

Arrangement of ncAA library for Figure 1, S1, and S2*

	1	2	3	4	5	6	7	8	9	10	11	12	
H	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H
G	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G
F	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F
E	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E
D	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B
A	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A
	1	2	3	4	5	6	7	8	9	10	11	12	

*The wells labeled in red color were set as control experiments with water. Codes in each well represent the different ncAAs in the library (see below).

GFP fluorescence measurements for the data shown in Figure 1 (AcKRS3)*

	1	2	3	4	5	6	7	8	9	10	11	12	
H	0	0	0	0	0	0	0	0	2 (0.1)	0	0	0	H
G	0	0	0	0	0	0	0	0	0	0	0	0	G
F	0	0	0	0	0	0	0	0	0	0	0	0	F
E	0	0	0	0	0	3 (0.5)	0	0	0	0	0	0	E
D	0	0	0	0	0	0	0	0	1 (0.1)	0	0	0	D
C	0	0	0	0	0	0	0	0	0	0	0	0	C
B	0	0	0	0	19 (2.0)	0	0	0	0	0	0	3 (0.2)	B
A	100 (6.0)	3(0.3)	0	0	0	0	0	0	0	0	0	0	A
	1	2	3	4	5	6	7	8	9	10	11	12	

*The wells labeled in red color were set as control experiments with water. Codes in each well represent the different ncAAs in the library (see below). Standard deviations for each above background measurement are given in parentheses.

GFP fluorescence measurements for the data shown in Figure S1 (AcKRS1)*

	1	2	3	4	5	6	7	8	9	10	11	12	
H	0	0	0	0	0	0	0	0	4 (0.2)	0	0	0	H
G	0	0	0	0	0	0	0	0	0	0	0	0	G
F	0	0	0	0	0	0	0	0	0	0	0	0	F
E	0	0	0	0	0	5 (0.1)	0	0	0	0	0	0	E
D	0	0	0	0	0	0	0	0	1 (0.1)	5 (0.4)	0	0	D
C	0	0	0	0	0	0	0	0	0	0	0	0	C
B	0	0	0	0	21 (2.1)	0	0	0	0	0	0	0	B
A	100 (5.0)	6 (0.3)	0	0	0	0	0	0	0	0	0	0	A
	1	2	3	4	5	6	7	8	9	10	11	12	

*The wells labeled in red color were set as control experiments with water. Codes in each well represent the different ncAAs in the library (see below). Standard deviations for each above background measurement are given in parentheses.

GFP fluorescence measurements for the data shown in Figure S2 (IFRS)*

	1	2	3	4	5	6	7	8	9	10	11	12	
H	0	1 (0.2)	12 (1.0)	0	0	0	0	0	0	0	0	0	H
G	0	0	8 (0.6)	1 (0.2)	0	0	1 (0.2)	0	0	0	0	0	G
F	0	0	1 (0.1)	0	0	9 (0.5)	0	0	0	0	46 (3.2)	0	F
E	0	0	0	0	0	68 (5.2)	2 (0.4)	0	0	0	0	0	E
D	0	9 (1.0)	1 (0.1)	0	0	0	0	0	7 (0.9)	0	0	0	D
C	0	0	0	0	0	0	0	0	0	0	0	0	C
B	0	0	0	0	48 (0.9)	0	0	0	0	0	0	0	B
A	100 (5.9)	53 (2.3)	0	0	0	0	0	30 (0.5)	0	0	0	0	A
	1	2	3	4	5	6	7	8	9	10	11	12	

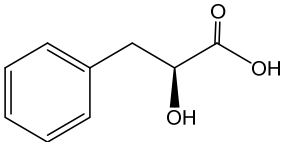
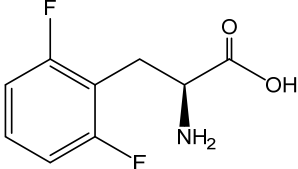
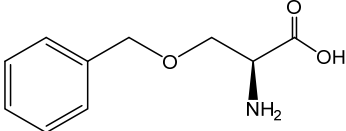
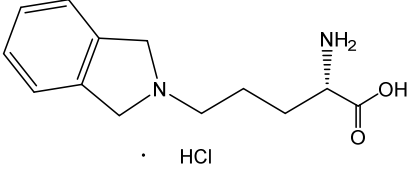
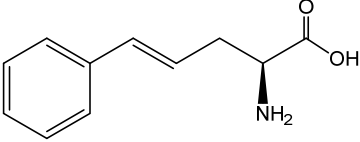
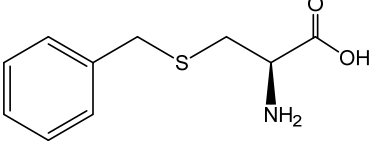
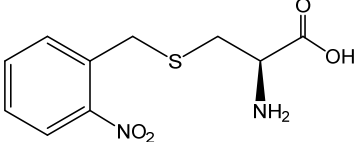
*The wells labeled in red color were set as control experiments with water. Codes in each well represent the different ncAAs in the library (see below). Standard deviations for each above background measurement are given in parentheses.

List of ncAAs used in this study for Figure 2, S1, and S2

Position	Name	NAA Name	IUPAC Name	CAS Number	Chemical Structure
A1	Control	-	-	-	-
B1	Pyl	<i>N</i> ^ε -(pyrrolyl)-L-lysine	<i>N</i> 6-[[<i>(2R,3R)</i> -3-methyl-3,4-dihydro-2H-pyrrol-2-yl]carbonyl]-L-lysine	448235-52-7	
C1	mK	<i>N</i> ^ε -Methyl-L-lysine hydrochloride	(<i>S</i>)-2-Amino-6-(methylamino)hexanoic acid hydrochloride	7622-29-9	
D1	2mK	<i>N</i> ^ε , <i>N</i> ^ε -Dimethyl-L-lysine hydrochloride	(<i>S</i>)-2-Amino-6-(dimethylamino)hexanoic acid hydrochloride	2259-86-1	
E1	3mK	<i>N</i> ^ε , <i>N</i> ^ε , <i>N</i> ^ε -Trimethyl-L-lysine chloride	(<i>S</i>)-2-Amino-6-(trimethylamino)hexanoic acid chloride	55528-53-5	
F1	CbzK	<i>N</i> ^ε -Carbobenzoxy-L-lysine	(<i>S</i>)-2-Amino-6-(phenylmethoxycarbonylamino)hexanoic acid	1155-64-2	
G1	NicoK	<i>N</i> ^ε -Nicotinyll-L-lysine	(<i>S</i>)-2-Amino-6-(nicotinylamino)hexanoic acid	158276-23-4	

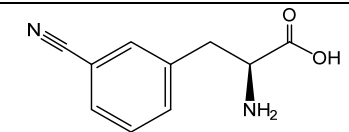
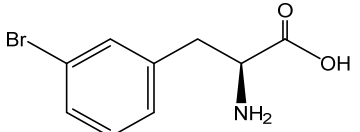
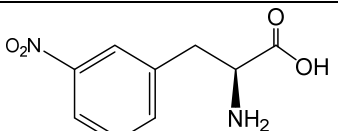
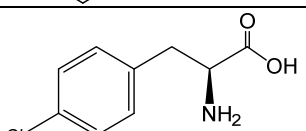
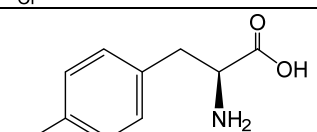
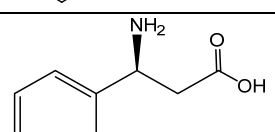
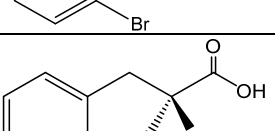
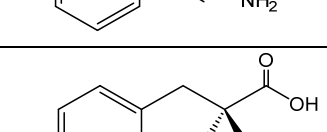
H1	AlocK	<i>N</i> ^ε - Allyloxycarbonyl - L-lysine	(<i>S</i>)-2-Amino-6-(allyloxycarbonyl amino)hexanoic acid	6298-03-9	
A2	3CF3F	3-Trifluoromethyl- L-phenylalanine	(<i>S</i>)-2-amino-3-(3-trifluoromethylphenyl)propanoic acid	14464-68-7	
B2	2BrF	L-2-Bromo phenylalanine	(<i>S</i>)-2-amino-3-(2-bromophenyl)propanoic acid	42538-40-9	
C2	2IF	L-2-Iodo phenylalanine	(<i>S</i>)-2-amino-3-(2-iodophenyl)propanoic acid	167817-55-2	
D2	2MeF	L-2-Methyl phenylalanine	(<i>S</i>)-2-amino-3-(2-methylphenyl)propanoic acid	80126-53-0	
E2	2CF3F	L-2-Trifluoromethyl phenylalanine	(<i>S</i>)-2-amino-3-(2-trifluoromethylphenyl)propanoic acid	119009-47-1	
F2	Cit	L-Citrulline	(<i>S</i>)-2-amino-5-ureidopentanoic acid	372-75-8	
G2	Orn	L-Ornithine	(<i>S</i>)-2,5-diaminopentanoic acid	70-26-8	

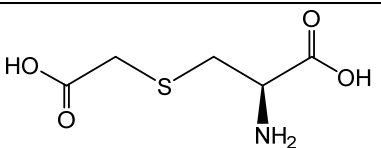
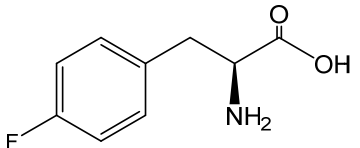
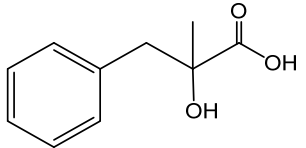
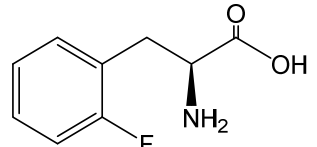
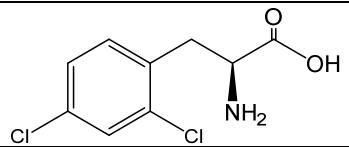
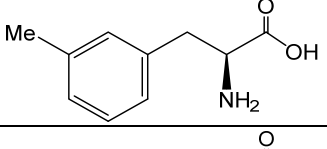
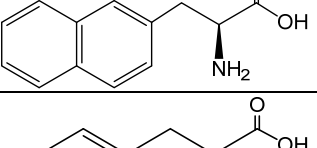
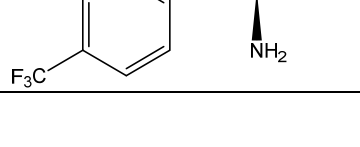
H2	2CBF	L-2-Carbamoyl phenylalanine	(S)-2-amino-3-(2-carbamoyl phenyl)propanoic acid	-	
A3	AcmC	S-(acetamidomethyl)-L-cysteine	3-(acetamidomethylsulfanyl)-2-aminopropanoic acid	19647-70-2	
B3	34MeF	L-3,4-Dimethoxyl phenylalanine	(S)-2-amino-3-(3,4-dimethoxyphenyl)propanoic acid	32161-30-1	
C3	24NiF	L-2,4-Dinitro phenylalanine	(S)-2-amino-3-(2,4-dinitrophenyl) propanoic acid	49607-21-8	
D3	ZY	O-Carbobenzoxy-L-tyrosine	(S)-2-amino-3-(4-(((benzyloxy)carbonyl)oxy)phenyl)propanoic acid	21106-04-7	
E3	35BrY	L-3,5-Dibromo tyrosine	(S)-2-amino-3-(3,5-dibromo-4-hydroxyphenyl)propanoic acid	300-38-9	
F3	26ClBzY	O-2,6-Dichlorobenzyl-L-tyrosine	(S)-2-amino-3-(4-((2,6-dichlorobenzyl)oxy)phenyl)propanoic acid	40298-69-9	

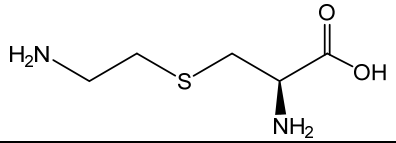
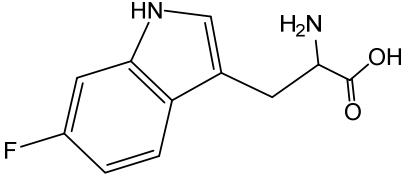
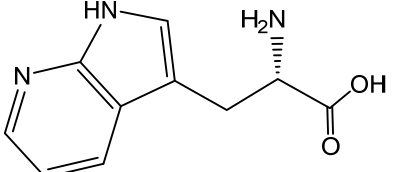
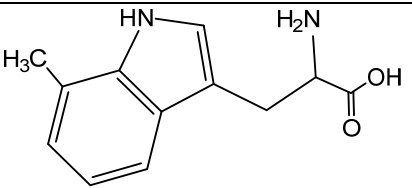
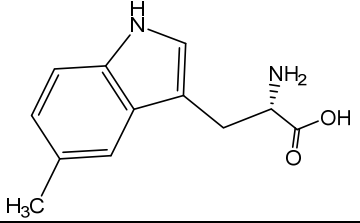
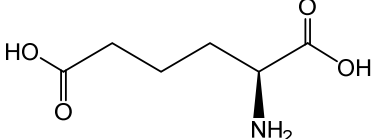
G3	FOH	L-β-Phenyllactic acid	(S)-2-hydroxy-3-phenylpropanoic acid	20312-36-1	
H3	26FF	L-2,6-Difluoro phenylalanine	(S)-2-amino-3-(2,6-difluorophenyl)propanoic acid	33787-05-2	
A4	BzS	O-Benzyl-L-serine	(2R)-2-amino-3-phenylmethoxypropanoic acid	4726-96-9	
B4	PhtOrn	N ^δ -Phthaloyl-L-ornithine hydrochloride	(S)-2-amino-5-(isoindolin-2-yl)pentanoic acid hydrochloride	-	
C4	AlStA	3-Styryl-L-alanine	(S,E)-2-amino-5-phenylpent-4-enoic acid	267650-37-3	
D4	BzC	S-benzyl-L-cysteine	(2R)-2-amino-3-benzylsulfanylpropanoic acid	3054-01-1	
E4	ONBC	S-(2-nitrobenzyl)-L-cysteine	(2R)-2-amino-3-(2-nitrobenzyl)sulfanylpropanoic acid	-	

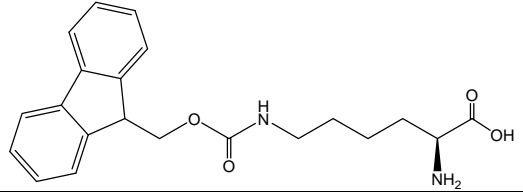
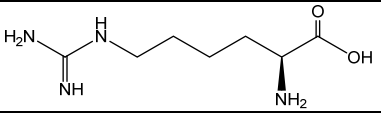
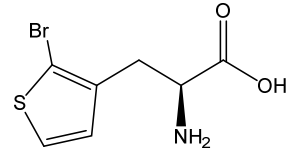
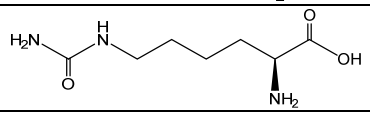
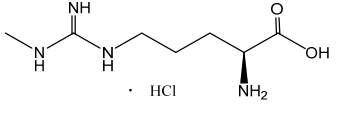
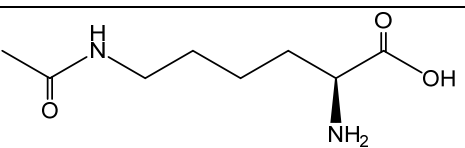
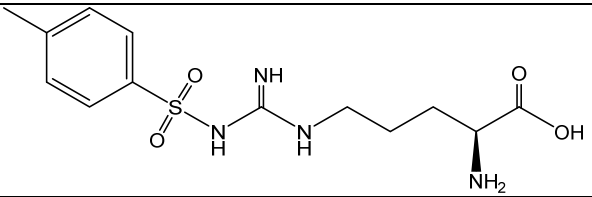
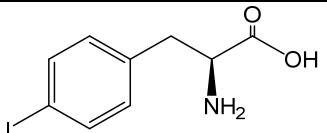
F4	BocK	<i>N</i> ^ε -(<i>tert</i> -butoxycarbonyl)-L-lysine	(<i>S</i>)-2-Amino-6-(<i>tert</i> -butoxycarbonylamino)hexanoic acid	2418-95-3	
G4	ForW	<i>N</i> ^α -Formyl-L-tryptophan hydrochloride	(<i>S</i>)-2-Amino-3-(1-formyl-1H-indol-3-yl)propanoic acid hydrochloride	38023-86-8	
H4	5BrW	5-Bromo-L-tryptophan	(<i>S</i>)-2-Amino-3-(5-bromo-1H-indol-3-yl)propanoic acid	25197-99-3	
A5	2ClZK	<i>N</i> ^ε -2-Chloro-carbobenzoxy-L-lysine	(<i>S</i>)-2-Amino-6-(2-chlorobenzylamino)hexanoic acid	42390-97-6	
B5	3IF	3-Iodo-L-phenylalanine	(<i>S</i>)-2-amino-3-(3-iodophenyl)propanoic acid	20846-39-3	
C5	ForK	<i>N</i> ^ε -Formyl-L-lysine	(<i>S</i>)-2-Amino-6-(formylamino)hexanoic acid	1190-48-3	
D5	4NO2K	<i>N</i> ^ε -4-Nitro-carbobenzoxy-L-lysine	(<i>S</i>)-2-Amino-6-(4-nitro-carbobenzoxyamino)hexanoic acid	3557-90-2	

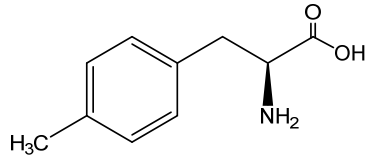
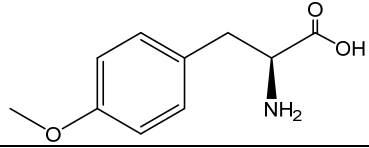
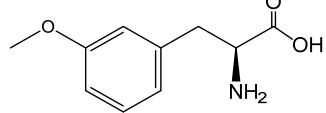
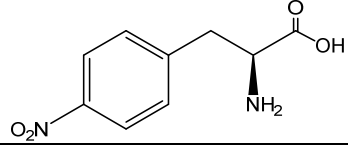
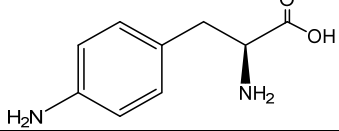
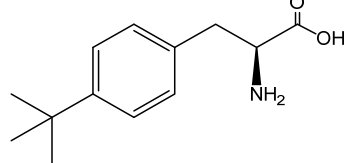
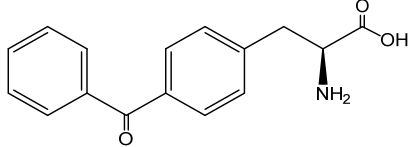
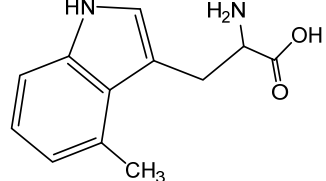
E5	5OHK	5-Hydroxylysine	(2 <i>S</i> ,5 <i>R</i>)-2,6-diamino-5-hydroxyhexanoic acid	30528-11-1	
F5	BioK	<i>N</i> ^ε -Biotinyl-L-lysine	(<i>S</i>)-2-Amino-6-(5-((3 <i>aS</i> ,4 <i>S</i> ,6 <i>aR</i>)-2-oxohexahydro-1 <i>H</i> -thieno[3,4- <i>d</i>]imidazol-4-yl)pentanoyl amino)hexanoic acid	576-19-2	
G5	AcNH2 K	DL-2-Acetylamino-6-N-Boc-amino-4-hexynoic acid·DCHA	2-acetamido-6-((tert-butoxycarbonyl)amino)hex-4-ynoic acid·DCHA	90102-79-7	
H5	TosK	<i>N</i> ^ε - <i>p</i> -Tosyl-L-lysine	(<i>S</i>)-2-Amino-6-(4-tosylamino)hexanoic acid	2130-76-9	
A6	6BrW	6-Bromo-DL-tryptophan	2-Amino-3-(6-bromo-1 <i>H</i> -indol-3-yl)propanoic acid	33599-61-0	
B6	BocW	<i>N</i> ^ε - <i>tert</i> -Butoxycarbonyl-L-tryptophan	(<i>S</i>)-2-Amino-3-(1- <i>tert</i> -butoxycarbonyl-1 <i>H</i> -indol-3-yl)propanoic acid	146645-63-8	
C6	5FW	5-Fluoro-DL-tryptophan	2-Amino-3-(5-fluoro-1 <i>H</i> -indol-3-yl)propanoic acid	154-08-5	

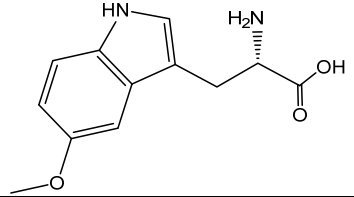
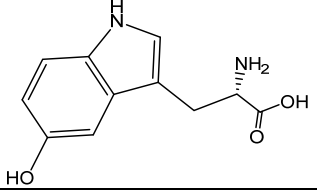
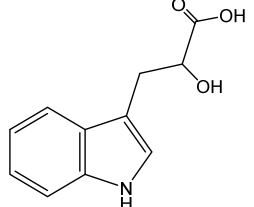
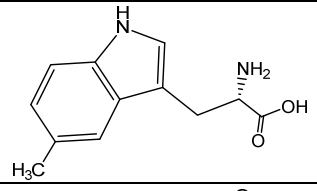
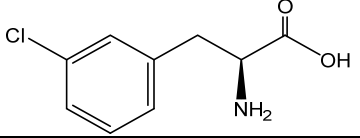
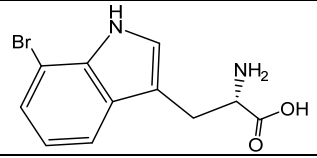
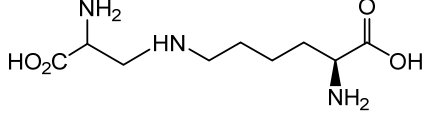
D6	3CNF	3-Cyano- L-phenylalanine	(S)-2-amino-3-(3-cyanophenyl)propanoic acid	57213-48-6	
E6	3BrF	L-3-Bromo phenylalanine	(S)-2-amino-3-(3-bromophenyl)propanoic acid	82311-69-1	
F6	3NO2F	3-Nitro- L-phenylalanine	(S)-2-amino-3-(3-nitrophenyl)propanoic acid	19883-74-0	
G6	4ClF	4-Chloro-L-phenylalanine	(S)-2-Amino-3-(4-chlorophenyl)propanoic acid	14173-39-8	
H6	4BrF	4-Bromo-L-phenylalanine	(S)-2-Amino-3-(4-bromophenyl)propanoic acid	24250-84-8	
A7	b2BrF	L-2-Bromo-β-phenylalanine	(S)-3-amino-3-(2-bromophenyl)propanoic acid	275826-34-1	
B7	aMeF	α-Methyl-L-phenylalanine	(S)-2-amino-2-methyl-3-phenylpropanoic acid	23239-35-2	
C7	aMe4F F	α-Methyl-L-4-fluorophenylalanine	(S)-2-amino-3-(4-fluorophenyl)-2-methylpropanoic acid	130855-57-1	

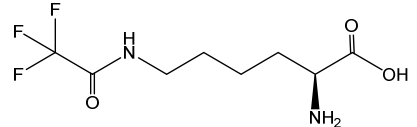
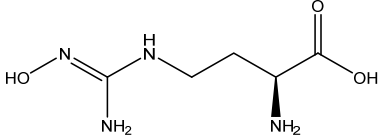
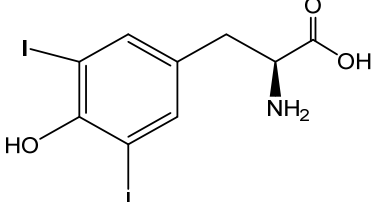
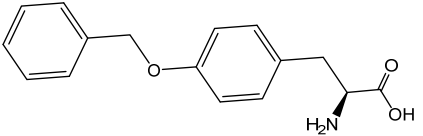
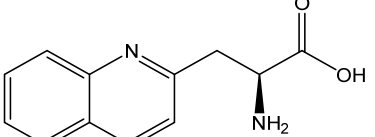
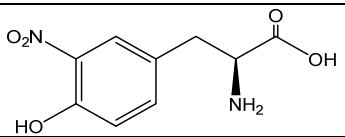
D7	CaMeC	S-(Carboxymethyl)-L-cysteine	(2S)-2-Amino-3-(carboxymethylsulfanyl)propanoic acid	50698-76-5	
E7	4FF	L-4-Fluorophenylalanine	(S)-2-amino-3-(4-fluorophenyl)propanoic acid	1132-68-9	
F7	HOaF	α -Phenyllactic acid	2-hydroxy-2-methyl-3-phenylpropanoic acid	515-30-0	
G7	2FF	L-2-Fluorophenylalanine	(S)-2-amino-3-(2-fluorophenyl)propanoic acid	19883-78-4	
H7	24ClF	L-2,4-Dichlorophenylalanine	(S)-2-amino-3-(2,4-dichlorophenyl)propanoic acid	111119-36-9	
A8	3MeF	3-Methyl-L-phenylalanine	(S)-2-amino-3-(3-methylphenyl)propanoic acid	114926-37-3	
B8	2NapA	3-(2-Naphthyl)-L-alanine	(S)-2-Amino-3-(naphthalen-2-yl)propanoic acid	58438-03-2	
C8	4CF3F	L-4-Trifluoromethylphenylalanine	(S)-2-amino-3-(4-trifluoromethylphenyl)propanoic acid	114926-38-4	

D8	AmEtC	S-(Aminoethyl)-L-cysteine	(S)-2-Amino-3-(2-aminoethylsulfanyl)propionic acid	2936-69-8	
E8	6FW	6-Fluoro-DL-tryptophan	2-Amino-3-(6-fluoro-1H-indol-3-yl)propanoic acid	7730-20-3	
F8	7AzW	L-7-Azatryptophan	(S)-2-Amino-3-(1H-pyrrolo[2,3-b]pyridin-3-yl)propanoic acid	1137-00-4	
G8	7MeW	7-Methyl-DL-tryptophan	2-Amino-3-(7-methyl-1H-indol-3-yl)propanoic acid	17332-70-6	
H8	5MeW	5-Methyl-L-tryptophan	(S)-2-Amino-3-(5-methyl-1H-indol-3-yl)propanoic acid	154-06-3	
A9	AmAdP	L-2-Amino adipic acid	(S)-2-amino hexanedioic acid	1118-90-7	

B9	FmocK	<i>N</i> ^ε -(Fmoc)-L-lysine	(<i>S</i>)-2-Amino-6-(9H-fluoren-9-yl)methoxy carbonylamino)hexanoic acid	84624-28-2	
C9	HomoR	L-Homoarginine	(<i>S</i>)-2-Amino-6-(carbamimidoyl amino)hexanoic acid	156-86-5	
D9	BrThiA	L-2-(5-Bromothieryl)alanine	(<i>S</i>)-2-amino-3-(2-bromothiophen-3-yl)propanoic acid	154593-58-5	
E9	HomoCit	L-Homocitrulline	(<i>S</i>)-2-Amino-6-(carbamoylamino)hexanoic acid	1190-49-4	
F9	MeR	<i>N</i> ^ω -methyl-L-arginine hydrochloride	(2 <i>S</i>)-2-amino-5-[(<i>N</i> ¹ -methylcarbamimidoyl)amino]pentanoic hydrochloride	156706-47-7	
G9	AcK	<i>N</i> ^ε -Acetyl-L-lysine	(<i>S</i>)-2-Amino-6-(acetylamino)hexanoic acid	692-04-6	
H9	TosR	<i>N</i> ^ω - <i>p</i> -Tosyl-L-arginine	(2 <i>S</i>)-2-amino-5-[[amino-[(4-methylphenyl)sulfonylamino]methylidene]amino]pentanoic acid	4353-32-6	
A10	4IF	L-4-Iodo phenylalanine	(<i>S</i>)-2-amino-3-(4-iodophenyl)propanoic acid	24250-85-9	

B10	4MeF	L-4-Methyl phenylalanine	(S)-2-amino-3-(4-methylphenyl)propanoic acid	1991-87-3	
C10	oMeY	L-4-Methoxy phenylalanine	(S)-2-amino-3-(4-methoxyphenyl)propanoic acid	6230-11-1	
D10	3MeOF	3-Methoxy-L-phenylalanine	(S)-2-amino-3-(3-methoxyphenyl)propanoic acid	33879-32-2	
E10	4NO2F	L-4-Nitro phenylalanine	(S)-2-amino-3-(4-nitrophenyl)propanoic acid	949-99-5	
F10	4NH2F	L-4-Amino phenylalanine	(S)-2-amino-3-(4-aminophenyl)propanoic acid	2410-24-4	
G10	4tBuF	L-4-tert-butyl phenylalanine	(S)-2-amino-3-(4-(tert-butyl)phenyl)propanoic acid	82372-74-5	
H10	4BzoF	L-4-benzoyl phenylalanine	(S)-2-amino-3-(4-benzoylphenyl)propanoic acid	104504-45-2	
A11	4MeW	4-Methyl-DL-tryptophan	2-Amino-3-(4-methyl-1H-indol-3-yl)propanoic acid	1954-45-6	

B11	5MeO W	5-Methoxy-L-tryptophan	(S)-2-amino-3-(5-methoxy-1H-indol-3-yl)propanoic acid	4350-09-8	
C11	5OHW	5-hydroxy-L-tryptophan	(S)-2-amino-3-(5-hydroxy-1H-indol-3-yl)propanoic acid	4350-09-8	
D11	HOW	DL-Indole-3-lactic acid	2-hydroxy-3-(1H-indol-3-yl)propanoic acid	1821-52-9	
E11	5MeW	5-Methyl-L-tryptophan	(S)-2-amino-3-(5-methyl-1H-indol-3-yl)propanoic acid	154-06-3	
F11	3ClF	L-3-Chloro phenylalanine	(S)-2-amino-3-(3-chlorophenyl)propanoic acid	80126-51-8	
G11	7BrW	7-Bromo-DL-tryptophan	2-Amino-3-(7-bromo-1H-indol-3-yl)propanoic acid	852391-45-8	
H11	AmCa EtK	N ^ε -(2-amino-2-carboxyethyl)-L-lysine	(2S)-2-Amino-6-[(2-amino-3-hydroxy-3-oxopropyl)amino]hexanoic acid	18810-04-3	
A12	Control	-	-	-	-

B12	TfAcK	<i>N</i> ^ε -(Trifluoroacetyl)-L-lysine	(<i>S</i>)-2-Amino-6-(trifluoroacetylamino)hexanoic acid	10009-20-8	
C12	dHONoR	ω -Hydroxy-nor-L-arginine	(<i>S</i>)-2-Amino-4-(2'-hydroxyguanidino)butyric acid	189302-40-7	
D12	35IY	3,5-Diiodo-L-tyrosine	(<i>S</i>)-2-Amino-3-(4-hydroxy-3,5-diiodophenyl)propanoic acid	300-39-0	
E12	OZY	<i>O</i> -Benzyl-L-tyrosine	(<i>S</i>)-2-amino-3-(4-(benzyloxy)phenyl)propanoic acid	16652-64-5	
F12	QuinolyA	3-(2-Quinoyl)-L-alanine	(<i>S</i>)-2-amino-3-(quinolin-2-yl)propanoic acid	161513-46-8	
G12	3NO2Y	L-3-Nitrotyrosine	(<i>S</i>)-2-Amino-3-(4-hydroxy-3-nitrophenyl)propanoic acid	621-44-3	
H12	3IY	L-3-Iodotyrosine	(<i>S</i>)-2-Amino-3-(4-hydroxy-3-iodophenyl)propanoic acid	2751-18-0	