Supporting Information

Site-Specific Incorporation of Multiple Thioamide Substitutions into a Peptide Backbone via Solid Phase Peptide Synthesis

Jinhua Yang[†], Changliu Wang[†], Chaochao Yao[†], Chunqiu Chen[†], Yafang Hu[†], Guifeng He[†] and Junfeng Zhao[†‡*]

†College of Chemistry and Chemical Engineering, Jiangxi Normal University, Nanchang, 330022, Jiangxi, P. R. China

‡Key Laboratory of Chemical Biology of Jiangxi Province, Nanchang, 330022, Jiangxi, P. R. China

Email: zhaojf@jxnu.edu.cn

Contents

1. The Copies of the NMR Spectrums for Compounds 3…….. S1
2. Optimization of Reaction Conditions for Thioamide Incorporation on a Solid Support…….. S14
3. Crude HPLC Traces and ESI-MS Spectra of Monothioamide Substituted peptides **5a-5v**…….. S20
4. Crude HPLC Traces and ESI-MS Spectra of Multi-Thioamide Substituted Peptides **7a-7q**…... S42

The Copies of the NMR Spectrums of Compounds 3

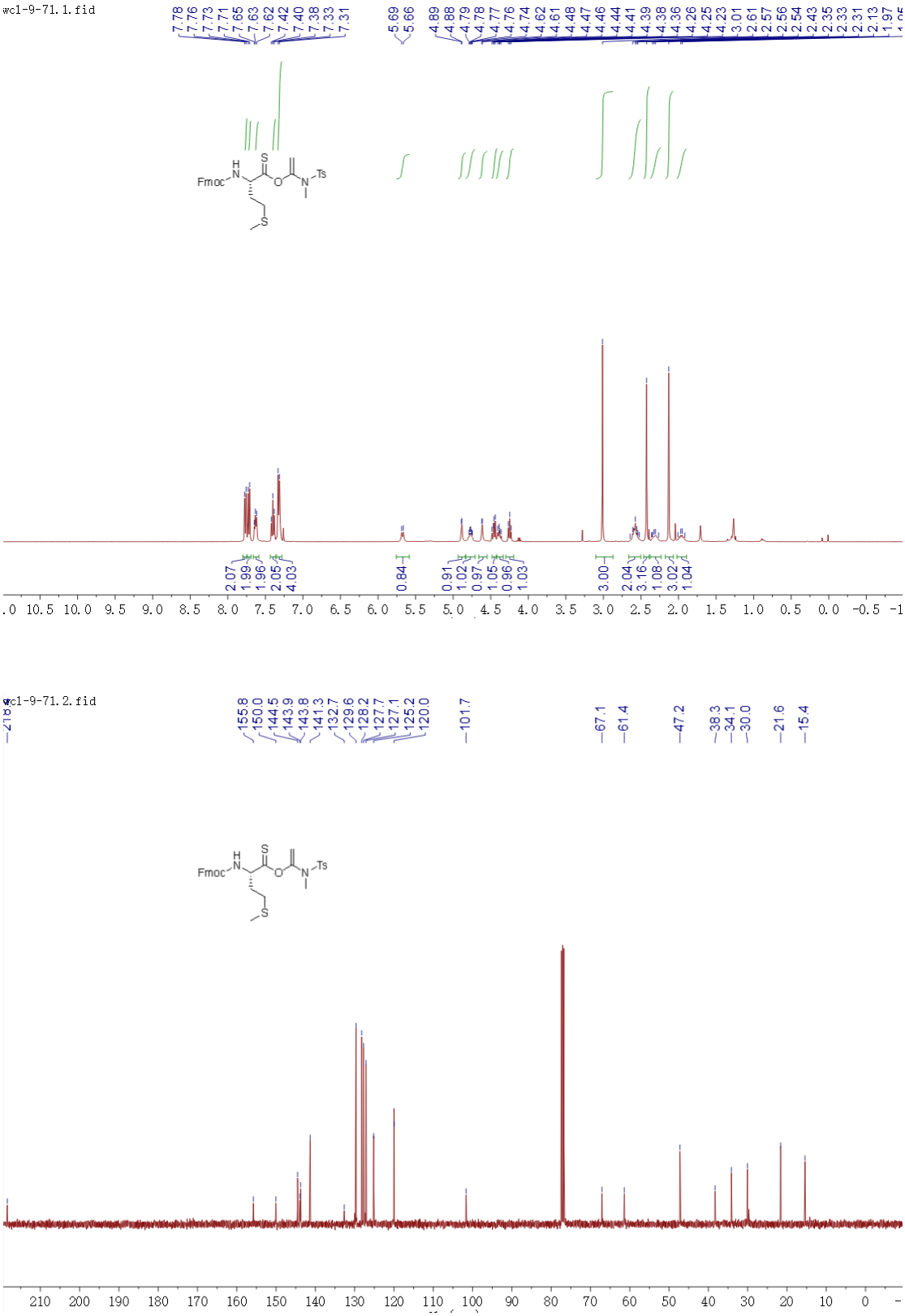
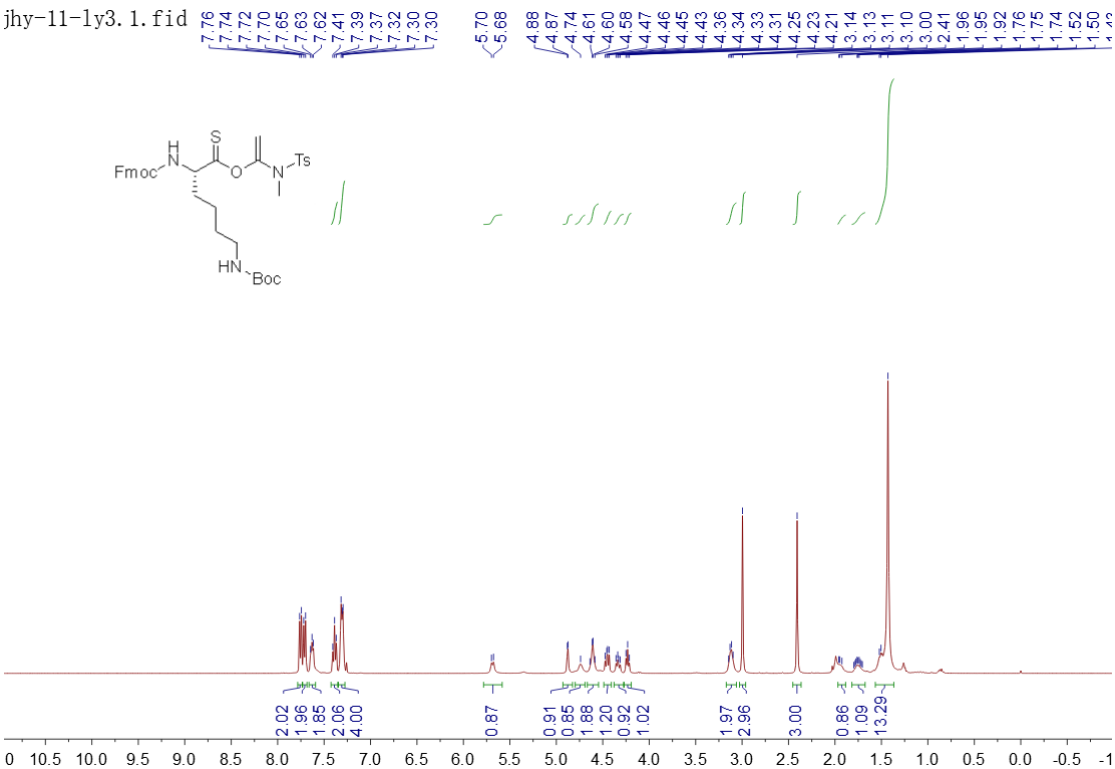


Figure S1. ^{1}H NMR and ^{13}C NMR spectrums of **3c**.



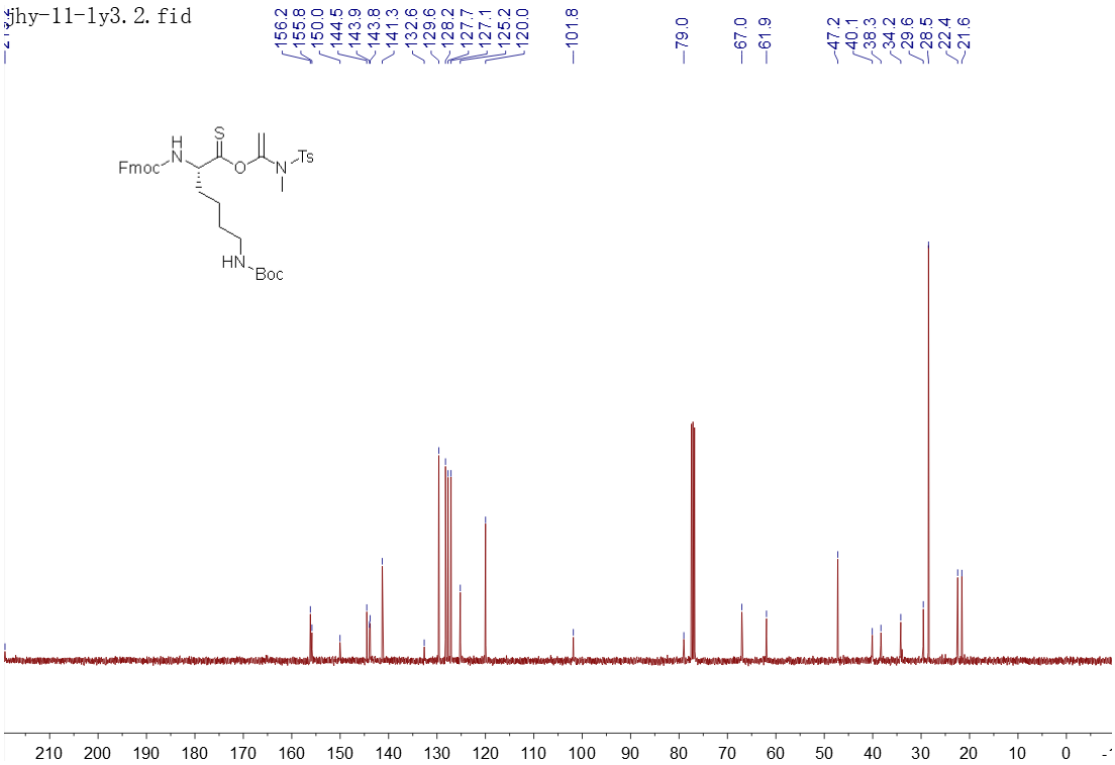


Figure S2. ^{1}H NMR and ^{13}C NMR spectrums of **3d**.

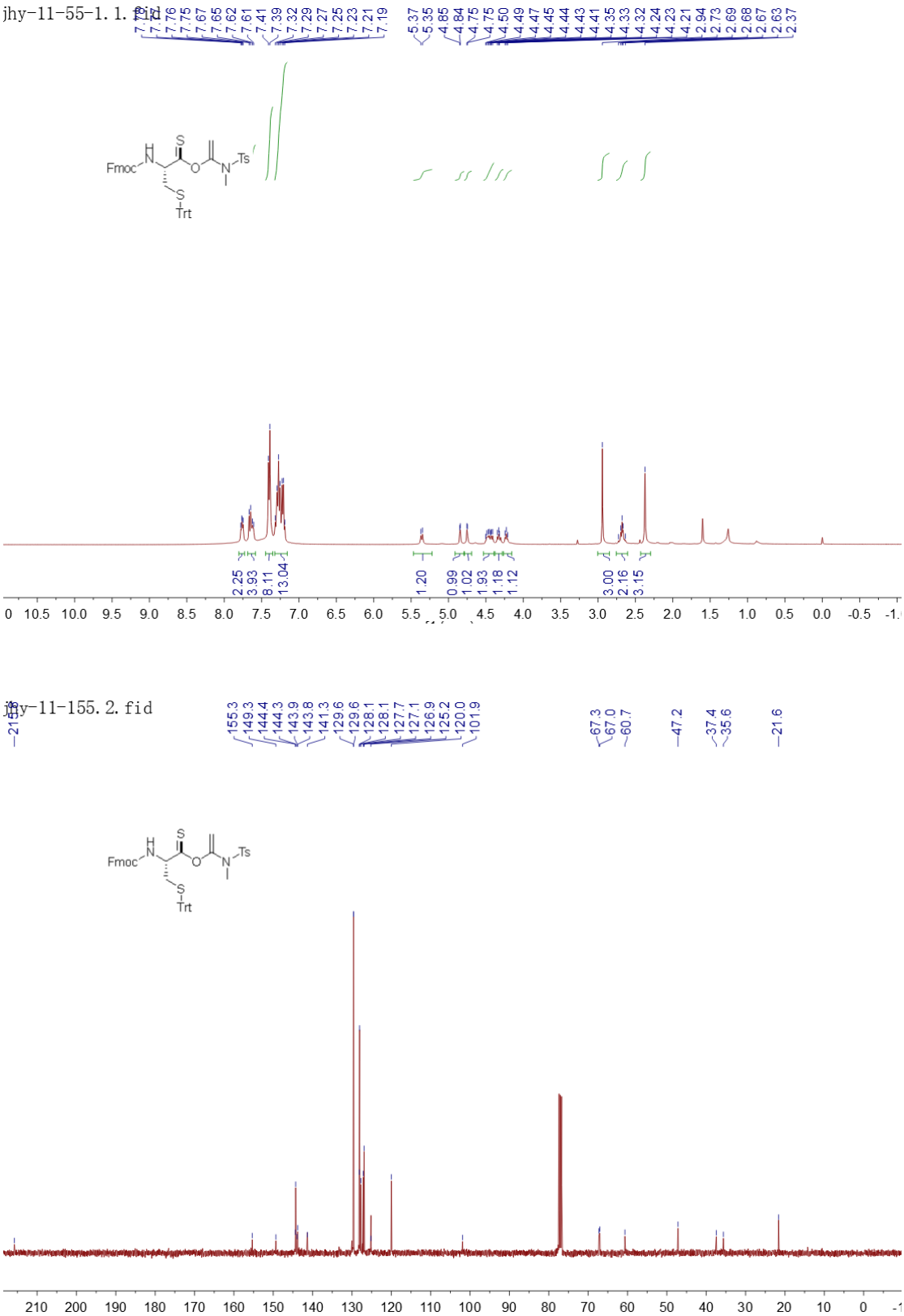
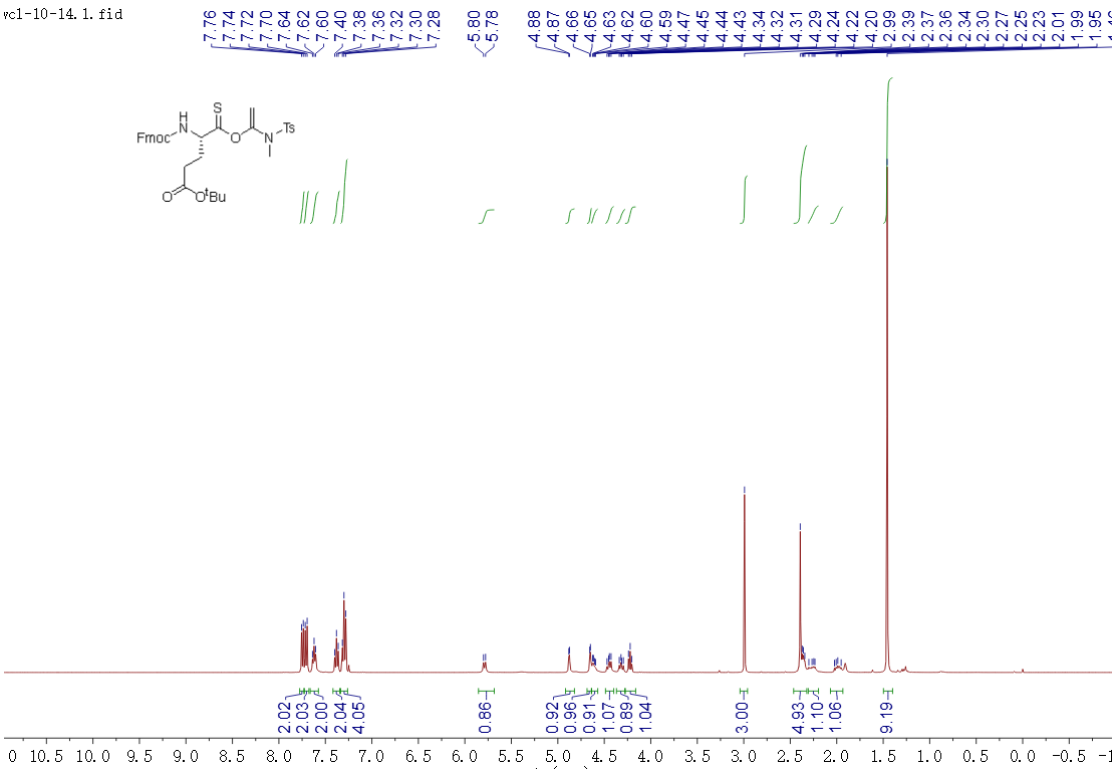


Figure S3. ^{1}H NMR and ^{13}C NMR spectrums of **3e**.



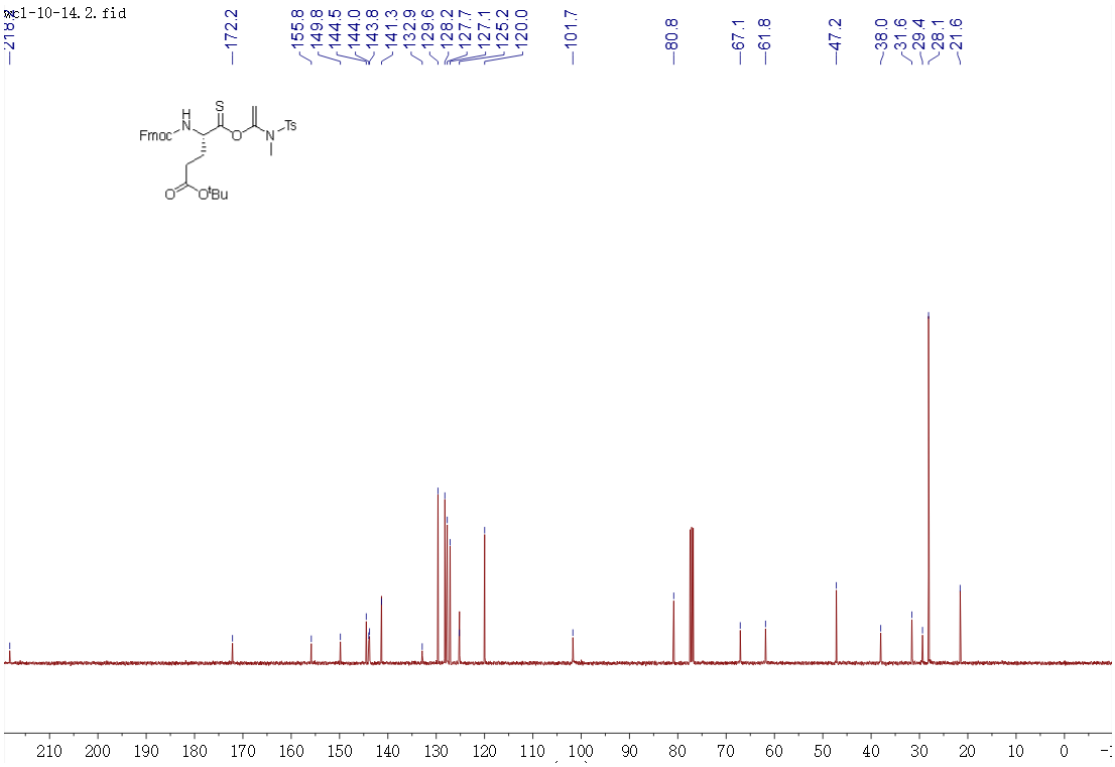


Figure S4. ^{1}H NMR and ^{13}C NMR spectrums of **3f**.

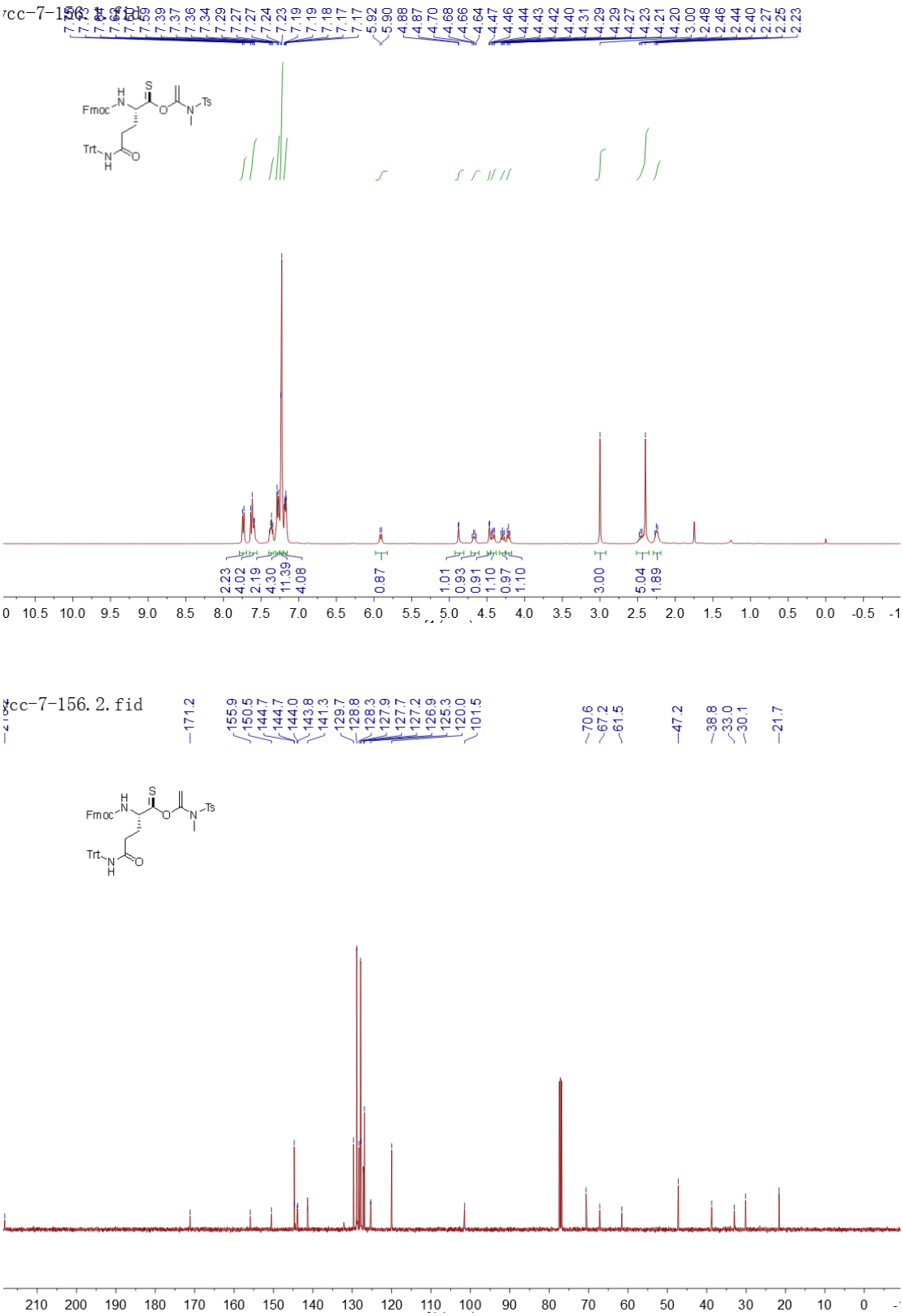
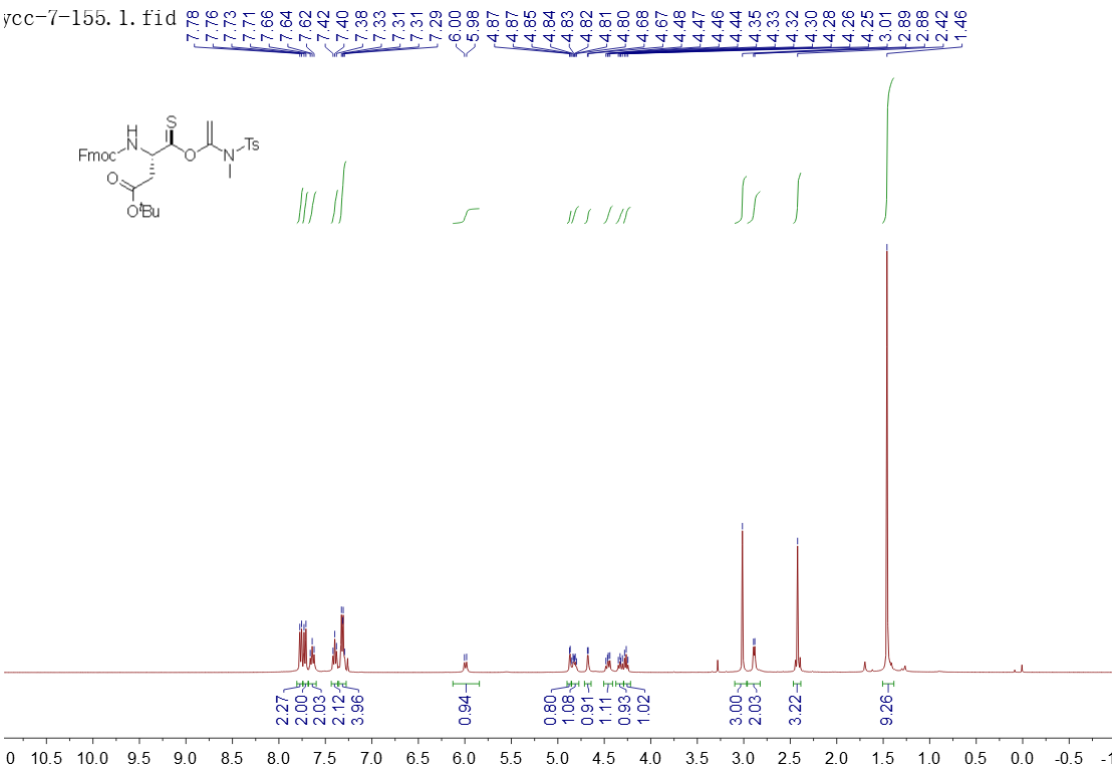


Figure S5. ^{1}H NMR and ^{13}C NMR spectrums of **3g**.



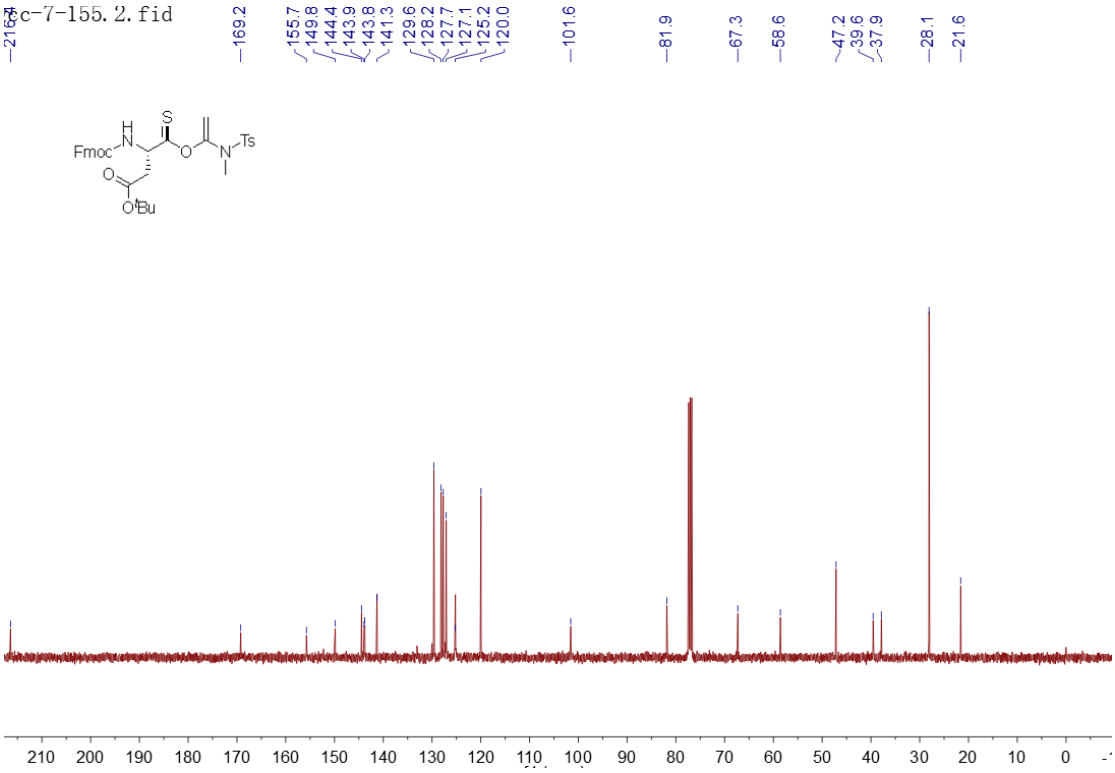


Figure S6. ^{1}H NMR and ^{13}C NMR spectrums of **3h**.

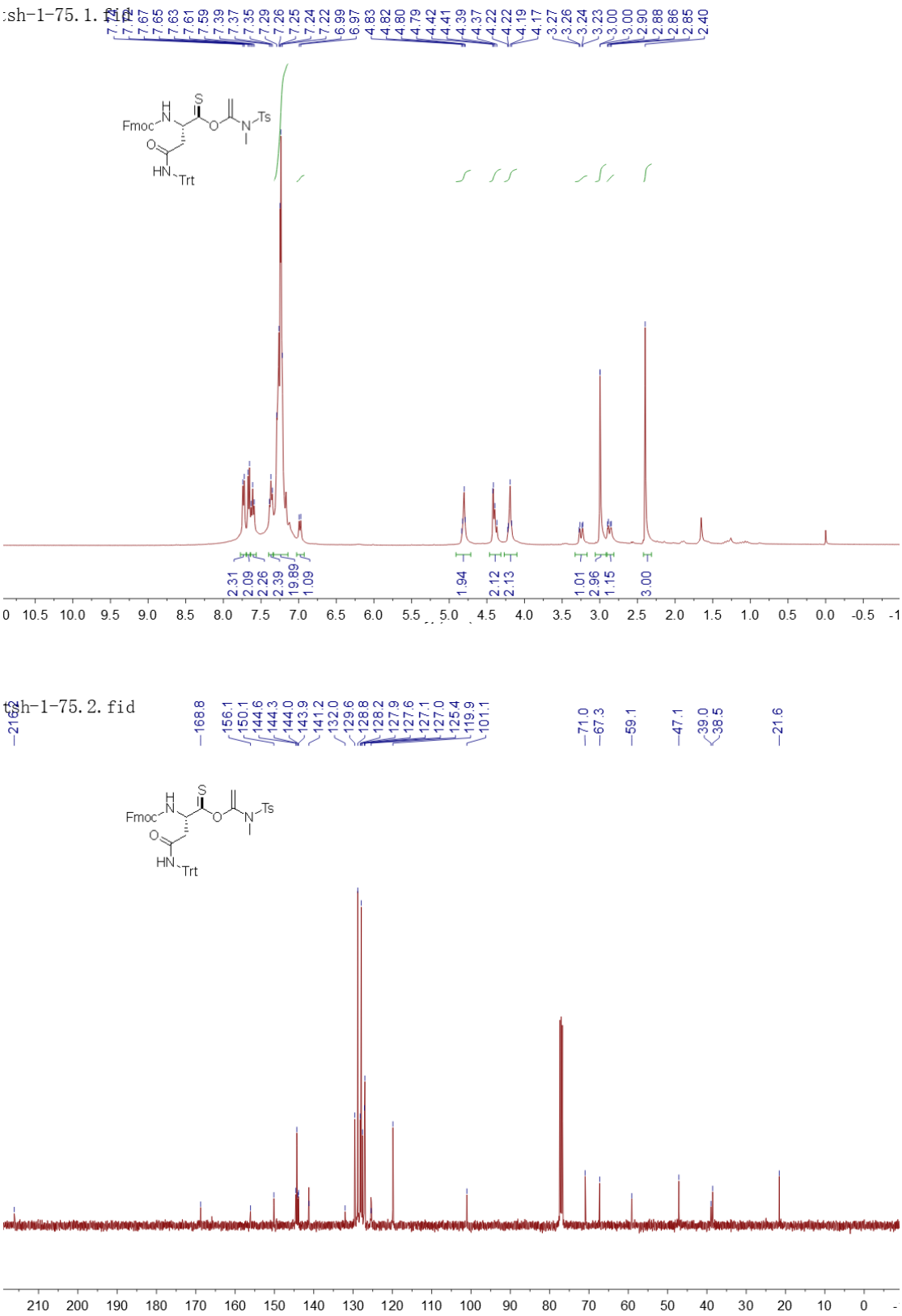


Figure S7. ^{1}H NMR and ^{13}C NMR spectrums of **3i**.

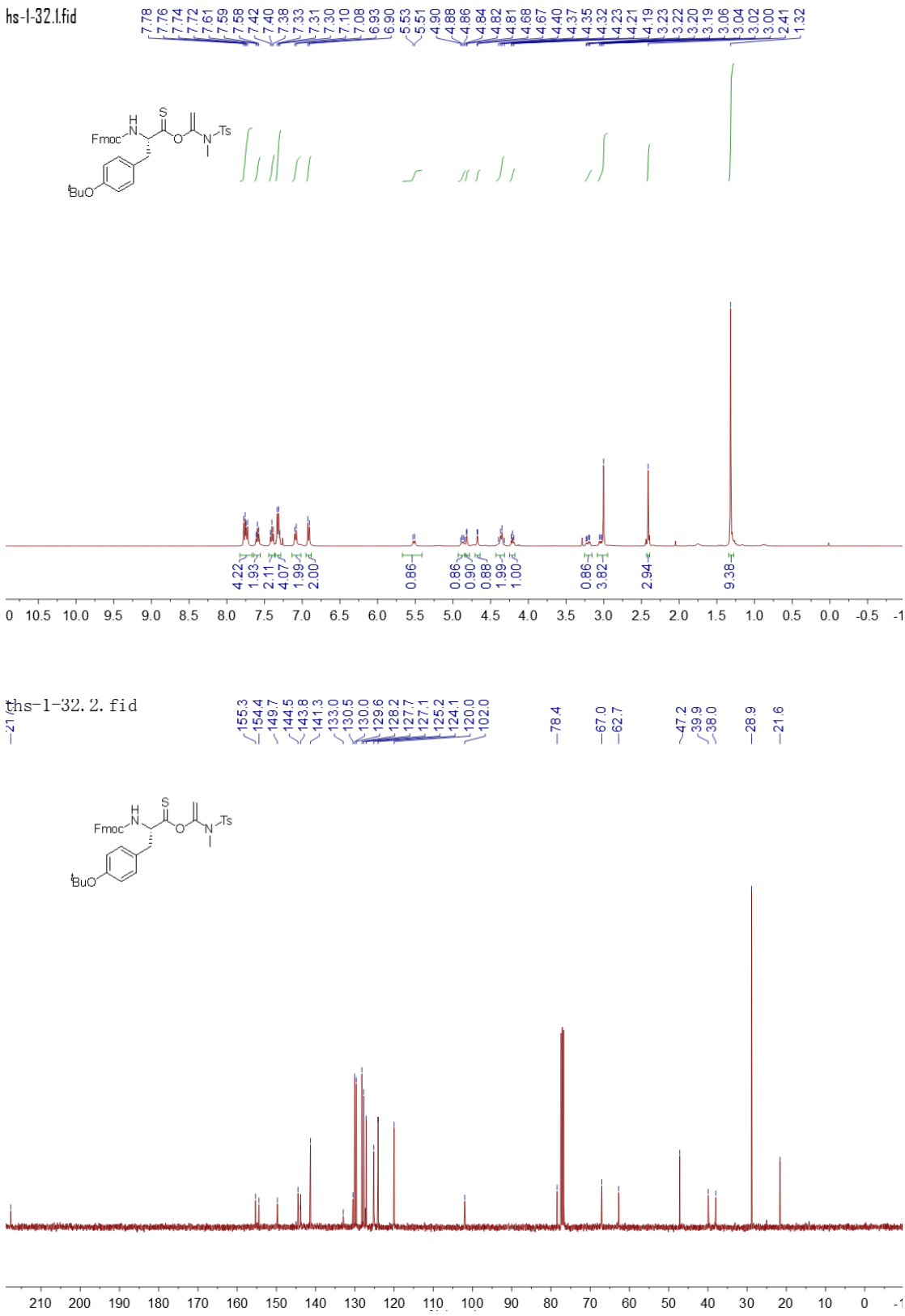


Figure S8. ^{1}H NMR and ^{13}C NMR spectrums of **3j**.

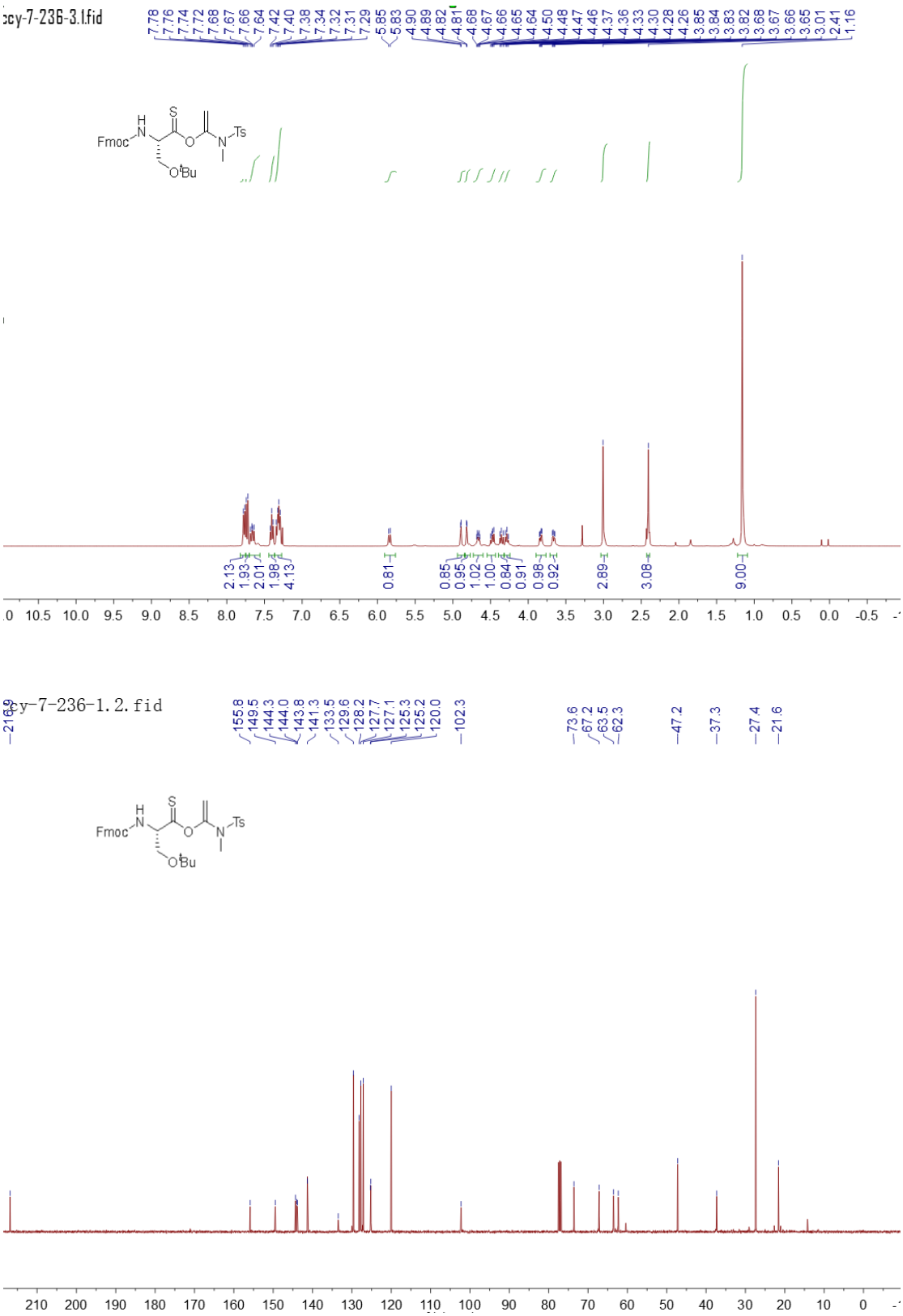
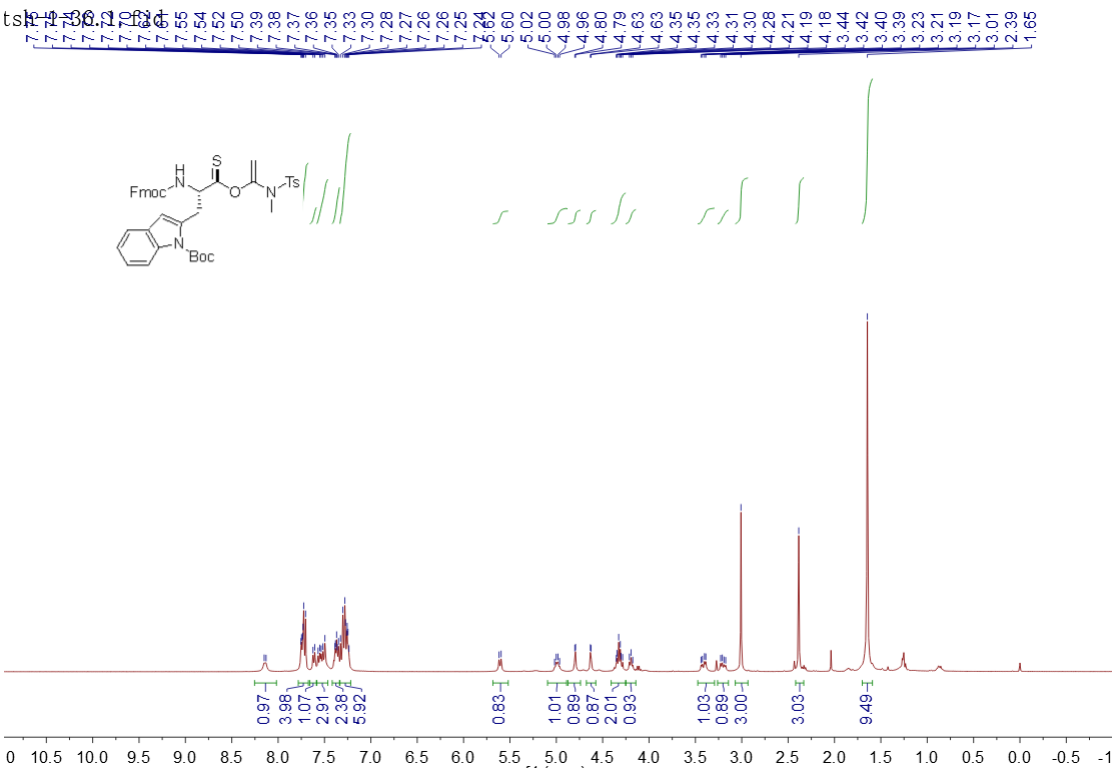


Figure S9. ^{1}H NMR and ^{13}C NMR spectrums of **3k**.



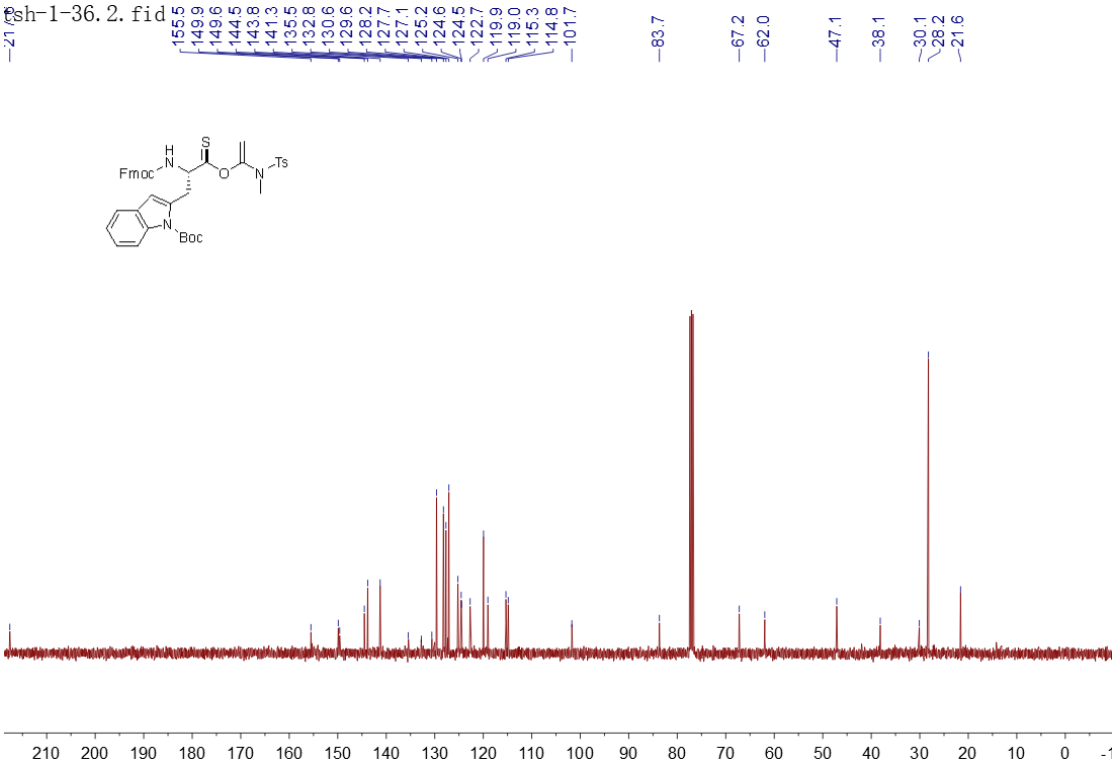


Figure S10. ^{1}H NMR and ^{13}C NMR spectrums of **3l**.

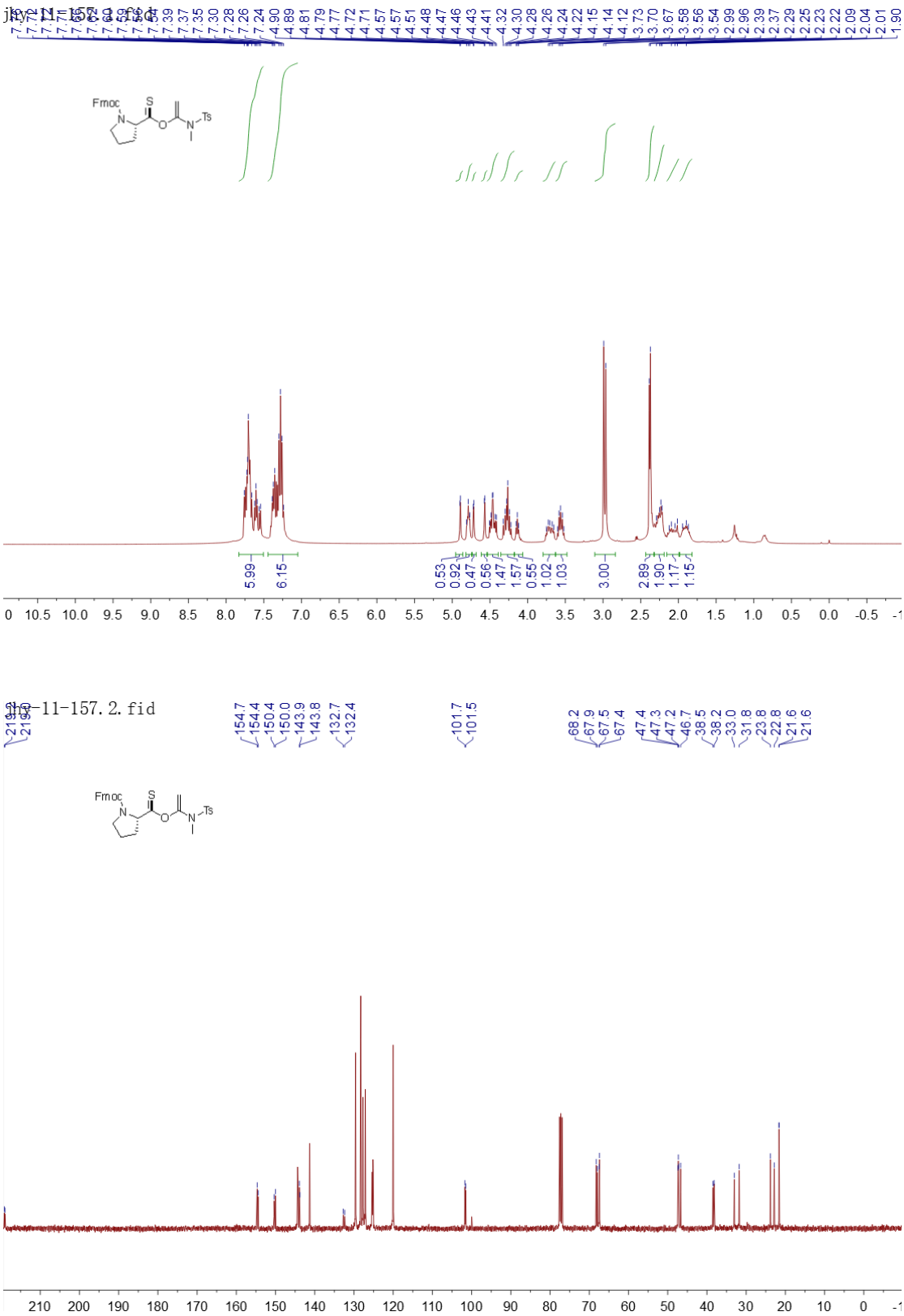


Figure S11. ^{1}H NMR and ^{13}C NMR spectrums of **3n**.

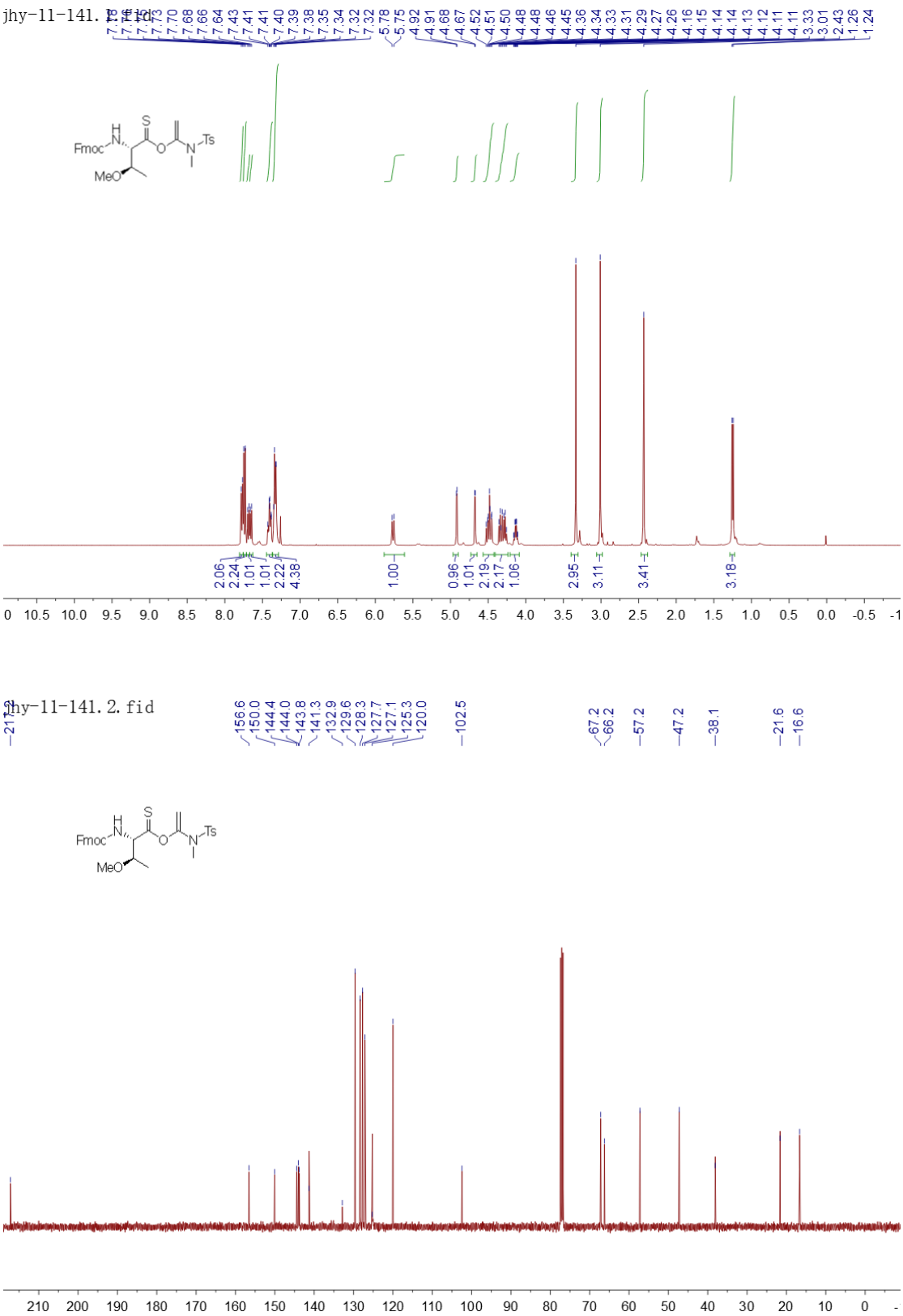


Figure S12. ^{1}H NMR and ^{13}C NMR spectrums of **3o**.

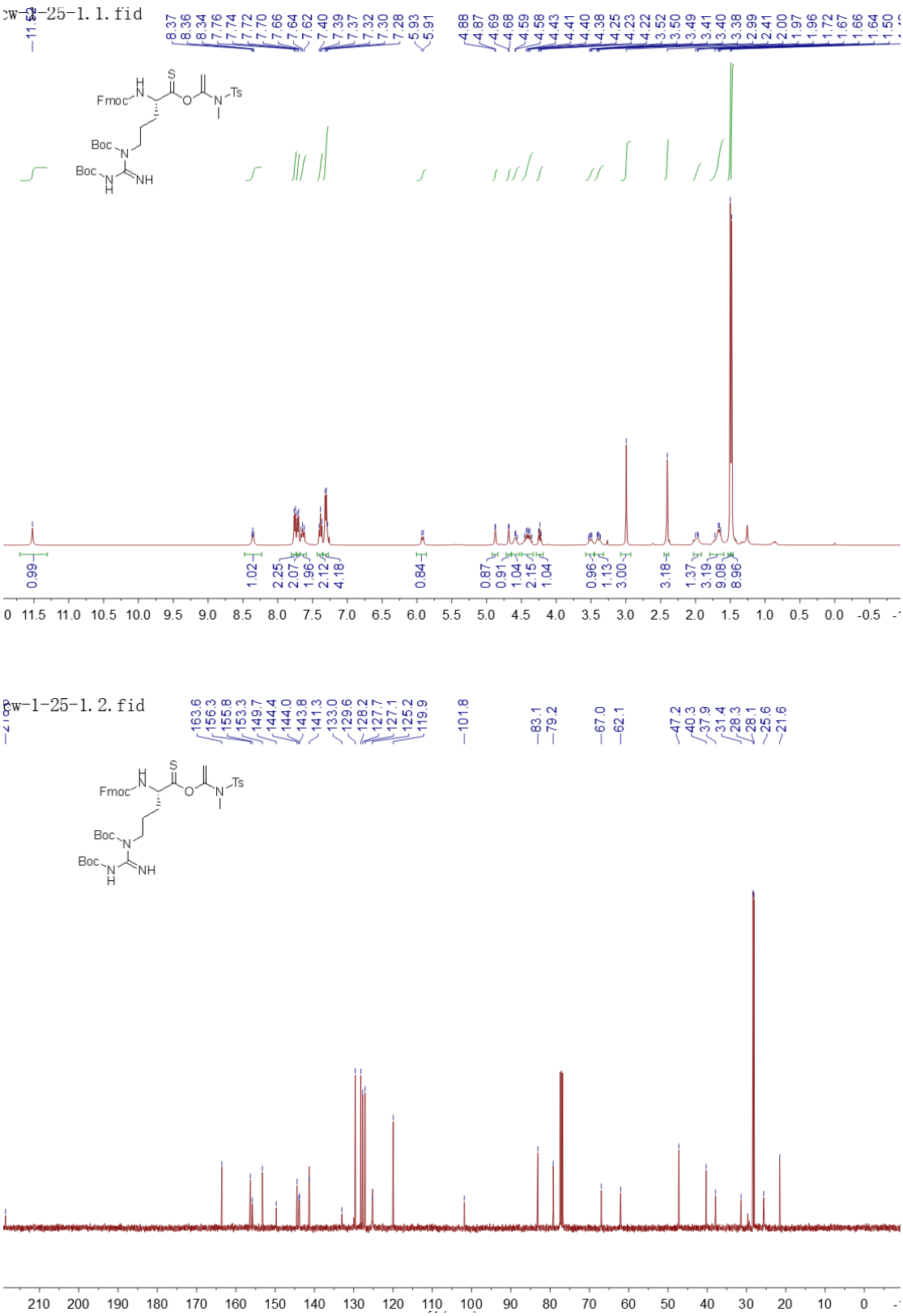
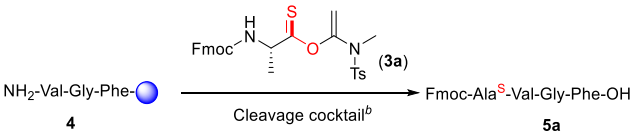


Figure S13. ^{1}H NMR and ^{13}C NMR spectrums of **3s**.

Optimization of Reaction Conditions for Thioamide Incorporation on a Solid Support

Effects of solvent, amount of α-thioacyloxyenamide, and coupling time on the reaction efficiency have been studied. The details of optimization for synthesis of thiopeptide **5a** are shown in Table S1. HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 50-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide.

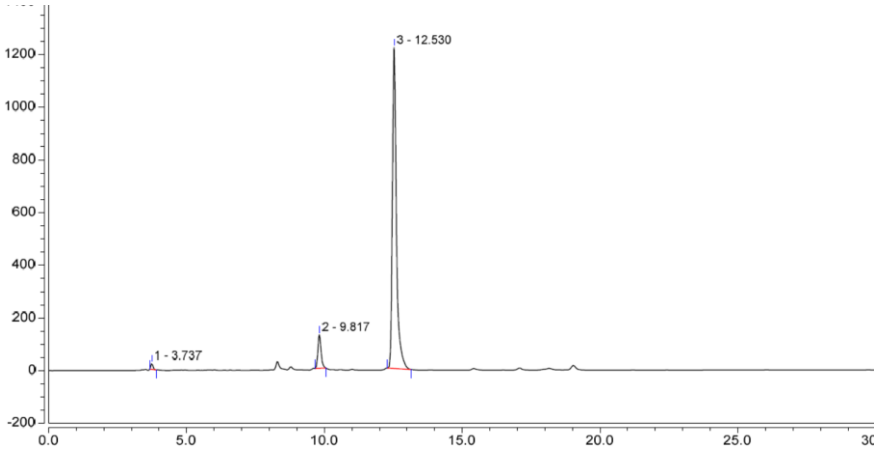
Table S1. Optimization of Reaction Conditions for Thioamide Incorporation on a Solid Support[a]



entry	equiv	time (h)	solvent	conversion (**4**/**5a**)[c]	dr[d]
1[e]	3	1	NMP	1:99	> 99:1
2	3	1	MeOH	69:31	98:2
3	3	1	CH_2Cl_2	3:97	95:5
4	3	1	THF	1:99	98:2
5[f]	3	1	DMF/CH_2Cl_2	1:99	> 99:1
6	**3**	**1**	**DMF**	**< 1:99**	**> 99:1**
7	1	1	DMF	2:98	> 99:1
8	2	1	DMF	1:99	> 99:1
9	3	0.5	DMF	1:99	> 99:1
10[g]	3	2	DMF	< 1:99	> 99:1

[a]Reactions were carried out with 0.03 mmol of **4** at rt. [b]Cleavage cocktail: TFE:AcOH:CH_2Cl_2 = 1:3:6. [c]Conversion (**4**/**5a** ratio) was calculated based on HPLC at 254 nm. [d]Product diastereomeric purity was determined by HPLC. [e]8% (oxoamide:thioamide) oxoamide was detected. [f]DMF/CH_2Cl_2 (v/v) = 1:1. [g]2% (oxoamide:thioamide) oxoamide was detected.

Crude HPLC Traces of Thiopeptide (5a): NMP (Table S1, entry 1): t_R = 12.530 min

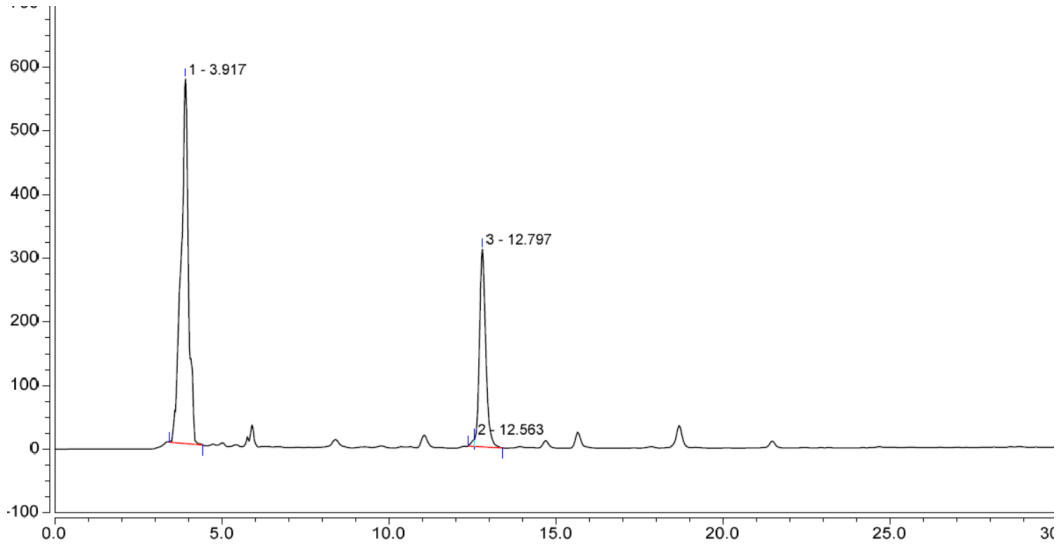


Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.88	1.992	3.737	22.337
2	7.53	16.969	9.817	127.725
3	91.58	206.337	12.530	1218.184
Total:	100	225.298		1368.247

Figure S14: Crude HPLC trace of **5a** after reaction in regular NMP.

MeOH (Table S1, entry 2): t_R = 12.797 min

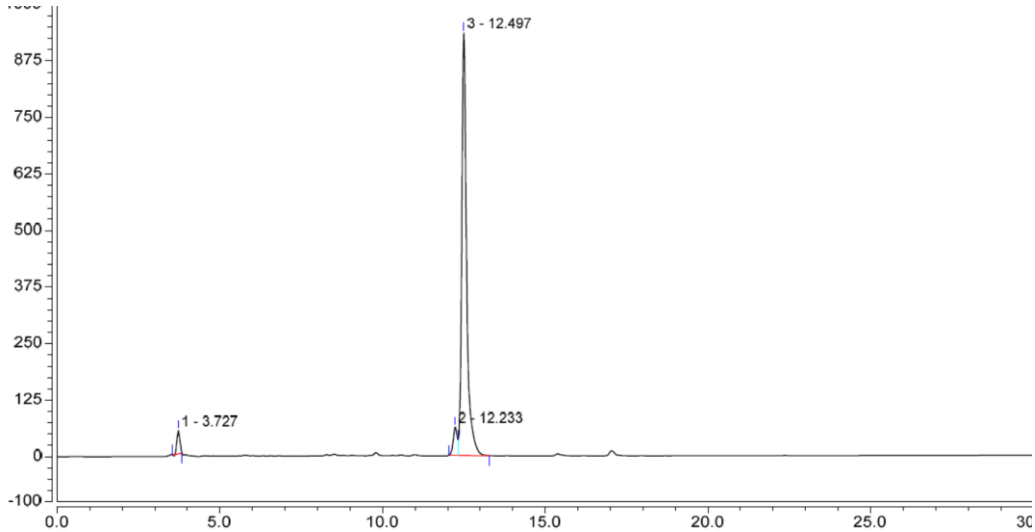


Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	68.99	151.905	3.917	573.019
2	0.52	1.141	12.563	11.169
3	30.50	67.153	12.797	310.335
Total:	100	220.199		894.523

Figure S15: Crude HPLC trace of **5a** after reaction in regular MeOH.

CH_2Cl_2 (Table S1, entry 3): t_R = 12.497 min

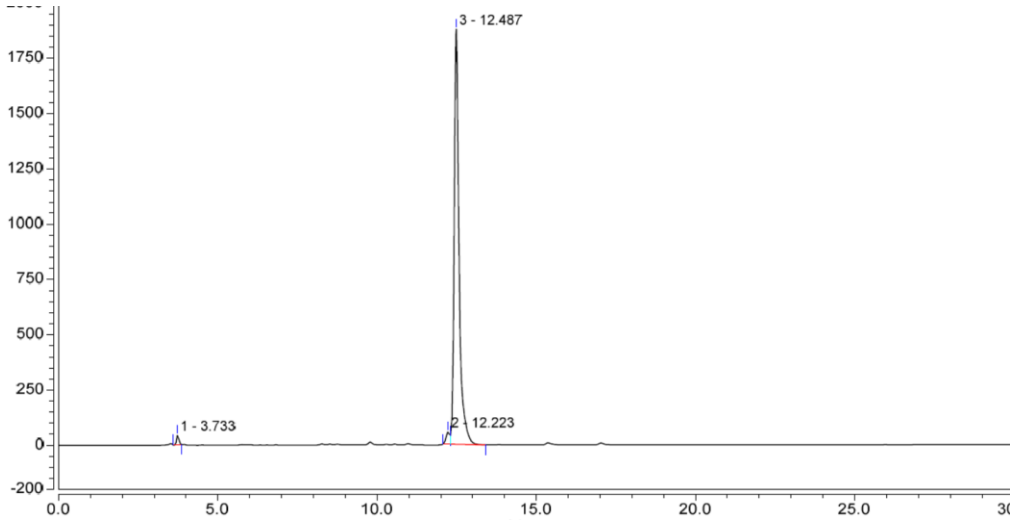


Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	3.10	5.464	3.727	51.748
2	5.06	8.920	12.233	63.112
3	91.84	161.967	12.497	934.236
Total:	100	176.350		1049.095

Figure S16: Crude HPLC trace of **5a** after reaction in regular CH_2Cl_2.

THF (Table S1, entry 4): t_R = 12.487 min

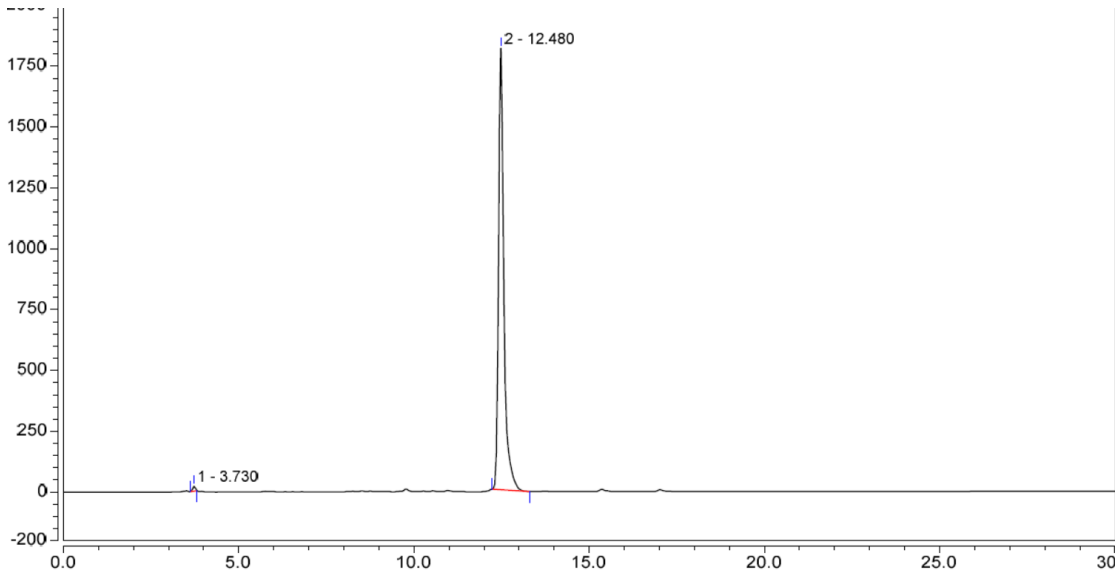


Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.98	3.405	3.733	42.646
2	2.05	7.107	12.223	55.634
3	96.97	336.042	12.487	1878.016
Total:	100	346.555		1976.296

Figure S17: Crude HPLC trace of **5a** after reaction in regular THF.

CH_2Cl_2/DMF (1:1, Table S1, entry 5): t_R = 12.480 min

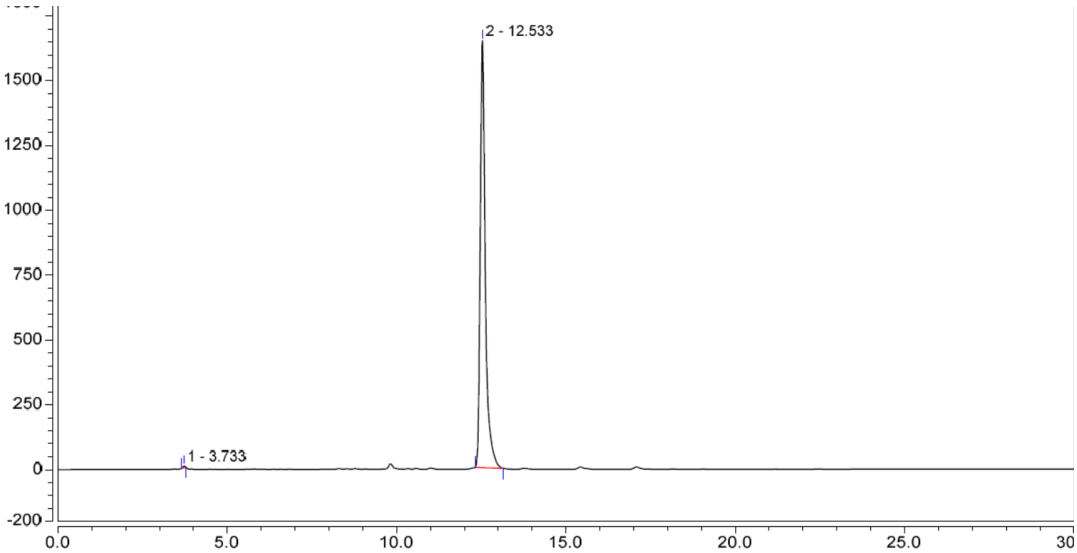


Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.59	1.906	3.730	20.994
2	99.41	320.836	12.480	1813.561
Total:	100	322.742		1834.555

Figure S18: Crude HPLC trace of **5a** after reaction in regular CH_2Cl_2/DMF.

DMF (Table S1, entry 6): t_R = 12.533 min

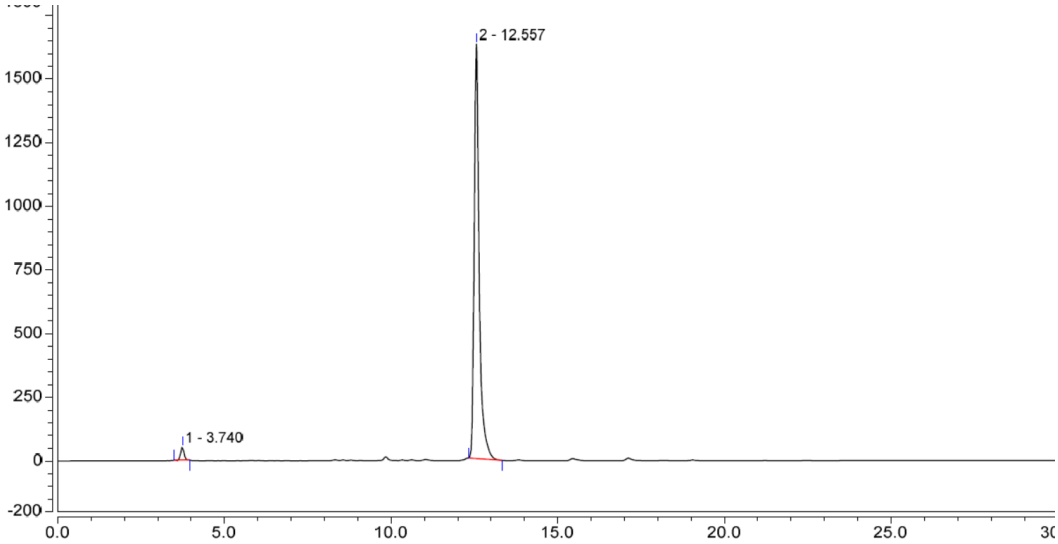


Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.19	0.538	3.733	7.479
2	99.81	320.836	12.533	1647.676
Total:	100	287.996		1655.155

Figure S19: Crude HPLC trace of **5a** after reaction in regular DMF.

1.0 equiv of 3a (Table S1, entry 7): t_R = 12.557 min

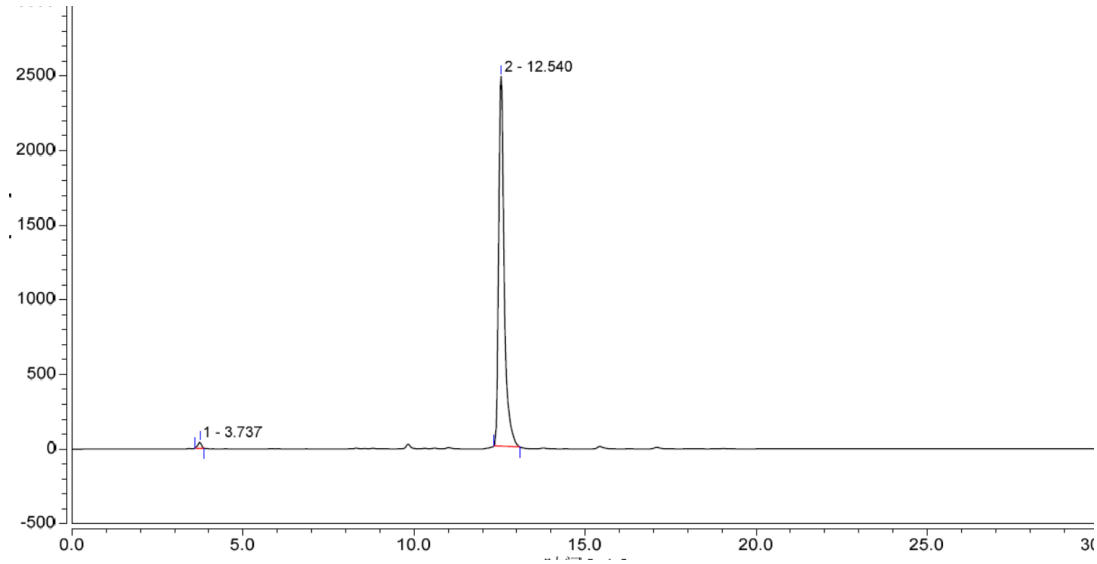


Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	1.99	5.746	3.740	50.999
2	98.01	283.645	12.557	1627.386
Total:	100	289.391		1678.385

Figure S20: Crude HPLC trace of **5a** after 1.0 equiv of **3a** was added.

2.0 equiv of 3a (Table S1, entry 8): t_R = 12.540 min

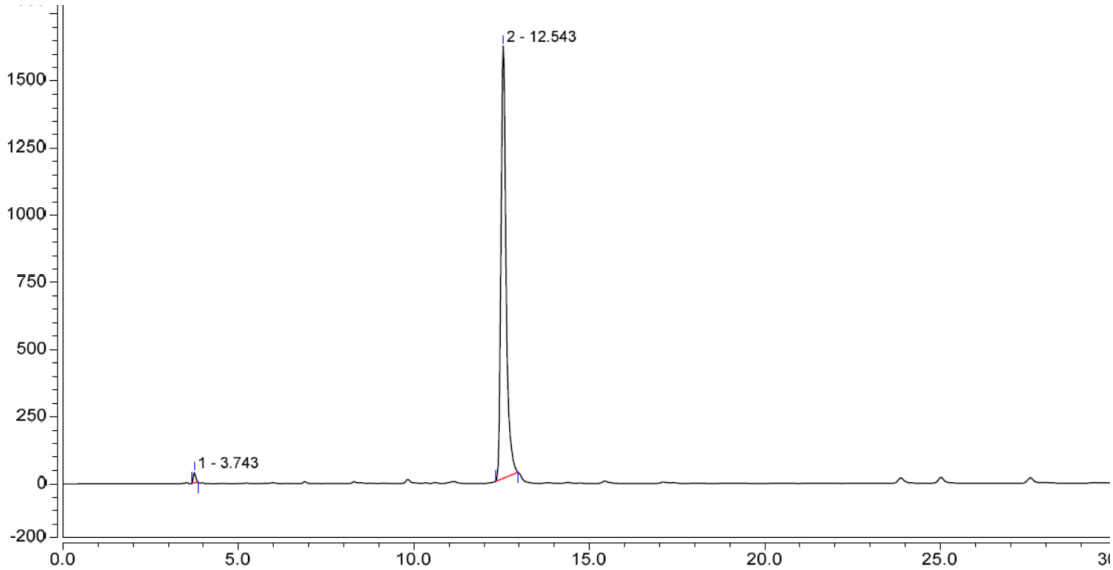


Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	1.02	4.808	3.737	41.395
2	98.98	466.029	12.540	2479.161
Total:	100	470.836		2520.556

Figure S21: Crude HPLC trace of **5a** after 2.0 equiv of **3a** was added.

0.5 h (Table S1, entry 9): t_R = 12.543 min

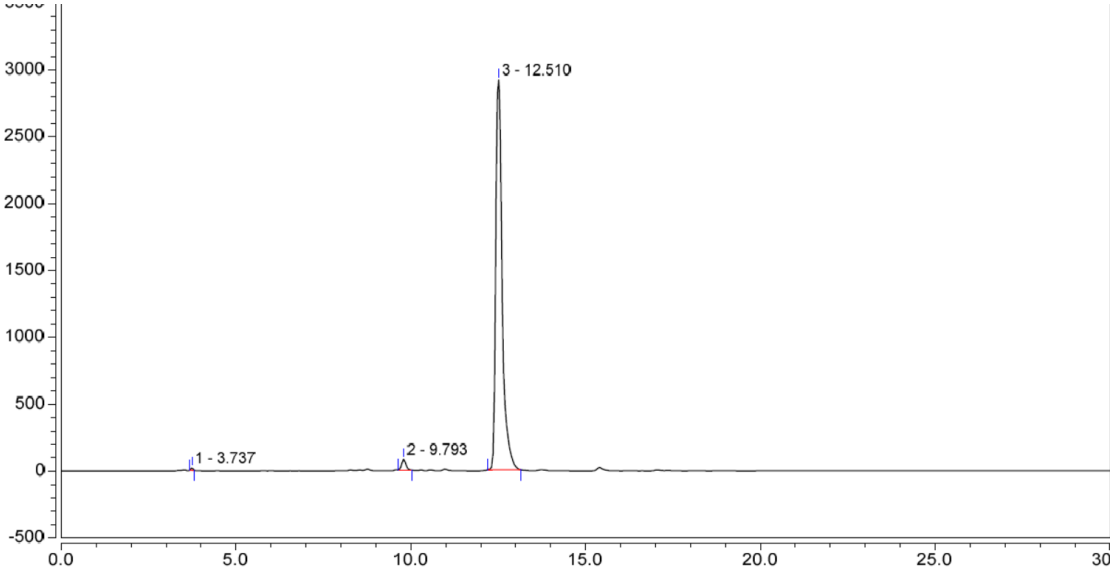


Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	1.11	3.020	3.743	38.821
2	98.89	268.658	12.543	1608.450
Total:	100	271.678		1647.270

Figure S22: Crude HPLC trace of **5a** after 0.5 h.

2 h (Table S1, entry 10): t_R = 12.510 min



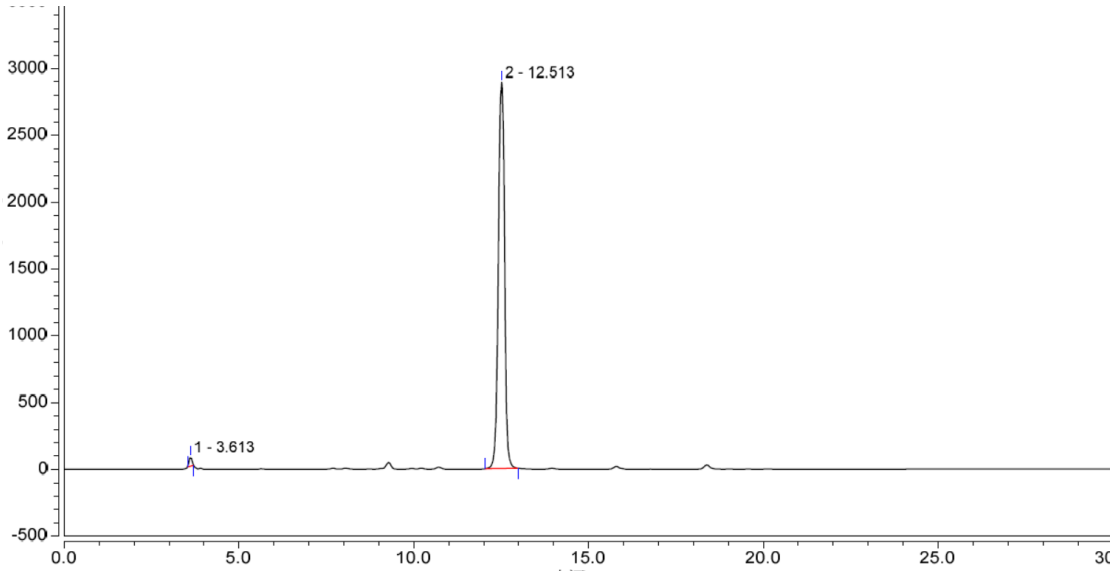
Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.21	1.348	3.737	18.557
2	1.72	10.919	9.793	80.039
3	98.06	621.473	12.510	2916.136
Total:	100	633.740		3014.732

Figure S23: Crude HPLC trace of **5a** after 2 h.

Crude HPLC Traces and ESI-MS Spectra of Monothiopeptides 5a-5v.

Fmoc-Ala^{S}-Val-Gly-Phe-OH (**5a**):



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	1.15	6.880	3.613	66.647
2	98.85	593.678	12.513	2890.773
Total:	100	600.558		2957.419

Figure S24: Crude HPLC trace of **5a** with optimized reaction conditions.

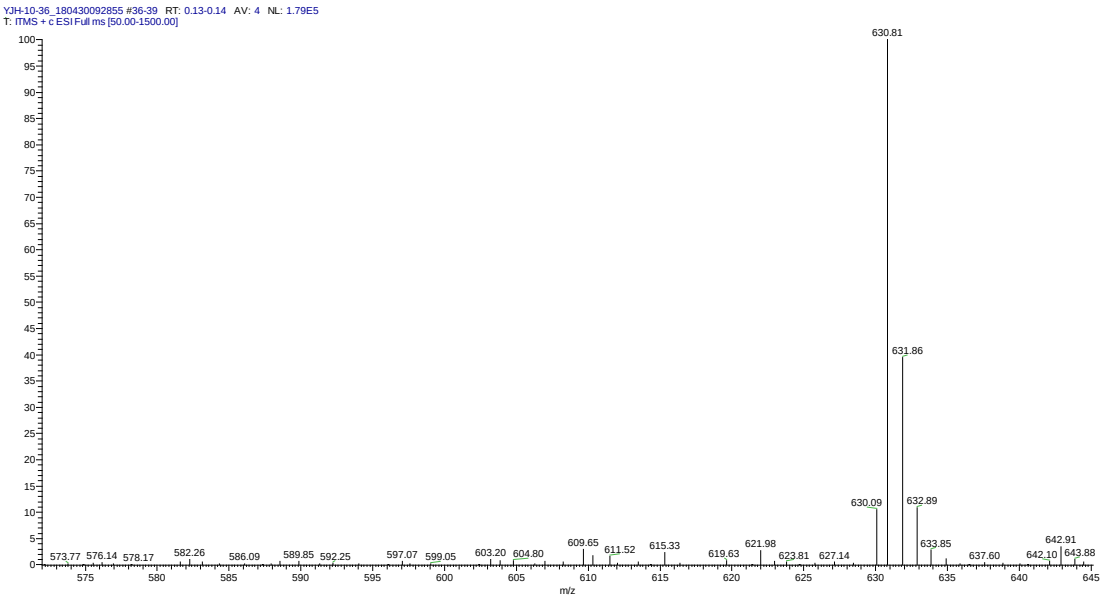
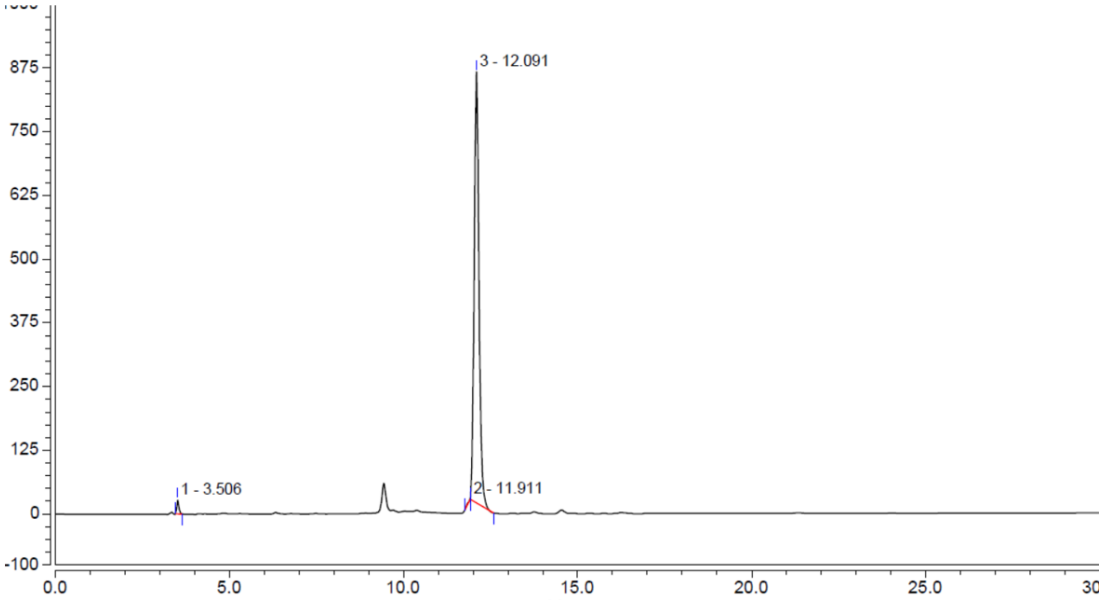


Figure S25: ESI-MS analysis of **5a**.

Fmoc-Phe^{S}-Val-Gly-Phe-OH (**5b**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	1.40	1.966	3.506	27.827
2	1.38	2.237	11.896	21.550
3	97.12	141.624	12.091	863.111
Total:	100	145.827		912.488

Figure S26: Crude HPLC trace of **5b** with optimized reaction conditions.

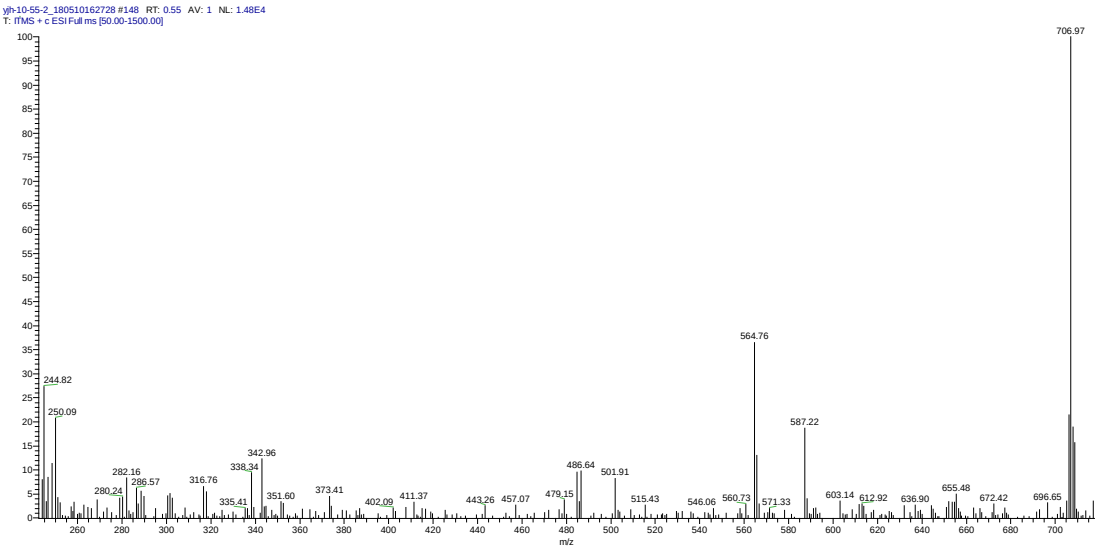
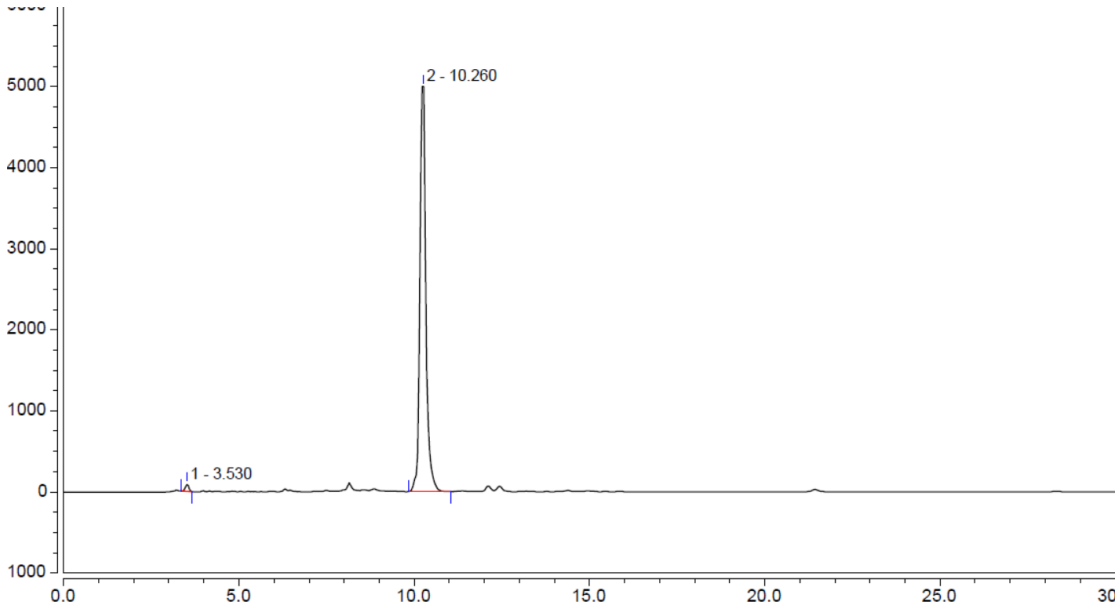


Figure S27: ESI-MS analysis of **5b**.

Fmoc-Met^{S}-Val-Gly-Phe-OH (**5c**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	1.00	10.023	3.530	91.529
2	99.00	996.380	10.260	4995.454
Total:	100	1006.403		5086.983

Figure S28: Crude HPLC trace of **5c** with optimized reaction conditions.

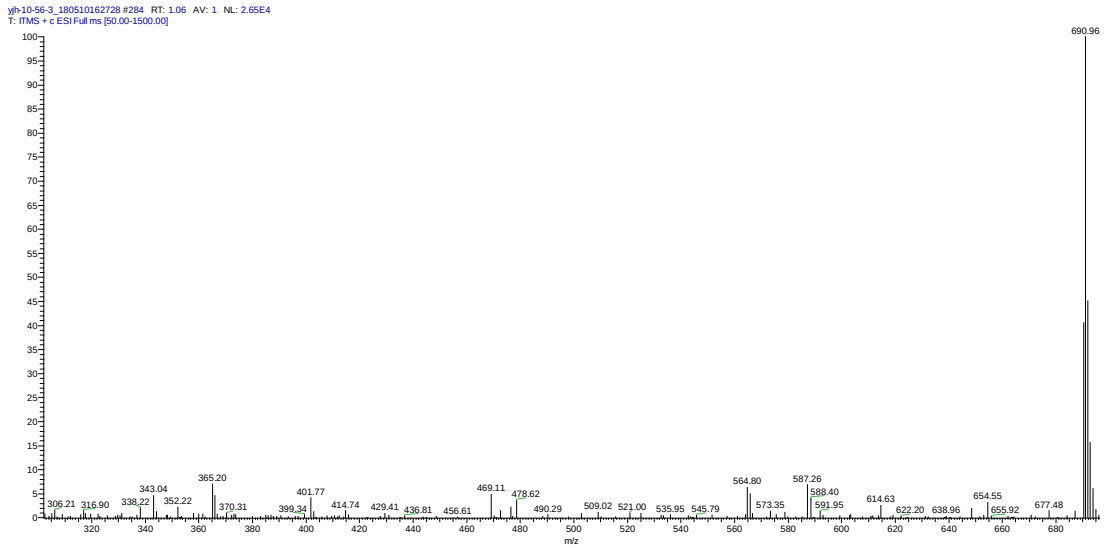
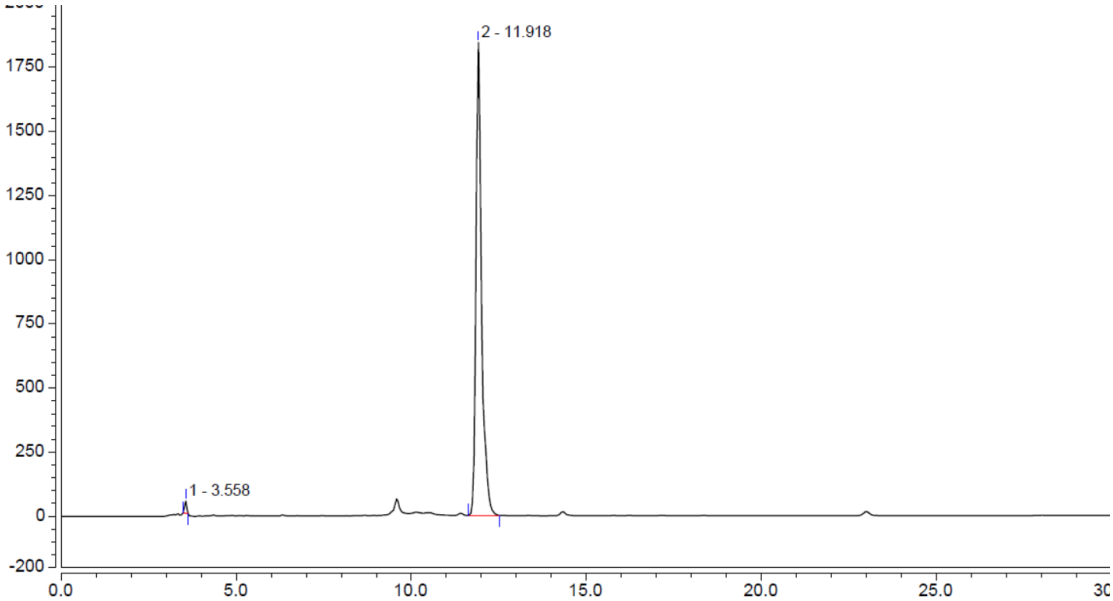


Figure S29: ESI-MS analysis of **5c**.

Fmoc-Lys(Boc)^{S}-Val-Gly-Phe-OH (**5d**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.97	3.354	3.558	48.337
2	99.03	342.162	11.918	1842.934
Total:	100	345.516		1891.272

Figure S30: Crude HPLC trace of **5d** with optimized reaction conditions.

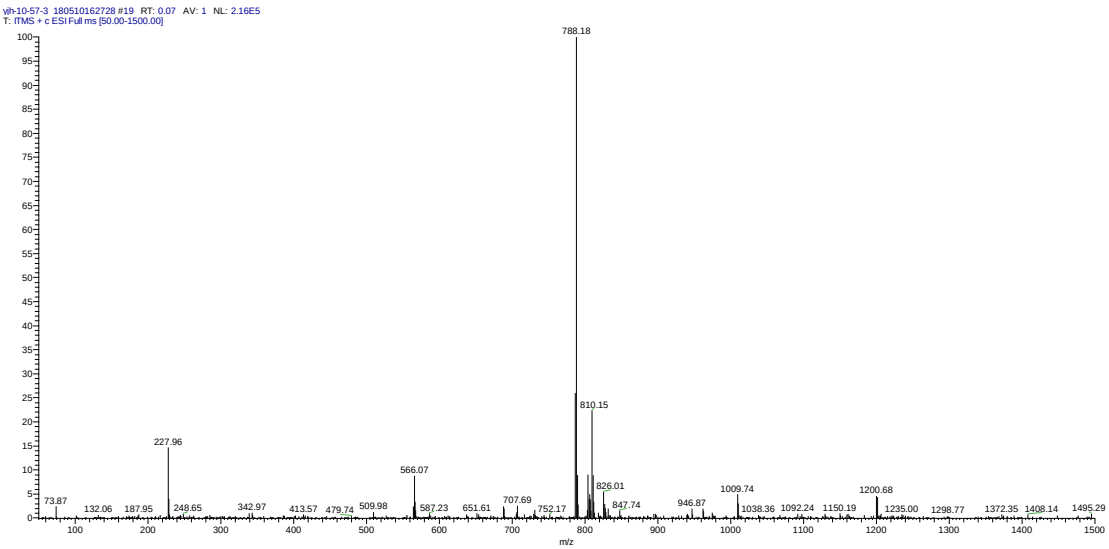
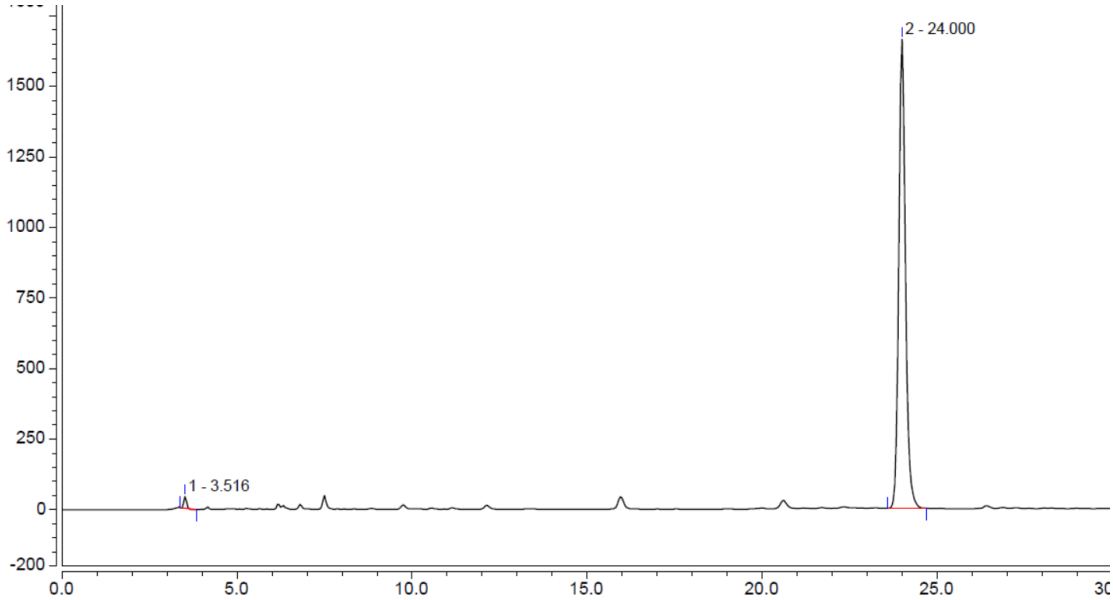


Figure S31: ESI-MS analysis of **5d**.

Fmoc-Cys(Trt)^{S}-Val-Gly-Phe-OH (**5e**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.98	3.665	3.516	42.859
2	99.02	369.252	24.000	1662.655
Total:	100	372.916		1705.513

Figure S32: Crude HPLC trace of **5e** with optimized reaction conditions.

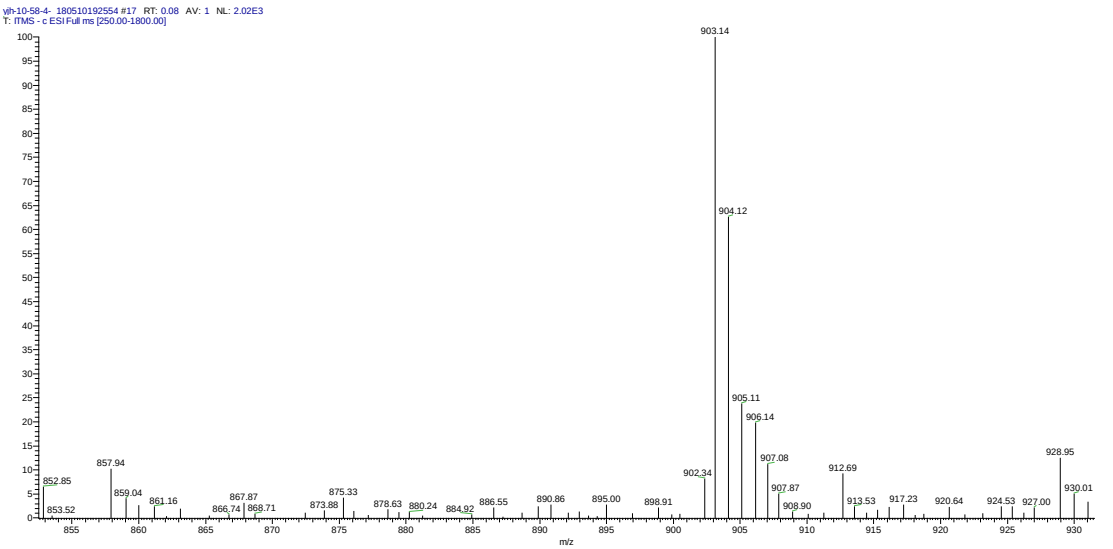
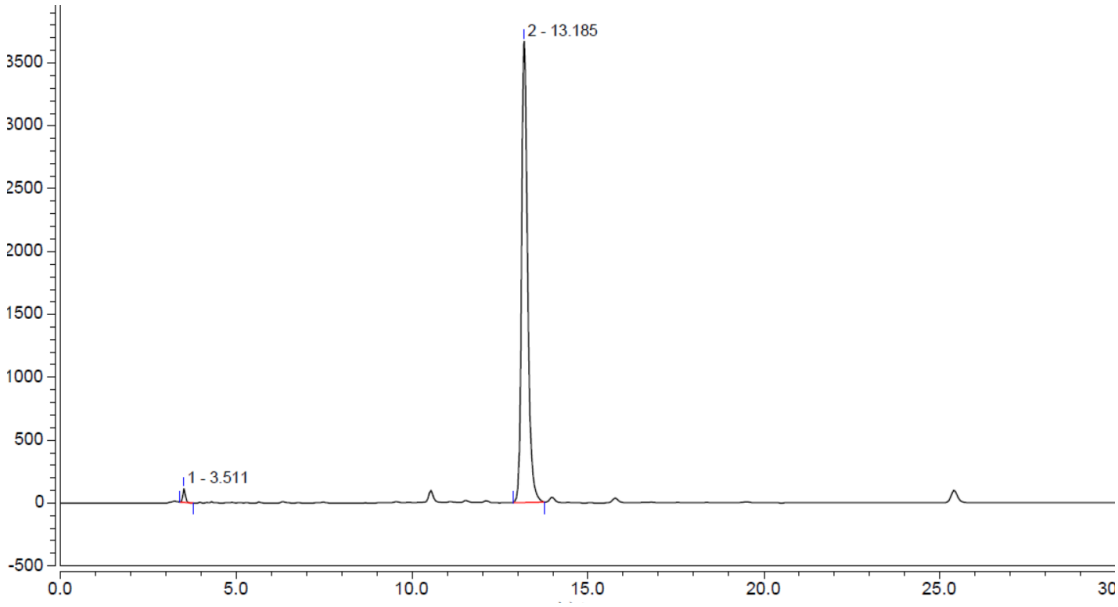


Figure S33: ESI-MS analysis of **5e**.

Fmoc-Glu(^{t}Bu)^{S}-Val-Gly-Phe-OH (**5f**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	1.20	8.921	3.551	110.210
2	98.80	737.467	13.185	3667.636
Total:	100	746.388		3777.846

Figure S34: Crude HPLC trace of **5f** with optimized reaction conditions.

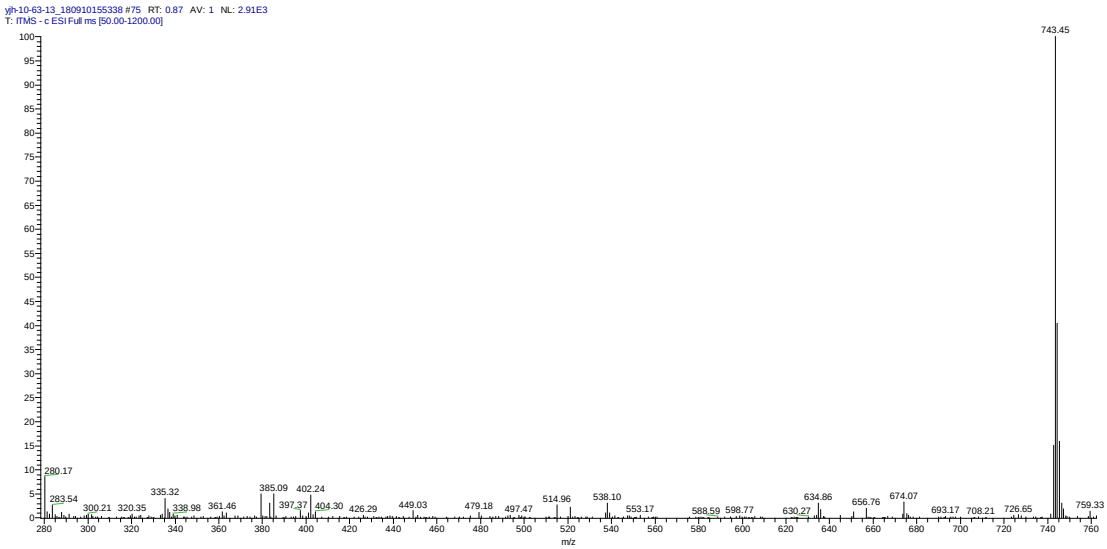
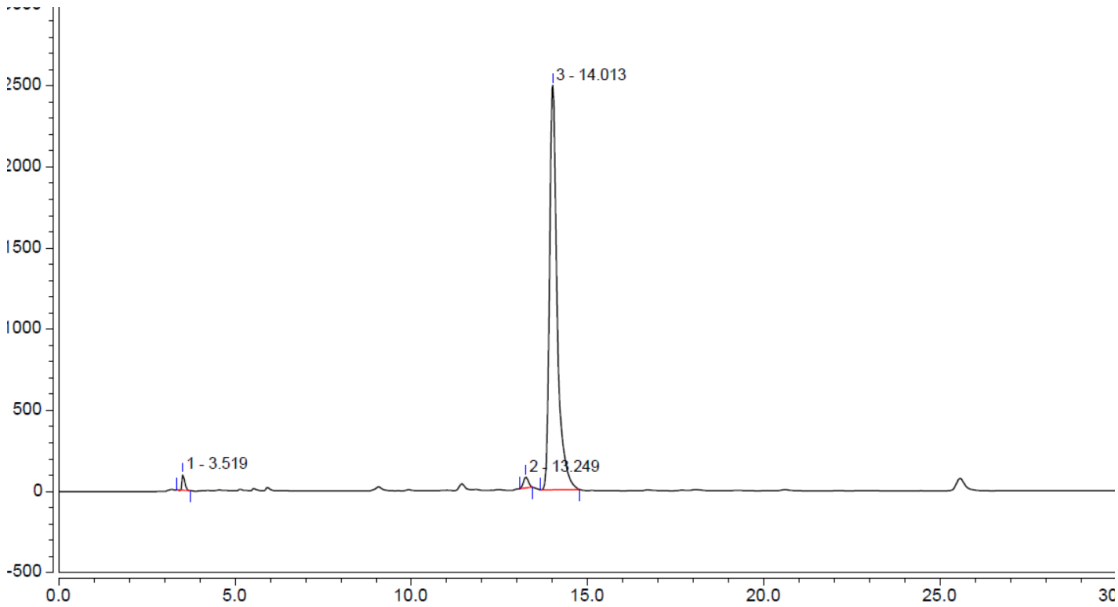


Figure S35: ESI-MS analysis of **5f**.

Fmoc-Gln(Trt)^{S}-Val-Gly-Phe-OH (**5g**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	1.30	8.360	3.519	98.131
2	1.74	11.224	13.249	68.417
3	96.96	625.536	14.013	2493.088
Total:	100	645.120		2659.636

Figure S36: Crude HPLC trace of **5g** with optimized reaction conditions.

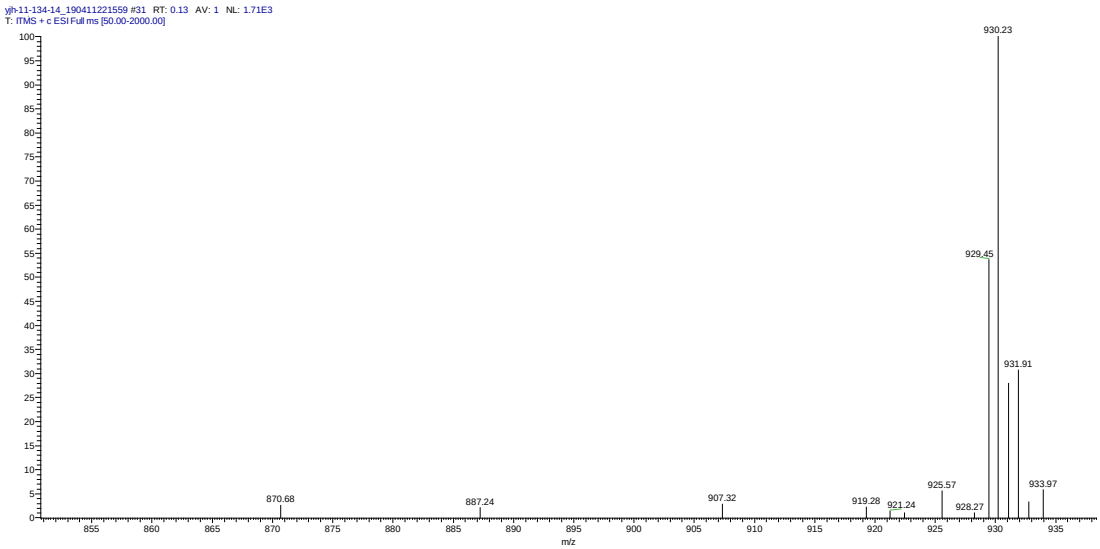
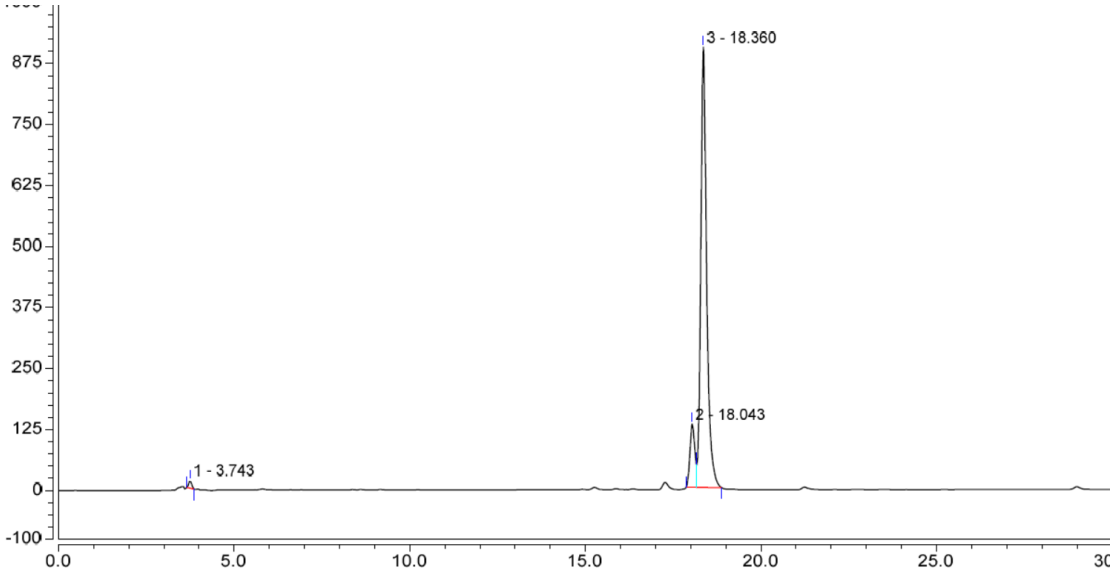


Figure S37: ESI-MS analysis of **5g**.

Fmoc-Asp(^{t}Bu)^S-Val-Gly-Phe-OH (**5h**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.77	1.510	3.743	15.342
2	10.37	20.307	18.043	129.545
3	88.86	174.075	18.360	902.017
Total:	100	195.891		1046.904

Figure S38: Crude HPLC trace of **5h** with optimized reaction conditions.

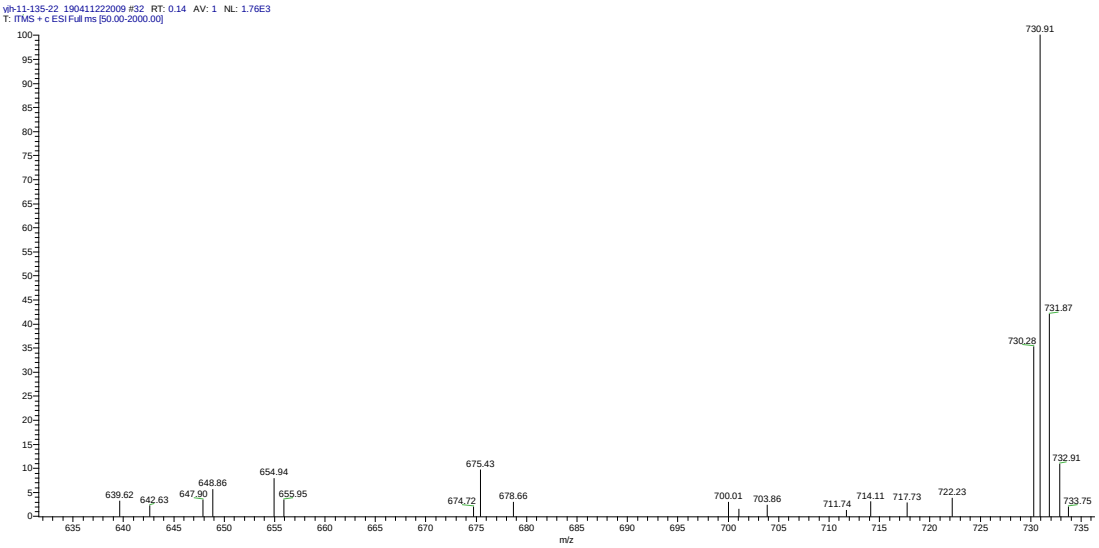
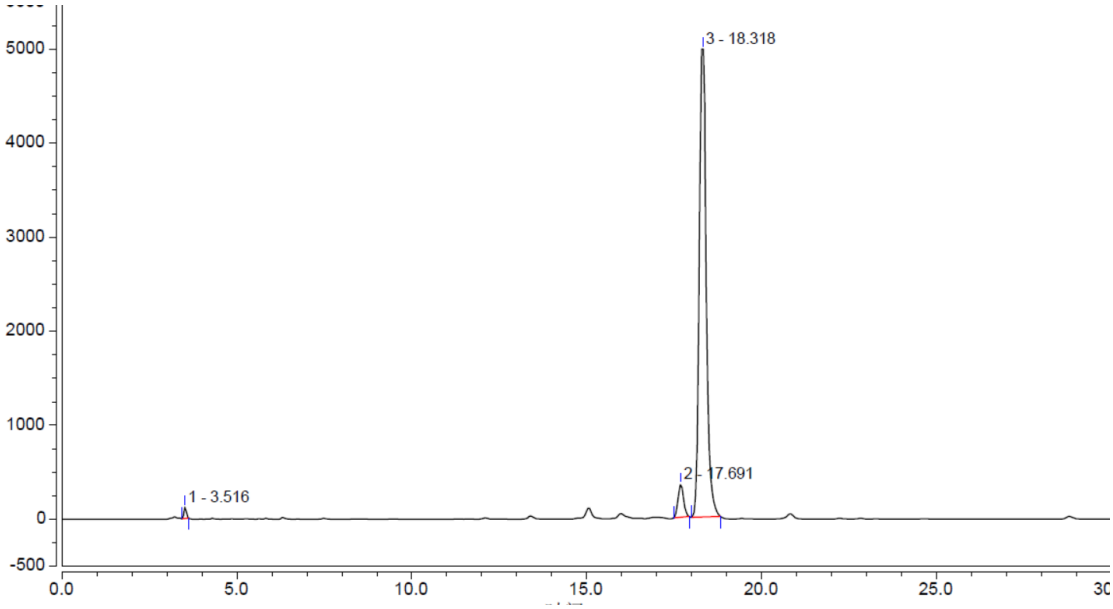


Figure S39: ESI-MS analysis of **5h**.

Fmoc-Asn(Trt)^{S}-Val-Gly-Phe-OH (**5i**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.81	9.988	3.516	122.619
2	5.19	64.074	17.691	350.658
3	94.00	1160.495	18.318	4974.494
Total:	100	1234.556		5447.771

Figure S40: Crude HPLC trace of **5i** with optimized reaction conditions.

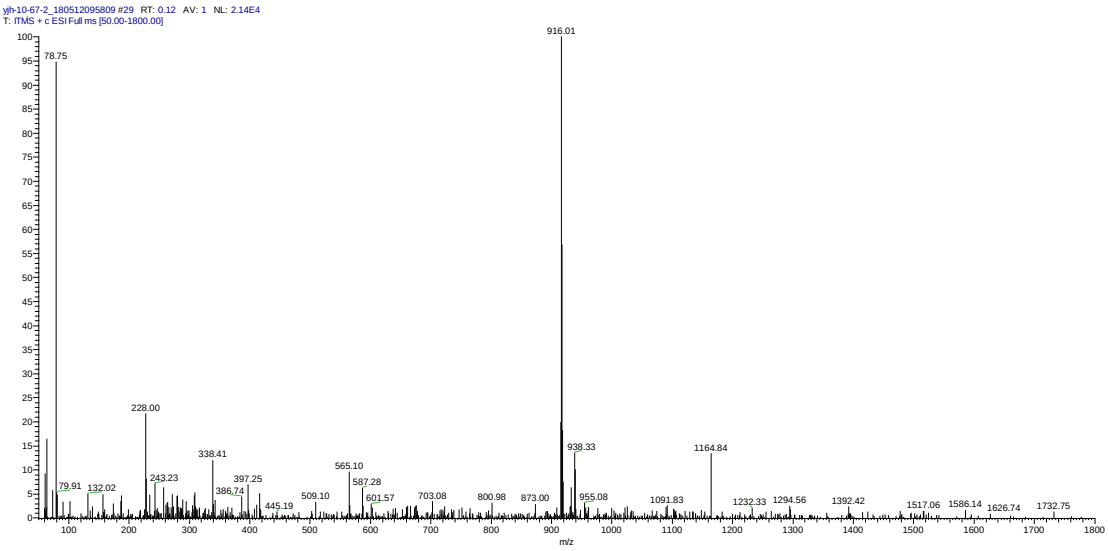
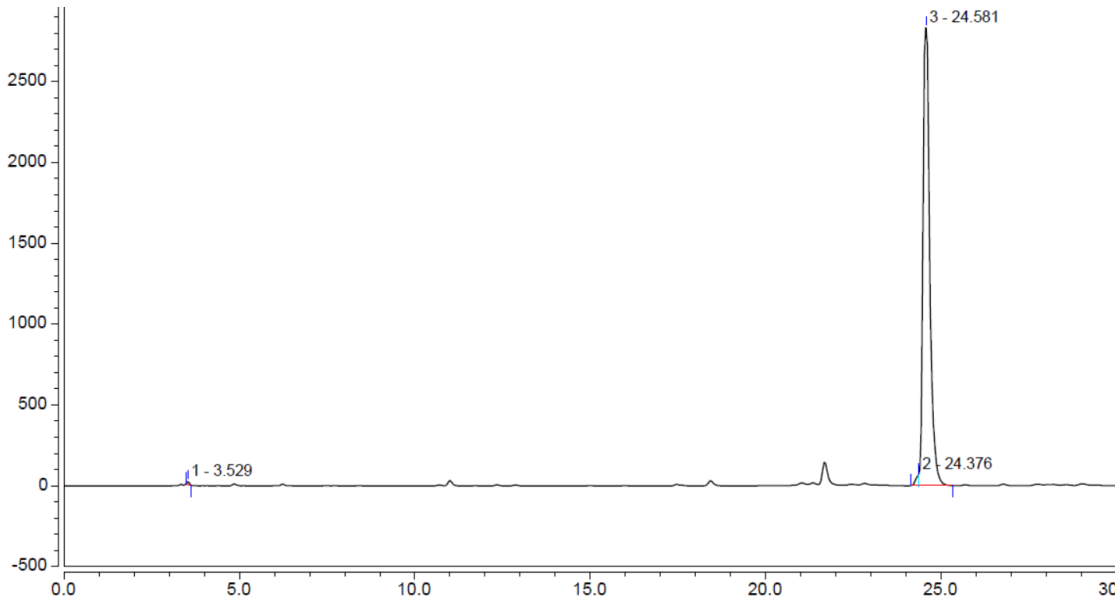


Figure S41: ESI-MS analysis of **5i**.

Fmoc-Tyr(^{t}Bu)^S-Val-Gly-Phe-OH (**5j**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.22	1.394	3.529	19.444
2	1.05	6.754	24.376	67.627
3	98.73	635.333	24.581	2833.548
Total:	100	643.480		2920.620

Figure S42: Crude HPLC trace of **5j** with optimized reaction conditions.

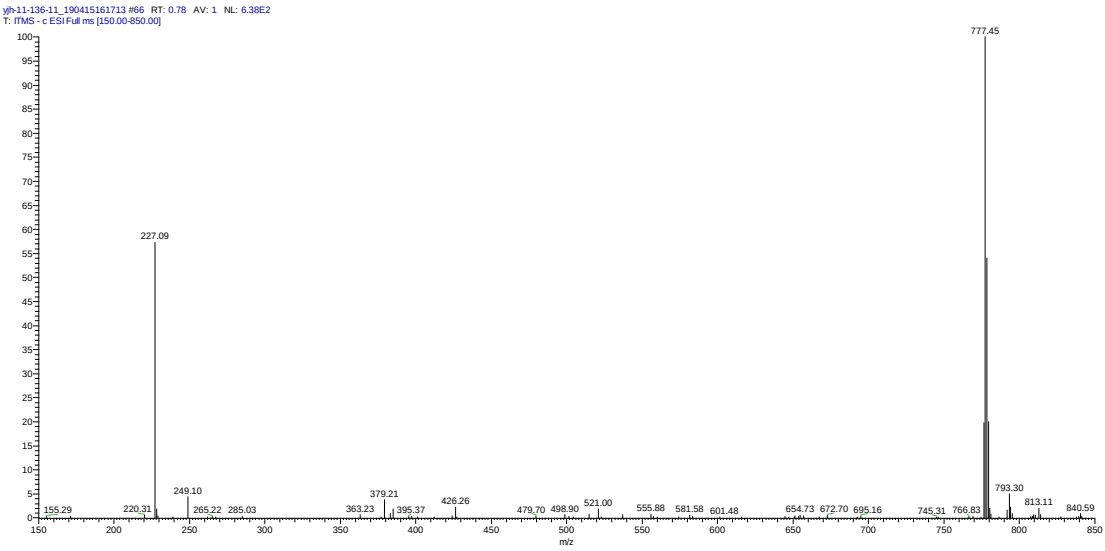
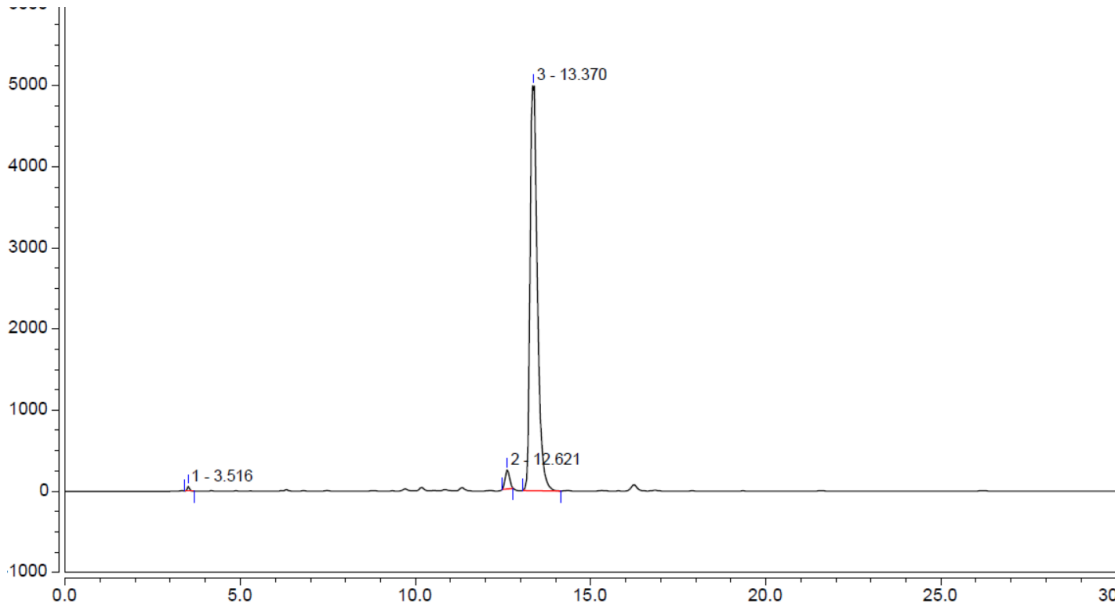


Figure S43: ESI-MS analysis of **5j**.

Fmoc-Ser(^{t}Bu)^S-Val-Gly-Phe-OH (**5k**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.36	4.486	3.516	62.009
2	2.81	35.066	12.621	240.548
3	96.83	1208.637	13.370	4991.976
Total:	100	1248.189		5294.533

Figure S44: Crude HPLC trace of **5k** with optimized reaction conditions.

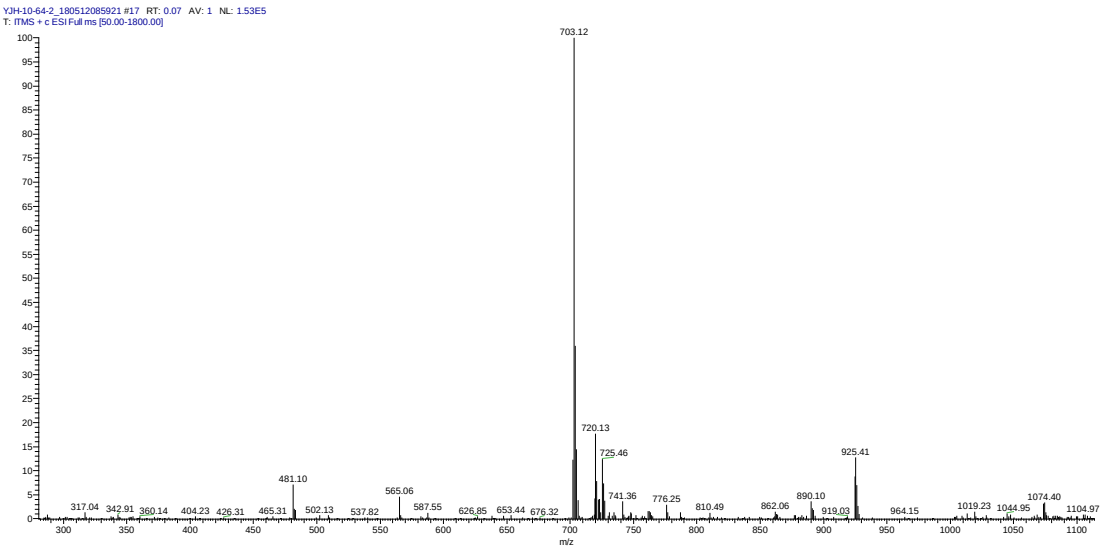
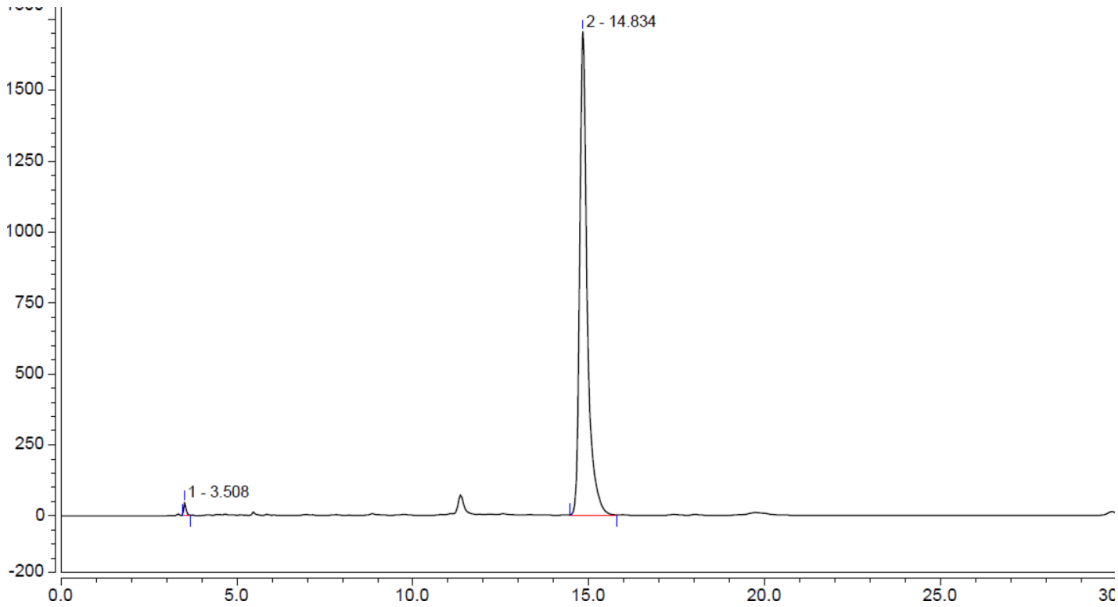


Figure S45: ESI-MS analysis of **5k**.

Fmoc-Trp(Boc)^{S}-Val-Gly-Phe-OH (**5l**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.83	3.568	3.508	46.597
2	99.17	424.195	14.834	1705.084
Total:	100	427.763		1751.681

Figure S46: Crude HPLC trace of **5l** with optimized reaction conditions.

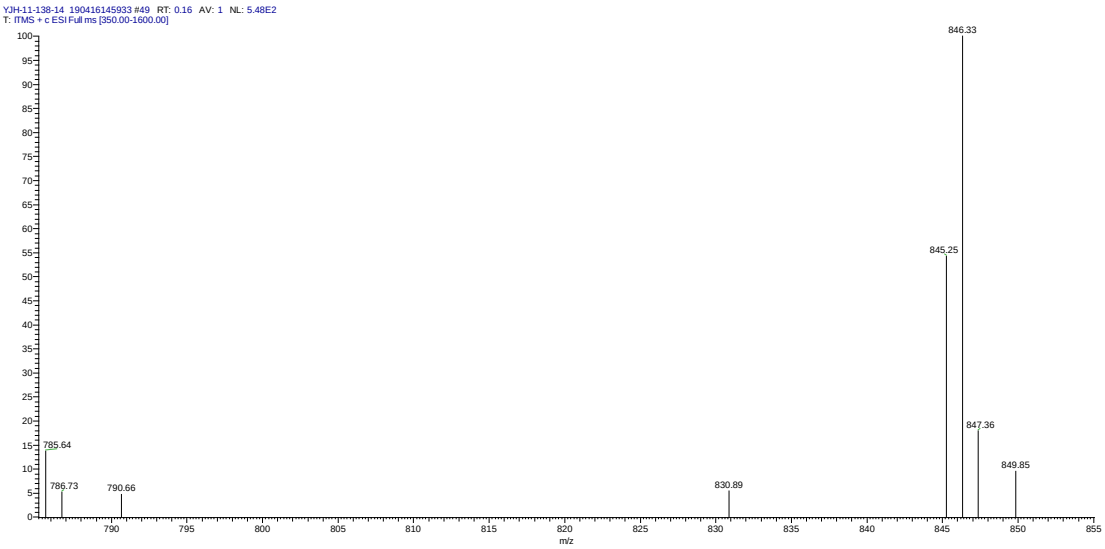
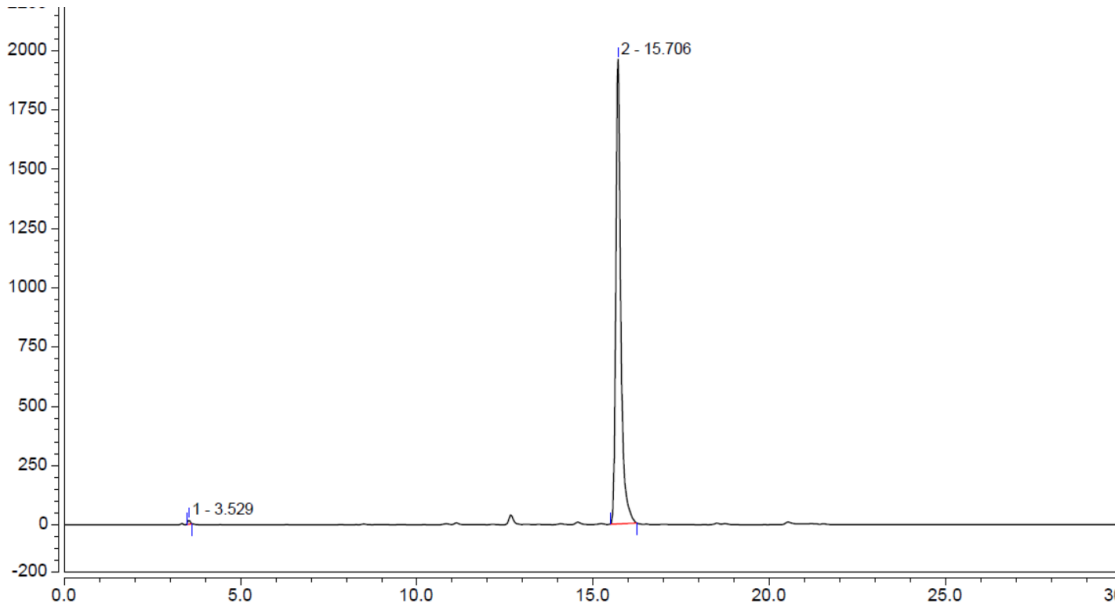


Figure S47: ESI-MS analysis of **5l**.

Fmoc-Gly^{S}-Val-Gly-Phe-OH (**5m**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.42	1.354	3.529	20.028
2	99.58	322.506	15.706	1960.325
Total:	100	323.859		1980.353

Figure S48: Crude HPLC trace of **5m** with optimized reaction conditions.

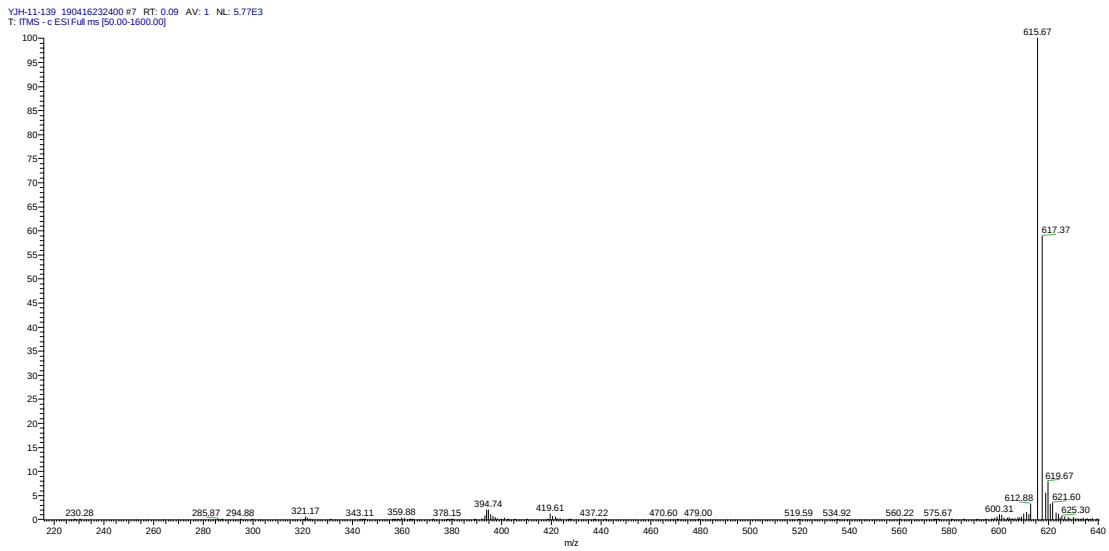
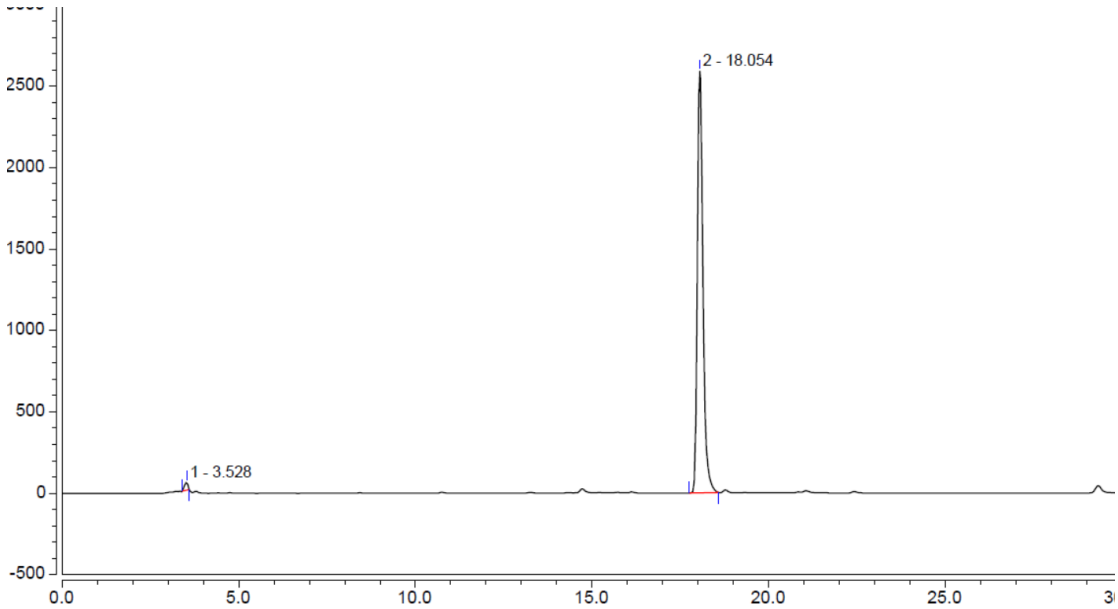


Figure S49: ESI-MS analysis of **5m**.

Fmoc-Pro^{S}-Val-Gly-Phe-OH (**5n**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	1.15	5.575	3.528	47.516
2	98.85	477.191	18.054	2590.887
Total:	100	482.766		482.766

Figure S50: Crude HPLC trace of **5n** with optimized reaction conditions.

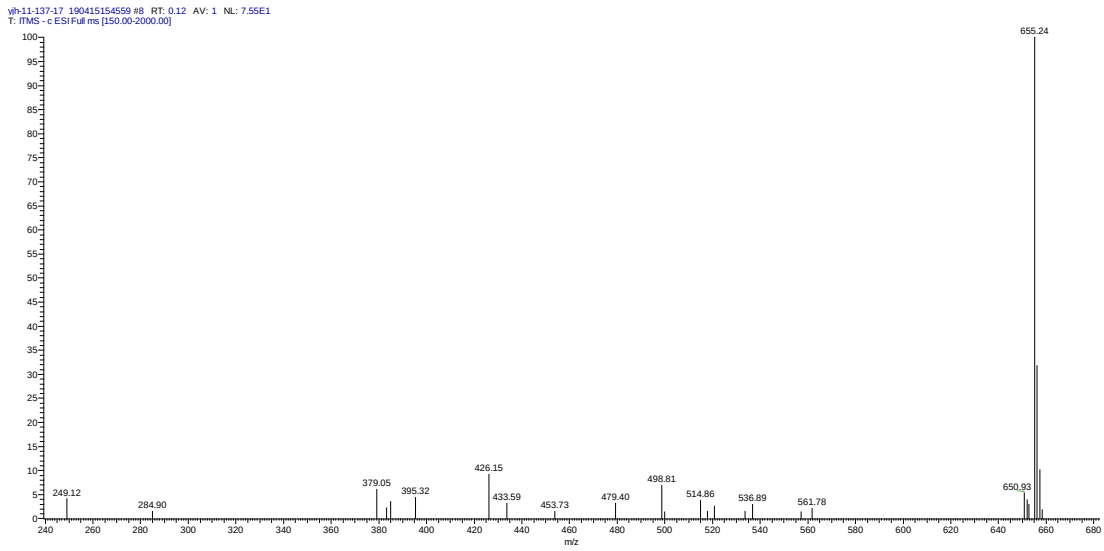
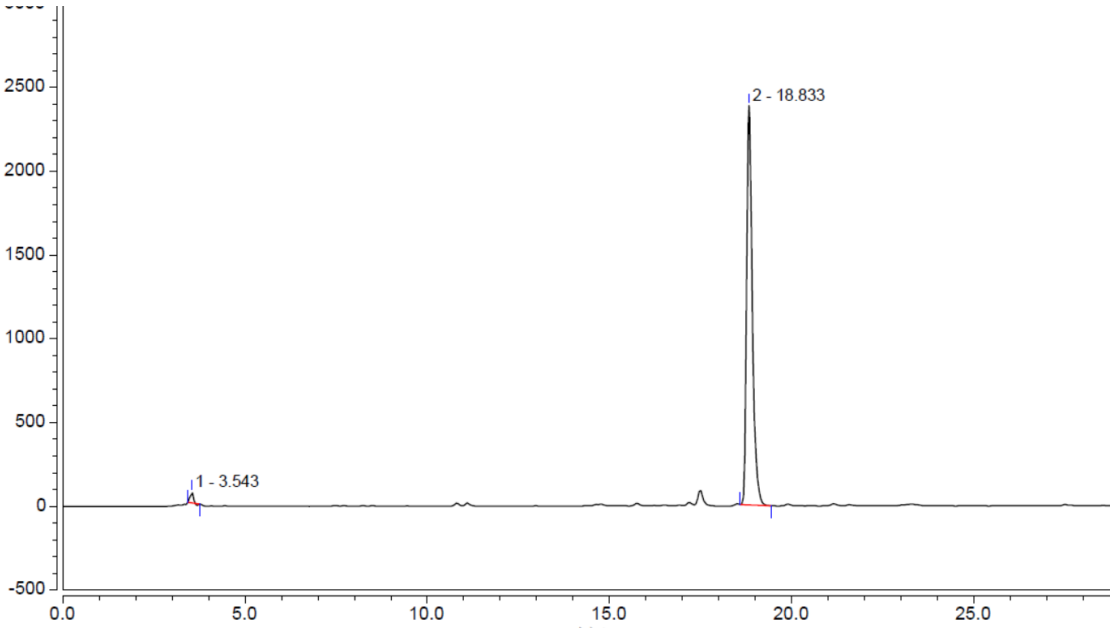


Figure S51: ESI-MS analysis of **5n**.

Fmoc-Thr(Me)^{S}-Val-Gly-Phe-OH (**5o**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	1.28	5.601	3.543	63.394
2	98.72	430.935	18.833	2383.883
Total:	100	436.536		2447.277

Figure S52: Crude HPLC trace of **5o** with optimized reaction conditions.

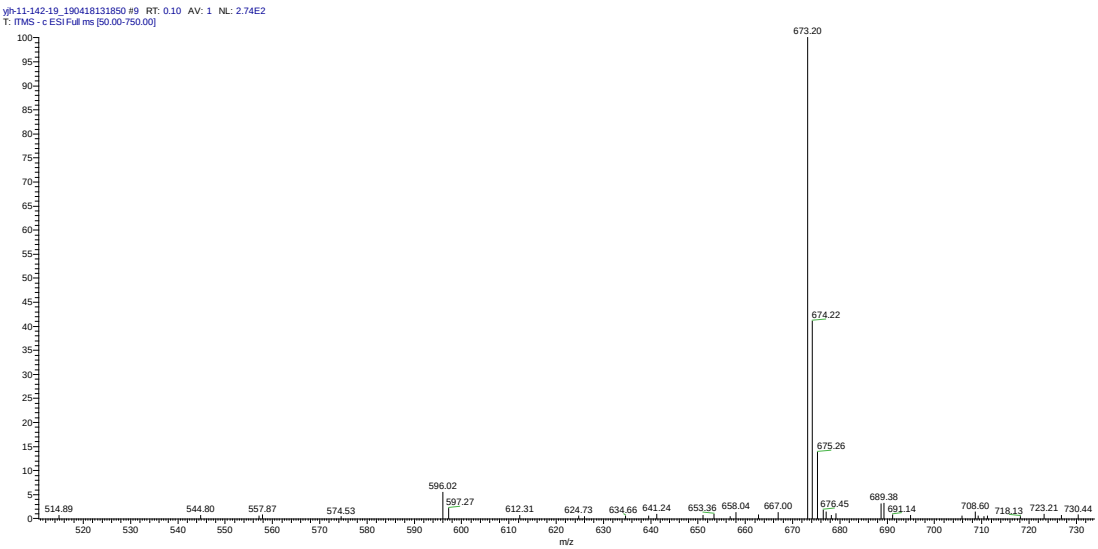
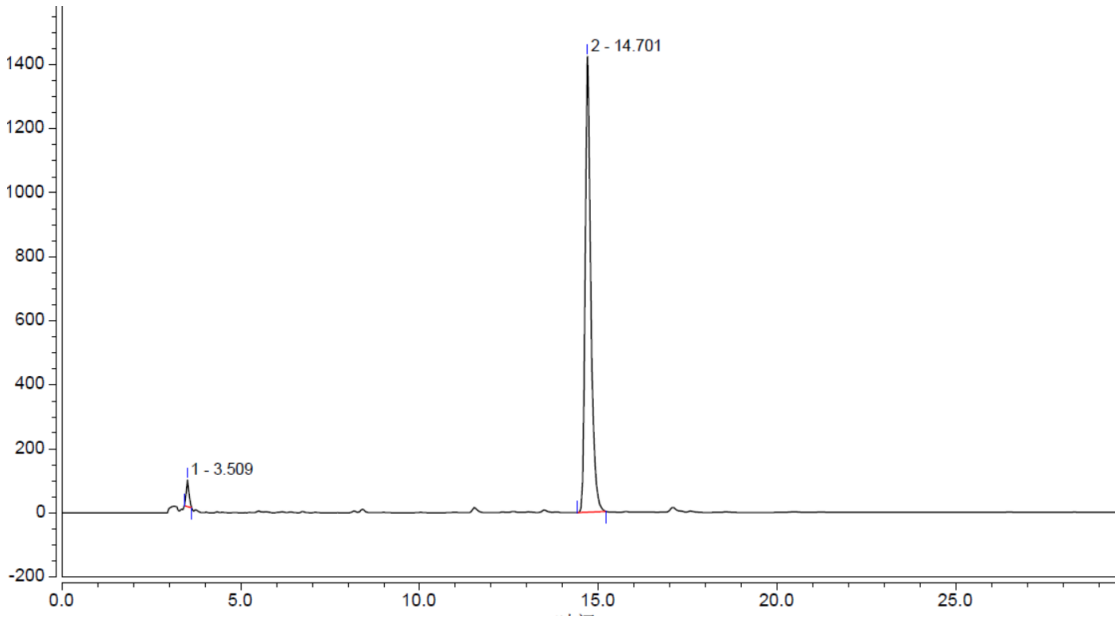


Figure S53: ESI-MS analysis of **5o**.

Fmoc-Ile^{S}-Val-Gly-Phe-OH (**5p**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	2.78	7.489	3.509	83.195
2	97.22	261.442	14.701	1423.602
Total:	100	268.931		1506.797

Figure S54: Crude HPLC trace of **5p** with optimized reaction conditions.

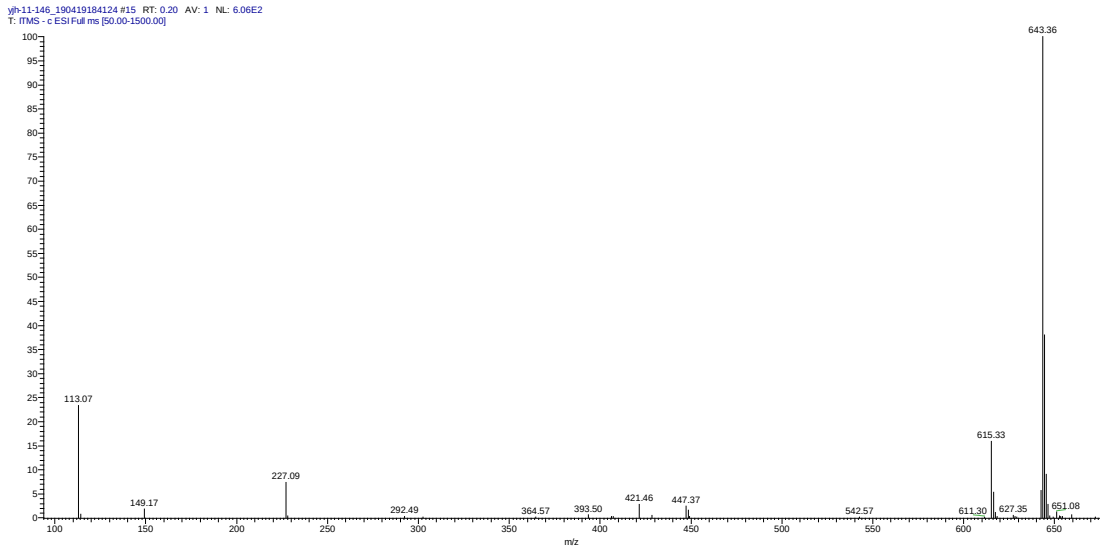
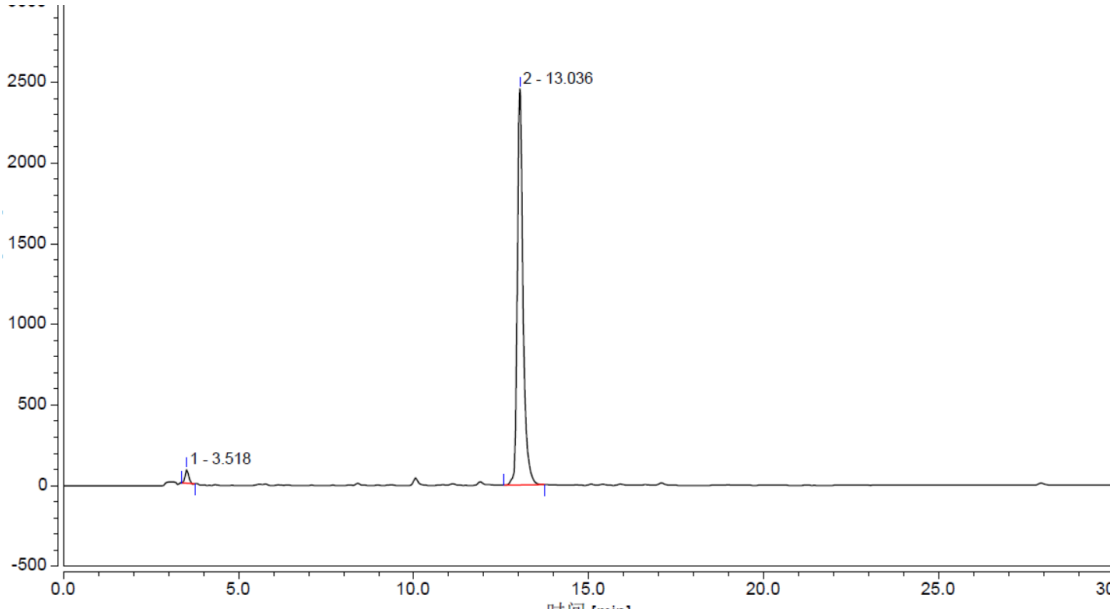


Figure S55: ESI-MS analysis of **5p**.

Fmoc-Val^{S}-Ala-Gly-Phe-OH (**5q**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	2.06	9.727	3.518	85.635
2	97.94	463.571	13.036	2458.533
Total:	100	473.298		2544.168

Figure S56: Crude HPLC trace of **5q** with optimized reaction conditions.

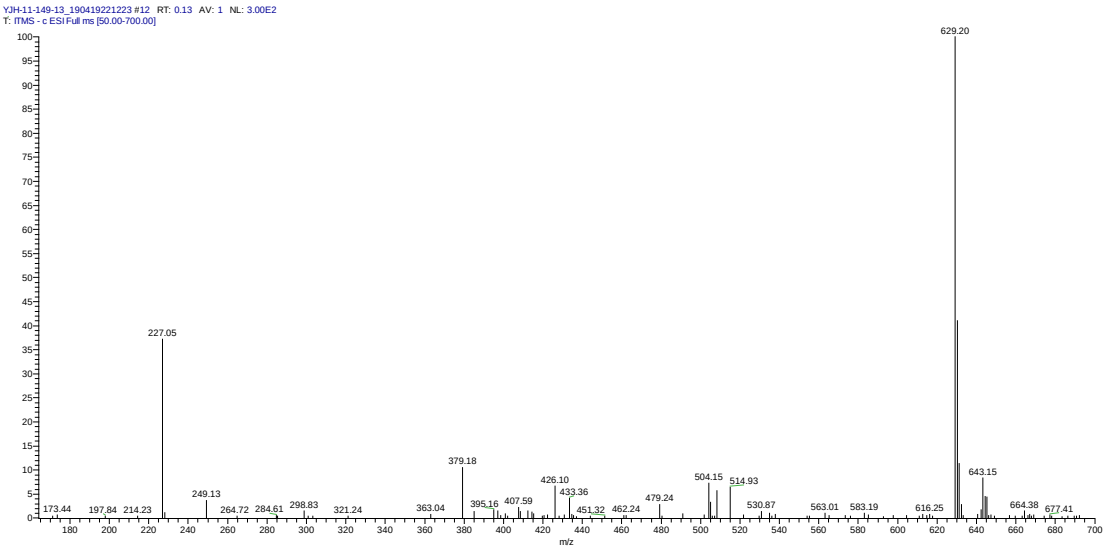
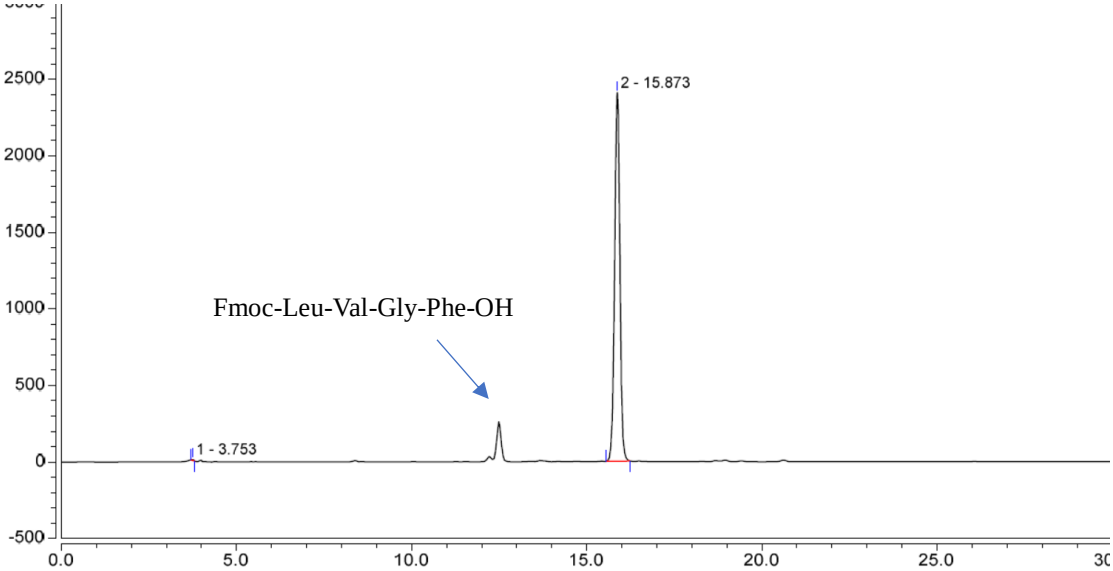


Figure S57: ESI-MS analysis of **5q**.

Fmoc-Leu^{S}-Val-Gly-Phe-OH (**5r**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.12	0.494	3.753	7.673
2	99.88	423.491	15.873	2409.545
Total:	100	423.986		2417.218

Figure S58: Crude HPLC trace of **5r** with optimized reaction conditions.

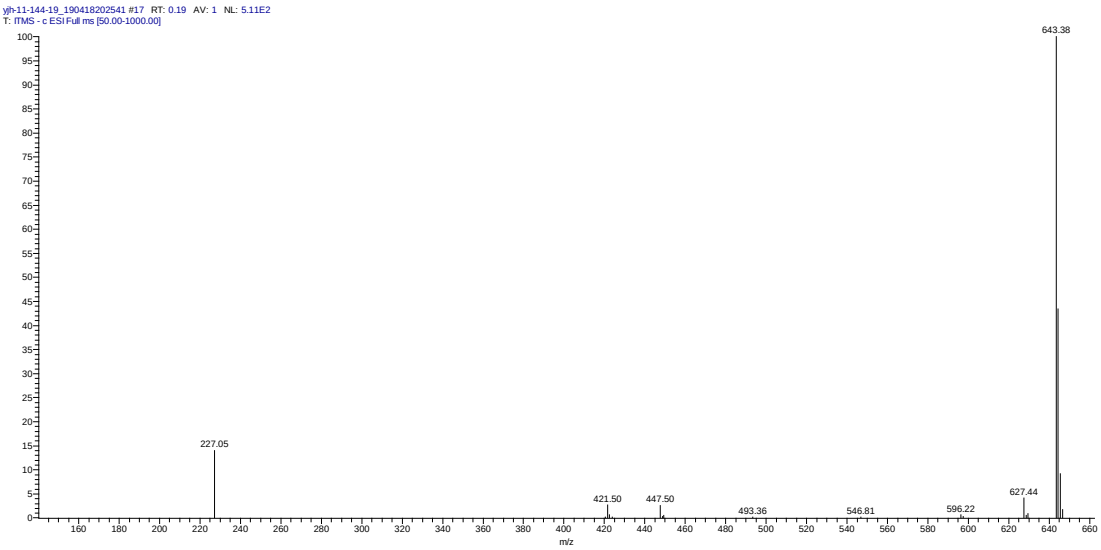
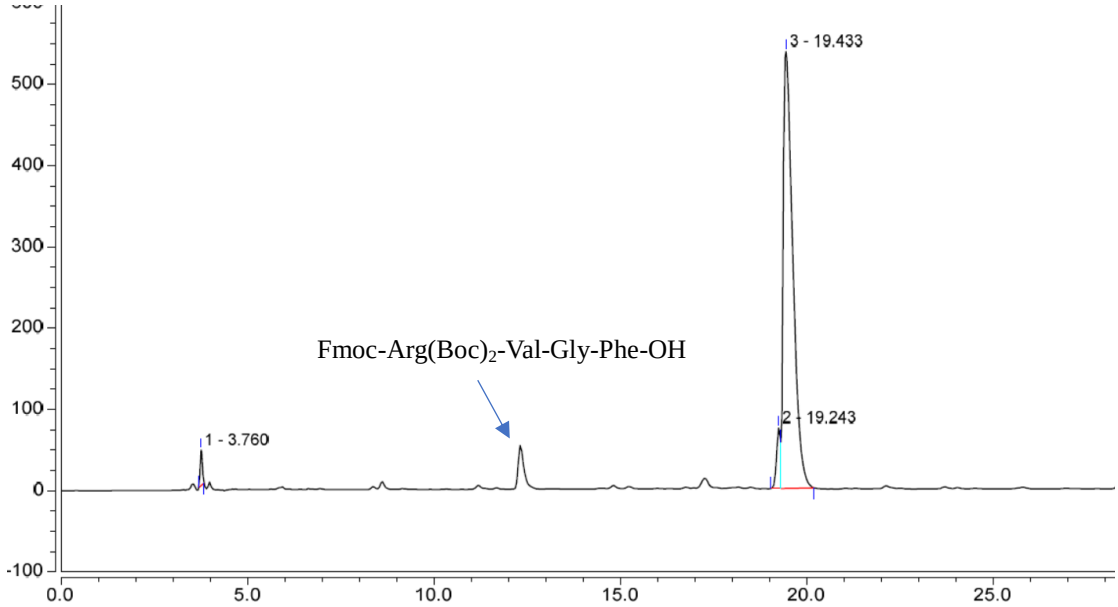


Figure S59: ESI-MS analysis of **5r**.

Fmoc-Arg(Boc)$_2^{S}$-Val-Gly-Phe-OH (**5s**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	1.67	2.861	3.760	43.755
2	5.27	9.040	19.243	74.478
3	93.06	159.644	19.433	537.545
Total:	100	171.545		2544.168

Figure S60: Crude HPLC trace of **5s** with optimized reaction conditions.

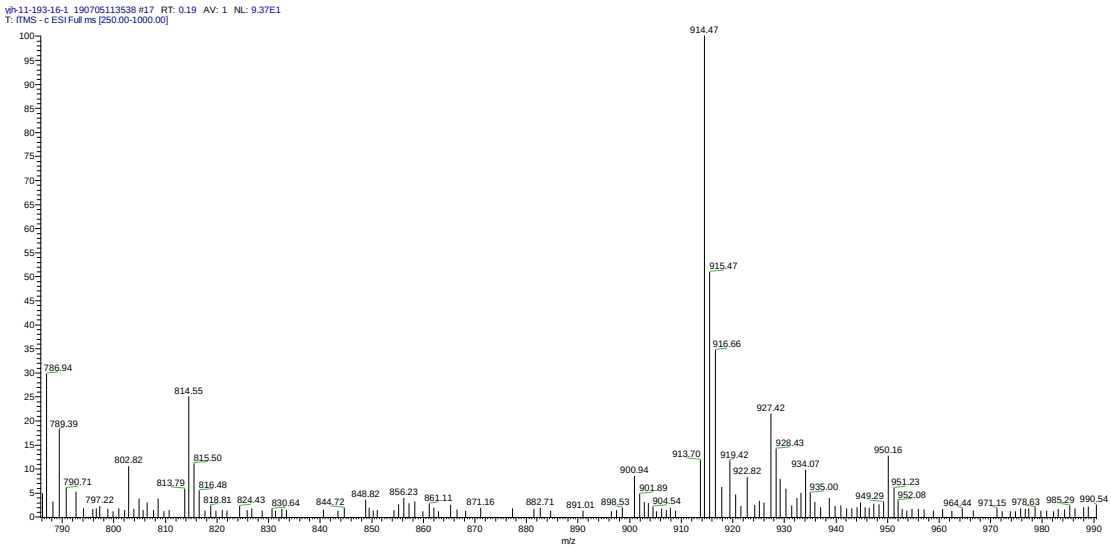
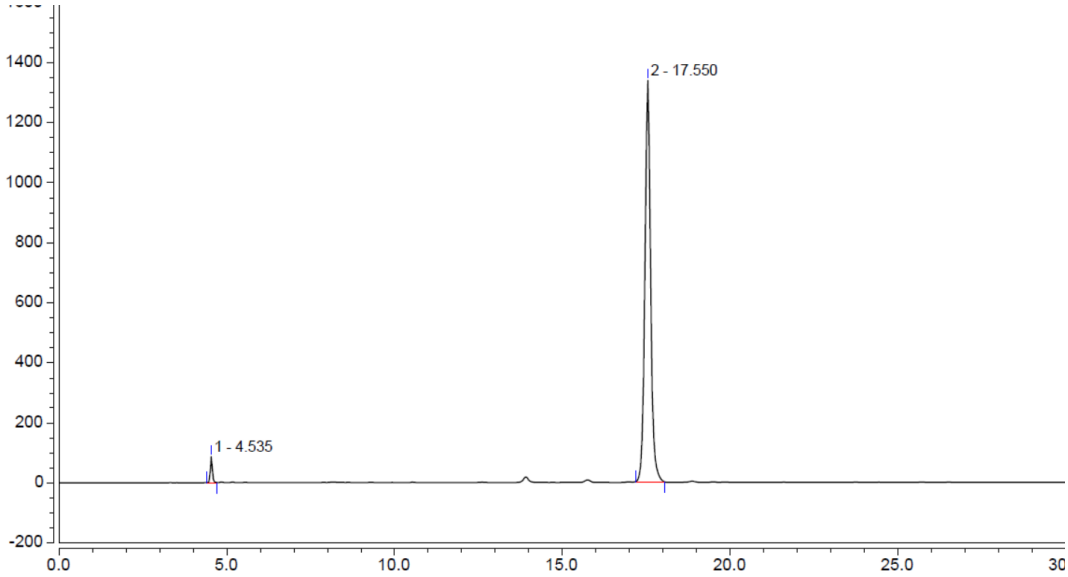


Figure S61: ESI-MS analysis of **5s**.

Fmoc-Ala^{S}-Val-Arg(pbf)-Gly-OH (**5t**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	1.99	5.412	4.535	86.231
2	98.01	266.010	17.550	1336.641
Total:	100	35.145		1422.871

Figure S62: Crude HPLC trace of **5t** with optimized reaction conditions.

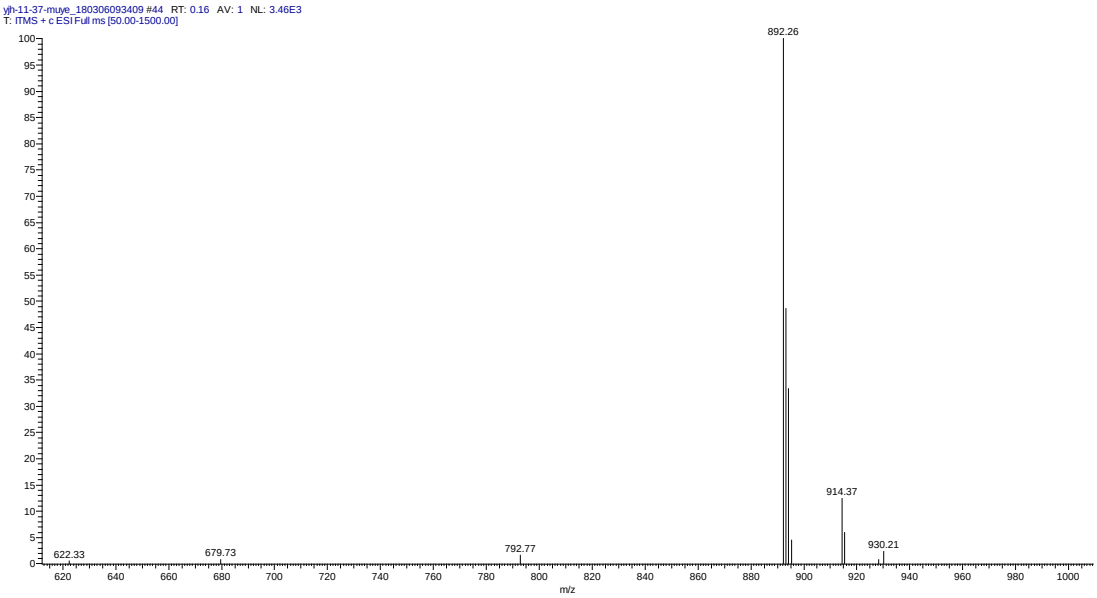
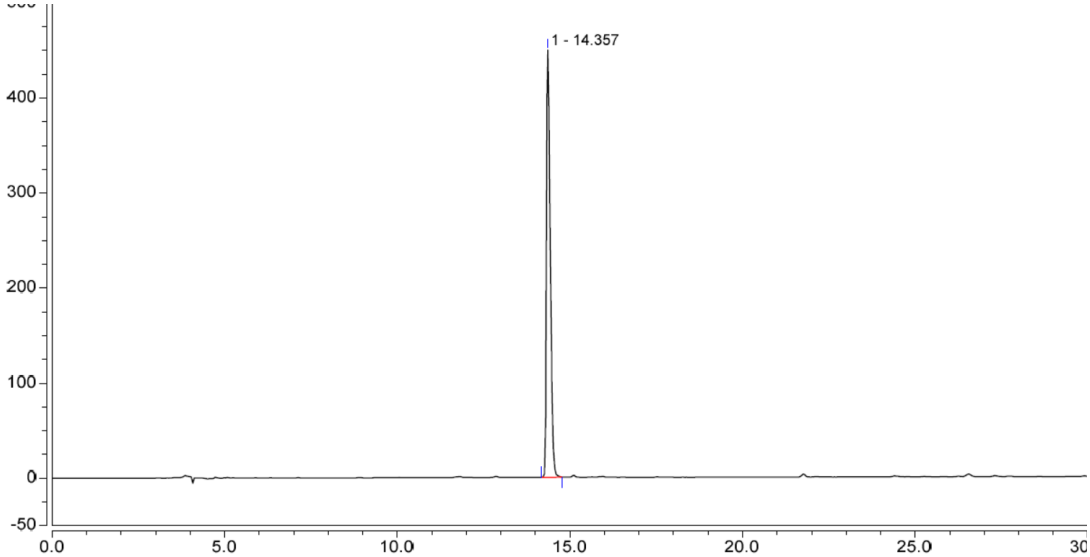


Figure S63: ESI-MS analysis of **5t**.

NH_2-Glu^S-Ala-Gln-Trp-Pro-Lys-OH (**5u**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	100	56.270	14.357	449.533
Total:	100	56.270		449.533

Figure S64: HPLC trace of **5u** with optimized reaction conditions.

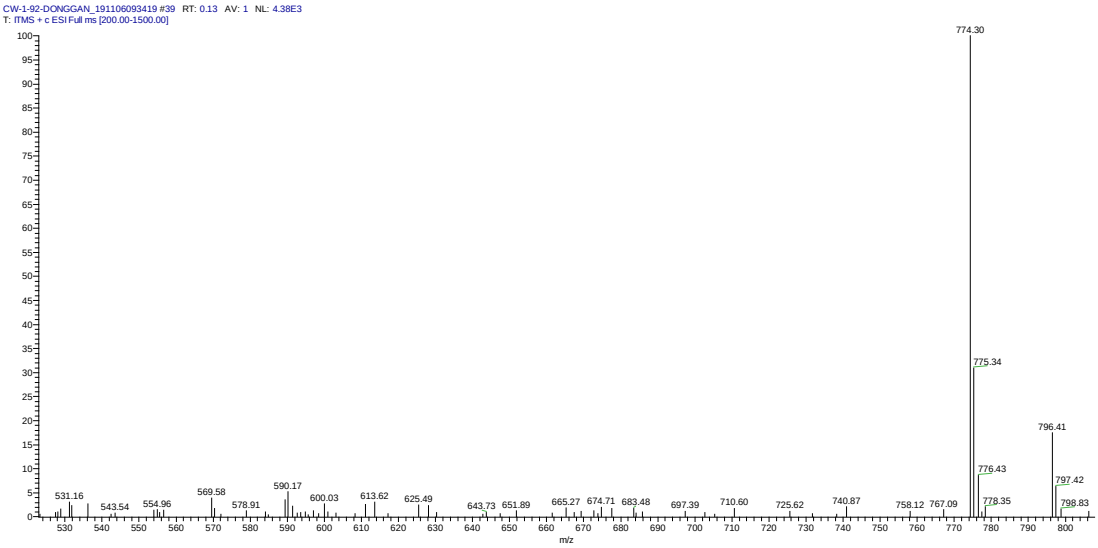
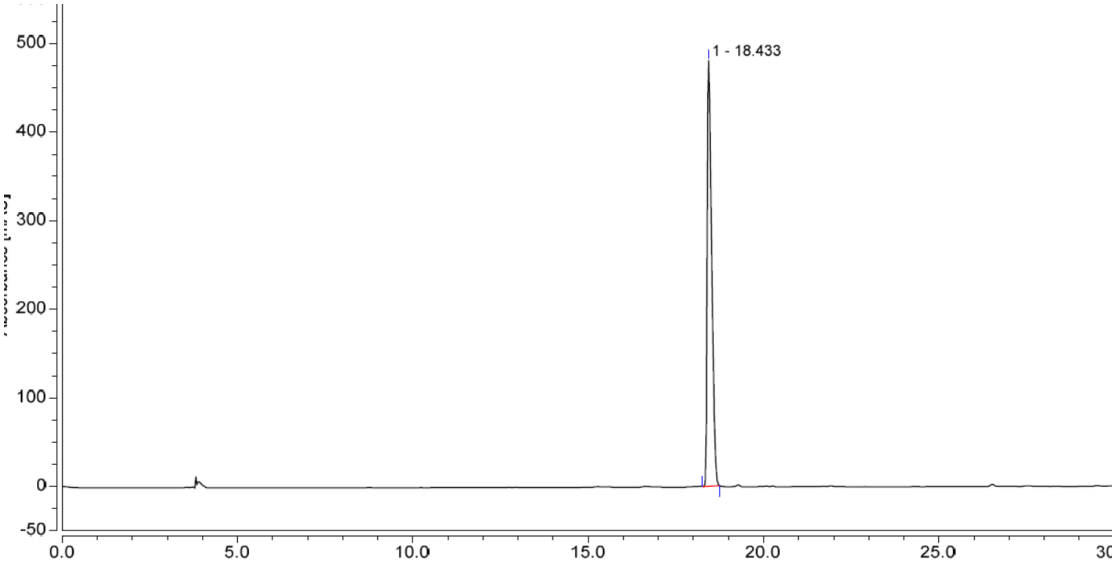


Figure S65: ESI-MS analysis of **5u**.

NH_2-Aal^S-Ala-Tyr-Asn-Phe-OH (**5v**):



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	100	171.842	26.020	810.946
Total:	100	171.842		810.946

Figure S66: HPLC trace of **5v** with optimized reaction conditions.

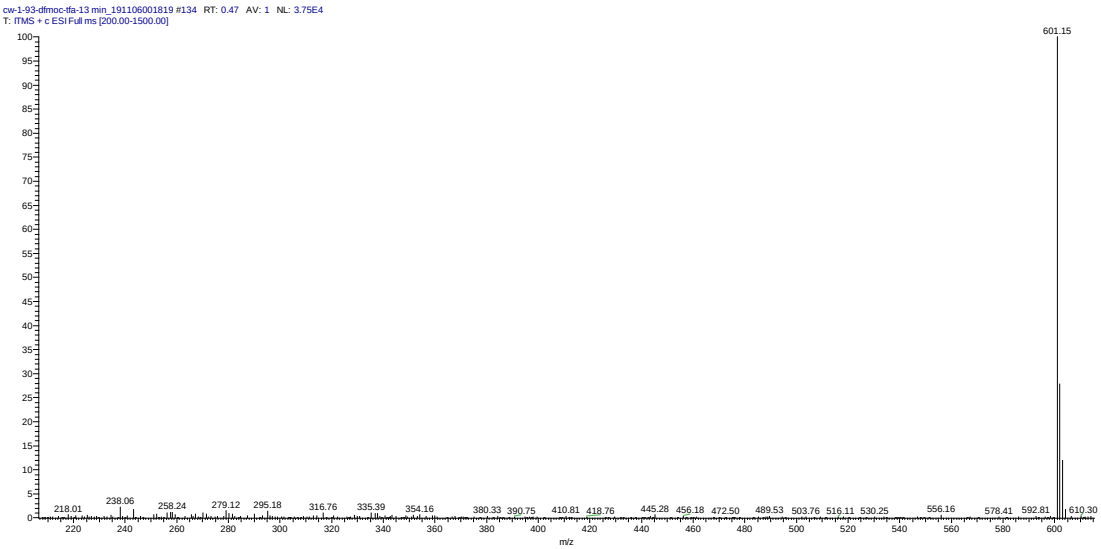
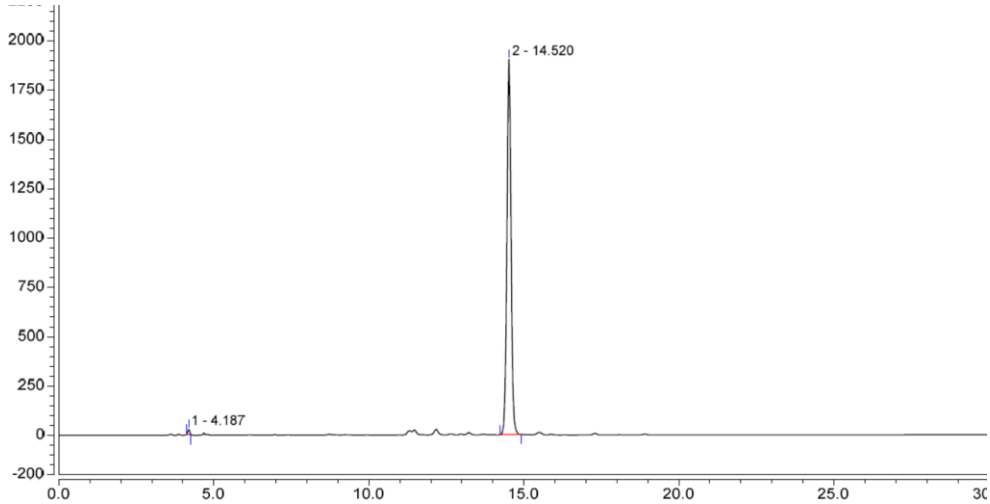


Figure S67: ESI-MS analysis of **5v**.

Crude HPLC Traces and ESI-MS Spectra of Multi-Thioamide Substituted Peptide 7a-7q

Fmoc-Ala^{S}-Ala^{S}-Val-Gly-Phe-OH (**7a**); HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 50-90% B (**10% H_2O and 0.039% TFA (v/v) in ACN**) with a flow rate of 1.0 mL/min over 30 min gave the target peptide. t_R =14.520 min.

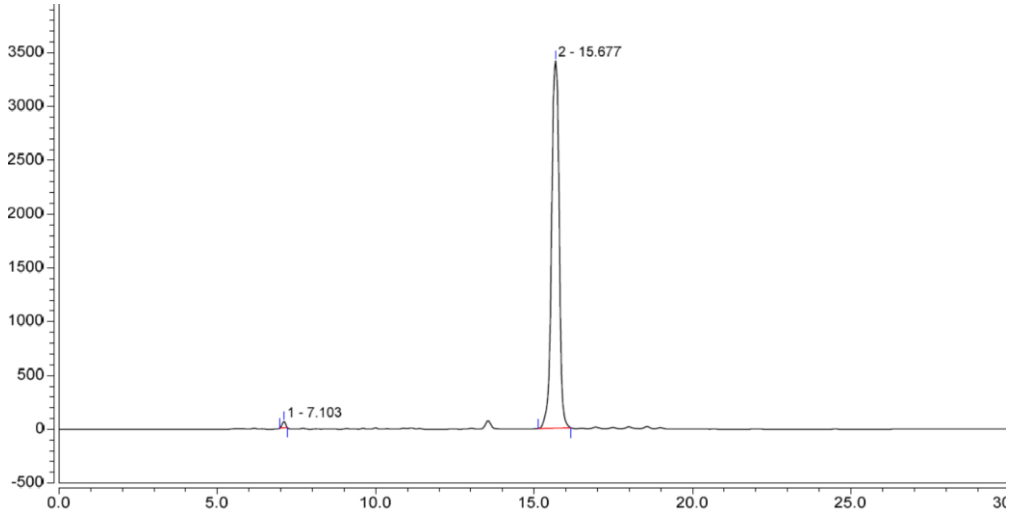


Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.58	1.724	4.187	26.240
2	99.42	297.318	14.520	1901.565
Total:	100	299.041		1927.805

Figure S68: Crude HPLC trace of **7a** with optimized reaction conditions.

Fmoc-Ala^{S}-Ala^{S}-Val-Gly-Phe-OH (**7a**); HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 80-100% B (**10% H_2O and 0.045% TFA in MeOH**) with a flow rate of 0.6 mL/min over 30 min gave the target peptide. t_R = 15.677 min.



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.74	7.091	7.103	60.973
2	99.26	945.369	15.677	3415.336
Total:	100	952.461		3476.309

Figure S69: Crude HPLC trace of **7a** with optimized reaction conditions.

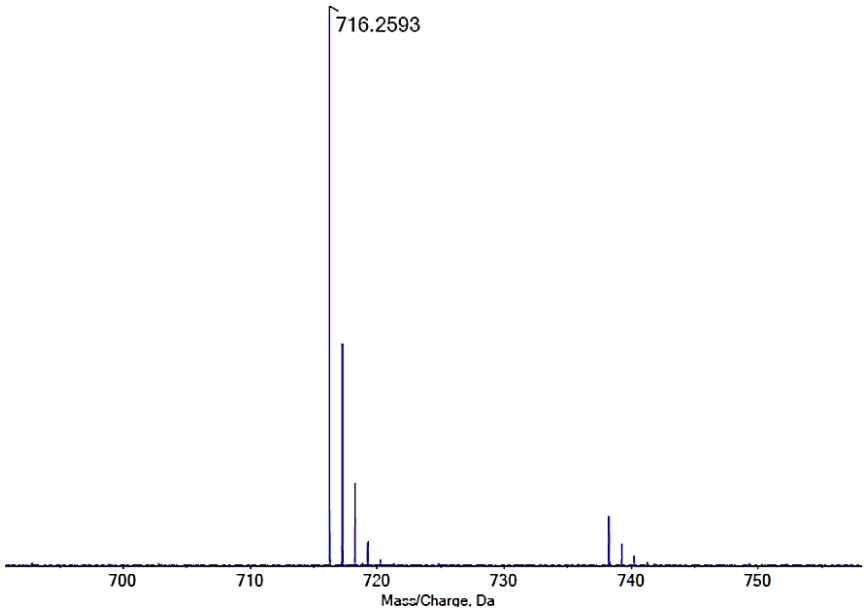
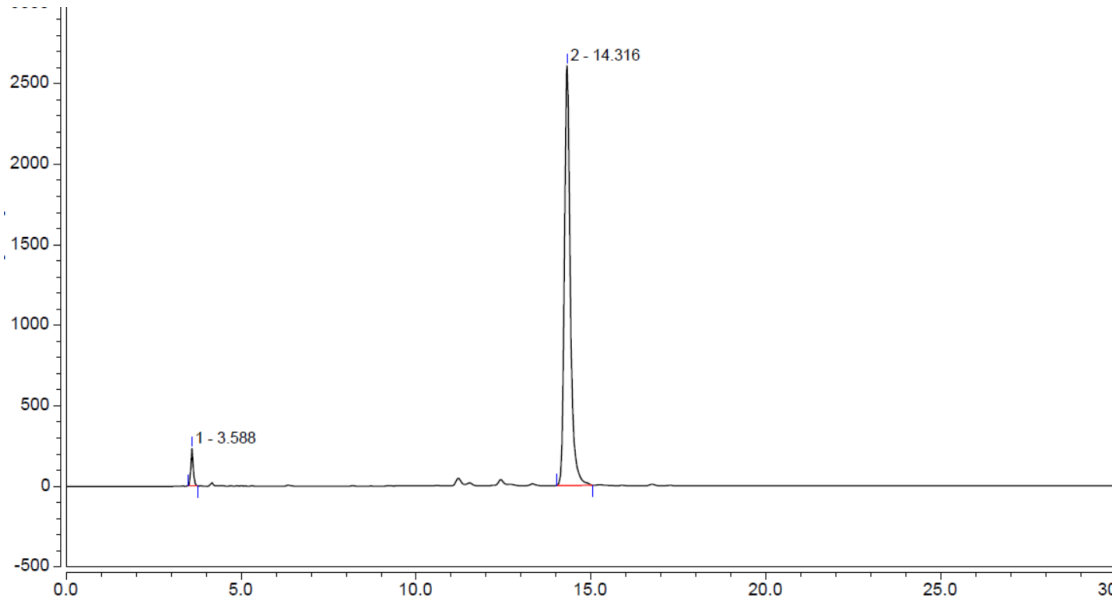


Figure S70: HRMS (ESI-TOF) analysis of **7a**.

Fmoc-Glu(^{t}Bu)^S-Ala^S-Val-Gly-Phe-OH (**7b**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	3.34	17.613	3.588	232.878
2	96.66	510.431	14.316	2608.269
Total:	100	528.044		2841.147

Figure S71: Crude HPLC trace of **7b** with optimized reaction conditions.

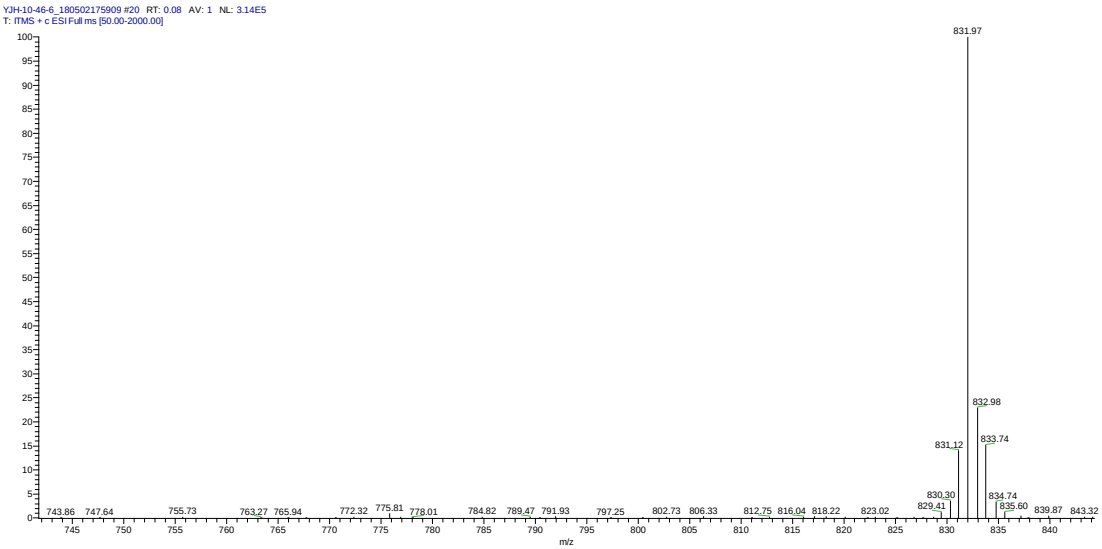
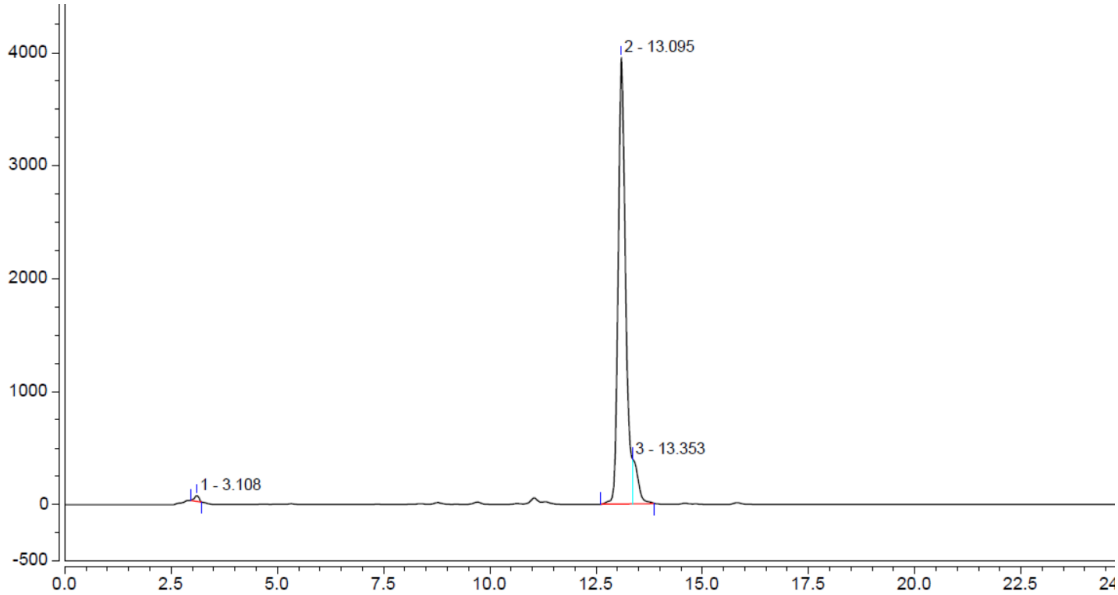


Figure S72: ESI-MS analysis of **7b**.

Fmoc-Ser(^{t}Bu)^S-Ala^S-Val-Gly-Phe-OH (**7c**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.75	6.415	3.108	51.941
2	91.94	790.305	13.095	3955.029
3	7.32	62.899	13.353	397.593
Total:	100	859.619		4404.563

Figure S73: Crude HPLC trace of **7c** with optimized reaction conditions.

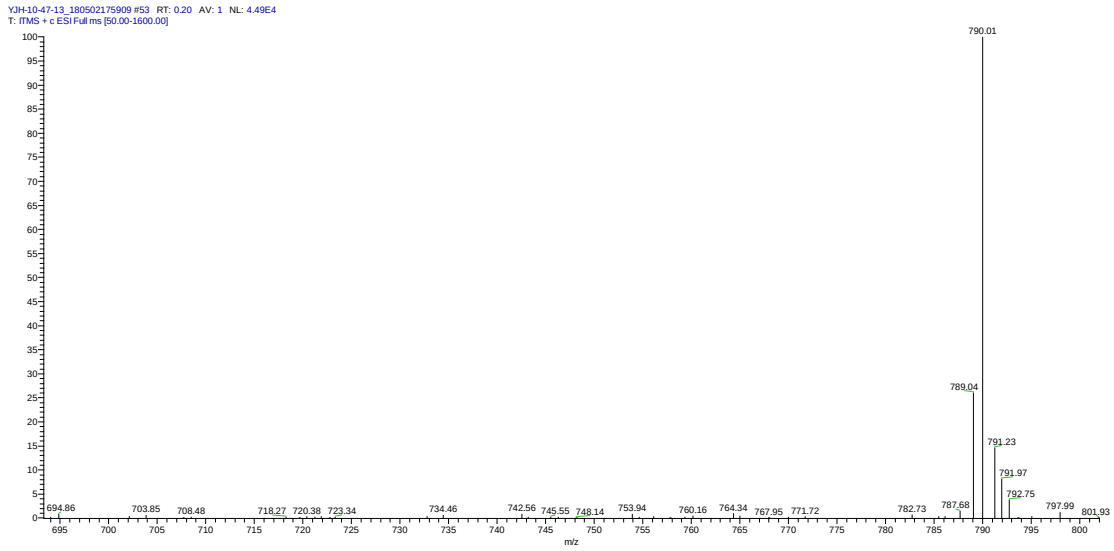
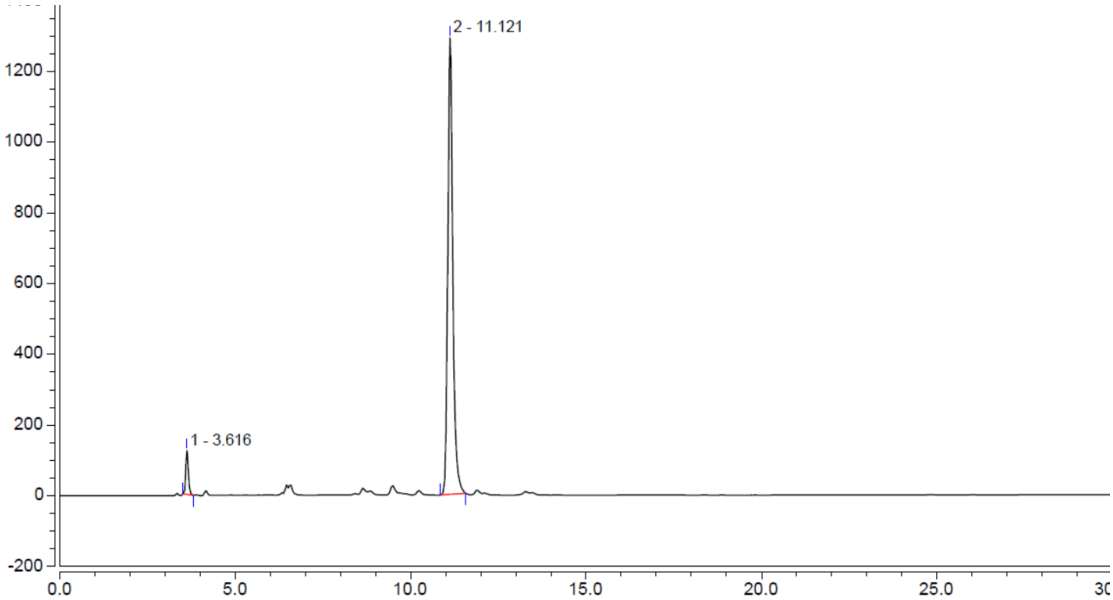


Figure S74: ESI-MS analysis of **7c**.

Fmoc-Met^{S}-Ala^{S}-Val-Gly-Phe-OH (**7d**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	4.49	9.963	3.616	124.583
2	95.51	211.905	11.121	1292.359
Total:	100	221.867		1416.942

Figure S75: Crude HPLC trace of **7d** with optimized reaction conditions.

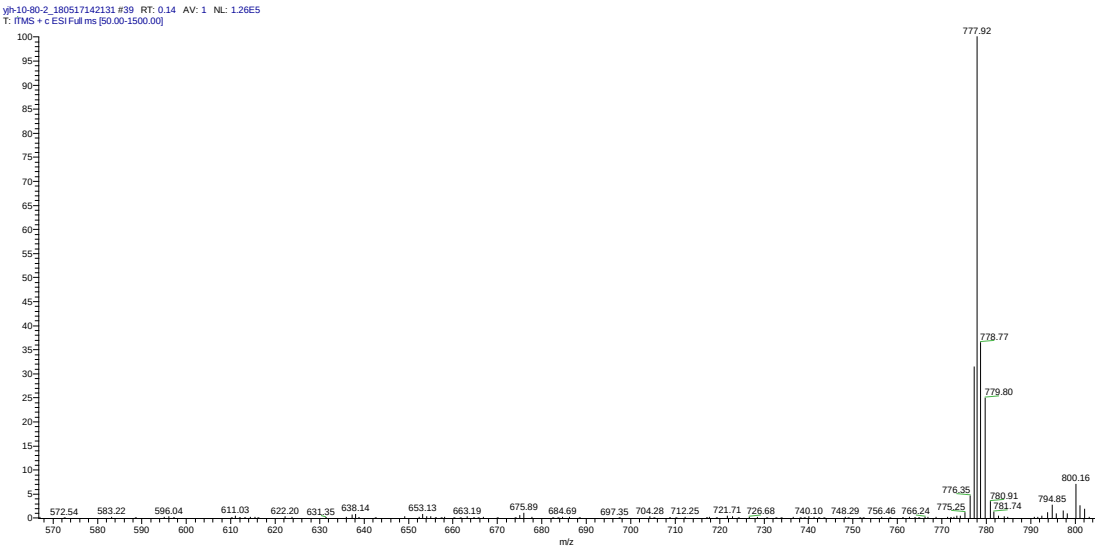
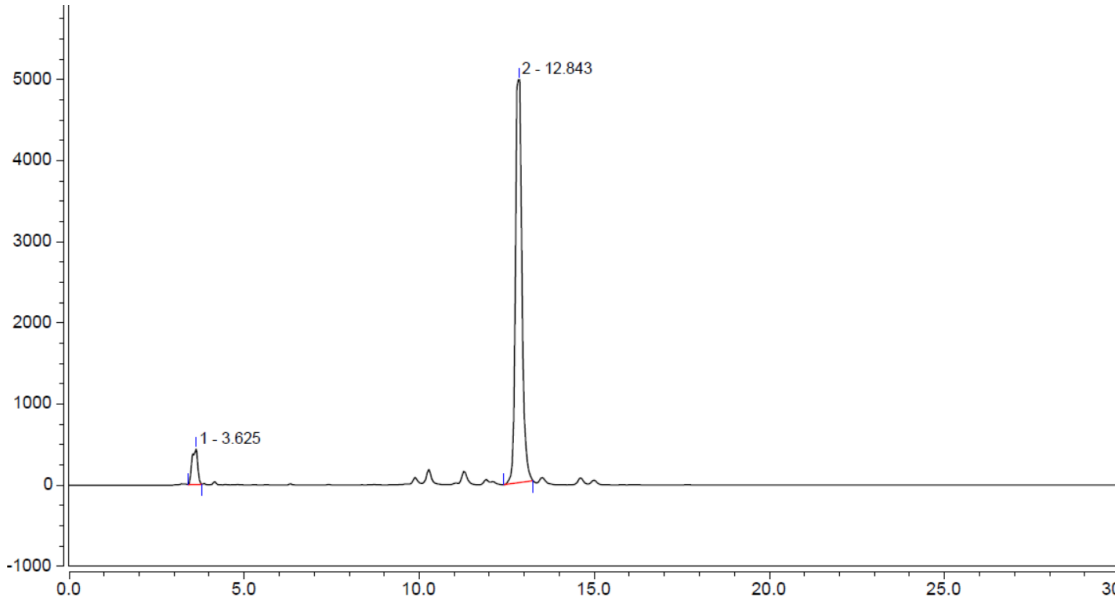


Figure S76: ESI-MS analysis of **7d**.

Fmoc-Lys(Boc)^{S}-Ala^{S}-Val-Gly-Phe-OH (**7e**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	7.08	81.249	3.625	435.637
2	92.92	1066.450	12.843	4969.695
Total:	100	1147.700		5405.332

Figure S77: Crude HPLC trace of **7e** with optimized reaction conditions.

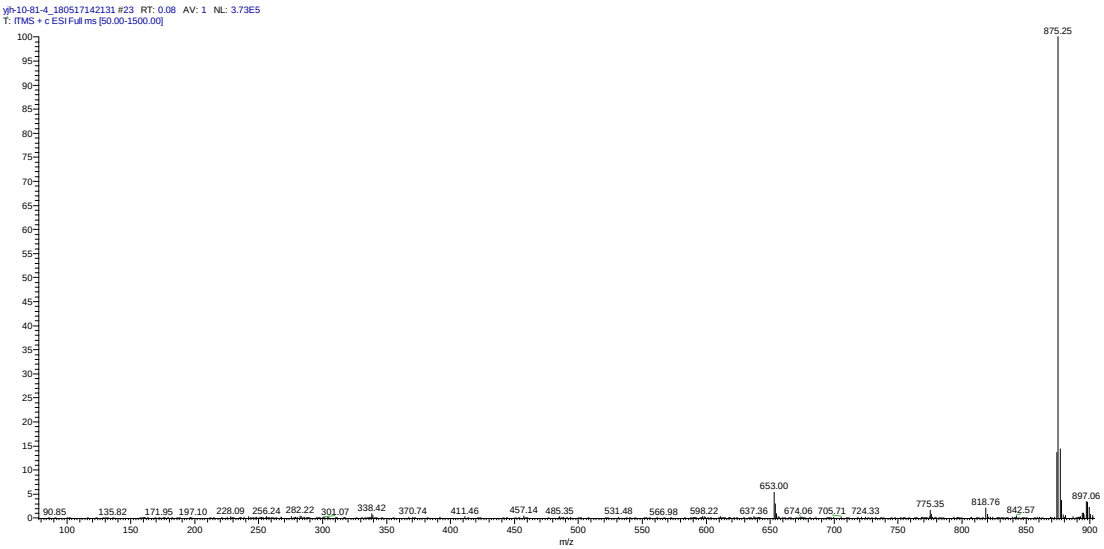
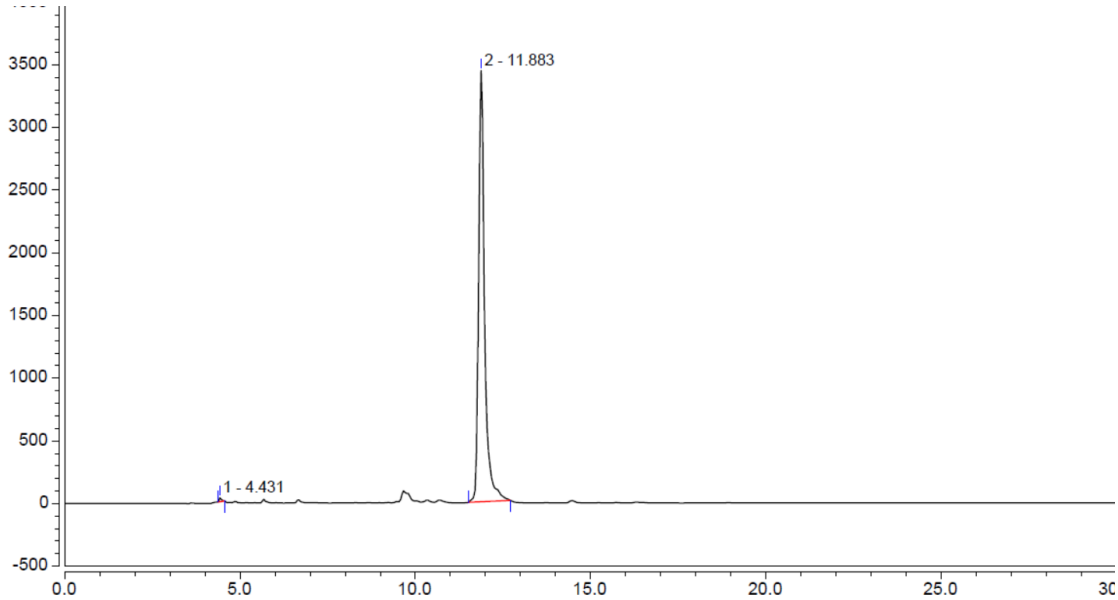


Figure S78: ESI-MS analysis of **7e**.

Fmoc-Ala^{S}-Leu-Ala^{S}-Val-Gly-Phe-OH (**7f**); HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B (**10% H_2O and 0.039% TFA (v/v) in ACN**) with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 99.63% conversion of crude HPLC trace; t_R = 11.883 min.

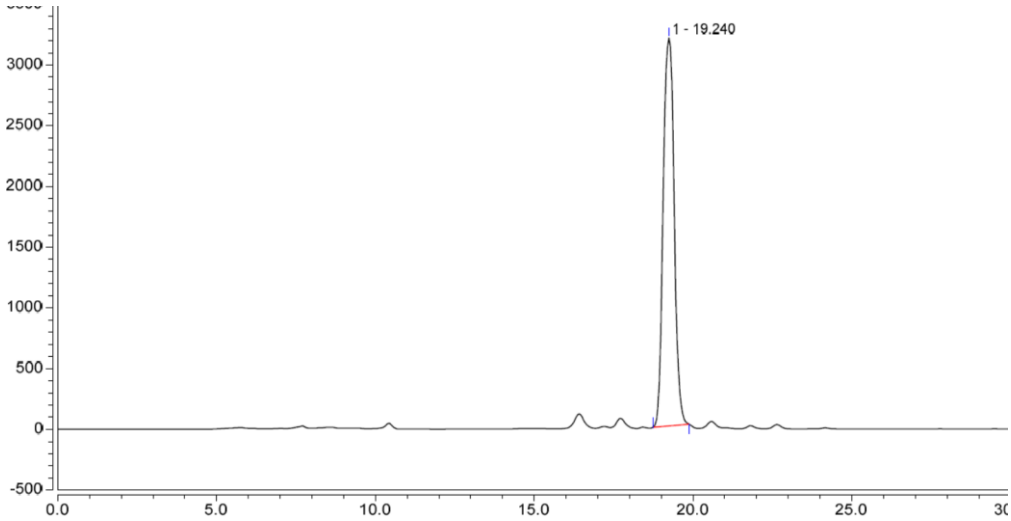


Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.37	2.512	4.431	33.072
2	99.63	679.757	11.883	3440.501
Total:	100	682.269		3473.573

Figure S79: Crude HPLC trace of **7f** with optimized reaction conditions.

Fmoc-Ala^{S}-Leu-Ala^{S}-Val-Gly-Phe-OH (**7f**); HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 80-100% B (**10% H_2O and 0.045% TFA in MeOH**) with a flow rate of 0.6 mL/min over 30 min gave the target peptide. t_R =19.240 min.



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	100	1272.272	19.240	3193.681
Total:	100	952.461		3476.309

Figure S80: Crude HPLC trace of **7f** with optimized reaction conditions.

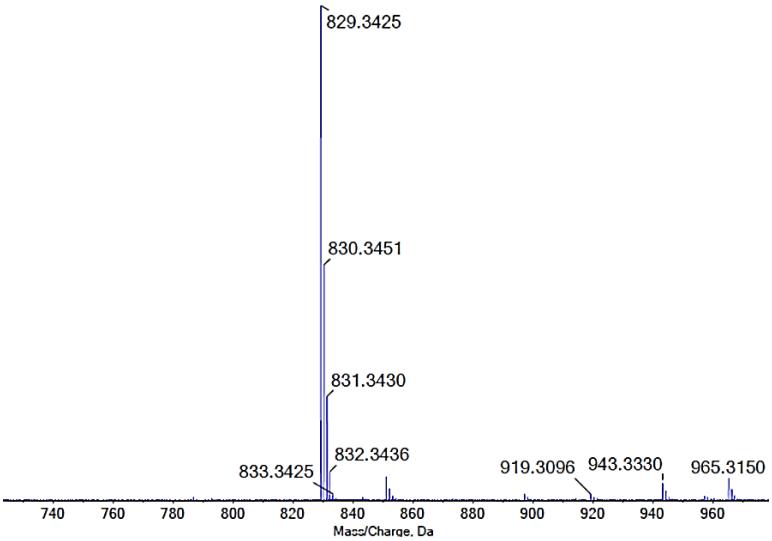
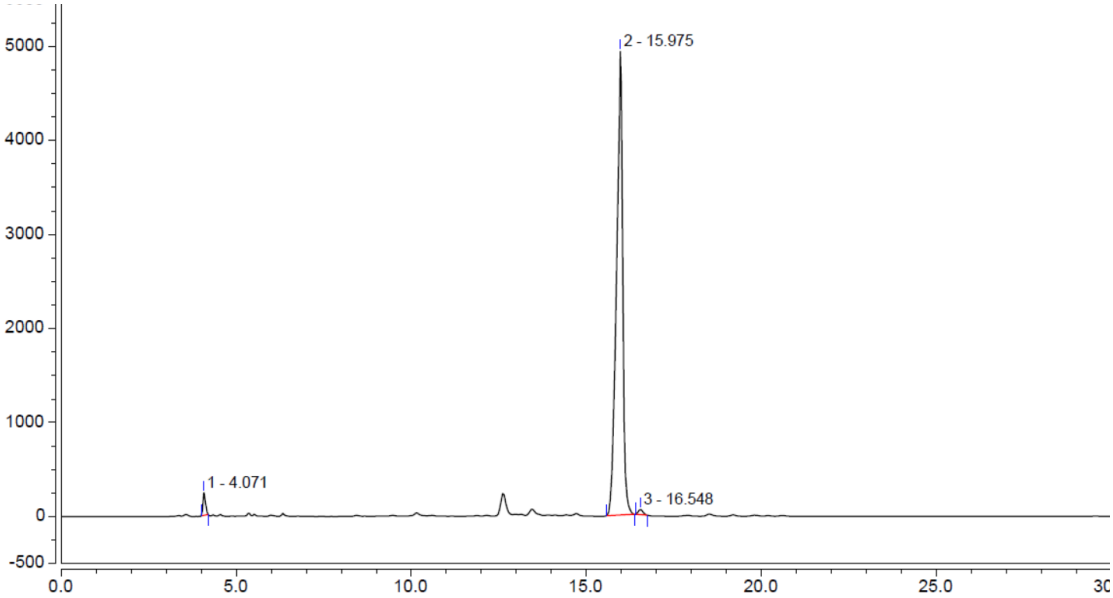


Figure S81: HRMS (ESI-TOF) analysis of **7f**.

Fmoc-Phe^{S}-Leu-Ala^{S}-Val-Gly-Phe-OH (**7g**); HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 60-90% B (**10% H_2O and 0.039% TFA (v/v) in ACN**) with a flow rate of 1.0 mL/min over 30 min gave the target peptide. 98.10% conversion of crude HPLC trace; t_R = 15.975 min (major).

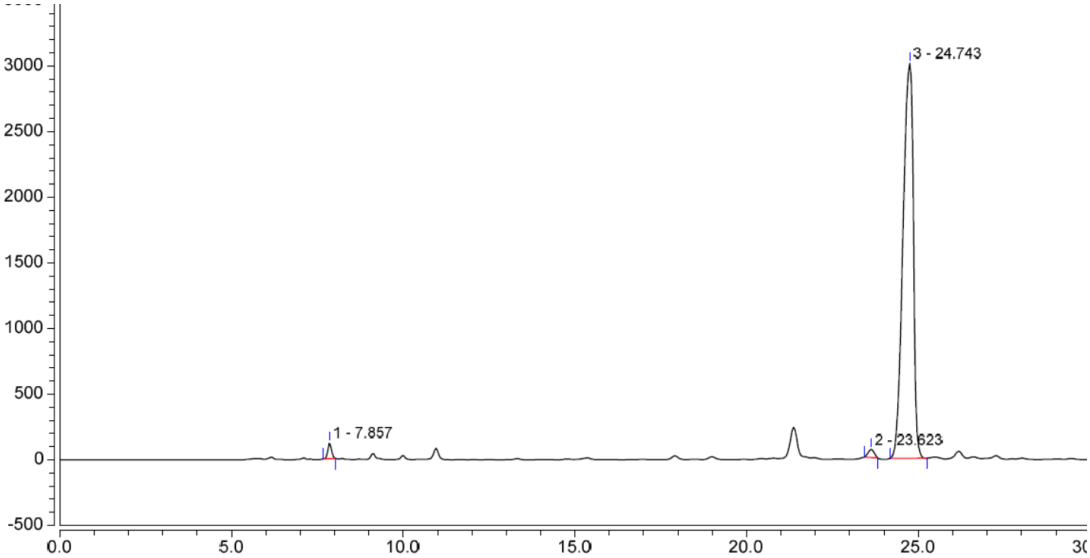


Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	1.90	19.371	4.071	239.158
2	97.27	989.923	15.975	4931.498
3	0.83	8.454	16.548	54.042
Total:	100	1017.748		5224.697

Figure S82: Crude HPLC trace of **7g** with optimized reaction conditions.

Fmoc-Phe^{S}-Leu-Ala^{S}-Val-Gly-Phe-OH (**7g**); HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 80-100% B (**10% H_2O and 0.045% TFA in MeOH**) with a flow rate of 0.6 mL/min over 30 min gave the target peptide. t_R = 24.743 min.



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	1.39	14.987	7.857	123.169
2	1.14	12.303	23.623	61.763
3	97.46	1047.484	24.743	3008.586
Total:	100	547.069		3193.519

Figure S83: Crude HPLC trace of **7g** with optimized reaction conditions.

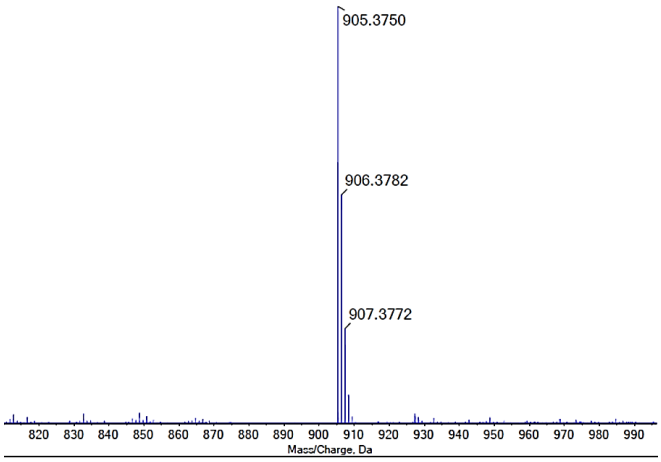
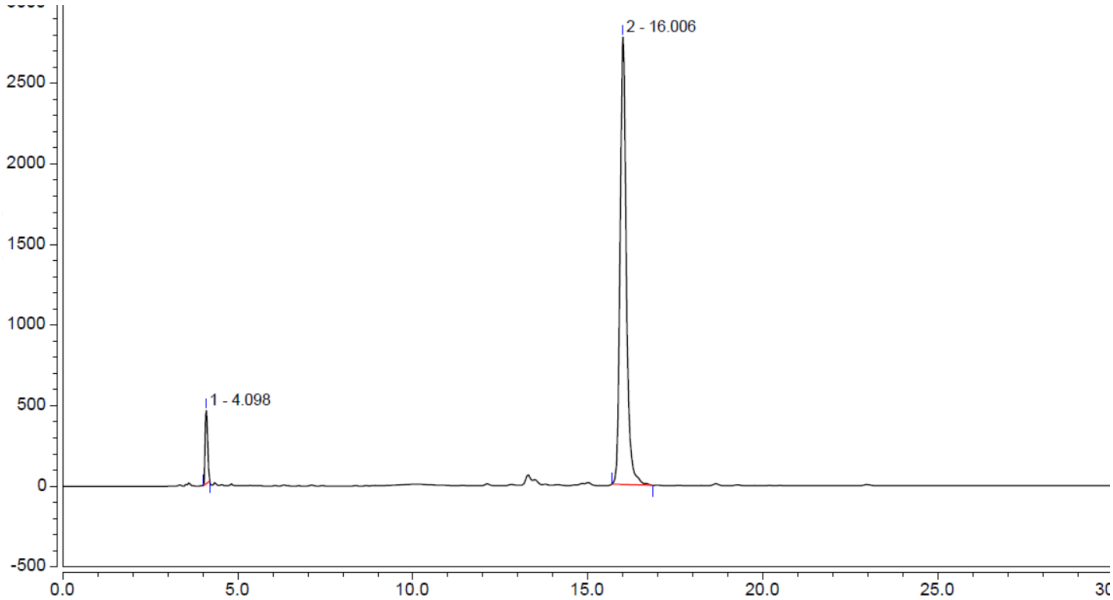


Figure S84: HRMS (ESI-TOF) analysis of **7g**.

Fmoc-Lys(Boc)^{S}-Leu-Ala^{S}-Val-Gly-Phe-OH (**7h**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	6.36	38.932	4.098	453.031
2	93.64	572.891	16.006	2775.984
Total:	100	611.823		3229.014

Figure S85: Crude HPLC trace of **7h** with optimized reaction conditions.

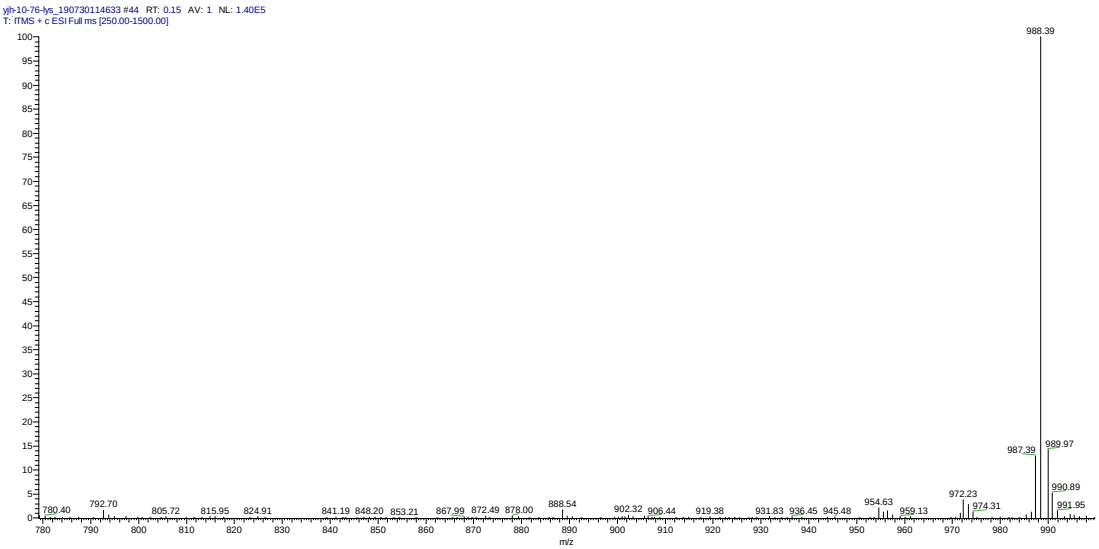
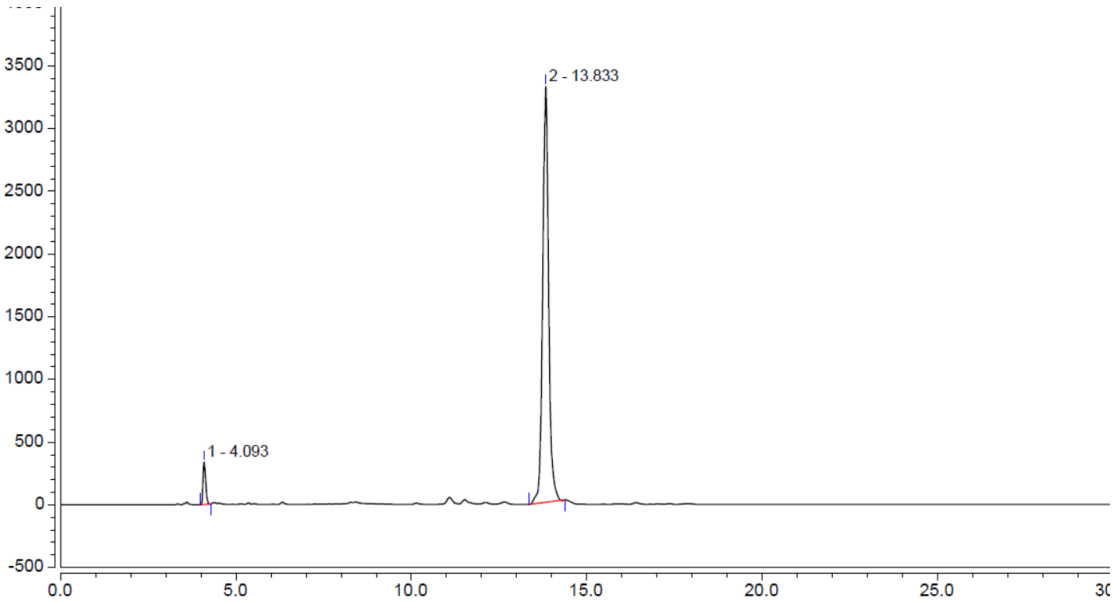


Figure S86: ESI-MS analysis of **7h**.

Fmoc-Met^{S}-Leu-Ala^{S}-Val-Gly-Phe-OH (**7i**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	4.38	29.895	4.093	338.875
2	95.62	653.372	13.833	3316.164
Total:	100	683.267		3655.038

Figure S87: Crude HPLC trace of **7i** with optimized reaction conditions.

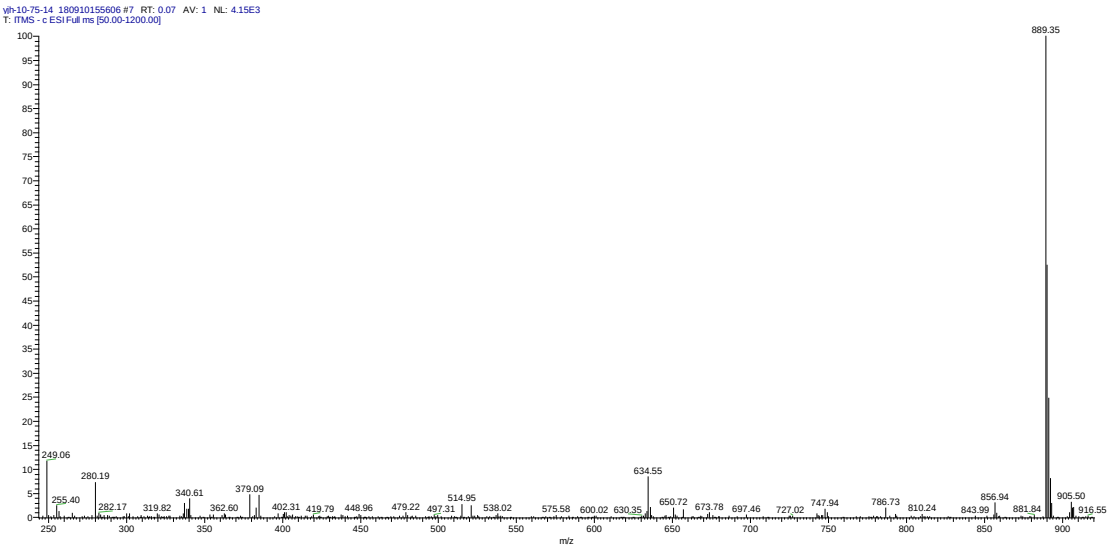
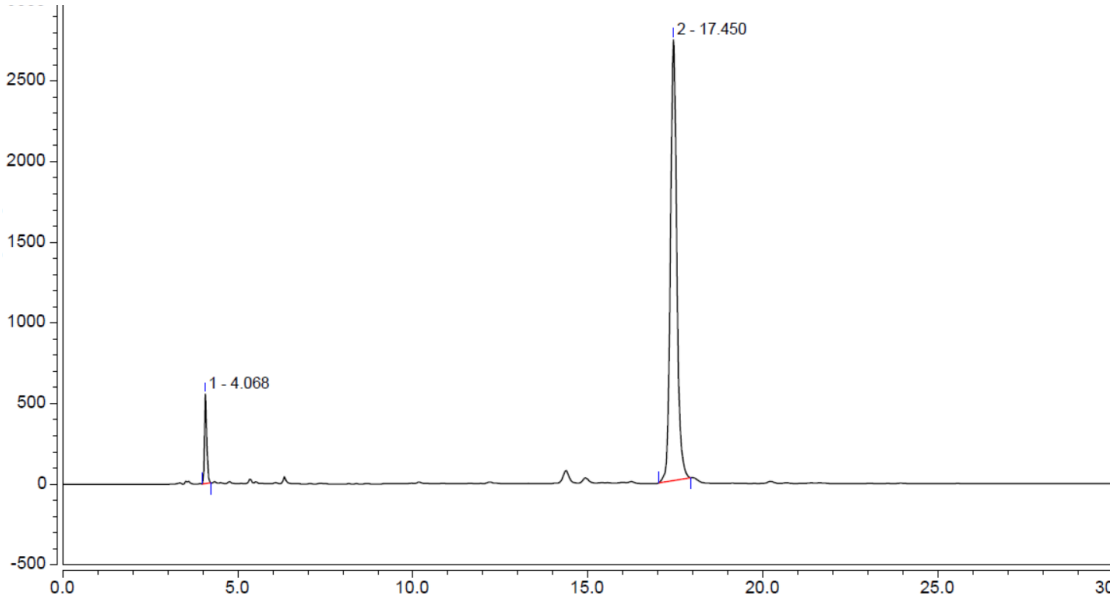


Figure S88: ESI-MS analysis of **7i.**

Fmoc-Glu(^{t}Bu)^S-Leu-Ala^S-Val-Gly-Phe-OH (**7j**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	6.66	40.924	4.068	555.334
2	93.34	573.187	17.450	2733.682
Total:	100	614.112		3289.016

Figure S89: Crude HPLC trace of **7j** with optimized reaction conditions.

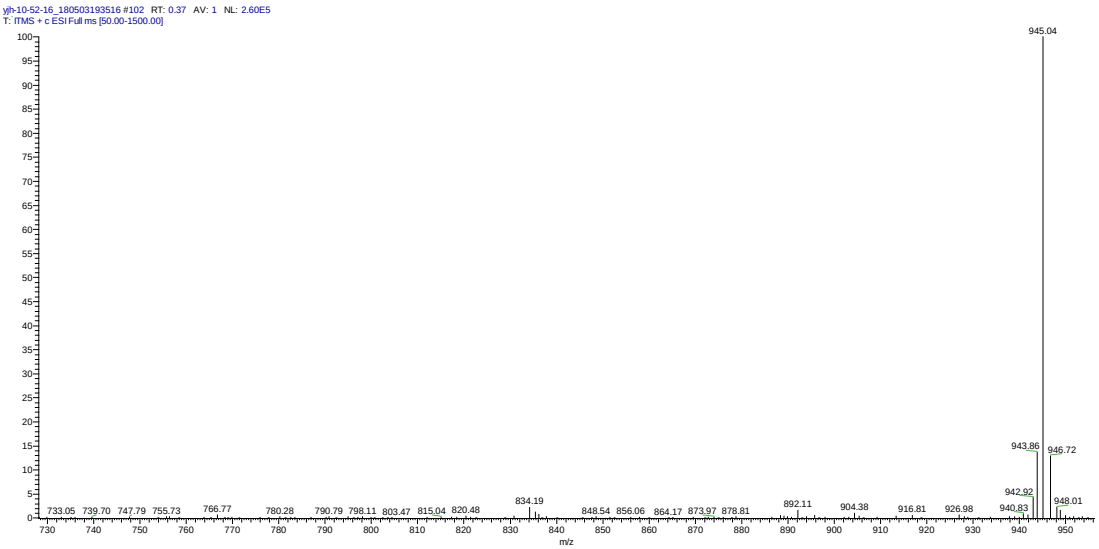
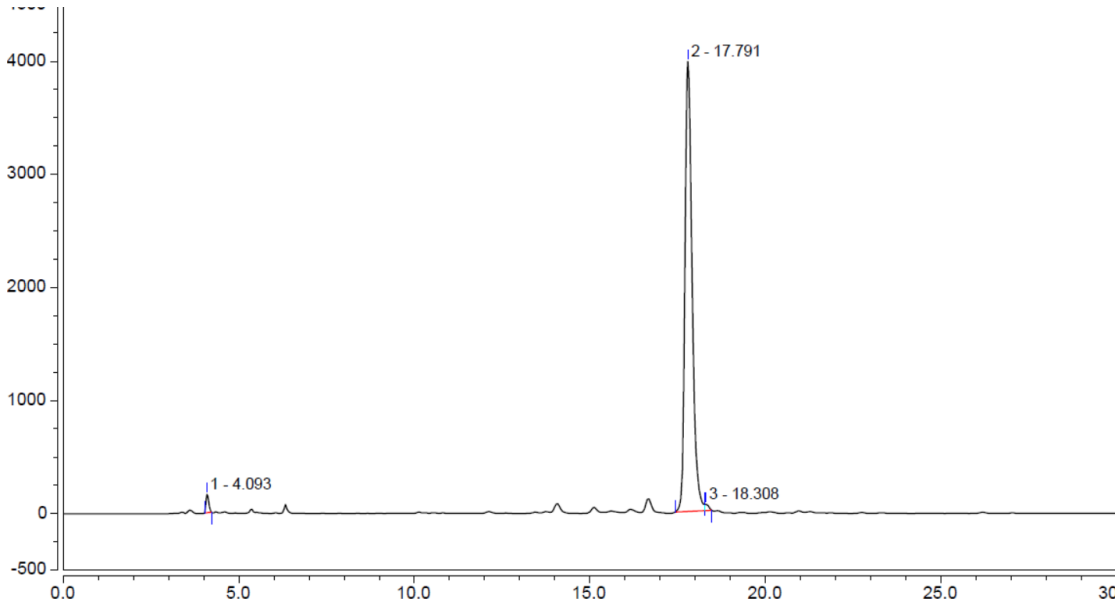


Figure S90: ESI-MS analysis of **7j**.

Fmoc-Ser(^{t}Bu)^S-Leu-Ala^S-Val-Gly-Phe-OH (**7k**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	1.46	14.028	4.093	161.550
2	97.81	943.017	17.791	3980.474
3	0.73	7.084	18.308	54.965
Total:	100	964.129		4196.989

Figure S91: Crude HPLC trace of **7k** with optimized reaction conditions.

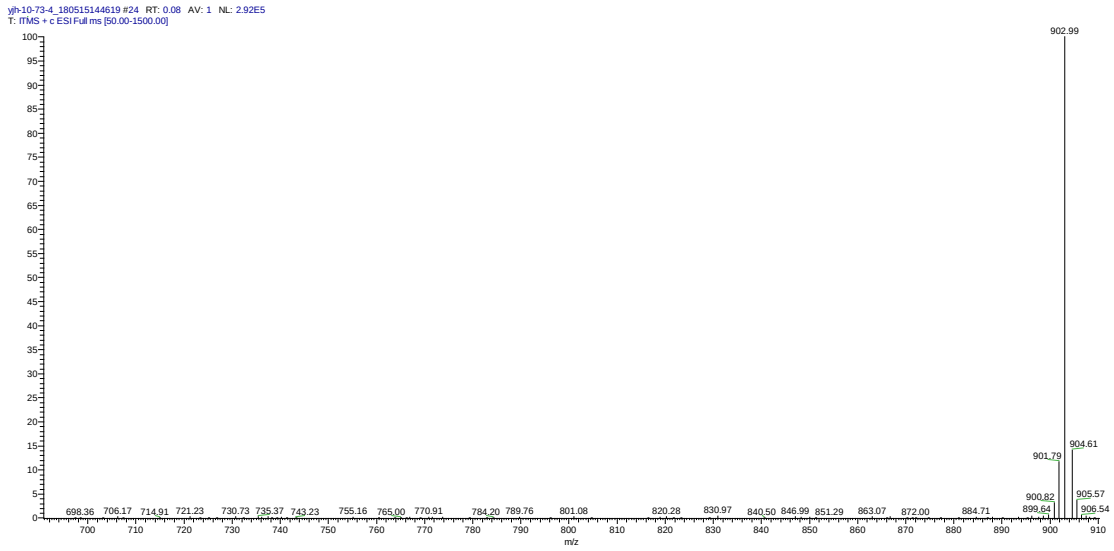
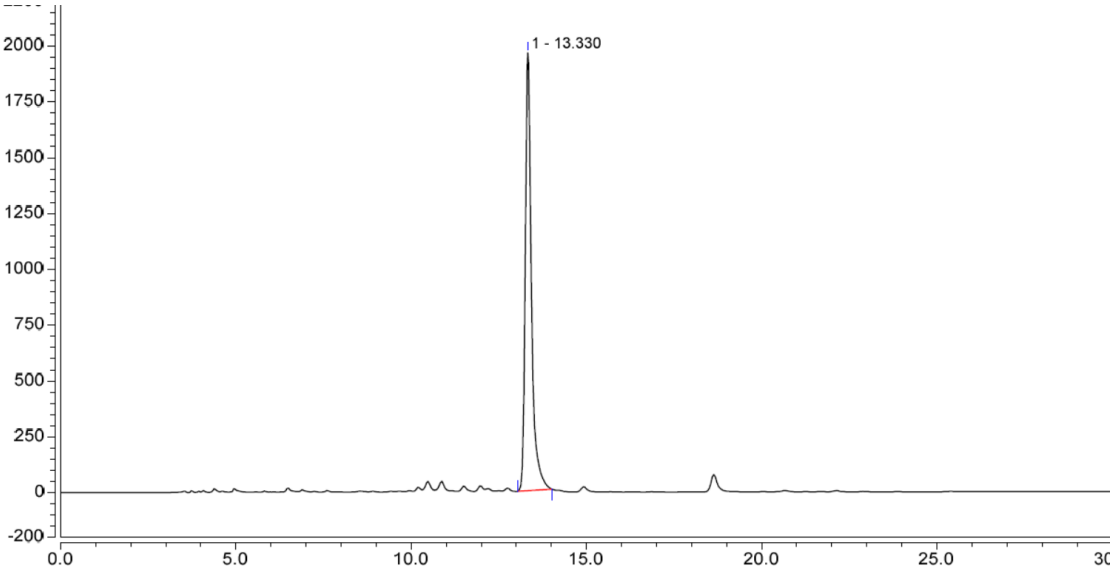


Figure S92: ESI-MS analysis of **7k**.

Fmoc- Ala^{S}-Leu-Ala-Ala^{S}-Val-Phe-OH (**7l**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	100.00	402.182	13.330	1960.486
Total:	100	402.182		1960.486

Figure S93: Crude HPLC trace of **7l** with optimized reaction conditions.

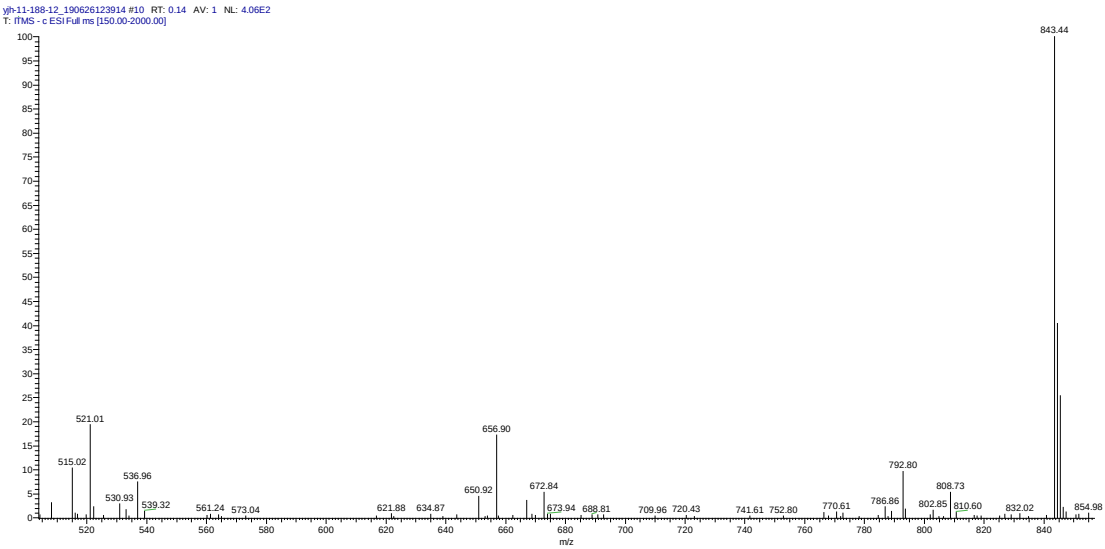
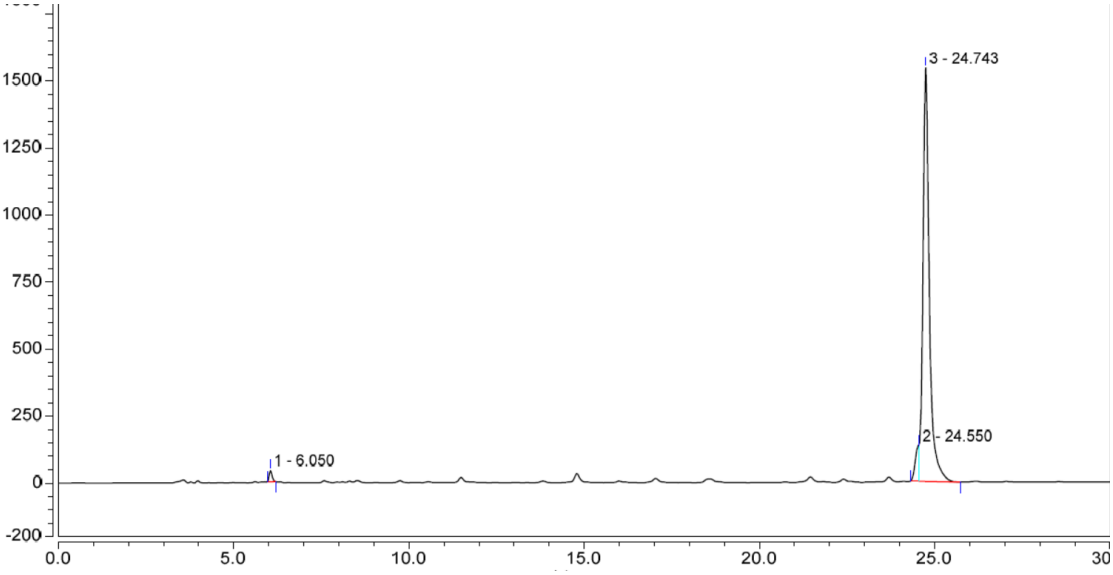


Figure S94: ESI-MS analysis of **7l**.

Fmoc- Met^{S}-Ala-Met-Tyr(^{t}Bu)^{S}-Ala-Leu-OH (**7m**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	1.11	3.874	6.050	41.285
2	4.38	15.858	24.550	132.835
3	94.51	325.796	24.743	1543.582
Total:	100	345.528		1717.701

Figure S95: Crude HPLC trace of **7m** with optimized reaction conditions.

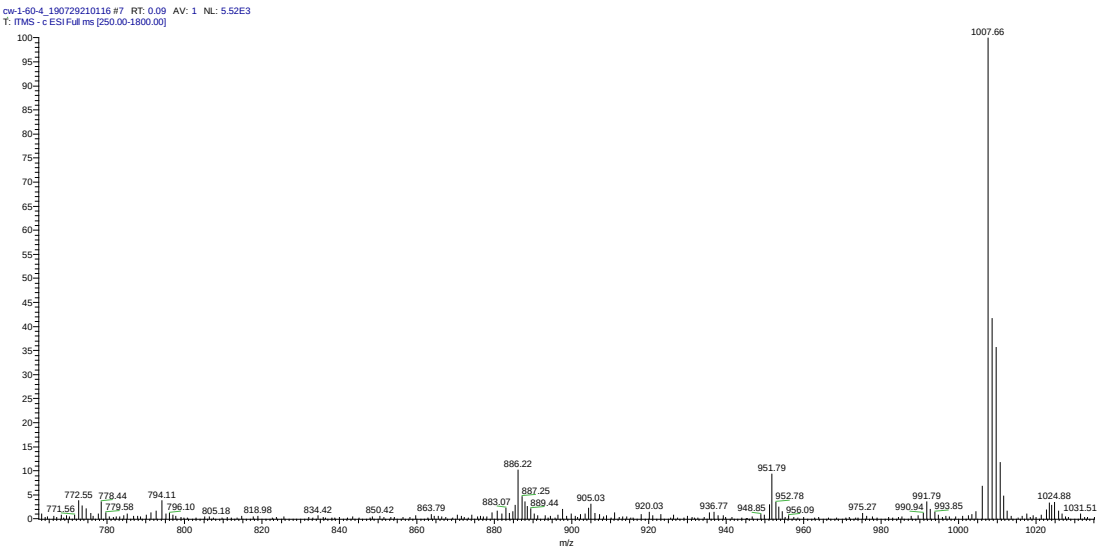
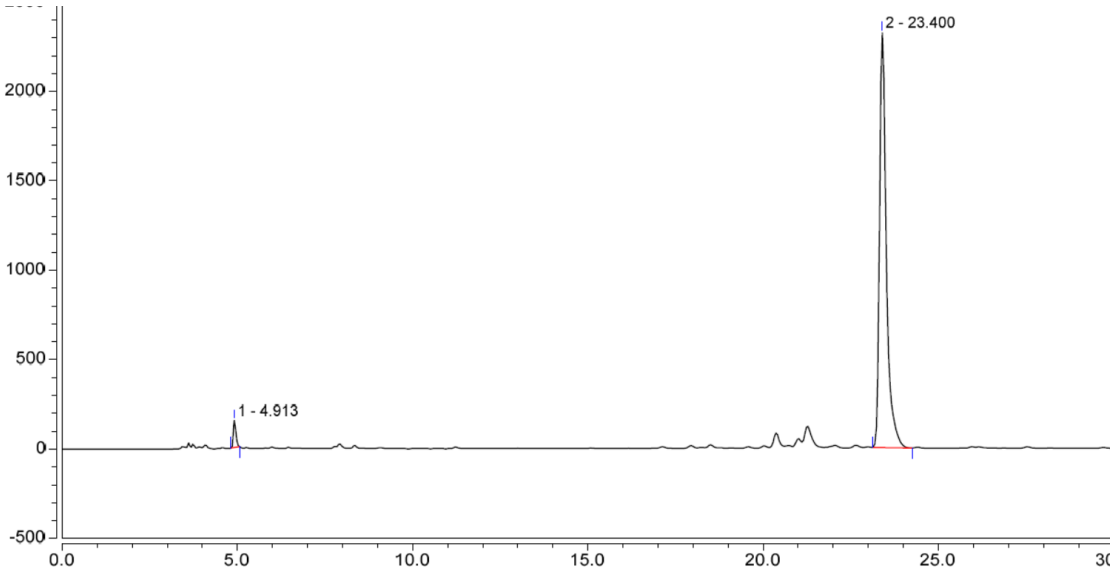


Figure S96: ESI-MS analysis of **7m**.

Fmoc-Lys(Boc)^{S}-Leu-Lys(Boc)-Ala^{S}-Ala-Gly-Phe-OH (**7n**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	2.32	12.538	4.913	151.406
2	97.68	527.603	23.400	2320.516
Total:	100	540.141		2471.922

Figure S97: Crude HPLC trace of **7n** with optimized reaction conditions.

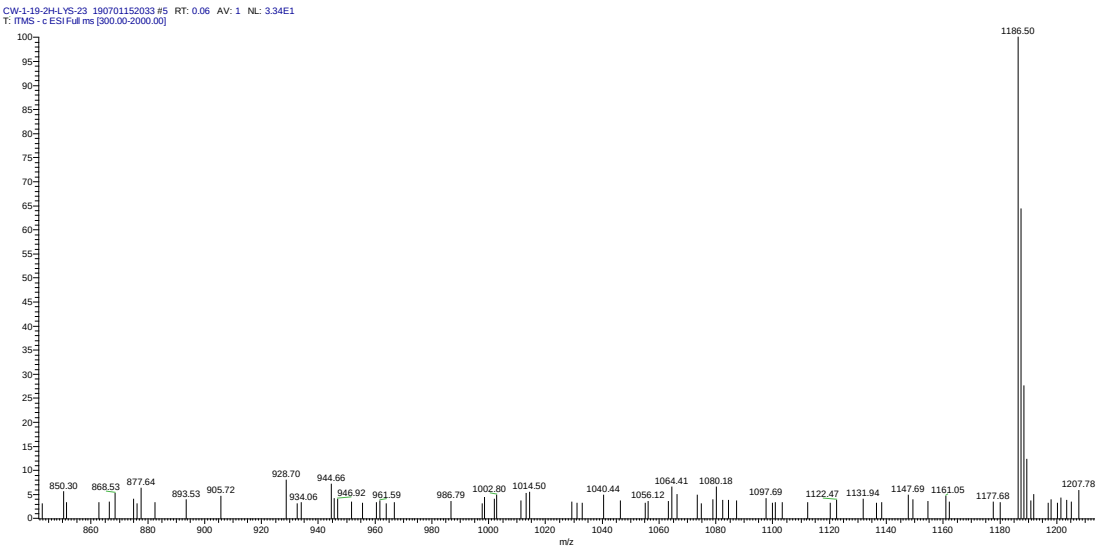
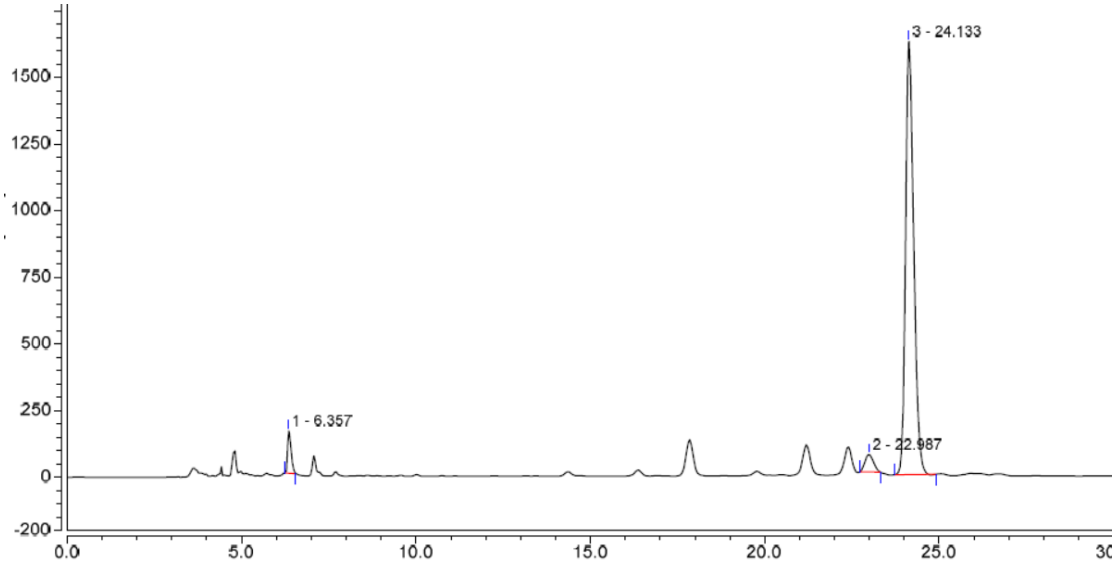


Figure S98: ESI-MS analysis of **7n**.

Fmoc-Glu(^{t}Bu)^S-Ser(^{t}Bu)-Val-Glu(^{t}Bu)^S-Ala-Pro-Phe-Asn(Trt)-Gly-Lys(Boc)-OH (**7o**)



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	3.83	18.324	6.357	158.556
2	3.90	18.687	22.987	65.730
3	92.27	441.849	24.133	1624.755
Total:	100	478.859		1849.040

Figure S99: Crude HPLC trace of **7o** with optimized reaction conditions.

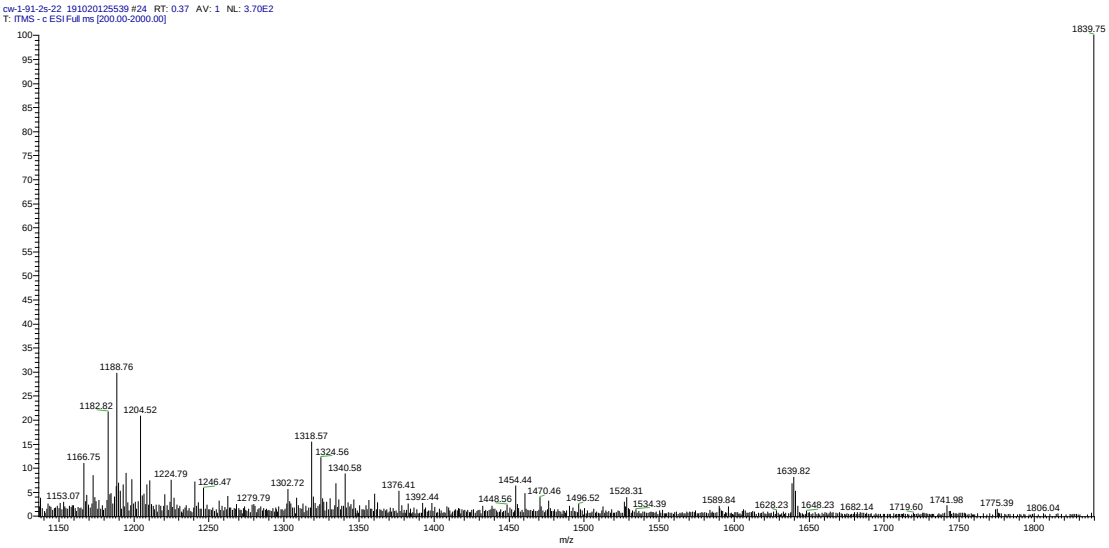
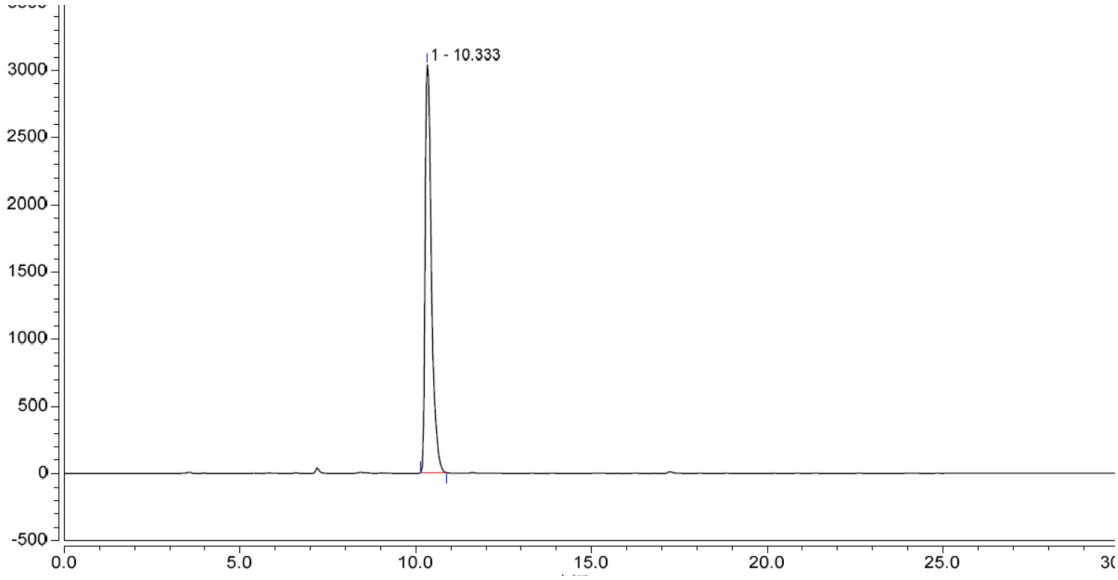


Figure S100: ESI-MS analysis of **7o**.

Fmoc-Ala^{S}-Ala-OH (**7p-1**), HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 50-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. t_R = 10.333 min, MS (ESI-TOF) calculated for $C_{21}H_{23}N_2O_4S^+$ m/z $[M+H]^+$: 399.14, Observed: 399.06.



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	100	632.385	10.333	3035.441
Total:	100	540.141		3035.441

Figure S101: Crude HPLC trace of **7p-1** with standard reaction condition.

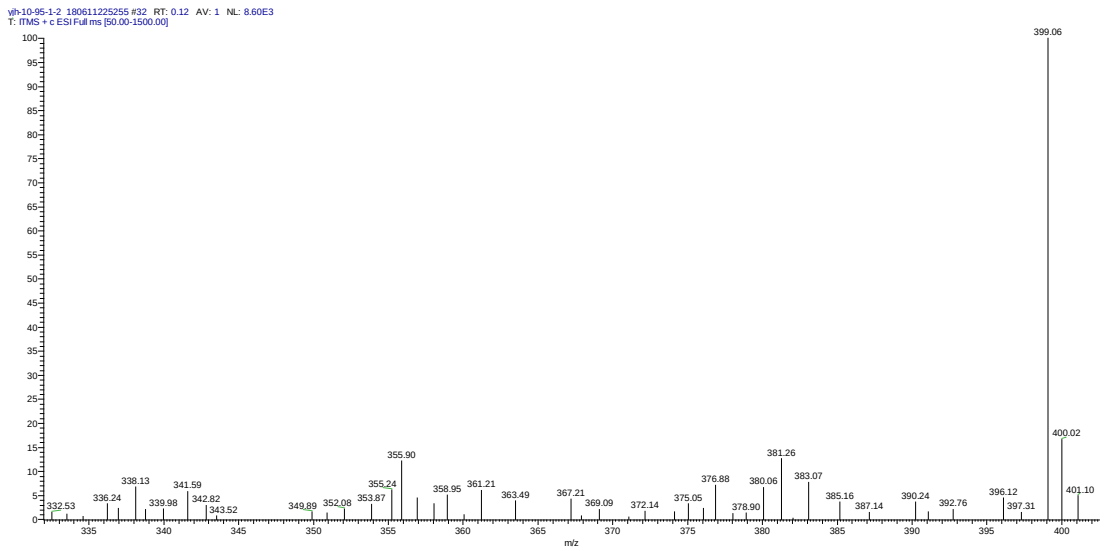
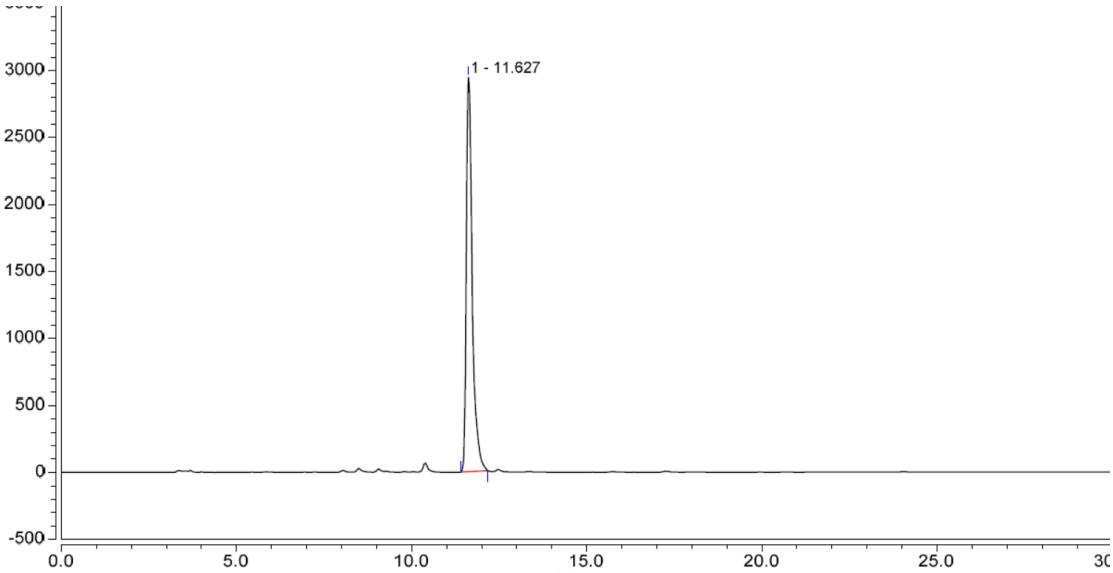


Figure S102: ESI-MS analysis of **7p-1**.

Fmoc-Ala^{S}-Ala^{S}-Ala-OH (**7p-2**), HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 50-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. t_R = 11.627 min, MS (ESI-TOF) calculated for $C_{24}H_{26}N_3O_4S_2^-$ calculated m/z $[M-H]^-$: 484.14, Observed: 484.25.



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	100	577.811	11.627	2944.753
Total:	100	577.811		2944.753

Figure S103: Crude HPLC trace of **7p-2** with standard reaction condition.

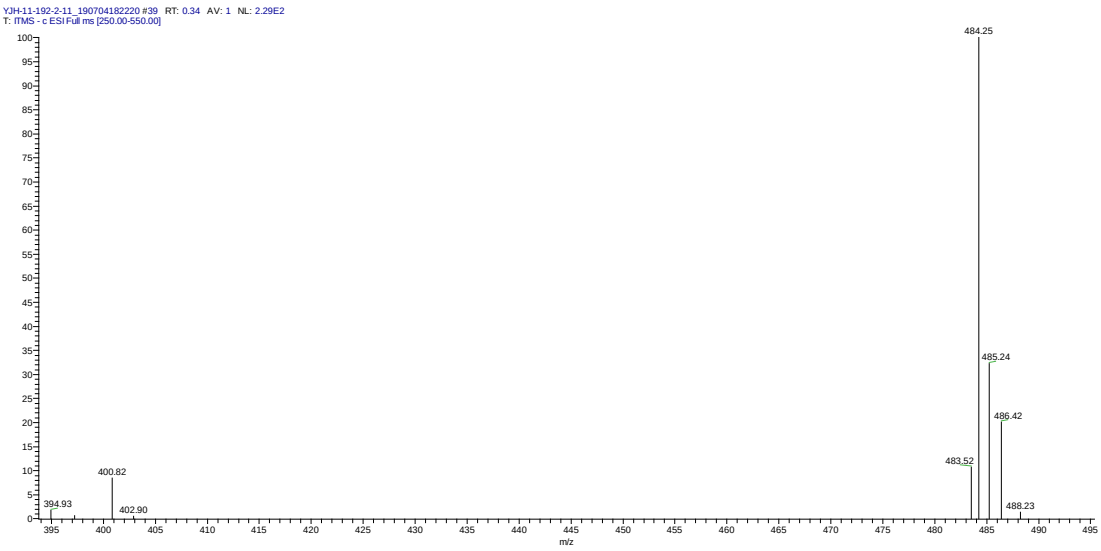
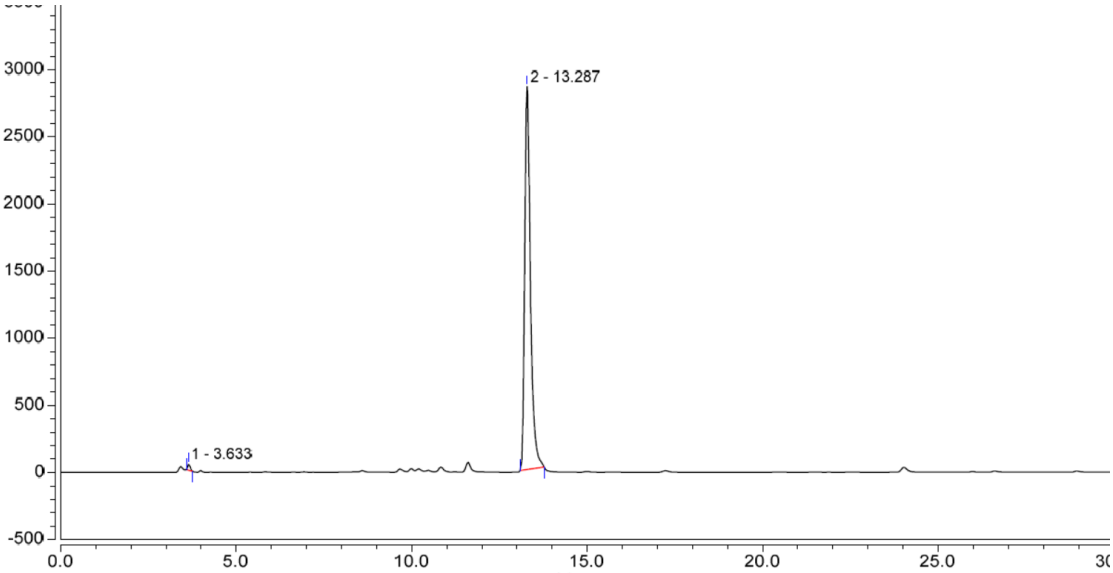


Figure S104: ESI-MS analysis of **7p-2**.

Fmoc-Ala^{S}-Ala^{S}-Ala^{S}-Ala-OH (**7p-3**), HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 50-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. t_R = 13.287 min, MS (ESI-TOF) calculated for $C_{27}H_{31}N_4O_4S_3^-$ calculated m/z $[M-H]^-$: 571.15, Observed: 571.36.



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.74	3.983	3.633	45.700
2	99.26	537.443	13.287	2851.804
Total:	100	541.426		2897.505

Figure S105: Crude HPLC trace of **7p-3** with standard reaction condition.

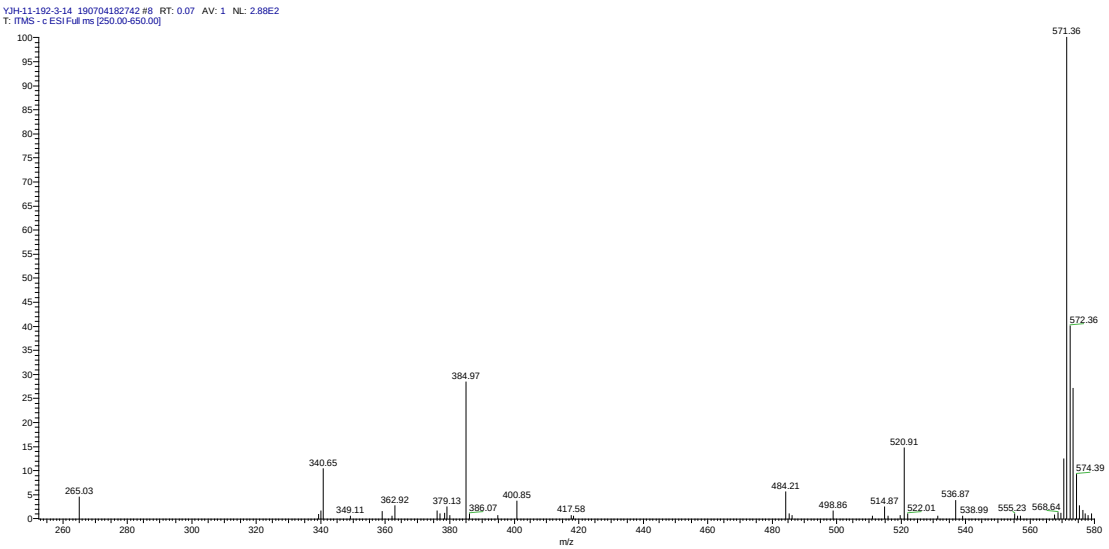
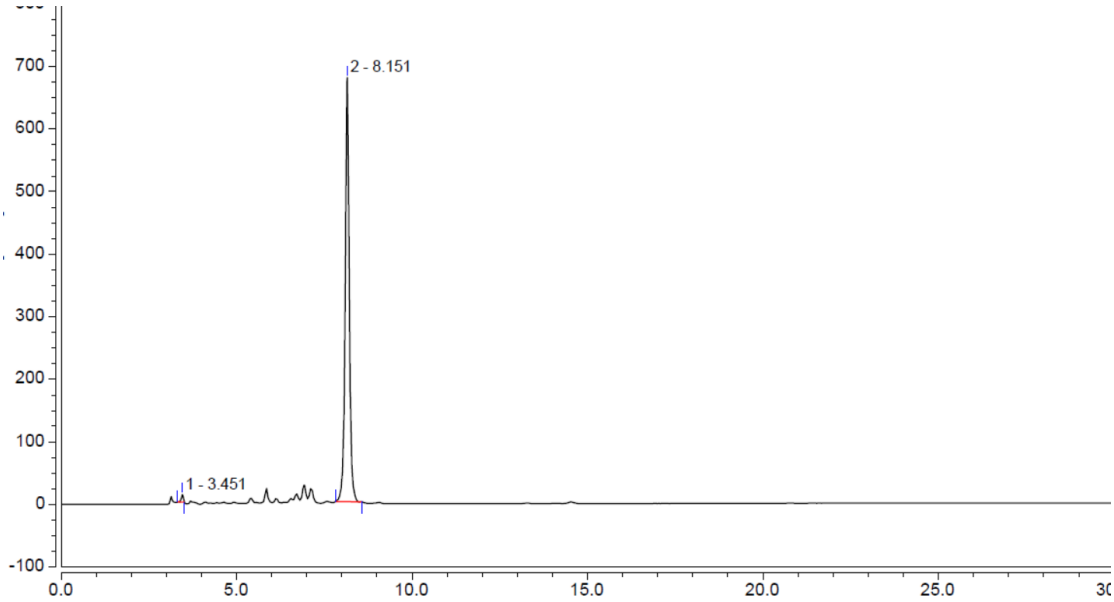


Figure S106: ESI-MS analysis of **7p-3**.

Fmoc-Met^{S}-Ala^{S}-Ala^{S}-Ala^{S}-Ala-OH (**7p**), HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 70-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. t_R = 8.151 min, MS (ESI-TOF) calculated for $C_{32}H_{40}N_5O_4S_5^-$ calculated m/z $[M-H]^-$: 718.17, Observed: 717.94.



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	0.76	0.685	3.451	11.985
2	99.24	90.039	8.151	677.828
Total:	100	90.724		689.813

Figure S107: Crude HPLC trace of **7p** with standard reaction condition.

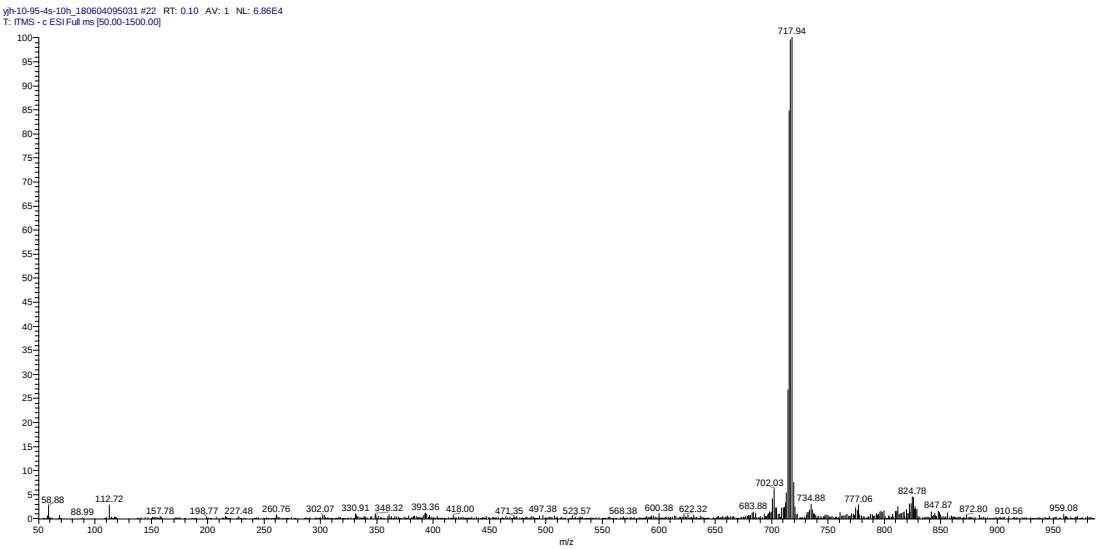
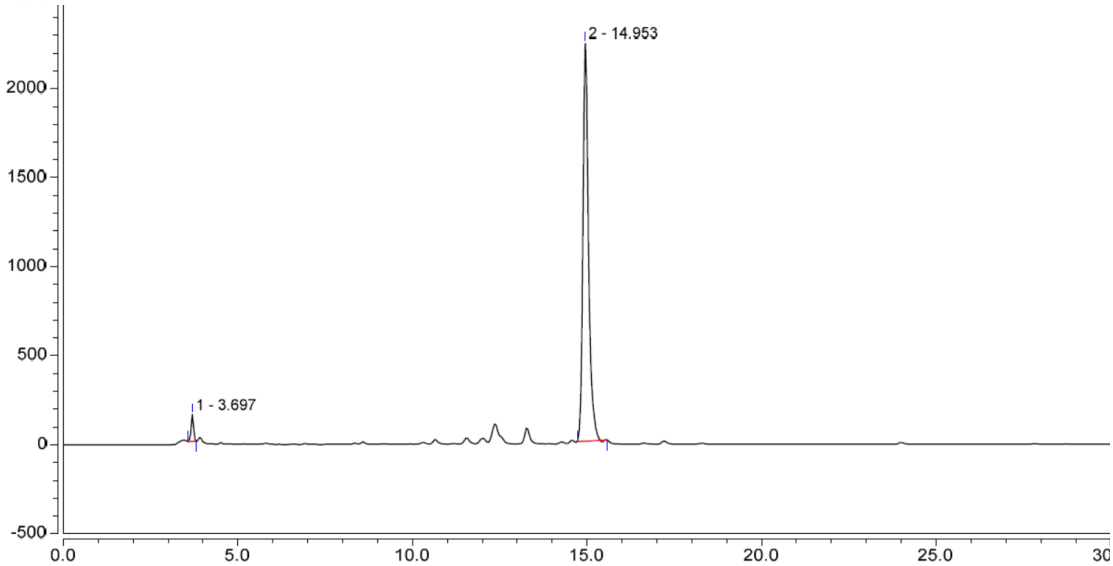


Figure S108: ESI-MS analysis of **7p**.

Fmoc-Ala^{S}-Ala^{S}-Ala^{S}-Ala^{S}-Ala-OH (**7q-1**), HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 50-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. t_R = 14.953 min, MS (ESI-TOF) calculated for $C_{30}H_{36}N_5O_4S_4^-$ calculated m/z $[M-H]^-$: 658.17, Observed: 658.24.



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	2.75	11.790	3.697	150.900
2	97.25	416.593	14.953	2235.206
Total:	100	428.382		2386.106

Figure S109: Crude HPLC trace of **7q-1** with standard reaction condition.

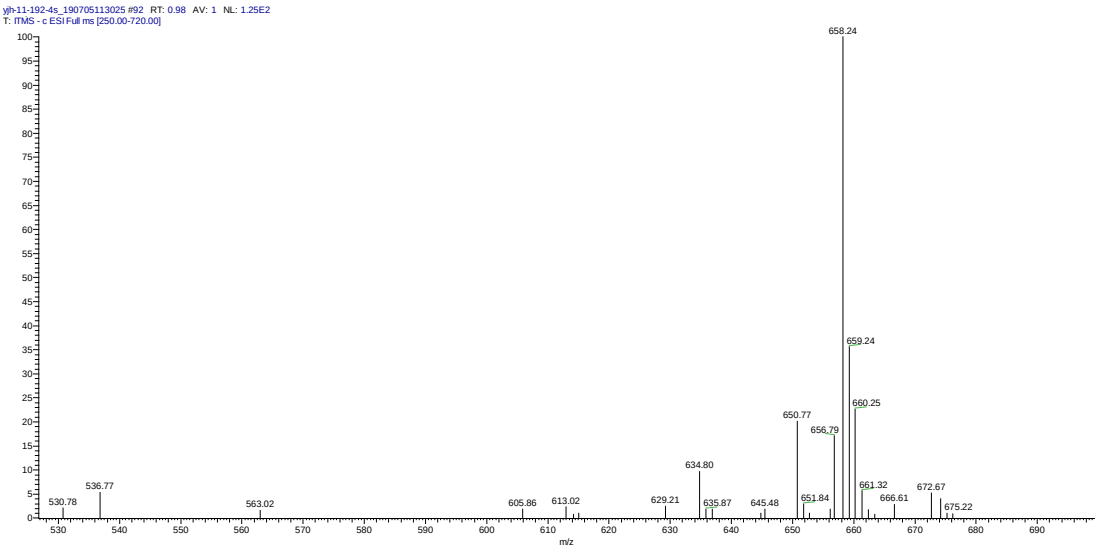
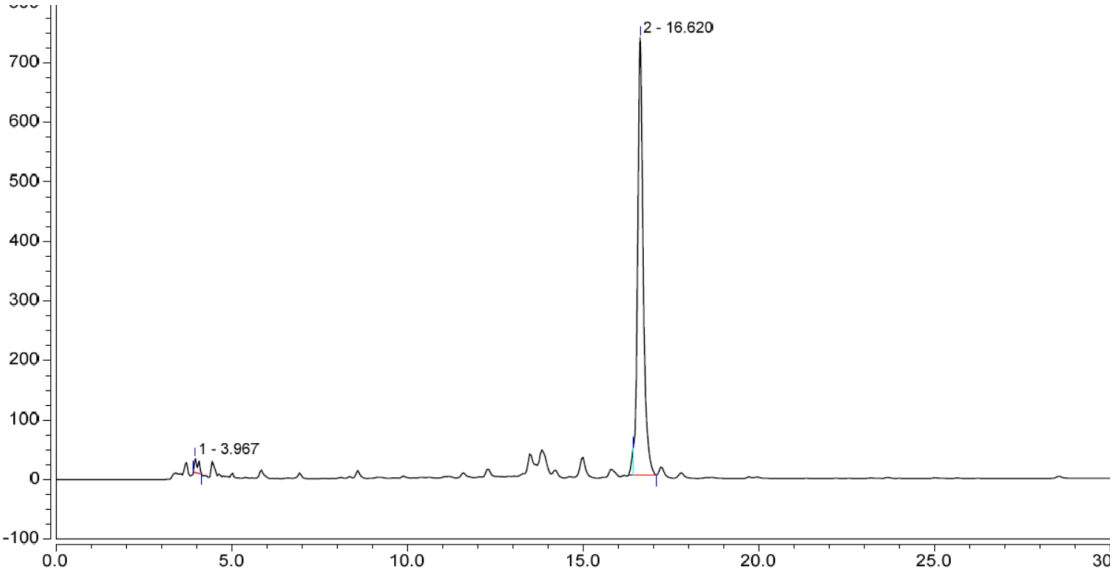


Figure S110: ESI-MS analysis of **7q-1**.

Fmoc-Ala^{S}-Ala^{S}-Ala^{S}-Ala^{S}-Ala^{S}-Ala-OH (**7q**), HPLC analysis was performed using C18 analytical column, 254 nm UV detection. Gradient elution with 50-90% B with a flow rate of 1.0 mL/min over 30 min gave the target peptide. t_R = 16.620 min, MS (ESI-TOF) calculated for $C_{33}H_{41}N_6O_4S_5^-$ calculated m/z $[M-H]^-$: 745.18, Observed: 745.25.



Peak Info:

Peak No	% Area	Area	RT (min)	Height (mAV)
1	2.25	3.075	3.967	23.542
2	97.75	133.857	16.620	735.377
Total:	100	136.933		758.919

Figure S111: Crude HPLC trace of **7q** with standard reaction condition.

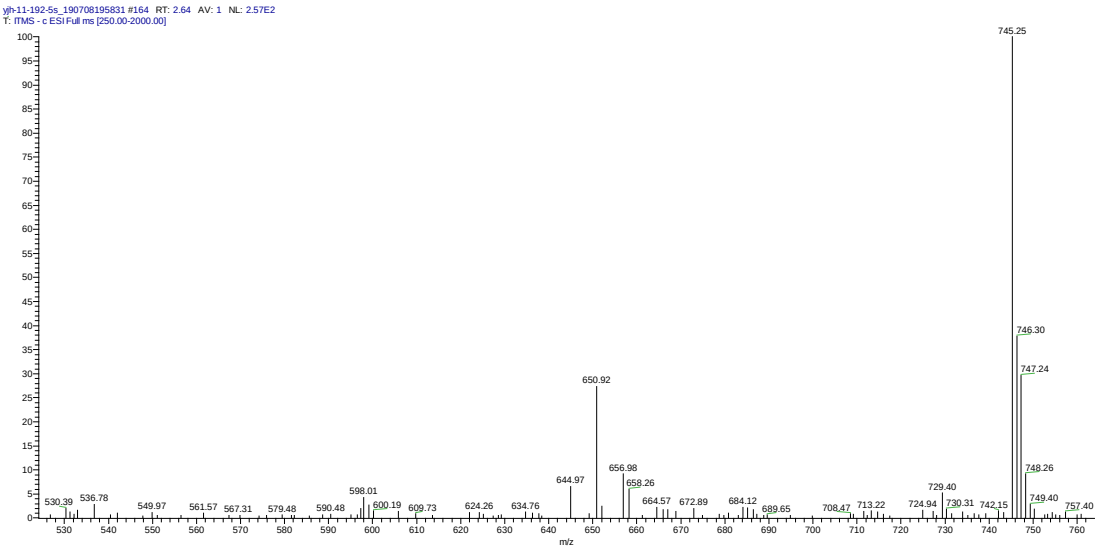


Figure S112: ESI-MS analysis of **7q**.