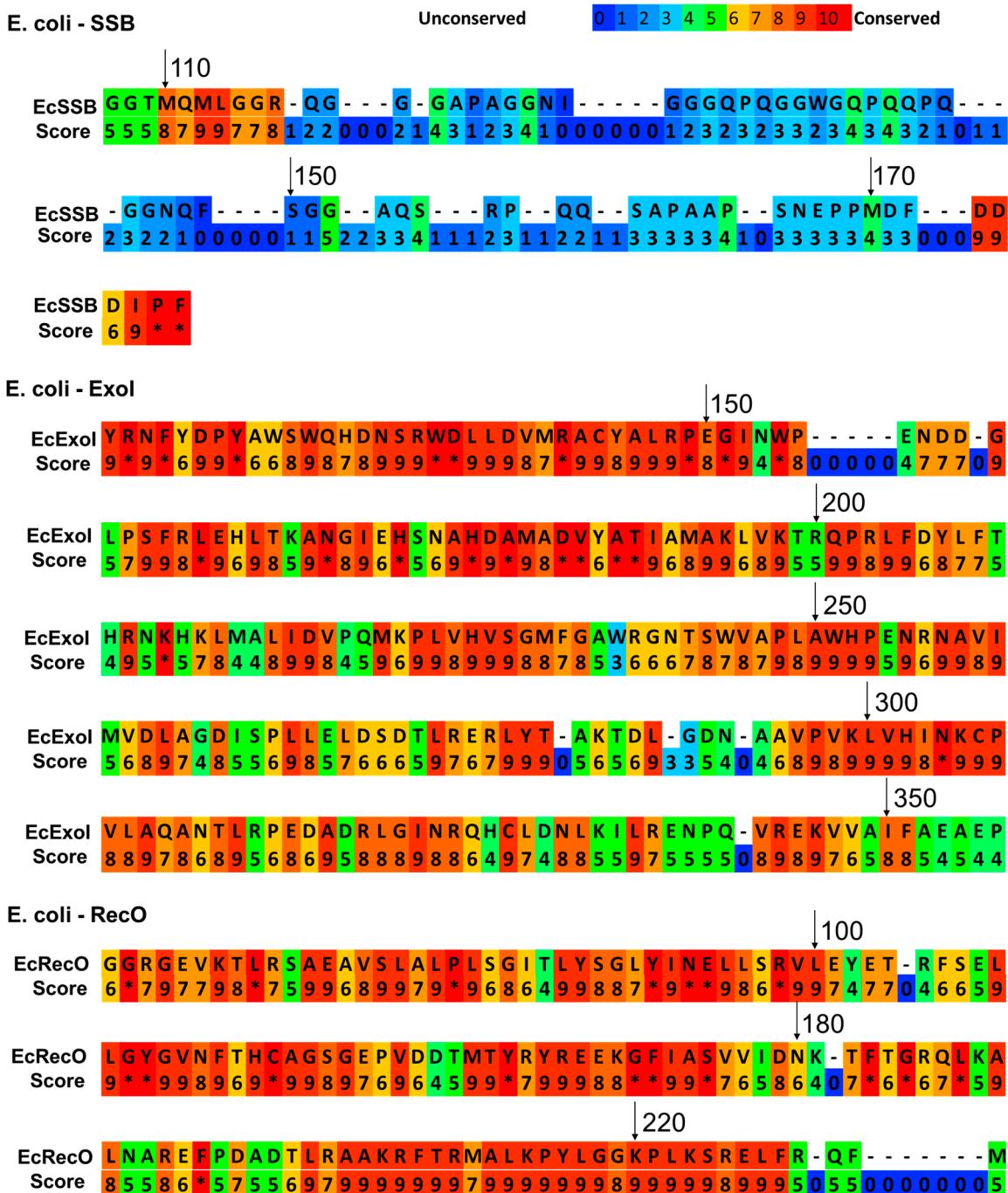


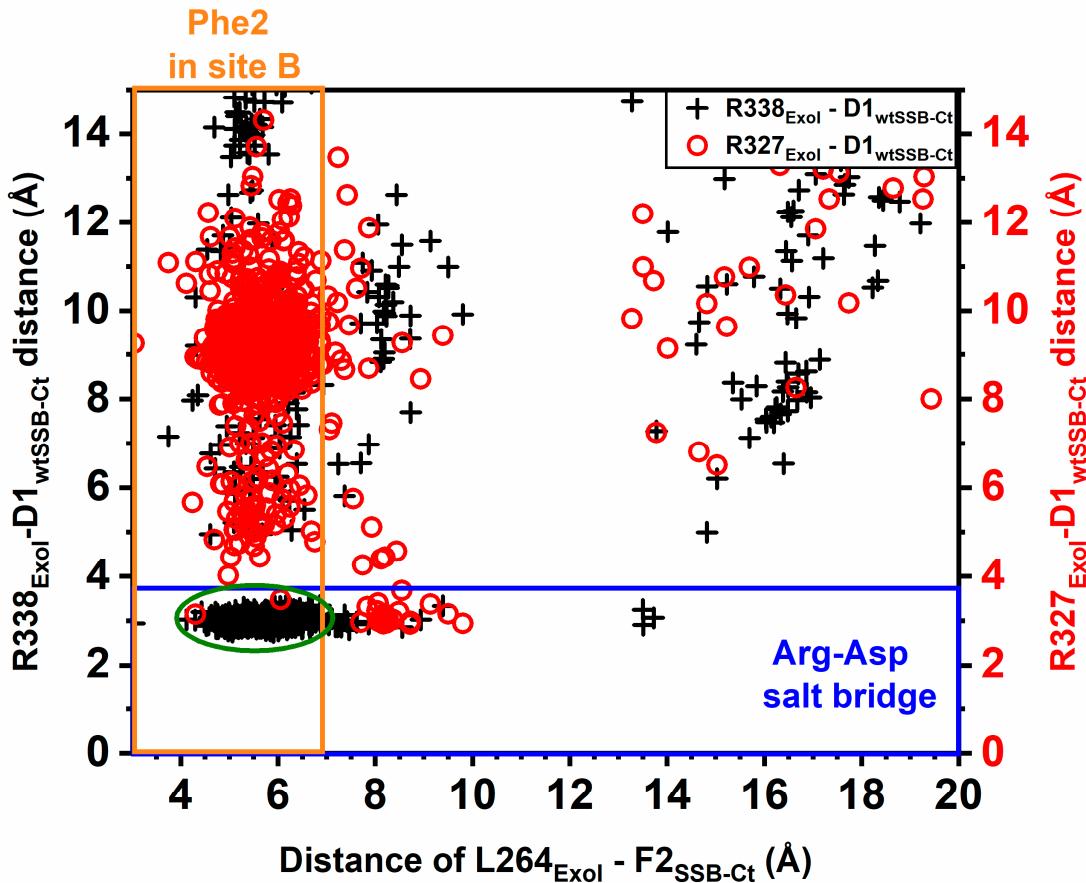
# Supplementary Materials: Structural adaptation of the single-stranded DNA-binding protein C-terminal to DNA metabolizing partners guides inhibitor design

Attila Tököli, Brigitta Bodnár, Ferenc Bogár,‡ Gábor Paragi, Anasztázia Hetényi, Éva Bartus, Edit Wéber† Zsófia Hegedüs, Zoltán Szabó, Gábor Kecskeméti, Gerda Szakonyi, and Tamás A. Martinek

Supplementary information:

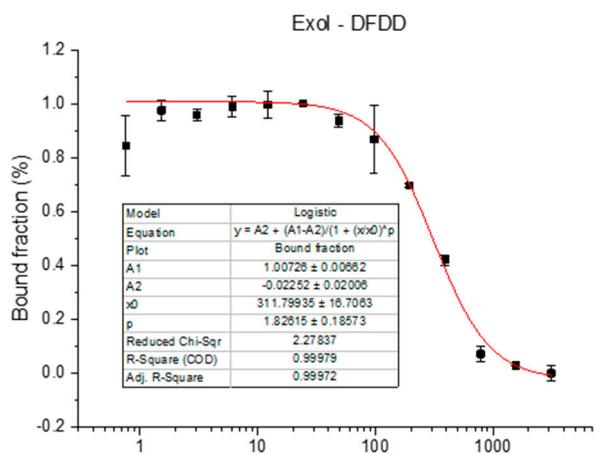
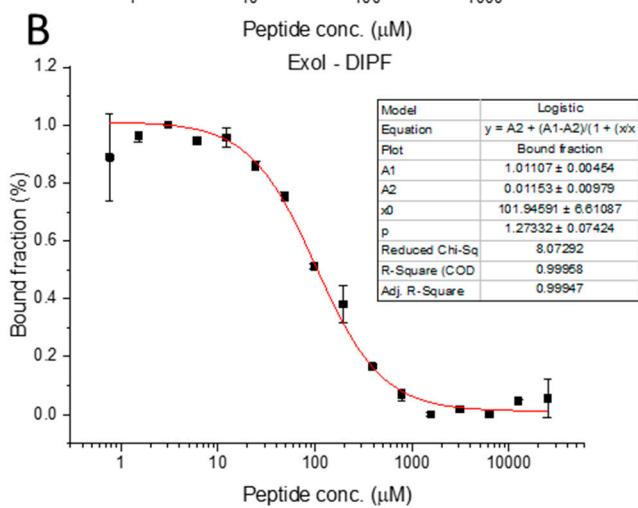
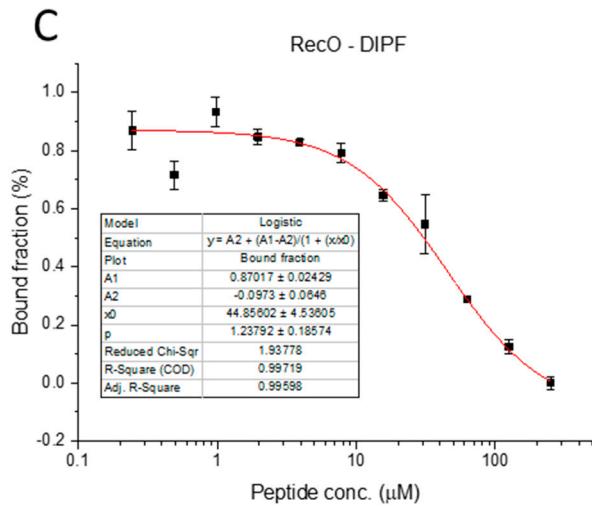


**Figure S1.** Sequence conservation across 250 eubacterial species shown for (A) SSB C-terminal tail, (B) ExoI, and (C) RecO focusing on segments important for SSB-SIP interactions. NCBI BLAST version 2.12.0. and Uniprot database (accession date: 28.11.2022.) was used for the search filtered for proteobacteria (taxid: 1224). The best 250 non-redundant sequences were further processed. The following parameters were used in PRALINE algorithm.<sup>1</sup> Exchange weights matrix: BLOSUM62, Associated gap penalties:12, Progressive alignment strategy: PSI-BLAST pre-profile processing (Homology-extended alignment). Structural features: DSSP-defined secondary structure search (YES), Secondary structure prediction (PSIPRED), Transmembrane structure prediction (NO). Output customization: Tree representation final alignment (NO), Customize alignment colours (NO), File format final alignment (FASTA).

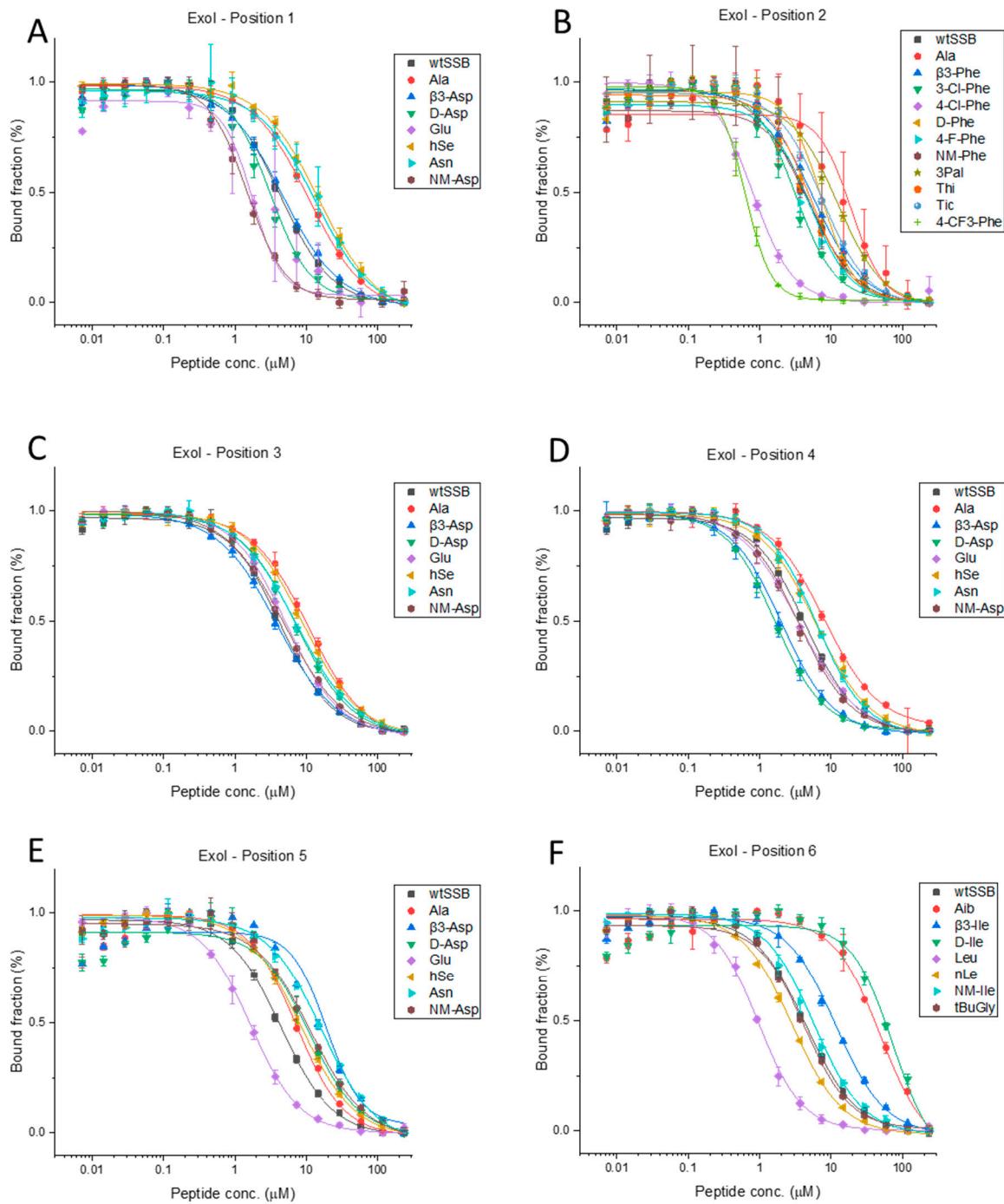


**Figure S2.** Distribution of the R338<sub>ExoI</sub>-D1<sub>wtSSB-Ct</sub> distance against the L264<sub>ExoI</sub>-F2<sub>wtSSB-Ct</sub> distance (black cross). Distribution of the R327<sub>ExoI</sub>-D1<sub>wtSSB-Ct</sub> distance against L264<sub>ExoI</sub>-F2<sub>wtSSB-Ct</sub> distance (red circle). Each symbol represents a trajectory point from the lowest temperature replica of the REST simulation. The population appearing within the green ellipse indicates conformations with Phe2 bound to site B and the concomitant stabilizing salt bridge formed by Asp1 of wtSSB-Ct. Low distance for R338<sub>ExoI</sub>-D1<sub>wtSSB-Ct</sub> is predominant over that for R327<sub>ExoI</sub>-D1<sub>wtSSB-Ct</sub>.

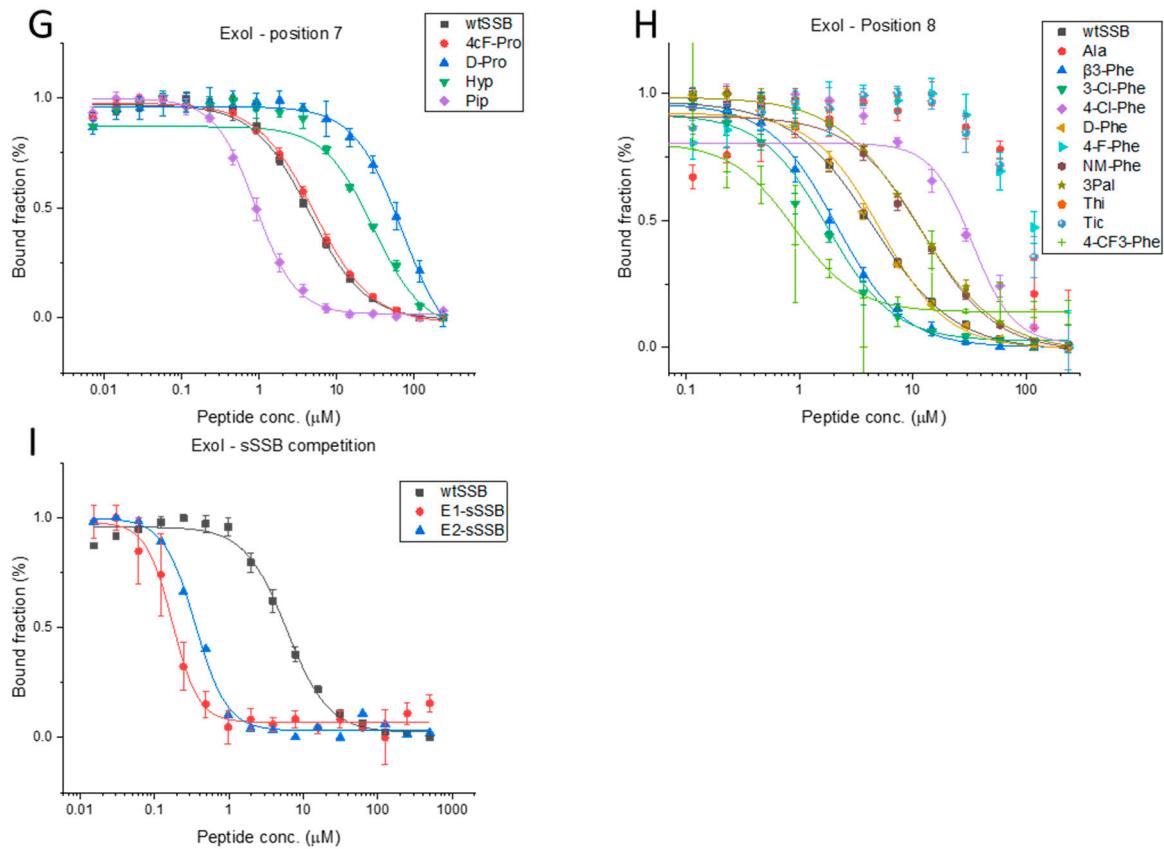
<sup>1</sup> Bawono and Heringa, "PRALINE."

**A****B****C**

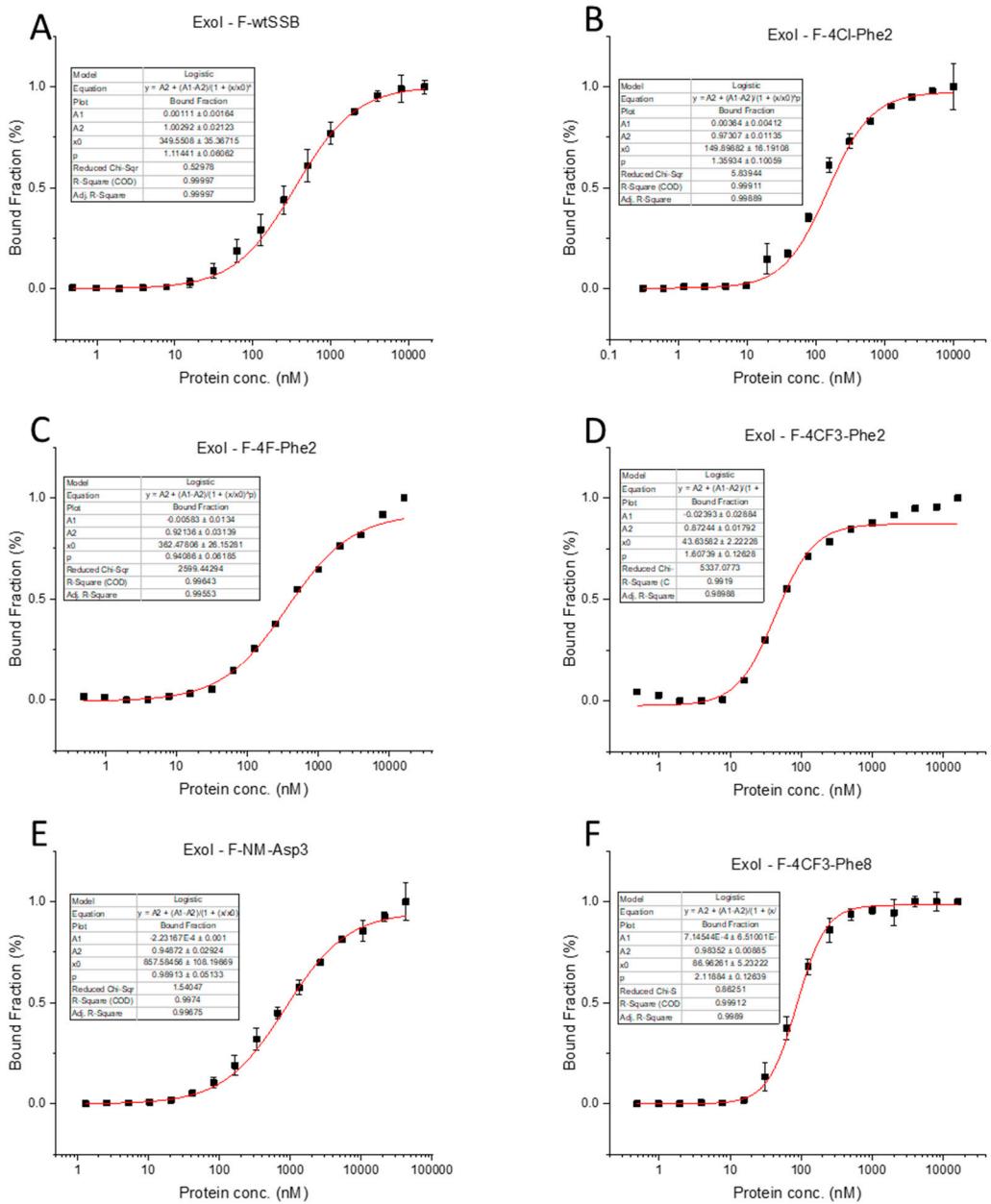
**Figure S3.** Competitive fluorescence anisotropy titration curves for the ExoI-DFDD (A), ExoI-DIPF (B) and RecO-DIPF (C) interactions.



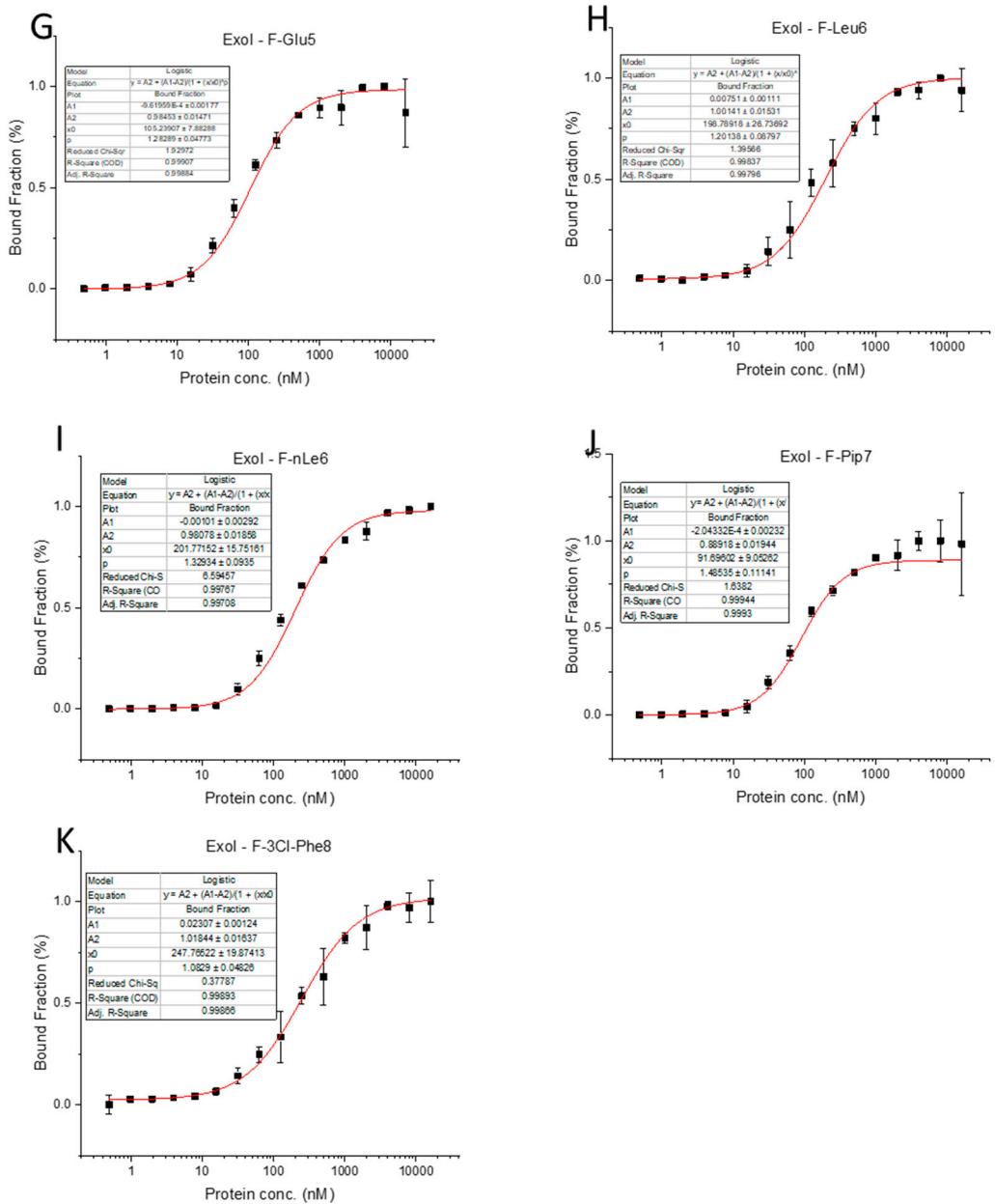
**Figure S4 (a).** ExoI-mSSB competitive fluorescence anisotropy titration curves for each position (A-F).



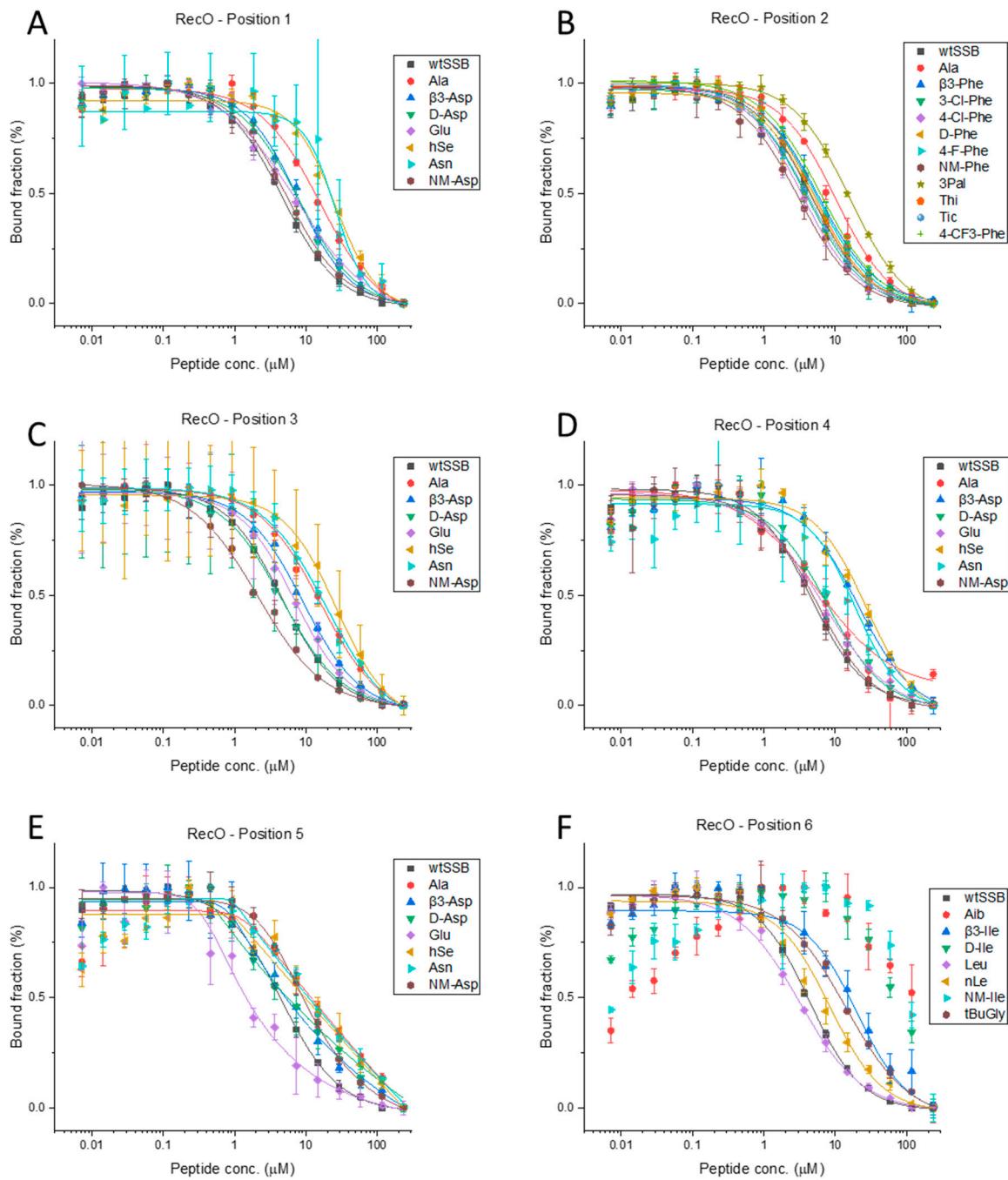
**Figure S4 (b).** ExoI-mSSB competitive fluorescence anisotropy titration curves for each position (G-H). ExoI-sSSB competition curves (I).



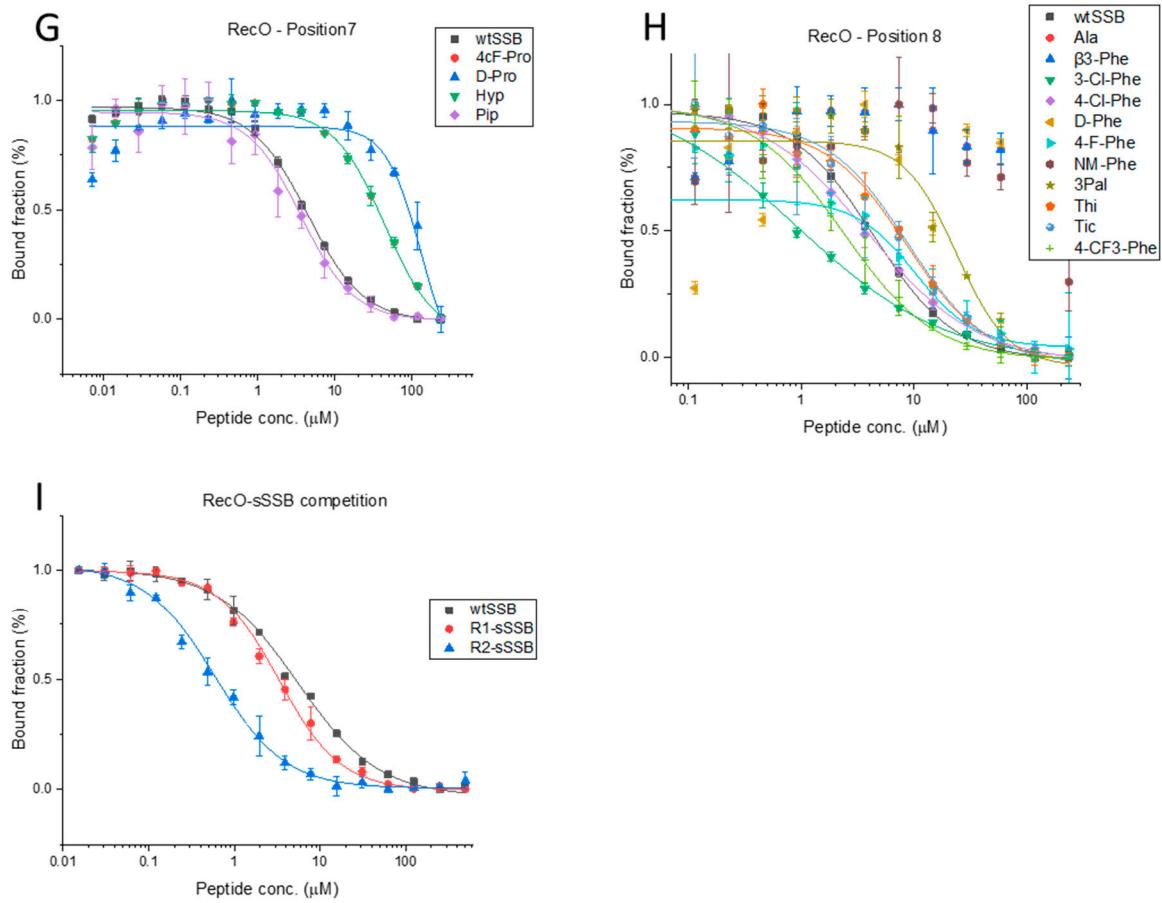
**Figure S5 (a).** Direct titration of F-mSSBs to ExoI. Validation of competitive fluorescence anisotropy hits.



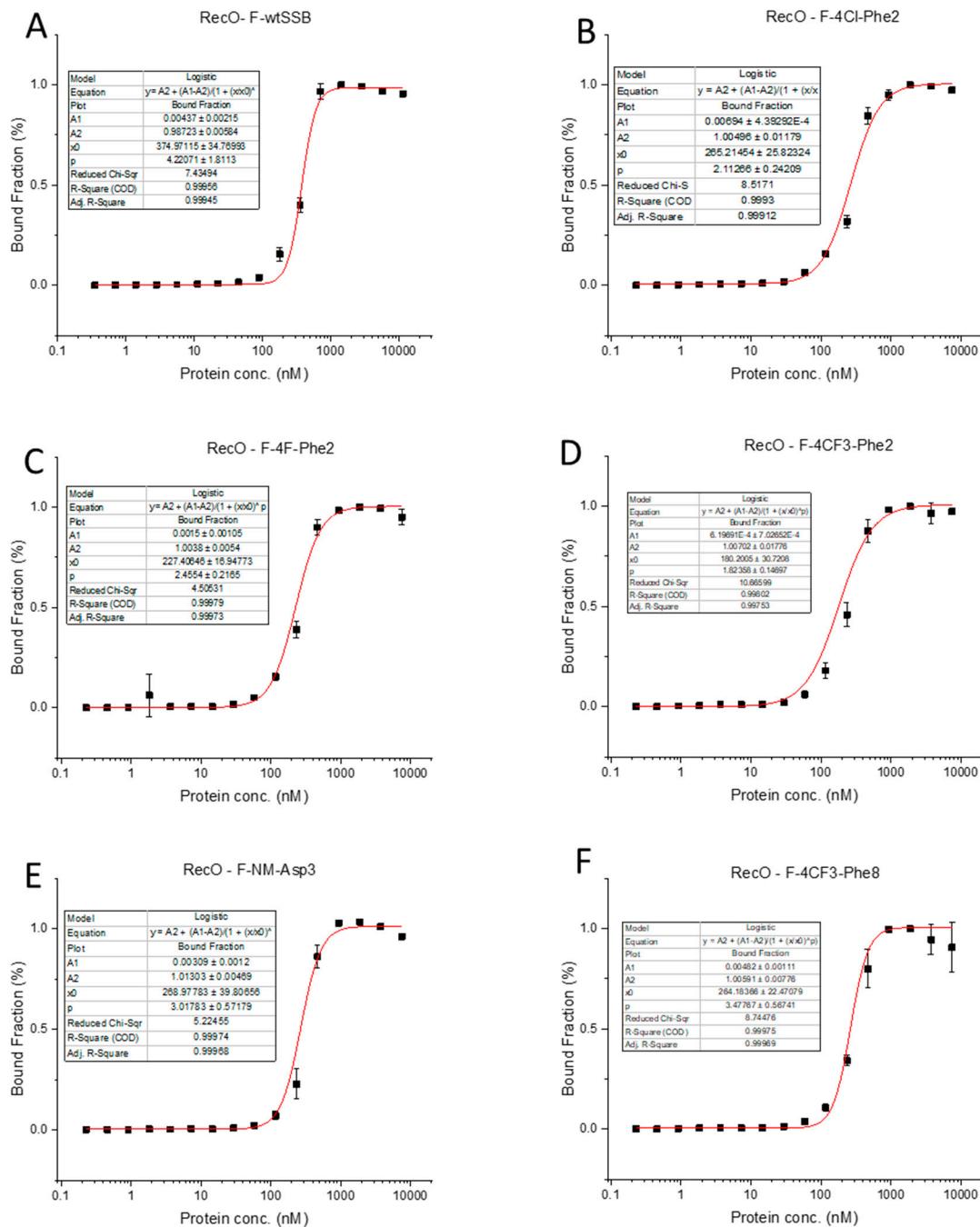
**Figure S5 (b).** Direct titration of F-mSSBs to ExoI. Validation of competitive fluorescence anisotropy hits.



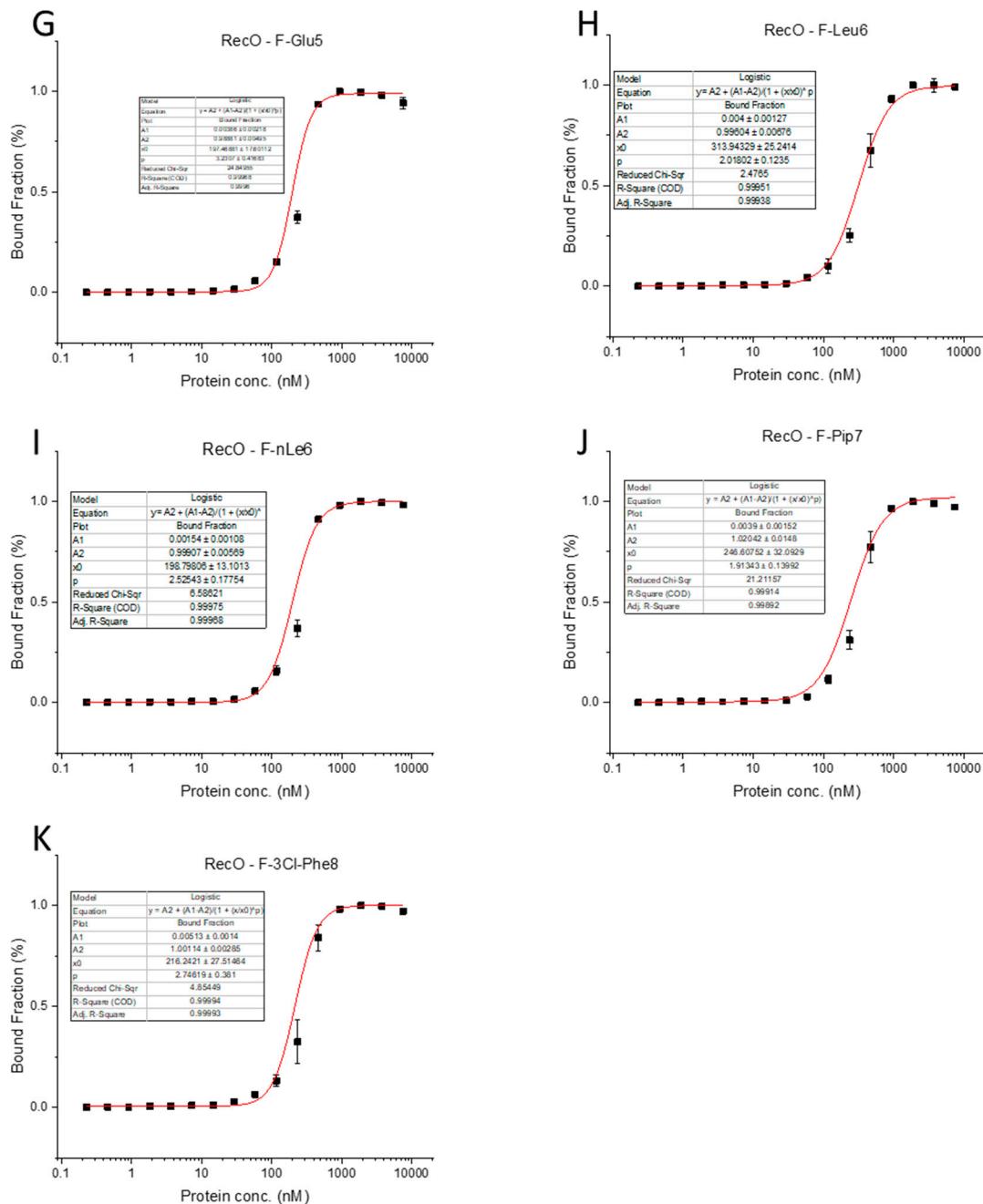
**Figure S6. a.** RecO-mSSB competitive fluorescence anisotropy titration curves for each position (A-F).



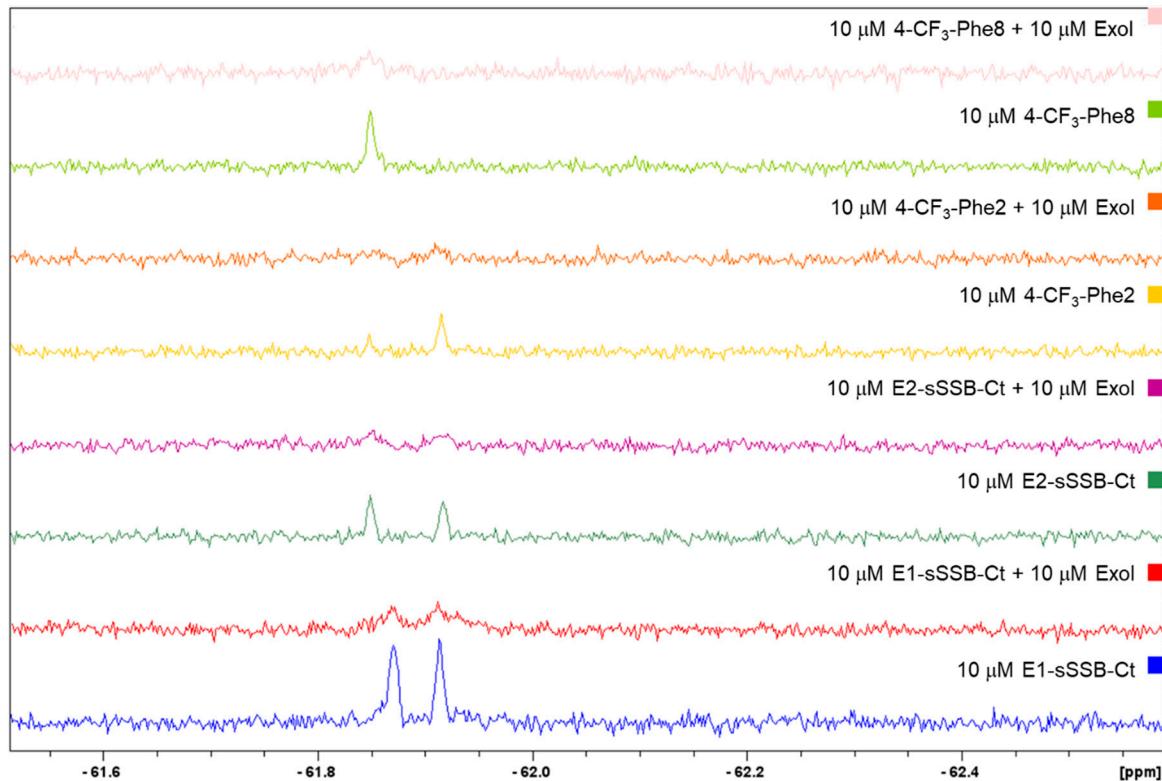
**Figure S6. b.** RecO-mSSB competitive fluorescence anisotropy titration curves for each position (G-H). RecO-sSSB competition curves (I).



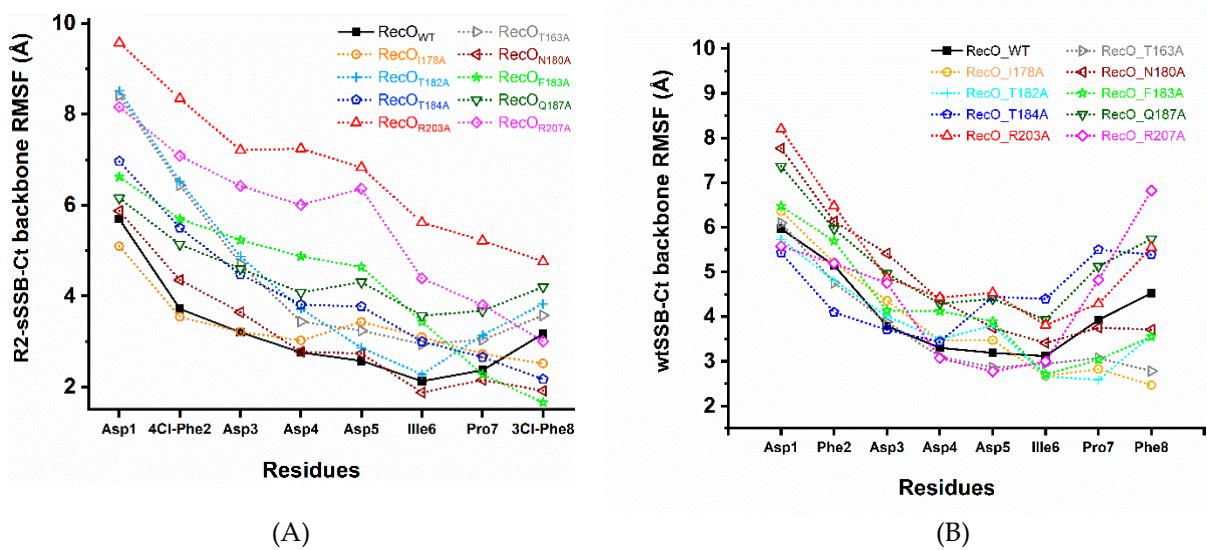
**Figure S7. a.** Direct titration of F-mSSBs to RecO. Validation of competitive fluorescence anisotropy hits.

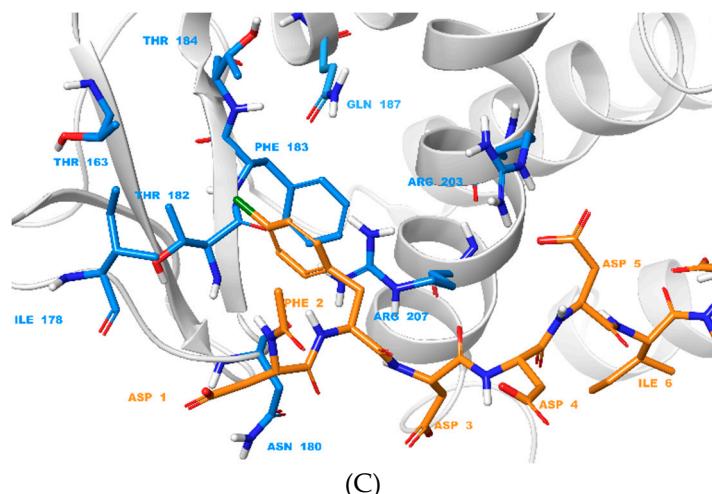


**Figure S7. b.** Direct titration of F-mSSBs to RecO. Validation of competitive fluorescence anisotropy hits.

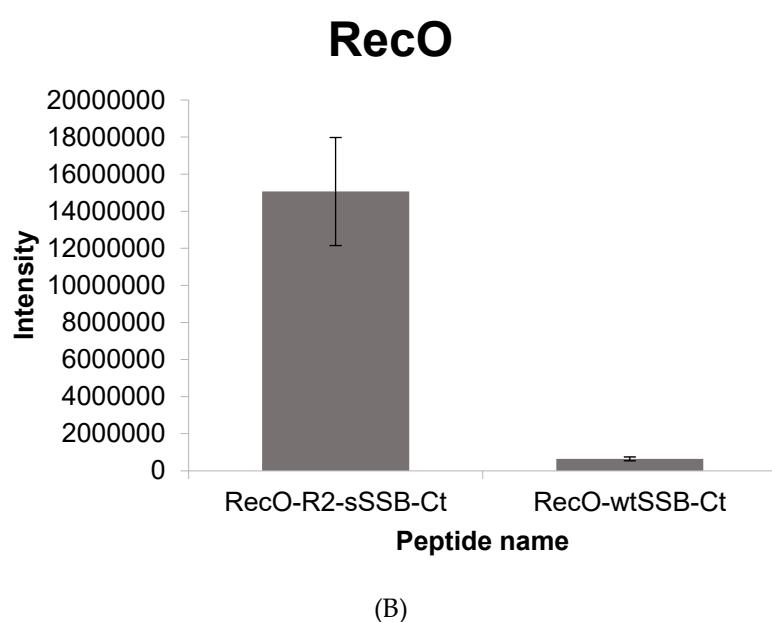
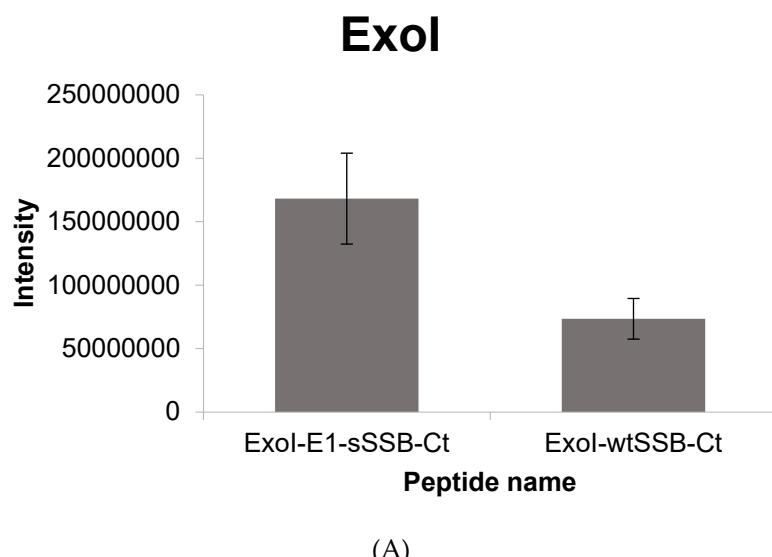


**Figure S8.**  $^{19}\text{F}$  NMR measurements carried out for E1-sSSB-Ct, and the single mutated derivatives 4-CF<sub>3</sub>-Phe8 and 4-CF<sub>3</sub>-Phe2 with and without ExoI.





**Figure S9.** RMSF values of backbone atom coordinates of A) R2-sSSB-Ct and B) wtSSB-Ct in complex with the wild-type and Ala-mutated RecO proteins. Mutated residues: Thr163, Ile178, Asn180, Thr182, Phe183, Thr184, Gln187, Arg203, and Arg207. C) A binding pose model of R2-sSSB-Ct (orange) in complex with RecO and the positions of the Ala-mutated residues (blue).



**Figure S10.** Protein enrichment values are shown as intensities from pull-down experiments using wtSSB-Ct and sSSB-Ct peptides. Recombinant protein (ExoI or RecO) containing lysate was incubated with peptides immobilized on streptavidin beads. Pull-down assay was performed using E1-sSSB-Ct, and R2-sSSB-Ct for ExoI and RecO, respectively. wtSSB-Ct was used as a control for both proteins. Protein-enriched beads were trypsinized, and tryptic peptides were measured using HPLC-MS. ExoI shows two-fold enrichment on E1-sSSB-Ct compared with wtSSB-Ct (A). RecO was enriched on R2-sSSB-Ct compared with wtSSB-Ct (B).

**Table S1.** Competitive fluorescence anisotropy data of ExoI. IC<sub>50</sub> values are shown in μM.

Pos	Peptide	IC <sub>50</sub> (μM)
	wt	4.54 ± 0.34
1	β <sup>3</sup> -Asp	4.47 ± 0.25
	D-Asp	5.55 ± 0.19
	Glu	1.33 ± 0.09
	Asn	9.31 ± 0.4
	NM-Asp	2.17 ± 0.15
2	β <sup>3</sup> -Phe	3.8 ± 0.11
	3-Cl-Phe	2.03 ± 0.51
	4-Cl-Phe	0.56 ± 0.03
	D-Phe	3.49 ± 2.41
	4-F-Phe	1.93 ± 0.81
	NM-Phe	5.37 ± 0.53
	3Pal	7.47 ± 0.88
	Thi	4.22 ± 0.81
	Tic	6.6 ± 0.55
	4-CF <sub>3</sub> -Phe	0.33 ± 0.02
3	β <sup>3</sup> -Asp	3.65 ± 0.16
	D-Asp	6.82 ± 0.53
	Glu	4.03 ± 0.24
	Asn	6.36 ± 0.36
	NM-Asp	3.88 ± 0.13
4	β <sup>3</sup> -Asp	1.54 ± 0.13
	D-Asp	3.08 ± 0.07
	Glu	3.58 ± 0.11
	Asn	6.02 ± 0.3
	NM-Asp	1.27 ± 0.14
5	β <sup>3</sup> -Asp	13.94 ± 6.11
	D-Asp	N/A
	Glu	1.49 ± 0.1
	Asn	7.92 ± 0.62
	NM-Asp	9.3 ± 0.66
6	Aib	27.55 ± 7.9
	β <sup>3</sup> -Ile	11.28 ± 0.03
	D-Ile	56.01 ± 18.42
	Leu	0.92 ± 0.06
	nLe	2.68 ± 0.13
	NM-Ile	3.95 ± 0.54
	tBuGly	2.87 ± 0.26
7	D-Pro	61.54 ± 16.18
	4-cF-Pro	3.79 ± 0.36
	Hyp	18.97 ± 3.07
	7Pip	0.89 ± 0.14

8	β-Phe	N/A
	3-Cl-Phe	3.4 ± 2.41
	4-Cl-Phe	1.41 ± 0.18
	D-Phe	N/A
	4-F-Phe	3.04 ± 0.64
	NM-Phe	N/A
	3Pal	19.91 ± 2.81
	Thi	7.72 ± 0.51
	Tic	96.06 ± 83.78
	4-CF3-Phe	0.39 ± 0.07
super	E1-sSSB	0.166 ± 0.11
	E2-sSSB	

**Table S2.** Competitive fluorescence anisotropy data of RecO. IC<sub>50</sub> values are shown in μM.

Pos	Peptide	IC <sub>50</sub> (μM)
	wt	4.66 ± 0.24
1	β3-Asp	8.03 ± 0.4
	D-Asp	6.56 ± 1.4
	Glu	5.17 ± 1.1
	Asn	13.85 ± 17.2
	NM-Asp	5.1 ± 0.64
2	β3-Phe	3.66 ± 0.61
	3-Cl-Phe	3.44 ± 0.14
	4-Cl-Phe	2.38 ± 0.27
	D-Phe	2.75 ± 0.59
	4-F-Phe	3.05 ± 0.28
	NM-Phe	3.33 ± 0.35
	3Pal	11.68 ± 0.8
	Thi	5.27 ± 0.28
	Tic	3.99 ± 0.1
	4-CF3-Phe	3.27 ± 0.42
3	β3-Asp	8.92 ± 0.39
	D-Asp	4.56 ± 1.53
	Glu	5.81 ± 1.65
	hSe	25.05 ± 10.02
	Asn	14.21 ± 1.8
	NM-Asp	1.88 ± 0.09
4	β3-Asp	19.59 ± 2.78
	D-Asp	5.92 ± 0.91
	Glu	5.68 ± 0.59
	Asn	15.7 ± 3.54
	NM-Asp	3.85 ± 1.03
5	β3-Asp	5.91 ± 0.68
	D-Asp	N/A
	Glu	1.41 ± 0.55
	Asn	16.01 ± 0.51
	NM-Asp	9.33 ± 4.3
6	Aib	N/A
	β3-Ile	19.21 ± 7.47

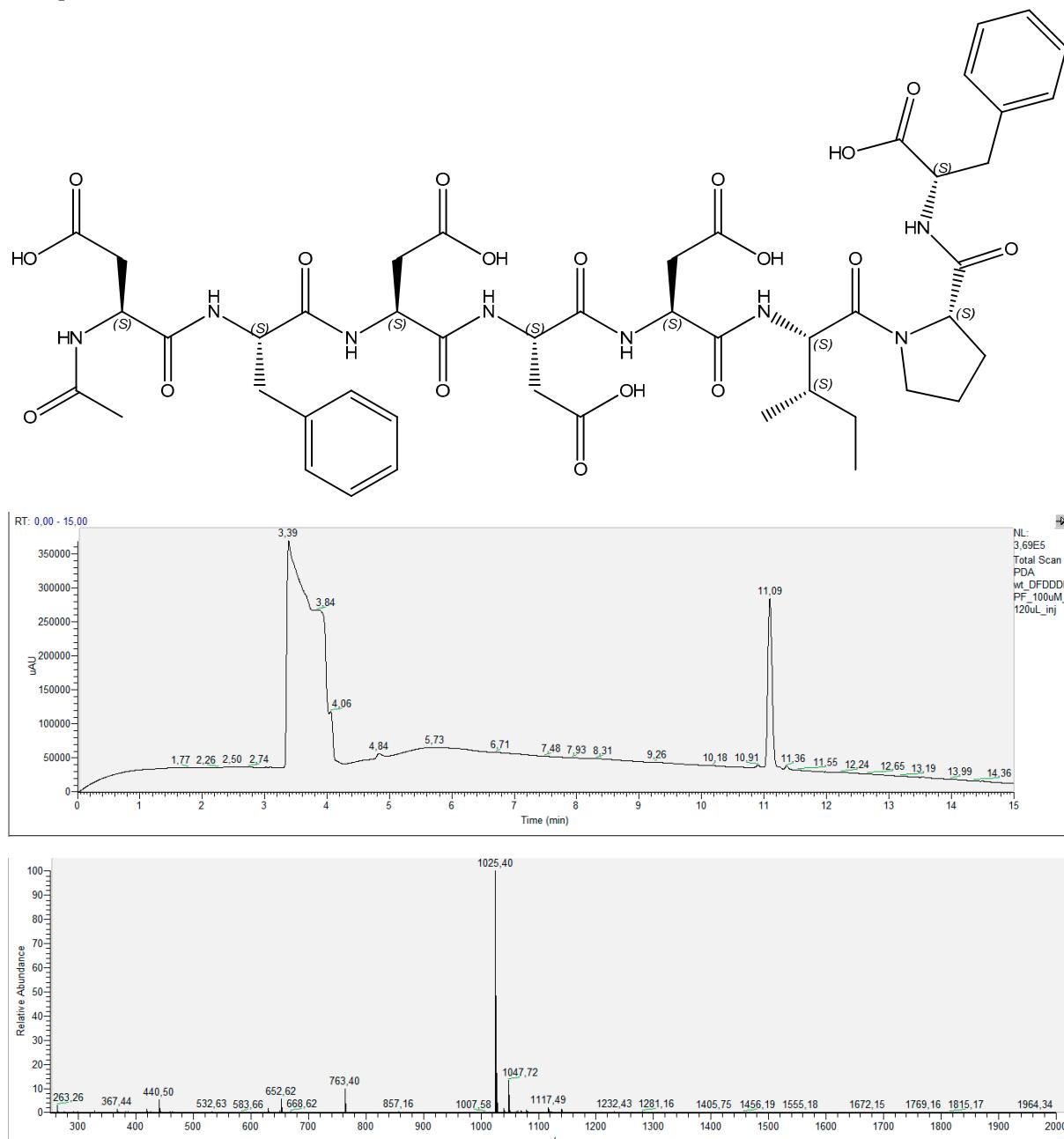
	D-Ile	N/A
	Leu	3.23 ± 0.16
	nLe	2.55 ± 0.23
	NM-Ile	9.36 ± 0.08
	tBuGly	10.42 ± 1.02
7	D-Pro	11.62 ± 10.94
	4-cF-Pro	240.77 ± 199.68
	Hyp	34.56 ± 10.94
	7Pip	3.31 ± 0.44
8	β3-Phe	N/A
	3-Cl-Phe	0.36 ± 0.03
	4-Cl-Phe	3.62 ± 0.84
	D-Phe	N/A
	4-F-Phe	4.07 ± 7.98
	NM-Phe	64.67 ± 18.92
	3Pal	31.87 ± 4.16
	Thi	8.59 ± 7.26
	Tic	141.27 ± 8.37
	4-CF3-Phe	5.97 ± 2.43
super	R1-sSSB	3.33 ± 0.28
	R2-sSSB	0.59 ± 0.05

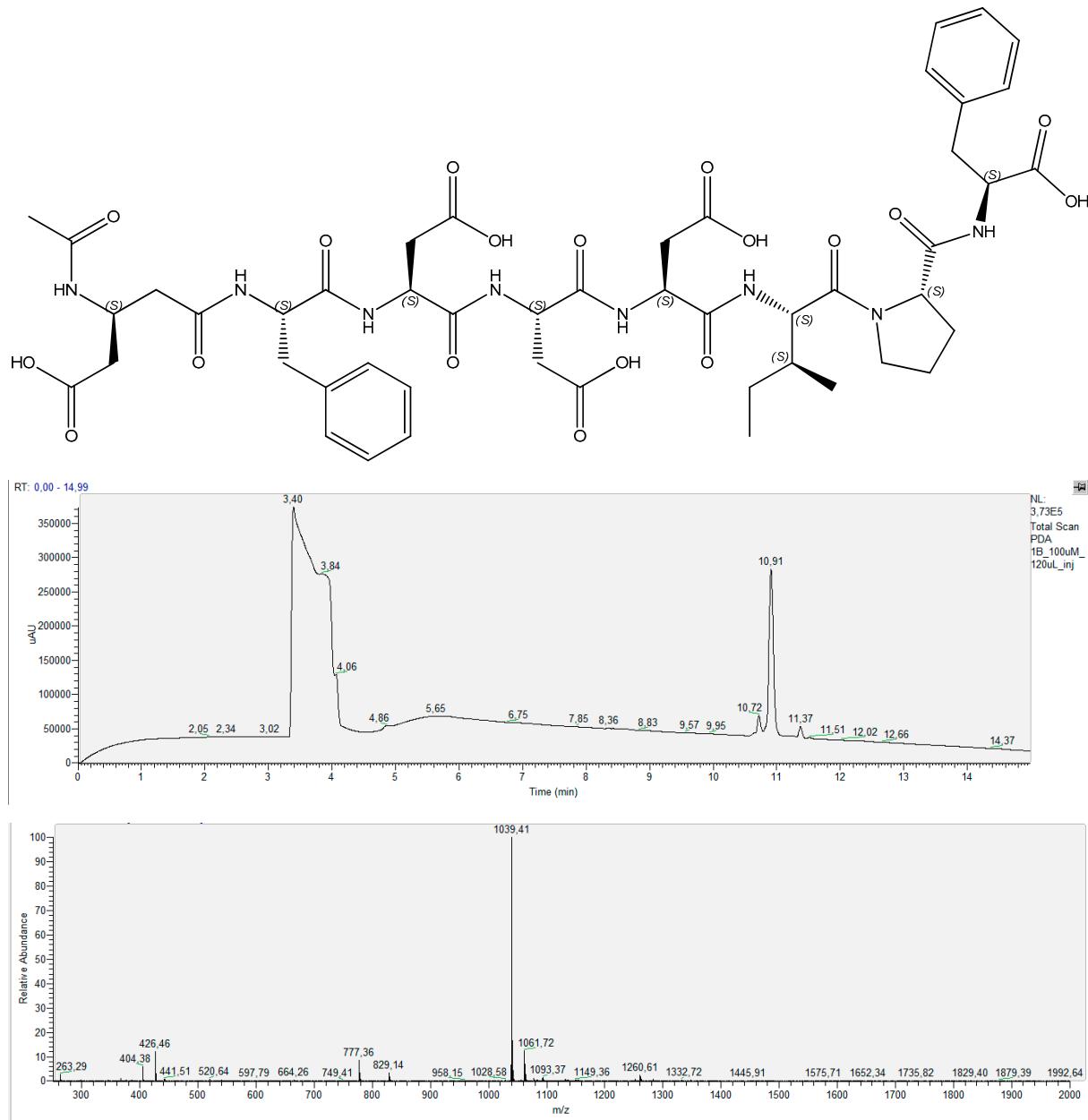
**Table S3.** Mass spectrometry data of SSB analogs.

	compound	MW	calculated m/z	detected m/z
1	wtSSB	1025.02	1024.40	1025.40
2	β <sup>3</sup> -Asp1	1039.05	1038.42	1039.41
3	D-Asp1	1025.02	1024.40	1025.13
4	Glu1	1039.05	1038.42	1039.25
5	Asn1	1024.04	1023.42	1024.15
6	NM-Asp1	1039.05	1038.42	1039.10
7	β <sup>3</sup> -Phe2	1039.05	1038.42	1039.38
8	3-Cl-Phe2	1059.47	1058.36	1059.21
9	4-Cl-Phe2	1059.47	1058.36	1059.07
10	D-Phe2	1025.02	1024.40	1025.08
11	4-F-Phe2	1043.01	1042.39	1043.16
12	NM-Phe2	1039.05	1038.42	1039.15
13	3Pal2	1026.01	1025.40	1026.29
14	Thi2	1031.05	1030.36	1031.09
15	Tic2	1037.03	1036.41	1037.11
16	4-CF3-Phe2	1093.02	1092.39	1093.08
17	β <sup>3</sup> -Asp3	1039.05	1038.42	1093.31
18	D-Asp3	1025.02	1024.40	1025.07
19	Glu3	1039.05	1038.42	1039.23
20	Asn3	1024.04	1023.42	1024.29
21	NM-Asp3	1039.05	1038.42	1039.11
22	β <sup>3</sup> -Asp4	1039.05	1038.42	1039.36
23	D-Asp4	1025.02	1024.40	1025.04
24	Glu4	1039.05	1038.42	1039.16
25	Asn4	1024.04	1023.42	1024.19

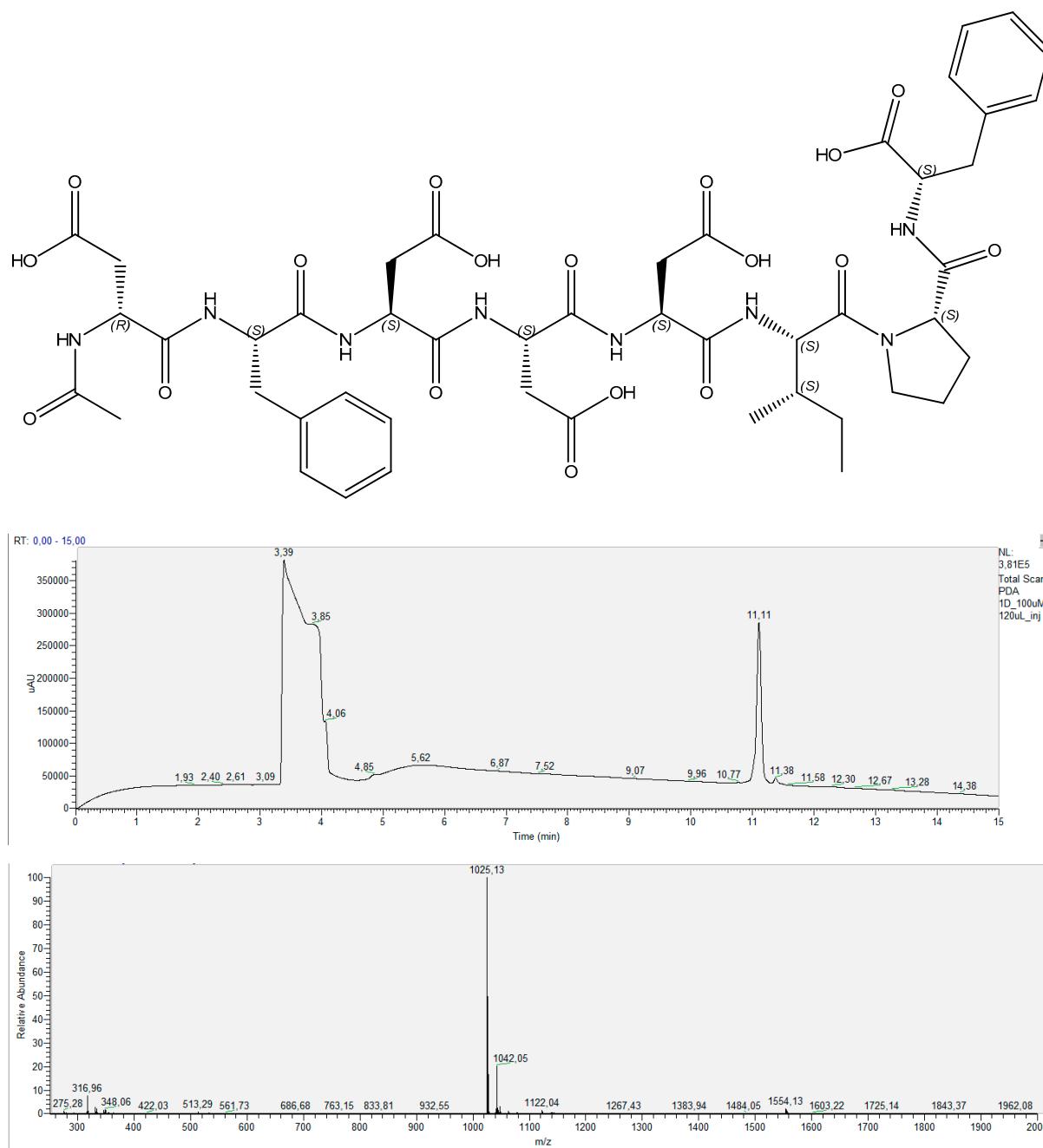
26	NM-Asp4	1039.05	1038.42	1039.05
27	$\beta^3$ -Asp5	1039.05	1038.42	1039.22
28	D-Asp5	1025.02	1024.40	1025.13
29	Glu5	1039.05	1038.42	1039.19
30	Asn5	1024.04	1023.42	1024.28
31	NM-Asp5	1039.05	1038.42	1039.22
32	Aib6	996.97	996.37	996.87
33	$\beta^3$ -Ile6	1039.05	1038.42	1039.06
34	D-Ile6	1025.02	1024.40	1025.04
35	Leu6	1025.02	1024.40	1025.17
36	NM-Ile6	1039.05	1038.42	1038.9
37	nLeu6	1025.02	1024.40	1025.32
38	tBuGly6	1025.02	1024.40	1025.20
39	4-cF-Pro7	1043.01	1042.39	1043.05
40	D-Pro7	1025.02	1024.40	1025.15
41	Hyp7	1041.02	1040.40	1041.18
42	Pip7	1039.05	1038.42	1039.29
43	$\beta^3$ -Phe8	1039.05	1038.42	1039.16
44	3-Cl-Phe8	1059.47	1058.36	1059.23
45	4-Cl-Phe8	1059.47	1058.36	1059.19
46	D-Phe8	1025.02	1024.40	1025.08
47	4-F-Phe8	1043.01	1042.39	1043.08
48	NM-Phe8	1039.05	1038.42	1039.20
49	3Pal8	1026.01	1025.40	1026.33
50	Thi8	1031.05	1030.36	1031.1
51	Tic8	1037.03	1036.41	1037.15
52	4-CF3-Phe8	1093.02	1092.39	1093.25
53	F-wtSSB-Ct	1455.39	1454.51	1455.76
54	F-4-Cl-Phe2	1490.84	1488.47	1489.42
55	F-4-F-Phe2	1474.39	1472.5	1473.5
56	F-4-CF3-Phe2	1523.39	1522.43	1523.53
57	F-Glu5	1469.39	1468.53	1469.55
58	F-Leu6	1455.39	1454.51	1455.56
59	F-nLe6	1455.39	1454.51	1455.41
60	F-Pip7	1469.39	1468.53	1469.47
61	F-3Cl-Phe8	1490.84	1488.47	1489.44
62	F-4CF3-Phe8	1523.39	1522.43	1523.45
63	E1-sSSB-Ct	1217.12	1216.44	1217.16
64	E2-sSSB-Ct	1161.02	1160.38	1161.38
65	R1-sSSB-Ct	1121.97	1120.36	1121.24
66	R2-sSSB-Ct	1093.91	1092.32	1093.11
67	K(Btn)-wtSSB-Ct	1337.45	1336.56	1337.80
68	K(Btn)-E1-sSSB-Ct	1529.56	1528.60	1530.07
69	K(Btn)-R2-sSSB-Ct	1406.34	1404.49	1407.67
70	Ac-DFDD-NH2	551.50	551.19	552.20
71	Ac-DIPF-OH	532.59	532.25	533.26

## Compound 1 – wtSSB

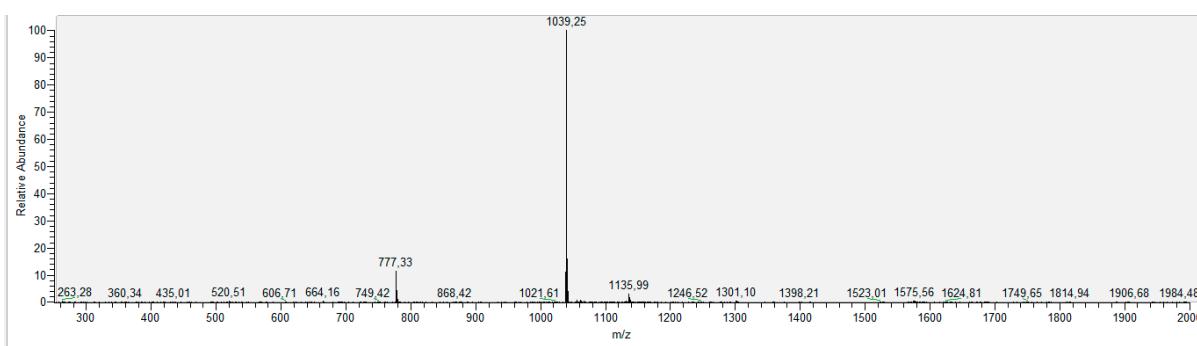
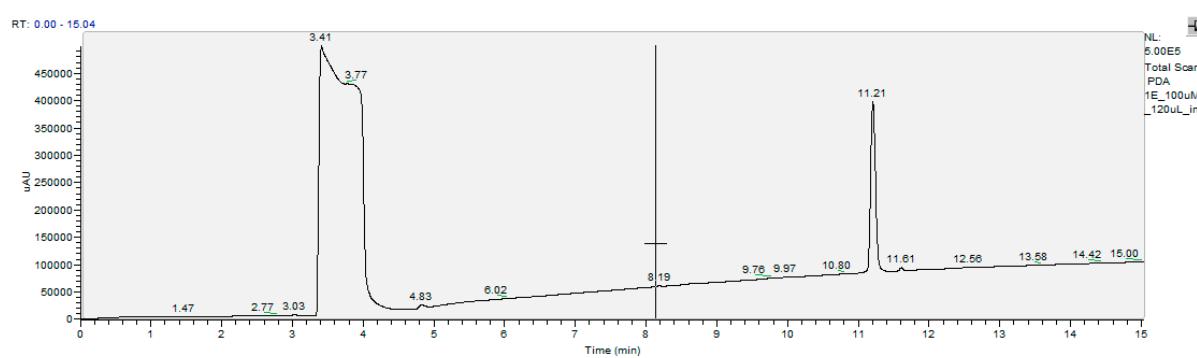
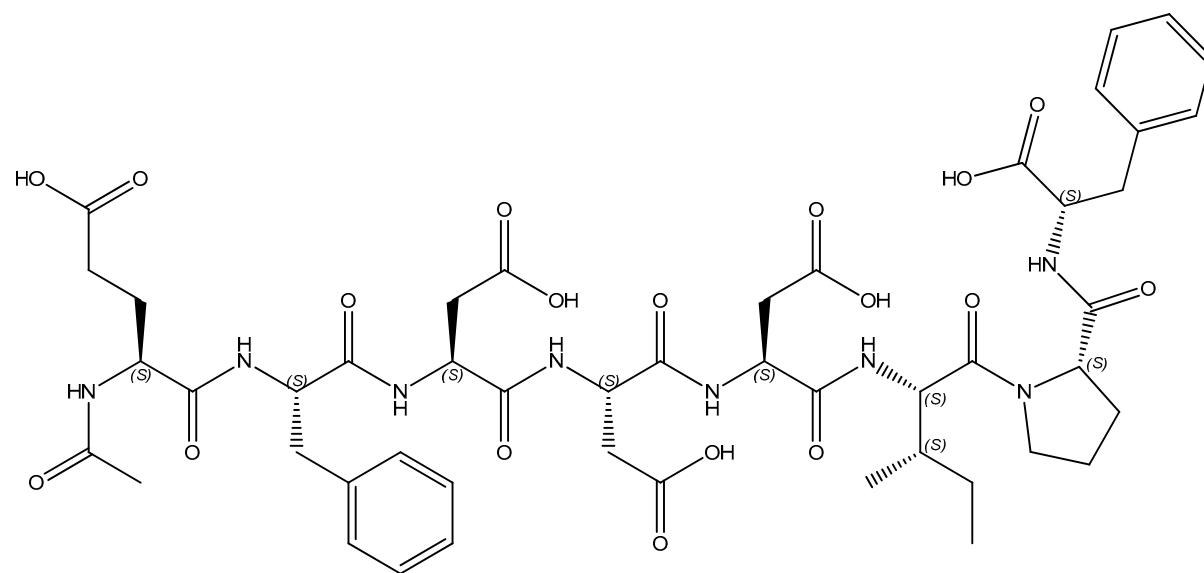


Compound 2 –  $\beta^3\text{-Asp1}$ 

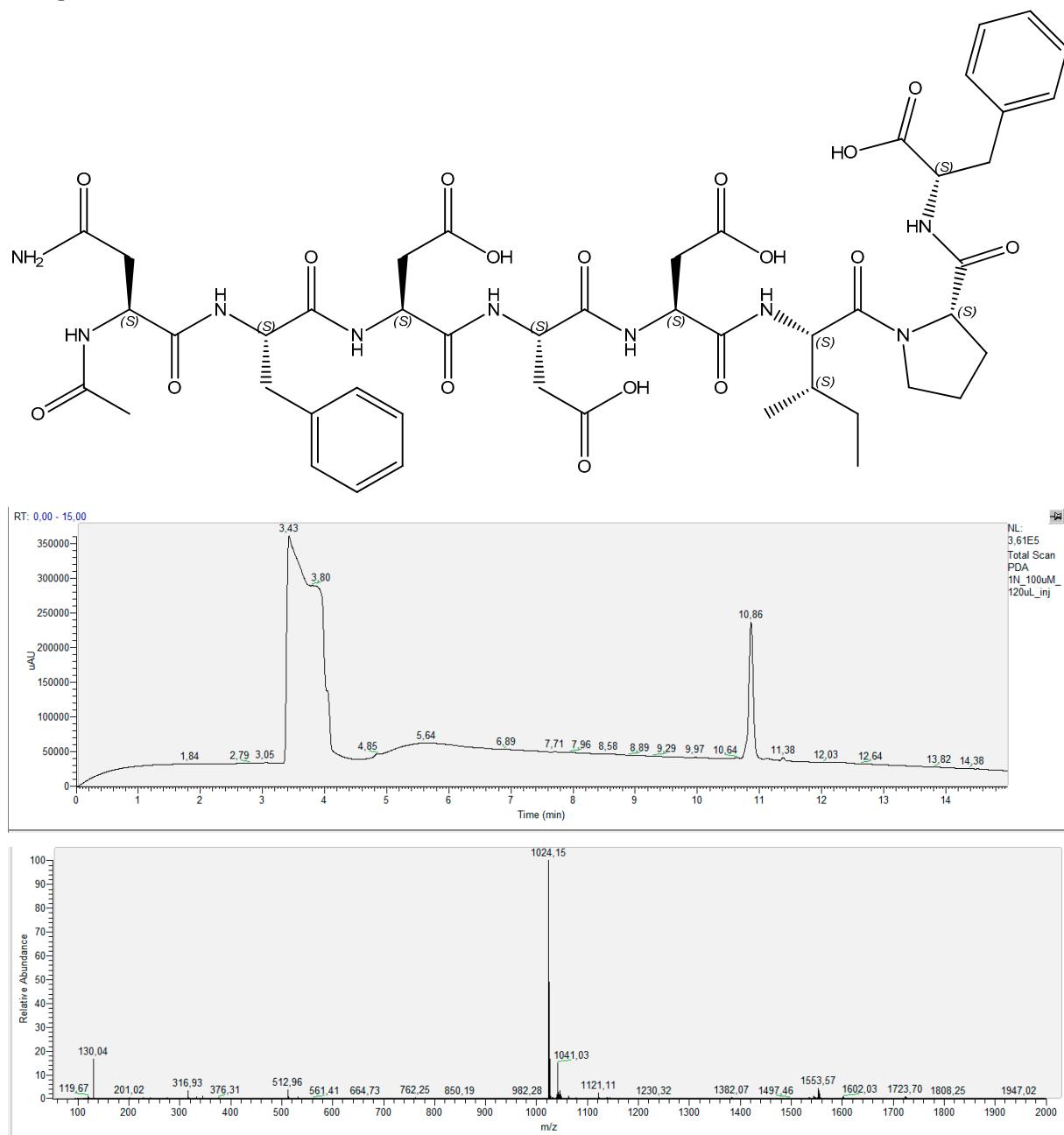
## Compound 3 – D-Asp1



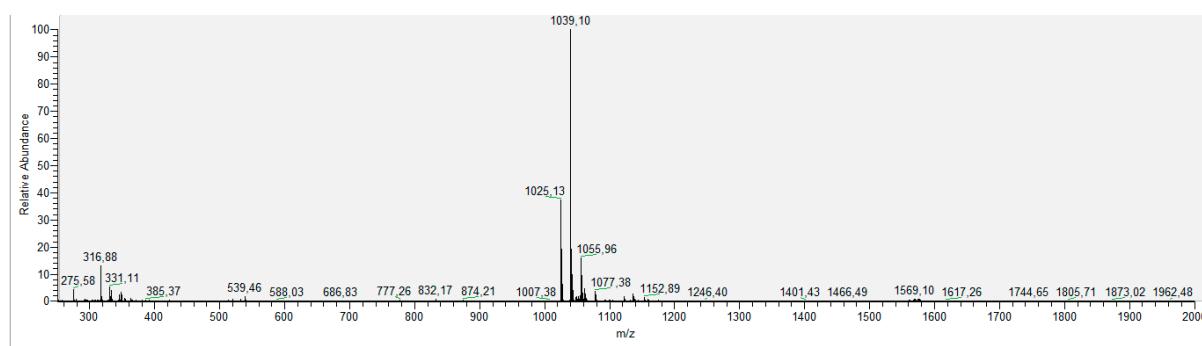
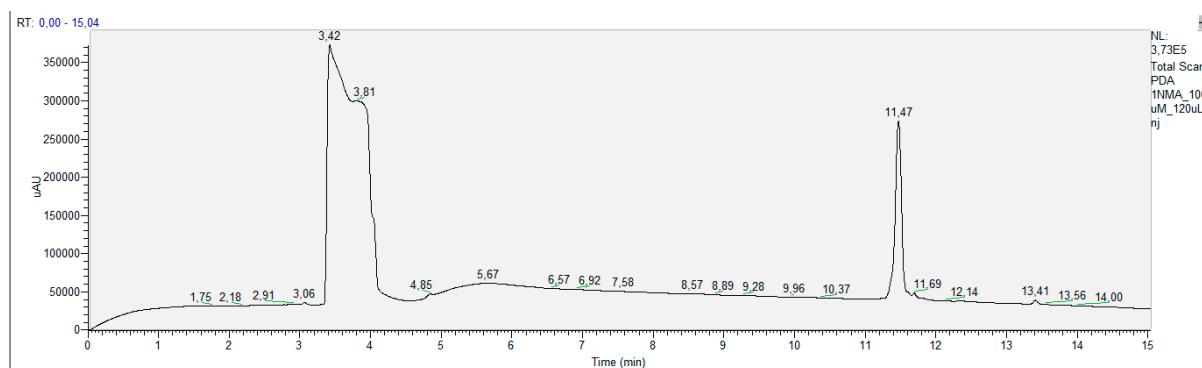
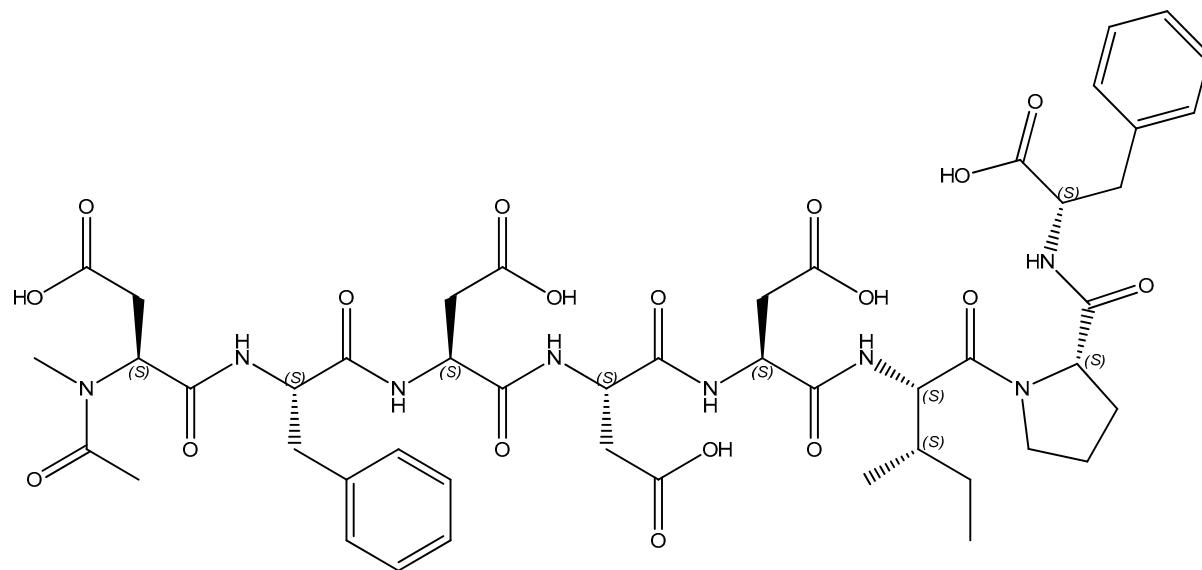
## Compound 4 – Glu1

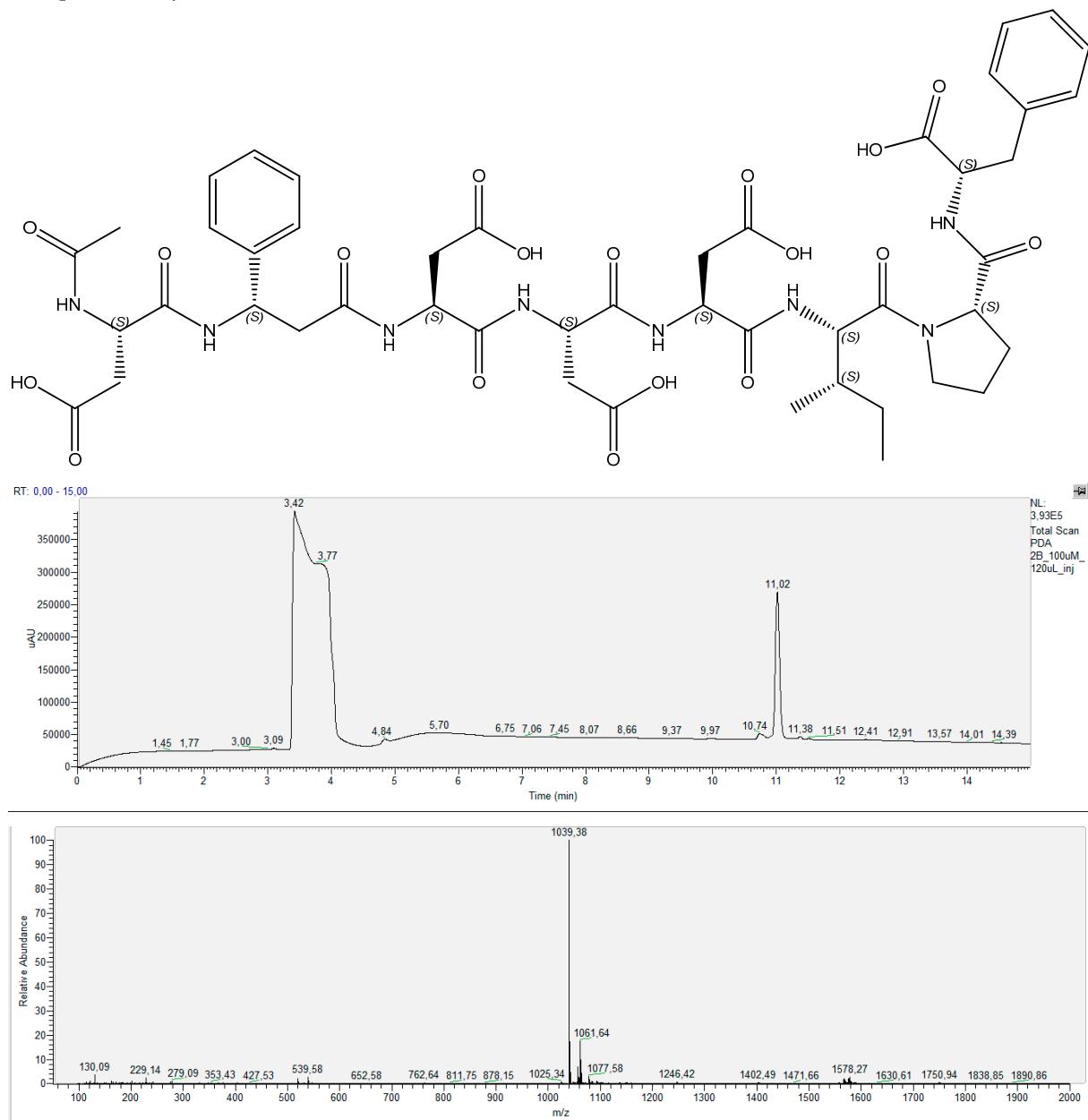


## Compound 5 – Asn1

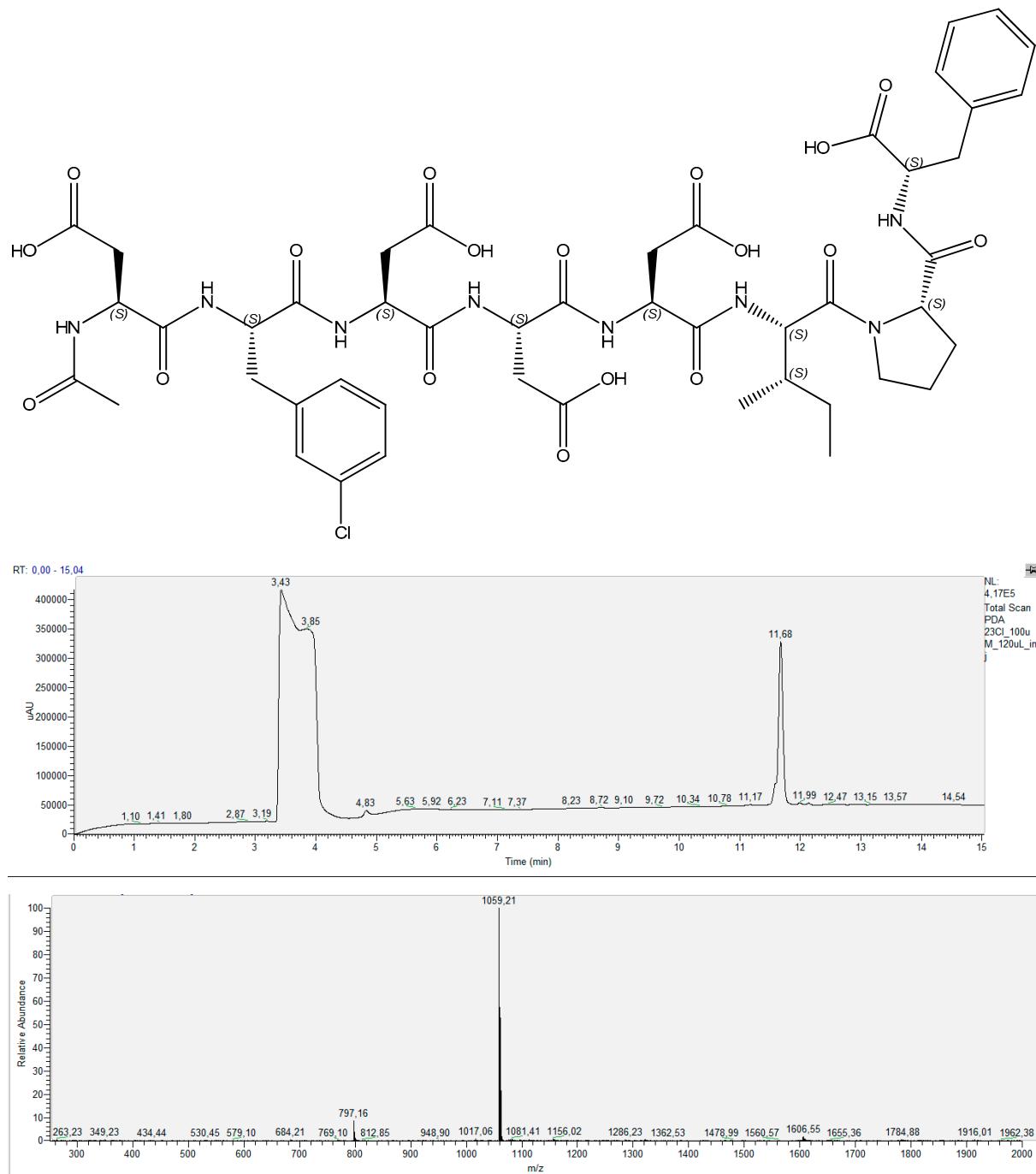


## Compound 6 – NM-Asp1

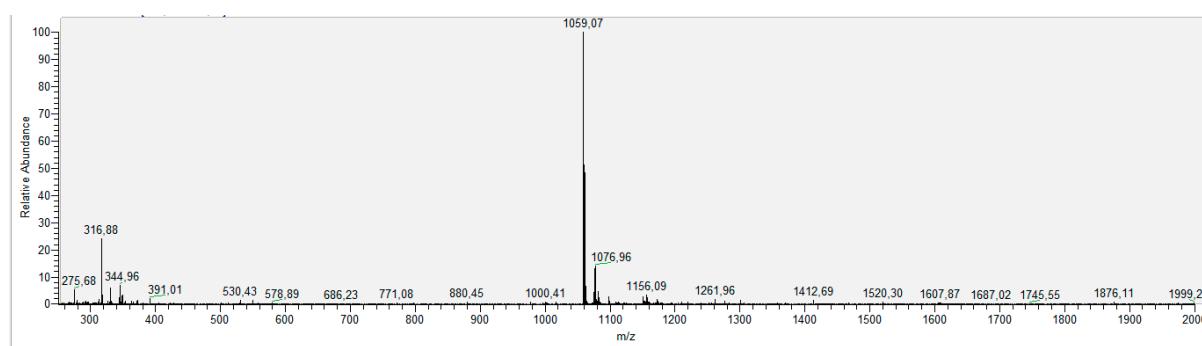
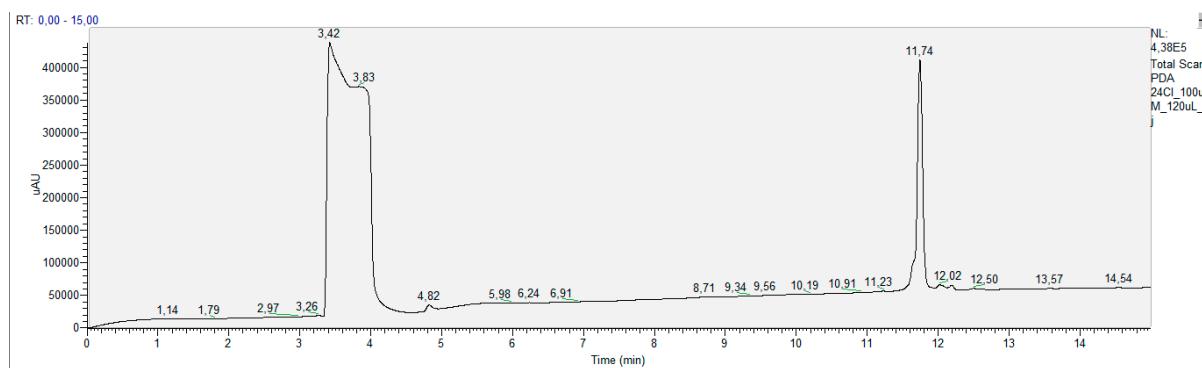
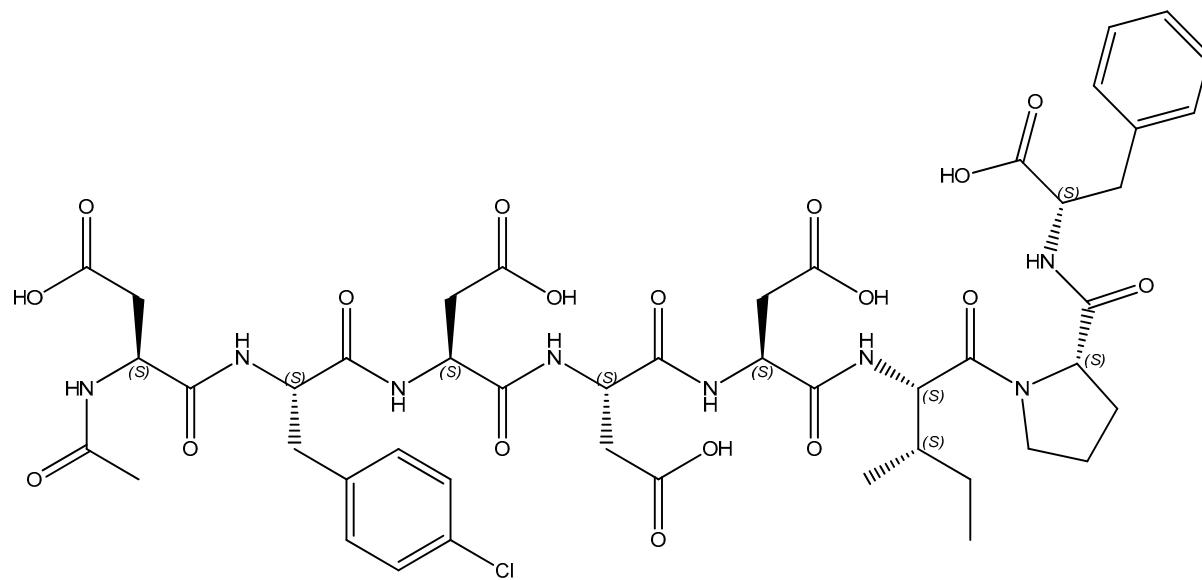


Compound 7 –  $\beta^3\text{-Phe2}$ 

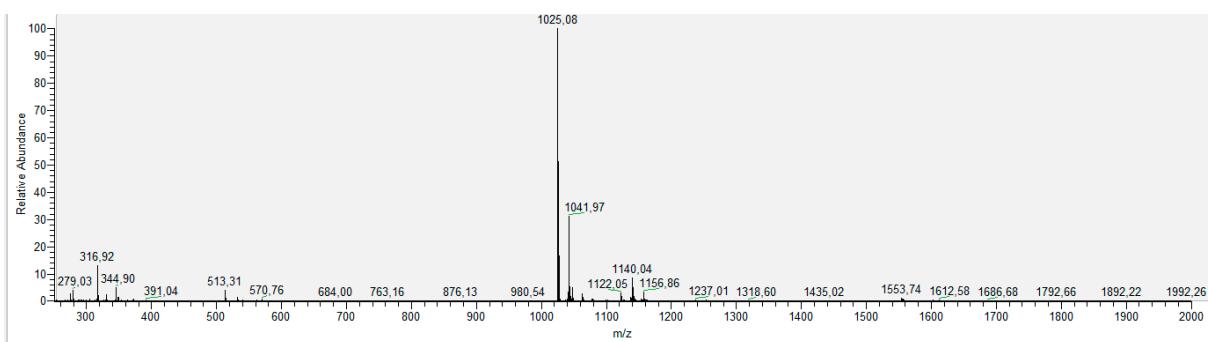
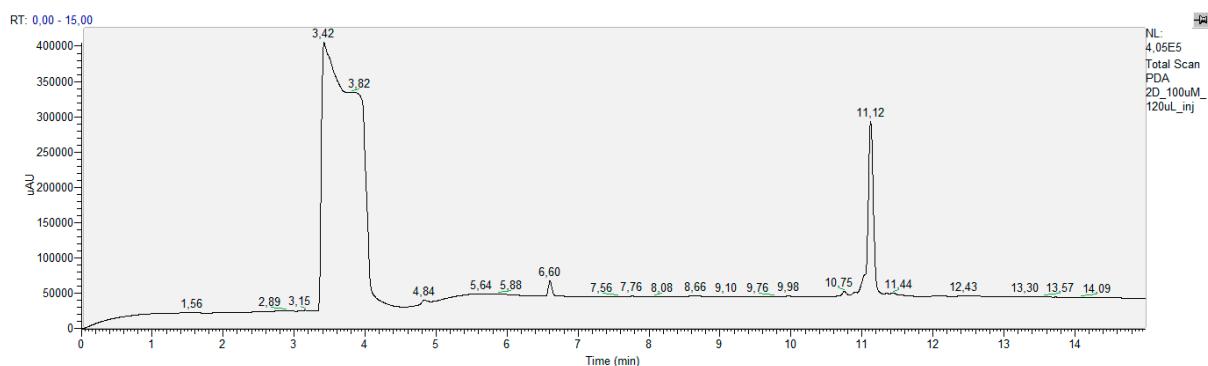
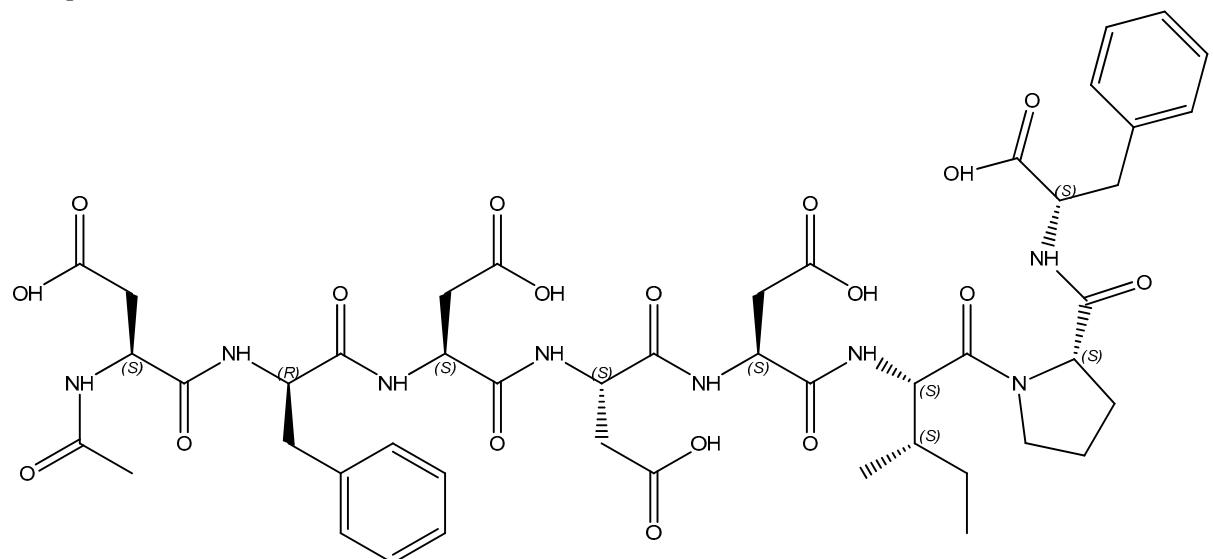
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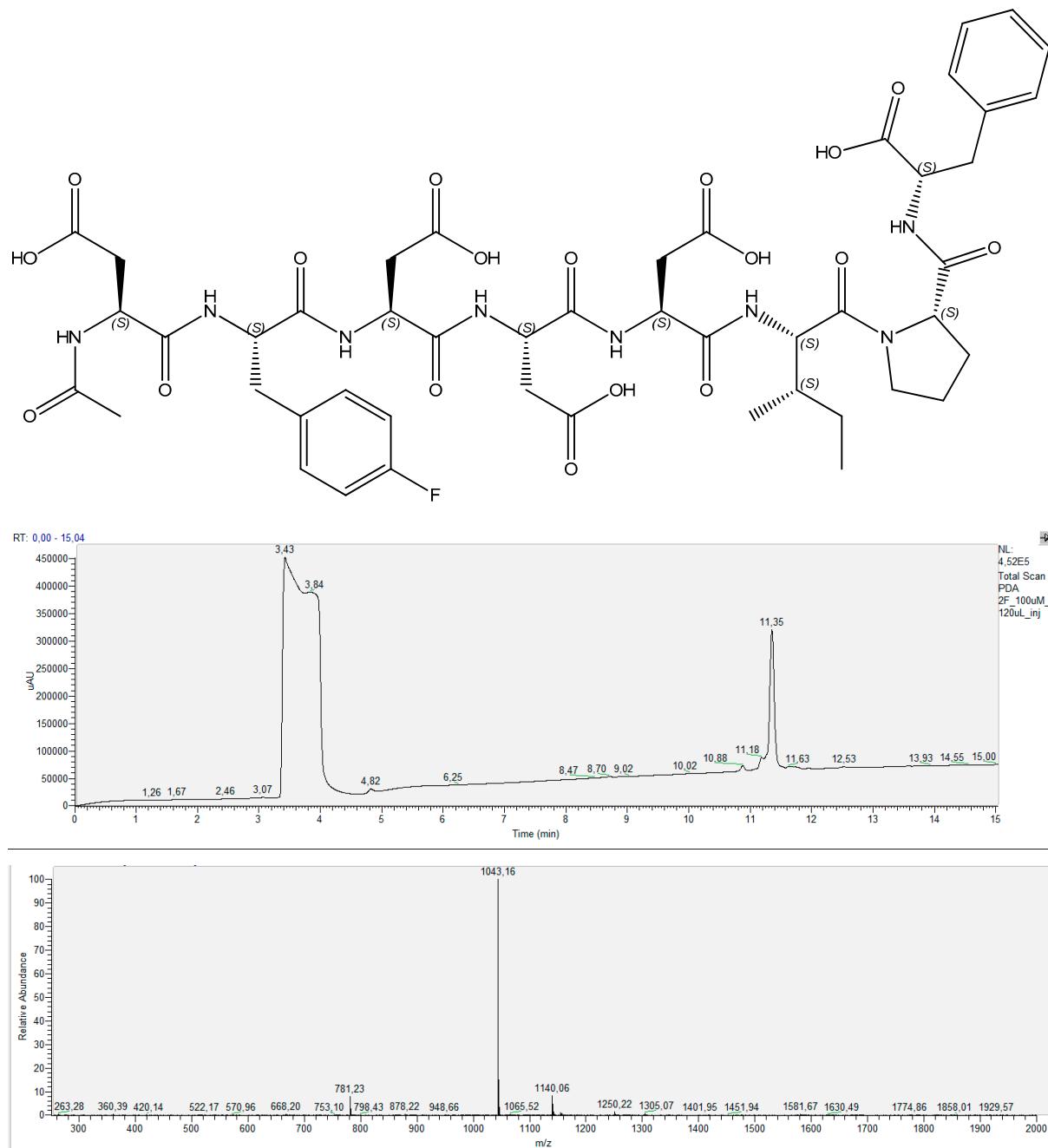
## Compound 9 – 4Cl-Phe2



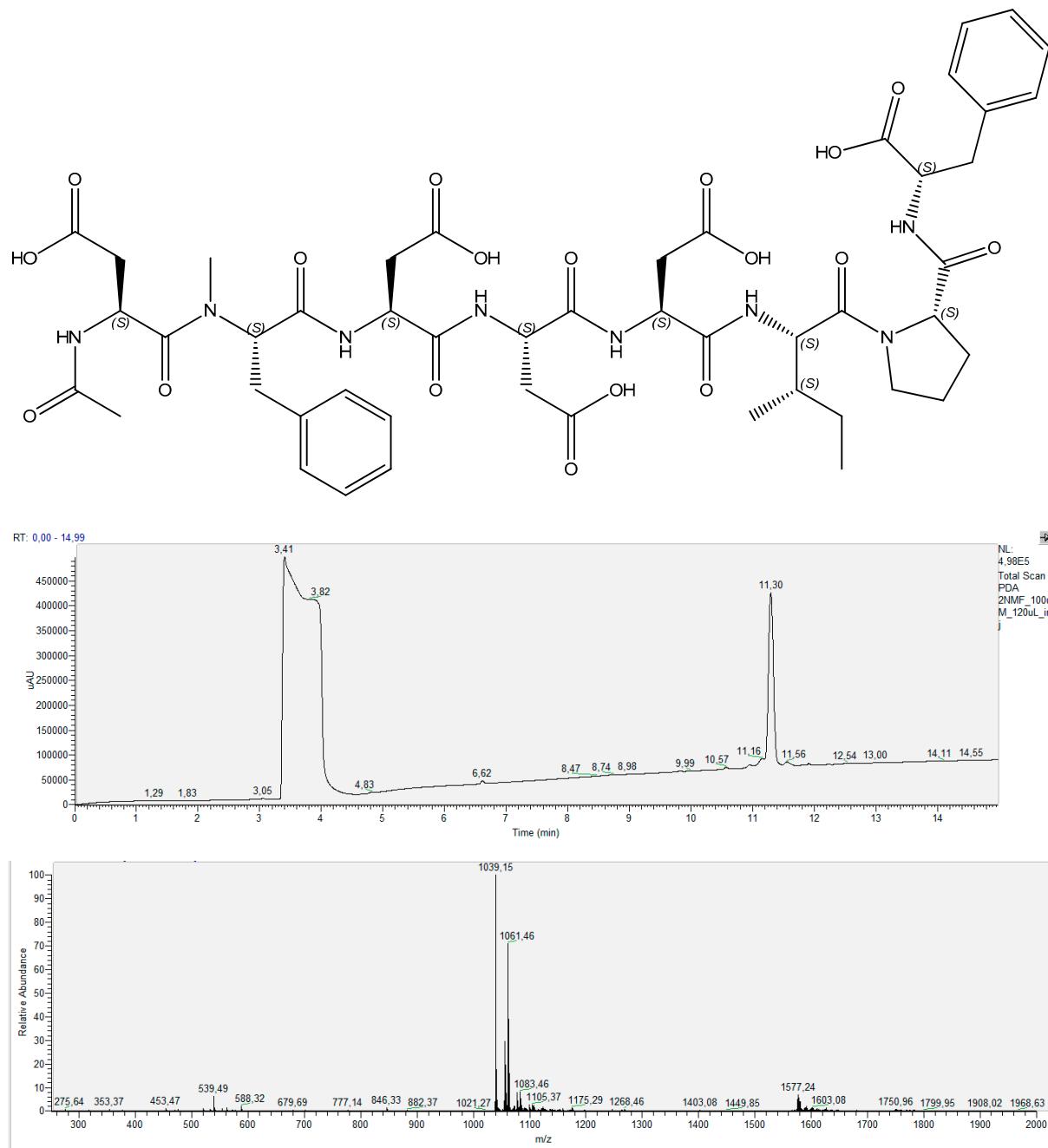
## Compound 10 – D-Phe2



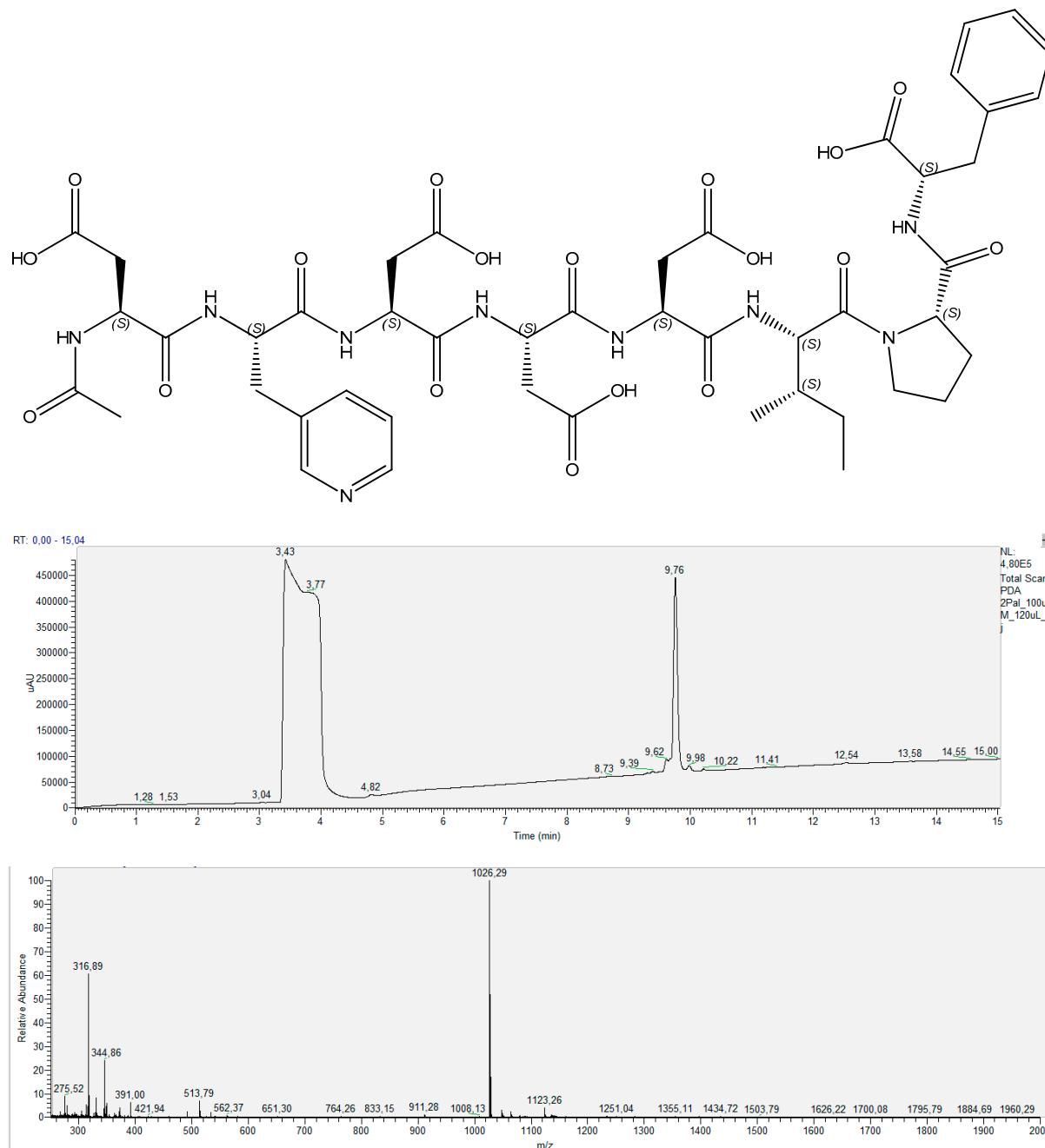
## Compound 11 – 4F-Phe2



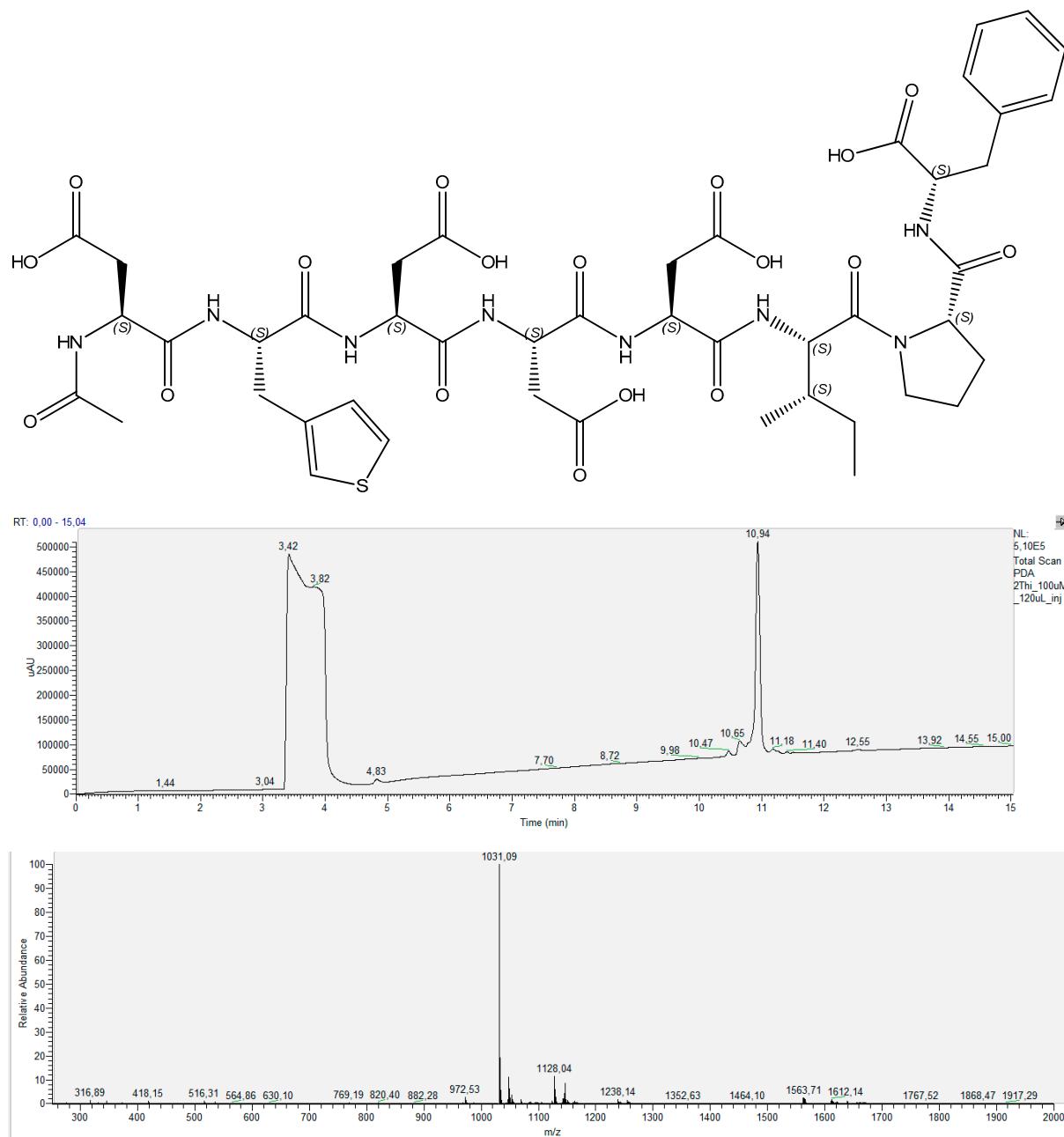
## Compound 12 – NM-Phe2



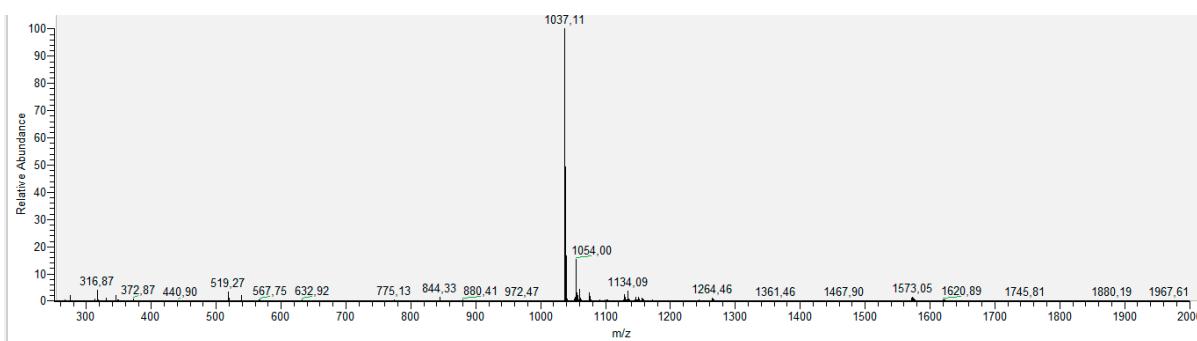
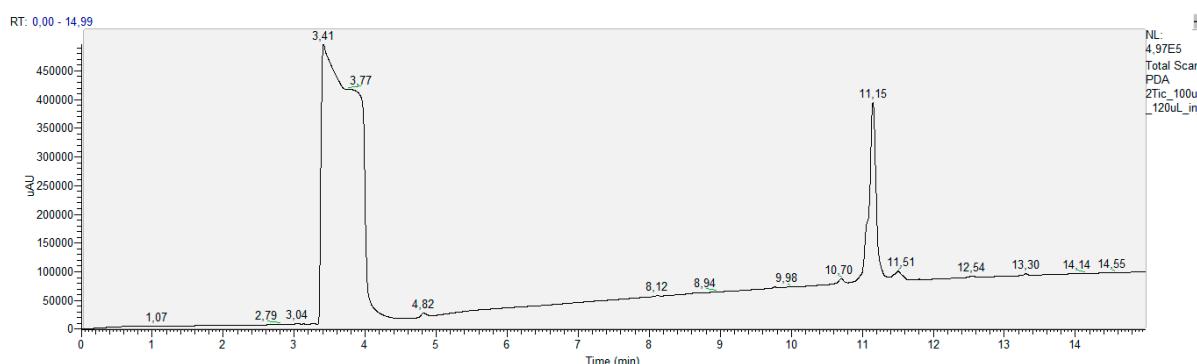
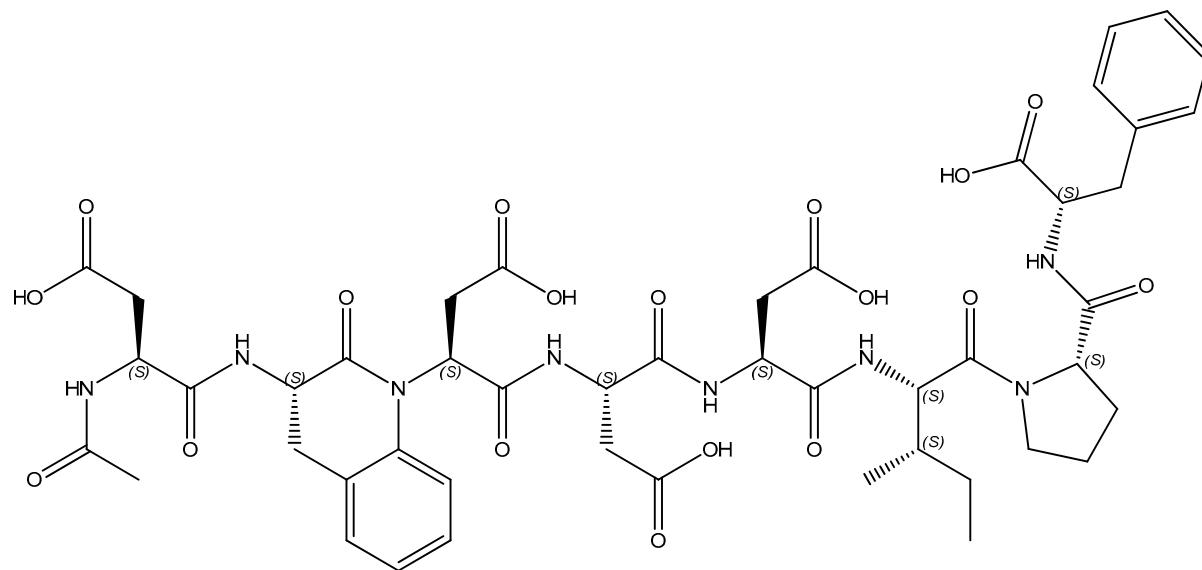
## Compound 13 – 3Pal2

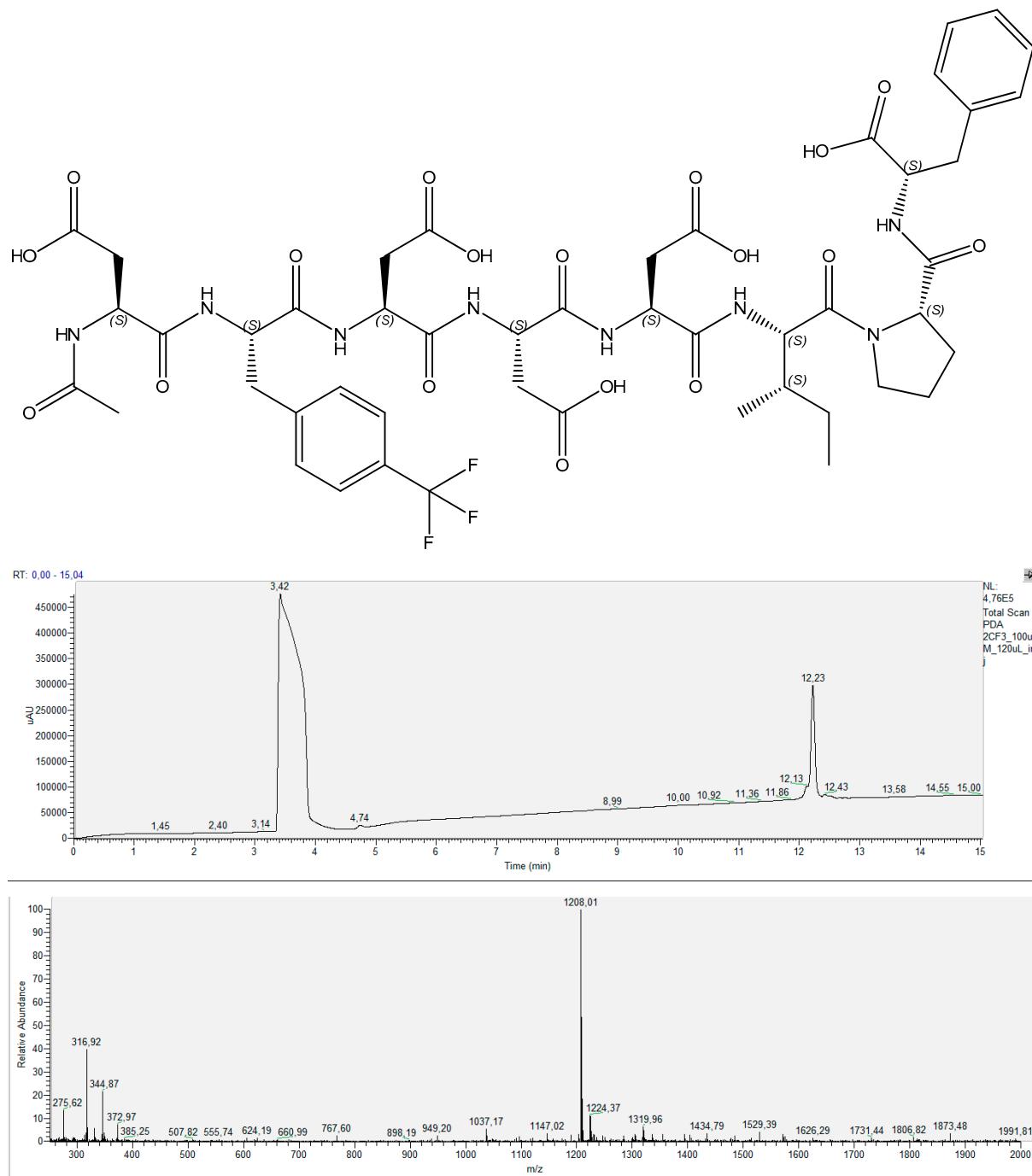


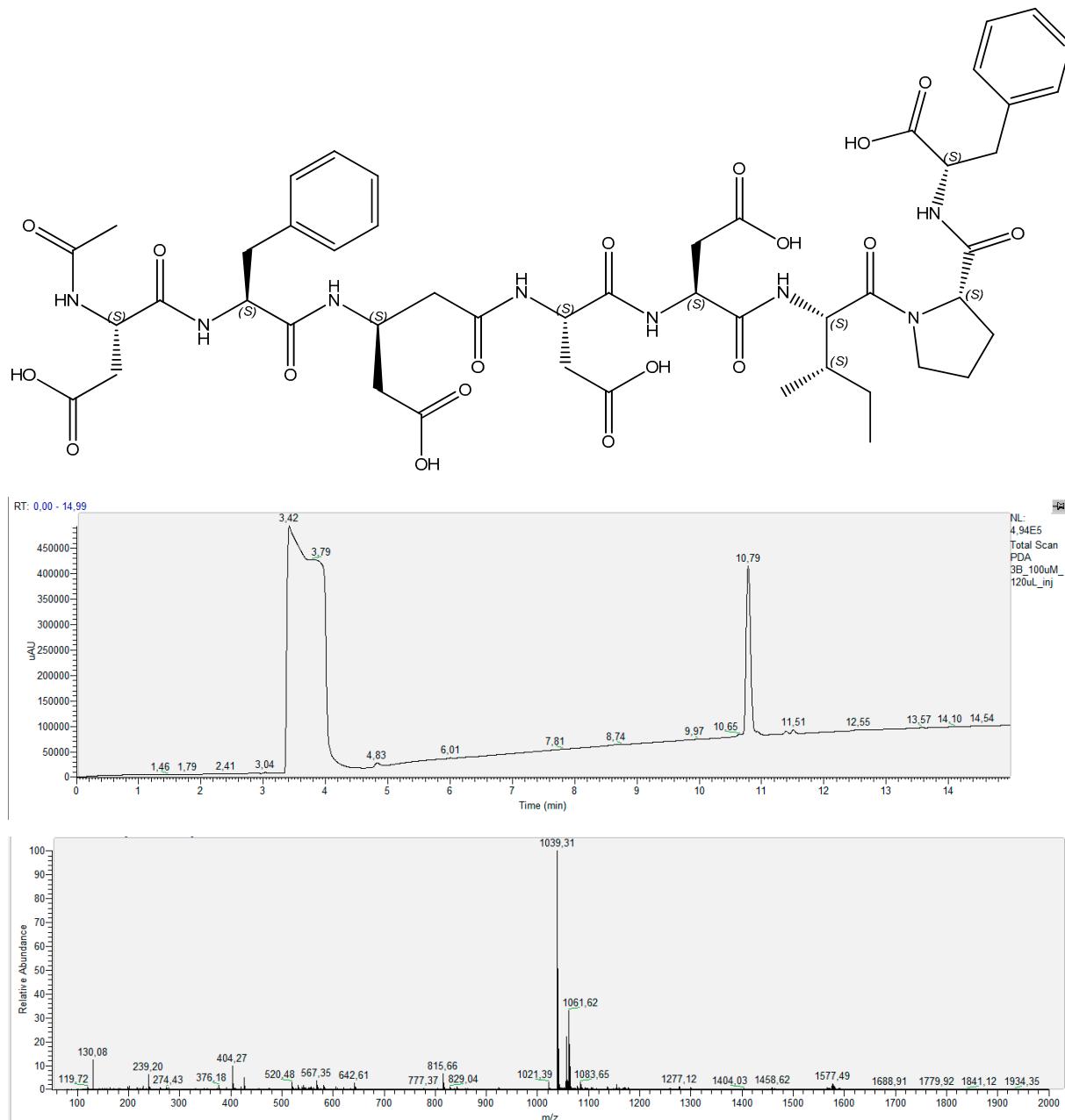
## Compound 14 – Thi2



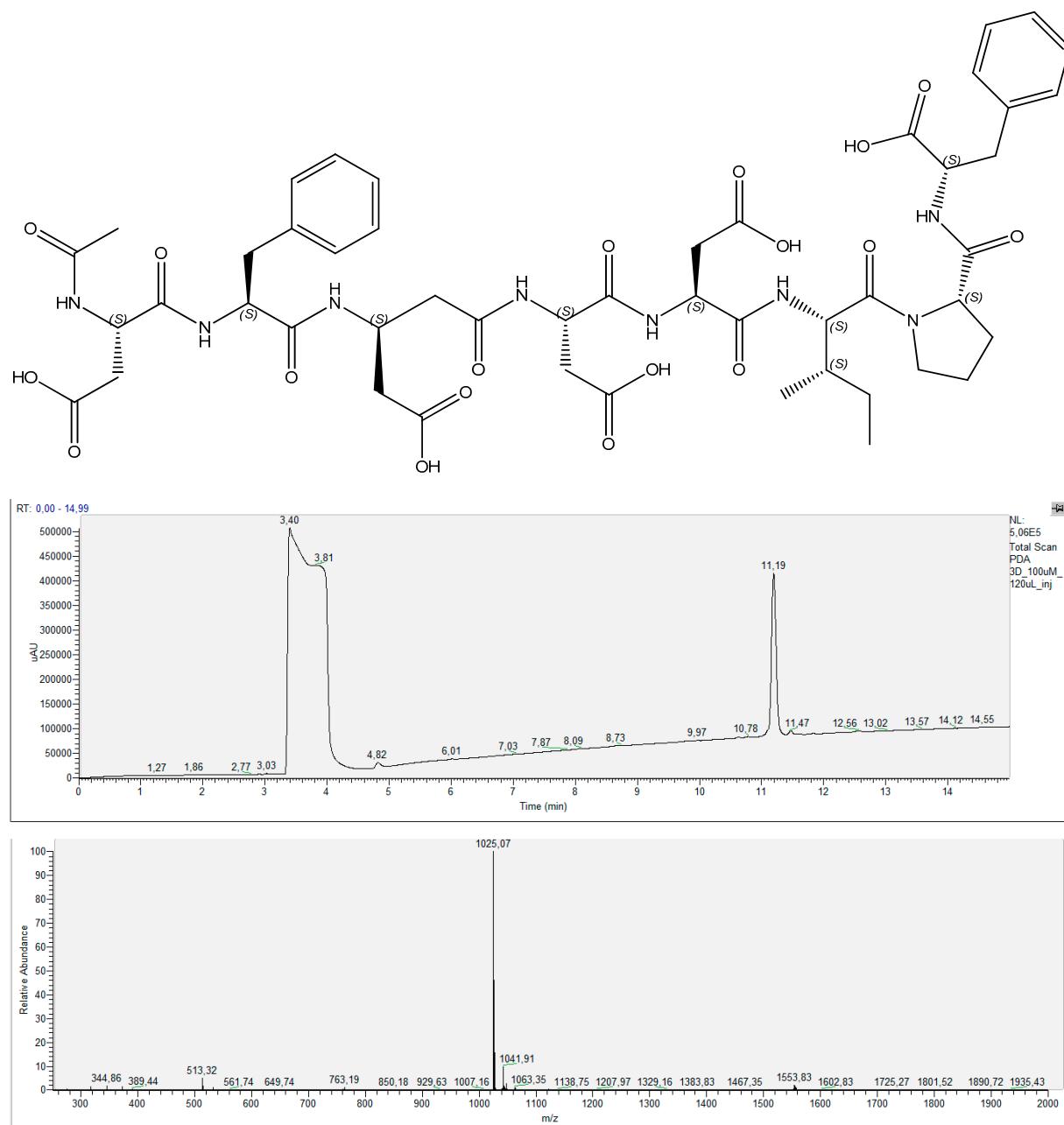
## Compound 15 – Tic2



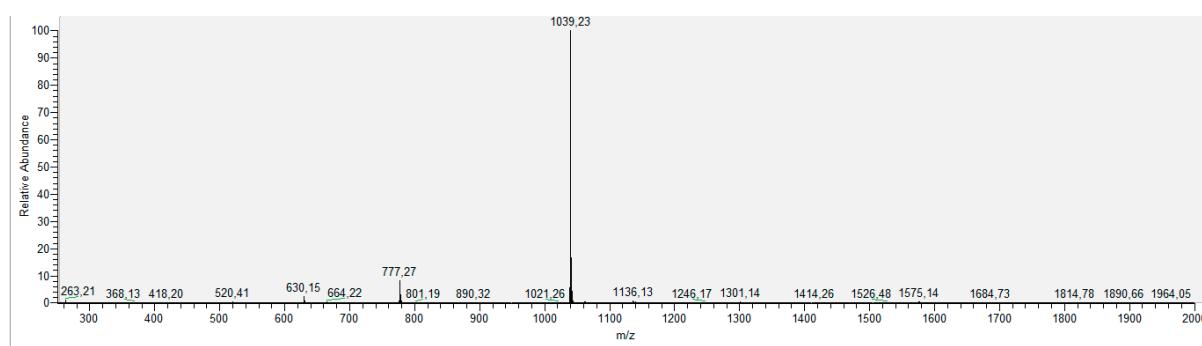
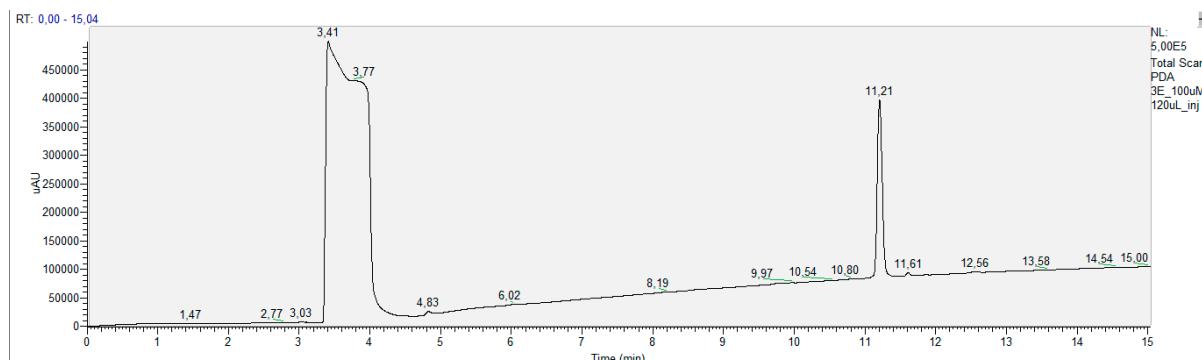
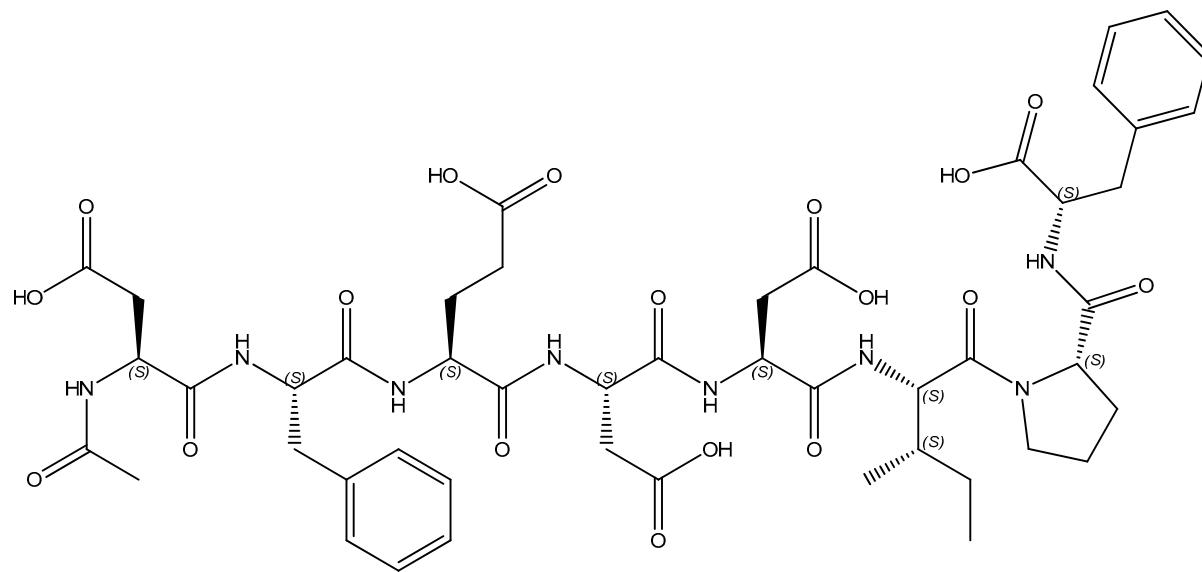
Compound 16 – 4CF<sub>3</sub>-Phe2

Compound 17 –  $\beta$ <sup>3</sup>-Asp3

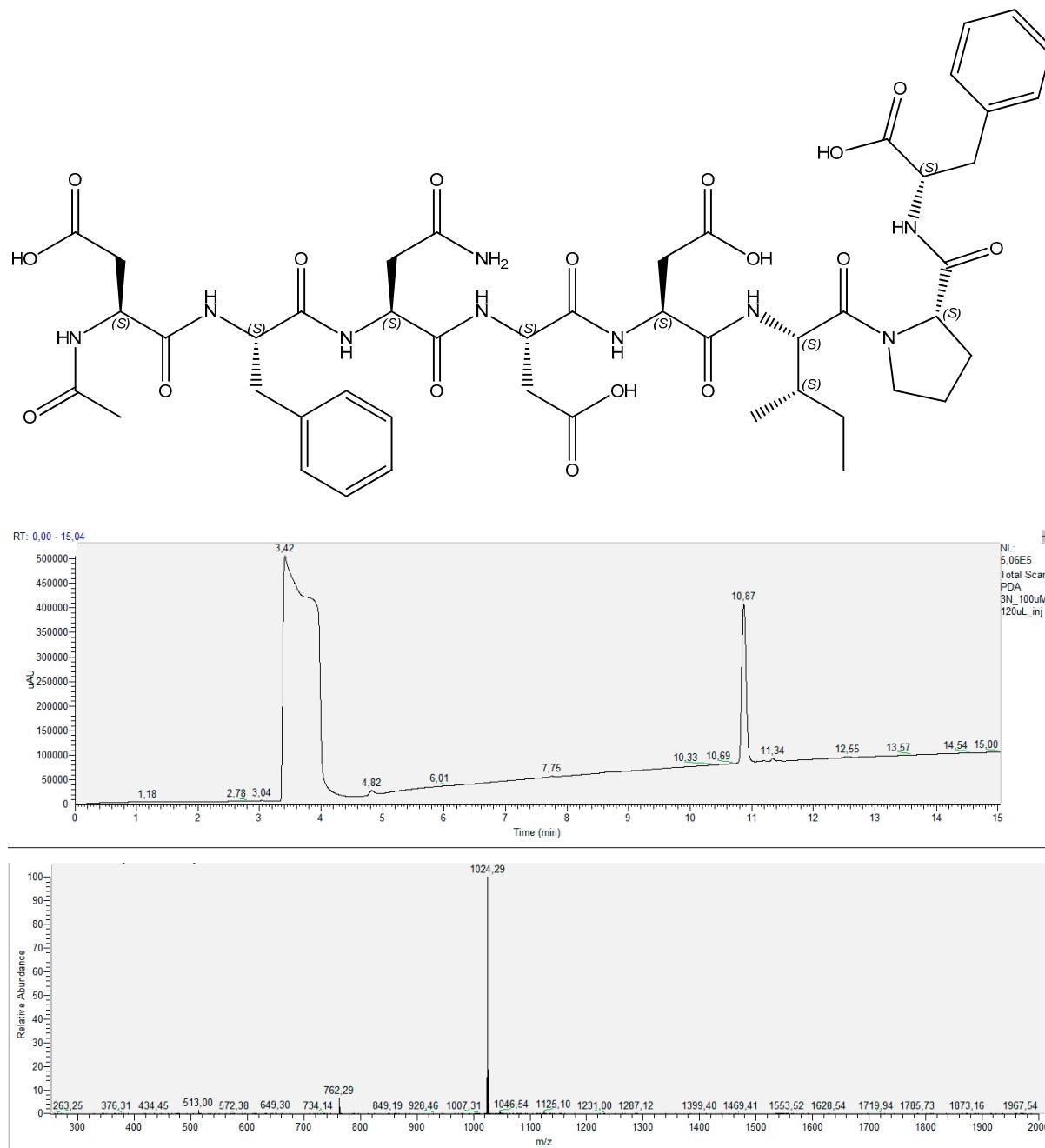
## Compound 18 – D-Asp3



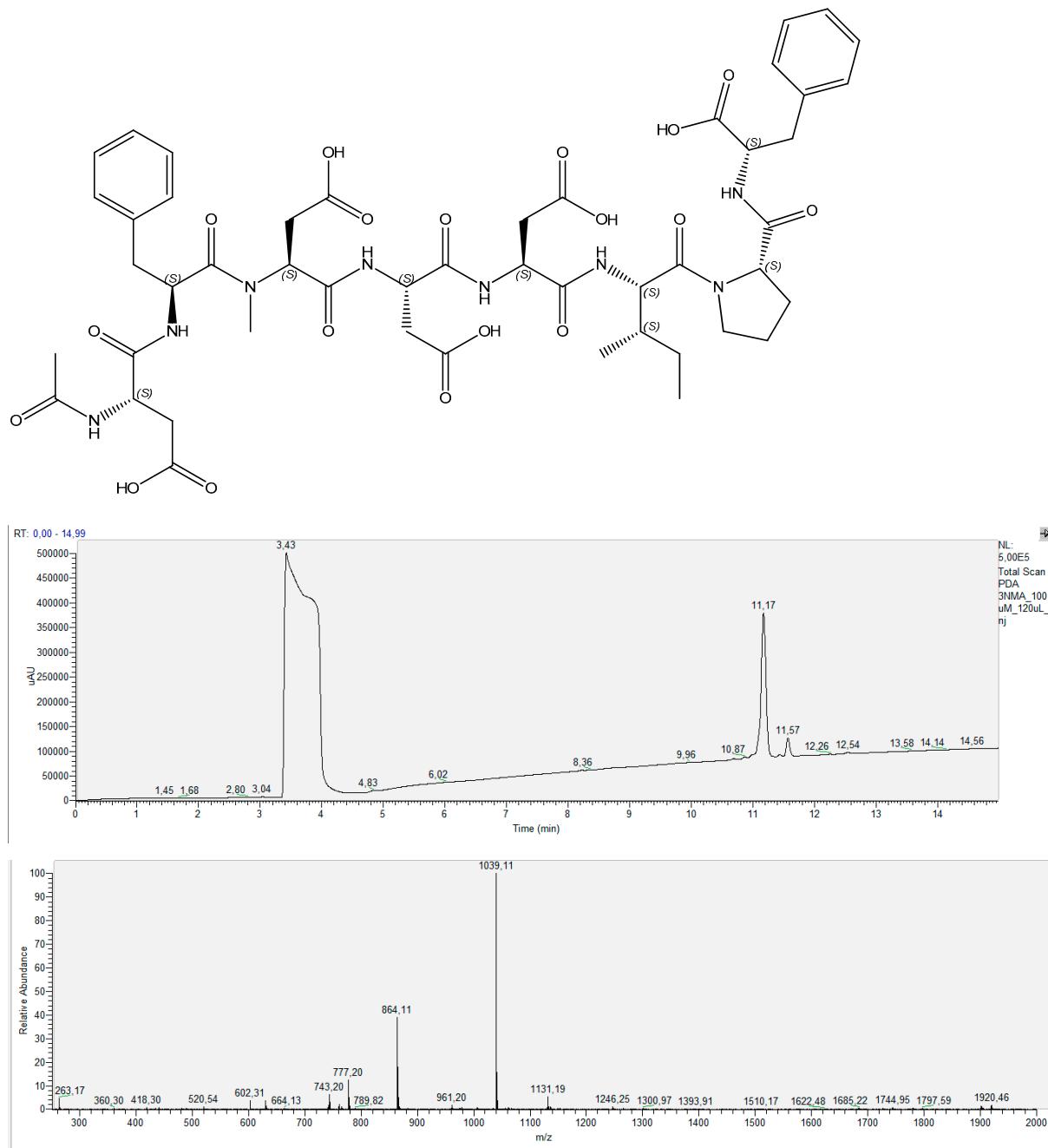
## Compound 19 – Glu3

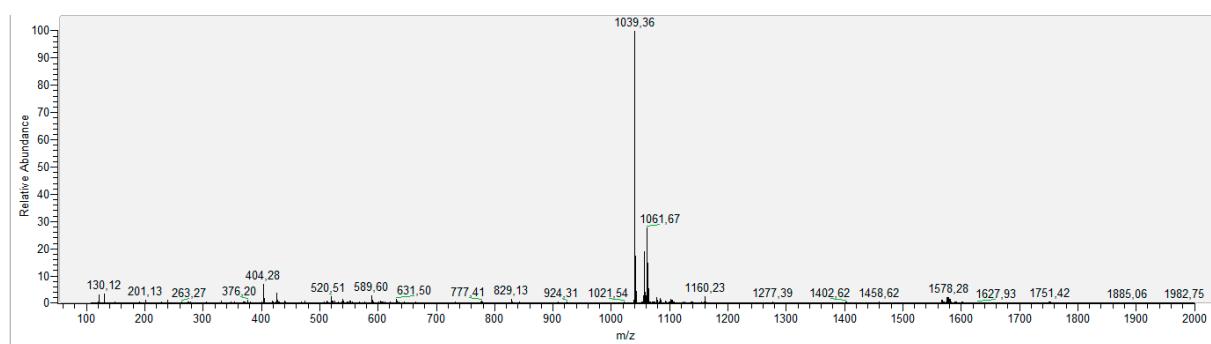
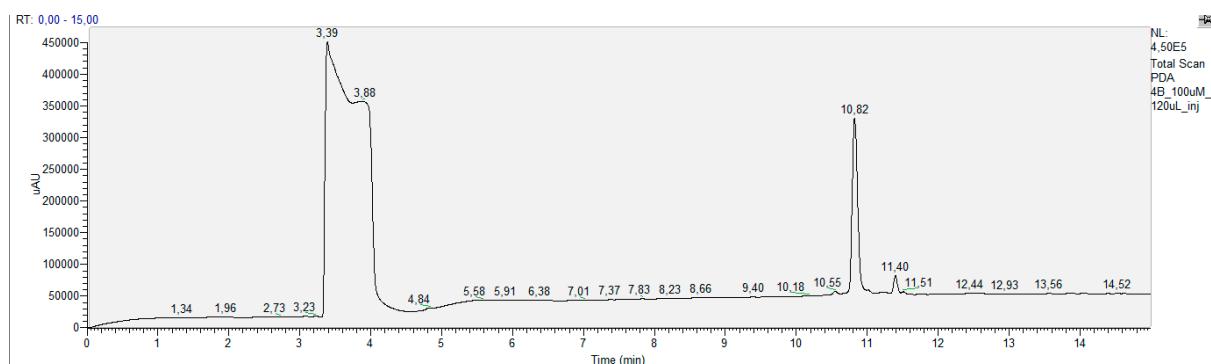
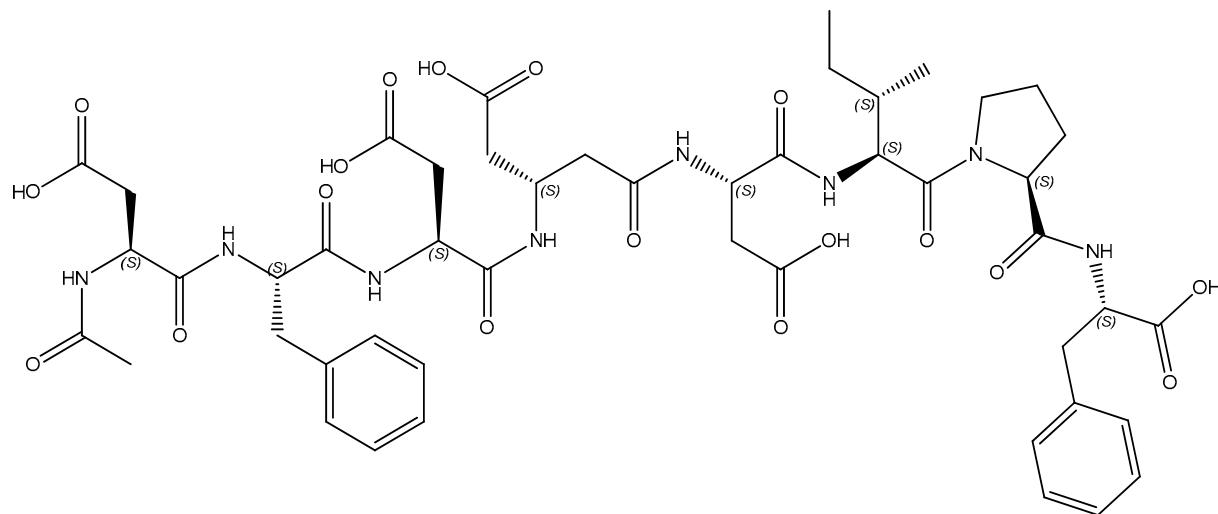


## Compound 20 – Asn3

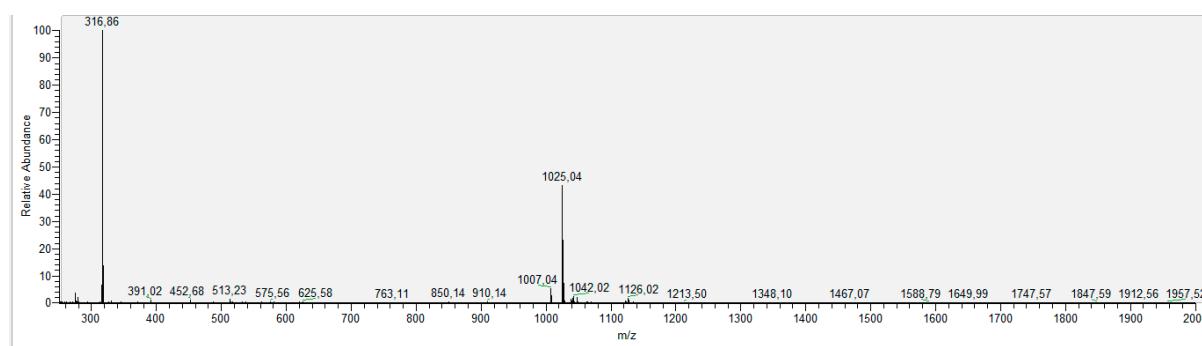
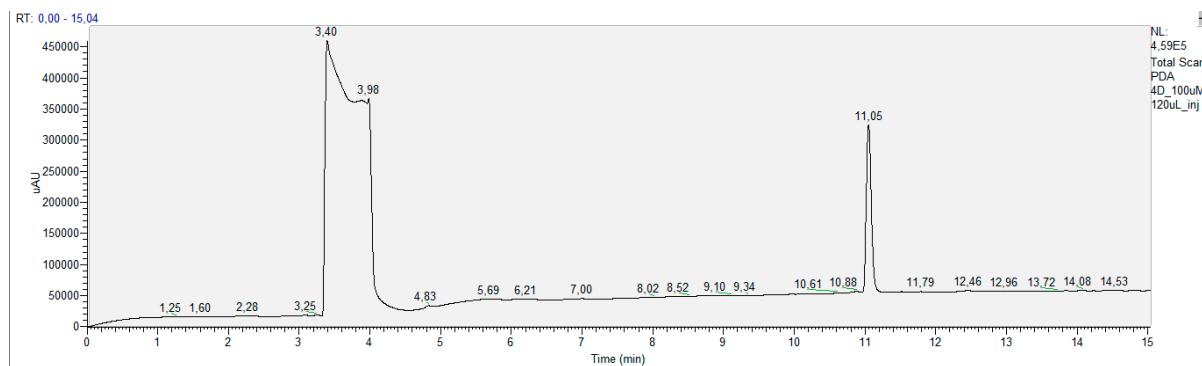
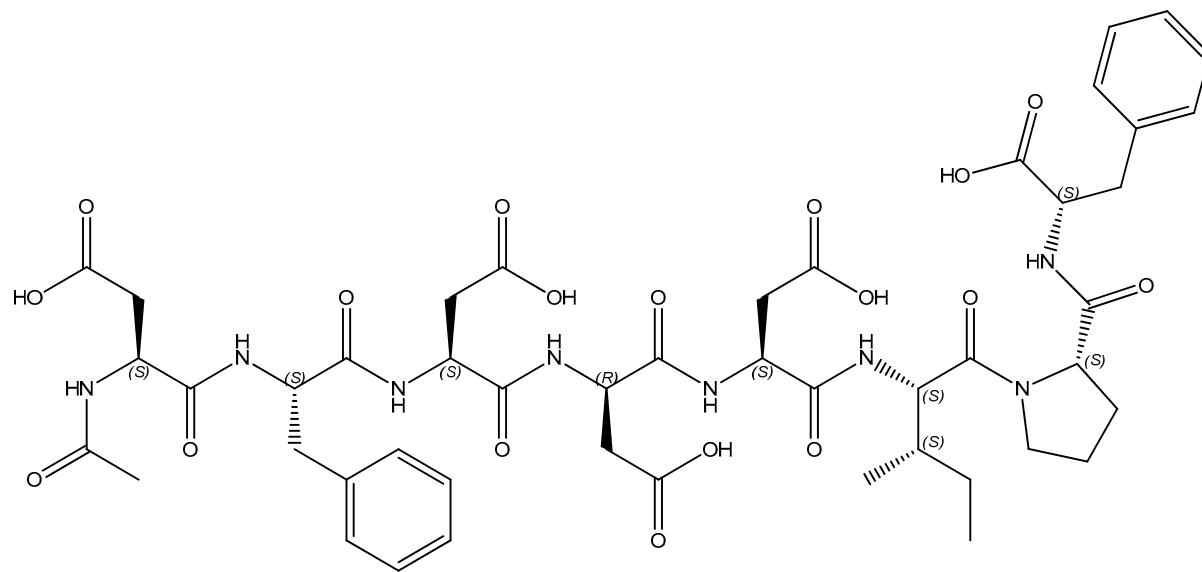


## Compound 21 – NM-Asp3

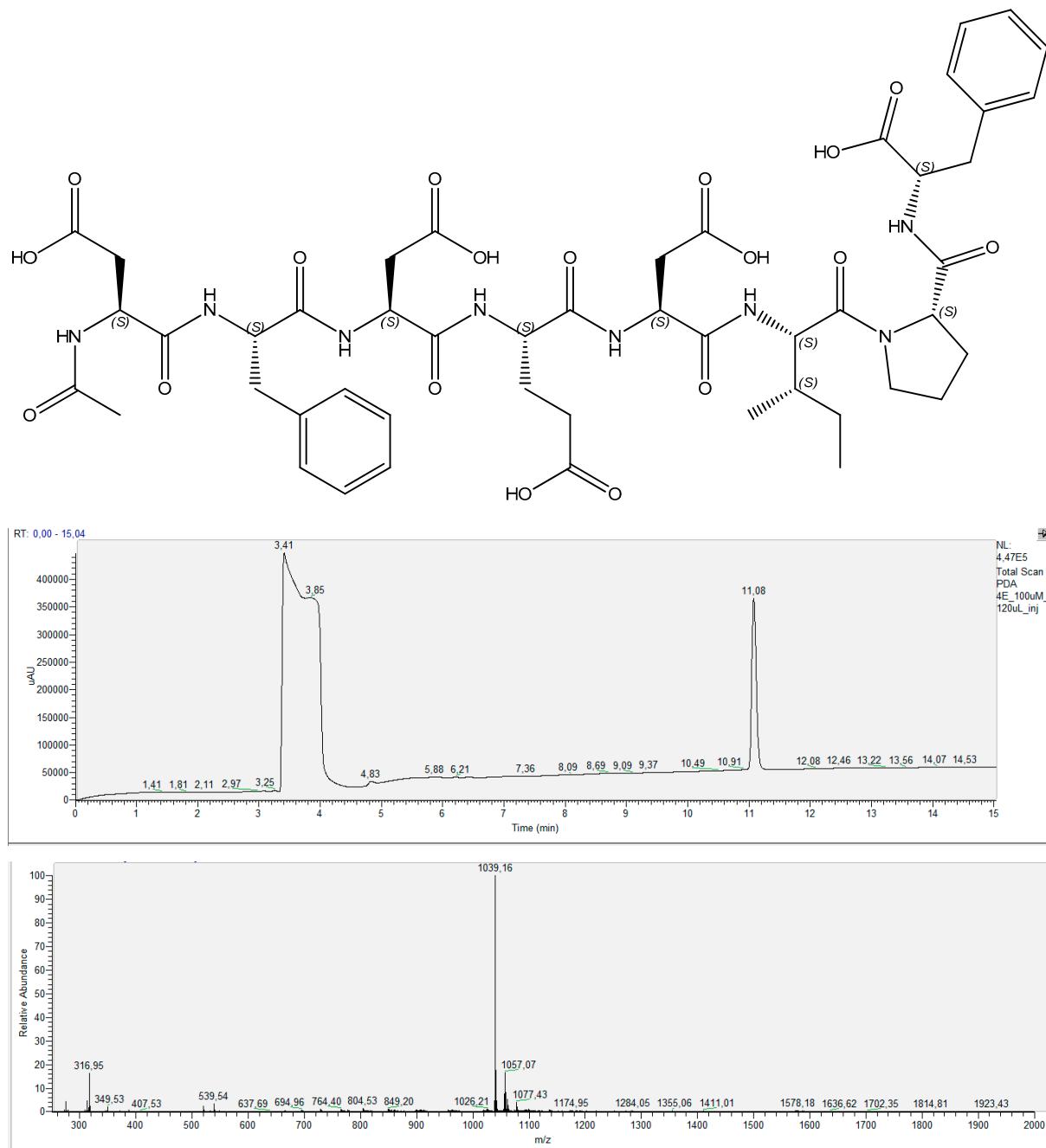


Compound 22 –  $\beta$ <sup>3</sup>-Asp4

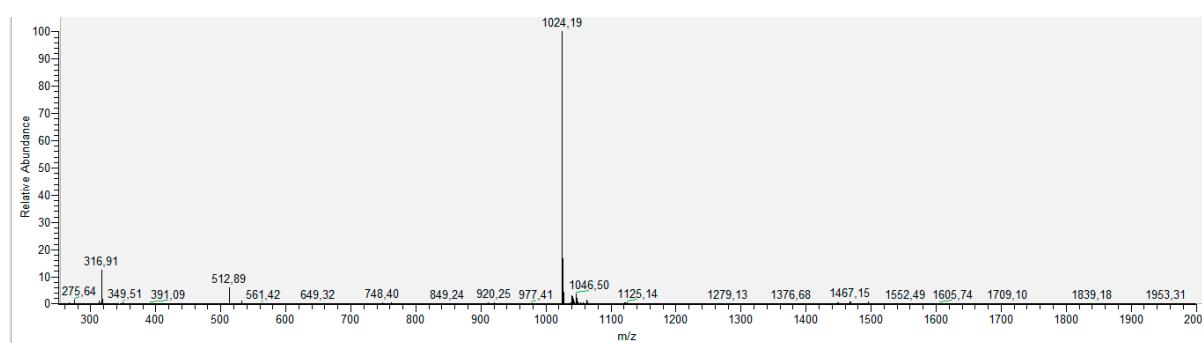
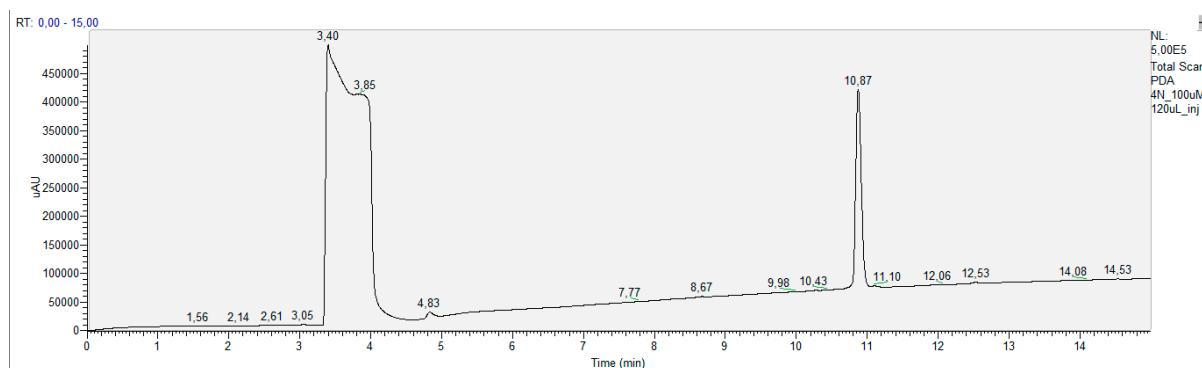
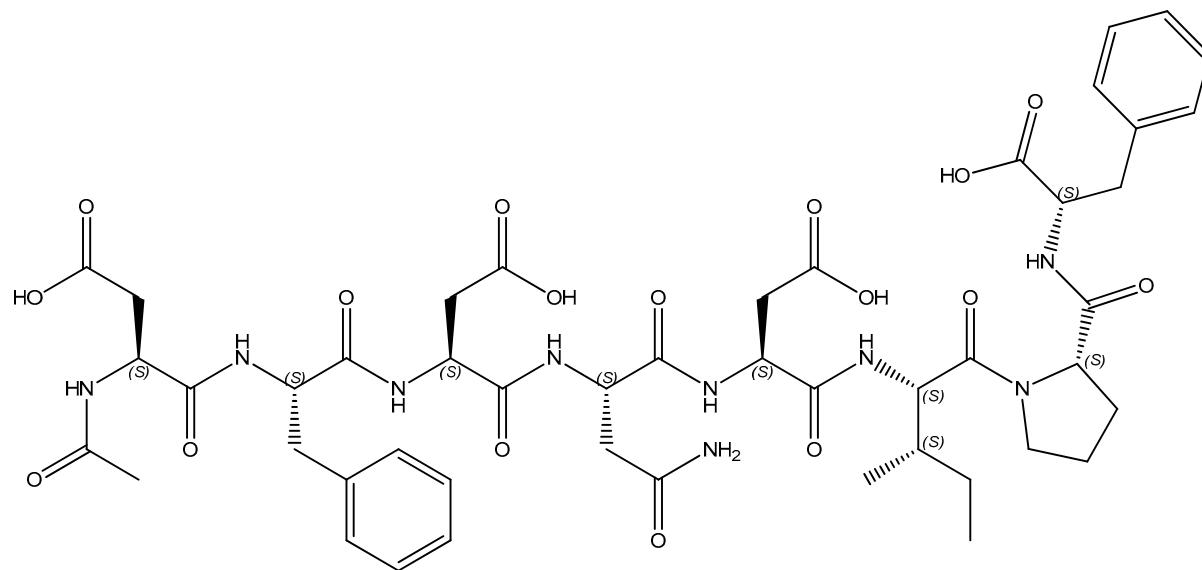
## Compound 23 – D-Asp4



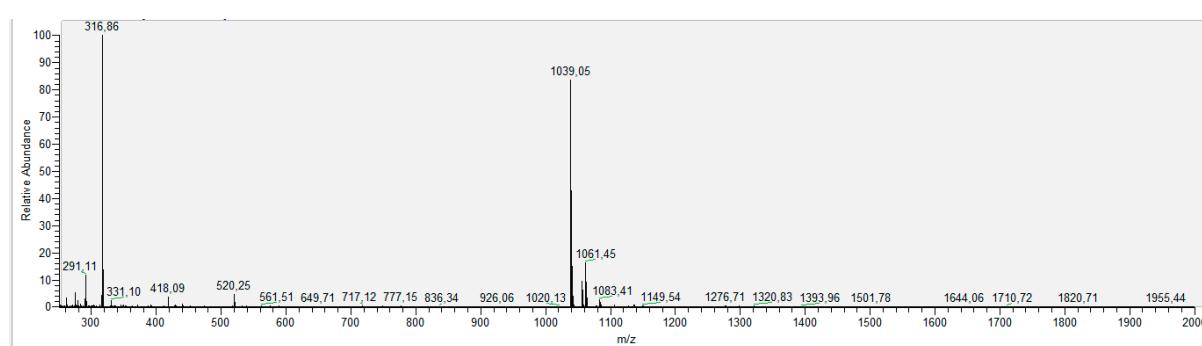
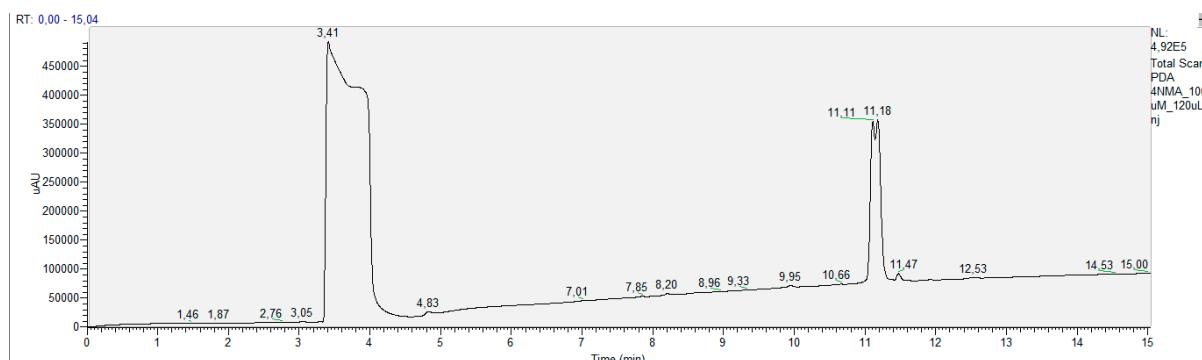
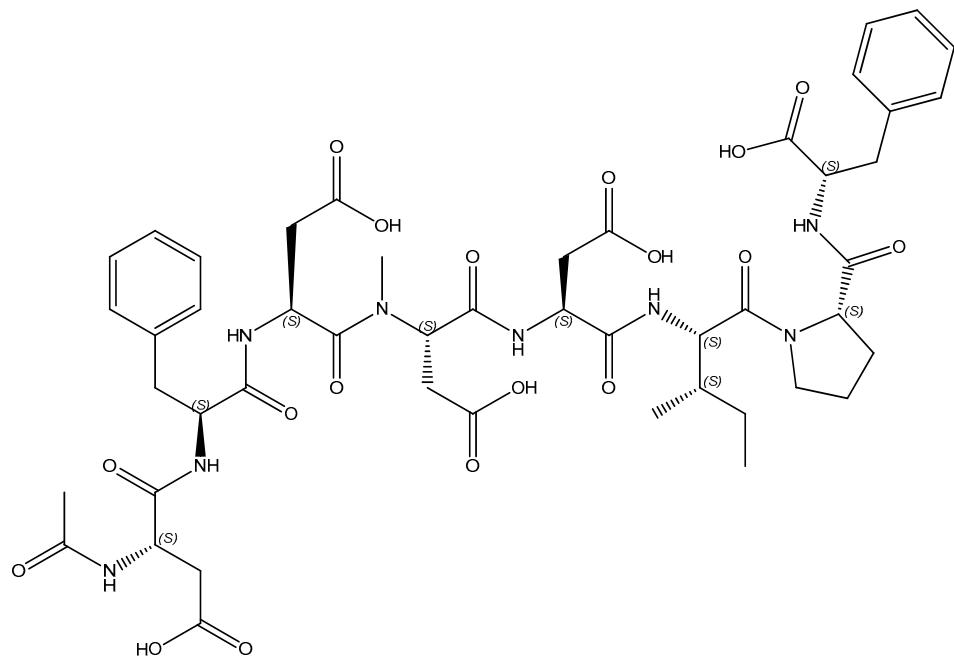
## Compound 24 – Glu4

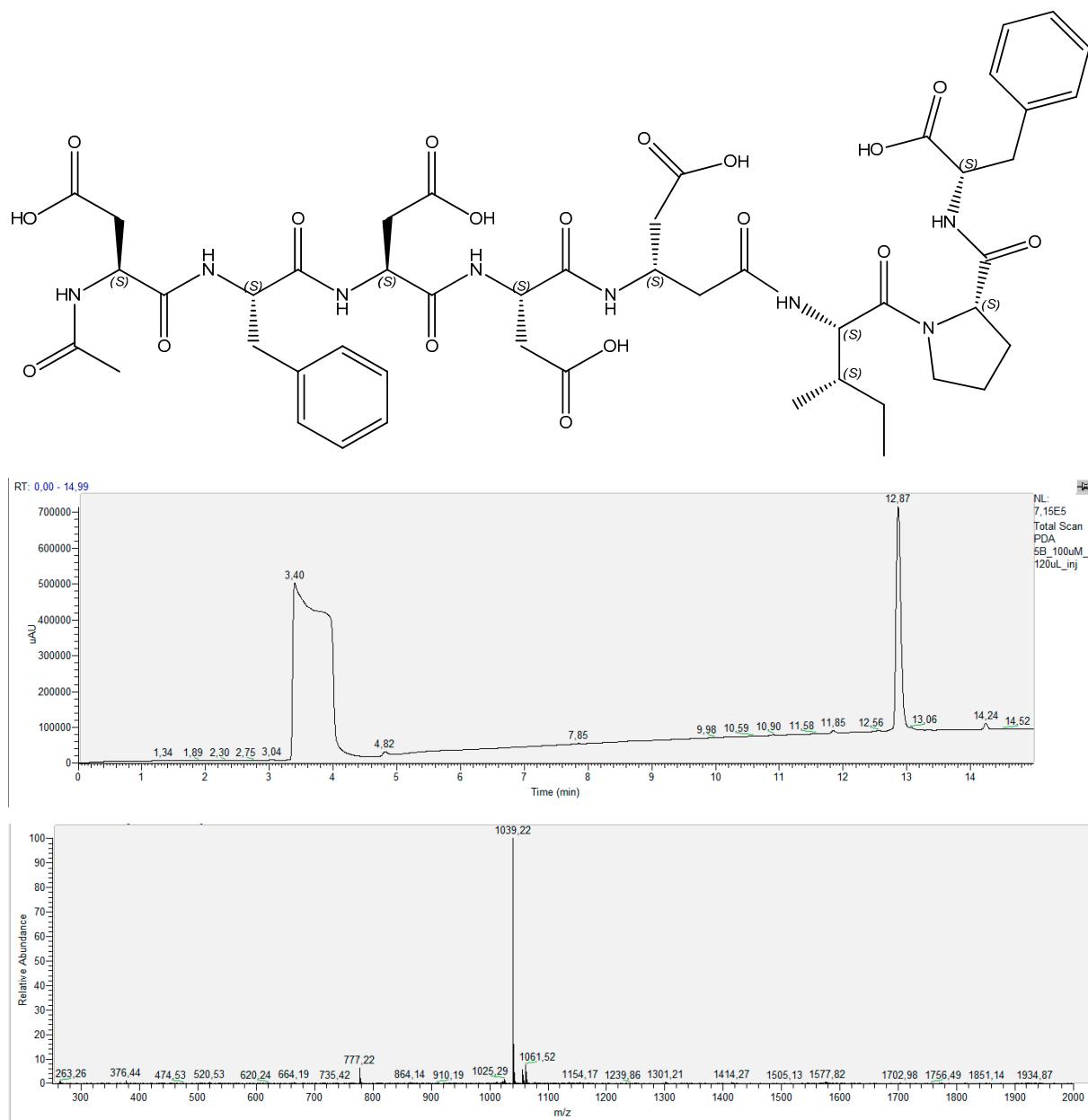


## Compound 25 – Asn4

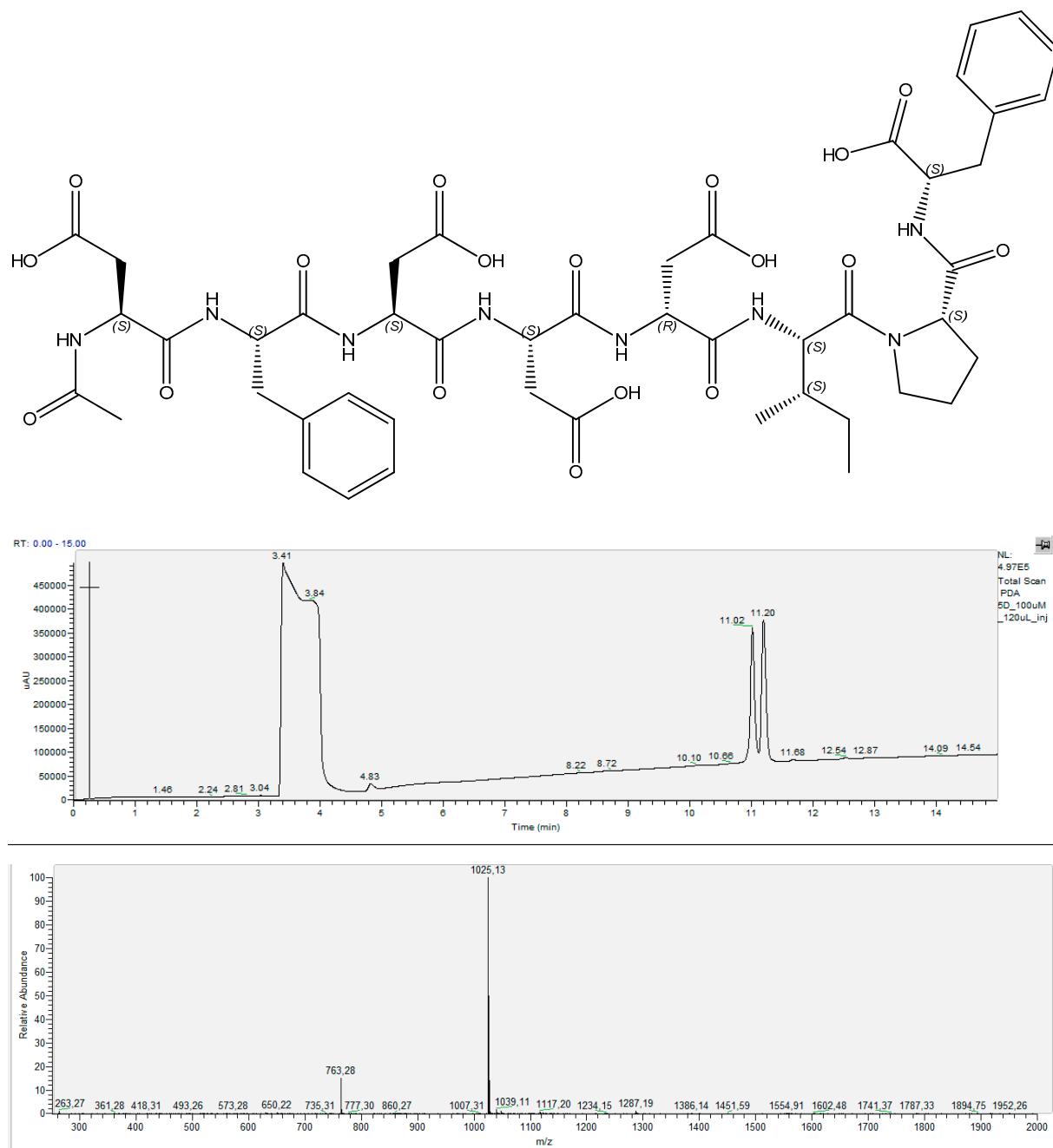


## Compound 26 – NM-Asp4

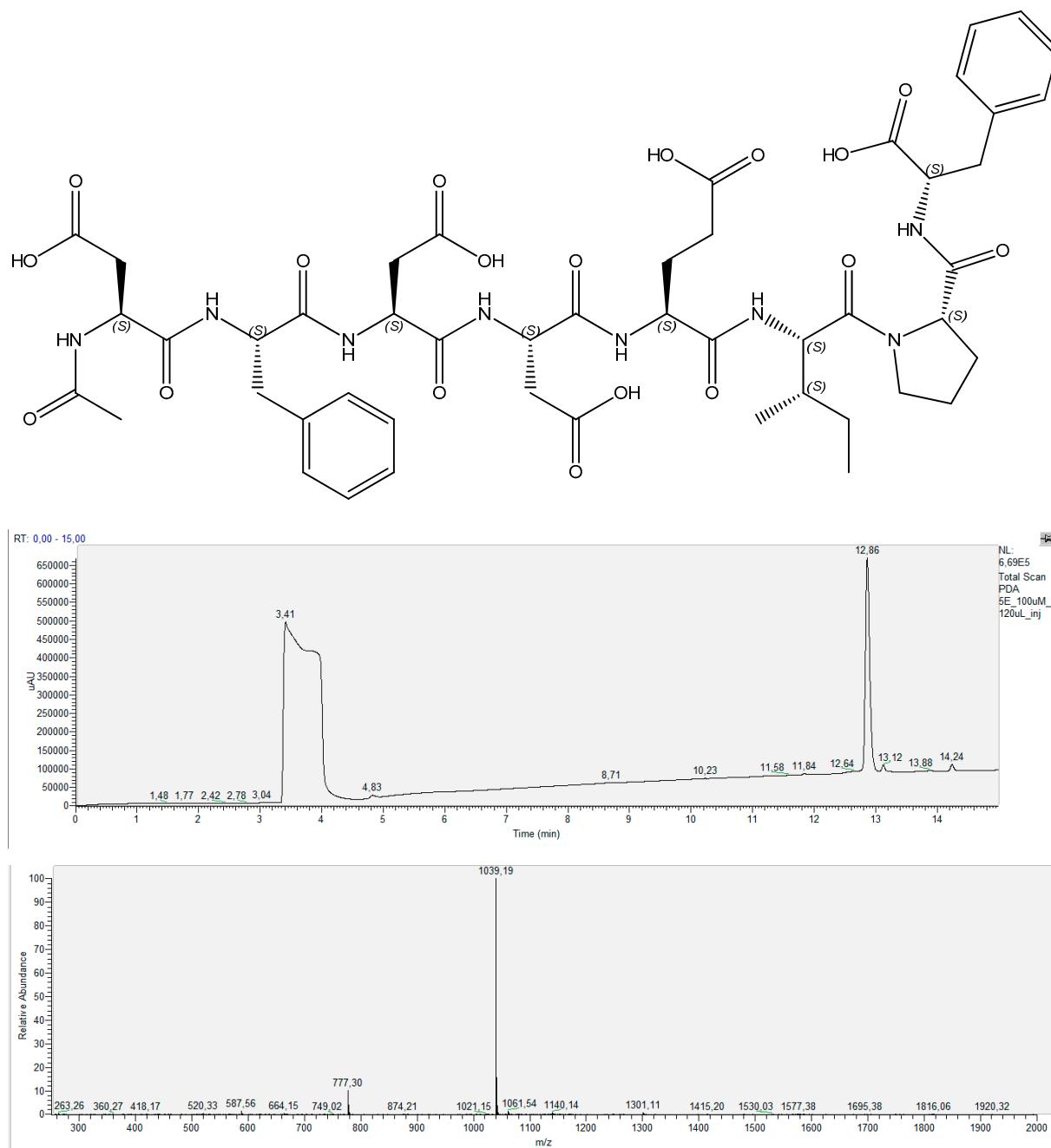


Compound 27 –  $\beta$ <sup>3</sup>-Asp5

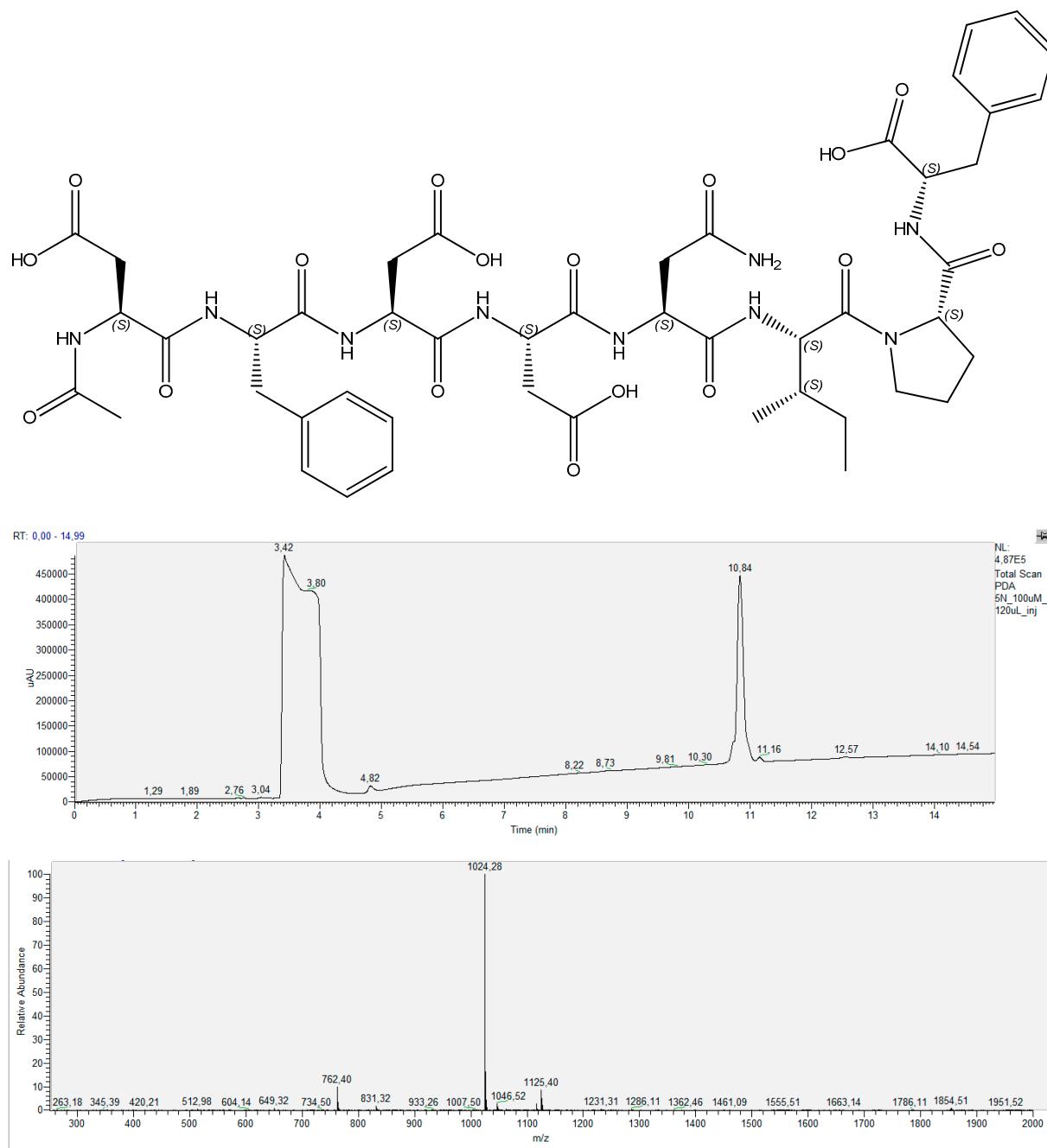
## Compound 28 – D-Asp5



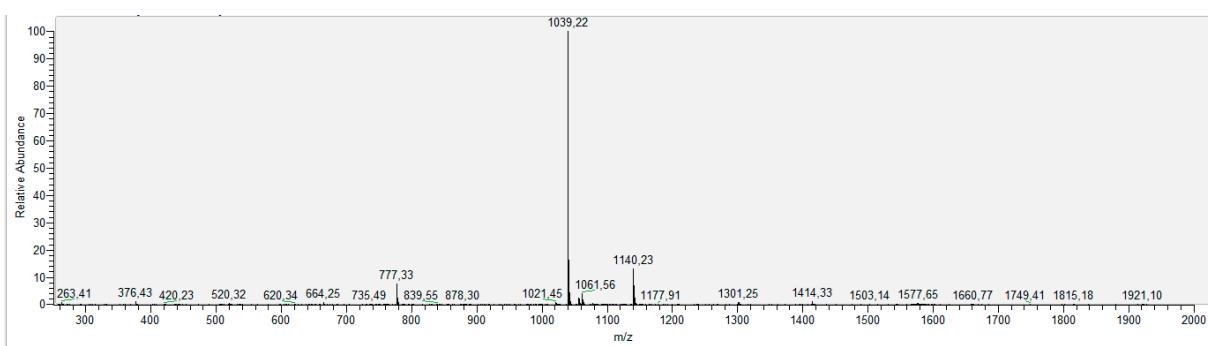
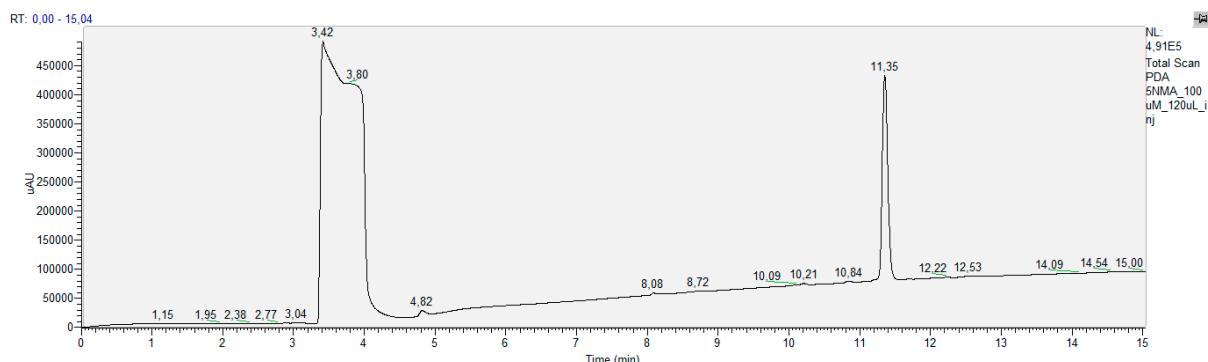
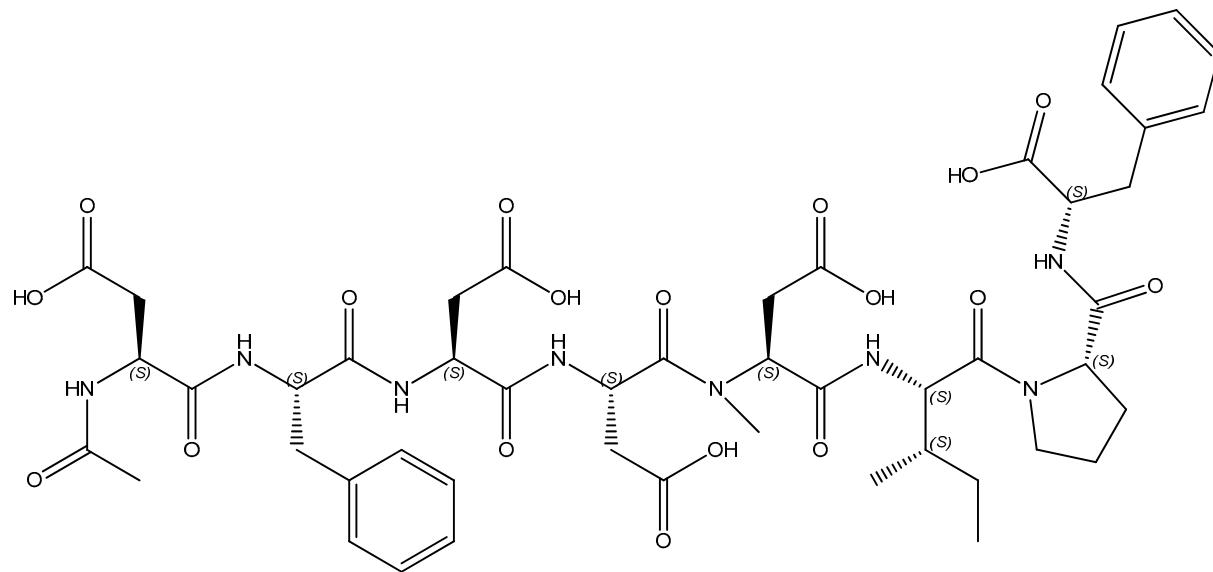
## Compound 29 – Glu5



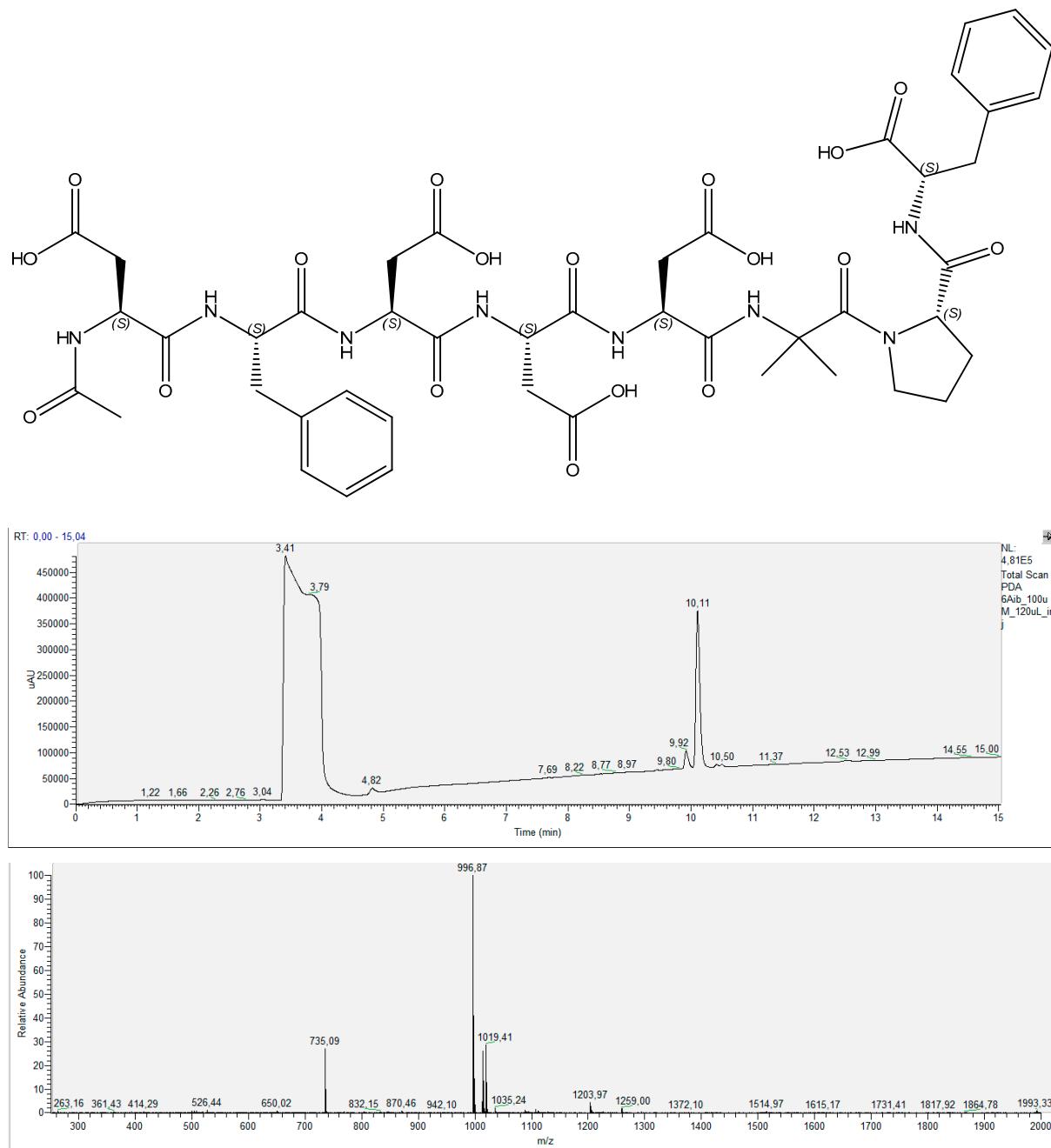
## Compound 30 – Asn5

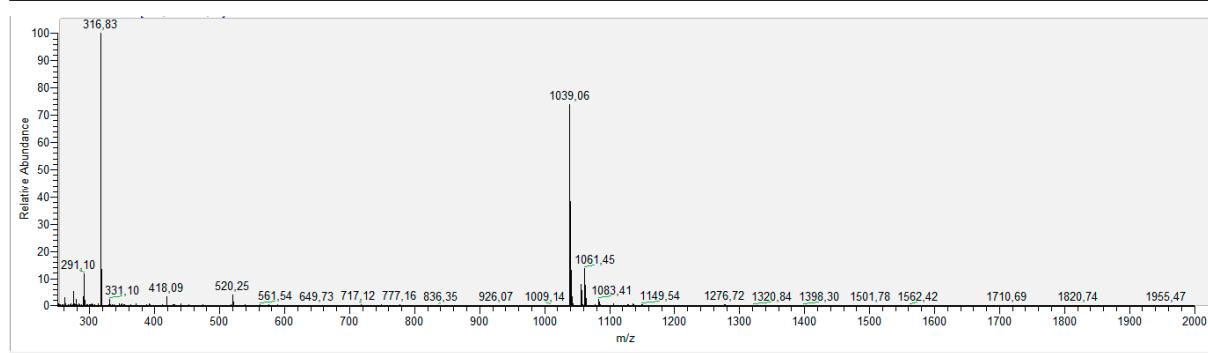
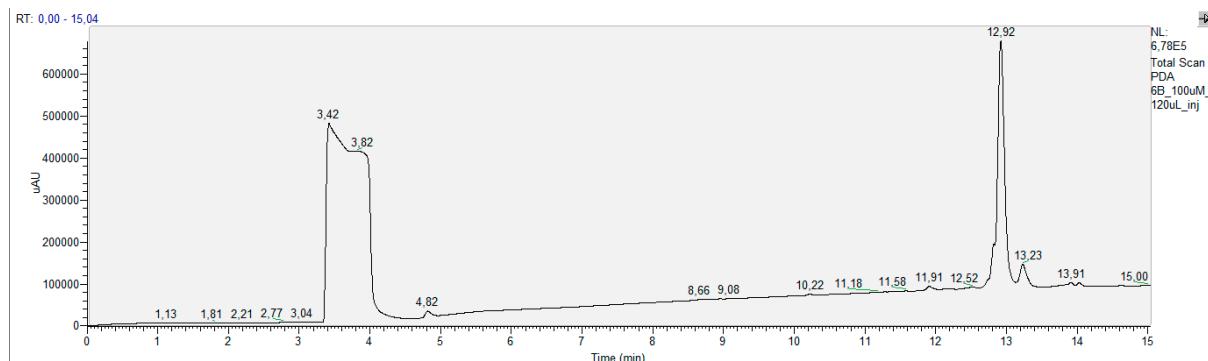
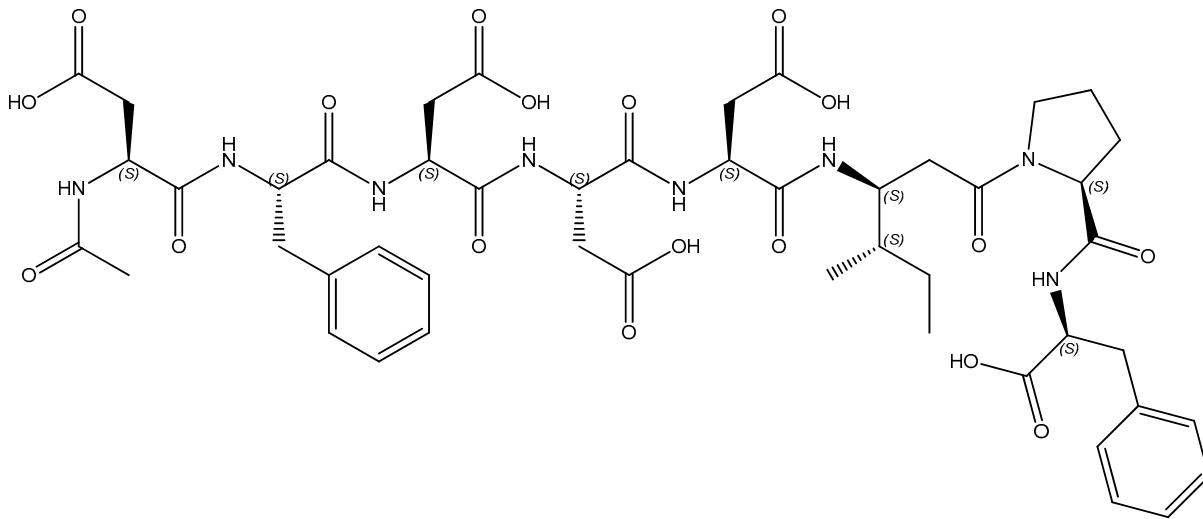


## Compound 31 – NM-Asp5

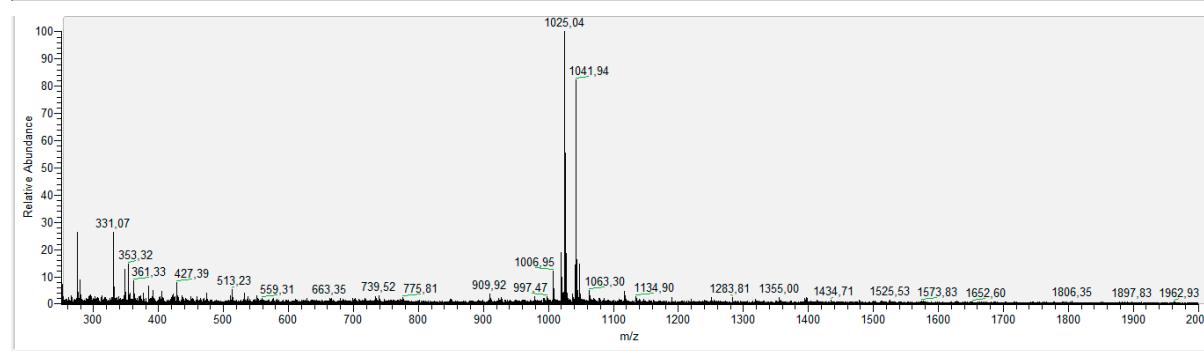
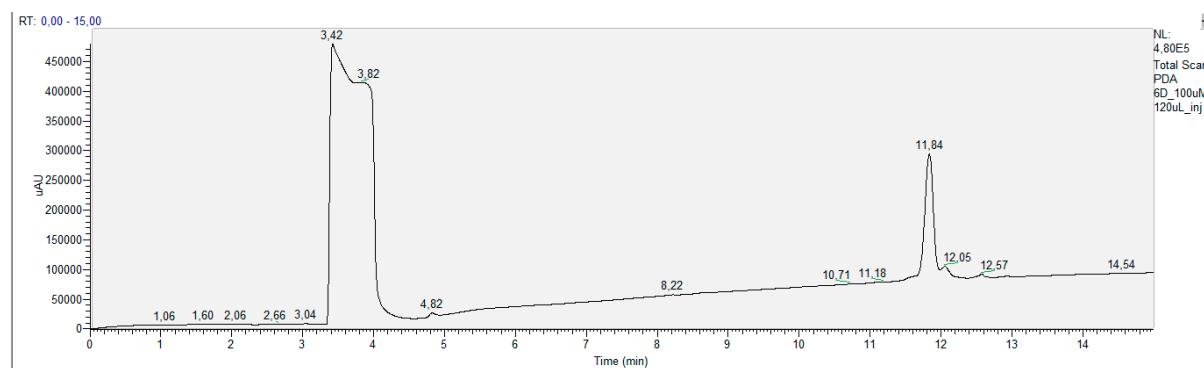
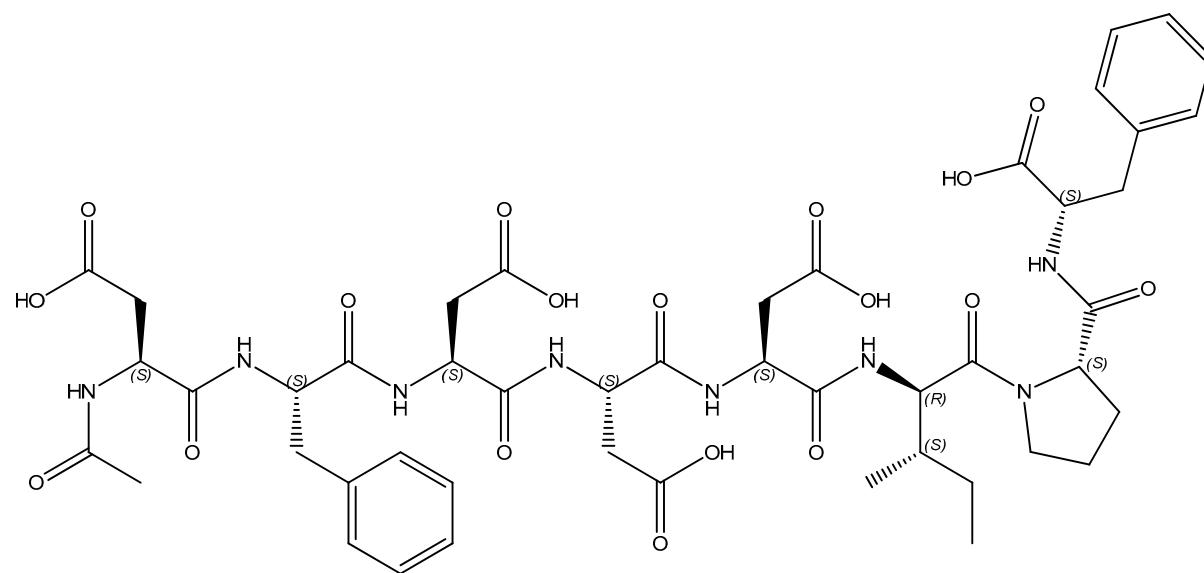


## Compound 32 – Aib6

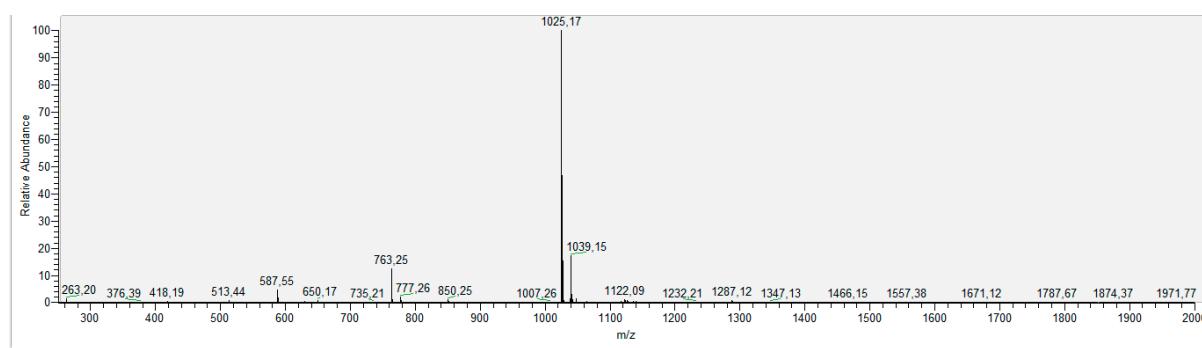
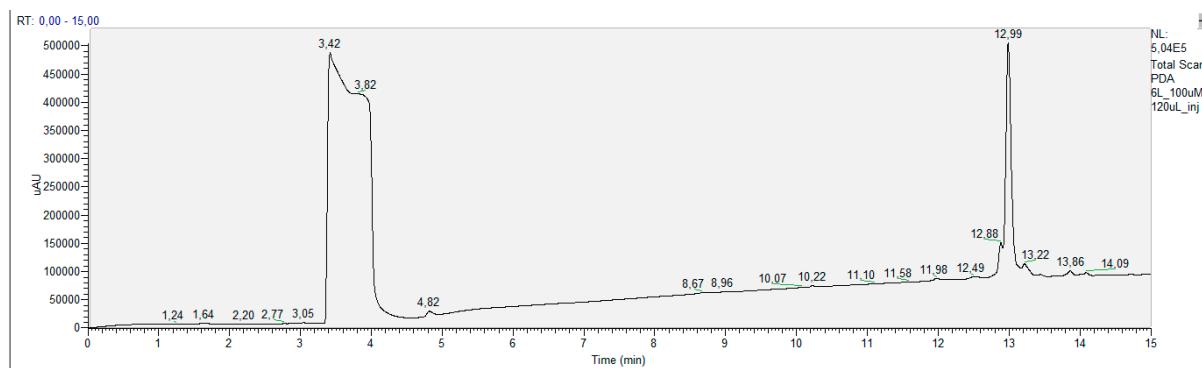
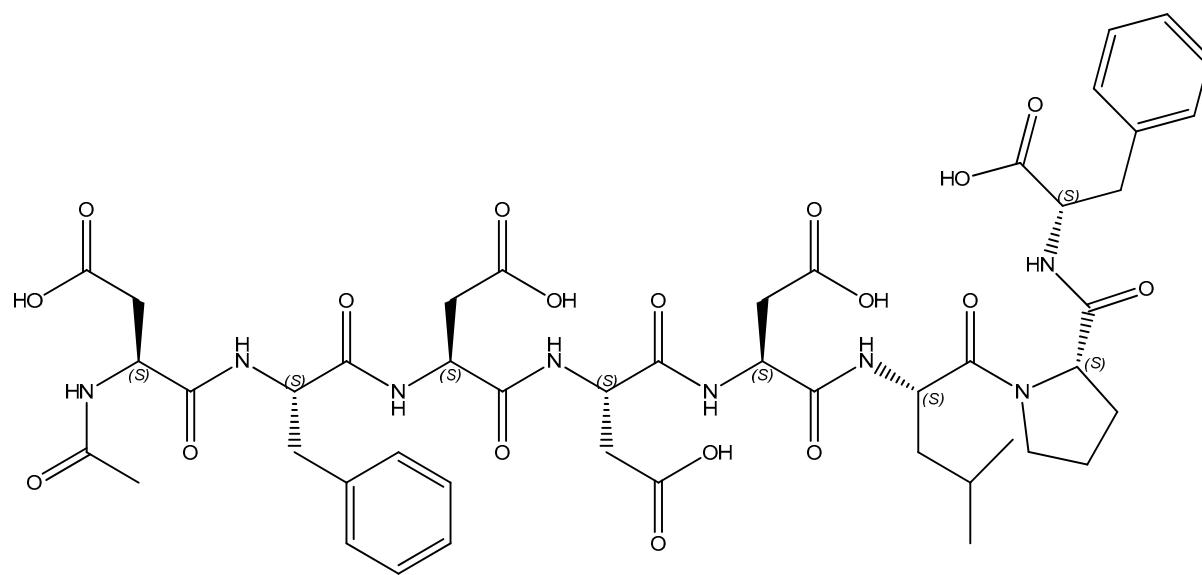


Compound 33 –  $\beta^3\text{-Ile6}$ 

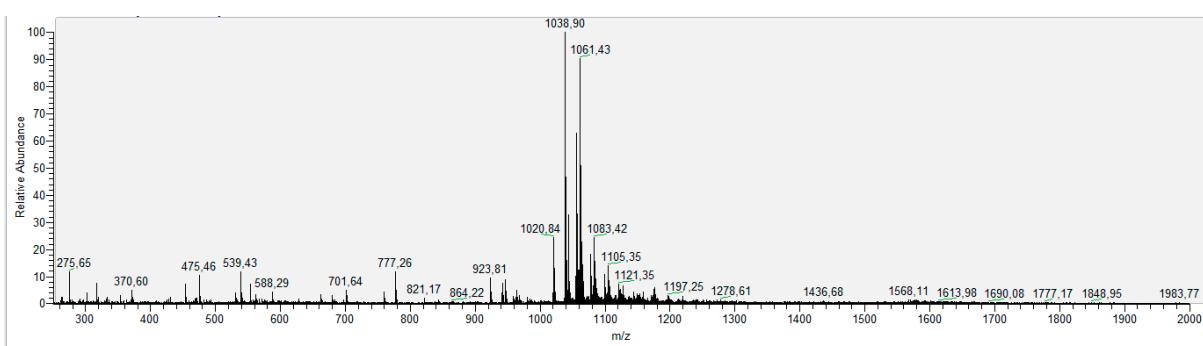
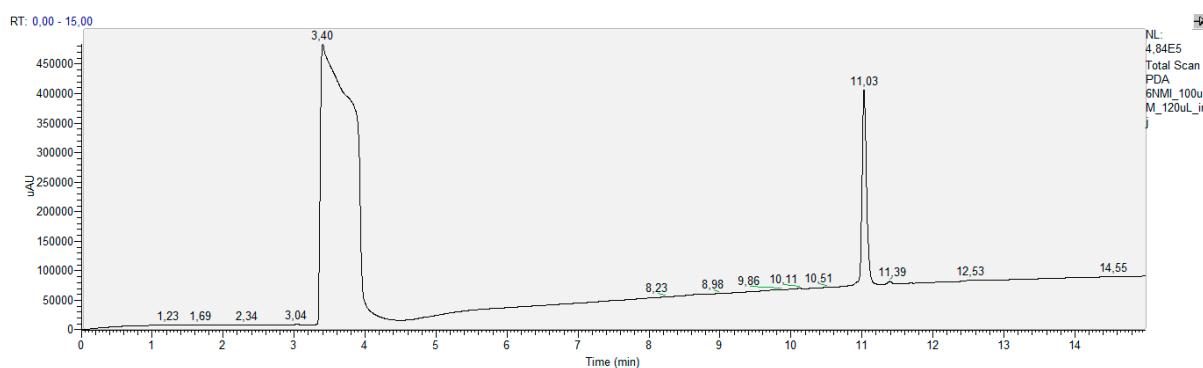
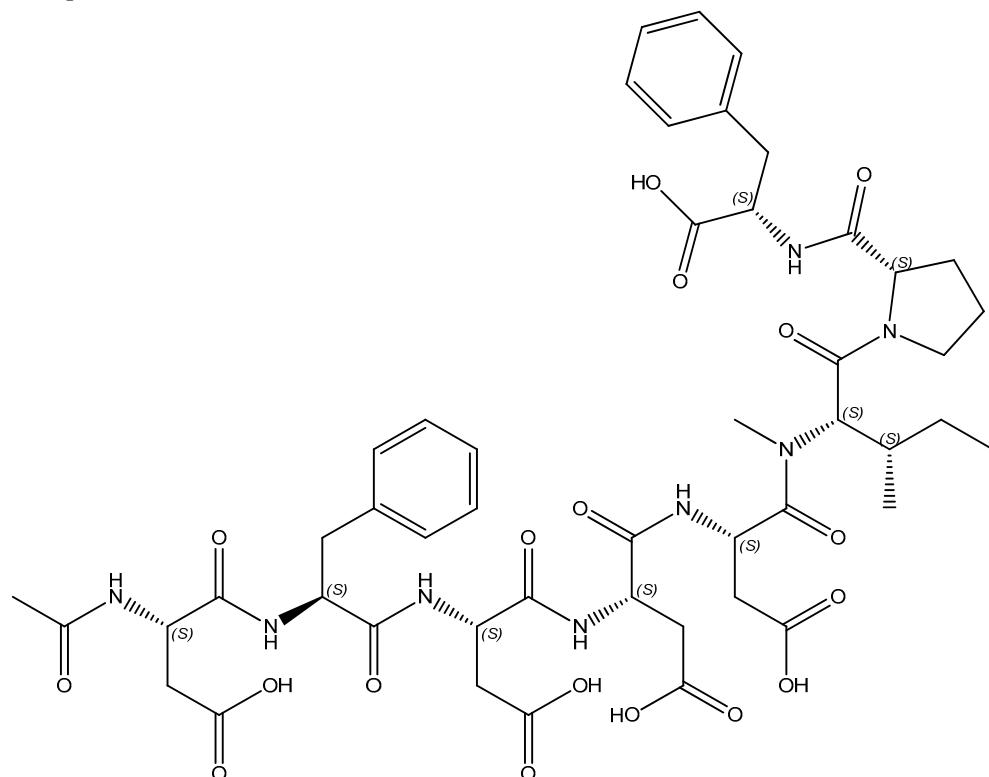
## Compound 34 – D-Ile6



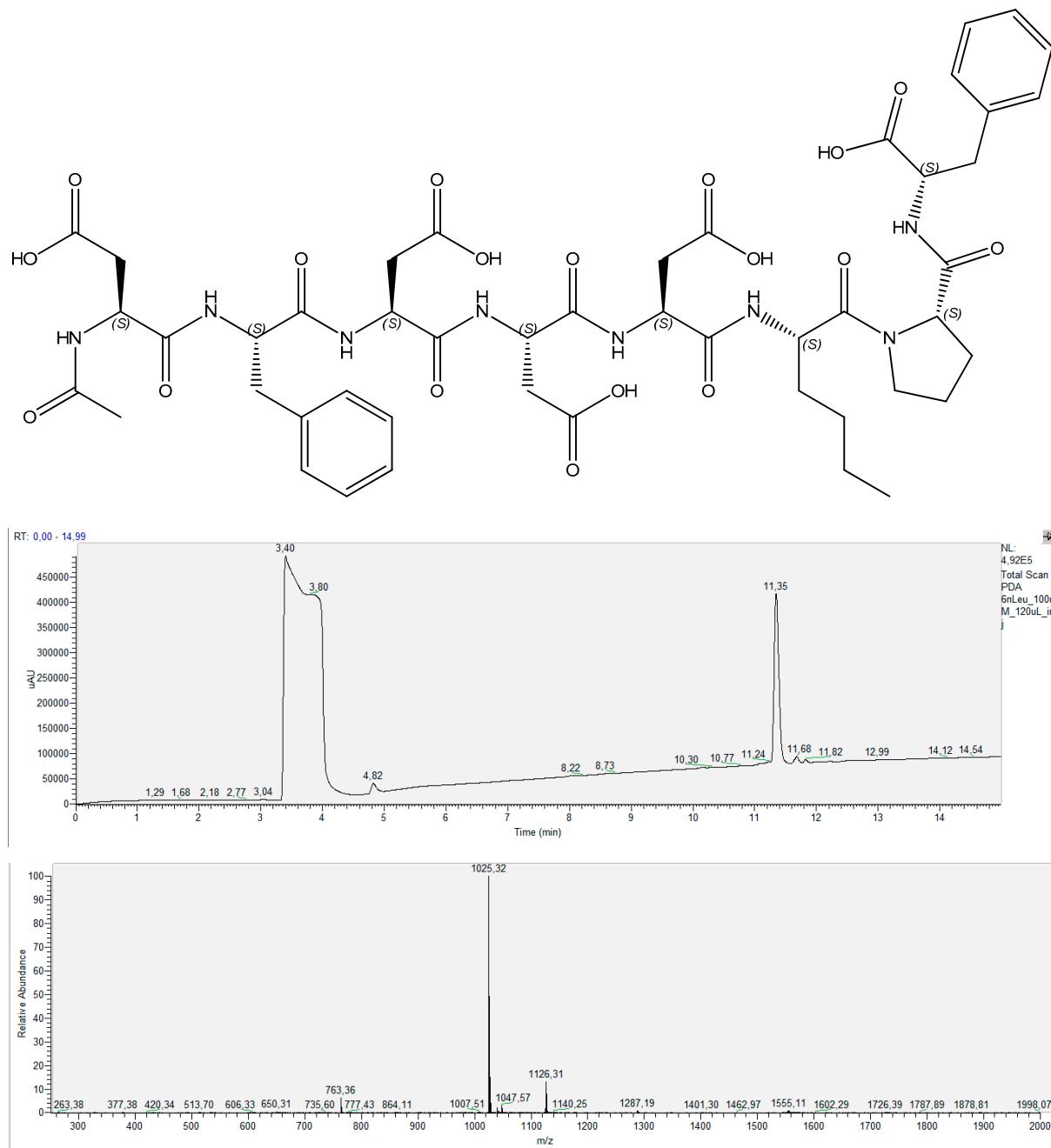
## Compound 35 – Leu6



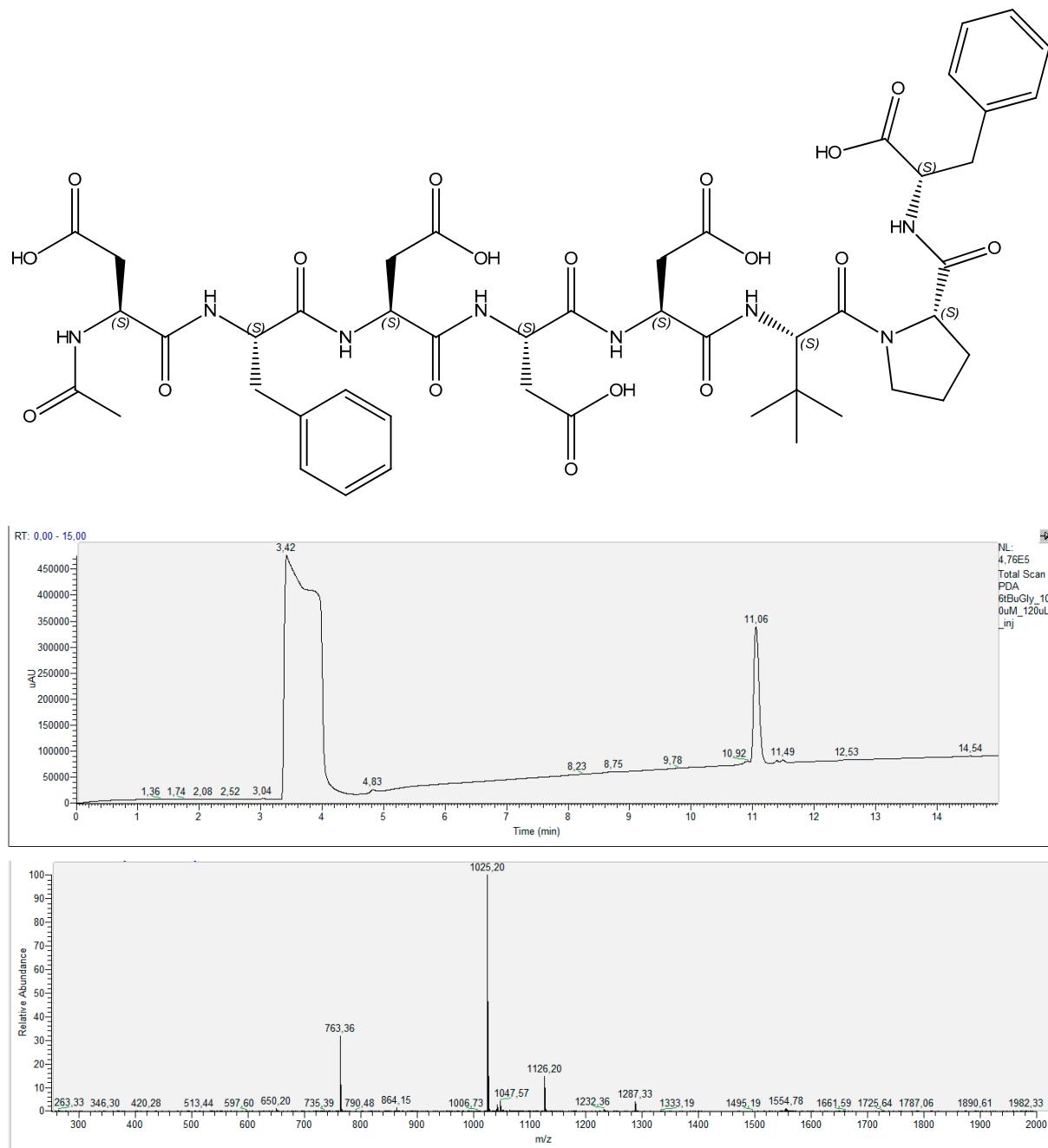
## Compound 36 – NM-Ile6



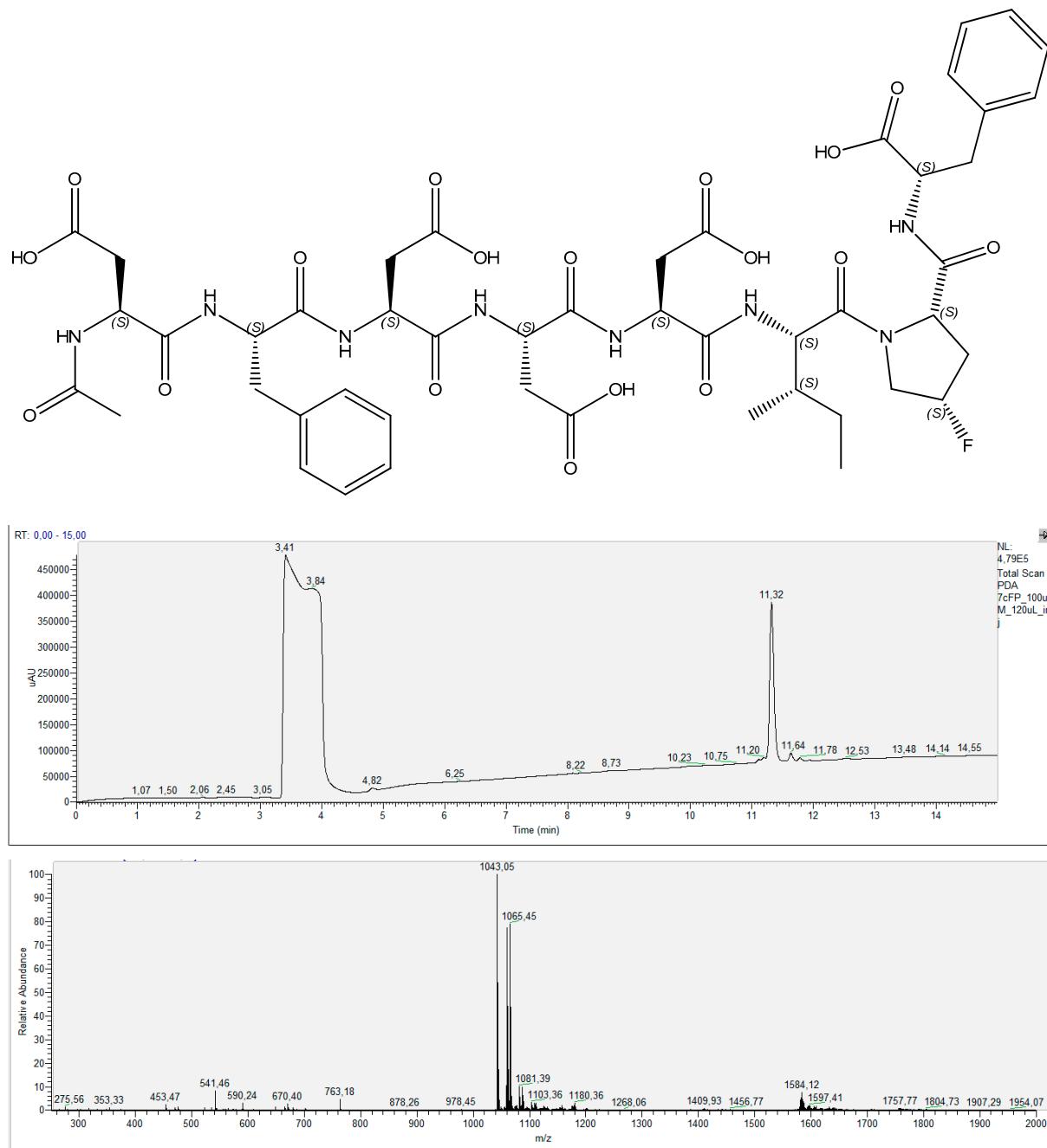
## Compound 37 – nLeu6



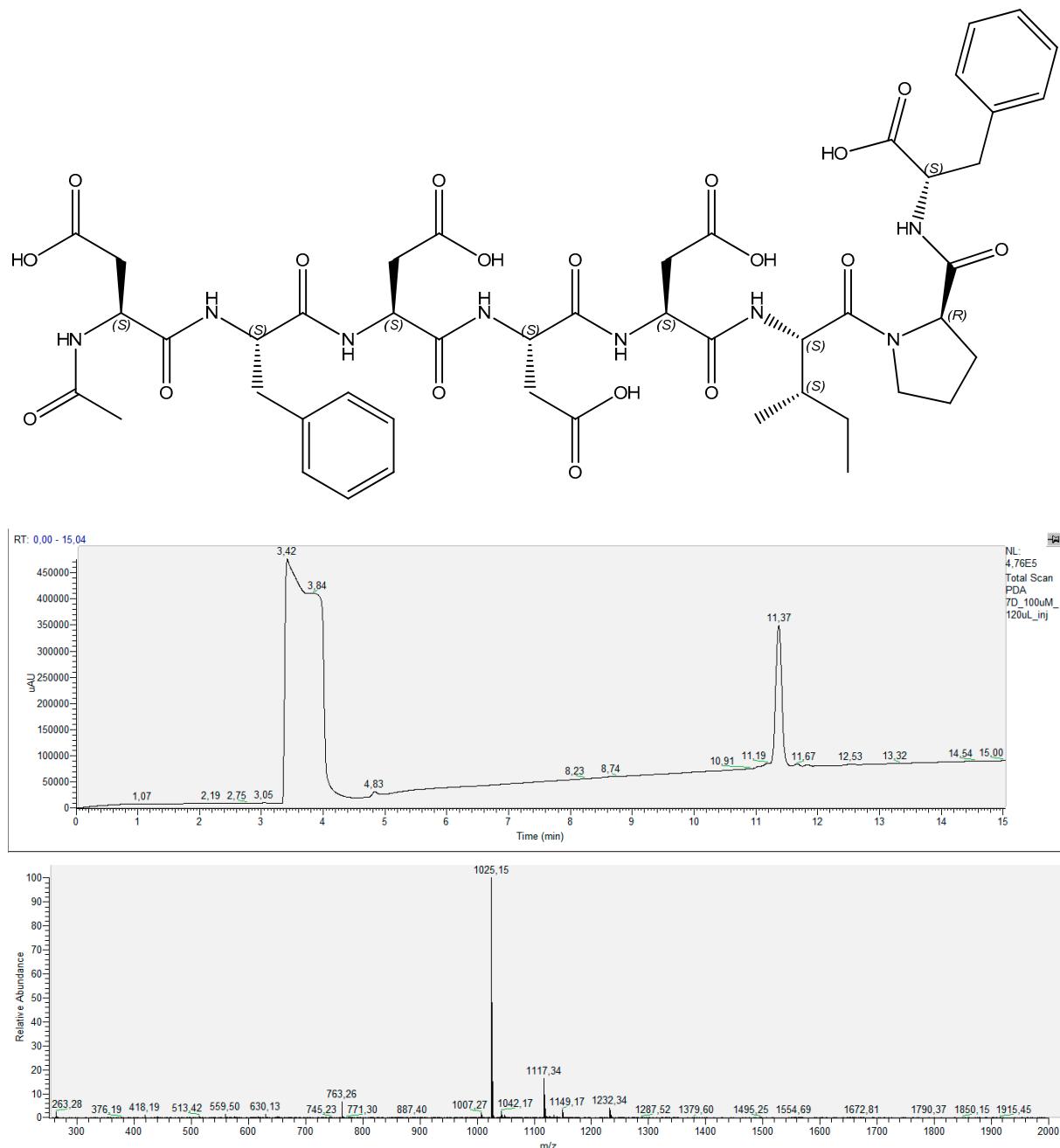
## Compound 38 – tBuGly6



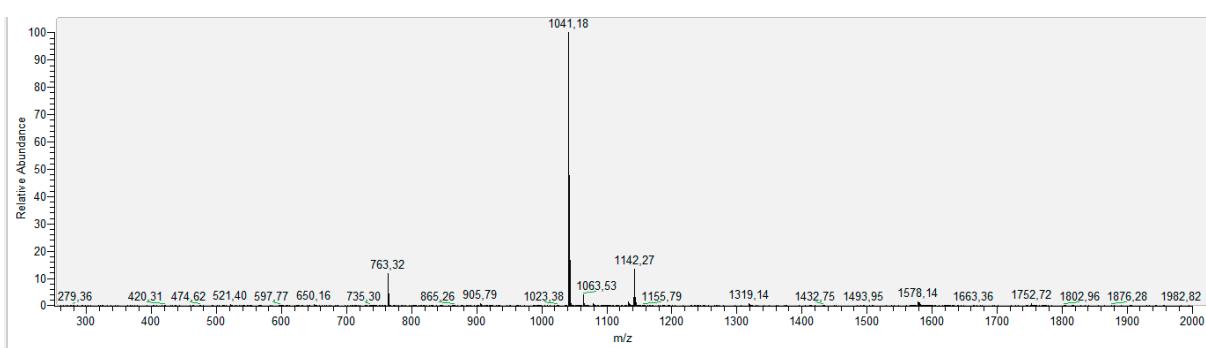
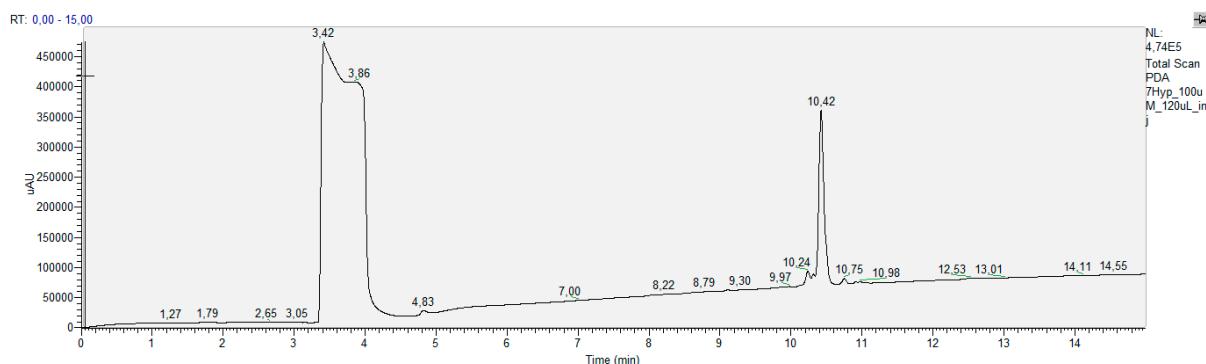
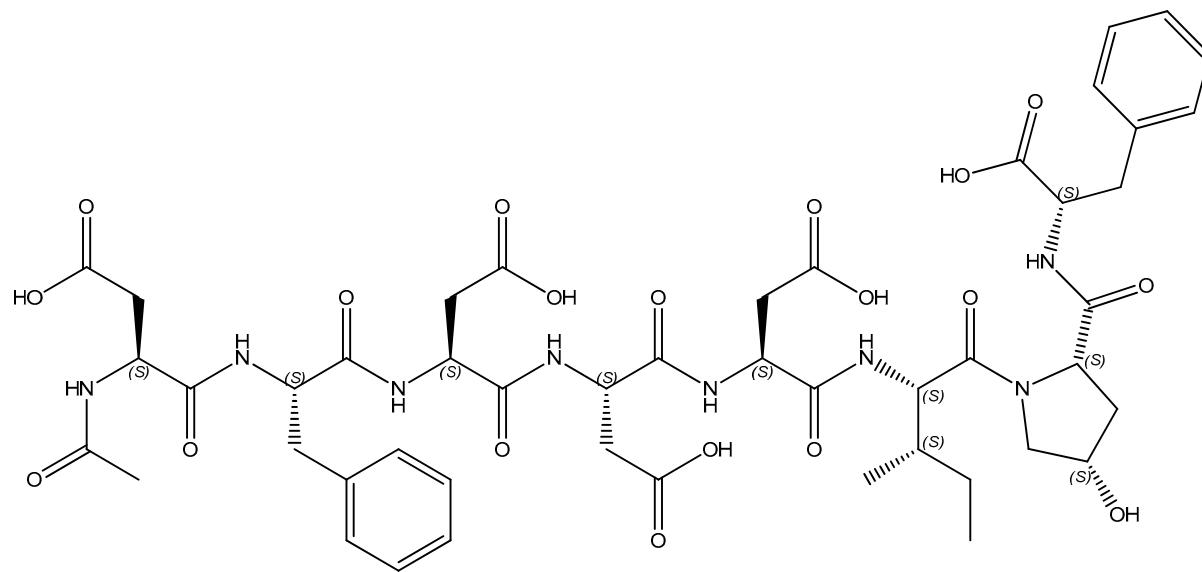
## Compound 39 – 4cF-Pro7



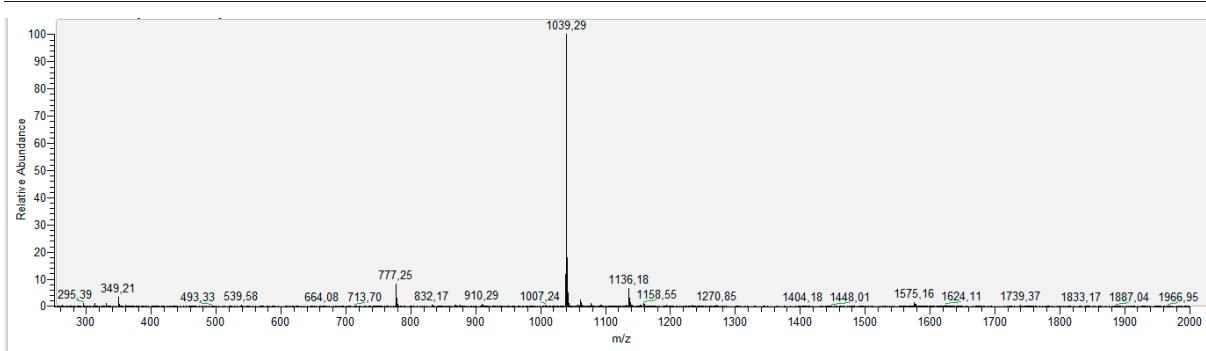
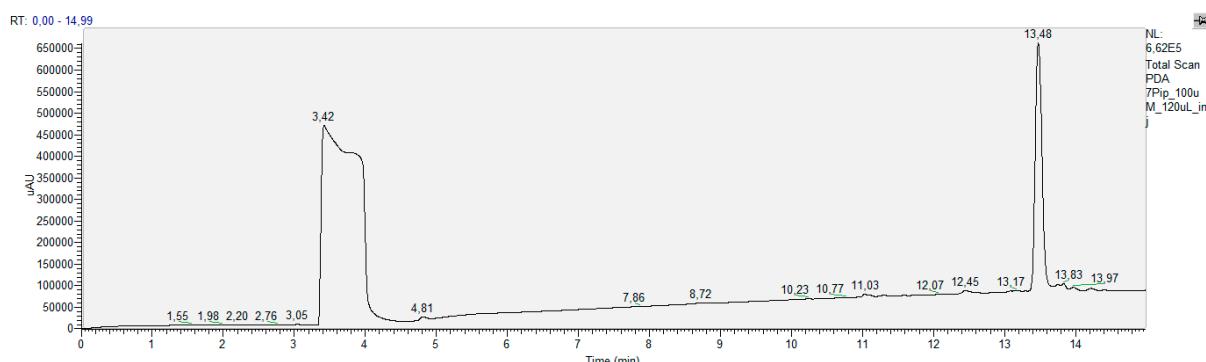
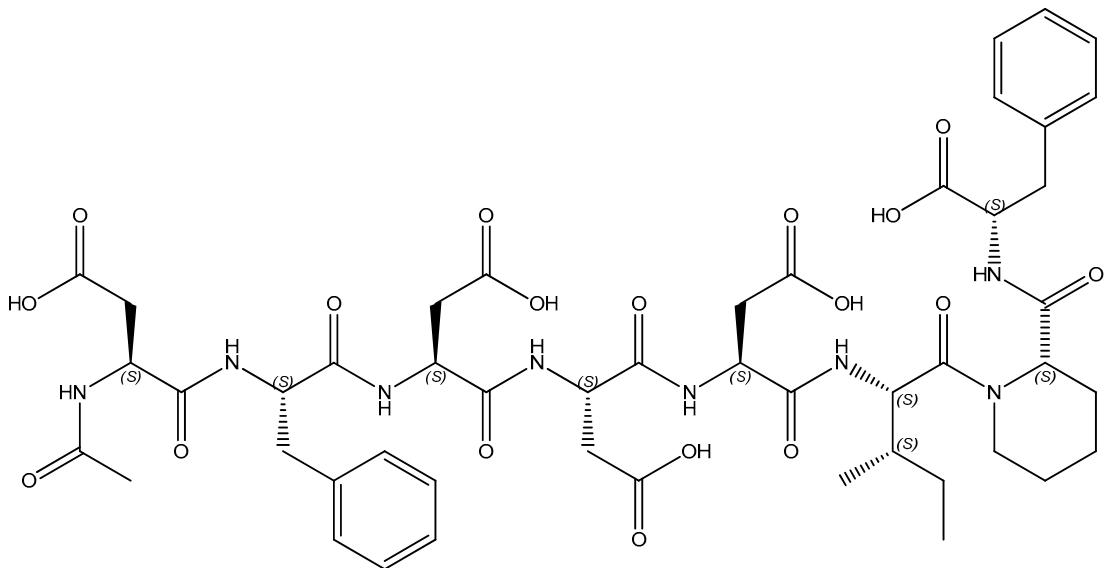
## Compound 40 – D-Pro7

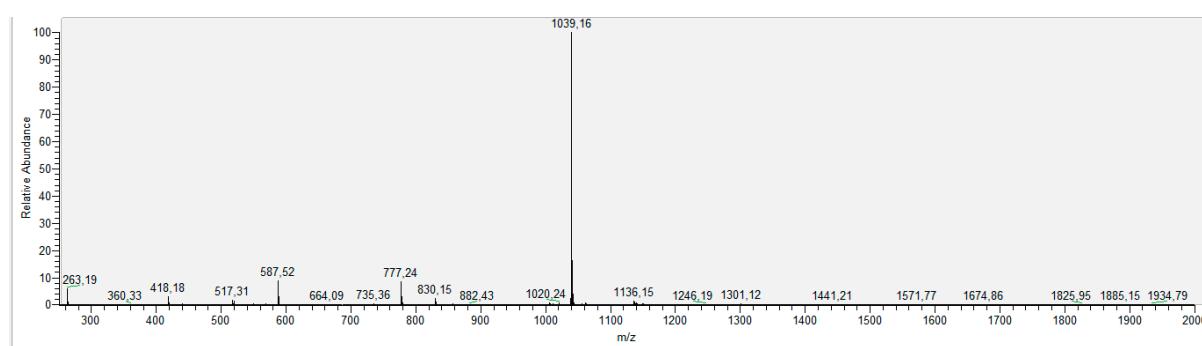
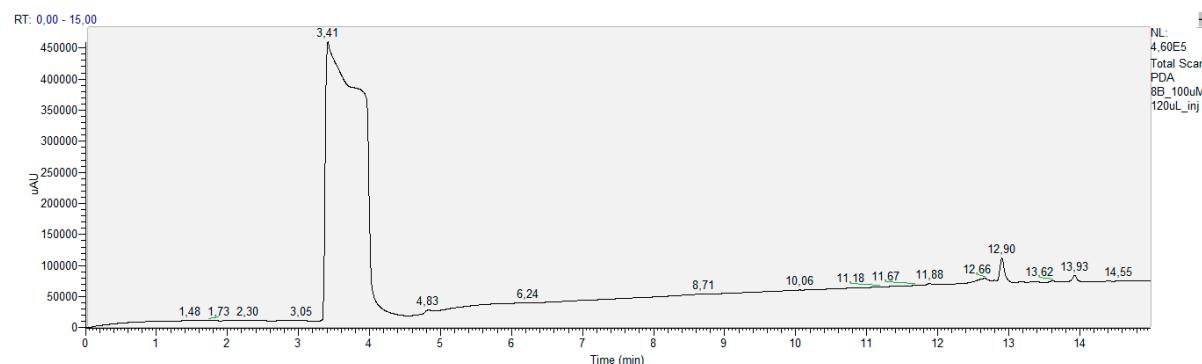
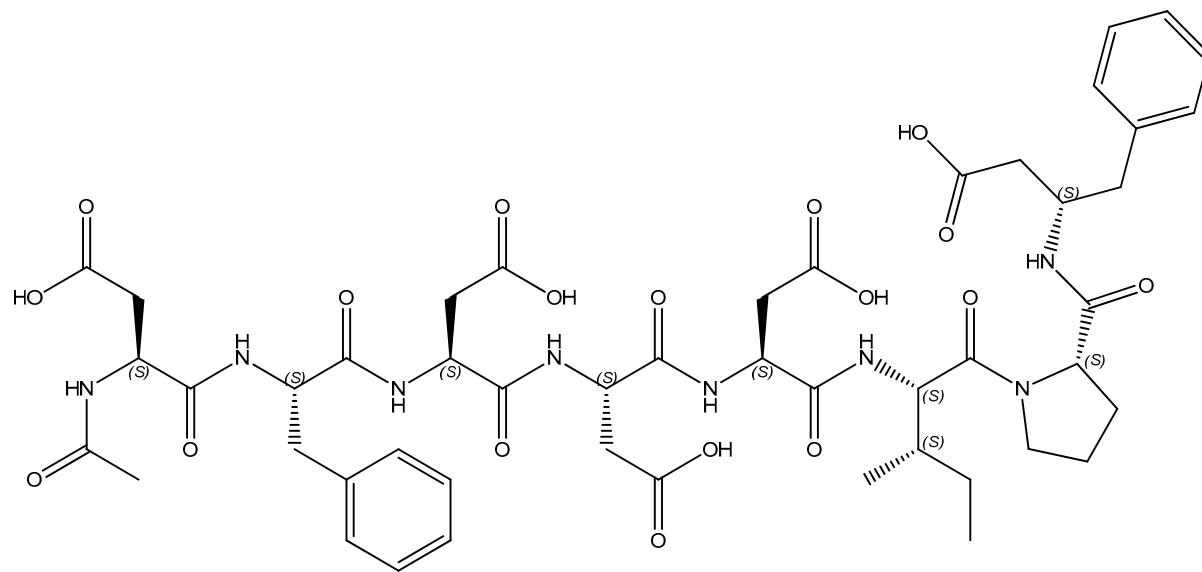


## Compound 41 – Hyp7

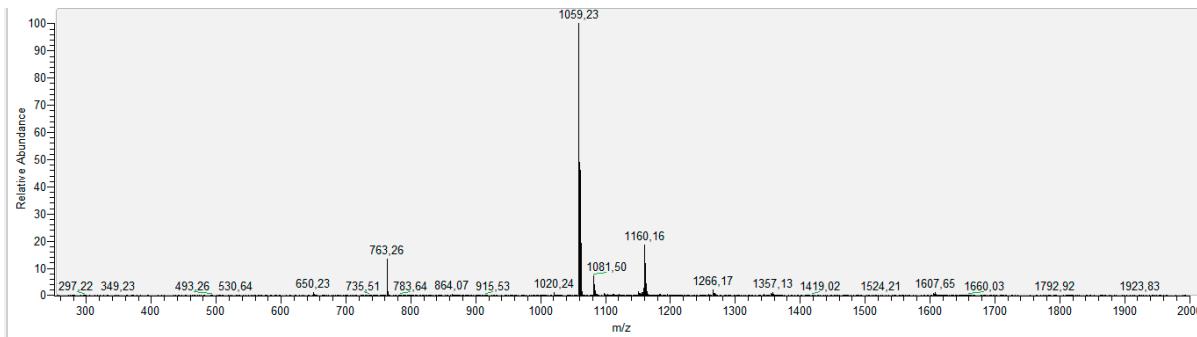
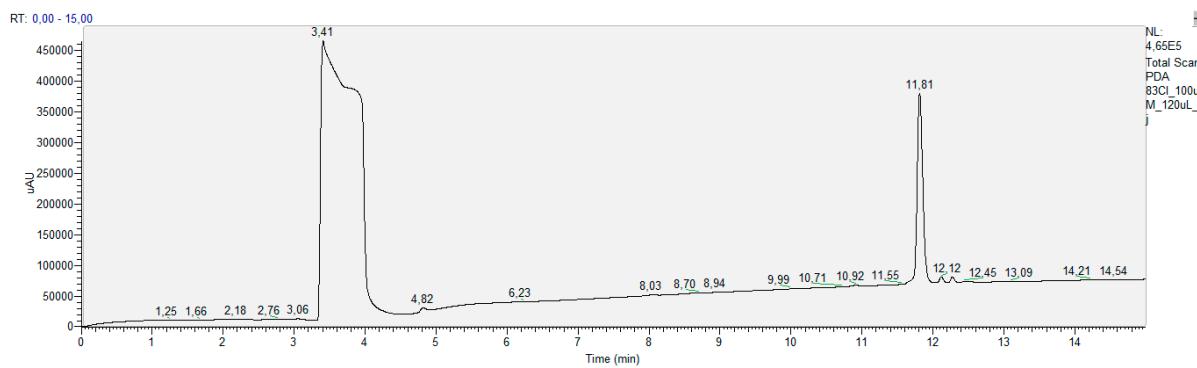
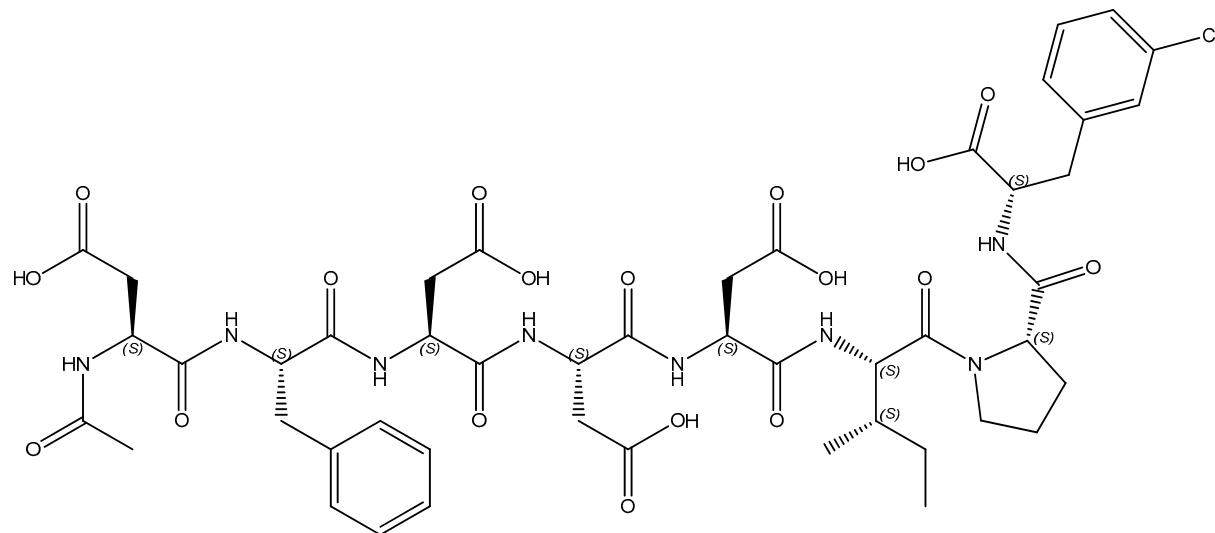


## Compound 42 – Pip7

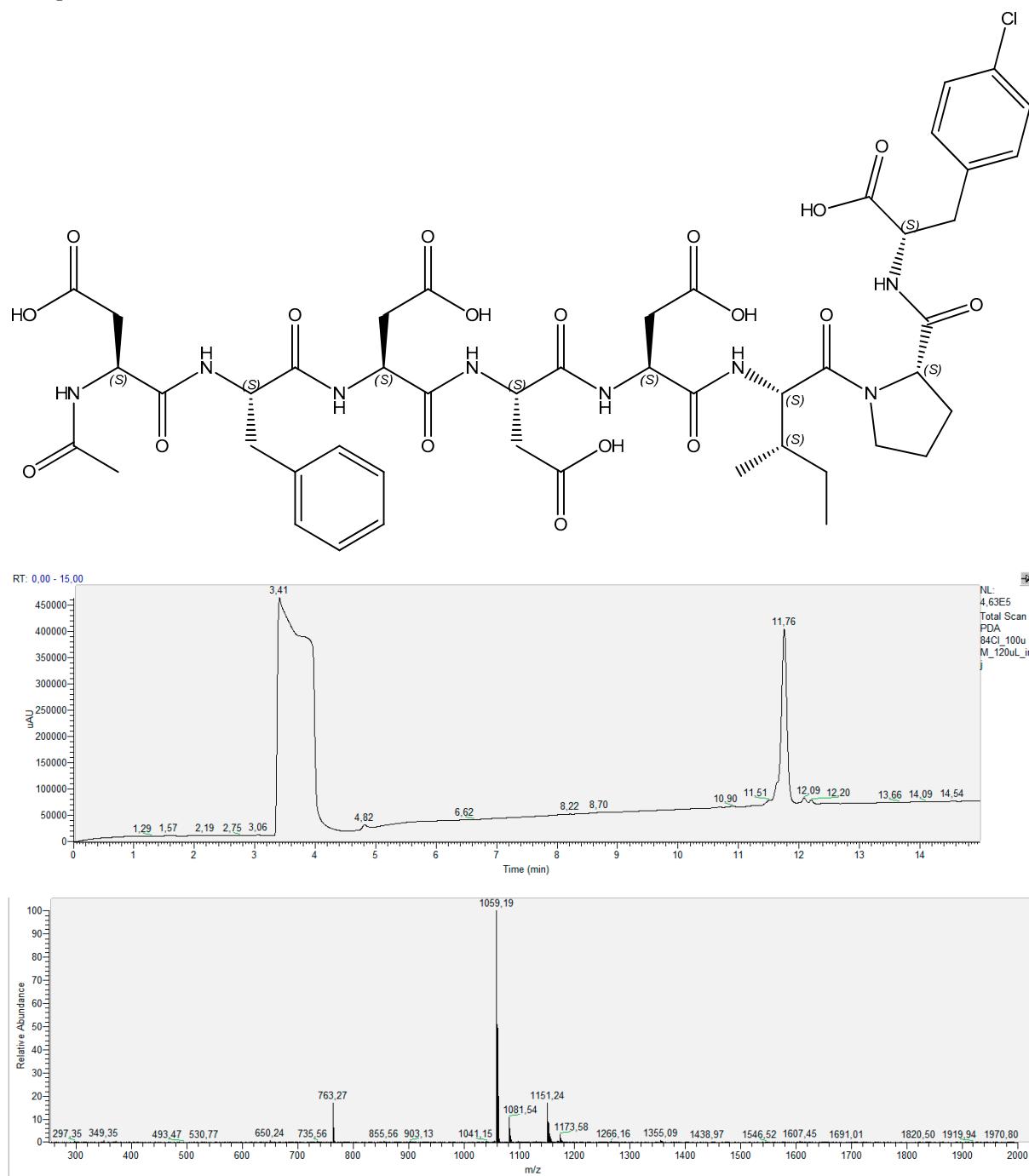


Compound 43 –  $\beta^3\text{-Phe8}$ 

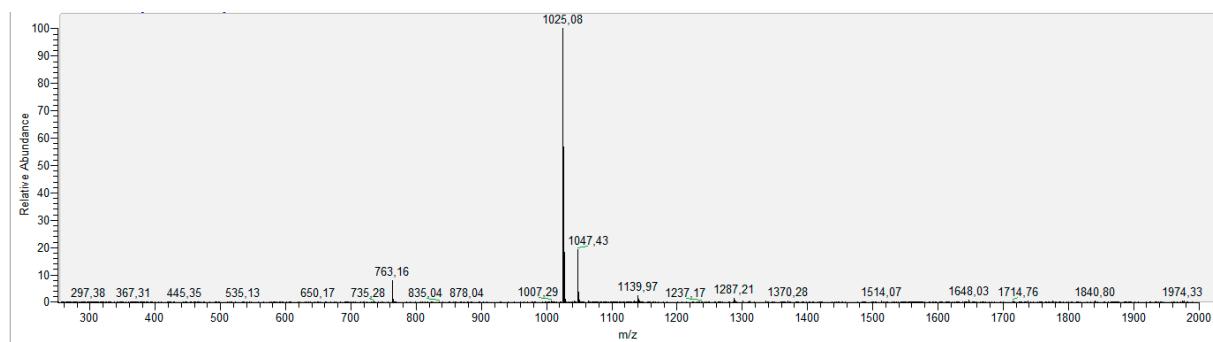
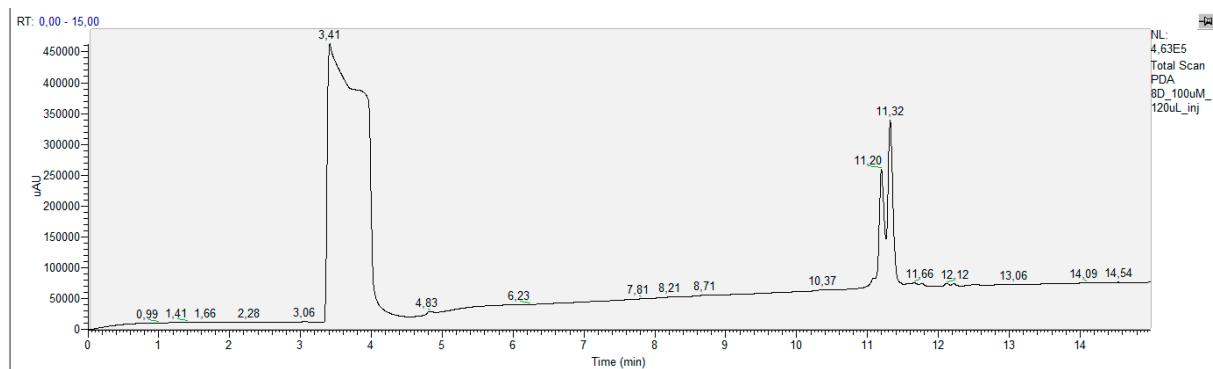
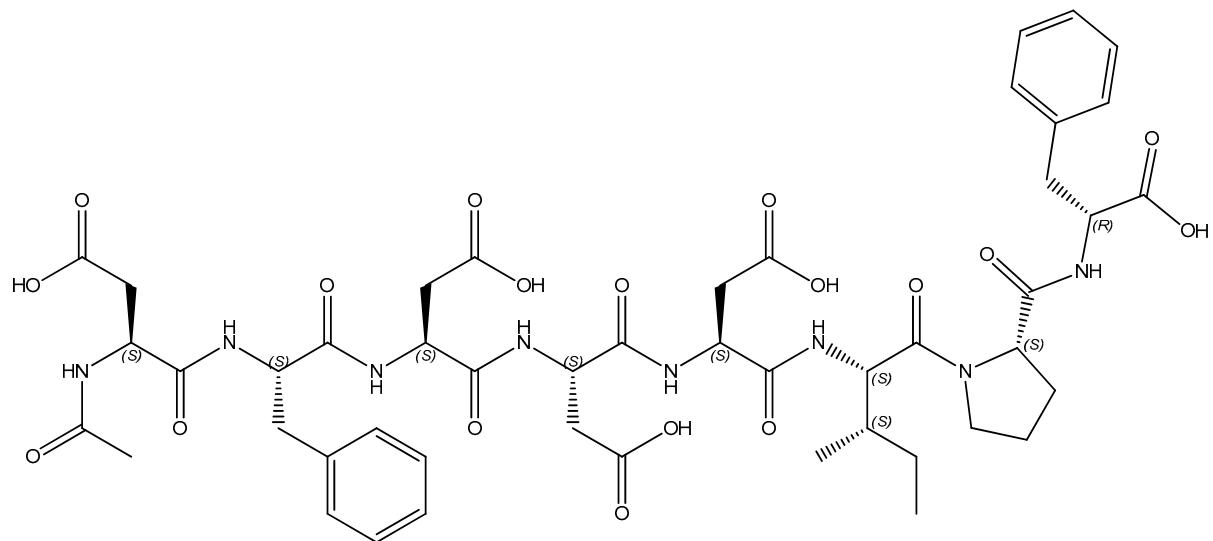
## Compound 44 – 3Cl-Phe8



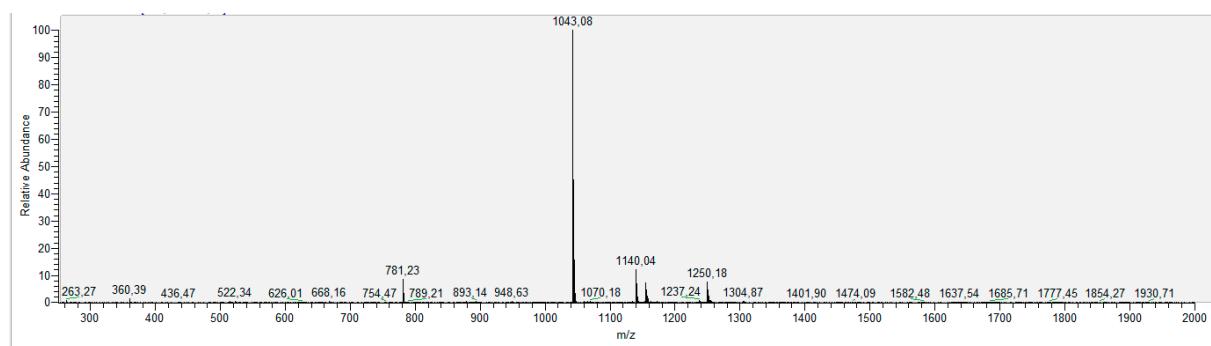
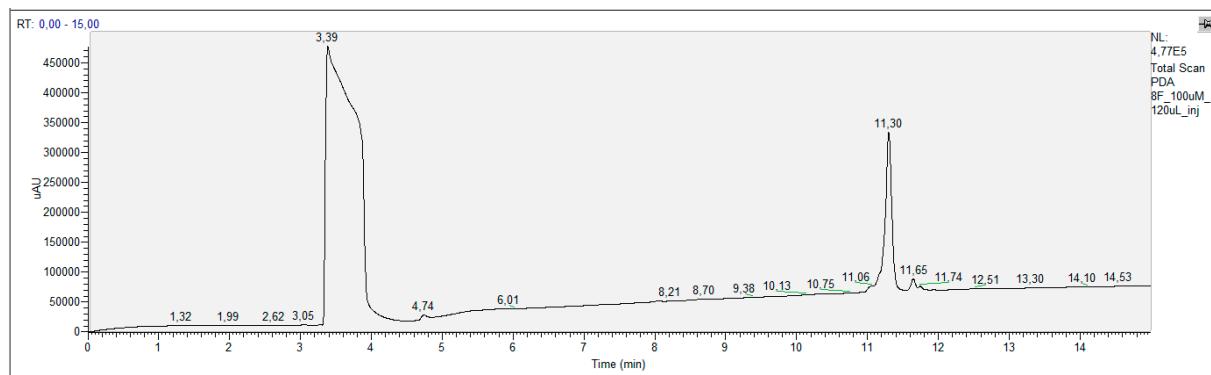
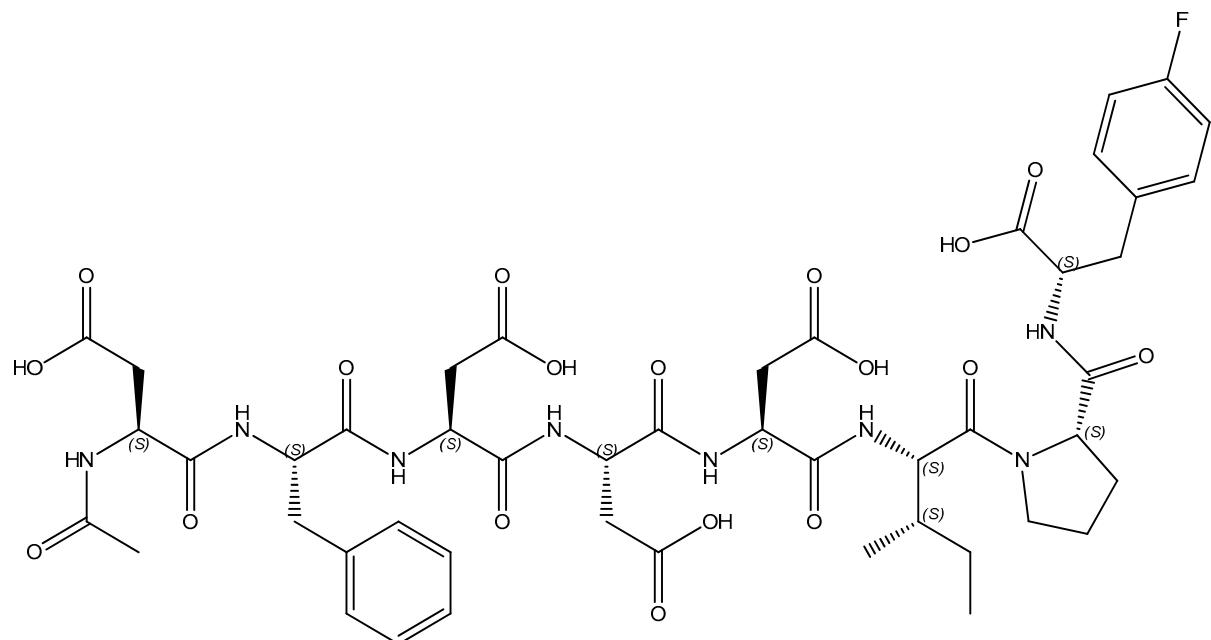
Compound 45 – 4Cl-Phe8



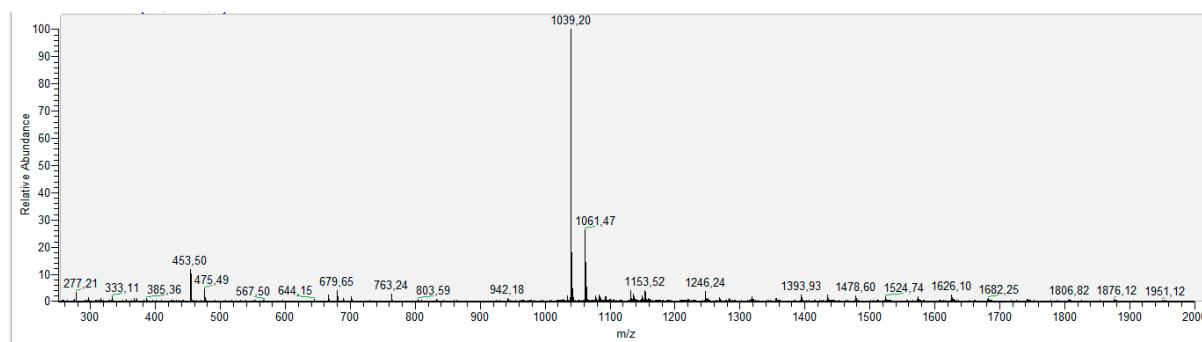
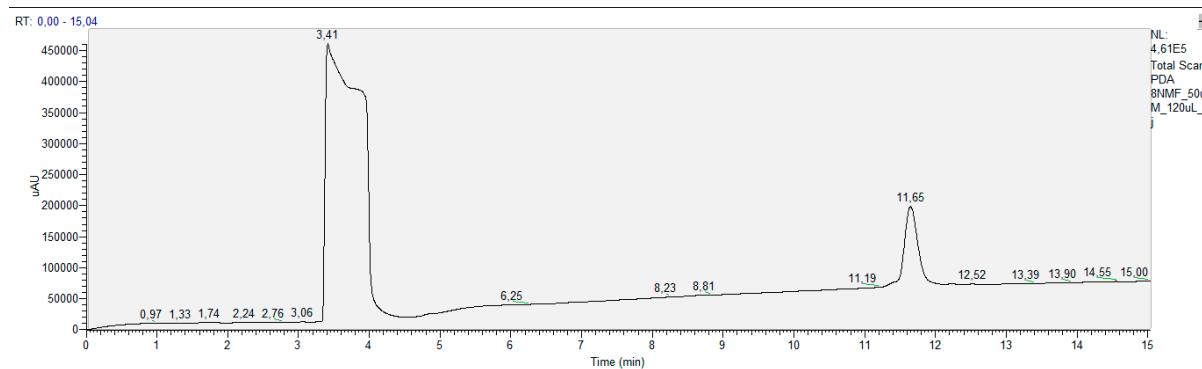
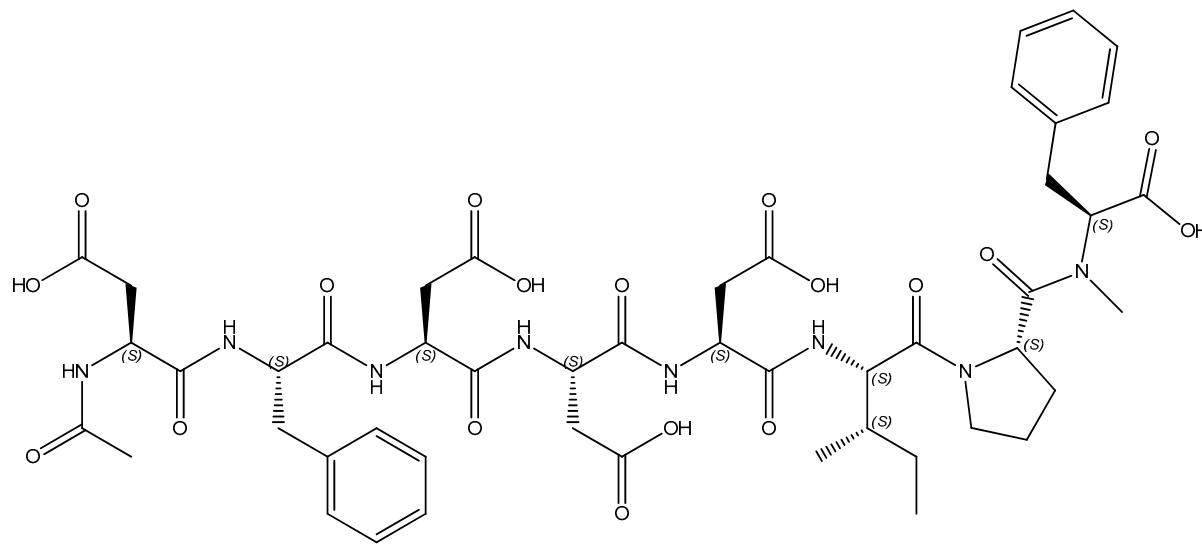
## Compound 46 – D-Phe8



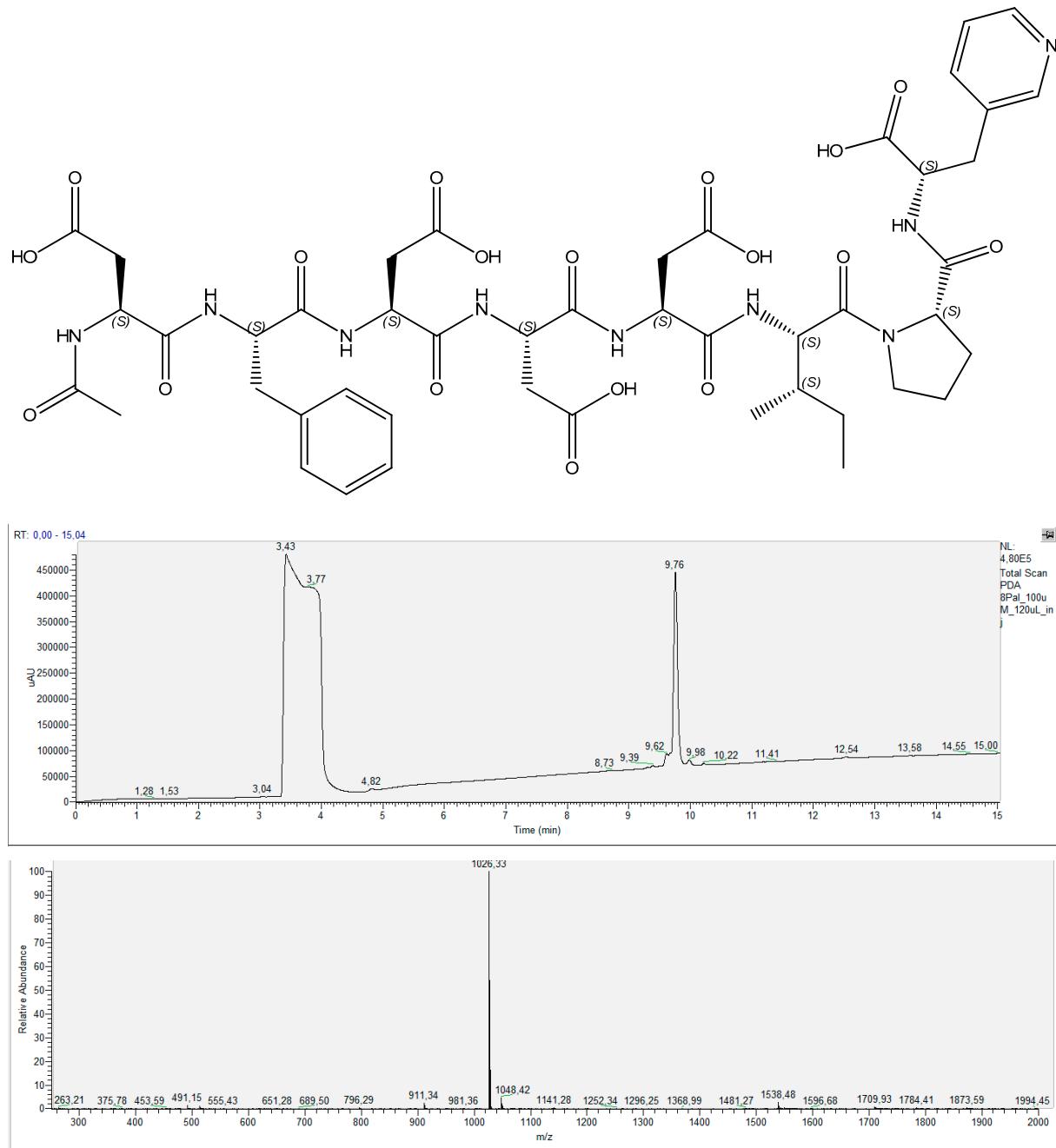
## Compound 47 – F-Phe8



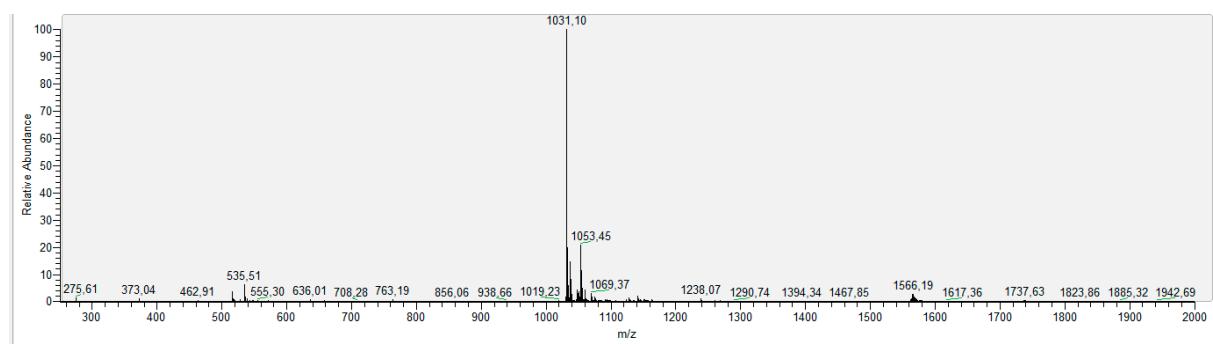
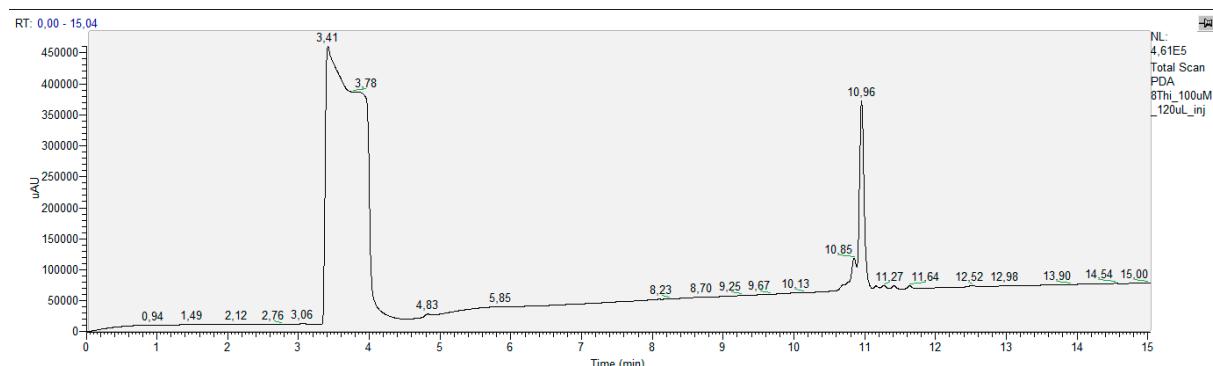
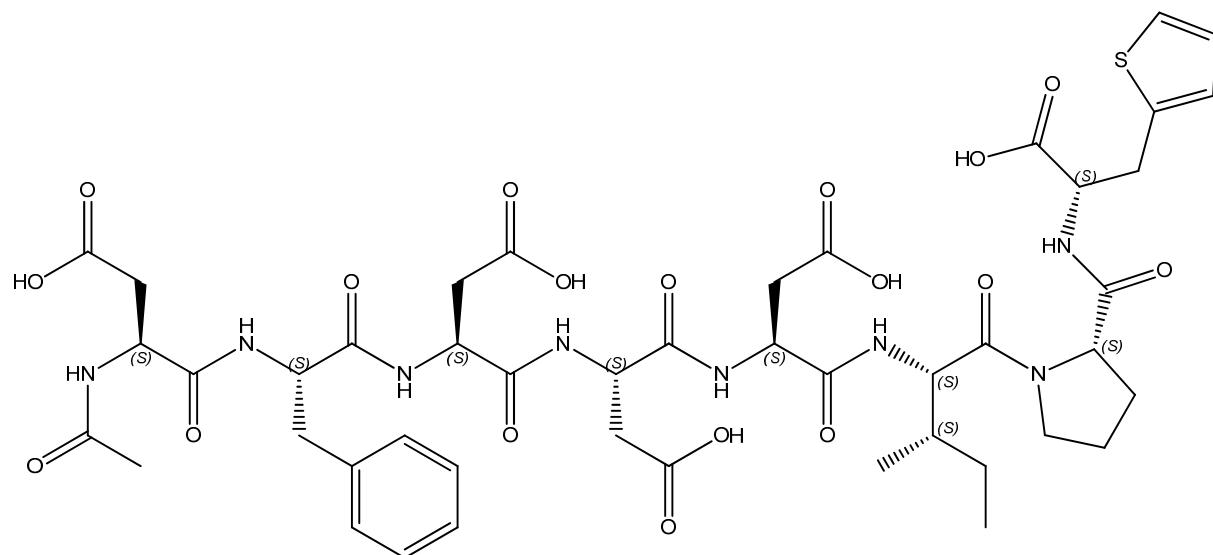
## Compound 48 – NM-Phe8



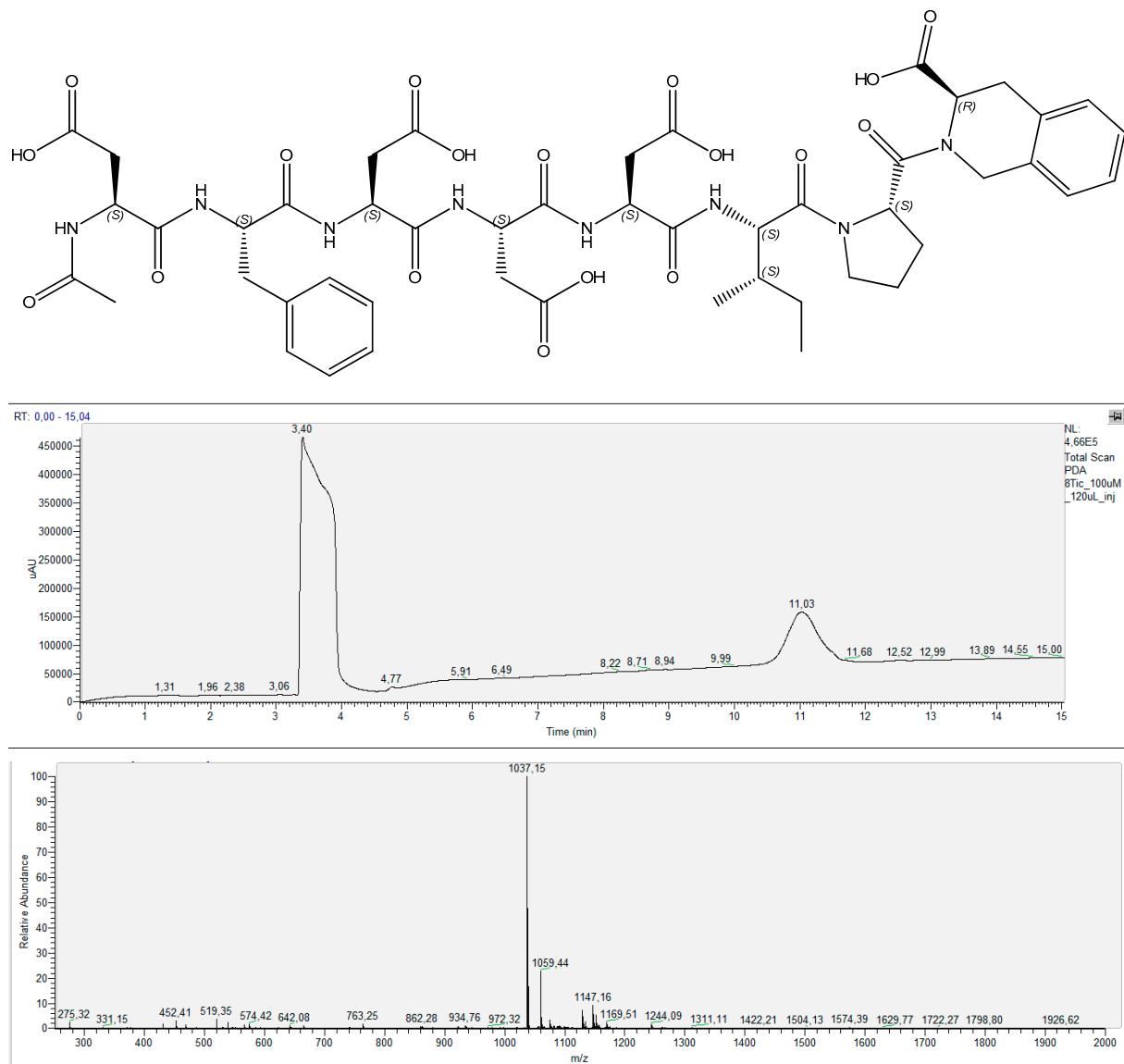
## Compound 49 – 3Pal8

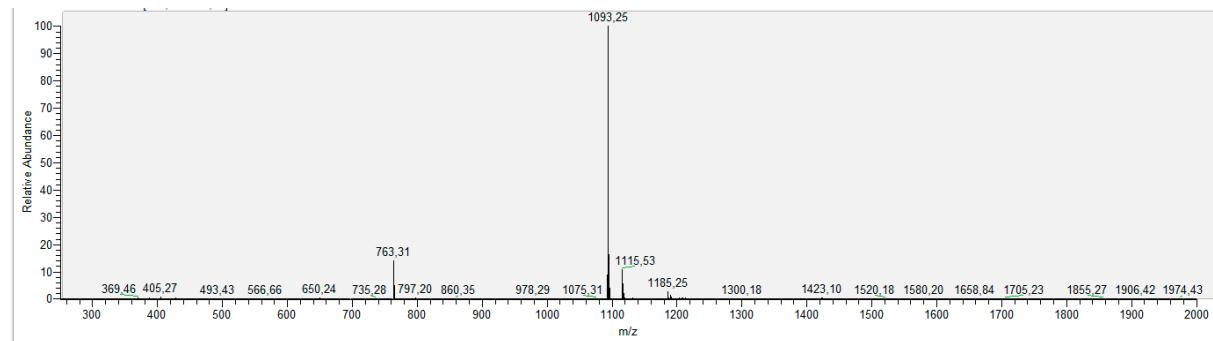
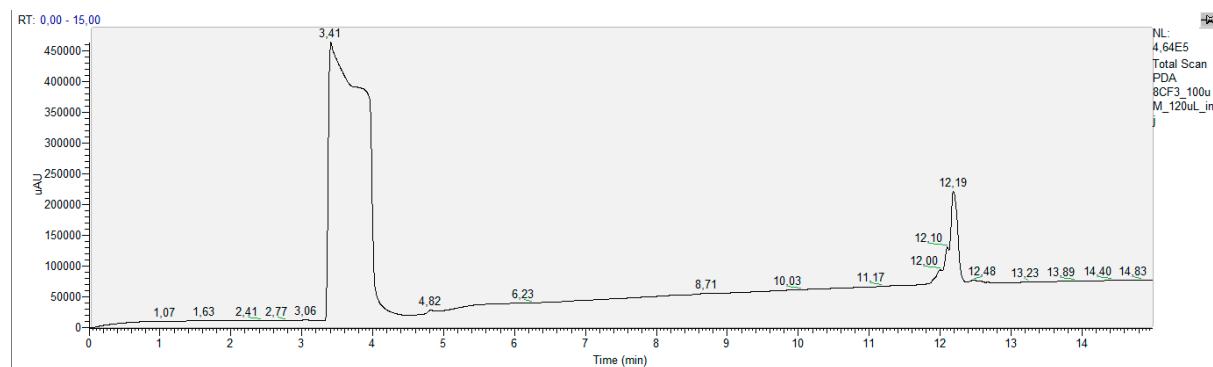
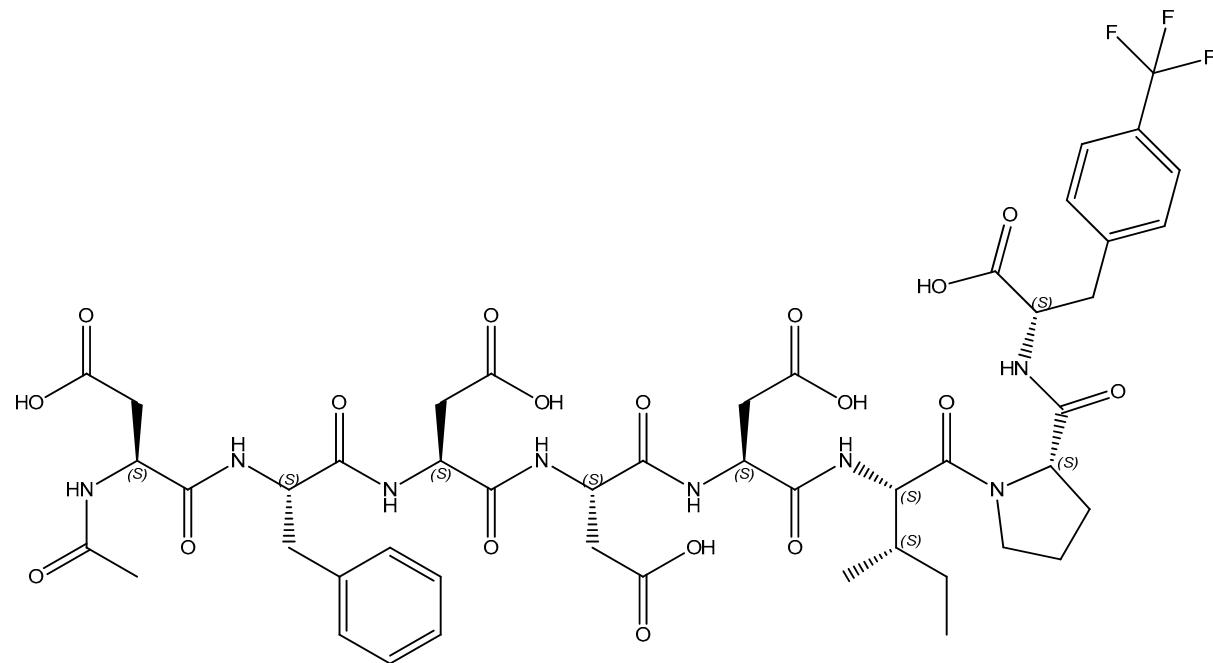


## Compound 50 – Thi8

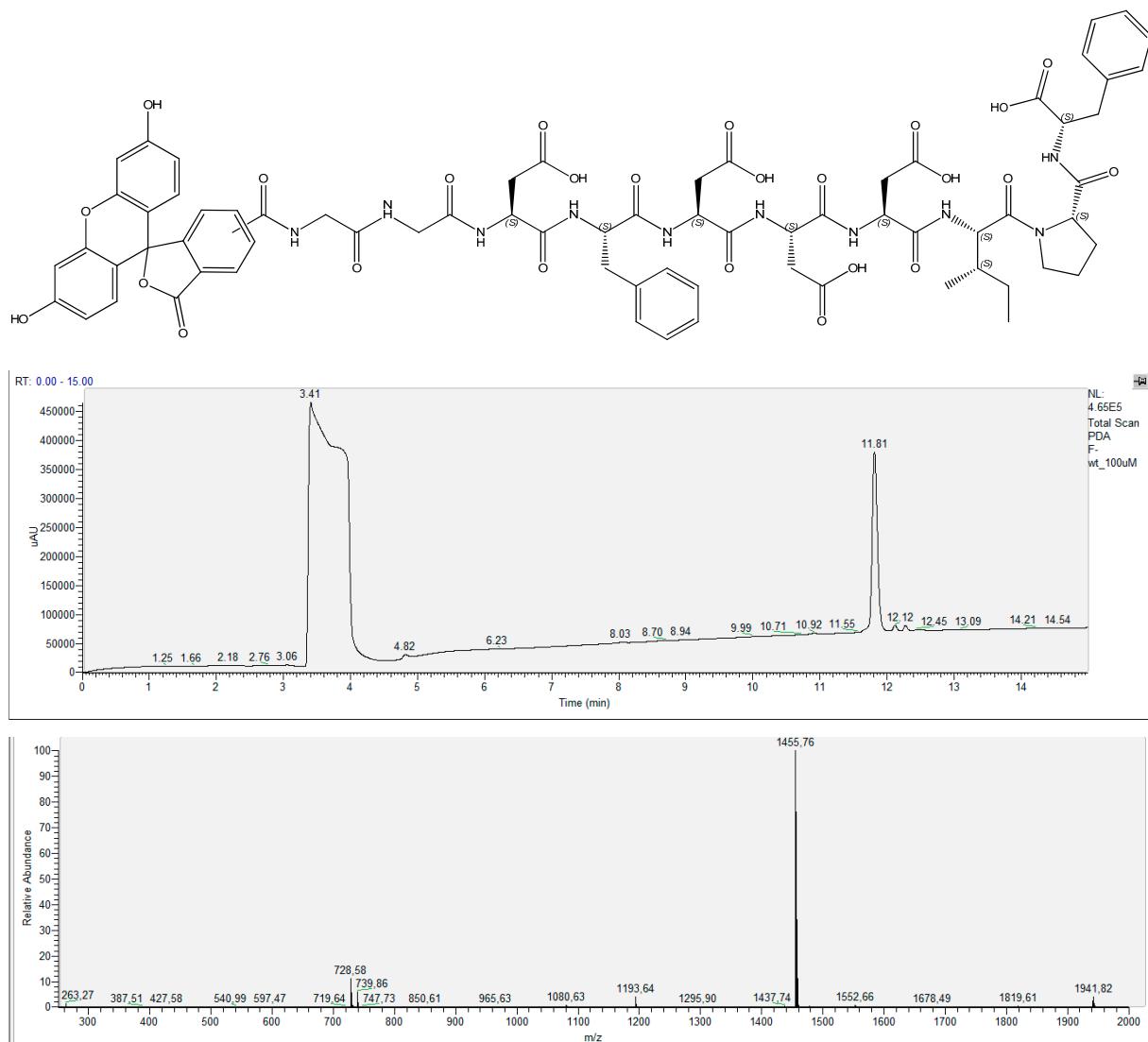


## Compound 51 – Tic8

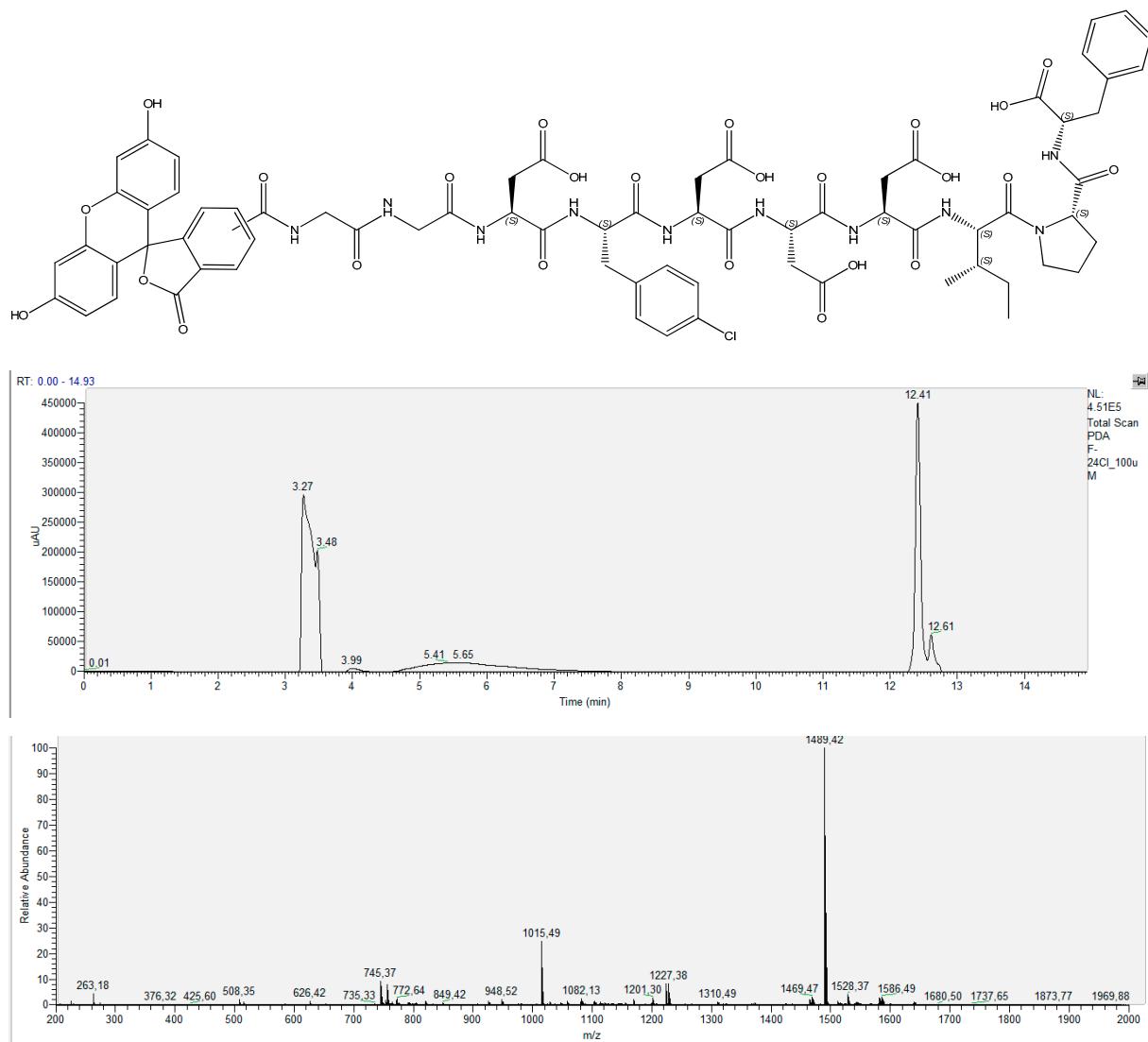


Compound 52 – 4CF<sub>3</sub>-Phe8

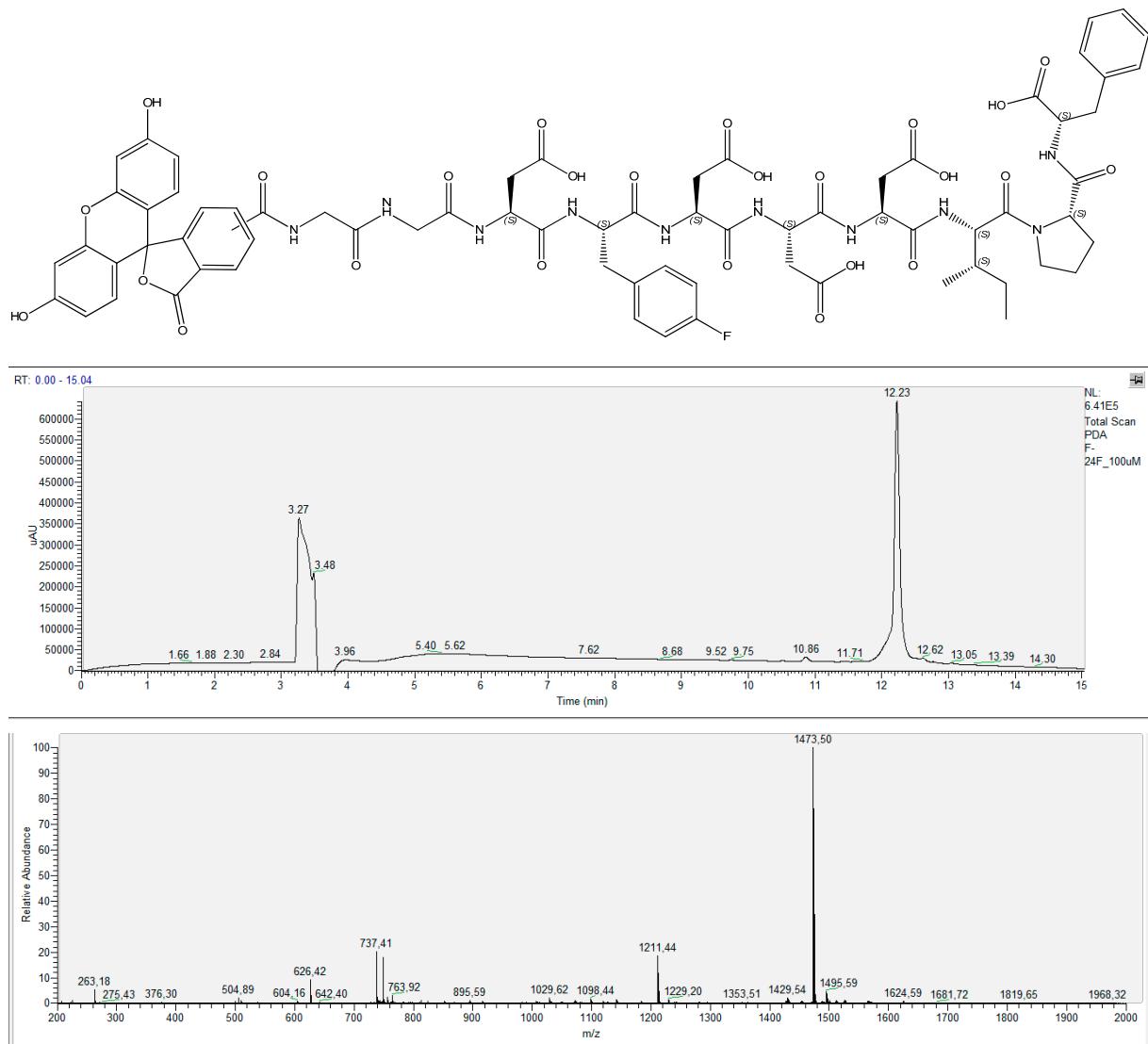
## Compound 53 – F-wtSSB-Ct

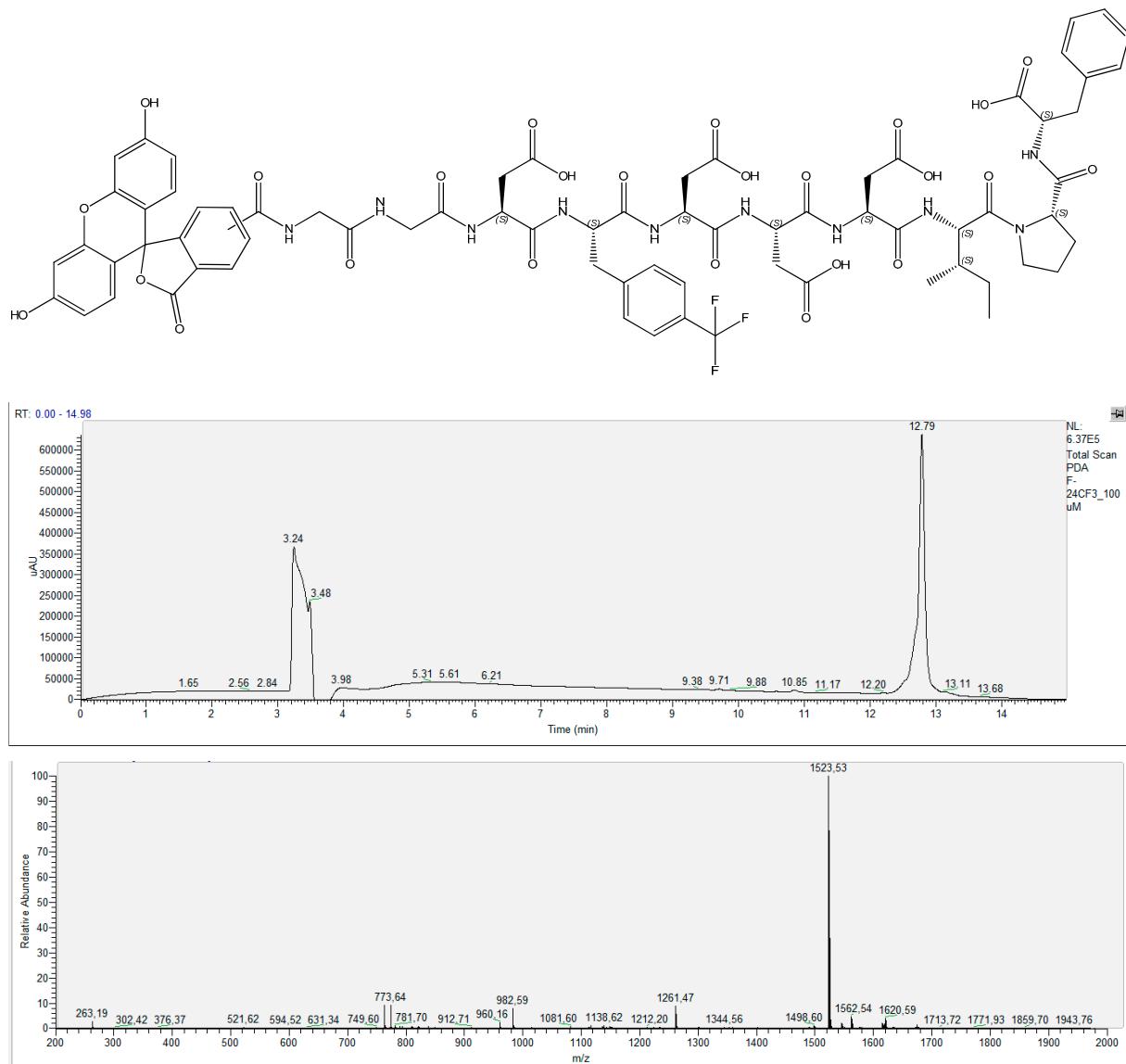


## Compound 54 – F-4Cl-Phe2

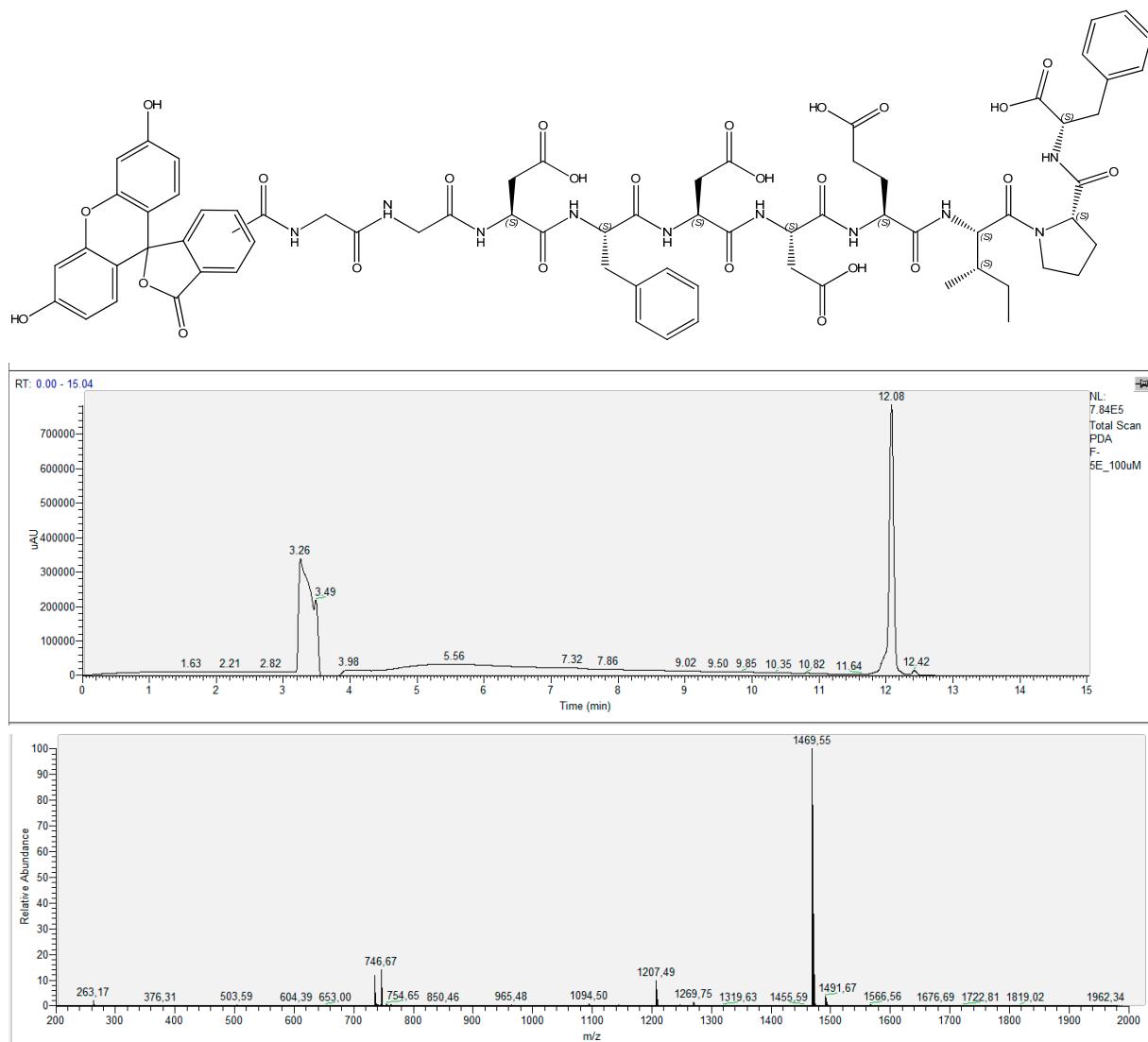


## Compound 55 – F-4F-Phe2

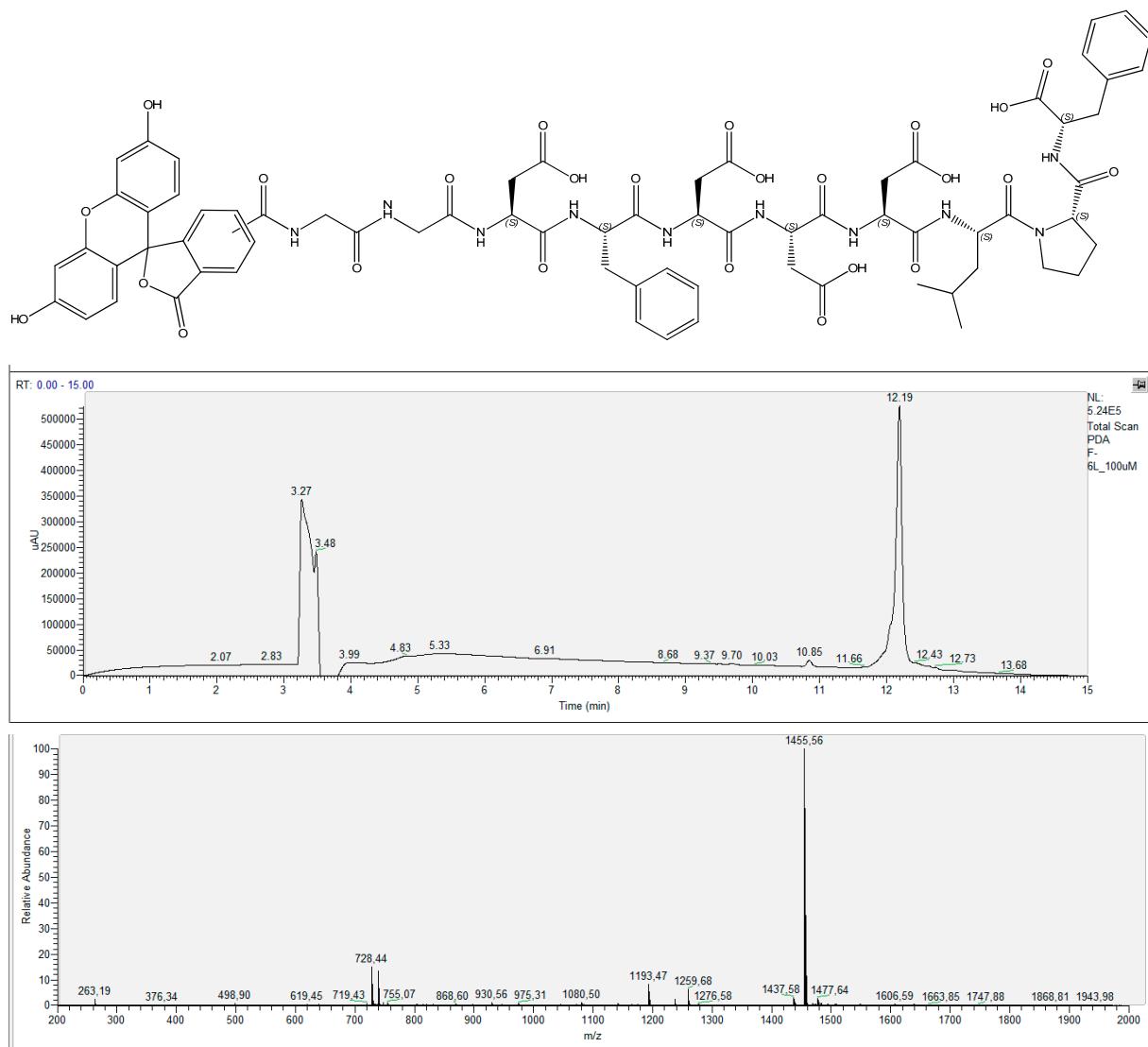


Compound 56 – F-4CF<sub>3</sub>-Phe2

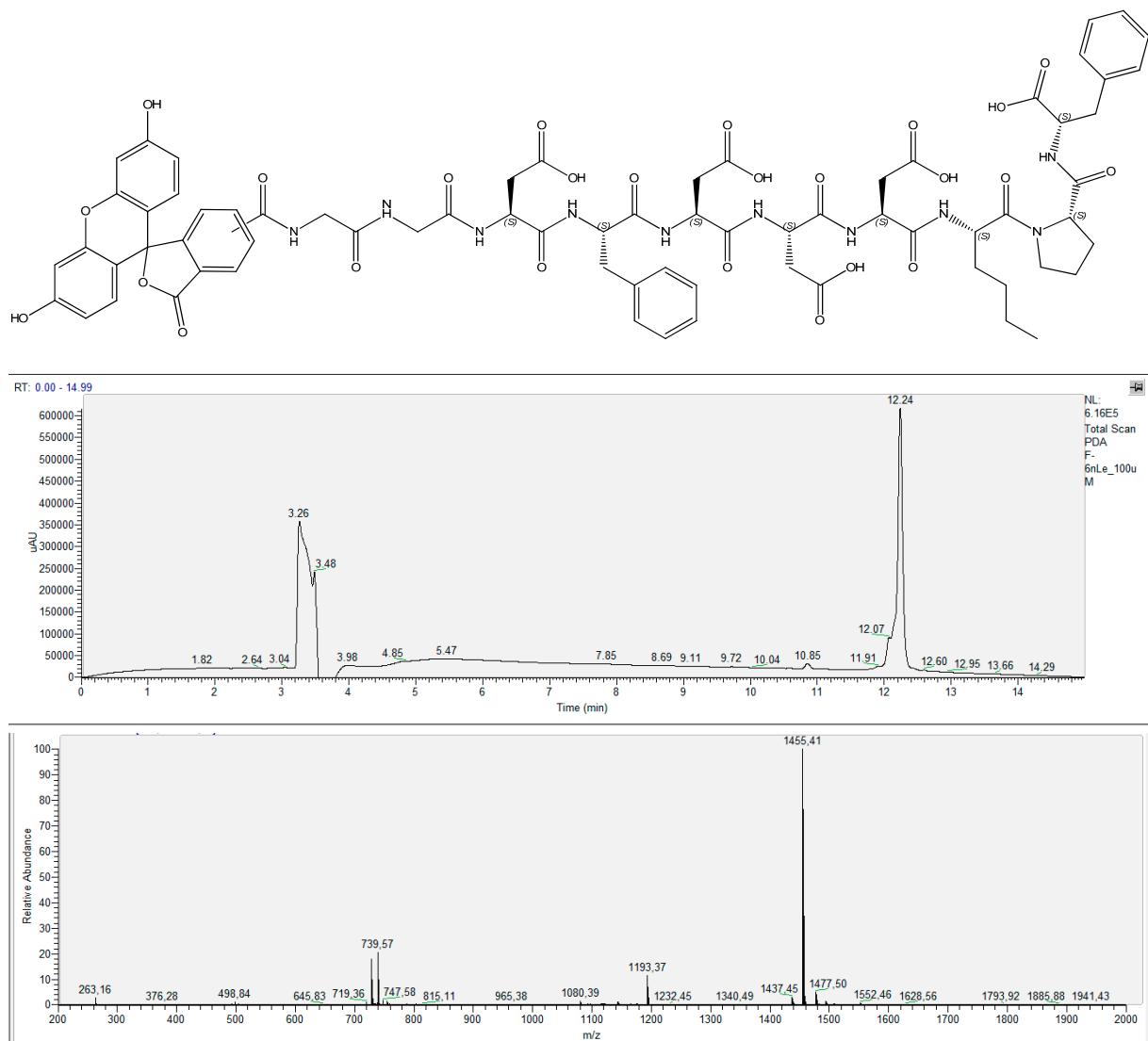
## Compound 57 – F-Glu5

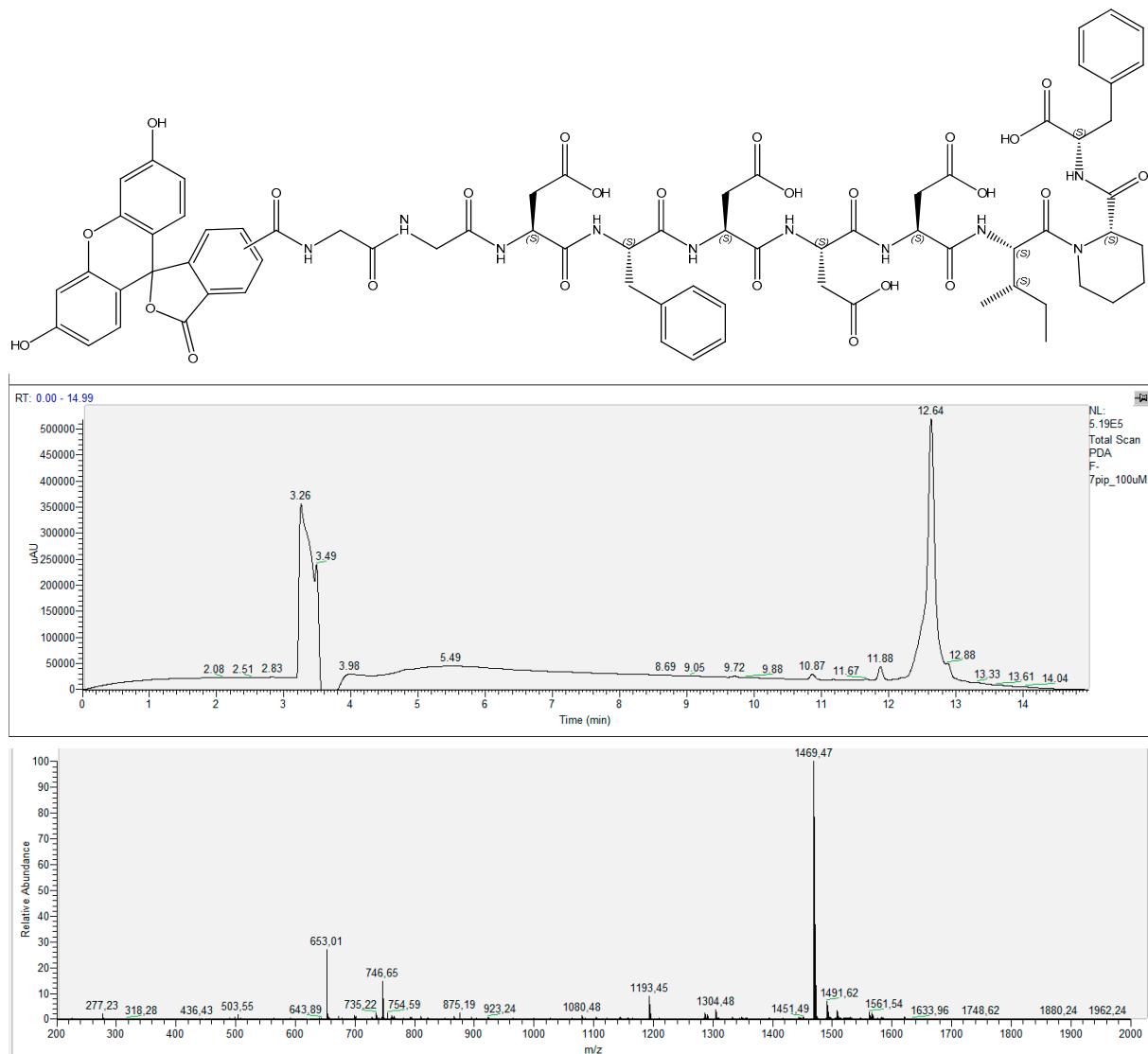


## Compound 58– F-Leu6

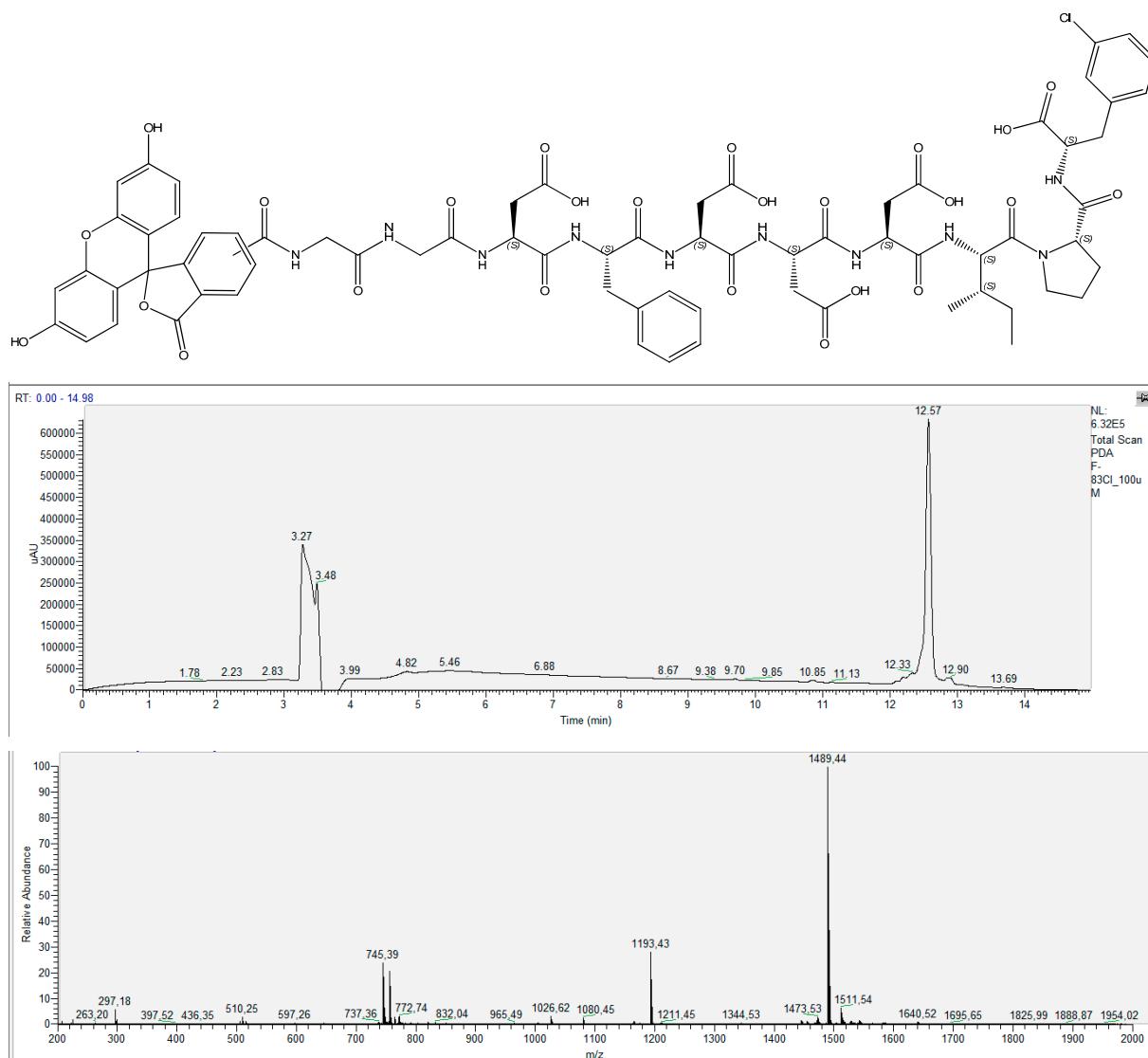


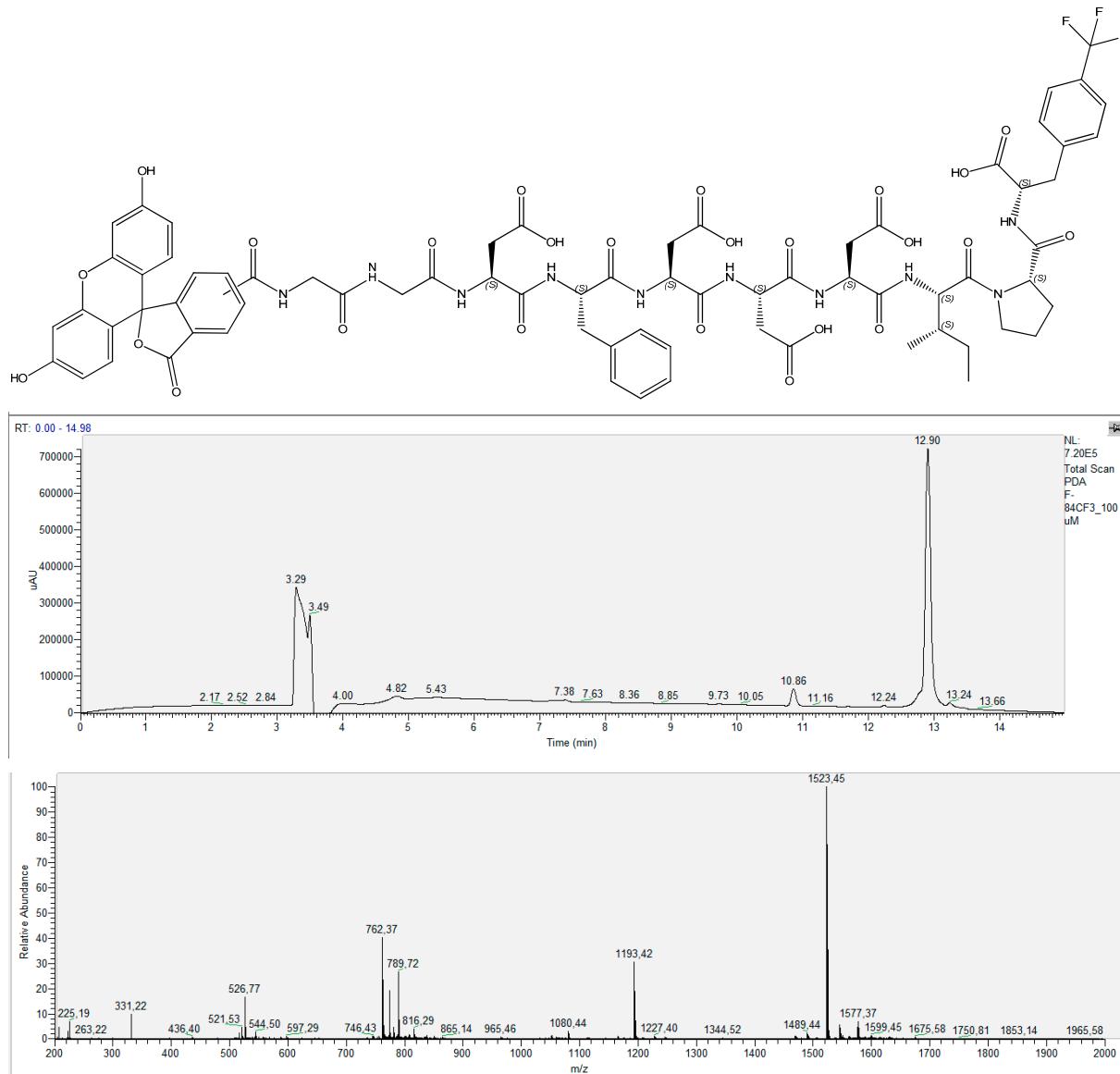
## Compound 59 – F-nLe6



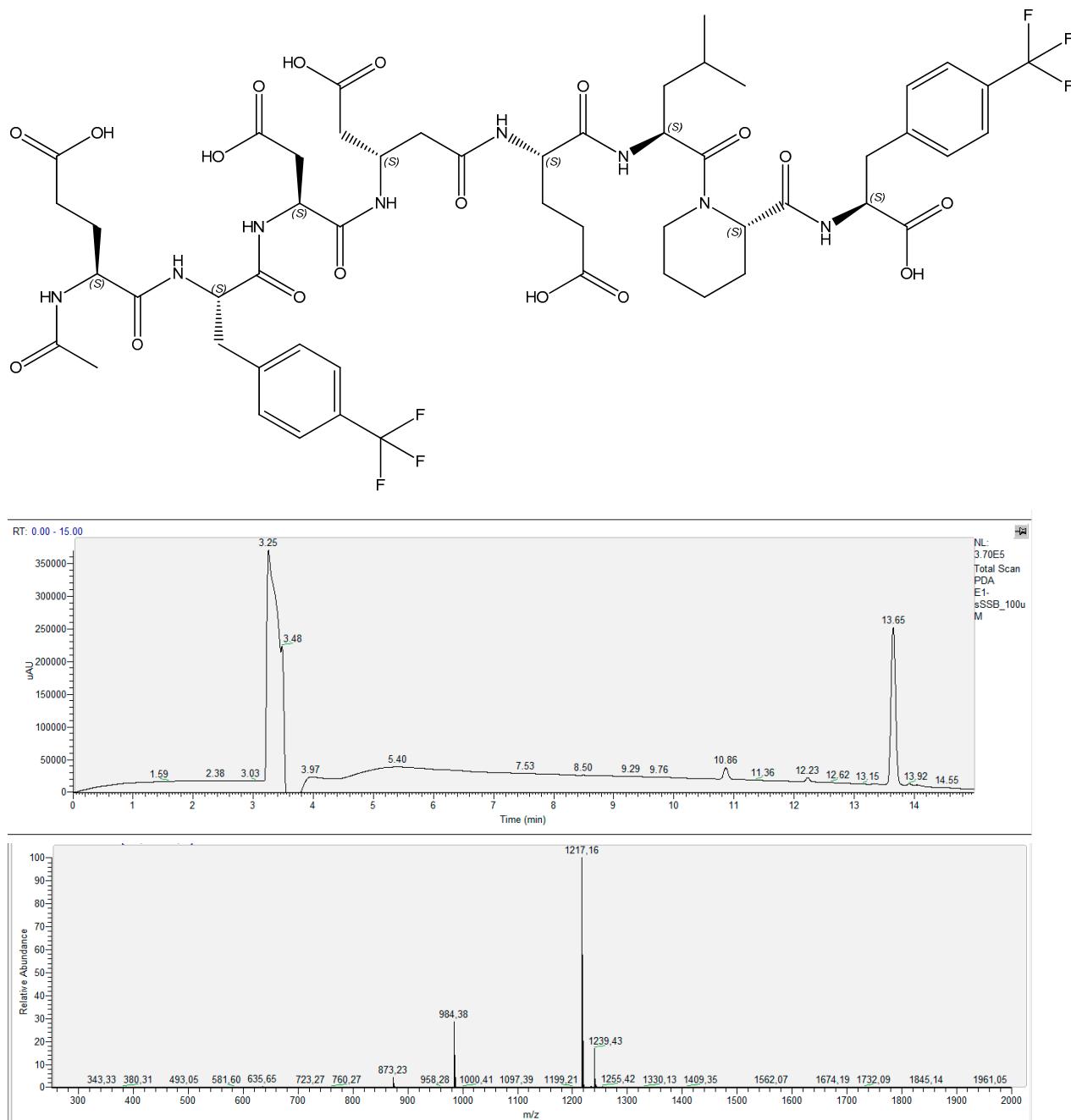
Compound 60 – F-Pip<sup>7</sup>

## Compound 61 – F-3Cl-Phe8

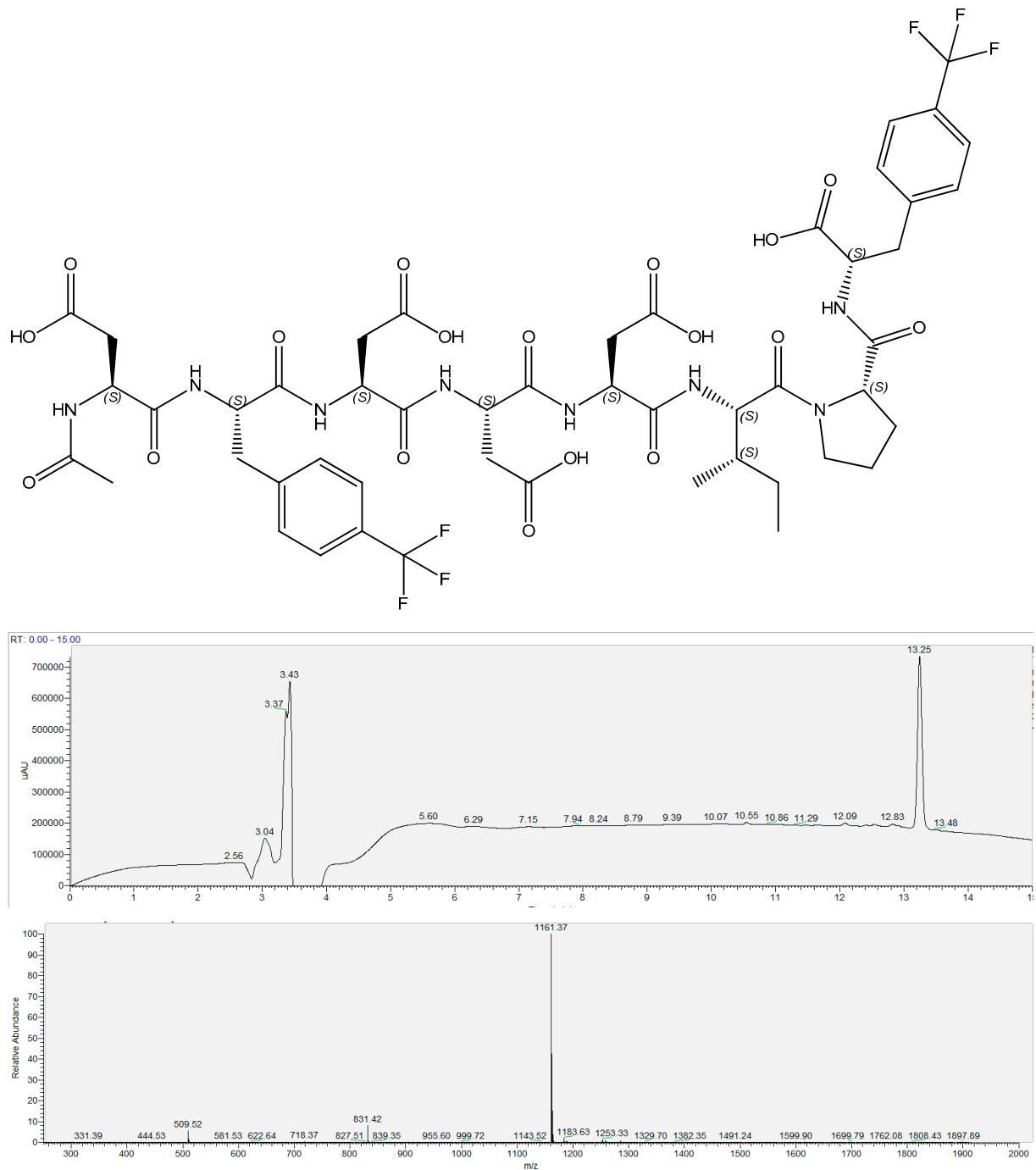


Compound 62 – F-4CF<sub>3</sub>-Phe8

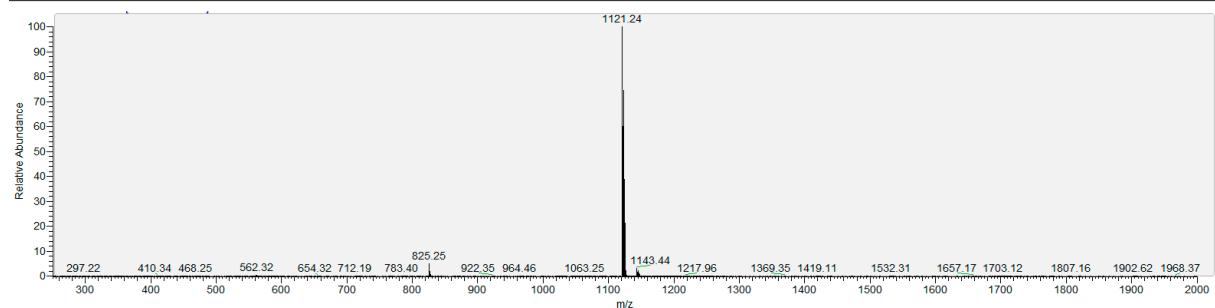
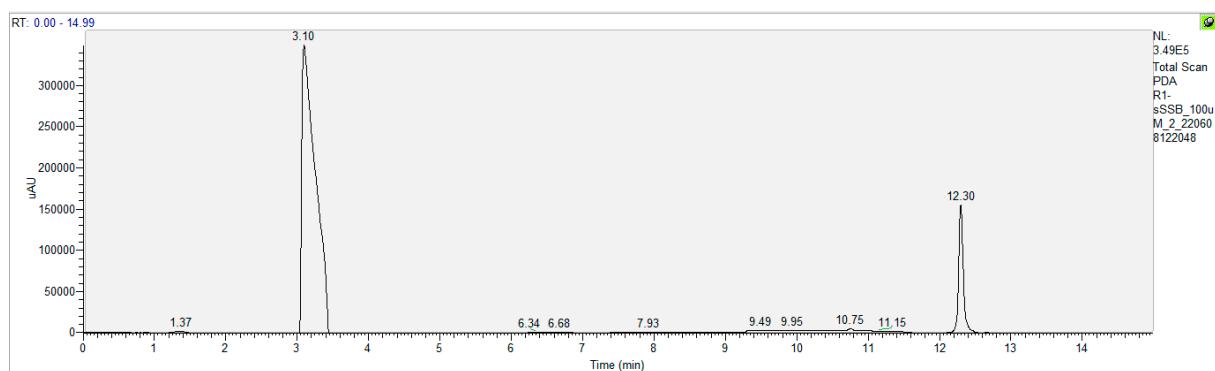
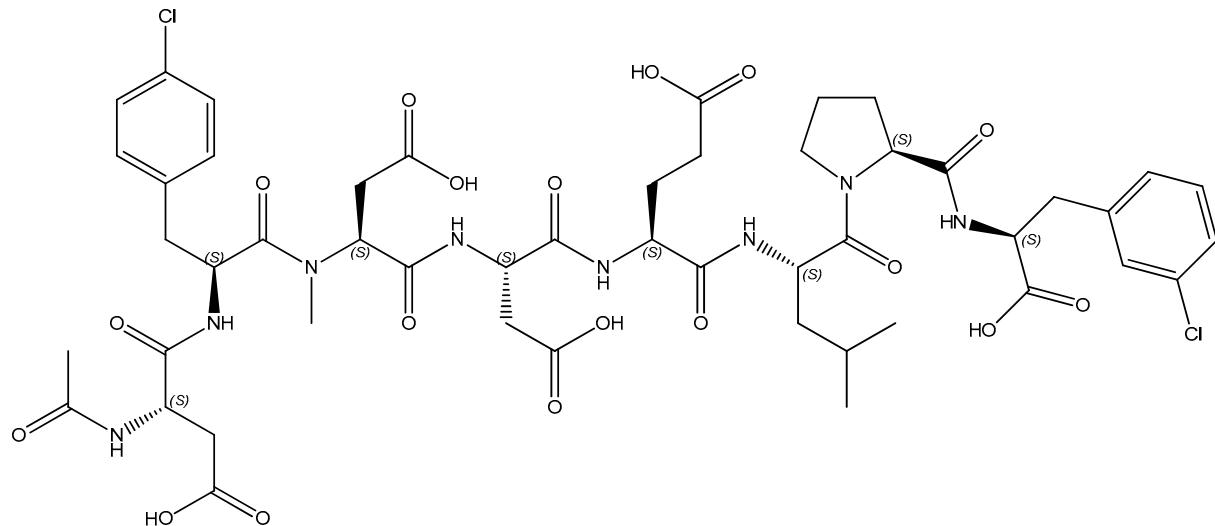
## Compound 63 – E1-sSSB-Ct



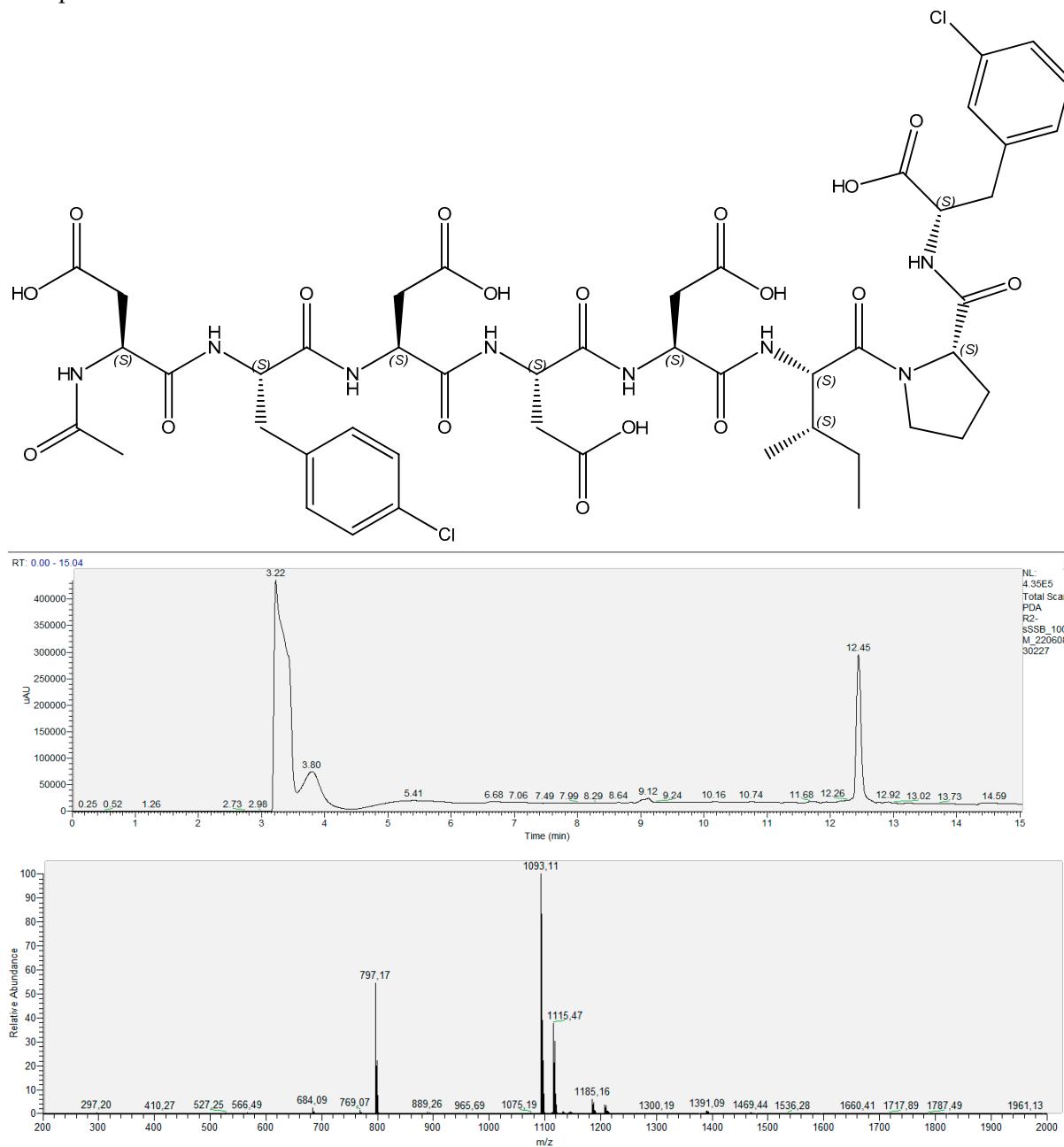
## Compound 64 – E2-sSSB-Ct



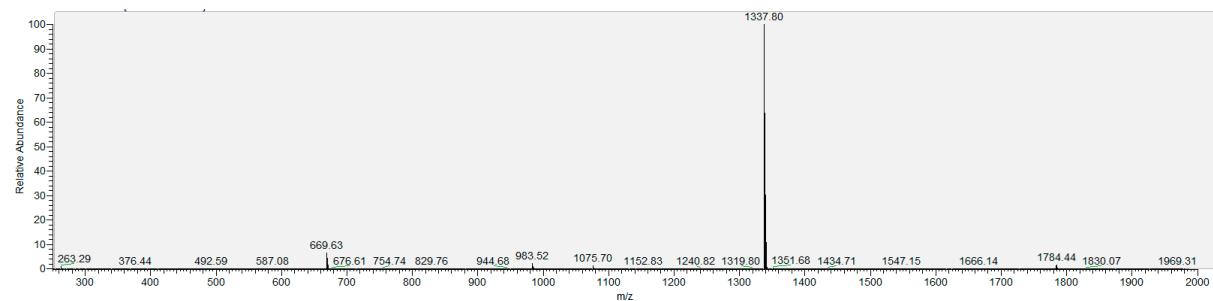
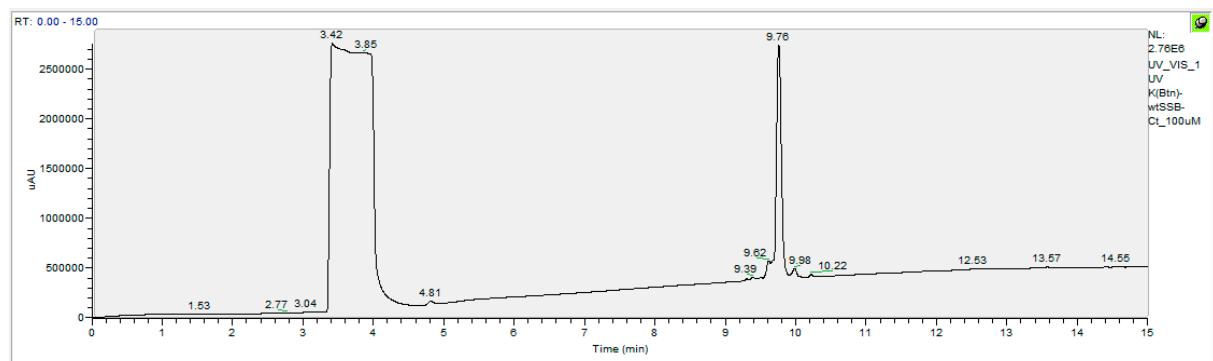
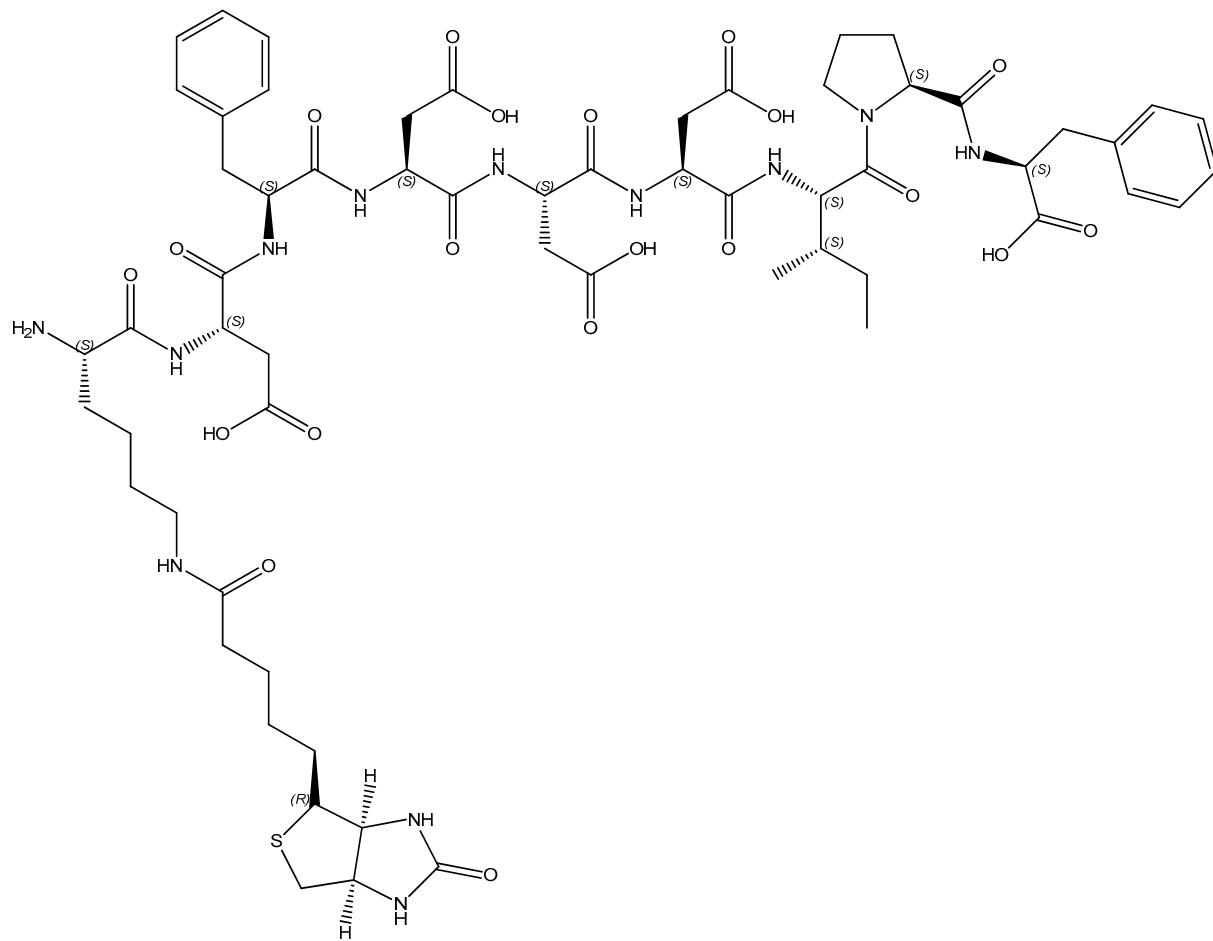
## Compound 65 – R1-sSSB-Ct



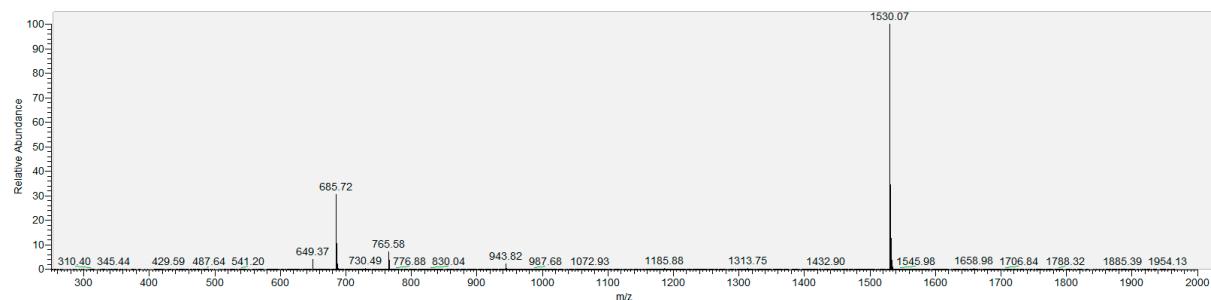
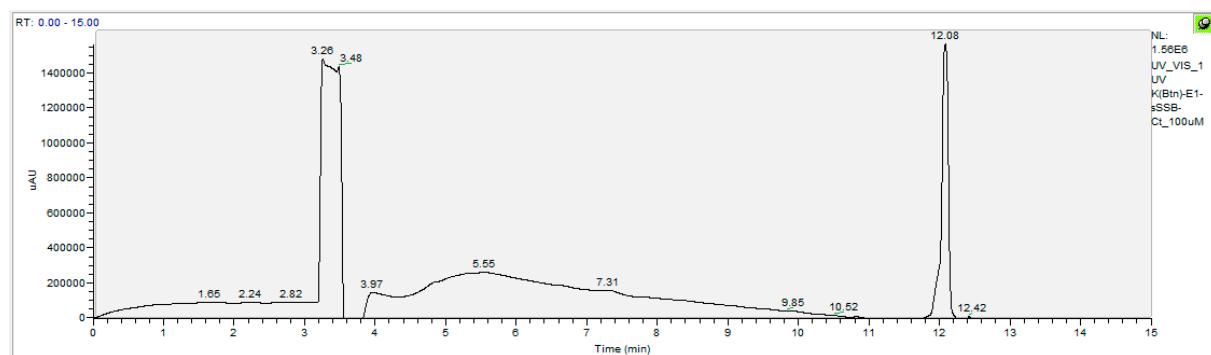
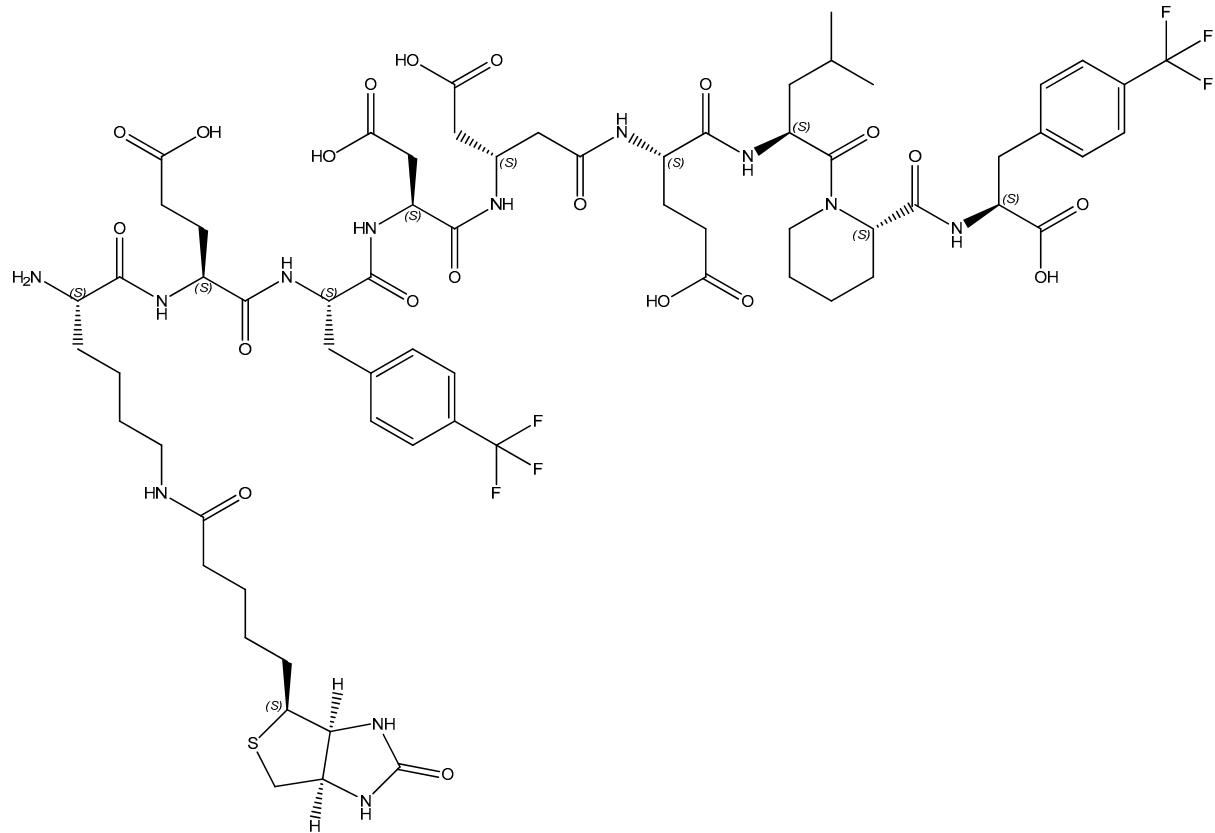
## Compound 66 – R2-sSSB-Ct



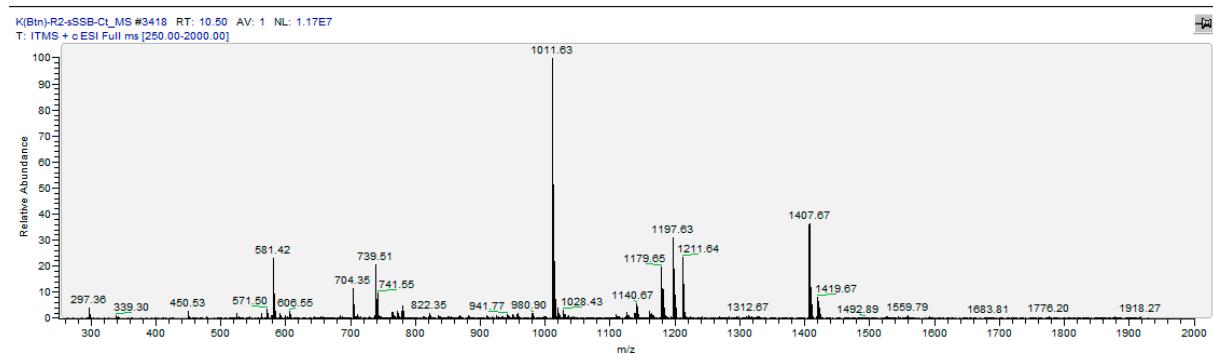
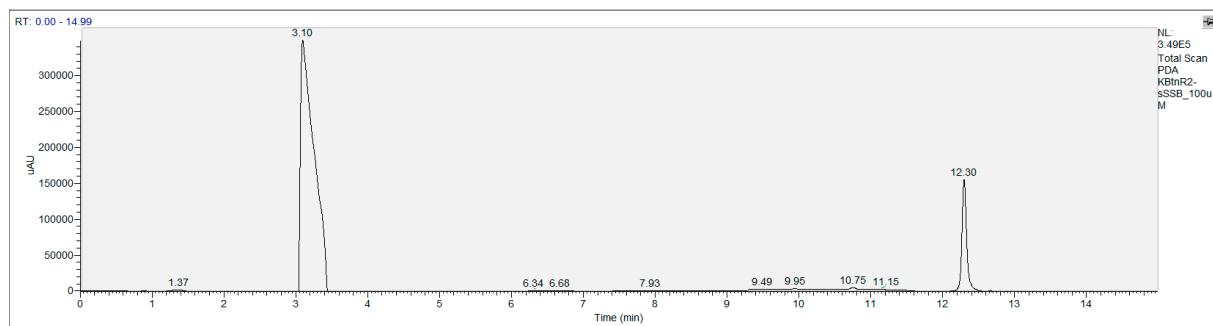
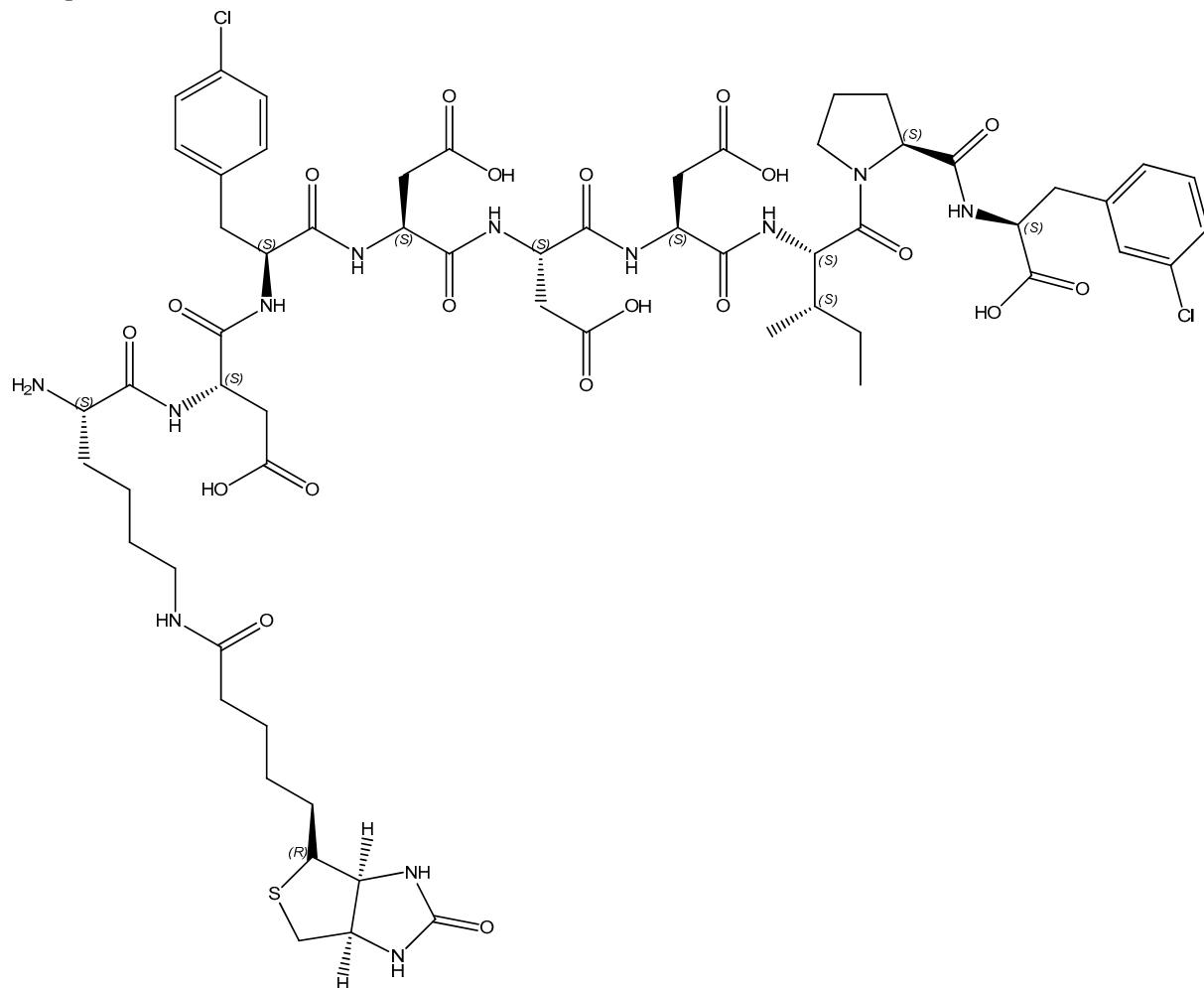
## Compound 67 – K(Btn)-wtSSB-Ct

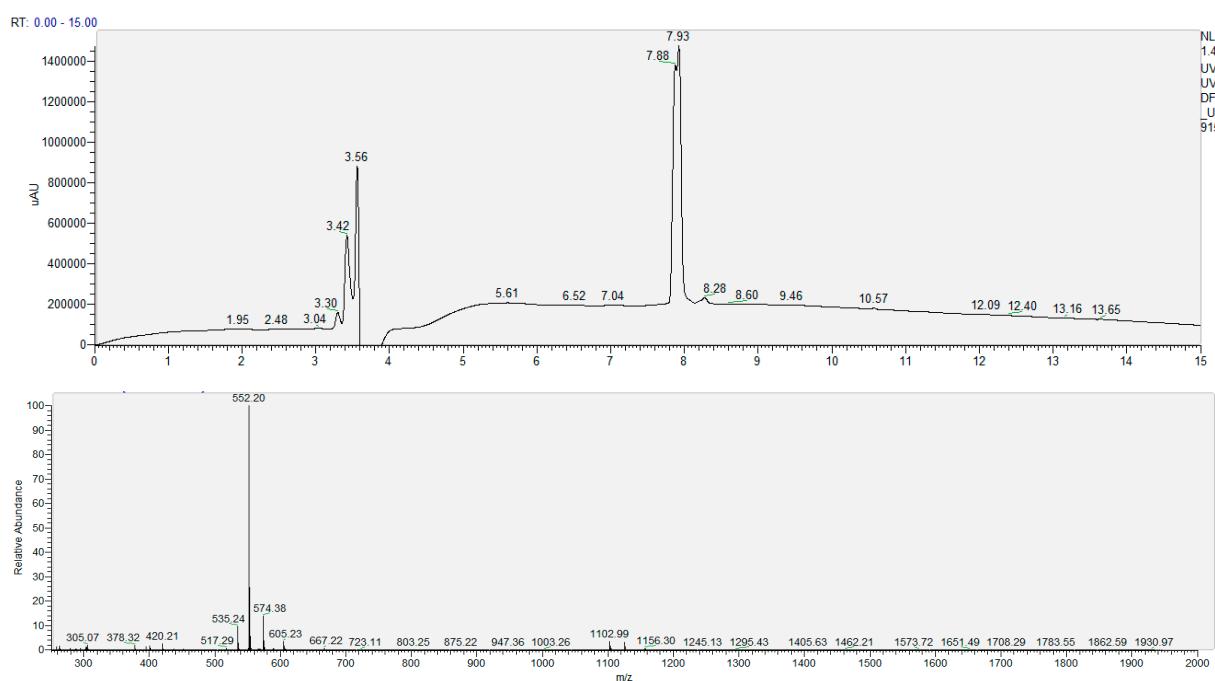
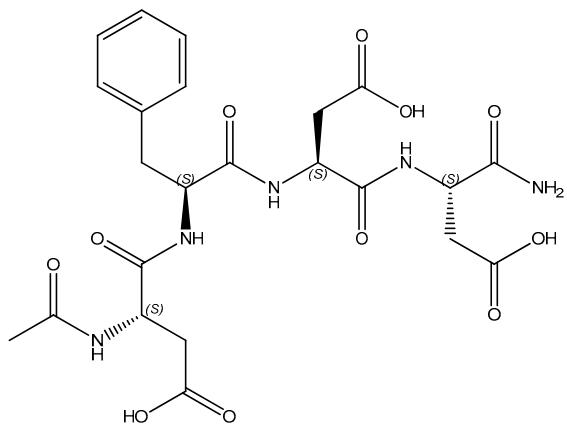


## Compound 68 – K(Btn)-E1-sSSB-Ct



## Compound 69 – K(Btn)-R2-sSSB-Ct



Compound 70 – Ac-DFDD-NH<sub>2</sub>

**Compound 71 – Ac-DIPF-OH**