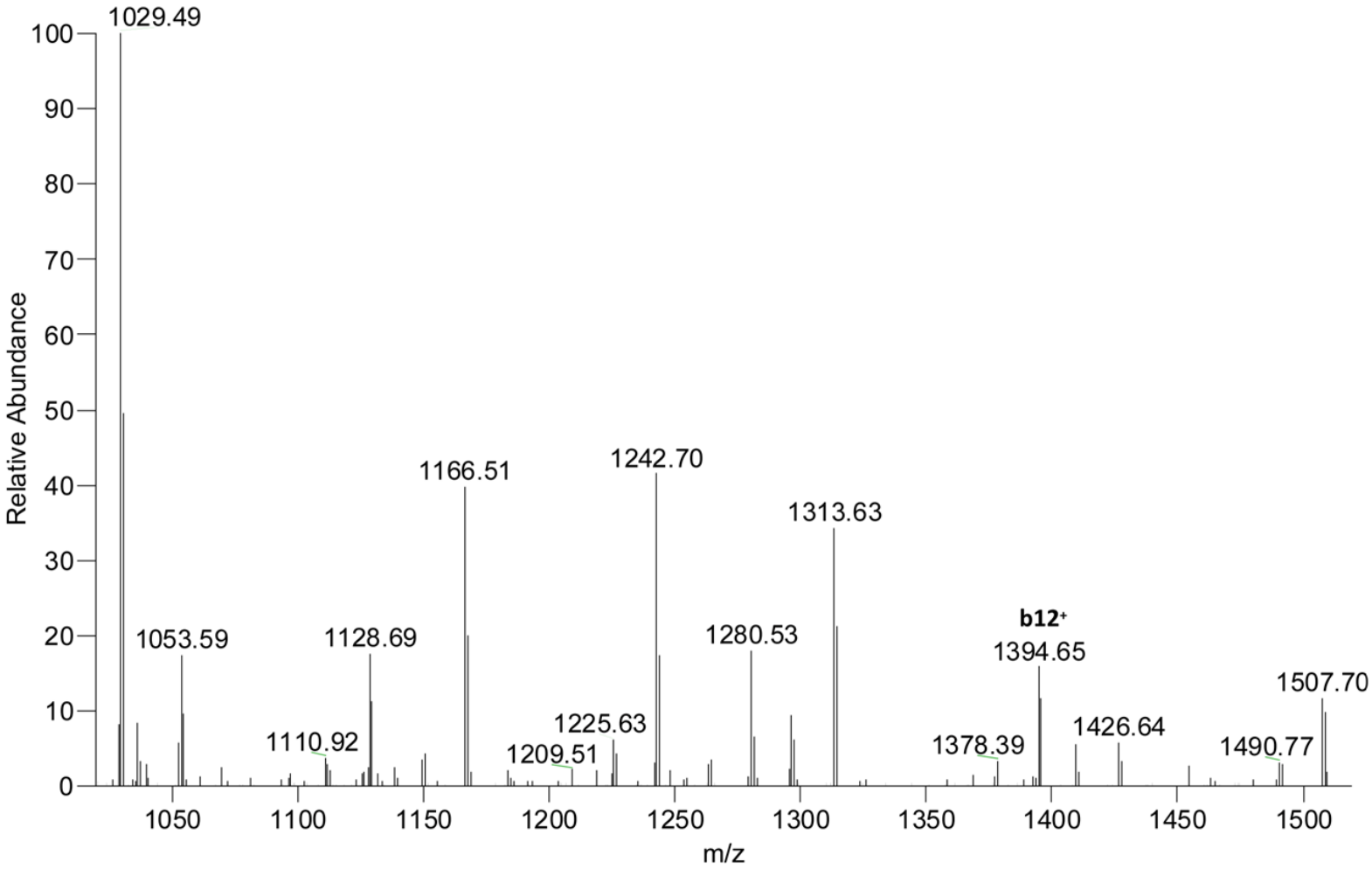


Figure S15b Zoom in (m/z range 1010-1510) of the MS/MS spectrum depicted in figure S15a.

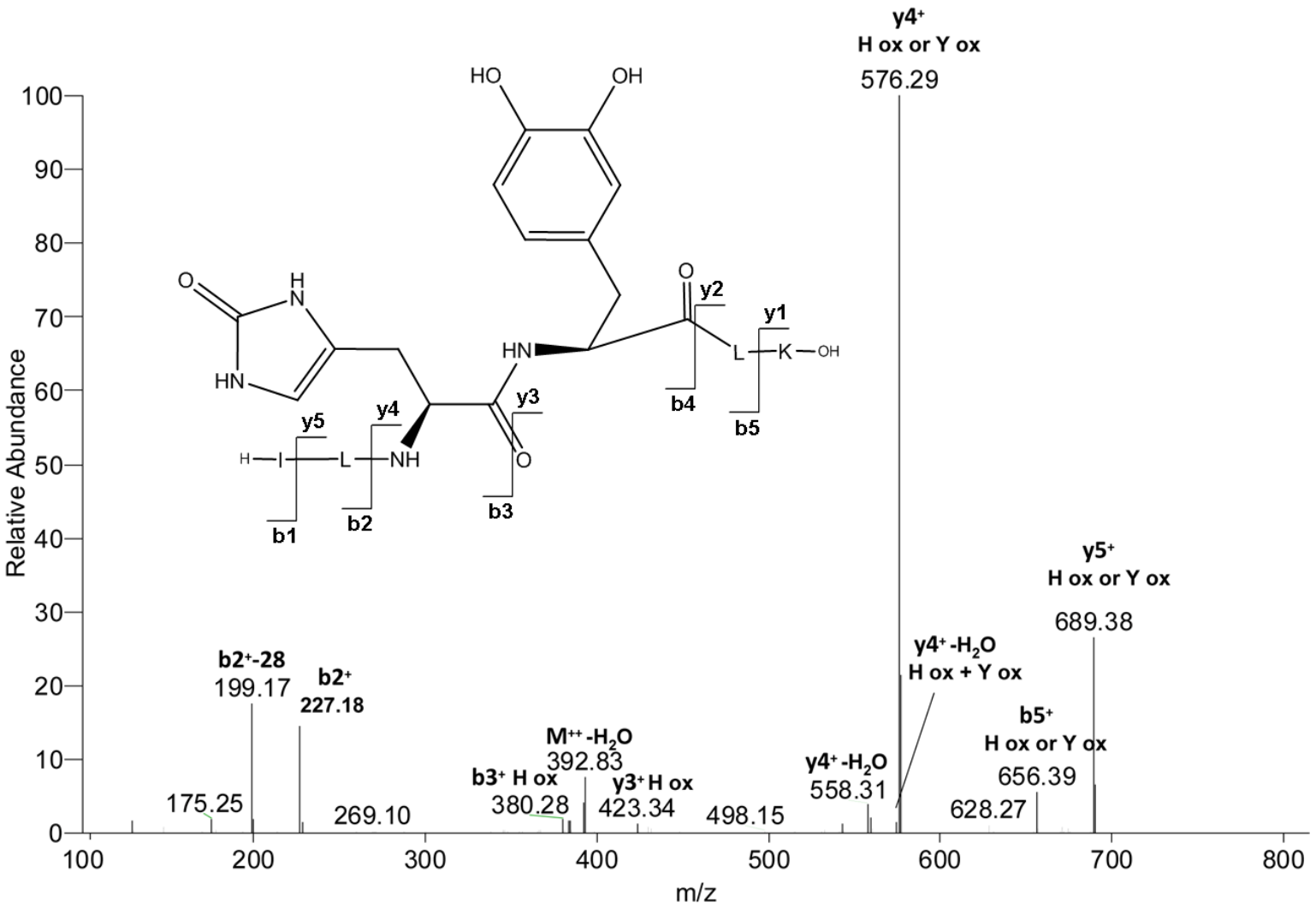


Figure S16a MS/MS spectrum and suggested chemical structure for the oxidized (+16 Da) sequence **I_{129}LHYLK$_{134}$** (theoretical *mass* 801.48, experimental *m/z* 401.74 $[M+H]^{2+}$, Δm from native parent peptide 0.00).

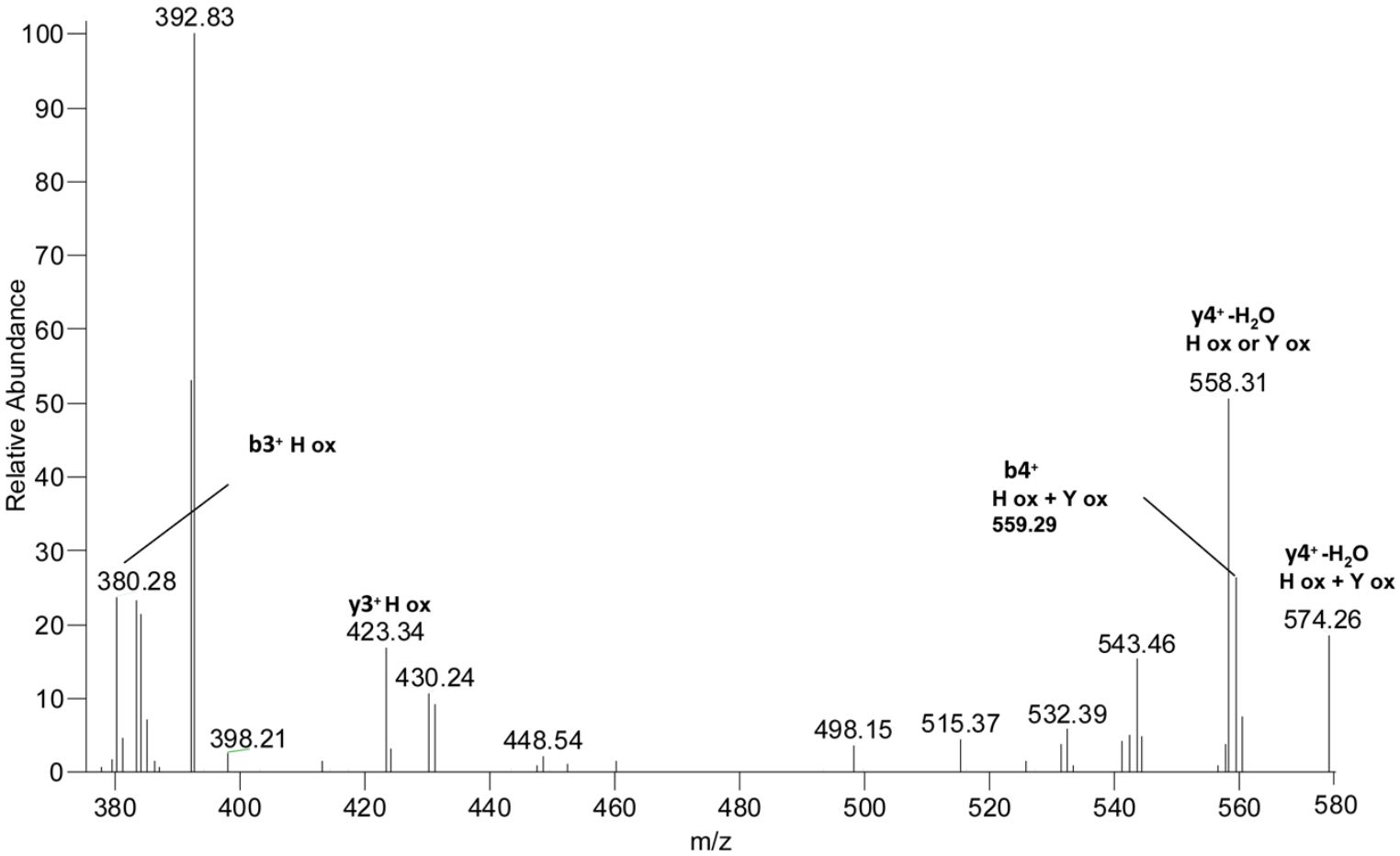


Figure S16b Zoom in (m/z range 375-580) of the MS/MS spectrum depicted in figure S16a.

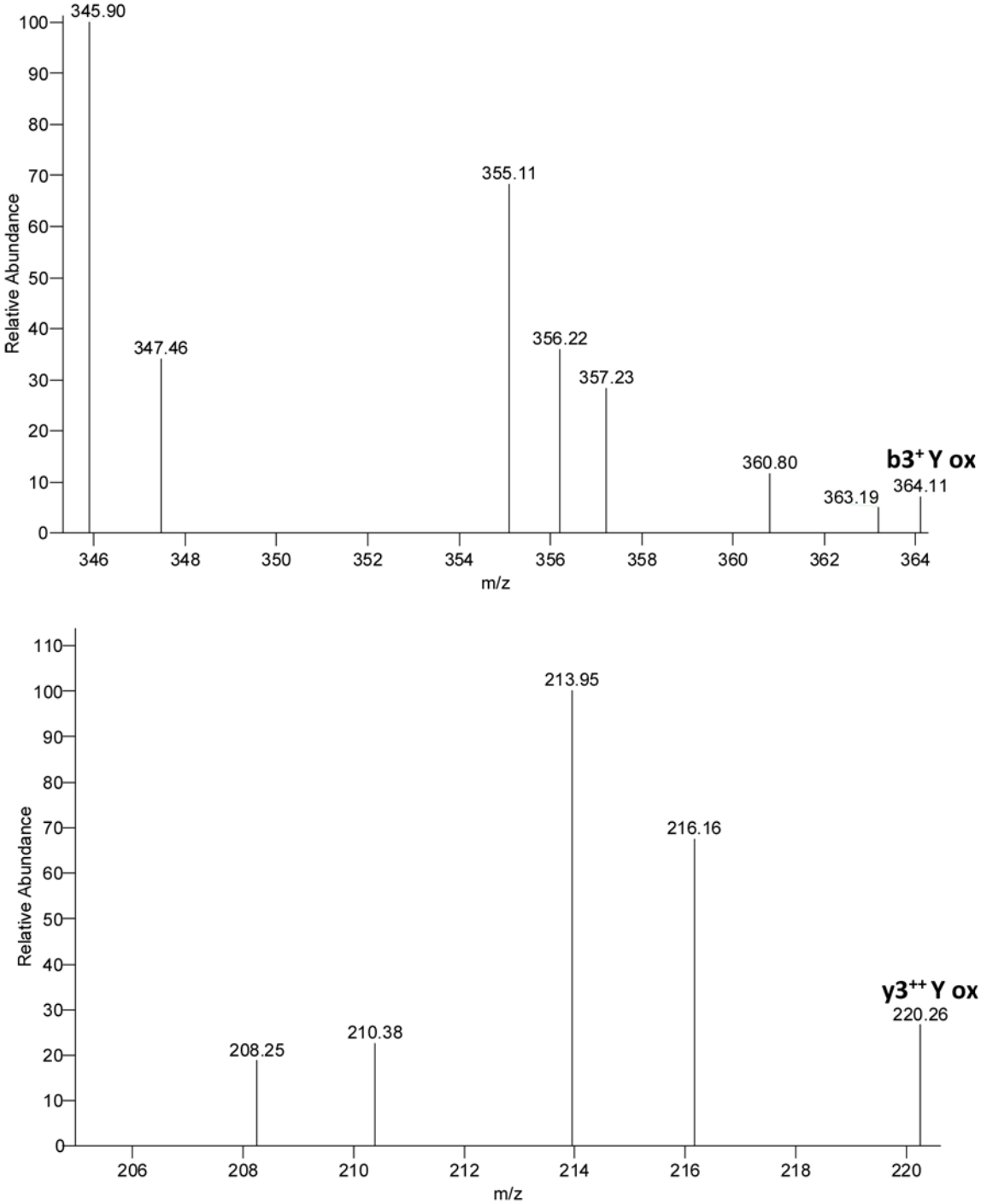


Figure S16c Zoom in (m/z range 345-365, upper panel, and 180-1400, lower panel) of the MS/MS spectrum depicted in figure S16a.

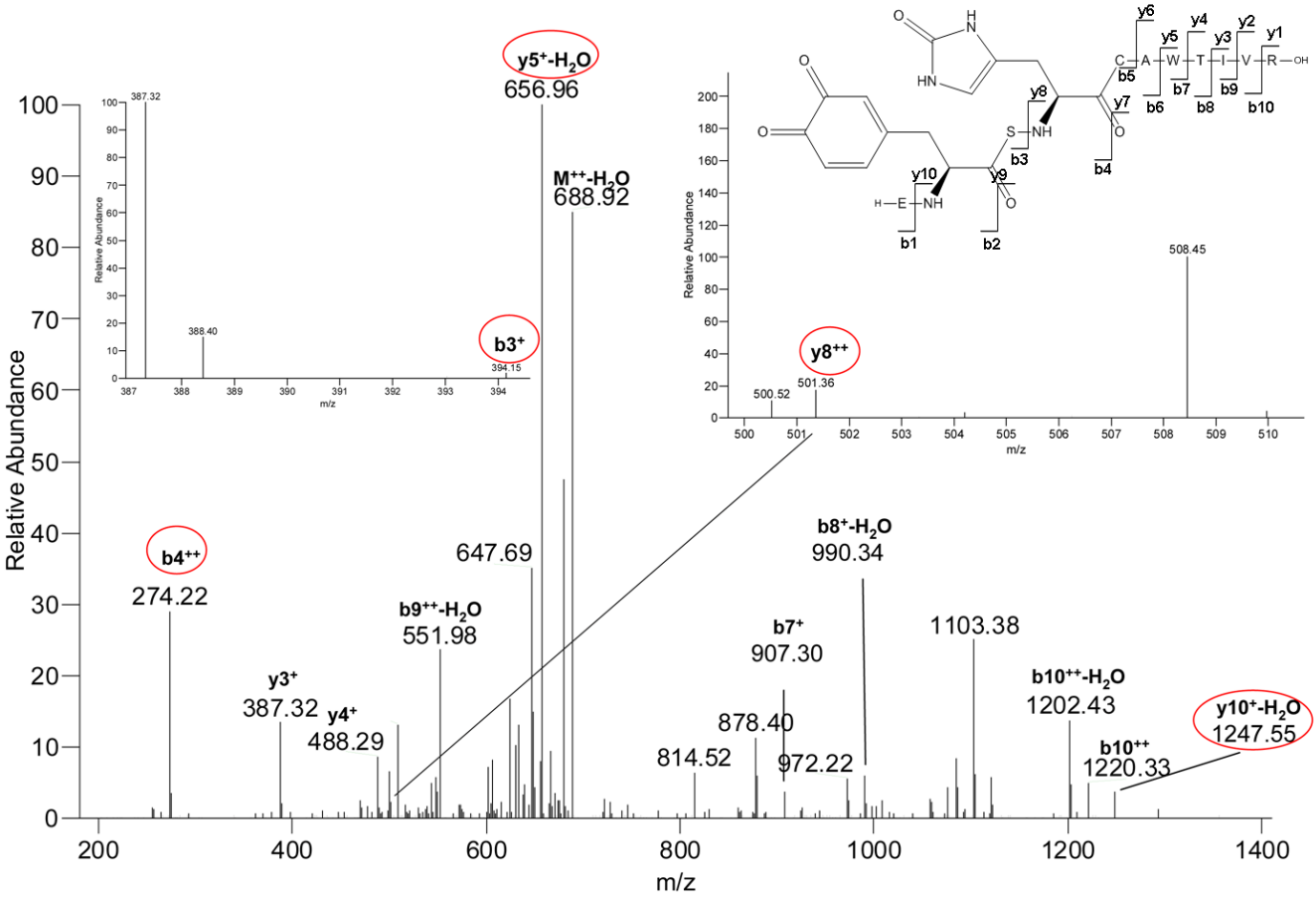


Figure S17 MS/MS spectrum and suggested chemical structure for the oxidized (+32 Da) sequence **E_{137}YSHCAWTIVR_{147}** (theoretical *mass* 1393.62, experimental *m/z* 697.81 $[M+H]^{2+}$, Δm from native parent peptide 0.00).

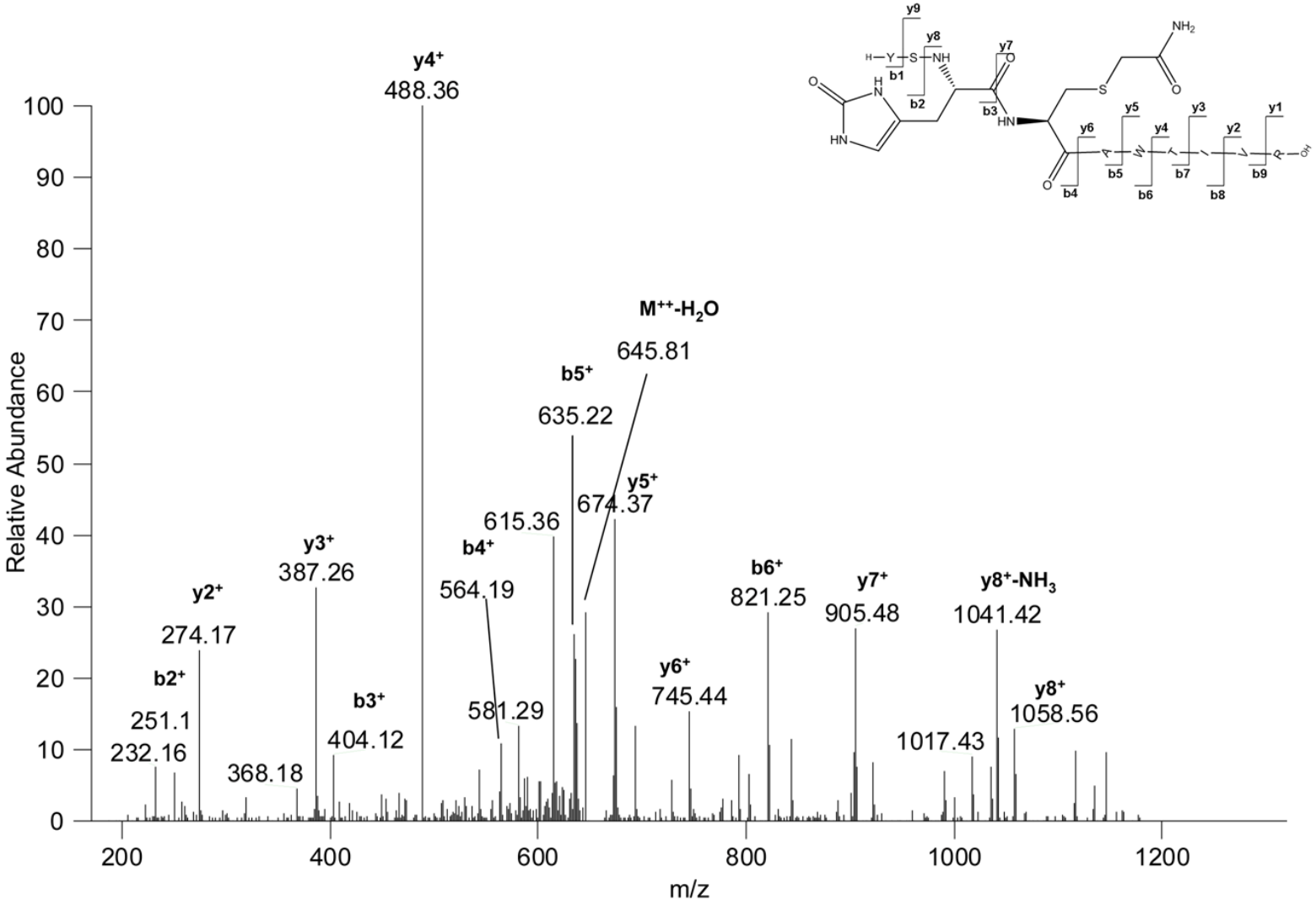


Figure S18 MS/MS spectrum and suggested chemical structure for the oxidized (+16 da) sequence **Y_{138}SHCAWTIVR_{147}** (theoretical *mass* 1307.62, experimental *m/z* 654.81 $[M+H]^{2+}$, Δm from native parent peptide 0.00).

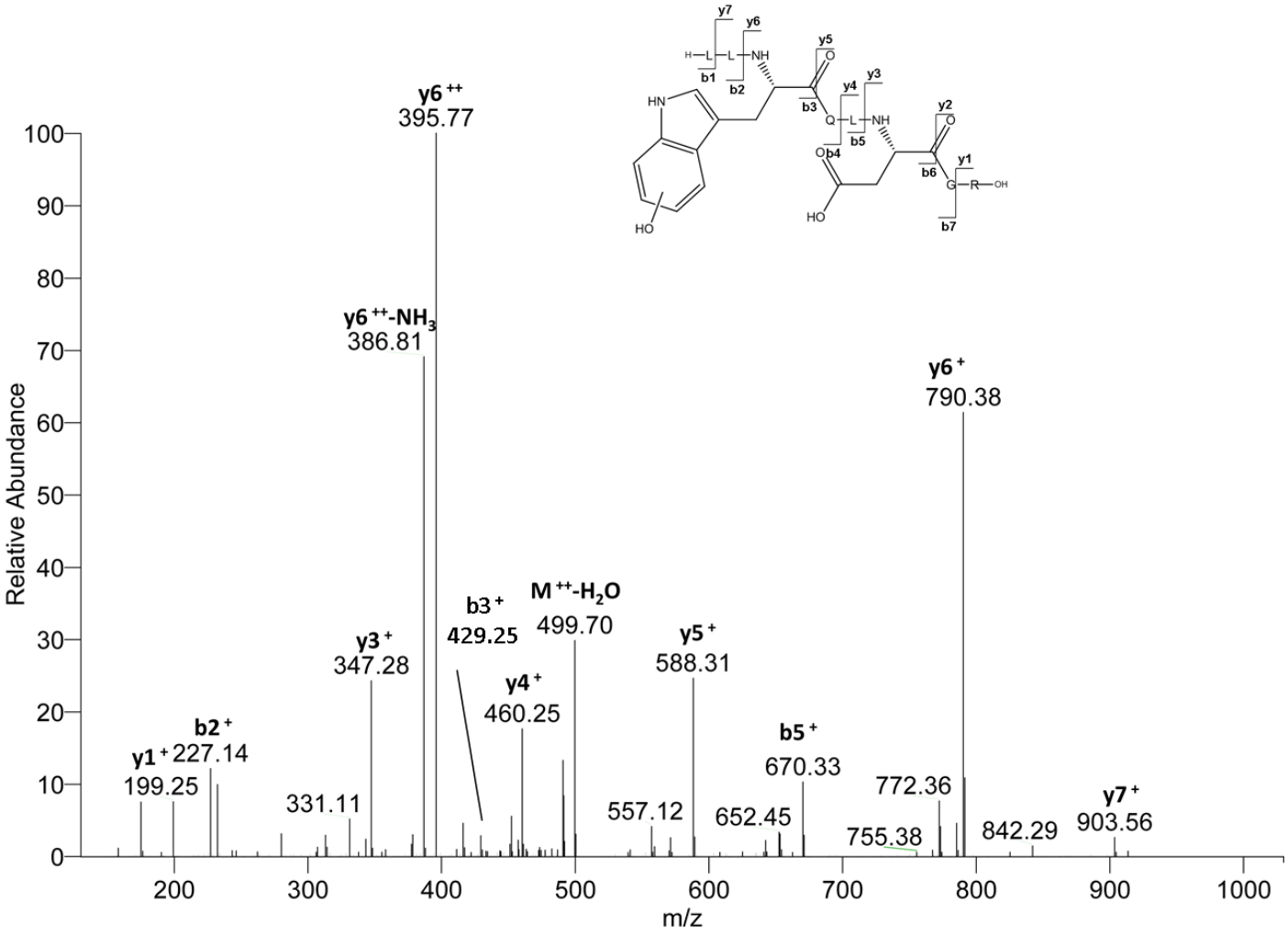


Figure S19 MS/MS spectrum and suggested chemical structure for the oxidized (+16 Da) sequence **L_{20}LWQLNGR$_{27}$** (theoretical *mass* 1015.55, experimental *m/z* 508.78, $[M+H]^{2+}$, Δm from native parent peptide +0.01).

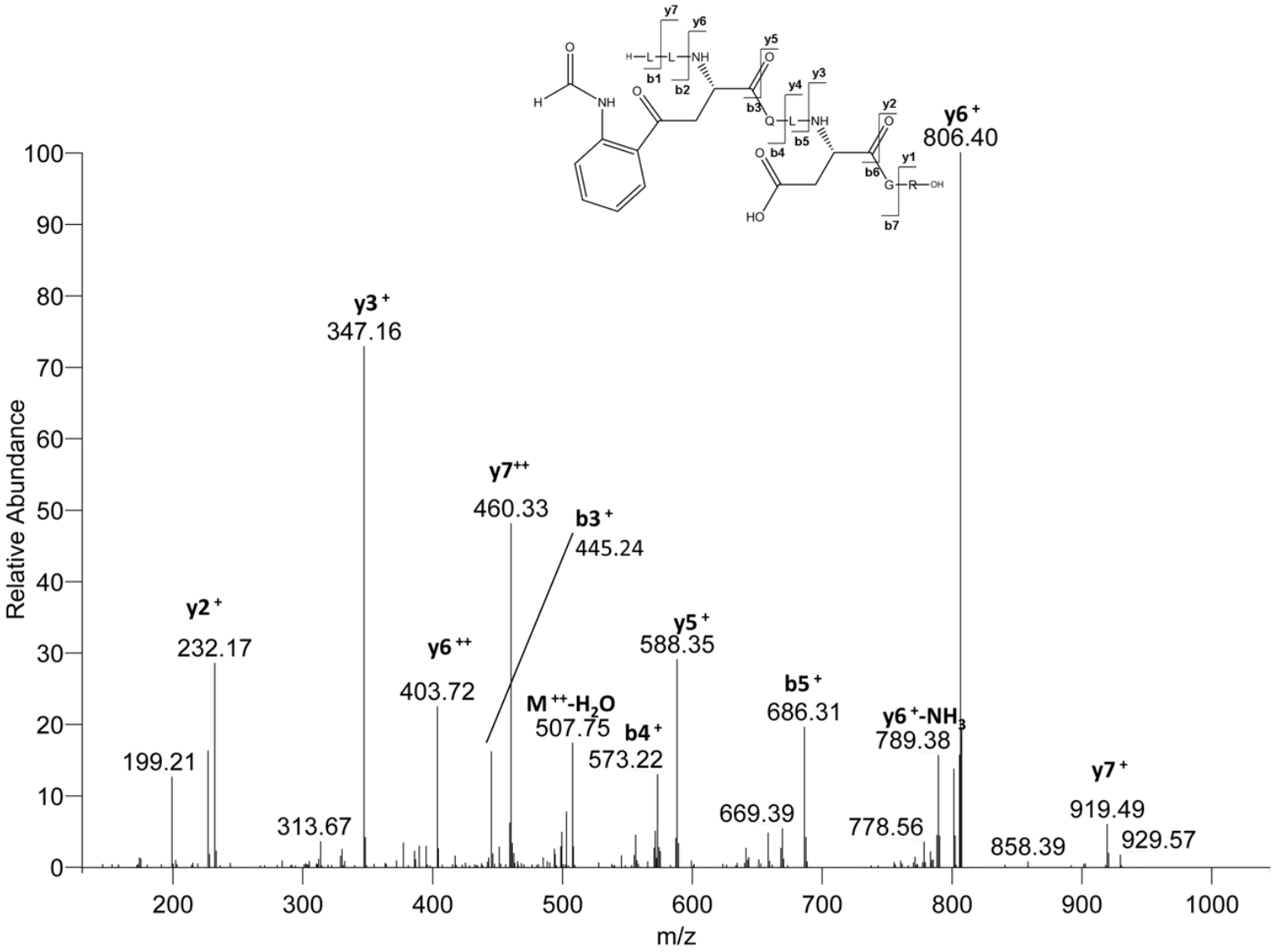


Figure S20 MS/MS spectrum and suggested chemical structure for the oxidized (+32 Da) sequence **L_{20}LWQLNGR$_{27}$** (theoretical *mass* 1031.55, experimental *m/z* 516.78, $[M+H]^{2+}$, Δm from native parent peptide +0.01).

Table S1 Potential chemical modifications of amino acid residues included in the settings of the database search employed to identify chemical changes in the primary structure of IFNβ1a after MCO.

Chemical modifications	Chemical addition - deletion	Δm (monoisotopic) in Da
Asn deamidation	-H, -N, +O	+0.984016
Arg to glutamic semialdehyde	-C, -5H, -3N, +O	-43.053433
Cys to sulfinic acid	+2O	31.989829
Cys to sulfonic acid	+3O	47.984744
Cys to oxoalanine	-2H, +O, -S	-17.992806
Cys to carbamidomethyl cysteine (iodoacetamide alkylation)	+2C, +3H, +N, +O	57.021464
Met to sulfoxide	+O	15.994915
Met to sulfone	+2O	31.989829
Met loss of CH3SO from oxidized Met	-C, -4H, -S	-48.003371
Gln deamidation	-H, -N, +O	+0.984016
Gln to pyroglutamic acid	-3H, -N	-17.026549
Glu to pyroglutamic acid	-2H, -O	-18.010565
His to 2-oxo histidine	+O	15.994915
His to asparagine	-2C, -H, -N, +O	-23.015984
His to aspartic acid	-2C, -2H, -2N, +2O	-22.031969

Lys to aminoadipic semialdehyde	-3H, -N ,+O,	-1.031634
Lys to alpha aminoadipic acid	-3H, -N, +2O,	14.963280
Lys peroxidation	+2O	31.989829
Lys to carbamidomethyl Lysine (iodoacetamide alkylation)	+2C, +3H, +N, +O	57.021464
Tyr to TOPA (2,4,5-trihydroxyphenylalanine)	+2O	31.989829
Thr to 2-amino-ketobutirric acid	-2H	-2.015650
Trp to hydroxytryptophan	+O	15.994915
Trp to kynurenine	-C, +O	3.994915
Trp to N-formyl kynurenine	+2O	31.989829
Phe to TOPA quinone (2,4,5-trihydroxyphenylalanine quinone)	-2H, +3O	45.969094
Phe to TOPA (2,4,5-trihydroxyphenylalanine)	+3O	47.984744
Phe to hydroxytyrosine	+O	15.994915
Phe to 3,4-dihydroxyphenylalanine (DOPA)	+2O	31.989829
Phe to DOCH	-2H, +2O	29.974179
Pro to Pyrrolidinone	-C, -2H, -O	-30.010565
Pro to pyroglutamic acid	-2H, +O	13.979265
Tyr to 3,4-dihydroxyphenylalanine (DOPA)	+O	15.994915
Tyr to DOCH	-2H, +O	13.979265
Tyr to TOPA quinone (2,4,5-trihydroxyphenylalanine quinone)	-2H, +2O	29.974179