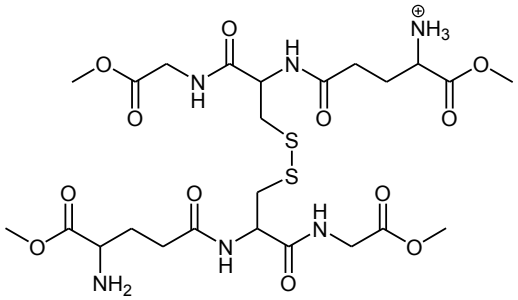
Supplemental Information for:

An Efficient Esterification of Oxidized L-Glutathione and Other Small Peptides

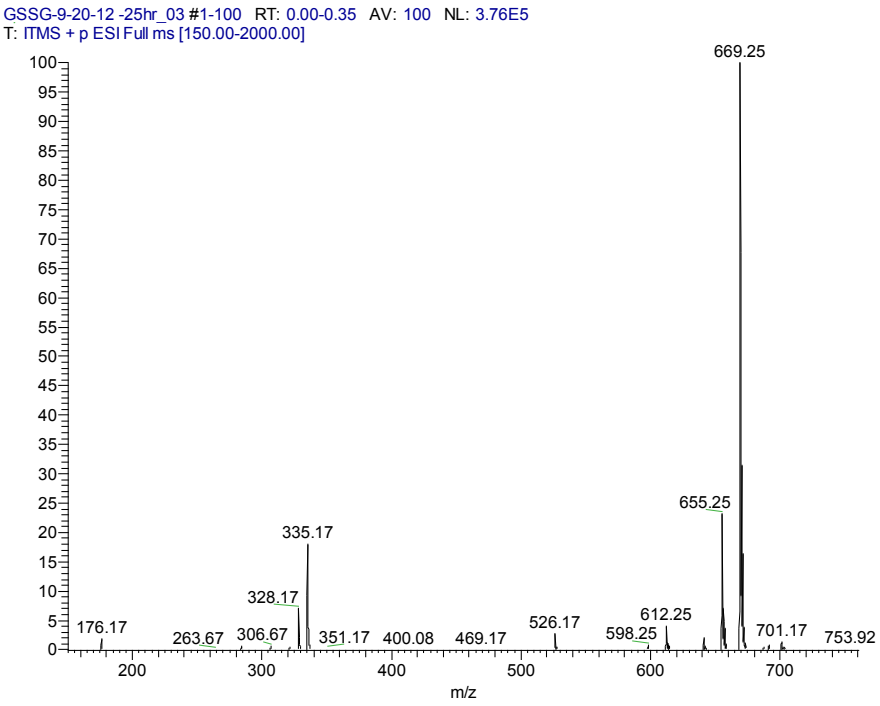
General Experimental Conditions

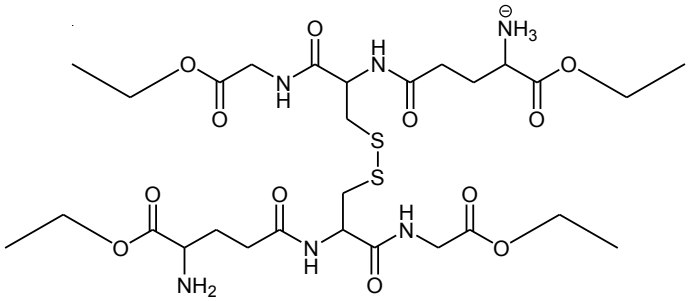
NMR spectra were acquired on a Bruker 400 MHz NMR in proton decoupled mode. The internal standard used in the NMR experiment was residual solvent signal for CD_3OD. ESI MS was carried out on a ThermoFisher LXQ ESI-Ion trap mass spectrometer using Optima LCMS grade methanol and water from Fisher Scientific. All samples were analyzed using a 1% acetic acid 1:1 methanol:water solution. The methanol used for the esterification reactions was distilled from calcium hydride, absolute ethanol was stored over molecular sieves, and 2-propanol distilled from sodium prior to use. L-oxidized glutathione and reduced glutathione was obtained from Sigma-Aldrich, and all other peptides were acquired from the American Peptide Company and used as received. Peptides furnished with Certificate of Analysis (COA) contained trace impurities and those masses were provided courtesy of American Peptide Company.



Glycine, L-γ-glutamyl-l-cysteinyl-1,3-dimethyl ester, bimol. (2→2′)-disulfide (2) ESI MS of $[M + H]^+$ calculated $[C_{24}H_{44}N_6O_{12}S_2]^+$, 669.22, found 669.2. ^{1}H-NMR and ^{13}C-NMR matched published data [1].

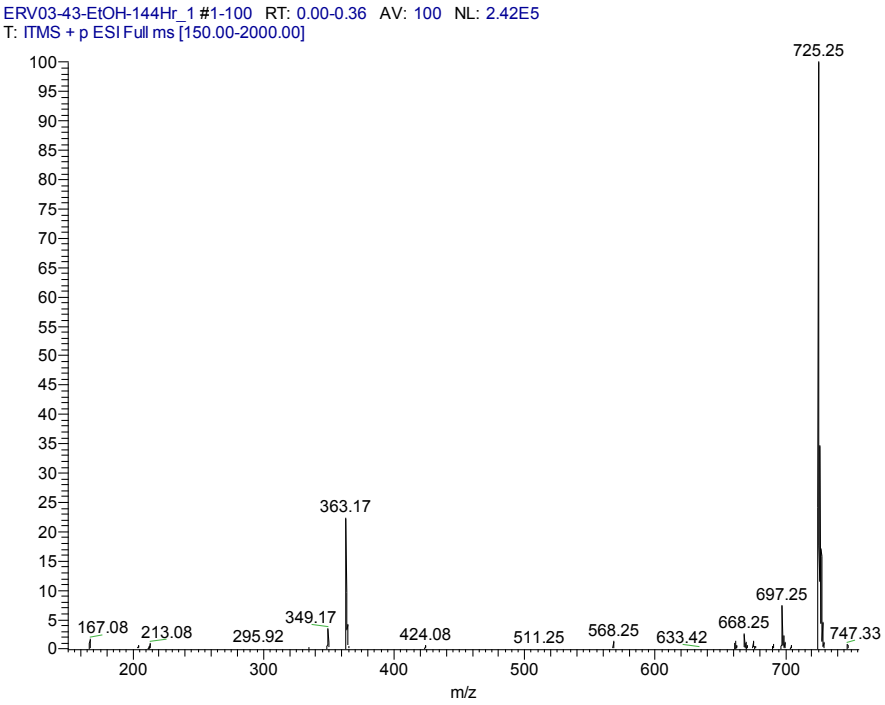
Observed Mass (*m/z*)	Relative Abundance (%)	Comments	Number of Esters
669.25	100.00%	$[M + H]^+$	4
655.25	24.50%	$[M + H]^+$	3
335.17	18.32%	$[M + 2H]^{2+}$	4
328.17	7.56%	$[M + 2H]^{2+}$	3

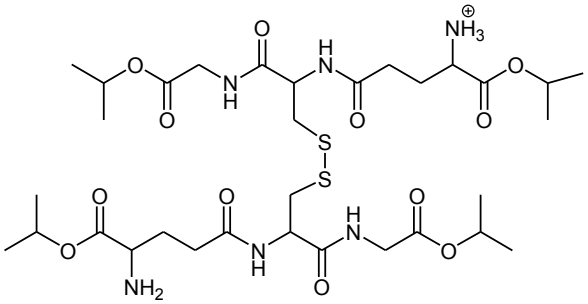




Glycine, L-γ-glutamyl-L-cysteinyl-1,3-diethyl ester, bimol. (2→2′)- ESI MS of $[M + H]^+$ calculated $[C_{28}H_{44}N_6O_{12}S_2]^+$, 725.28, found 725.25. ^{1}H-NMR and ^{13}C-NMR matched published data [2].

Observed Mass (*m/z*)	Relative Abundance (%)	Comments	Number of Esters
725.25	100%	$[M + H]^+$	4
363.17	24.00%	$[M + 2H]^{2+}$	4
697.25	7.05%	$[M + H]^+$	3

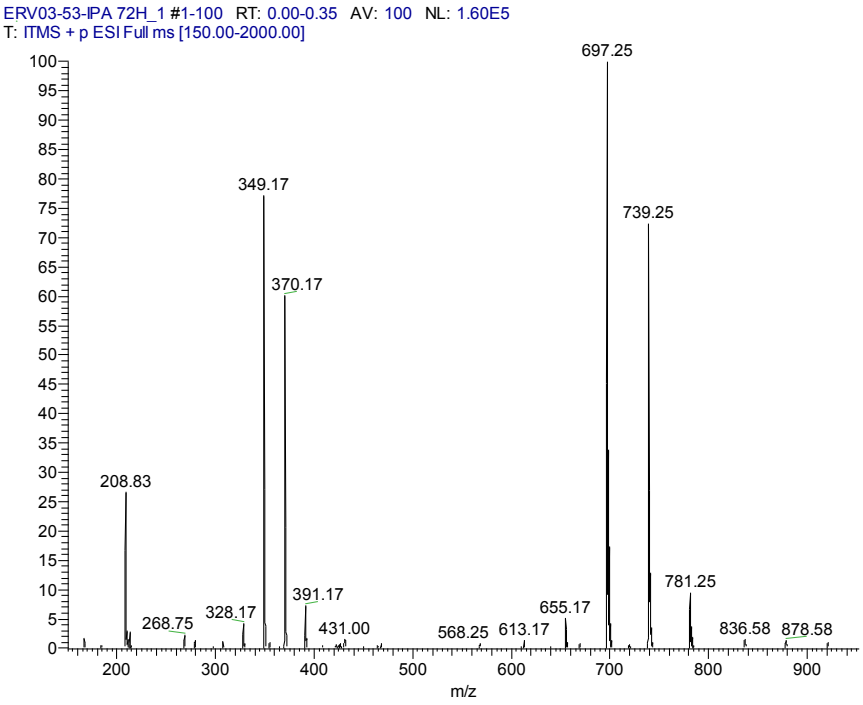


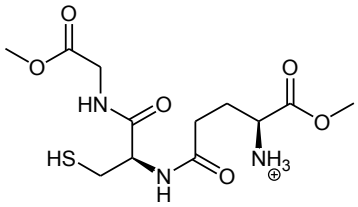


Glycine, L-γ-glutamyl-L-cysteinyl-1,3-diisopropyl ester, bimol. (2→2′)- ESI MS of $[M + H]^+$ calculated $[C_{32}H_{57}N_6O_{12}S_2]^+$, 781.35, found 781.25.Expected mass not present in significant quantities so no NMR data was recorded.

Observed Mass (*m/z*)	Relative Abundance (%)	Comments	Number of Esters
697.25	100.00%	$[M + H]^+$	2
349.17	78.04%	$[M + 2H]^{2+}$	2
739.25	74.30%	$[M + H]^+$	3
370.17	62.30%	$[M + 2H]^{2+}$	3
208.83	27.00%	UK	UK
781.25	9.00%	$[M + H]^+$	4
391.17	7.50%	$[M + H]^{2+}$	4

UK = unknown.

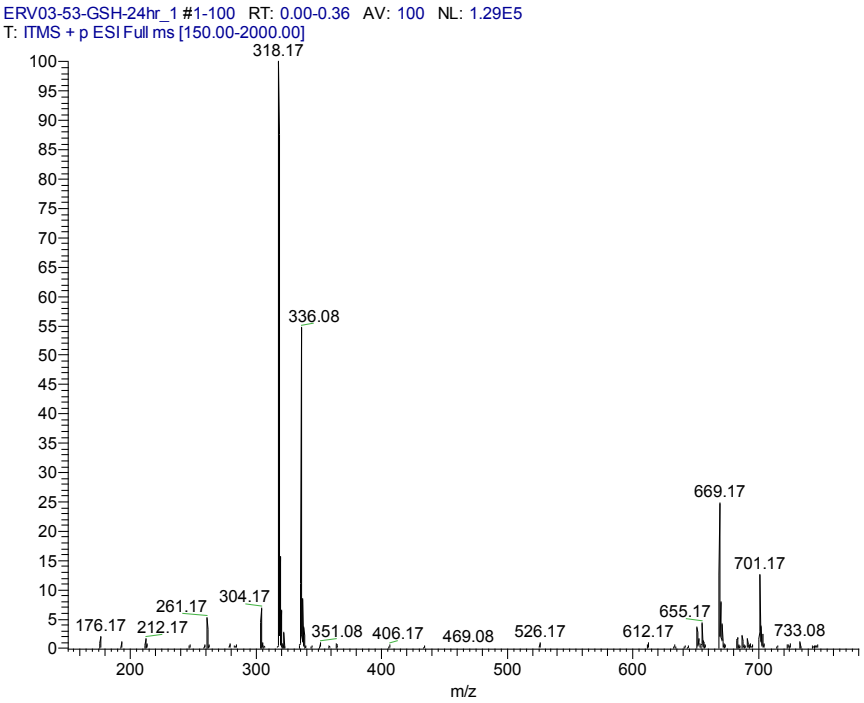




ESI MS of $[M + H]^+$ calculated $[C_{12}H_{22}N_3O_6S]^+$, 336.12, found 336.08. Sample contained multiple impurities so no NMR analysis was performed.

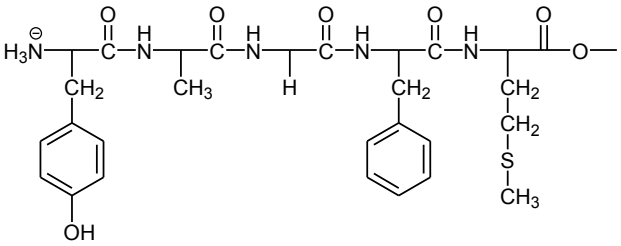
Observed Mass (*m/z*)	Relative Abundance (%)	Comments	Number of Esters
318.17	100%	UK	UK
336.08	56.70%	$[M + H]^+$	2
669.17	26.40%	$[*M + H]^+$	4
701.17	13.25%	UK	UK
304.17	8.30%	UK	UK
655.164	5.80%	$[*M + H]^+$	3
261.17	5.50%	UK	UK

*M = $[C_{24}H_{44}N_6O_{12}S_2]^+$, Oxidized Glutathione; UK = unknown.



[DAla2,DMet5] Enkephalin

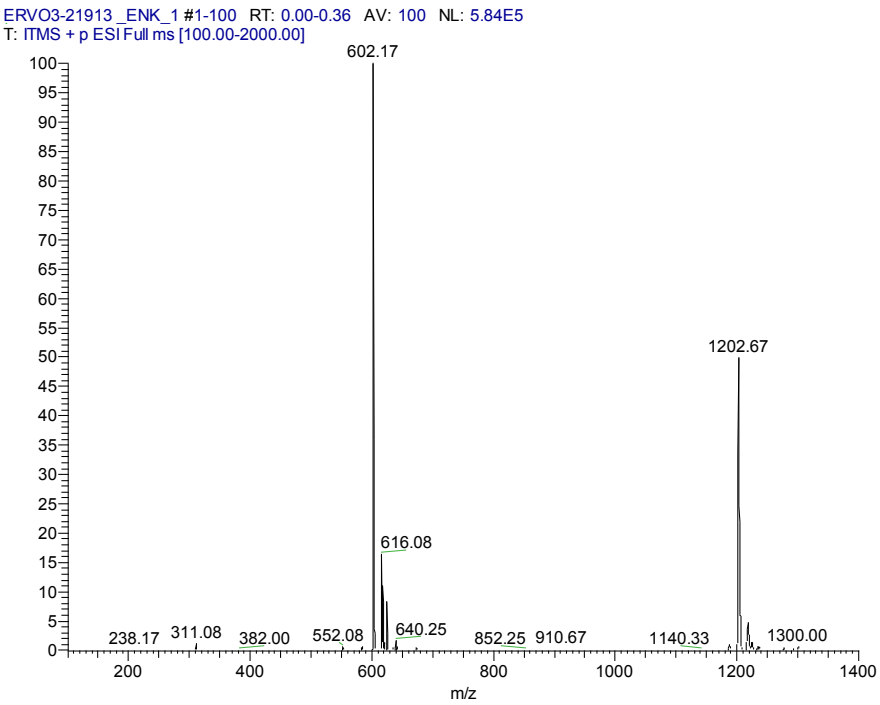
Tyr-D Ala-Gly-Phe-D Met



ESI MS of $[M + H]^+$ calculated $[C_{29}H_{40}N_5O_7S]^+$, 602.26, found 602.1. Masses present before reaction: 411 (8%), 587 (100%), 610 (23%), 1174 (42%).

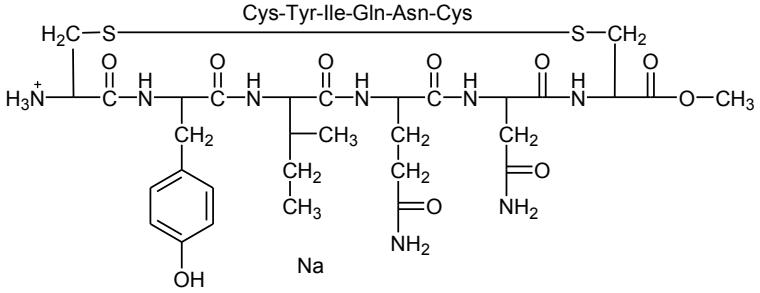
Observed Mass (*m/z*)	Relative Abundance (%)	Comments
602.17	100%	$[M + H]^+$
1202.67	54.00%	$[2M + H]^+$
616.08	16.54%	UK

UK = unknown.



[Ile3]-Pressinoic Acid

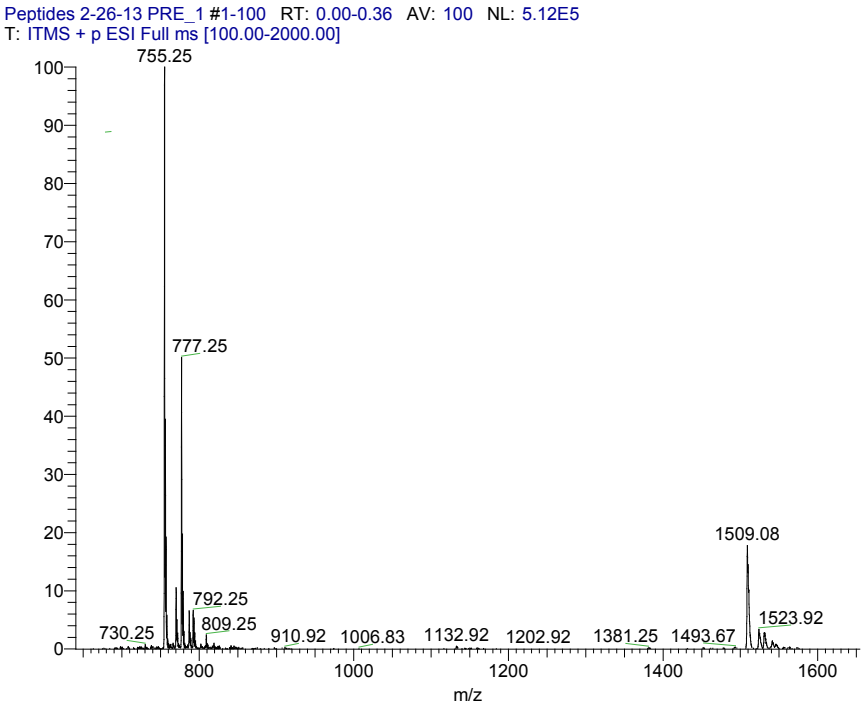
Cys-Tyr-Ile-Gln-Asn-Cys



ESI MS of $[M + H]^+$ calculated $[C_{31}H_{47}N_8O_{10}S_2]^+$, 755.29, found 755.22. Masses present before reaction: 726 (10%), 742 (100%), 764 (21%), 781 (16%), 1483 (10%), 1522 (20%).

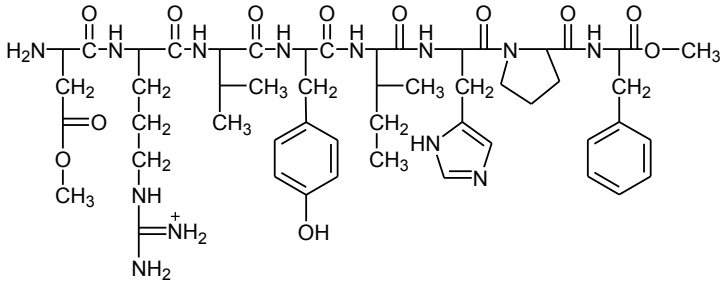
Observed Mass (*m/z*)	Relative Abundance (%)	Comments
755.25	100.00%	$[M + H]^+$
777.25	50.00%	$[M+ Na]^+$
1509.08	24.55%	$[2M+ H]^+$
770.212	10.54%	UK

UK = unknown.



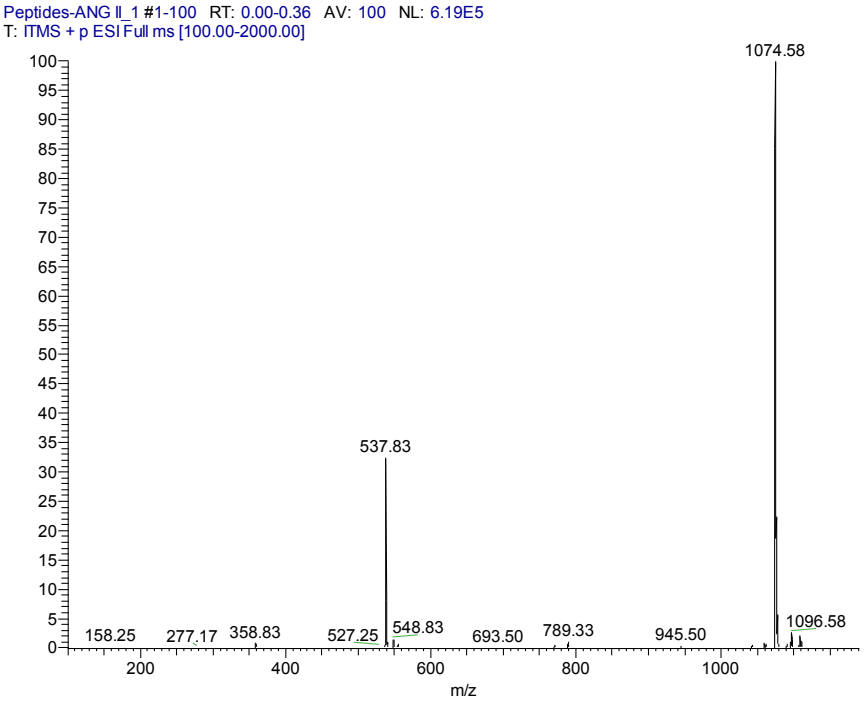
Angiotensin II, human

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe



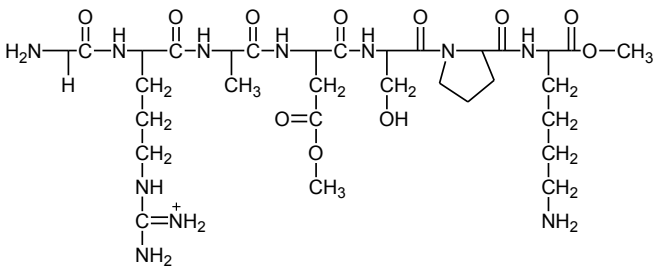
ESI MS of $[M + H]^+$ calculated $[C_{52}H_{76}N_{13}O_{12}]^+$, 1074.57, found 1074.6 *m/z*.

Observed Mass (*m/z*)	Relative Abundance (%)	Comments
1074.58	100.00%	$[M + H]^+$
537.83	35.34%	$[M+ 2H]^{2+}$



Fibronectin

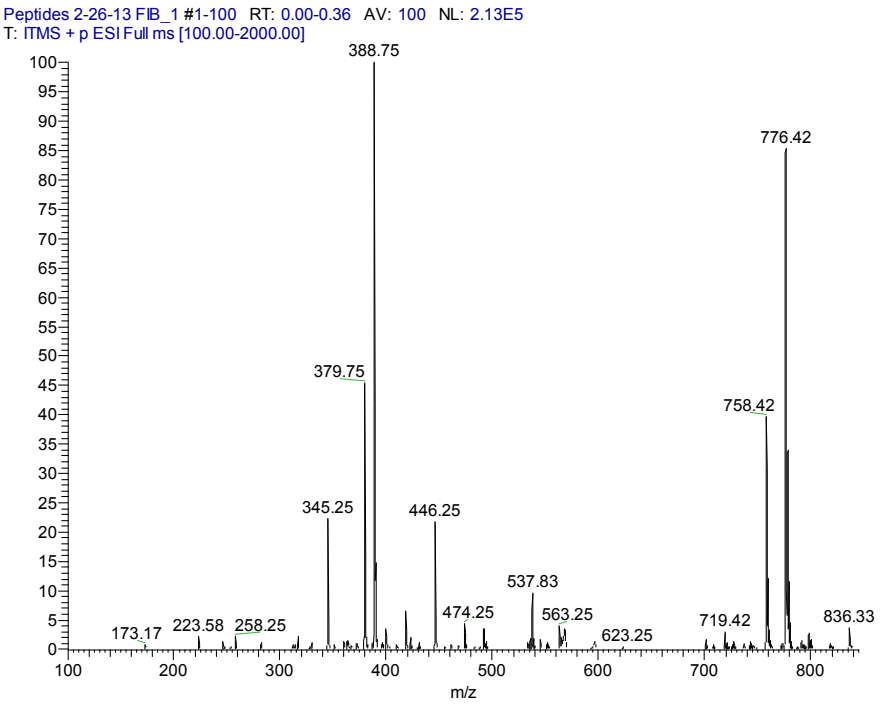
Gly-Arg-Ala-Asp-Ser-Pro-Lys



ESI MS of $[M + H]^+$ Calculated $[C_{31}H_{56}N_{11}O_{11}]^+$, 758.42, Found 758.42.

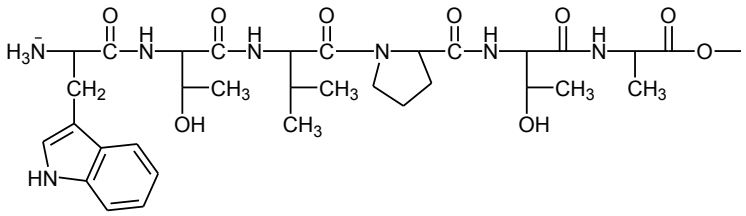
Observed Mass (*m/z*)	Relative Abundance (%)	Comments
388.75	100.00%	$[*M + 2H]^{2+}$
776.42	85.40%	$[*M + H]^+$, ^{35}Cl
758.42	39.66%	$[M + H]^+$
778.365	33.97%	$[*M + H]^+$, ^{37}Cl
379.75	25.28%	$[M + 2H]^{2+}$
345.25	22.36	UK
446.25	20.94	UK
537.83	9.43%	UK

*M = $C_{31}H_{55}ClN_{11}O_{10}$; UK = unknown.



Necrofibrin, rat

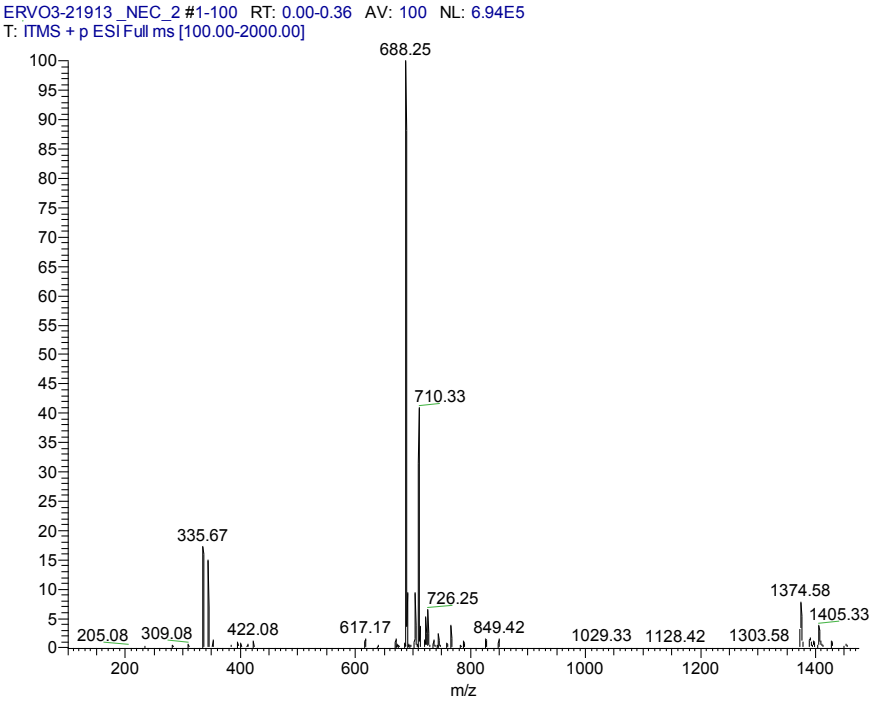
Trp-Thr-Val-Pro-Thr-Ala



ESI MS of $[M + H]^+$ calculated $[C_{33}H_{50}N_7O_9]^+$, 688.37; found 688.20. Masses present in starting material: 674 (55%), 696 (100%), 1346 (19%), 1368 (10%).

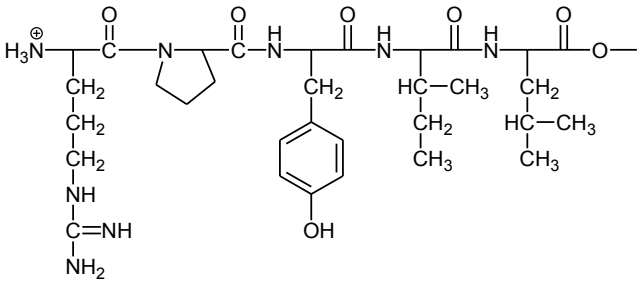
Observed Mass (*m/z*)	Relative Abundance (%)	Comments
688.25	100.00%	$[M + H]^+$
* 710.33	37.07%	$[*M+ H]^+$
335.678	18.46%	$[*M+ 2H]^{2+}$
344.51	16.51%	$[M+ 2H]^{2+}$
1374.58	9.4%	$[**M+ H]^+$

* Impurity from starting material undergoing methyl esterification *m/z* = 696; ** Impurity from starting material undergoing two methyl esterifications *m/z* = 1346.



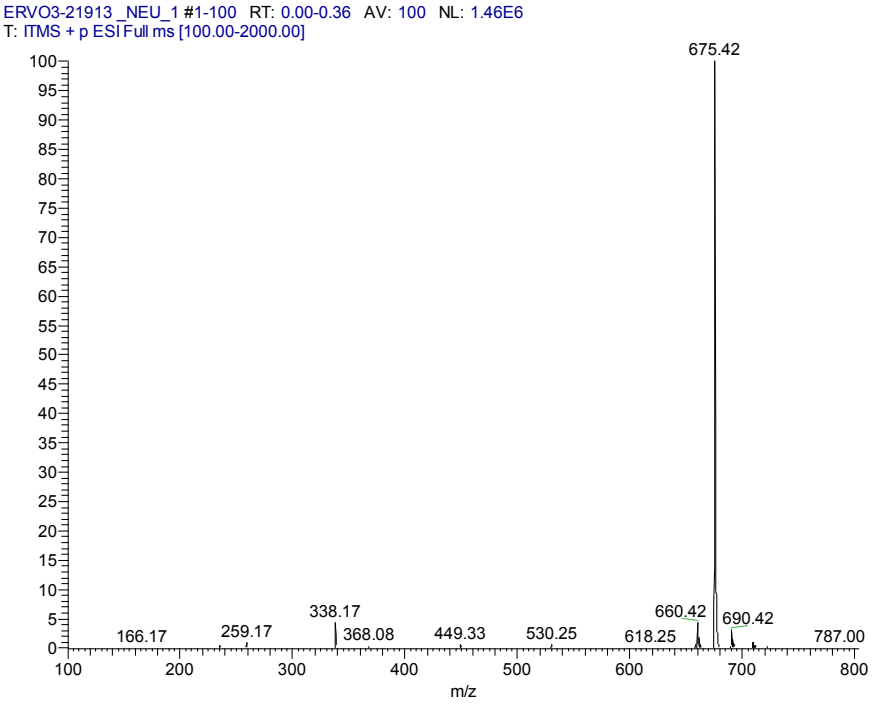
Neurotensin (9-13)

Arg-Pro-Tyr-Ile-Leu



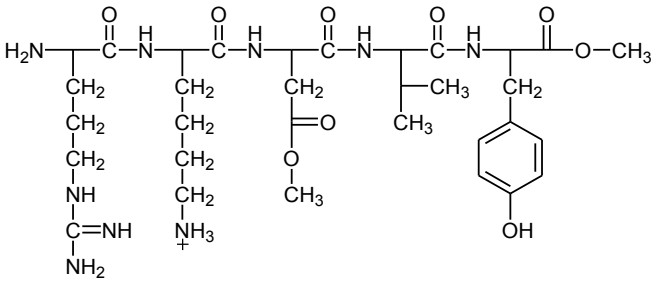
ESI MS of $[M + H]^+$ calculated $[C_{33}H_{55}N_8O_7]^+$, 675.42; found 675.39. Masses present in starting material: 661 (100%), 1320 (56%), 1322 (40%).

Observed Mass (*m/z*)	Relative Abundance (%)	Comments
675.42	100.00%	$[M + H]^+$



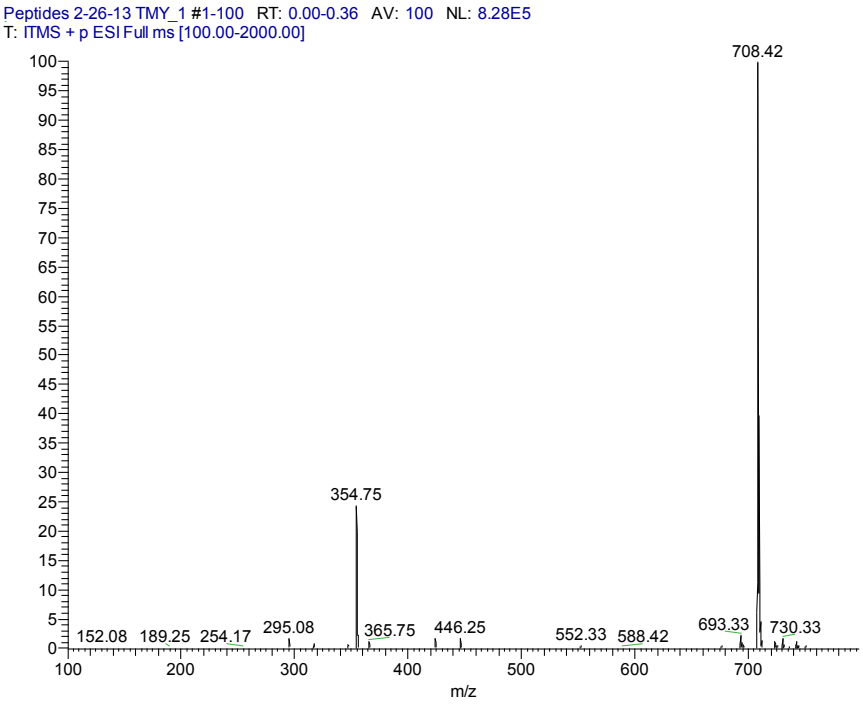
Thymopentin (TP-5)

Arg-Lys-Asp-Val-Tyr



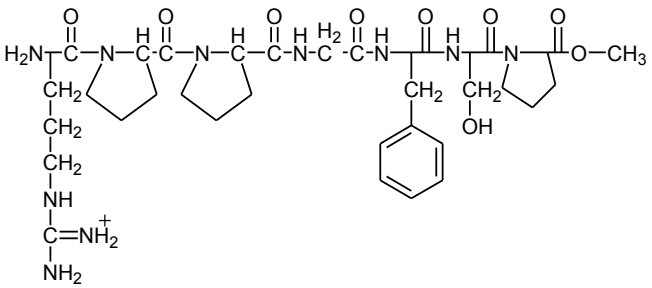
ESI MS of $[M + H]^+$ calculated $[C_{32}H_{54}N_9O_9]^+$, 708.40, found 708.47. Masses present in starting material: 341(14%), 680 (100%), 1358(8%).

Observed Mass (*m/z*)	Relative Abundance (%)	Comment
708.42	100.00%	$[M + H]^+$
354.76	25.40%	$[M+ 2H]^{2+}$



Bradykinin

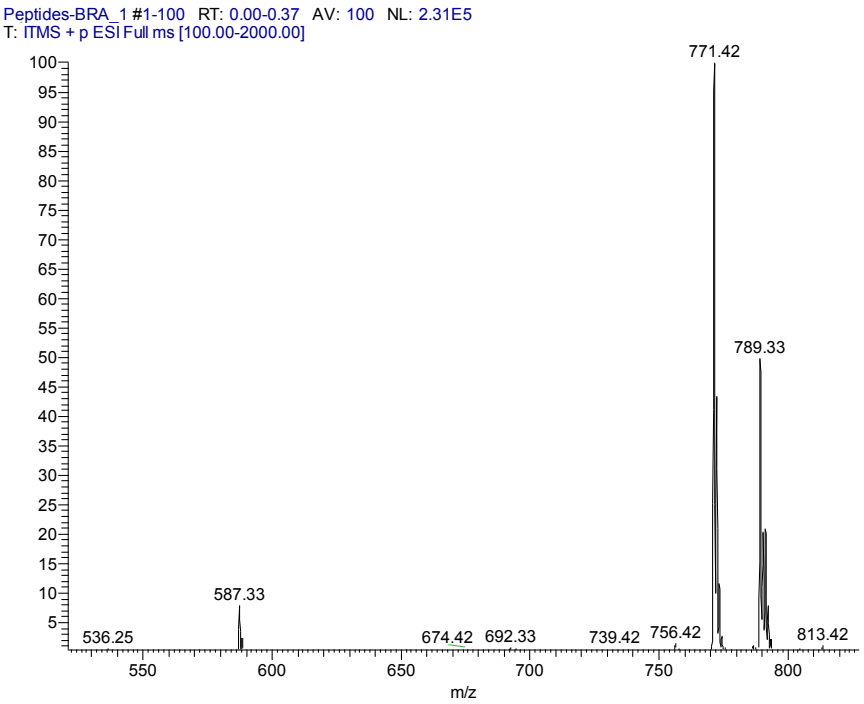
Arg-Pro-Pro-Gly-Phe-Ser-Pro



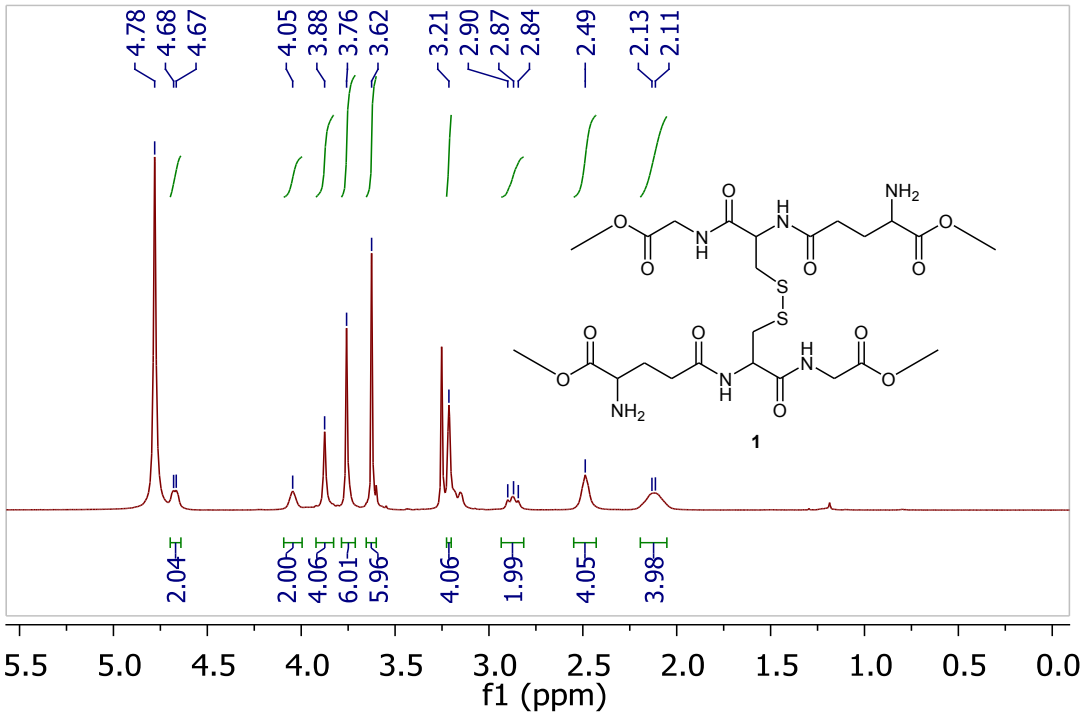
ESI MS of $[M + H]^+$ calculated $[C_{36}H_{55}N_{10}O_9]^+$, 771.41, found 771.38.

Observed Mass (*m/z*)	Relative Abundance (%)	Comment
771.42	100.00%	$[M + H]^+$
789.33*	49.97%	$[*M + H]^+$, ^{35}Cl
791.34*	21.34%	$[*M + H]^+$, ^{37}Cl
587.33	7.26%	UK

*M = $C_{36}H_{54}ClN_{10}O_8$; UK = unknown.



NMR DATA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4.67
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3.88
3.76
3.62
3.21
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2.87
2.84
2.49
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2.11
2.04
2.00
4.06
6.01
5.96
4.06
1.99
4.05
3.98
5.5
5.0
4.5
4.0
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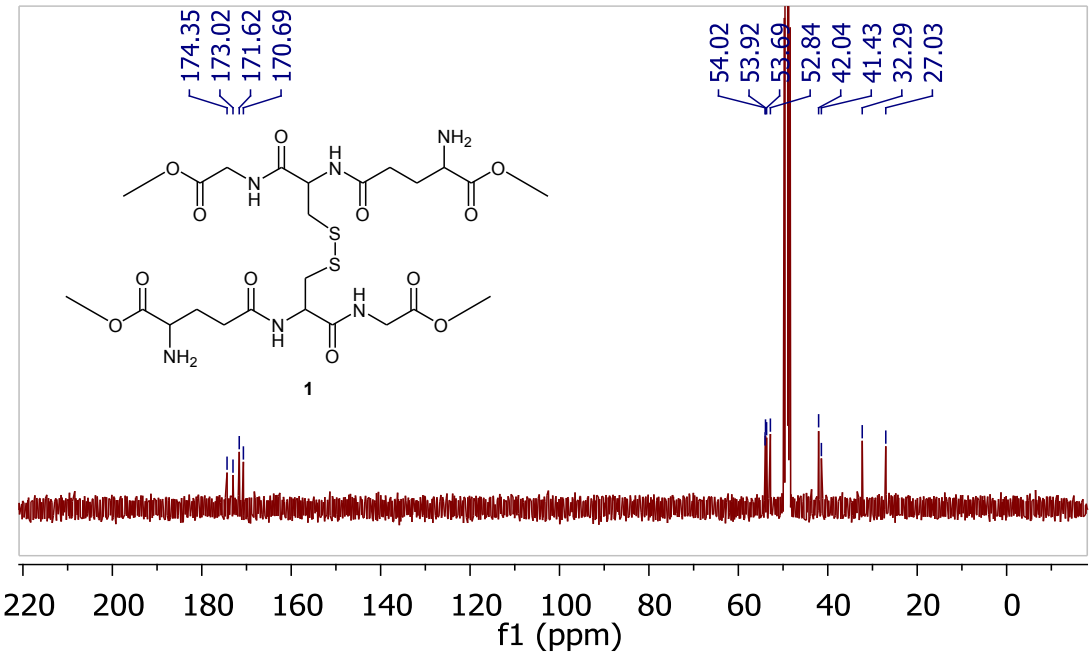
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)
NH2
1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41.43
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100
80
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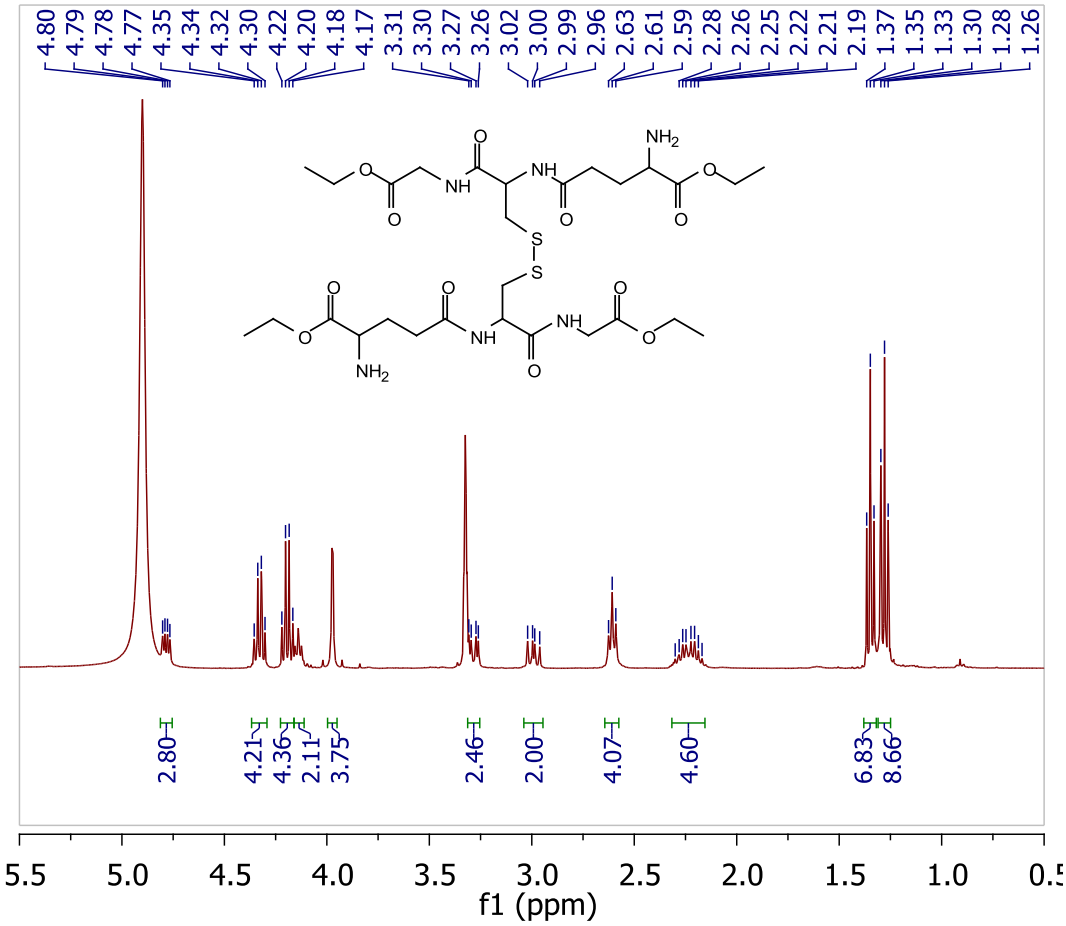
f1 (ppm)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2.80 4.21 4.36 2.11 3.75 2.46 2.00 4.07 4.60 6.83 8.66
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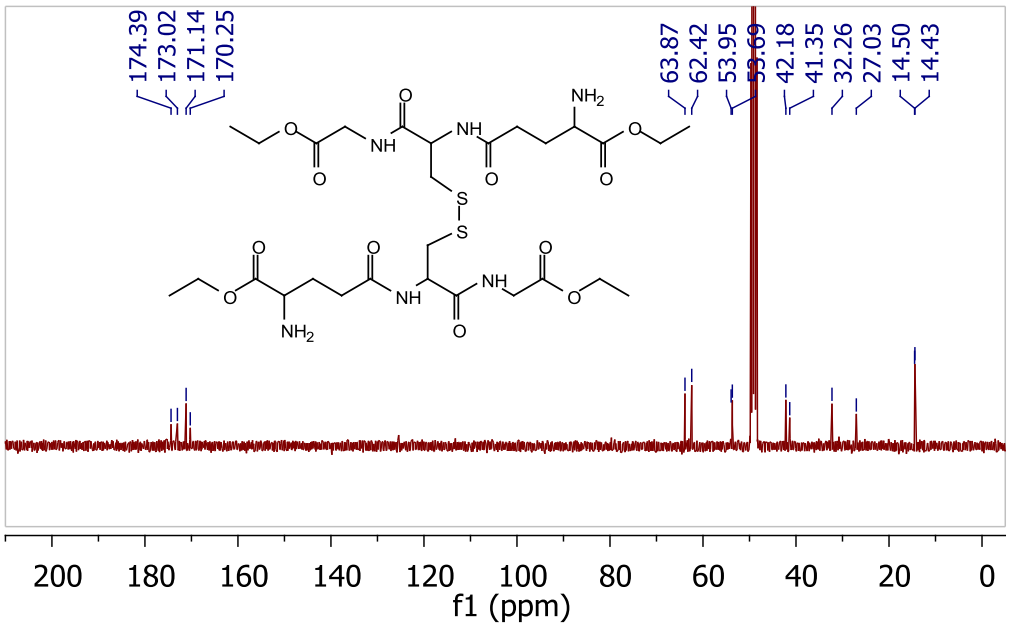
f1 (ppm)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f1 (ppm)

Reference

1. Gatterdam, V.; Ramadass, R.; Stoess, T.; Fichte, M.A.H.; Wachtveitl, J.; Heckel, A.; Tampé, R. Three-Dimensional Protein Networks Assembled by Two-Photon Activation. *Angew. Chem. Int. Ed.* **2014**, *53*, 5680–5684.
2. McCulloch, M.W.B.; Coombs, G.S.; Banerjee, N.; Bugni, T.S.; Cannon, K.M.; Harper, M.K.; Veltri, C.A.; Virshup, D.M.; Ireland, C.M. Psammaplin A as a general activator of cell-based signaling assays via HDAC inhibition and studies on some bromotyrosine derivatives. *Bioorg. Med. Chem.* **2009**, *17*, 2189–2198.