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Supporting Information

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Simone Mariani, Claudia Honisch, Paolo Ruzza, and Stefano Tartaggia*

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Supporting information

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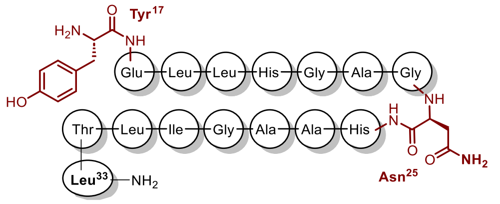
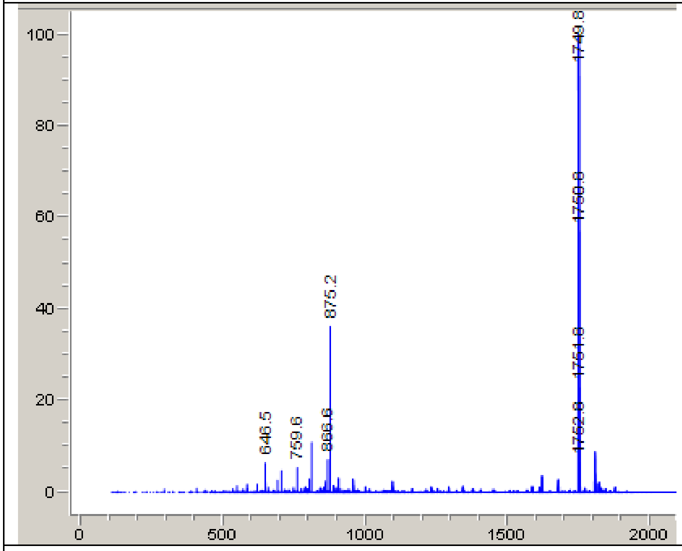
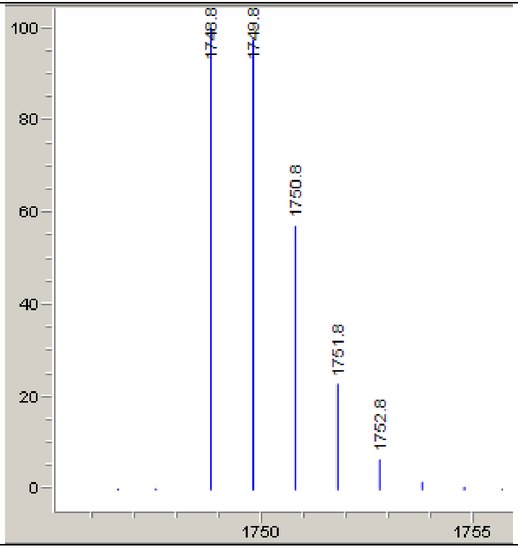
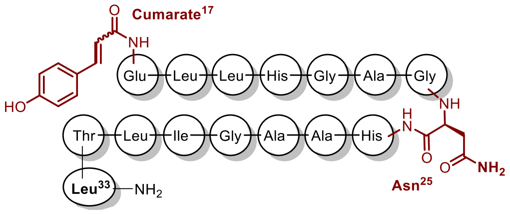
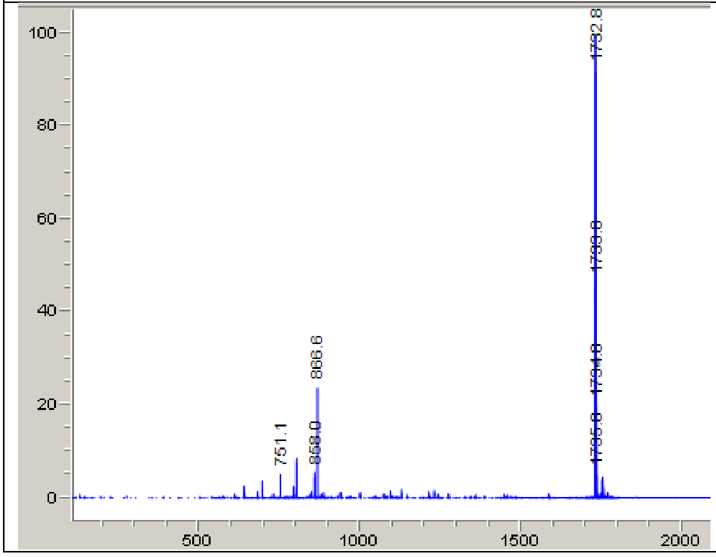
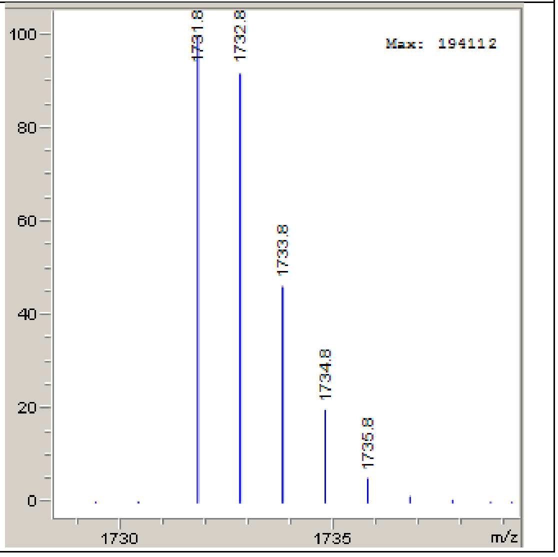
Entry 1. Peptide **1**: Tyr^{17}-Leu^{33}(Asn^{25}) m/z (M+H) = 1748.8	
	
Entry 2. Peptide (*E*/*Z*)-**4**: (*E*/*Z*)-*p*-Coumarate^{17}-Leu^{33}(Asn^{25}) from irradiation experiment: m/z (M+H) = 1731.8	
	

Table S1. ESI-MS of peptides 1 and (*E*/*Z*)-*4* with zoomed molecular ion peak (continued).

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Entry 3. Peptide (*E*/*Z*)-**4**: (*E*/*Z*)-*p*-Coumarate^{17}-Leu^{33}(Asn^{25}) from SPPS: m/z (M+H) = 1731.8

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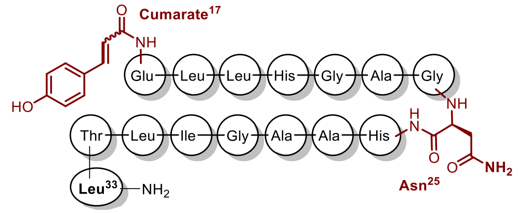

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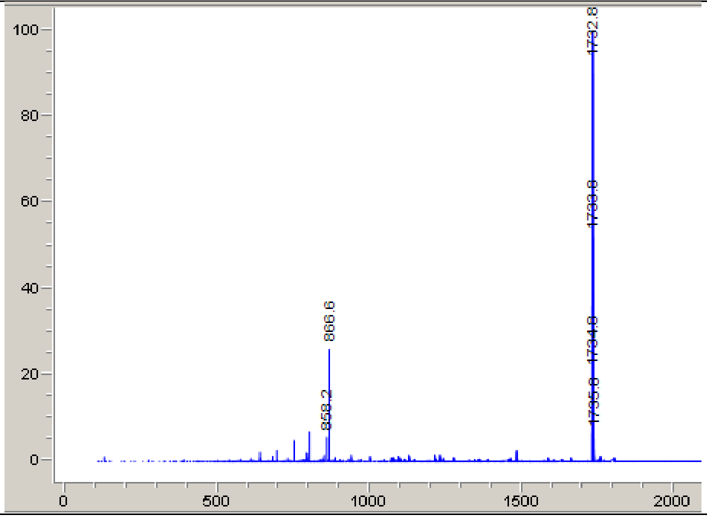

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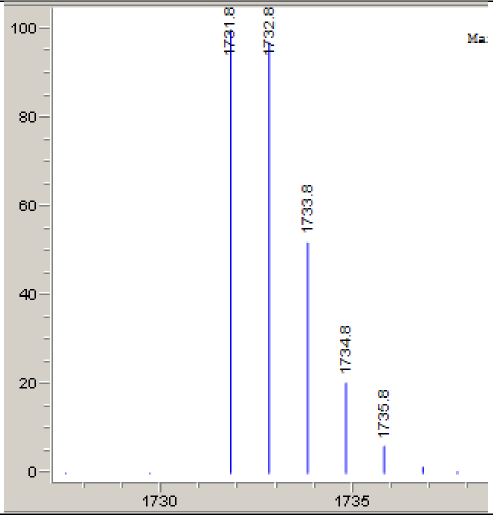

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Table S2. ESI-MS of peptides 2 and (*E*/*Z*)-*5* with zoomed molecular ion peak

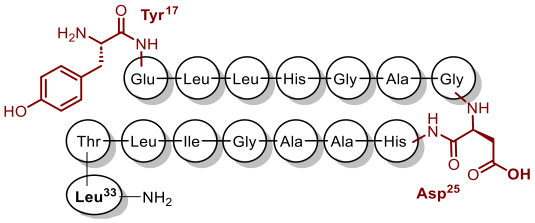
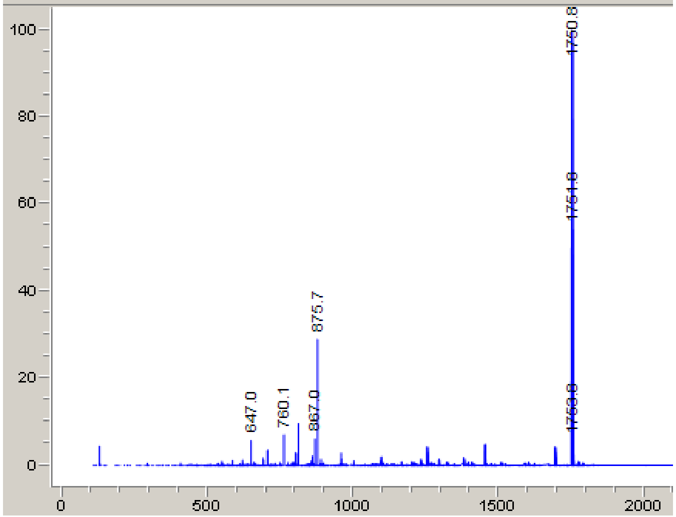
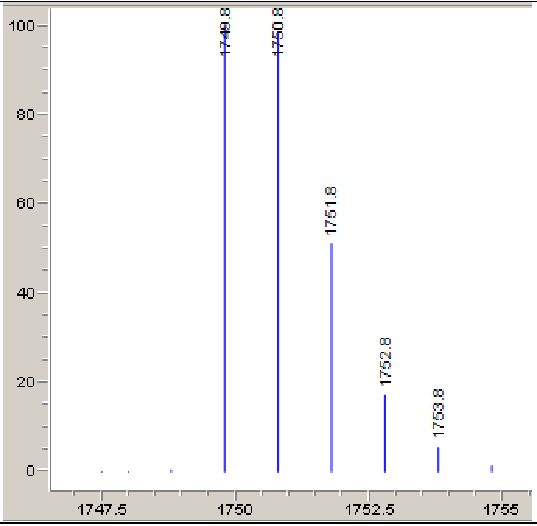
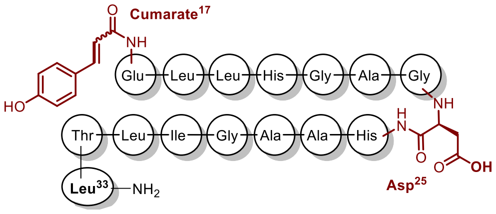
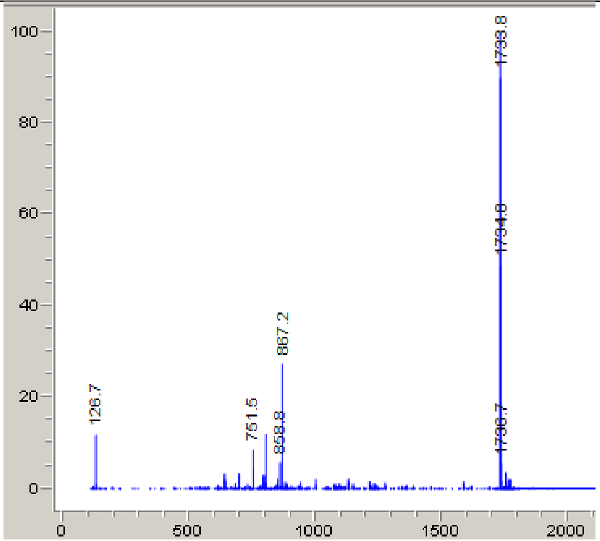
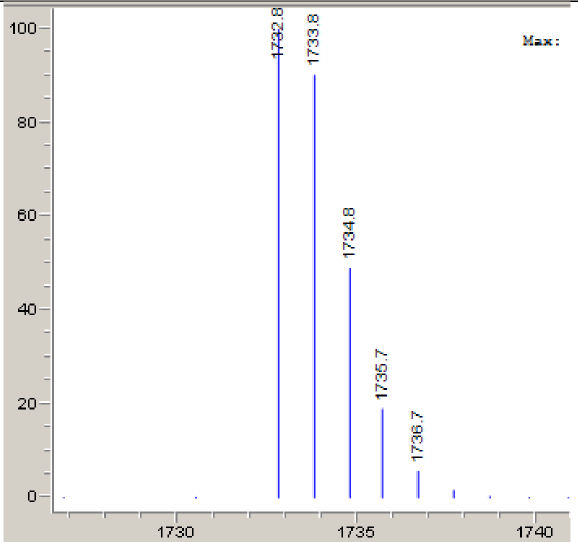
Entry 1. Peptide **2**: Tyr^{17}-Leu^{33}(Asp^{25}) m/z (M+H) = 1749.8	
	
Entry 2. Peptide (*E*/*Z*)-**5**: (*E*/*Z*)-*p*-Coumarate^{17}-Leu^{33}(Asp^{25}) from irradiation experiment: m/z (M+H) = 1732.8	
	

Table S3. ESI-MS of peptides 3. and (*E*/*Z*)-*6* with zoomed molecular ion peak

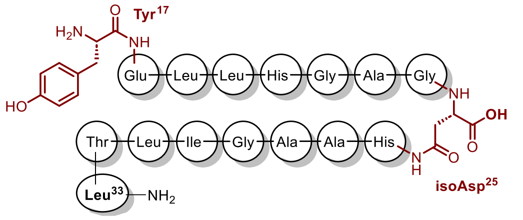
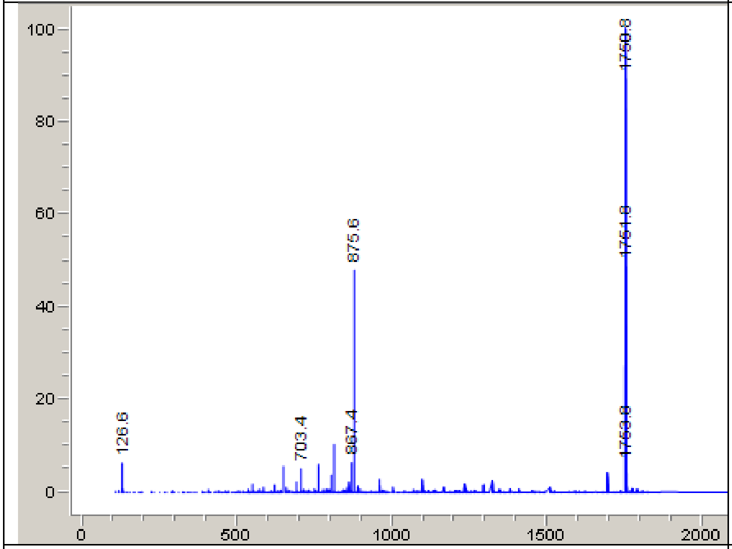
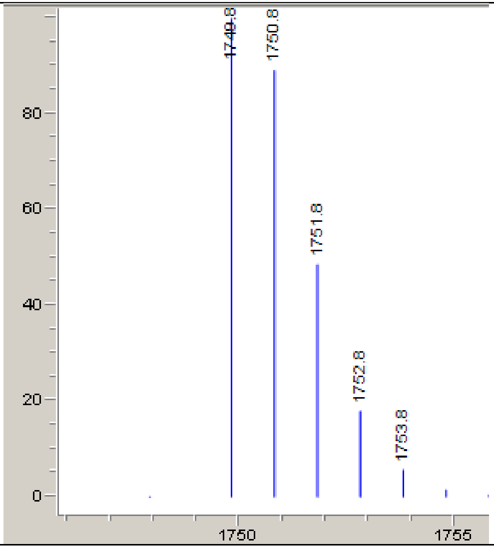
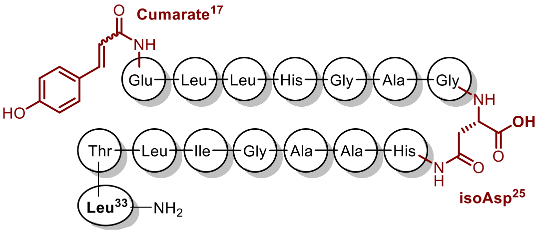
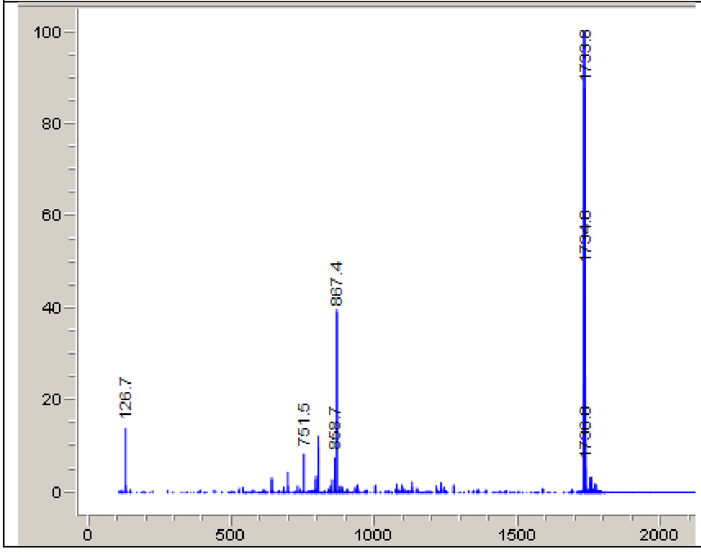
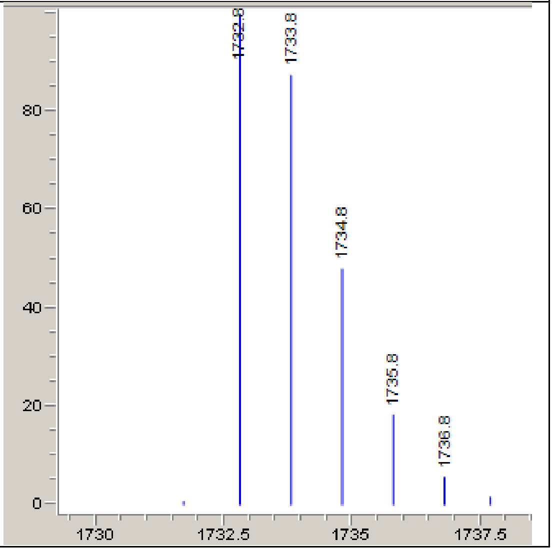
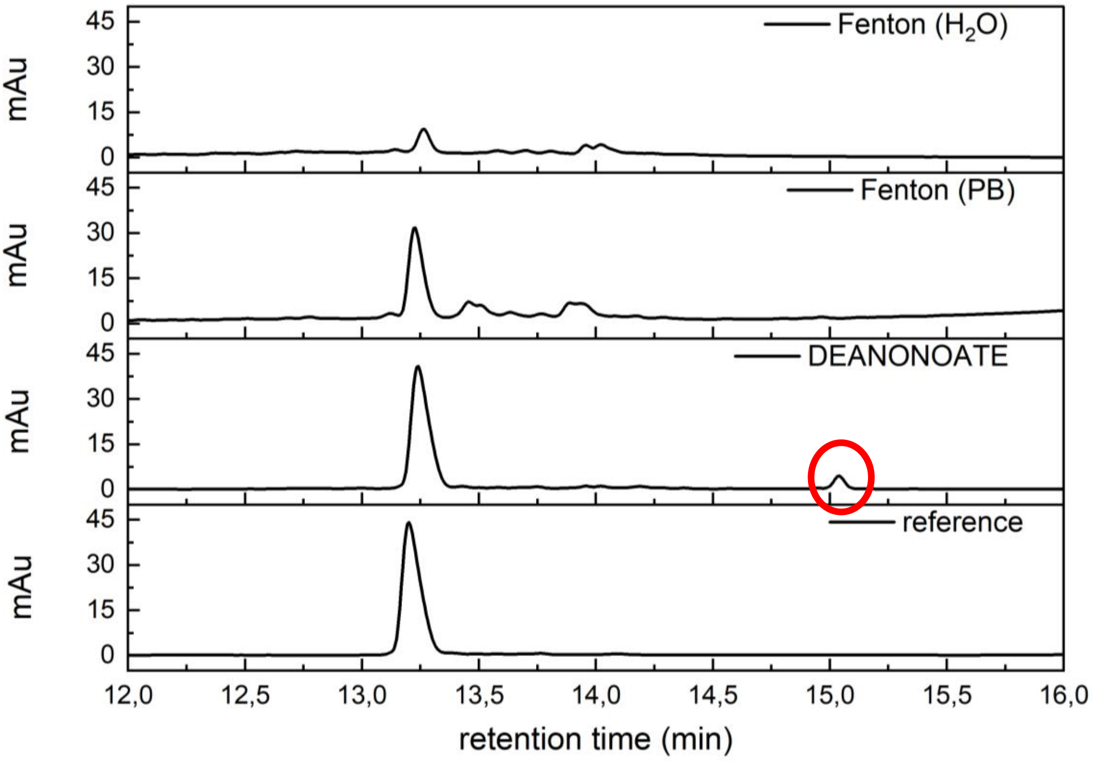
Entry 1. Peptide **3**: Tyr^{17}-Leu^{33}(isoAsp^{25}) m/z (M+H) = 1749.8	
	
Entry 2. Peptide (E/Z)-**6**: (*E*/*Z*)-*p*-Coumarate^{17}-Leu^{33}(isoAsp^{25}) from irradiation experiment: m/z (M+H) = 1732.8	
	

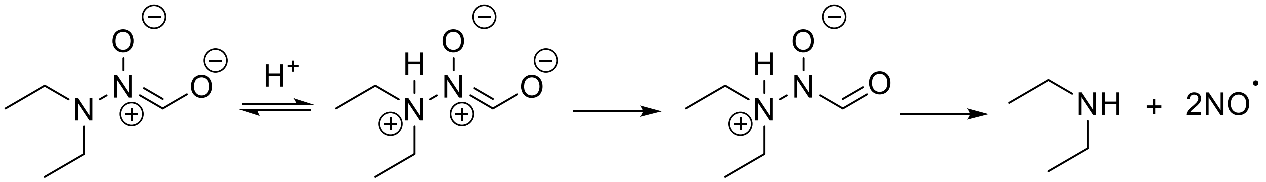
Table S4. Degradation of peptide 1 after treatment with Fenton reagent for 2 h. The experiments were performed by diluting peptide **1** (1 mg/mL in H_2O) with different solvents as indicated in the table, and mixing with variable amount of Fe(SO_4) (2 mM in H_2O) and H_2O_2 (20 mM in H_2O) to obtain a final peptide concentration of 0.15 mg/mL (86 μM) in a final volume of 200 μL and a variable ratio between the reagents as indicated in the table. The solutions were prepared in 0.5 mL eppendorf vials and were mixed for two hours under dark conditions. Afterwards, reactions mixtures were injected and analyzed by HPLC.

Entry	$FeSO_4$ (eq.)	H_2O_2 (eq.)	solvent	Temperature (°C)	Residual peptide (%)
1	2	0	H_2O	25	100
2	0	20	H_2O	25	100
3	0	200	H_2O	25	87
4	0,2	20	H_2O	25	19
5	1	20	H_2O	25	11
6	2	20	H_2O	25	7,4
7	2	20	H_2O	0	8,6
8	0	200	PB (10 mM)	25	100
9	1	200	PB (10 mM)	25	91
10	2	200	PB (10 mM)	25	52
11	5	200	PB (10 mM)	25	32
12	5	20	PB (10 mM)	25	61

Figure S1. HPLC profiles of peptide 1 treated with DEA NONOate and Fenton reagent. Peptide **1** (0.15 mg/mL, 86 µM) appears at RT of 13.2 min (reference) and provided a detectable amount of (*E*/*Z*)-**4** (RT 15.0 min) after treatment with DEA NONOate (100 eq in PB 10 mM pH 7.0). Instead, chromatograms of **1** treated with Fenton reagent in PB (2 eq. of Fe(II) sulfate and 200 eq. of H_2O_2) and H_2O (2 eq. of Fe(II) sulfate and 20 eq. of H_2O_2) show a consumption of **1** due to an unselective degradation of the peptide.



Scheme S1. Mechanism of NO radical formation from DEA NONOate.



Scheme S2. Simplified mechanism of oxygen of radical formation from Fenton reaction[38] and UV irradiation of water molecules.[39]

Fenton chemistry

$$Fe_2^+ + H_2O_2 \longrightarrow Fe_3^+ + OH^\bullet + OH^-$$

$$Fe_3^+ + H_2O_2 \longrightarrow Fe_2^+ + OOH^\bullet + H^+$$

Irradiation of aqueous media

$$H_2O \xrightarrow{hv} H_2O^* \longrightarrow OH^\bullet + H^\bullet$$

$$H_2O \xrightarrow{hv} H_2O^\bullet \longrightarrow OH^\bullet + H^+ + e^-$$

$$2HO^\bullet \longrightarrow H_2O_2$$

$$e^-_{aq} + O_2 \longrightarrow O_2^{\bullet -}$$

$$e^-_{aq} + O_2 + H^+ \longrightarrow HO_2^{\bullet -}$$

$$H^\bullet + O_2 \longrightarrow HO_2^\bullet$$

$$h\nu + {}^3O_2 \longrightarrow {}^1O_2$$

References

[54] P. Wardman, L. P. Candeias, “Fenton Chemistry: An Introduction” *Radiat. Res.* **1996**, *145*, 523-531. DOI:10.2307/3579270.

[55] G.H. Xu, M.R. Chance, “Hydroxyl radical-mediated modification of proteins as probes for structural proteomics” *Chem. Rev.* **2007**, *107*, 3514–3543. DOI: 10.1021/cr0682047.

Table S5. Estimated Secondary structure (%) of synthetized peptides in PB (10 mM, pH 7.0) and TFE evaluated with CDApps software (SELCOM3 algorithm).

peptide	α-helix	β-sheet	turn	unordered
	PB			
1	20	13	22	45
2	14	15	26	45
3	17	15	23	45
(*E*/*Z*)-***4***	11	18	25	46
	TFE			
1	80	7	4	9
2	82	4	4	10
3	79	8	4	9
(*E*/*Z*)-***4***	80	9	2	9

Table S6. Estimated Secondary structure (%) of 1 in PB/TFE mixtures evaluated with CDApps software (SELCOM3 algorithm).

TFE (%)	α-helix	β-sheet	turn	unordered
0	20	13	22	45
10	25	16	22	37
20	40	16	17	27
30	55	9	16	20
40	73	4	12	11
50	75	4	10	11
60	75	5	10	10
70	78	4	8	10
80	78	4	7	11
90	80	7	4	9

Figure S2. **CD spectra of 1 (0.15 mg/mL) under UV irradiation (254 nm) in (A) PB (10 mM, pH 7.0) and (B) TFE.**

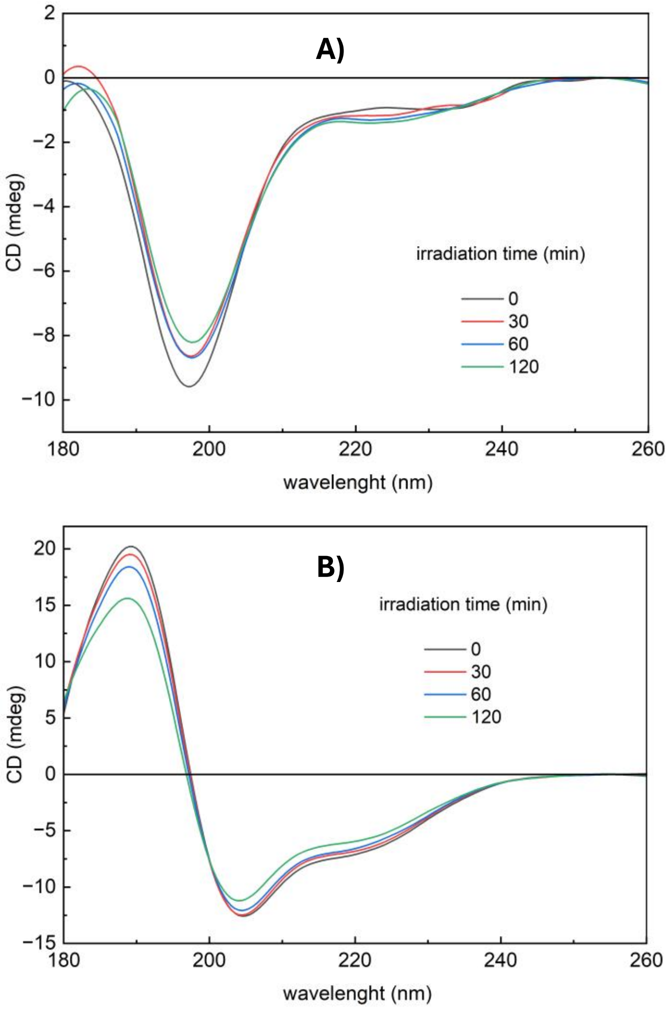


Table S7. Estimated Secondary structure (%) of 1 after UV irradiation in PB (10 mM, pH 7.0) and TFE evaluated with CDApps software (SELCOM3 algorithm).

Irradiation time (min)	α-helix	β-sheet	turn	unordered
	PB			
0	20	13	22	45
30	20	12	24	46
60	23	7	25	45
120	22	7	24	47
	TFE			
0	80	7	4	9
30	81	6	4	9
60	79	6	5	10
120	79	7	4	10

Figure S3 COSY spectra of (*E*/*Z*)-*4* and significant portion showing aryl, E and Z vinyl proton interactions.

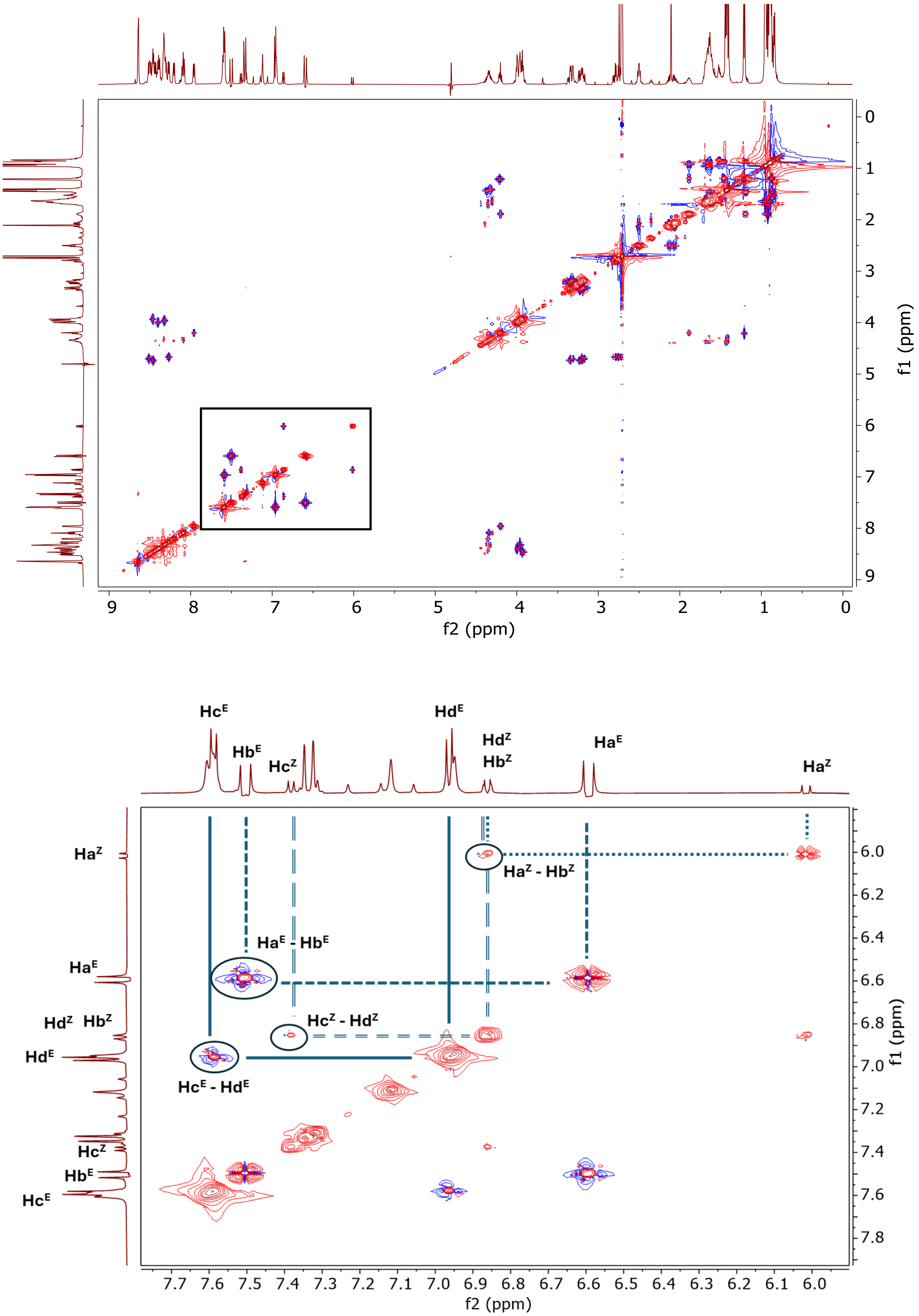


Figure S4. ROESY spectra of (*E*/*Z*)-*4* and significant portion showing aryl-vinyl proton interactions.

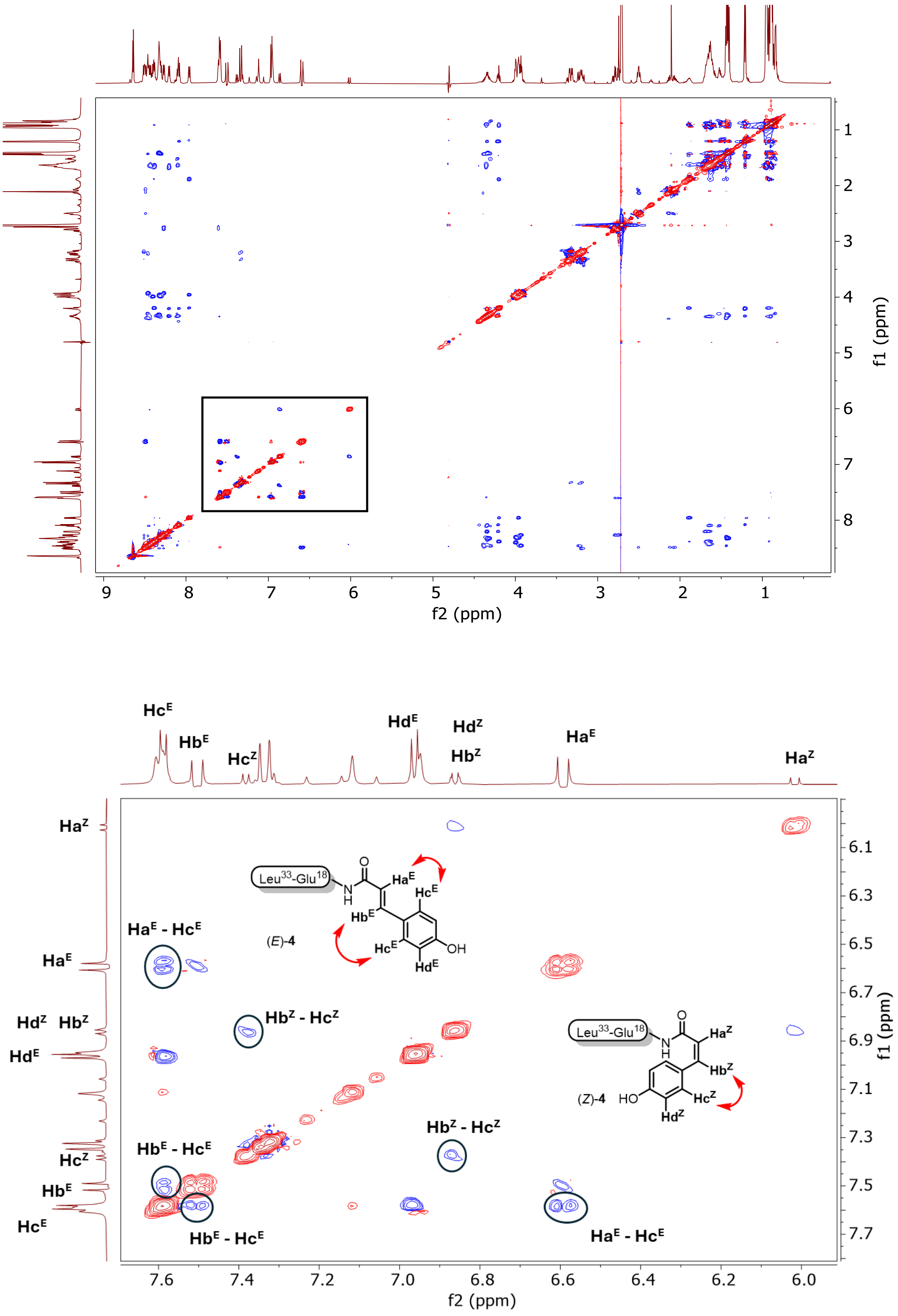


Figure S5. TOCSY spectra of peptide 1 and significant portion showing interaction of amide NH with alfa CH protons for assignment of amino acid residues.

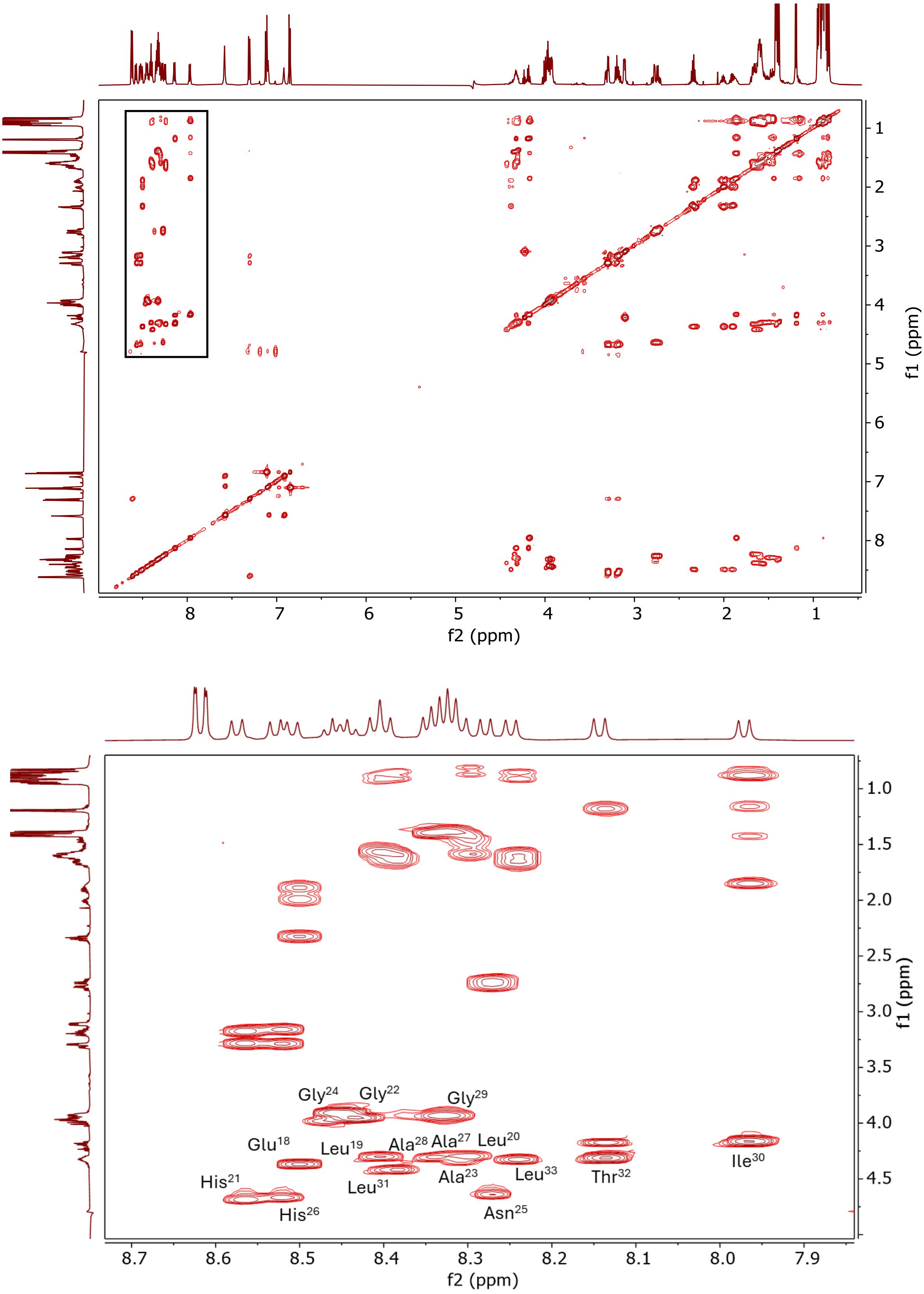


Table S8. Chemical shift (ppm) of peptide 1 dissolved in H_2O+D_2O (90/10)

Peptide **1**	NH	CHα	Others
Tyr^{17}	/	4.23	3.12 (2H), 6.85 (2H), 7.12 (2H)
Glu^{18}	8.50	4.38	1.88 (1H), 1.99 (1H), 2.32 (2H)
Leu^{19}	8.40	4.32	0.89 (6H), 1.56 (3H)
Leu^{20}	8.30	4.30	0.84 (6H), 1.58 (3H)
His^{21}	8.57	4.69	3.17 (1H), 3.29 (1H), 7.30 (1H), 8.61 (1H)
Gly^{22}	8.44	3.96	/
Ala^{23}	8.31	4.35	1.46 (3H)
Gly^{24}	8.46	3.92	/
Asn^{25}	8.27	4.62	2.74 (2H)
His^{26}	8.53	4.68	3.17 (1H), 3.29 (1H), 7.31 (1H), 8.61 (1H)
Ala^{27}	8.32	4.31	1.41 (3H)
Ala^{28}	8.35	4.31	1.39 (3H)
Gly^{29}	8.33	3.94	/
Ile^{30}	7.97	4.17	0.88 (3H), 1.18 (3H), 1.43 (2H), 1.86 (1H)
Leu^{31}	8.39	4.43	0.87 (6H), 1.65 (3H)
Thr^{32}	8.14	4.32	1.18 (3H), 4.18 (1H)
Leu^{33}	8.25	4.32	0.89 (6H), 1.64 (3H)

Figure S6. TOCSY spectra of peptide (*E*/*Z*)-4 and significant portion showing interaction of amide NH with alfa CH protons for assignment of amino acid residues.

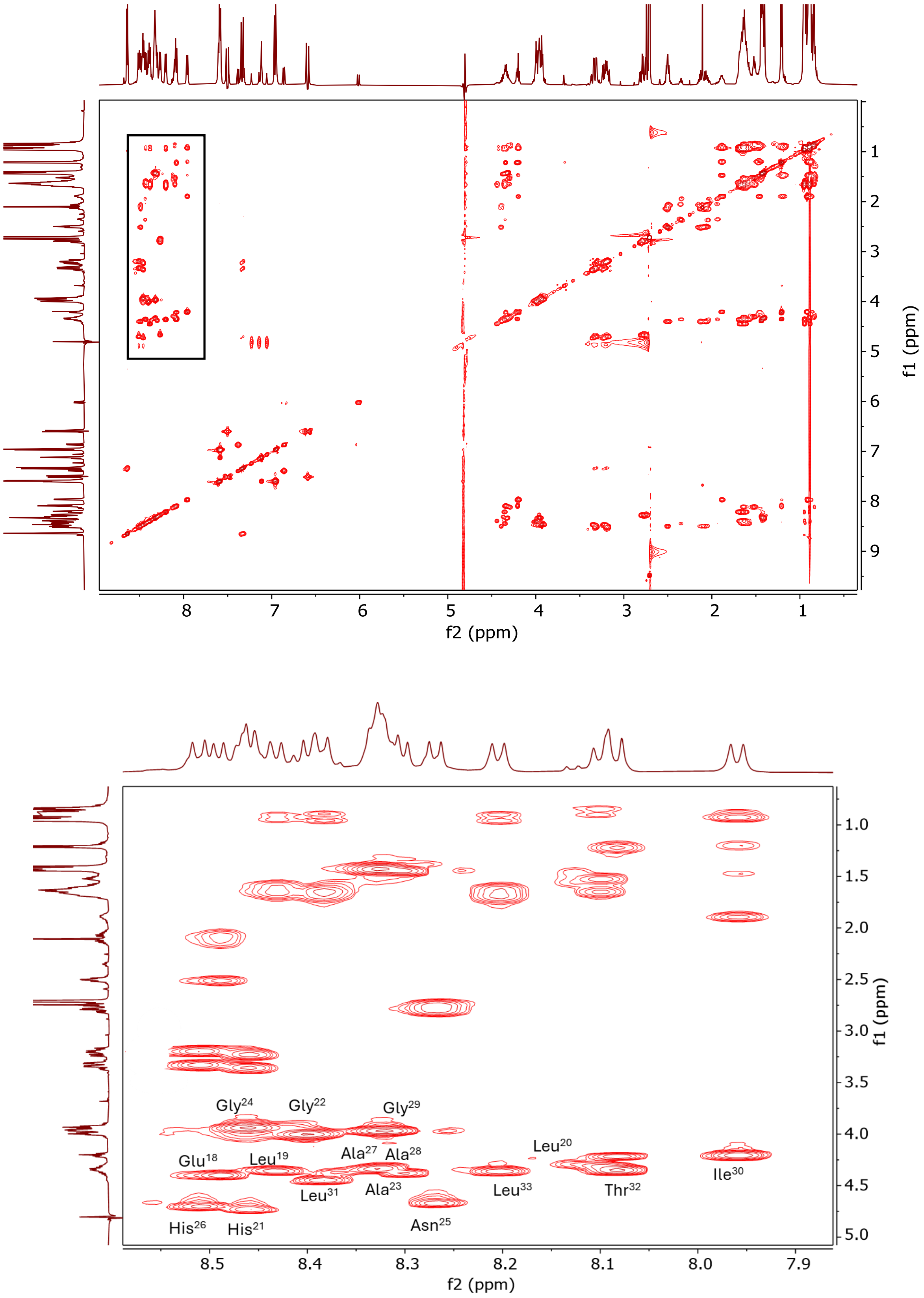


Table S9. Chemical shift (ppm) of peptide (*E*/*Z*)-4 dissolved in H_2O+DMSOd_6 (70/30)

Peptide (*E*/*Z*)-**4**	NH	CHα	Others
Coumarate[17]	/	/	*E*: 6.59 (1H), 7.50 (1H), 6.96 (2H), 7.59 (2H)
			Z: 6.02 (1H), 6.86 (3H), 7.38 (2H)
Glu[18]	8.49	4.39	1.88 (1H), 2.10 (1H), 2.51 (2H)
Leu[19]	8.44	4.36	0.94 (6H), 1.63 (3H)
Leu[20]	8.11	4.29	0.89 (6H), 1.60 (3H)
His[21]	8.46	4.74	3.23 (1H), 3.36 (1H), 7.35 (1H), 8.65 (1H)
Gly[22]	8.40	4.01	/
Ala[23]	8.30	4.29	1.46 (3H)
Gly[24]	8.46	3.96	/
Asn[25]	8.27	4.66	2.78 (2H)
His[26]	8.51	4.71	3.20 (1H), 3.34 (1H), 7.32 (1H), 8.64 (1H)
Ala[27]	8.34	4.34	1.44 (3H)
Ala[28]	8.32	4.33	1.44 (3H)
Gly[29]	8.33	3.97	/
Ile[30]	7.96	4.21	0.92 (3H), 1.21 (3H), 1.48 (2H), 1.90 (1H)
Leu[31]	8.38	4.43	0.93 (6H), 1.69 (3H)
Thr[32]	8.08	4.34	1.22 (3H), 4.22 (1H)
Leu[33]	8.20	4.35	0.92 (6H), 1.65 (3H)