Supplemental Materials

Design, Synthesis, and Optimization of Novel Epoxide Incorporating Peptidomimetics as Selective Calpain Inhibitors

Isaac T. Schiefer[†], *Subhasish Tapadar*[†], *Vladislav Litosh*[†], *Marton Siklos*[†], *Rob Scism*[†], *Gihani T. Wijewickrama*[†], *Esala P. Chandrasena*[†], *Vaishali Sinha*[†], *Ehsan Tavassoli*[†], *Michael Brunsteiner*[†], *Mauro Fa'*[‡], *Ottavio Arancio*[‡] *Pavel Petukhov*[†], *and Gregory R. J. Thatcher*[†]*

[†]Department of Medicinal Chemistry and Pharmacognosy, University of Illinois College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612-7231

[‡]Department of Pathology & Cell Biology, The Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University, 630W 168th St., New York, NY 10032

E-mail: thatcher@uic.edu

* To whom correspondence about the manuscript should be sent: Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago (MC 781), 833 S. Wood St., Chicago, IL 60612-7231. Tel: 312-355-5282. Fax: 312 996 7107.

APPENDIX

Cal1$_{cat}$ expression and purification

Expression: *E. coli* strain BL21(DE3) was transformed with a pET24d-based plasmid construct containing active domains I-II of μ-calpain, the creation of which is described elsewhere.[1] 1 L cultures were incubated in LB medium containing 30 μg/mL kanamycin at 37 °C with shaking at 225 rpm, and induced with 0.5 mM IPTG after reaching an OD_{600} of 0.6. Following an additional 3 h, cell pellets were generated by centrifugation of 0.5 L aliquots (4 °C, 13,000 rpm, 30 min) and stored at -80 °C prior to use.

Growth conditions for Cal1cat expression : *E. coli* strain BL21(DE3) was transformed with a pET24d-based plasmid construct containing active domains I-II of μ-calpain, the creation of which is described elsewhere.[1] 1 L cultures were incubated in LB medium containing 30 μg/mL kanamycin at 37 °C with shaking at 225 rpm, and induced with 0.5 mM IPTG after reaching an OD_{600} of 0.6. Following an additional 3 h, cell pellets were generated by centrifugation of 0.5 L aliquots (4 °C, 13,000 rpm, 30 min) and stored at -80 °C prior to use.

Purification of Cal1$_{cat}$: Frozen cell pellets were resuspended in 25 mL Lysis Buffer (500 mM NaCl, 50 mM HEPES, pH 7.6, 20 mM imidazole, 1 mM PMSF, 100 ug/mL lysozyme) and incubated on ice for 30 min. Cells were lysed by sonication and clarified by centrifugation (4 °C, 13,000 rpm, 20 min). Lysate was applied to Ni^{2+}-affinity columns (HisTrap FF crude, 5 mL, GE Healthcare) for purification. Proteins were eluted with a linear gradient of 100:0 (A:B) to 0:100 (A:B) (Buffer A = 500 mM NaCl, 50 mM HEPES, pH 7.6, 20 mM imidazole. Buffer B = 500 mM NaCl, 250 mM imidazole, 50 mM HEPES, pH 7.6.) at 5 mL/min and collected in 5 mL

fractions containing 200 µL Receiving Buffer (50 mM HEPES, pH 7.6, 4 mM EDTA, 1 mM TCEP; final concentration). Eluted protein was concentrated by 10K molecular weight filter (Millipore), and exchanged into Storage Buffer (150 mM NaCl, 50 mM HEPES, pH 7.6, 5% glycerol (v/v), 1 mM TCEP, 100 µM EDTA) via gel filtration (HiTrap Desalting, 5 mL, GE Healthcare). Concentrated enzyme was stored in 20 µL aliquots at -80 °C. Final purified enzyme protein concentration was determined by bicinchoninic acid assay (Thermo Scientific) following the manufacturer's protocol against BSA standards, diluted with Storage Buffer. Proteins were analyzed by 4-12 % polyacrylamide gel (NuPAGE) loaded with 7 µL marker (Precision Plus Protein Kaleidoscope Standard, BioRad Inc), Gels were stained with Bio SafeCoomassie (BioRad, Inc).

µ I-II Sequence:

MGRHENAIKYLGQDYENLRARCLQNGVLFQDDAFPPVSHSLGFKELGPNSSKTYGIKW
KRPTELLSNPQFIVDGATRTDICQGALGDCWLLAAIASLTLNETILHRVVPYGQSFQEGY
AGIFHFQLWQFGEWVDVVVDDLLPTKDGKLVFVHSAQGNEFWSALLEKAYAKVNGSY
EALSGGCTSEAFEDFTGGVTEWYDLQKAPSDLYQIILKALERGSLLGCSINISDIRDLEAIT
FKNLVRGHAYSVTDAKQVTYQGQRVNLIRMRNPWGEVEWKGPWSDNSYEWNKVDPY
EREQLRVKMEDGEFWMSFRDFIREFTKLEICNLTPDLEHHHHHH

Figure S1. Cal1$_{cat}$ Sequence Alignment

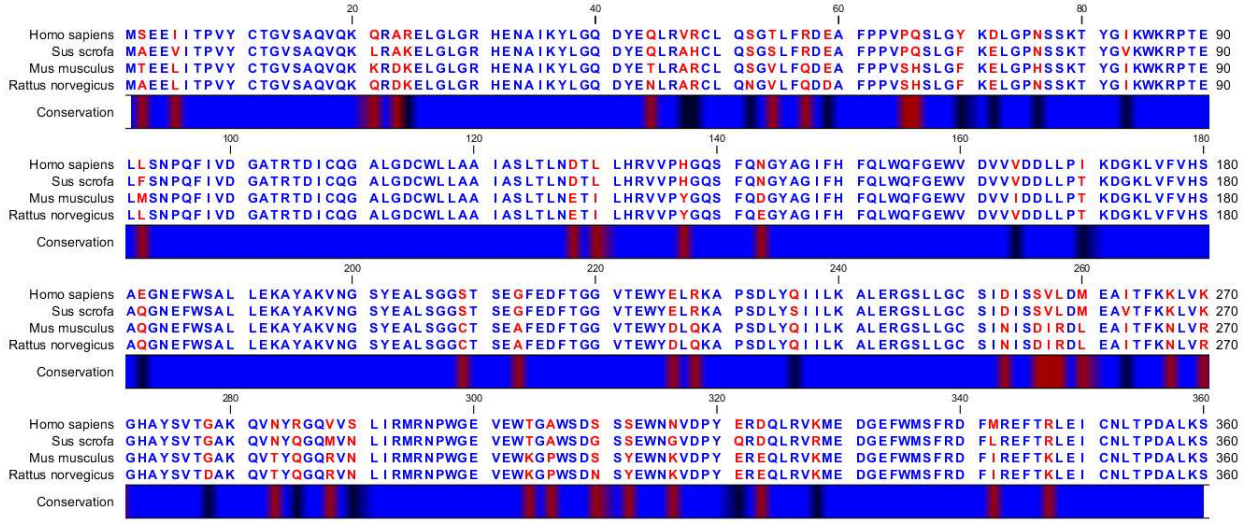


Figure S1. Active site cysteine is 115 in the "mini calpains alignment" and 132 in "whole calpains alignment". Alignment was performed with CLC Sequence viewer (free version) using sequences downloaded from pubmed.

Calpain and Papain Inhibition Kinetics

Full length porcine calpain (156 nM), or papain (236 pM) was added to a solution of 100 mM NaCl, 50 mM HEPES, pH 7.6, 1 mM TCEP, 30 μM Suc-LLVY-AMC substrate, and inhibitor (0.5 to 50 μM). Calpain reactions also contained $CaCl_2$ (1 mM and 100 mM for porcine and rat respectively). Both substrate and inhibitors were dissolved in acetonitrile/DMSO (1:1) with the exception of E-64, dissolved in water. Organic solvent remained < 2 % in all reactions, and most often < 1 %. Reactions were carried out in microtiter 96-well plates, with 150 μL per well, 30 °C, and product formation was monitored over time by fluorescence (Ex/Em 346/444 nm, with 420 nm cutoff filter). Kinetic values of k_{obs} presented in the body of the manuscript were determined

via non-linear regression using one-phase association analysis and linear plots of 1/ k_{obs} vs. 1/[I] provided kinetic constants k_i and K_I from the data shown below (Figure S1-S2).

LC-MS/MS examination of inhibitor modified $Cal1_{cat}$ active site

The recombinant $Cal1_{cat}$ (5 µM) was activated via addition of $CaCl_2$ (10 mM) and incubated with **E-64** and/or **22a** (5 µM) for 30 min. The reaction was quenched with EDTA (10 µM) and reaction mixture ran on a SDS PAGE gel. The $Cal1_{cat}$ containing band was cut from the gel and submitted to in-gel alkylation with IAA (100 mM) for 60 min, followed by trypsin digestion. LC-MS/MS was carried out on an Agilent 6300 Ion-Trap LC/MS. The resulting TIC and m/z of modified peptide fragments are described below.

Calculated m/z for the active site peptide:

TDICQGALGDCWLLAAIASLTLNETILHR

Theoretical m/z = 3113.6 (+3) MW:1038.8, (+4) MW 779.2.

Calculated m/z for IAA modified active site peptide sequence:

TDIC(Carbamidomethyl)QGALGDC(Carbamidomethyl)WLLAAIASLTLNETILHR

Theoretical m/z = 3227.8 (+3); 1076.8, (+4); MW 807.6.

Observed m/z = (+3) 1076.5; (+4) 807.7.

Calculated m/z for E-64 and IAA modified active site peptide sequence: Sequence-

TDIC(Carbamidomethyl)QGALGDC(E64)WLLAAIASLTLNETILHR

Theoretical m/z = 3528.1 (+3); 1176.1, (+4); MW 882.1.

Observed m/z = (+3); 1176.4, (+4) MW 882.8.

Figure S2. Comparison of TIC from digest of Cal1 and Cal1$_{cat}$

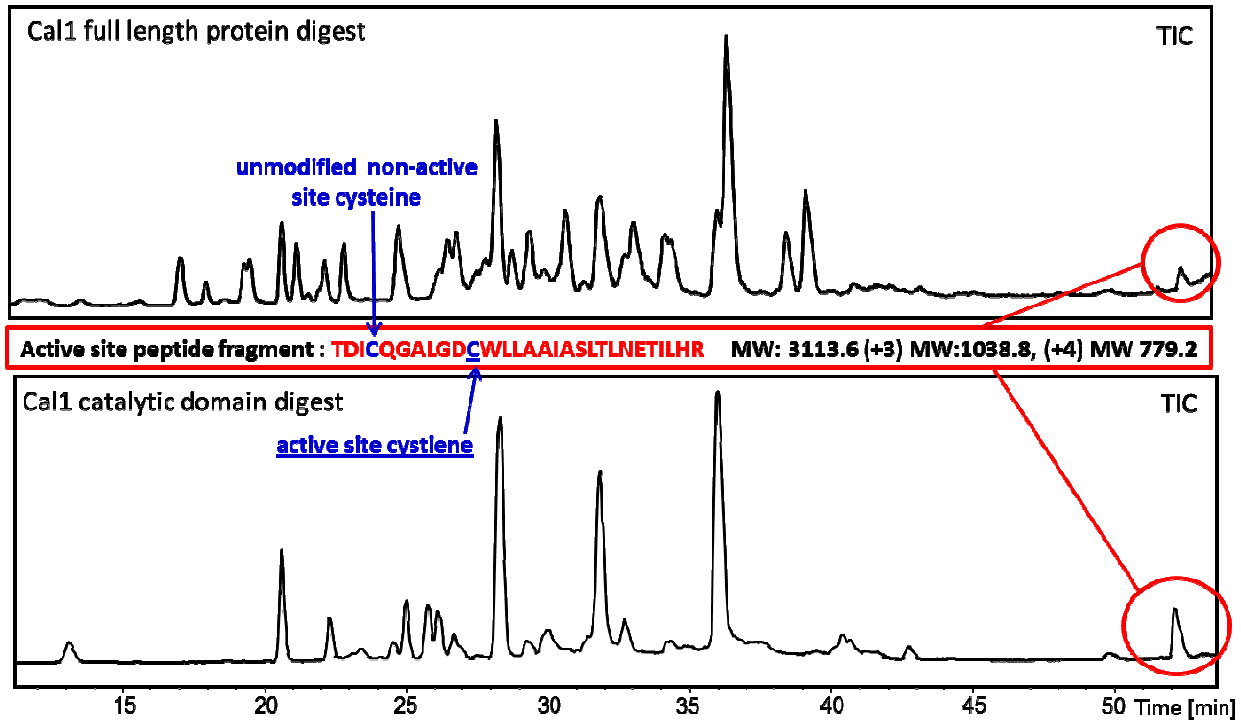


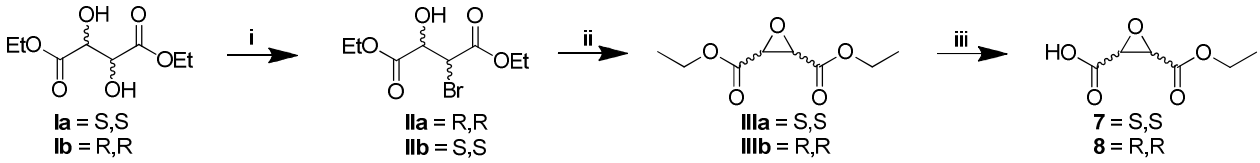
Figure S2. Total ion chromatograms of the resulting peptides from tryptic digest of Cal full length protein (top) and recombinant Cal1 catalytic domain (bottom). Digest of each protein affords an identical active site peptide ion (circled in red), which contains the active site cysteine, and one unmodified non-active site cysteine.

Supplementary Synthesis

General Methods: Unless stated otherwise, all reactions were carried out under an atmosphere of dry argon in oven-dried glassware. Indicated reaction temperatures refer to those of the reaction bath, while room temperature (rt) is noted as 25 °C. Dichloromethane (CH_2Cl_2) was distilled over CaH_2, and THF distilled over Na(s). All other solvents were of anhydrous quality purchased from Aldrich Chemical Co. and used as received. Pure reaction products were

typically dried under high vacuum in the presence of phosphorus pentoxide. Commercially available starting materials and reagents were purchased from Aldrich, TCI and Fisher Scientific and were used as received unless specified otherwise. Analytical thin layer chromatography (TLC) was performed with (5 x 20 cm, 60 Å, 250 μm). Visualization was accomplished using a 254 nm UV lamp. ^{1}H and ^{13}C NMR spectra were recorded on either a Bruker Avance 400 MHz spectrometer or Bruker DPX 400 MHz spectrophotometer. Chemical shifts are reported in ppm with the solvent resonance as internal standard ([$CDCl_3$ 7.27 ppm, 77.23 ppm] [DMSO-d_6 2.5 ppm, 39.51 ppm] and [MeODd_4 4.78, 49.0] for ^{1}H, ^{13}C respectively). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, br = broad, m = multiplet, abq = ab quartet), number of protons, and coupling constants. Low-resolution mass spectra (LRMS) were acquired on an Agilent 6300 Ion-Trap LC/MS. High resolution mass spectral data was collected in-house using a Shimadzu QTOF 6500.

Scheme S1. Epoxide warhead synthesis



Reagents: i) HBr (33 %) in acetic acid, r.t, 12 h, then acetyl chloride, reflux, 12 h, 75-80 %; ii) DBU, Et_2O, 0°C, 12 h, 70-80 %; iii) Ethanolic KOH, 0°C, 6 h, 75-85 %.

General procedure for large scale production of the bromohydrin (IIa & IIb). HBr (33 % in acetic acid, 175 ml, 970 mmol) was added dropwise to D-DET (50 g, 242 mmol) at 0 °C over 1 h,

and the reaction allowed to warm to r.t. and stirred for 12 h. The reaction mixture was then poured into ice (~500 g), and the resulting aqueous layer extracted with Et2O (4 x 200 ml). The combined organic extracts were then washed with dH2O (2 x 300 ml), brine (2 x 200 ml), dried over Na2SO4 and concentrated under reduced pressure. The resulting pale yellow oil was dissolved in anhydrous EtOH (300 mL) and acetyl chloride (8 ml, 242 mmol) was added dropwise. The solution was heated under gentle reflux for 7 h, cooled to r.t., and concentrated under reduced pressure to give yellow oil. The crude product was purified by under reduced pressure by fractional vacuum distillation to afford the desired bromohydrins as clear colorless oils (**2a**, 54.0 g, 99.1 %; **2b**, 51.2 g, 94.0 %).

(2R,3R)-diethyl 2-bromo-3-hydroxysuccinate (IIa). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.73-4.72 (d, 1H, 4.0 Hz); 4.69-4.68 (bt, 1H); 4.34-4.24 (m, 4H); 3.43-3.41 (d, 1H, J = 7.2 Hz); 1.35-1.31 (t, 6H, J = 14.4 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): 170.27, 166.62, 72.51, 62.84, 62.62, 47.70, 14.03, 13.92.

(2S,3S)-diethyl 2-bromo-3-hydroxysuccinate (IIb). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.73-4.72 (d, 1H, 4.0 Hz); 4.69-4.68 (bt, 1H); 4.34-4.24 (m, 4H); 3.43-3.41 (d, 1H, J = 7.2 Hz); 1.35-1.31 (t, 6H, J = 14.4 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): 170.27, 166.62, 72.51, 62.84, 62.62, 47.70, 14.03, 13.92.

(2S,3S)-diethyl oxirane-2,3-dicarboxylate (IIIa). IIa (38.1 g, 142 mmol) was dissolved in anhydrous Et_2O (100 ml) and DBU (32.3 g, 212.4 mmol, in Et_2O [50 ml]) was added dropwise at 0 °C for 1 h. The reaction was allowed to warm to r.t. and stirred for an additional 4 h. Reaction was quenched with cold 1N HCl to pH ~5. The resulting aqueous layer was extracted with Et_2O (3 x

150 ml). The combined organic extracts were washed with brine (2 x 100 ml), dried over Na_2SO_4, and concentrated to give the pure product as faintly yellow oil (21.5 g, 80.7 % yield). Product purity typically reflects that of the bromohydrin starting material. If necessary, fractional vacuum distillation can be used for purification. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.36-4.22 (m, 4H); 3.67 (s, 2H); 1.35-1.31 (t, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): 166.75, 62.21, 52.01, 14.01.

(2R,3R)-diethyl oxirane-2,3-dicarboxylate (IIIb). Followed general procedure for **IIIa** using the following quantities: **IIb** (34. 6 g, 129 mmol); Et_2O (100 ml); DBU (29.4 g, 193 mmol, in Et_2O [50 ml]); yielded **IIIb** as a colorless oil (18.1 g, 74.8 %).^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.36-4.22 (m, 4H); 3.67 (s, 2H); 1.35-1.31 (t, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): 166.75, 62.21, 52.01, 14.01.

(2S,3S)-3-(ethoxycarbonyl)oxirane-2-carboxylic acid (7). An ethanolic solution of KOH (4.2 g, 64 mmol, in EtOH [50 ml]) was added dropwise to the **IIIa** (9.7 g, 63 mmol) in EtOH (20 ml) at 0 °C for 30 min, and then the reaction was continued at r.t. for 8 h. The resulting viscous solution was diluted with ice water (150 ml), extracted with EA (2 x 200 ml). The aqueous layer was acidified with 1N HCl to pH~4, and extracted with EA (3 x 150 ml). Combined organic extracts washed with brine (2 x 50 ml), dried over Na_2SO_4, and concentrated to afford the pure epoxide monoester **7** as colorless oil (8.9 g, 86.9 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.57 (br, 1H); 4.35-4.24 (m, 2H); 3.73(s, 1H); 3.72 (s, 1H); 1.36-1.32 (t, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): 171.61, 166.40, 62.45, 52.20, 51.45, 13.89.

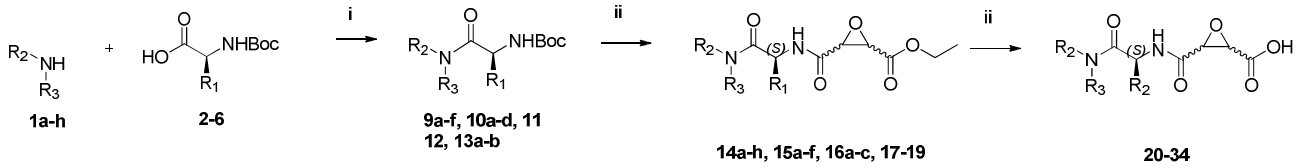
(2R,3R)-3-(ethoxycarbonyl)oxirane-2-carboxylic acid (8). Followed general procedure for **7** using the following quantities: **IIIb** (15.2 g, 81 mmol); KOH (4.53 g, 81 mmol, in EtOH [50 ml]); yielded **8** as a colorless oil (9.5 g, 73.5 %).^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.57 (br, 1H); 4.35-4.24

(m, 2H); 3.73(s, 1H); 3.72 (s, 1H); 1.36-1.32 (t, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): 171.61, 166.40, 62.45, 52.20, 51.45, 13.89.

Optimized procedure for coupling of the epoxide monoester with the peptidomimetic amine

TFA (10 eq) was added to a suspension of compound the boc protected peptidomimetic (1 eq) in freshly distilled CH_2Cl_2 (10 ml/ 1 mmol peptidomimetic) at 0 °C and the reaction mixture stirred at the same temperature for 6 h. If needed, additional TFA (5 eq) was added at 0 °C every 30 min until no starting material remained on TLC. The reaction was then concentrated under vacuo and the residual TFA-salt was dissolved in MeOH/H_2O and brought to pH of ~ 7 using a sat. $NaHCO_3$ solution, followed by extraction with CH_2Cl_2 (3 x 50 ml) and removal of solvent in vacuo to afford the pure free amine, which was dried under high vacuum and used in the next reaction without further purification. A round bottom was charged with the epoxide monoacid (1.0 eq) dissolved in minimal DMF (~2 ml/mmol) with DIPEA (1.1 eq) under argon and maintained at 0 ºC. EDCI (1.0 eq) was then added in one portion and the suspension stirred until homogenous (typically < 5 min), followed by the addition of HOBt (1.2 eq). After stirring for an additional 15 min, the free amine (1.0 eq) in DMF (~3 ml/mmol) was added dropwise, and the reaction allowed to warm to room temperature, monitored by TLC. After 12 h, the reaction was quenched acidified to pH ~ 4 with 1 N HCl, extracted CH_2Cl_2 (3x). Combined organic extracts were washed with 1 N HCl (2x), and brine (1x), dried over Na_2SO_4, concentrated in vacuo, and purified by column chromatography to give the desired peptidomimetic scaffolds.

Scheme S2. Synthesis of 1st and 2nd generation epoxide esters



Reagents: i) EDCI, HOBT, DIPEA, CH_2Cl_2, 0°C; ii) **7** or **8**, EDCI, HOBT, DIPEA, DMF, 0°C; iii) LiOH, THF/MeOH/H_2O, 0°C.

Table S1. Structure designations for epoxide esters 14-18.

Compound	R1	R2	R3	Stereochemistry
14a	L-Leucine	Phenyl	H	*S,S,S*
14b	L-Leucine	2,6 difluorophenyl	H	*S,S,S*
14c	L-Leucine	2,6 difluorophenyl	H	*S,R,R*
14d	L-Leucine	4-(4-Fluorophenyl)thiazol-2-amine	H	*S,S,S*
14e	L-Leucine	4-(4-Fluorophenyl)thiazol-2-amine	H	*S,R,R*
14f	L-Leucine	4-F-Phenyl-SO2-NH-(CH2)4-NH2	H	*S,S,S*
14g	L-Leucine	Lipoyl-NH-(CH2)4-NH2	H	*S,S,S*
14h	L-Leucine	D-Biotin-NH-(CH2)4-NH2	H	*S,S,S*
15a	L-Histidine	Phenyl	H	*S,S,S*
15b	L-Histidine	1,3,5 trimethylaniline	H	*S,S,S*
15c	L-Histidine	4-(4-Fluorophenyl)thiazol-2-amine	H	*S/R,S,S*
15d	L-Histidine	4-(4-Fluorophenyl)thiazol-2-amine	H	*S/R,R,R*
15e	L-Histidine	6-fluorobenzo[d]thiazol-2-amine	H	*S/R,S,S*
15f	L-Histidine	6-fluorobenzo[d]thiazol-2-amine	H	*S/R,R,R*
16a	L-Ala(4-thiazoyl)-OH	Phenyl	H	*S,S,S*
16b	L-Ala(4-thiazoyl)-OH	4-(4-Fluorophenyl)thiazol-2-amine	H	*S,S,S*
16c	L-Ala(4-thiazoyl)-OH	4-(4-Ethynyl)thiazol-2-amine	H	*S,S,S*

17	L-His(2-Me)-OH	4-(4-Fluorophenyl)thiazol-2-amine	H	*S,S,S*
18	L-His(4-Me)-OH	4-(4-Fluorophenyl)thiazol-2-amine	H	*S,S,S*
19	L-Ala(4-thiazoyl)-OH	Propargylamine	H	*S,S,S*

(2S,3S)-ethyl-3-((S)-4-methyl-1-oxo-1-(phenylamino)pentan-2-ylcarbamoyl)oxirane-2-carboxylate (14a). General procedure using: Boc-protected peptidomimetic (570 mg, 1.1 mmol); (2*S*, 3*S*)-epoxysuccinic acid monoester (126 mg, 0.93 mmol), DIPEA (0.16 mL, 0.93 mmol), and HOBT (126 mg, 0.93 mmol) CH_2Cl_2 (10 ml)/DMF (2 ml); EDCI (220 mg, 1.1 mmol) in DMF (1 mL) gave the title compound (100 mg, 30.6 %) as white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.57-7.55 (d, 2H, J = 7.67 Hz); 7.34-7.30 (t, 2H); 7.13-7.10 (t, 1H); 4.63-4.61 (m, 1H); 4.30-4.24 (m, 2H); 3.729-3.725 (d, 1H, J = 1.67 Hz); 3.62-3.61 (d, 1H, J = 1.72 Hz); 1.76-1.63 (m, 3H); 1.33-1.29 (t, 3H); 1.02-1.00 (t, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): 171.22, 169.07, 167.15, 137.98, 128.41, 124.12, 120.14, 52.90, 52.58, 52.50, 51.70, 40.70, 29.50, 24.65, 22.04, 20.60.

(2S,3S)-ethyl-3-((S)-1-(2,6-difluorophenylamino)-4-methyl-1-oxopentan-2-ylcarbamoyl)oxirane-2-carboxylate (14b). General procedure using: Boc-protected peptidomimetic (600 mg, 1.7 mmol); (2*S*, 3*S*)-epoxysuccinic acid monoester (280 mg, 1.7 mmol), DIPEA (0.45 mL, 2.6 mmol), in CH_2Cl_2 (10 ml)/DMF (5 ml); EDCI (280 mg, 1.7 mmol) in DMF (1 mL) gave the title compound (210 mg, 31.6 %) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (s, 1H); 7.18-7.16 (t, 1H); 6.94-6.90 (t, 2H); 6.72-6.70 (d, 1H); 4.86-4.85 (q, 1H); 4.29-4.23 (m, 2H**);** 3.83-3.80 (d, 1H, J = 1.69 Hz); 3.72-3.55 (d, 1H, J = 1.69 Hz); 1.84-1.70

(m, 3H); 1.30-1.26 (t, 3H); 1.01-0.99(d, 6H). ^{13}C NMR (DMSO-d_6, 100 MHz): 170.10, 166.5, 166.39, 158.92, 156.46, 127.73, 111.88, 111.54, 164.9, 62.27, 53.9, 52.9, 51.01, 41.2, 24.74, 22.5, 14.0.

(2R,3R)-ethyl-3-((S)-1-(2,6-difluorophenylamino)-4-methyl-1-oxopentan-2-ylcarbamoyl)oxirane-2-carboxylate (14c). General procedure using: Boc-protected peptidomimetic (550 mg, 1.6mmol); (2*S*, 3*S*)-epoxysuccinic acid monoester (250 mg, 1.6 mmol), DIPEA (0.65 mL, 3.8 mmol), and HOBT (250 mg, 1.9 mmol) in CH_2Cl_2 (15 ml)/DMF (5 ml); EDCI (300 mg, 2.0 mmol) in DMF (1 mL) gave the title compound (25 mg, 41.6 %) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (s, 1H); 7.19-7.17 (t, 1H); 6.94-6.90 (t, 2H); 6.72-6.70 (d, 1H); 4.86-4.85 (q, 1H); 4.31-4.25 (m, 2H); 3.84-3.81 (d, 1H, J = 1.69 Hz); 3.73-3.56 (d, 1H, J = 1.69 Hz); 1.84-1.69 (m, 3H); 1.32-1.28 (t, 3H); 1.02-1.00 (d, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): 170.10, 166.67, 166.39, 158.92, 156.46, 127.73, 111.78, 111.54, 62.28, 53.67, 52.69, 51.21, 40.81, 24.74, 22.74, 22.11, 13.97.

(2S,3S)-ethyl-3-((S)-1-(4-(4-fluorophenyl)thiazol-2-ylamino)-4-methyl-1-oxopentan-2-ylcarbamoyl)oxirane-2-carboxylate (14d). General procedure using: Boc-protected peptidomimetic (410 mg, 1.0 mmol); (2*S*, 3*S*)-epoxysuccinic acid monoester (160 mg, 1.0 mmol), DIPEA (0.18 mL, 1.0 mmol), and HOBT (163 mg, 1.2 mmol) in CH_2Cl_2 (10 ml); EDCI (211 mg, 1.1 mmol) in DMF (0.5 mL) gave the title compound (203 mg, 40.1 %) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.96 (s, 1H); 7.79-7.76 (q, 2H); 7.11-7.07 (m, 3H); 6.57-6.55 (d, 1H, J = 8.4 Hz); 4.80-4.78 (1, 1H); 4.29-4.23 (m, 2H); 3.868-3.864 (d, 1H, J = 1.60 Hz); 3.522-3.581 (d, 1H, J = 1.60 Hz); 1.86-1.81 (m, 1H); 1.66-1.61 (m, 2H); 1.28-1.24 (m, 4H); 0.98-0.94

(t, 6H). ^{13}C NMR (DMSO-d_6, 100 MHz): 170.28, 167.48, 165.78, 163.43, 161.00, 158.23, 148.34, 135.36, 131.32, 128.17, 116.13, 108.52, 62.01, 53.71, 53.36, 51.82, 14.33. ESI-LRMS (m/z): $[M+H]^+$ = 450.

(2R,3R)-ethyl-3-((S)-1-(4-(4-fluorophenyl)thiazol-2-ylamino)-4-methyl-1-oxopentan-2-ylcarbamoyl)oxirane-2-carboxylate (14e). General procedure using: Boc-protected peptidomimetic (290 mg, 0.71 mmol); (2*S*, 3*S*)-epoxysuccinic acid monoester (113 mg, 0.71 mmol), DIPEA (0.13 mL, 0.71 mmol), and HOBT (115 mg, 0.85 mmol) in DMF (3 ml); EDCI (150 mg, 0.78 mmol) in DMF (0.5 mL) gave the title compound (100 mg, 31.5 %) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.96 (s, 1H); 7.79-7.76 (q, 2H); 7.11-7.06 (m, 3H); 6.48-6.46 (d, 1H, J = 8.4 Hz); 4.71-4.69 (1, 1H); 4.25-4.22 (m, 2H); 3.78-3.77 (d, 1H, J = 1.20 Hz); 3.60-3.59 (d, 1H, J = 1.20 Hz); 1.86-1.81 (m, 1H); 1.71-1.60 (m, 2H); 1.30-1.25 (m, 4H); 0.98-0.96 (t, 6H). ^{13}C NMR (DMSO-d_6, 100 MHz): 170.24, 167.46, 165.58, 163.43, 161.00, 158.20, 148.34, 135.36, 131.29, 128.09, 115.92, 108.52, 62.01, 53.52, 53.41, 51.85, 14.33. ESI-LRMS (m/z): $[M+H]^+$ = 450.

(2S,3S)-ethyl-3-((S)-1-(4-(4-fluorophenylsulfonamido)butylamino)-4-methyl-1-oxopentan-2-ylcarbamoyl)oxirane-2-carboxylate (14f). General procedure using: Boc-protected peptidomimetic (442 mg, 1.0 mmol); (2*S*, 3*S*)-epoxysuccinic acid monoester (154.1 mg, 0.96 mmol), DIPEA (0.17 mL, 0.96 mmol), and HOBT (157 mg, 1.15 mmol) in DMF (5 ml); EDCI (203 mg, 1.05 mmol) in DMF (0.5 mL) gave the title compound (321 mg, 60.5 %) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89-7.86 (m, 2H); 7.21-7.19 (t, 2H); 6.87-6.85 (d, 1H, J = 8.40 Hz); 6.32 (bs, 1H); 5.25 (t, 1H); 4.27-4.25 (q, 1H); 4.22-4.19 (m, 2H); 3.71-3.70 (d, 1H. J = 1.60

Hz); 3.48-3.47 (d, 1H, J = 1.60 Hz); 3.28-3.12 (m, 2H); 2.95-2.93 (d, 2H); 1.62-1.45 (m, 9H); 1.33-1.30 (t, 3H); 0.94-0.90 (t, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): 171.52, 166.67, 166.63, 166.51, 163.98, 130.01, 129.92, 116.66, 116.44, 62.65, 53.99, 53.23, 51.75, 42.98, 41.20, 39.15, 26.80, 26.63, 25.06, 23.06, 22.19, 14.25. ESI-LRMS (m/z): $[M+H]^+$ = 502.

(2S,3S)-ethyl-3-((2S)-1-((5-(1,2-dithiolan-3-yl)pentanamido)methylamino)-4-methyl-1-oxopentan-2-ylcarbamoyl)oxirane-2-carboxylate (14g). General procedure using: Boc-protected peptidomimetic (170 mg, 0.35 mmol); (2*S*, 3*S*)-epoxysuccinic acid monoester (56 mg, 0.35 mmol), DIPEA (0.06 mL, 0.35 mmol), and HOBT (56 mg, 0.42 mmol) in DMF (2 ml); EDCI (73mg, 1.2 mmol) in DMF (0.5 mL) gave the title compound (53 mg, 28 %) as white solid. ^1H NMR ($CDCl_3$, 400 MHz): δ 7.23-7.21 (d, 1H, J = 8.4Hz); 7.18-7.15 (t, 1H); 6.33 (t, 1H); 4.51-4.49 (q, 1H); 4.28-4.24 (m, 2H); 3.717-3.713 (d, 1H, J = 1.60 Hz); 3.60-3.57 (m, 1H); 3.524-3.520 (d, 1H, J = 1.60 Hz); 3.18-3.11 (m, 7H); 2.47-2.45 (m, 1H); 2.22-2.18 (t, 2H); 1.93-1.90 (m, 1H); 1.67-1.52 (m, 15H); 1.33-1.30 (dd, 4H); 0.94-0.90 (t, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz):173.66, 171.94, 167.13, 166.52, 62.85, 56.95, 56.92, 54.28, 53.28, 52.01, 41.89, 40.73, 40.10, 39.72, 39.40, 39.23, 38.94, 36.87, 35.07, 29.38, 29.36, 27.65, 27.50, 26.89, 26.27, 25.90, 25.32, 23.41, 22.48, 14.52. ESI-LRMS (m/z): $[M+H]^+$ = 532.

(2S,3R)-ethyl-3-((S)-4-methyl-1-oxo-1-(4-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)butylamino)pentan-2-ylcarbamoyl)oxirane-2-carboxylate (14h). General procedure using: Boc-protected peptidomimetic (570 mg, 1.1 mmol); (2*S*, 3*S*)-epoxysuccinic acid monoester (176 mg, 1.1 mmol), DIPEA (0.2 mL, 1.1 mmol), and HOBT (178 mg, 1.3 mmol) in DMF (5 ml); EDCI (232 mg, 1.2 mmol) in DMF (1 mL) gave the title

compound (624 mg, 85 %) as white solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.62 (d, 1H); 8.08 (t, 1H); 7.74 (t, 1H); 6.42 (s, 1H); 6.36 (s, 1H); 4.31 (m, 2H); 4.18 (m, 3H); 3.71 (d, 1H); 3.59 (d, 1H); 3.12 (m, 1H); 3.01 (m, 4H); 2.92 (dd, 1H); 2.58 (d, 1H); 2.04 (t, 2H); 1.49 (m, 7H); 1.35 (m, 6H); 1.23 (t, 3H); 0.89 (d, 3H); 0.83 (d, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): 171.7; 170.9; 167.1; 164.4; 162.6; 61.4; 60.9; 59.1; 55.3; 52.8; 51.1; 41.0; 38.2; 37.9; 35.1; 28.1; 27.9; 26.5; 26.4; 25.2; 24.1; 22.8; 21.5; 13.8. ESI-LRMS (m/z): $[M+H]^+$ = 570.

(2S,3S)-ethyl-3-((S)-3-(1H-imidazol-5-yl)-1-oxo-1-(phenylamino)propan-2-ylcarbamoyl)oxirane-2-carboxylate (15a). General procedure using: Boc-protected peptidomimetic (463 mg, 1.4 mmol); (2*S*, 3*S*)-epoxysuccinic acid monoester (220 mg, 1.4 mmol), DIPEA (0.24 mL, 1.4 mmol), and HOBT (230 mg, 1.7 mmol) in DMF (5 ml); EDCI (300 mg, 1.5 mmol) in DMF (0.5 mL) gave the title compound (180 mg, 34.5 %) as white solid.^{1}H NMR (400 MHz, MeOD-d^4): δ 7.63 (s, 1H); 7.52-7.50 (d, 2H, J = 7.72 Hz); 7.33-7.31 (t, 2H); 7.13-7.11 (t, 1H); 6.90 (s, 1H); 4.76-4.75 (t, 1H); 4.29-4.24 (q, 2H); 3.69-3.68 (d, 1H, J = 1.56 Hz); 3.64-3.63 (d, 1H, J =1.56 Hz); 3.17-3.05 (qd, 2H) ; 1.33-1.29 (t, 3H). ^{13}C NMR (MeOD-d^4, 100 MHz): 169.75, 167.25, 166.90, 137.85, 134.98, 127.36, 124.19, 119.88, 61.73, 54.15, 52.90, 51.60, 12.88.

(2S,3S)-ethyl-3-((S)-3-(1H-imidazol-5-yl)-1-(mesitylamino)-1-oxopropan-2-ylcarbamoyl)oxirane-2-carboxylate (15b). General procedure using: Boc-protected peptidomimetic (180 mg, 0.47 mmol); (2*S*, 3*S*)-epoxysuccinic acid monoester (75 mg, 0.47 mmol), DIPEA (0.12 mL, 0.7 mmol), and HOBT (76 mg, 0.56 mmol) in DMF (5 ml); EDCI (99 mg, 0.52 mmol) in DMF (0.5 mL) gave the title compound (55 mg, 28.3 %) as white solid. ^{1}H

NMR (400 MHz, MeOD-d^4): δ 7.66 (s, 1H); 6.96 (s, 1H) ; 6.88 (s,1H); 4.27-4.25 (q, 2H); 3.68 (s, 1H); 3.32 (s, 1H); 3.21-3.07 (m, 2H); 2.25 (s, 3H); 2.06 (s, 6H); 1.32-1.29 (t, 3H). ^{13}C NMR (MeOD): ^{13}C NMR (MeOD-d^4, 100 MHz): 167.22, 136.74, 135.14, 128.25, 61.74, 52.98, 51.80, 19.53, 16.77, 12.88. MS (ESI) *m*/*z* 415.2 $(M+H)^+$.

(2S,3S)-ethyl-3-(1-(4-(4-fluorophenyl)thiazol-2-ylamino)-3-(1H-imidazol-4-yl)-1-oxopropan-2-ylcarbamoyl)oxirane-2-carboxylate (15c). General procedure using: the racemic Boc-protected peptidomimetic (400 mg, 1.2 mmol); (2*S*, 3*S*)-epoxysuccinic acid monoester (160 mg, 1.0 mmol), DIPEA (0.43 mL, 2.5 mmol) and HOBT (76 mg, 0.56 mmol) in DMF (5 ml); EDCI (210 mg, 1.1 mmol) in DMF (0.5 mL) gave the title compound (217 mg, 45.8 %) as a racemic mixture in the form of a white solid. ^{1}H NMR (DMSO-d^6, 400 MHz): δ 12.50 (bs, 1H); 11.90 (bs, 1H); 8.85-8.76 (dd, 1H, J = 7.14 Hz); 7.95-7.91 (q, 2H); 7.64-7.58 (m, 2H); 7.28-7.24 (t, 2H); 6.84 (bs, 1H); 4.79-4.75 (q, 1H); 4.22-4.18 (q, 2H); 3.74-3.73 (dd, 1H, J = 1.80 Hz); 3.63-3.62 (dd, 1H; J = 1.80 Hz); 3.17-3.08 (m, 2H); 1.25-1.21 (t, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): 169.53, 168.87, 168.81, 165.98, 165.83, 162.89, 160.47, 157.65, 147.82, 134.58, 132.10, 127.64, 127.56, 116.40, 115.60, 115.40, 108.04, 52.98, 52.75, 51.90, 28.55, 28.42.

(2R,3R)-ethyl-3-(1-(4-(4-fluorophenyl)thiazol-2-ylamino)-3-(1H-imidazol-4-yl)-1-oxopropan-2-ylcarbamoyl)oxirane-2-carboxylate (15d). General procedure using: the racemic Boc-protected peptidomimetic (400 mg, 1.0 mmol); (2*S*, 3*S*)-epoxysuccinic acid monoester (149 mg, 0.93 mmol), DIPEA (0.16 mL, 0.93 mmol) and HOBT (151 mg, 1.1 mmol) in DMF (5 ml); EDCI (196 mg, 1.0 mmol) in DMF (0.5 mL) gave the title compound (240 mg, 50.8 %) as a racemic mixture in the form of a white solid. ^{1}H NMR (DMSO-d^6, 400 MHz): δ 12.52 (bs, 1H);

11.86 (bs, 1H); 8.85-8.77 (dd, J = 7.21 Hz; J = 7.48 Hz); 7.95-7.91 (q, 2H); 7.62 (s, 1H); 7.57 (s, 1H); 7.28-7.24 (t, 2H); 6.84 (s, 1H); 4.81-4.76 (m, 1H); 4.23-4.15 (m, 1H); 3.76-3.74 (dd, 1H, J = 1.8 Hz; J = 1.8 Hz); 3.64-3.61 (dd, 1H, J = 1.8 Hz; J = 1.8 Hz); 3.08-2.99 (m, 2H); 1.25-1.17 (t, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): 170.28 & 170.24, 167.48 & 167.46, 165.78 & 165.58, 163.43, 161.01, 158.53 & 158.20, 135.37, 131.32 & 131.29, 128.17 & 128.09, 116.13 & 115.92, 108.52, 62.01, 53.72 & 53.52, 53.41 & 53.36, 51.85 & 51.82, 14.33.

(2S,3S)-ethyl-3-(1-(6-fluorobenzo[d]thiazol-2-ylamino)-3-(1H-imidazol-4-yl)-1-oxopropan-2-ylcarbamoyl)oxirane-2-carboxylate (15e). General procedure using: the racemic Boc-protected peptidomimetic (402 mg, 0.99 mmol); (2*S*, 3*S*)-epoxysuccinic acid monoester (159 mg, 0.99 mmol), DIPEA (0.18 mL, 0.99 mmol) and HOBT (161 mg, 1.18 mmol) in DMF (5 ml); EDCI (209 mg, 1.09 mmol) in DMF (0.5 mL) gave the title compound (229 mg, 51.5 %) as a racemic mixture in the form of a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.96 (bs, 1H); 7.76-7.73 (m, 2H); 7.52-7.50 (d, 1H); 7.18-7.14 (m, 1H); 6.94 (s, 1H); 4.93 (bs, 1H); 4.32-4.27 (m, 2H); 3.78 (s, 1H): 3.63 (s, 1H); 3.26-3.22 (dd, 1H); 3.10-3.06 (dd, 1H); 1.36-1.32 (t, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): 172.15, 168.52 & 168.50, 166.88 & 166.70, 161.31, 159.19 & 158.91, 146.53, 136.25, 134.04 & 133.93, 122.94 & 122.84, 115.55 & 115.30, 109.41 & 109.14, 62.40, 55.67, 54.20 & 53.98, 53.72 & 53.66, 52.17 & 52.12, 14.42.

(2R,3R)-ethyl-3-(1-(6-fluorobenzo[d]thiazol-2-ylamino)-3-(1H-imidazol-4-yl)-1-oxopropan-2-ylcarbamoyl)oxirane-2-carboxylate (15f). General procedure using: Boc-protected peptidomimetic (420 mg, 1.0 mmol); (2*S*, 3*S*)-epoxysuccinic acid monoester (162 mg, 1.01 mmol), DIPEA (0.18 mL, 1.01 mmol) and HOBT (164 mg, 1.21 mmol) in DMF (5 ml); EDCI

(213 mg, 1.11 mmol) in DMF (0.5 mL) gave the title compound (226 mg, 50.5 %) as a racemic mixture in the form of a white solid. ^{1}H NMR (DMSO-d^{6}, 400 MHz): δ 11.85 (bs, 1H); 8.89-8.81 (dd, 1H, J = 7.21 Hz; J = 7.42 Hz); 7.91-7.88 (dd, 1H, J = 2.5 Hz; J = 2.5 Hz); 7.77-7.74 (q, 1H); 7.57 (s, 1H); 7.32-7.27 (td, 1H, J = 2.5 Hz; J = 2.5 Hz; J = 2.5 Hz); 6.85 (s, 1H); 4.82-4.76 (m, 1H); 4.23-4.16 (m, 2H); 3.76-3.74 (dd, 1H, J = 1.8 Hz; J = 1.8 Hz); 3.65-3.61 (, 1H, J = 1.8 Hz; J = 1.8 Hz); 3.17-2.98 (m, 2H); 1.25-1.17 (t, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): 171.13 & 171.09, 167.47 & 167.45, 165.83 & 165.65, 160.32, 158.21 & 157.93, 145.64, 135.40, 133.23 & 133.12, 122.17 & 122.08, 114.83 & 114.59, 108.74 & 108.47, 62.01, 53.86 & 53.65, 53.40 & 53.33, 51.86 & 51.81, 14.34.

(2S,3S)-ethyl-3-((S)-1-oxo-1-(phenylamino)-3-(thiazol-4-yl)propan-2-ylcarbamoyl)oxirane-2-carboxylate (16a). General procedure using: Boc-protected peptidomimetic (230 mg, 0.66 mmol); (2*S*, 3*S*)-epoxysuccinic acid monoester (106 mg, 0.66 mmol), DIPEA (0.12 mL, 0.66 mmol) and HOBT (110 mg, 0.79 mmol) in CH_2Cl_2 (5 ml); EDCI (130 mg, 0.66 mmol) in DMF (0.5 mL) gave the title compound (84 mg, 32.5 %) as white solid. ^{1}H NMR (MeOD-d^{4}, 400 MHz): δ 8.94 (s, 1H); 7.52-7.50 (d, 2H); 7.33 (s, 1H); 7.30-7.26 (t, 3H); 7.11-7.07 (t, 1H); 4.98- 4.95 (t, 1H); 4.24-4.20 (q, 2H); 3.69 (s, 1H); 3.54 (s, 1H); 3.43-3.28 (m, 2H); 1.28-1.24 (t, 3H). ^{13}C NMR (MeOD-d^{4}, 100 MHz):169.43, 167.21, 166.84, 153.89, 152.24, 137.82, 128.45, 124.23, 120.24, 116.10, 61.82, 53.60, 53.11, 51.92, 32.94, 12.99.

(2S,3S)-ethyl-3-((S)-1-(4-(4-fluorophenyl)thiazol-2-ylamino)-1-oxo-3-(thiazol-4-yl)propan-2-ylcarbamoyl)oxirane-2-carboxylate (16b). Synthesized following the optimized coupling procedure using: (2*S*, 3*S*)-epoxysuccinic acid monoester (622 mg, 3.88 mmol), DIPEA (0.75 mL,

4.2 mmol) in DMF (5 ml); EDCI (890 mg, 4.7 mmol); HOBt (730 mg, 5.4 mmol); deprotected free amine (1.35 g, 3.9 mmol) in DMF (5 ml); after column chromatography afforded the title compound (isolated yield = 1.38 g, 72.4 %) as white solid. ^{1}H NMR (400 MHz, MeOD-d^4): δ 8.98-8.98 (d, 1H, J = 1.75 Hz); 7.92-7.90 (q, 2H); 7.37 (s, 1H); 7.36 (s, 1H); 7.15-7.10 (t, 2H); 5.06-5.02 (q, 1H); 4.27-4.24 (q, 2H); 3.69-3.68 (d, 1H, J = 1.66 Hz); 3.55-3.54 (d, 1H, J = 1.66 Hz); 3.56-3.29 (m, 2H); 1.33-1.29. ^{13}C NMR (DMSO-d_6, 100 MHz): 169.17, 167.17, 157.69, 154.00, 151.90, 148.90, 130.95, 127.55, 116.15, 114.99, 114.77, 107.20, 61.76, 52.97, 52.91, 51.82, 32.35, 12.89. ESI-HRMS (m/z): $[M-H]^+$ calcd. for $C_{21}H_{19}FN_4O_5S_2$: 489.0781, observed: 489.0731.

(2S,3S)-ethyl 3-((S)-1-(4-(4-ethynylphenyl)thiazol-2-ylamino)-1-oxo-3-(thiazol-4-yl)propan-2-ylcarbamoyl)oxirane-2-carboxylate (16c). ^{1}H NMR (DMSO-d^6, 400 MHz) δ = 12.56 (s, 1H); 9.04-9.03 (d, 1H, J = 1.8 Hz); 8.84-8.82 (d, 1H, J = 7.7 Hz); 7.93-7.91 (d, 2H, J = 8.3 Hz); 7.76 (s, 1H); 7.55-7.53 (d, 2H, J = 8.3 Hz); 7.45-7.44 (d, 1H, J = 1.6 Hz); 4.99-4.93 (q, 1H); 4.26 (s, 1H); 4.22-4.16 (m, 2H); 3.72-3.71 (d, 1H, J = 1.7 Hz); 3.56-3.55 (d, 1H, J = 1.7 Hz); 3.67-3.22 (m, 2H); 1.25-1.19 (t, 3H).^{13}C NMR (DMSO-d^6, 100 MHz): 170.04, 167.47, 165.62, 158.24, 154.25, 152.62, 148.47, 134.95, 132.61, 126.26, 121.33, 116.67, 110.21, 83.87, 81.99, 62.02, 53.36, 53.11, 51.81, 33.11, 14.33. ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{23}H_{20}N_4O_5S_2$: 497.0948; observed, 497.0949; HPLC method 2: Purity = 96.7 %, R_t = 14.5 min (Ion-Trap HPLC).

(2S,3S)-ethyl-3-((S)-1-(4-(4-fluorophenyl)thiazol-2-ylamino)-3-(1-methyl-1H-imidazol-5-yl)-1-oxopropan-2-ylcarbamoyl)oxirane-2-carboxylate (17). General procedure using: Boc-

protected peptidomimetic (200 mg, 0.45 mmol); (2*S*, 3*S*)-epoxysuccinic acid monoester (72 mg, 0.44 mmol), DIPEA (0.078 mL, 0.44 mmol) and HOBT (73 mg, 0.53 mmol) in DMF (4 ml); EDCI (95 mg, 0.49 mmol) in DMF (0.5 mL) gave the title compound (152 mg, 69.3 %) as white solid . ^{1}H NMR (DMSO-d^6, 400 MHz): δ 12.59 (bs, 1H); 8.97-8.95 (d, 1H, J = 7.82 Hz); 7.95-7.92 (q, 2H); 7.64 (s, 1H); 7.50 (s, 1H); 7.28-7.24 (t, 2H); 6.67 (s, 1H); 4.87-4.81 (q, 1H); 4.21-4.15 (m, 2H); 3.74-3.73 (d, 1H, J = 1.79 Hz); 3.59 (s, 3H), 3.55-3.54 (d, 1H, J = 1.79 Hz); 3.16-2.97 (m, 2H); 1.24-1.17 (t, 3H). ^{13}C NMR (DMSO-d^6, 100 MHz): 169.95, 167.48, 165.56, 163.45, 161.02, 148.41, 138.56, 131.27, 128.18, 128.09, 127.82, 127.00, 116.16, 115.95, 108.70, 62.01, 53.24, 52.36, 51.70, 31.30, 26.40, 14.33. ESI-LRMS (m/z): $[M+H]^+$ calcd. for $C_{22}H_{22}FN_5O_5S$: 487.5, observed: 488.0.

(2S,3S)-ethyl-3-((S)-1-(4-(4-fluorophenyl)thiazol-2-ylamino)-3-(1-methyl-1H-imidazol-4-yl)-1-oxopropan-2-ylcarbamoyl)oxirane-2-carboxylate (18). General procedure using: Boc-protected peptidomimetic (185.5mg, 0.41mmol); (2*S*, 3*S*)-epoxysuccinic acid monoester (67mg, 0.41mmol), DIPEA (0.07mL, 0.41mmol) and HOBT (68mg, 0.49mmol) in DMF (3ml); EDCI (88mg, 0.45mmol) in DMF (0.5mL) gave the title compound (104mg, 50.2%) as white solid. . ^{1}H NMR (DMSO-d^6, 400 MHz): δ 12.49 (bs, 1H); 8.74-8.72 (d, 1H, J = 7.4 Hz); 7.95-7.92 (q, 2H); 7.62 (s, 1H); 7.49 (s, 1H); 7.27-7.24 (t, 2H); 6.89 (s, 1H); 4.77-4.74 (q, 1H); 4.22-4.17 (m, 2H); 3.75-3.74 (d, 1H, J = 1.8 Hz); 3.62-3.61 (d, 1H, J = 1.8 Hz); 3.58 (s, 3H); 3.02-2.91 (m, 2H); 1.26-1.22 (t, 3H). ^{13}C NMR (DMSO-d^6, 100 MHz): 170.31, 167.48, 165.56, 163.43, 161.00, 148.32, 137.89, 136.98, 131.31, 128.11 (d, J = 8.1 Hz); 118.45, 116.14, 115.92, 108.49, 62.01, 53.54, 53.40, 51.87, 33.24, 30.55, 14.34. ESI-LRMS (m/z): $[M+H]^+$ calcd. for $C_{22}H_{22}FN_5O_5S$: 487.5, observed: 488.0.

Supplemental Refere nces

1. Davies, P. L.; Moldoveanu, T.; Hosfield, C. M.; Lim, D.; Elce, J. S.; Jia, Z. C. A Ca2+ switch aligns the active site of calpain. *Cell* 2002, 108, 649-660.