Unnatural amino acids increase activity and specificity of synthetic substrates for human and malarial cathepsin C

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Supplemental data

Table S1 Kinetic parameters (K_m, k_{cat}, k_{cat}/K_m) of two fluorogenic substrates determined in a three different pH conditions. Each experiment was repeated at least three times.

	pH = 5.0	**pH = 5.5**	**pH = 6.0**
NH_2-Abu-Nle(OBzl)-ACC			
K_m, μM	1.62 ± 0.09	1.88 ± 0.11	2.52 ± 0.21
k_{cat}, s^{-1}	11.4 ± 0.32	17.8 ± 0.56	24.3 ± 1.20
k_{cat}/K_m, $s^{-1}M^{-1}$	71.4 ± 3.56	94.5 ± 0.34	96.6 ± 1.23
Pip-Lys-ACC			
K_m, μM	87.1 ± 0.89	77.4 ± 5.35	87.6 ± 2.85
k_{cat}, s^{-1}	5.65 ± 0.21	7.45 ± 0.41	9.27 ± 0.22
k_{cat}/K_m, $s^{-1}M^{-1}$	0.62 ± 0.02	0.96 ± 0.07	1.03 ± 0.11

Table S2 Kinetic parameters (K_m, k_{cat}, k_{cat}/K_m) of two fluorogenic substrates determined in a four different DTT concentrations. Each experiment was repeated at least three times.

	[DDT] = 0 mM	**[DDT] = 1 mM**	**[DDT] = 5 mM**	**[DDT] = 10 mM**
NH_2-Abu-Nle(OBzl)-ACC				
K_m, μM	2.47 ± 0.35	2.47 ± 0.18	1.88 ± 0.11	1.94 ± 0.12
k_{cat}, s^{-1}	0.65 ± 0.12	15.5 ± 0.25	17.8 ± 0.56	18.3 ± 0.22
k_{cat}/K_m, $s^{-1}M^{-1}$	26.3 ± 1.95	62.7 ± 3.41	94.5 ± 0.34	94.4 ± 0.69
Pip-Lys-ACC				
K_m, μM	64.5 ± 0.35	92.9 ± 2.56	77.4 ± 5.35	95.8 ± 5.65
k_{cat}, s^{-1}	0.82 ± 0.09	9.27 ± 0.52	7.45 ± 0.41	11.7 ± 0.12
k_{cat}/K_m, $s^{-1}M^{-1}$	0.13 ± 0.02	0.99 ± 0.10	0.96 ± 0.07	1.13 ± 0.01

Table S3 Kinetic parameters (K_m, k_{cat}, k_{cat}/K_m) of two fluorogenic substrates determined in a five different NaCl concentrations. Each experiment was repeated at least three times.

	[NaCl] = 0 mM	**[NaCl] = 5 mM**	**[NaCl] = 30 mM**	**[NaCl] = 100 mM**	**[NaCl] = 500 mM**	**[NaCl] = 1000 mM**
NH_2-Abu-Nle(OBzl)-ACC						
K_m, μM	> 10	3.87 ± 0.23	1.79 ± 0.08	1.88 ± 0.11	2.63 ± 0.21	3.09 ± 0.15
k_{cat}, s^{-1}	< 5	19.3 ± 1.10	17.5 ± 0.33	17.8 ± 0.56	22.3 ± 1.55	21.8 ± 1.25
k_{cat}/K_m, $s^{-1}M^{-1}$	4.51 ± 0.56	49.7 ± 3.92	97.8 ± 6.32	94.5 ± 0.34	84.8 ± 5.64	70.5 ± 6.35
Pip-Lys-ACC						
K_m, μM	102.8 ± 12.3	101.1 ± 5.21	83.1 ± 5.56	77.4 ± 5.35	114.7 ± 9.63	127.8 ± 5.64
k_{cat}, s^{-1}	1.11 ± 0.08	5.03 ± 0.26	7.60 ± 0.68	7.45 ± 0.41	6.27 ± 0.88	5.25 ± 0.22
k_{cat}/K_m, $s^{-1}M^{-1}$	0.11 ± 0.02	0.49 ± 0.06	1.09 ± 0.12	0.96 ± 0.07	0.55 ± 0.05	0.39 ± 0.05

Table S4 Kinetic parameters (K_m, k_{cat}, k_{cat}/K_m) of two fluorogenic substrates determined in two different buffers.

	Human cathepsin C buffer	***Plasmodium falciparum* DPAP1 buffer**
NH_2-Abu-Nle(OBzl)-ACC		
K_m, μM	1.88 ± 0.11	1.86 ± 0.58
k_{cat}, s^{-1}	17.8 ± 0.56	22.1 ± 1.59
k_{cat}/K_m, $s^{-1}M^{-1}$	94.5 ± 0.34	108.5 ± 5.68
Pip-Lys-ACC		
K_m, μM	77.4 ± 5.35	69.4 ± 5.64
k_{cat}, s^{-1}	7.45 ± 0.41	8.47 ± 1.10
k_{cat}/K_m, $s^{-1}M^{-1}$	0.96 ± 0.07	1.08 ± 0.08

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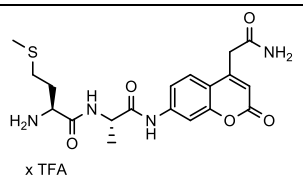
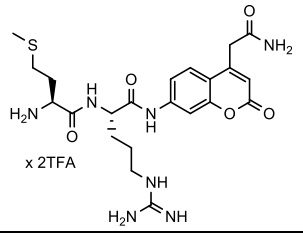
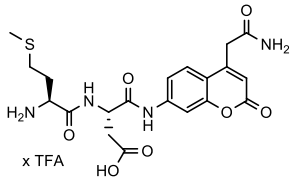
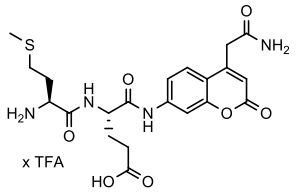
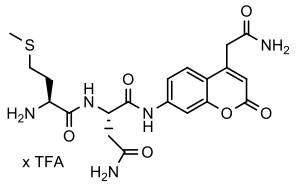
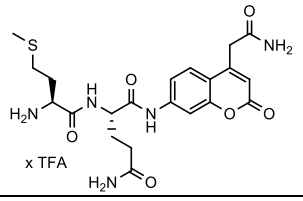
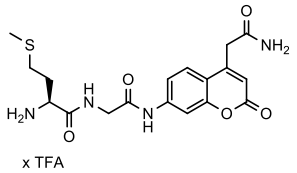
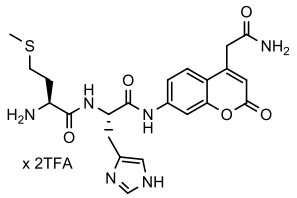
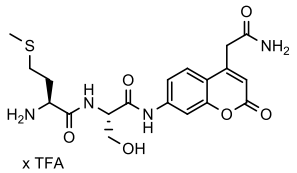
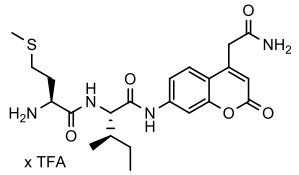
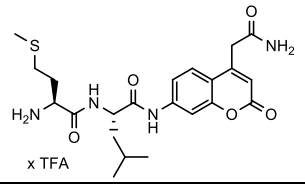
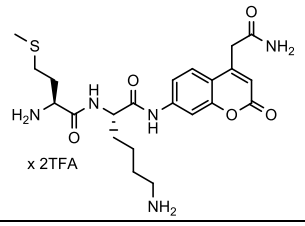
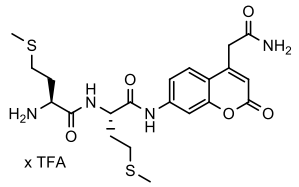
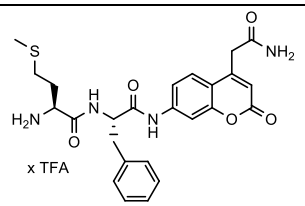
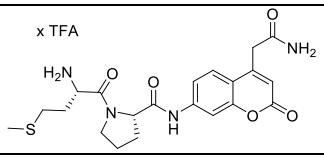
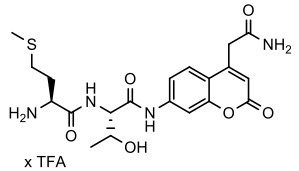
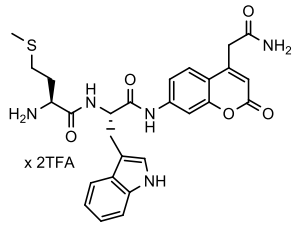
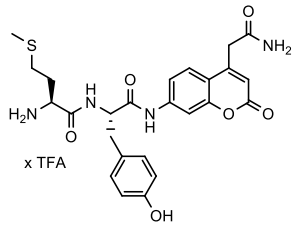
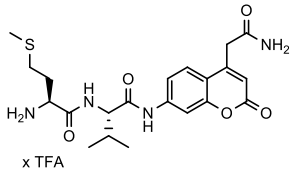
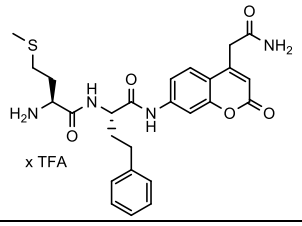
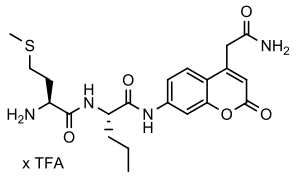
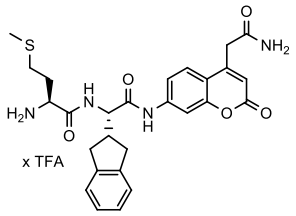
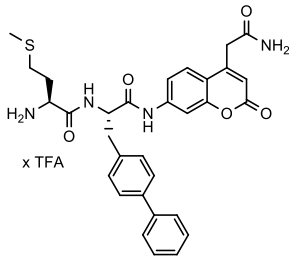
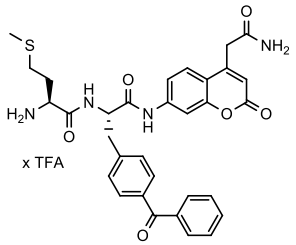
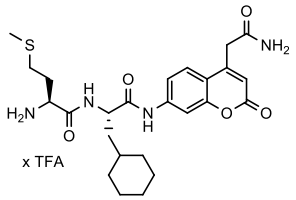
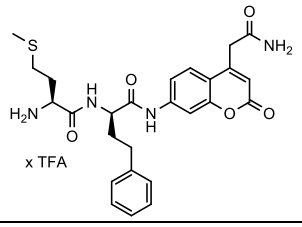
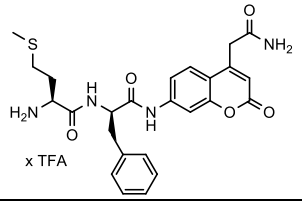
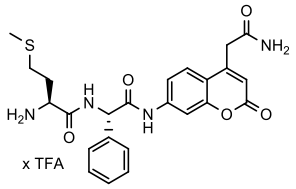
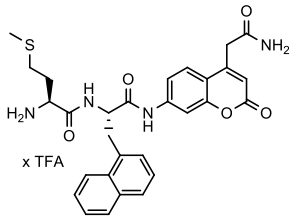
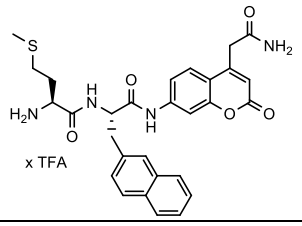
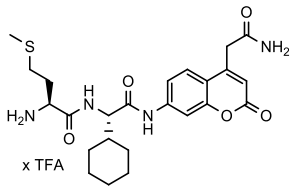
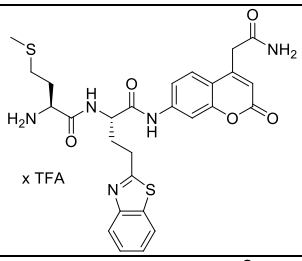
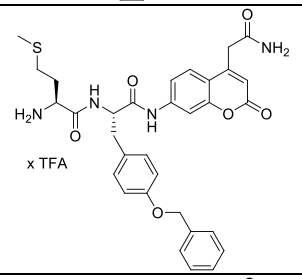
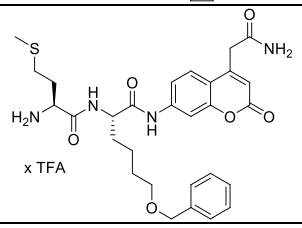
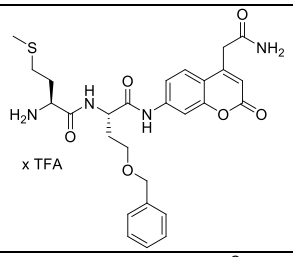
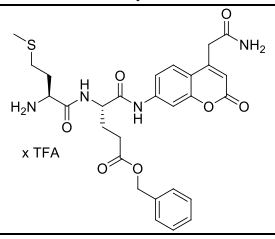
Entry	Structure	Name	M.W.	P1 side chain
1.		**NH_2-*L*-Met-*L*-Ala-ACC**	**534.51**	*L*-alanine
2.		**NH_2-*L*-Met-*L*-Arg-ACC**	**733.64**	*L*-arginine
3.		**NH_2-*L*-Met-*L*-Asp-ACC**	**578.52**	*L*-aspartic acid
4.		**NH_2-*L*-Met-*L*-Glu-ACC**	**592.54**	*L*- glutamic acid
5.		**NH_2-*L*-Met-*L*-Asn-ACC**	**577.53**	*L*-asparagine
6.		**NH_2-*L*-Met-*L*-Gln-ACC**	**591.56**	*L*-glutamine
7.		**NH_2-*L*-Met-Gly-ACC**	**520.48**	glycine
8.		**NH_2-*L*-Met-*L*-His-ACC**	**714.59**	*L*-histidine
9.		**NH_2-*L*-Met-*L*-Ser-ACC**	**550.51**	*L*-serine

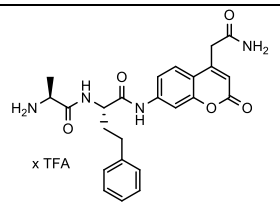
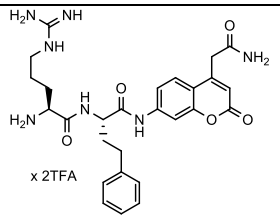
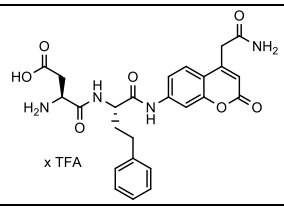
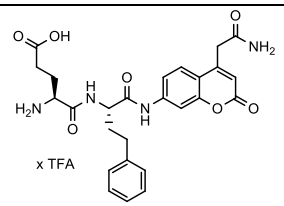
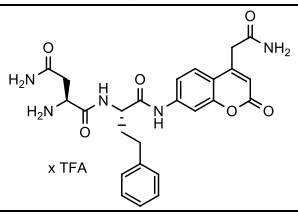
10.		**NH_2-*L*-Met-*L*-Ile-ACC**	**576.59**	*L*-isoleucine
11.		**NH_2-*L*-Met-*L*-Leu-ACC**	**576.59**	*L*-leucine
12.		**NH_2-*L*-Met-*L*-Lys-ACC**	**705.62**	*L*-lysine
13.		**NH_2-*L*-Met-*L*-Met-ACC**	**594.62**	*L*-methionine
14.		**NH_2-*L*-Met-*L*-Phe-ACC**	**610.60**	*L*-phenylalanine
15.		**NH_2-*L*-Met-*L*-Pro-ACC**	**560.54**	*L*-proline
16.		**NH_2-*L*-Met-*L*-Thr-ACC**	**564.53**	*L*-threonine
17.		**NH_2-*L*-Met-*L*-Trp-ACC**	**763.66**	*L*-tryptophan
18.		**NH_2-*L*-Met-*L*-Tyr-ACC**	**626.60**	*L*-tyrosine

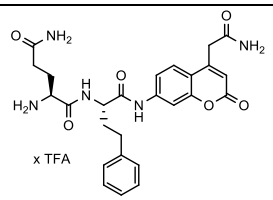
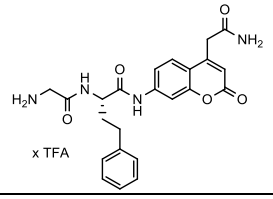
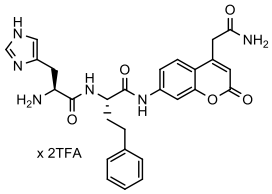
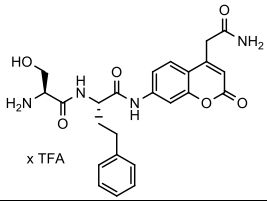
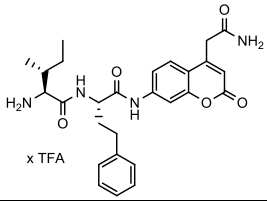
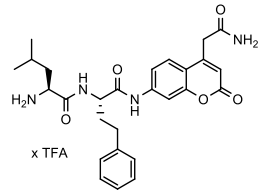
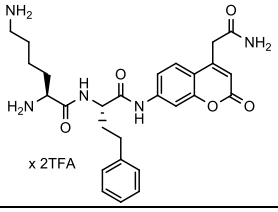
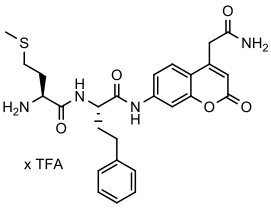
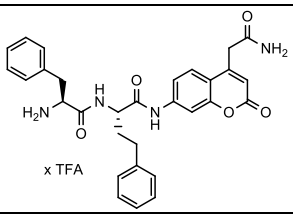
19.		**NH_2-*L*-Met-*L*-Val-ACC**	**562.56**	*L*-valine
20.		**NH_2-*L*-Met-*L*-hPhe-ACC**	**624.63**	*L*-homophenylalanine
21.		**NH_2-*L*-Met-*L*-Nva-ACC**	**562.56**	*L*-norvaline
22.		**NH_2-*L*-Met-*L*-Igl-ACC**	**636.64**	*L*-indanylglycine
23.		**NH_2-*L*-Met-*L*-Bip-ACC**	**686.70**	*L*-biphenylalanine
24.		**NH_2-*L*-Met-*L*-Bpa-ACC**	**714.71**	*L*-(4-benzoyl)-phenylalanine
25.		**NH_2-*L*-Met-*L*-Cha-ACC**	**616.65**	*L*-cyclohexylalanine
26.		**NH_2-*L*-Met-*D*-hPhe-ACC**	**624.63**	*D*-homophenylalanine

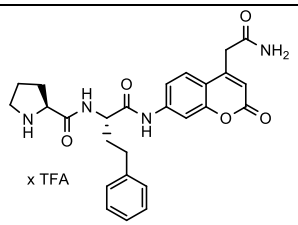
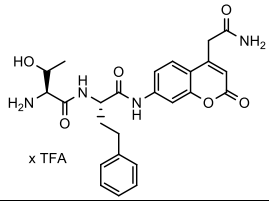
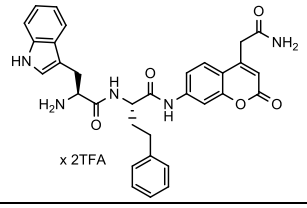
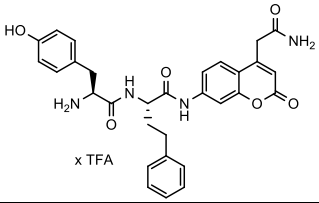
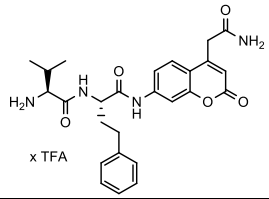
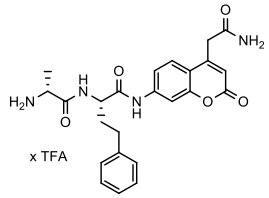
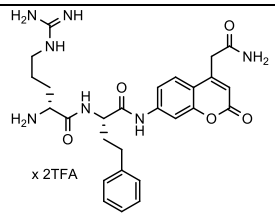
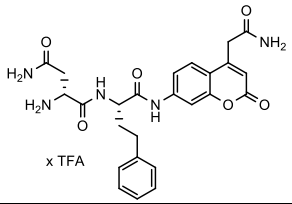
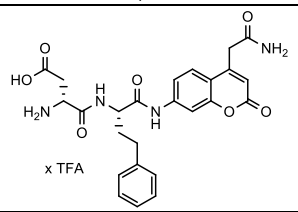
27.		**NH_2-*L*-Met-*D*-Phe-ACC**	**610.6**	*D*-phenylalanine
28.		**NH_2-*L*-Met-*L*-Phg-ACC**	**596.58**	*L*-phenylglycine
29.		**NH_2-*L*-Met-*L*-1-Nal-ACC**	**660.66**	*L*-(1-naphthyl)-alanine
30.		**NH_2-*L*-Met-*L*-2-Nal-ACC**	**660.66**	*L*-(2-naphthyl)-alanine
31.		**NH_2-*L*-Met-*L*-Chg-ACC**	**602.62**	*L*-cyclohexylglycine
32.		**NH_2-*L*-Met-*L*-Abu(Bht)-ACC**	**681.70**	*L*-(4-benzothiazol-2-yl)homoalanine
33.		**NH_2-*L*-Met-*L*-Tyr(Bzl)-ACC**	**716.72**	*L*-tyrosine *O*-benzyl ester
34.		**NH_2-*L*-Met-*L*-Nle(6-*O*-Bzl)-ACC**	**682.71**	*L*-(6-benzyloxy)-norleucine

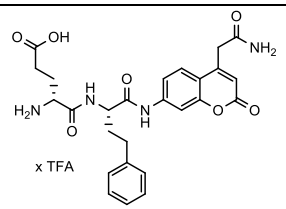
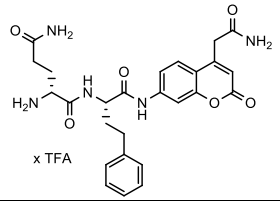
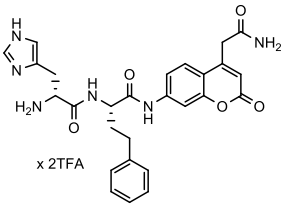
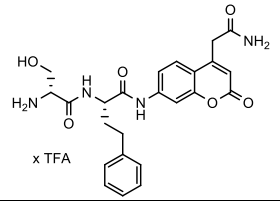
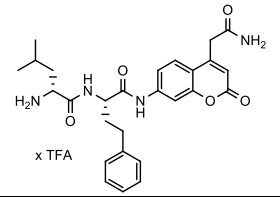
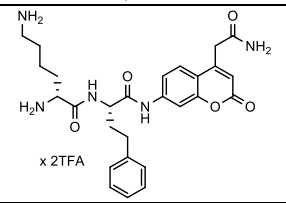
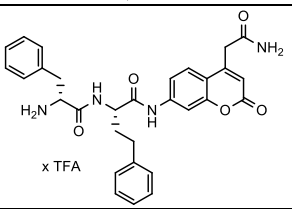
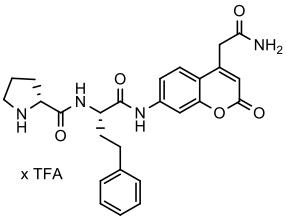
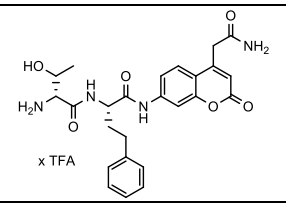
35.		**NH_2-*L*-Met-*L*-Hse(Bzl)-ACC**	**654.65**	*L*-homoserine-*O*-benzyl ester
36.		**NH_2-*L*-Met-*L*-Glu(Bzl)-ACC**	**682.66**	*L*-glutamic acid *O*-benzyl ester

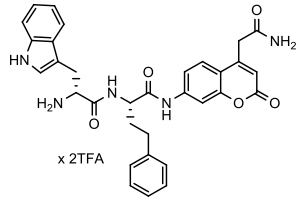
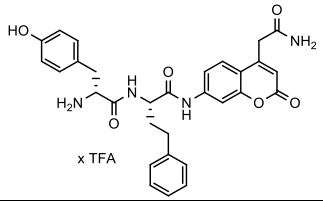
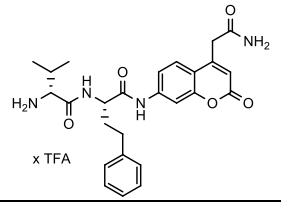
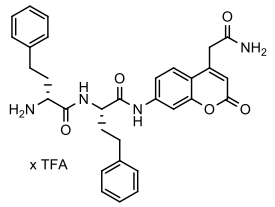
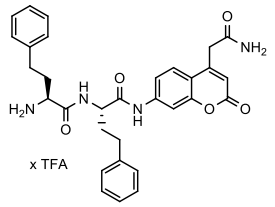
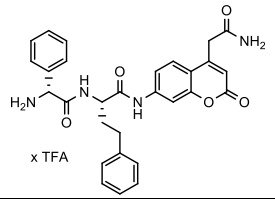
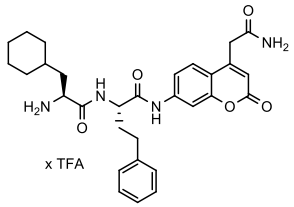
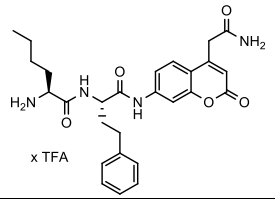
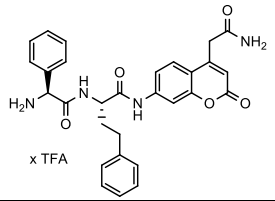
P2 LIBRARY

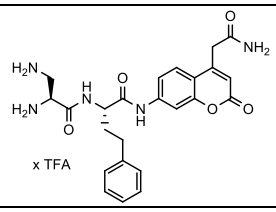
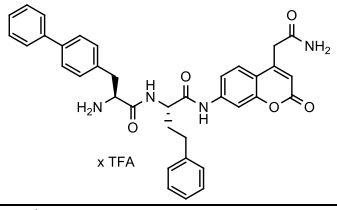
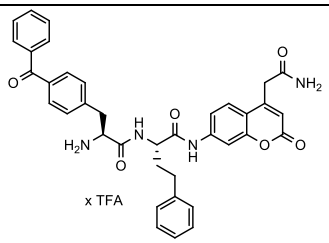
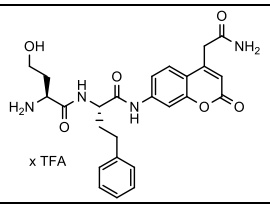
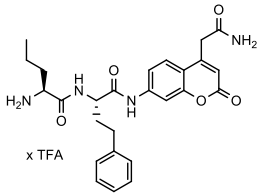
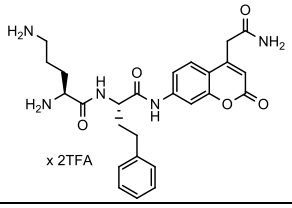
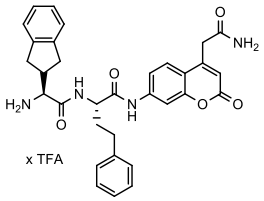
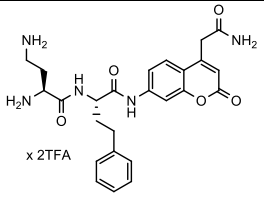
Entry	Structure	Name	M.W.	P2 side chain
1.		**NH_2-*L*-Ala-*L*-hPhe-ACC**	**564.51**	*L*-alanine
2.		**NH_2-*L*-Arg-*L*-hPhe-ACC**	**763.64**	*L*-arginine
3.		**NH_2-*L*-Asp-*L*-hPhe-ACC**	**608.52**	*L*-aspartic acid
4.		**NH_2-*L*-Glu-*L*-hPhe-ACC**	**622.55**	*L*-glutamic acid
5.		**NH_2-*L*-Asn-*L*-hPhe-ACC**	**607.54**	*L*-asparagine

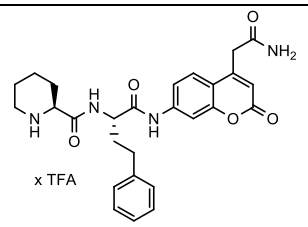
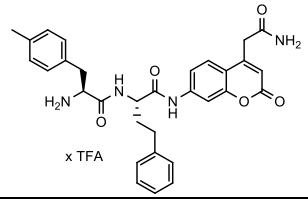
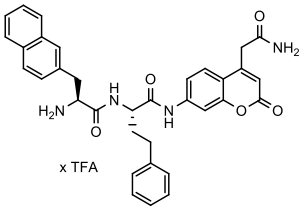
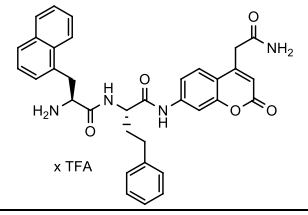
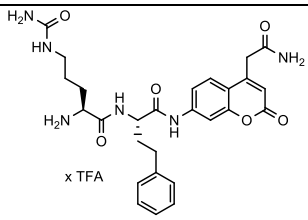
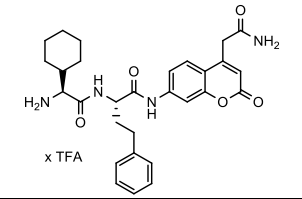
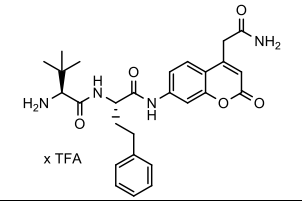
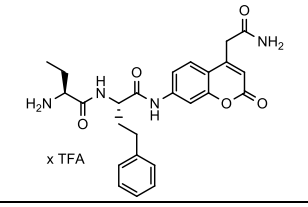
6.		**NH_2-*L*-Gln-*L*-hPhe-ACC**	**621.56**	*L*-glutamine
7.		**NH_2-Gly-*L*-hPhe-ACC**	**550.48**	glycine
8.		**NH_2-*L*-His-*L*-hPhe-ACC**	**744.60**	*L*-histidine
9.		**NH_2-*L*-Ser-*L*-hPhe-ACC**	**580.51**	*L*-serine
10.		**NH_2-*L*-Ile-*L*-hPhe-ACC**	**606.59**	*L*-isoleucine
11.		**NH_2-*L*-Leu-*L*-hPhe-ACC**	**606.59**	*L*-leucine
12.		**NH_2-*L*-Lys-*L*-hPhe-ACC**	**735.63**	*L*-lysine
13.		**NH_2-*L*-Met-*L*-hPhe-ACC**	**624.63**	*L*-methionine
14.		**NH_2-*L*-Phe-*L*-hPhe-ACC**	**640.61**	*L*-phenylalanine

15.		***L*-Pro-*L*-hPhe-ACC**	**590.55**	*L*-proline
16.		**NH_2-*L*-Thr-*L*-hPhe-ACC**	**594.54**	*L*-threonine
17.		**NH_2-*L*-Trp-*L*-hPhe-ACC**	**793.67**	*L*-tryptophan
18.		**NH_2-*L*-Tyr-*L*-hPhe-ACC**	**656.61**	*L*-tyrosine
19.		**NH_2-*L*-Val-*L*-hPhe-ACC**	**592.56**	*L*-valine
20.		**NH_2-*D*-Ala-*L*-hPhe-ACC**	**564.51**	*D*-alanine
21.		**NH_2-*D*-Arg-*L*-hPhe-ACC**	**763.64**	*D*-arginine
22.		**NH_2-*D*-Asn-*L*-hPhe-ACC**	**607.54**	*D*-asparagine
23.		**NH_2-*D*-Asp-*L*-hPhe-ACC**	**608.52**	*D*-aspartic acid

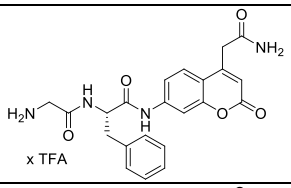
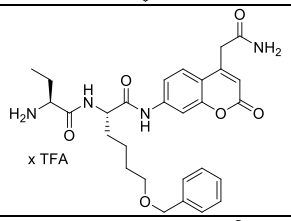
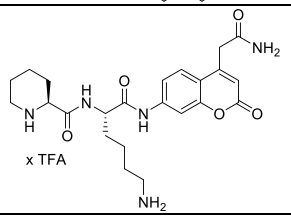
24.		**NH_2-*D*-Glu-*L*-hPhe-ACC**	**622.55**	*D*-glutamic acid
25.		**NH_2-*D*-Gln-*L*-hPhe-ACC**	**621.56**	*D*-glutamine
26.		**NH_2-*D*-His-*L*-hPhe-ACC**	**744.60**	*D*-histidine
27.		**NH_2-*D*-Ser-*L*-hPhe-ACC**	**580.51**	*D*-serine
28.		**NH_2-*D*-Leu-*L*-hPhe-ACC**	**606.59**	*D*-leucine
29.		**NH_2-*D*-Lys-*L*-hPhe-ACC**	**735.63**	*D*-lysine
30.		**NH_2-*D*-Phe-*L*-hPhe-ACC**	**640.61**	*D*-phenylalanine
31.		***D*-Pro-*L*-hPhe-ACC**	**590.55**	*D*-proline
32.		**NH_2-*D*-Thr-*L*-hPhe-ACC**	**594.54**	*D*-threonine

33.		**NH_2-*D*-Trp-*L*-hPhe-ACC**	**793.67**	*D*-tryptophan
34.		**NH_2-*D*-Tyr-*L*-hPhe-ACC**	**656.61**	*D*-tyrosine
35.		**NH_2-*D*-Val-*L*-hPhe-ACC**	**592.56**	*D*-valine
36.		**NH_2-*D*-hPhe-*L*-hPhe-ACC**	**654.63**	*D*-homophenylalanine
37.		**NH_2-*L*-hPhe-*L*-hPhe-ACC**	**654.63**	*L*-homophenylalanine
38.		**NH_2-*D*-Phg-*L*-hPhe-ACC**	**626.58**	*D*-phenylglycine
39.		**NH_2-*L*-Cha-*L*-hPhe-ACC**	**646.65**	*L*-cyclohexylalanine
40.		**NH_2-*L*-Nva-*L*-hPhe-ACC**	**606.59**	*L*-norleucine
41.		**NH_2-*L*-Phg-*L*-hPhe-ACC**	**626.58**	*L*-phenylglycine

42.		**NH_2-*L*-Dap-*L*-hPhe-ACC**	**693.55**	*L*-aminoalanine
43.		**NH_2-*L*-Bip-*L*-hPhe-ACC**	**716.70**	*L*-biphenylalanine
44.		**NH_2-*L*-Bpa-*L*-hPhe-ACC**	**744.71**	*L*-(4-benzoyl)-phenylalanine
45.		**NH_2-*L*-Hse-*L*-hPhe-ACC**	**594.54**	*L*-homoserine
46.		**NH_2-*L*-Nva-*L*-hPhe-ACC**	**592.56**	*L*-norvaline
47.		**NH_2-*L*-Orn-*L*-hPhe-ACC**	**721.60**	*L*-ornithine
48.		**NH_2-*L*-Igl-*L*-hPhe-ACC**	**666.64**	*L*-indanylglycine
49.		**NH_2-*L*-Dab-*L*-hPhe-ACC**	**707.57**	*L*-aminohomoalanine

50.		***L*-Pip-*L*-hPhe-ACC**	**640.57**	*L*-piperidine
51.		**NH_2-*L*-4-CH_3-Phe-*L*-hPhe-ACC**	**654.63**	*L*-(4-methyl)-phenylalanine
52.		**NH_2-*L*-2-Nal-*L*-hPhe-ACC**	**690.67**	*L*-(2-naphthyl)-alanine
53.		**NH_2-*L*-1-Nal-*L*-hPhe-ACC**	**690.67**	*L*-(1-naphthyl)-alanine
54.		**NH_2-*L*-Cit-*L*-hPhe-ACC**	**650.60**	*L*-citrulline
55.		**NH_2-*L*-Chg-*L*-hPhe-ACC**	**632.63**	*L*-cyclohexylglycine
56.		**NH_2-*L*-Tle-*L*-hPhe-ACC**	**606.59**	*L*-*tert*-leucine
57.		**NH_2-*L*-Abu-*L*-hPhe-ACC**	**578.54**	*L*-homoalanine

Additional fluorogenic substrates

Entry	Structure	Name	M.W.	P2/P1 side chains
1.		**NH_2-Gly-*L*-Phe-ACC**	**536.46**	P1: *L*-phenylalanine P2: glycine
2.		**NH_2-*L*-Abu-*L*-Nle-(*O*-Bzl)-ACC**	**636.62**	P1: *L*-(6-benzyloxy)-norleucine P2: *L*-homoalanine
3.		***L*-Pip-*L*-Lys-ACC**	**571.55**	P1: *L*-lysine P2: *L*-piperidine