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for

A Chemical Library Approach to Organic-Modified Peptide Ligands for PDZ Domain Proteins: A Synthetic, Thermodynamic and Structural Investigation

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1.1 Materials

Novabiochem: Preloaded Val-Wang resin (Fmoc-Val-Wang), Fmoc-Lys(Dde)-OH, Fmoc-Dap(ivDde)-OH, all standard Fmoc-L-amino acids, hydroxybenzotriazole (HOBt), benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphoniumhexafluorophosphate (BOP), PyBOP (benzotriazole-1yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate), 2-(1H-benzo-triazole-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate (HBTU).

Note: The acronym "Dpr" is sometimes used instead of "Dap" for diaminopropionic acid.

Fisher Scientific/Fisherbiochem: diisopropylcarbodiimide (DICPI), diethyl ether, methanol, dichloromethane (DCM).

Aldrich: hydrazine, diisopropylethylamine (DIPEA) and all organic acids used in library synthesis (see below for full listing).

Alfa Aesar: trifluoroacetic acid (TFA), dimethyl formamide (DMF), piperidine, dimethyl sulfoxide (DMSO).

Avocado Research Chemicals: biotin

Acros: *N*-Methyl morpholine (NMM), triisopropyl silane (TIS), thioanisole

PALL Life Sciences: AcroPrep 96 (w/ valves) Pre-filter plates (1.0 mL)

Whatman: UNIPLATE 96 well, 750 µL multichem round bottom plates

Pierce: Start block blocking buffer, ImmunoPure Anti-Glutathione *S*-Transferase Mouse Monoclonal primary antibody, ImmunoPure antibody Mouse IgG (H+L) Alkaline phosphatase conjugated secondary antibody, ImunoPure PNPP tablets, Diethanolamine substrate buffer, Reacti-Bind Streptavidin Coated High binding Capacity (HBC) Clear 96-Well Plates, StartingBlock (TBS) Blocking Buffer

1.2 Methods

1.2.1 Synthesis of Library I (N-Biotin-(Ahx)$_2$-Tyr-Lys-Gln-Thr-((N-e-COR)Lys)-Val)

1. A single solid-phase peptide synthesis reaction vessel was loaded with Fmoc-Val-Wang resin (*n* mol). Dichloromethane (DCM) was used to swell the resin (45 min) with shaking.
2. Piperidine/DMF (10x resin volume, 20% v/v) was added to the resin to remove the Fmoc group. After shaking for 10 min, the piperidine/DMF was drawn off and the procedure repeated with fresh solvent. The solvent was removed, and the resin was washed with DMF (10 times, 10x resin volume).
3. Fmoc-L-Lys(Dde)-OH (3n mol), DIPCI (4n mol), HOBt (6n mol) and DMF (5x resin volume) were added to the reaction vessel, which was gently shaken for 2 h. A small amount of resin sample was used for the Kaiser test to confirm completion of coupling.
4. Steps 2 and 3 were repeated using the required Fmoc-L-amino acids to synthesize the full length protected hexapeptide (Tyr-Lys-Gln-Thr-Lys-Val), followed by two rounds of the same conditions using Fmoc-6-aminohexanoic acid (Ahx). The terminal Fmoc (from the second Ahx residue added) was removed as described in Step 2.
5. Biotin (10*n*) was dissolved in DMF:DMSO (5 mL, 1:1), with warming to dissolve the biotin fully. This solution was treated with 0.45 M HBTU/0.45 M HOBt in DMF (2.1 mL), followed by addition of DIEA (0.3 mL). This activated biotin solution was added to the resin and allowed to react overnight (ca. 12 h).

6. After coupling was completed, the reaction solution was drawn off and the resin sequentially washed with DMF (10 x 10x resin volume), DMF:DMSO (1:1, 3 x 5x resin volume) and DCM:methanol (1:1, 2 x 5x resin volume). The resin was thoroughly dried under vacuum.

7. Hydrazine (2% in DMF, 10x resin volume) was added and shaken for 10 min (to effect removal of Dde from Lys). This procedure was repeated two more times with fresh hydrazine-DMF solution.

8. After washing the resin with DMF (10 x 10x resin volume), an isopycnic resin solution (a solution of DMF:DCM which gives homogeneous distribution of resin as a slurry; see *Note 1* below) was prepared. Equal volumes (calculated resin amounts ~5 mg; see *Note 2* below) of this slurry were distributed in the PALL 96-well filter plates. The solvent was removed by centrifugation (2000 *g*) with a collection 96-well plate at the bottom.

Note 1: Solvent ratio for generating an isopycnic slurry was empirically determined by first suspending the resin in an arbitrary minimal amount of DMF, and then adding aliquots of DCM until the resins distributed equally.

Note 2: For 480 mg (96 x 5) of resin suspended in 4.8 mL of the isopycnic solution, 50 µL of the slurry was added to each well.

9. PyBOP (5*n* mol, 0.3 M), HOBt (5*n* mol, 0.3 M) and NMM (25*n* mol) in DMF were combined, and an aliquot of the solution (75 µL) was added to each well using a multichannel pipette. Aliquots (50 µL) of organic acids (10 nmol, 0.6 M in DMF) from a pre-prepared 96-well stock plate (containing 92 different acids) were added to each well. The plates were shaken overnight (ca. 12 h), attached to an orbital shaker to complete the coupling reaction.

10. Each well was washed sequentially with DMF (3 x 200 µL), isopropyl alcohol (3 x 200 µL) and DCM (3 x 200 µL). Solvent removal after each step was performed by centrifugation (2000 *g*) with a collection 96-well plate at the bottom.

11. Into each well was added TFA cleavage solution (100 µL; TFA/triisopropyl silane (TIS)/thioanisole/water (94:2:2:2)), and allowed to incubate for 2 h.

12. After completion of the cleavage, the compounds in TFA solution were collected into Whatman UNIPLATE 96-well, 750 µL multichem round bottom plates by centrifugation (3000 *g*). Fresh pure TFA (2 x 25 µL) was placed in each well and

again collected into the same plate as described above. Cold diethyl ether (500 µL) was added to each well to precipitate out the modified peptides, and the plate immediately centrifuged (3000 *g*) to collect the precipitates. The solvent was removed, and fresh cold ether (500 µL) was added to wash the precipitate, followed again by centrifugation. This cold ether washing procedure was repeated for a third round to completely remove any remaining TFA.

13. Modified peptides in each well were dissolved in deionized water (400 µL), and the plate stored at -20 °C until needed for screening.

1.2.2 Synthesis of Library II (N-Biotin-(Ahx)$_2$-Tyr-Lys-Gln-Thr-((N-e-COR)Dap)-Val)

All steps were performed as per the procedure for Library I synthesis, with the following two exceptions: 1) Fmoc-L-Dap(ivDde)-OH was used instead of Fmoc-L-Lys-(Dde)-OH in Step 3; and 2) 186 instead of 92 organic acids were used in Step 9.

1.2.3. ELISA Screening

Microplate composition:

Library I — One 96-well plate; 92 organic acids (wells #1-92) and 4 control wells
Well #93: unmodified ligand (YKQTKV)
Well #94: known strong binding peptide (KKETAV)
Well #95: known weak binding peptide (KKGTGV)
Well #96: no ligand

Library II — Two 96-well plates; 186 organic acids (93 organic acids on each; wells #1-93) and 3 control wells on each
Well #94: unmodified ligand (YKQTKV)
Well #95: known strong binding peptide (KKETAV)
Well #96: known weak binding peptide (KKGTGV)

Note: Known strong and weak binding sequences obtained from: Biochemistry (2007) *46*, 6340-52

Assay procedure:

1. Streptavidin-coated plates were treated with start block blocking buffer for several minutes and discarded.

2. Biotinylated modified peptides (from the library, in parallel) in start block blocking buffer was added to each microplate well (100 µL) and allowed to incubate for 1 h at room temperature.

Note: Modified peptides from the library were initially dissolved at high concentration in water for the stock plates (ca. 1 mg/mL in 400 µL water), and applied to the well in several-hundred equivalent excess of the rated streptavidin equivalents (125 pmol/well). In this manner, it is assumed that all binding sites in all wells are saturated with biotinylated ligand.

3. The solution was removed with a multichannel pipettor and the plate washed with TBST buffer (3 x 100 µL).
4. PDZ3-GST fusion protein (100 µL of 5 µM solution) in start block blocking buffer was added to each well and allowed to bind overnight at 4 °C. (PDZ3-GST fusion protein was prepared in a manner consistent with the procedure reported in Saro et al, *Biochemistry* **2007**, *46*, 6340-52.)
5. The solution was removed with a multichannel pipettor and the plate washed with TBST buffer (3 x 100 µL).
6. Anti-GST primary antibody (100 µL of 1:1000 dilution) in start block blocking buffer was added to each well and incubated for 1 h at room temperature.
7. The solution was removed with a multichannel pipettor and the plate washed with TBST buffer (3 x 100 µL).
8. Alkaline phosphatase conjugated secondary antibody (100 µL of 1:5000 dilution) in start block blocking buffer was added to each well and incubated for 0.5 h at room temperature.
9. The wells were washed thoroughly (5 x with TBST buffer ((4 x 100 µL) and a final wash of 1 x 200 µL)).
10. *p*-Nitrophenyl phosphate, disodium salt (PNPP) substrate solution (100 µL) was added to each well, and the absorbance values (λ = 405 nm) were measured after 15 min using a Tecan Spectrafluor platereader.

1.2.4 Individual (Preparative Scale) Synthesis of Selected Modified Peptides

1. Each of the selected compounds (Figure 2 and Table 1 of the main paper) was synthesized on Val-Wang resin using standard Fmoc solid-phase synthesis procedures (as described in section 1.2.1, Steps 1-3).

2. After removing the Dde (or ivDde) protecting group from the side chain of the P_{-1} residue (Lys-Dde in Library 1, Dap-ivDde in Library 2) the specific organic acid was coupled by using PyBOP (5*n* mol, 0.3 M), HOBt (5*n* mol, 0.3 M) and NMM (25*n* mol) in DMF and allowed to shake overnight (ca. 12 h).
3. The crude acylated peptide was isolated by adding TFA cleavage solution (4 mL) TFA/triisopropyl silane (TIS)/thioanisole/water (94:2:2:2)) and incubating for 2 h, followed by precipitation with cold ether, redissolving in water and lyopilization.

1.2.5 Purification and Analysis of Organic Modified Peptides

A small portion of the lyophilized powder was used for preliminary mass (by MALDI-TOF and ESI-MS) and purity (analytical RP-HPLC) characterization. For the latter, ca. 1.5 mg of the powder was dissolved in H_2O (0.5 mL). In determining an appropriate ratio for the methanol: H_2O (0.1% TFA) solvent system used for HPLC separation (C18 RP-HPLC Phenomenex column), small injections (20 µL) were made at different ratios. After a satisfactory solvent system was found, the purification was performed at a preparative scale (50 mg/mL of crude peptide). The major single peak fractions of each run was collected and combined, frozen and lyophilized to a powdered solid. The purified material was subjected to mass spectral analysis, and an analytical scale RP-HPLC was performed again to confirm a single peak. If the analysis displayed more than one peak, a repurification was performed until a single peak was observed.

1.2.6 Isothermal Titration Calorimetry (ITC)

Preparation of isolated (non-fusion form) PDZ3 and standard ITC binding experiments were conducted with minimal deviations from the methods as previously described (*Biochemistry* **2007**, *46*, 6340-52) using a VP-ITC microcalorimeter (Microcal). Briefly, PDZ3 subcloned from PSD-95 protein (residues 302-402) was expressed in *E. coli* BLR-Gold as a glutathione-*S*-transferase (GST) fusion protein, then isolated after trypsin cleavage from the GST and chromatographic purification steps. After PDZ3 was extensively dialyzed (24 h) in the chosen buffer (20 mM MES, 10 mM NaCl, pH 6.00), the dialysis buffer was saved and used to dissolve the ligands to be titrated (thereby insuring no buffer mismatch between samples). The pH of the PDZ3 sample was measured and the peptide ligand solution was adjusted to the same value (with an allowed difference of <0.02 units). The protein and peptide samples were separately degassed for 20-30 min under vacuum without stirring.

In a typical ITC experiment, 50-120 μM PDZ3 protein (1.4 mL) was loaded into the sample cell and 0.8-1.4 mM peptide (290 μL) was placed in the syringe. Typically 30 or 59 injections were programmed, with the first injection volume set at 1 μL (2 s injection) and the remaining at 10 μL (in 30 injections of 20 s each) or 5 μL (in 59 injections of 10 s each). The spacing between injections was 180 s. The reference power was 10 μcal/s with an initial delay of 1 min. Experiments were conducted at 25 °C with a stirring speed of 270 rpm. The raw data were collected and analyzed by ORIGIN software (version 5.0, Microcal Inc.). Thermodynamic parameters were determined by non-linear least squares fitting using a One Set of Binding Sites model.

1.2.7 HSQC Chemical Shift Perturbation

Original resonance assignments and structure determination of PSD-95 PDZ3 by NMR spectroscopy are reported in the Ph.D. (Chemistry) dissertation of Dr. Tao Li (*"Studies of Ligand-Protein Interaction for the Third PDZ Domain (PDZ3) of Mammalian Post-synaptic Density-95 (PSD-95)"*; Wayne State University, 2005).

Chemical shift perturbation NMR experiments were performed to identify the PDZ3 amino residues affected by the presence of peptide ligands. Data were collected on a Varian Inova 600 MHz spectrometer equipped with a triple-resonance ^{1}H, ^{13}C and ^{15}N cryoprobe with z-axis pulsed field gradients. ^{15}N-labelled PDZ3 domains were generated from minimal media bacterial expression (*J. Biomol. NMR* **2004**, *30*, 111-112) at a concentration of 0.6-1.0 mM in NMR buffer (20 mM sodium phosphate (pH 5.5) and 40 mM NaCl). Two dimensional ^{1}H,^{15}N HSQC experiments were performed with ^{15}N-labeled PDZ3 containing 10% (*v/v*) $^{2}H_2O$ as an internal lock. The spectral data were acquired with 1024 x 512 complex data points and a spectral width of 8000 Hz for the ^{1}H dimension and 3000 Hz for the ^{15}N dimension.

For the two dimensional ^{1}H,^{15}N HSQC experiments of PDZ3 saturated with ligand (either YKQTKV or compound **1**), the spectra were recorded at different protein:ligand ratios ranging from 1:0.1 to 1:1.6 in 9 steps (1:0.1, 1:0.2, 1:0.35, 1:0.5, 1:0.65, 1:0.75, 1:0.9, 1:1 and 1:1.6). All the spectral data were processed using NMRpipe (*J. Biomol. NMR* **1995**, *6*, 277-293) and analyzed by Sparky (TD Goddard and DG Kneller, SPARKY 3, University of California, San Francisco). PDZ3 residues whose HSQC peak shifted from the apo-protein (uncomplexed/absence of ligand) on titration with ligand were assigned by superimposing the HSQC spectra in the absence and presence of peptide in different ratios. The degree of perturbation was measured by

normalized chemical shift index calculated by the formula [$d = 25[(d_{HN})^2 + (d_N/5)^2]^{0.5}$] (*Nat. Struct. Biol.* **1996**, *3*, 340-345). The normalized differential chemical shift obtained for compound 1 compared to peptide YKQTKV for each residues were plotted in Figure S1. A normalized amide chemical shift index value of 1 or larger was considered a substantial change.

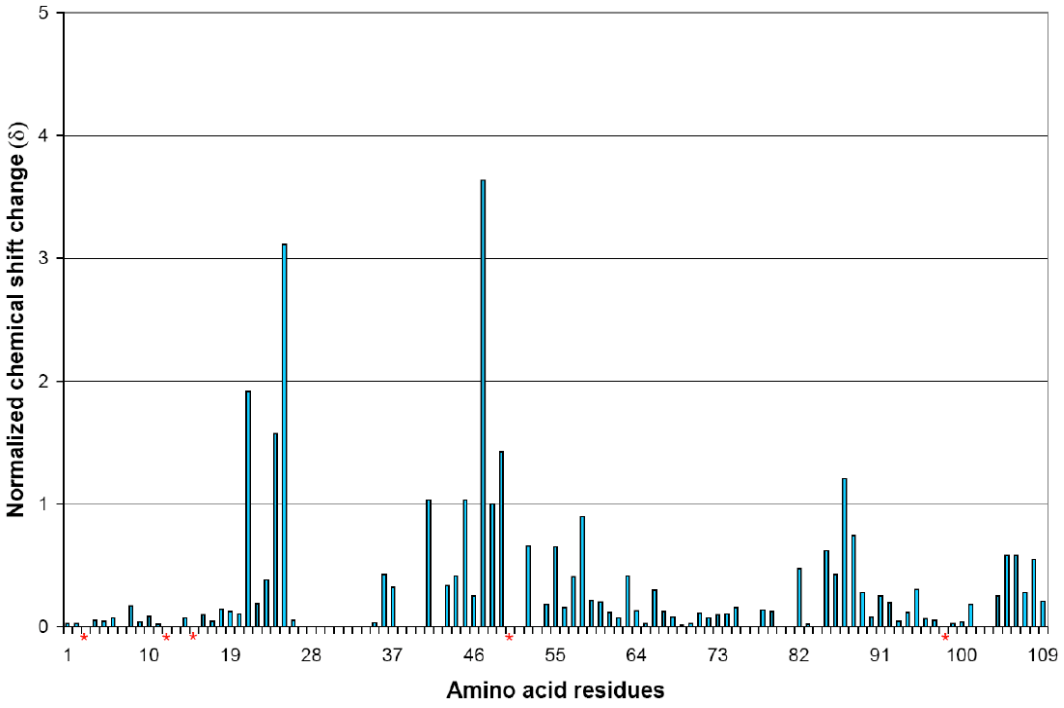
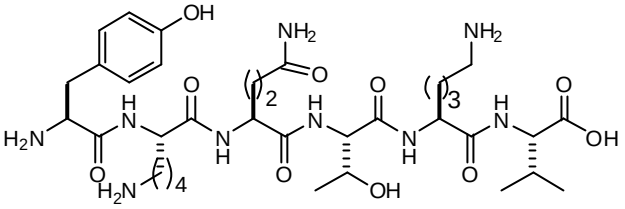


Figure S1. Normalized differential chemical shift change for compound **1** compared to YKQTKV. The red asterisk denotes proline residues (which lack an amide proton and cannot be tracked), while residues whose resonance disappeared during titration are represented by blank columns in the chart.

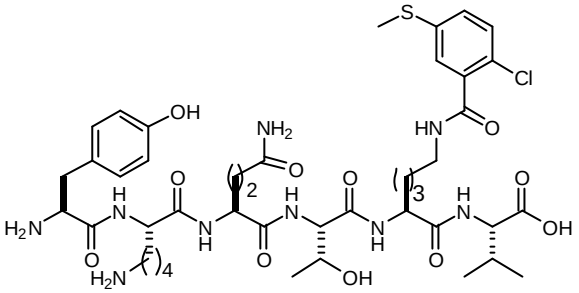
Appendix 1. Structures and Mass Spectral Data for Modified Peptides

YKQTKV (unmodified parent peptide)



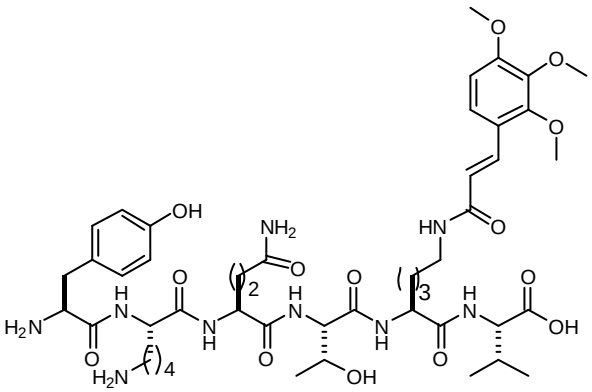
Calculated exact mass: 765.44; mass found (MALDI-TOF): 766.66 $[M+H]^+$, 788.54 $[M+Na]^+$, 804.47 $[M+K]^+$

Compound 1 (2-chloro-5-(methylthio)benzoic acid modification)



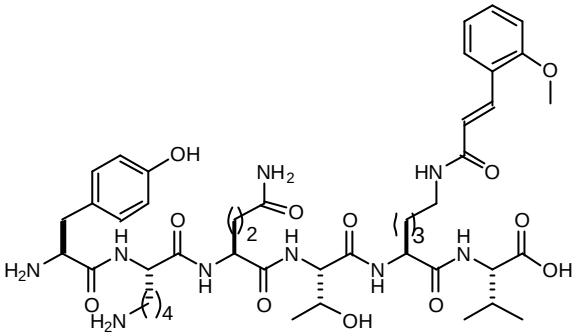
Calculated exact mass: 949.41; mass found (MALDI-TOF): 950.40 $[M+H]^+$, 972.41 $[M+Na]^+$

Compound 2 (*trans*-2,3,4-trimethoxycinnamic acid modification)

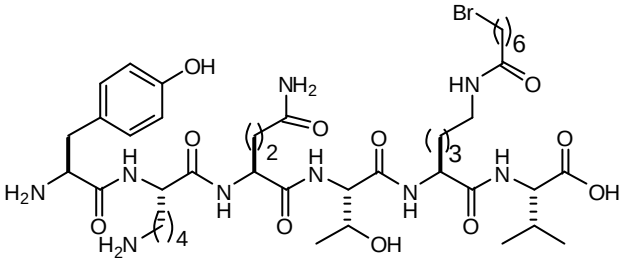


Calculated exact mass: 985.51; mass found (MALDI-TOF): 986.53 $[M+H]^+$, 1008.59 $[M+Na]^+$

Compound 3 (*trans*-2-methoxycinnamicacid modification)



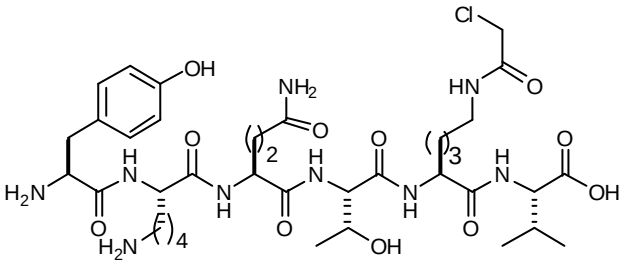
Calculated exact mass: 925.49; mass found (MALDI-TOF): 926.46 $[M+H]^+$, 948.48 $[M+Na]^+$



Compound 4 (6-bromohexanoic acid modification)

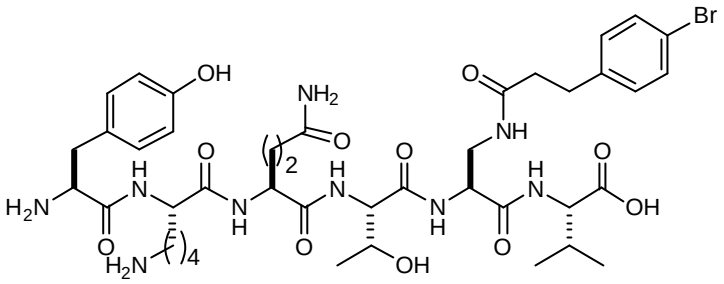
Calculated exact mass: 941.42; mass found (MALDI-TOF): 942.36 $[M+H]^+$, 964.58 $[M+Na]^+$

Compound 5 (chloroacetic acid modification)



Calculated exact mass: 841.41; mass found (MALDI-TOF): 842.44 $[M+H]^+$, 864.45 $[M+Na]^+$

Compound 6 (3-(4-bromophenyl) propionic acid modification)



Calculated exact mass: 934.97; mass found (MALDI-TOF): 936.47 $[M+H]^+$

Appendix 2. Organic Acids Used in Modified Peptide Library Synthesis

No.	Compound Name	MW	Molecular Formula
1	2,6-dichlorobenzoic acid	191.01	$Cl_2C_6H_3CO_2H$
2	2-Amino-3-methylbenzoic acid	151.16	$C_8H_9NO_2$
3	2-Bromopropionic acid	152.98	$CH_2CH(Br)CO_2H$
4	2-Chloro-5-(methylthio)benzoic acid	202.66	$ClC_6H_3(SCH_3)CO_2H$
5	2-Hydroxyisobutyric acid	104.11	$(CH_3)_2C(OH)CO_2H$
6	2-Iodobenzoic acid	248.02	$C_7H_5IO_2$
7	3,4-Dimethoxycinnamic acid	208.21	$C_{11}H_{12}O_4$
8	3,5-Dimethoxybenzoic acid	182.18	$C_9H_{10}O_4$
9	3-chloropropionic acid	108.52	$ClCH_2CH_2CO_2H$
10	4-Benzoylbutyric acid	192.21	$C_{11}H_{12}O_3$
11	4-Fluorobenzoic acid	140.11	$FC_6H_4CO_2H$
12	4-Hydroxyphenylacetic acid	152.15	$HOC_6H_4CH_2CO_2H$
13	5-Hexynoic acid	112.13	$CHC(CH_3)_3CO_2H$
14	6-Bromohexanoic acid	195.05	$C_6H_{11}BrO_2$
15	Acetoxyacetic acid	118.09	$CH_3CO_2CH_2CO_2H$
16	Bromoacetic acid	138.95	$C_2H_3BrO_2$
17	Chloroacetic acid	94.5	$ClCH_2CO_2H$
18	*cis*-2-Methoxycinnamic acid	178.19	$CH_3OC_6H_4CH=CHCO_2H$
19	Cyanoacetic acid	85.06	$NCCH_2CO_2H$
20	Cyclohexanecarboxylic acid	128.17	$C_{10}H_{16}O_4$
21	Glycolic acid	76.05	$C_2H_4O_3$
22	Indole-2-carboxylic acid	161.16	$C_9H_7NO_2$
23	Lactic acid	90.08	$CH_3CH(OH)CO_2H$
24	Malonic Acid	104.07	$CH_2(CO2H)_2$
25	*o*-Methoxycinnamic acid (predominantly *trans*)	178.19	$C_{10}H_{10}O_3$
26	Palmitic acid	256.43	$CH_3(CH_2)_{14}CO_2H$
27	Picolinic acid	123.11	$C_6H_5NO_2$
28	*trans*-2,3,4-Trimethoxycinnamic acid	238.24	$C_{12}H_{14}O_5$
29	Trichloroacetic acid, sodium salt	185.37	$C_2Cl_3NO_2$
30	1-Adamantanecarboxylic	180.25	$C_{11}H_{16}O_2$
31	2,4-Dichlorophenoxyacetic acid	221.04	$C_9H_{10}O_4$
32	2-Hydroxycinnamic acid	164.16	$OHC_6H_4CH=CHCO_2H$
33	3(-Furyl)acrylic acid	138.12	$C_7H_6O_3$

No.	Compound Name	MW	Molecular Formula
34	D-Camphoric acid	200.3	$C_{10}H_{16}O_4$
35	Fumaric acid	116.1	COOH-CH=CHCOOH
36	Nitrilotriacetic acid	191.14	$C_6H_9NO_6$
37	Oxalic acid	126.07	$(COOH)_2 .2H_2O$
38	Phenyl malonic acid	180.16	$C_6H_5CH(CO_2H)_2$
39	*p*-Methoxycinnamic acid	178.19	$C_{10}H_{10}O_3$
40	Suberic acid	174.2	$HO_2C(CH_2)_6CO_2H$
41	Tribromoacetic acid	296.76	Br_3CCO_2H
42	a-Bromo-*p*-toluic acid	215.05	$C_8H_7BrO_2$
43	(-)-2-Oxo-4-thiazolidinecarboxylic acid	147.15	$C_4H_5NO_3S$
44	(±)-2-Phenylbutyric acid	164.2	$CH_3CH_2CH(C_6H_5)COOH$
45	(1-Naphthoxy)acetic acid	202.21	$C_{10}H_7OCH_2CO_2H$
46	(1*R*,3*R*,4*R*,5*R*)-(-)-Quinic acid	192.17	$(HO)_4C_6H_7CO_2H$
47	(2,4-Dimethoxyphenyl)acetic acid	196.2	$(CH_3O)_2C_6H_3CH_2CO_2H$
48	(2-Hydroxyphenoxy)acetic acid	168.15	$HOC_6H_4OCH_2CO_2H$
49	(2-Pyrimidylthio)acetic acid	170.19	$C_6H_6N_2O_2S$
50	(3,5-Dimethoxyphenyl)acetic acid	196.2	$(CH_3O)_2C_6H_3CH_2CO_2H$
51	(4-Pyridylthio)acetic acid	169.2	$C_7H_7NO_2S$
52	(*S*)-(+)-a-Hydroxy-1,3-dioxo-2-isoindolinebutyric acid	249.22	$C_{12}H_{11}NO_5$
53	1-(4-Methoxyphenyl)ethyliminoxyacetic acid	223.23	$C_{11}H_{13}NO_4$
54	10-Undecynoic acid	182.26	$HC{\equiv}C(CH_2)_8CO_2H$
55	1*H*-Tetrazole-5-acetic acid	128.09	$C_3H_4N_4O_2$
56	1-Methylindole-3-carboxylic acid	175.18	$C_{10}H_9NO_2$
57	2,2-Bis(hydroxymethyl)propionic acid	134.13	$(HOCH_2)_2C(CH_3)COOH$
58	2,3,4-Trihydroxybenzoic acid	170.12	$(HO)_3C_6H_2CO_2H$
59	2,3-Dihydro-3-oxo-4*H*-1,4-benzoxazine-4-propionic acid	221.21	$C_{11}H_{11}NO_4$
60	2,3-Dimethoxybenzoic acid	182.17	$(CH_3O)_2C_6H_3CO_2H$
61	2,4-Dihydroxypyrimidine-5-carboxylic acid	156.1	$C_5H_4N_2O_4$
62	2,6-Dichloro-5-fluoro-3-pyridinecarboxylic acid	209.99	$C_6H_2Cl_2FNO_2$
63	2,6-Diphenylisonicotinic acid	275.3	$C_{18}H_{13}NO_2$
64	2-Acetamidoacrylic acid	129.11	$CH_2{=}C(NHCOCH_3)COOH$
65	2-Acetylbenzoic acid	164.16	$C_9H_8O_3$
66	2-Benzimidazolepropionic acid	190.2	$C_{10}H_{10}N_2O_2$
67	2-Benzoylbenzoic acid	226.23	$C_6H_5COC_6H_4CO_2H$
68	2-Butynoic acid	84.07	$CH_3C{\equiv}CCO_2H$
69	2-Chloromandelic acid	186.59	$ClC_6H_4CH(OH)CO_2H$
70	2-Ethoxybenzoic acid	166.17	$C_2H_5OC_6H_4CO_2H$
71	2-Ethylbutyric acid	116.16	$(C_2H_5)_2CHCOOH$
72	2-Hydroxy-3-isopropylbenzoic acid	180.2	$(CH_3)_2CHC_6H_3(OH)CO_2H$
73	2-Hydroxy-5-(1H-pyrrol-1-yl)benzoic acid	203.19	$C_{11}H_9NO_3$
74	2-Hydroxy-5-methoxybenzoic acid	168.15	$CH_3OC_6H_3(OH)CO_2H$

No.	Compound Name	MW	Molecular Formula
75	2-Mercaptonicotinic acid	155.17	$C_6H_5NO_2S$
76	2-Methoxynicotinic acid	153.14	$C_7H_7NO_3$
77	2-Methoxyphenylacetic acid	166.17	$CH_3OC_6H_4CH_2CO_2H$
78	2-Methyl-4-oxo-4-phenylbutyric acid	192.21	$C_6H_5COCH_2CH(CH_3)CO_2H$
79	2-Methylhippuric acid	193.2	$CH_3C_6H_4CONHCH_2CO_2H$
80	2-Naphthoxyacetic acid	202.21	$C_{10}H_7OCH_2CO_2H$
81	2-Phenoxybenzoic acid	214.22	$C_6H_5OC_6H_4CO_2H$
82	2-Phenoxypropionic acid	166.17	$C_6H_5OCH(CH_3)CO_2H$
83	2-Phenyl-4-quinolinecarboxylic acid	249.26	$C_{16}H_{11}NO_2$
84	2-Pyrrolidone-5-carboxylic acid	129.11	$C_5H_7NO_3$
85	2-Thiopheneacetic acid	142.18	$C_6H_6O_2S$
86	Fmoc-Gly-OH	297.3	$C_5H_4O_2S$
87	2-Thiophenepropionic acid	156.2	$C_7H_8O_2S$
88	3-(2,4-Dimethoxyphenyl)propionic acid	210.23	$(CH_3O)_2C_6H_3CH_2CH_2CO_2H$
89	3-(2-Hydroxyphenyl)propionic acid	166.17	$HOC_6H_4CH_2CH_2CO_2H$
90	3-(2-Methoxyphenyl)propionic acid	180.2	$CH_3OC_6H_4CH_2CH_2CO_2H$
91	3-(4-Bromophenyl)propionic acid	229.07	$BrC_6H_4CH_2CH_2CO_2H$
92	3-(4-Hydroxyphenyl)propionic acid	166.17	$HOC_6H_4CH_2CH_2CO_2H$
93	3-(*p*-Tolyl)propionic acid	164.2	$CH_3C_6H_4CH_2CH_2CO_2H$
94	3,3-Dimethylacrylic acid	100.12	$(CH_3)_2C=CHCOOH$
95	3,3-Dimethylbutyric acid	116.16	$(CH_3)_3CCH_2COOH$
96	3,4-(Methylenedioxy)phenylacetic acid	180.16	$C_9H_8O_4$
97	3,4-Dihydroxyphenylacetic acid	168.15	$(HO)_2C_6H_3CH_2CO_2H$
98	3,4-Dimethylbenzoic acid	150.17	$(CH_3)_2C_6H_3CO_2H$
99	3,5-Dimethylisoxazole-4-carboxylic acid	141.12	$C_6H_7NO_3$
100	3,6-Dichloropyridazine-4-carboxylic acid	192.99	$C_5H_2Cl_2N_2O_2$
101	3,7-Dihydroxy-2-naphthoic acid	204.18	$(HO)_2C_{10}H_5CO_2H$
102	3-[5-(2-(Trifluoromethyl)phenyl)furan-2-yl]-acrylic acid, predominantly trans	282.21	$C_{14}H_9F_3O_3$
103	3-[5-(4-Chlorophenyl)furan-2-yl]acrylic acid	352.12	$C_{13}H_9ClO_3$
104	3-Benzoyl-2-pyridinecarboxylic acid	227.22	$C_{13}H_9NO_3$
105	3-Chloro-benzo[b]thiophene-2-carboxylic acid	212.65	$C_9H_5ClO_2S$
106	3-Chlorobenzoic acid	156.57	$ClC_6H_4CO_2H$
107	3-Chloroisonicotinic acid	157.55	$C_6H_4ClNO_2$
108	3-Fluoroisonicotinic acid	141.1	$C_6H_4FNO_2$
109	3-Hydroxy-2-quinoxalinecarboxylic acid	190.16	$C_9H_6N_2O_3$
110	3-Hydroxypicolinic acid	139.11	$C_6H_5NO_3$
111	3-Indolepropionic acid	189.21	$C_{11}H_{11}NO_2$
112	3-Methyl-4-pentenoic acid	114.14	$CH_2=CHCH(CH_3)CH_2CO_2H$
113	3-Methyl-5-isoxazoleacetic acid	141.12	$C_6H_7NO_3$
114	3-Methylbenzofuran-2-carboxylic acid	176.17	$C_{10}H_8O_3$
115	3-Pyridinepropionic acid	151.16	$C_8H_9NO_2$

No.	Compound Name	MW	Molecular Formula
116	3-Thiophenecarboxylic acid	128.15	$C_5H_4O_2S$
117	4-(1*H*-Imidazole-1yl)benzoic acid	188.18	$C_{10}H_8N_2O_2$
118	4-(2,5-Dimethoxyphenyl)butyric acid	224.25	$(CH_3O)_2C_6H_3(CH_2)_3CO_2H$
119	4-(2-Thienyl)butyric acid	170.23	$C_8H_{10}O_2S$
120	4-(3-Methyl-5-oxo-2-pyrazolin-1-yl)benzoic acid	218.21	$C_{11}H_{10}N_2O_3$
121	4-(Diethylamino)benzoic acid	193.24	$(C_2H_5)_2NC_6H_4CO_2H$
122	4-(Phenylazo)benzoic acid	226.23	$C_6H_5N=NC_6H_4CO_2H$
123	4-Acetamidobenzoic acid	179.17	$CH_3CONHC_6H_4CO_2H$
124	4-Acetyl-3,5-dimethyl-2-pyrrolecarboxylic acid	181.19	$C_9H_{11}NO_3$
125	4-Acetylbenzoic acid	164.16	$CH_3COC_6H_4CO_2H$
126	4-Chlorophenoxyacetic acid	186.59	$C_8H_7ClO_3$
127	4-Ethoxyphenylacetic acid	180.2	$C_2H_5OC_6H_4CH_2CO_2H$
128	4-Guanidinobutyric acid	145.16	$C_5H_{11}O_2N_3$
129	4-Hydroxy-7-trifluoromethyl-3-quinolinecarboxylic acid	257.17	$C_{11}H_6F_3NO_3$
130	4-Imidazoleacrylic acid	138.12	$C_6H_6N_2O_2$
131	4-Methyl-5-thiazolecarboxylic acid	143.16	$C_5H_5NO_2S$
132	4-Methylhippuric acid	193.2	$CH_3C_6H_4CONHCH_2CO_2H$
133	4-Oxo-4*H*-1-benzopyran-2-carboxylic acid	190.15	$C_{10}H_6O_4$
134	5-Benzimidazolecarboxylic acid	162.15	$C_8H_6N_2O_2$
135	5-Bromo-2-thiophenecarboxylic acid	207.05	$C_5H_3BrO_2S$
136	5-Chlorothiophene-2-carboxylic acid	162.59	$C_5H_3ClO_2S$
137	5-Fluoroindole-2-carboxylic acid	179.15	$C_9H_6FNO_2$
138	5-Methyl-3-phenylisoxazole-4-carboxylic acid	203.19	$C_{11}H_9NO_3$
139	5-Phenyl-2-furoic acid	188.18	$C_{11}H_8O_3$
140	6-Acetamidohexanoic acid	173.21	$CH_3CONH(CH_2)_5CO_2H$
141	6-Bromopicolinic acid	202.01	$C_6H_4BrNO_2$
142	6-Phenylhexanoic acid	192.25	$C_6H_5(CH_2)_5CO_2H$
143	7-Chloro-4-hydroxy-3-quinolinecarboxylic acid	223.61	$C_{10}H_6ClNO_3$
144	7-Hydroxycoumarinyl-4-acetic acid	220.18	$C_{11}H_8O_5$
145	9,10-Difluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid	281.21	$C_{13}H_9F_2NO_4$
146	9-Anthracenecarboxylic acid	222.24	$C_{15}H_{10}O_2$
147	Allantoic acid	176.13	$C_4H_8N_4O_4$
148	Benzilic acid	228.24	$(C_6H_5)_2C(OH)COOH$
149	Benzotriazole-5-carboxylic acid	163.13	$C_7H_5N_3O_2$
150	Biphenyl-2-carboxylic acid	198.22	$C_6H_5C_6H_4CO_2H$
151	Butyric acid	88.11	$CH_3CH_2CH_2COOH$
152	Citrazinic acid	155.11	$C_6H_5NO_4$
153	Coumarin-3-carboxylic acid	190.15	$C_{10}H_6O_4$
154	Crotonic acid	86.09	$CH_3CH=CHCOOH$

No.	Compound Name	MW	Molecular Formula
155	Cyclobutanecarboxylic acid	100.12	$C_4H_7CO_2H$
156	Dibenzofuran-4-carboxylic acid	212.2	$C_{13}H_8O_3$
157	DL-4-Hydroxy-3-methoxymandelic acid	198.17	$HOC_6H_3(OCH_3)CH(OH)CO_2H$
158	Fluorene-9-carboxylic acid	210.23	$C_{14}H_{10}O_2$
159	Indole-3-butyric acid	203.24	$C_{12}H_{13}NO_2$
160	Isonicotinic acid	123.11	$C_6H_5NO_2$
161	Isoquinoline-1-carboxylic acid	173.17	$C_{10}H_7NO_2$
162	Mefenamic acid	241.29	$C_{15}H_{15}NO_2$
163	Methoxyacetic acid	90.08	CH_3OCH_2COOH
164	Nalidixic acid	232.24	$C_{12}H_{12}N_2O_3$
165	*N*-Phenylanthranilic acid	213.23	$2\text{-}(C_6H_5NH)C_6H_4CO_2H$
166	*p*-Coumaric acid	164.16	$HOC_6H_4CH{=}CHCO_2H$
167	Pentadecanoic acid	242.4	$CH_3(CH_2)_{13}COOH$
168	Pyrazinecarboxylic acid	124.1	$C_5H_4N_2O_2$
169	Thianaphthene-2-carboxylic acid	178.21	$C_9H_6O_2S$
170	*trans*-2,3-Dimethylacrylic acid	100.12	$CH_3CH{=}C(CH_3)COOH$
171	*trans*-3-(3-Pyridyl)acrylic acid	149.15	$C_8H_7NO_2$
172	*trans*-3-(3-Thienyl)acrylic acid	154.19	$C_7H_6O_2S$
173	*trans*-4-Cotininecarboxylic acid	220.22	$C_{11}H_{12}N_2O_3$
174	Xanthene-9-carboxylic acid	226.23	$C_{14}H_{10}O_3$
175	Xanthurenic acid	205.17	$C_{10}H_7NO_4$
176	a,4-Dimethylphenylacetic acid	164.2	$CH_3C_6H_4CH(CH_3)CO_2H$
177	a-Acetamidocinnamic acid	205.21	$C_6H_5CH{=}C(NHCOCH_3)COOH$
178	4-Phenyl Butyric acid	164.2	$C_{10}H_{12}O_2$
178	4-Phenyl Butyric acid	164.2	$C_{10}H_{12}O_2$
179	Fmoc-6-aminonhexanoic acid	353.4	$C_{21}H_{23}NO_4$
180	Glutaric acid	132.12	$C_5H_8O_4$
181	Hydrocinnamic acid	150.18	$C_9H_{10}O_2$
182	Isophthalic acid	166.13	$C_8H_6O_4$
183	Isopropyl malonic acid	146.14	$C_6H_{10}O_4$
184	*N*-β-Fmoc-β-alanine	311.3	$C_{18}H_{17}NO_4$
185	*N*-γ-Fmoc-?-aminobutyric acid	325.4	$C_{19}H_{19}O_4$
186	Pyruvic acid	88.06	$C_3H_4O_3$