SUPPORTING INFORMATION

Targeted amino acid substitutions in a *Trichoderma* peptaibol confer activity against fungal plant pathogens and protect host tissues from *Botrytis cinerea* infection

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Table of contents

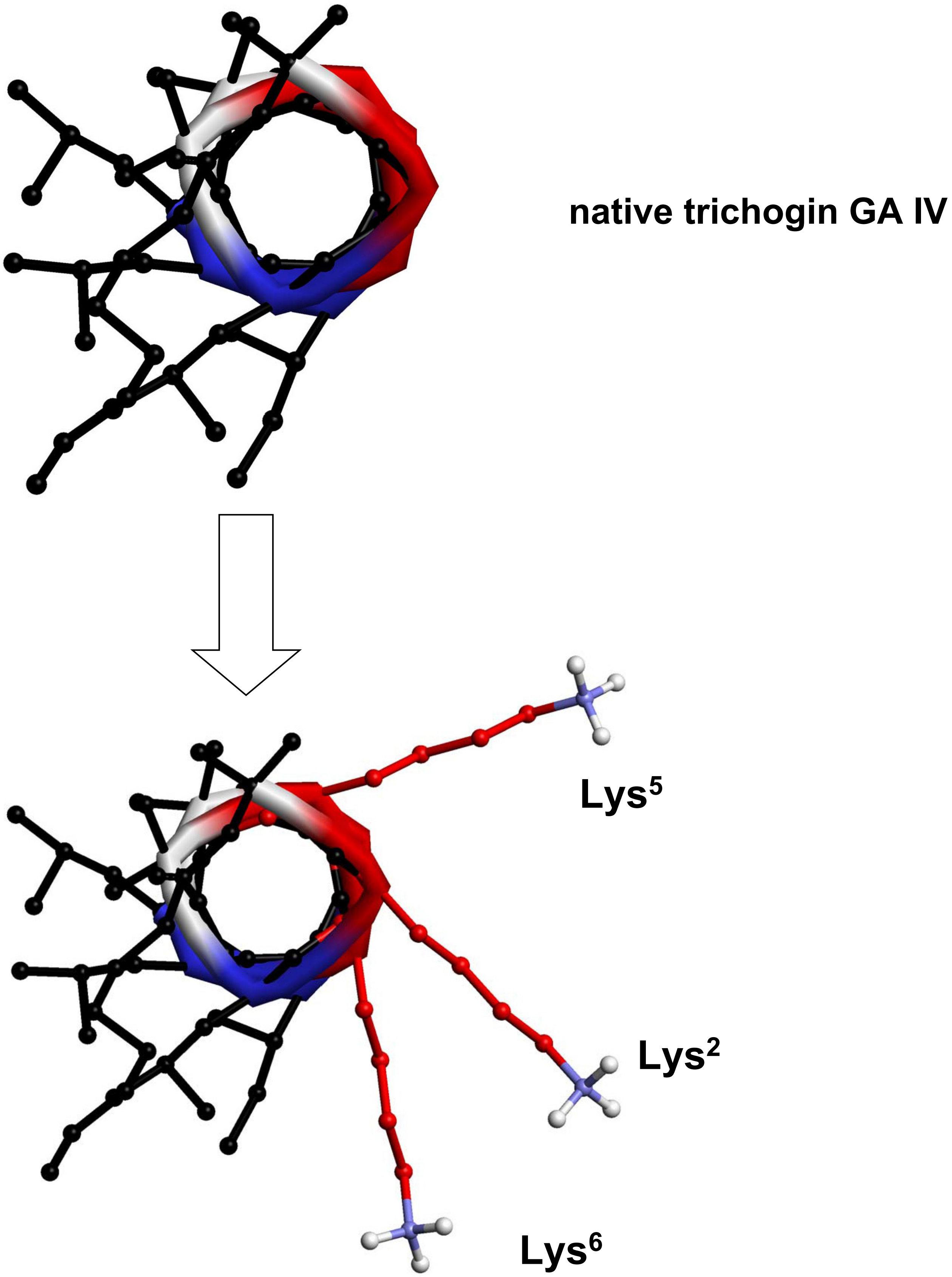


Figure S1. 3D-structure for compounds **1-5** and **7**. View along the helical axis.

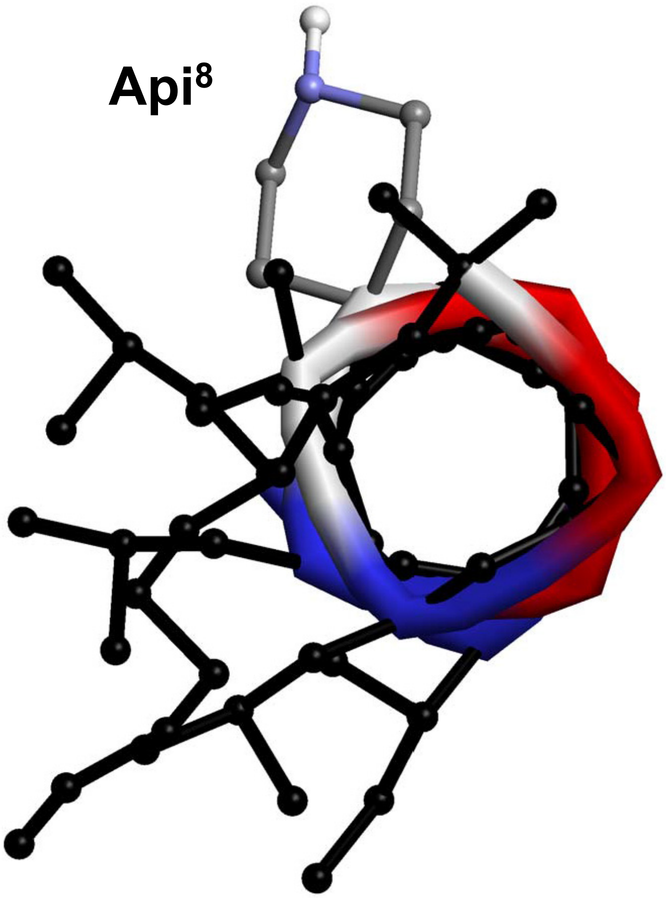


Figure S2. 3D-structure for compound **8**. View along the helical axis.

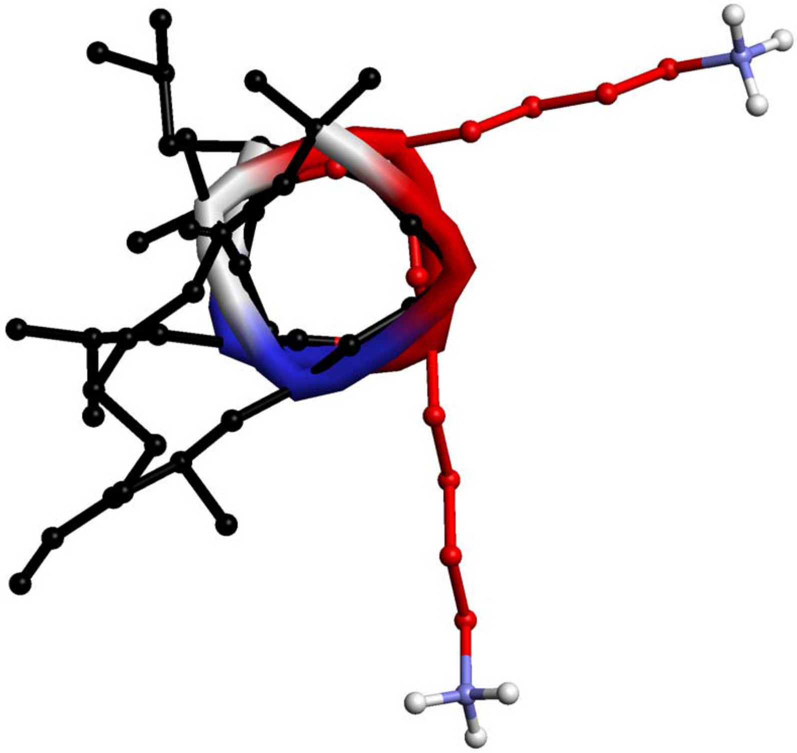


Figure S3. 3D-structure for compounds **4c** and **4c1**. View along the helical axis.

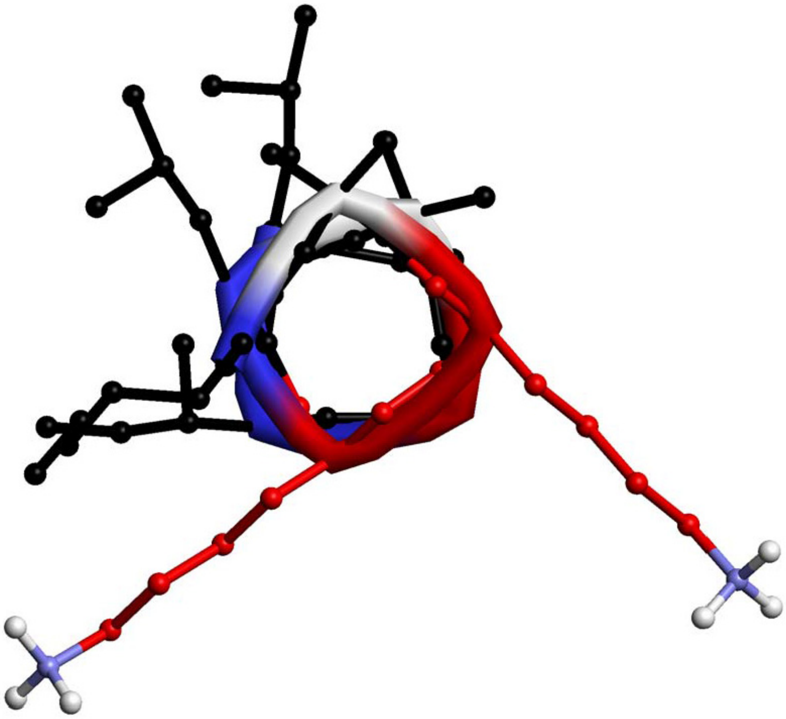


Figure S4. 3D-structure for compound **4c2**. View along the helical axis.

Untreated control **50 µM peptide 4** **15 µM peptide 4**

Fig. S5. Representative symptoms produced by *Botrytis cinerea* on common bean leaves (Borlotto var. Teggia) untreated or treated with the peptide **4** at 50 µM or 15 µM at 7 days post inoculation (dpi). Leaves were arranged on moist towels in plastic trays and inoculated on two or three points with 10 µL of a conidial suspension containing 2.5 x 10^6 mL^{-1} *B. cinerea* conidia in potato dextrose broth (PDB).

Untreated control **50 µM peptide 4**

Fig. S6. Representative symptoms produced by *Botrytis cinerea* on grape berries (cv. Glera) untreated or treated with the peptide **4** at 50 µM at 4 days post inoculation (dpi). Ripe grape berries were surface-sterilized, rinsed with sterile water, separately arranged on a plastic tray and sprayed with *B. cinerea* conidia at a concentration of 1.85 x 10^6 mL^{-1}.

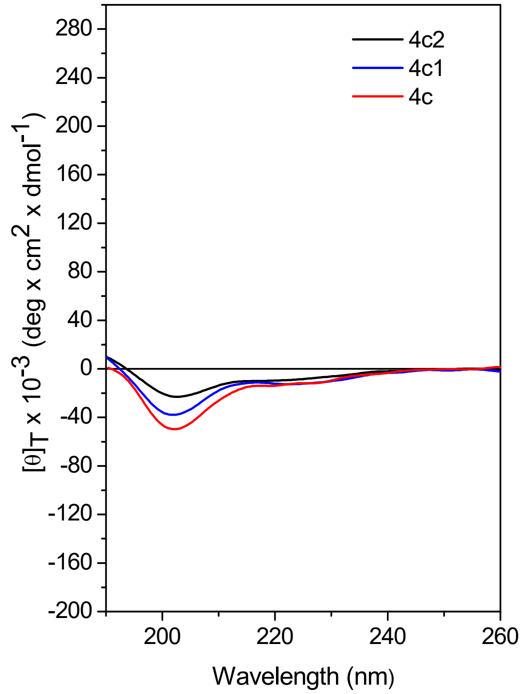


Fig. S7. CD profiles acquired for peptides **4c**, **4c1** and **4c2** in aqueous solution (peptide concentration: 10^{-4} M).

Peptide Characterization (HPLC, ESI-HRMS, ^{1}H NMR)

Table S1. Sequences of the synthesized trichogin analogs.

n°	**peptide sequence**^{a}
1	*n*Oct-Aib-**Lys**^{2}-Leu-Aib-**Lys**^{5}-Gly-Leu-Aib-Gly-Ile-Lol
2	*n*Oct-Aib-**Lys**^{2}-Leu-Aib-Gly^5-Gly-Leu-Aib-Gly-Ile-Lol
3	*n*Oct-Aib-Gly^2-Leu-Aib-**Lys**^{5}-Gly-Leu-Aib-Gly-Ile-Lol
4	*n*Oct-Aib-Gly^2-Leu-Aib-**Lys-Lys**-Leu-Aib-Gly-Ile- Lol
4c	*n*Oct-Aib - **Lys**^{2}-**Lys**-Leu-Aib-Gly-Ile- Lol
4r	*n*Oct-Aib-Gly^2-Leu-Aib-**Lys**^{5}-**Lys**-Leu-Aib-Gly-Ile-**Leu-NH$_2$**
4c1	*n*Oct-Aib-Gly^2-Leu-Aib-**Lys**^{5}-**Lys**-Leu - **Leu-NH$_2$**
4c2	*n*Oct-Aib - **Lys**^{2}-**Lys**-Leu-Aib-Gly-Ile- **Leu-NH$_2$**
5	*n*Oct-Aib-Gly^2-Leu-Aib-Gly^5-**Lys**-Leu-Aib-Gly-Ile- Lol
6	*n*Oct-Aib-Gly^2-Leu-Aib-**Lys**^{5}-**Aib**-Leu-Aib-Gly-Ile- Lol
7	*n*Oct-Aib-**Lys**^{2}-Leu-Aib-Gly^5-**Lys**-Leu-Aib-Gly-Ile- Lol
8	*n*Oct-Aib-Gly^2-Leu-Aib-Gly^5-Gly-Leu-**Api^8**-Gly-Ile-Lol

[a] *n*Oct, *n*-octanoyl; Aib, α-aminoisobutyric acid; Lol, leucinol; Api, 4-aminopiperidine-4-carboxylic acid.

Abbreviations:

ESI, Electron Spray Ionization

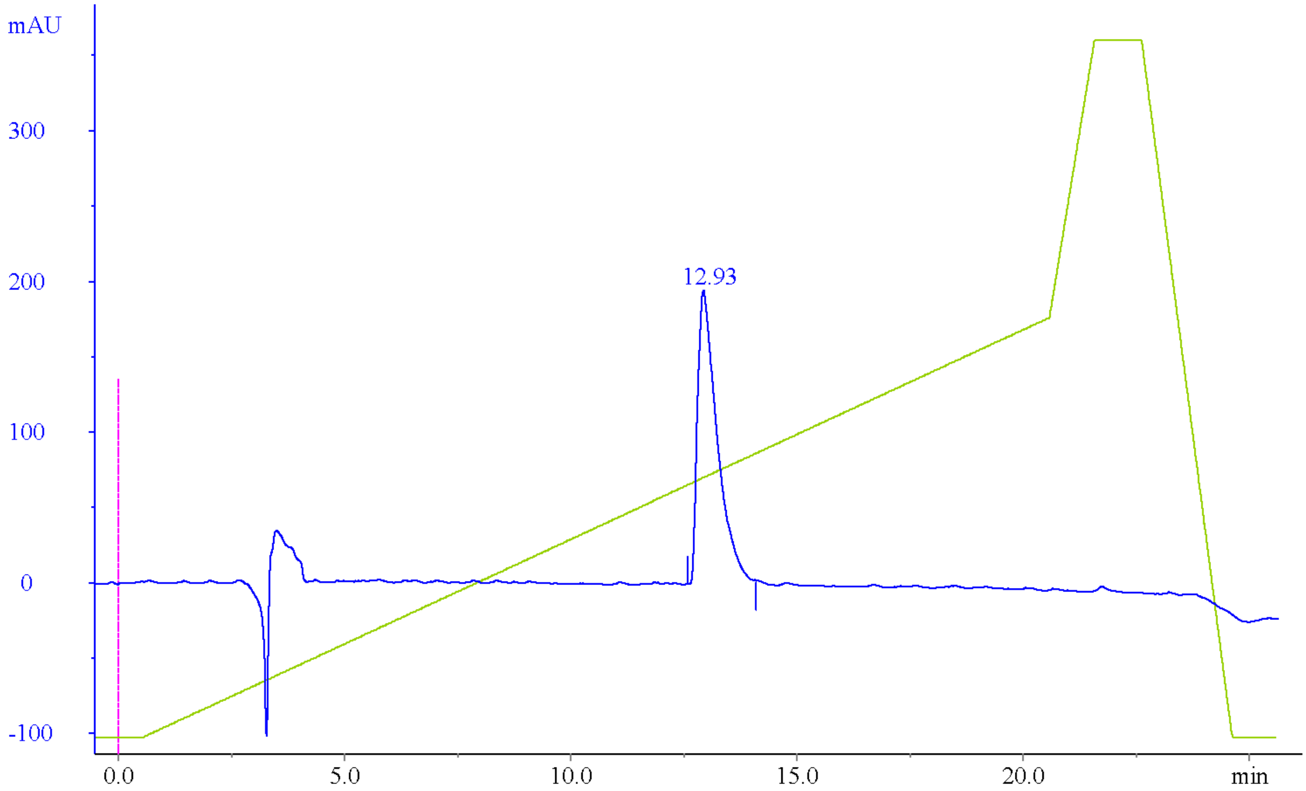
HRMS, High Resolution Mass Spectrometry

Eluants for HPLC:

A: H_2O/CH_3CN 9:1 + 0.05% TFA

B: CH_3CN/H_2O 9:1 + 0.05% TFA

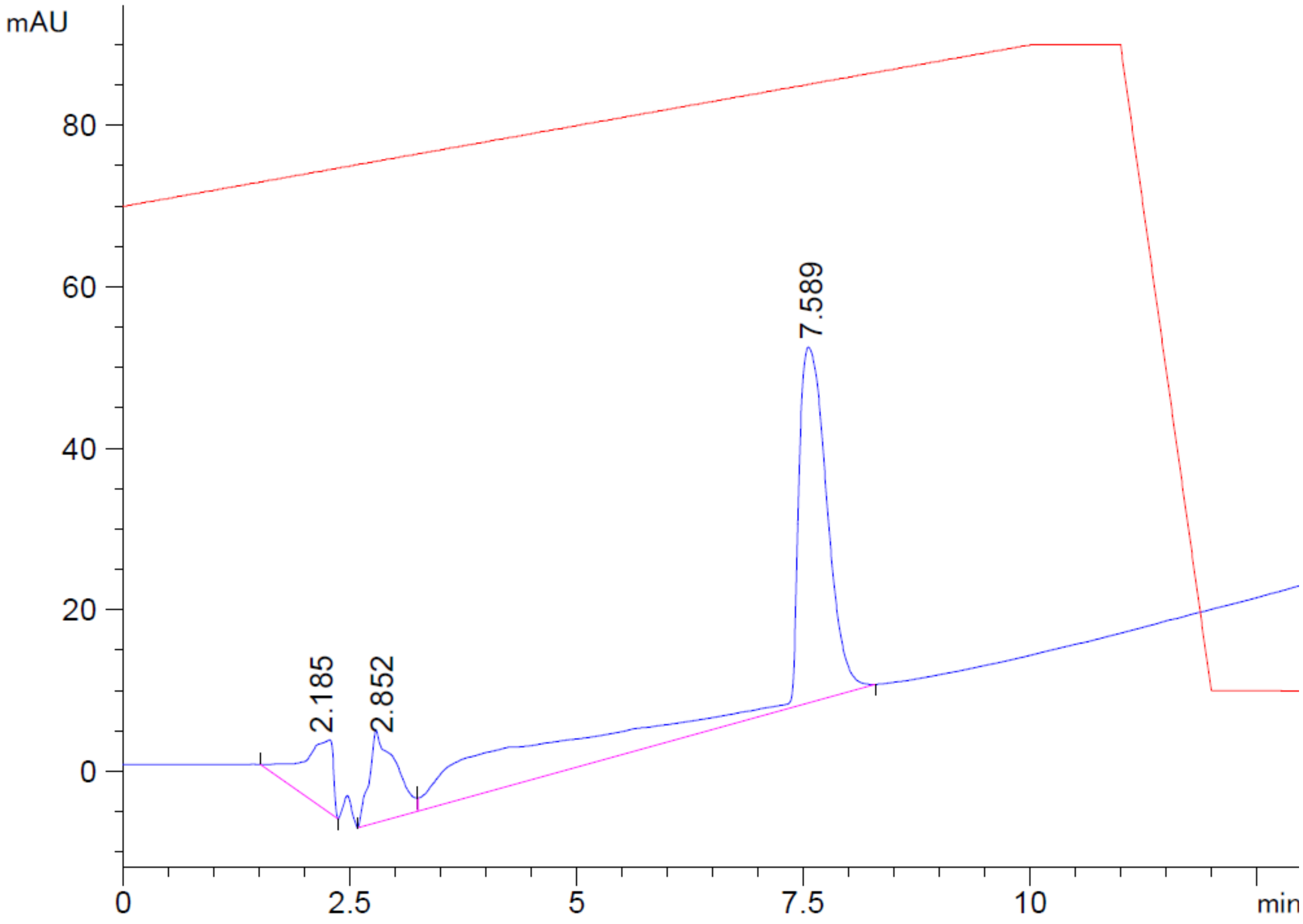
peptide **1** (**K25**)



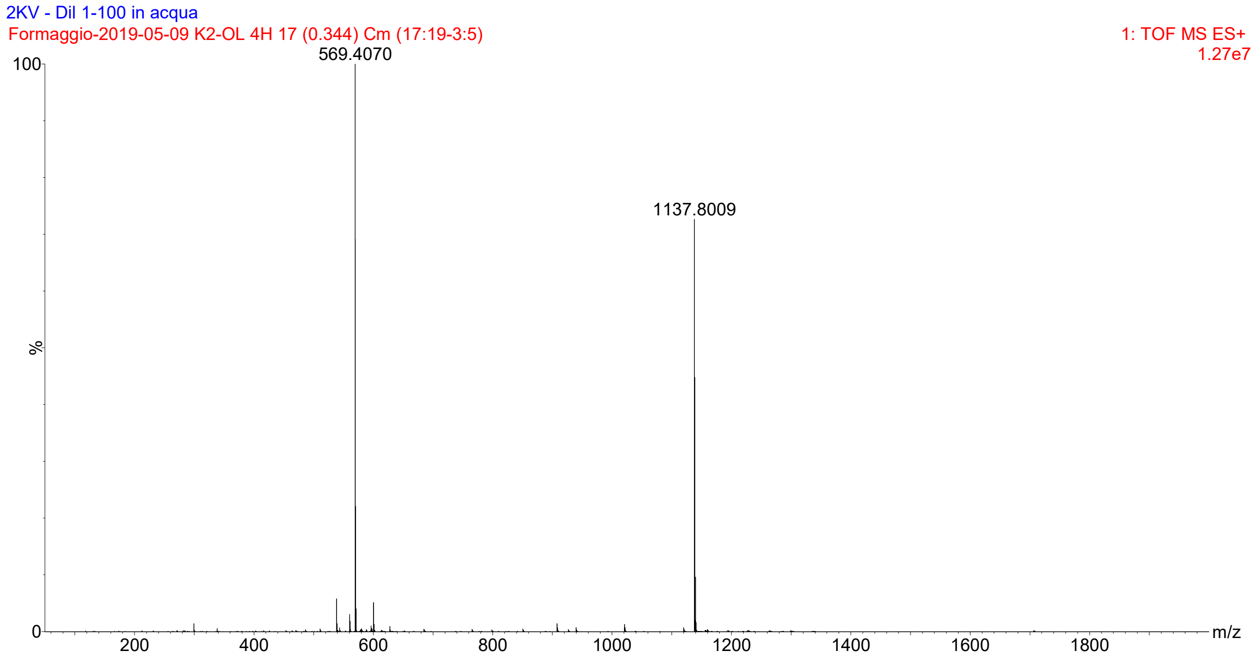
HPLC profile obtained for peptide **1** (gradient: 50→80%B [CH_3CN/H_2O 9:1 + 0.05% TFA] in 20 minutes; flow rate: 1mL/min; C_{18} Phenomenex 300Å) Retention time (R_t) = 12.93 min. Detection Wavelength: 216 nm.

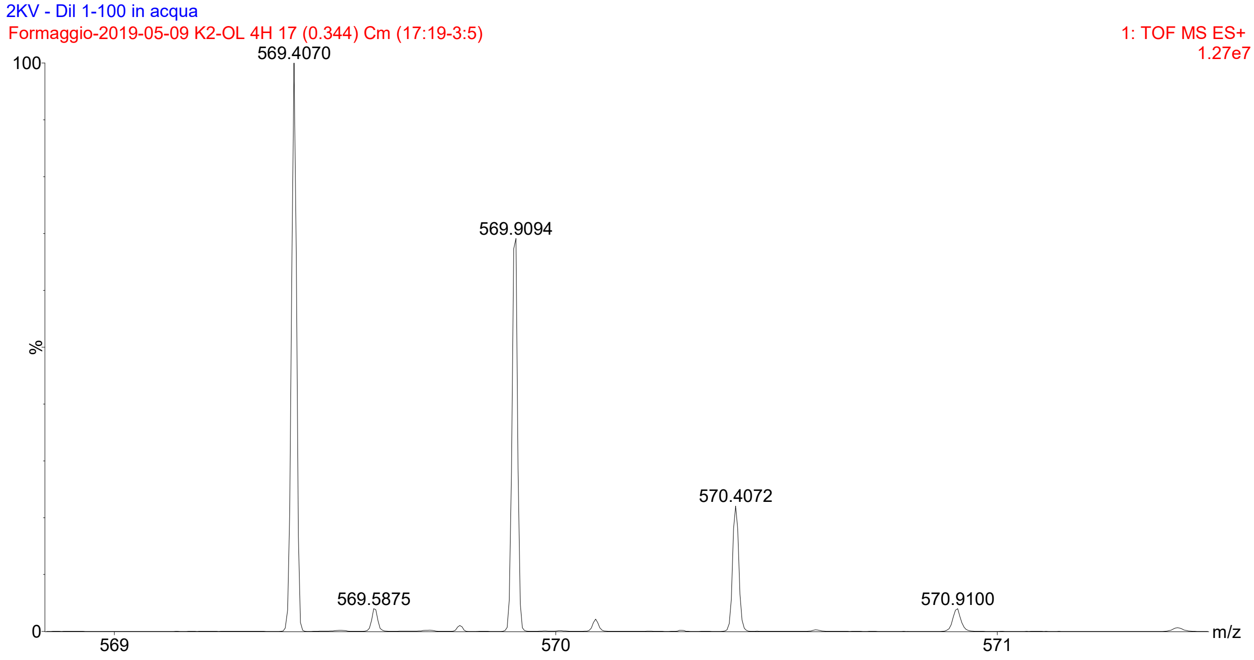
ESI-HRMS for peptide **1**: $[M+H]^+_{calcd.}$ 1208.8632; $[M+H]^+_{found}$: 1208.8896 (*monoisotopic*), $[M+2H]^{2+}_{found}$: 604.9448.

peptide **2** (**K2**)



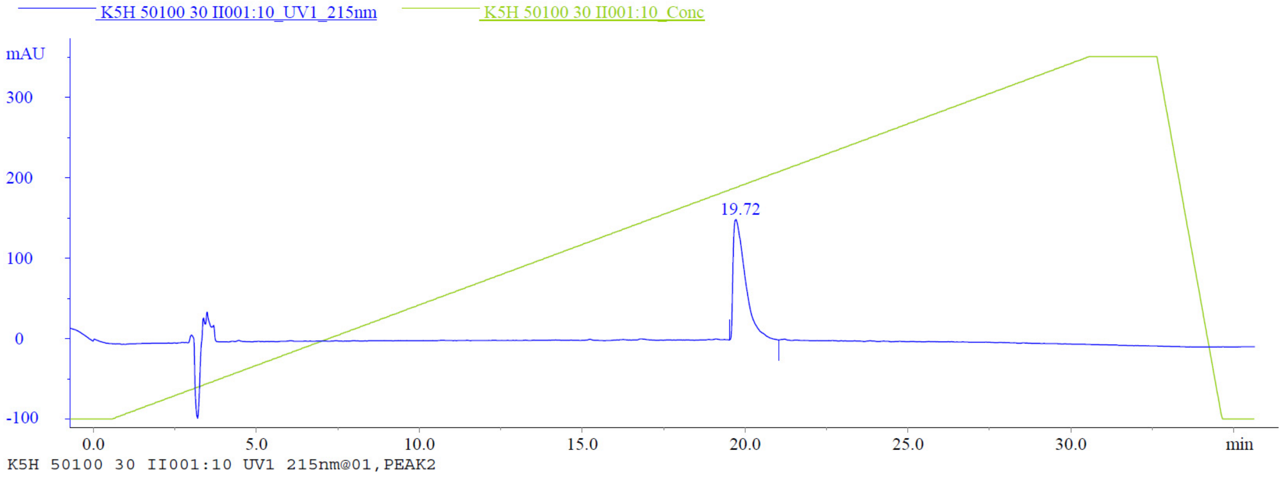
HPLC profile obtained for peptide **2** (gradient: 70→90%B [B: CH_3CN/H_2O 9:1 + 0.05% TFA] in 10 minutes; flow rate: 1mL/min; C_{18} Phenomenex 100Å) Retention time (R_t) = 7.59 min. Detection Wavelength: 226 nm.



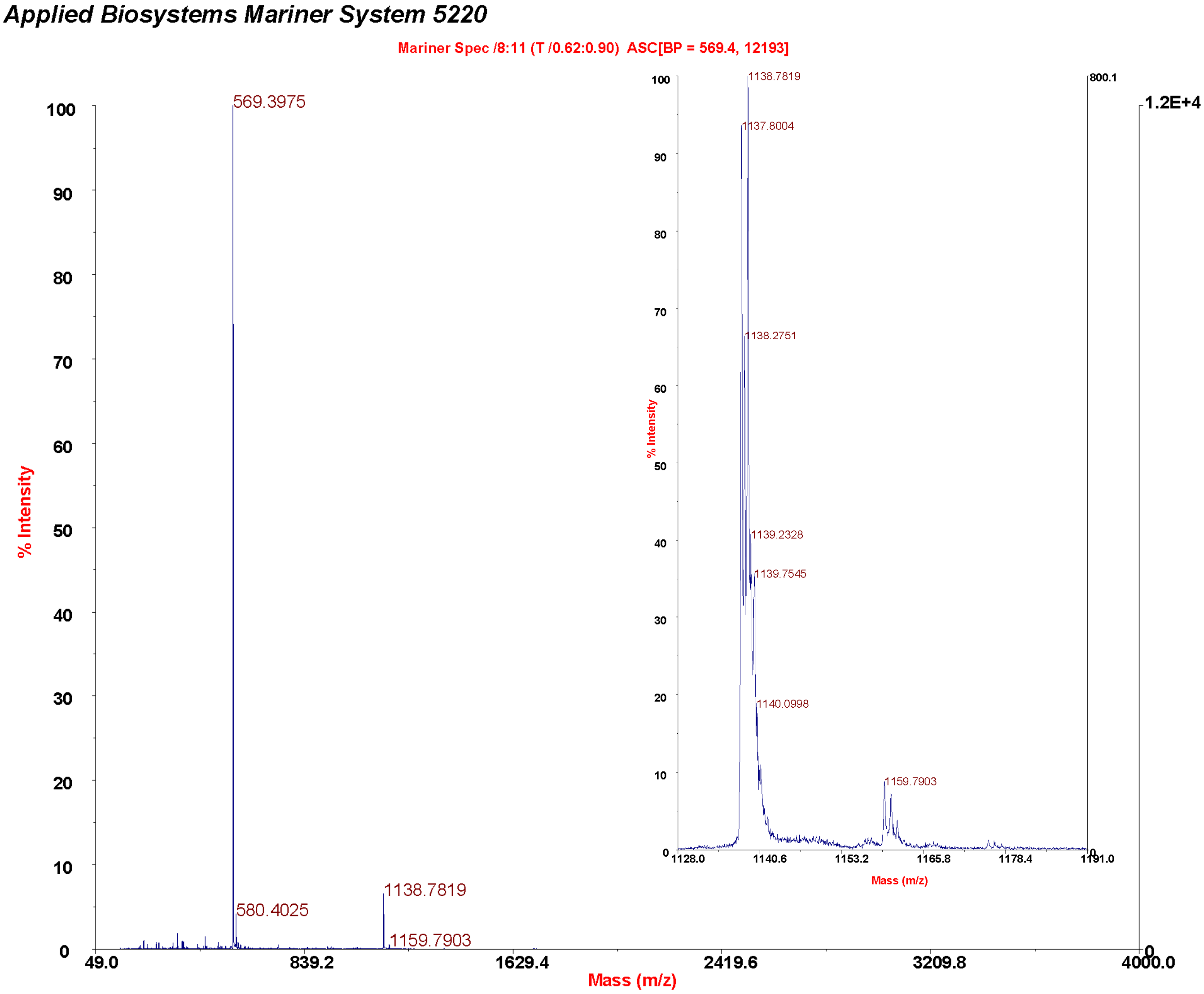


ESI-HRMS for peptide **2**: $[M+H]^{+}_{calcd.}$ 1137.7997; $[M+H]^{+}_{found}$: 1137.8009 (*monoisotopic*), $[M+2H]^{2+}_{found}$: 569.4070.

peptide **3** (**K5**)

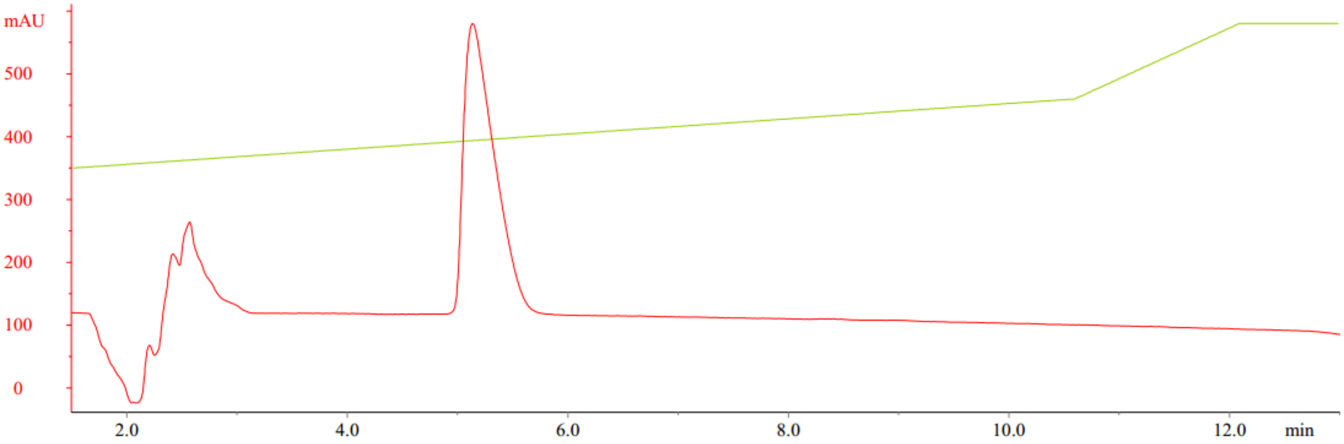


HPLC profile obtained for peptide **3** (gradient: 50→100%B [B: CH_3CN/H_2O 9:1 + 0.05% TFA] in 30 minutes; flow rate: 1mL/min; C_{18} Phenomenex 300Å) Retention time (R_t) = 19.72 min. Detection Wavelength: 215 nm.

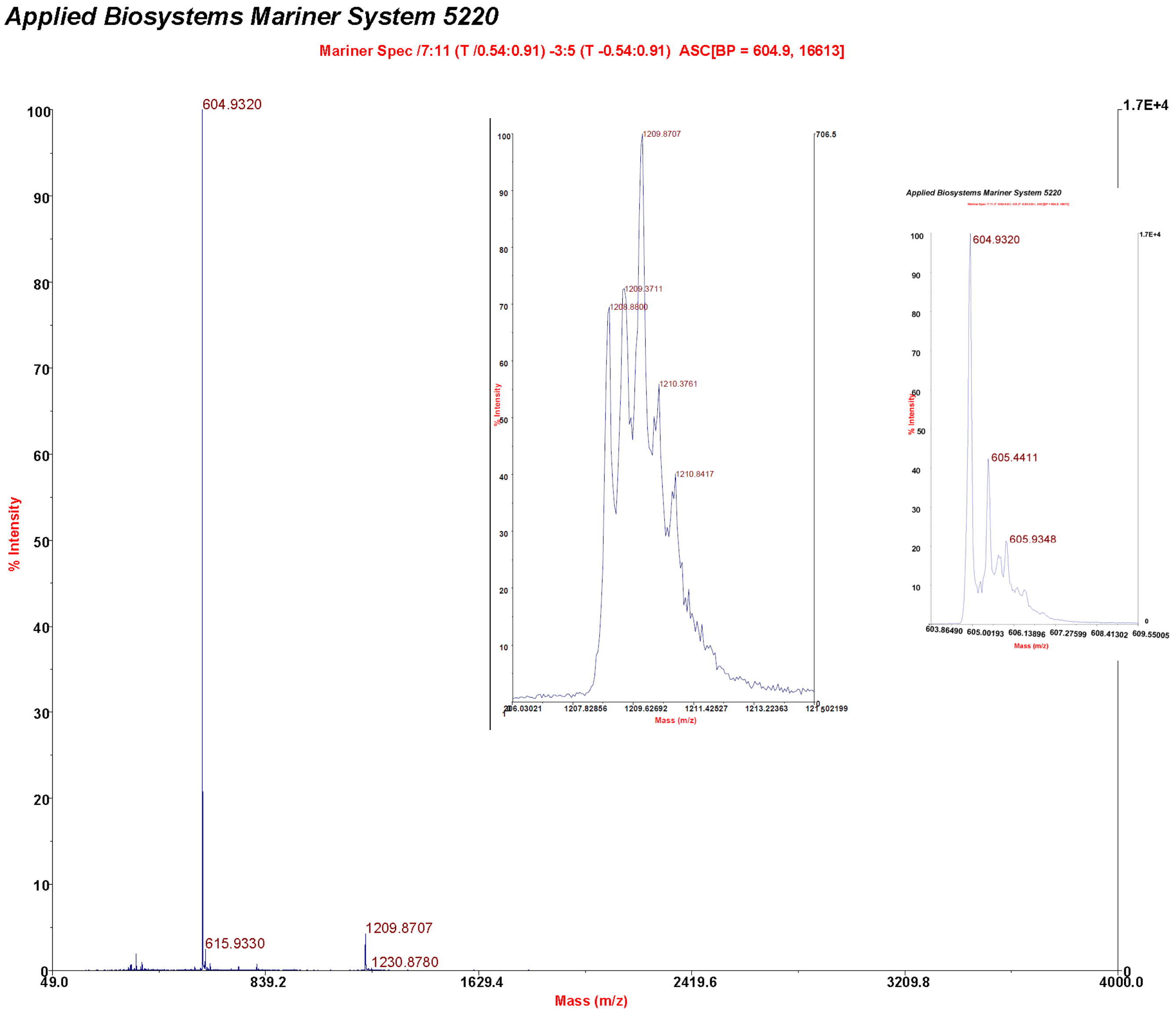


ESI-HRMS for peptide **3**: $[M+H]^+_{calcd.}$ 1137.7997; $[M+H]^+_{found}$: 1137.8004 (*monoisotopic*), $[M+Na]^+_{found}$: 1159.7903 (*monoisotopic*), $[M+2H]^{2+}_{found}$: 569.3975.

peptide **4** (**K56**)

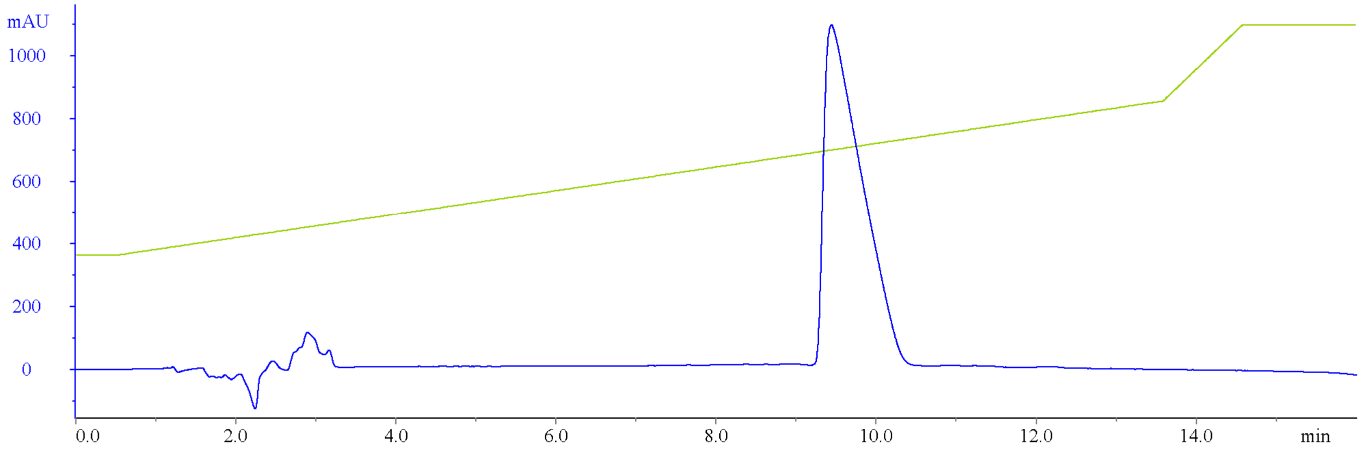


HPLC profile obtained for peptide **4** (gradient: 60→80%B [B: CH_3CN/H_2O 9:1 + 0.05% TFA] in 10 minutes; flow rate: 1mL/min; C_{18} Agilent Zorbax RX-C18) Retention time (R_t) = 5.14 min. Detection Wavelength: 226 nm.

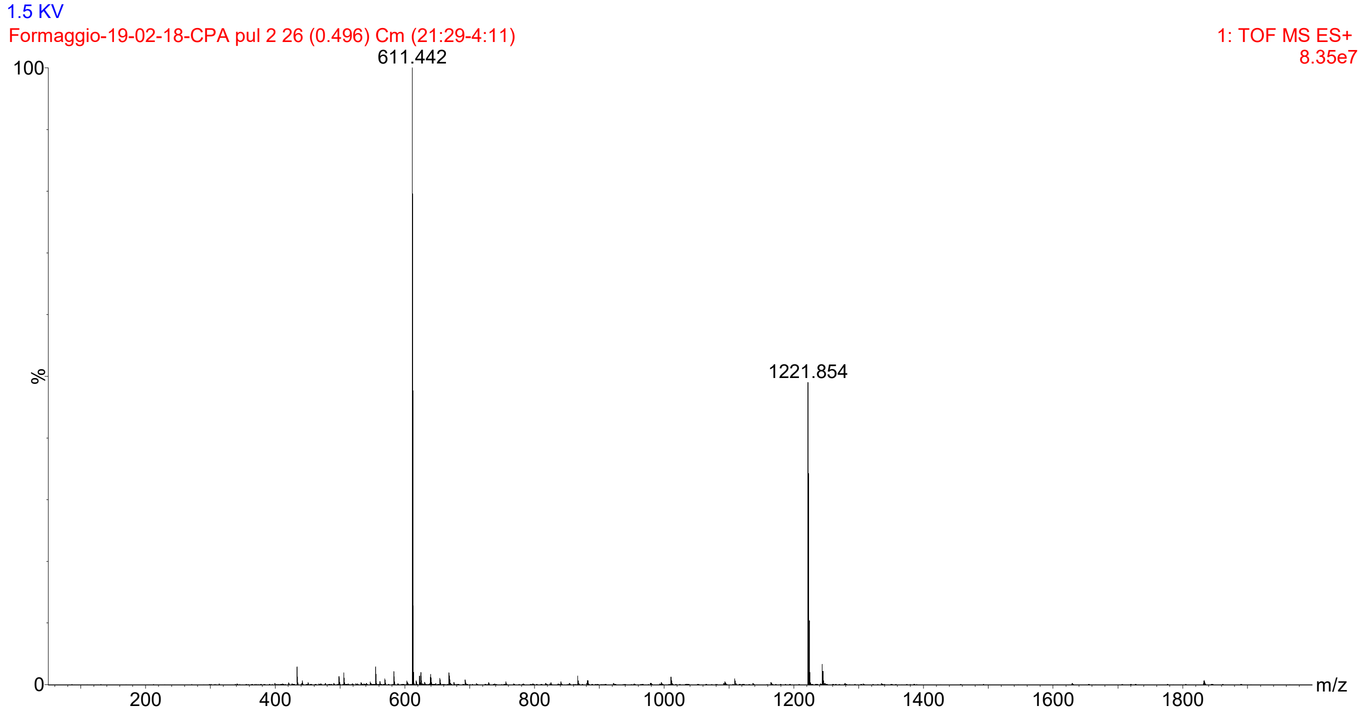


ESI-HRMS for peptide **4**: $[M+H]^+_{calcd.}$ 1208.8732; $[M+H]^+_{found}$: 1208.8800 (*monoisotopic*), $[M+Na]^+_{found}$: 1230.8780 (*monoisotopic*), $[M+2H]^{2+}_{found}$: 604.9320.

peptide **4r** (**K56-NH2**)

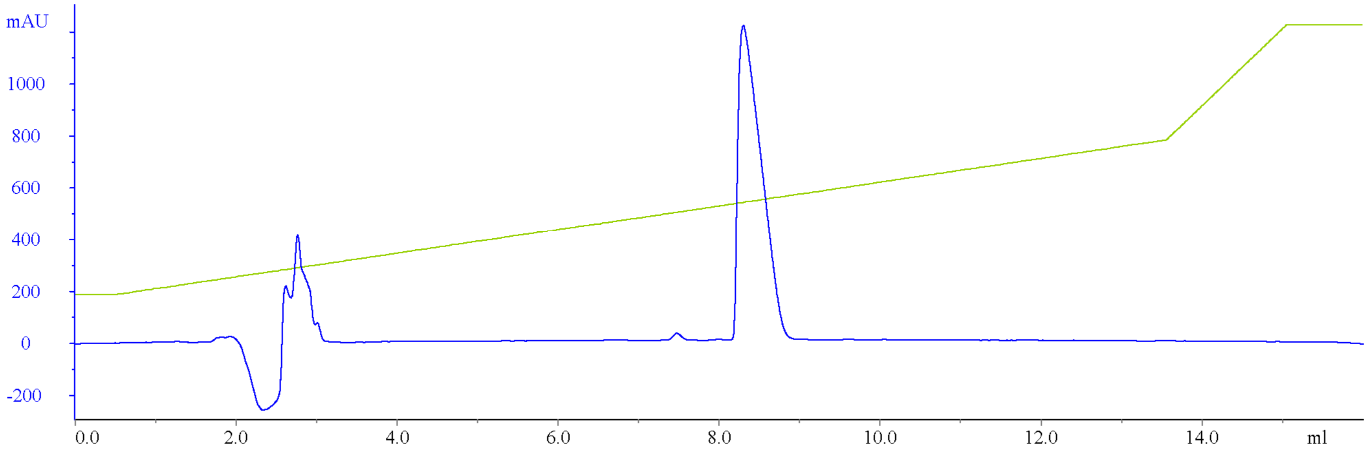


HPLC profile obtained for peptide **4r** (gradient: 40→80%B [CH_3CN/H_2O 9:1 + 0.05% TFA] in 15 minutes; flow rate: 1mL/min; C_{18} Phenomenex 300Å) Retention time (R_t) = 9.44 min. Detection Wavelength: 208 nm.

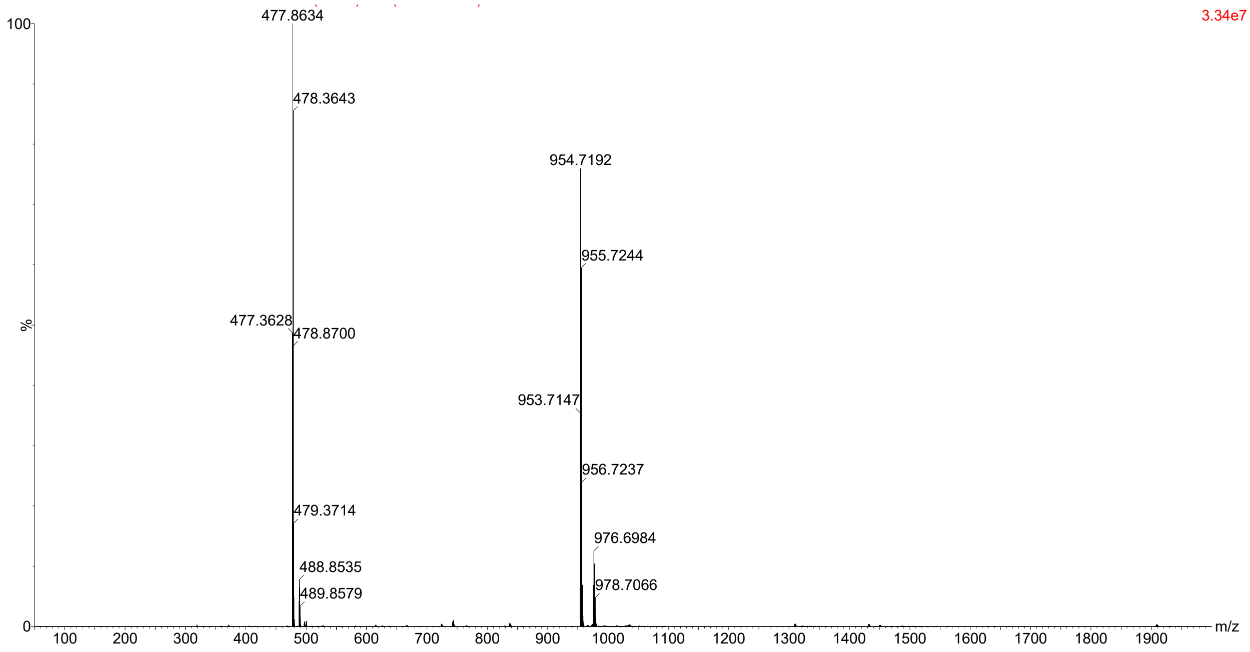


ESI-HRMS for peptide **4r**: $[M+H]^+_{calcd.}$ 1221.8584; $[M+H]^+_{found}$: 1221.8540 (*monoisotopic*), $[M+2H]^{2+}_{found}$: 611.4420.

peptide **4c**

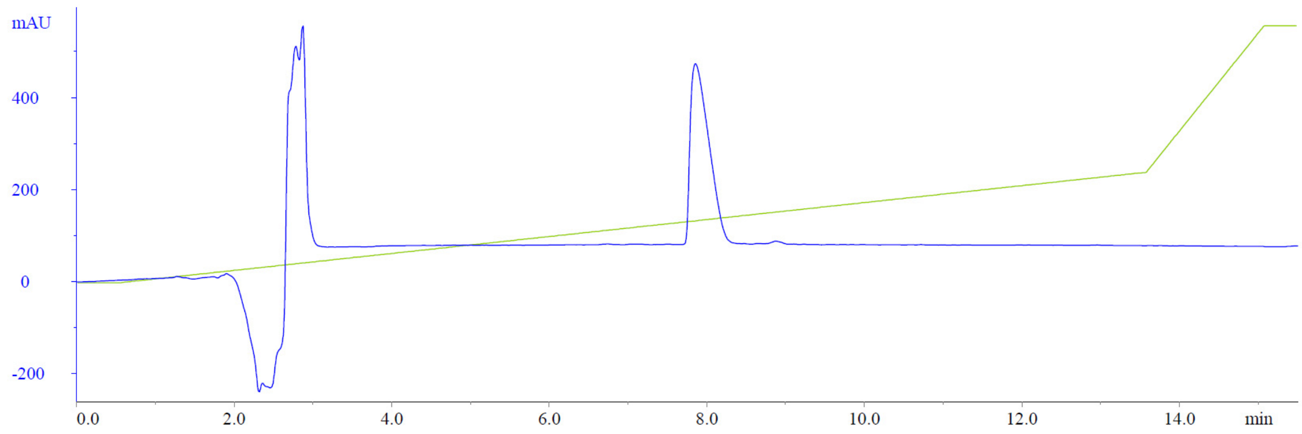


HPLC profile obtained for peptide **4c** (gradient: 30→70%B [CH_3CN/H_2O 9:1 + 0.05% TFA] in 15 minutes; flow rate: 1mL/min; C_{18} Phenomenex 300Å) Retention time (R_t) = 8.31 min. Detection Wavelength: 208 nm.

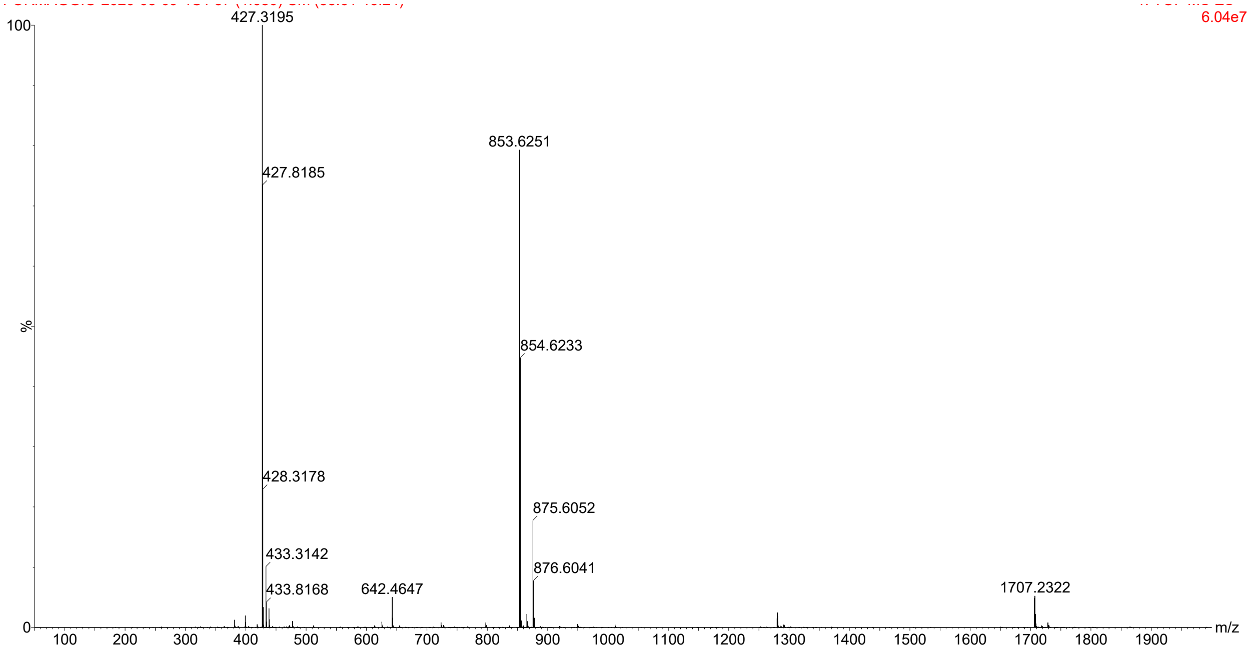


ESI-MS for peptide **4c**: $[M+H]^+_{calcd.}$ 953.7049; $[M+H]^+_{found}$: 953.7147 (*monoisotopic*) $[M+Na]^+_{found}$: 975.6951, $[M+2H^{2+}]_{found}$: 477.3628.

peptide **4c1** (**Oct-UGLUKKL-NH$_2$**)

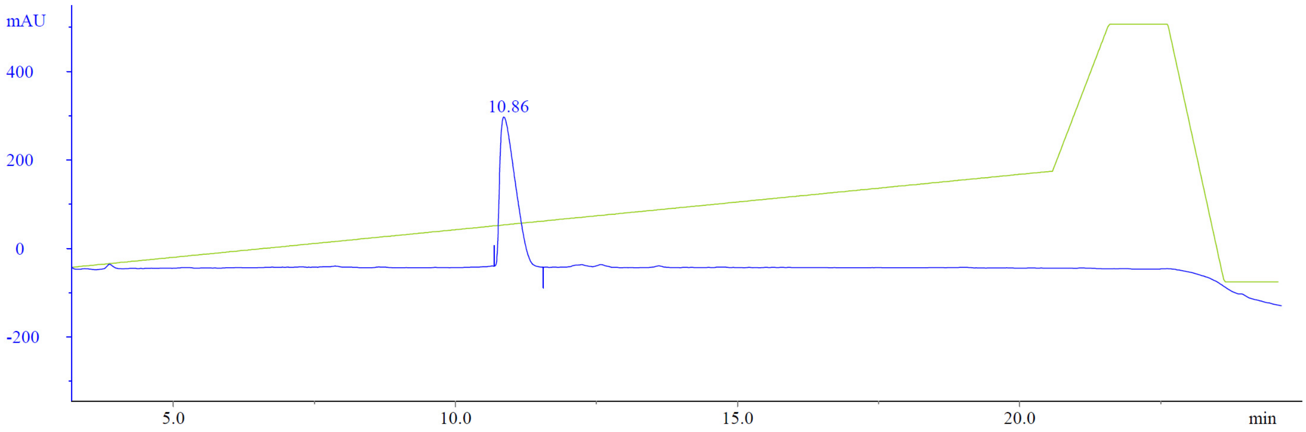


HPLC profile obtained for peptide **4c1** (gradient: 30→60%B [CH_3CN/H_2O 9:1 + 0.05% TFA] in 14 minutes; flow rate: 1 mL/min; C_{18} Phenomenex 300Å) Retention time (R_t) = 7.85 min. Detection Wavelength: 208 nm.

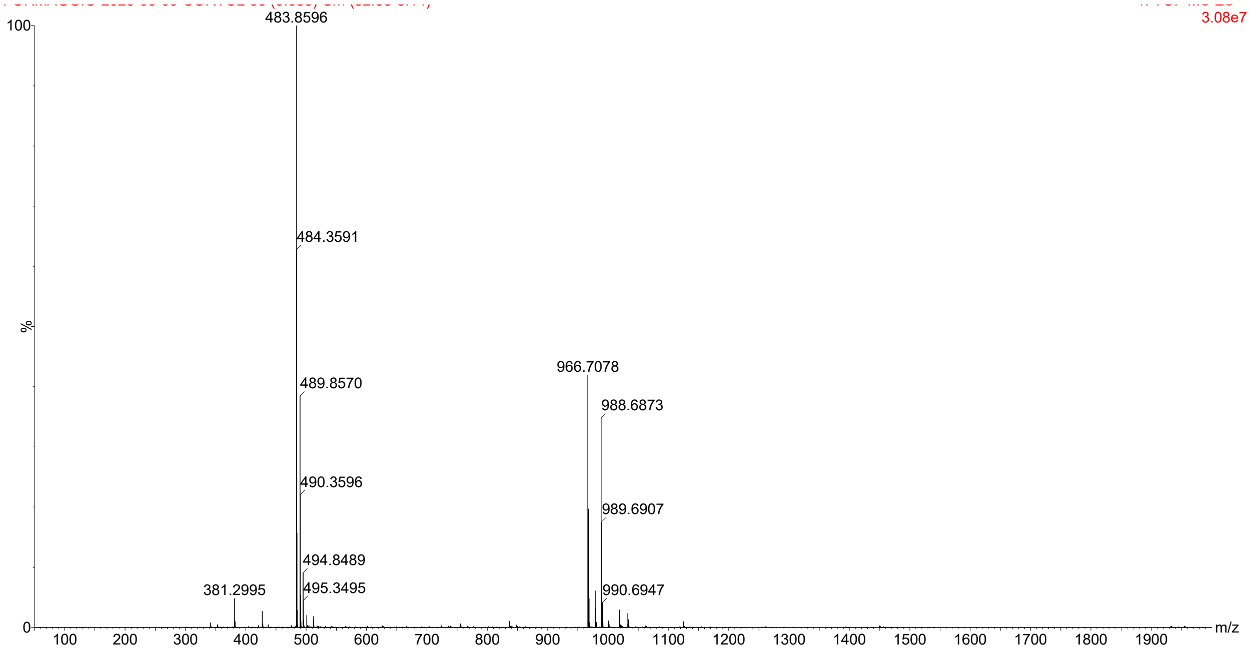


ESI-MS for peptide **4c1**: $[M+H]^+_{calcd.}$: 853.6261; $[M+H]^+_{found}$: 853.6251, $[M+2H^{2+}]_{found}$: 427.3195. $[2M+H]^+_{found}$: 1707.2322.

peptide **4c2** (**Oct-AibLLAibGIL-NH$_2$**)

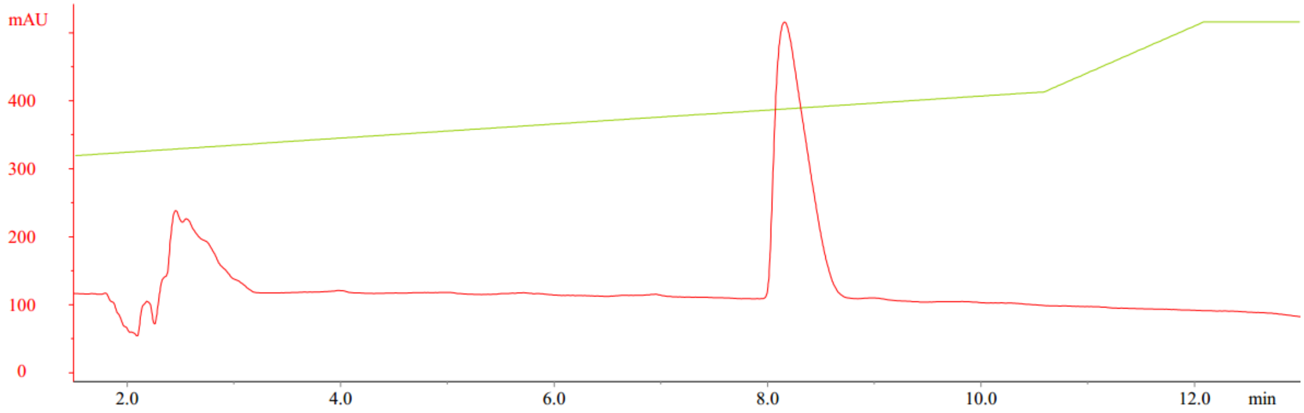


HPLC profile obtained for peptide **4c2** (gradient: 30→60%B [CH_3CN/H_2O 9:1 + 0.05% TFA] in 20 minutes; flow rate: 1mL/min; C_{18} Phenomenex 300Å) Retention time (R_t) = 10.86 min. Detection Wavelength: 208 nm.

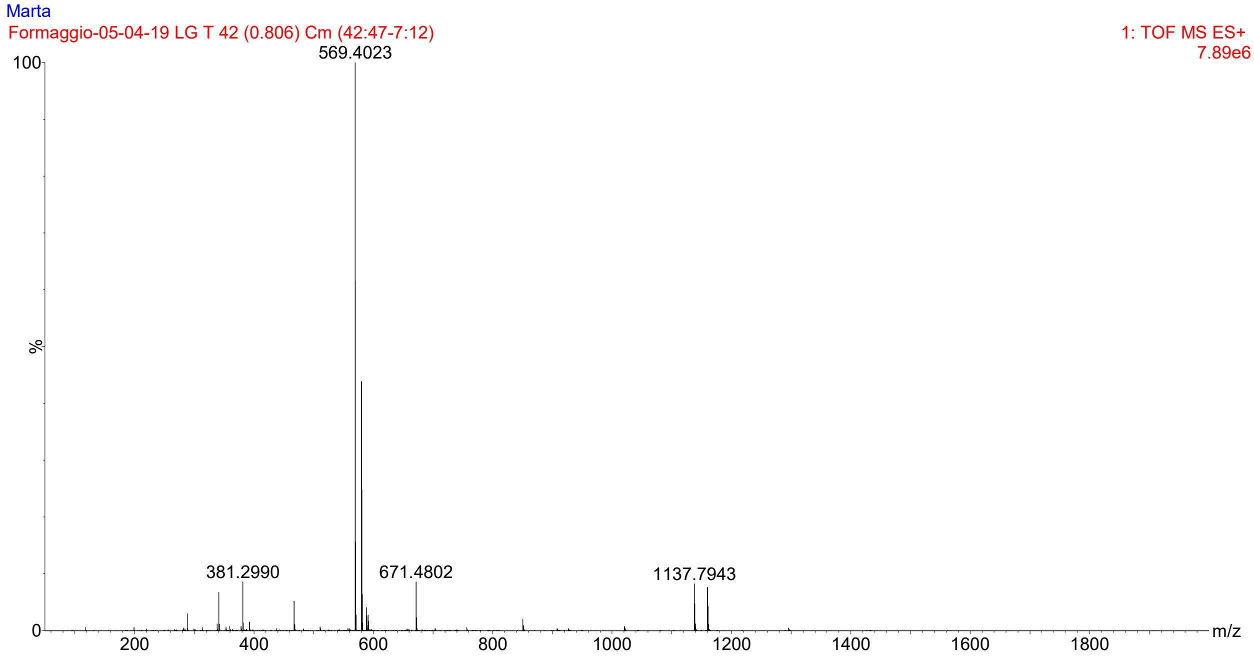


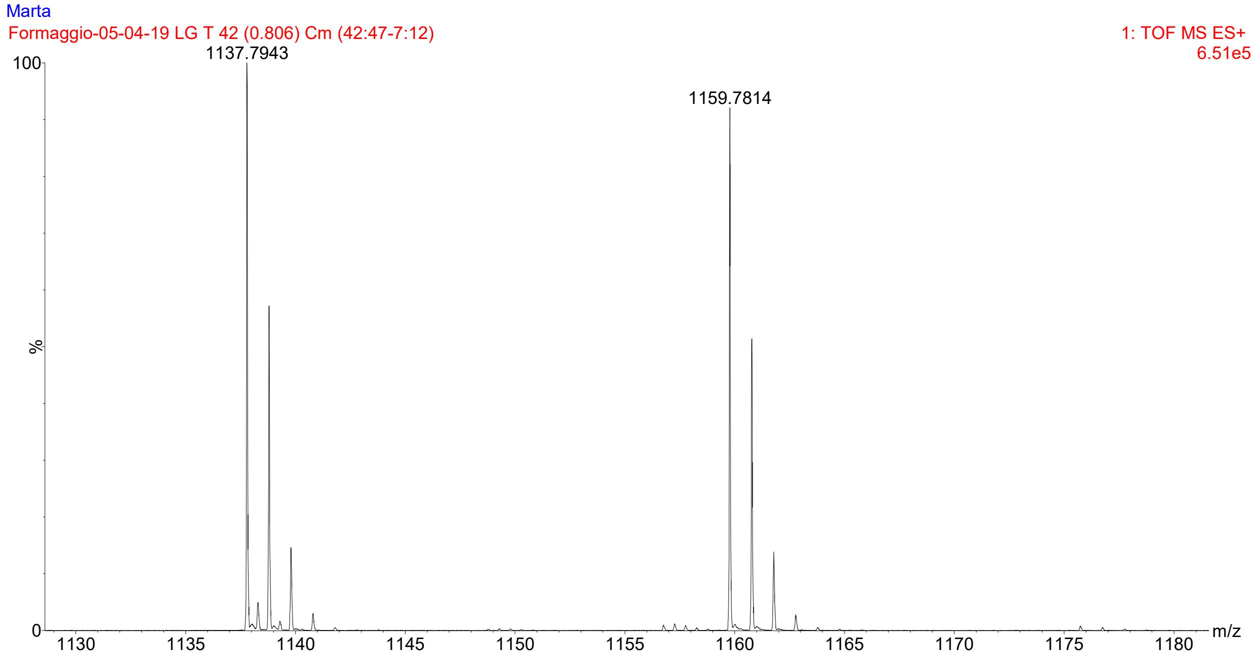
ESI-MS for peptide **4c2**: $[M+H]^+_{calcd.}$ 966.7001; $[M+H]^+_{found}$: 966.7078, $[M+Na]^+_{found}$: 988.6873, $[M+2H^{2+}]_{found}$: 483.8596.

peptide **5** (**K6**)



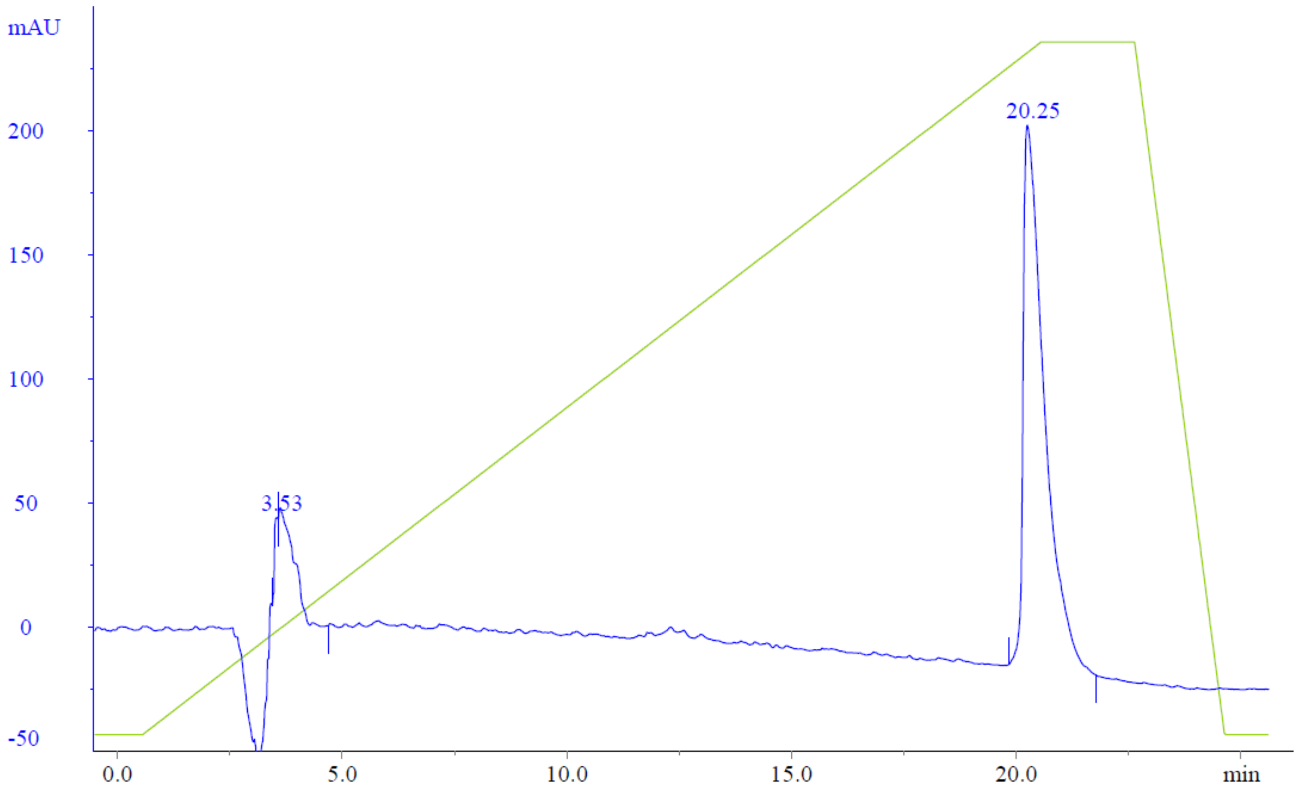
HPLC profile obtained for peptide **5** (gradient: 60→80%B [CH_3CN/H_2O 9:1 + 0.05% TFA] in 10 minutes; flow rate: 1mL/min; C_{18} Agilent Zorbax RX-C18) R_t = 8.16 min. Detection Wavelength: 216 nm.



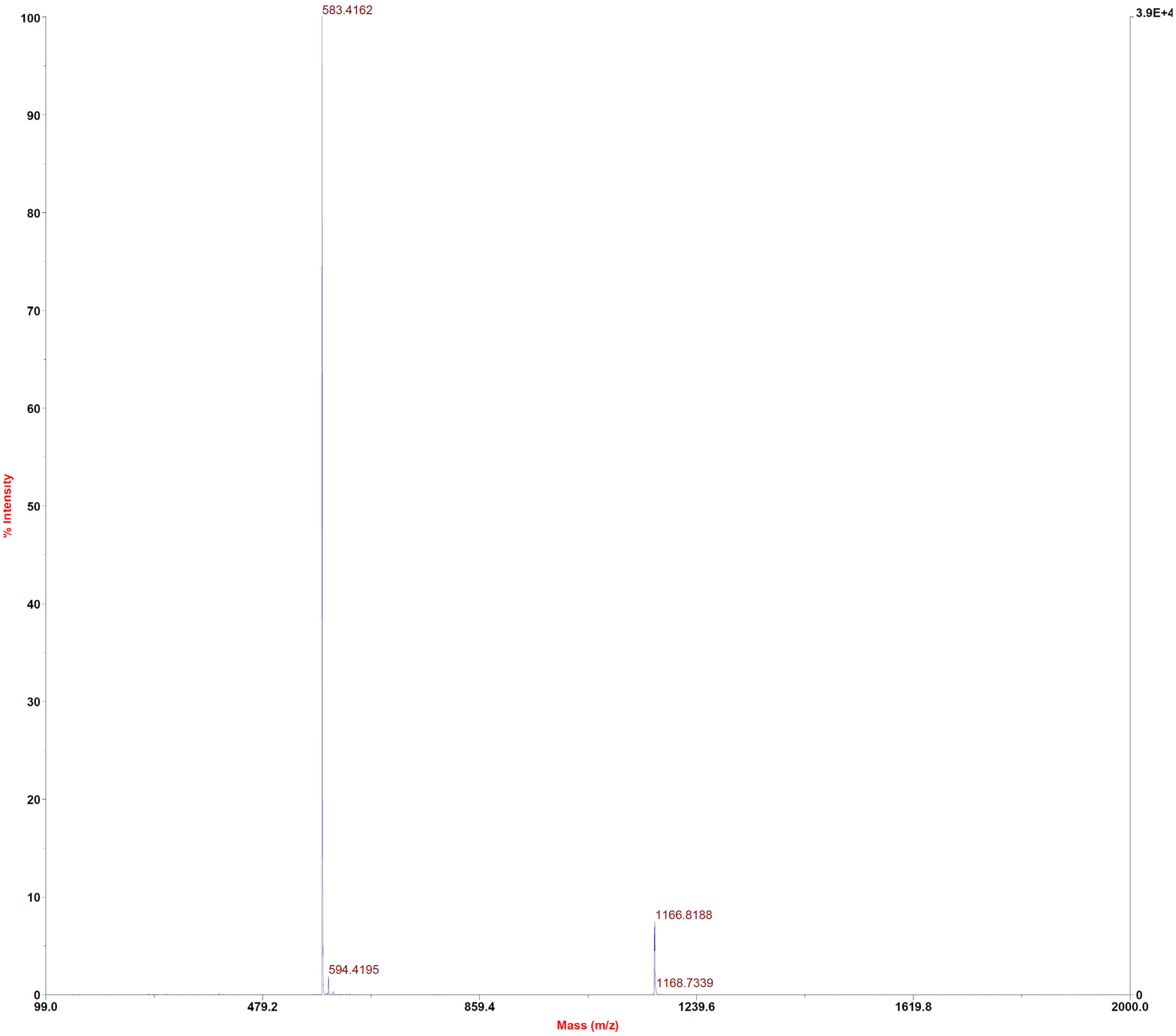


ESI-HRMS for peptide **5**: [M+H]$^{+}_{\text{calcd.}}$ 1137.7997; [M+H]$^{+}_{\text{found}}$: 1137.7943 (*monoisotopic*), [M+Na]$^{+}_{\text{found}}$: 1159.7814 (*monoisotopic*), [M+2H]$^{2+}_{\text{found}}$:569.4023. *Peaks at 381.2990 and 671.4802 are impurities from samples injected before (the instrument was contaminated).*

peptide **6** (**K5U6**)

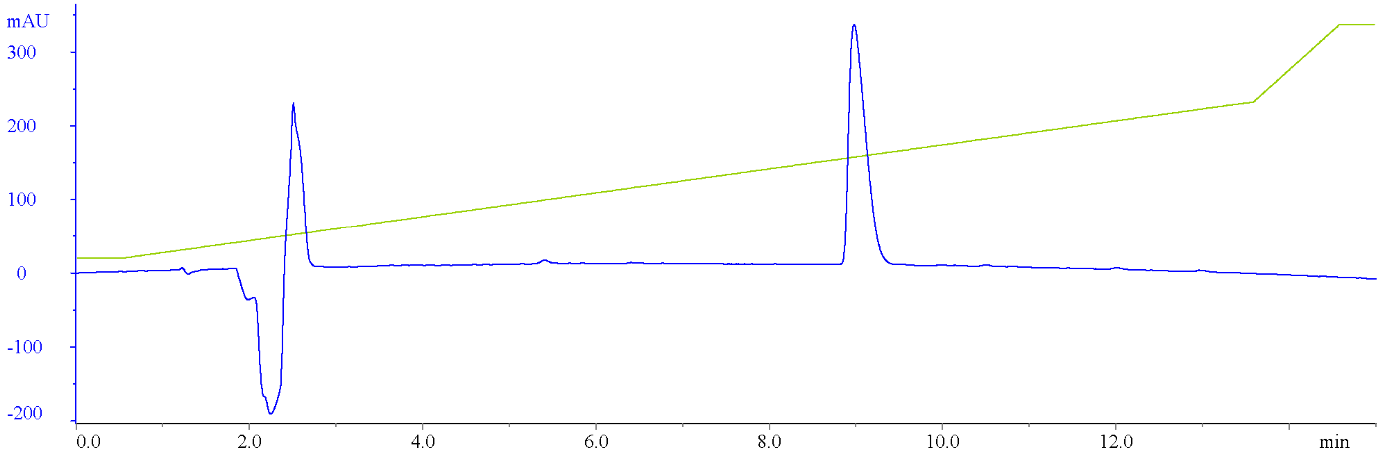


HPLC profile obtained for peptide **6** (gradient: 60→100%B [CH_3CN/H_2O 9:1 + 0.05% TFA] in 20 minutes; flow rate: 1mL/min; C_{18} Phenomenex 300Å) R_t = 20.25 min. Detection Wavelength: 216 nm.

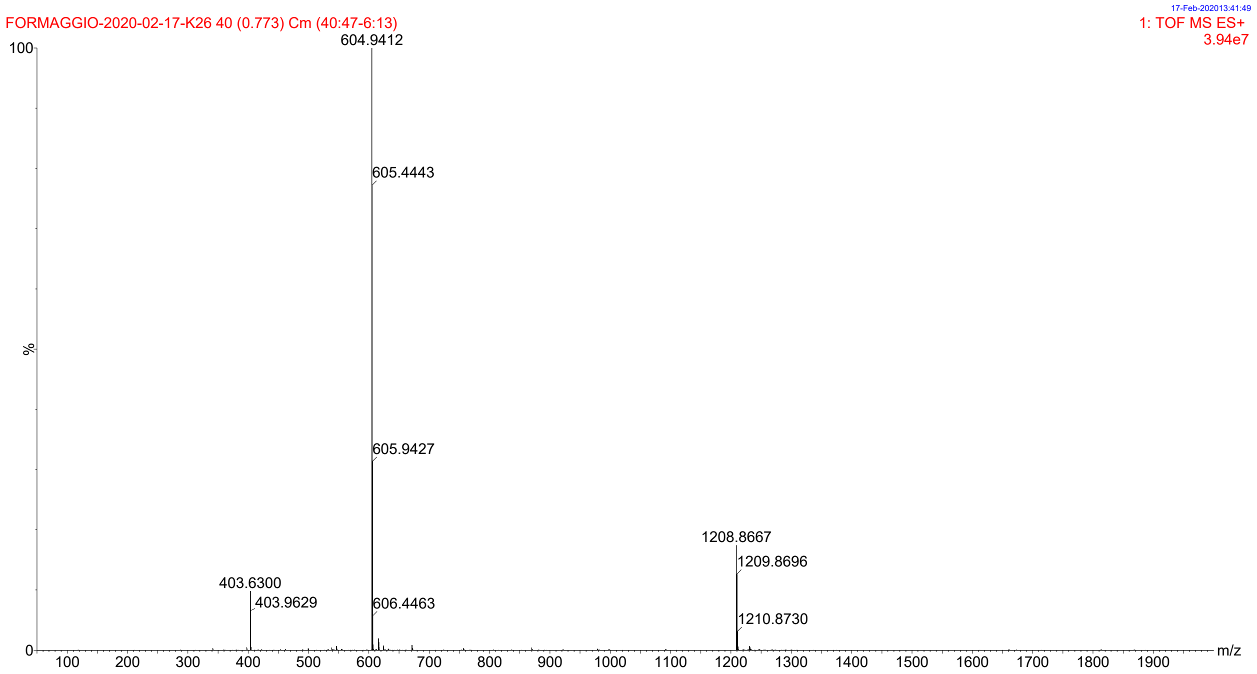


ESI-MS for peptide **6**: $[M+H]_{\text{calcd.}}$ 1165.8210; $[M+H]_{\text{found}}$: 1165.8271 (*monoisotopic*), $[M+2H^{2+}]_{\text{found}}$:583.4162, $[M+H+Na^{2+}]_{\text{found}}$: 594.4195.

peptide **7** (**K26**)

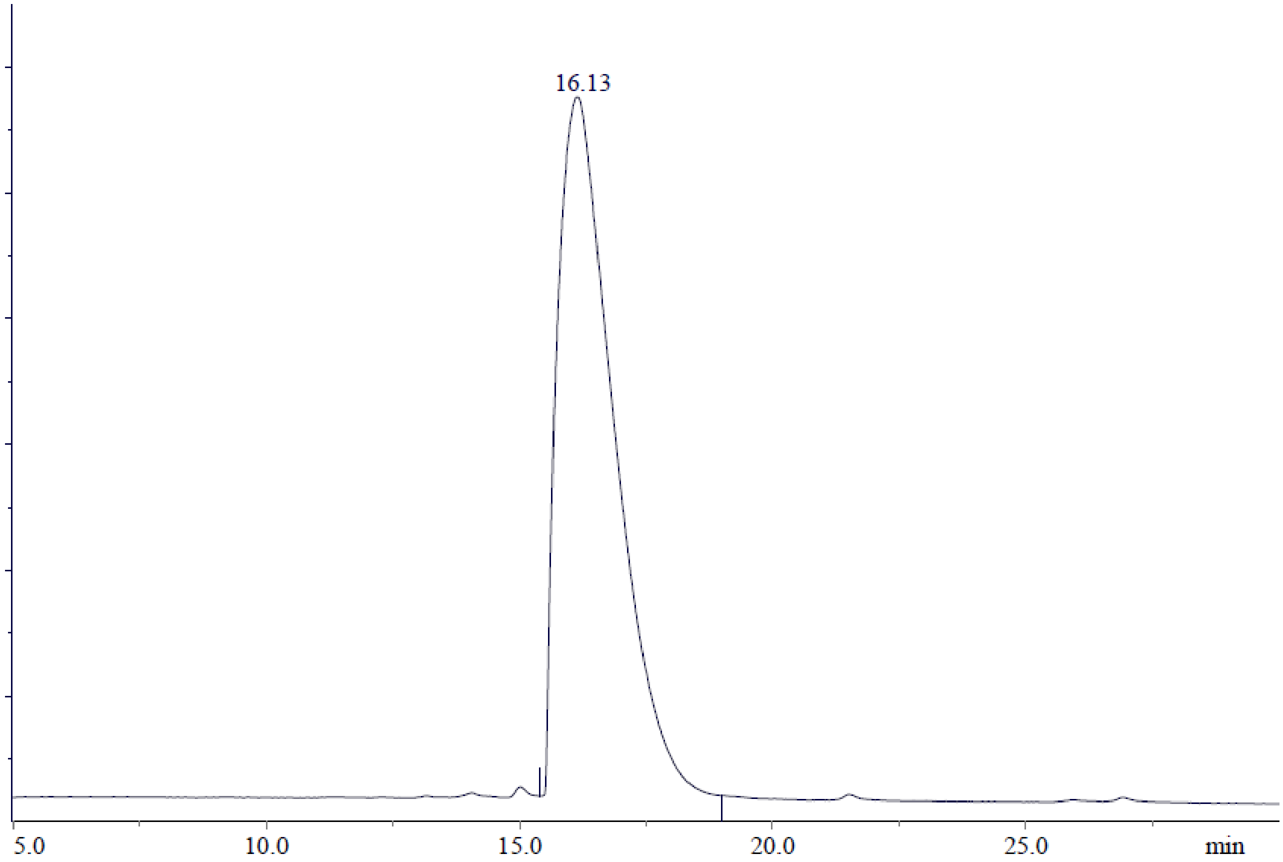


HPLC profile obtained for peptide **7** (gradient: 40→80%B [CH_3CN/H_2O 9:1 + 0.05% TFA] in 15 minutes; flow rate: 1mL/min; C_{18} Phenomenex 300Å) R_t = 8.97 min. Detection Wavelength: 208 nm.



ESI-MS for peptide **7**: $[M+H]_{calcd.}$ 1208.8632; $[M+H]_{found}$: 1208.8667, $[M+2H^{2+}]_{found}$:604.9412; $[M+3H^{3+}]_{found}$: 403.6300.

peptide **8** (**Api8**)

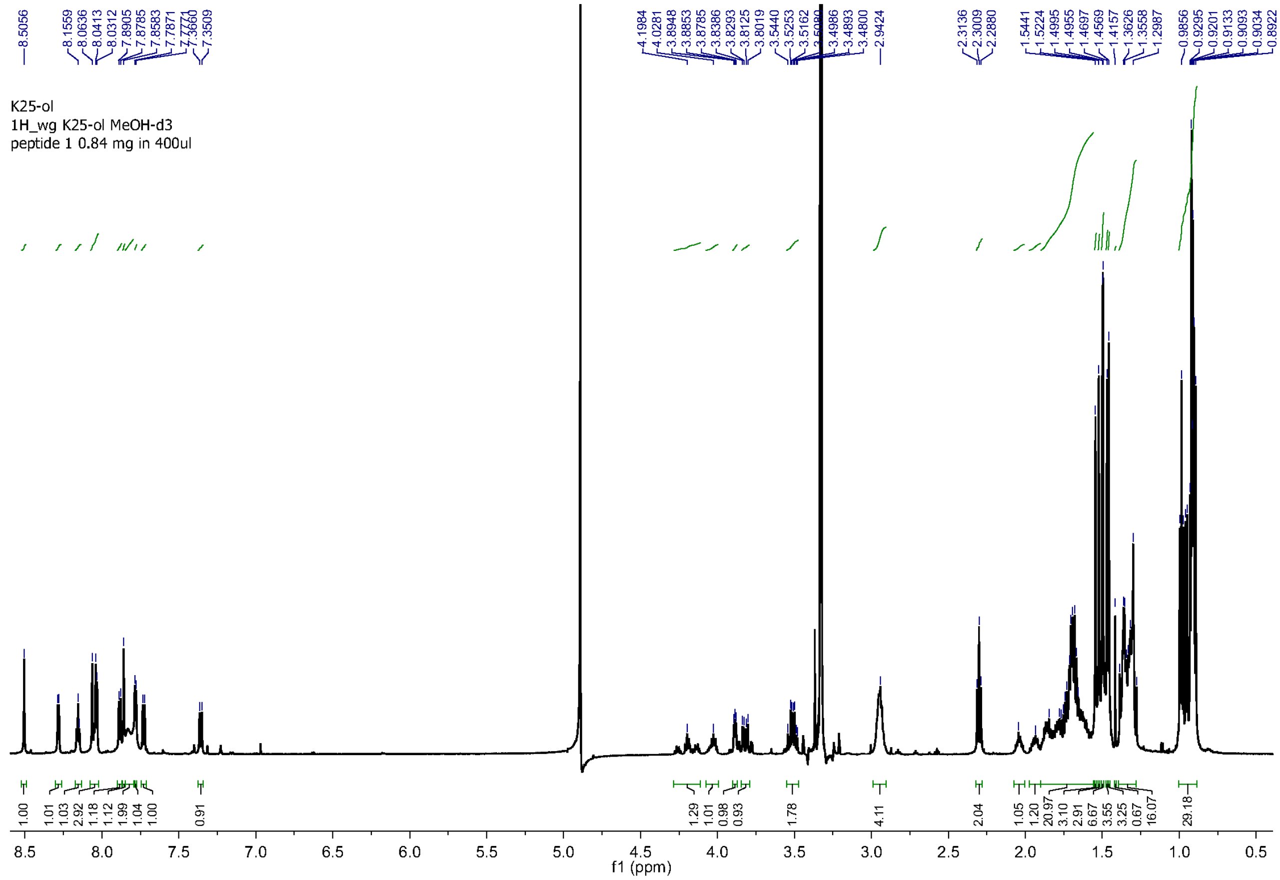


HPLC profile obtained for peptide **8** ([Api^8]-Tric) (gradient: 45→75% B [CH_3CN/H_2O 9:1 + 0.05% TFA] in 30 minutes; flow rate: 1mL/min; C_{18} Phenomenex 300Å); R_t = 16.13 min.

ESI-MS for peptide **8**: $[M+H]_{calcd.}$ 1107.7427; $[M+H]_{found}$: 1107.7760, $[M+2H^{2+}]_{found}$: 554.3865.

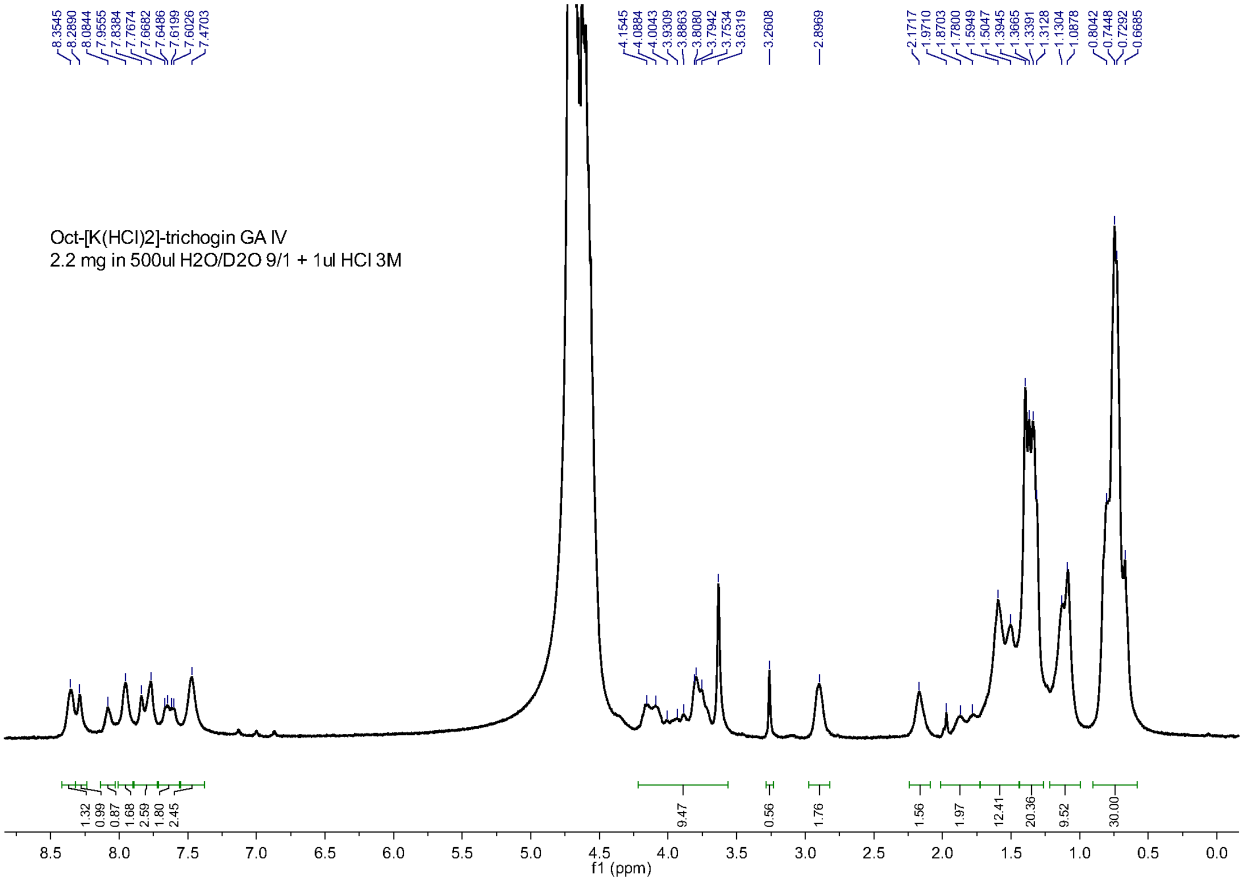
^{1}H NMR characterizations

peptide **1** (**K25**)



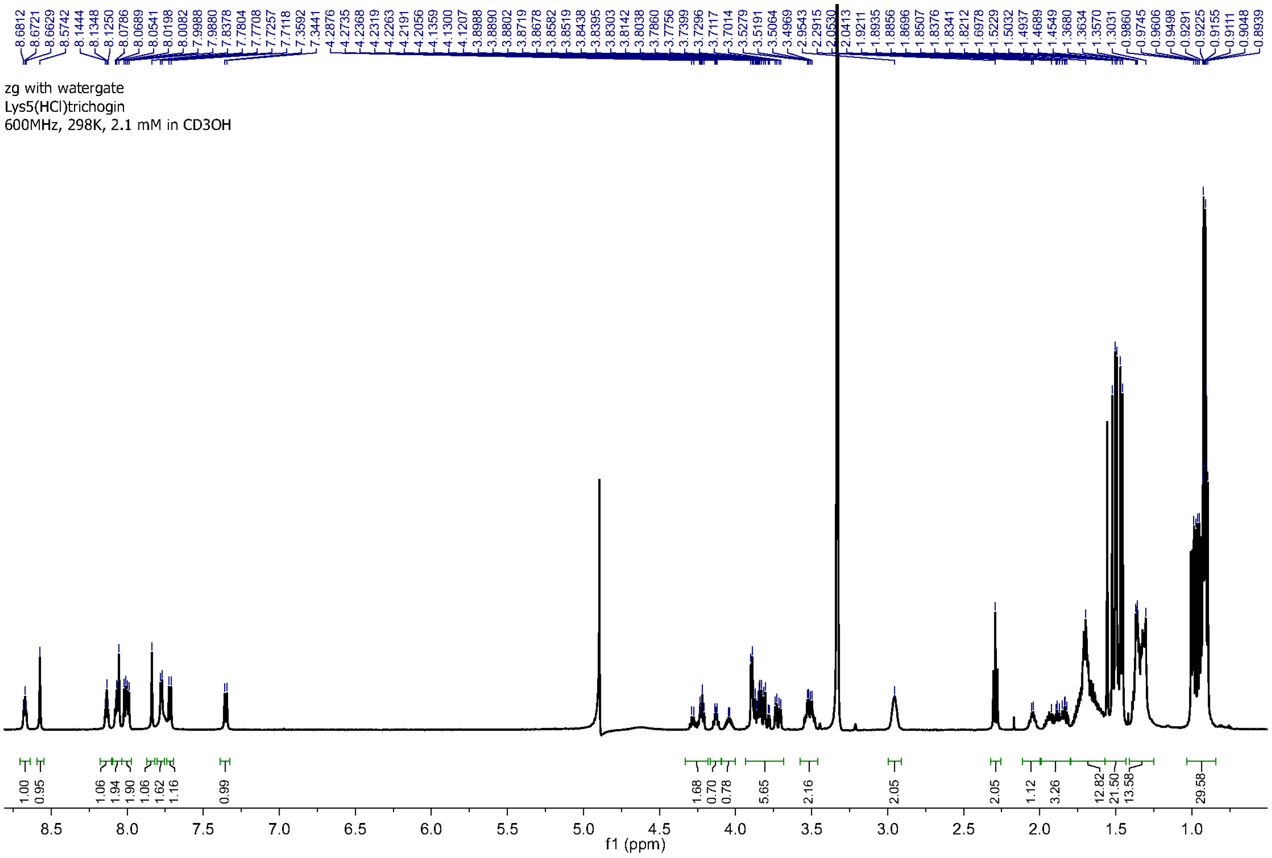
1D ^{1}H NMR spectrum for peptide **1** in CD_3OH, 298K, 600MHz (peptide concentration ca. 2 mM).

peptide **2** (**K2**)



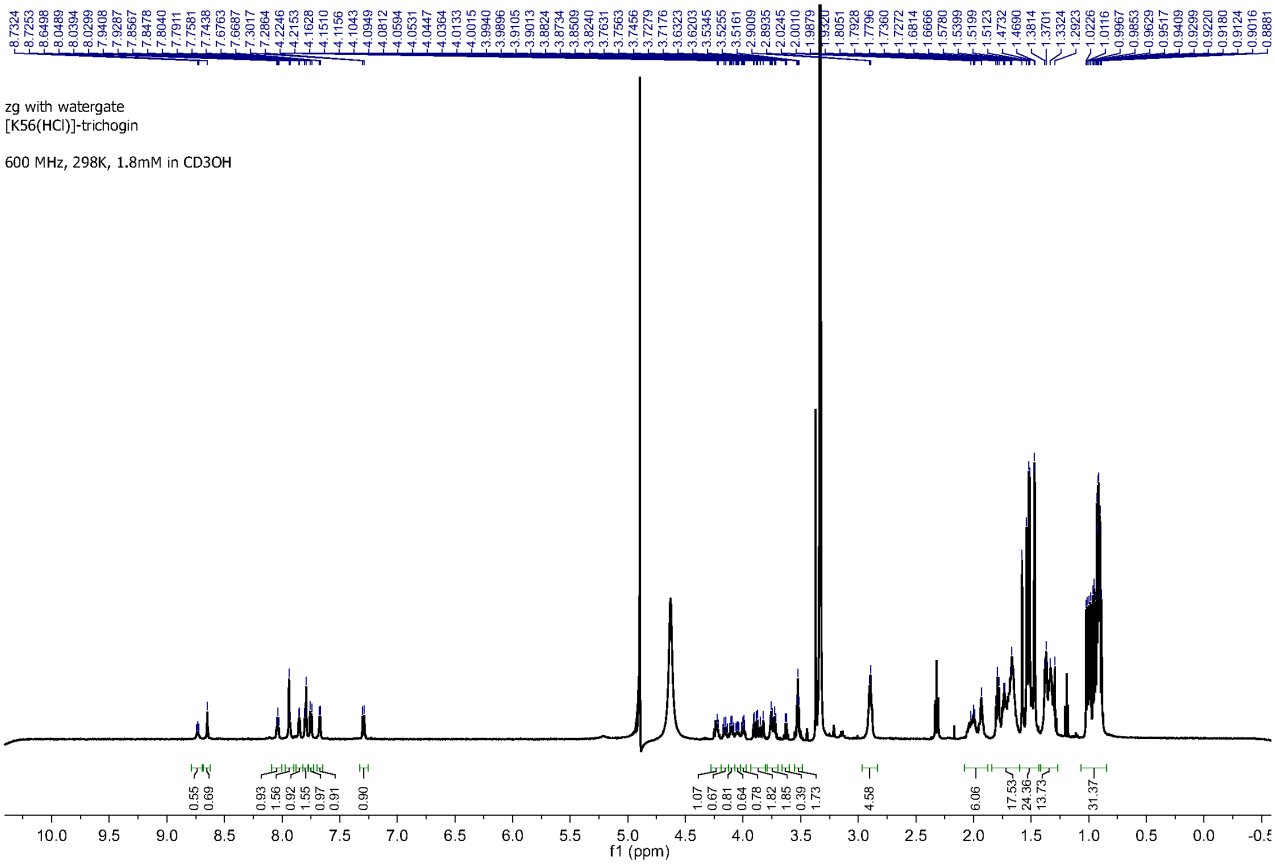
1D ^{1}H NMR spectrum for peptide **2** in H_2O/D_2O 9:1(+ 1μl HCl 3 M), 298K, 400MHz (peptide concentration ca. 4 mM).

peptide **3** (**K5**)

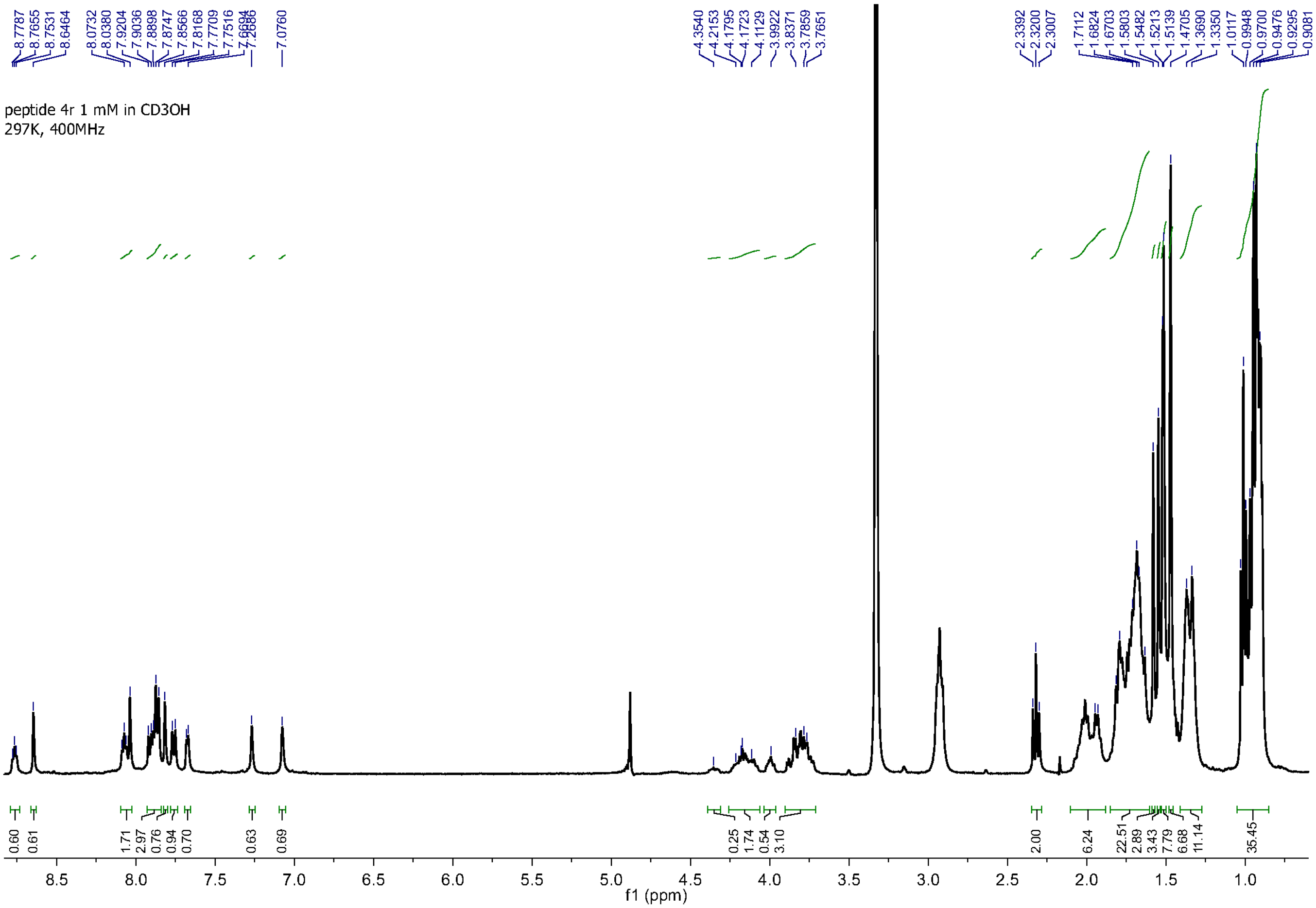


1D ^{1}H NMR spectrum for peptide **3** in CD_3OH, 298 K, 600MHz (peptide concentration ca. 2.1 mM).

peptide **4** (**K56**)

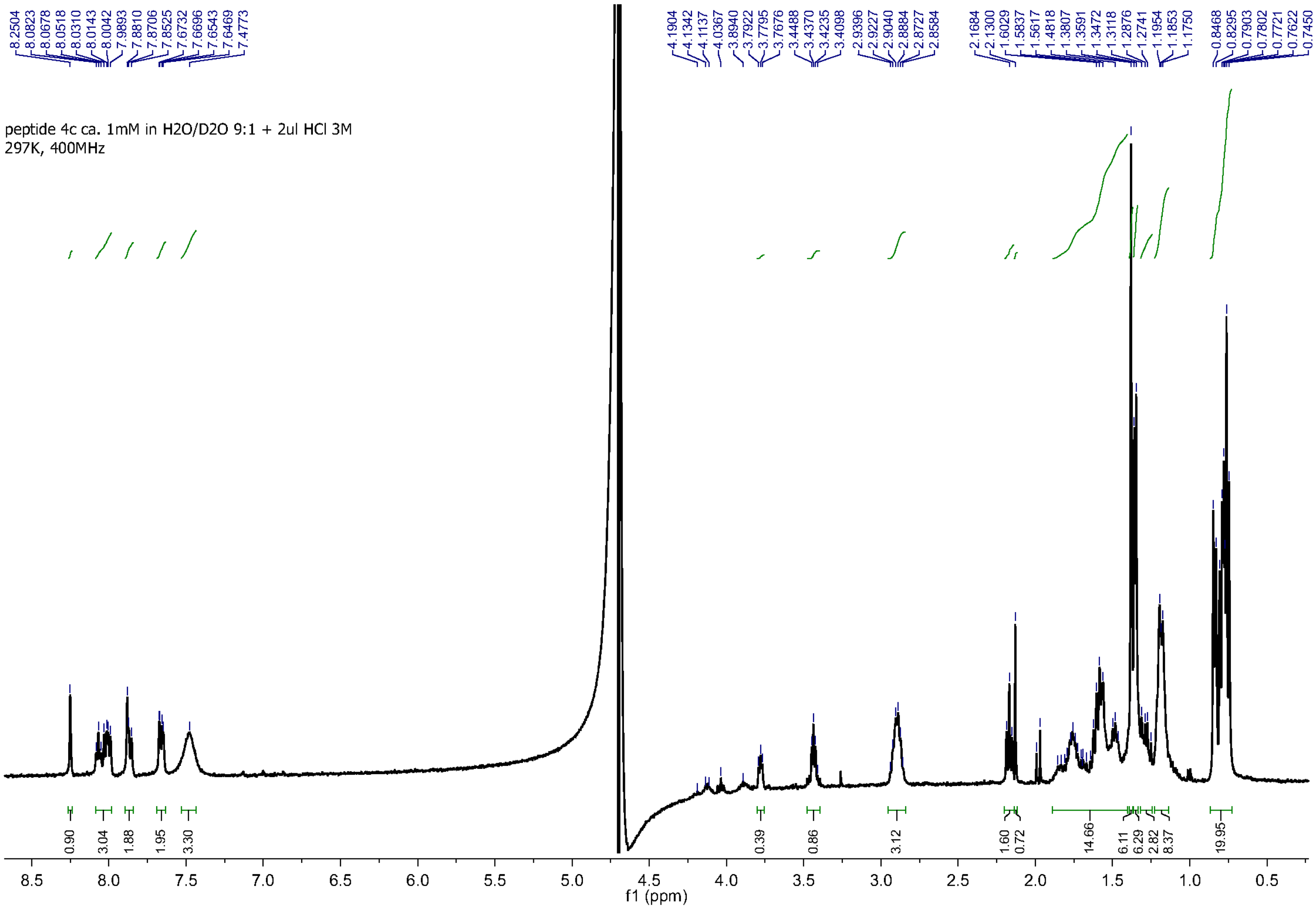


1D ^1H NMR spectrum for peptide **4** in CD_3OH, 298K, 600MHz (peptide concentration ca. 1.8 mM).



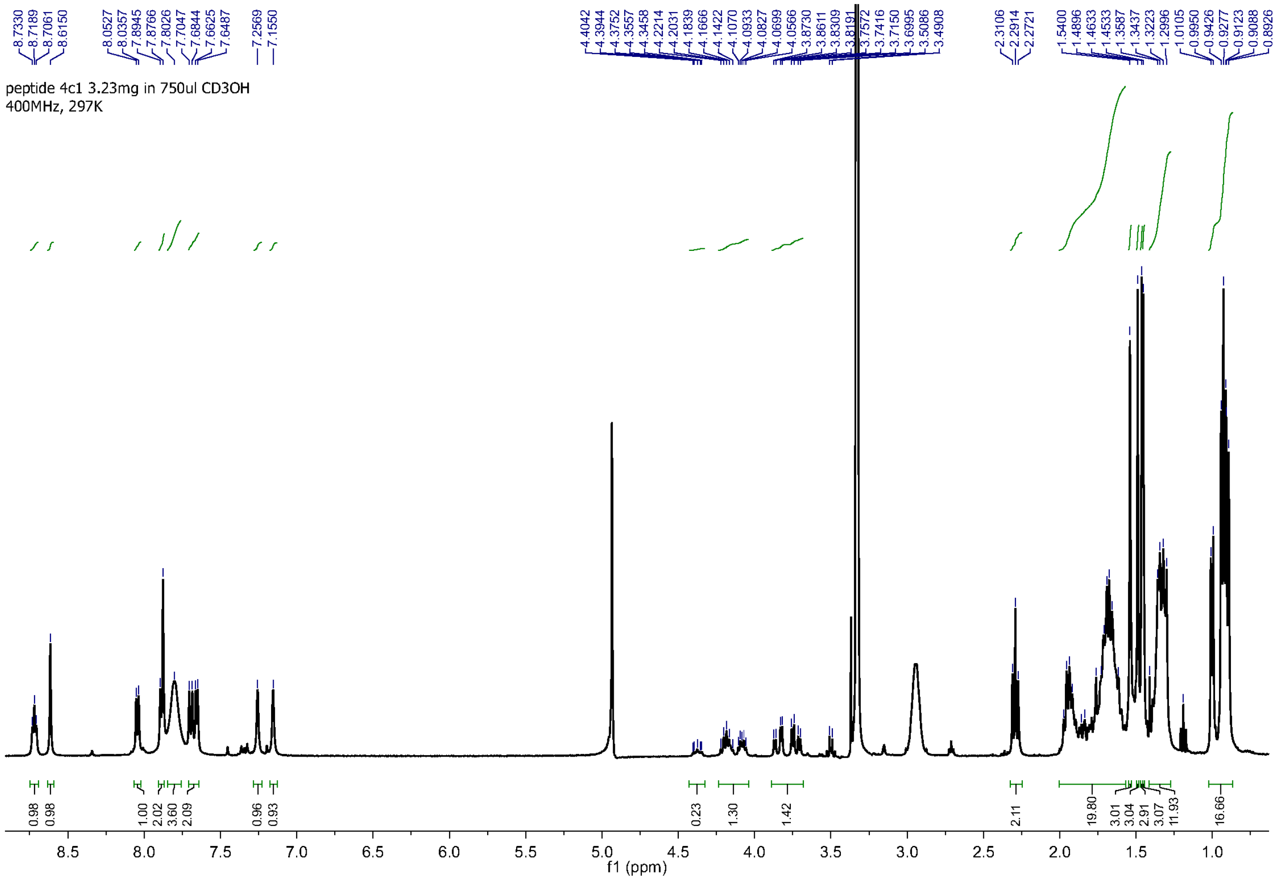
1D ^{1}H NMR spectrum for peptide **4r** in CD_3OH, 297K, 400MHz (peptide concentration 1 mM).

peptide **4c**



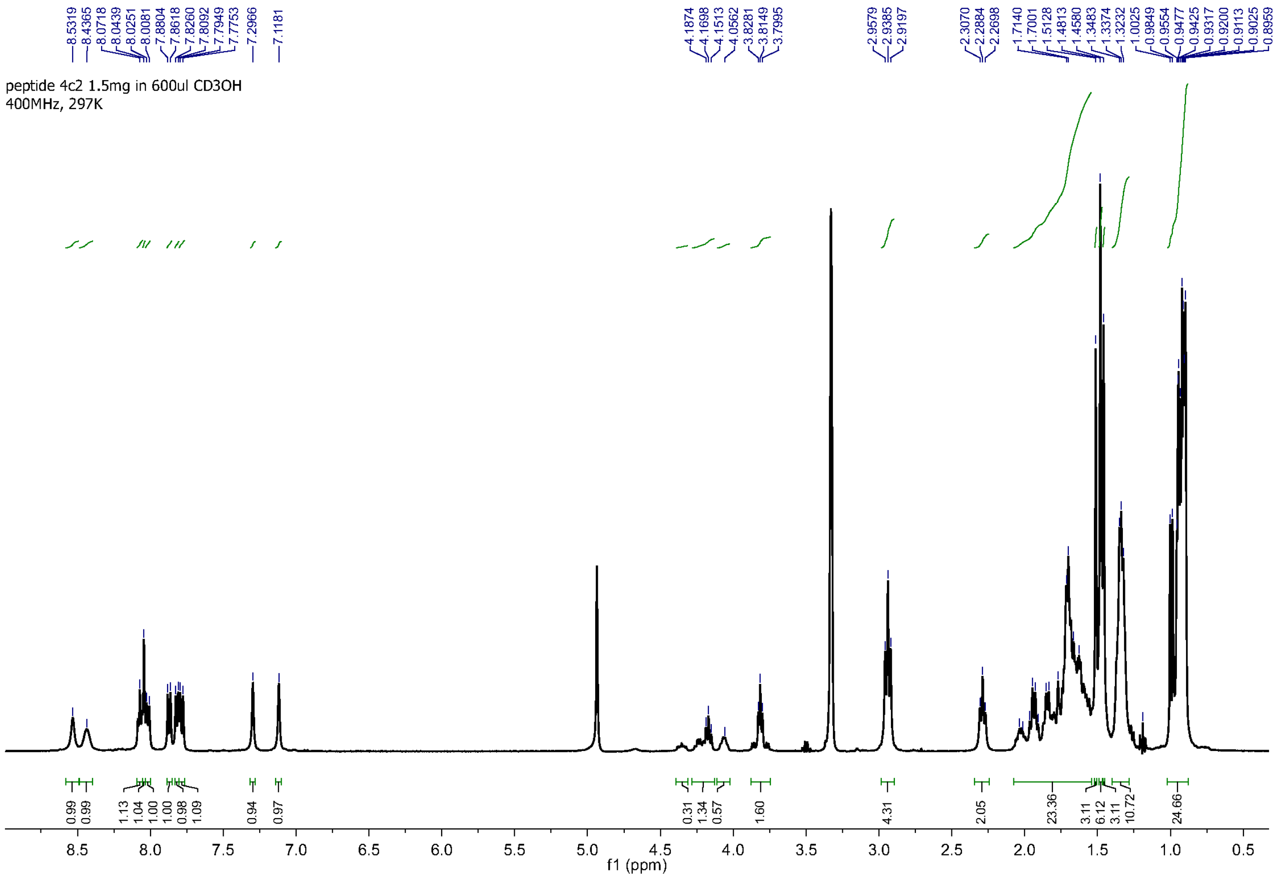
1D ^{1}H NMR spectrum for peptide **4c** in H_2O/D_2O 9:1 (+2µl HCl 3M in D_2O), 297K, 400MHz (peptide concentration 1 mM).

peptide **4c1**



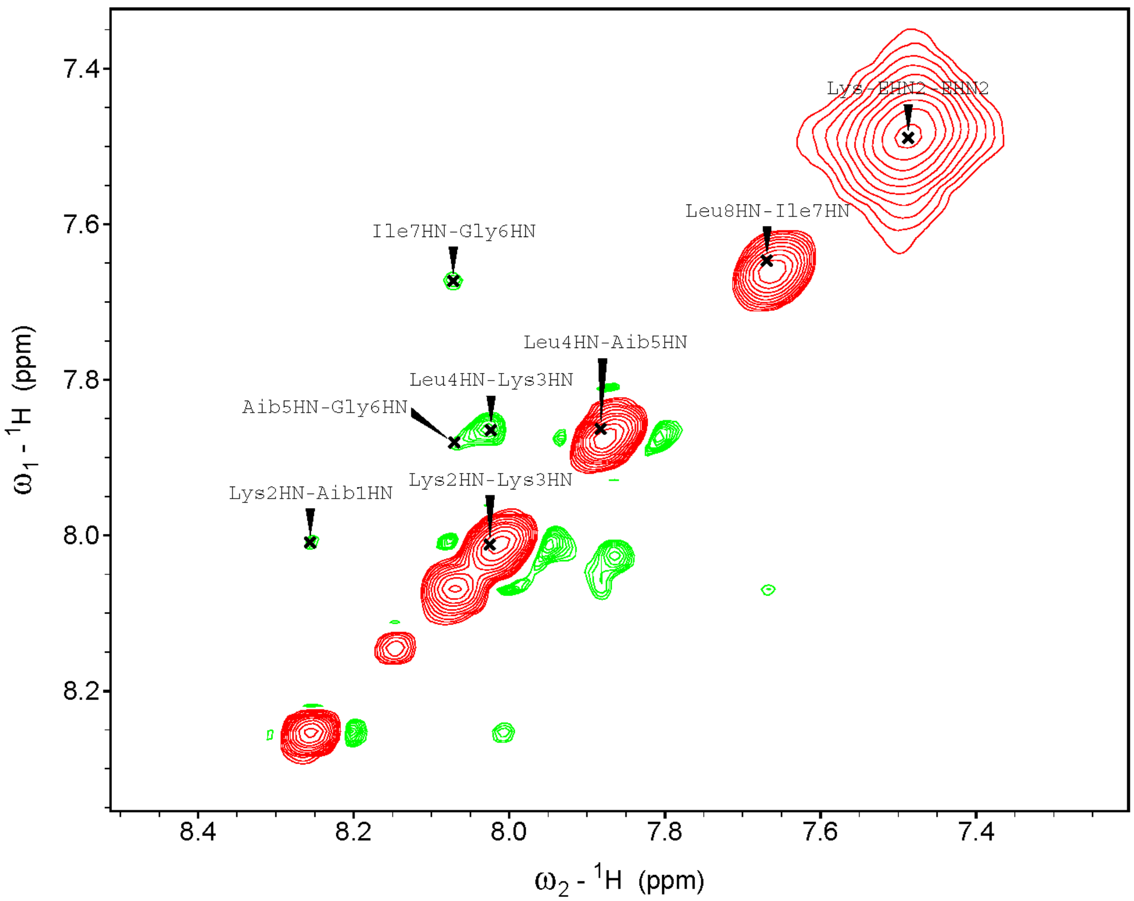
1D ^{1}H NMR spectrum for peptide **4c1** in CD$_3$OH, 297K, 400MHz (peptide concentration ca. 5 mM).

peptide **4c2**



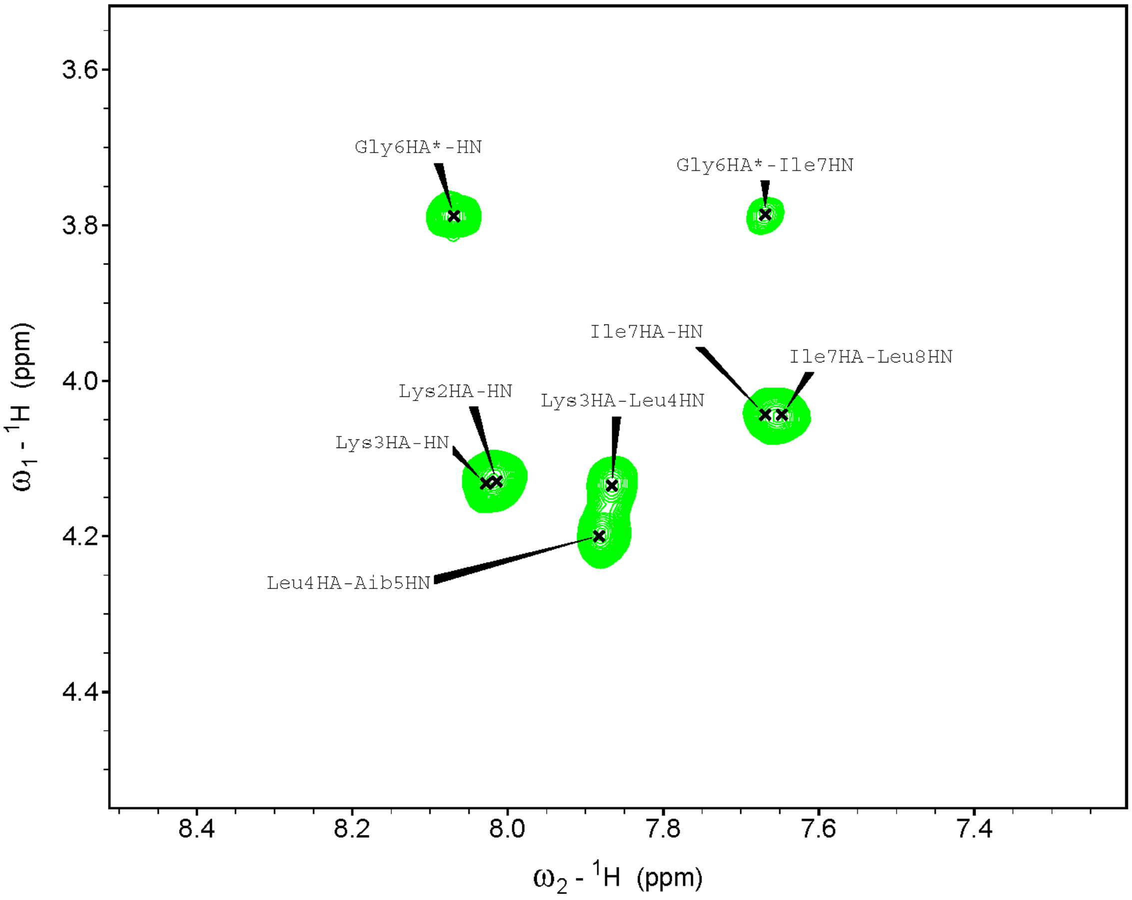
1D ^{1}H NMR spectrum for peptide **4c2** in CD$_3$OH, 297K, 400MHz (peptide concentration ca. 2.6 mM).

2D-NMR peptide **4c**



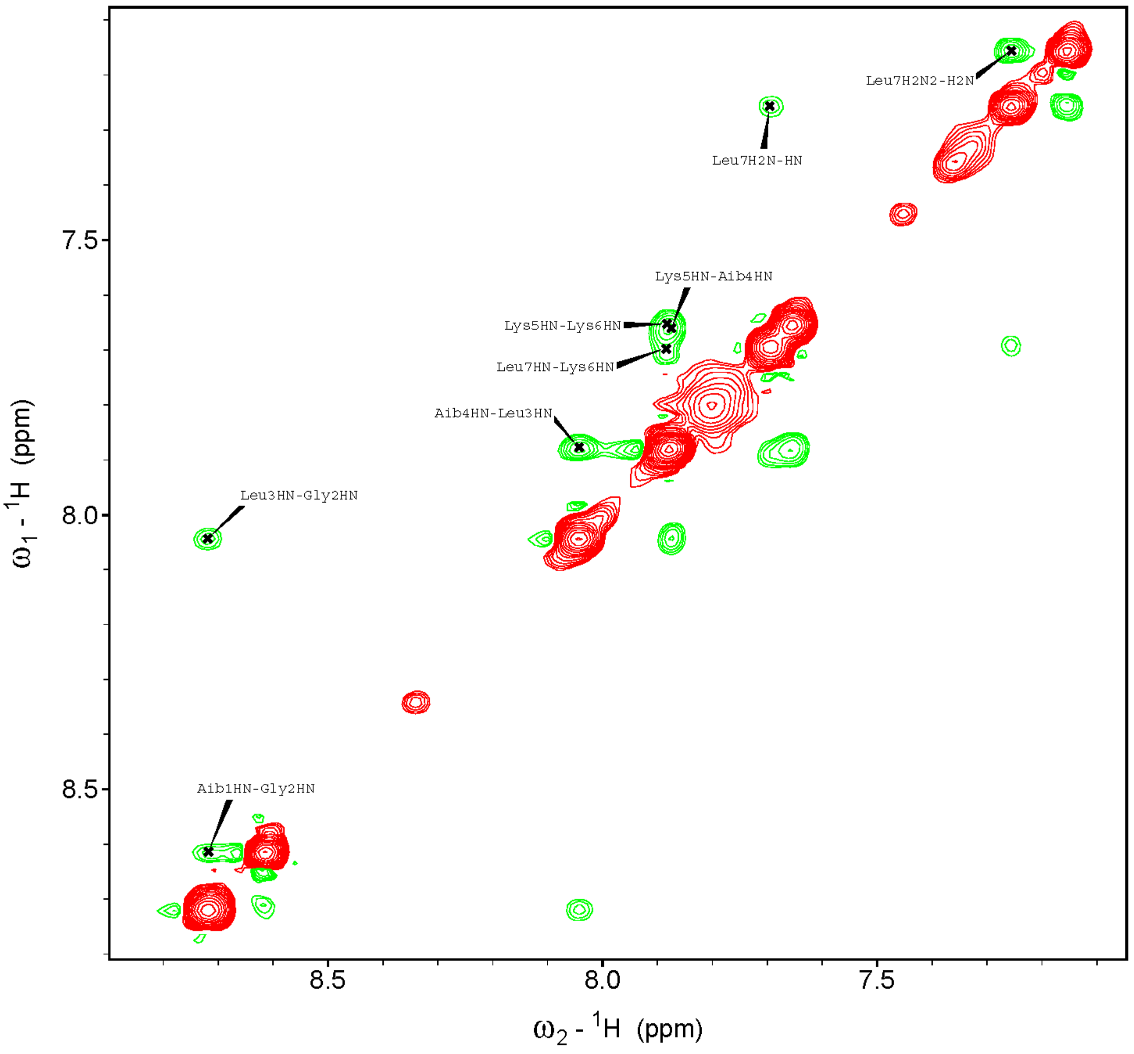
2D ^{1}H NMR for peptide **4c** in H_2O/D_2O 9:1 (+3µl HCl 3M in D_2O), 297K, 400MHz (peptide concentration 1 mM). NH-NH (amide) cross-peaks region of the ROESY spectrum. All sequential $NH_i \rightarrow NH_{i+1}$ are detectable (apart from those falling under the diagonal).

2D-NMR peptide **4c**



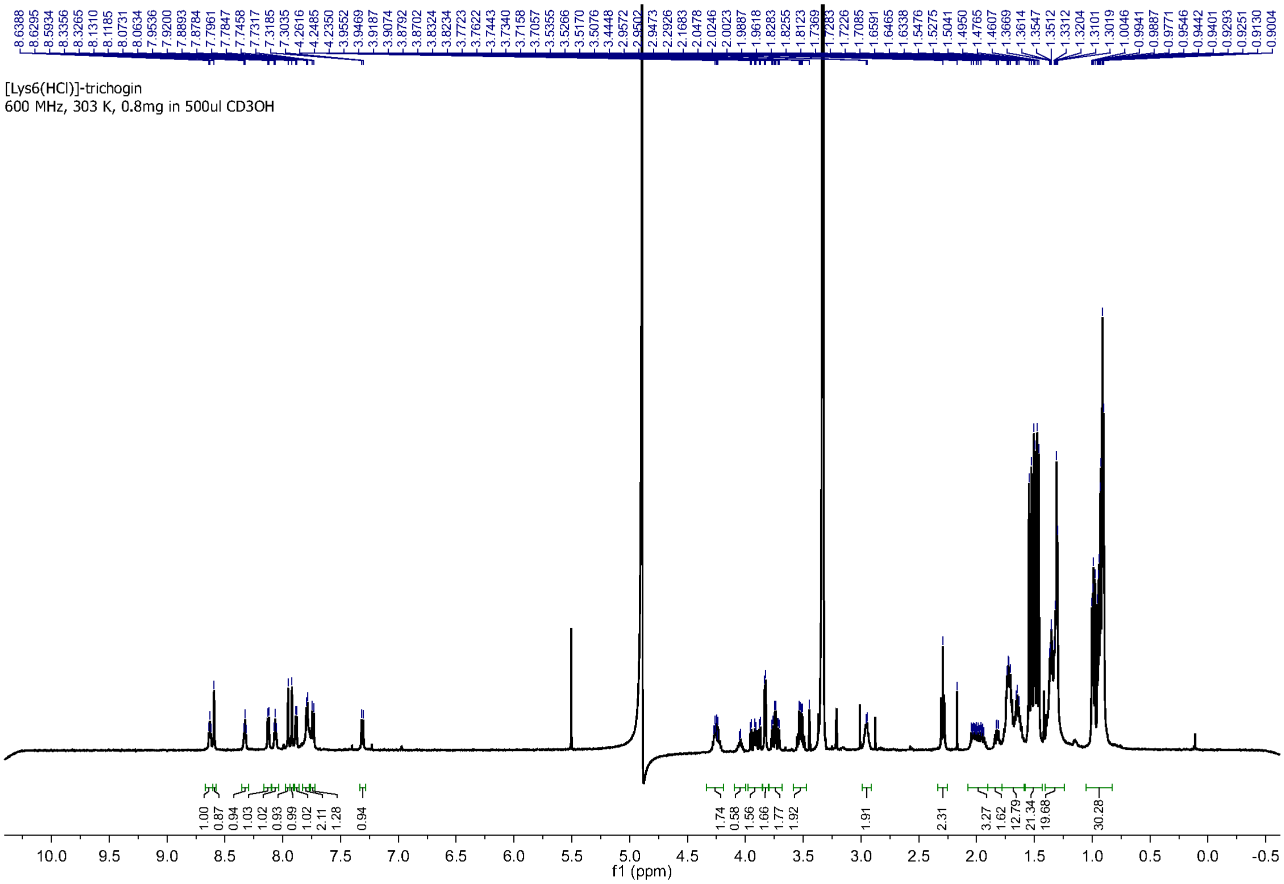
2D ^{1}H NMR for peptide **4c** in H_2O/D_2O 9:1 (+3µl HCl 3M in D_2O), 297K, 400MHz (peptide concentration 1 mM). αH-NH cross-peaks region (*fingerprint region*) of the ROESY spectrum. All sequential $\alpha H_i \rightarrow NH_{i+1}$ are detectable. There are no long-range correlations ($\alpha H_i \rightarrow NH_{i+n}$). This observation is (mainly) due to the extensive peak overlap, but it is usually associated with a somewhat flexible helical conformation, in agreement with CD findings.

2D-NMR peptide **4c1**



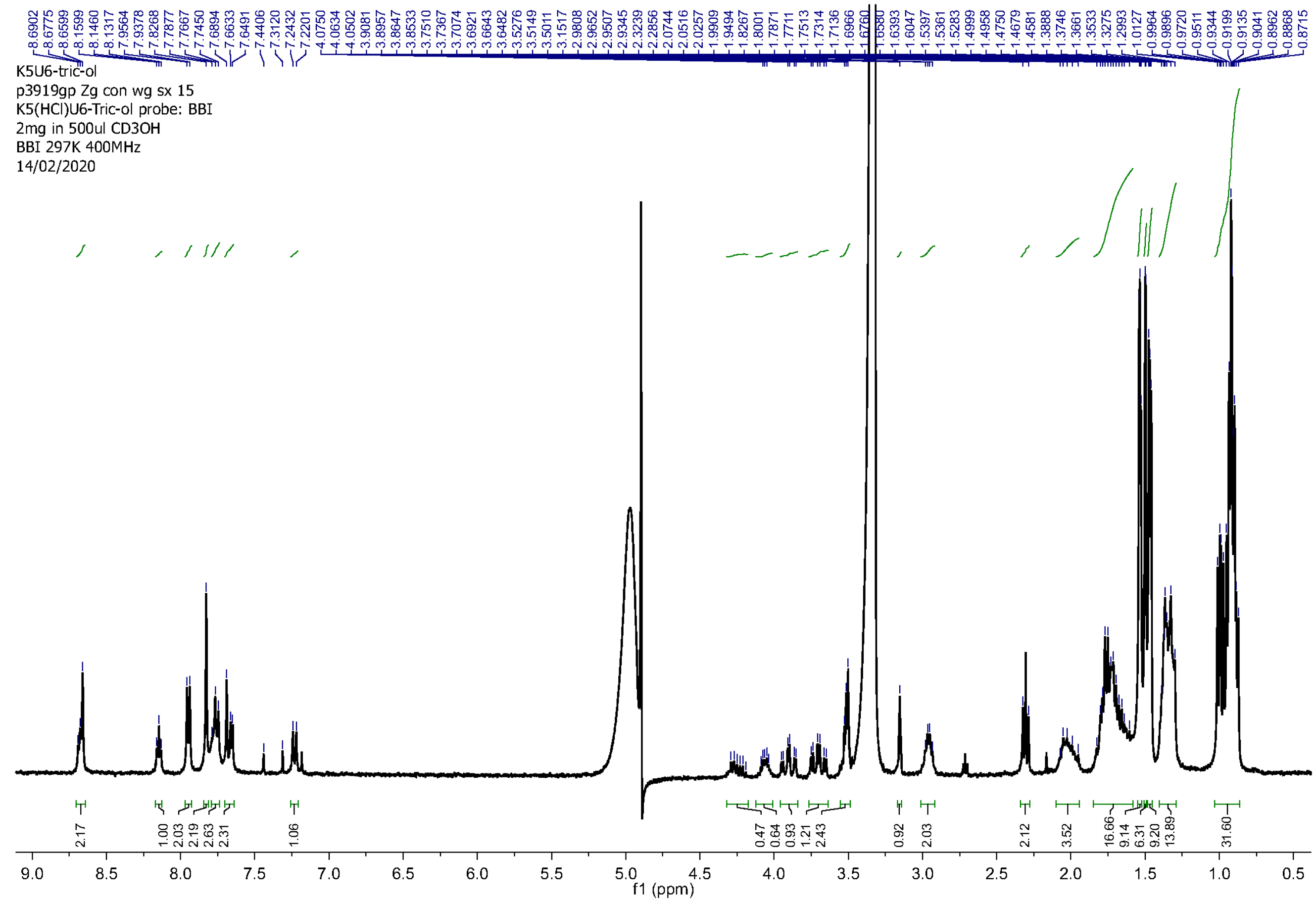
2D ^{1}H NMR for peptide **4c1** in CD_3OH, 297K, 400MHz (peptide concentration about 5 mM). The figure reports the NH-NH (amide) portion of the ROESY spectrum. All sequential $NH_i \rightarrow NH_{i+1}$ are detectable.

peptide **5** (**K6**)



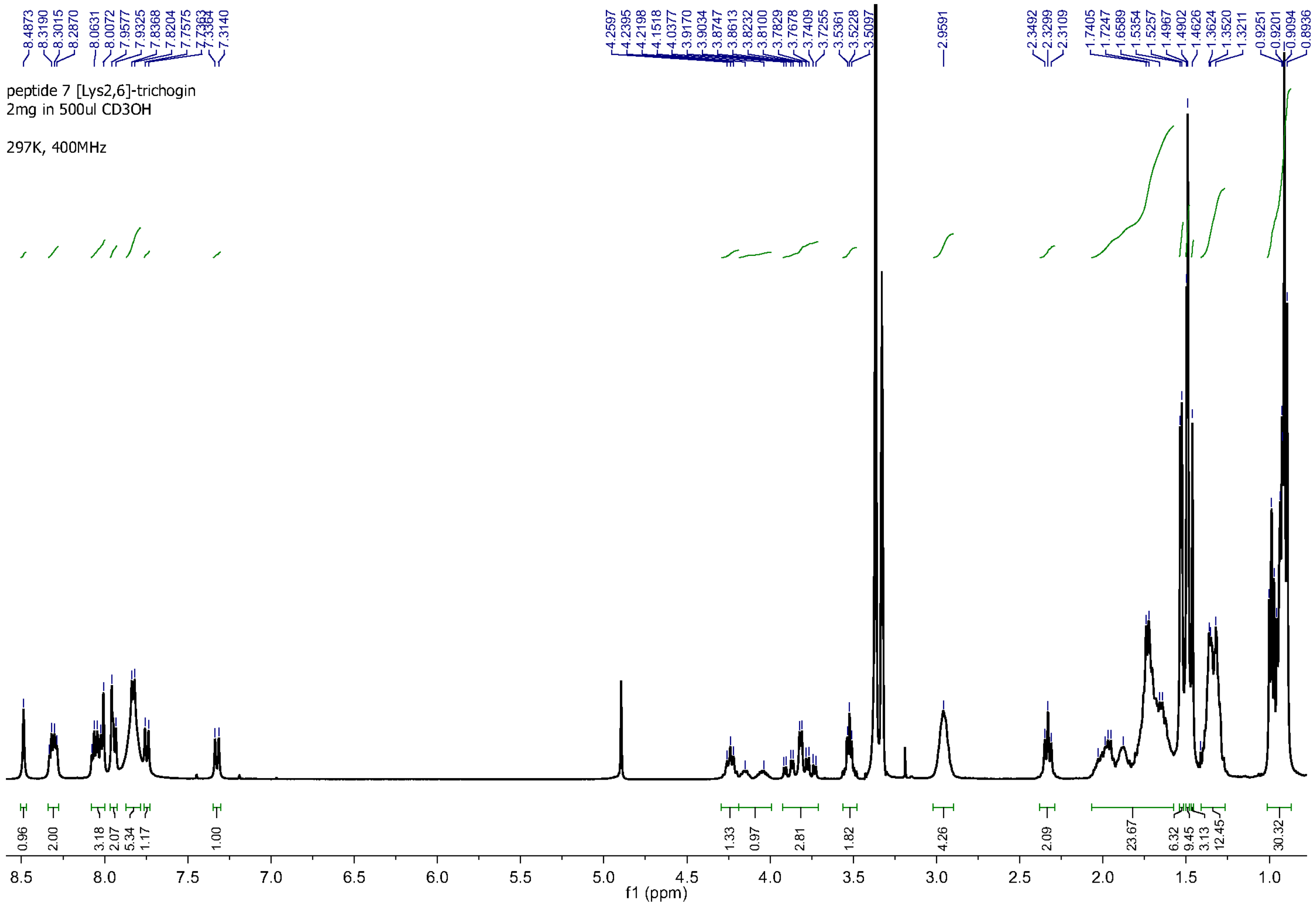
1D ^{1}H NMR spectrum for peptide **5** in CD_3OH, 298K, 600MHz (peptide concentration ca. 1.3 mM).

peptide **6** (**K5U6**)



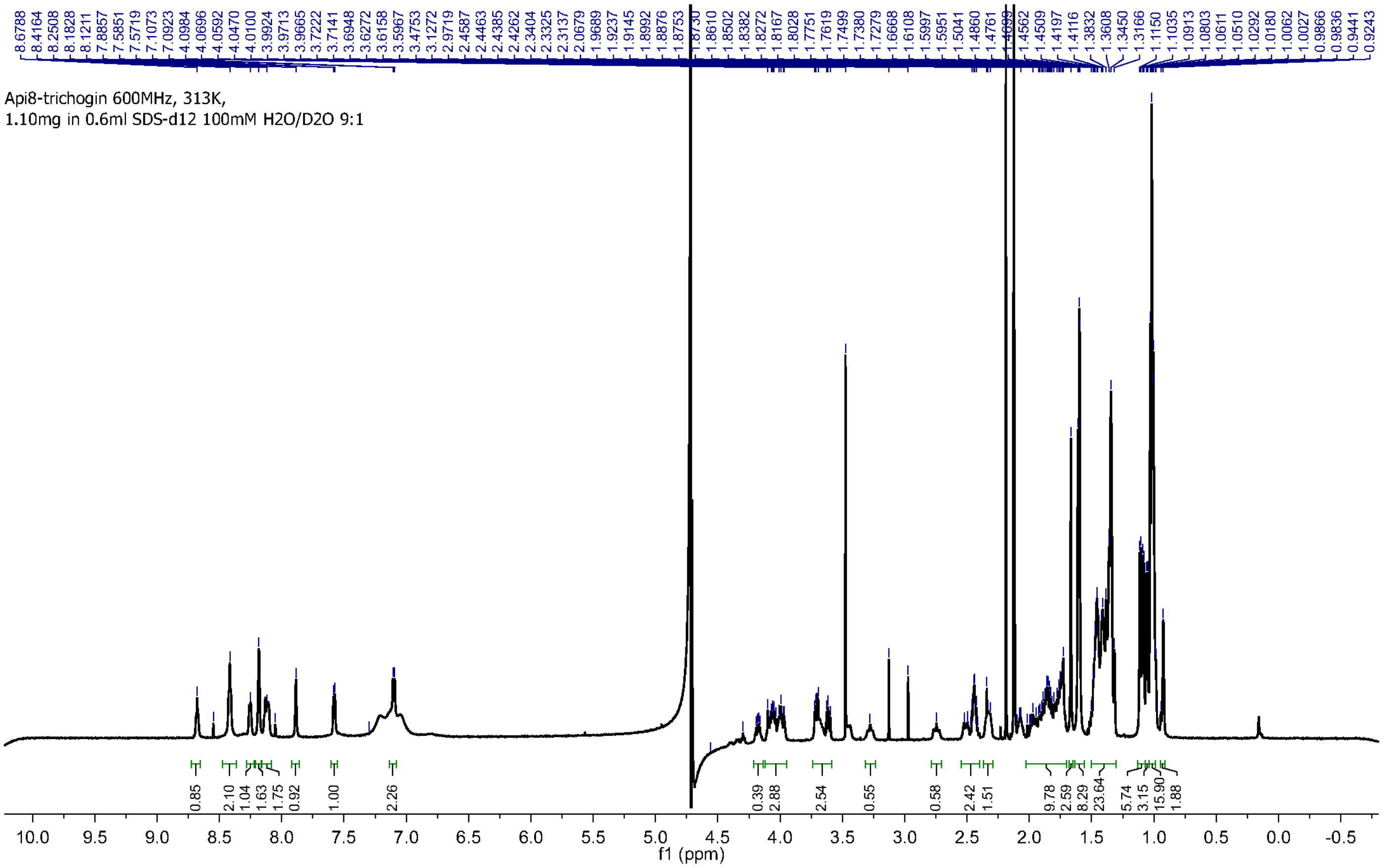
1D ^{1}H NMR spectrum for peptide **6** in CD_3OH, 297K, 400MHz (peptide concentration ca. 3 mM).

peptide **7** (**K26**)



1D ^{1}H NMR spectrum for peptide **7** in CD_3OH, 297K, 400MHz (peptide concentration ca. 3 mM).

peptide **8** (**Api8**)



1D ^{1}H NMR spectrum for peptide **8** in SDS-d_{12} 100mM in H_2O/D_2O 9:1, 313K, 600MHz (peptide concentration ca. 2 mM).