

Supporting Materials

Investigation of P1' and P2' sites of IQF substrates on their selectivity towards 20s proteasome subunits.

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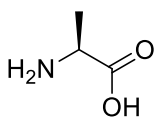
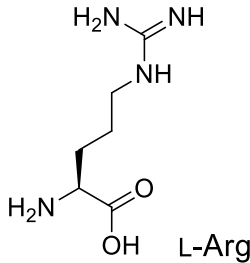
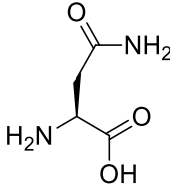
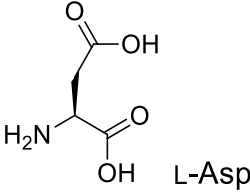
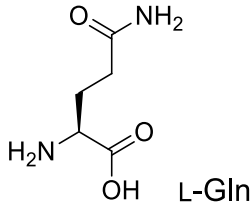
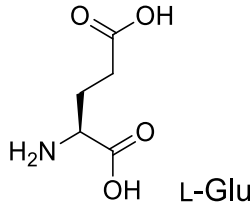
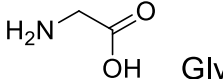
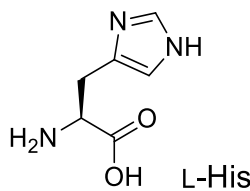
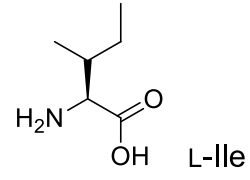
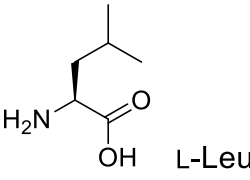
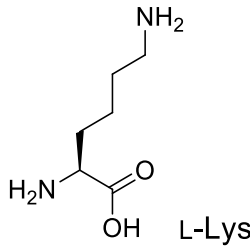
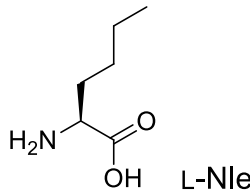
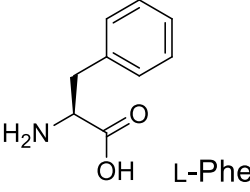
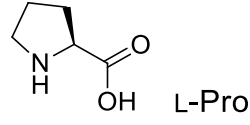
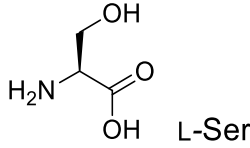
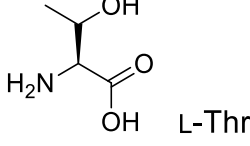
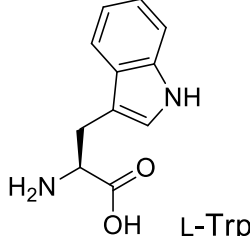
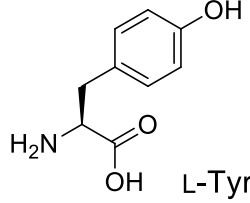
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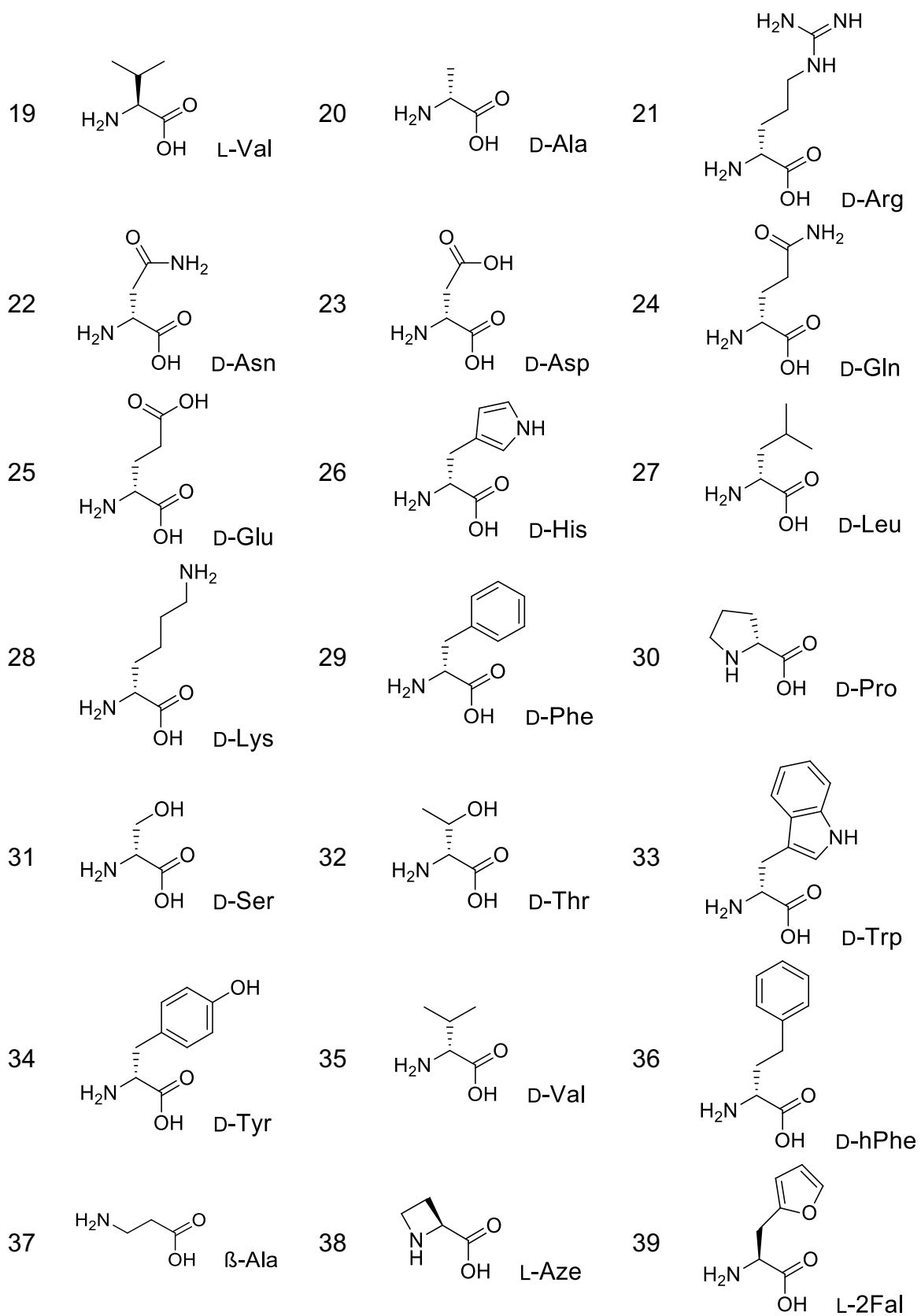
Contents of Supporting Materials

<i>Supplementary Tables</i>	2
Table S1. Structures of natural and unnatural amino acids used in P1' and P2' positions.....	2
Table S2. List of PR1_P1' substrates with their calculated and found (M+H) ²⁺ values.....	5
Table S3. List of PR1_P2' substrates with their calculated and found (M+H) ²⁺ values.....	6
Table S4. List of PR2_P1' substrates with their calculated and found (M+H) ²⁺ values.....	8
Table S5. List of PR2_P2' substrates with their calculated and found (M+H) ²⁺ values.....	9
Table S6. List of PR5_P1' substrates with their calculated and found (M+H) ²⁺ values.....	11
Table S7. List of PR5_P2' substrates with their calculated and found (M+H) ²⁺ values.....	13
Table S8. List of specific substrates, their sequence, calculated and found (M+H) ² value.....	14
<i>Supplementary Figures</i>	16
Figure S1. Graphs representing kinetics of IQF selective substrates.	16
Figure S2. PR1_S1 structure, chromatogram and cleavage site analysis.	17
Figure S3. PR1_S2 structure, chromatogram and cleavage site analysis.	18
Figure S4. PR2_S1 structure, chromatogram and cleavage site analysis.	21
Figure S5. PR2_S2 structure, chromatogram and cleavage site analysis.	22
Figure S6. PR5_S1 structure, chromatogram and cleavage site analysis.	25
Figure S7. PR5_S2 structure, chromatogram and cleavage site analysis.	25

Supplementary Tables

Table S1. Structures of natural and unnatural amino acids used in P1' and P2' positions.

No.	Structure + code	No.	Structure + code	No.	Structure + code
1	 L-Ala	2	 L-Arg	3	 L-Asn
4	 L-Asp	5	 L-Gln	6	 L-Glu
7	 Gly	8	 L-His	9	 L-Ile
10	 L-Leu	11	 L-Lys	12	 L-Nle
13	 L-Phe	14	 L-Pro	15	 L-Ser
16	 L-Thr	17	 L-Trp	18	 L-Tyr



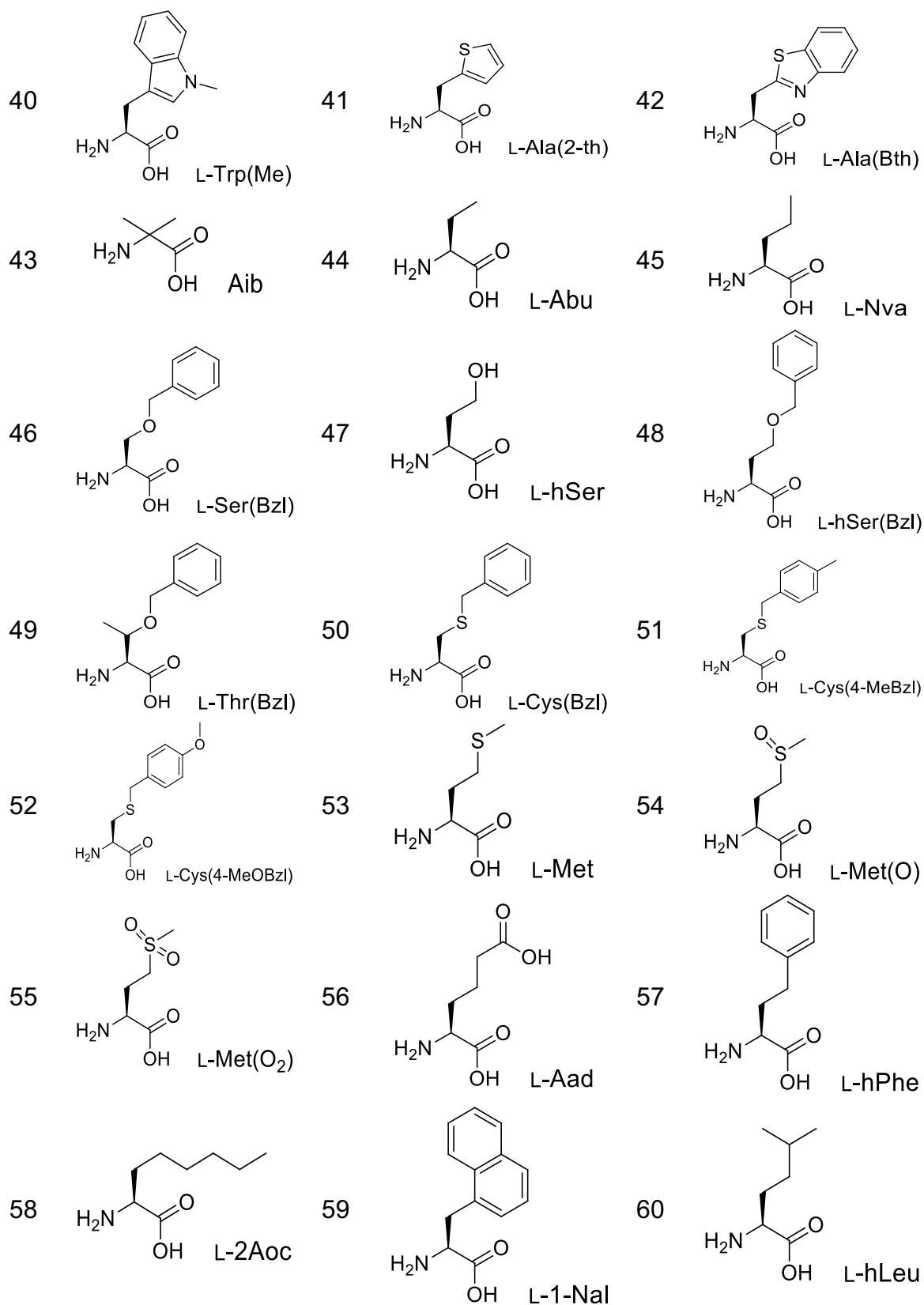
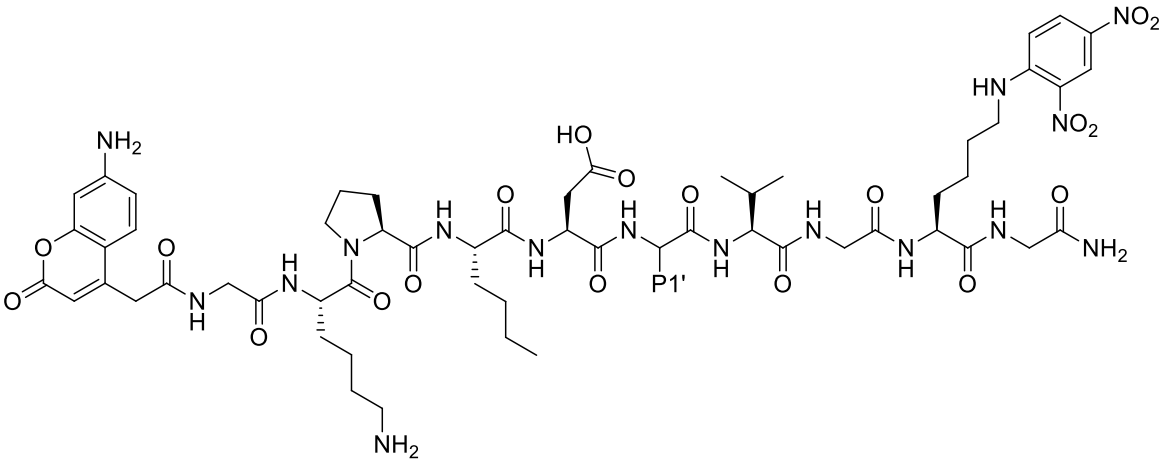
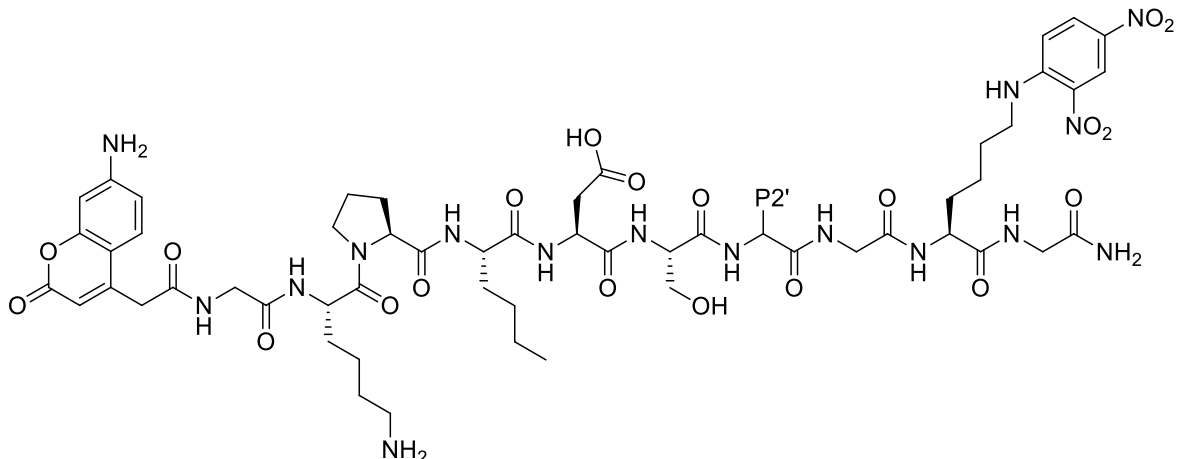


Table S2. MS analysis of PR1_P1' substrates library.

Substrate structure: ACC-Gly-Lys-Pro-Nle-Asp-P1'-Val-Gly-Lys(Dnp)-Gly-NH ₂			
			
Name	Amino acid in P1'	(M+H) ²⁺ calculated	(M+H) ²⁺ found
PR1_P1'_1	L-Ala	654.29	654.48
PR1_P1'_2	L-Arg	696.83	697.08
PR1_P1'_3	L-Asn	675.81	676.04
PR1_P1'_4	L-Asp	676.29	676.50
PR1_P1'_5	L-Glu	683.33	683.51
PR1_P1'_6	L-Gln	682.82	682.98
PR1_P1'_7	Gly	647.29	647.45
PR1_P1'_8	L-His	687.31	687.50
PR1_P1'_9	L-Ile	675.32	675.56
PR1_P1'_10	L-Leu	675.32	675.57
PR1_P1'_11	L-Lys	682.82	683.05
PR1_P1'_12	L-Nle	675.32	675.57
PR1_P1'_13	L-Phe	692.32	692.53
PR1_P1'_14	L-Pro	667.3	667.41
PR1_P1'_15	L-Ser	662.29	662.57
PR1_P1'_16	L-Thr	669.33	669.55
PR1_P1'_17	L-Trp	711.82	712.13
PR1_P1'_18	L-Tyr	700.31	700.62
PR1_P1'_19	L-Val	668.32	668.57
PR1_P1'_20	D-Ala	654.29	654.42
PR1_P1'_21	D-Arg	696.83	697.02
PR1_P1'_22	D-Asn	675.84	675.93
PR1_P1'_23	D-Asp	676.29	676.44
PR1_P1'_24	D-Gln	682.82	682.92
PR1_P1'_25	D-Glu	683.33	683.45
PR1_P1'_26	D-His	687.31	687.46
PR1_P1'_27	D-Leu	675.32	675.43
PR1_P1'_28	D-Lys	682.82	682.97
PR1_P1'_29	D-Phe	692.32	692.48
PR1_P1'_30	D-Pro	667.33	667.44
PR1_P1'_31	D-Ser	662.29	662.42
PR1_P1'_32	D-Thr	669.3	669.42
PR1_P1'_33	D-Trp	711.82	712.00

PR1_P1' 34	D-Tyr	700.31	700.50
PR1_P1' 35	D-Val	668.32	668.40
PR1_P1' 36	D-hPhe	699.32	699.49
PR1_P1' 37	β -Ala	654.29	654.30
PR1_P1' 38	L-Aze	660.29	680.42
PR1_P1' 39	L-2-Fal	687.33	687.48
PR1_P1' 40	L-Trp(Me)	718.77	719.03
PR1_P1' 41	L-Ala(2-th)	695.29	695.49
PR1_P1' 42	L-Ala(Bth)	721.81	721.01
PR1_P1' 43	Aib	661.31	661.42
PR1_P1' 44	L-Abu	661.32	661.43
PR1_P1' 45	L-Nva	668.32	668.45
PR1_P1' 46	L-Ser(Bzl)	707.32	707.45
PR1_P1' 47	L-hSer	669.33	669.44
PR1_P1' 48	L-hSer(Bzl)	714.33	714.52
PR1_P1' 49	L-Thr(Bzl)	714.33	714.52
PR1_P1' 50	L-Cys(Bzl)	715.31	715.61
PR1_P1' 51	L-Cys(MeBzl)	722.32	722.60
PR1_P1' 52	L-Cys(4-MeOBzl)	730.31	730.58
PR1_P1' 53	L-Met	684.33	684.47
PR1_P1' 54	L-Met(O)	692.29	692.48
PR1_P1' 55	L-Met(O)2	700.29	700.48
PR1_P1' 56	L-2-Aad	690.32	690.58
PR1_P1' 57	L-hPhe	699.32	699.50
PR1_P1' 58	L-2-Aoc	680.33	680.54
PR1_P1' 59	L-1-Nal	717.32	717.54
PR1_P1' 60	L-hLeu	682.33	682.49

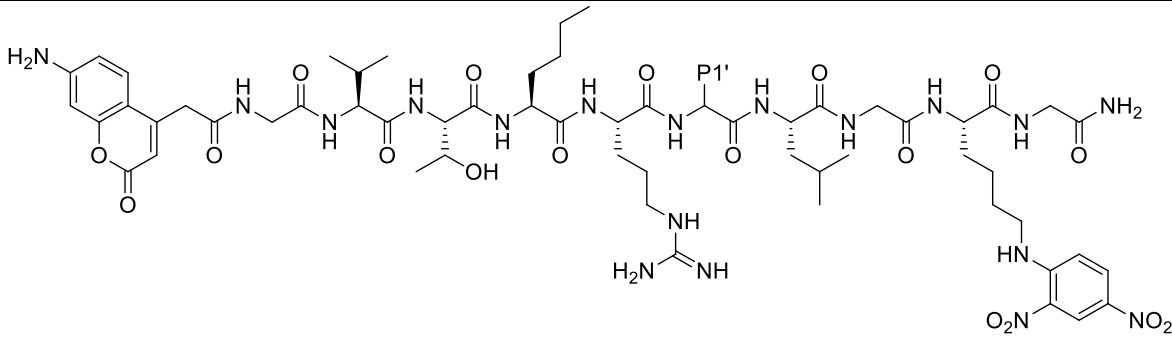
Table S3. MS analysis of PR1_P2' substrates library.

Substrate structure: ACC-Gly-Lys-Pro-Nle-Asp-Ser-P2'-Gly-Lys(Dnp)-Gly-NH ₂			
			
Name	Amino acid in P1'	(M+H) ²⁺ calculated	(M+H) ²⁺ found
PR1_P2' 1	L-Ala	647.78	648.42
PR1_P2' 2	L-Arg	690.81	691.01
PR1_P2' 3	L-Asn	669.29	669.92
PR1_P2' 4	L-Asp	670.28	670.40
PR1_P2' 5	L-Glu	677.28	677.43

PR1_P2'_6	L-Gln	676.79	676.94
PR1_P2'_7	Gly	640.27	641.40
PR1_P2'_8	L-His	680.29	681.46
PR1_P2'_9	L-Ile	669.33	669.44
PR1_P2'_10	L-Leu	669.32	669.44
PR1_P2'_11	L-Lys	676.81	676.96
PR1_P2'_12	L-Nle	669.30	669.46
PR1_P2'_13	L-Phe	686.29	686.51
PR1_P2'_14	L-Pro	661.29	661.45
PR1_P2'_15	L-Ser	656.28	656.40
PR1_P2'_16	L-Thr	663.29	663.42
PR1_P2'_17	L-Trp	705.82	706.15
PR1_P2'_18	L-Tyr	694.29	694.49
PR1_P2'_19	L-Val	662.29	662.44
PR1_P2'_20	D-Ala	647.78	648.45
PR1_P2'_21	D-Arg	690.81	691.32
PR1_P2'_22	D-Asn	669.29	669.95
PR1_P2'_23	D-Asp	670.28	670.44
PR1_P2'_24	D-Gln	677.28	676.99
PR1_P2'_25	D-Glu	676.79	677.46
PR1_P2'_26	D-His	680.29	681.50
PR1_P2'_27	D-Leu	669.33	661.48
PR1_P2'_28	D-Lys	676.81	656.45
PR1_P2'_29	D-Phe	686.29	686.33
PR1_P2'_30	D-Pro	661.29	661.16
PR1_P2'_31	D-Ser	656.28	656.44
PR1_P2'_32	D-Thr	663.29	663.46
PR1_P2'_33	D-Trp	705.82	706.06
PR1_P2'_34	D-Tyr	694.29	662.51
PR1_P2'_35	D-Val	662.29	694.63
PR1_P2'_36	D-hPhe	693.31	693.30
PR1_P2'_37	β -Ala	655.29	648.31
PR1_P2'_38	L-Aze	654.28	654.21
PR1_P2'_39	L-2-Fal	674.28	681.51
PR1_P2'_40	L-Trp(Me)	712.82	713.06
PR1_P2'_41	L-Ala(2-th)	689.29	689.52
PR1_P2'_42	L-Ala(Bth)	714.78	714.53
PR1_P2'_43	Aib	655.29	655.92
PR1_P2'_44	L-Abu	655.29	655.44
PR1_P2'_45	L-Nva	662.29	662.4
PR1_P2'_46	L-Ser(Bzl)	701.30	701.98
PR1_P2'_47	L-hSer	663.29	663.34
PR1_P2'_48	L-hSer(Bzl)	708.31	708.59
PR1_P2'_49	L-Thr(Bzl)	708.29	708.58
PR1_P2'_50	L-Cys(Bzl)	709.29	709.58
PR1_P2'_51	L-Cys(MeBzl)	716.29	716.83
PR1_P2'_52	L-Cys(4-MeOBzl)	724.28	724.56
PR1_P2'_53	L-Met	678.28	678.52

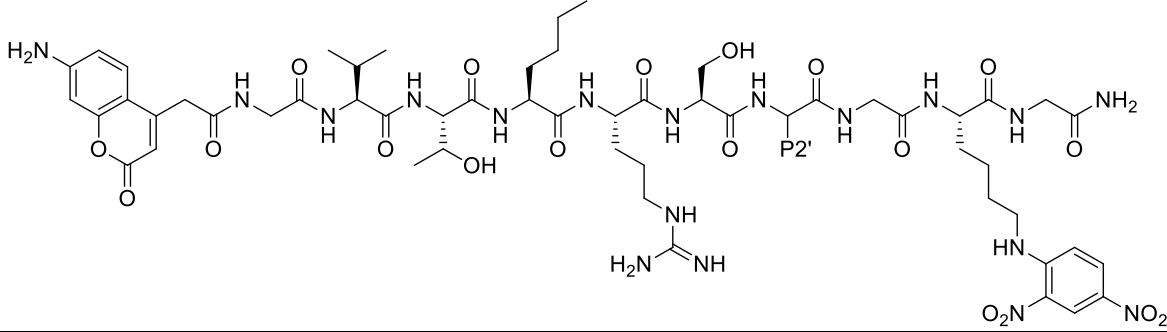
PR1_P2' 54	L-Met(O)	686.28	686.54
PR1_P2' 55	L-Met(O)2	694.28	694.52
PR1_P2' 56	L-2-Aad	685.29	684.84
PR1_P2' 57	L-hPhe	693.33	693.57
PR1_P2' 58	L-2-Aoc	863.32	863.52
PR1_P2' 59	L-1-Nal	711.31	711.57
PR1_P2' 60	L-hLeu	676.31	676.47

Table S4. MS analysis of P2_P1' substrates library.

Substrate structure: ACC-Gly-Val-Thr-Nle-Arg-P1'-Leu-Gly-Lys(Dnp)-Gly-NH ₂			
			
Name	Amino acid in P1'	(M+H) ²⁺ calculated	(M+H) ²⁺ found
PR2_P1' 1	L-Ala	669.62	669.56
PR2_P1' 2	L-Arg	712.10	712.13
PR2_P1' 3	L-Asn	692.44	692.93
PR2_P1' 4	L-Asp	691.52	691.44
PR2_P1' 5	L-Glu	698.74	698.46
PR2_P1' 6	L-Gln	698.44	698.58
PR2_P1' 7	Gly	662.32	622.42
PR2_P1' 8	L-His	702.33	702.50
PR2_P1' 9	L-Ile	690.35	690.47
PR2_P1' 10	L-Leu	690.35	690.44
PR2_P1' 11	L-Lys	697.85	698.00
PR2_P1' 12	L-Nle	690.35	690.43
PR2_P1' 13	L-Phe	707.34	707.48
PR2_P1' 14	L-Pro	682.32	682.44
PR2_P1' 15	L-Ser	677.33	677.44
PR2_P1' 16	L-Thr	684.33	684.43
PR2_P1' 17	L-Trp	726.84	726.99
PR2_P1' 18	L-Tyr	715.34	715.49
PR2_P1' 19	L-Val	683.44	683.44
PR2_P1' 20	D-Ala	669.24	669.40
PR2_P1' 21	D-Arg	712.11	712.06
PR2_P1' 22	D-Asn	691.25	690.94
PR2_P1' 23	D-Asp	692.08	691.41
PR2_P1' 24	D-Gln	698.33	697.96
PR2_P1' 25	D-Glu	698.74	698.41
PR2_P1' 26	D-His	702.33	702.53
PR2_P1' 27	D-Leu	690.35	690.57
PR2_P1' 28	D-Lys	697.85	698.12

PR2_P1'_29	D-Phe	707.34	707.46
PR2_P1'_30	D-Pro	682.32	682.42
PR2_P1'_31	D-Ser	677.33	677.43
PR2_P1'_32	D-Thr	700.33	700.47
PR2_P1'_33	D-Trp	684.33	684.47
PR2_P1'_34	D-Tyr	726.84	727.06
PR2_P1'_35	D-Val	715.34	715.54
PR2_P1'_36	D-hPhe	683.34	683.44
PR2_P1'_37	β -Ala	714.35	715.38
PR2_P1'_38	L-Aze	669.32	669.58
PR2_P1'_39	L-2-Fal	702.33	702.42
PR2_P1'_40	L-Trp(Me)	733.81	734.15
PR2_P1'_41	L-Ala(2-th)	710.32	710.45
PR2_P1'_42	L-Ala(Bth)	735.82	736.32
PR2_P1'_43	Aib	676.33	676.45
PR2_P1'_44	L-Abu	676.33	676.47
PR2_P1'_45	L-Nva	683.34	683.57
PR2_P1'_46	L-Ser(Bzl)	722.35	722.46
PR2_P1'_47	L-hSer	684.33	684.43
PR2_P1'_48	L-hSer(Bzl)	729.35	729.02
PR2_P1'_49	L-Thr(Bzl)	729.35	729.47
PR2_P1'_50	L-Cys(Bzl)	730.33	730.44
PR2_P1'_51	L-Cys(MeBzl)	737.34	737.50
PR2_P1'_52	L-Cys(4-MeOBzl)	745.34	745.51
PR2_P1'_53	L-Met	699.31	699.41
PR2_P1'_54	L-Met(O)	707.35	707.56
PR2_P1'_55	L-Met(O) ₂	715.33	715.46
PR2_P1'_56	L-2-Aad	705.33	705.55
PR2_P1'_57	L-hPhe	714.33	714.50
PR2_P1'_58	L-2-Aoc	704.67	704.55
PR2_P1'_59	L-1-Nal	732.35	732.51
PR2_P1'_60	L-hLeu	697.52	697.83

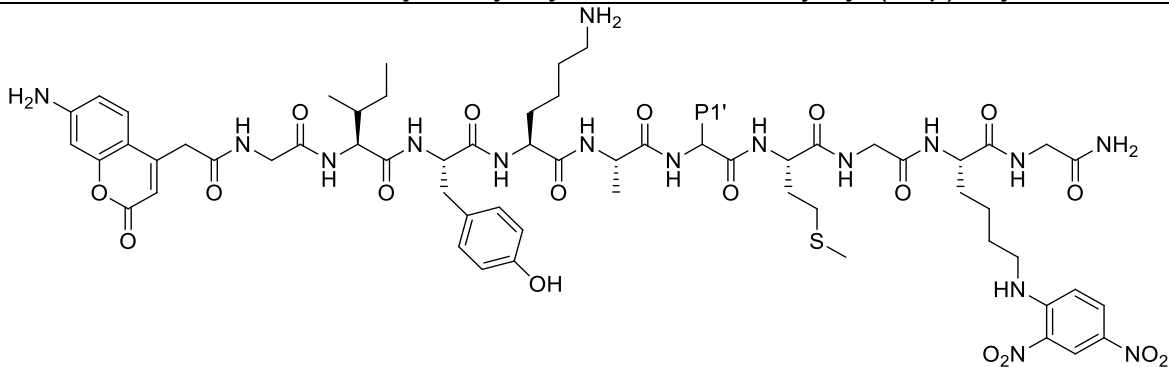
Table S5. MS analysis of P2_P2' substrates library.

Substrate structure: ACC-Gly-Val-Thr-Nle-Arg-Ser-P2'-Gly-Lys(Dnp)-Gly-NH ₂			
			
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PR2_P2'_1	L-Ala	656.30	656.42
PR2_P2'_2	L-Arg	698.83	698.70

PR2_P2'_3	L-Asn	677.81	677.92
PR2_P2'_4	L-Asp	678.29	678.40
PR2_P2'_5	L-Glu	685.31	685.38
PR2_P2'_6	L-Gln	684.86	684.91
PR2_P2'_7	Gly	649.29	649.39
PR2_P2'_8	L-His	689.31	689.36
PR2_P2'_9	L-Ile	677.33	677.41
PR2_P2'_10	L-Leu	677.33	677.44
PR2_P2'_11	L-Lys	684.83	684.07
PR2_P2'_12	L-Nle	677.33	677.38
PR2_P2'_13	L-Phe	694.32	694.31
PR2_P2'_14	L-Pro	669.31	669.31
PR2_P2'_15	L-Ser	664.30	664.30
PR2_P2'_16	L-Thr	670.80	669.42
PR2_P2'_17	L-Trp	713.82	714.03
PR2_P2'_18	L-Tyr	702.32	702.47
PR2_P2'_19	L-Val	670.32	670.44
PR2_P2'_20	D-Ala	656.30	656.41
PR2_P2'_21	D-Arg	698.83	698.60
PR2_P2'_22	D-Asn	677.81	667.82
PR2_P2'_23	D-Asp	678.29	678.34
PR2_P2'_24	D-Gln	684.86	684.80
PR2_P2'_25	D-Glu	685.31	685.30
PR2_P2'_26	D-His	689.31	689.41
PR2_P2'_27	D-Leu	677.33	677.35
PR2_P2'_28	D-Lys	684.83	684.87
PR2_P2'_29	D-Phe	694.31	694.47
PR2_P2'_30	D-Pro	669.31	669.43
PR2_P2'_31	D-Ser	664.30	664.43
PR2_P2'_32	D-Thr	671.31	671.44
PR2_P2'_33	D-Trp	713.82	713.81
PR2_P2'_34	D-Tyr	702.32	702.51
PR2_P2'_35	D-Val	670.32	670.44
PR2_P2'_36	D-hPhe	701.33	701.47
PR2_P2'_37	β-Ala	663.31	656.30
PR2_P2'_38	L-Aze	662.30	662.33
PR2_P2'_39	L-2-Fal	689.31	689.43
PR2_P2'_40	L-Trp(Me)	720.83	721.03
PR2_P2'_41	L-Ala(2-th)	696.29	697.47
PR2_P2'_42	L-Ala(Bth)	722.80	722.84
PR2_P2'_43	Aib	663.31	663.46
PR2_P2'_44	L-Abu	663.31	663.33
PR2_P2'_45	L-Nva	670.32	670.39
PR2_P2'_46	L-Ser(Bzl)	702.32	702.34
PR2_P2'_47	L-hSer	671.28	671.44
PR2_P2'_48	L-hSer(Bzl)	716.55	716.58
PR2_P2'_49	L-Thr(Bzl)	715.88	716.52
PR2_P2'_50	L-Cys(Bzl)	717.31	717.52

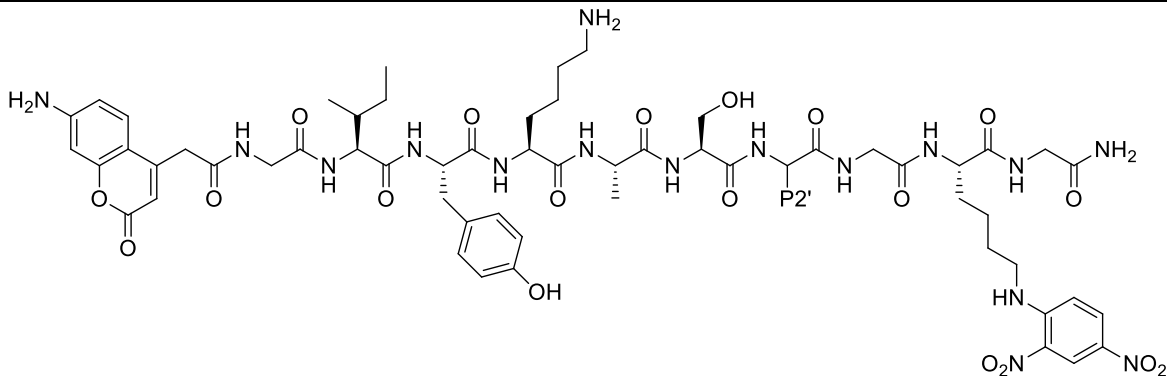
PR2_P2'_51	L-Cys(MeBzl)	724.32	724.56
PR2_P2'_52	L-Cys(4-MeOBzl)	732.32	732.53
PR2_P2'_53	L-Met	686.30	686.46
PR2_P2'_54	L-Met(O)	694.30	694.45
PR2_P2'_55	L-Met(O) ₂	701.79	702.46
PR2_P2'_56	L-2-Aad	691.81	692.55
PR2_P2'_57	L-hPhe	701.33	701.41
PR2_P2'_58	L-2-Aoc	691.34	691.56
PR2_P2'_59	L-1-Nal	719.33	719.79
PR2_P2'_60	L-hLeu	684.33	684.42

Table S6. MS analysis of P5_P1' substrates library.

Substrate structure: ACC-Gly-Ile-Tyr-Lys-Ala-P1'-Met-Gly-Lys(Dnp)-Gly-NH ₂			
			
Name	Amino acid in P1'	(M+H) ²⁺ calculated	(M+H) ²⁺ found
PR5_P1'_1	L-Ala	681.29	681.19
PR5_P1'_2	L-Arg	723.73	723.81
PR5_P1'_3	L-Asn	702.79	702.88
PR5_P1'_4	L-Asp	703.28	703.43
PR5_P1'_5	L-Glu	710.29	710.37
PR5_P1'_6	L-Gln	709.81	709.76
PR5_P1'_7	Gly	674.29	674.26
PR5_P1'_8	L-His	714.30	714.40
PR5_P1'_9	L-Ile	702.31	702.27
PR5_P1'_10	L-Leu	702.31	702.27
PR5_P1'_11	L-Lys	709.82	709.83
PR5_P1'_12	L-Nle	702.31	702.29
PR5_P1'_13	L-Phe	719.30	719.39
PR5_P1'_14	L-Pro	694.32	694.28
PR5_P1'_15	L-Ser	689.29	689.31
PR5_P1'_16	L-Thr	696.29	696.29
PR5_P1'_17	L-Trp	738.81	738.91
PR5_P1'_18	L-Tyr	727.31	727.38
PR5_P1'_19	L-Val	695.30	695.24
PR5_P1'_20	D-Ala	681.29	681.34
PR5_P1'_21	D-Arg	723.73	723.87
PR5_P1'_22	D-Asn	702.79	702.56

PR5_P1'_23	D-Asp	703.28	703.24
PR5_P1'_24	D-Gln	709.80	709.71
PR5_P1'_25	D-Glu	710.29	710.59
PR5_P1'_26	D-His	714.31	714.34
PR5_P1'_27	D-Leu	714.30	702.34
PR5_P1'_28	D-Lys	702.31	710.26
PR5_P1'_29	D-Phe	719.30	720.01
PR5_P1'_30	D-Pro	694.32	694.22
PR5_P1'_31	D-Ser	689.29	689.24
PR5_P1'_32	D-Thr	696.29	712.24
PR5_P1'_33	D-Trp	738.81	738.85
PR5_P1'_34	D-Tyr	727.31	727.15
PR5_P1'_35	D-Val	695.30	695.90
PR5_P1'_36	D-hPhe	726.31	726.26
PR5_P1'_37	β -Ala	681.29	680.85
PR5_P1'_38	L-Aze	724.31	724.29
PR5_P1'_39	L-2-Fal	714.30	714.60
PR5_P1'_40	L-Trp(Me)	745.82	745.67
PR5_P1'_41	L-Ala(2-th)	747.79	747.86
PR5_P1'_42	L-Ala(Bth)	688.31	688.37
PR5_P1'_43	Aib	688.31	688.45
PR5_P1'_44	L-Abu	754.79	755.14
PR5_P1'_45	L-Nva	734.31	734.78
PR5_P1'_46	L-Ser(Bzl)	696.29	696.39
PR5_P1'_47	L-hSer	741.32	741.67
PR5_P1'_48	L-hSer(Bzl)	741.32	741.52
PR5_P1'_49	L-Thr(Bzl)	742.34	742.39
PR5_P1'_50	L-Cys(Bzl)	749.31	749.43
PR5_P1'_51	L-Cys(MeBzl)	757.30	757.39
PR5_P1'_52	L-Cys(4-MeOBzl)	711.29	711.35
PR5_P1'_53	L-Met	719.29	719.42
PR5_P1'_54	L-Met(O)	727.28	727.34
PR5_P1'_55	L-Met(O)2	712.34	712.43
PR5_P1'_56	L-2-Aad	728.31	728.36
PR5_P1'_57	L-hPhe	715.32	715.45
PR5_P1'_58	L-2-Aoc	709.32	709.55
PR5_P1'_59	L-1-Nal	744.32	744.60
PR5_P1'_60	L-hLeu	734.31	734.45

Table S7. MS analysis of P5_P2' substrates library.

Substrate structure: ACC-Gly-Ile-Tyr-Lys-Ala-Ser-P2'-Gly-Lys(Dnp)-Gly-NH ₂			
			
Name	Amino acid in P1'	(M+H) ²⁺ calculated	(M+H) ²⁺ found
PR5_P2'_1	L-Ala	659.51	659.44
PR5_P2'_2	L-Arg	702.23	701.84
PR5_P2'_3	L-Asn	681.19	681.56
PR5_P2'_4	L-Asp	680.68	681.32
PR5_P2'_5	L-Glu	688.70	688.28
PR5_P2'_6	L-Gln	688.20	687.77
PR5_P2'_7	Gly	652.33	652.24
PR5_P2'_8	L-His	692.31	692.39
PR5_P2'_9	L-Ile	680.32	680.32
PR5_P2'_10	L-Leu	680.32	680.74
PR5_P2'_11	L-Lys	687.82	687.94
PR5_P2'_12	L-Nle	680.31	679.71
PR5_P2'_13	L-Phe	697.33	698.83
PR5_P2'_14	L-Pro	672.31	672.77
PR5_P2'_15	L-Ser	667.29	667.24
PR5_P2'_16	L-Thr	674.29	674.59
PR5_P2'_17	L-Trp	716.81	716.89
PR5_P2'_18	L-Tyr	705.31	705.29
PR5_P2'_19	L-Val	673.31	673.45
PR5_P2'_20	D-Ala	659.29	659.51
PR5_P2'_21	D-Arg	702.23	701.86
PR5_P2'_22	D-Asn	681.19	681.12
PR5_P2'_23	D-Asp	680.68	682.64
PR5_P2'_24	D-Gln	688.27	687.96
PR5_P2'_25	D-Glu	688.70	688.44
PR5_P2'_26	D-His	692.30	692.66
PR5_P2'_27	D-Leu	680.32	680.81
PR5_P2'_28	D-Lys	687.82	687.87
PR5_P2'_29	D-Phe	697.33	697.41
PR5_P2'_30	D-Pro	672.31	672.24
PR5_P2'_31	D-Ser	667.29	667.35
PR5_P2'_32	D-Thr	674.29	674.49
PR5_P2'_33	D-Trp	716.81	717.21
PR5_P2'_34	D-Tyr	705.31	705.65
PR5_P2'_35	D-Val	673.31	673.82
PR5_P2'_36	D-hPhe	704.13	704.42

PR5_P2'_37	β -Ala	659.56	659.64
PR5_P2'_38	L-Aze	665.29	666.03
PR5_P2'_39	L-2-Fal	691.25	692.27
PR5_P2'_40	L-Trp(Me)	723.82	724.06
PR5_P2'_41	L-Ala(2-th)	700.29	700.49
PR5_P2'_42	L-Ala(Bth)	725.79	726.58
PR5_P2'_43	Aib	666.31	666.54
PR5_P2'_44	L-Abu	666.31	666.48
PR5_P2'_45	L-Nva	673.31	673.55
PR5_P2'_46	L-Ser(Bzl)	712.31	712.33
PR5_P2'_47	L-hSer	674.29	674.35
PR5_P2'_48	L-hSer(Bzl)	719.32	719.11
PR5_P2'_49	L-Thr(Bzl)	719.32	719.38
PR5_P2'_50	L-Cys(Bzl)	720.33	720.31
PR5_P2'_51	L-Cys(MeBzl)	727.33	727.73
PR5_P2'_52	L-Cys(4-MeOBzl)	735.32	735.44
PR5_P2'_53	L-Met	689.29	689.99
PR5_P2'_54	L-Met(O)	697.29	697.29
PR5_P2'_55	L-Met(O) ₂	705.29	705.27
PR5_P2'_56	L-2-Aad	695.50	695.32
PR5_P2'_57	L-hPhe	703.31	704.39
PR5_P2'_58	L-2-Aoc	694.33	694.51
PR5_P2'_59	L-1-Nal	722.32	722.45
PR5_P2'_60	L-hLeu	687.32	687.24

Table S8. List of specific substrates, their sequence, calculated and found (M+H)²⁺ value.

Name	Dedicated subunit	Amino acid sequence	(M+H) ²⁺ calculated	(M+H) ²⁺ found	Purity
PR1_S1	β 1	ACC-Gly-Lys-Pro-Nle-Asp-2-Fal-hPhe-Gly-Lys(Dnp)-Gly-NH ₂	719.32	718.65	>97%
PR1_S2	β 1	ACC-Gly-Lys-Pro-Nle-Asp-Aad-Nva-Gly-Lys(Dnp)-Gly-NH ₂	690.32	690.52	>98%
PR1_S3	β 1	ACC-Gly-Val-Thr-Nle-Asp-hSer(Bzl)-Trp(Me)-Gly-Lys(Dnp)-Gly-NH ₂	752.33	752.57	>93%
PR1_S4	β 1	ACC-Gly-Ile-Tyr-Lys-Asp-2-Aze-Cys(Bzl)-Gly-Lys(Dnp)-Gly-NH ₂	740.29	740.53	>98%

PR2_S1	$\beta 2$	ACC-Gly-Val-Thr-Nle-Arg-hSer(Bzl)-Trp(Me)-Gly-Lys(Dnp)-Gly-NH ₂	773.37	773.14	>99%
PR2_S2	$\beta 2$	ACC-Gly-Val-Thr-Nle-Arg-1-Nal-Cys(Bzl)-Gly-Lys(Dnp)-Gly-NH ₂	772.89	772.68	>93%
PR2_S3	$\beta 2$	ACC-Gly-Lys-Pro-Nle-Arg-Aad-Nva-Gly-Lys(Dnp)-Gly-NH ₂	711.86	711.03	>98%
PR2_S4	$\beta 2$	ACC-Gly-Ile-Tyr-Lys-Arg-2-Aze-Cys(Bzl)-Gly-Lys(Dnp)-Gly-NH ₂	760.84	761.16	>98%
PR5_S1	$\beta 5$	ACC-Gly-Ile-Tyr-Lys-Ala-2-Aze-Cys(Bzl)-Gly-Lys(Dnp)-Gly-NH ₂	718.31	718.81	>97%
PR5_S2	$\beta 5$	ACC-Gly-Ile-Tyr-Lys-Ala-2-Aad-2-Aoc-Gly-Lys(Dnp)-Gly-NH ₂	722.82	722.57	>97%
PR5_S3	$\beta 5$	ACC-Gly-Lys-Pro-Nle-Ala-Aad-Nva-Gly-Lys(Dnp)-Gly-NH ₂	669.32	668.47	>98%
PR5_S4	$\beta 5$	ACC-Gly-Val-Thr-Nle-Ala-hSer(Bzl)-Trp(Me)-Gly-Lys(Dnp)-Gly-NH ₂	730.33	730.59	>93%

Supplementary Figures

Figure S1. Graphs representing kinetics of IQF selective substrates.

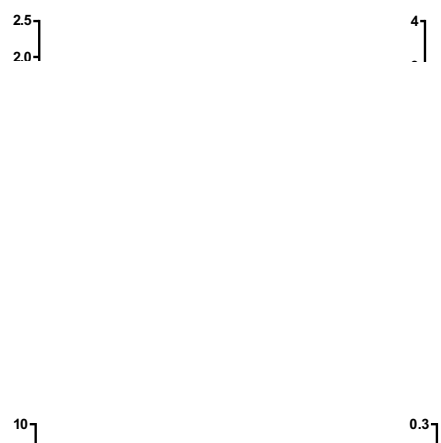


Figure S2. PR1_S1 structure, chromatogram and cleavage site analysis.

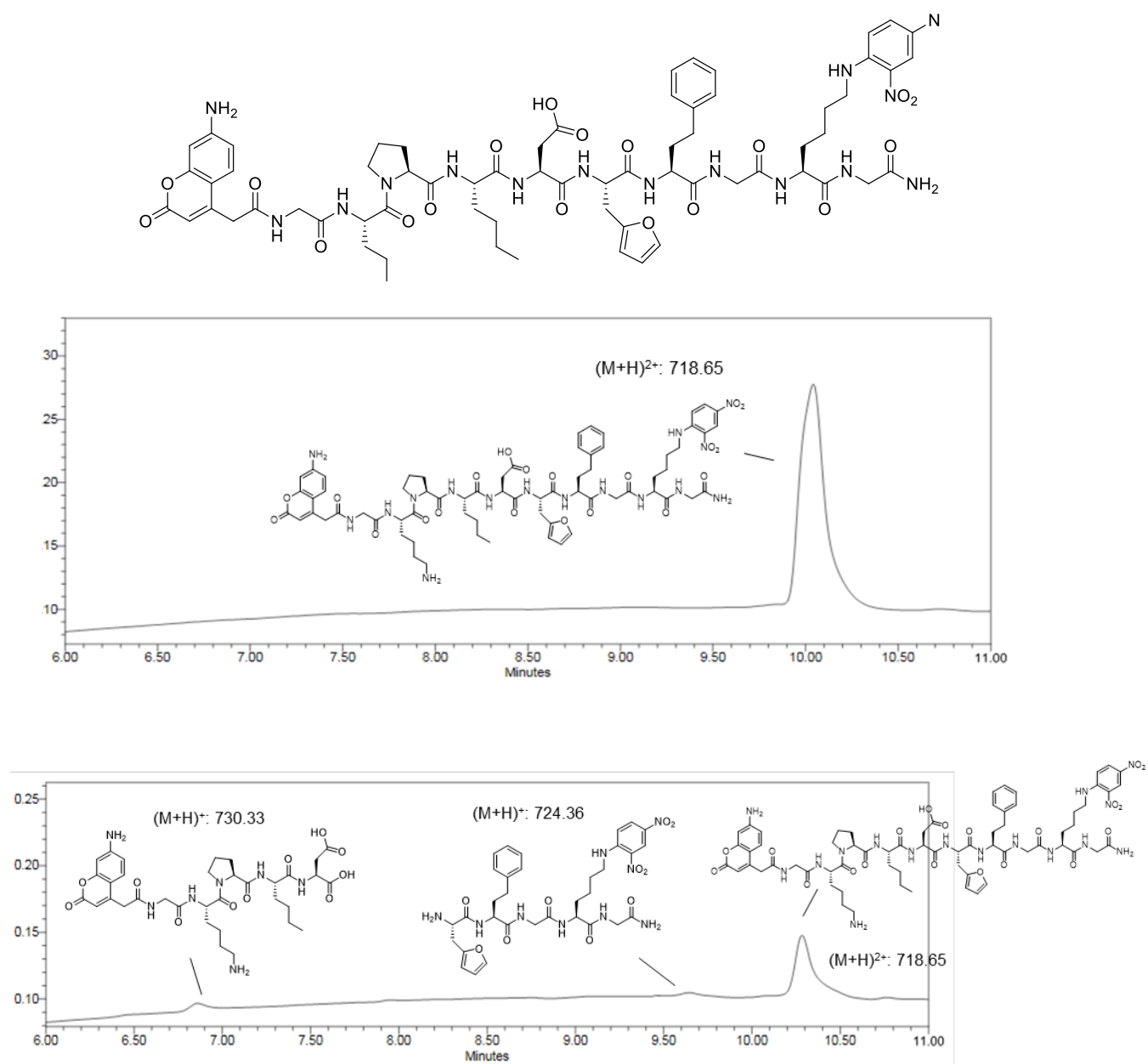


Figure S3. PR1_S2 structure, chromatogram and cleavage site analysis.

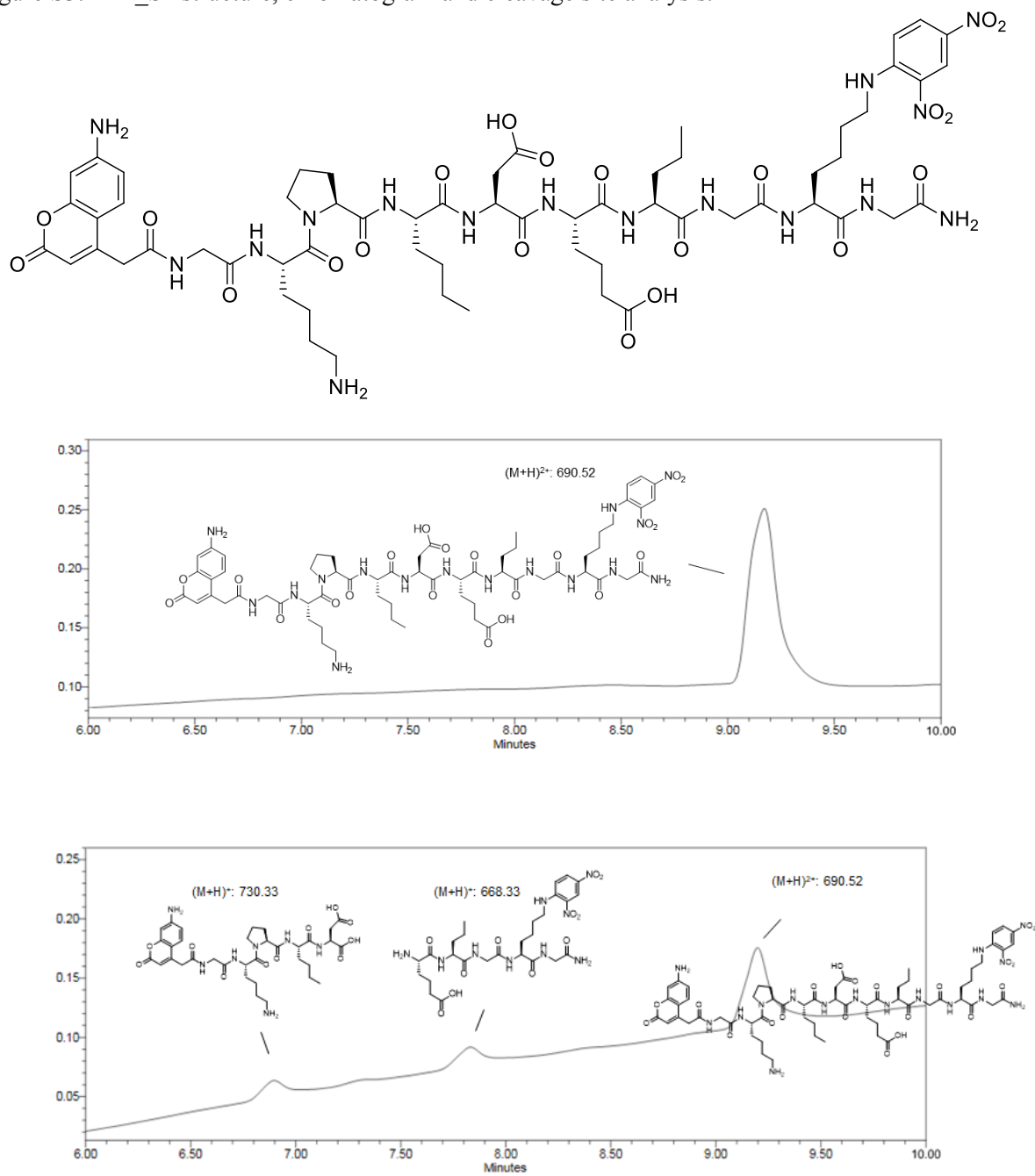


Figure S4. PR1_S3 structure, chromatogram and cleavage site analysis.

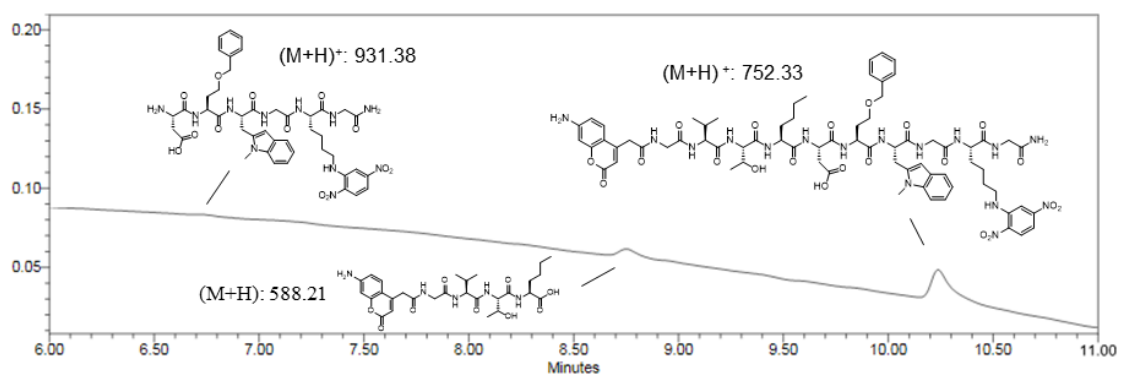
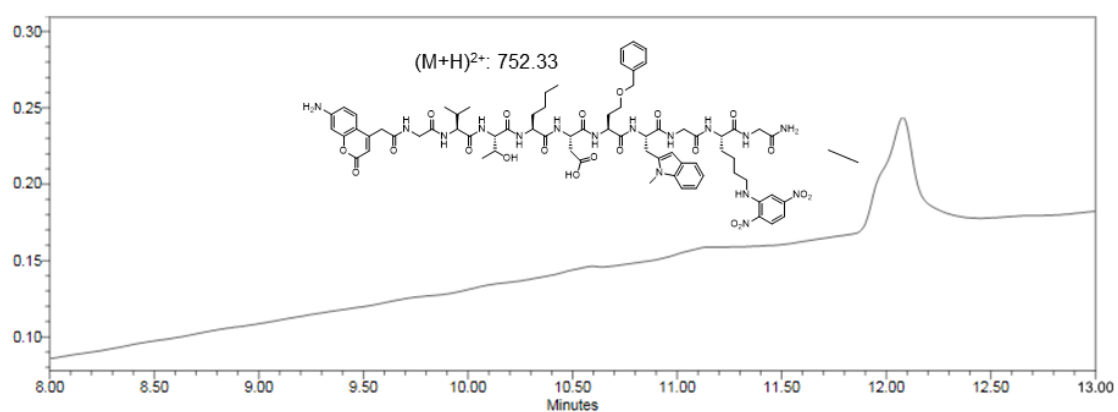
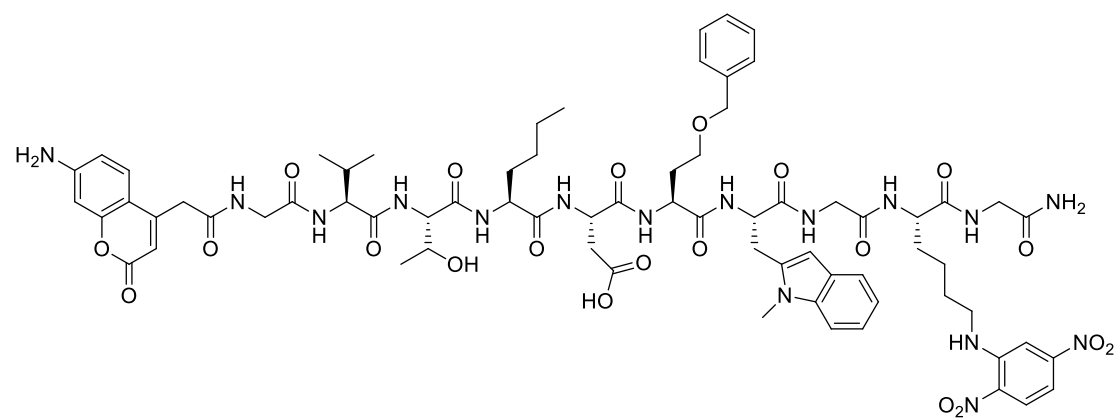


Figure S5. PR1_S4 structure, chromatogram and cleavage site analysis.

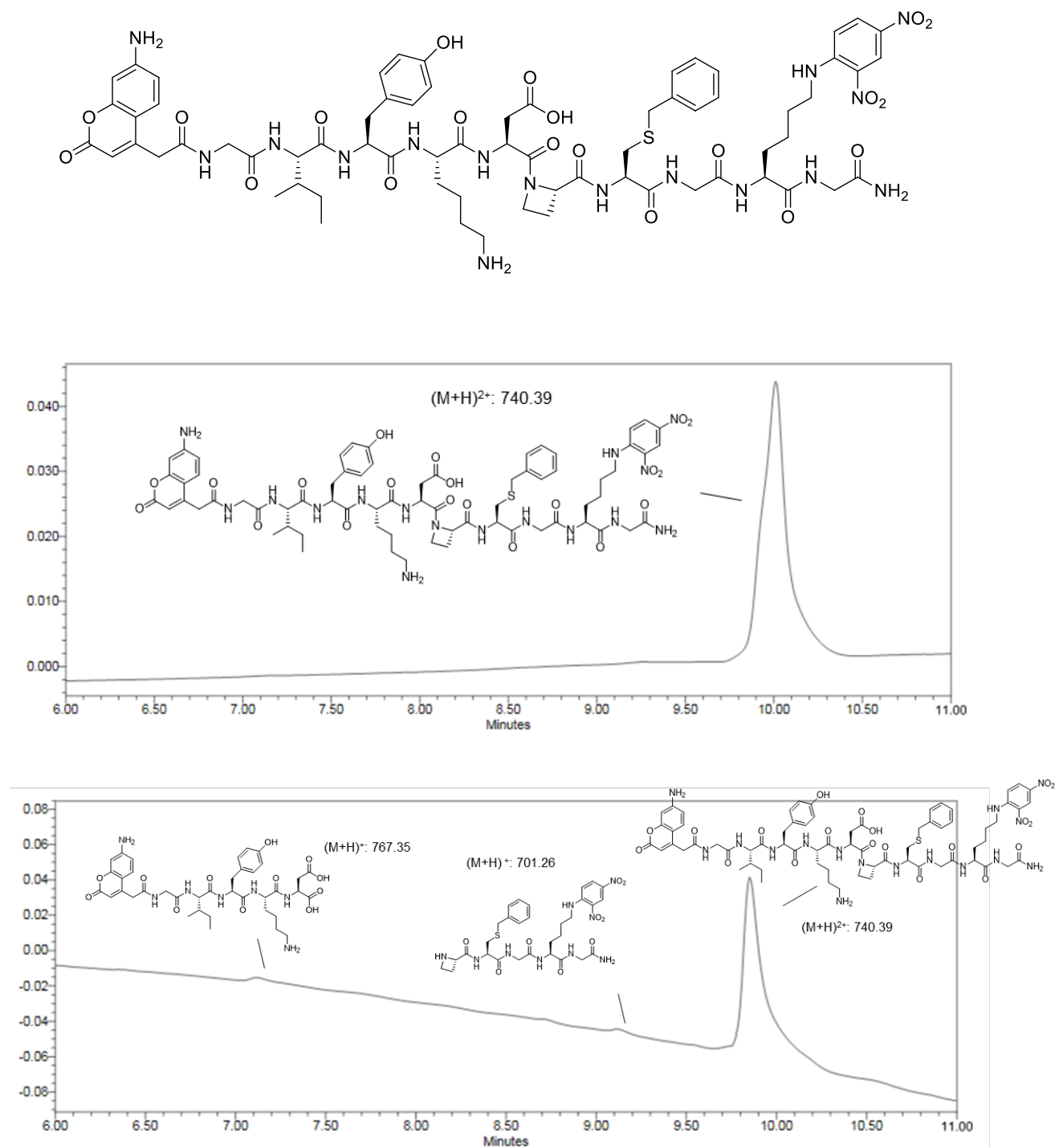
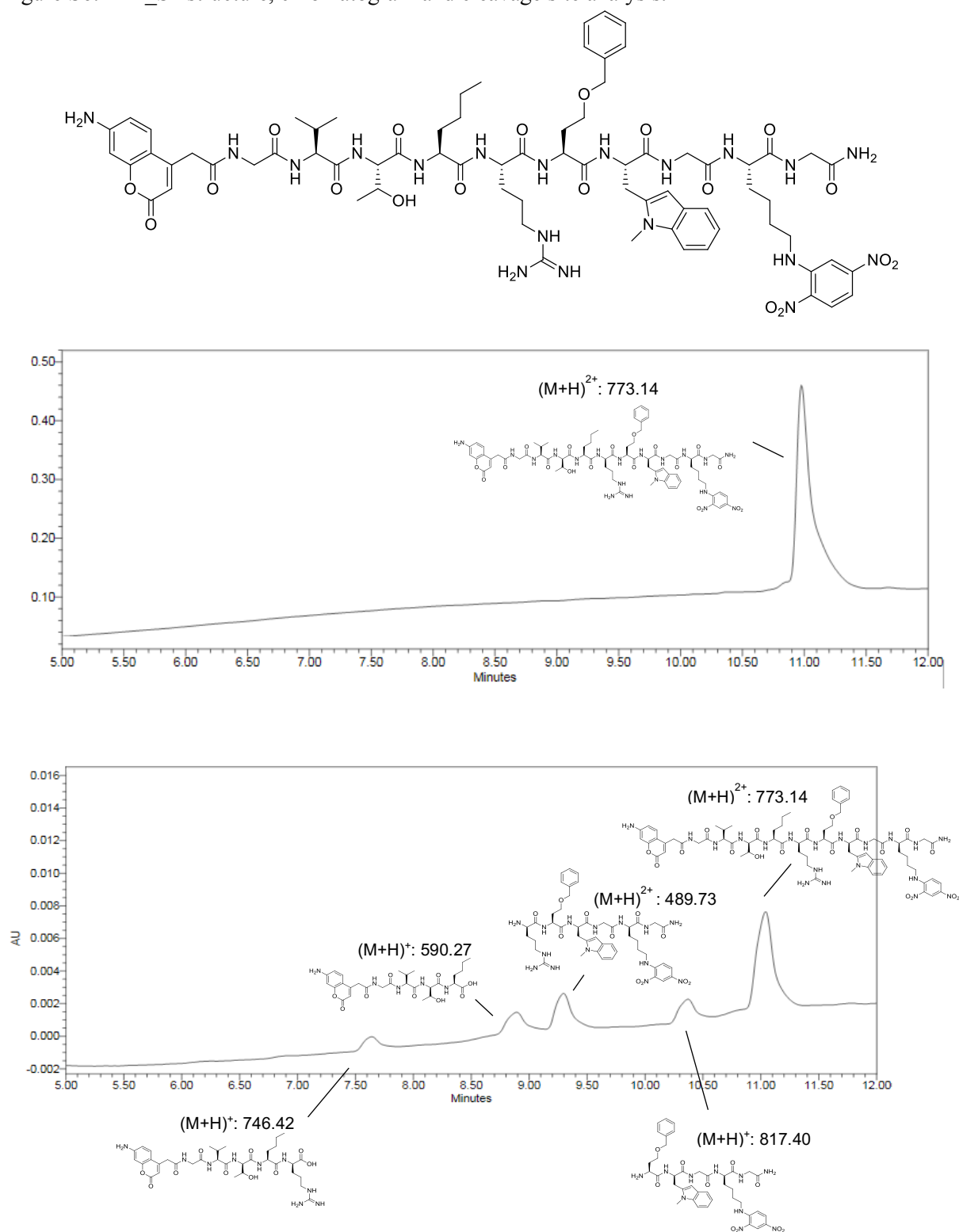


Figure S6. PR2_S1 structure, chromatogram and cleavage site analysis.



In case of PR2_S1 substrate there are two cleavage sites - main after Arg and additional after Nle. We performed additional screens with subunit inhibitors and discovered that PR2_S1 substrate is cleaved after Nle by $\beta 2$ subunit of 20s proteasome (data not shown).

Figure S7. PR2_S2 structure, chromatogram and cleavage site analysis.

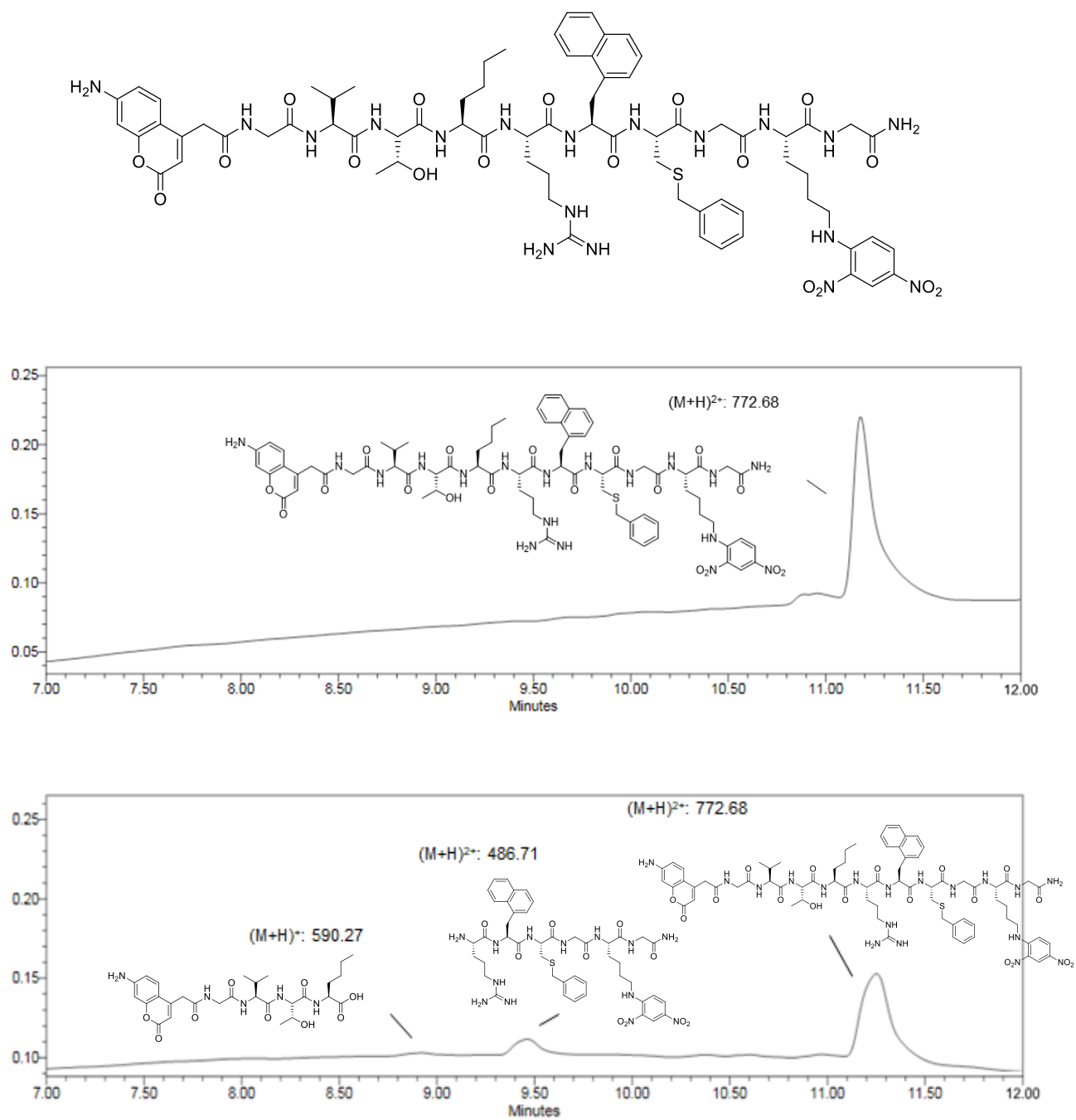


Figure S8. PR2_S3 structure, chromatogram and cleavage site analysis.

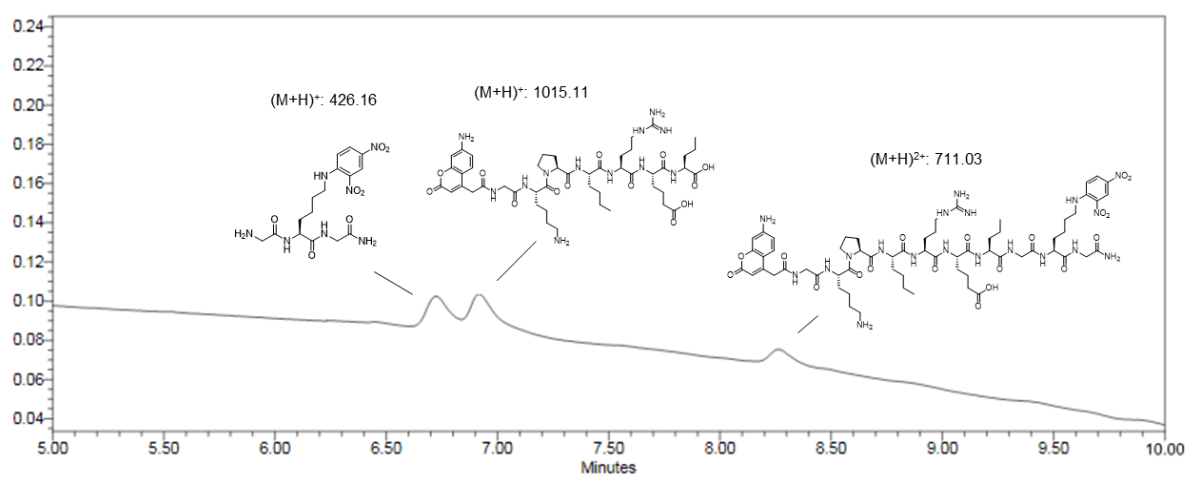
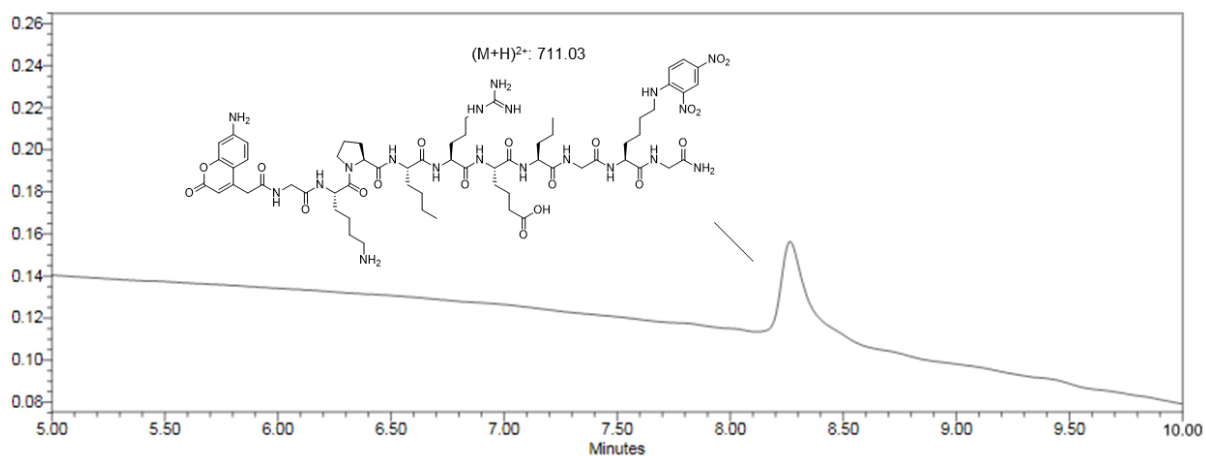
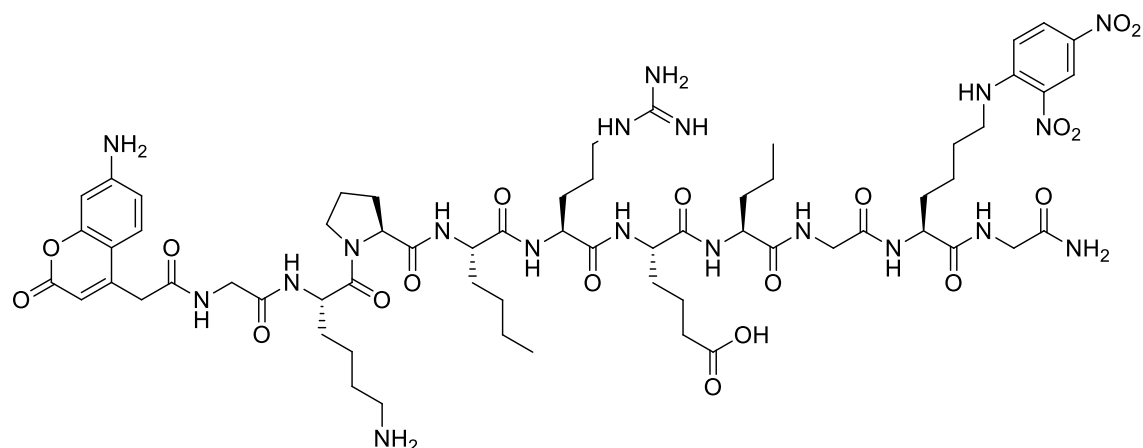


Figure S9. PR2_S4 structure, chromatogram and cleavage site analysis.

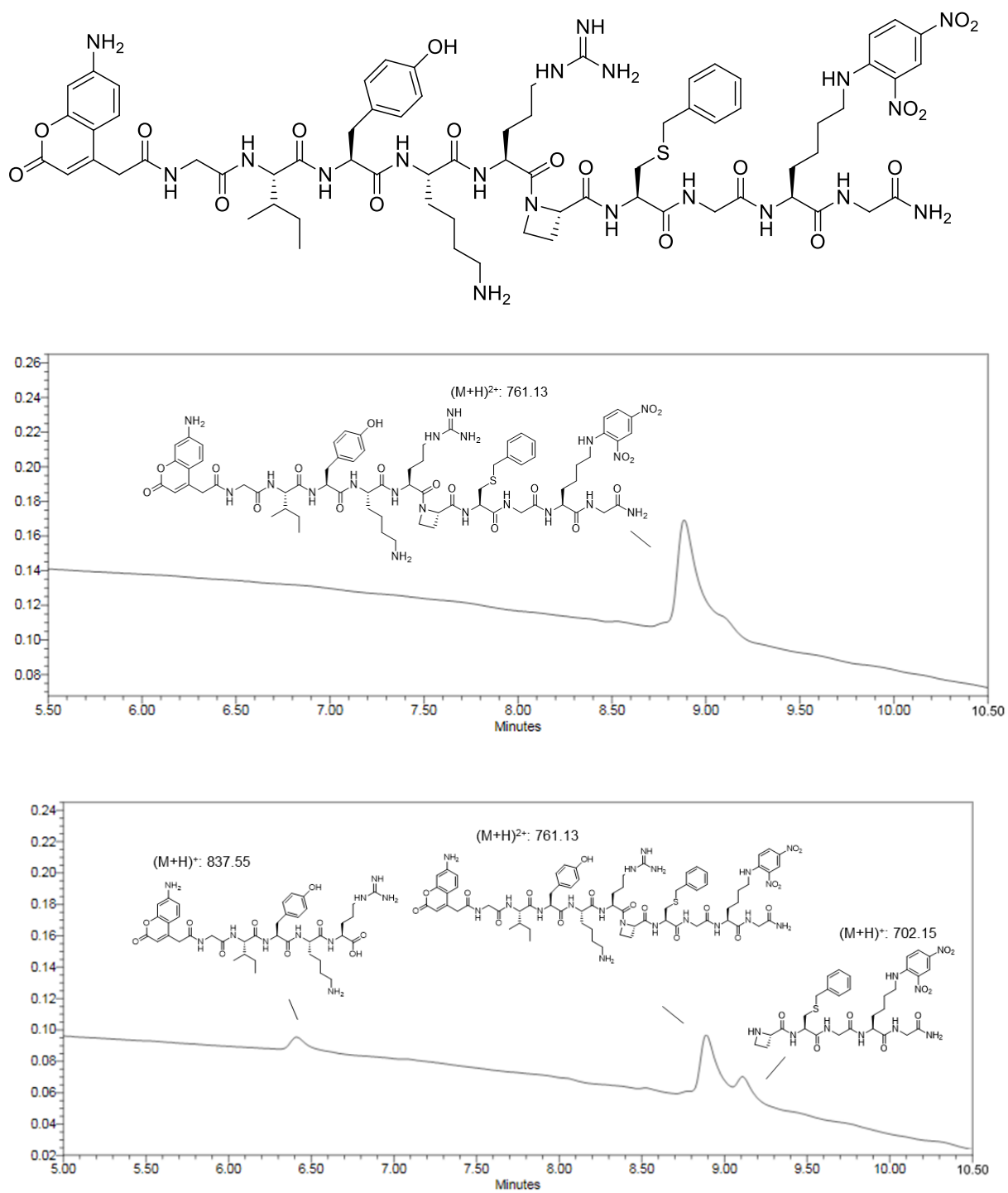


Figure S10. PR5_S1 structure, chromatogram and cleavage site analysis.

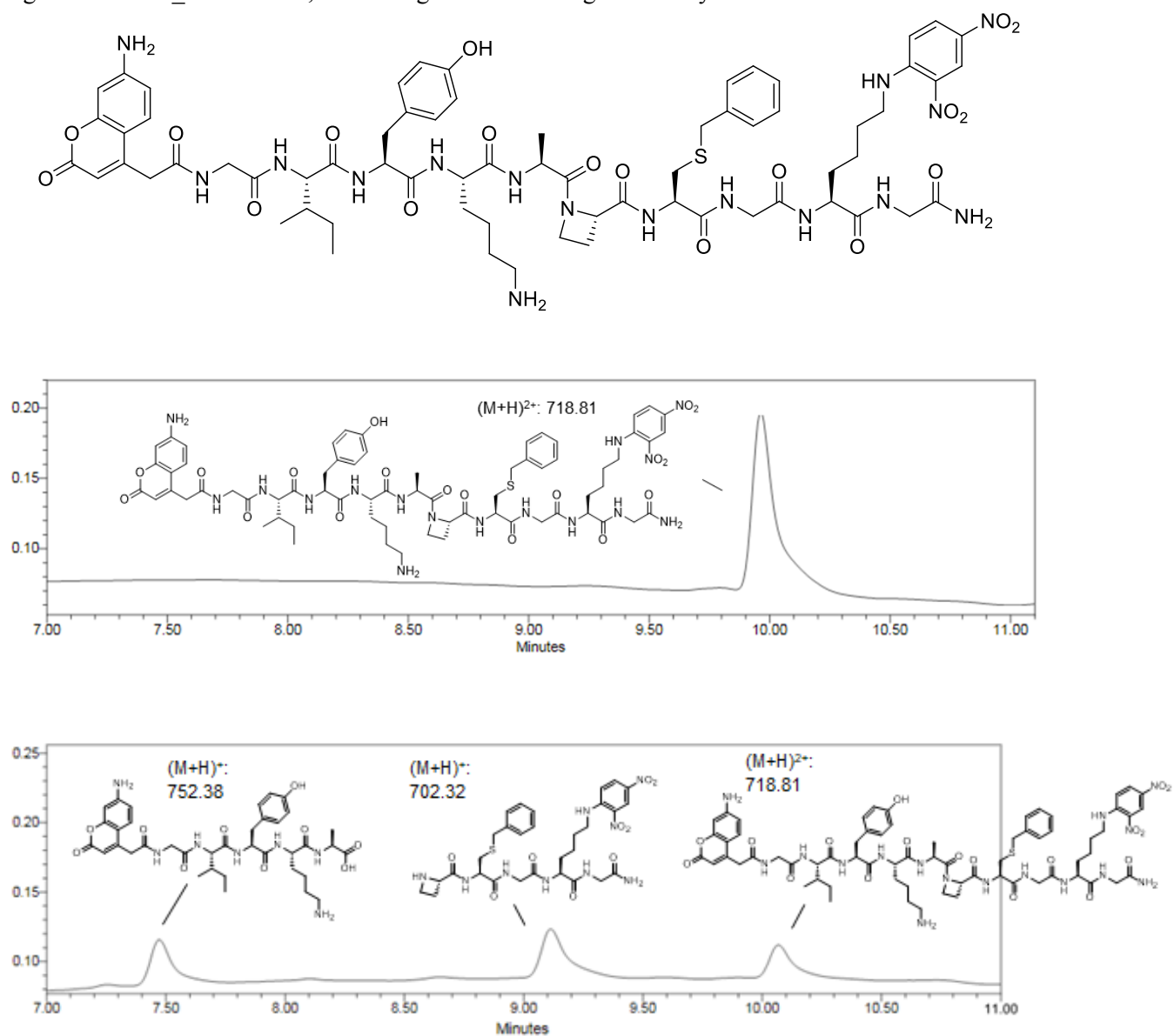
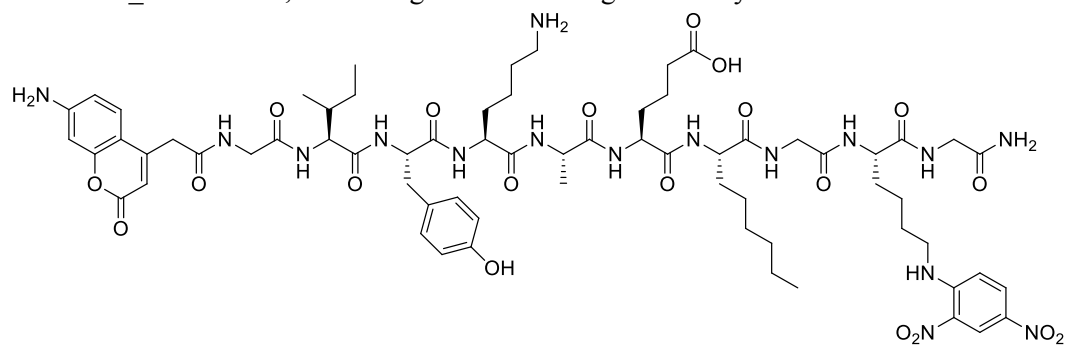


Figure S11. PR5_S2 structure, chromatogram and cleavage site analysis.



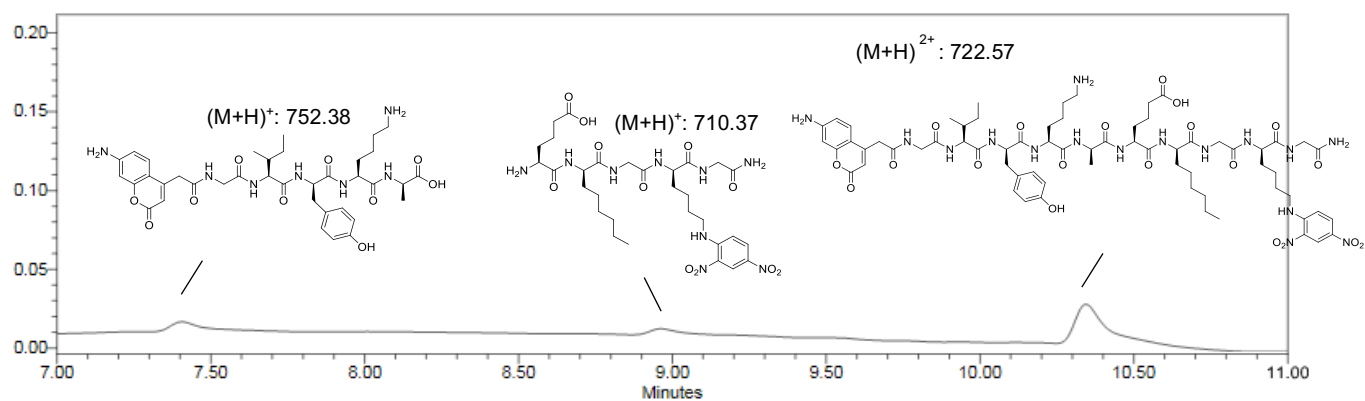
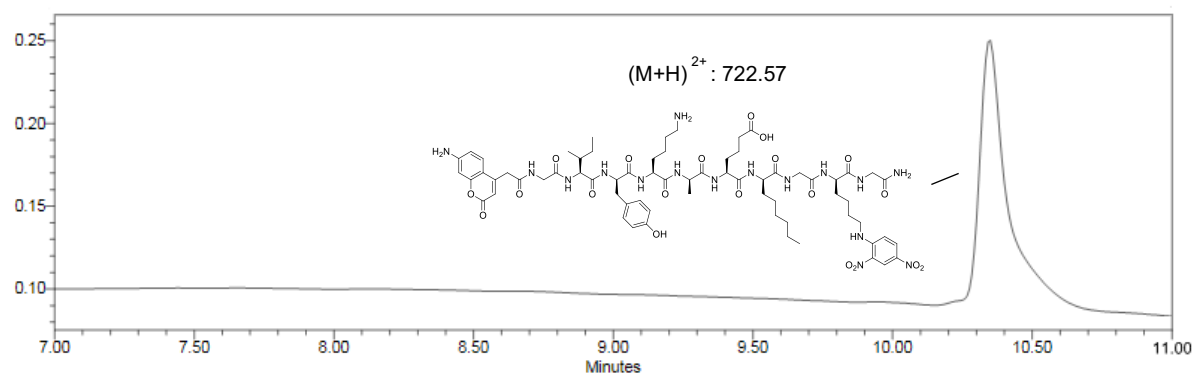


Figure S12. PR5_S3 structure, chromatogram and cleavage site analysis.

