

Disulfide Bond Assignments by Mass Spectrometry of Native Natural Peptides.

Cysteine Pairing in Disulfide Bonded Conotoxins.

Kallol Gupta¹, Mukesh Kumar², Padmanabhan Balaram^{1*}

1. Molecular Biophysics Unit, Indian Institute of Science, Bangalore-560012, India

2. National Centre for Biological Sciences, Tata Institute of Fundamental Research,
Bangalore-560065, India

Supporting Figures S-1 - S-6, S-9, S-10, S-12, S-14 and S-15 show mass spectrometric data and Supporting Figures S-7, S-8, S-11 and S-13 show schemes rationalizing observed product ions and Supporting Table S-1 summarizes the disulfide connectivity of different conotoxins obtained by various techniques.

Table S-1: Summary of the different disulfide bonding schemes in conotoxins determined using various methods

Name ^(ref)	Sequence	Disulfide Connectivity	Name ^(ref)	Sequence	Disulfide Connectivity
1. Selective chemical synthesis followed by co elution			Vi1361 ¹⁷	ZC ₁ C ₂ PTMPEC ₃ C ₄ RI*	1-3, 2-4 (MS)
α -MI ¹⁹	GRC ₁ C ₂ HPAC ₃ GKNYSC* ₄	1-3, 2-4	Tx3c ¹⁴	C ₁ C ₂ RTC ₃ FGC ₄ TOC ₅ C ₆ *	1-6, 2-4, 3-5 (Edmann)
α -SIA ²¹	YC ₁ C ₂ HPAC ₃ GKNFDC* ₄	1-3, 2-4	Mr3a ¹⁴	GC ₁ C ₂ GSFAC ₃ RFGC ₄ VOC ₅ C ₆ V	1-6, 2-4, 3-5 (Edmann)
α -MII ²²	GRC ₁ C ₂ SNPVC ₃ HLEHSNLC* ₄	1-3, 2-4	3.NMR		
λ -MrVIA ²³	VC ₁ C ₂ GYKLC ₃ HOC ₄	1-4, 2-3	A-GI ²⁷	EC ₁ C ₂ NPAC ₃ GRHYSC* ₄	1-3, 2-4
λ -MrVIB ^{23,24}	NGVC ₁ C ₂ GYKLC ₃ HOC ₄	1-4, 2-3	A-MII ²⁸	GRC ₁ C ₂ SNPVC ₃ HLEHSNLC* ₄	1-3, 2-4
λ -MrX ²³	GIC ₁ C ₂ GVSFC ₃ YOC ₄	1-4, 2-3	E-TXIX ²⁹	γ C ₁ C ₂ γ NGWC ₃ C ₄ TAAO	1-3, 2-4
α -GIC ²⁵	GC ₁ C ₂ SHPAC ₃ AGNNQHIC* ₄	1-3, 2-4	α -SI ³⁰	IC ₁ C ₂ NPAC ₃ GPKYSC* ₄	1-3, 2-4
λ -MrIe ²⁶	C ₁ C ₂ HSSWC ₃ KHLC* ₄	1-4, 2-3	α -GID ³¹	IRD γ C ₁ C ₂ SNPAC ₃ RVNNOHVC* ₄	1-3, 2-4
2. Selective Reduction & Alkylation followed by Sequencing (MS/ Edmann)			SxIIIA ³²	RC ₁ C ₂ TGKKGSC ₃ SGRAC ₄ KNLKC ₅ C ₆ A*	1-4, 2-5, 3-6
α -ImI ¹²	GC ₁ C ₂ SDPRC ₃ LAYRC* ₄	1-3, 2-4 (Edmann)	SxIIIB ³³	ZKC ₁ C ₂ TGKKGSC ₃ SGRAC ₄ KNLKC ₅ C ₆ A*	1-4, 2-5, 3-6
α -EpI ¹³	GC ₁ C ₂ SDPRC ₃ NMNNPDYC* ₄	1-3, 2-4 (MS)	4. X-ray		
Mo1274 ¹⁵	GNWC ₁ C ₂ SARVC ₃ C* ₄	1-3, 2-4 (MS)	α -PnIA ⁷	GC ₁ C ₂ SLPPC ₃ AANNPDYC* ₄	1-3, 2-4
Vc1.1 ¹⁶	GC ₁ C ₂ SDPRC ₃ NYDHPEIC* ₄	1-3, 2-4 (Edmann)	α -PnIB ⁸	GC ₁ C ₂ SLPPC ₃ ALSNPDYC* ₄	1-3, 2-4
Vi1359 ¹⁷	ZC ₁ C ₂ ITIPEC ₃ C ₄ RI*	1-3, 2-4 (MS)	α -PnIC ⁹	GC ₁ C ₂ SRPPC ₃ IANNPDLC* ₄	1-3, 2-4

*, amidated C-terminus; Z, Pyroglutamic acid; W, Bromotryptophan;

γ , gama carboxyglutamic acid; O, Hydroxyproline

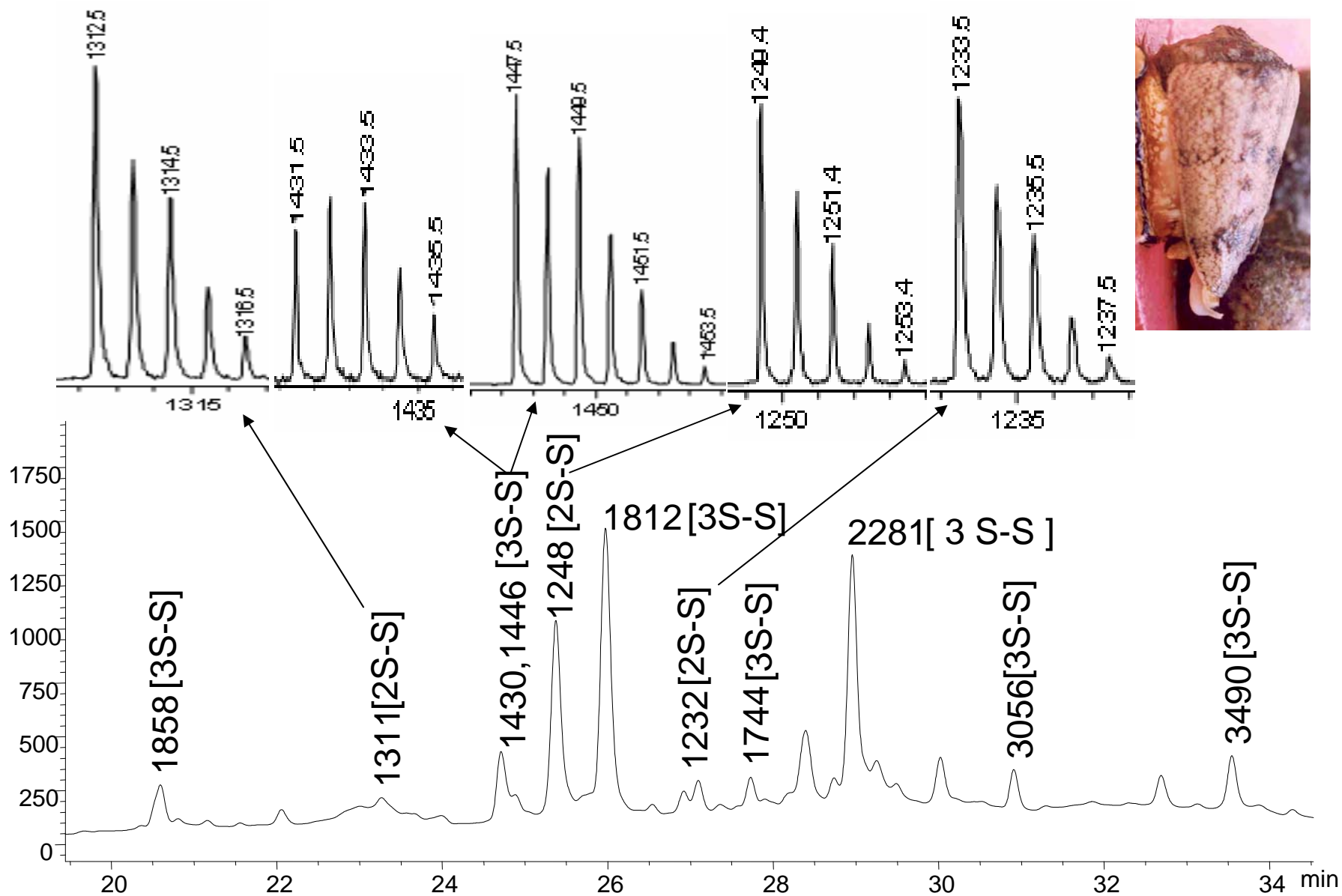


Figure S-1. Expanded segment of the HPLC profile of crude venom from *C. araneosus*. Molecular mass and number of disulfide bonds, determined by global reduction and alkylation (in parenthesis), are shown over selected peaks. Expanded region of the mass spectra corresponding to the m/z region of $[M+H]^+$ are shown for peptide Ar1232, Ar1248, Ar1311, Ar1430 and Ar1446.

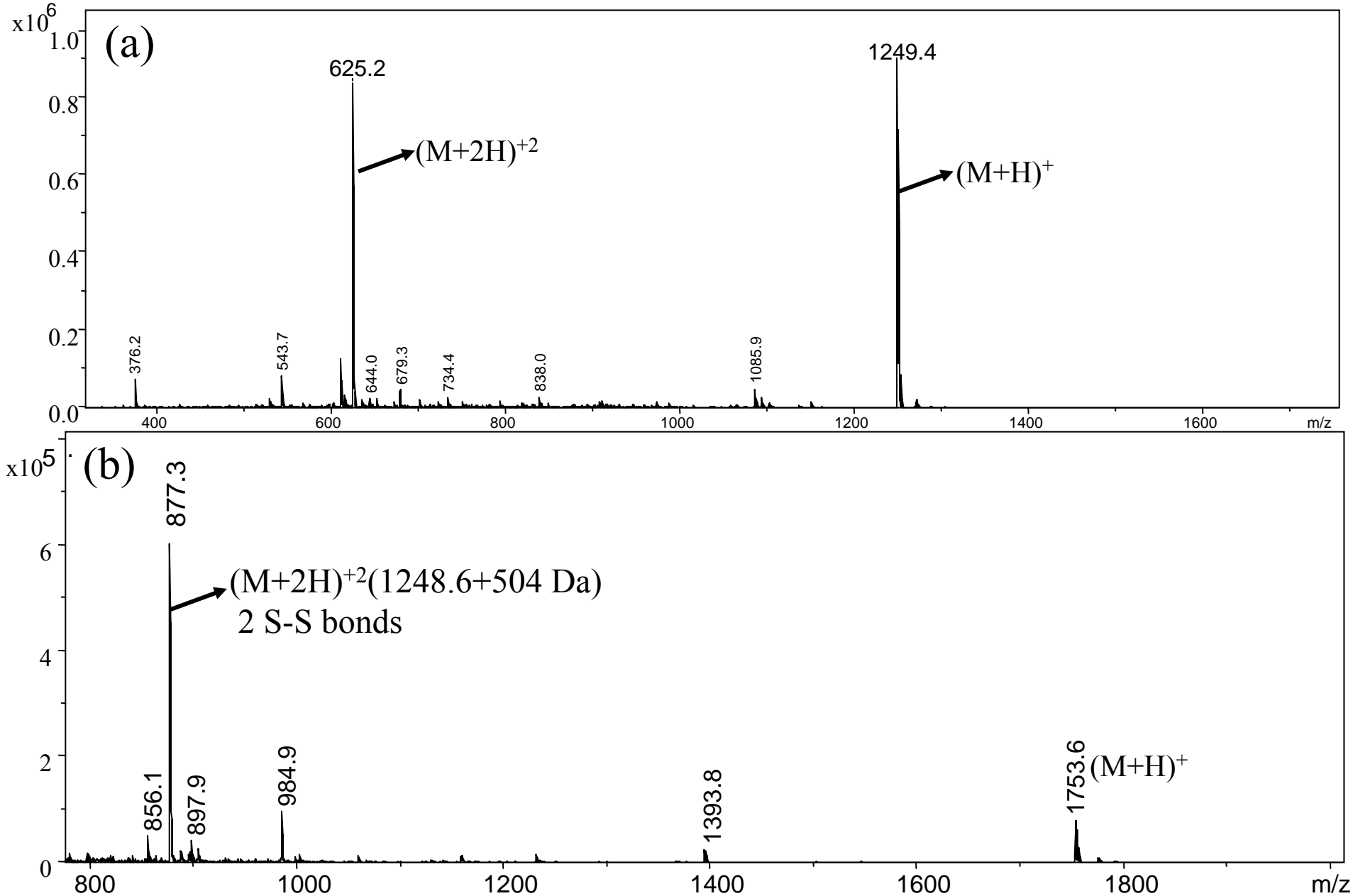


Figure S-2: ESI MS of (a) native Ar1248 and (b) reduced and alkylated species. Reduction was carried out with Tris (carboxyethyl) phosphine, followed by alkylation with N-ethylmaleimide. The mass increase of 504Da reveals the presence of two disulfide bonds.

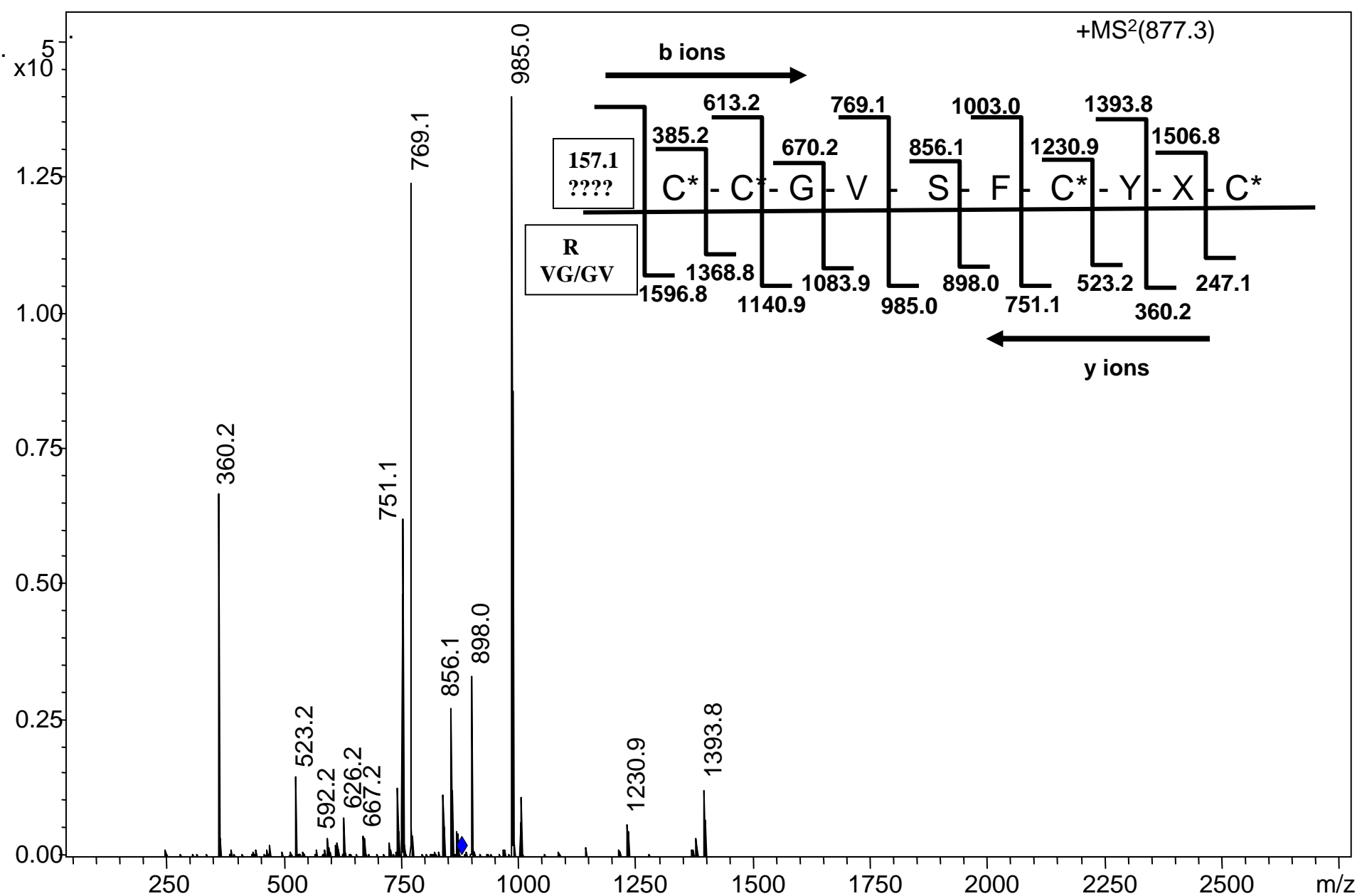


Figure S-3: Positive ion CID MS² spectrum of the doubly charged reduced and alkylated Ar1248. Fragment ions observed and sequence obtained are schematically shown. The identity of the N-terminus fragment, with m/z 157.1, could not be unambiguously established through CID MS². (See Fig S-3 for ETD results) C*=ethylmaleiamidocysteine

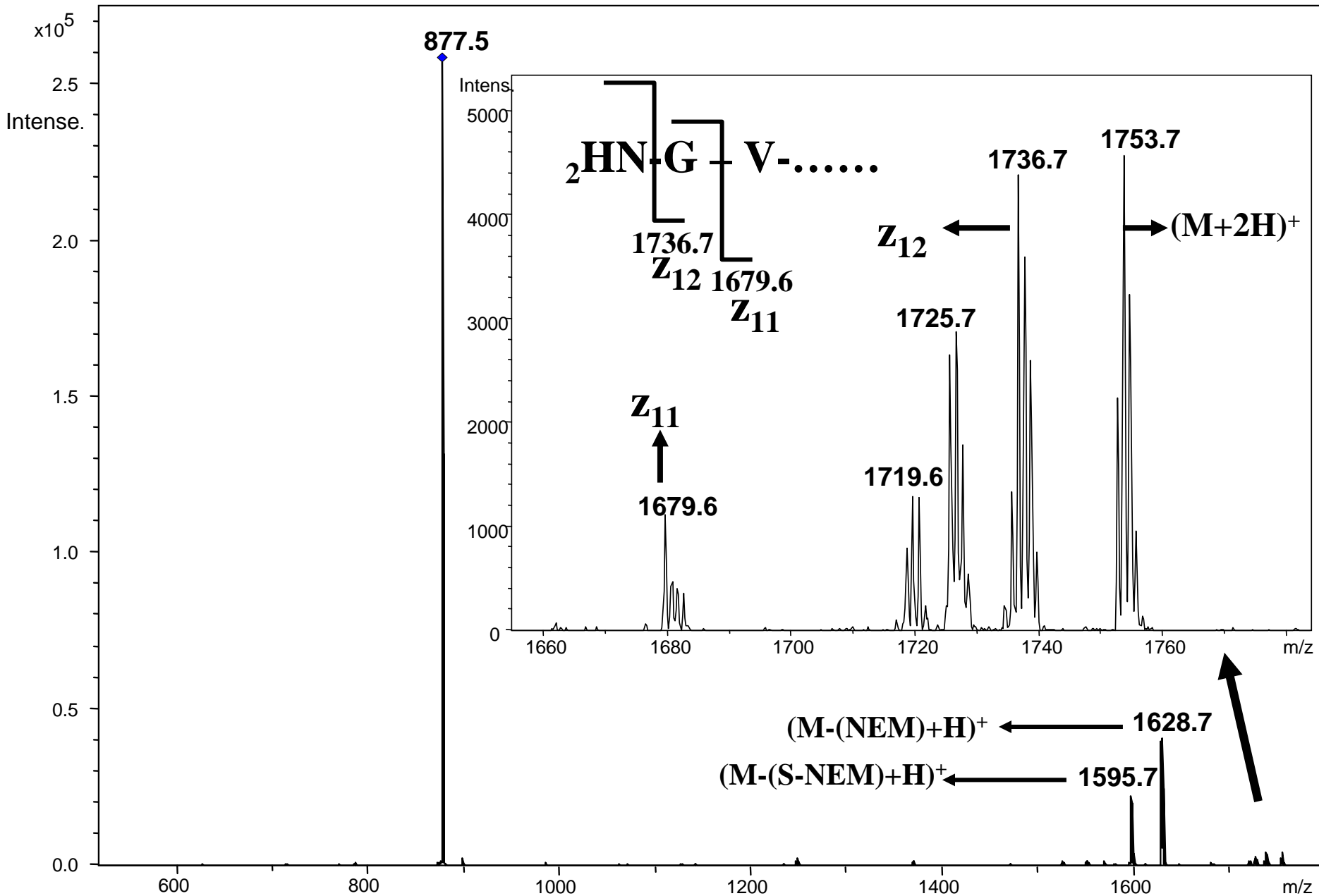


Figure S-4: ETD MS² spectrum of the doubly charged reduced and alkylated Ar1248. An expansion of the m/z region 1650-1780 shows the critical z_{11} and z_{12} ions that reveal the identity of the N-terminus residues.

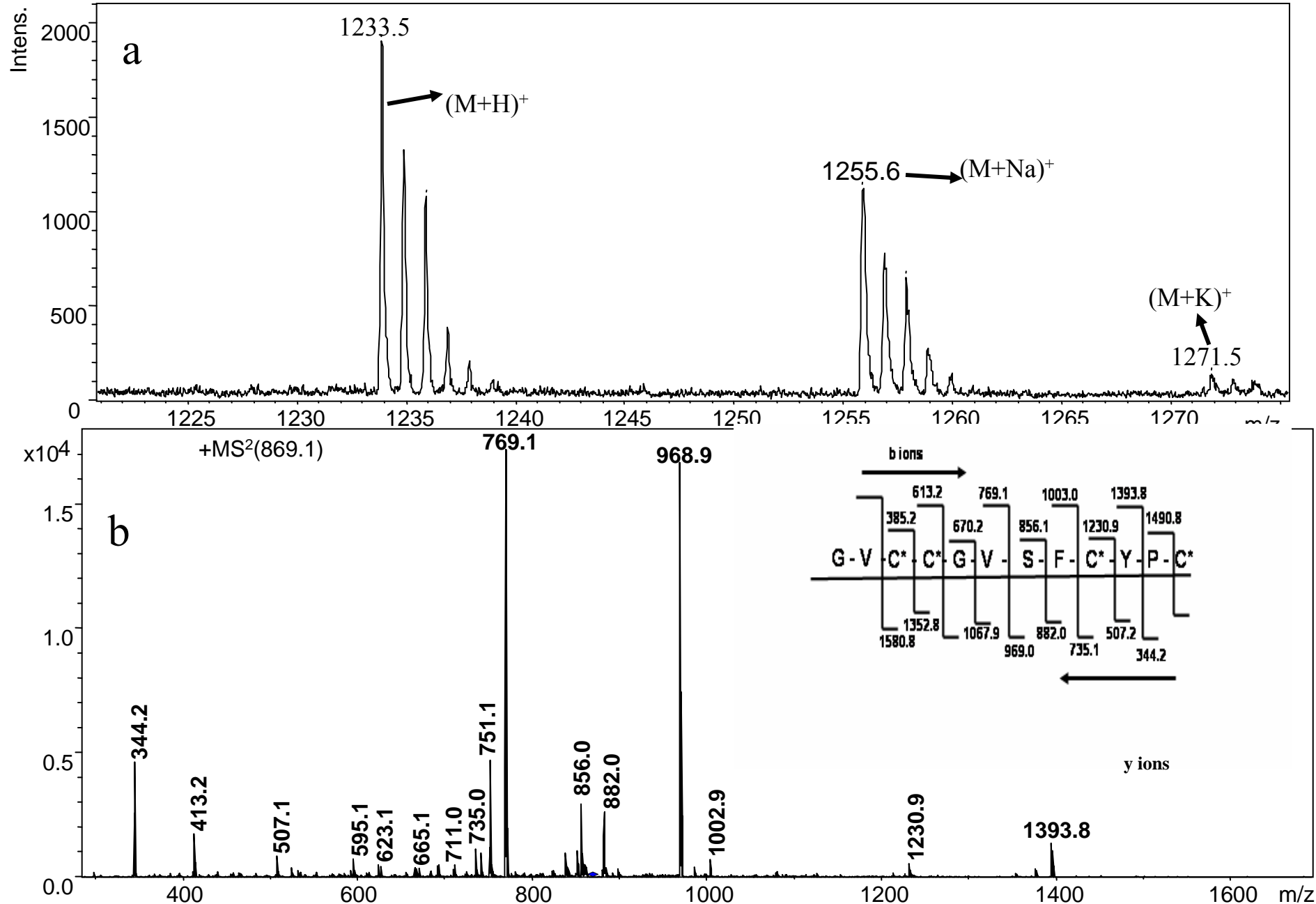


Figure S-5: MALDI spectrum of the related peptide (a) Ar1232 and (b) CID MS² spectrum of the reduced and alkylated doubly charged species.

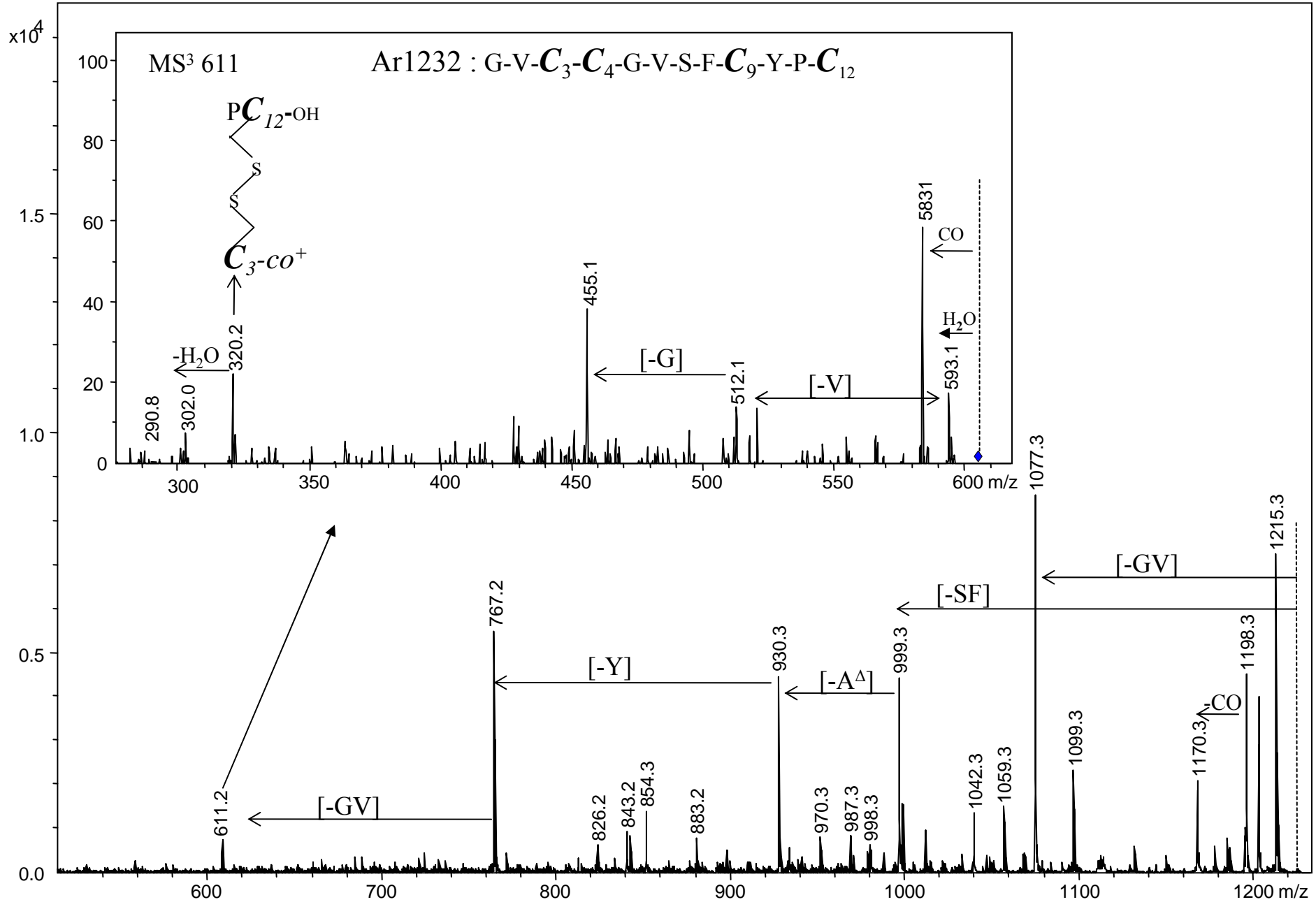


Figure S-6: Positive ion CID MS² spectrum of the native disulfide bonded Ar1232. Inset shows the MS³ spectrum of the ion at m/z 611.2.

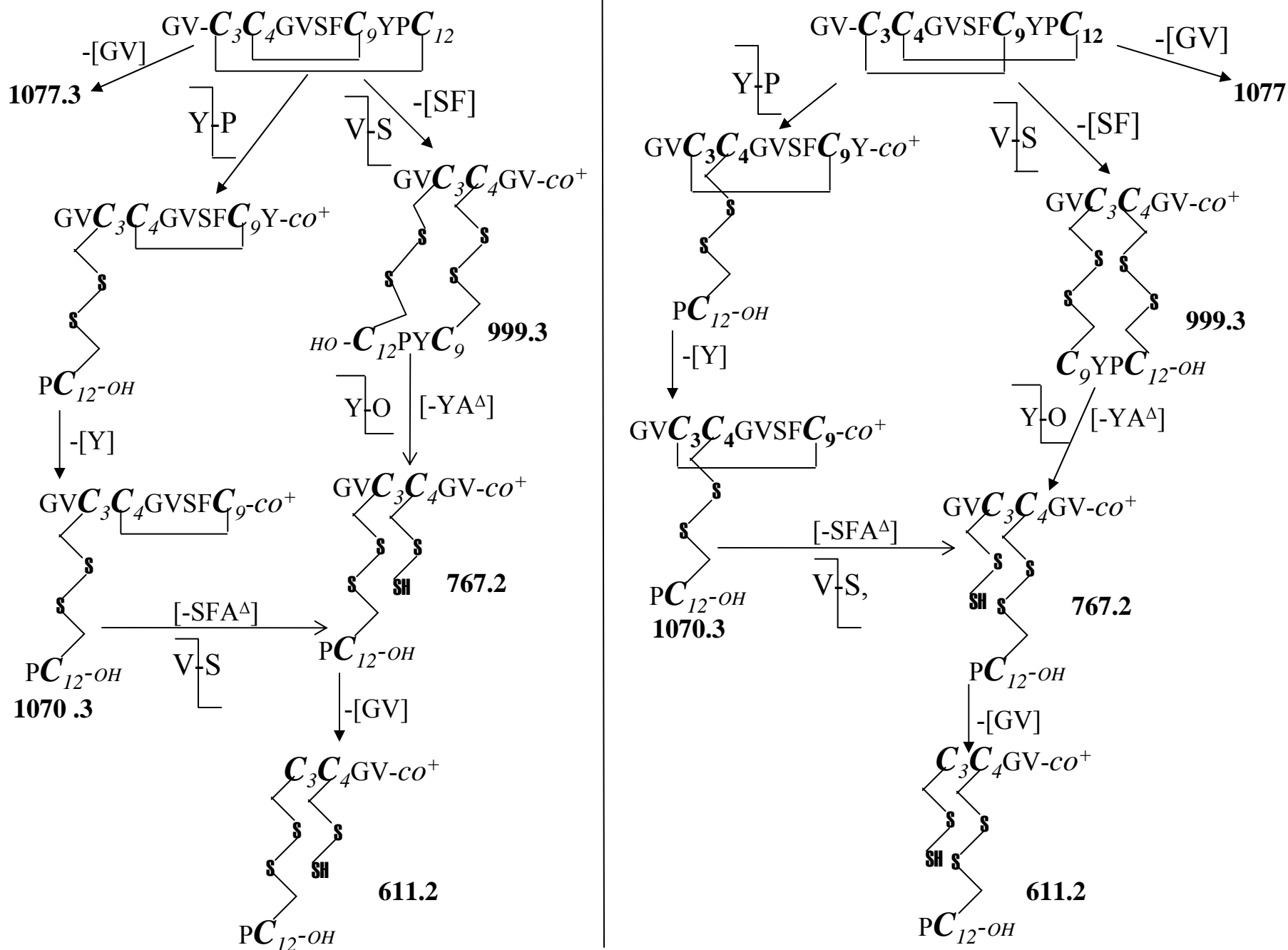


Figure S-7: Probable structures of product ions, observed in the CID MS² spectrum of Ar1232, for two possible disulfide bonded isomers.

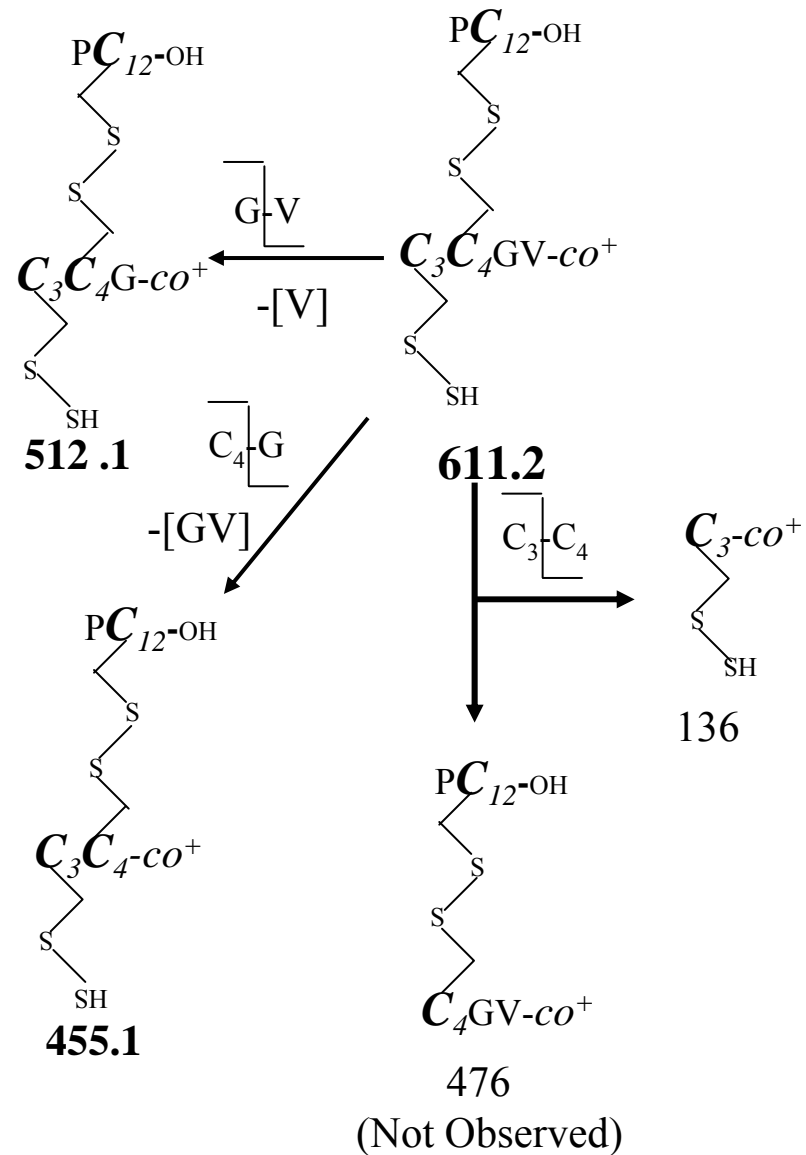
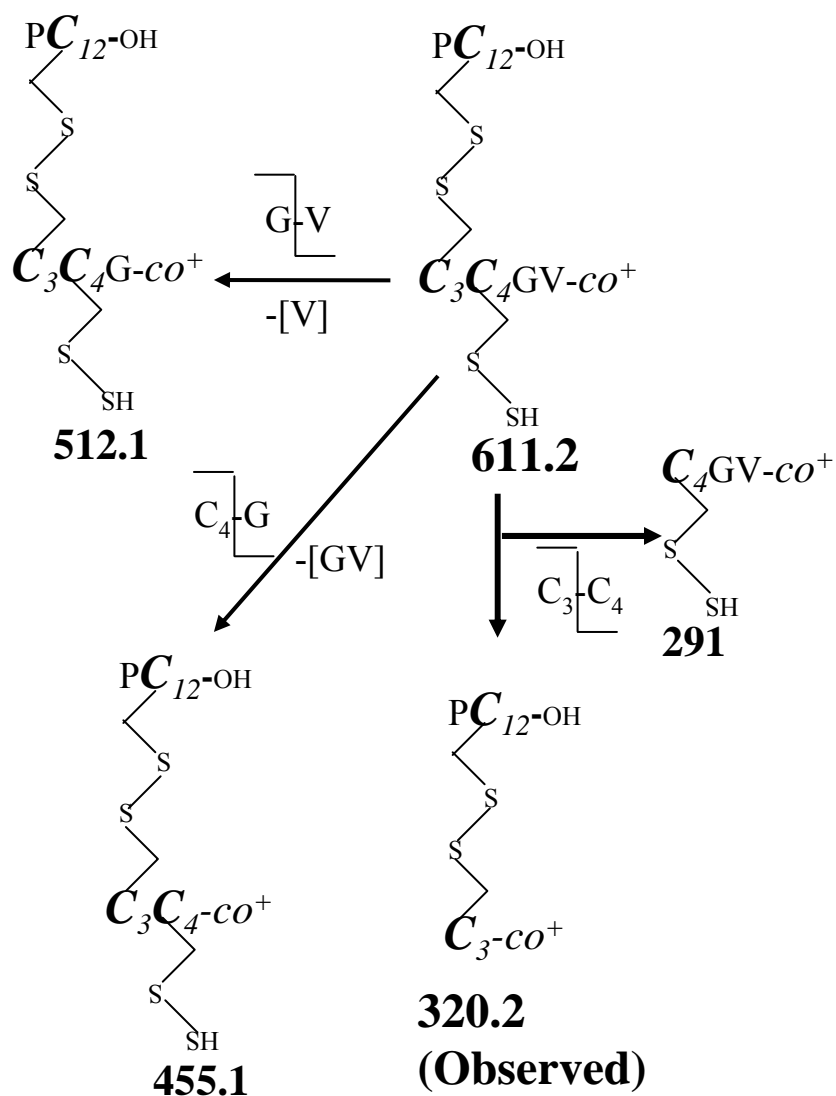


Figure S-8: Probable diagnostic MS³ fragment ions for two possible structures of the ion at m/z 611.2

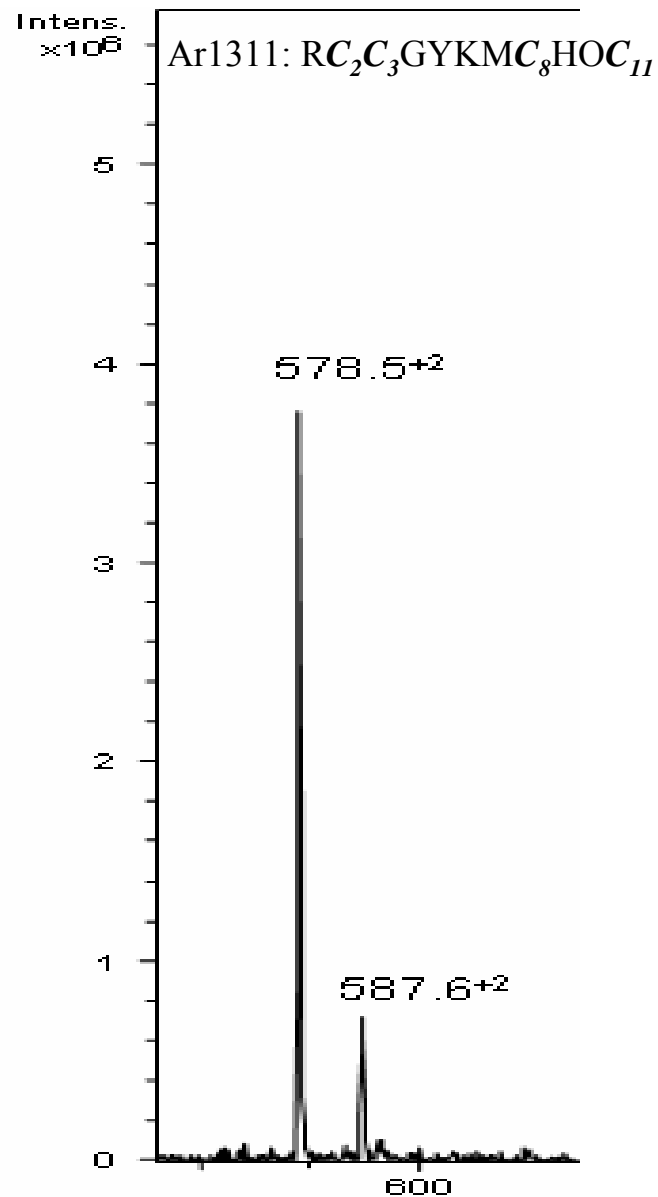


Figure S-9: ESI MS of the trypsin digest of Ar1311. The doubly charged ion at 578.5 shows the presence of Arg cleaved species and the doubly charged ion at 587.5 represents the Arg cleaved and Lys₆-Met₇ bond nicked peptide.

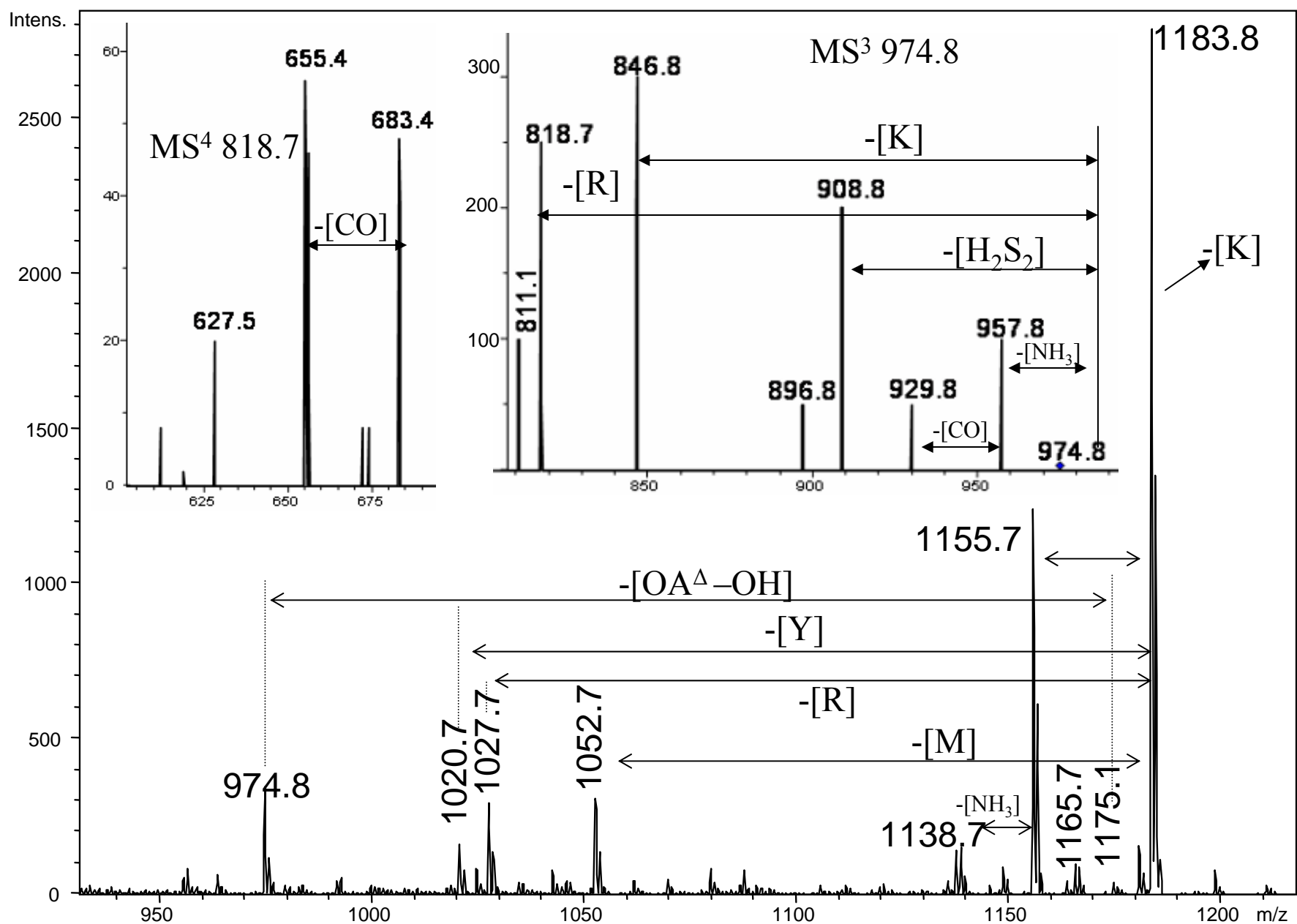
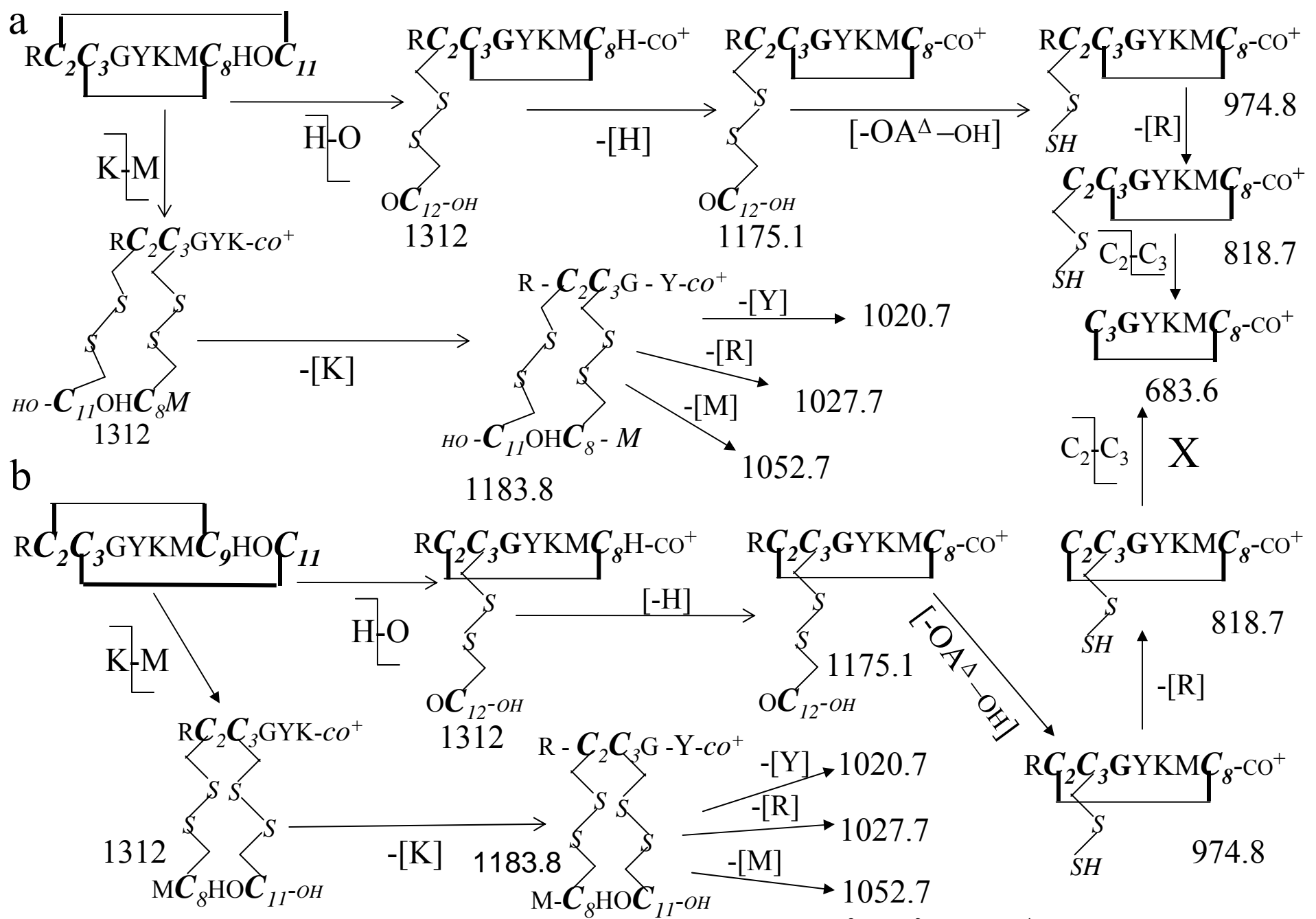


Figure S-10: Positive ion CID MS² spectrum of native disulfide bonded Ar1311. Insets show the MS³ spectrum of the fragment ion at m/z 974.8, present in the MS² spectrum of native Ar1311 and the MS⁴ spectra of the ion at m/z 818.7, present in the MS³ spectrum of 974.8.



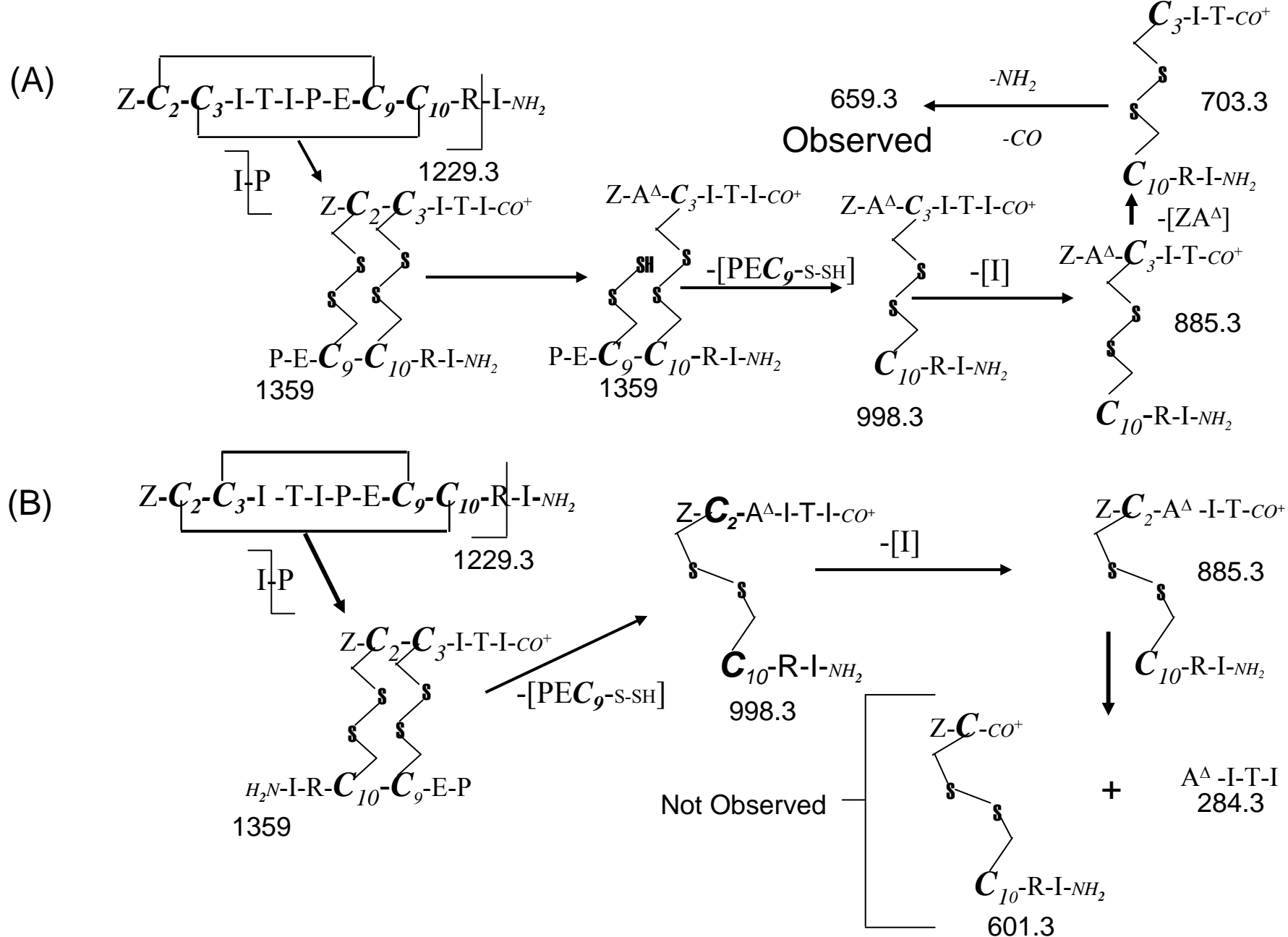


Figure S-13: Scheme shows the probable structures and m/z of the product ions that can arise through the same fragmentation route for the two probable disulfide bonded isomers of Vi1358. Note that it is the ion at m/z 659.3, corresponding to the structure (a) is observed, whereas the diagnostic ion for the alternative structure (b) at m/z 601.3 is not observed.

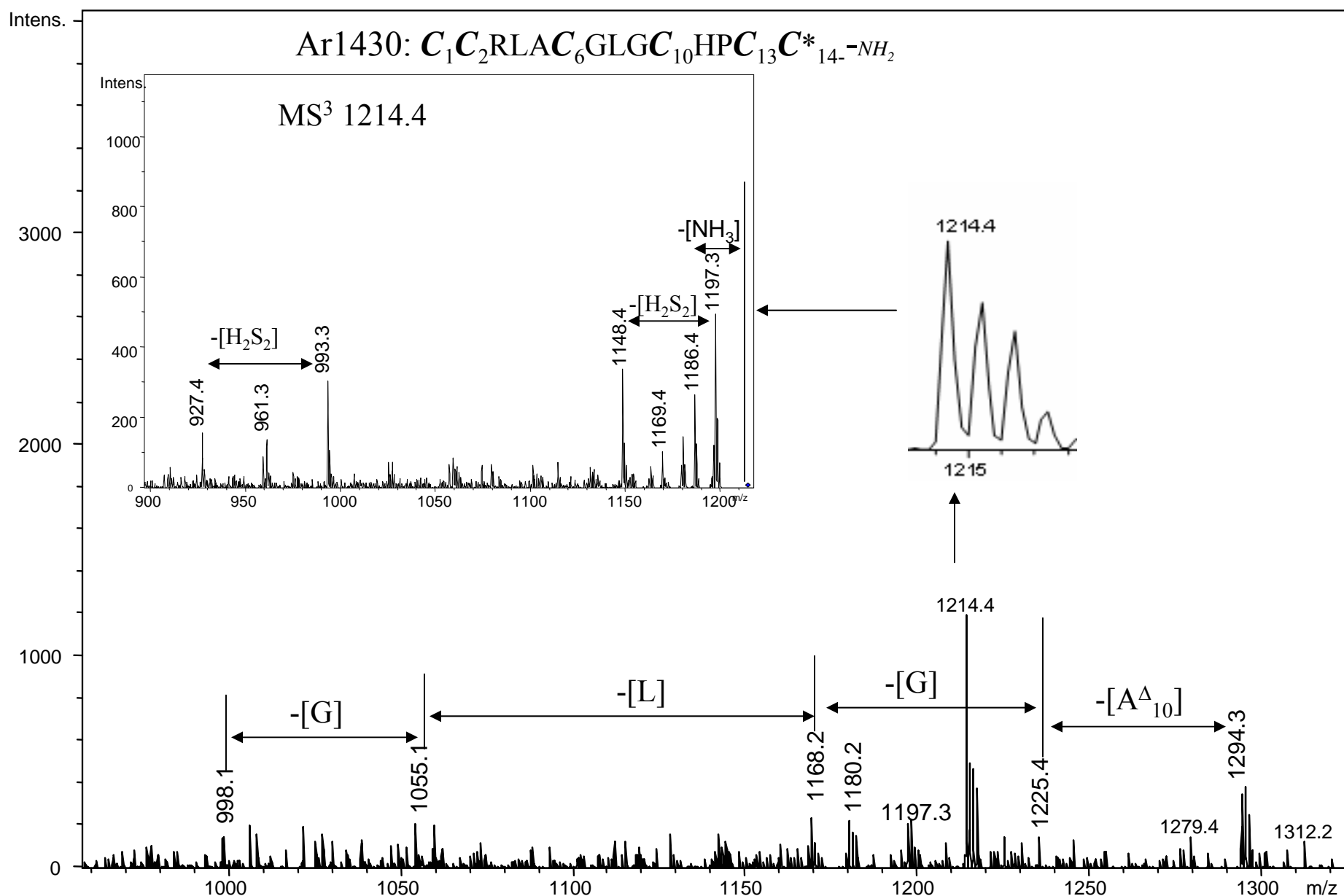


Figure S-14: Positive ion CID MS² spectrum of disulfide bonded native Ar1430. Inset shows the CID MS³ spectrum of the ion at m/z 1214.4. An absolute identity of the MS³ spectrum of this ion at m/z 1214.4 with the one present in the MS² spectrum of Ar1446, having same m/z , confirms the structural identity between the two 1214.4 species present in the MS² spectra of two peptides.

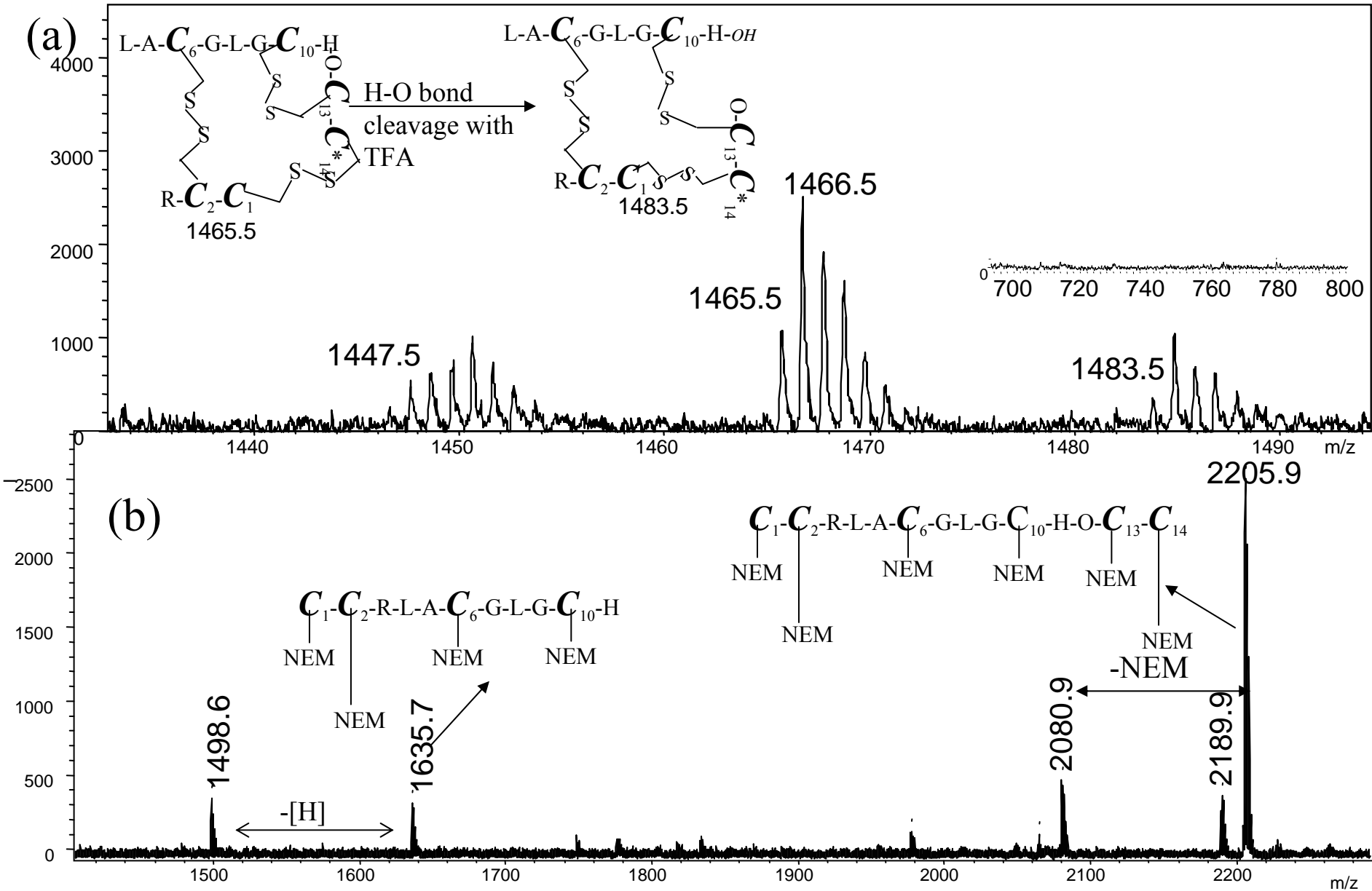


Figure S-15: (a) MALDI MS of the trypsin nicked Ar1446 followed by trifluoroacetic acid (TFA) cleavage. The unusually high intensity of the second isotope for the ions at m/z 1465 and 1483 is a consequence of the C-terminal amide hydrolysis upon prolonged incubation with TFA, whereas the isotopic distribution in the m/z range 1447 reveals the presence of native Ar1446, C-terminally hydrolyzed Ar1446 (mass 1447) and trypsin cleaved Ar1430 (mass 1448). A control experiment carried out on the reduced and alkylated Ar1446, shown in panel (b), under identical conditions further proves the preferential susceptibility of the Xxx-Pro/Hyp peptide bond to TFA cleavage.