Extensive peptide and natural protein substrate screens reveal that mouse caspase-11 has much narrower substrate specificity than caspase-1

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[*]MLG and MP contributed equally to this work

Running Title – Inflammatory caspases

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SUPPLEMENTARY INFORMATION

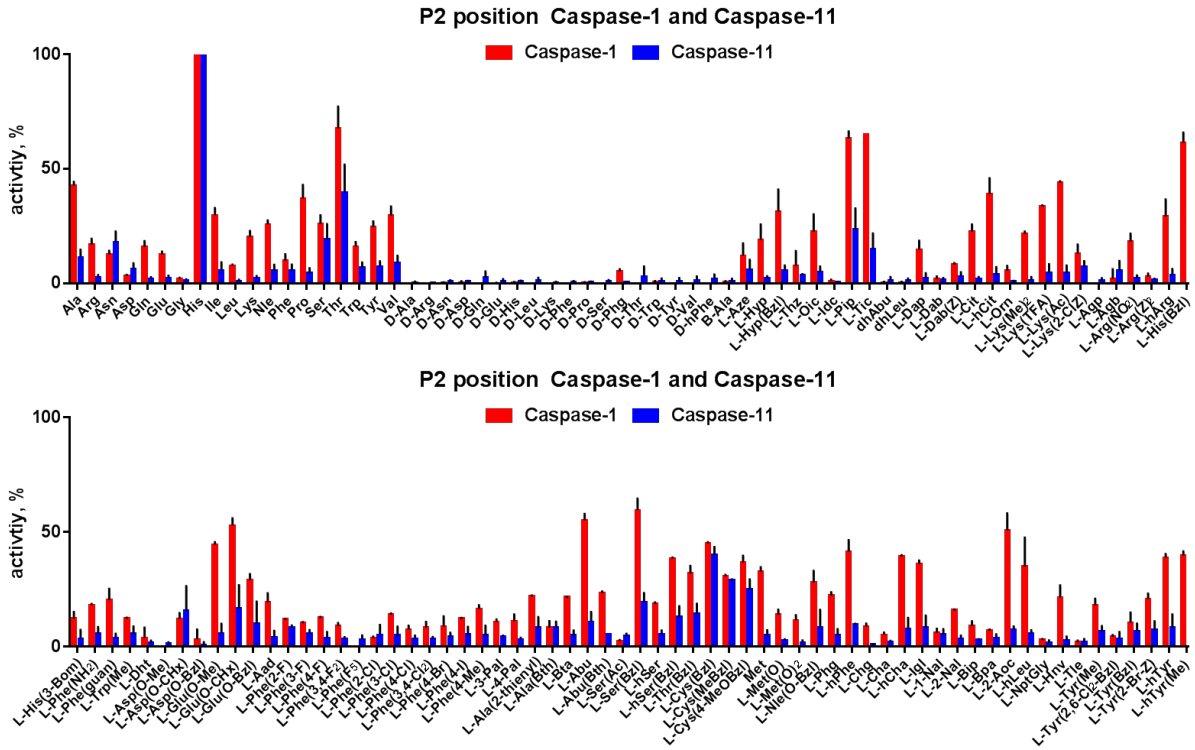


Figure S1 Mouse Caspase-1 and Caspase-11 substrate specificity in the P_2 position determined using HyCoSuL. Y axis shows relative activity, and x axis displays abbreviated natural and unnatural amino acids. Error bars represent standard deviations from three replicates (one biological replicate and two technical replicates).

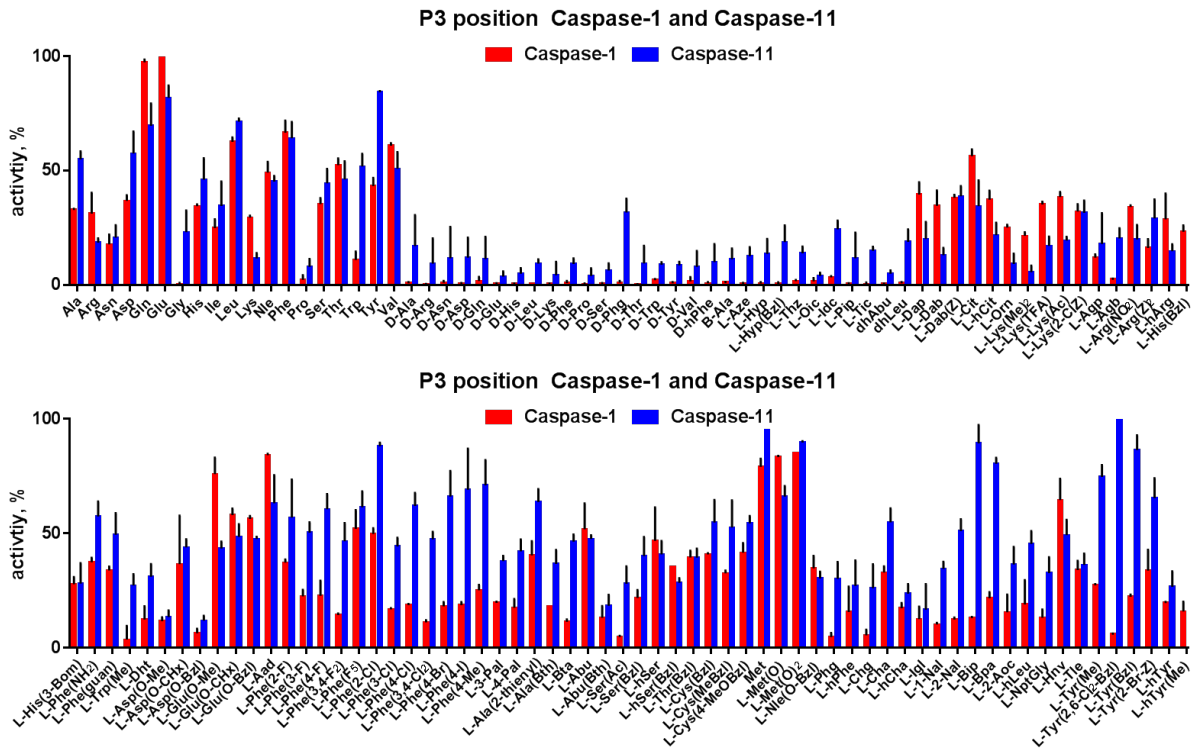


Figure S2 Mouse Caspase-1 and Caspase-11 substrate specificity in the P_3 position determined using HyCoSuL. Y axis shows relative activity, and x axis displays abbreviated natural and unnatural amino acids. Error bars represent standard deviations from three replicates (one biological replicate and two technical replicates).

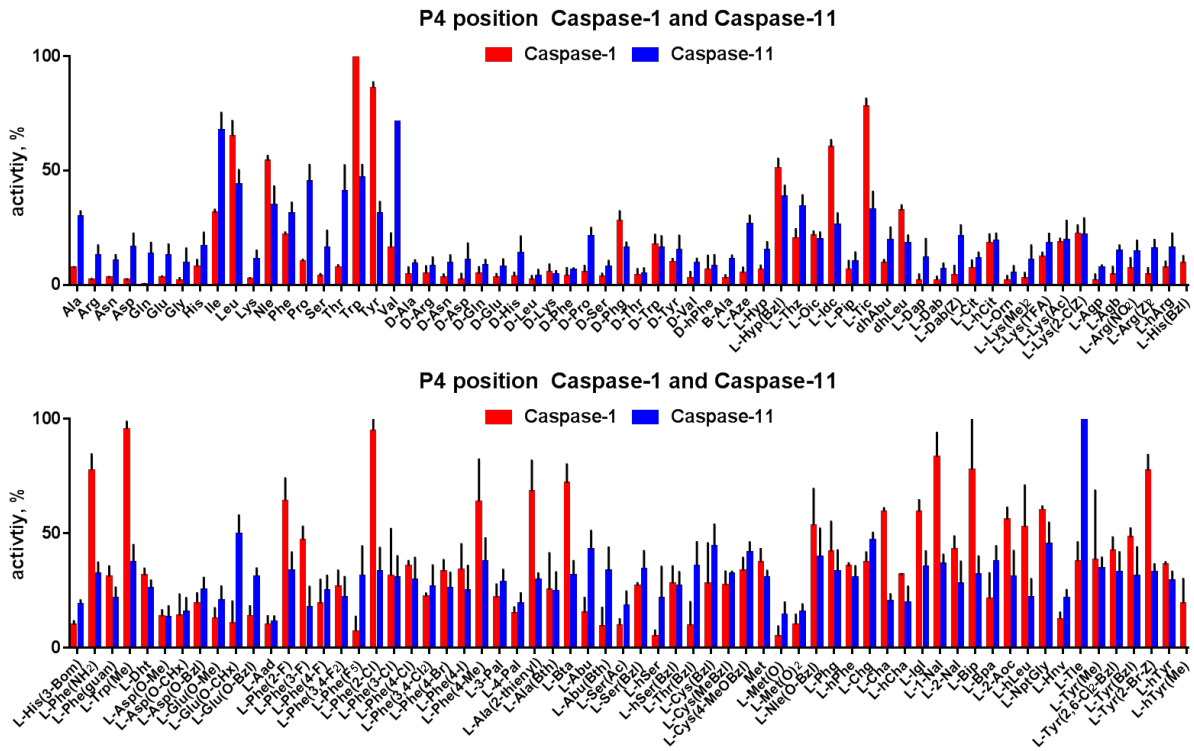


Figure S3 Mouse Caspase-1 and Caspase-11 substrate specificity in the P_4 position determined using HyCoSuL. Y axis shows relative activity, and x axis displays abbreviated natural and unnatural amino acids. Error bars represent standard deviations from three replicates (one biological replicate and two technical replicates).

Substrate sequence Ac-P_4-P_3-P_2-P_1-ACC		k_{cat}/K_M, $M^{-1}s^{-1}$		Selectivity factor
		Caspase-1	Caspase-11	
WQPD	Trp-Gln-Pro-Asp	257,000	< 50	> 5,140
FEAD	Phe-Glu-Ala-Asp	171,000	< 50	> 3,420
YQPD	Tyr-Gln-Pro-Asp	148,000	< 50	> 2,960
WTPD	Trp-Thr-Pro-Asp	138,000	< 50	> 2,760
YEKD	Tyr-Glu-Lys-Asp	106,000	< 50	> 2,120
WQVD	Trp-Gln-Val-Asp	328,000	244	1,344
YTID	Tyr-Thr-Ile-Asp	67,000	< 50	> 1,340
WKQD	Trp-Lys-Gln-Asp	46,200	< 50	> 924
YKAD	Tyr-Lys-Ala-Asp	38,400	< 50	> 768
YVHD	Tyr-Val-His-Asp	207,000	415	499
YKMD	Tyr-Lys-Met-Asp	29,200	152	192
FLTD	Phe-Leu-Thr-Asp	141,000	829	170
LESD	Leu-Glu-Ser-Asp	78,800	534	147
WEHD	Trp-Glu-His-Asp	522,000	4,100	127
LEHD	Leu-Glu-His-Asp	179,000	3,850	47
VWHD	Val-Trp-His-Asp	31,500	4,600	6.9
VAHD	Val-Asp-His-Asp	29,500	4,890	6.0
PYHD	Pro-Tyr-His-Asp	22,400	7,070	3.2
TWHD	Thr-Trp-His-Asp	5,030	1,620	3.1
PMHD	Pro-Met-His-Asp	16,100	7,200	2.2

Table S1 Kinetic parameters (k_{cat}/K_M) of ACC-substrates with natural amino acids measured toward mouse caspase-1 and caspase-11. Substrates were sorted by a decreasing selectivity factor, which was calculated by dividing k_{cat}/K_M(Caspase-1) by k_{cat}/K_M (Caspase-11). Each experiment was repeated at least three times, and the k_{cat}/K_M values are presented as an average. Standard deviations were below 15%.

Substrate sequence Ac-P_4-P_3-P_2-P_1-ACC	k_{cat}/K_M, $M^{-1}s^{-1}$		Selectivity factor
	Caspase-1	Caspase-11	
Trp(Me)-Glu-hLeu-Asp	212,000	165	1,280
Tyr-Val-Lys(tfa)-Asp	52,200	52	1,000
Cha-Glu-Tic-Asp	544,000	609	893
Trp(Me)-Gln-Tic-Asp	455,000	621	732
Trp(Me)-Glu(Me)-hCit-Asp	212,000	348	609
Phe(2Cl)-Glu-Tic-Asp	525,000	1,010	519
Trp-Tle-His-Asp	316,000	635	498
Trp(Me)-Dab-Pip-Asp	23,300	52	448
1Nal-Gln-Abu-Asp	226,000	510	443
Tyr-Gln-2Aoc-Asp	21,800	52	419
Phe(2Cl)-Lys(ac)-His-Asp	139,000	52	267
Tyr(Me)-Gln-Thr-Asp	118,000	568	208
Phe(2Cl)-Glu-Pip-Asp	388,000	2,420	160
Trp-Thr(Bzl)-His-Asp	488,000	3,370	145
Phe(2Cl)-Met(O2)-Pip-Asp	321,000	2,270	141
Cha-Glu-His-Asp	289,000	2,300	126
Tle-Phg-His-Asp	6,000	52	115
Cha-Glu-His-Asp	295,000	2,580	114
Trp(Me)-Glu-His(Bzl)-Asp	524,000	4,900	107
Tyr-Phe(F5)-His-Asp	229,000	2,200	104
Tyr-Thr(Bzl)-His-Asp	99,800	1,180	85
Trp-Met(O2)-His-Asp	512,000	7,140	72
2Nal-Thr-His-Asp	99,800	1,580	63
Phe(Me)-Met(O2)-His-Asp	275,000	5,740	48
Val-Hyp(Bzl)-His-Asp	2,470	52	48
Val-hGlu-Thr(Bzl)-Asp	34,500	1,030	34
Tyr-Tyr(2,6Cl2Bzl)-His-Asp	19,000	1,010	19
Glu(Ochx)-Bip-His(Bzl)-Asp	745	52	14
Tle-hGlu-Thr(Bzl)-Asp	34,000	2,580	13
Tle-Met-His-Asp	163,000	22,800	7.2
Tle-Phe(2Cl)-His-Asp	149,000	61,600	2.4
Val-Tyr(Bzl)-His-Asp	35,400	16,600	2.1
Val-Tyr(2,6Cl2Bzl)-His-Asp	8,860	4,550	2.0
Tle-Phe(4-Cl)-His-Asp	81,000	59,000	1.3
Val-Bip-His-Asp	13,000	15,000	0.87
Tle-Bpa-His(Bzl)-Asp	89,000	112,000	0.80
Tle-Bip-His-Asp	80,200	109,000	0.74
Pro-Bip-His(Bzl)-Asp	25,900	69,000	0.38

Table S2 Kinetic parameters (k_{cat}/K_M) of ACC-substrates with natural and unnatural amino acids measured toward mouse caspase-1 and caspase-11. Substrates were sorted by a decreasing selectivity factor, which was calculated by dividing k_{cat}/K_M(Caspase-1) by k_{cat}/K_M(Caspase-11). Each experiment was repeated at least three times, and the k_{cat}/K_M values are presented as an average. Standard deviations were below 15%.

Substrate sequence: Ac-P4-P3-P2-P1-ACC		k_{cat}/K_M, $M^{-1}s^{-1}$								
		Mouse caspases		**Human caspases**						
		Caspase-1	Caspase-11	Caspase-1	Caspase-4	Caspase-5	Caspase-3	Caspase-7	Caspase-8	Caspase-9
reference	WEHD	522,000	4,100	554,000	18,000	26,800	8,970	413	14,900	8,470
	LEHD	179,000	3,850	97,500	8,950	21,500	9,670	730	18,600	12,700
Mouse caspase-1 preferred	WQPD	257,000	<50	124,000	3,470	5,420	1,410	71	2,480	192
	FEAD	171,000	<50	57,100	1,870	6,720	1,540	182	3,890	526
	WQVD	328,000	244	125,000	4,290	5,090	12,100	1,300	3,870	217
Mouse caspase-11 preferred	PYHD	22,400	7,070	10,900	327	1,110	2,100	88	4,490	11,000
	PMHD	16,100	7,200	13,200	463	129	3,160	95	3,570	12,900

Table S3 Kinetic parameters (k_{cat}/K_M) of ACC-substrates with natural amino acids measured toward mouse caspase-1 and caspase-11. Based on screening of a natural amino acid HyCoSuL library, single substrates were synthesized focusing on mouse caspase-1 and caspase-11 hits. Each substrate was assayed with either mouse caspase-1 or caspase-11, or the indicated human caspases. All synthesized natural amino acid substrates were preferred by caspase-1, even those that had the highest catalytic numbers for caspase-11. Each experiment was repeated at least three times, and the k_{cat}/K_M values are presented as an average. Standard deviations were below 15%.

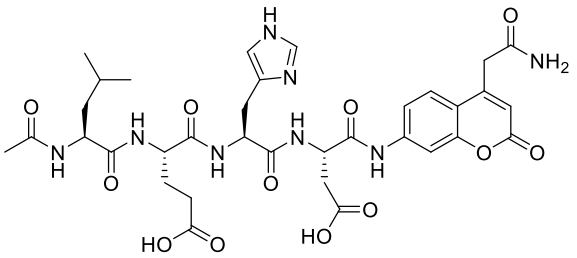
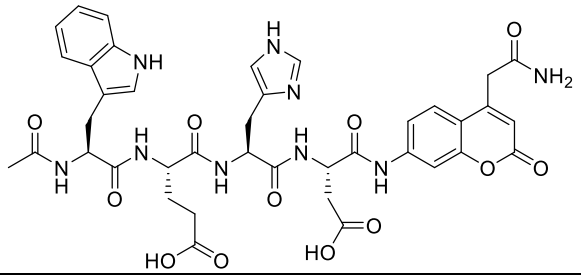
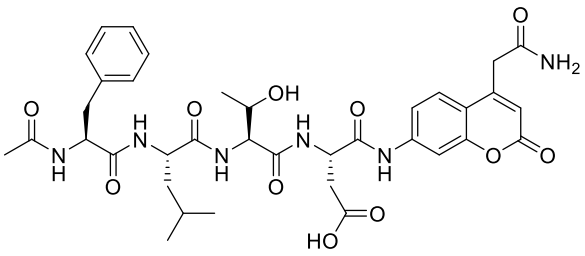
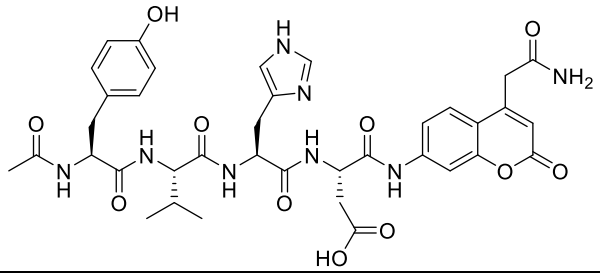
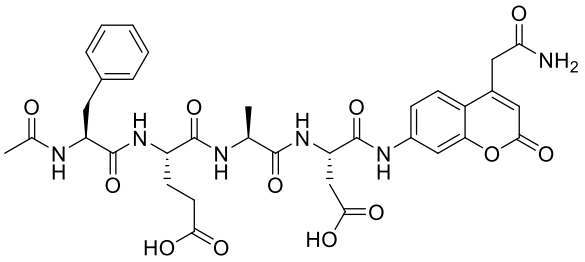
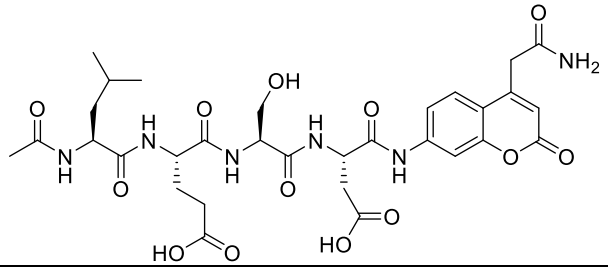
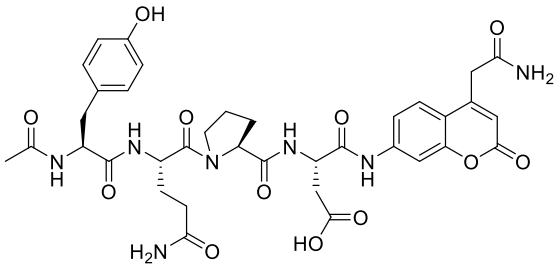
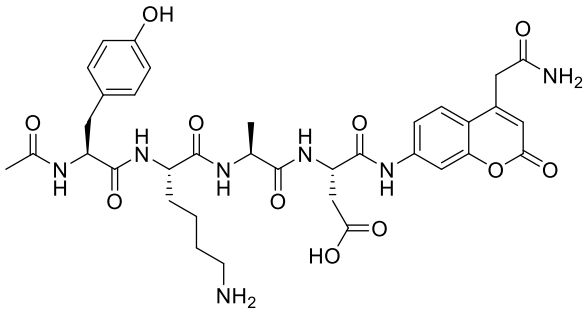
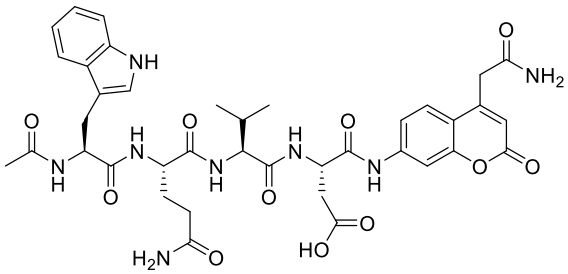
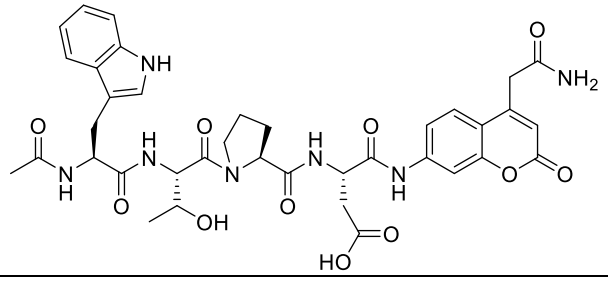
Substrate sequence: Ac-P4-P3-P2-P1-ACC		k_{cat}/K_M, $M^{-1}s^{-1}$								
		Mouse caspases		**Human caspases**						
		Caspase -1	Caspase -11	Caspase -1	Caspase -4	Caspase -5	Caspase -3	Caspase -7	Caspase -8	Caspase -9
Mouse caspase-1 preferred	Cha-Glu-Tic-Asp	544,000	609	159,000	37,900	28,900	10,500	953	7,860	7,760
	Phe(2Cl)-Glu-Tic-Asp	525,000	1,010	244,000	15,200	16,800	75,900	10,100	14,200	5,400
	Trp(Me)-Glu-His(Bzl)-Asp	524,000	1,700	624,000	15,800	20,100	6,300	278	7,620	6,670
	Trp-Met(O_2)-His-Asp	512,000	7,140	548,000	9,600	15,400	5,000	60	2,400	6,700
Mouse caspase-11 preferred	Tle-Bpa-His(Bzl)-Asp	89,000	112,000	25,200	4,400	2,650	23,300	100	6,400	4,200
	Tle-Bip-His-Asp	80,200	109,000	10,800	2,800	5,280	16,900	163	11,700	8,400
	Pro-Bip-His(Bzl)-Asp	25,900	69,000	13,800	590	314	1,260	41	2,750	6,990

Table S4 Kinetic parameters (k_{cat}/K_M) of ACC-substrates with natural amino acids measured toward mouse caspase-1 and caspase-11. Based on screening of an unnatural amino acid HyCoSuL library, single substrates were synthesized focusing on mouse caspase-1 and caspase-11 hits. Each substrate was assayed with either mouse caspase-1 or caspase-11, or the indicated human caspases. All synthesized natural amino acid substrates were preferred by caspase-1, even those that had the highest catalytic numbers for caspase-11. Each experiment was repeated at least three times, and the k_{cat}/K_M values are presented as an average. Standard deviations were below 15%.

fluorogenic substrate	$[m/z + H]^+$ calc.	$[m/z + H]^+$ measured
Substrates with natural amino acids		
Ac-Leu-Glu-His-Asp-ACC	755.2995	755.2998
Ac-Trp-Glu-His-Asp-ACC	828.2947	828,2958
Ac-Phe-Leu-Thr-Asp-ACC	759.2966 (with Na^+)	759.2957 (with Na^+)
Ac-Tyr-Val-His-Asp-ACC	775.3046	775.3040
Ac-Phe-Glu-Ala-Asp-ACC	723.2620	723.2638
Ac-Leu-Glu-Ser-Asp-ACC	705.2726	705.2736
Ac-Tyr-Gln-Pro-Asp-ACC	764.2886	764.2653
Ac-Tyr-Lys-Ala-Asp-ACC	738.3093	738.3094
Ac-Trp-Gln-Val-Asp-ACC	789.3202	789.3213
Ac-Trp-Thr-Pro-Asp-ACC	760.2937	760.2942
Ac-Tyr-Thr-Ile-Asp-ACC	753.3090	753.3113
Ac-Tyr-Glu-Lys-Asp-ACC	796.3148	796.3146
Ac-Trp-Lys-Gln-Asp-ACC	818.3468	818.3476
Ac-Trp-Gln-Pro-Asp-ACC	787.3046	787.3046
Ac-Tyr-Lys-Met-Asp-ACC	798.3127	798.3141
Ac-Thr-Trp-His-Asp-ACC	800.2998	800.3013
Ac-Val-Trp-His-Asp-ACC	798.3206	798.3236
Ac-Pro-Tyr-His-Asp-ACC	773.2889	773.2880
Ac-Val-Asp-His-Asp-ACC	727.2682	727.2677
Ac-Pro-Met-His-Asp-ACC	741.2661	741.2664
Substrates with natural and unnatural amino acids		
Ac-Tyr(Me)-Gln-Thr-Asp-ACC	782.2992	782.3013
Ac-2Nal-Thr-His-Asp-ACC	811.3046	811.3060
Ac-Phe(Me)-Met(O_2)-His-Asp-ACC	837.2872	837.2888
Ac-Tyr-Thr(Bzl)-His-Asp-ACC	867.3308	867.3317
Ac-Tyr-Phe(F_5)-His-Asp-ACC	913.2575	913.2579
Ac-Trp-Met(O_2)-His-Asp-ACC	862.2824	862.2839
Ac-Trp-Thr(Bzl)-His-Asp-ACC	890.3468	890.3487
Ac-Cha-Glu-Tic-Asp-ACC	817.3403	817.3427
Ac-Cha-Glu-His-Asp-ACC	795.3308	795.3336
Ac-Trp-Tle-His-Asp-ACC	812.3362	812.3362
Ac-Trp(Me)-Glu-His(Bzl)-Asp-ACC	932.3573	932.3574
Ac-Phe(2Cl)-Glu-Tic-Asp-ACC	845.2544	845.2551
Ac-Val-Glu-Thr(Bzl)-Asp-ACC	795.3196	795.3202
Ac-Tle-Glu-Thr(Bzl)-Asp-ACC	809.3352	809.3356
Ac-Phe(2Cl)-Met(O_2)-Pip-Asp-ACC	831.2421	831.2431
Ac-Trp(Me)-Gln-Tic-Asp-ACC	863.3359	863.3361
Ac-Phe(2Cl)-Glu-Pip-Asp-ACC	797.2544	797.2551
Ac-Trp(Me)-Glu(Me)-hCit-Asp-ACC	890.3679	890.3671
Ac-Tyr-Gln-2-Aoc-Asp-ACC	808.3512	808.3495
Ac-Tyr-Val-Lys(TFA)-Asp-ACC	862.3229	862.3230
Ac-Trp(Me)-Glu-hLeu-Asp-ACC	832.3512	832.3523

Ac-Phe(2Cl)-Lys(Ac)-His-Asp-ACC	864.3078	864.3097
Ac-Trp(Me)-Dab-Pip-Asp-ACC	787.3410	787.3382
Ac-1Nal-Gln-Abu-Asp-ACC	786.3093	786.3090
Ac-Val-Bip-His-Asp-ACC	835.3410	835.3412
Ac-Tle-Met-His-Asp-ACC	757.2974	757.2979
Ac-Tle-Phe(2Cl)-His-Asp-ACC	807.2863	807.2892
Ac-Tyr-Tyr(2,6ClBzl)-His-Asp-ACC	997.2685	997.2703
Ac-Val-Tyr(Bzl)-His-Asp-ACC	865.3515	865.3509
Ac-Val-Tyr(2,6ClBzl)-His-Asp-ACC	933.2736	933.2735
Ac-Tle-Bip-His-Asp-ACC	849.3566	849.3562
Ac-Tle-Bpa-His(Bzl)-Asp-ACC	967.3985	967.3997
Ac-Pro-Bip-His(Bzl)-Asp-ACC	923.3723	923.3732
Ac-Val-Hyp(Bzl)-His-Asp-ACC	815.3367	815.3359
Ac-Glu(Chx)-Bip-His(Bzl)-Asp-ACC	1037.4403	1037.4426
Ac-Tle-Phg-His-Asp-ACC	759.3097	759.3102
Ac-Tle-Phe(4Cl)-His-Asp-ACC	807.2863	807.2868

Table S5 High Resolution – Mass Spectrometry analysis of ACC-substrates.

Ac-LEHD-ACC 	Ac-WEHD-ACC 
Ac-FLTD-ACC 	Ac-YVHD-ACC 
Ac-FEAD-ACC 	Ac-LESD-ACC 
Ac-YQPD-ACC 	Ac-YKAD-ACC 
Ac-WQVD-ACC 	Ac-WTPD-ACC 

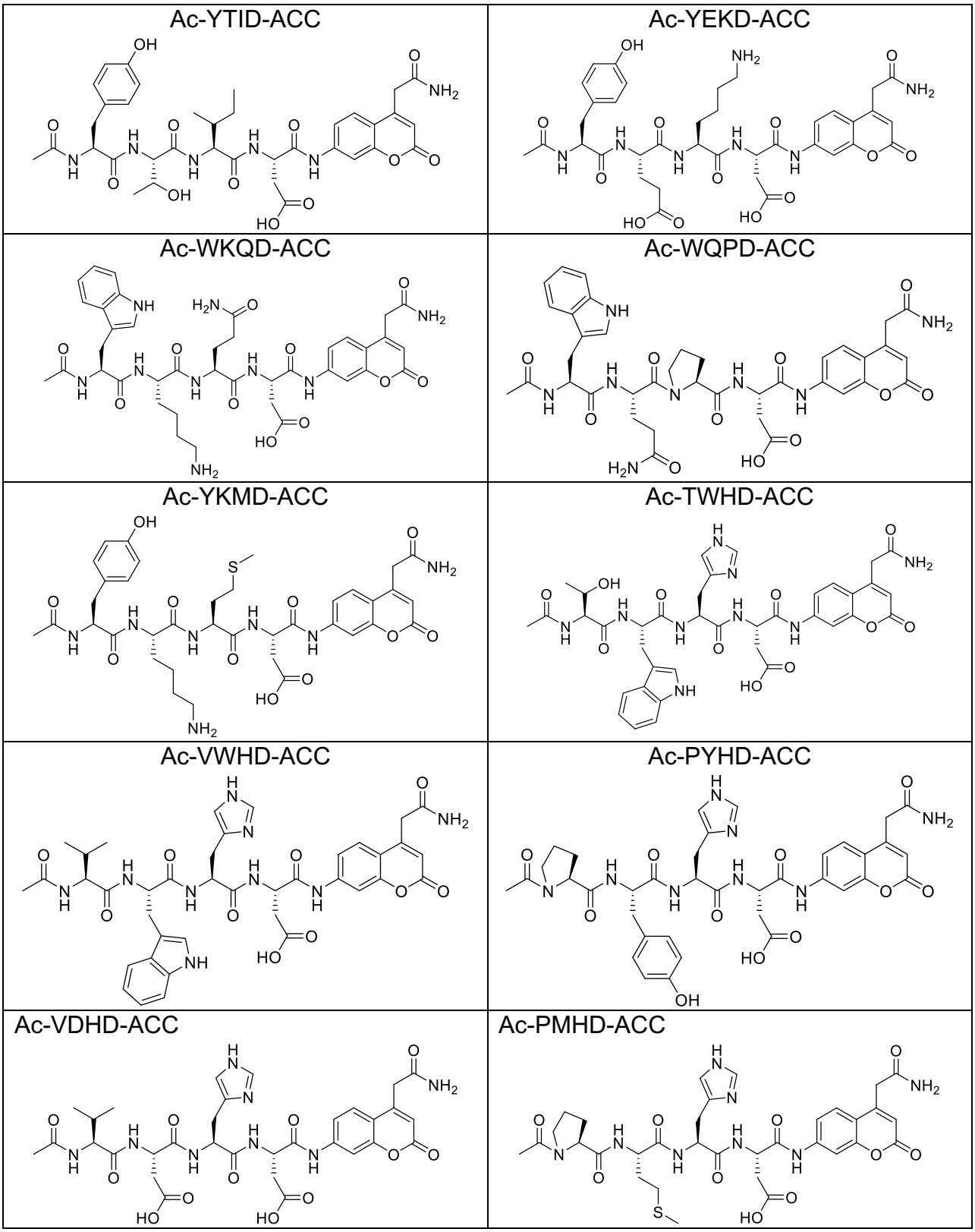
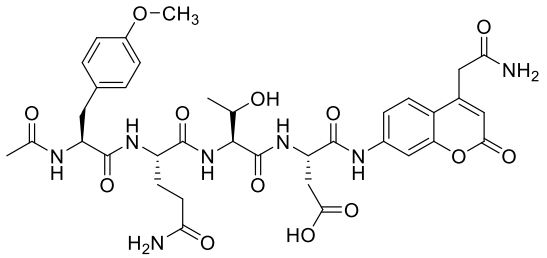
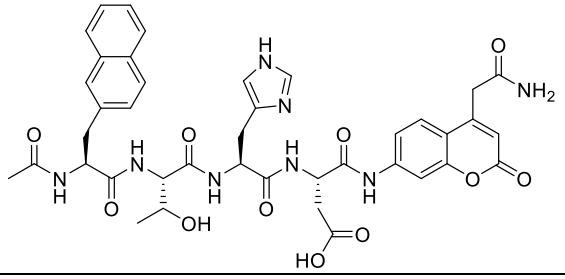
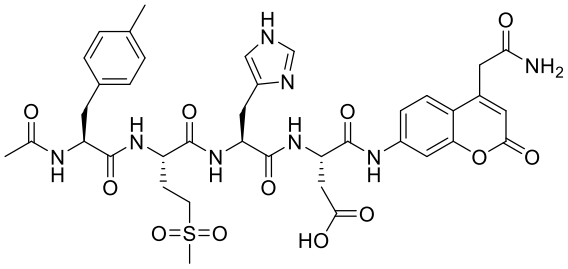
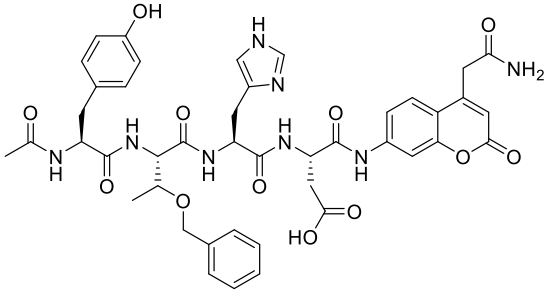
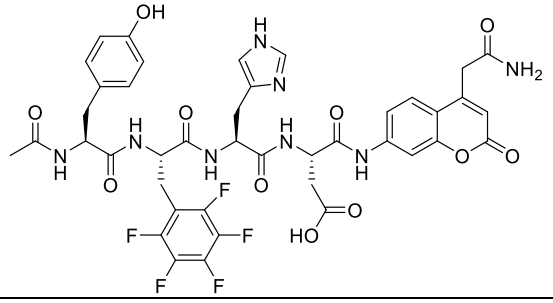
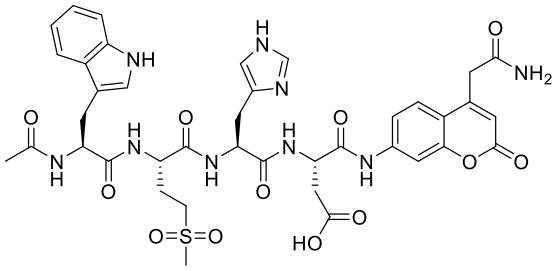
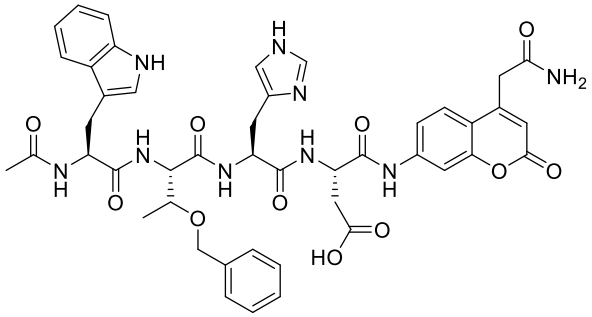
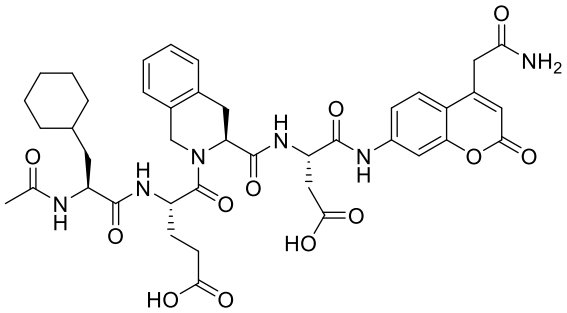
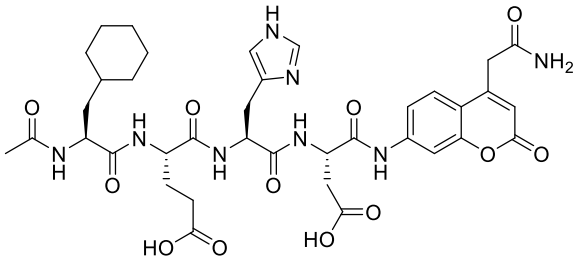
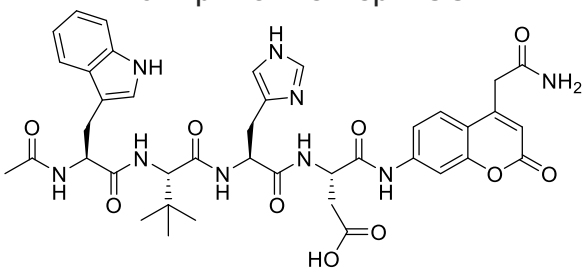
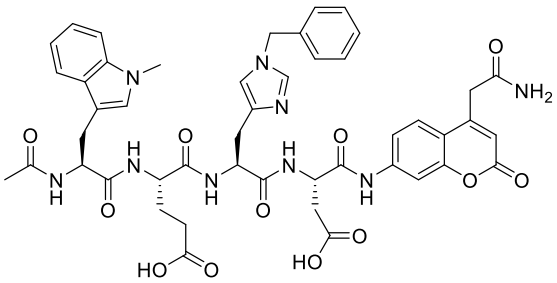
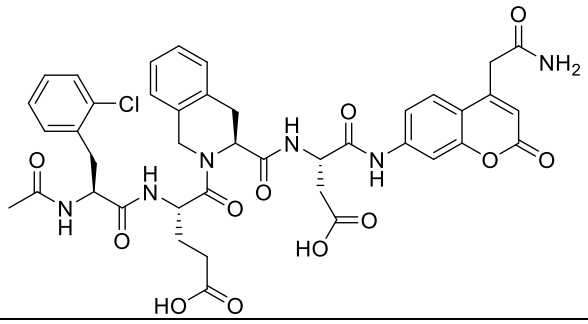


Table S4 Structures of ACC-substrate with natural amino acids.

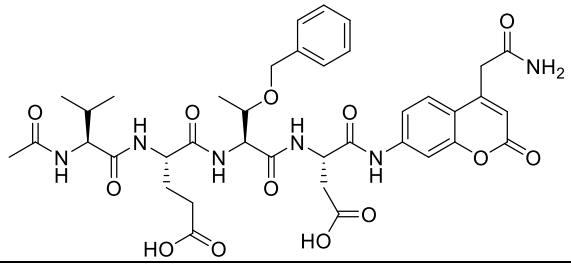
Ac-Tyr(Me)-Gln-Thr-Asp-ACC 	Ac-2Nal-Thr-His-Asp-ACC 
Ac-Phe(Me)-Met(O_2)-His-Asp-ACC 	Ac-Tyr-Thr(Bzl)-His-Asp-ACC 
Ac-Tyr-Phe(F_5)-His-Asp-ACC 	Ac-Trp-Met(O_2)-His-Asp-ACC 
Ac-Trp-Thr(Bzl)-His-Asp-ACC 	Ac-Cha-Glu-Tic-Asp-ACC 
Ac-Cha-Glu-His-Asp-ACC 	Ac-Trp-Tle-His-Asp-ACC 

Ac-Trp(Me)-Glu-His(Bzl)-Asp-ACC	Ac-Phe(2Cl)-Glu-Tic-Asp-ACC

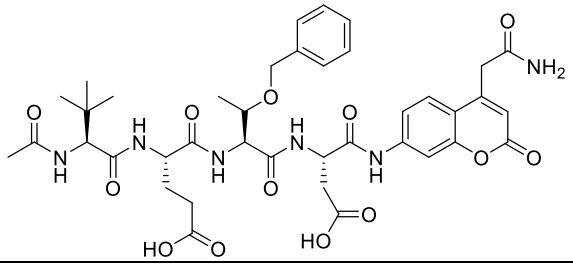




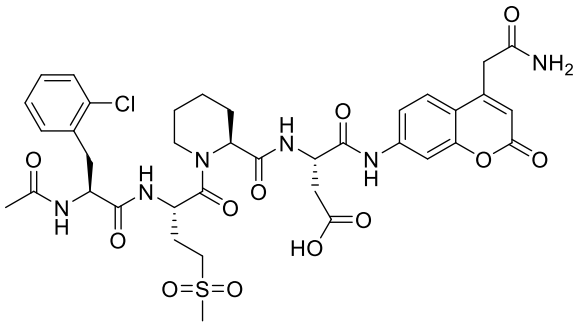
Ac-Val-Glu-Thr(Bzl)-Asp-ACC



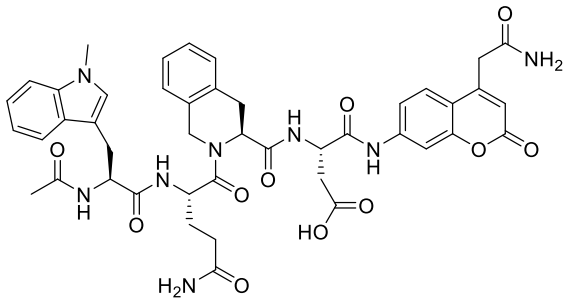
Ac-Tle-Glu-Thr(Bzl)-Asp-ACC



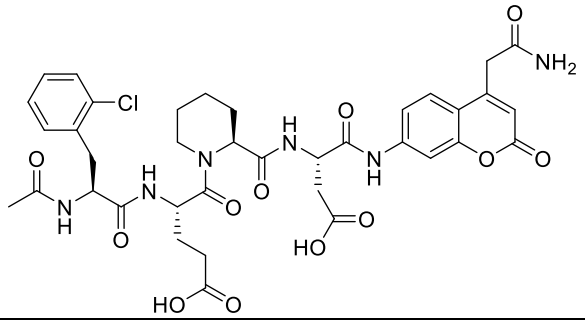
Ac-Phe(2Cl)-Met(O_2)-Pip-Asp-ACC



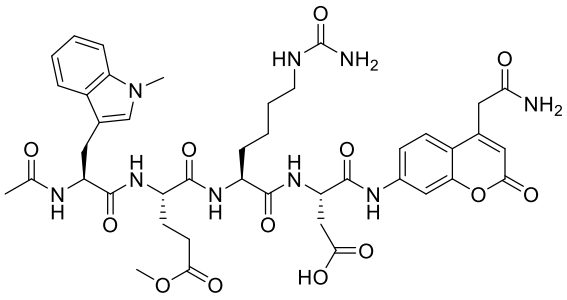
Ac-Trp(Me)-Gln-Tic-Asp-ACC

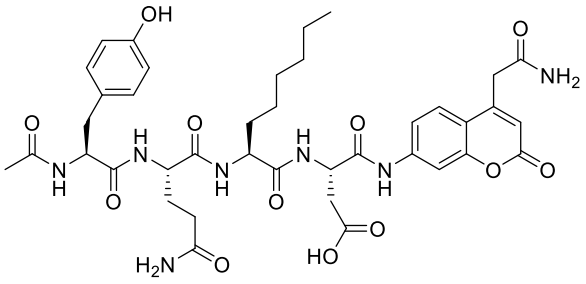
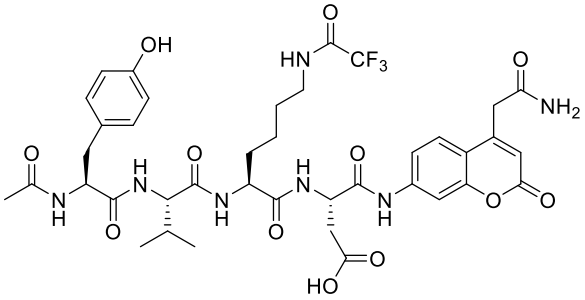
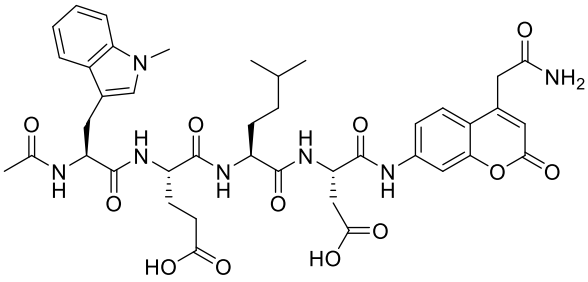
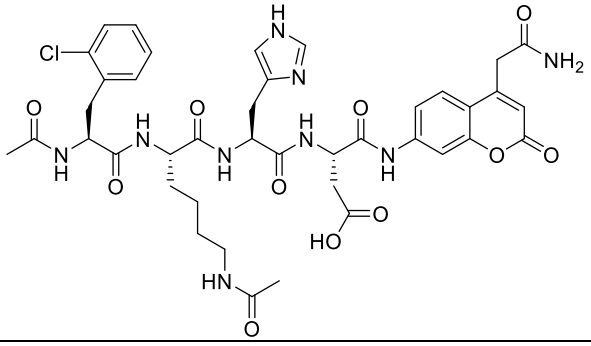
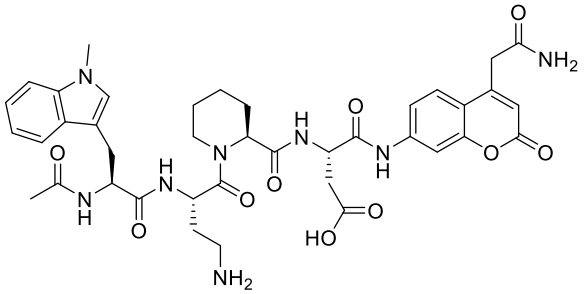
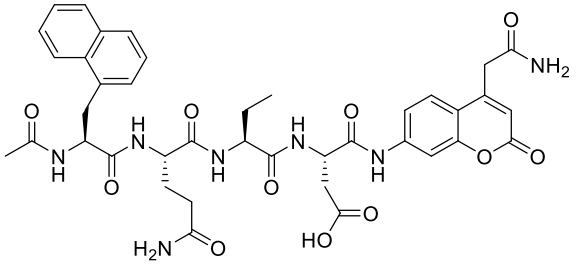
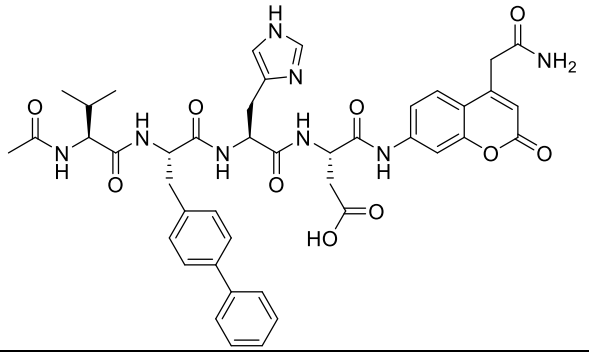
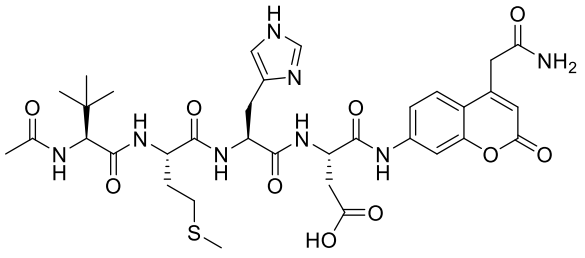


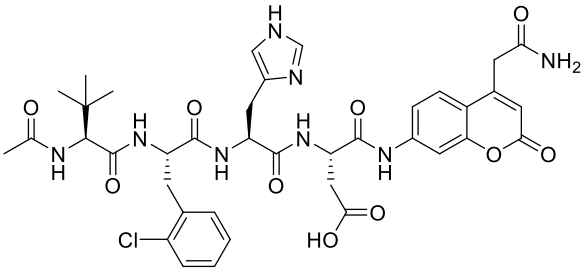
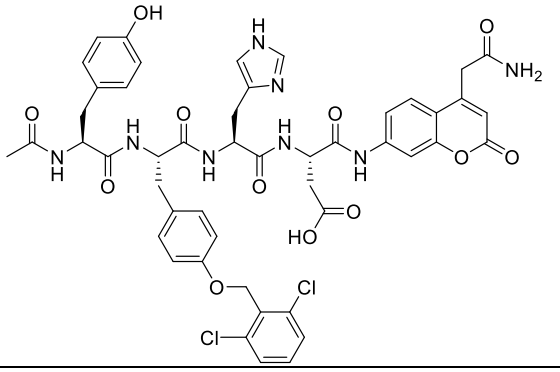
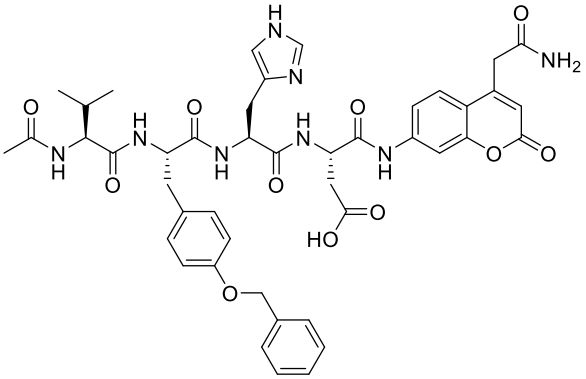
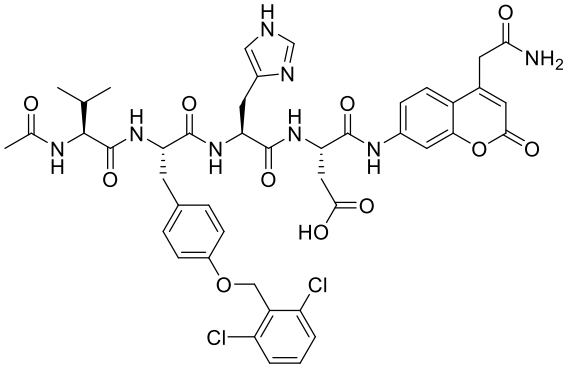
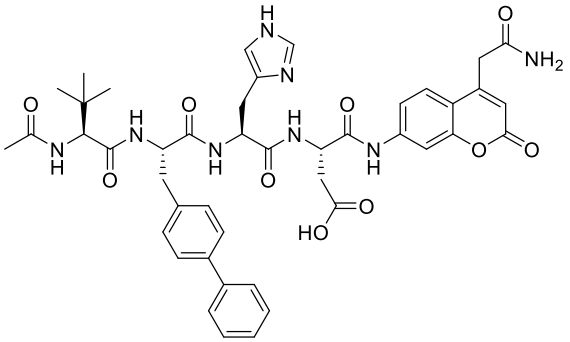
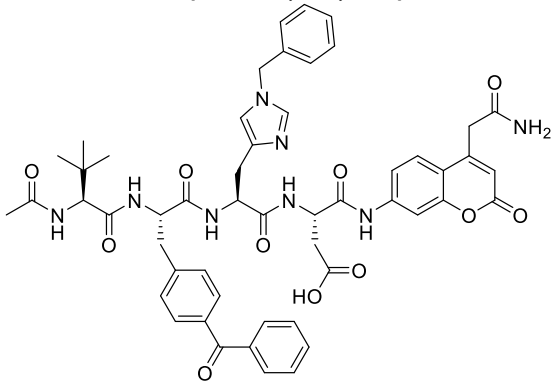
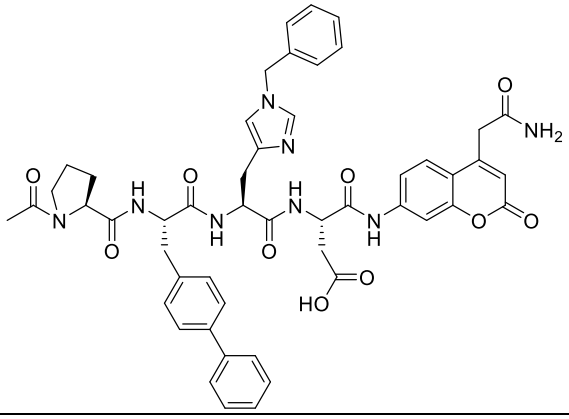
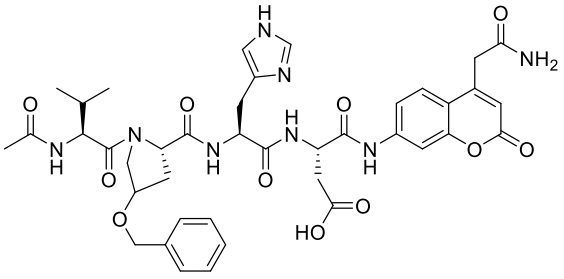
Ac-Phe(2Cl)-Glu-Pip-Asp-ACC



Ac-Trp(Me)-Glu(Me)-hCit-Asp-ACC



Ac-Tyr-Gln-2-Aoc-Asp-ACC	Ac-Tyr-Val-Lys(TFA)-Asp-ACC
	
Ac-Trp(Me)-Glu-hLeu-Asp-ACC	Ac-Phe(2Cl)-Lys(Ac)-His-Asp-ACC
	
Ac-Trp(Me)-Dab-Pip-Asp-ACC	Ac-1Nal-Gln-Abu-Asp-ACC
	
Ac-Val-Bip-His-Asp-ACC	Ac-Tle-Met-His-Asp-ACC
	

Ac-Tle-Phe(2-Cl)-His-Asp-ACC	Ac-Tyr-Tyr(2,6ClBzl)-His-Asp-ACC
	
Ac-Val-Tyr(Bzl)-His-Asp-ACC	Ac-Val-Tyr(2,6ClBzl)-His-Asp-ACC
	
Ac-Tle-Bip-His-Asp-ACC	Ac-Tle-Bpa-His(Bzl)-Asp-ACC
	
Ac-Pro-Bip-His(Bzl)-Asp-ACC	Ac-Val-Hyp(Bzl)-His-Asp-ACC
	

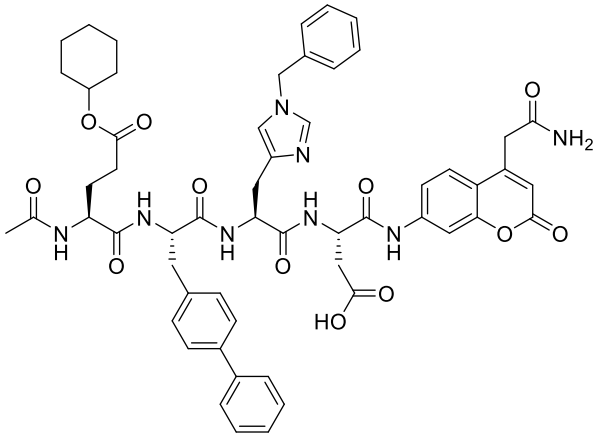
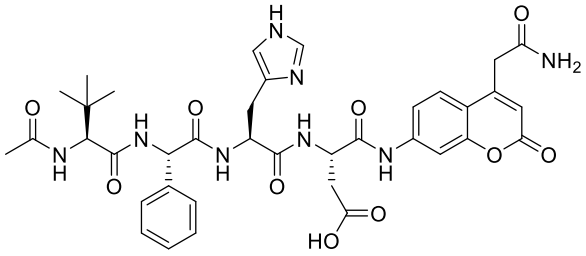
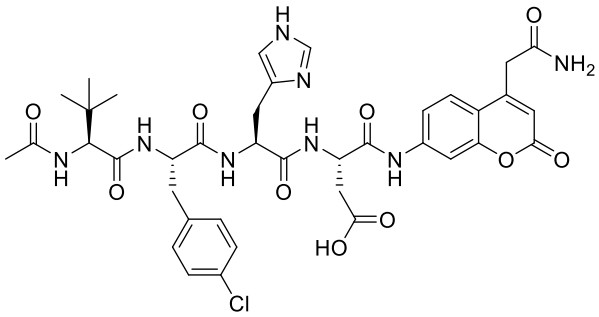
Ac-Glu(Chx)-Bip-His(Bzl)-Asp-ACC 	Ac-Tle-Phg-His-Asp-ACC 
Ac-Tle-Phe(4Cl)-His-Asp-ACC 	

Table S6 Structures of ACC-substrates with natural and unnatural amino acids.