Supporting information

Peptide Ligands for Targeting the Extracellular Domain of EGFR: Comparison Between Linear and Cyclic Peptides.

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Table 1. Chemical shift, coupling constant and temperature dependence of amide resonances of 1H NMR resonances for CycloL1 in DMSO-d6.

	Chemical shift in ppm (δ)						
Sequence	NH	CH	CH	CH	Other	ΔδΔ/T ppb/K	$^{3}J_{HN\alpha}$
K1	7.78	4.26	1.63	1.54		N.D	N.D
L2	8.30	4.22	1.73	1.49	0.88	3.5	↓
A3	8.10	4.06	1.26			4.9	↓
R4	8.42	3.69	1.85	1.47	3.14	4	↓
L5	7.79	4.34	1.62	1.54	0.85	2.6	↓
L6	7.77	4.15	1.63	1.55	0.86	2.3	↓
T7	8.50	4.27	3.90	1.04		4.3	↑

*Upside arrows represent the coupling constant ≥ 6 Hz and downward arrows indicate coupling constants ≤ 5Hz. N.D not determined.

Table 2. Chemical shift, coupling constant and temperature dependence of amide resonances of of 1H NMR resonances for CycloL1.1 in DMSO-d6.

	Chemical shift in ppm (δ)						
Sequence	NH	CH	CH	CH	Other	ΔδΔ/T ppb/K	$^3J_{HN\alpha}$
K1	8.04	3.93	1.75	1.49	3.11	5.1	↑
l2	8.22	4.33	1.64	1.50	0.88	4.3	↓
a3	7.59	4.01	1.07			5.1	↓
r4	8.16	4.09	1.81	1.57		5.7	↑
l5	7.90	4.05	1.82	1.60	0.89	4.8	↑
l6	7.90	4.33	1.64	1.50	0.88	0.7	↑
t7	8.23	4.16	1.35			3.3	↓

*Upside arrows represent the coupling constant ≥ 6 Hz and downward arrows indicate coupling constants ≤ 5Hz. L amino acid is represented by capital letter and D amino acids by small letter.

Table 3. 1H NMR NOESY data with cross-peak connectivities indicating spatial connectivities between the protons for **Cyclo.L1.**

Connectivity between the protons		**Connectivity between the protons**	
Nα (i,i)		**βN (i,i)**	
K1NH	K1αH	K1NH	K1βH
L2NH	L2αH	L2NH	L2βH
A3NH	A3αH	A3NH	A3βH
R4NH	R4αH	R4NH	R4βH
L5NH	L5αH	L5NH	L5βH
L6NH	L6αH	L6NH	L6βH
T7NH	T7αH	T7NH	T7βH
αN (i,i+1)		**Other**	
K1αH	L2NH	K1NH	K1γH
L2αH	A3NH	L2NH	L2γH
A3αH	R4NH	L2NH	L2δH
R4αH	L5NH	R4NH	R4γH
L6αH	T7NH	L5NH	L5γH
NN (i,i+1)		L5NH	L5δH
T7NH	K1NH	L6NH	L6γH
R4NH	L5NH	L6NH	L6δH
R4 NH(Guanidine)	L5NH	K1αH	K1 βH
NH-side chain long range		L2αH	L2βH
K1NH	T7βH	L2αH	L2γH
T7NH	L6βH	L2αH	L2δH
R4NH	A3βH	R4αH	R4βH
A3NH	L2β,γ,δH	T7αH	T7βH
		T7αH	T7γH
		A3αH	A3βH
		L5αH	L5β, γH

Table 4. 1H NMR NOESY data with cross-peak connectivities indicating spatial connectivities between the protons for **Cyclo.L1.1.**

Connectivity between the protons		**Connectivity between the protons**	
Nα (i,i)		**βN (i,i)**	
K1NH	K1αH	K1NH	K1βH
l2NH	l2αH	l2NH	l2βH
a3NH	a3αH	a3NH	a3βH
r4NH	r4αH	r4NH	r4βH
l5NH	l5αH	l5NH	l5βH
l6NH	l6αH	l6NH	l6βH
t7NH	t7αH		
αN (i,i+1)		**Other**	
K1αH	l2NH	K1NH	K1γH
a3αH	r4NH	l2NH	l2γH
r4αH	l5NH	l2NH	l2δH
l6αH	t7NH	r4NH	r4γH
t7αH	K1NH	l5NH	l5γH
NN (i,i+1)		l5NH	l5δH
t7NH	l6NH	l6NH	l6γH
l2NH	K1NH	l6NH	l6δH
NN (long range)		r4αH	r4βH
l2NH	l5/6NH	r4αH	r4γH
K1NH	K1εH	l2αH	l2βH
NH-side chain long range		l2αH	l2γH
K1NH	t7βH	l2αH	l2δH
r4NH	a3βH	t7αH	t7γH
a3NH	l2β,γ H	l6αH	l6βH
		l6αH	l6δH

*L amino acids are represented by capital letters and D amino acids are represented by smallletters**.**

Figure legends for supporting information

Figure S1. HR-MS for Cyclic peptides A) Cyclo(KLARLLT) and B) Cyclo(KN3)larllt).

Figure S2. SPR analysis of linear peptide GLARLLT. EGFR was immobilized on CM5 sensor chip and different concentrations of peptide was injected to obtain the SPR response. Binding of peptide GLARLLT to EGFR was observed with increasing concentration of the peptide.

Figure S3. Curve fitting of SPR sensorgram for **Cyclo.L1.1** using 1:1 Langmuir interaction describing the single exponential of the data. Black lines are experimental data and different colored lines represent fitted lines. K_D values reported were from *k*on and *k*off calculations.

Figure S4. Curve fitting of SPR sensorgram for **L1.7** using 1:1 Langmuir interaction describing the single exponential of the data. Black lines are experimental data and different colored lines represent fitted lines.

Figure S5. HPLC for peptide **L1**.

Figure S6. HPLC for peptide **L1.3**.

Figure S7. HPLC for peptide **L1.4**.

Figure S8. HPLC for peptide **L1.5**.

Figure S9. HPLC for peptide **L1.6**.

Figure S10. HPLC for peptide **L1.7**.

Figure S11. HPLC for peptide L1

Figure S12. MALDI-TOF of peptide L1.3

Figure S13. MALDI-TOF of peptide L1.5

Figure S14. MALDI-TOF of peptide L1.6

Figure S15. MALDI-TOF of peptide L1.7

Figure S16. MALDI-TOF-TOF (MSMS) of peptide L1

Figure S17. MALDI-TOF-TOF (MSMS) of peptide L1.3

Figure S18. MALDI-TOF-TOF (MSMS) of peptide L1.5

Figure S19. MALDI-TOF-TOF (MSMS) of peptide L1.6

Figure S20. MALDI-TOF-TOF (MSMS) of peptide L1.7

Figure S21. ^{1}HNMR spectrum for peptide **L1**.

Figure S22. ^{1}HNMR spectrum for peptide **L1.1.**

Figure S23. ^{1}HNMR spectrum for peptide **L1.2**.

Figure S24. [1]HNMR spectrum for peptide **L1**.3.

Figure S25. [1]HNMR spectrum for peptide **L1.4**.

Figure S26. [1]HNMR spectrum for peptide **L1**.5.

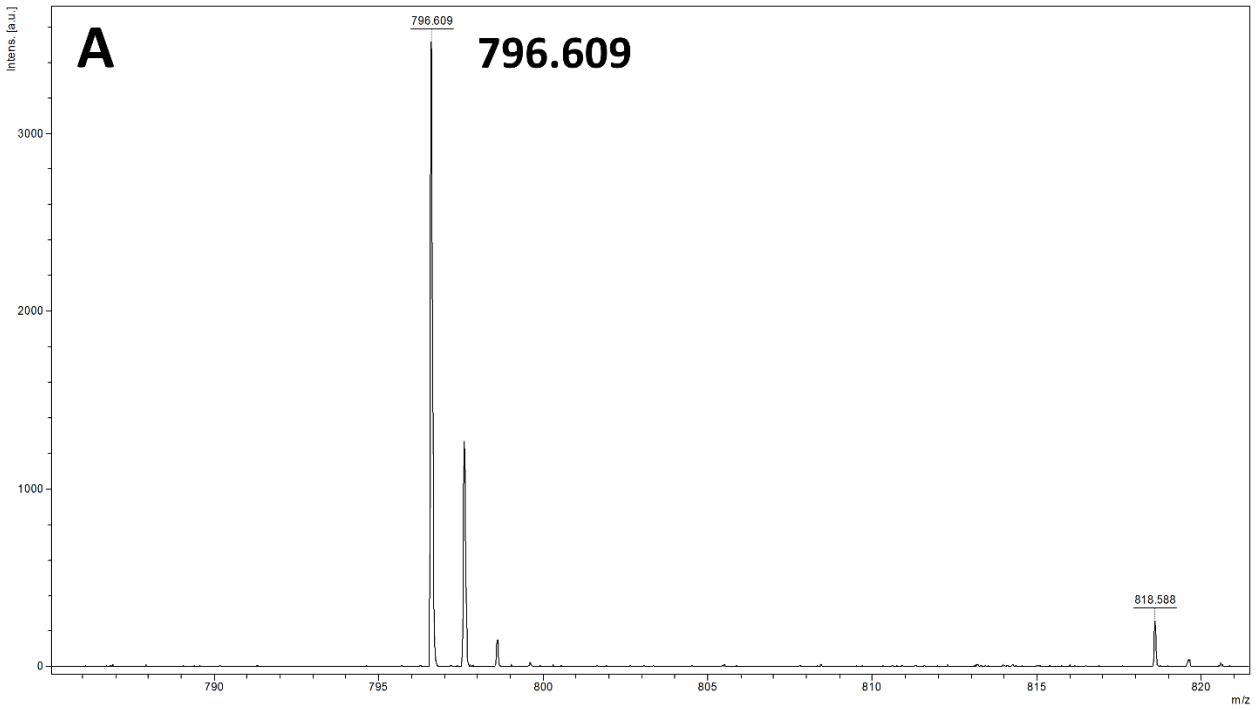
Figure S27. [1]HNMR spectrum for peptide **L1**.6.

Figure S28. [1]HNMR spectrum for peptide **L1**.7.

Figure S29. HSQC spectrum for peptide **L1**.

Figure S30. HSQC spectrum for peptide **L1**.5.

Figure S31. CD spectra of linear and cyclic peptides in methanol. 1 mg/mL of peptide was dissolved in methanol and CD spectra was acquired at 25 °C, and the spectra were average of 4 scans.



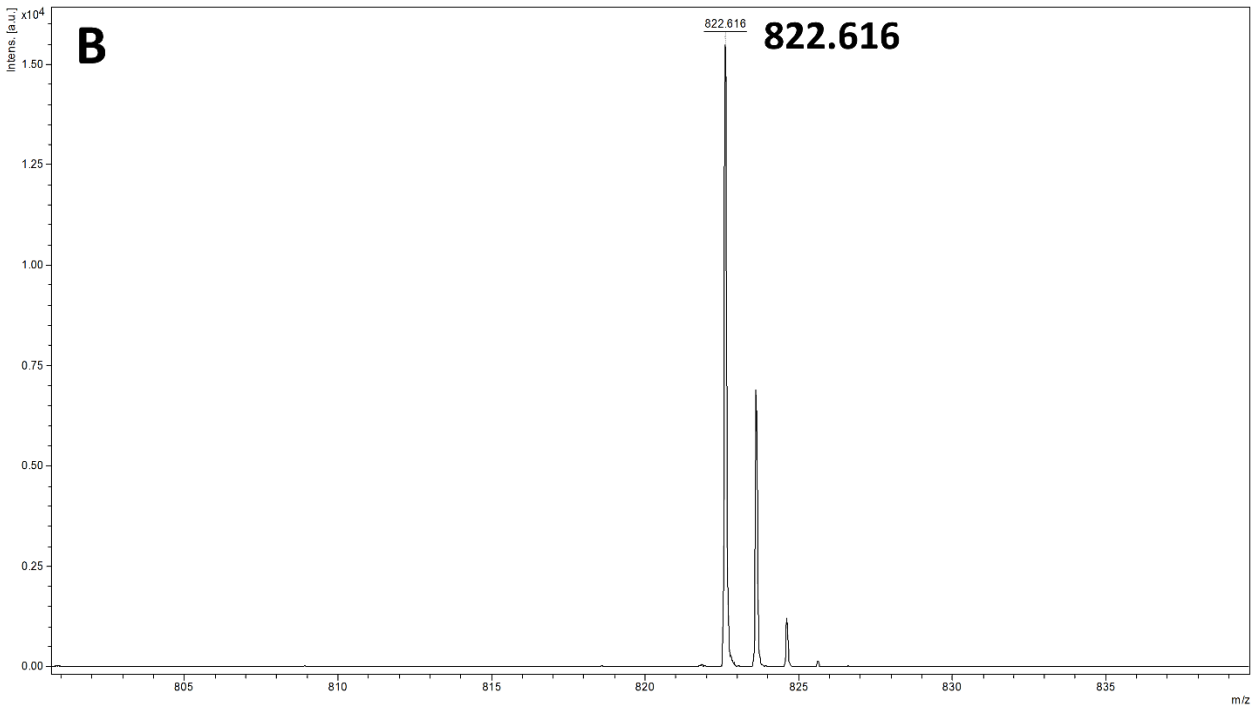


Figure S1. HR-MS for Cyclic peptide A) Cyclo(KLARLLT) and B) Cyclo(KN3)larllt).

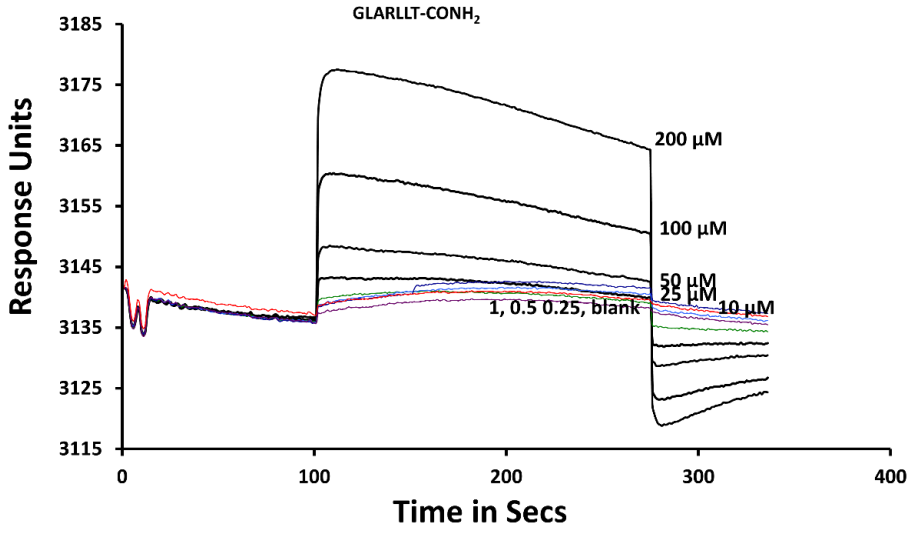


Figure S2. SPR analysis of linear peptide GLARLLT. EGFR was immobilized on CM5 sensor chip and different concentrations of peptide was injected to obtain the SPR response. Binding of peptide GLARLLT to EGFR was observed with increasing concentration of the peptide.

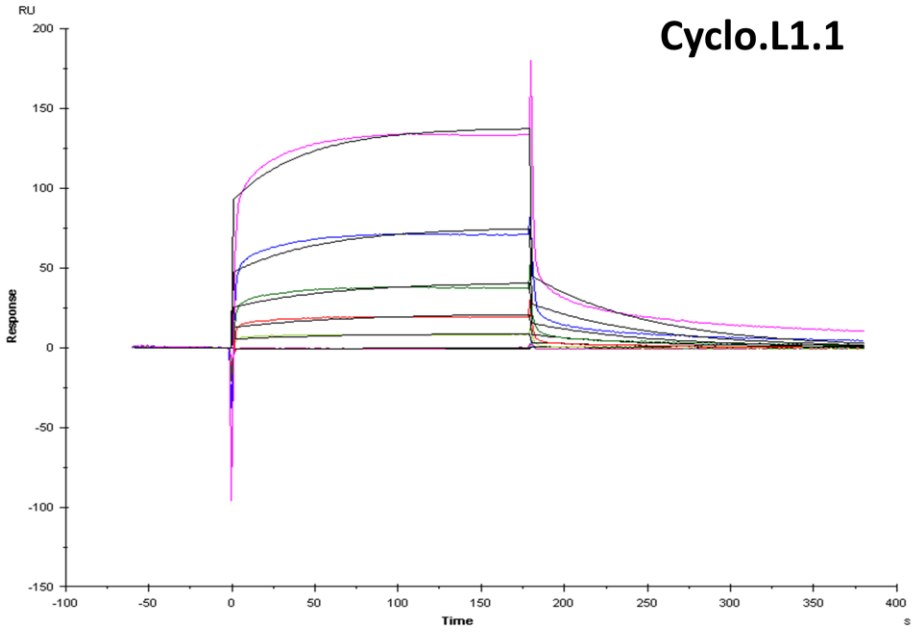


Figure S3. Curvefitting of SPR sensorgram for **Cyclo.L1.1** using 1:1 Langmuir interaction describing the single exponential of the data. Black lines are experimental data and different colored lines represent fitted lines. K_D values reported were from *k*on and *k*off calculations.

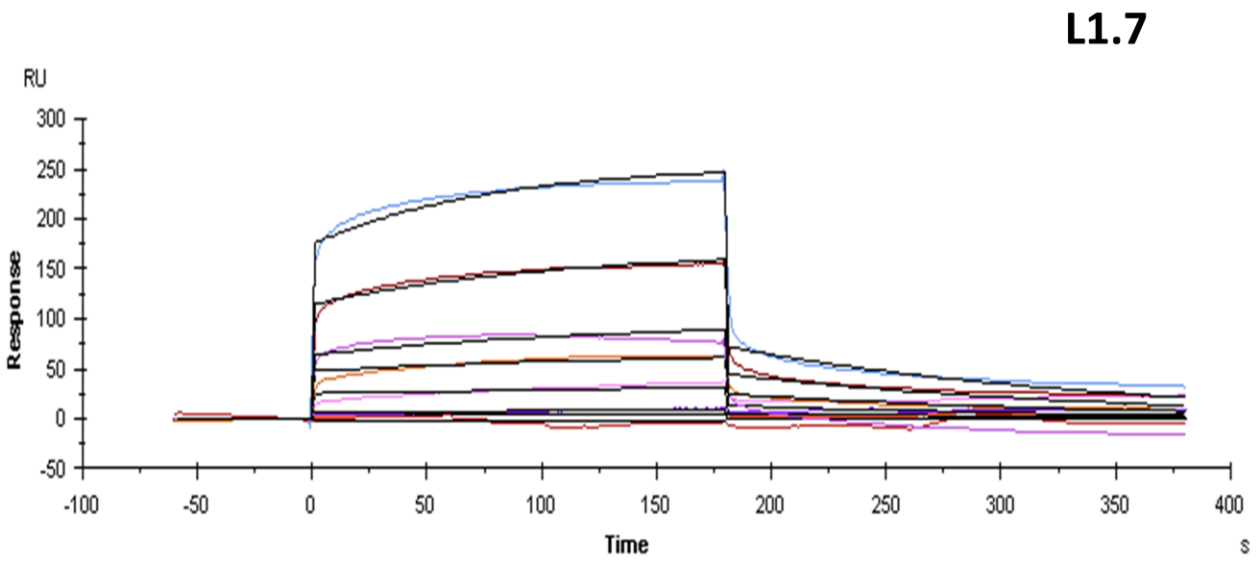


Figure S4. Curvefitting of SPR sensorgram for **L1.7** using 1:1 Langmuir interaction describing the single exponential of the data. Black lines are experimental data and different colored lines represent fitted lines.

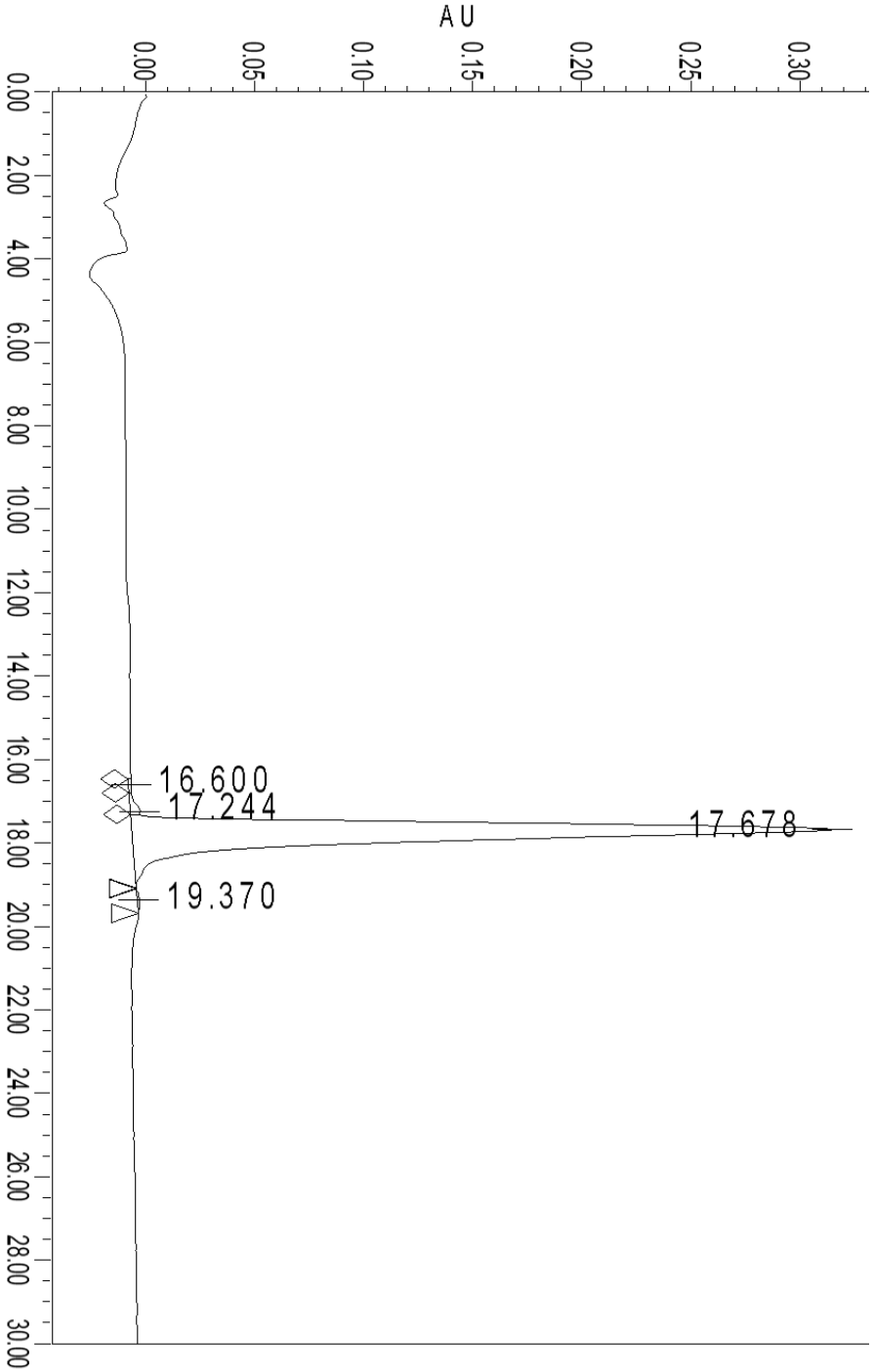


Figure S5. HPLC for peptide **L1**.

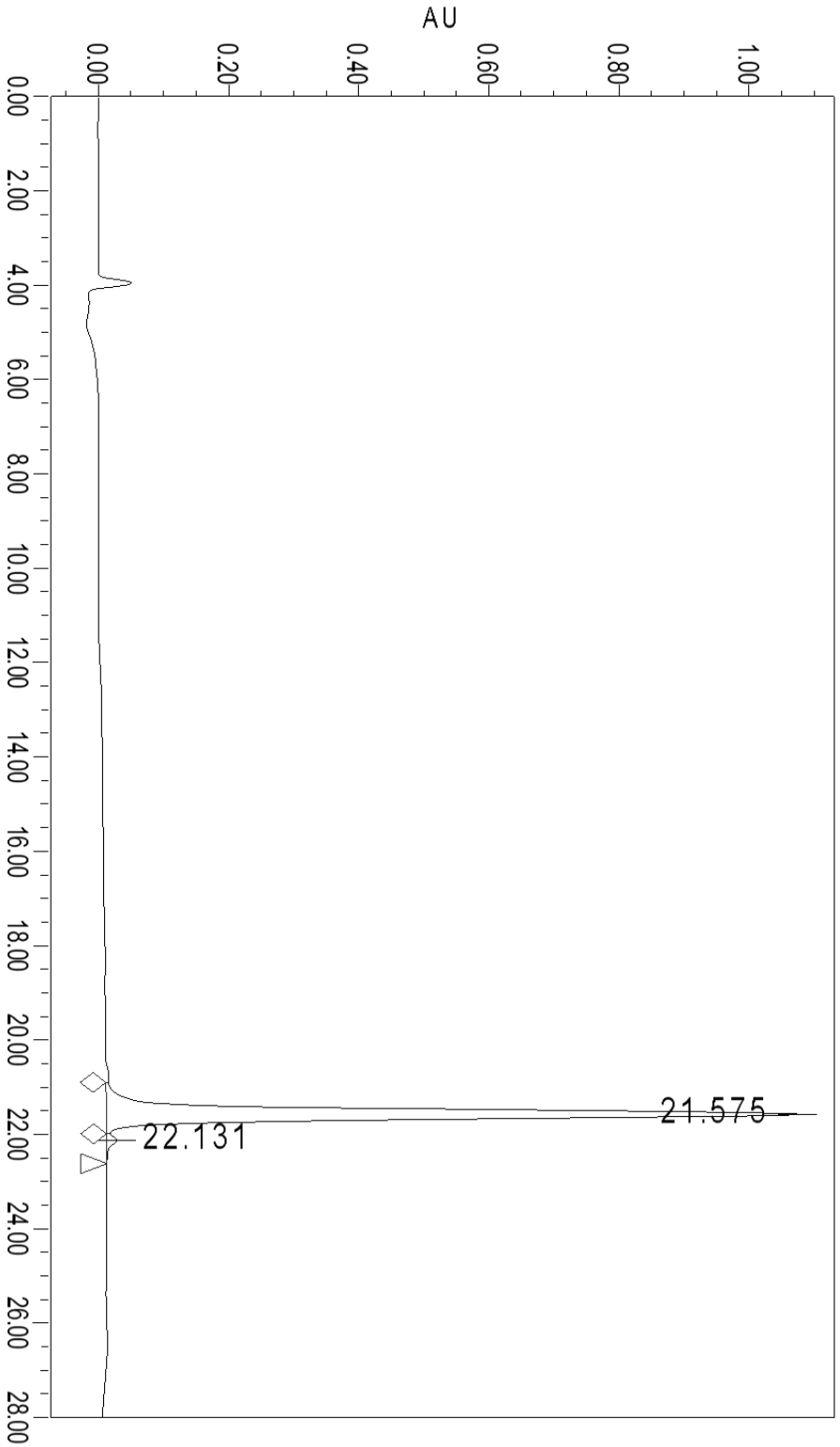


Figure S6. HPLC of peptide **L1.3**.

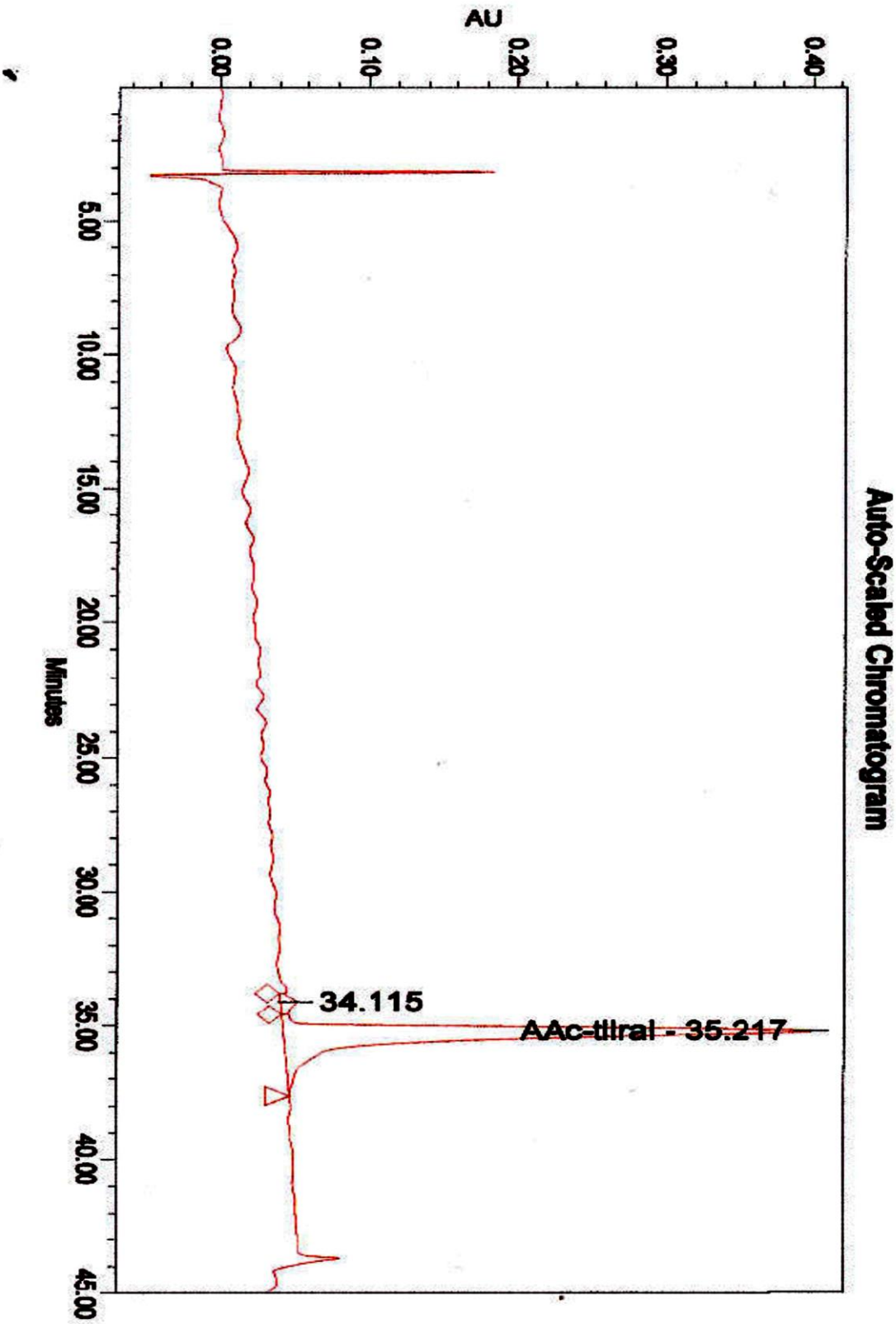


Figure S7. HPLC for peptide **L1.4**.

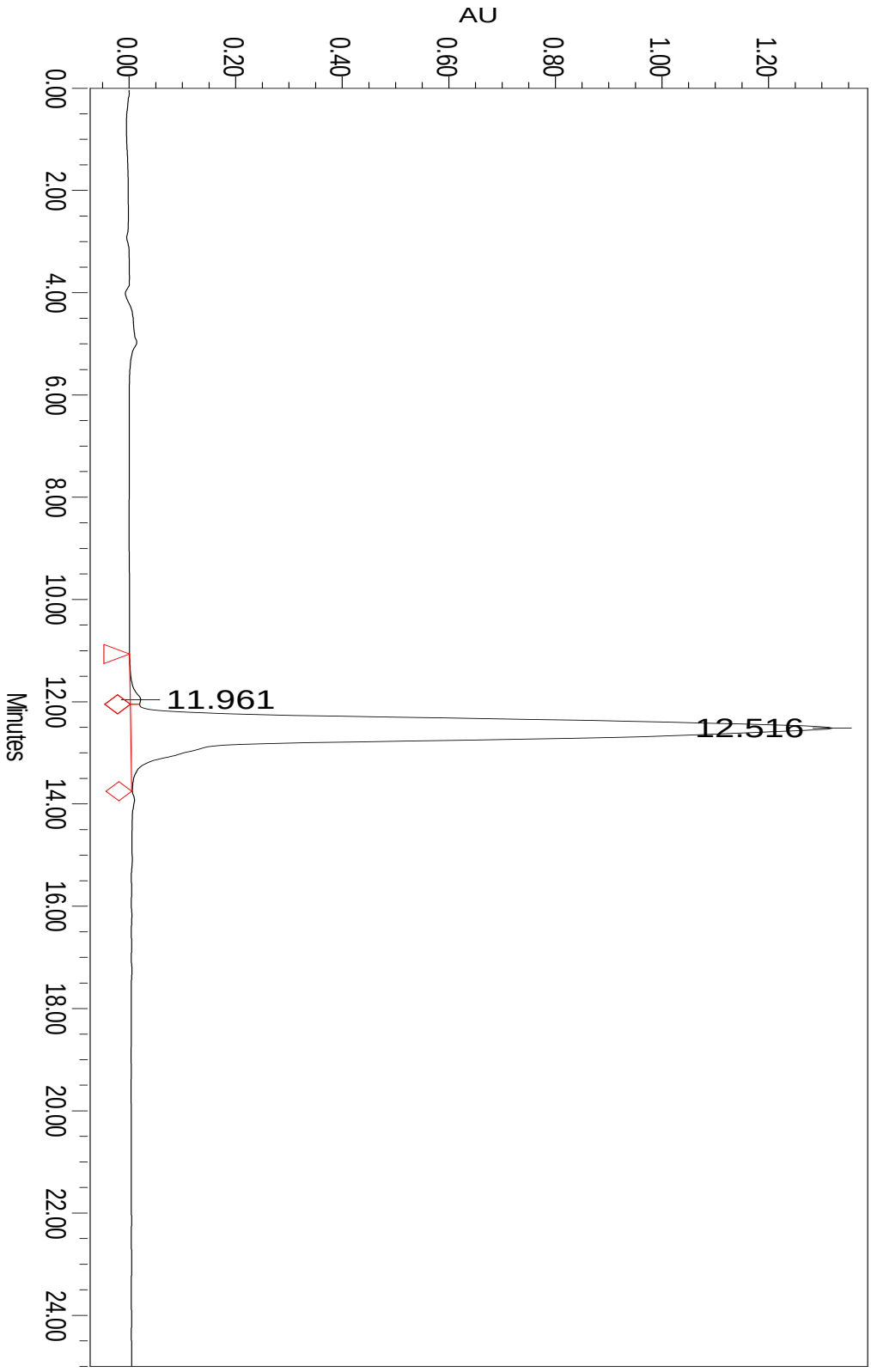


Figure S8. HPLC for peptide **L1.5**.

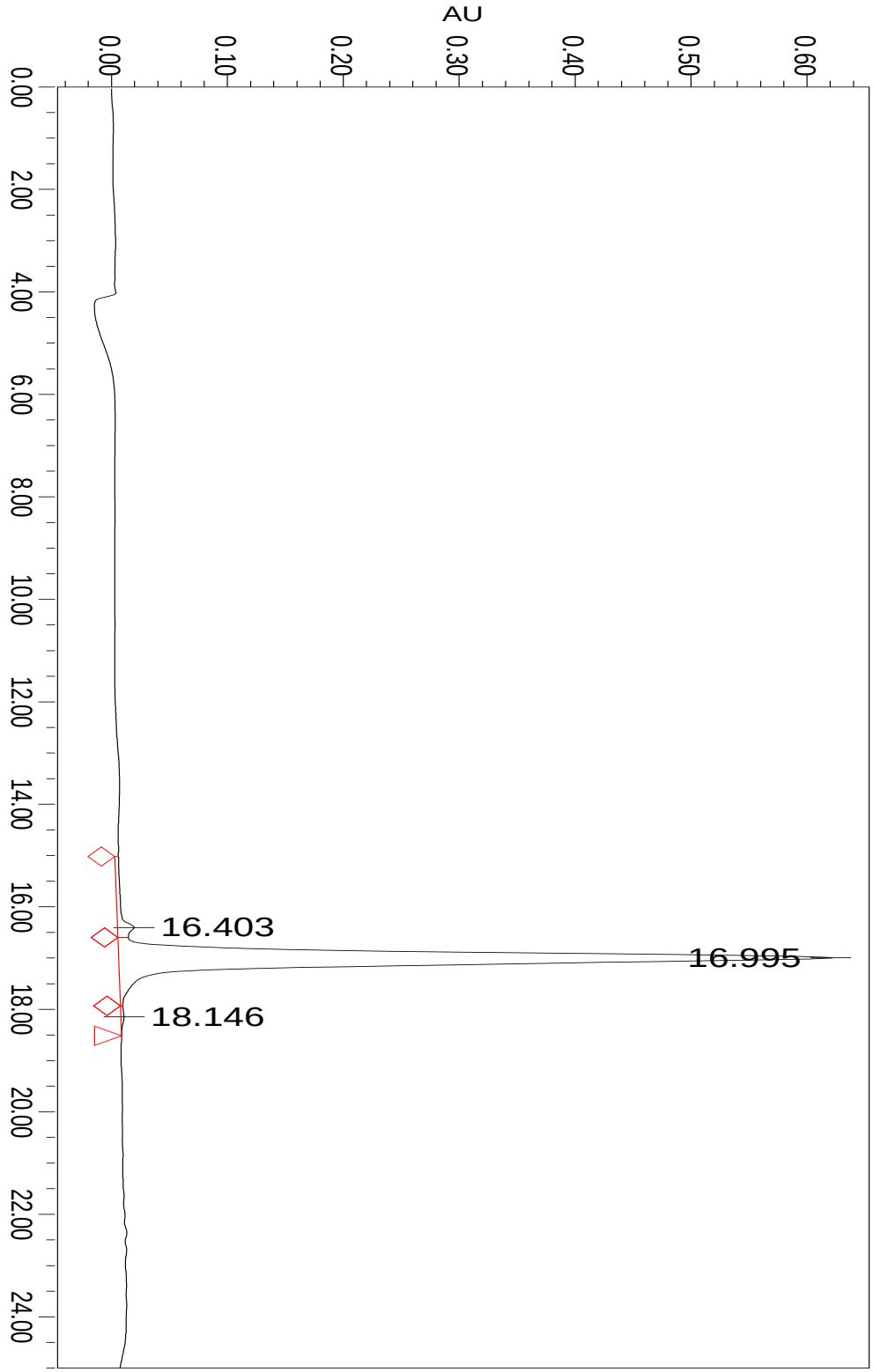


Figure S9. HPLC for peptide **L1.6**.

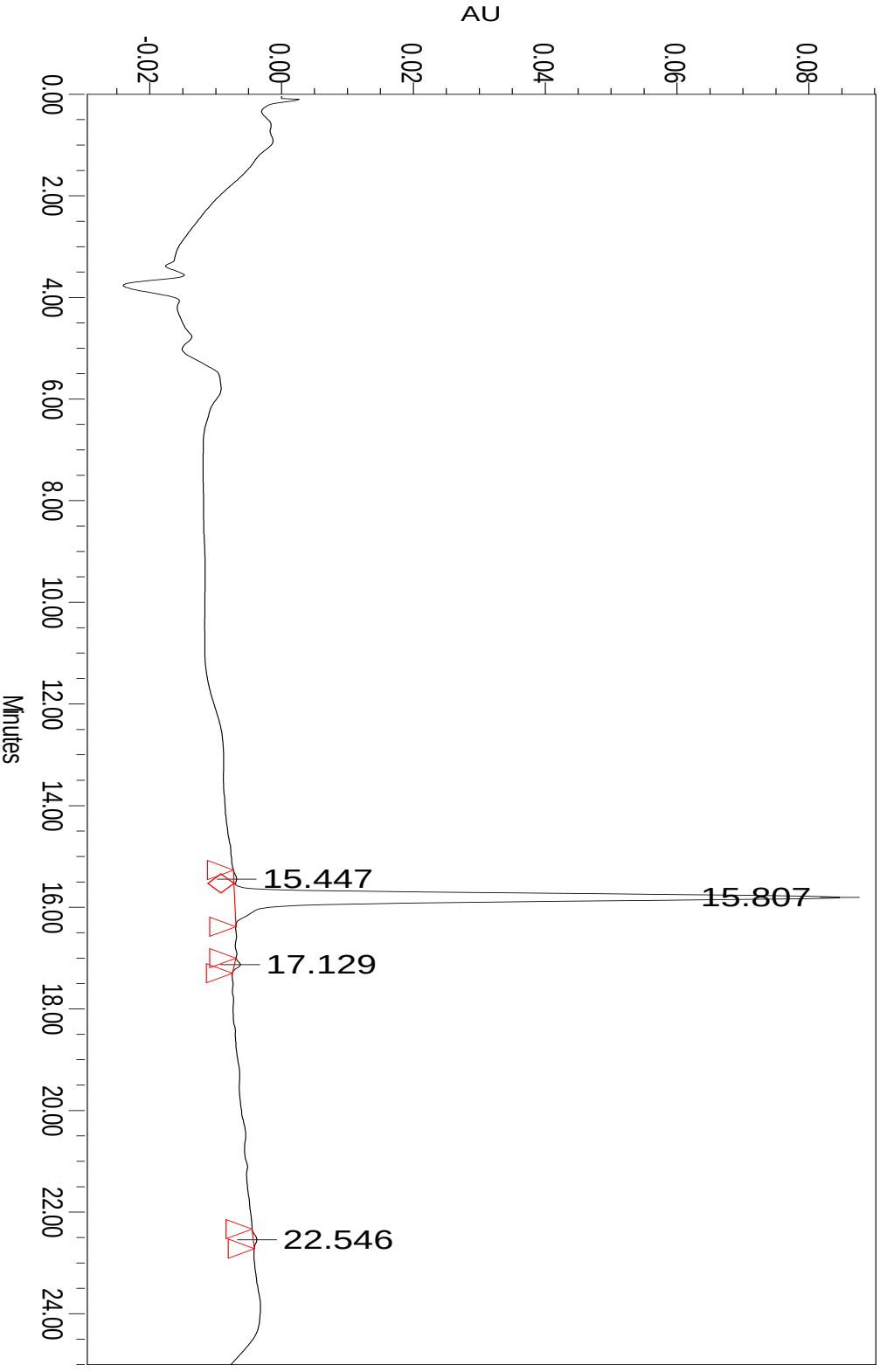


Figure S10. HPLC for peptide **L1.7**.

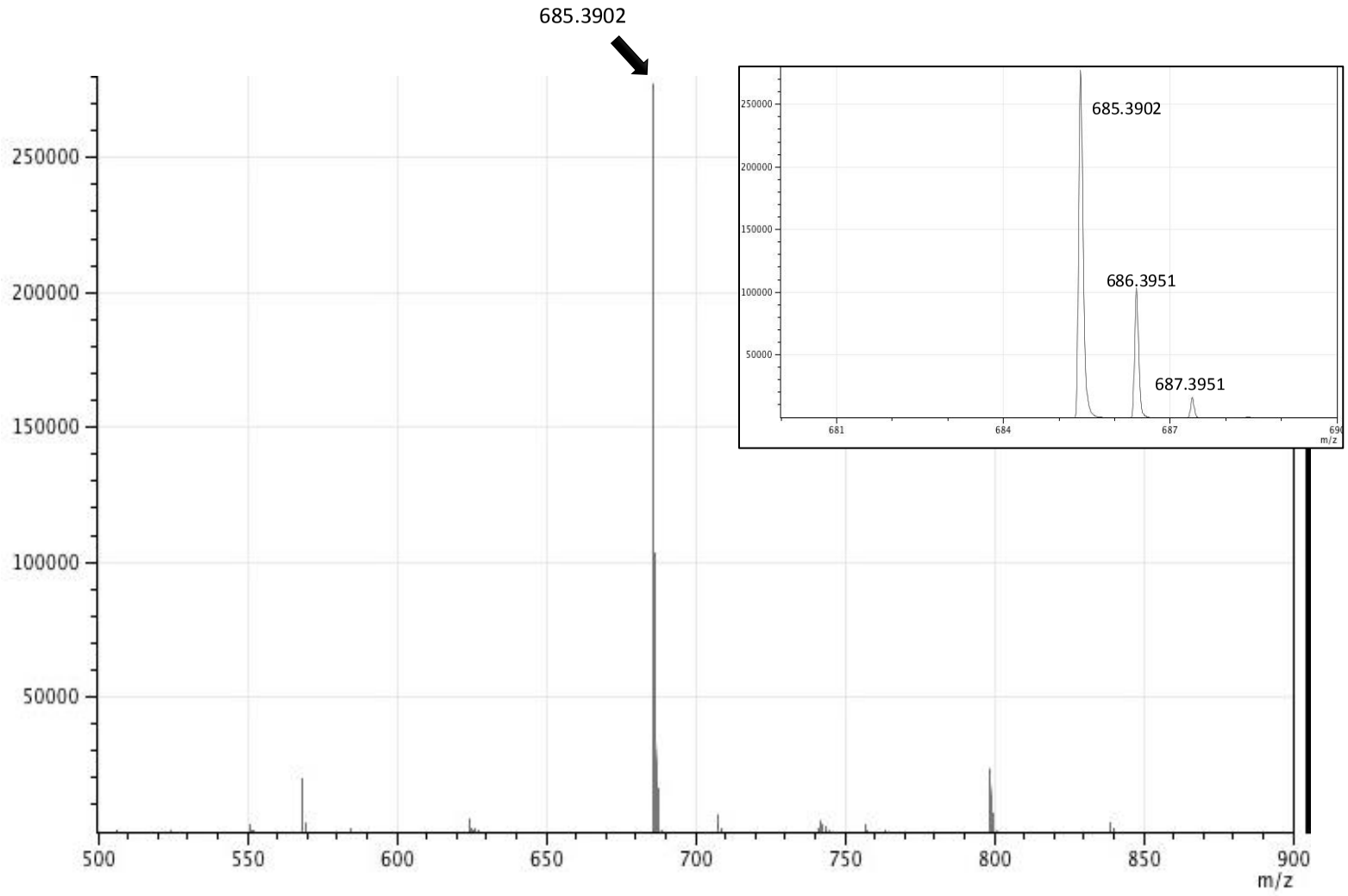


Figure S11. HPLC for peptide L1

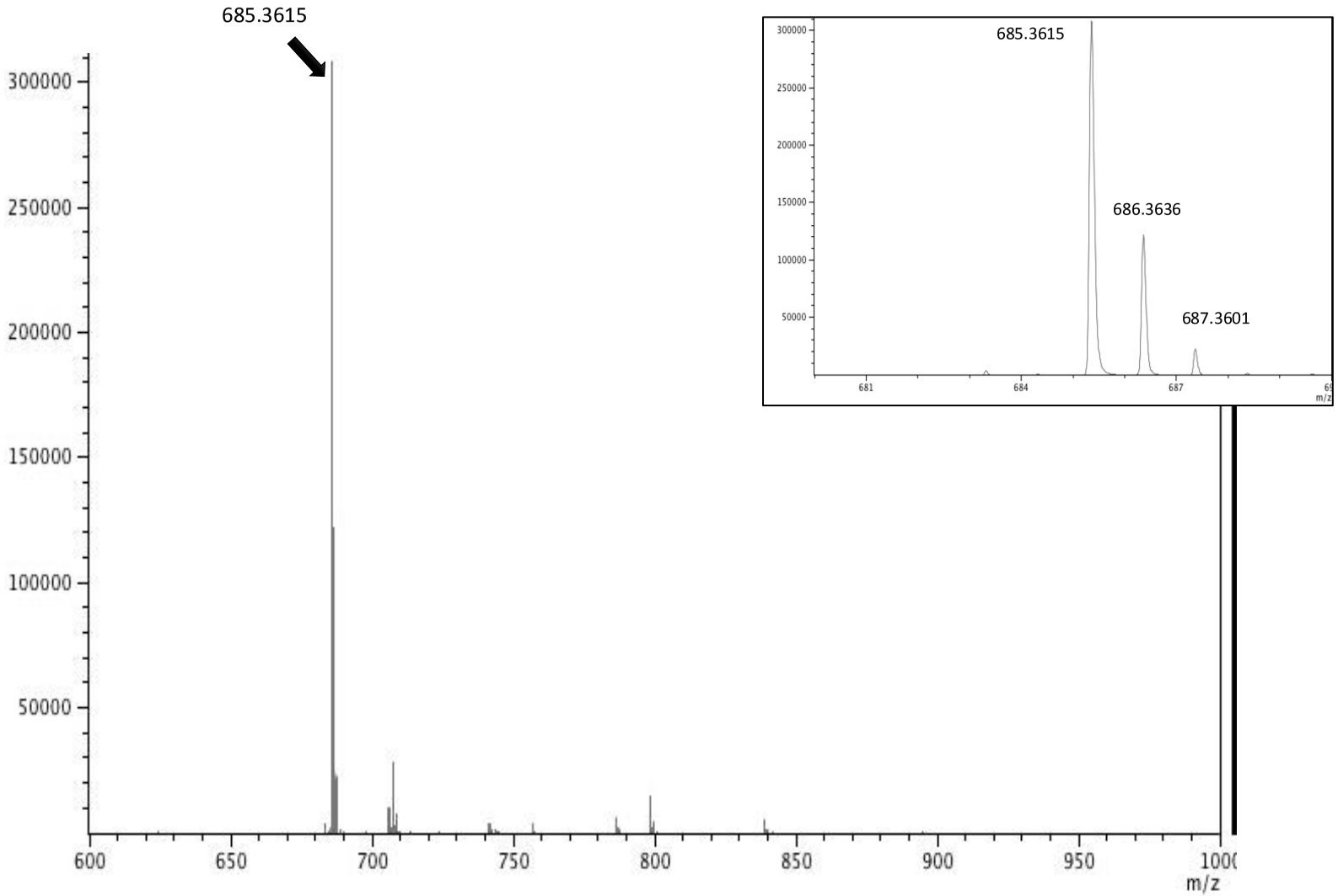


Figure S12. MALDI-TOF of peptide L1.3

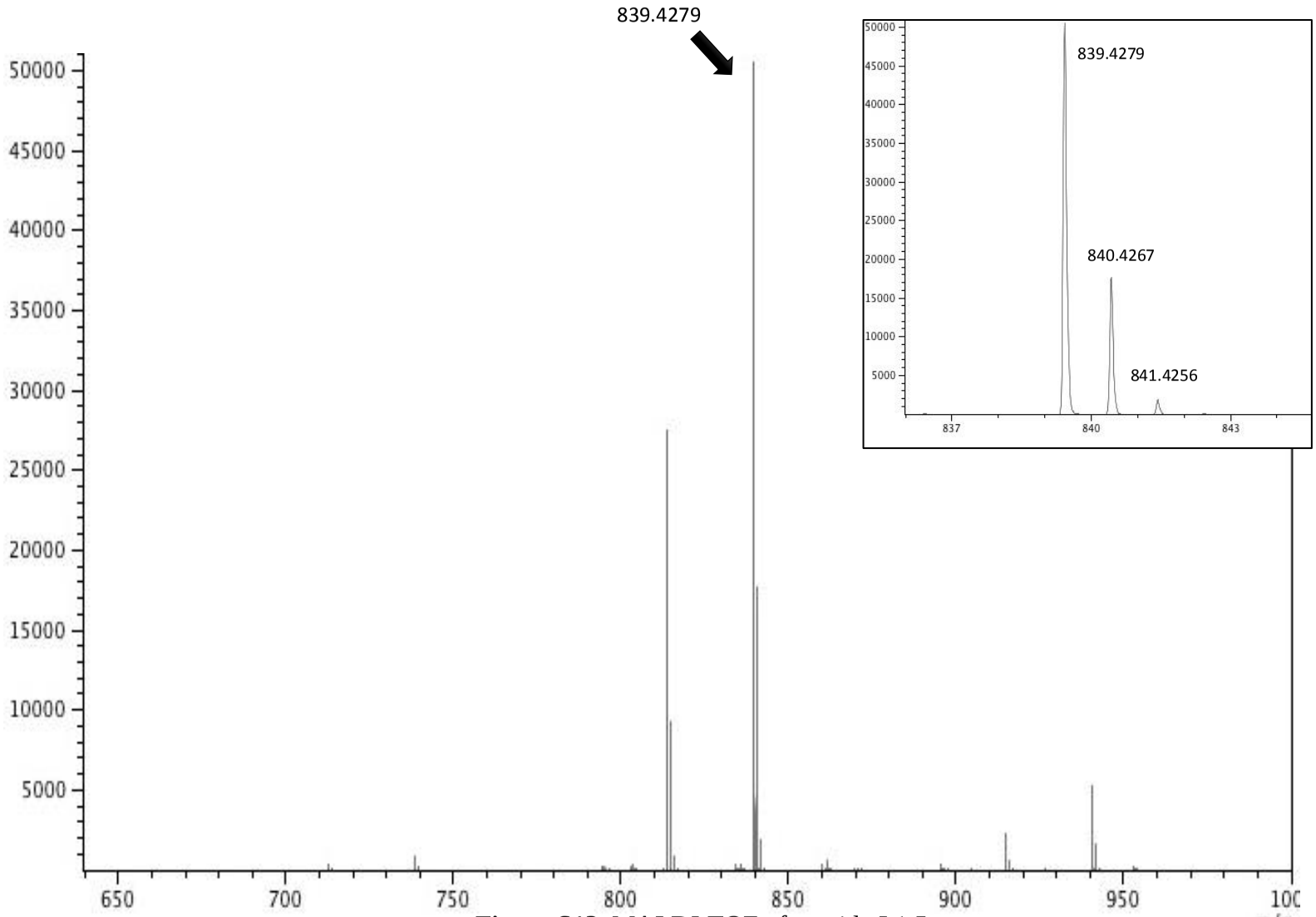


Figure S13. MALDI-TOF of peptide L1.5

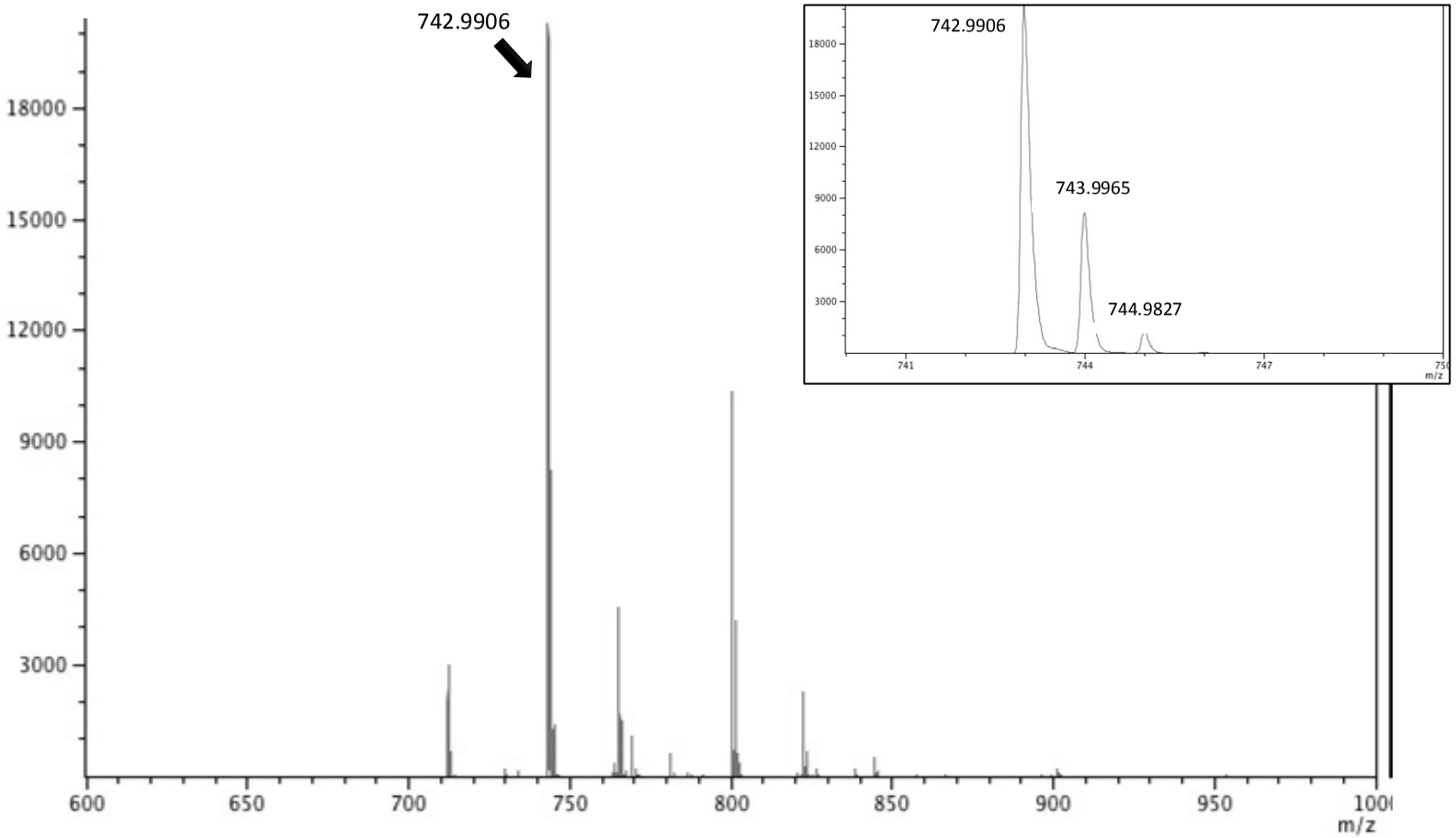


Figure S14. MALDI-TOF of peptide L1.6

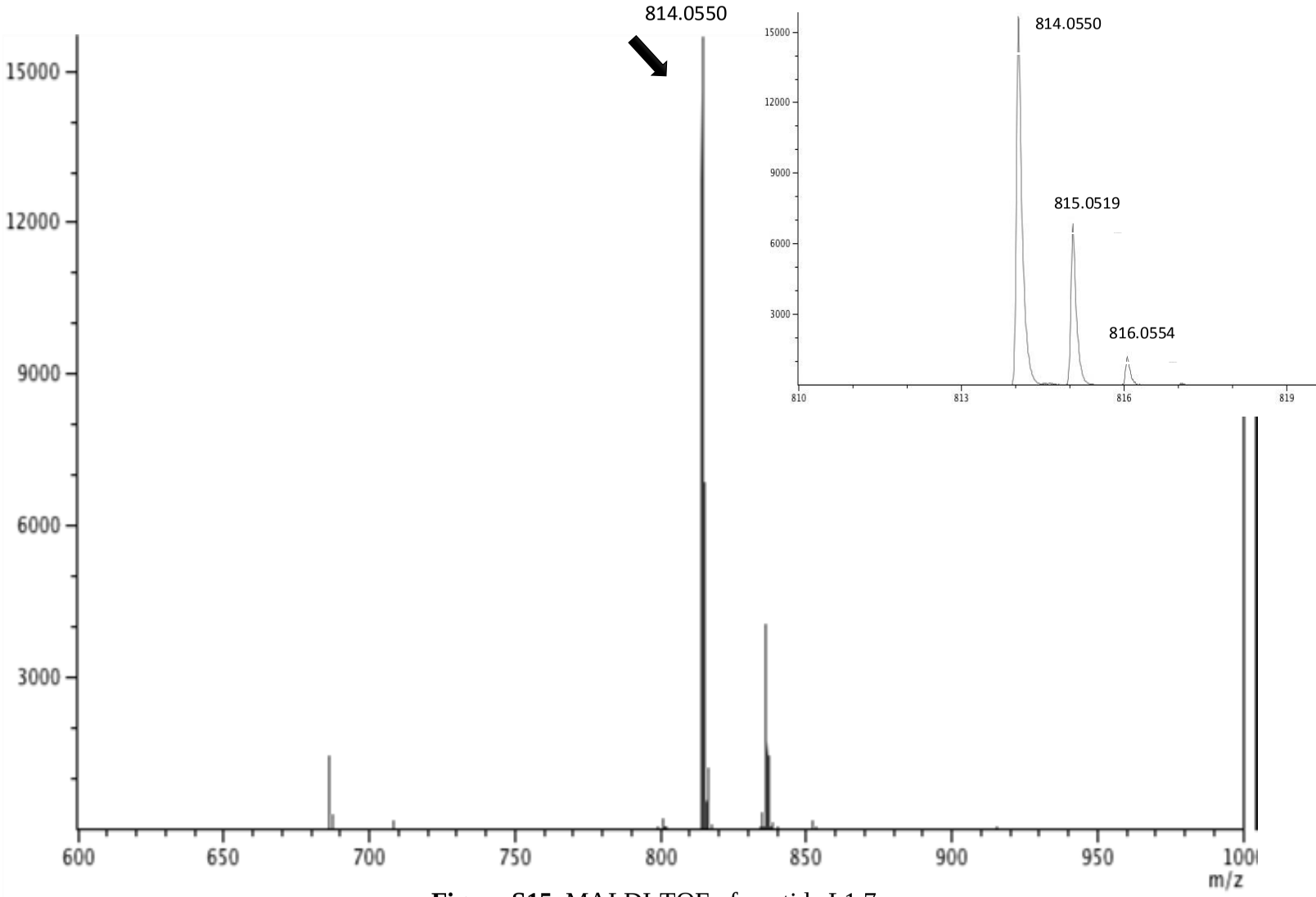


Figure S15. MALDI-TOF of peptide L1.7

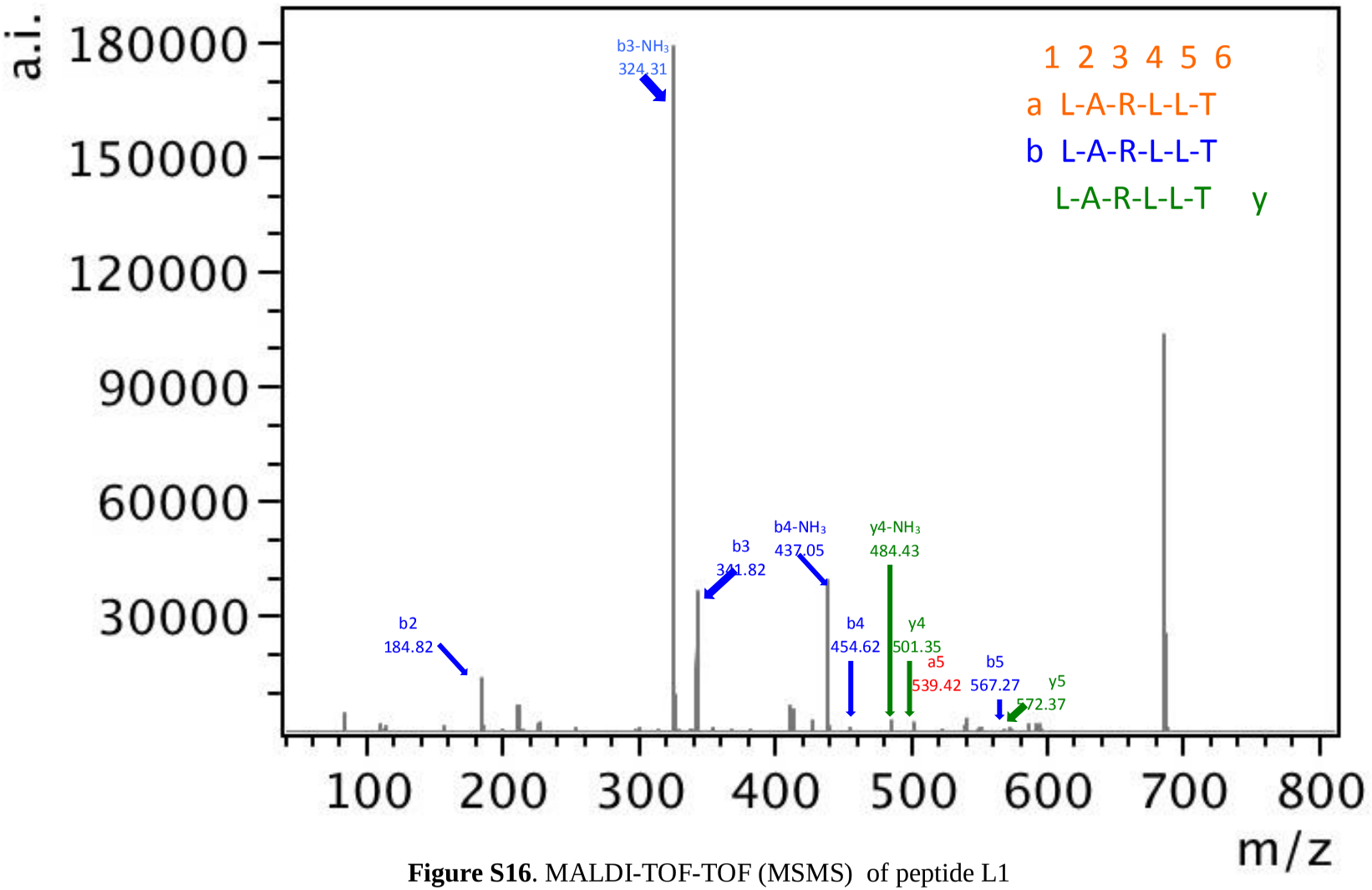


Figure S16. MALDI-TOF-TOF (MSMS) of peptide L1

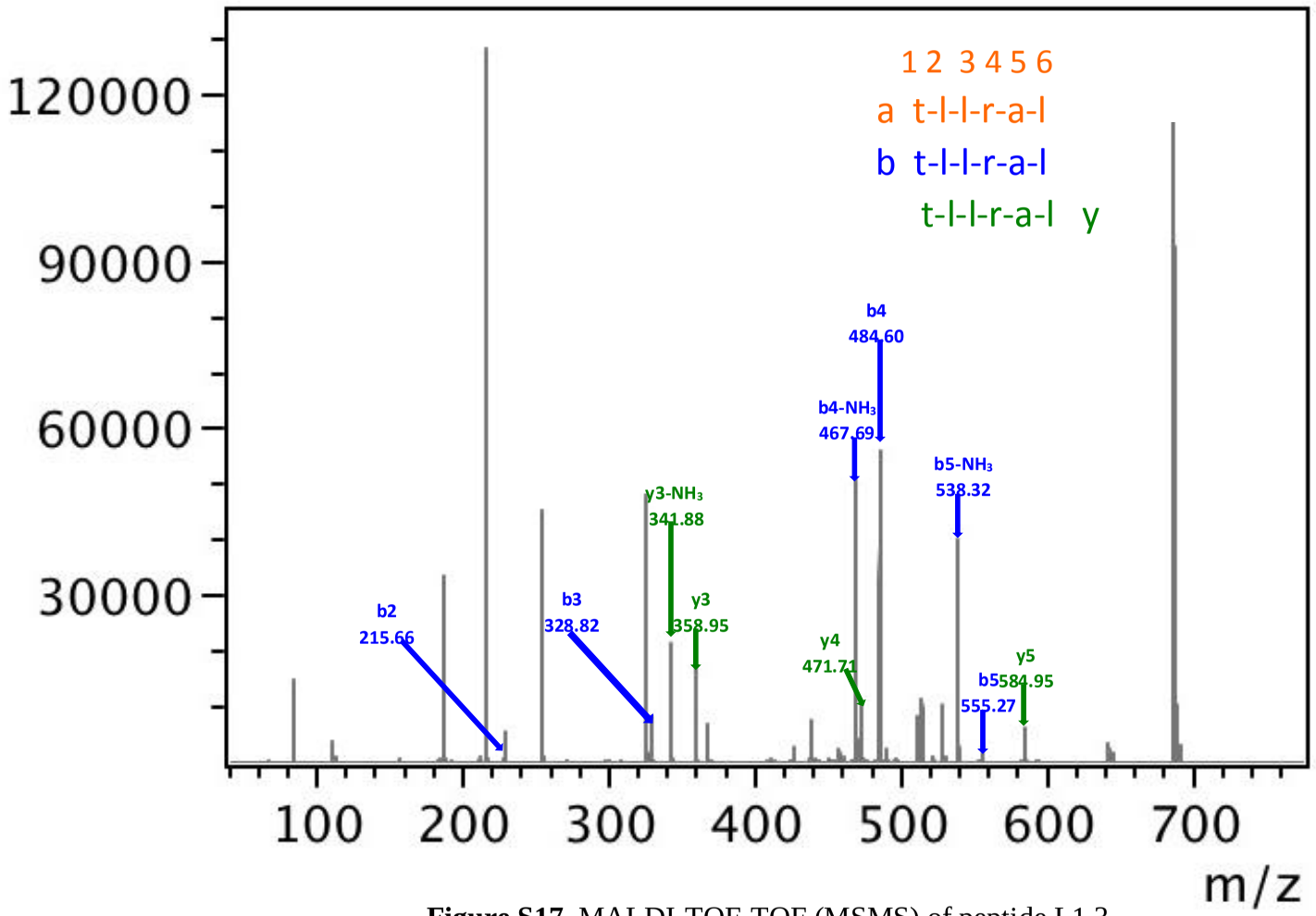


Figure S17. MALDI-TOF-TOF (MSMS) of peptide L1.3

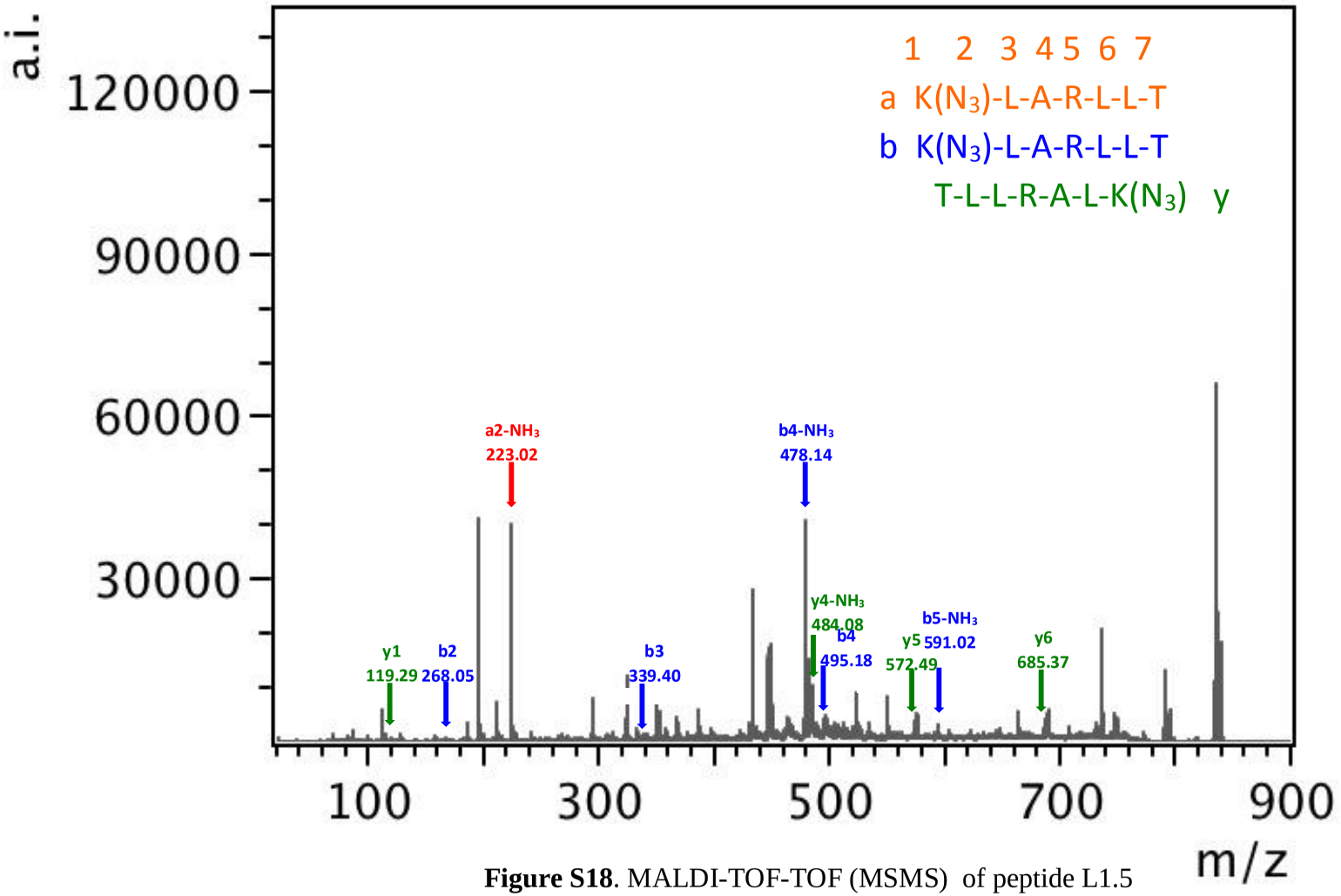


Figure S18. MALDI-TOF-TOF (MSMS) of peptide L1.5

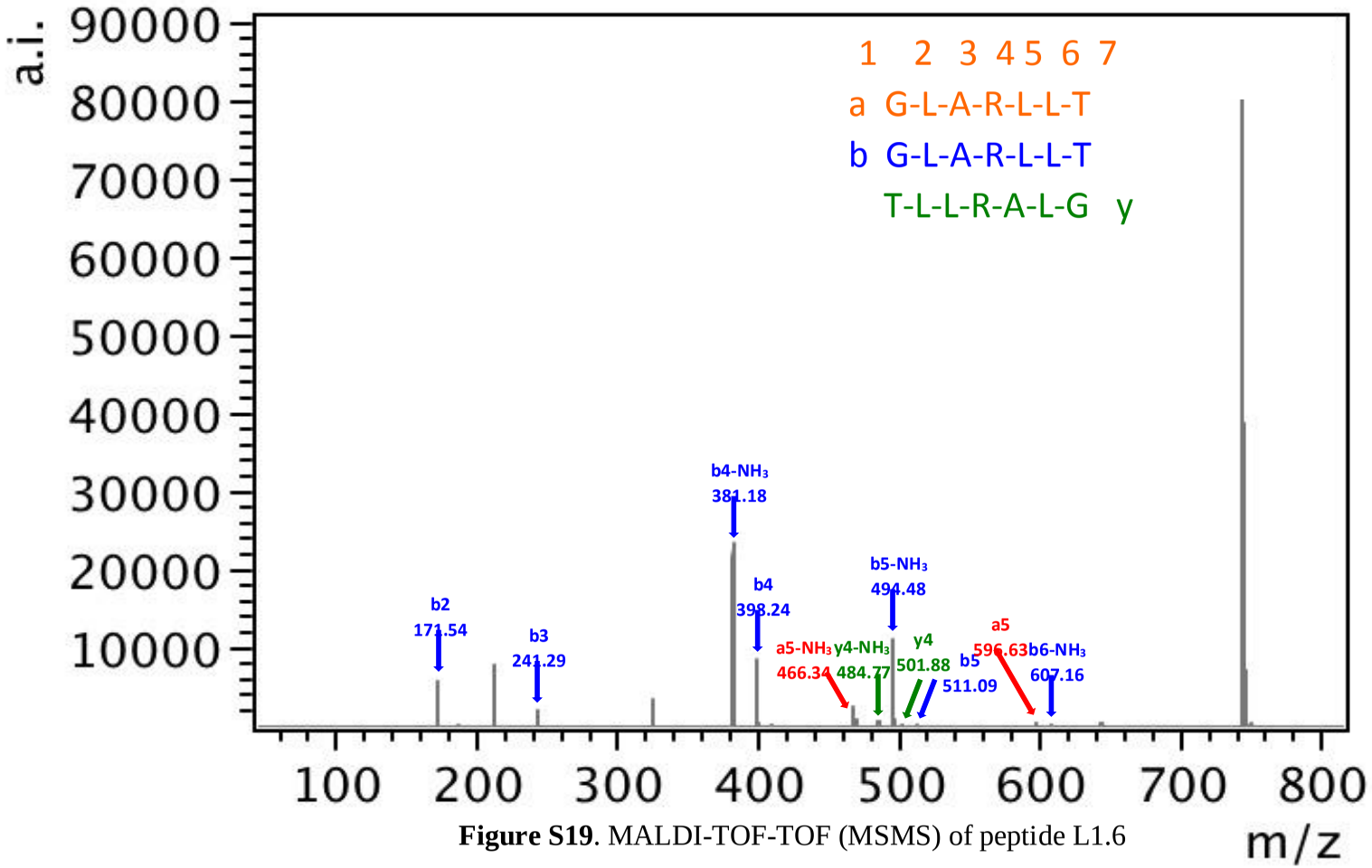


Figure S19. MALDI-TOF-TOF (MSMS) of peptide L1.6

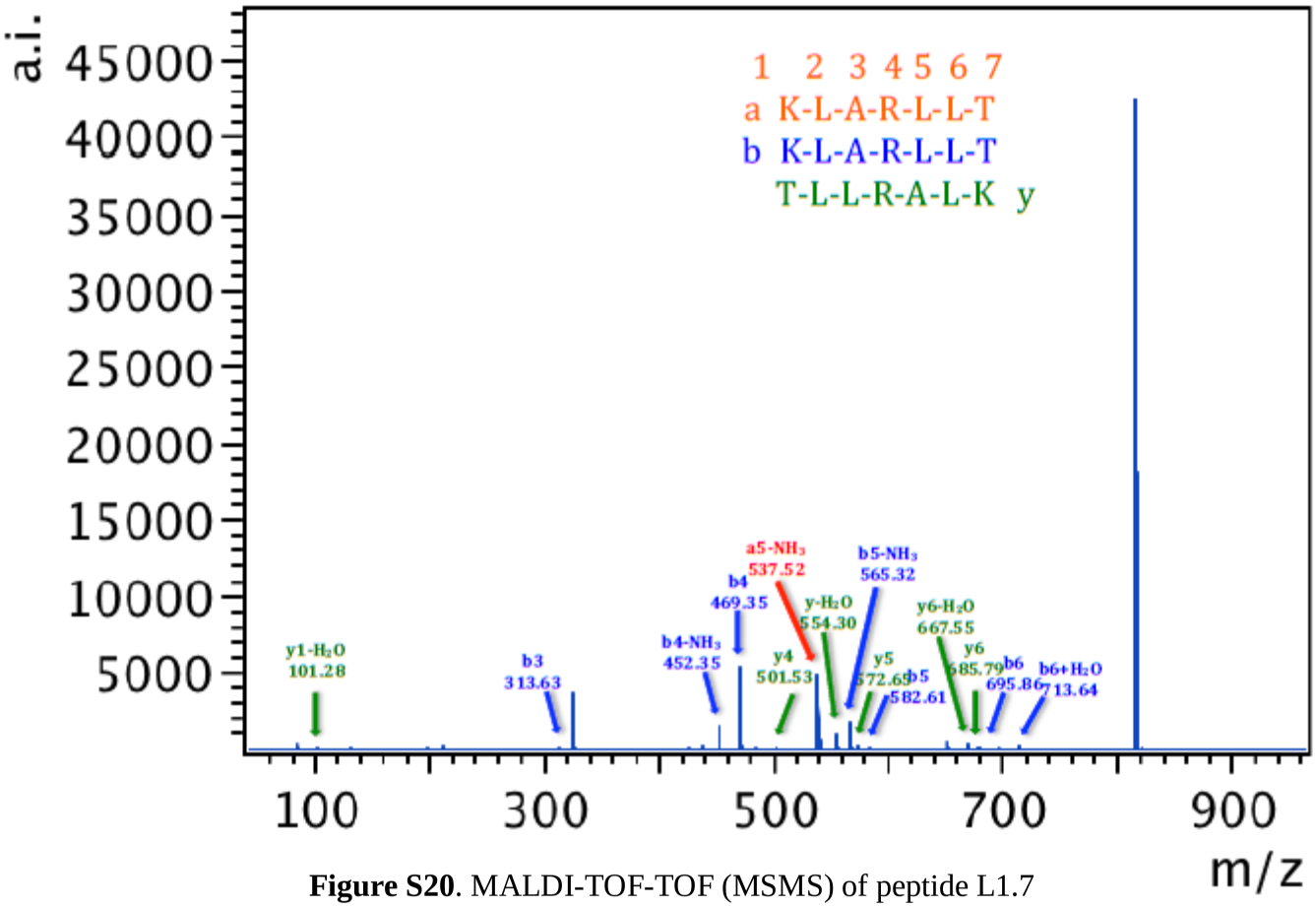


Figure S20. MALDI-TOF-TOF (MSMS) of peptide L1.7

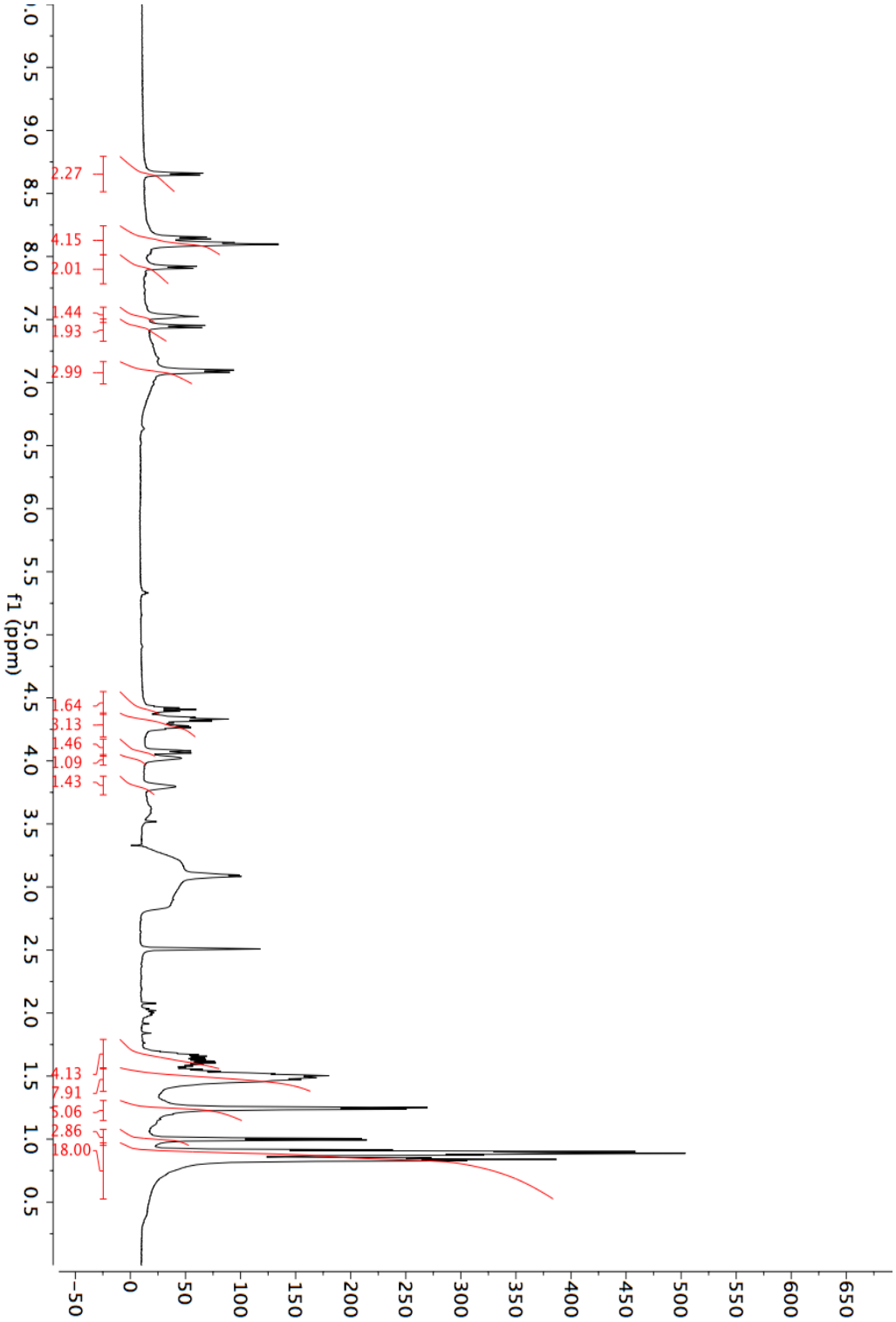


Figure S21. ^{1}HNMR spectrum for peptide **L1**.

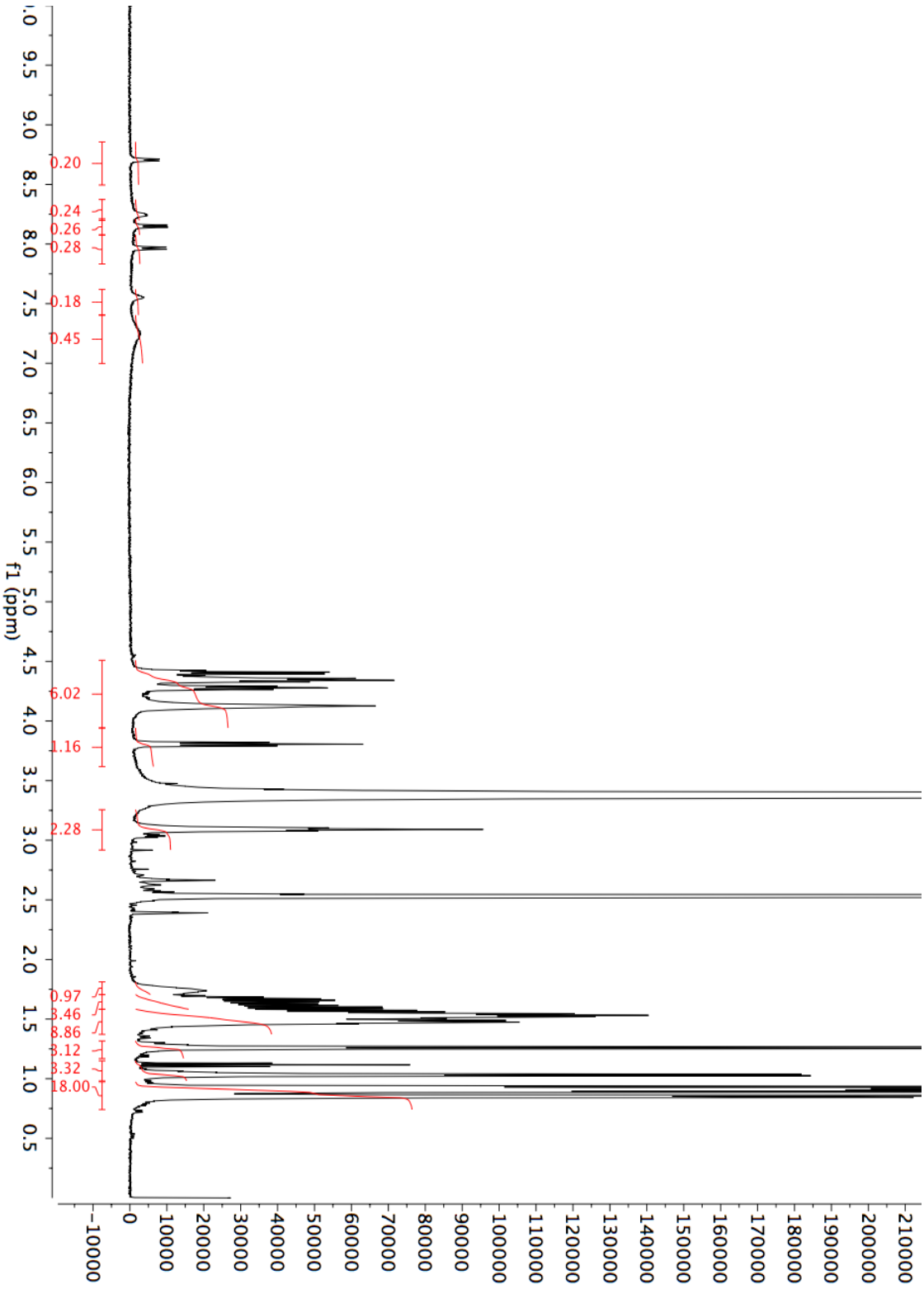


Figure S22. ^{1}HNMR spectrum for peptide **L1.1.**

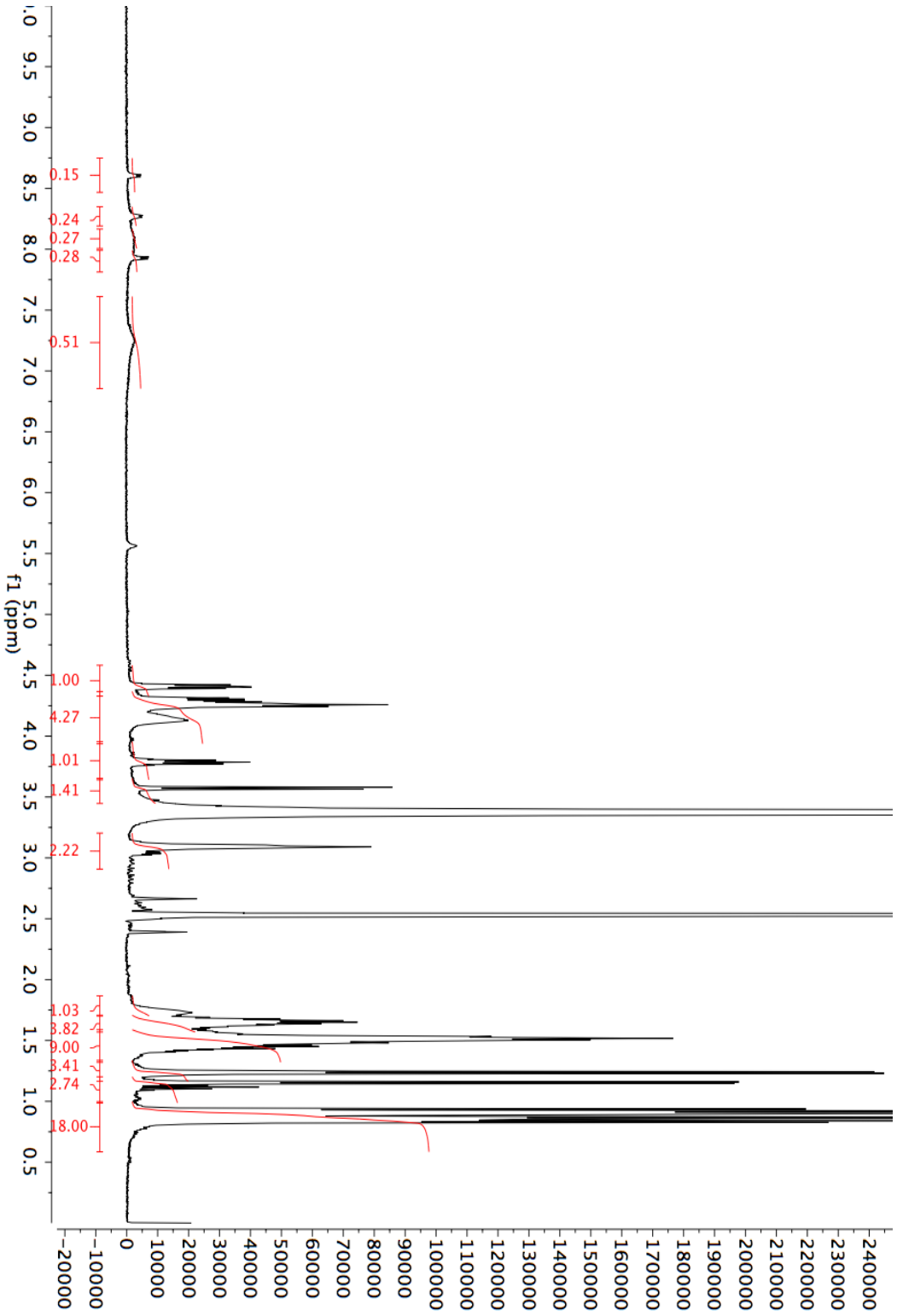


Figure S23. ^{1}HNMR spectrum for peptide **L1.2**.

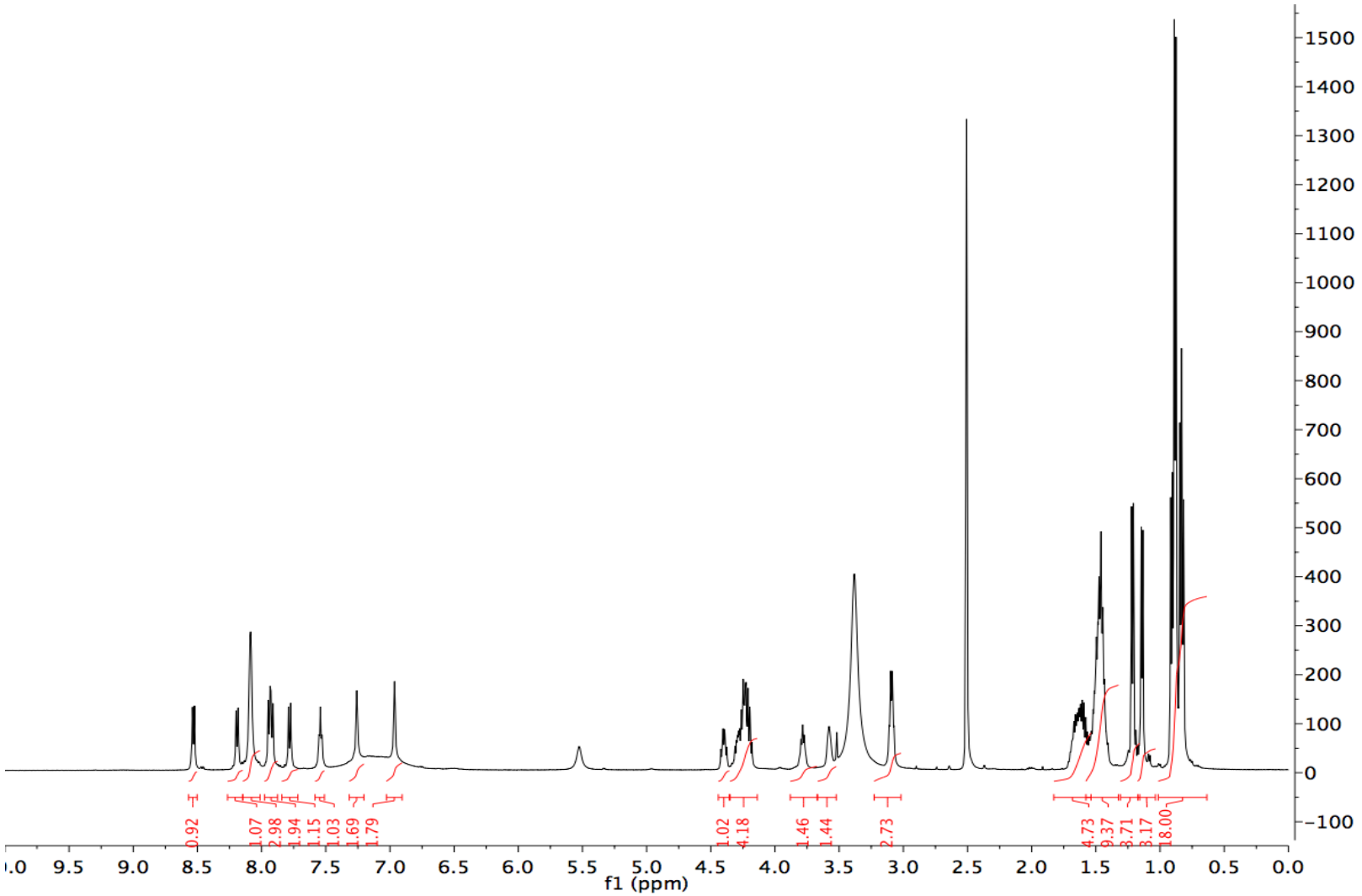


Figure S24. [1]HNMR spectrum for peptide **L1**.3.

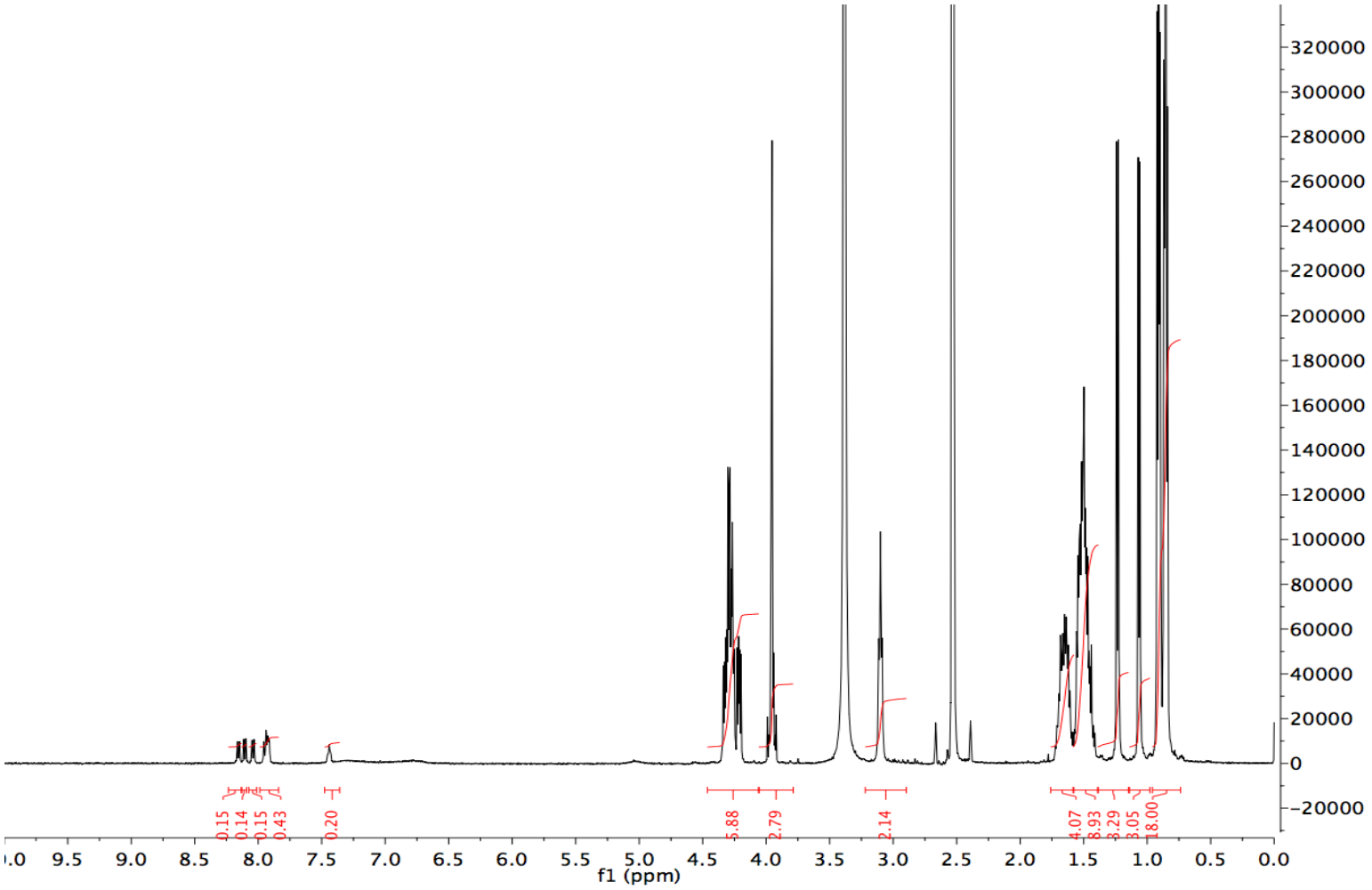


Figure S25. [1]HNMR spectrum for peptide **L1.4**.

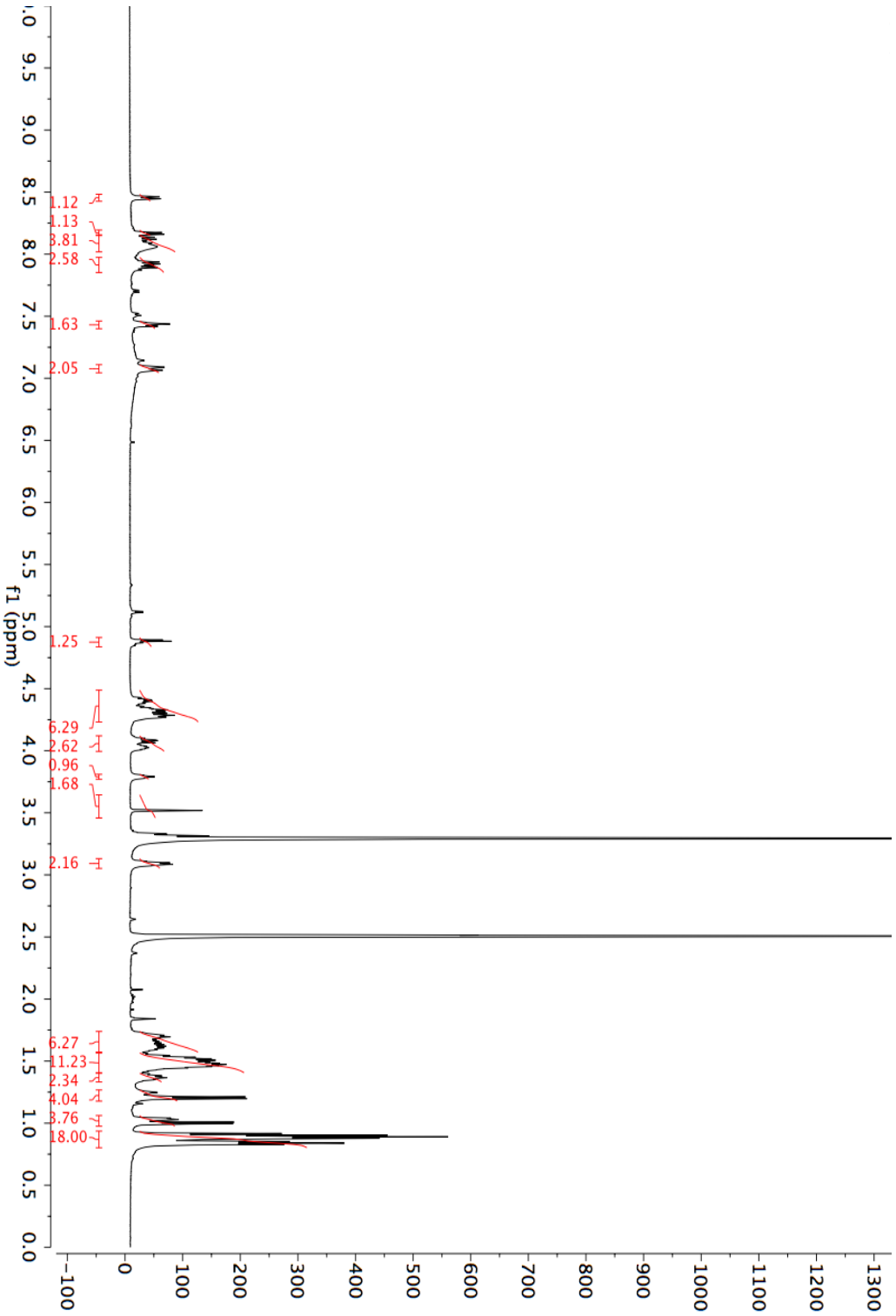


Figure S26. ^{1}HNMR spectrum for peptide **L1**.5.

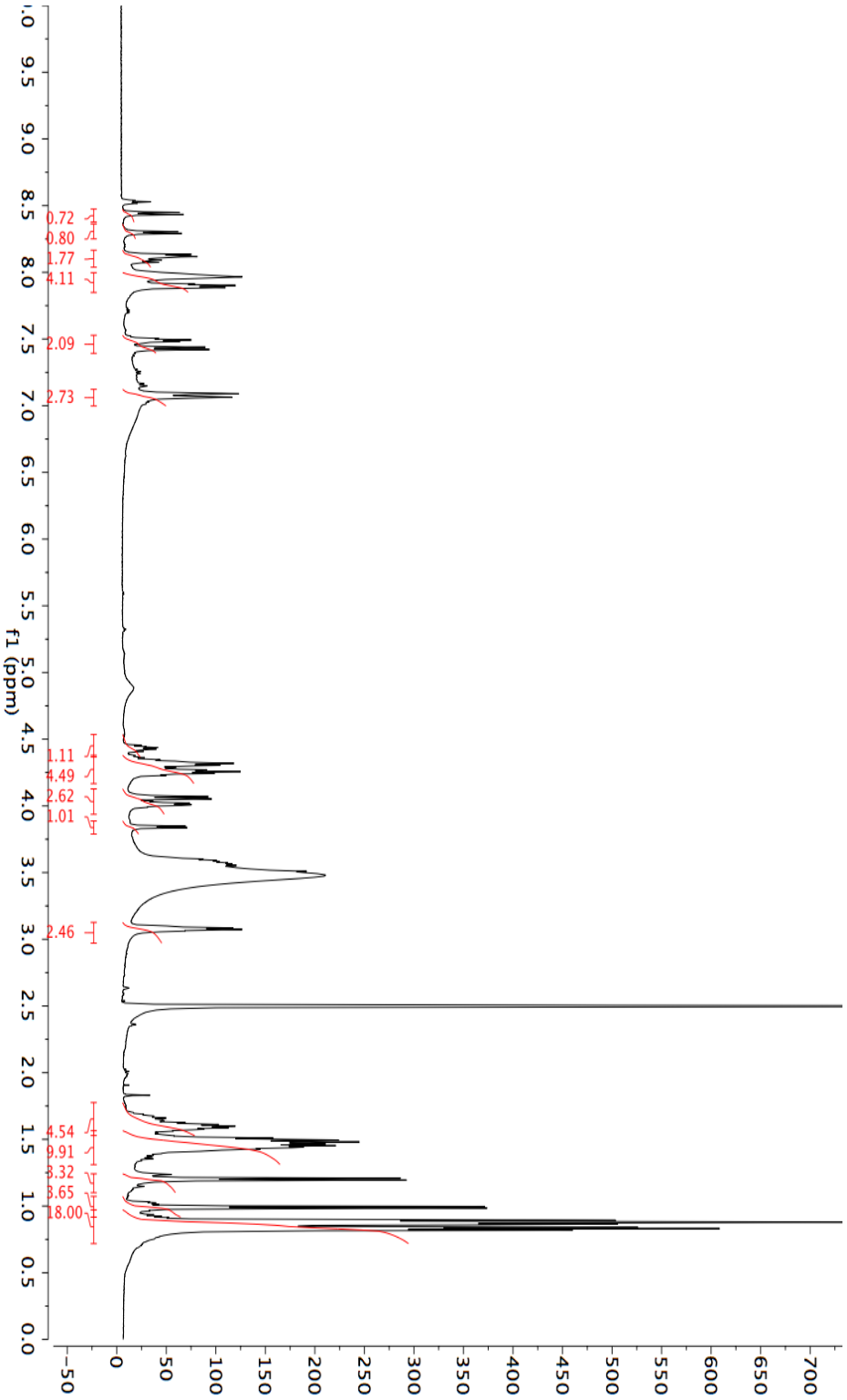


Figure S27. ^{1}HNMR spectrum for peptide **L1.6**.

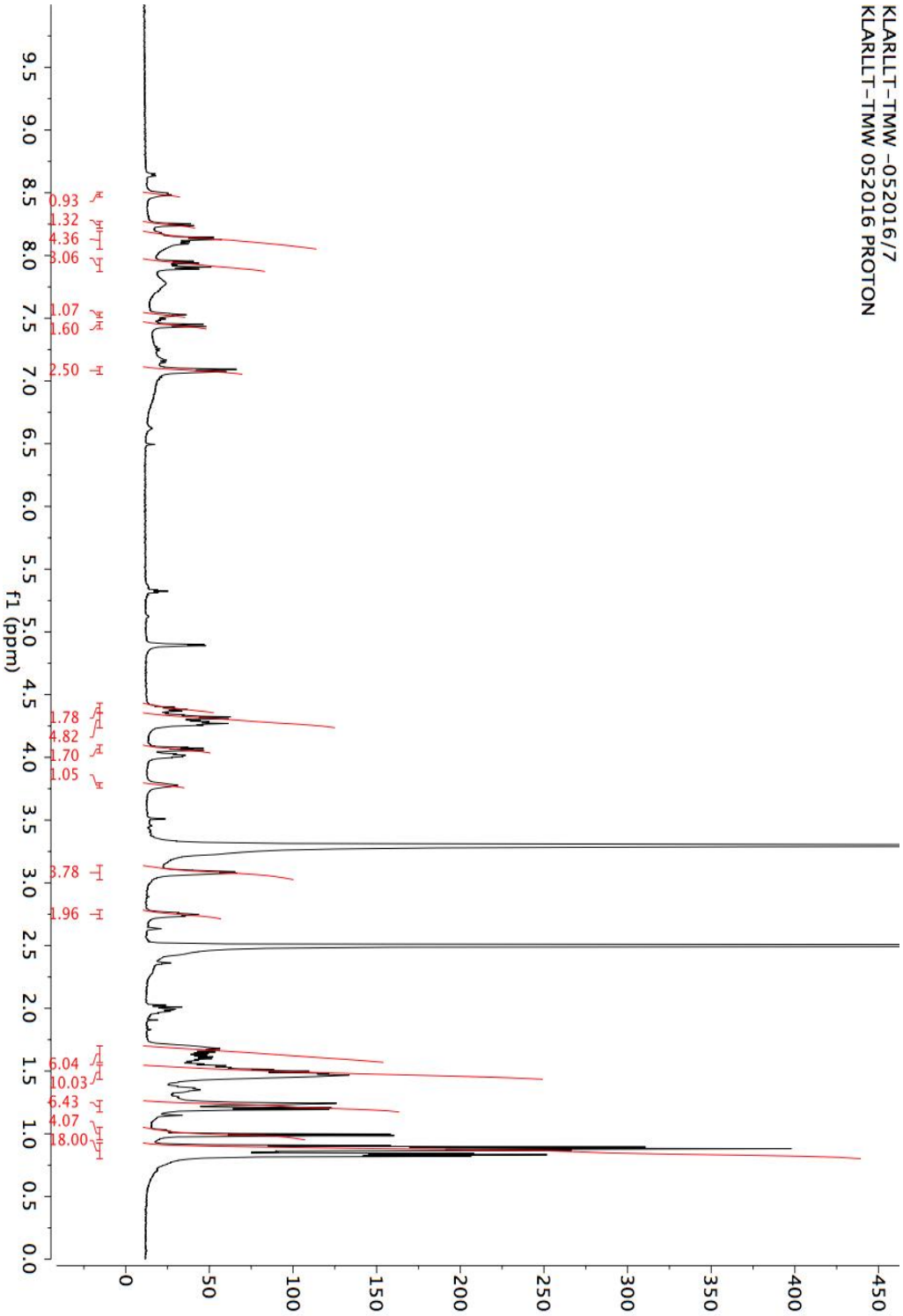


Figure S28. ^{1}HNMR spectrum for peptide **L1.7**.

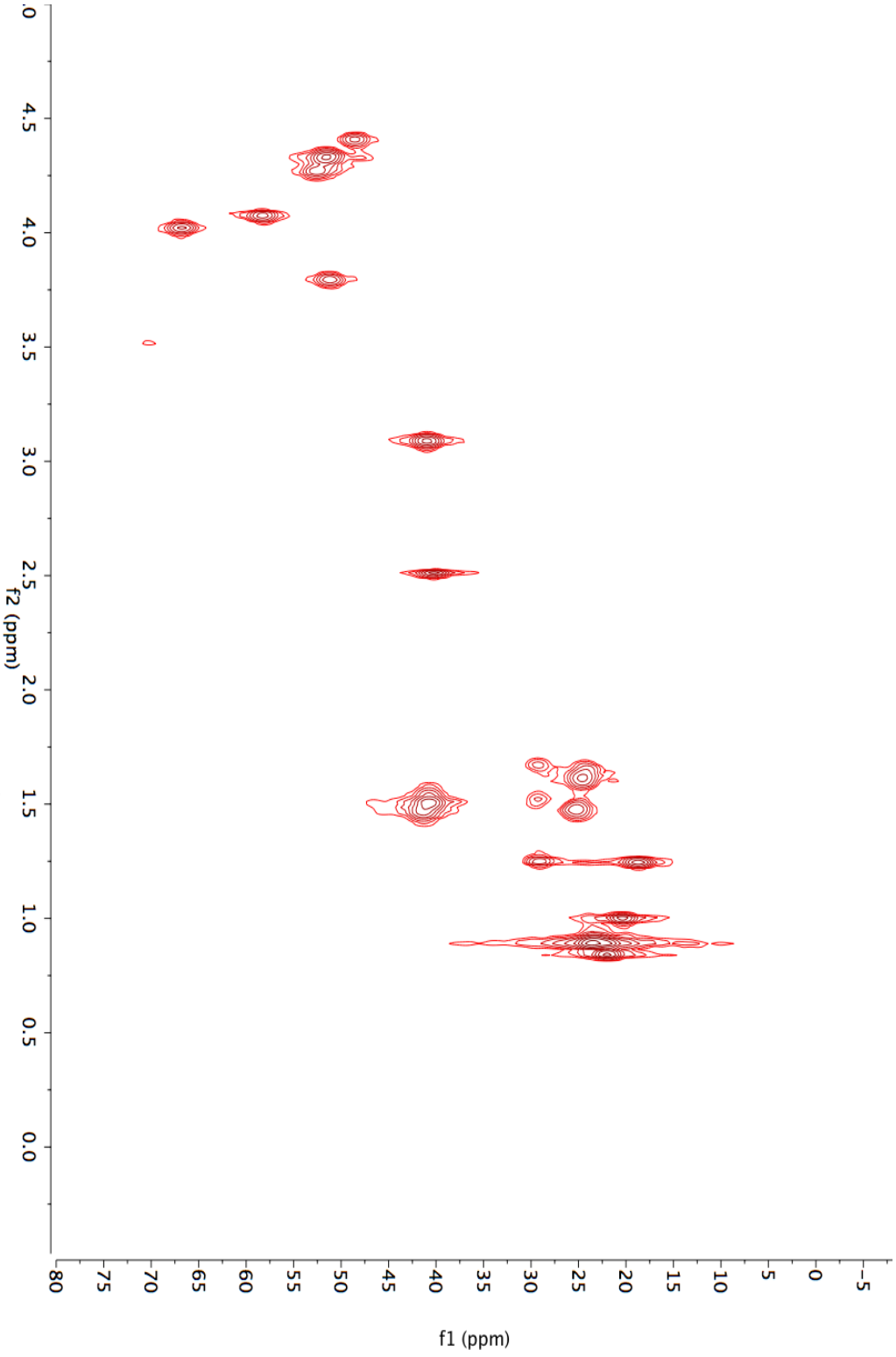


Figure S29. HSQC spectrum for peptide **L1**.

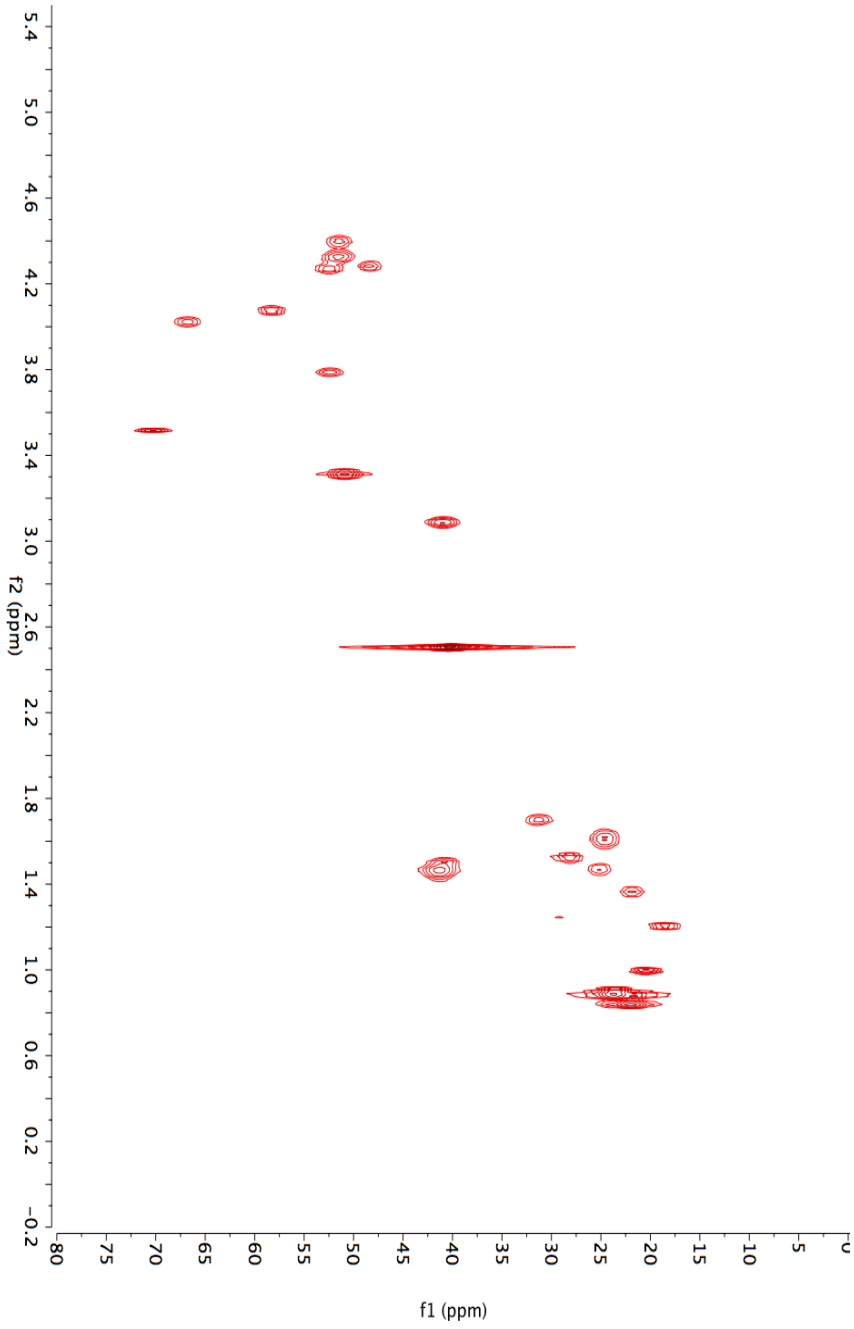


Figure S30. HSQC spectrum for peptide **L1.5**.

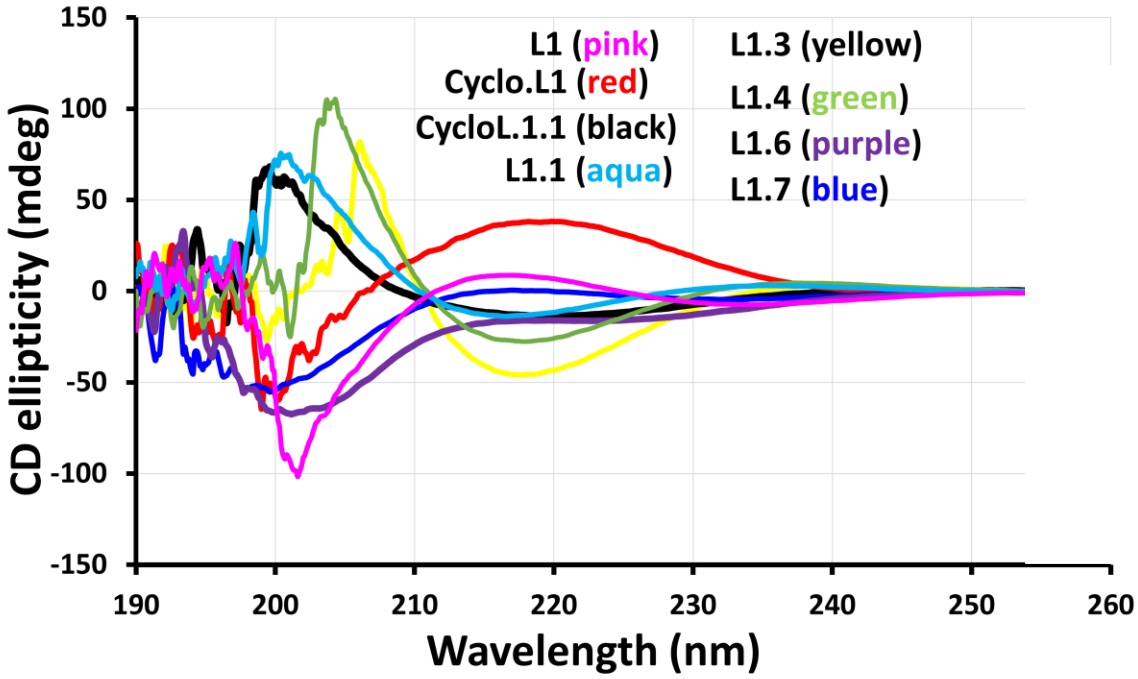


Figure S31. CD spectra of linear and cyclic peptides in methanol. 1 mg/mL of peptide was dissolved in methanol and CD spectra was acquired at 25 °C, and the spectra were average of 4 scans.