Supporting Information

Tryptic stability of synthetic bactenecin derivatives is determined by the side chain length of cationic residues and the peptide conformation

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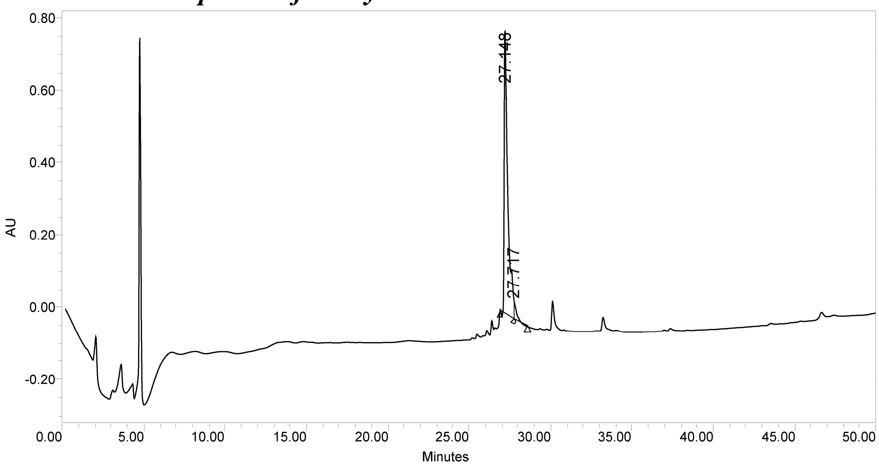
Table S1. The sequences of the synthetic bactenecin peptides and their analogues

Peptide code	Sequence
1	Lys-Arg-Trp-Trp-Lys-Trp-Ile-Arg-Trp-NH_2
2[a]	Lys-Arg-Trp-Trp-Lys-Trp-Ile-Arg-Trp-NH_2
3	Trp-Arg-Ile-Trp-Lys-Trp-Trp-Arg-Lys-NH_2
4	Arg-Lys-Trp-Trp-Arg-Trp-Ile-Lys-Trp-NH_2
5	Dab-Dab-Trp-Trp-Dab-Trp-Ile-Dab-Trp-NH_2
6	Orn-Orn-Trp-Trp-Orn-Trp-Ile-Orn-Trp-NH_2
7	Har-Har-Trp-Trp-Har-Trp-Ile-Har-Trp-NH_2
8	Dab-Har-Trp-Trp-Dab-Trp-Ile-Har-Trp-NH_2
9	Orn-Har-Trp-Trp-Orn-Trp-Ile-Har-Trp-NH_2
10	Lys-Arg-Trp-Trp-Lys-Trp-Trp-Arg-Arg-NH_2
11	Lys-Arg-Trp-Trp-Lys-Trp-Trp-Arg-Arg-NH_2
12	Arg-Arg-Trp-Trp-Lys-Trp-Trp-Arg-Lys-NH_2
13	Arg-Lys-Trp-Trp-Arg-Trp-Trp-Lys-Lys-NH_2
14	Dab-Dab-Trp-Trp-Dab-Trp-Trp-Dab-Dab-NH_2
15	Orn-Orn-Trp-Trp-Orn-Trp-Trp-Orn-Orn-NH_2
16	Har-Har-Trp-Trp-Har-Trp-Trp-Har-Har-NH_2
17	Dab-Har-Trp-Trp-Dab-Trp-Trp-Har-Har-NH_2
18	Orn-Har-Trp-Trp-Orn-Trp-Trp-Har-Har-NH_2

[a] *D*-amino acids are underlined.

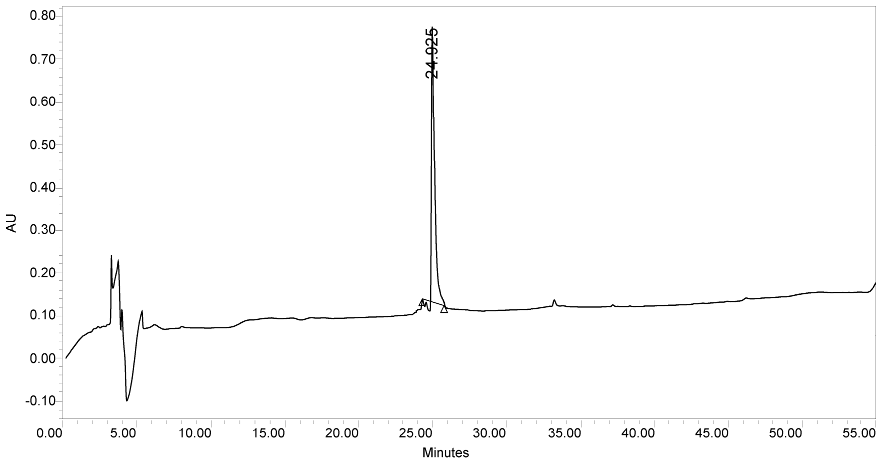
__Figure S1. RP-HPLC spectra of the synthetic bactenecin-derived AMPs__

1



	RT (min)	Area (μV*sec)	% Area	Height (μV)	% Height
1	27.148	11883913	96.35	783609	94.49
2	27.717	449558	3.65	45676	5.51

4



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1	24.925	12428055	100.00	650492	100.00

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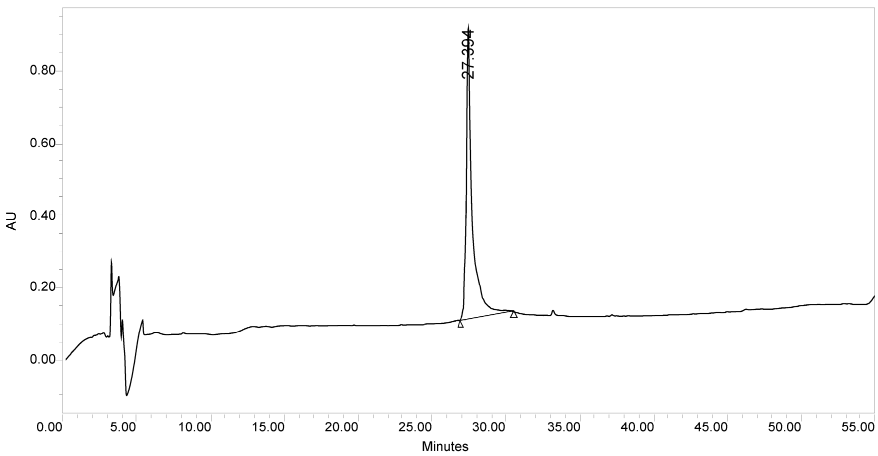

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1	27.394	31186141	100.00	809985	100.00

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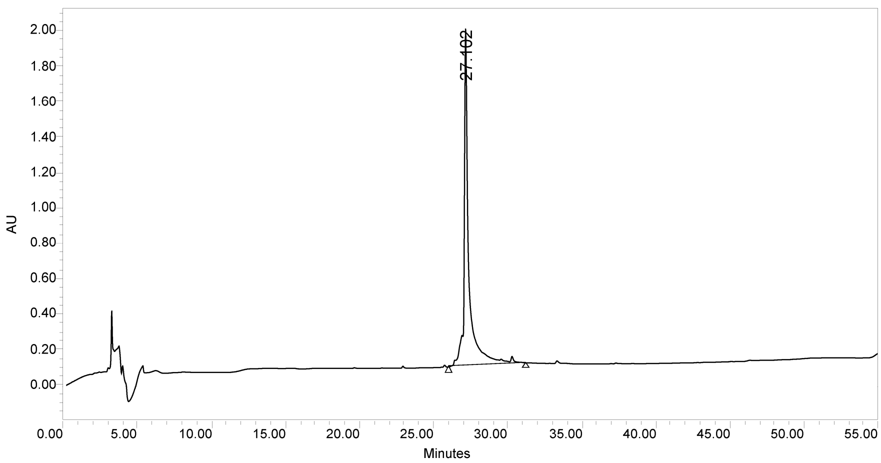

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1	27.102	41467130	100.00	1902051	100.00

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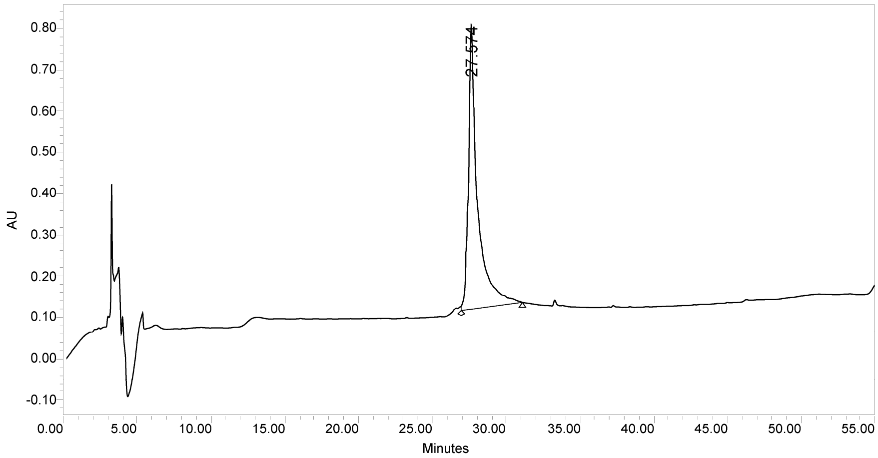

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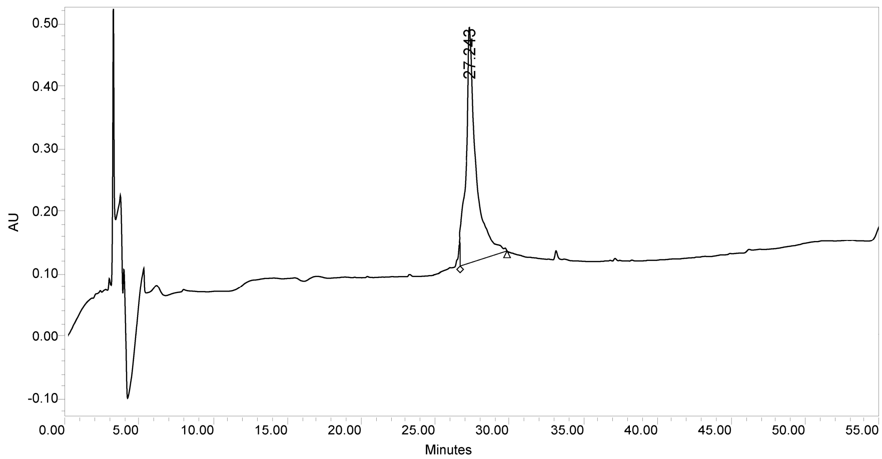
1	27.574	36064407	100.00	690649	100.00

8



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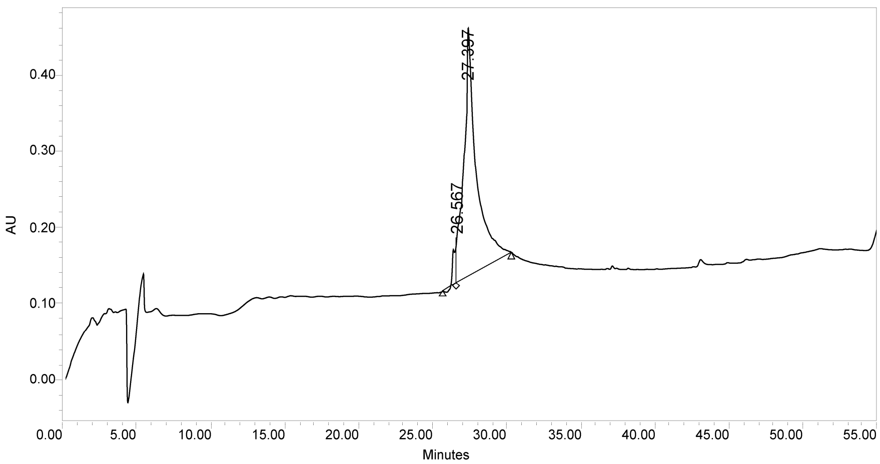
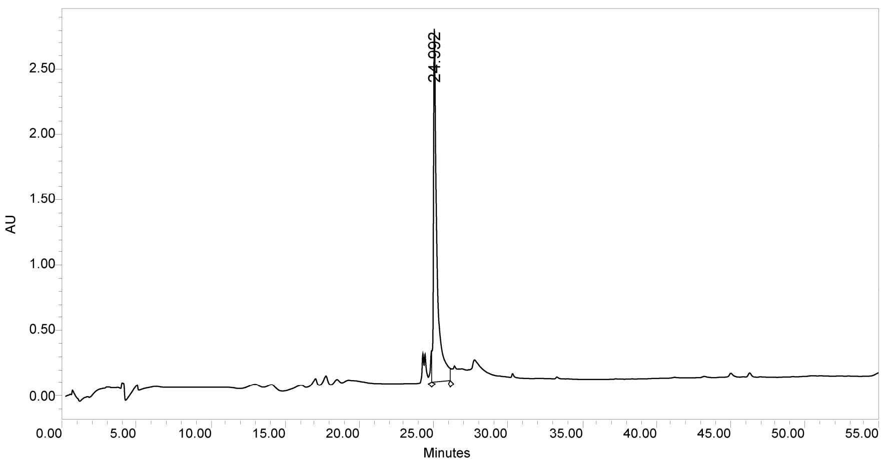
1	27.243	19238552	100.00	379505	100.00

9



	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	26.567	975534	4.79	60729	15.68
2	27.397	19388831	95.21	326690	84.32

10



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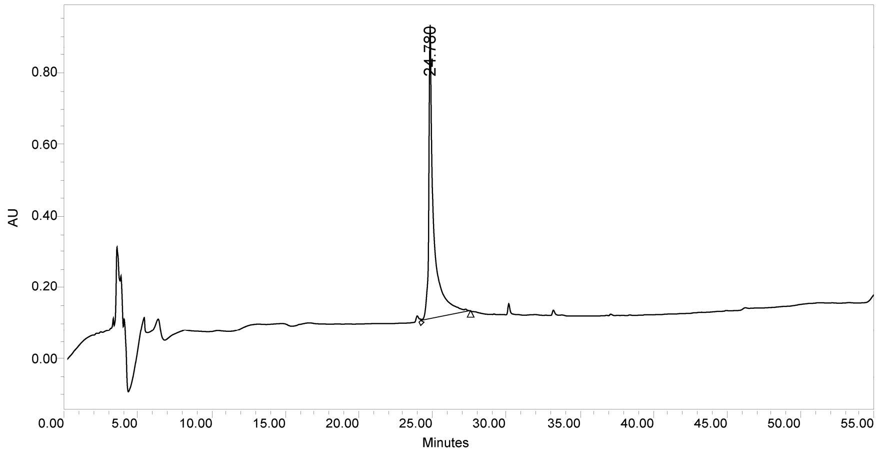
1	24.992	43856370	100.00	2732073	100.00

11



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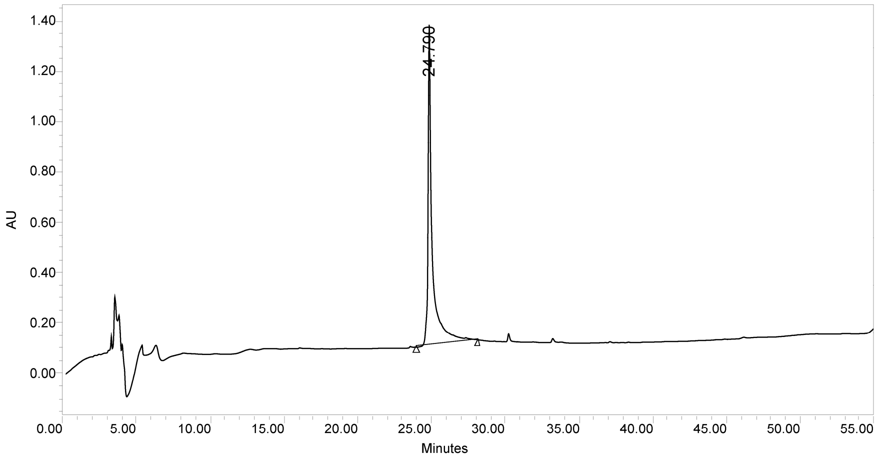
1	24.780	19350236	100.00	820719	100.00

12



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1	24.790	25655886	100.00	1278975	100.00

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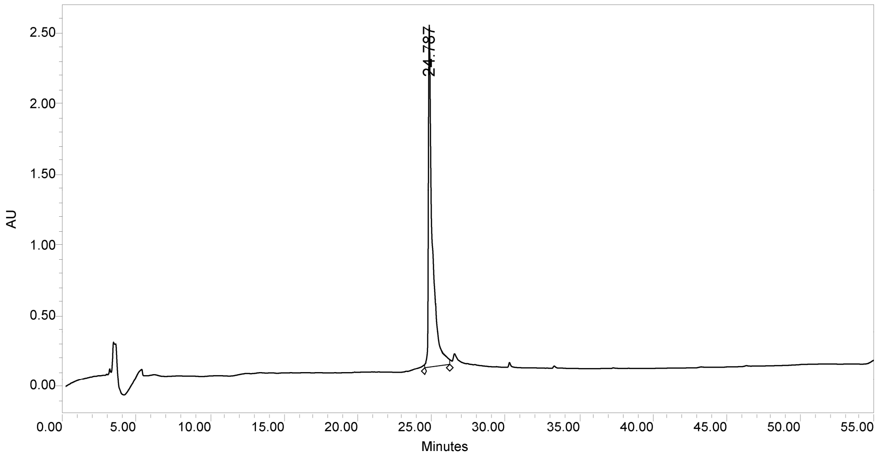

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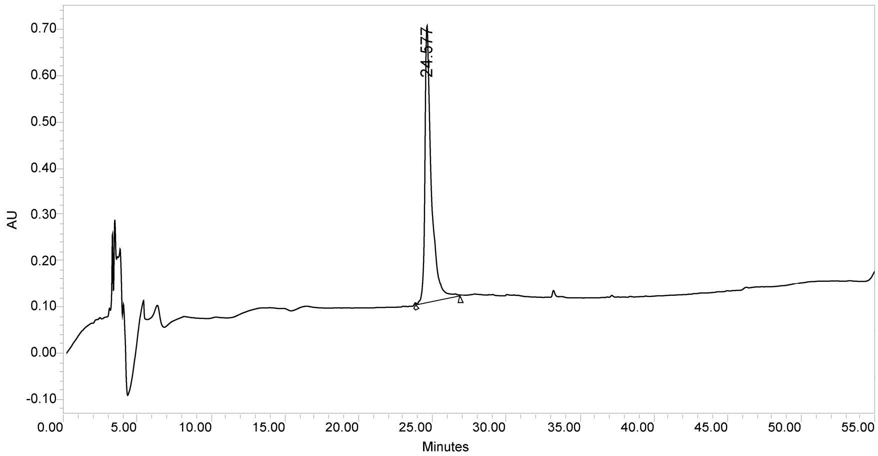
1	24.787	48101892	100.00	2437496	100.00

14



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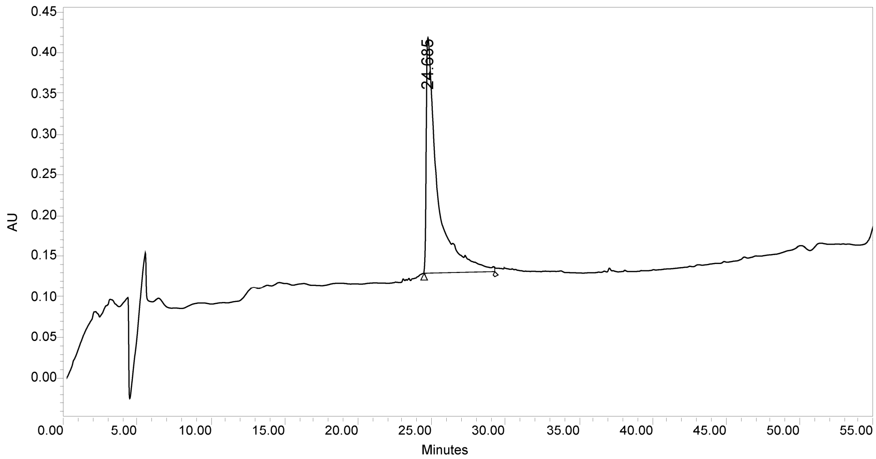
1	24.577	24794329	100.00	599472	100.00

15



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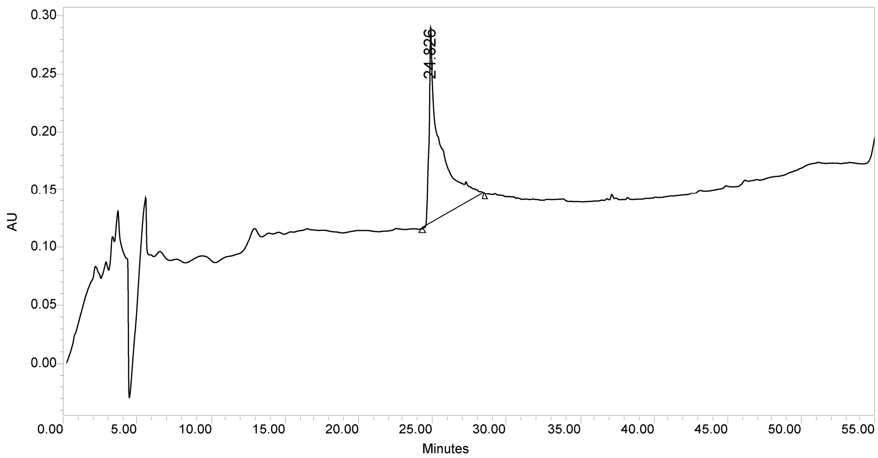
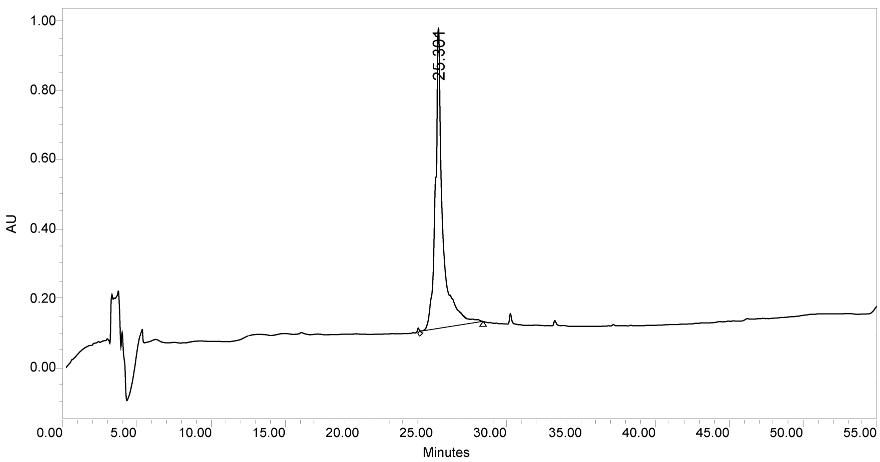
1	24.685	15778358	100.00	290499	100.00

16



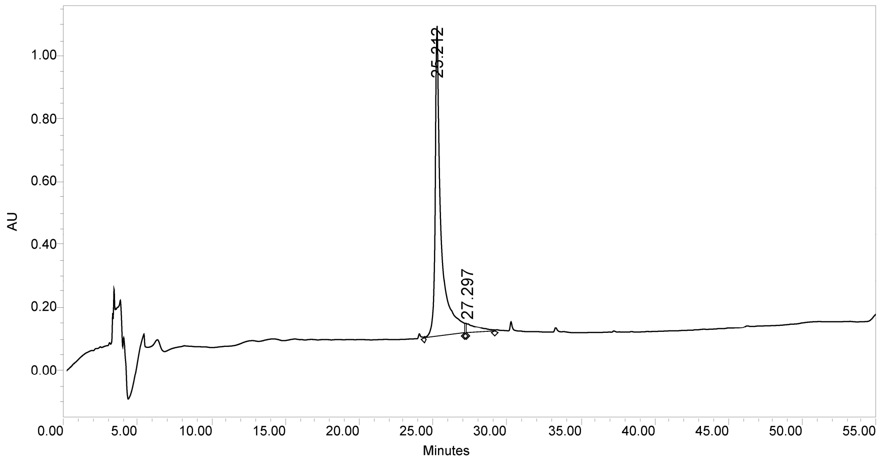
	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	24.826	9022603	100.00	170362	100.00

17



	RT (min)	Area (□V*sec)	% Area	Height (□V)	% Height
1	25.301	31638622	100.00	866338	100.00

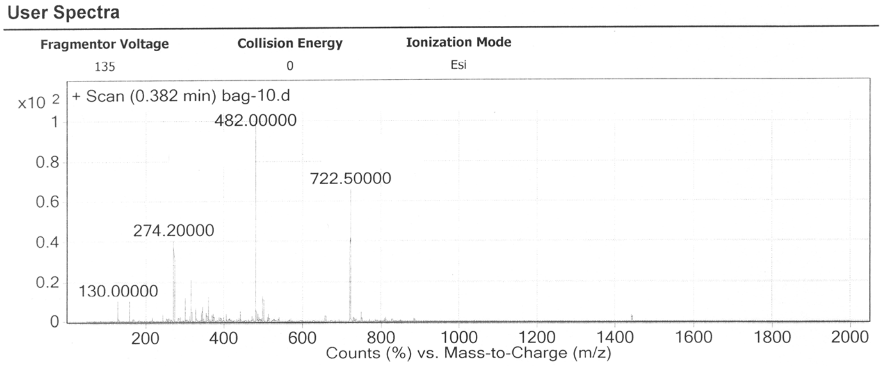
18



	RT (min)	Area (□V*sec)	% Area	Height (□V)	% Height
1	25.212	27873057	94.05	989471	96.82
2	27.297	1764761	5.95	32505	3.18

Figure S2. Mass spectra of synthetic bactenecin-derived AMPs. The values are monoisotopic masses in positive mode.

1



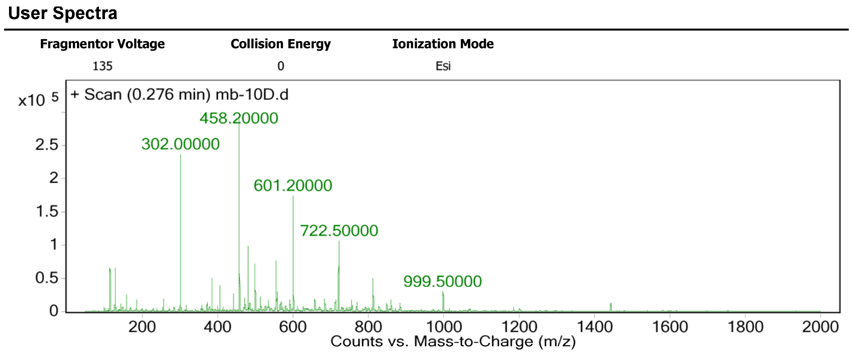
Peak List

m/z	z	Abund.
130		77893
158.9		77841
274.2	1	311022
302.2		91912
318.1		161996
362		94240
482		763461
499.2		98036
501.1		88568
722.5		505432

$[M+H^{2+}]^{2+} = 722.4^{a}$, $[M+H^{3+}]^{3+} = 482.0$

[a] These values are the calculated mass values for the respective peptides.

2



Peak List

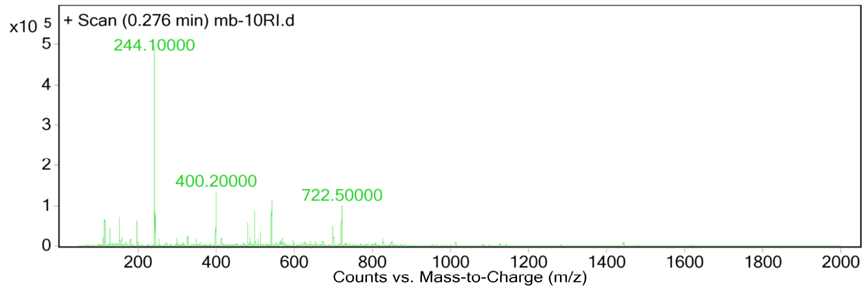
m/z	z	Abund.
302		234748
458.2	1	288131
459.2	1	91252
482		98099
499.1		71367
500.3		66172
557.2		77102
601.2	1	172559
602.2	1	69376
722.5		106466

$[M+H^{2+}]^{2+} = 722.4$, $[M+H^{3+}]^{3+} = 482.0$, $[M+2TFA+H^{3+}]^{3+} = 557.9^{b}$

[b] The calculated masses may reflect the mass of the peptides with TFA adduct ion; e.g., $[M+xTFA+nH]^{n+}$, where x is the number of TFA adduct ion, and n represents multiple-charge ionization of peptides from protonation. The monoisotopic mass of TFA is 113.99 Da.

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



3

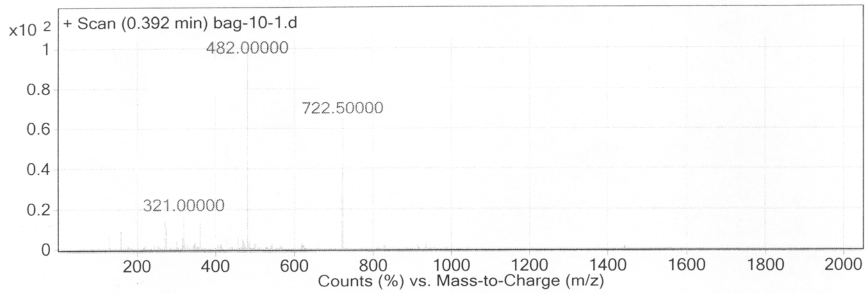
Peak List

m/z	z	Abund.
116.1		63614
154		70912
199		61460
244.1	1	495043
245.1	1	80116
400.2	1	133999
482		56105
499.2		85819
543.2	1	111402
722.5		99026

$[M+H^{2+}]^{2+} = 722.4$, $[M+H^{3+}]^{3+} = 482.0$

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



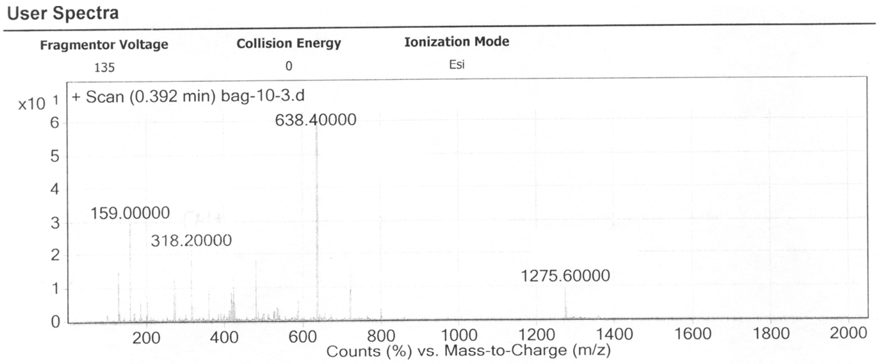
4

Peak List

m/z	Abund.
130	64455
159	86977
274.1	135066
318.2	62181
321	156159
361.8	123469
458.3	53090
471.1	50700
482	924981
722.5	598406

$[M+H^{2+}]^{2+} = 722.4$, $[M+H^{3+}]^{3+} = 482.0$

5

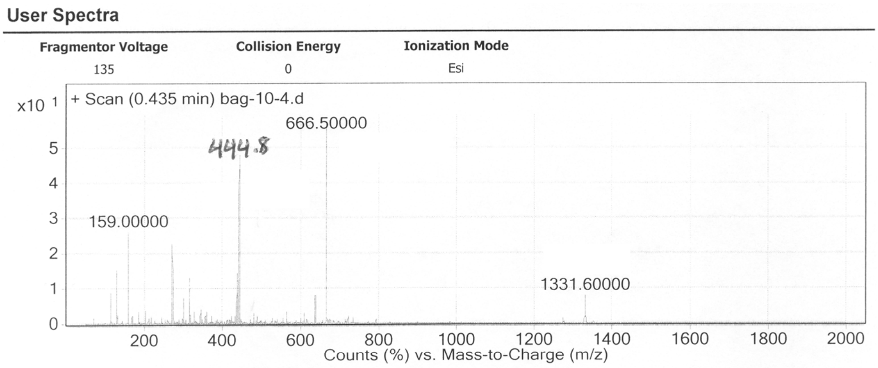


Peak List

m/z	z	Abund.
130		138730
159	1	273182
274.2		122067
318.2	1	194631
362.1		87964
425.9		123172
482.1		167167
638.4		555045
722.5		137598
1275.6	1	88806

$[M+H^{1+}]^{1+}$ =1275.8, $[M+H^{2+}]^{2+}$ = 638.4, $[M+H^{3+}]^{3+}$ = 425.9

6

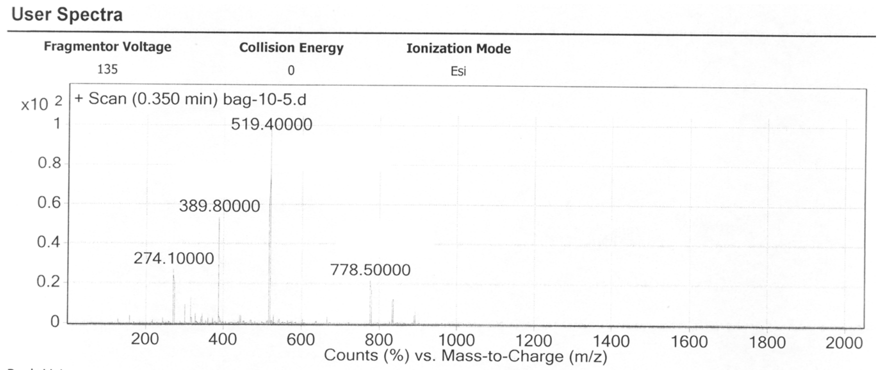


Peak List

m/z	z	Abund.
115		81442
130		144632
159	1	240092
274.1		211178
318.1		122252
439		133479
444.6		449990
638.5		75134
666.5		524785
1331.6	1	72481

$[M+H^{1+}]^{1+}$ =1331.8, $[M+H^{2+}]^{2+}$ = 666.4, $[M+H^{3+}]^{3+}$ = 444.6

7

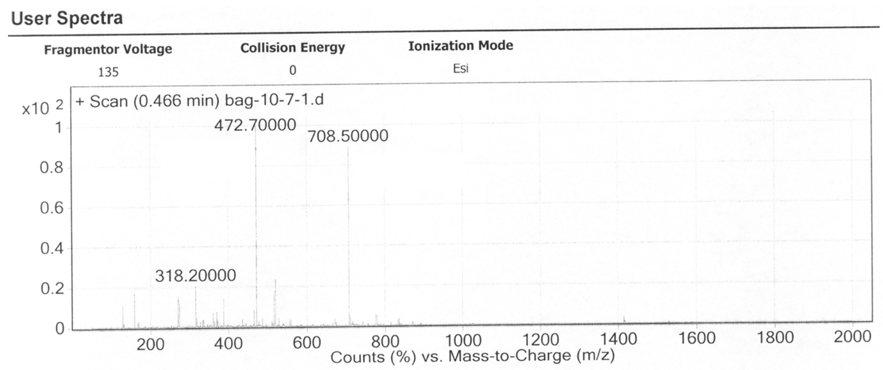


Peak List

m/z	Abund.
274.1	271624
302.2	92172
318.1	129798
389.8	531360
519.4	996489
778.5	213639
835.5	123206
892.5	58584

$[M+H^{2+}]^{2+} = 778.4$, $[M+H^{3+}]^{3+} = 519.3$, $[M+H^{4+}]^{4+} = 389.7$

8

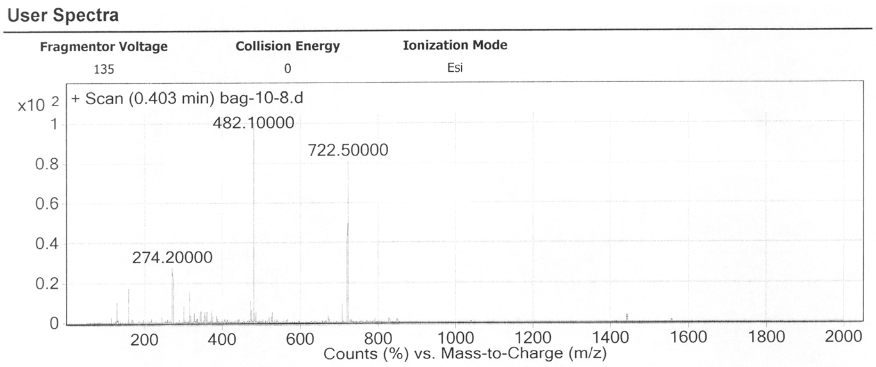


Peak List

m/z	Abund.
130	71799
159	117997
274.2	109054
318.2	142649
372.2	50444
389.9	97588
467	58062
472.7	685163
519.4	164256
708.5	612096

$[M+H^{2+}]^{2+} = 708.4$, $[M+H^{3+}]^{3+} = 472.6$

9

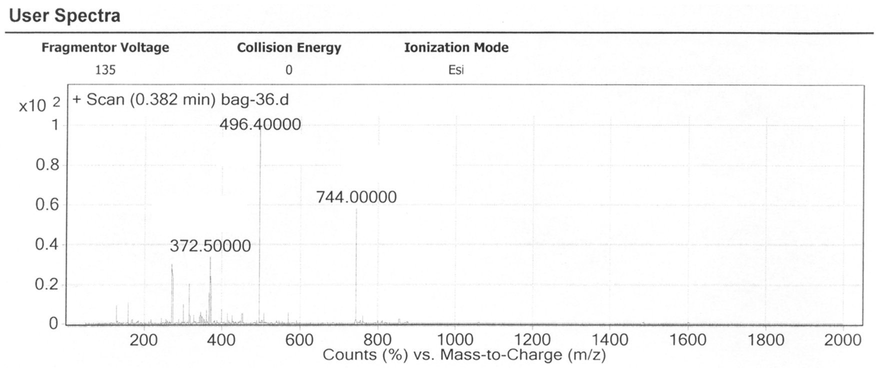


Peak List

m/z	Abund.
130	76560
159	130697
274.2	212212
302.1	65525
318.2	118411
472.6	80871
476.3	51301
482.1	761430
708.5	70294
722.5	617793

$[M+H^{2+}]^{2+} = 722.4$, $[M+H^{3+}]^{3+} = 481.9$

10

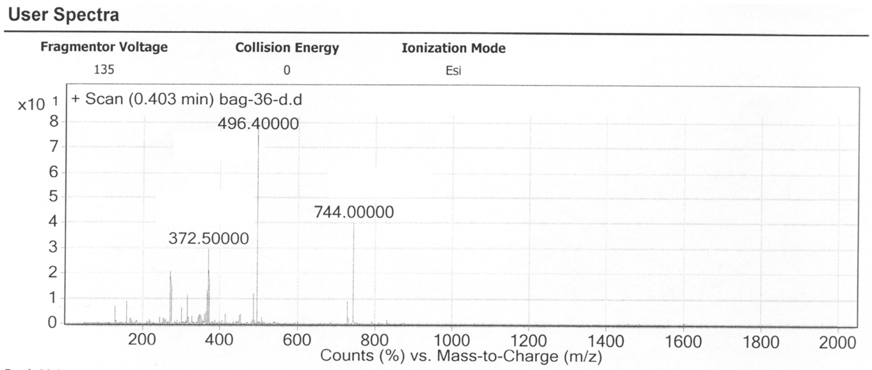


Peak List

m/z	z	Abund.
130		38998
159		44139
274.2	1	124167
302.2		39967
318.2		85810
368.3		66223
372.5		141317
400.7		32226
496.4		413198
744		241710

$[M+H^{2+}]^{2+} = 743.9$, $[M+H^{3+}]^{3+} = 496.3$, $[M+H^{4+}]^{4+} = 372.5$

11

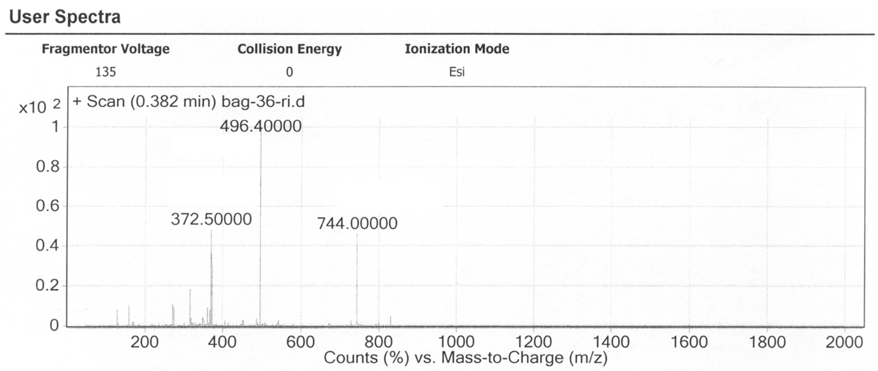


Peak List

m/z	Abund.
129.9	64249
159	83677
274.2	189870
318.2	103588
368.3	120922
372.5	269382
487.1	109489
496.4	719993
730	83255
744	364734

$[M+H^{2+}]^{2+}$ = 743.9, $[M+H^{3+}]^{3+}$ = 496.3, $[M+H^{4+}]^{4+}$ = 372.5

12

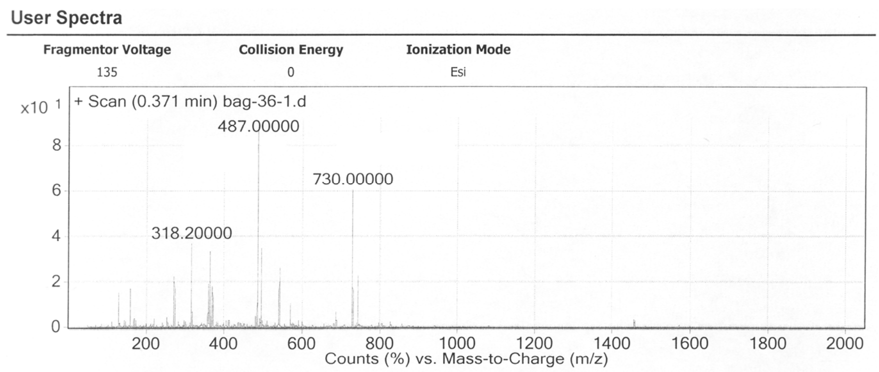


Peak List

m/z	Abund.
130	83148
159	102751
274.1	109280
318.2	189455
362.2	96044
368.3	83049
372.5	494158
496.4	1024810
744	472133

$[M+H^{2+}]^{2+}$ = 743.9, $[M+H^{3+}]^{3+}$ = 496.3, $[M+H^{4+}]^{4+}$ = 372.5

13

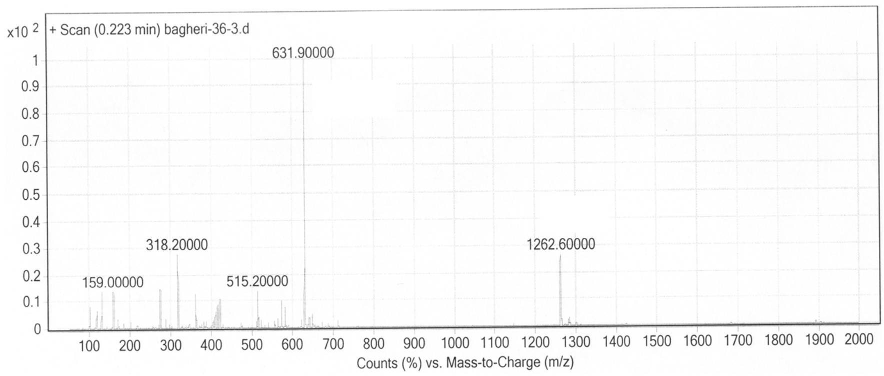


Peak List

m/z	z	Abund.
274.2		92613
318.2	1	151813
362.2		78777
365.5		138746
372.6		74897
487		364602
496.4		144475
543.2		108075
730		249741
744.1		94798

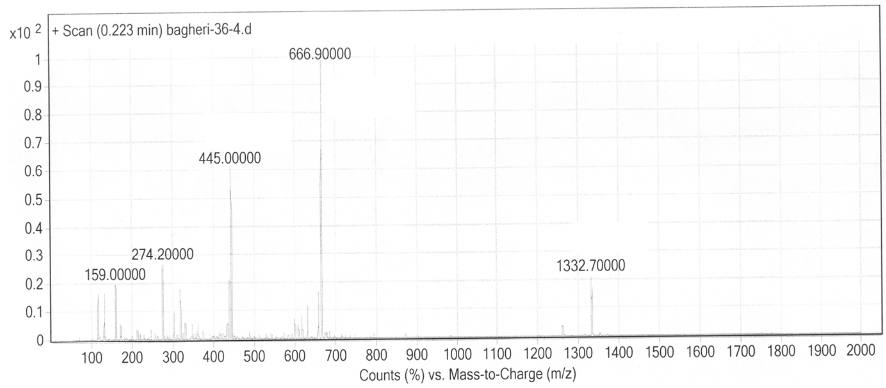
$[M+H^{2+}]^{2+} = 729.9$, $[M+H^{3+}]^{3+} = 487.0$, $[M+H^{4+}]^{4+} = 365.5$

14



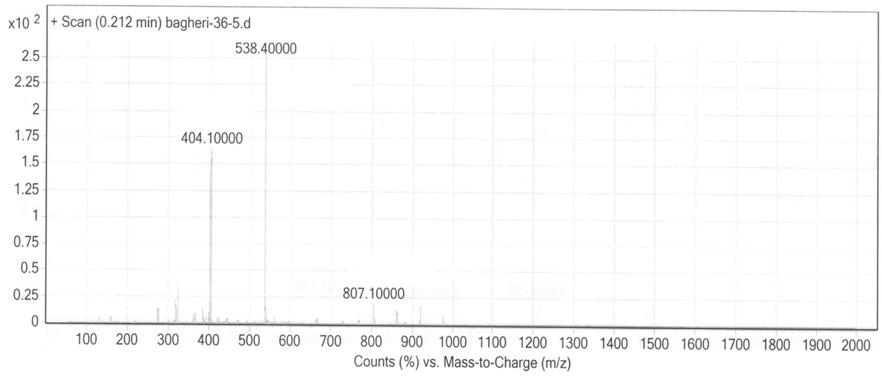
$[M+H^{1+}]^{1+} = 1262.8$, $[M+H^{2+}]^{2+} = 631.9$

15



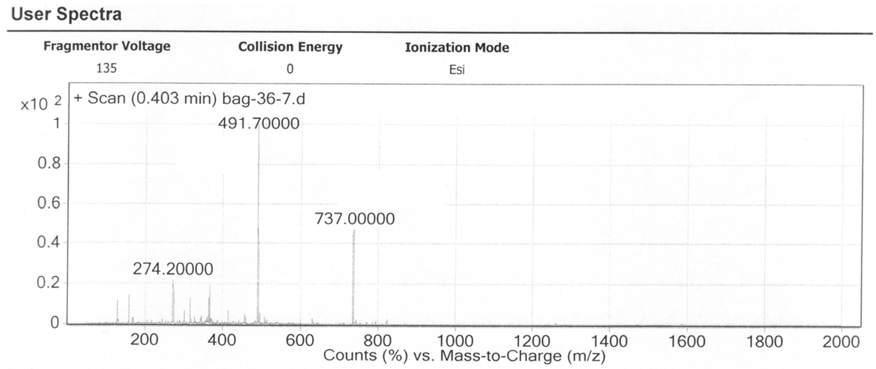
$[M+H^{1+}]^{1+} = 1332.8$, $[M+H^{2+}]^{2+} = 666.9$, $[M+H^{4+}]^{4+} = 444.9$

16



$[M+H^{2+}]^{2+} = 806.9$, $[M+H^{3+}]^{3+} = 538.4$, $[M+H^{4+}]^{4+} = 404.0$

17

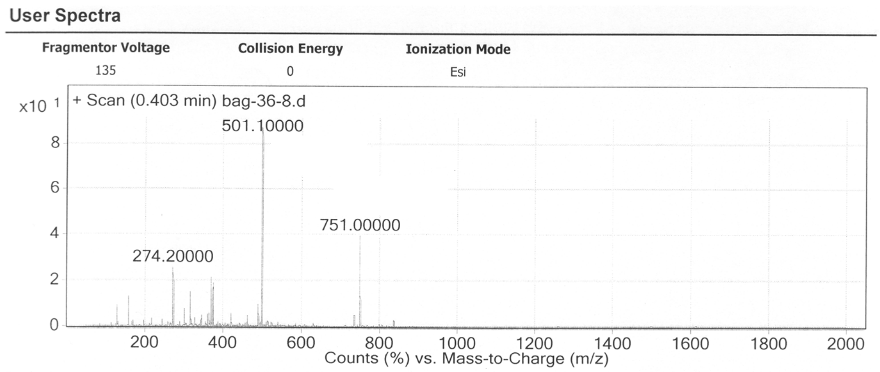


Peak List

m/z	Abund.
130	104002
159	128531
274.2	198299
302.2	61607
318.2	117253
364.7	119193
369	175936
415.8	59778
491.7	912051
737	432823

$[M+H^{2+}]^{2+}$ = 736.9, $[M+H^{3+}]^{3+}$ = 491.6, $[M+H^{4+}]^{4+}$ = 369.0

18



Peak List

m/z	z	Abund.
130		81220
159		121537
274.2	1	231058
302.2		68492
318.2		138385
371.8		195480
376		170543
491.7		85886
501.1		795609
751		362752

$[M+H^{2+}]^{2+}$ = 750.9, $[M+H^{3+}]^{3+}$ = 501.0, $[M+H^{4+}]^{4+}$ = 376.0

Figure S3. Ramachandran plots of each amino acid residues in the 1 derived AMPs for the structure with the highest percentage of occurrence in water. The three-letter amino acid codes from left to right represent the residues in the sequences starting from the 2nd residue at the peptide *N*-terminal to the 8th at the *C*-terminal.

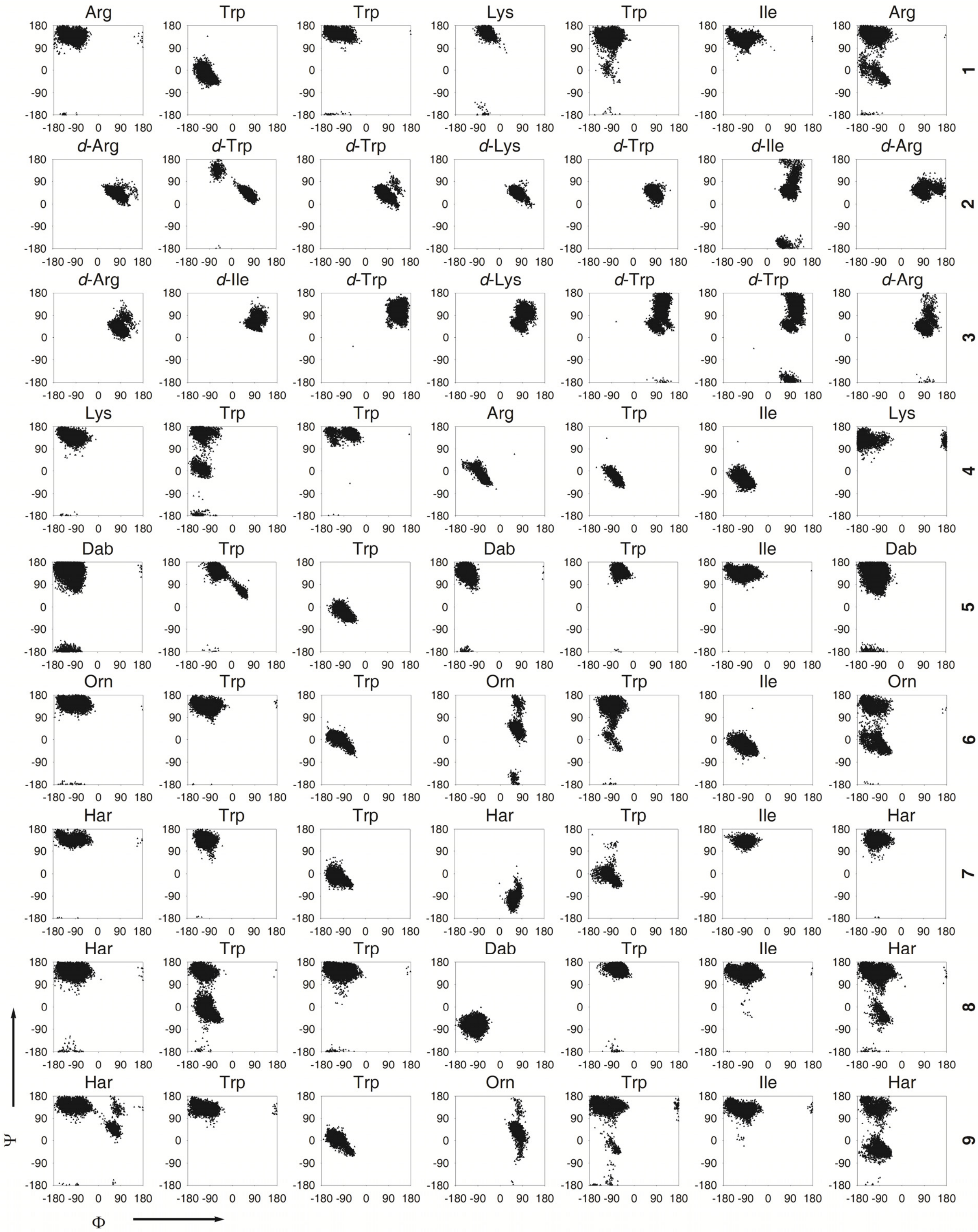


Figure S4. Ramachandran plots of each amino acid residues in the 10 derived AMPs for the structure with the highest percentage of occurrence in water. The three-letter amino acid codes from left to right represent the residues in the sequences starting from the 2nd residue at the peptide *N*-terminal to the 8th at the *C*-terminal.

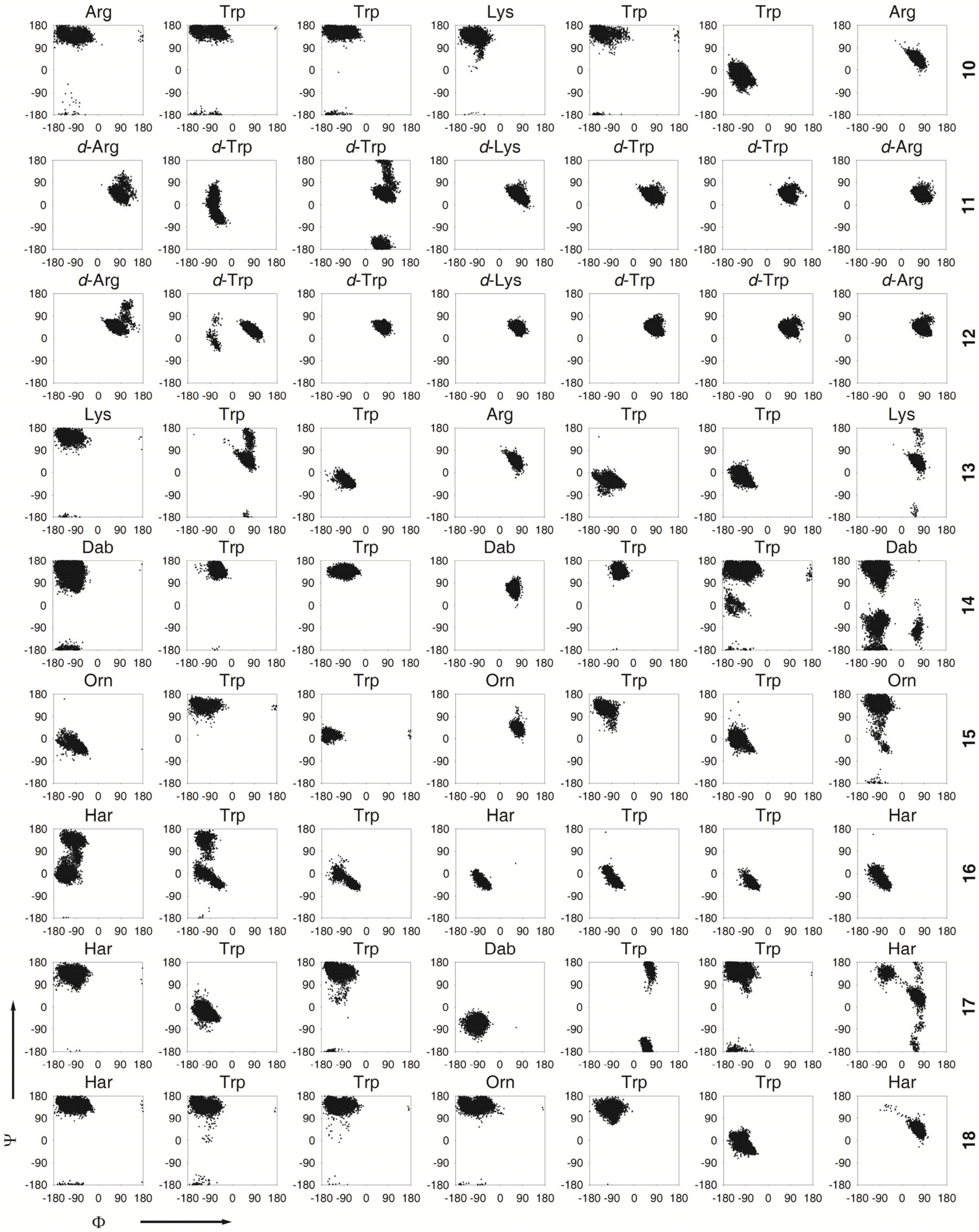
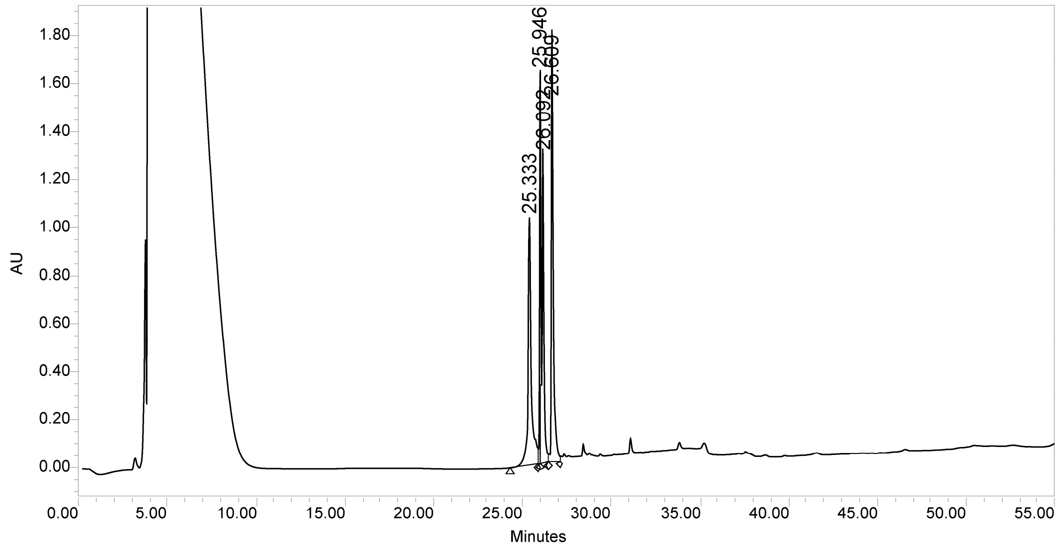


Figure S5. RP-HPLC spectra of the reaction mixture of each individual synthetic bactenecin-derived AMPs incubated with trypsin. Left and right spectra were recorded after 2 h and 24 h incubation, respectively.

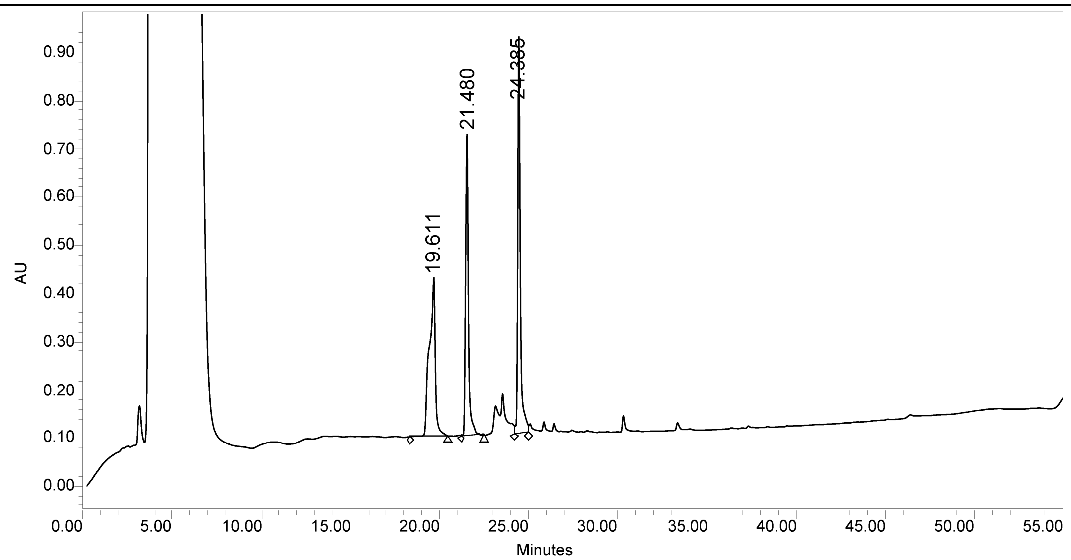
1

Not determined

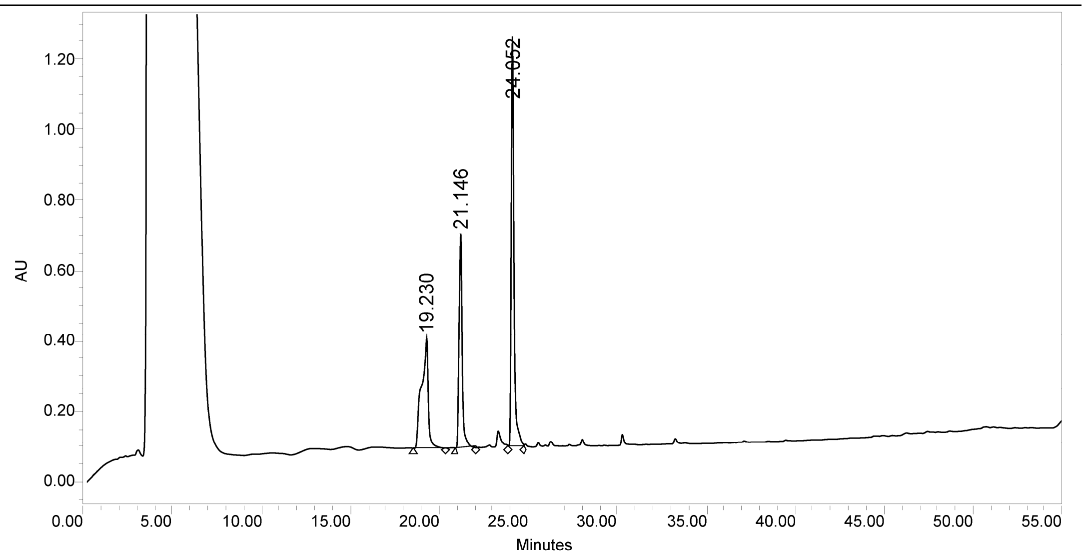


	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	25.333	13001191	29.48	1031427	17.80
2	25.946	8405109	19.06	1625079	28.05
3	26.092	8621698	19.55	1310912	22.62
4	26.609	14070998	31.91	1826946	31.53

4

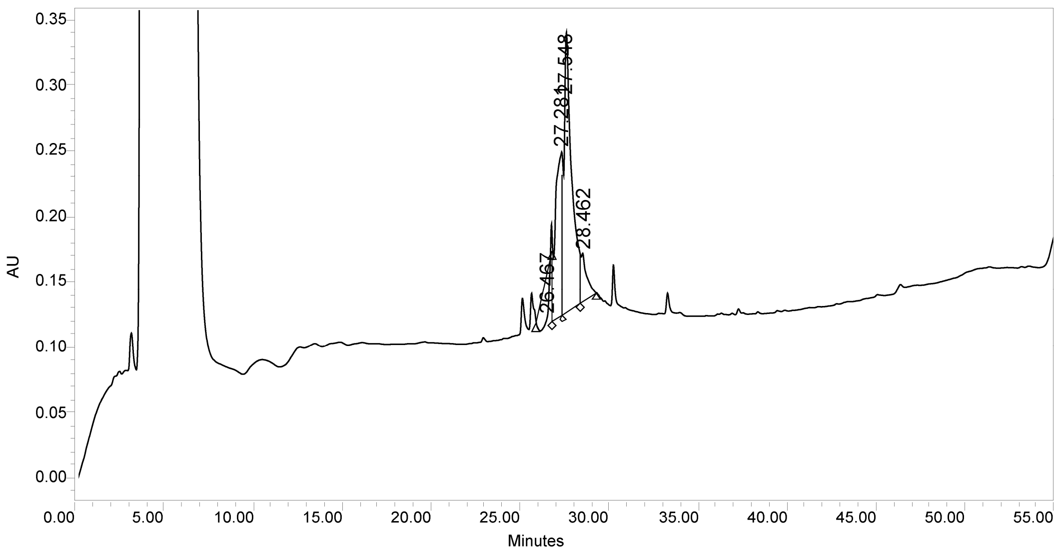


	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	19.611	7670579	33.04	330780	18.58
2	21.480	7060761	30.42	626051	35.17
3	24.385	8483124	36.54	823271	46.25

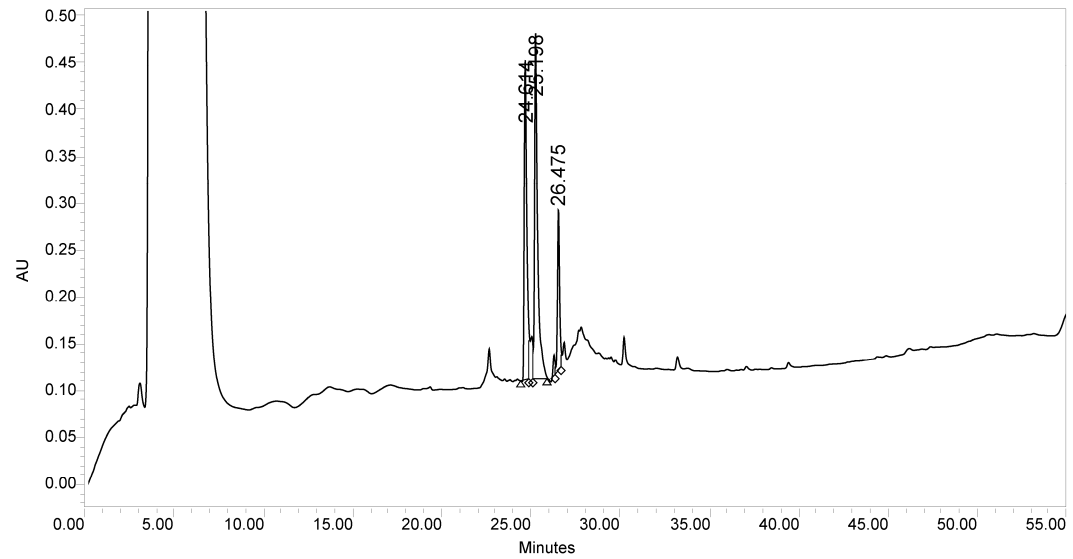


	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	19.230	8154749	28.69	312060	14.97
2	21.146	7554488	26.58	608124	29.17
3	24.052	12711148	44.73	1164805	55.87

5

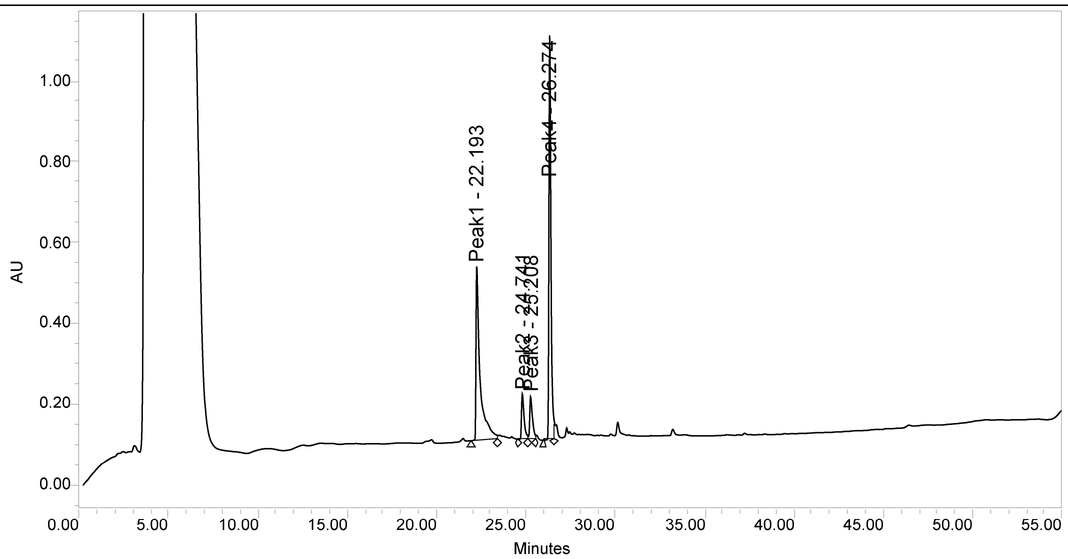


	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	26.467	1011273	8.63	-30416	7.41
2	27.281	3645777	31.12	126062	30.72
3	27.548	6209156	53.00	215372	52.49
4	28.462	849982	7.25	38479	9.38

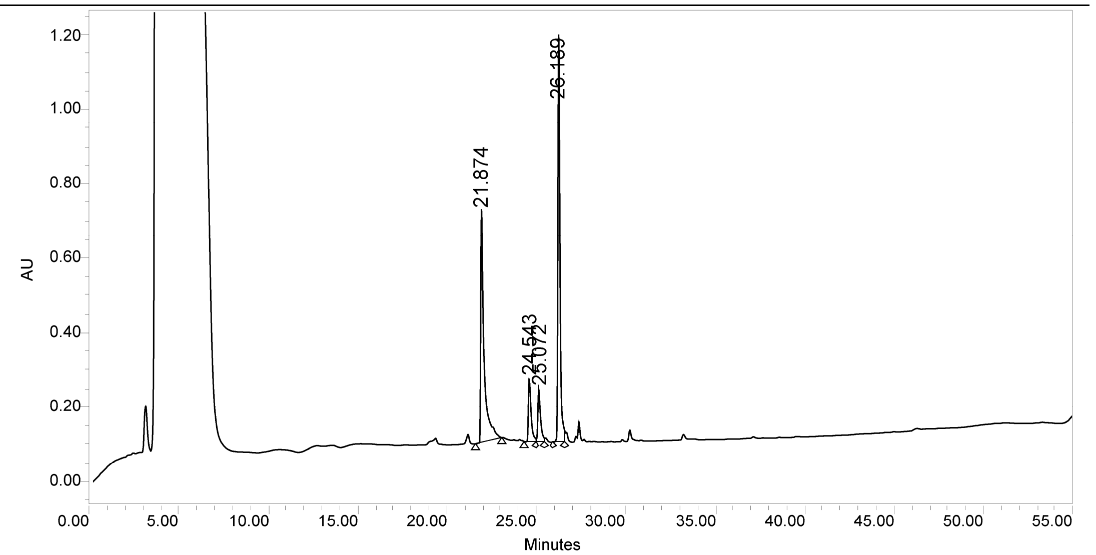


	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	24.614	3583893	37.68	342403	38.58
2	25.198	4421324	46.48	369775	41.66
3	26.475	1507111	15.84	175323	19.75

6

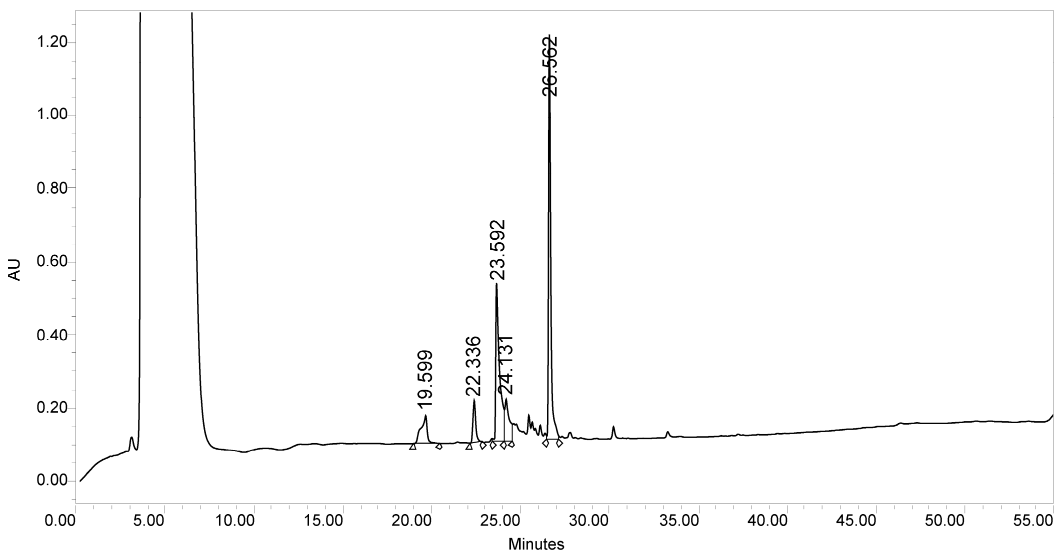


	Peak Name	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	Peak1	22.193	7135925	40.26	437584	26.26
2	Peak2	24.741	1246444	7.03	115327	6.92
3	Peak3	25.208	1183228	6.68	107751	6.47
4	Peak4	26.274	8160046	46.04	1006000	60.36

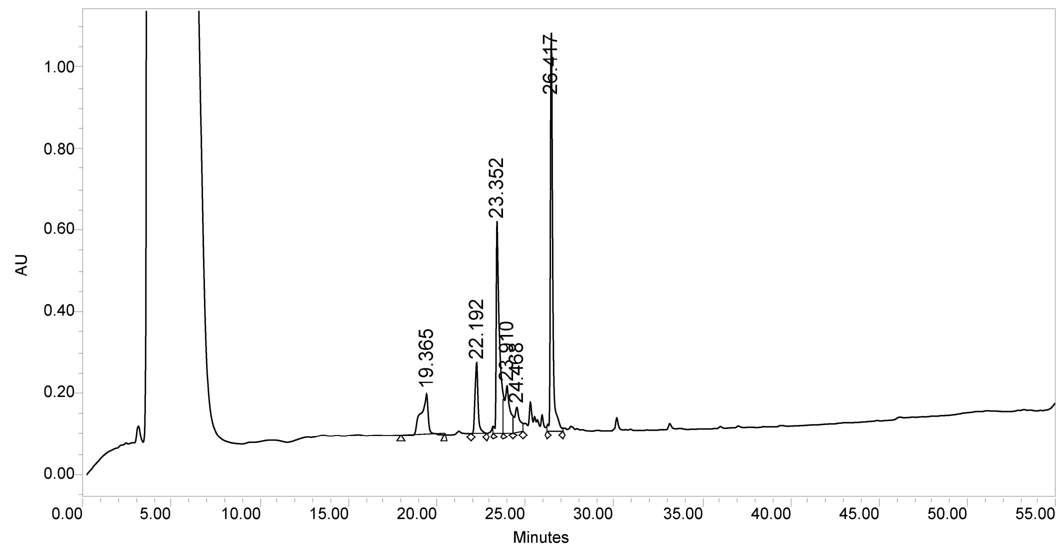


	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	21.874	8212554	39.66	630385	30.90
2	24.543	1749270	8.45	169927	8.33
3	25.072	1530707	7.39	140956	6.91
4	26.189	9216205	44.50	1098972	53.86

7

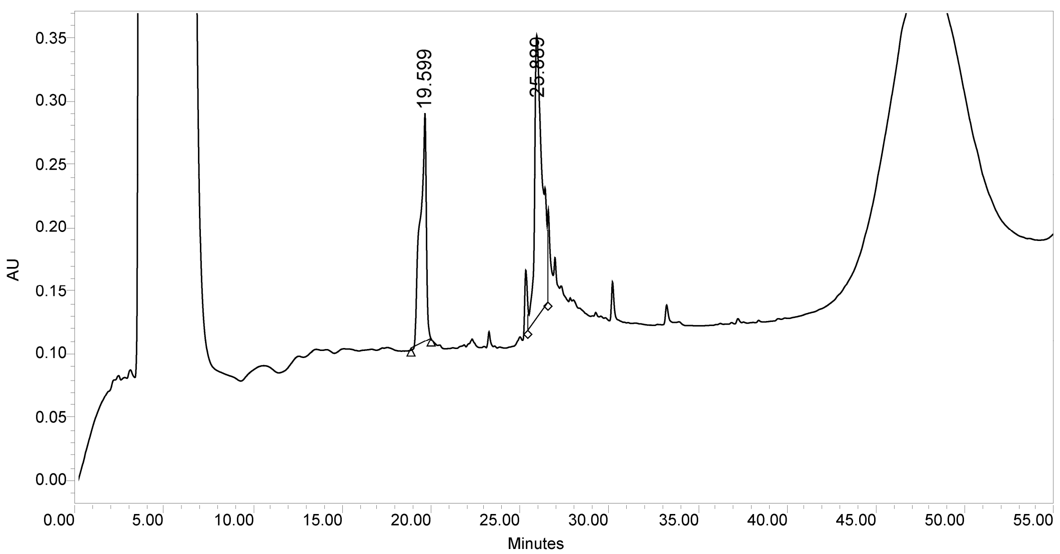


	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	19.599	1838440	8.43	75763	4.08
2	22.336	1284397	5.89	114686	6.17
3	23.592	6627827	30.39	440533	23.70
4	24.131	2104371	9.65	116065	6.24
5	26.562	9957598	45.65	1111509	59.80

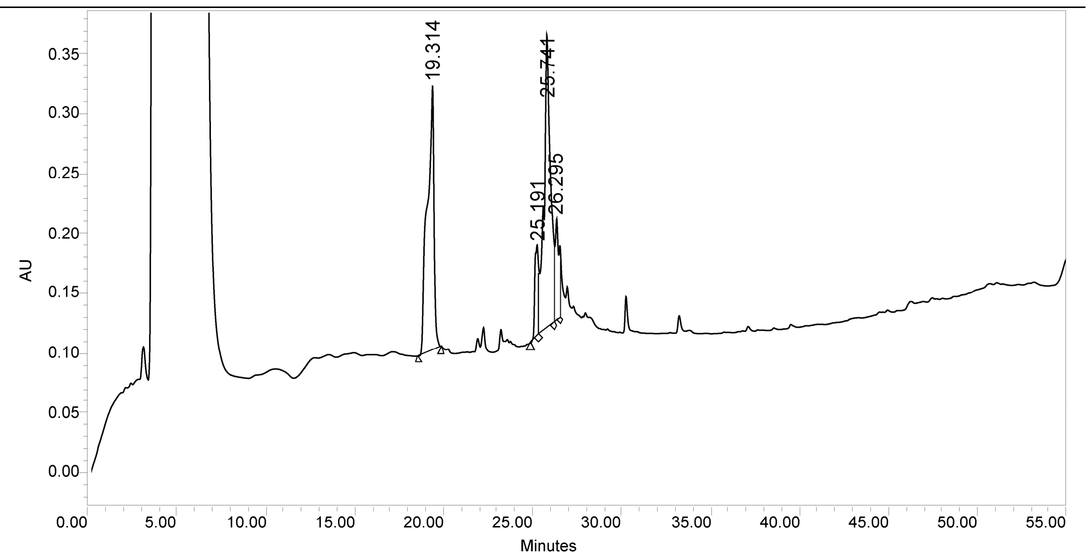


	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	19.365	2849178	11.73	99453	5.10
2	22.192	2166225	8.92	175777	9.01
3	23.352	7024702	28.92	520351	26.66
4	23.910	2240115	9.22	115563	5.92
5	24.468	1324039	5.45	62887	3.22
6	26.417	8688648	35.77	977804	50.10

8

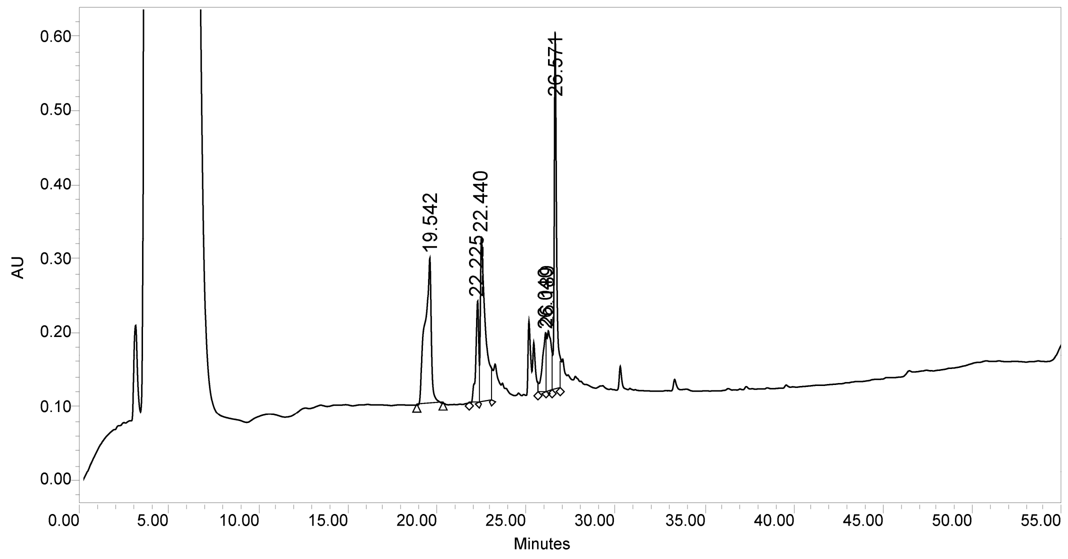


	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	19.599	4343694	38.17	182080	44.67
2	25.889	7036083	61.83	225524	55.33

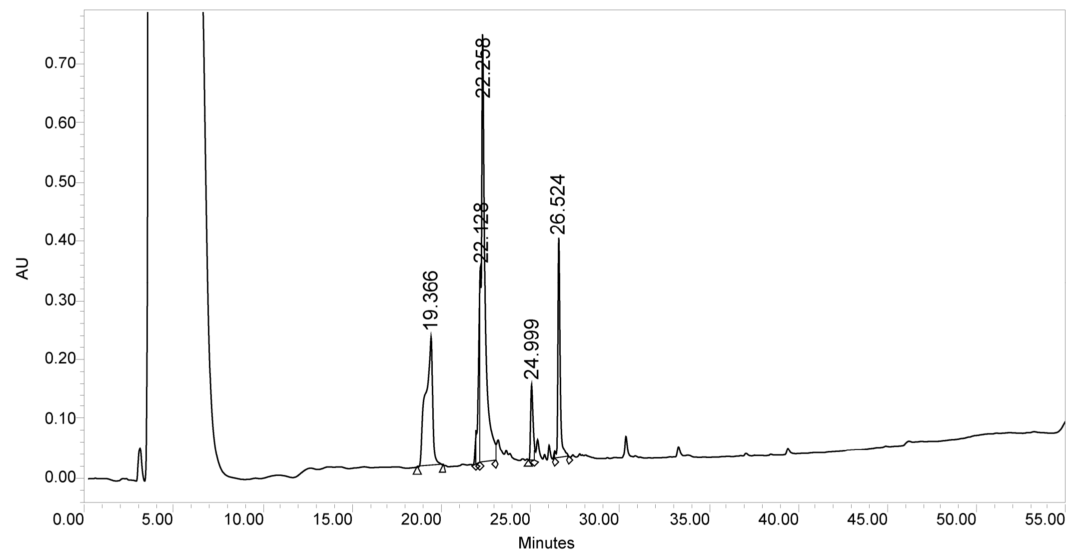


	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	19.314	5810375	39.59	221698	35.11
2	25.191	1270333	8.66	77277	12.24
3	25.741	6210353	42.31	246802	39.09
4	26.295	1386016	9.44	85613	13.56

9

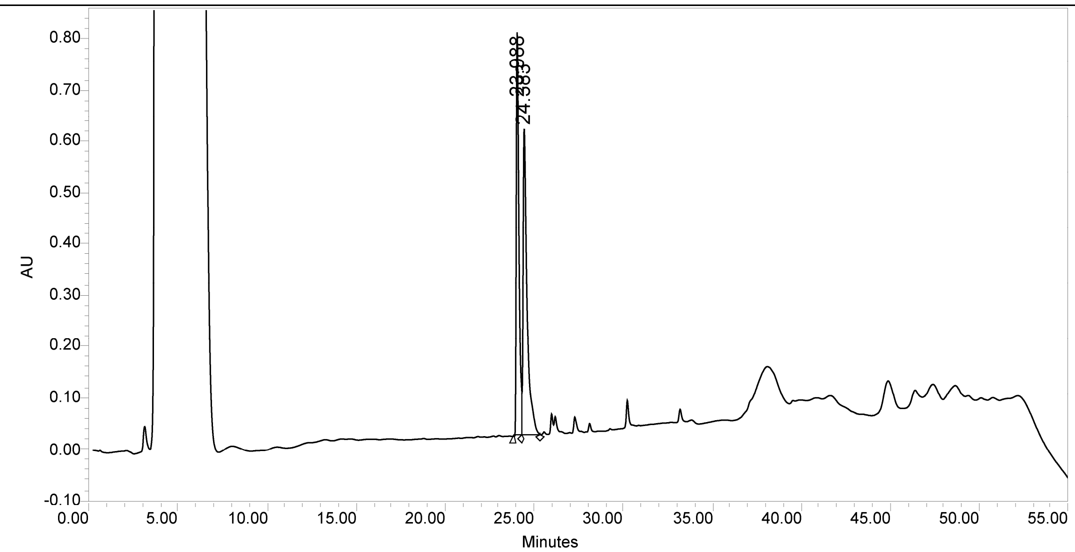


	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	19.542	4824240	26.56	197840	16.41
2	22.225	1562293	8.60	136987	11.37
3	22.440	4512258	24.84	224417	18.62
4	26.040	1294132	7.13	79748	6.62
5	26.189	1308022	7.20	81545	6.77
6	26.571	4662302	25.67	484746	40.22

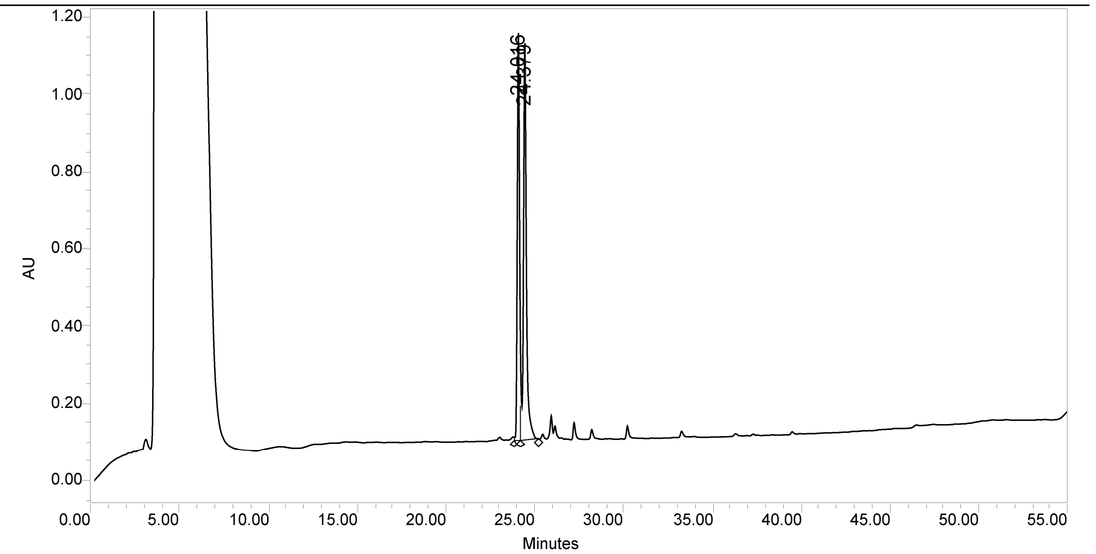


	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	19.366	5909889	25.37	218908	12.25
2	22.128	2837168	12.18	332424	18.61
3	22.258	9929396	42.63	731003	40.92
4	24.999	1272203	5.46	127670	7.15
5	26.524	3342753	14.35	376363	21.07

10



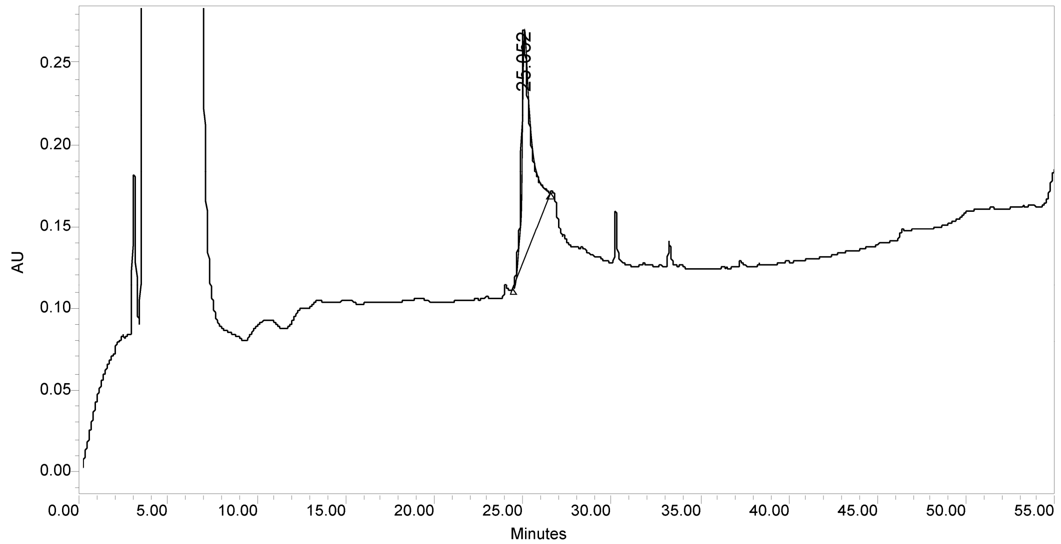
	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	23.988	8347216	47.05	788496	56.86
2	24.383	9393036	52.95	598115	43.14



	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height
1	24.016	9992755	46.19	1053376	50.58
2	24.379	11642428	53.81	1029111	49.42

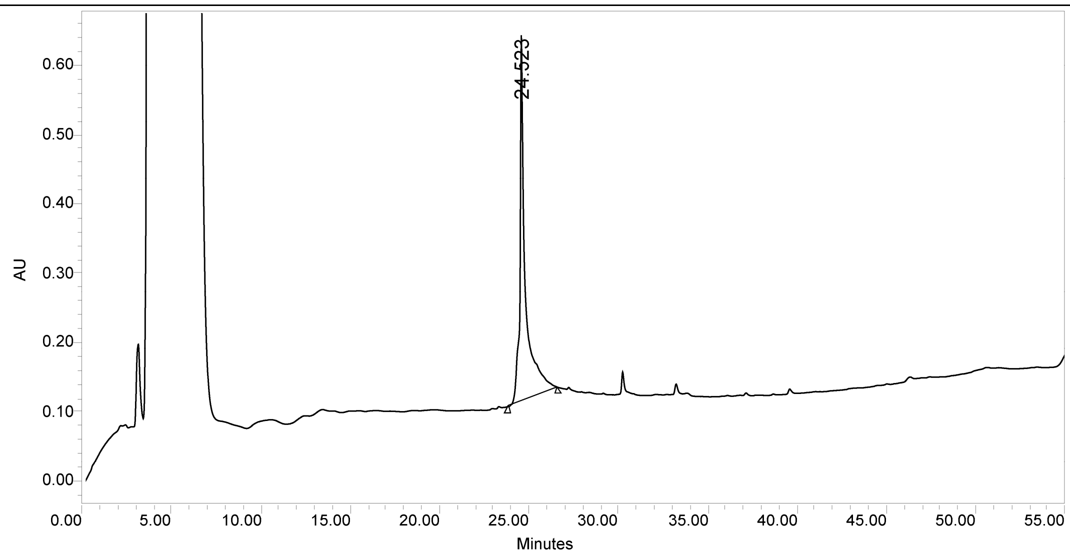
11

<u>Not determined</u>

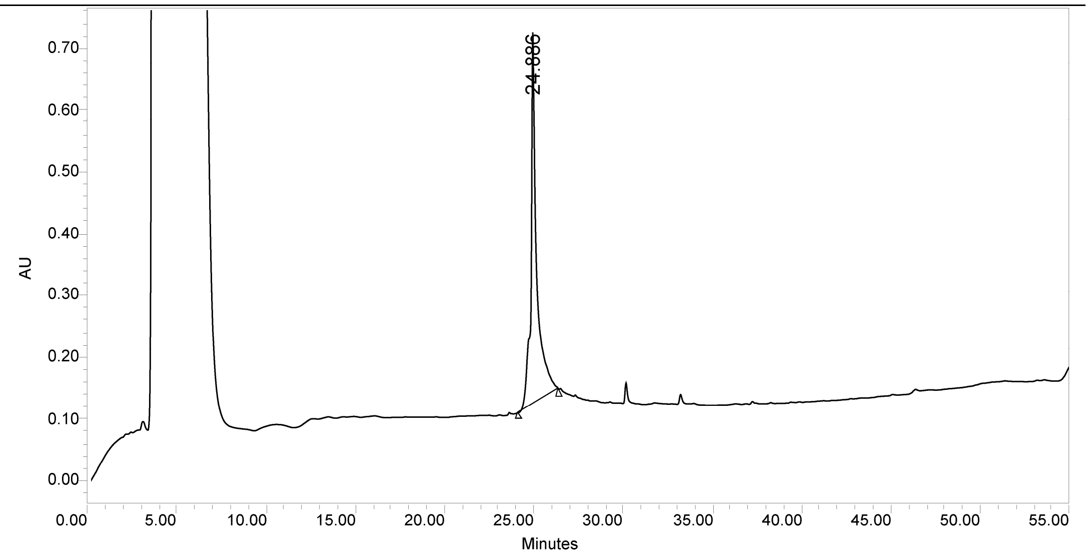


	RT (min)	Area (μV*sec)	% Area	Height (μV)	% Height
1	25.052	5455848	100.00	142245	100.00

12

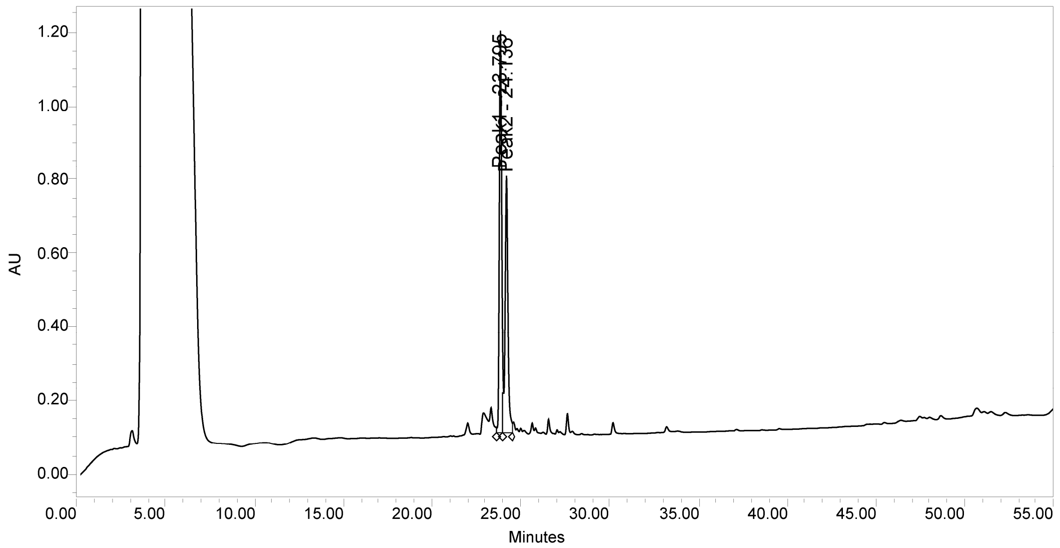


	RT (min)	Area (μV*sec)	% Area	Height (μV)	% Height
1	24.523	11192574	100.00	530664	100.00

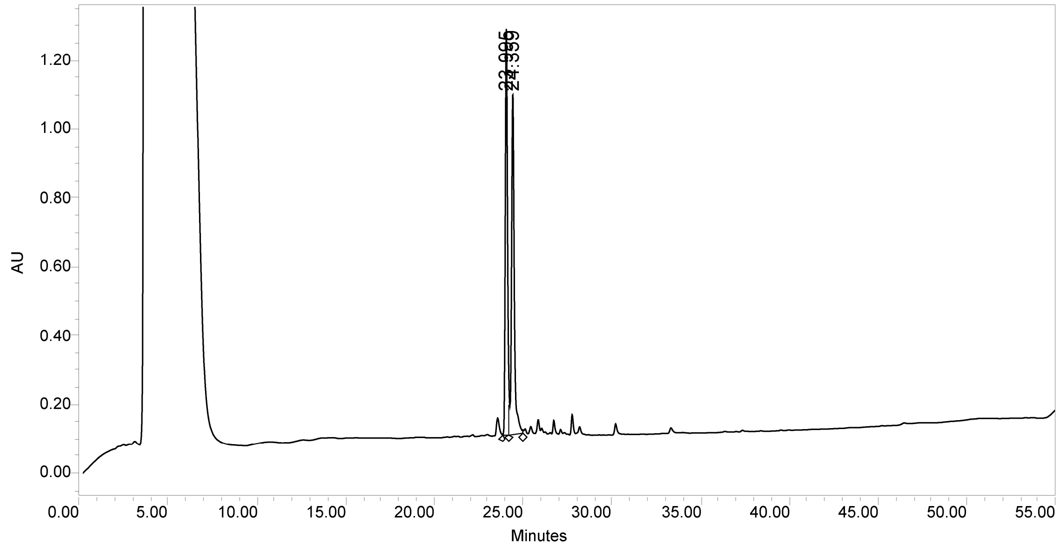


	RT (min)	Area (μV*sec)	% Area	Height (μV)	% Height
1	24.886	13375788	100.00	601280	100.00

13

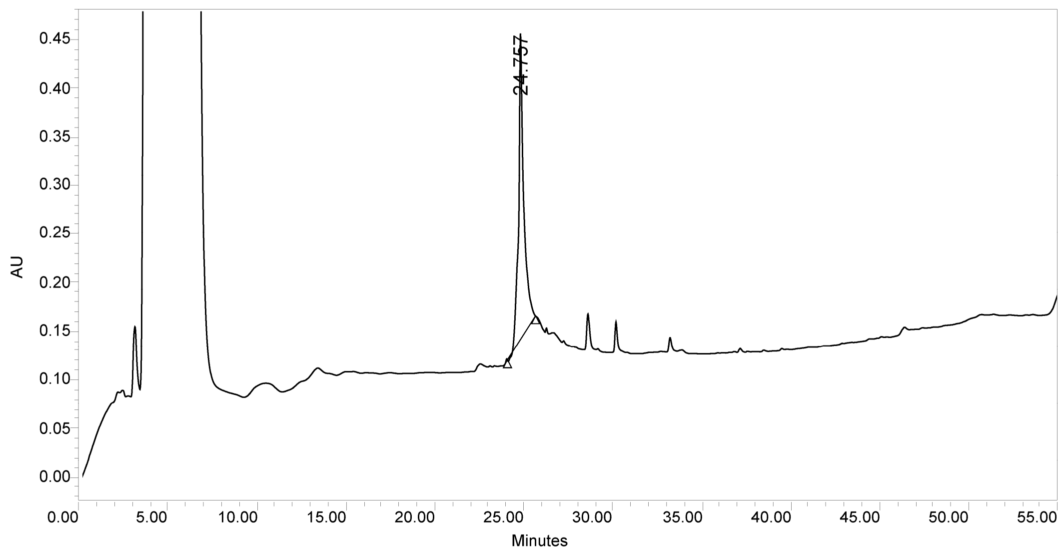


	Peak Name	RT (min)	Area (μV*sec)	% Area	Height (μV)	% Height
1	Peak1	23.795	10270094	57.63	1094543	61.02
2	Peak2	24.136	7550774	42.37	699079	38.98

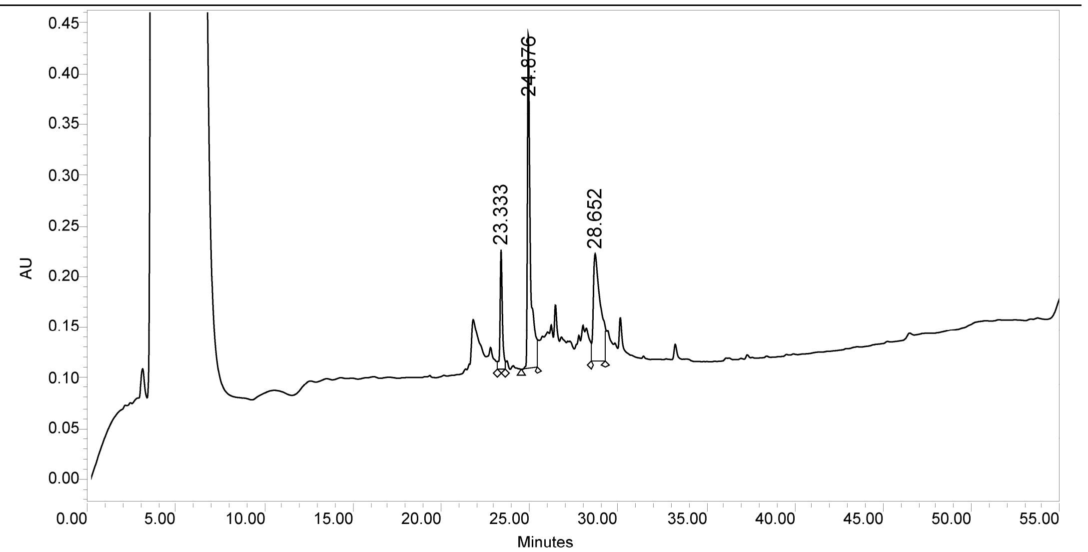


	RT (min)	Area (μV*sec)	% Area	Height (μV)	% Height
1	23.995	10837650	50.21	1183637	54.32
2	24.359	10749133	49.79	995544	45.68

14

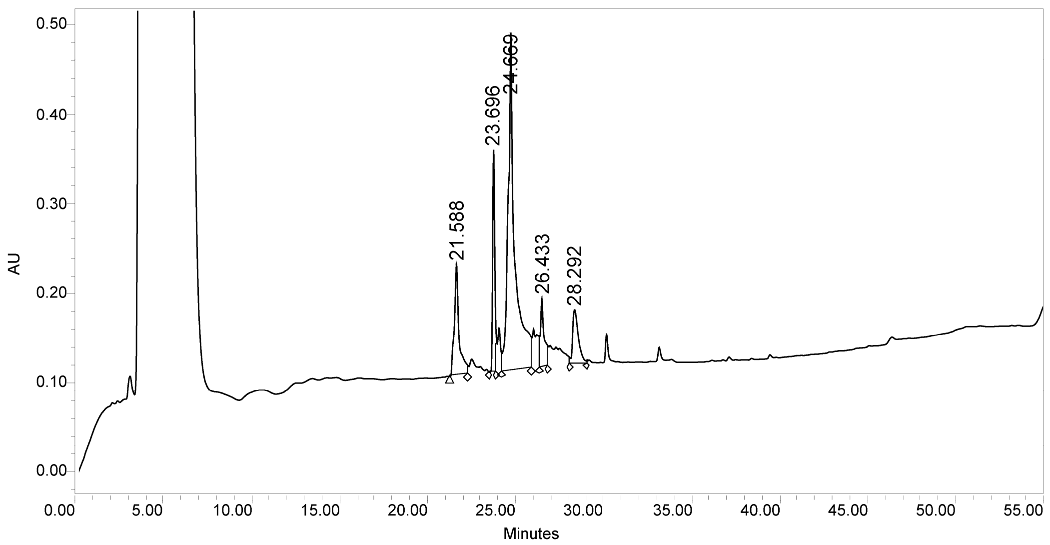


	RT (min)	Area (μV*sec)	% Area	Height (μV)	% Height
1	24.757	6742896	100.00	319308	100.00

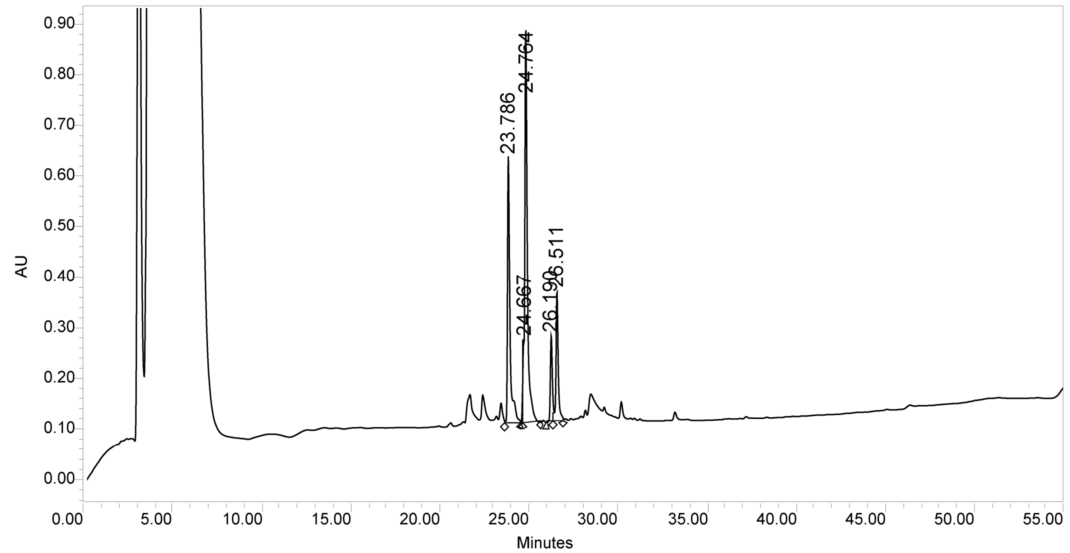


	RT (min)	Area (μV*sec)	% Area	Height (μV)	% Height
1	23.333	1082974	13.15	119029	21.25
2	24.876	4009100	48.69	333464	59.54
3	28.652	3142690	38.16	107571	19.21

15

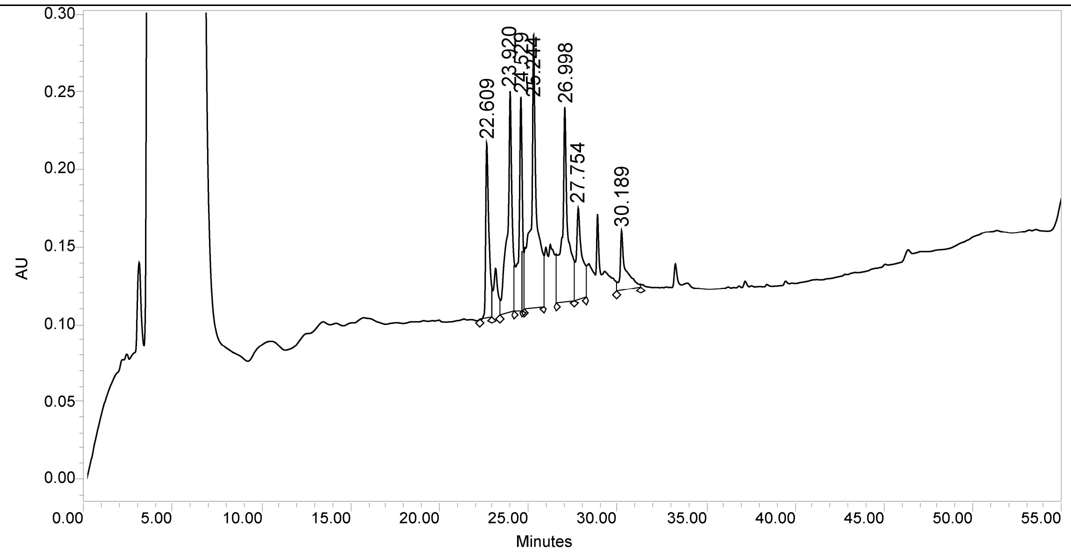


	RT (min)	Area (□V*sec)	% Area	Height (□V)	% Height
1	21.588	2395919	13.81	125205	14.08
2	23.696	2269585	13.08	249398	28.04
3	24.669	10122206	58.32	377370	42.44
4	26.433	1200915	6.92	76213	8.57
5	28.292	1366805	7.88	61101	6.87

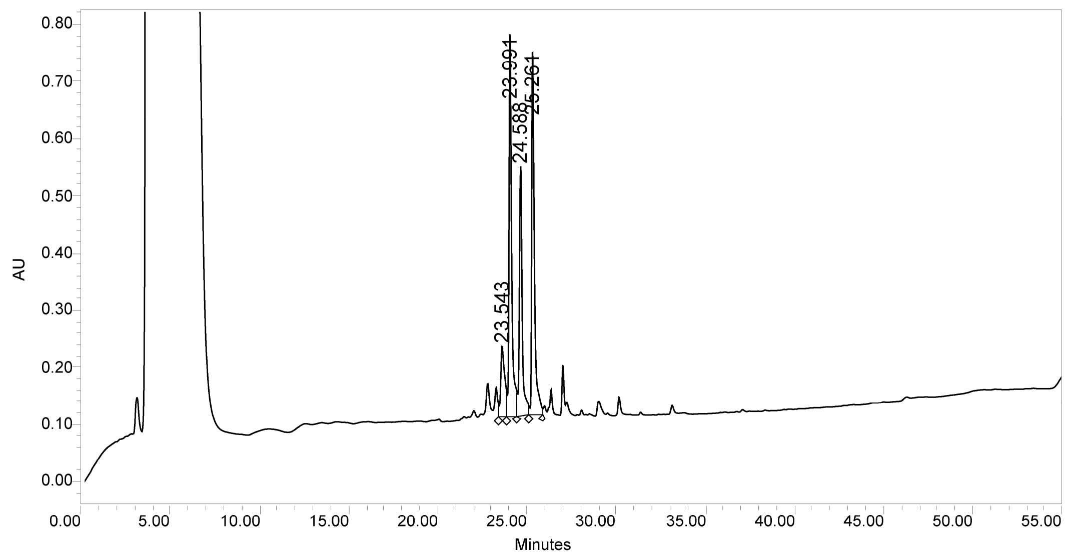


	RT (min)	Area (□V*sec)	% Area	Height (□V)	% Height
1	23.786	5056478	29.38	528096	27.93
2	24.667	823040	4.78	162693	8.60
3	24.764	7737352	44.95	775338	41.00
4	26.190	1390224	8.08	169667	8.97
5	26.511	2204924	12.81	255138	13.49

16

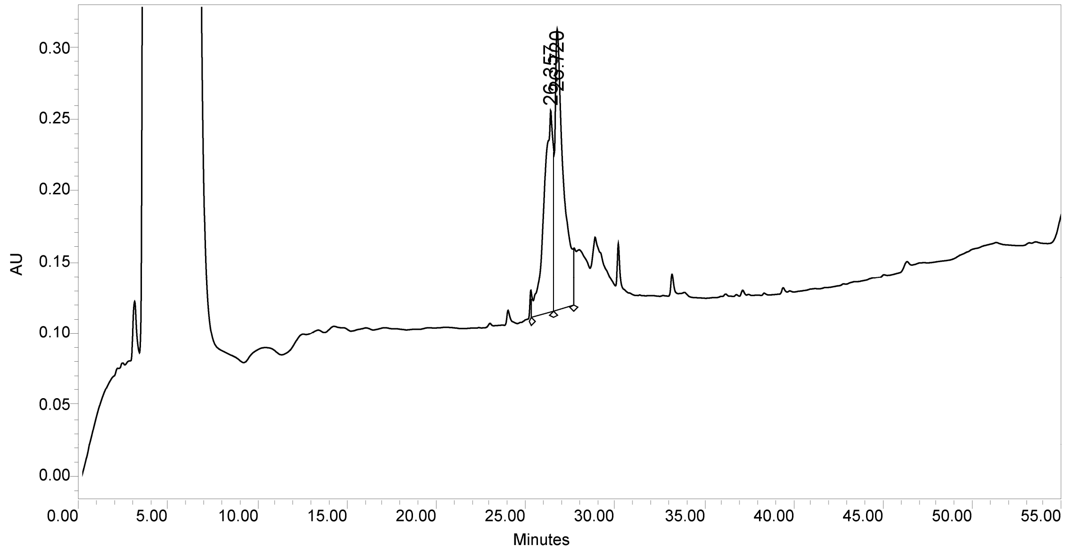


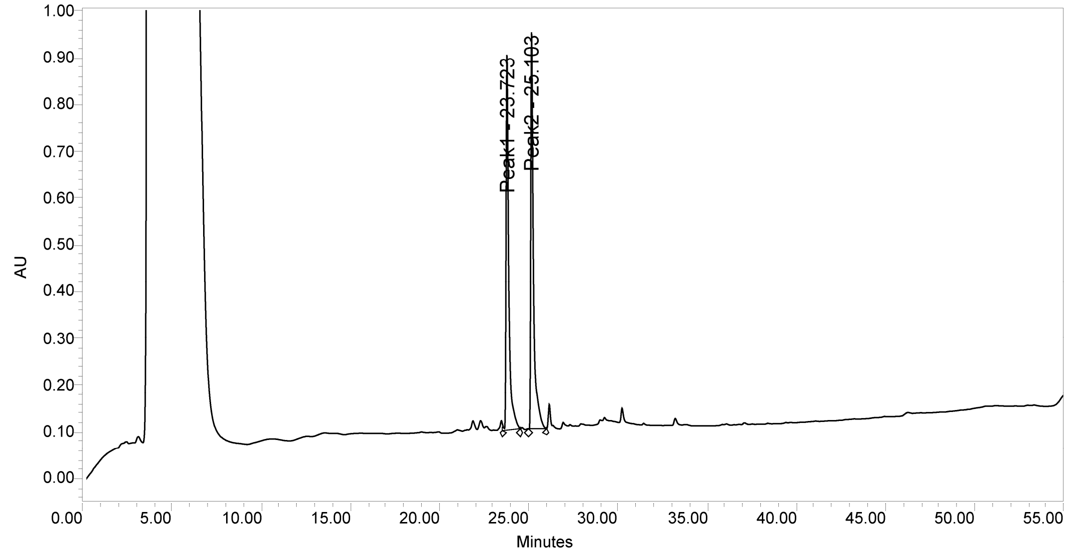
	RT (min)	Area (□V*sec)	% Area	Height (□V)	% Height
1	22.609	1498640	10.08	113879	14.23
2	23.920	2713264	18.24	143865	17.98
3	24.529	1793021	12.05	139000	17.37
4	25.244	3913721	26.31	177577	22.19
5	26.998	2789413	18.75	126568	15.82
6	27.754	1292310	8.69	59680	7.46
7	30.189	874233	5.88	39544	4.94



	RT (min)	Area (□V*sec)	% Area	Height (□V)	% Height
1	23.543	2036062	9.87	127334	6.76
2	23.991	7121377	34.51	676470	35.94
3	24.588	4785831	23.20	439482	23.35
4	25.261	6689465	32.42	639190	33.95

17

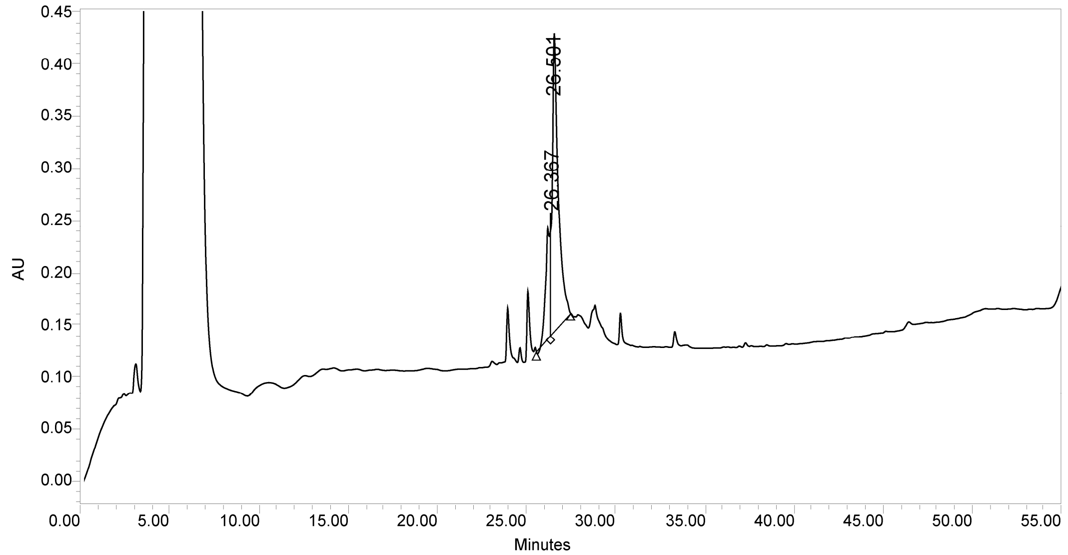


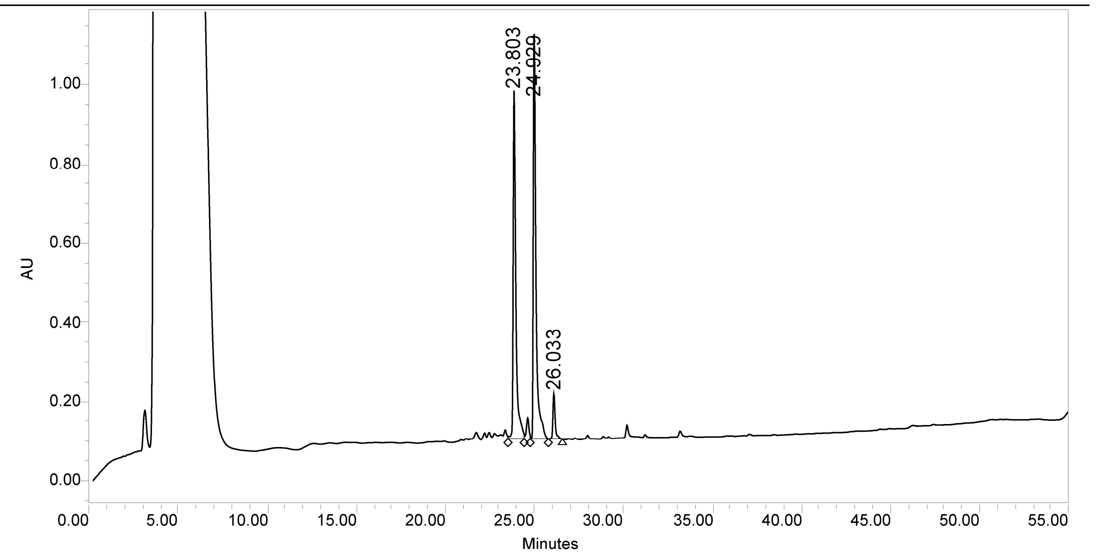


	RT (min)	Area (□V*sec)	% Area	Height (□V)	% Height
1	26.357	5180863	43.77	141521	41.78
2	26.720	6654384	56.23	197231	58.22

	Peak Name	RT (min)	Area (□V*sec)	% Area	Height (□V)	% Height
1	Peak1	23.723	8492882	48.15	807463	48.71
2	Peak2	25.103	9145389	51.85	850250	51.29

18



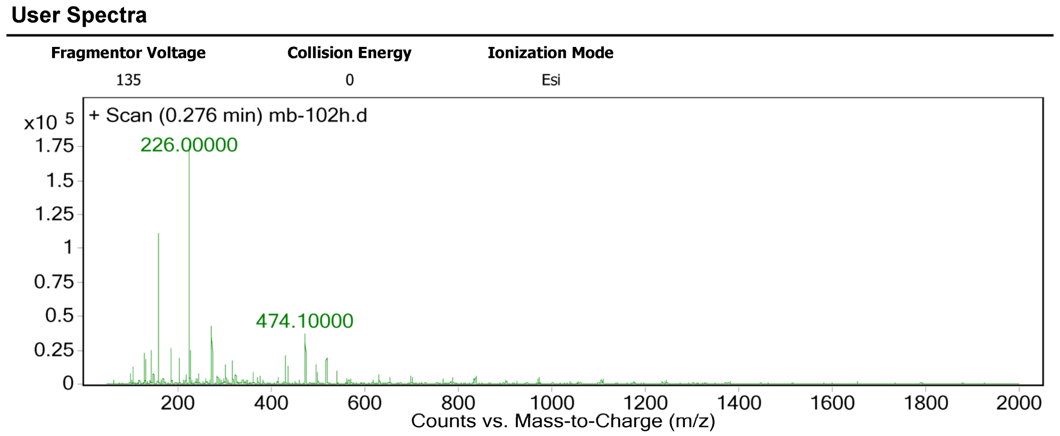


	RT (min)	Area (□V*sec)	% Area	Height (□V)	% Height
1	26.367	2491002	27.98	117938	28.98
2	26.501	6413279	72.02	288963	71.02

	RT (min)	Area (□V*sec)	% Area	Height (□V)	% Height
1	23.803	9228135	44.22	883963	43.65
2	24.929	10625166	50.92	1027216	50.73
3	26.033	1014661	4.86	113741	5.62

Figure S6. Mass spectra of the reaction mixture of each individual synthetic bactenecin-derived AMPs incubated with trypsin. Left and right spectra were recorded after 2 h and 24 h incubation, respectively. The values are monoisotopic masses in positive mode.

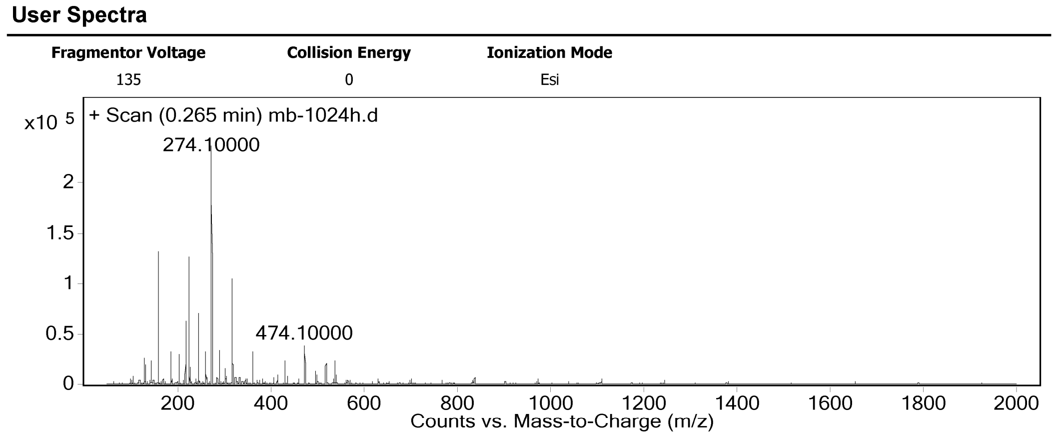
1



Peak List

m/z	z	Abund.
130		22915
144		24850
158.9		110347
186.9		26227
204		19372
226	1	174911
227	1	24455
274.1		42964
430.8		20408
474.1	1	36484

Trp-Ile-Arg-OH;
$[M+H]^+_{found} = 474.1$, $[M+H]^+_{calculated} = 474.3$

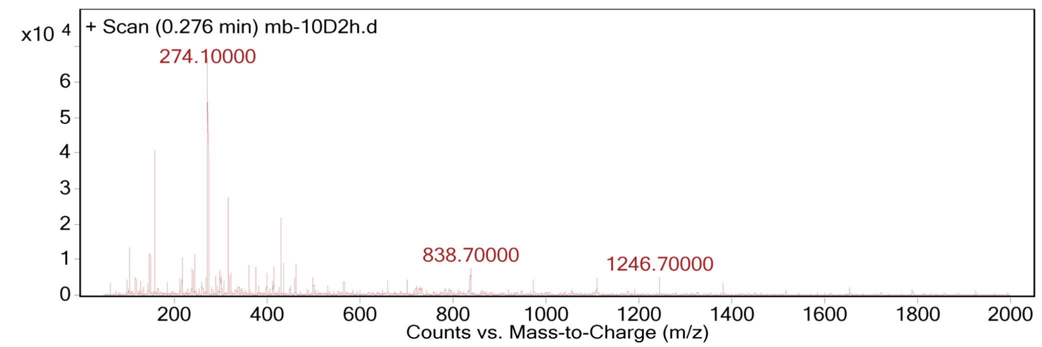


Peak List

m/z	z	Abund.
158.9		132174
218.1		62102
226	1	126188
246.1		70328
262.1		32493
274.1	1	235975
275.1	1	45791
290.1		32627
318.1	1	105356
474.1		38808

Trp-Ile-Arg-OH;
$[M+H]^+_{found} = 474.1$, $[M+H]^+_{calculated} = 474.3$
Trp-Trp-Lys-OH;
$[M+HOAc+2H]^{2+}_{found} = 290.1^a$, $[M+HOAc+2H]^{2+}_{calculated} = 290.1$
^{a} Monoisotopic mass of HOAc is 60.02 Da.

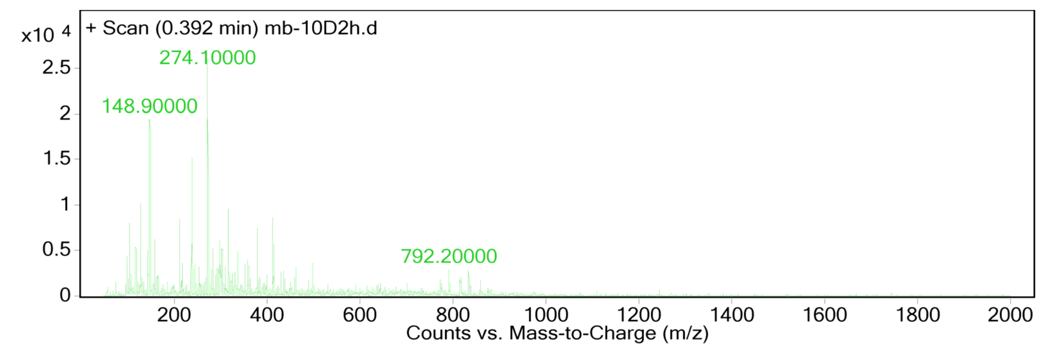
2



Peak List

m/z	z	Abund.
104.9		13188
148.9		11487
158.9		40282
218.1		10581
246.1		11461
274.1		66709
275.2		10741
318.1	1	27425
430.8		21746
437		8979

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
105		8058
130.1		10069
148.9		19474
158.9		6139
214.1	1	8381
239	1	15270
274.1	1	26010
318.1	1	9595
381.1		7493
413.1	1	8611

Intact **2**[b];

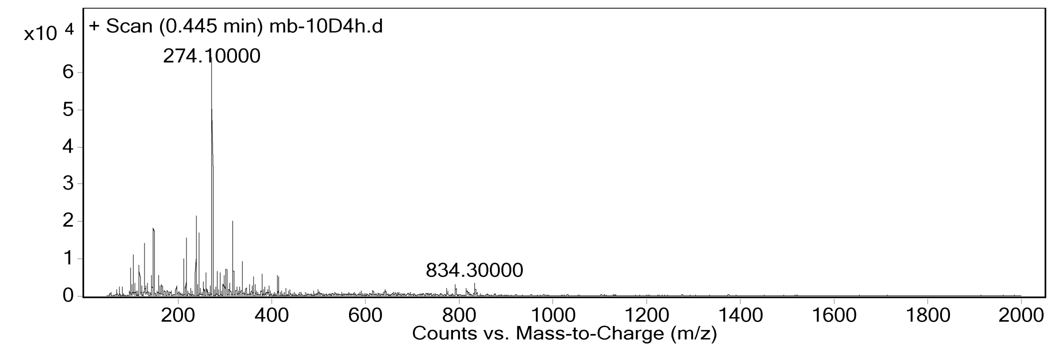
$[M+2HOAc+TFA+2H]^{2+}_{found} = 838.7$, $[M+2HOAc+TFA+2H]^{2+}_{calculated} = 839.4$

$[M+6HOAc+5TFA+3H]^{3+}_{found} = 792.2$, $[M+6HOAc+5TFA+3H]^{3+}_{calculated} = 791.9$

$[M+5HOAc+4H]^{4+}_{found} = 437.0$, $[M+5HOAc+4H]^{4+}_{calculated} = 436.7$

[b] $^{\text{all-}D}$HHC-10 and $^{\text{Retro-inverso}}$HHC-10 were in impure forms.

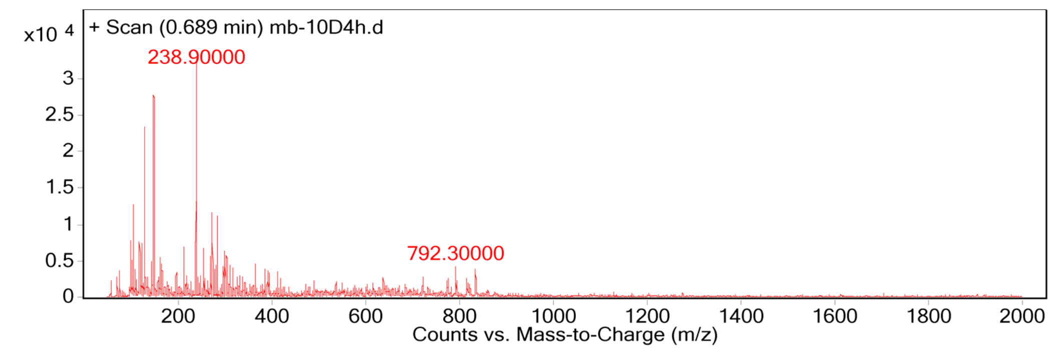
continued in next page



Peak List

m/z	Abund.
105	11122
130.1	14337
148.9	18060
214.1	9875
218.1	15576
238.9	21395
246.1	16825
274.1	64010
275.2	12533
318.1	20204

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

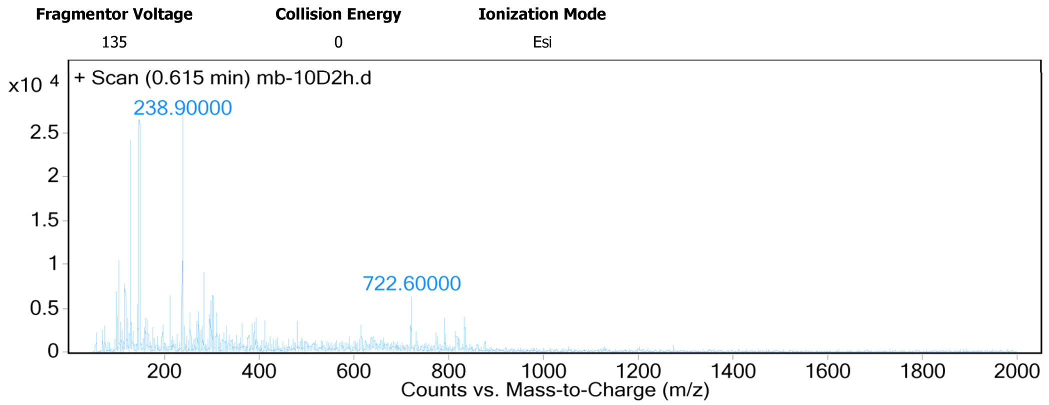
m/z	z	Abund.
100		7834
105		12716
119	1	7552
123		7496
130.1		23420
148.9		27757
214		7007
238.9	1	32790
274.1	1	11756
284.2		11104

Intact **2**;

$[M+10HOAc+4TFA+3H]^{3+}_{found} = 834.3$, $[M+10HOAc+4TFA+3H]^{3+}_{calculated} = 834.0$

$[M+6HOAc+5TFA+3H]^{3+}_{found} = 792.3$, $[M+6HOAc+5TFA+3H]^{3+}_{calculated} = 791.9$

2



Peak List

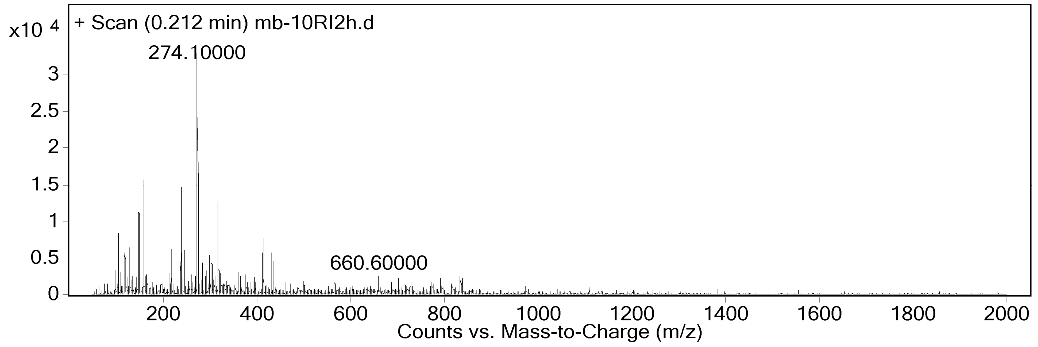
m/z	z	Abund.
100		6915
105	1	10539
119		7769
130	1	24113
148.9		26434
214.1		6367
238.9	1	27635
284.2	1	9176
304.1	1	6442
722.6		6260

Intact **2**;
$[M+2H]^{2+}_{found}$ = 722.6, $[M+2H]^{2+}_{calculated}$ = 722.4

3

User Spectra

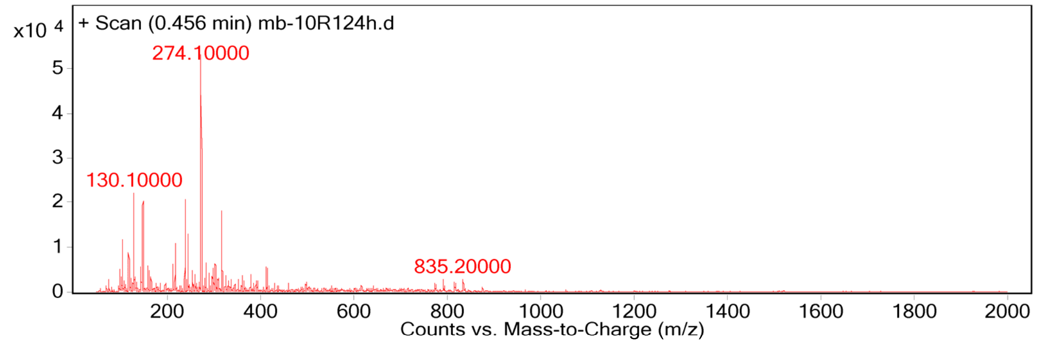
Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
105		8358
130		6383
148.9		11181
158.9		15700
218.1		6134
238.9	1	14529
246.1		6117
274.1		32820
318.1	1	12681
415.1		7555

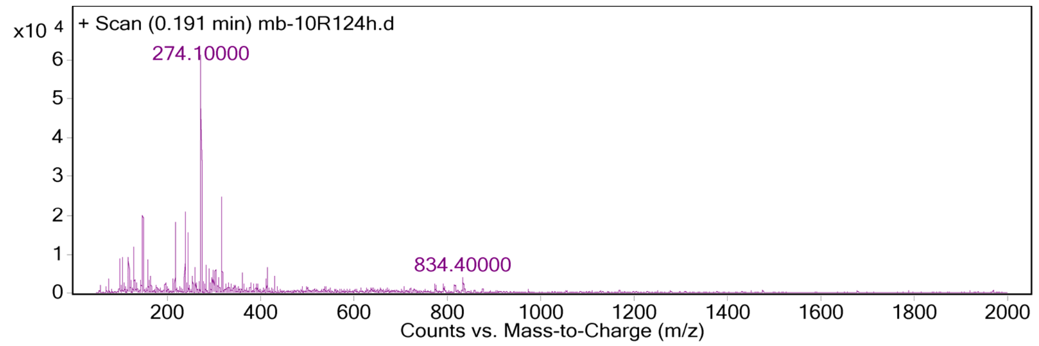
Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
105		11866
119		8747
130.1	1	22209
149		20062
218.1	1	10805
239	1	20799
246.1		12892
274.1		53057
275.2		10139
318.2		18131

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



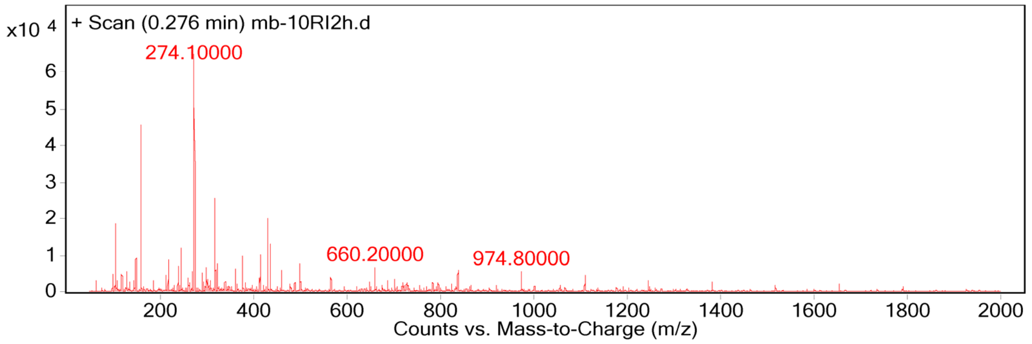
Peak List

m/z	z	Abund.
105.1		9111
119		9217
130.1	1	11969
148.9		19896
218.1	1	18140
239	1	20707
246.1		15701
274.1	1	61183
275.1	1	11864
318.2		24801

Intact **3**;
$[M+7HOAc+TFA+3H]^{3+}_{found}$ = 660.6, $[M+7HOAc+TFA+3H]^{3+}_{calculated}$ = 660.0

continued in next page

Intact **3**;
$[M+12HOAc+3TFA+3H]^{3+}_{found}$ = 835.2, $[M+12HOAc+3TFA+3H]^{3+}_{calculated}$ = 836.0
$[M+10HOAc+4TFA+3H]^{3+}_{found}$ = 834.4, $[M+10HOAc+4TFA+3H]^{3+}_{calculated}$ = 834.0

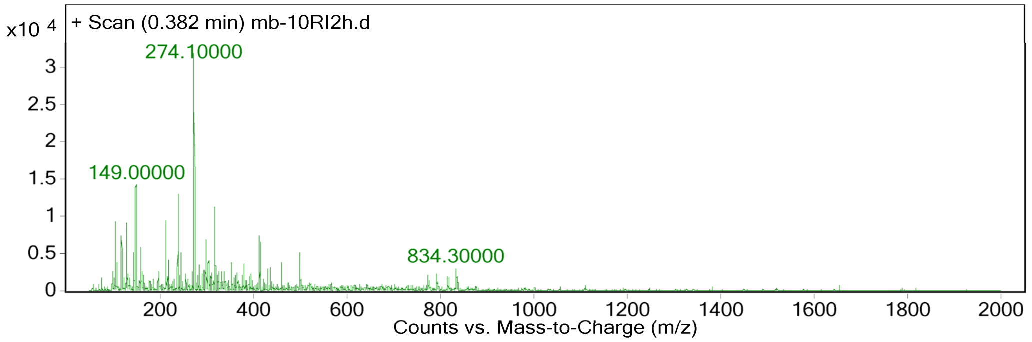


Peak List

m/z	z	Abund.
104.9		18756
158.9		45649
246.1		11887
274.1	1	64861
275.1	1	13726
318.2	1	25454
376.8		9946
415.1		10270
430.8		20170
437.1		13012

3

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
105.1		9345
119		7457
130	1	9147
149		14101
214.2		9520
238.9		12925
274.1	1	31874
300.9		6835
318.1		11280
413.1	1	7494

Intact **3**;

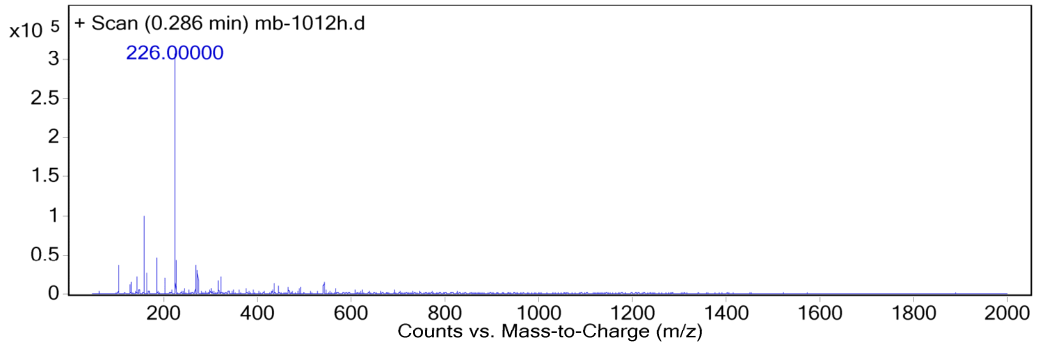
$[M+7HOAc+TFA+3H]^{3+}_{found}$ = 660.6, $[M+7HOAc+TFA+3H]^{3+}_{calculated}$ = 660.0

$[M+10HOAc+4TFA+3H]^{3+}_{found}$ = 834.3, $[M+10HOAc+4TFA+3H]^{3+}_{calculated}$ = 834.0

4

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



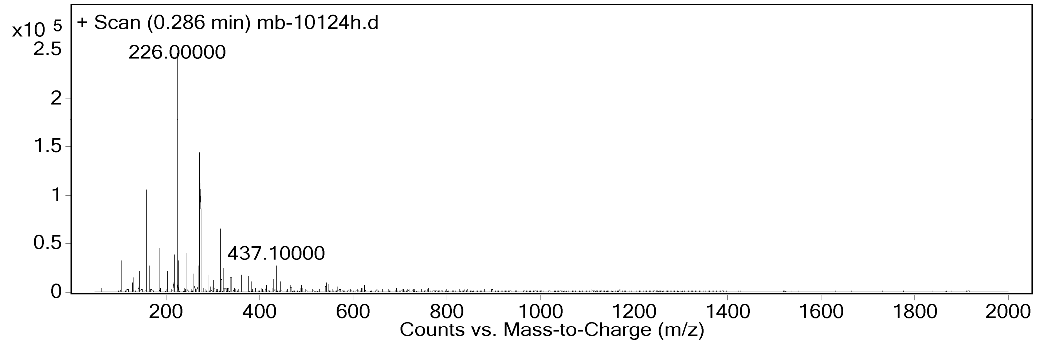
Peak List

m/z	z	Abund.
104.9		36393
144		21334
159		99205
164.8		26517
186.9		46810
226	1	305688
227	1	42299
228.8		22764
268.8		35942
274.1		30513

not detectable

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

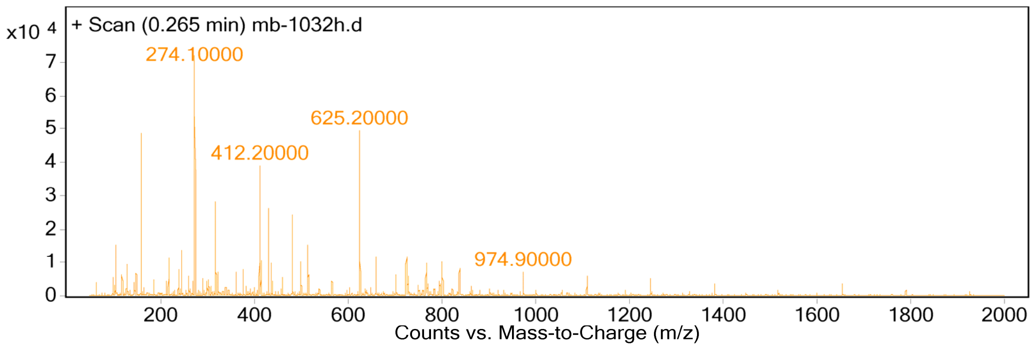
m/z	z	Abund.
104.9		32507
159		104871
186.9		45780
218.1		38048
226	1	246173
227	1	31928
246.1		39594
274.2		144258
275.1		28305
318.1		65216

Lys-Trp-Trp-Arg-OH;
$[M+3HOAc+4TFA+3H]^{3+}_{found} = 437.1$, $[M+3HOAc+4TFA+3H]^{3+}_{calculated} = 437.8$

5

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



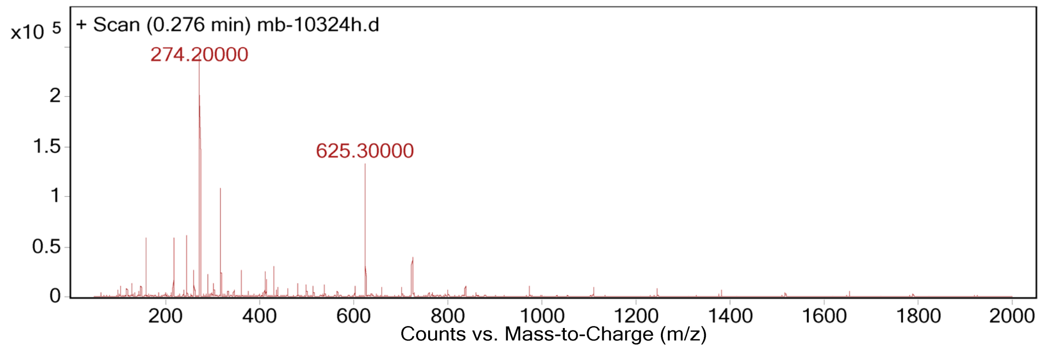
Peak List

m/z	z	Abund.
105		15343
158.9		48512
274.1	1	71857
318.2		28090
412.2		38701
430.8		26058
481.1	1	24143
515.2	1	15096
625.2		49371
626.1		17521

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



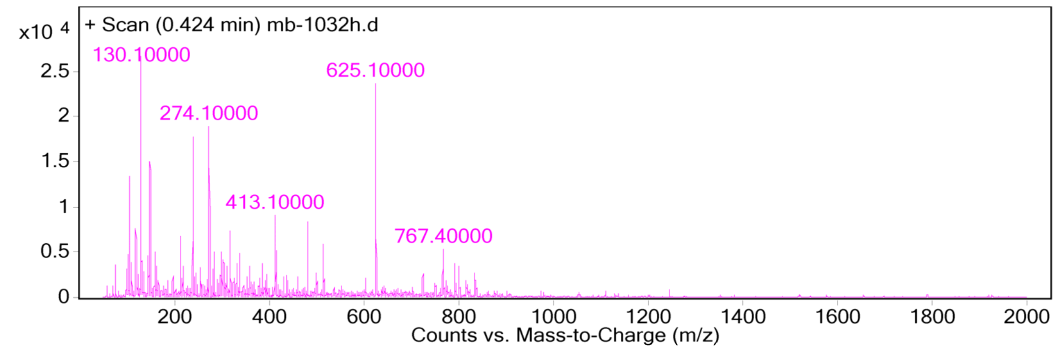
Peak List

m/z	z	Abund.
158.9		59020
218.1		58034
246.1		60990
274.2		240381
275.1		41059
318.1	1	107777
430.8		30477
625.3		132801
626.2		49911
725.3	1	39327

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi

continued in next page

Dab-Trp-Trp-Dab-Trp-Ile-Dab-Trp-NH_2;
$[M+1HOAc+3H]^{3+}_{found}$ = 412.2, $[M+1HOAc+3H]^{3+}_{calculated}$ = 412.6
Trp-Trp-Dab-Trp-Ile-Dab-Trp-NH_2;
$[M+HOAc+TFA+2H]^{2+}_{found}$ = 625.2, $[M+HOAc+TFA+2H]^{2+}_{calculated}$ = 625.3



5

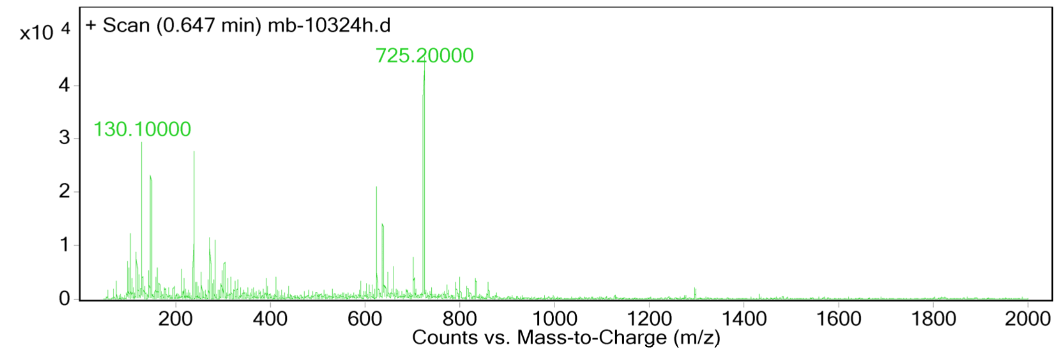
Peak List

m/z	z	Abund.
105	1	13482
119		7691
130.1	1	26659
148.9		15038
239		17818
274.1		18874
413.1		9052
481.2		8323
625.1		23710
626.2		10030

Dab-Trp-Trp-Dab-Trp-Ile-Dab-Trp-NH_2;
$[M+HOAc+3H]^{3+}_{found}$ = 412.2, $[M+HOAc+3H]^{3+}_{calculated}$ = 412.6
Trp-Trp-Dab-Trp-Ile-Dab-Trp-NH_2;
$[M+HOAc+TFA+2H]^{2+}_{found}$ = 625.2, $[M+HOAc+TFA+2H]^{2+}_{calculated}$ = 625.3

continued in next page

Trp-Trp-Dab-Trp-Ile-Dab-Trp-NH_2;
$[M+1HOAc+1TFA+2H]^{2+}_{found}$ = 625.3, $[M+1HOAc+1TFA+2H]^{2+}_{calculated}$ = 625.3
Trp-Trp-Dab-OH;
$[M+2HOAc+TFA+H]^{+}_{found}$ = 725.3, $[M+2HOAc+TFA+H]^{+}_{calculated}$ = 725.2



Peak List

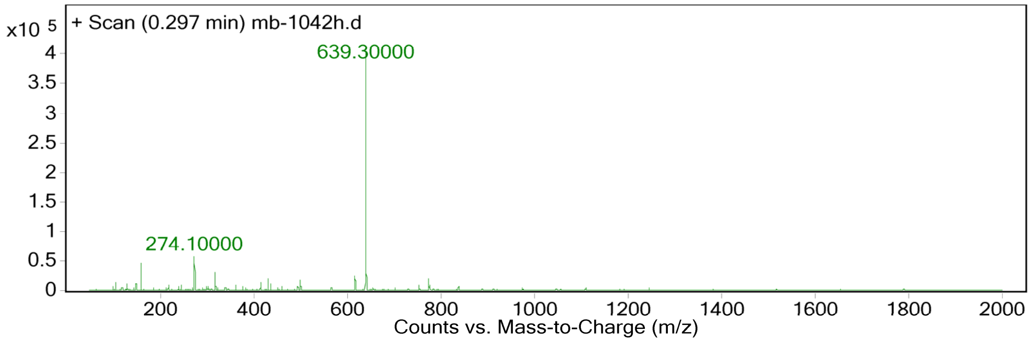
m/z	z	Abund.
105		12208
130.1	1	29339
148.9	1	22926
239	1	27537
274.1		11537
284.1	1	11052
625.2	1	21105
638.4		13982
725.2		45205
726.3		19599

Trp-Trp-Dab-OH;
$[M+2HOAc+TFA+H]^{+}_{found}$ = 725.2, $[M+2HOAc+TFA+H]^{+}_{calculated}$ = 725.2

6

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	Abund.
158.9	46568
274.1	55679
318.2	29759
617.3	24570
639.3	400349
640.2	148592
641.3	32216

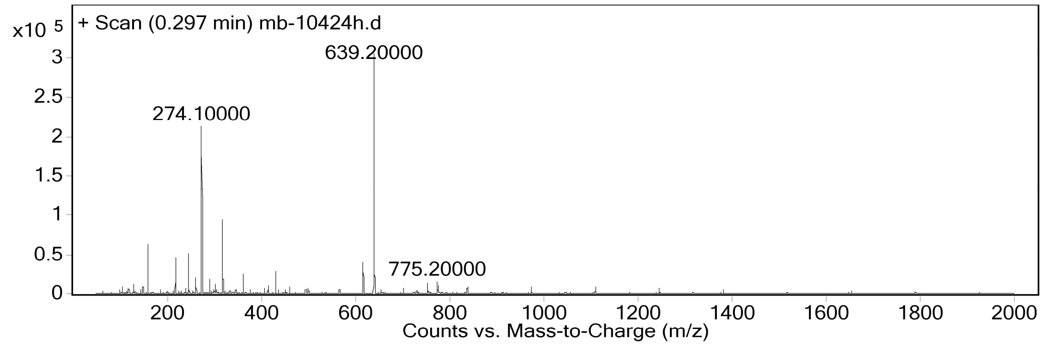
Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi

Orn-Trp-Trp-Orn-Trp-Ile-Orn-Trp-NH_2;
$[M+HOAc+2H]^{2+}{}_{found} = 639.3$, $[M+HOAc+2H]^{2+}{}_{calculated} = 639.4$
Trp-Ile-Orn-Trp-NH_2;
$[M+H]^{+}{}_{found} = 617.3$, $[M+H]^{+}{}_{calculated} = 617.4$

continued in next page

User Spectra

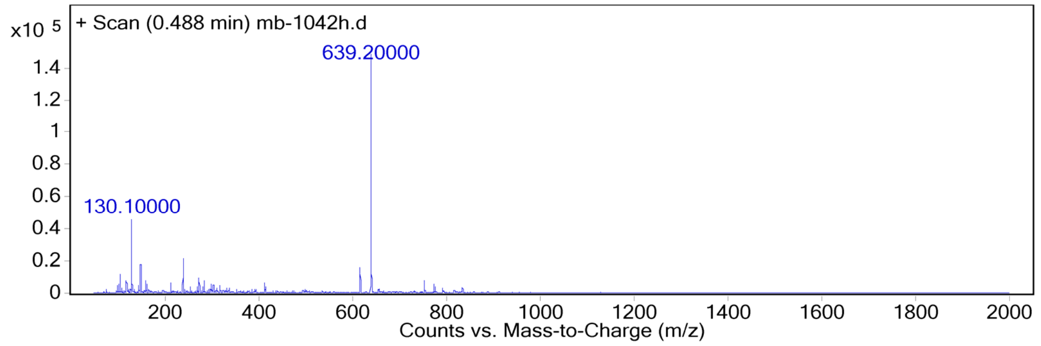
Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
158.9		62484
218.1		46096
246.1		51049
274.1	1	213089
275.1	1	38333
318.2		94464
430.8		27584
617.2		39439
639.2		304568
640.3	1	126566

Orn-Trp-Trp-Orn-Trp-Ile-Orn-Trp-NH_2;
$[M+HOAc+2H]^{2+}{}_{found} = 639.3$, $[M+HOAc+2H]^{2+}{}_{calculated} = 639.4$
Trp-Ile-Orn-Trp-NH_2;
$[M+H]^{+}{}_{found} = 617.2$, $[M+H]^{+}{}_{calculated} = 617.4$

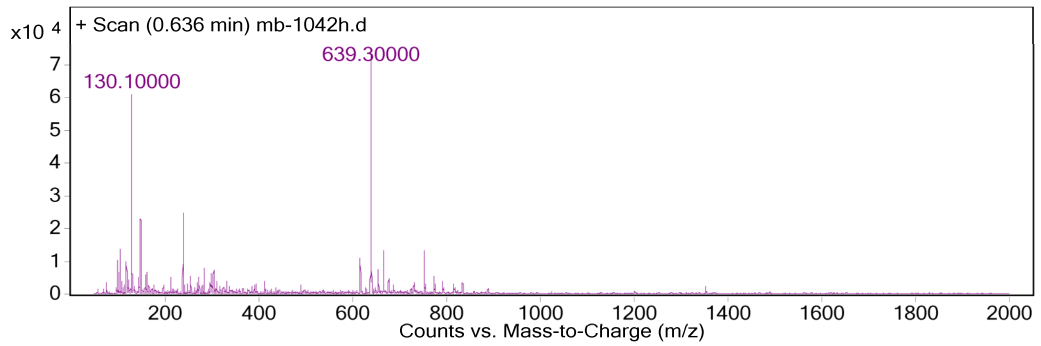


Peak List

m/z	z	Abund.
105.1		12106
130.1		45853
148.9		17802
158.9		8238
238.9		22081
274.1		10008
617.2	1	16366
639.2	1	148387
640.2	1	57112
641.3		12933

6

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

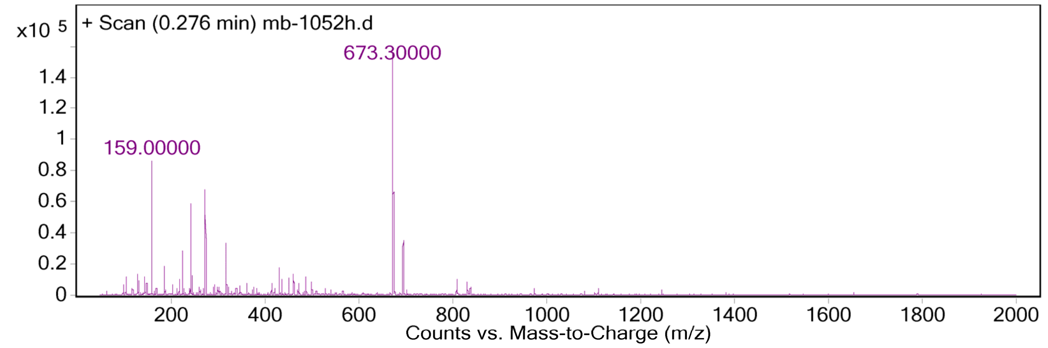
m/z	z	Abund.
100		10384
105		13925
130.1		60756
148.9		22933
239		24601
617.3		11015
639.3	1	72503
640.3	1	29998
666.5		13199
753.3	1	13469

Orn-Trp-Trp-Orn-Trp-Ile-Orn-Trp-NH_2;
$[M+HOAc+2H]^{2+}_{found}$ = 639.3, $[M+HOAc+2H]^{2+}_{calculated}$ = 639.4
Trp-Trp-Orn-Trp-Ile-Orn-Trp-NH_2;
$[M+2TFA+2H]^{2+}_{found}$ = 666.5, $[M+2TFA+2H]^{2+}_{calculated}$ = 666.3
Trp-Ile-Orn-Trp-NH_2;
$[M+H]^{+}_{found}$ = 617.3, $[M+H]^{+}_{calculated}$ = 617.4

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi

7



Peak List

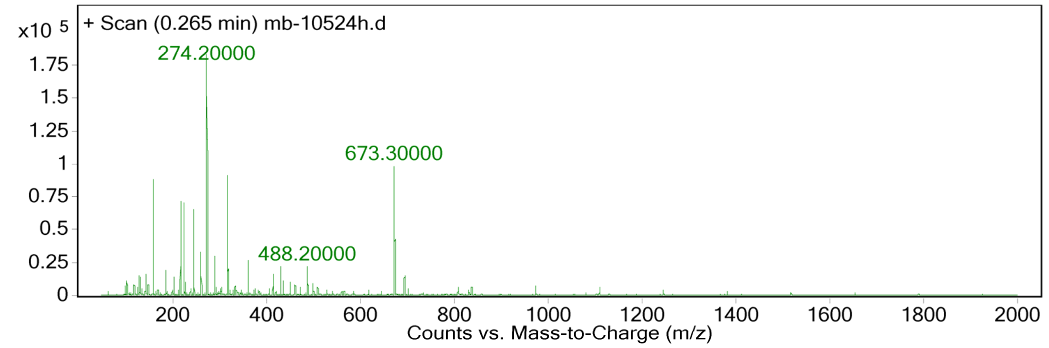
m/z	z	Abund.
159		86371
187		18517
226		28246
242.2	1	58201
274.1	1	68127
318.2		33413
430.8		17197
673.3	1	154456
674.3	1	65626
695.3	1	35312

Trp-Ile-Har-Trp-NH_2;

$[M+H]^+_{found}$ = 673.3, $[M+H]^+_{calculated}$ = 673.4

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

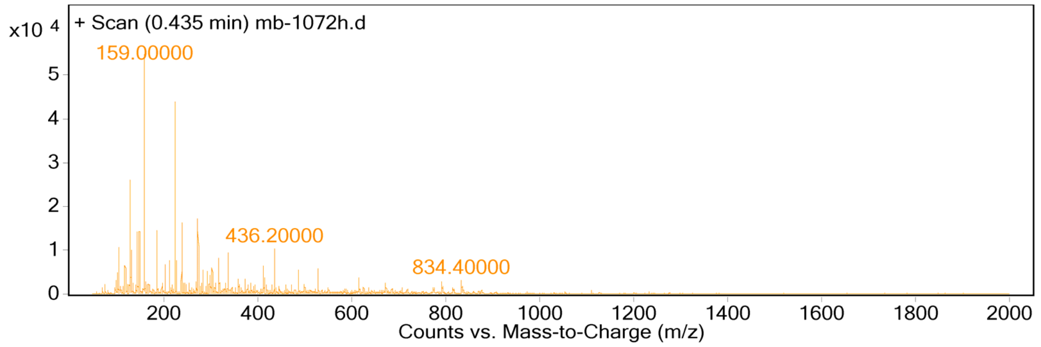
m/z	z	Abund.
158.9		88112
218.1	1	71241
226	1	70132
246.1	1	64854
262.1		33045
274.2		182694
290.1		29752
318.1		90749
673.3	1	98165
674.3	1	42186

Trp-Ile-Har-Trp-NH_2;

$[M+H]^+_{found}$ = 673.3, $[M+H]^+_{calculated}$ = 673.4

Trp-Ile-Har-OH;

$[M+H]^+_{found}$ = 488.2, $[M+H]^+_{calculated}$ = 488.3

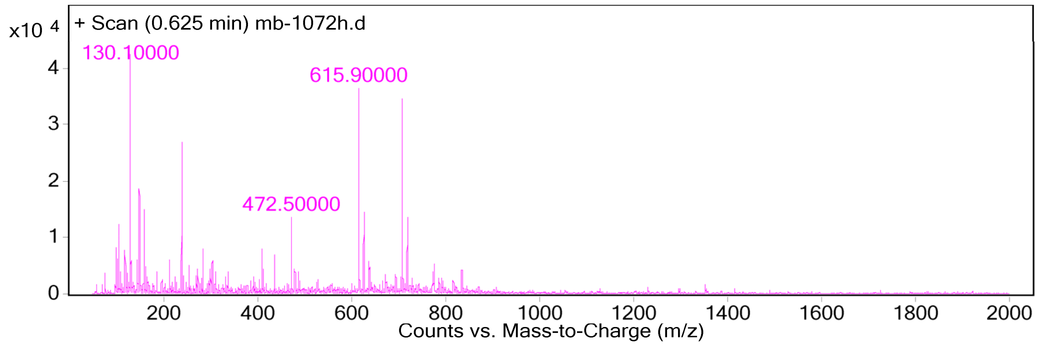


Peak List

m/z	z	Abund.
105		10569
130	1	25913
144		14329
145		10833
148.9		14247
159		54671
187		14595
226	1	43732
239		16303
274.1	1	17219

8

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



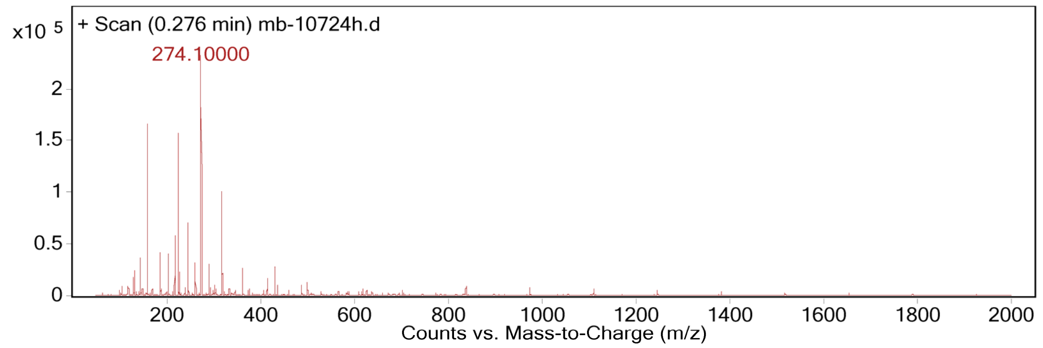
Peak List

m/z	z	Abund.
105		12287
130.1	1	42417
148.9		18524
158.9		14812
238.9		26805
472.5		13492
615.9		36534
626.9		14578
708.5		34568
719.4		13497

Dab-Har-Trp-Trp-Dab-Trp-Ile-Har-OH;
$[M+2H]^{2+}_{found} = 615.9$, $[M+2H]^{2+}_{calculated} = 615.9$
Har-Trp-Trp-Dab-Trp-Ile-Har-OH;
$[M+HOAc+TFA+3H]^{3+}_{found} = 436.2$, $[M+HOAc+TFA+3H]^{3+}_{calculated} = 435.5$
Trp-Trp-Dab-Trp-Ile-Har-OH;
$[M+4TFA+3H]^{3+}_{found} = 472.5$, $[M+4TFA+3H]^{3+}_{calculated} = 472.8$
Trp-Trp-Dab-OH
$[M+2TFA+H]^{+}_{found} = 719.4$, $[M+2TFA+H]^{+}_{calculated} = 719.3$

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

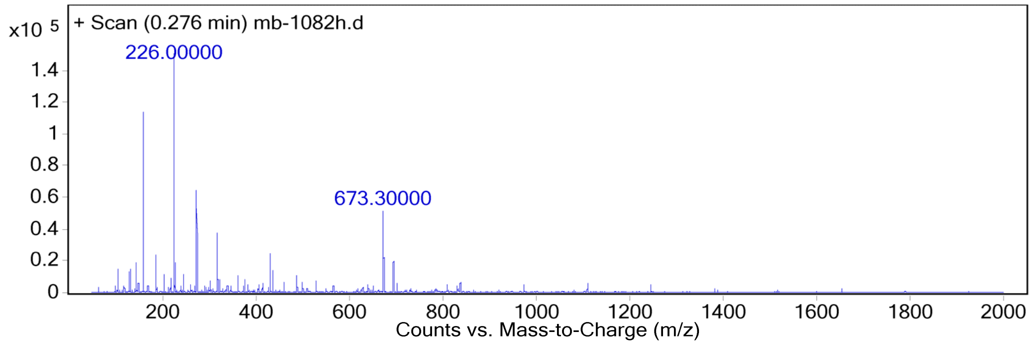
m/z	z	Abund.
144		36610
159	1	165379
187		41805
204		40082
218.1		58049
226	1	156687
246.1		69855
274.1	1	231713
275.1	1	40409
318.1		99774

<u>not detectable</u>

9

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
144		18578
158.9		114045
186.9		23781
226	1	149993
274.2		64521
318.1		37732
430.7		24489
673.3	1	51295
674.3	1	21648
695.2	1	19486

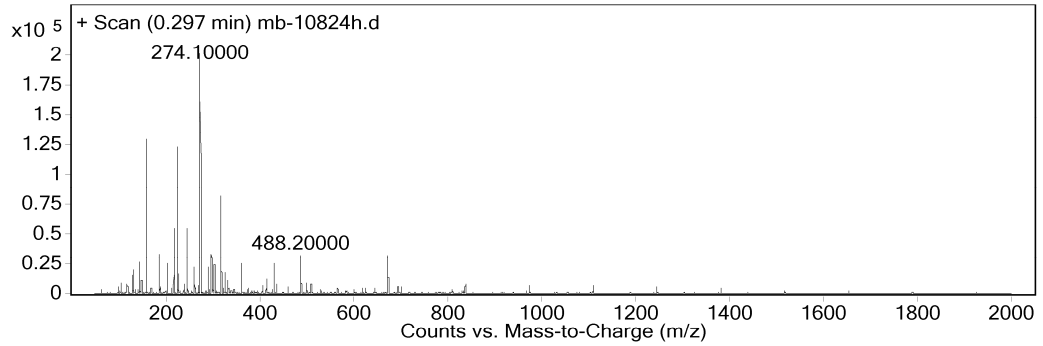
Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi

Trp-Ile-Har-Trp-NH_2;
$[M+H]^+_{found}$ = 673.3, $[M+H]^+_{calculated}$ = 673.4
Trp-Trp-Orn-OH
$[M+4HOAc+TFA+2H]^{2+}_{found}$ = 430.7, $[M+4HOAc+TFA+2H]^{2+}_{calculated}$ = 430.1

continued in next page

User Spectra

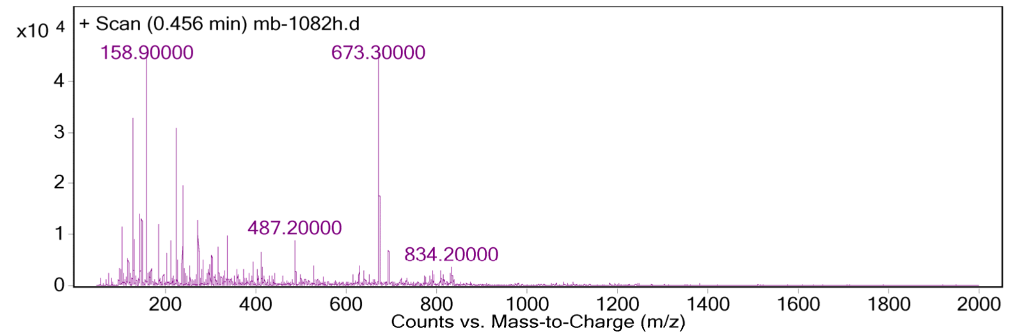
Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
158.9		129238
187		32472
218.1		54361
226	1	122973
246.1		54630
274.1		200416
275.2		33953
298.2		32169
318.1	1	81798
488.2		31792

Trp-Trp-Orn-Trp-Ile-Har-OH;
$[M+2H]^{2+}_{found}$ = 488.2, $[M+2H]^{2+}_{calculated}$ = 487.8

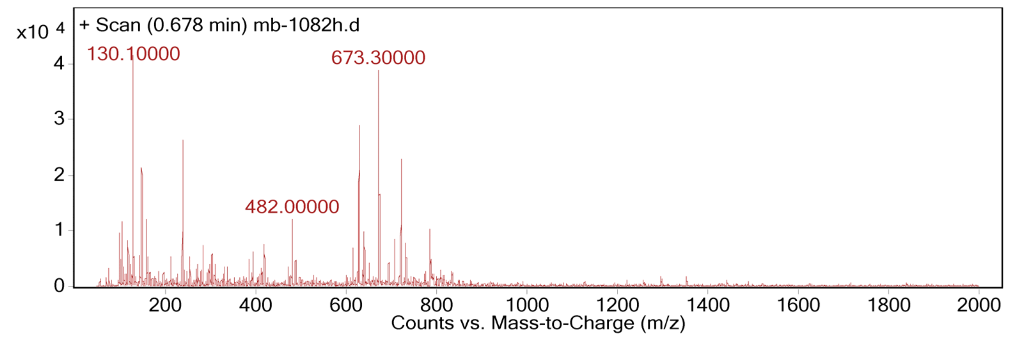


Peak List

m/z	z	Abund.
130.1		32844
144		13946
148.9		13061
158.9		44868
187		12020
226	1	30920
238.9	1	19617
274.1	1	12622
673.3		45282
674.2		17375

9

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
105.1		11720
130.1	1	41510
148.9	1	21319
159		12172
238.9		26234
482		12155
629.9		29021
673.3	1	38920
674.3	1	16672
722.5		22885

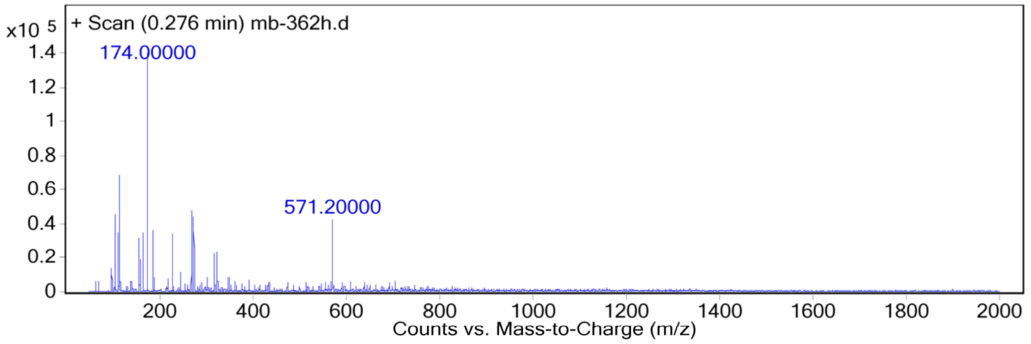
Orn-Har-Trp-Trp-Orn-Trp-Ile-Har-OH;
$[M+2H]^{2+}_{found} = 629.9$, $[M+2H]^{2+}_{calculated} = 629.9$
Har-Trp-Trp-Orn-Trp-Ile-Har-OH;
$[M+5HOAc+3H]^{3+}_{found} = 482.0$, $[M+5HOAc+3H]^{3+}_{calculated} = 482.2$
Trp-Trp-Orn-Trp-Ile-Har-OH;
$[M+2H]^{2+}_{found} = 487.2$, $[M+2H]^{2+}_{calculated} = 487.8$
Trp-Ile-Har-Trp-NH_2;
$[M+H]^{+}_{found} = 673.3$, $[M+H]^{+}_{calculated} = 673.3$
Trp-Ile-Har-OH

$[M+2HOAc+TFA+H]^{+}_{found} = 722.5$, $[M+2HOAc+TFA+H]^{+}_{calculated} = 722.3$

10

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



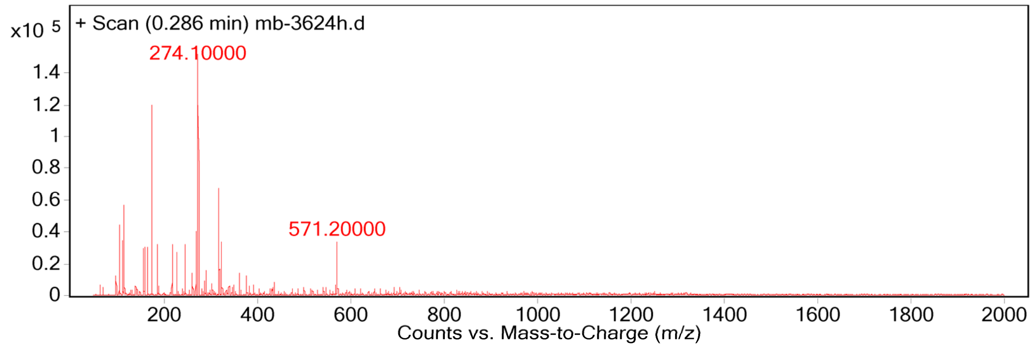
Peak List

m/z	z	Abund.
104.9		44913
112		34323
115		68670
164.9		34488
174	1	139034
186.8		35878
228.9		33600
268.9		47772
274.1	1	43597
571.2	1	42278

not detectable

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

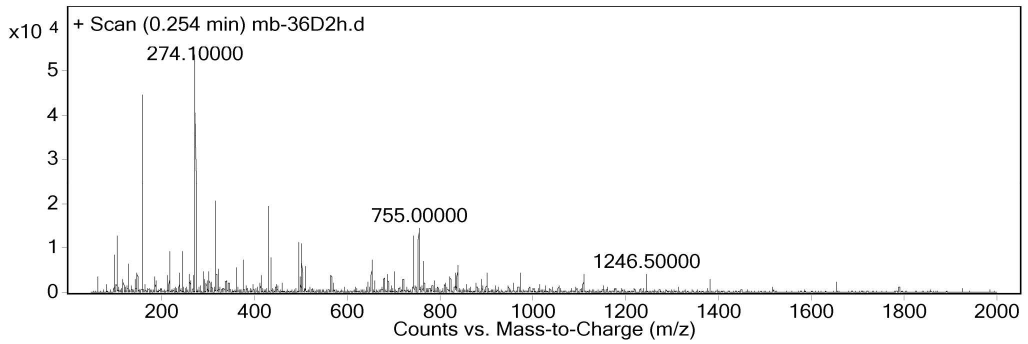
m/z	z	Abund.
105		44320
112		34119
115		56881
174	1	120091
246.1		32379
268.9		40174
274.1		151502
318.1		66843
322.8		33631
571.2		33841

not detectable

11

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



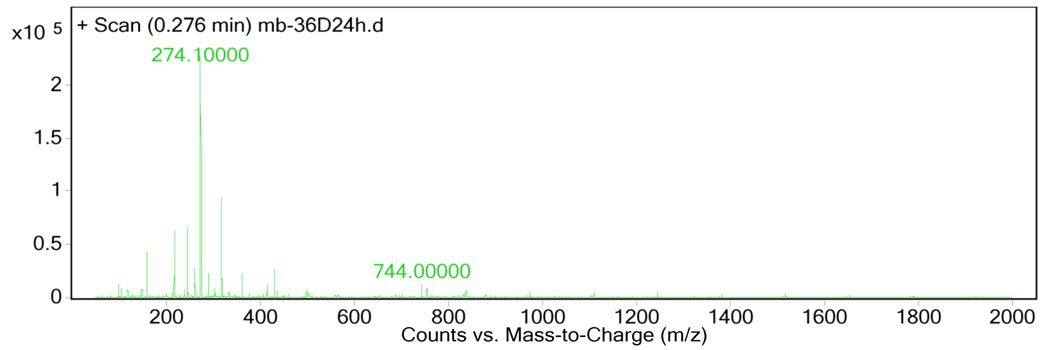
Peak List

m/z	Abund.
105	12700
158.9	44555
274.1	53511
275.2	12037
318.1	20657
430.8	19412
496.5	11372
503.7	10988
744	12604
755	14479

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
158.9		43194
218.1		62435
246.1		66799
262.1		27273
274.1		226489
275.2		37674
290.2		21976
318.2	1	93508
362.2		21850
430.8		25967

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi

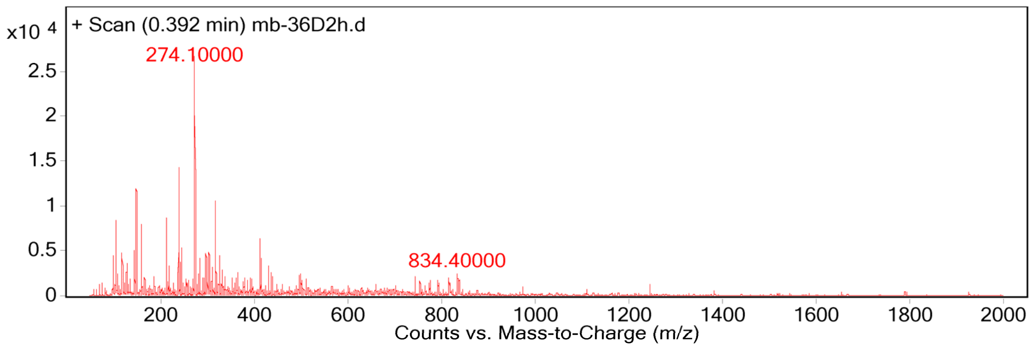
continued in next page

Intact **11**;

$[M+2H]^{2+}_{found} = 744.0$, $[M+2H]^{2+}_{calculated} = 743.9$

$[M+3H]^{3+}_{found} = 496.5$, $[M+3H]^{3+}_{calculated} = 496.3$

$[M+3HOAc+3TFA+4H]^{4+}_{found} = 503.7$, $[M+3HOAc+3TFA+4H]^{4+}_{calculated} = 503.0$

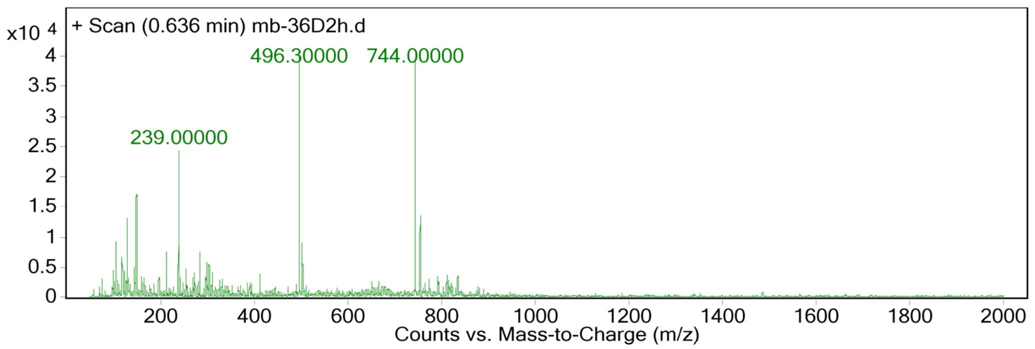


Peak List

m/z	z	Abund.
104.9		8413
148.9		11849
158.8		7933
214.1	1	8696
238.9		14318
246.1		5335
274.1		26652
275.2		5903
318.2		10523
413		6388

11

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
105		9305
130.1	1	13058
149		16925
214.1		7493
239		24260
284.1	1	7491
496.3		39770
503.6		9120
744		39527
755.1	1	13519

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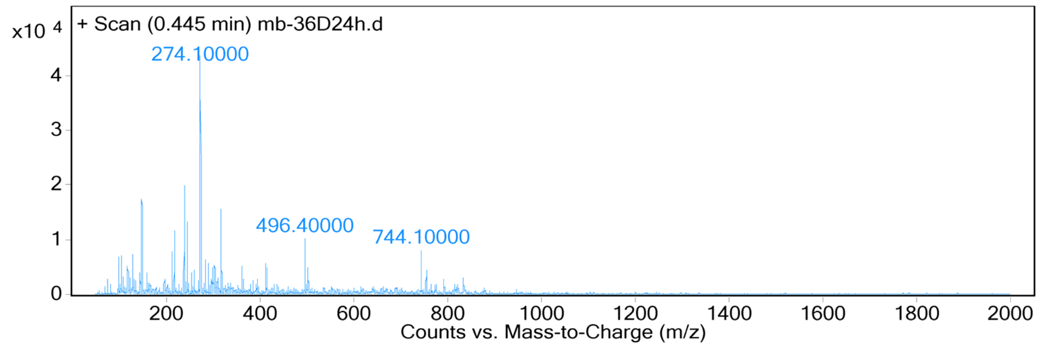
Intact **11**;

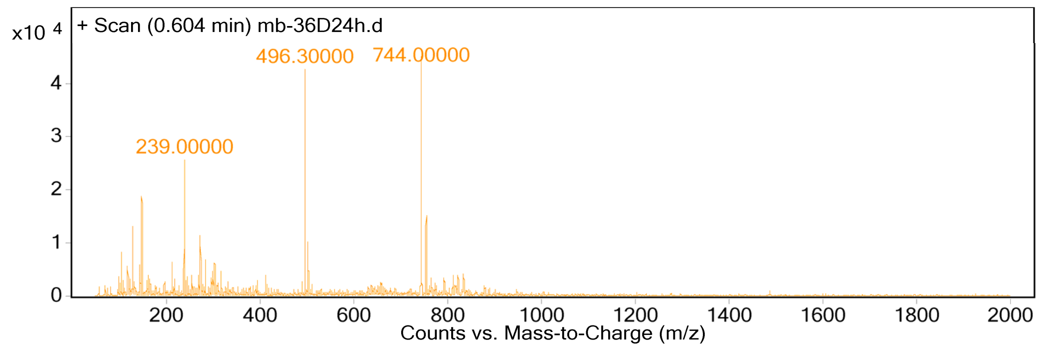
$[M+2H]^{2+}_{found} = 744.0$, $[M+2H]^{2+}_{calculated} = 743.9$



Peak List

m/z	z	Abund.
148.9	1	17284
214.1		7729
218.1		11631
238.9		19844
246.1		13108
274.1	1	43637
275.1	1	8248
318.2		15704
496.4		10250
744.1	2	8095

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
105		8259
130		13118
148.9		18449
239		25597
274.2	1	11483
284.1		6914
496.3		42725
503.6		10218
744		45063
755		15130

Intact **11**;

$[M+3HOAc+2H]^{2+}_{found}$ = 834.4, $[M+3HOAc+2H]^{2+}_{calculated}$ = 833.9

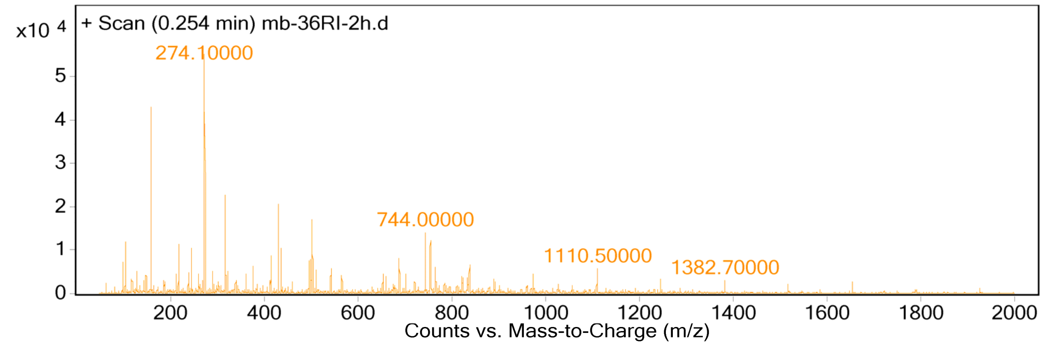
$[M+2H]^{2+}_{found}$ = 744.0, $[M+2H]^{2+}_{calculated}$ = 743.9

$[M+3H]^{3+}_{found}$ = 496.3, $[M+3H]^{3+}_{calculated}$ = 496.3

$[M+3HOAc+3TFA+4H]^{4+}_{found}$ = 503.6, $[M+3HOAc+3TFA+4H]^{4+}_{calculated}$ = 503.0

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
104.9		12069
158.9		43007
218.1		11333
246.1		10526
274.1		55096
318.1	1	22808
430.8		20631
503.7		17074
744		14139
755		12274

12

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi

Intact **12**;

$[M+2H]^{2+}_{found}$ = 744.0, $[M+2H]^{2+}_{calculated}$ = 743.9

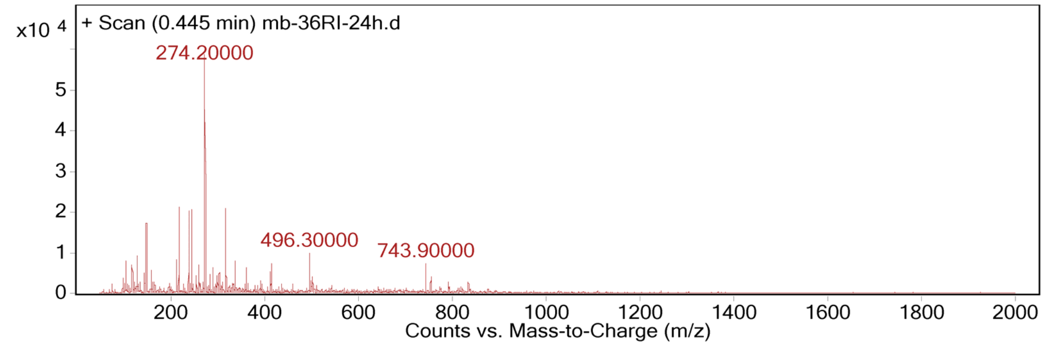
$[M+3HOAc+3TFA+4H]^{4+}_{found}$ = 503.7, $[M+3HOAc+3TFA+4H]^{4+}_{calculated}$ = 503.0

Intact **11**;

$[M+2H]^{2+}_{found}$ = 744.0, $[M+2H]^{2+}_{calculated}$ = 743.9

$[M+3H]^{3+}_{found}$ = 496.3, $[M+3H]^{3+}_{calculated}$ = 496.3

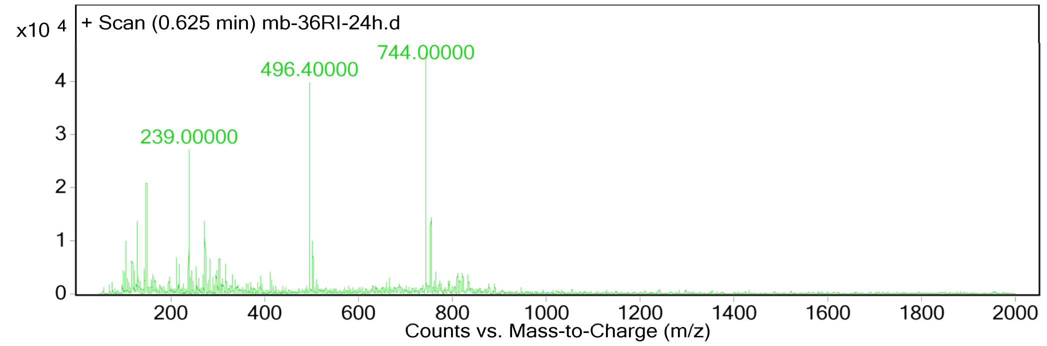
$[M+3HOAc+3TFA+4H]^{4+}_{found}$ = 503.6, $[M+3HOAc+3TFA+4H]^{4+}_{calculated}$ = 503.0



Peak List

m/z	z	Abund.
130.1		9246
148.9		17201
214.1		8394
218.1	1	21201
238.9	1	20400
246.1	1	20781
274.2	1	58770
275.2	1	10144
318.2		20848
496.3		9782

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
105		9906
130.1		13683
148.9	1	20845
214.1		6850
239		27078
274.1	1	13639
496.4		39813
503.7		10071
744		45170
755		14388

Intact **12**;

$[M+2H]^{2+}_{found}$ = 744.0 or 743.9, $[M+2H]^{2+}_{calculated}$ = 743.9

$[M+3H]^{3+}_{found}$ = 496.4, $[M+3H]^{3+}_{calculated}$ = 496.3

$[M+3HOAc+3TFA+4H]^{4+}_{found}$ = 503.7, $[M+3HOAc+3TFA+4H]^{4+}_{calculated}$ = 503.0

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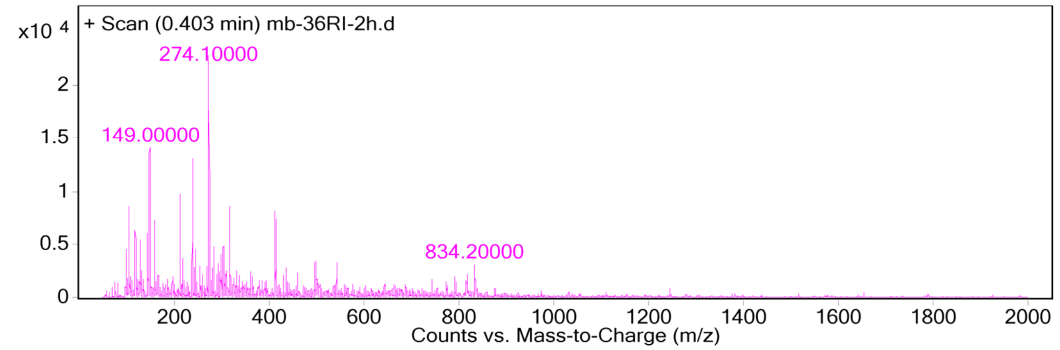
12



Peak List

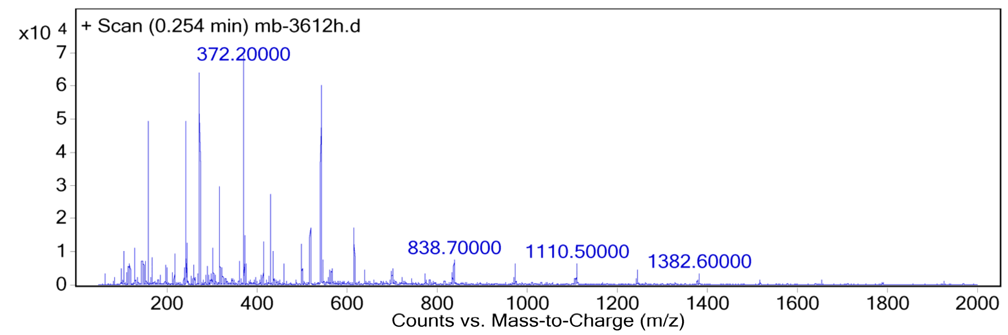
m/z	z	Abund.
105	1	8599
119.1		6241
149		13999
158.8		7288
214.2		9710
239	1	13106
274.1	1	22745
318.2		8567
413.1		8173
415	1	7445

Intact **12**;
$[M+3HOAc+2H]^{2+}_{found}$ = 834.2, $[M+3HOAc+2H]^{2+}_{calculated}$ = 833.9

13

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
158.9		49406
244.1		49392
274.1	1	63981
318.2		29443
372.2	1	69040
430.8		27398
519.2		17232
543.2	1	60078
544.2	1	20240
617.2		17232

Trp-Trp-Arg-Trp-Trp-Lys-Lys-NH_2;
$[M+HOAc+2H]^{2+}_{found}$ = 617.2, $[M+HOAc+2H]^{2+}_{calculated}$ = 617.8

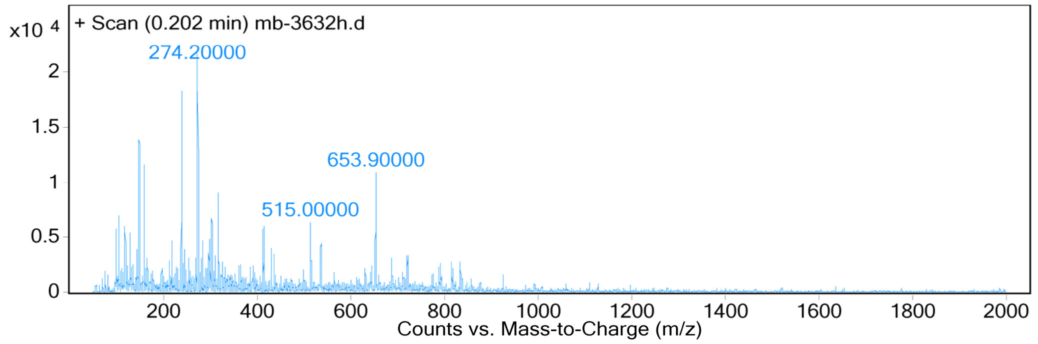
not determined

Trp-Trp-Arg-Trp-Trp-Lys-OH;
$[M+HOAc+5TFA+2H]^{2+}_{found}$ = 838.7, $[M+ HOAc+5TFA+2H]^{2+}_{calculated}$ = 839.3
Trp-Trp-Lys-OH
$[M+H]^{+}_{found}$ = 519.2, $[M+H]^{+}_{calculated}$ = 519.3

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi

14



Peak List

m/z	z	Abund.
105	1	6912
119		5996
148.9		13828
158.9		11541
239	1	18246
274.2		21626
304.1	1	6696
318.1	1	8995
515		6343
653.9		10891

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi

Intact **14**;
$[M+4HOAc+4TFA+3H]^{3+}_{found}$ = 653.9, $[M+4HOAc+4TFA+3H]^{3+}_{calculated}$ = 653.6

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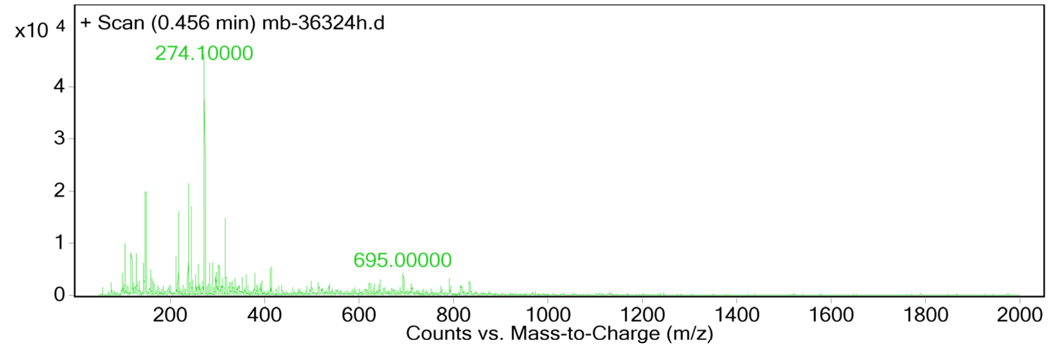
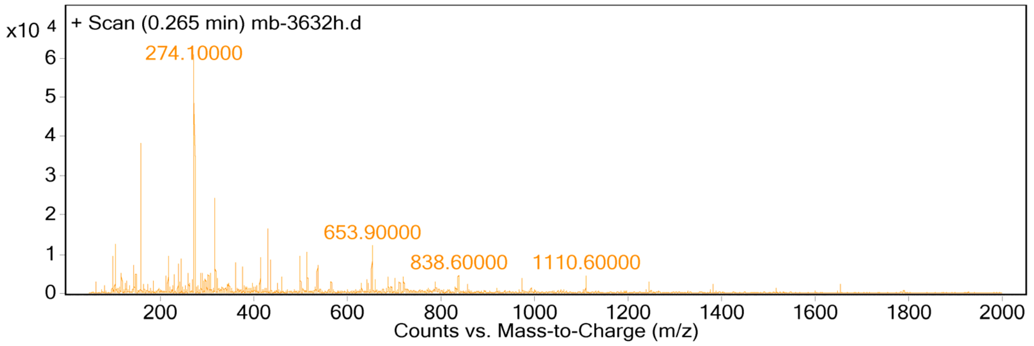


Peak List

m/z	z	Abund.
105	1	10080
119.1		8041
130		8123
149		19830
218.1	1	16086
239	1	21594
246.1		16917
274.1	1	46206
275.1	1	10225
318.2		14637

Dab-Trp-Trp-Dab-Trp-Trp-Dab-Dab-NH_2;
$[M+2TFA+2H]^{2+}_{found}$ = 695.0, $[M+2TFA+2H]^{2+}_{calculated}$ = 695.9

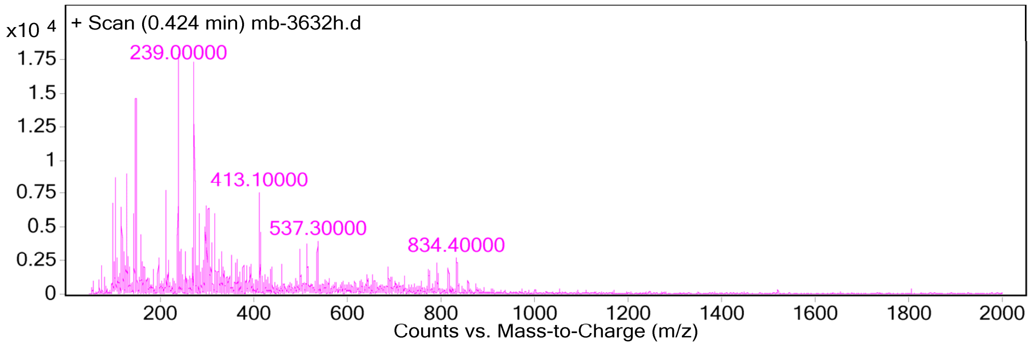


Peak List

m/z	z	Abund.
104.9		12476
158.9		38270
218		9530
274.1		60814
275.2		11339
318.1	1	24482
430.8		16521
500.1	1	9535
515.1		10651
653.9		12090

14

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
99		6752
105		8740
118.9		6512
130.1		8939
149		14635
214.2		7778
239	1	17853
274.1	1	17286
300.9		6617
413.1	1	7494

Intact **14**;

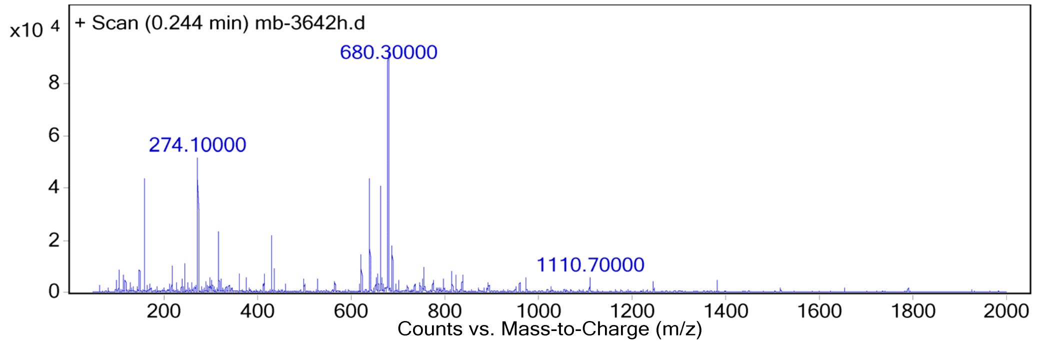
$[M+5HOAc+TFA+2H]^{2+}_{found} = 838.6$, $[M+5HOAc+TFA+2H]^{2+}_{calculated} = 838.9$

$[M+2HOAc+2TFA+3H]^{3+}_{found} = 537.3$, $[M+2HOAc+2TFA+3H]^{3+}_{calculated} = 537.6$

15

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
158.9		43360
274.1		51614
318.1		23108
430.8		21864
640.2		43533
641.3		18125
663.2		40659
680.3	1	91396
681.3	1	35169
689		17705

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi

Trp-Trp-Orn-Trp-Trp-Orn-Orn-NH_2;
$[M+HOAc+TFA+2H]^{2+}_{found}$ = 640.2, $[M+HOAc+TFA+2H]^{2+}_{calculated}$ = 639.8
Trp-Trp-Orn-OH
$[M+HOAc+TFA+H]^{+}_{found}$ = 680.3, $[M+HOAc+TFA+H]^{+}_{calculated}$ = 679.6

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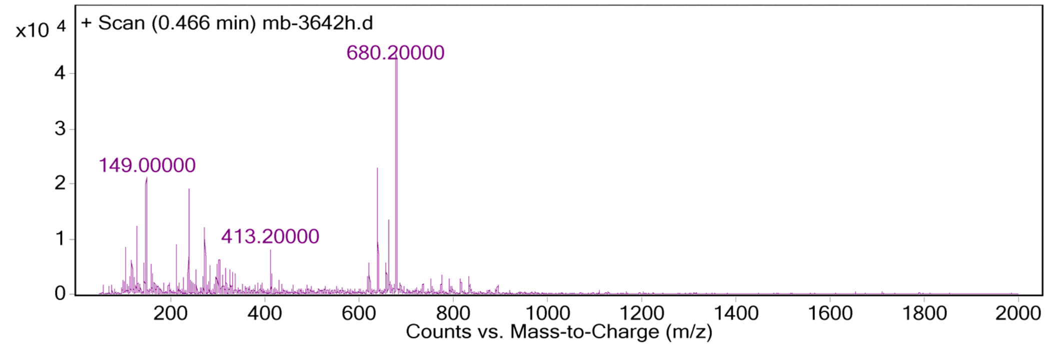
not determined

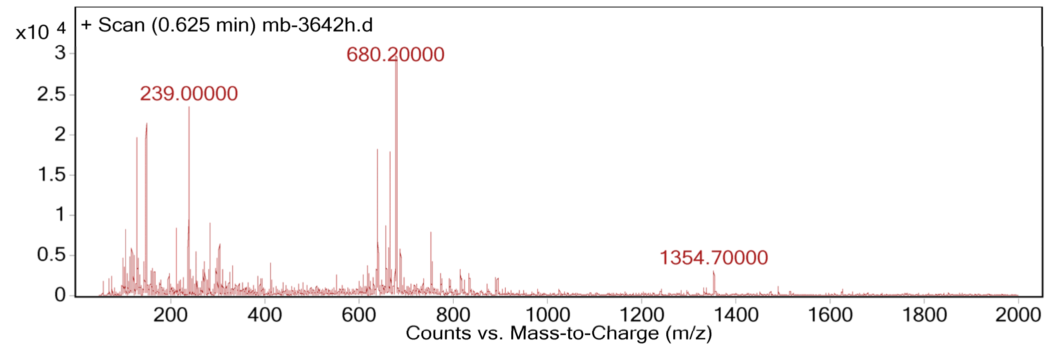


Peak List

m/z	z	Abund.
130		12303
149		20908
214.1		8900
239		19033
274.2		12059
640.3		22762
641.2	1	11760
663.3		13416
680.2		43540
681.3	1	19795

15

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
130.1	1	19591
149		21385
214.1		8326
239	1	23443
284.1	1	9027
640.2	1	18154
658.4		8640
666.9		17781
680.2	1	29717
681.2	1	13959

Orn-Trp-Trp-Orn-Trp-Trp-Orn-Orn-NH_2;
$[M+TFA+2H]^{2+}_{found} = 666.9$, $[M+TFA+2H]^{2+}_{calculated} = 666.9$
Trp-Trp-Orn-Trp-Trp-Orn-Orn-NH_2;
$[M+HOAc+TFA+2H]^{2+}_{found} = 640.3$, $[M+HOAc+TFA+2H]^{2+}_{calculated} = 639.8$
Trp-Trp-Orn-OH
$[M+HOAc+TFA+H]^{+}_{found} = 680.2$, $[M+HOAc+TFA+H]^{+}_{calculated} = 679.6$

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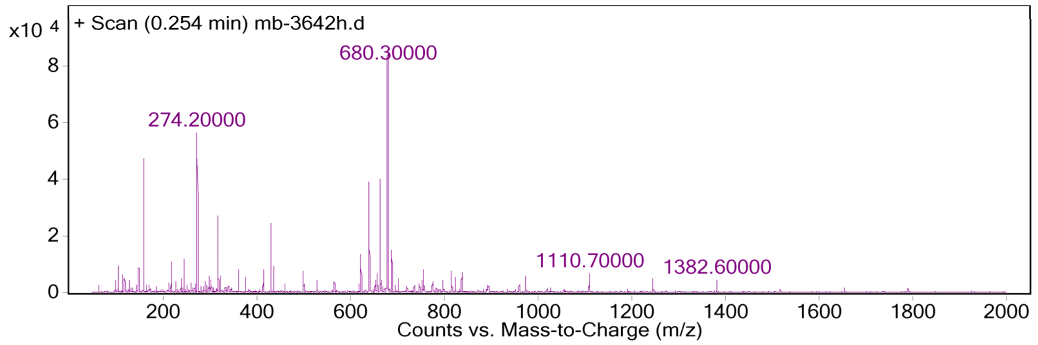
Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi

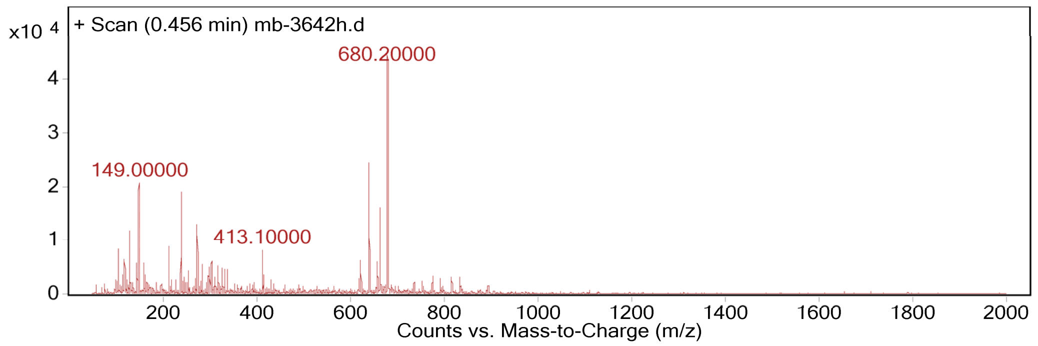


Peak List

m/z	z	Abund.
158.9		47573
274.2		56424
318.1	1	27324
430.8		24628
640.2		39148
641.3		16785
663.2		39965
664.3		15356
680.3	1	84209
681.3	1	32535

15

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
130		11793
149		20537
214.2		8922
239	1	18835
274.2		13034
640.2	1	24351
641.2	1	12392
663.3		16151
680.2		44274

Trp-Trp-Orn-Trp-Trp-Orn-Orn-NH_2;
$[M+HOAc+TFA+2H]^{2+}_{found}$ = 640.2, $[M+HOAc+TFA+2H]^{2+}_{calculated}$ = 639.8
Trp-Trp-Orn-OH
$[M+HOAc+TFA+H]^{+}_{found}$ = 680.2, $[M+HOAc+TFA+H]^{+}_{calculated}$ = 679.6

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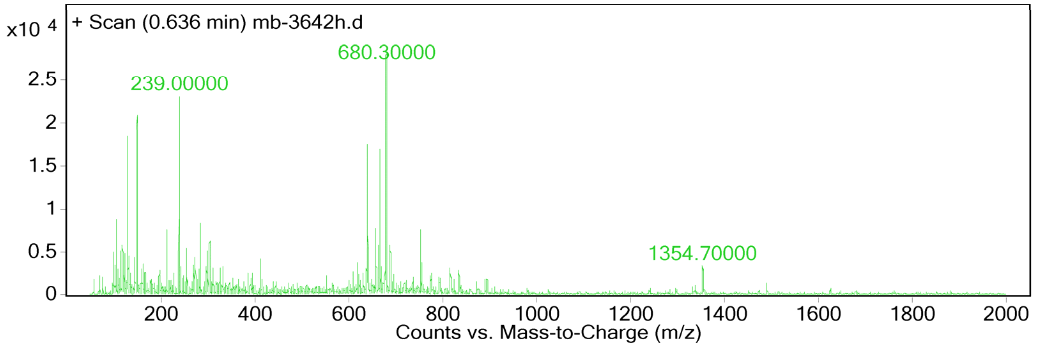
m/z	z	Abund.
681.3		20300

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi

15



Peak List

m/z	z	Abund.
105.1		8752
130.1	1	18471
149		20965
239	1	23024
284.1	1	8407
640.2	1	17534
641.2	1	7830
666.9		17049
680.3		28012
681.2		12378

Orn-Trp-Trp-Orn-Trp-Trp-Orn-Orn-NH_2;

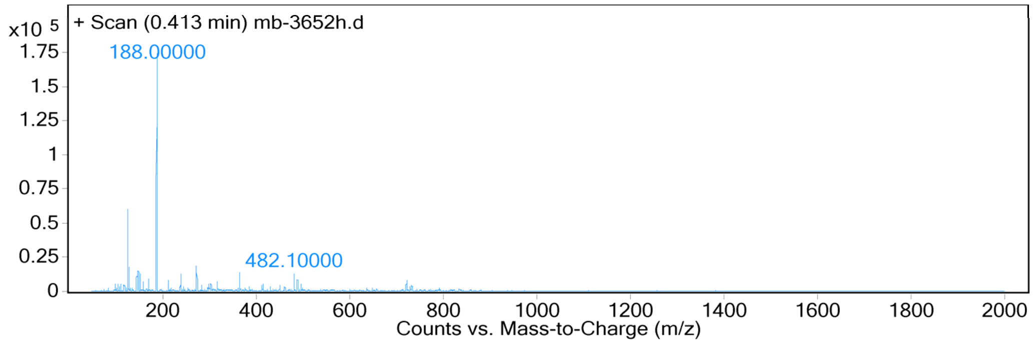
$[M+TFA+2H]^{2+}_{found}$ = 666.9, $[M+TFA+2H]^{2+}_{calculated}$ = 666.9

Trp-Trp-Orn-OH

$[M+HOAc+TFA+H]^{+}_{found}$ = 680.3, $[M+HOAc+TFA+H]^{+}_{calculated}$ = 679.6

16

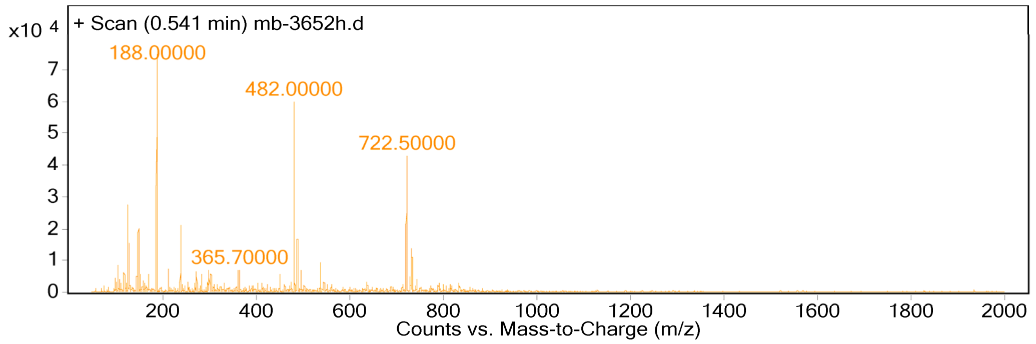
Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
126		60351
129		18184
148.9		15212
154		12995
188	1	173942
189	1	18031
239		13608
274.1		18946
365.6		13977
482.1		12858

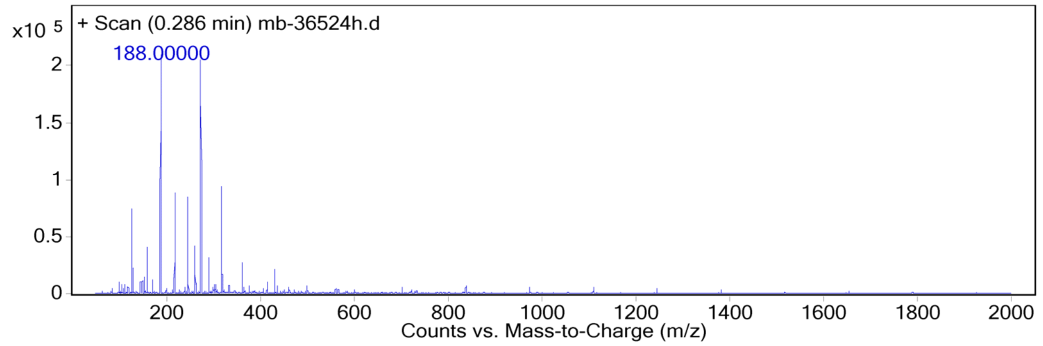
Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
126		27467
130		15580
149		19790
188		74769
239		21040
482		59862
489.3		16765
722.5		42756
733.5	2	13800

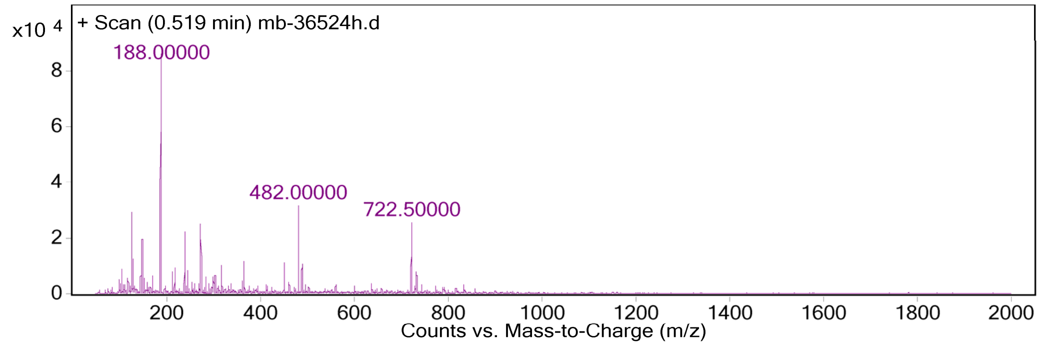
Har-Trp-Trp-Har-Trp-Trp-Har-Har-NH_2
$[M+2H]^{2+}{}_{found}$ = 722.5, $[M+2H]^{2+}{}_{calculated}$ = 721.9
$[M+3H]^{3+}{}_{found}$ = 482.0, $[M+3H]^{3+}{}_{calculated}$ = 481.6
Trp-Trp-Har-Trp-Trp-Har-OH;
$[M+3TFA+2H]^{2+}{}_{found}$ = 722.5, $[M+3TFA+2H]^{2+}{}_{calculated}$ = 723.3
$[M+3TFA+3H]^{3+}{}_{found}$ = 482.1, $[M+3TFA+3H]^{3+}{}_{calculated}$ = 482.5
Trp-Trp-Har-OH;
$[M+HOAc+3TFA+2H]^{2+}{}_{found}$ = 482.0, $[M+HOAc+3TFA+2H]^{2+}{}_{calculated}$ = 482.1



Peak List

m/z	z	Abund.
126		74206
158.9		40544
188	1	209267
218.1	1	88428
246.1	1	84950
262.1		41827
274.1	1	205203
275.1	1	37247
290.1		31525
318.2		94437

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
126.1		29242
130.1		12620
149		19658
188		85881
238.9		22141
274.1	1	25242
365.8		11540
451.2		11218
482		31634
722.5		25793

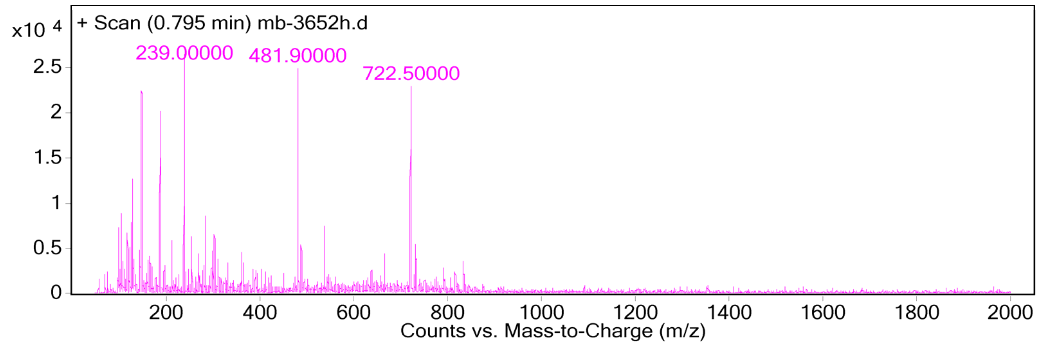
Har-Trp-Trp-Har-Trp-Trp-Har-Har-NH_2
$[M+2H]^{2+}{}_{found}$ = 722.5, $[M+2H]^{2+}{}_{calculated}$ = 721.9
$[M+3H]^{3+}{}_{found}$ = 482.0, $[M+3H]^{3+}{}_{calculated}$ = 481.6
Trp-Trp-Har-Trp-Trp-Har-OH;
$[M+3TFA+2H]^{2+}{}_{found}$ = 722.5, $[M+3TFA+2H]^{2+}{}_{calculated}$ = 723.3
$[M+3TFA+3H]^{3+}{}_{found}$ = 482.1, $[M+3TFA+3H]^{3+}{}_{calculated}$ = 482.5
Trp-Trp-Har-OH;
$[M+HOAc+3TFA+2H]^{2+}{}_{found}$ = 482.0, $[M+HOAc+3TFA+2H]^{2+}{}_{calculated}$ = 482.1

continued in next page

m/z	z	Abund.
734	2	10996

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi

16



Peak List

m/z	z	Abund.
105		8786
126.1		7853
130	1	12711
148.9	1	22254
188		20133
239	1	26364
284.2		8605
481.9		24837
722.5	2	22853
723	2	17229

Har-Trp-Trp-Har-Trp-Trp-Har-Har-NH_2

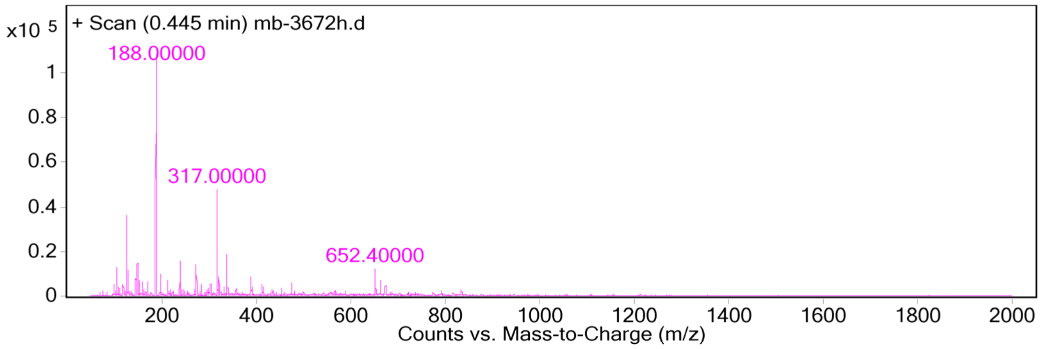
$[M+2H]^{2+}_{found}$ = 722.5, $[M+2H]^{2+}_{calculated}$ = 721.9

$[M+3H]^{3+}_{found}$ = 481.9, $[M+3H]^{3+}_{calculated}$ = 481.6

Trp-Trp-Har-Trp-Trp-Har-Har-NH_2;

$[M+HOAc+TFA+2H]^{2+}_{found}$ = 723.0, $[M+HOAc+TFA+2H]^{2+}_{calculated}$ = 723.8

17



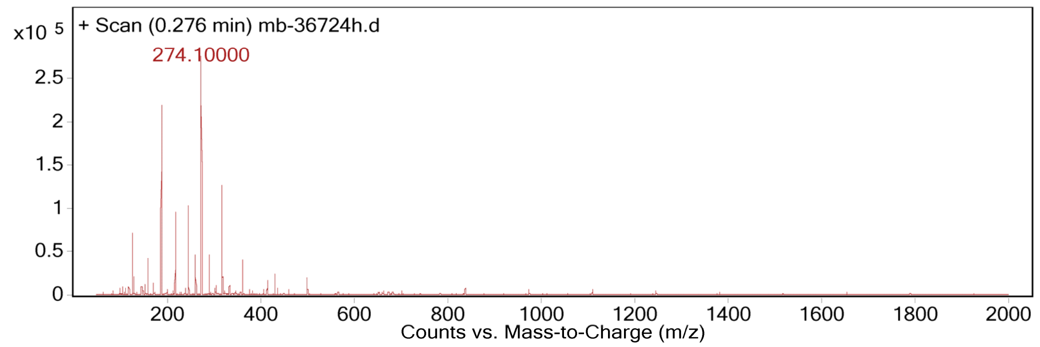
Peak List

m/z	z	Abund.
104.9		13078
126		36271
149		14654
188		108014
238.9		15594
274.1		13797
317	1	47991
318	1	16663
339		18842
652.4		12271

Har-Trp-Trp-Dab-Trp-Trp-Har-Har-NH_2;
$[M+4HOAc+3TFA+3H]^{3+}_{found}$ = 652.4, $[M+4HOAc+3TFA+3H]^{3+}_{calculated}$ = 652.5

User Spectra

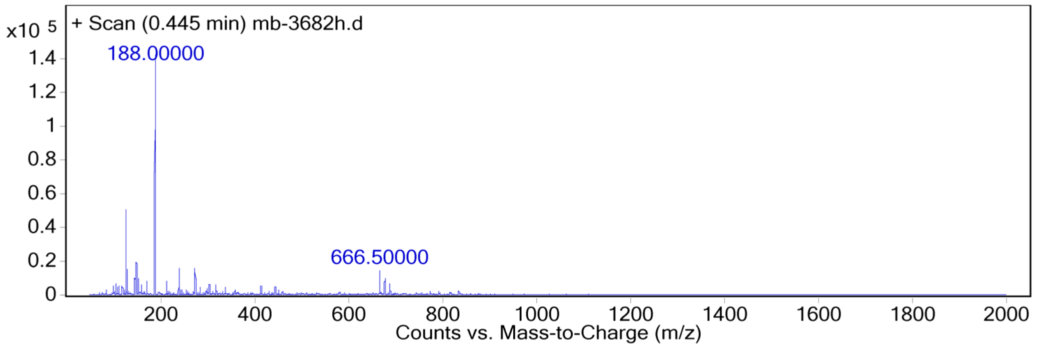
Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
126		72231
158.9		41894
188		219302
218.1	1	96189
246.2		103248
262.1		45554
274.1		275734
275.2		47507
290.1		46185
318.2	1	126357

<u>not detectable</u>

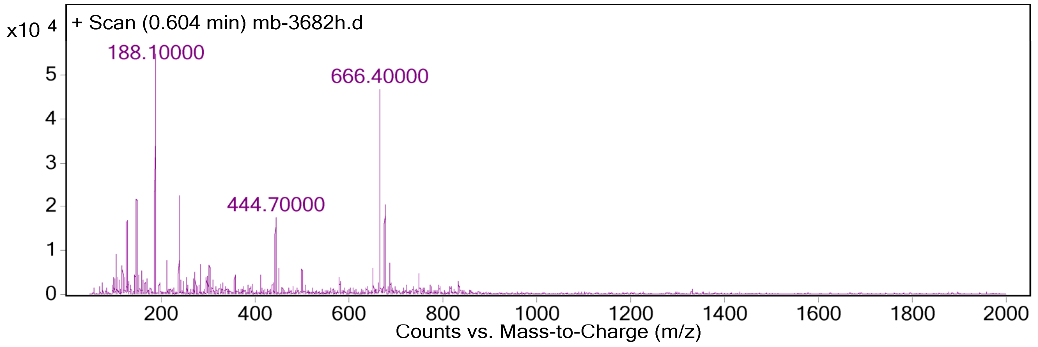


Peak List

m/z	Abund.
126	50693
129.1	15643
130.1	10685
148.9	18988
188	141974
189.1	13791
239	16049
274.2	16111
666.5	14218
677.5	9940

18

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi



Peak List

m/z	z	Abund.
105.1		9017
126.1		16422
130.1		16893
148.9		21439
188.1		54612
214.2		7571
239	1	22380
444.7		17575
666.4		46592
677.5		20403

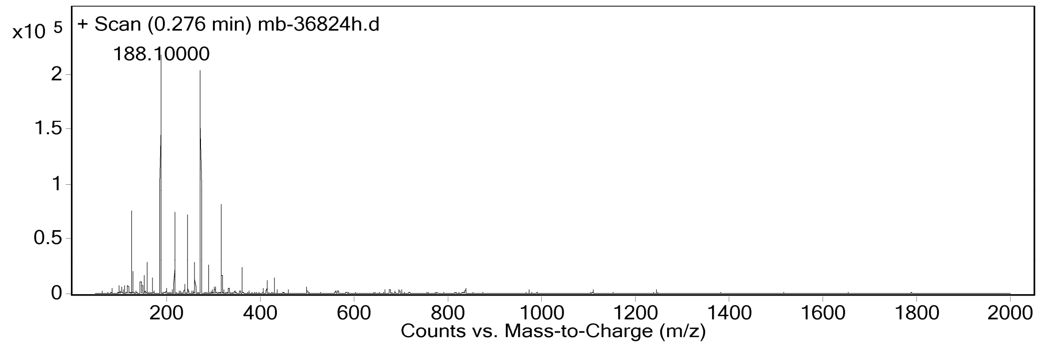
Trp-Trp-Orn-Trp-Trp-Har-Har-NH_2

$[M+TFA+2H]^{2+}_{found} = 666.4$, $[M+TFA+2H]^{2+}_{calculated} = 666.2$

$[M+TFA+3H]^{3+}_{found} = 444.7$, $[M+TFA+3H]^{3+}_{calculated} = 444.5$

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	Esi

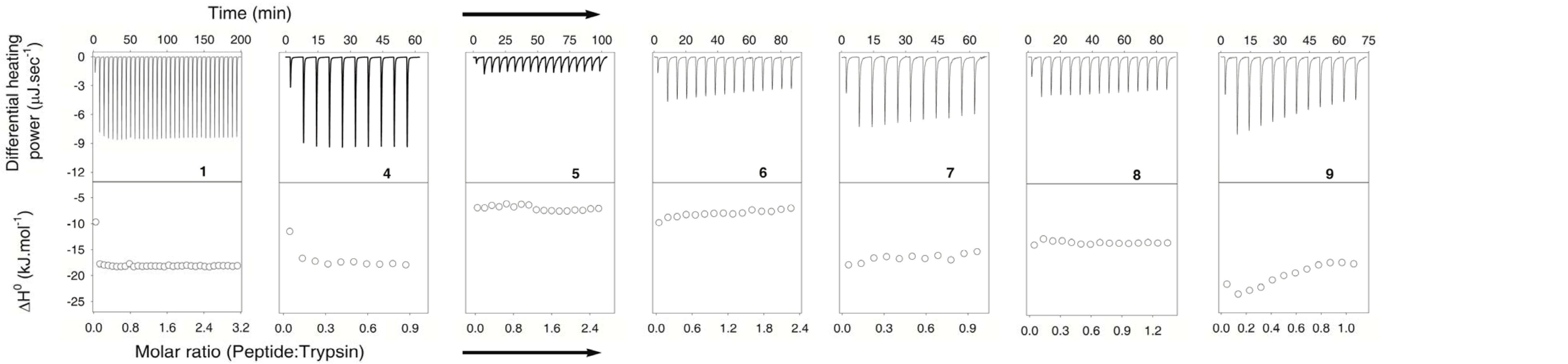


Peak List

m/z	z	Abund.
126.1		75077
158.9		28554
188.1		217254
218.1	1	73959
246.2		71250
262.1		28134
274.1	1	203287
275.1	1	37156
290.1		26440
318.1	1	80980

<u>not detectable</u>

Figure S7. The raw ITC data (power vs. time) and the integrated data for injection of the 1 and 10 derived peptides into the calorimeter cell containing trypsin at 310 °K. The fit parameters are $\Delta H° = -34.6$ kJ.mol^{-1}, $T\Delta S° = -1.2$ kJ.mol^{-1}, $K° = 2.4 \times 10^{+4}$ M^{-1}, n = 1.3 for **11** and $\Delta H° = -49.4$ kJ.mol^{-1}, $T\Delta S° = -3.3$ kJ.mol^{-1}, $K° = 1.1 \times 10^{+4}$ M^{-1}, n = 0.7 for **12**.



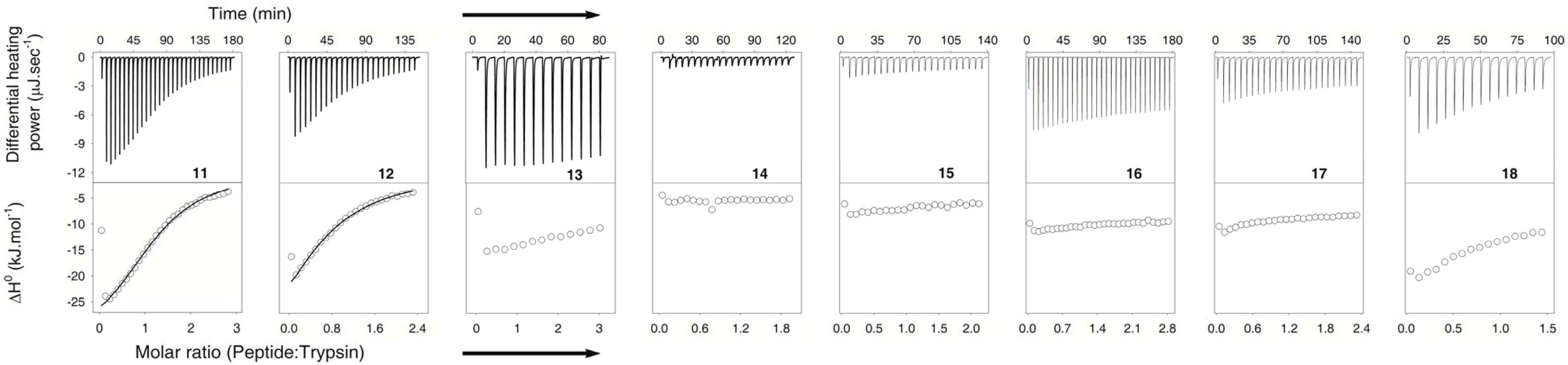


Figure S8. The representative structure of trypsin used in the docking studies. The structure with the highest percentage of occurrence generated from cluster analysis of trypsin (PDB ID: 4I8G) MD simulation in water for 20 ns. The left image shows enzyme binding pockets; e.g., S1 (composed of amino acid residues 189-192, 214-216, 224-228), S1' (composed of amino acid residues 41-45), S2 (composed of amino acid residues 57, 215, 99) and S2' (composed of amino acid residues 142-143, 151) in red, yellow, pink and orange colors, respectively. The right image shows the position of histidine-57, aspartate-102 and serin-195 in the enzyme catalytic triad with respect to aspartate-189 in the S1 binding site.

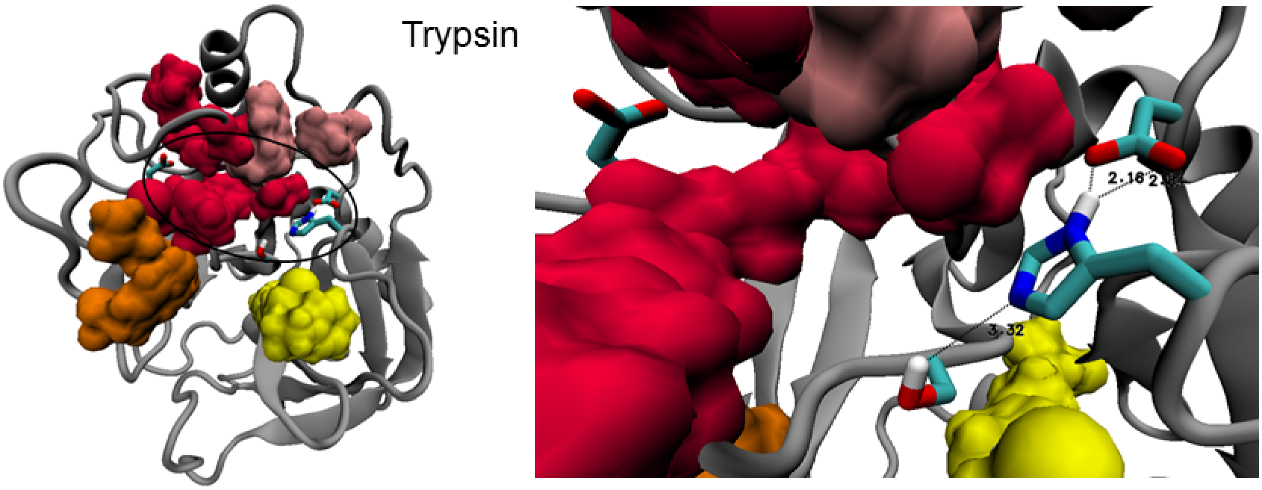
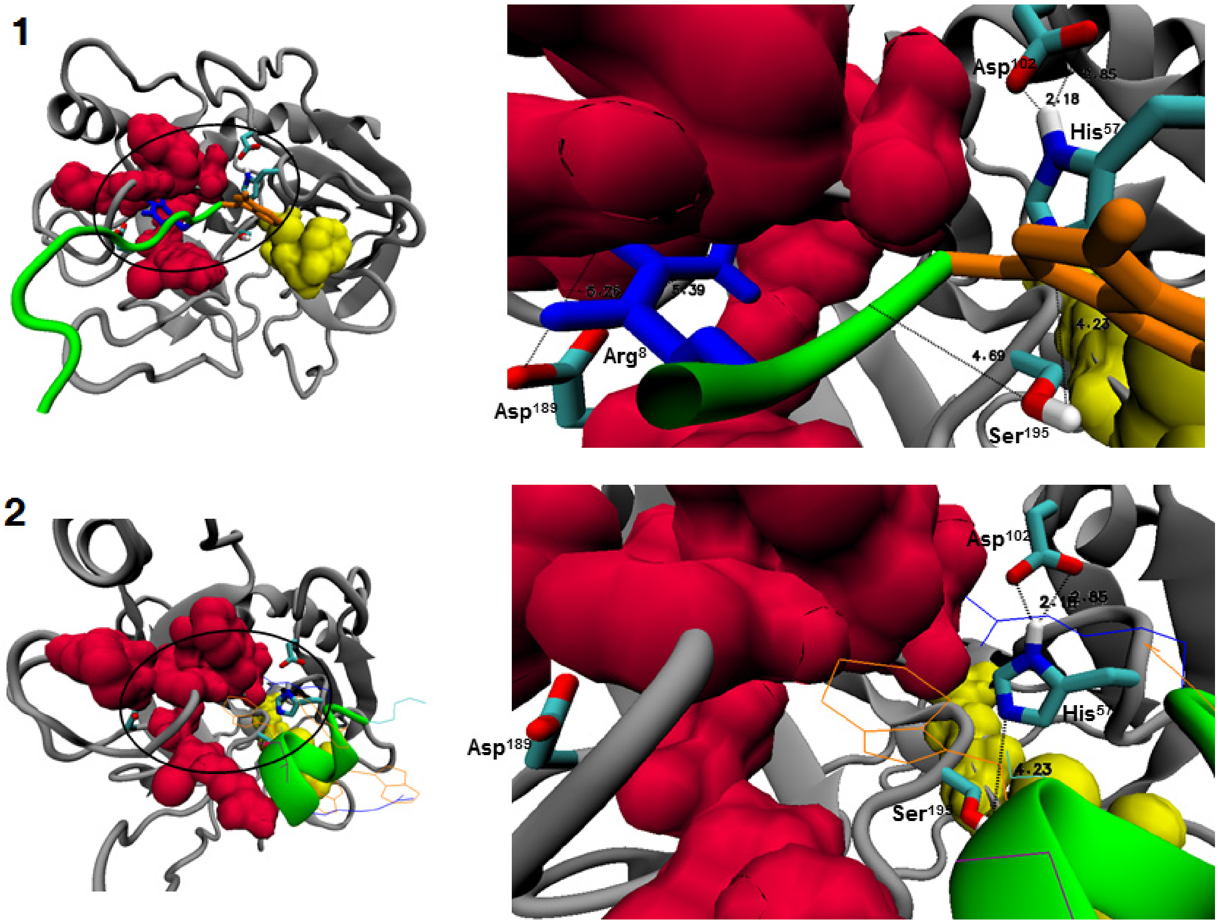
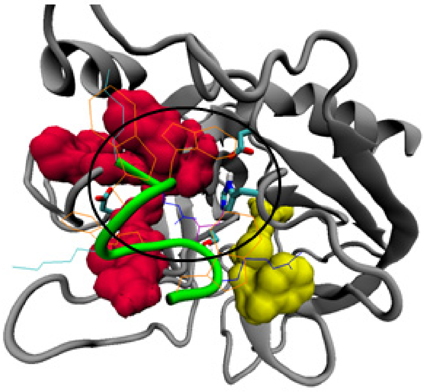
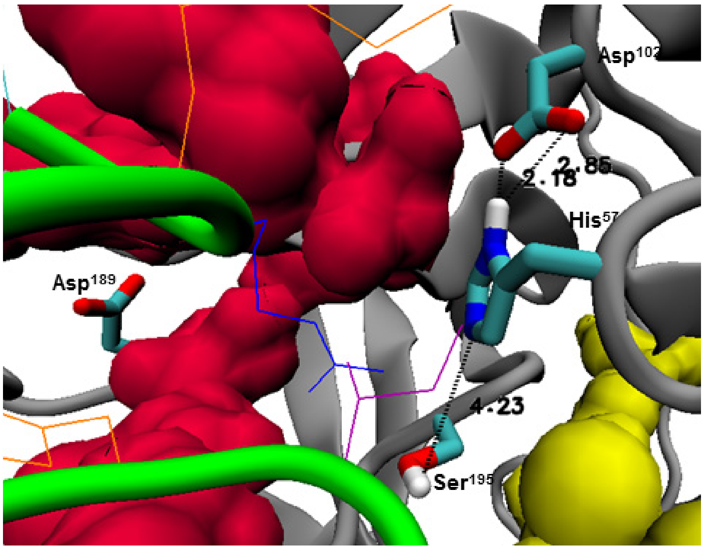


Figure S9. The molecular docked models of synthetic bactenecin 1 and its analogues with trypsin. The left image is the interaction mode between the peptides and trypsin and right panel is the inset shows the interaction from a close view. The enzyme binding pockets S1 (red color) and S1' (yellow color) are shown here. The colors in the peptide structures define as follow: Trp (orange), Ile (violet), Arg/Har (blue), Lys/Dab/Orn (cyan), and the peptide backbone (green).

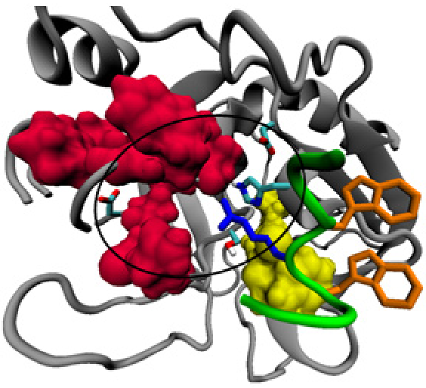


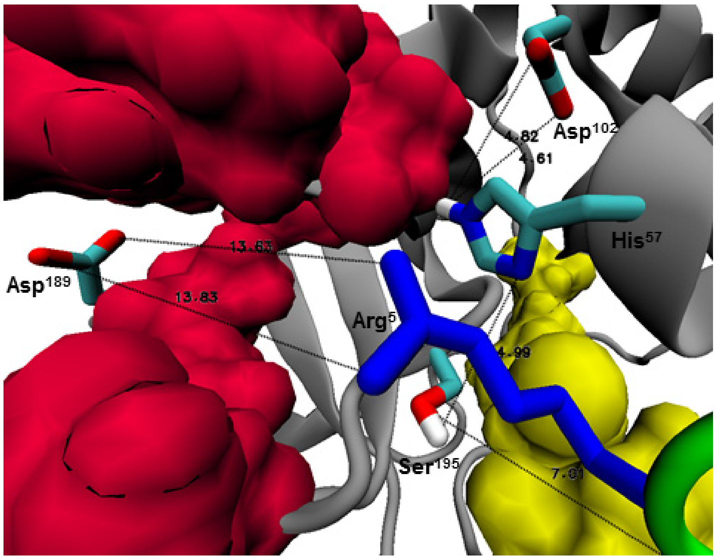
3



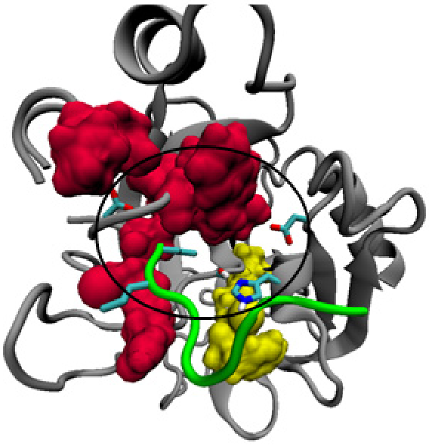

Asp^{102}
2.85
2.18
His^{57}
Asp^{189}
4.23
Ser^{195}

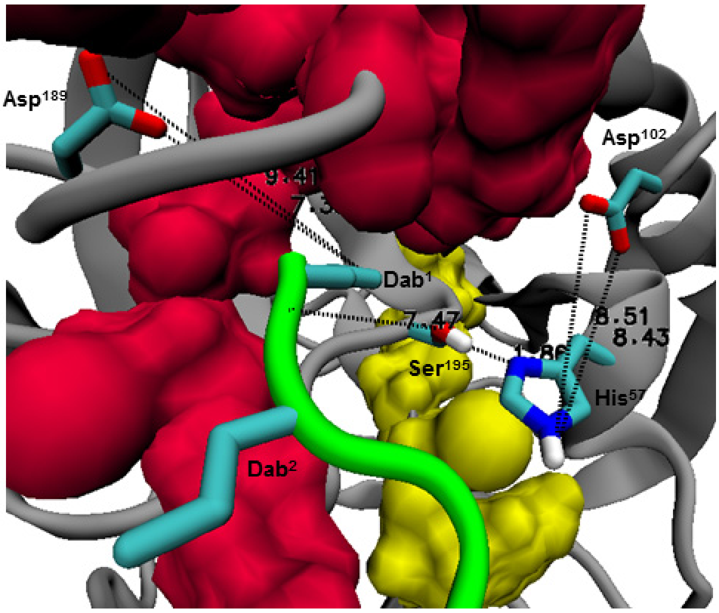
4



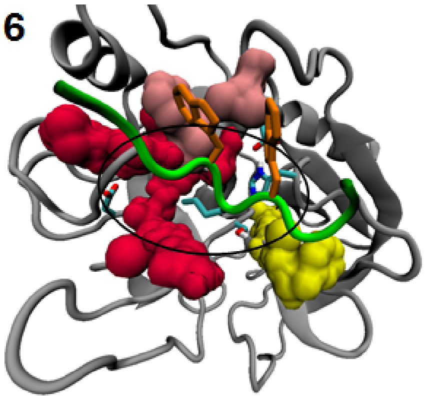

4.82
Asp^{102}
His^{57}
Asp^{189}
13.83
Arg^{5}
Ser^{195}

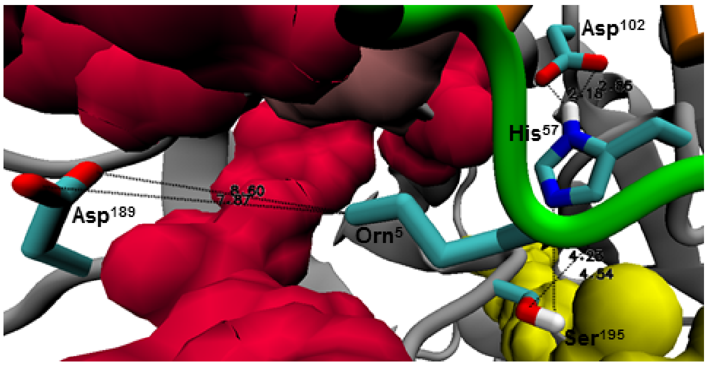
5



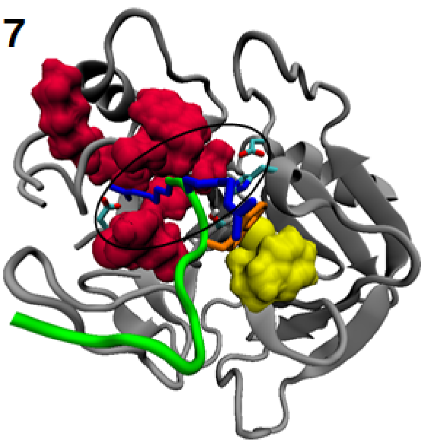

Asp^{189}
Asp^{102}
9.41
Dab^{1}
7.47
8.51
8.43
Ser^{195}
His^{57}
Dab^{2}

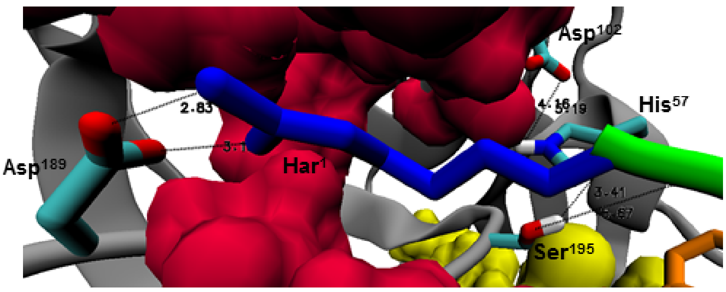
6



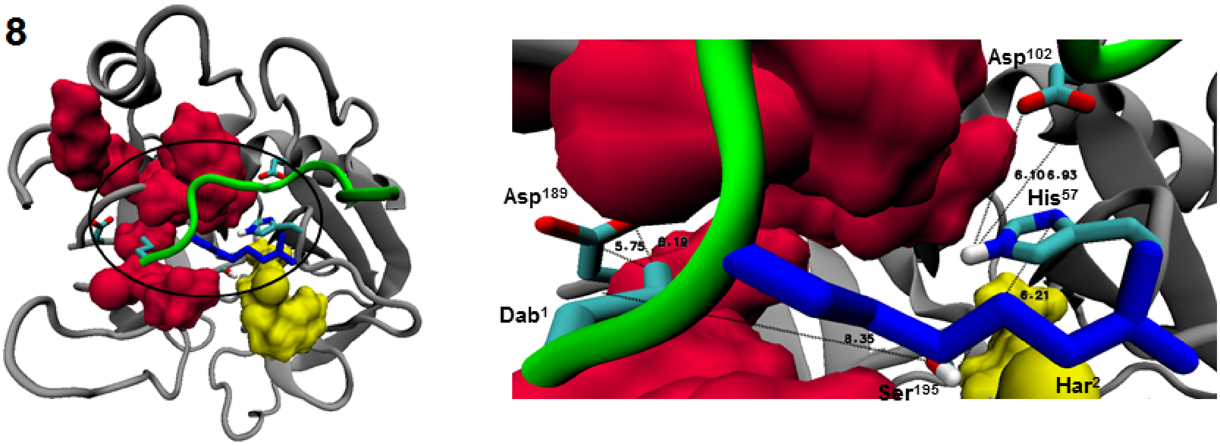

Asp^{102}
His^{57}
8.60
7.87
Asp^{189}
Orn^{5}
4.54
Ser^{195}

7




Asp^{102}
His^{57}
2.83
Asp^{189}
Har^{1}
3.41
Ser^{195}

8


Asp102
Asp189
5.75
6.19
6.10
6.93
His57
6.21
Dab1
8.35
Ser195
Har2

9

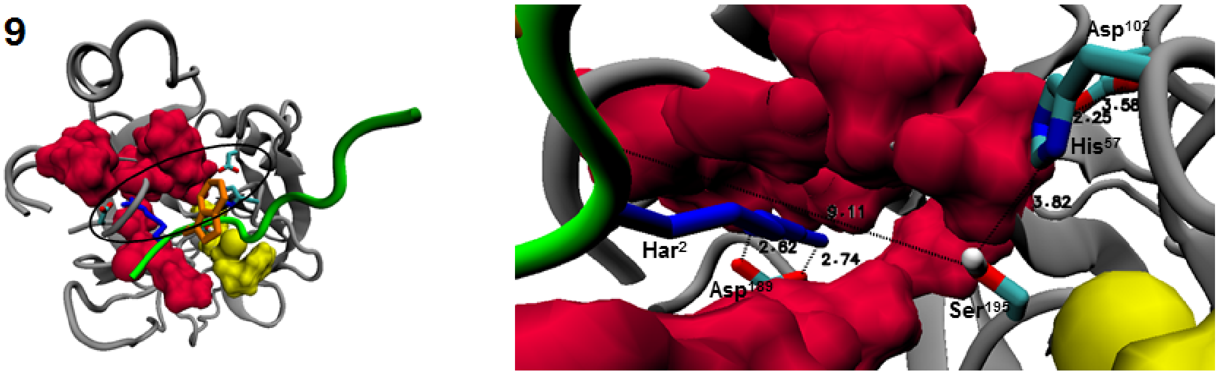
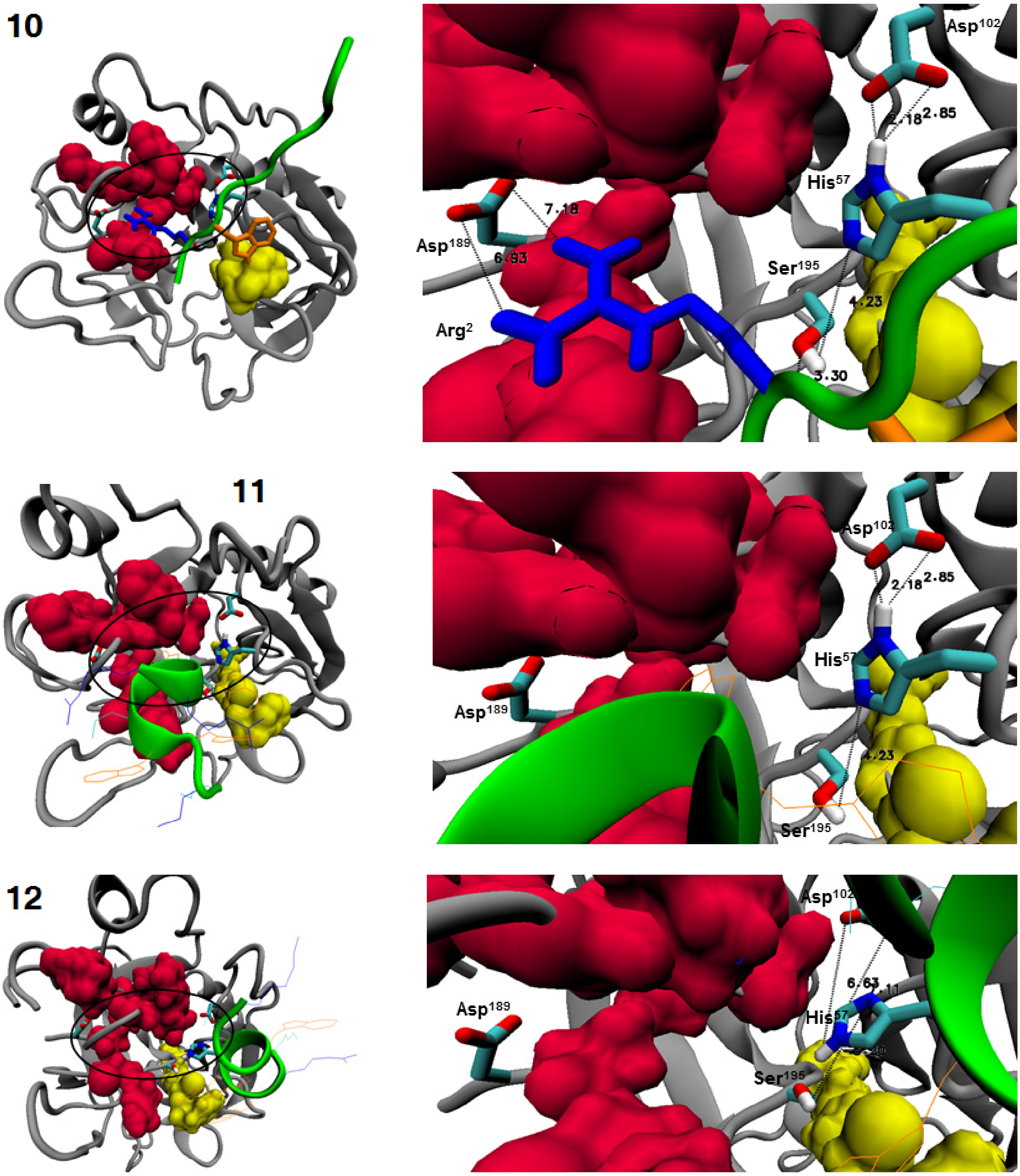
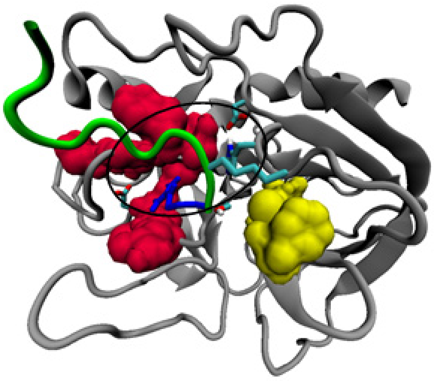
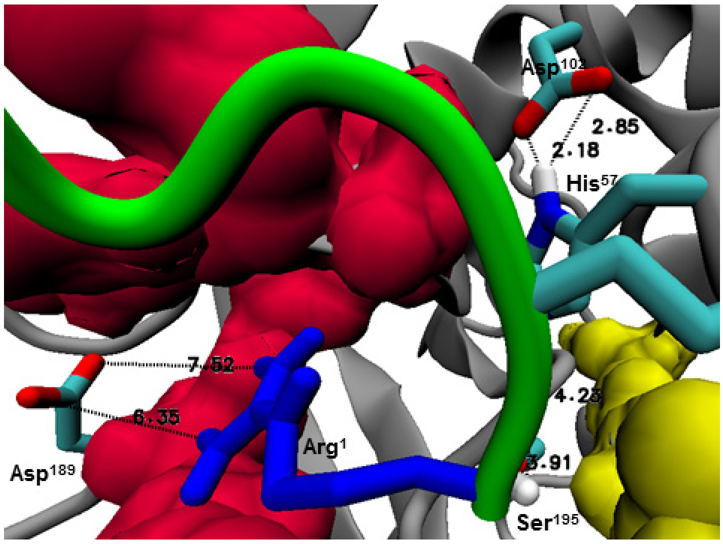

Asp102
3.58
2.25
His57
9.11
3.82
Har2
2.82
2.74
Asp189
Ser195

Figure S10. The molecular docked model of synthetic bactenecin 10 and its analogues with trypsin. The left image is the interaction mode between the peptides and trypsin and right panel is the inset shows the interaction from a close view. The enzyme binding pockets S1 (red color) and S1' (yellow color) are shown here. The colors in the peptide structures define as follow: Trp (orange), Arg/Har (blue), Lys/Dab/Orn (cyan) and the peptide backbone (green).



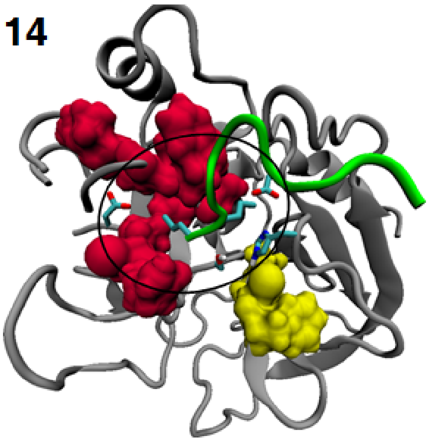
13

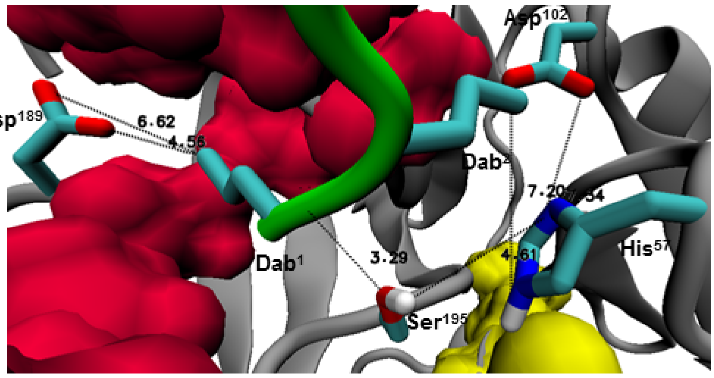




Asp102
2.85
2.18
His57
7.52
6.35
Arg1
4.23
3.91
Asp189
Ser195

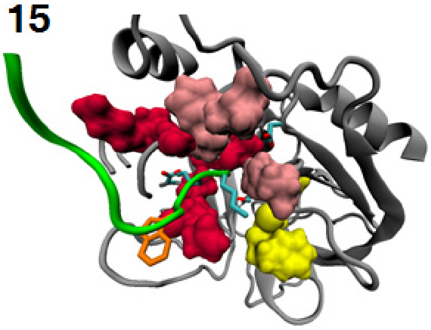
14

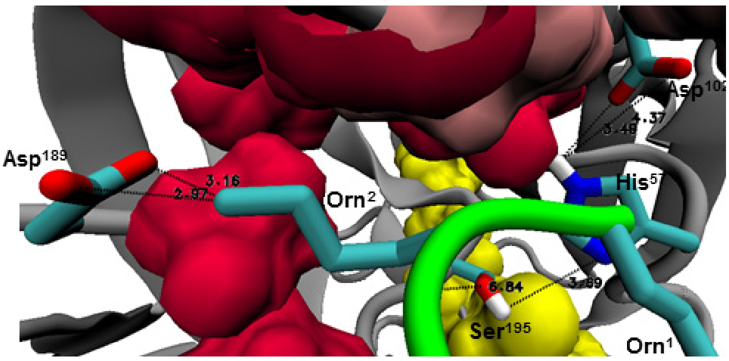




Asp102
Asp189
6.62
4.56
Dab2
7.20
Dab1
3.29
4.61
His57
Ser195

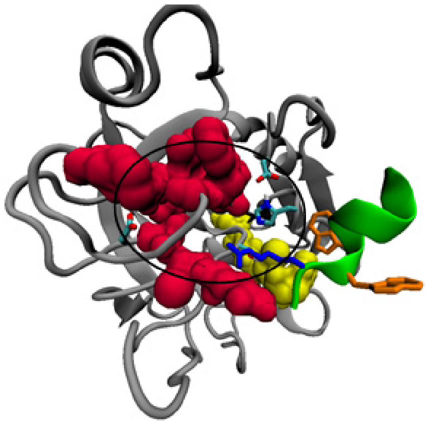
15

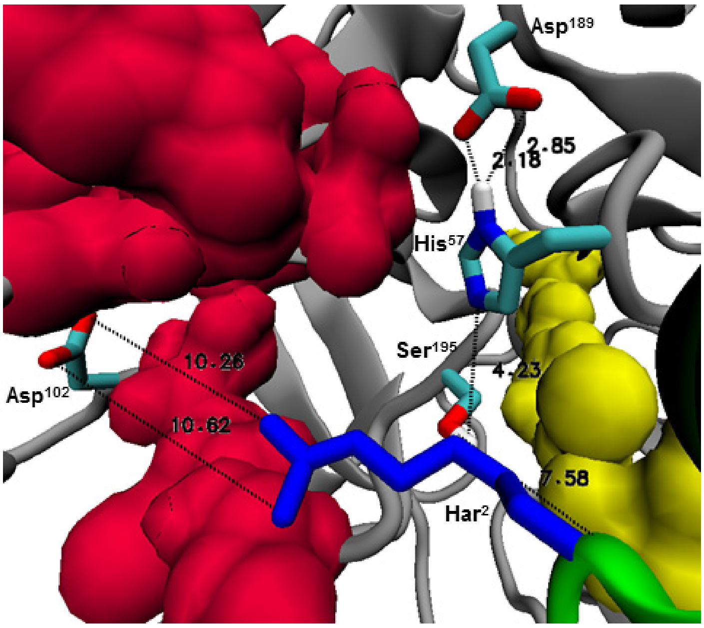




Asp102
4.37
Asp189
3.16
2.97
Orn2
His57
Ser195
Orn1

16

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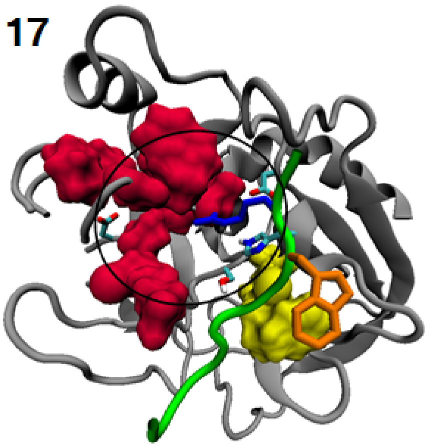
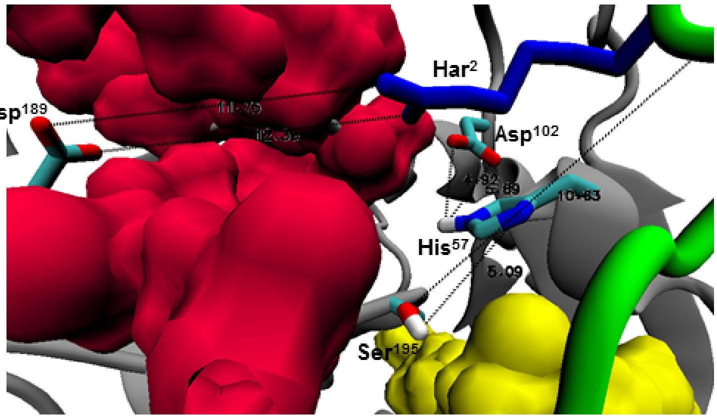
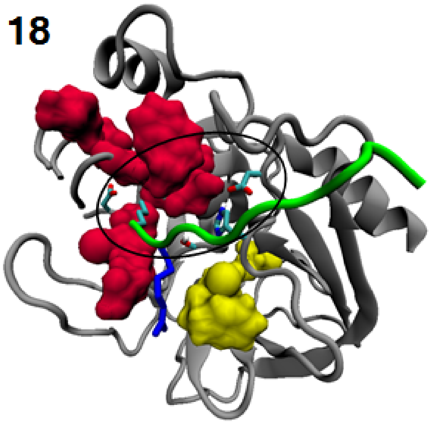
2.85
2.18
His^{57}
Ser^{195}
4.23
Asp^{102}
10.26
10.62
7.58
Har^{2}

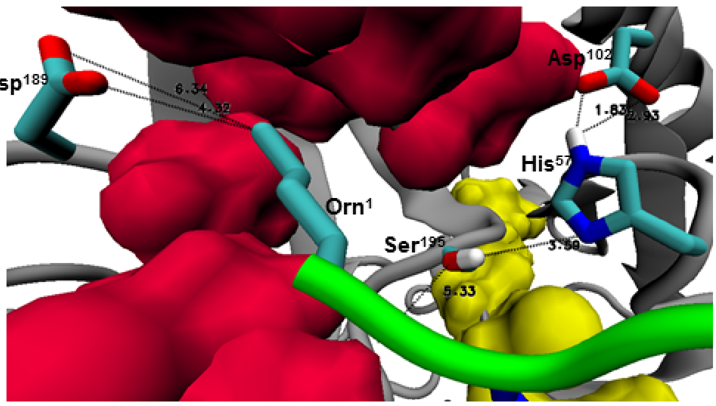
17




Har^{2}
Asp^{189}
Asp^{102}
His^{57}
5.09
Ser^{195}

18




Asp^{189}
6.34
4.32
Asp^{102}
His^{57}
Orn^{1}
Ser^{195}
5.33