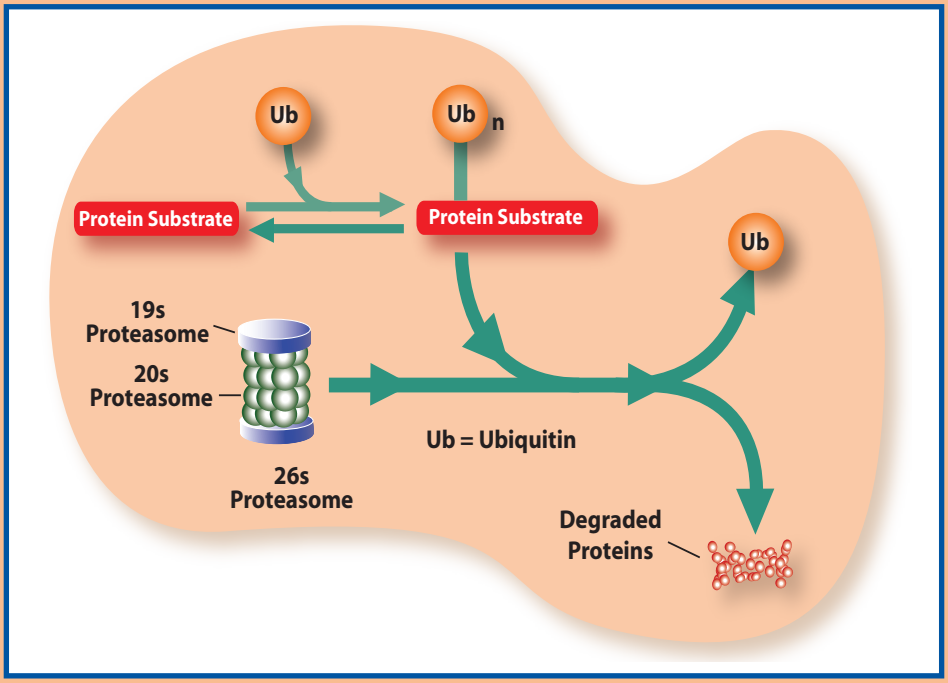
PEPTIDES INTERNATIONAL

Enzyme Inhibitors & Substrates

Cellular Housekeeping Peptides

The Ubiquitin-Proteasome Pathway

Accumulation of abnormal or damaged intracellular proteins in the body can disrupt normal functions such as cell cycle regulation and inflammatory processes, as well as contribute to neurogenerative diseases such as Alzheimer's. The ubiquitin-proteasome pathway is responsible for degrading intra-cellular protein that have been ubiquitin tagged.[1] Impairment of this pathway in individuals with Alzheimer's leads to accumulation of proteins which contributes to early onset of disease.[2-3]



The Ubitiquitin-Proteasome Pathway

It has also been recognized that inhibiting the ubiquitin-proteasomepathway may be beneficial. For example, inhibiting proteasomes in cancer cells can disrupt protein regulation, which can ultimately lead to apoptosis or programmed cell death of the malignant cells.[4] In addition, blocking proteasome activity reduces neuron and astrocyte degeneration and neutrophil infiltration and, therefore, could be potential therapy for stroke.[5,6] Studies have also shown that proteasome inhibitors may be beneficial in autoimmune encephalomyelitis and asthma as well.[7,8]

The peptide aldehydes, **MG 132**, **MG 115**, and **PSI** inhibit the proteasome complex's chymotrypsin-like activity in a potent but reversible manner. **Lactacystin** is a natural, irreversible, nonpeptide, cell permeable inhibitor that is more selective than peptide aldehydes but less selective than peptide boronates, another class of proteasome inhibitors.

Epoxomicin, originally isolated from a species of *Actinomycetes*, is cell-permeable, reversible and a relatively selective proteasome inhibitor. It is more potent than lactacystin and inhibits the chymotrypsin-like, trypsin-like, and peptidylglutamyl peptide-hydrolyzing activities of the proteasome.

Epoxoketones, such as epoxomicin, are the most selective because they not only react with the hydroxyl group but also the *N*-terminal threonine at the proteasome's active site. Current research efforts are aimed at evaluating the efficacy of epoxomicin in mouse models of myeloma and could prove valuable as an anticancer therapy.

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8. C.L. Vanderlugt, S.M. Rahbe, P.J. Elliott, M.C. Dal-Canto, And S.D. Miller, *J. Autoimmun.*,**14**, 205 (2000).

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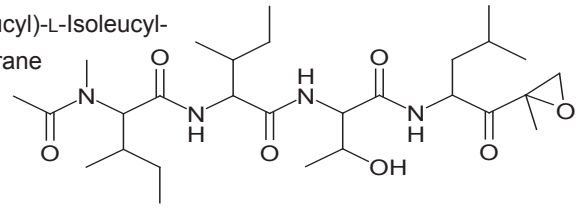
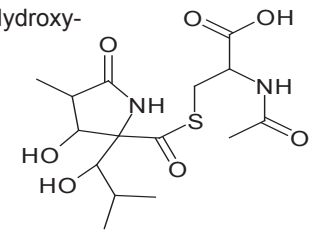
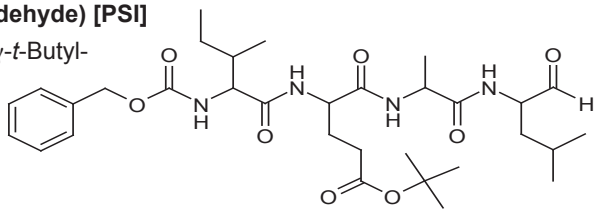
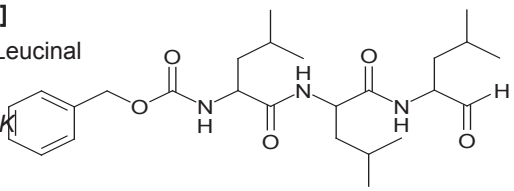
Proteasome Inhibitors Quick Reference

CODE	PRODUCT	QTY
IEP-4381-v	**Epoxomicin** (2*R*)-2-[Acetyl-(*N*-Methyl-L-Isoleucyl)-L-Isoleucyl-L-Threonyl-L-Leucyl]-2-Methyloxirane (M.W. 554.72) $C_{28}H_{50}N_4O_7$ *Inhibitor for Proteasome* Synthetic Product 	0.2 mg vial
ILC-4368-v	**Lactacystin** *N*-Acetyl-L-Cysteine, *S*-[2*R*,3*S*,4*R*]-3-Hydroxy-2-[(1*S*)-1-Hydroxy-2-Methylpropyl]-4-Methyl-5-Oxo-2-Pyrrolidinecarbonyl] (M.W. 376.43) $C_{15}H_{24}N_2O_7S$ *Inhibitor for Proteasome* Microbial Product 	0.2 mg vial
IUB-3207-v	**Ubiquitin Aldehyde (aldehyde)** Met*-Gln-Ile-Phe-Val-Lys-Thr-Leu-Thr-Gly-Lys-Thr-Ile-Thr-Leu-Glu-Val-Glu-Pro-Ser-Asp-Thr-Ile-Glu-Asn-Val-Lys-Ala-Lys-Ile-Gln-Asp-Lys-Glu-Gly-Ile-Pro-Pro-Asp-Gln-Gln-Arg-Leu-Ile-Phe-Ala-Gly-Lys-Gln-Leu-Glu-Asp-Gly-Arg-Thr-Leu-Ser-Asp-Tyr-Asn-Ile-Gln-Lys-Glu-Ser-Thr-Leu-His-Leu-Val-Leu-Arg-Leu-Arg-Gly-Gly-H (aldehyde) (M.W. 8564.7) $C_{378}H_{629}N_{105}O_{118}S$ *Inhibitor for Deubiquitinating Enzyme* Semi-synthetic Product J.R. Shaeffer and R.E. Cohen, *Biochemistry*, **35**, 10886 (1996). F. Melandri, L. Grenier, L. Plamondon, W.P. Huskey, and R.L. Stein, *Biochemistry*, **35**, 12893 (1996). S.H. Baek, K.S. Choi, Y.J. Yoo, J.M. Cho, R.T. Baker, K. Tanaka, and C.H. Chung, *J. Biol. Chem.*, **272**, 25560 (1997). L.C. Dang, F.D. Melandri, and R.L. Stein, *Biochemistry*, **37**, 1868 (1998).	50 µg vial
IAT-3169-v	**Z-Ile-Glu(OtBu)-Ala-Leu-H (aldehyde) [PSI]** Benzyloxycarbonyl-L-Isoleucyl-γ-*t*-Butyl-L-Glutamyl-L-Alanyl-L-Leucinal (M.W. 618.77) $C_{32}H_{50}N_4O_8$ *Inhibitor for Proteasome* Synthetic Product 	5 mg vial
IZL-3175-v	**Z-Leu-Leu-Leu-H (aldehyde) [MG 132]** Benzyloxycarbonyl-L-Leucyl-L-Leucyl-L-Leucinal (M.W. 475.63) $C_{26}H_{41}N_3O_5$ *Inhibitor for Proteasome and Cathepsin K* Synthetic Product 	5 mg vial

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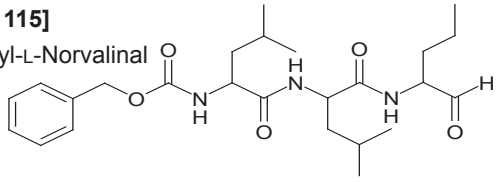
Ubiquitin-Proteasome Pathway

CODE	PRODUCT	QTY
IAT-3170-v	**Z-Leu-Leu-Nva-H (aldehyde) [MG 115]** Benzyloxycarbonyl-L-Leucyl-L-Leucyl-L-Norvalinal (M.W. 461.60) $C_{25}H_{39}N_3O_5$ *Inhibitor for Proteasome* Synthetic Product 	5 mg vial

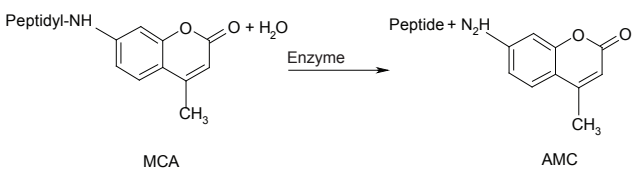
Proteasome Substrates Quick Reference

CODE	PRODUCT	QTY
MLR-3140-v	**Boc-Leu-Arg-Arg-MCA** *Substrate for Carboxyl Side of Paired Basic Residue Cleaving Enzyme and Proteasome*	5 mg
MLL-3120-v	**Suc-Leu-Leu-Val-Tyr-MCA** *Substrate for Chymotrypsin, Ingensin / Proteasome and Calpain*	5 mg
MLG-3176-v	**Z-Leu-Arg-Gly-Gly-MCA** *Substrate for Isopeptidase T*	5 mg
MLG-3179-v	**Z-Leu-Leu-Glu-MCA** *Substrate for Proteasome*	5 mg
MLL-3177-v	**Z-Leu-Leu-Leu-MCA** *Substrate for Proteasome*	5 mg
MPF-3096-v	**Pro-Phe-Arg-MCA** *Substrate for Pancreatic / Urinary Kallikrein and Proteasome*	5 mg
MAE-3160-v	**Suc-Ala-Glu-MCA** *Substrate for Ingensin / Proteasome*	5 mg
MVM-3156-v	**Z-Val-Lys-Met-MCA** *Substrate for Amyloid A4-Generating Enzyme and Proteasome*	5 mg

Please note: Carbobenzoxy (Cbz) = Benzyloxycarbonyl (Z) protecting group in peptide chemistry
AMC = 7-Amino-4-Methyl-Coumarin ≡ MCA = 4-Methyl - Coumaryl - Amide

Principle of Using Peptidyl-MCA Substrates

Protease activity leads to the cleavage of the peptide substrate from MCA, yielding fluorogenic AMC. Increase in AMC concentration due to cleavage can be monitored 1) flurometrically at λ ex = 380 nm and λ em = 460 nm and 2) photometrically at 370 nm.



MCA AMC

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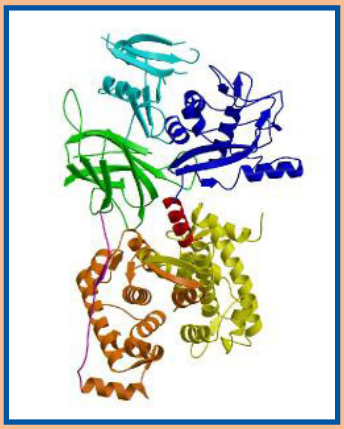
Calpain Inhibitors and Substrates: ALLN & ALLM

Calpain, a calcium-activated cysteine protease present in most mammalian tissues, is a potential therapeutic target for neurodegeneration prevention. Calpain has been implicated in neural cell death associated with cerebral ischemia, neurodegenerative processes such as Alzheimer's disease, myocardial ischemia, and cataract formation.[1]



Ac-Leu-Leu-Nle-H and Ac-Leu-Leu-Met-H aldehydes are non-specific cysteinyl protease inhibitors.[2] These cell-permeable aldehydes are most effective against the chymotrypsin-like proteolytic activity of the proteasomes. Both ALLN and ALLM should be useful for calpain function studies since this remains poorly understood.

CODE	INHIBITORS	QTY
IAL-3671-PI	**Ac-Leu-Leu-Nle-H (aldehyde)** ALLN, MG 101 Acetyl-L-Leucyl-L-Leucyl-L-Norleucinal (M.W. 383.54) $C_{20}H_{37}N_3O_4$ *Inhibitor for Calpain I and Proteasome*	5 mg
IAL-3678-PI	**Ac-Leu-Leu-Met-H (aldehyde)** ALLM Acetyl-L-Leucyl-L-Leucyl-L-Methioninal (M.W. 401.57) $C_{19}H_{35}N_3O_4S$ *Inhibitor for Calpain II and Proteasome*	5 mg
IES-4096	**E-64**	25 mg*
IEC-4320-v	**E-64-c**	5 mg
IED-4321-v	**E-64-d (Proinhibitor)**	5 mg
IZL-3178-v	**Z-Leu-Leu-H (aldehyde)**	5 mg
	SUBSTRATES	
MVL-3104-v	**Boc-Val-Leu-Lys-MCA**	5 mg
MLL-3120-v	**Suc-Leu-Leu-Val-Tyr-MCA**	5 mg

* Other packaging is available.

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