Supporting Information

(2S,4S,5S)-5-t-butoxycarbonylamino-4-t-butyldimethylsiloxy-2-isopropyl-7-methyloctanoic acid ***(46)***: The procedure for the synthesis of the Leu-Val hydroxyethylene intermediate (2S, 4S, 5S)-5-t-butoxycarbonylamino-4-t-butyldimethylsiloxy-2-isopropyl-7-methyloctanoic acid (**46**), has been previously described by Pals, DT; Saneii, HH; Sawyer, TK; Schostarez, HJ; TenBrink, RE; Thaisrivo*ngs,* S. Peptides. *European Patent Application 173481* and by Wuts, PGM; Putt, SR; Ritter, AR.. *J. Org. Chem.* ***1988****, 53, 4503-4508.*

O-benzyl-boc-4(S)-amino-3(S)-O-acetyl-6-methylheptanoate (**48**) & *Boc-4(S)-amino-3(S)-O-acetyl-6 methylheptanoic acid* (**49**): The synthesis of the statine-ester (**48**) and the statine-acid (**49**) have been previously described by Rittle, K.E.; Homnick, C.F.; Ponticello, G.S.; and Evans, B.E. *J. Org. Chem.* ***1982****, 47, 3016-1018* and by McConnell, R.M.; Frizzell, D; Camp, A; Evans, A; Jones, W; Cagle, C. *J.Med. Chem,* ***1991****, 34, 2298-2300.*

Peptide synthesis

The substrate and inhibitor peptides were synthesized in a peptide synthesizer using boc-protected amino acids for chain assembly. All chemicals, reagents, and boc amino acids were purchased from Applied Biosystems (ABI; Foster City, CA) with the exception of dichloromethane and N,N-dimethylformamide which were obtained from Burdick and Jackson and boc-statine that was purchased from Neosystems. The starting resin, boc-Phe-OCH_2-Pam resin was also purchased from ABI. All amino acids were coupled following preactivation to the corresponding HOBT ester using 1.0 equivalent of 1-hydroxybenzotriazole (HOBT), and 1.0 equivalent of N,N-dicyclohexylcarbodiimide (DCC) in dimethylformamide. The boc protecting group on the amino acid -amine was removed with 50% trifluoroacetic acid in dichloromethane after each coupling step and prior to Hydrogen Fluoride cleavage. Amino acid side chain protection was as follows: Glu(Bzl), Lys(Cl-CBZ), Ser(OBzl), Thr(OBzl). All other amino acids were used with no further side chain protection including boc-Statine.

After the sequential addition of all fourteen residues the P_{10}-$P_{4'}$sta(D->V) peptide has the sequence NH_2-KTEEISEVN[sta]VAEF-COOH (**22**), where "sta" represents a statine moiety. The side chain protected peptide resin was deprotected and cleaved from the resin by reacting with anhydrous hydrogen fluoride (HF) at 0°C for one hour. This generated the fully deprotected crude peptide as a C-terminal carboxylic

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acid. [*Abbreviations: (* Bzl) benzyl, (CBZ) carbobenzoxy, (Cl-CBZ) chlorocarbobenzoxy, (OBzl) O-benzyl]

HPLC purification

Following HF treatment, the peptide was extracted from the resin in acetic acid and lyophilized. The crude peptide was then purified using preparative reverse phase HPLC on a Vydac C4, 330Å, 10μm column 2.2cm I.D. x 25cm in length. The solvent system used with this column was 0.1% TFA / H2O ([A] buffer) and 0.1% TFA / CH3CN ([B] buffer) as the mobile phase. Typically the peptide was loaded onto the column in 2 % [B] at 8-10 mL/min. and eluted using a linear gradient of 2% [B] to 60% [B] in 174 minutes. The purified peptide was subjected to mass spectrometry, and analytical reverse phase HPLC to confirm its composition and purity. All the peptides shown were single peaks by analytical HPLC and had purities > 99% as determined by the peak area on the analytical HPLC.

Mass Spectrometric analysis:

Peptide **1:** Prepared as described above MH+ = 1623.7

Peptide **2:** Prepared by above procedure MH+ = 2067.2

Peptide **3:** Prepared by above procedure MH+ = 2053.1

Peptide **4:** Prepared by above procedure MH+ = 2140.4

Peptide **5:** Prepared by above procedure MH+ = 2217.4

Peptide **6**: Prepared by above procedure MH+ = 2152.3

Peptide **7:** Prepared by above procedure MH+ = 2125.3

Peptide **8:** Prepared by above procedure MH+ = 2095.3

Peptide **9**: Prepared by above procedure MH+ = 2097.2

Peptide **10**: Prepared by above procedure, MH+ = 2133.3

Peptide **11**: Prepared by above procedure, MH+ = 2050.1

Peptide **12**: Prepared by above procedure, MH+ = 2105.8

Peptide **13**: Prepared by above procedure, MH+ = 2092.8

Peptide **14**: Prepared by above procedure, MH+ = 2121.1

Peptide **15**: Prepared by above procedure, MH+ = 2141.1

Peptide **16**: Prepared by above procedure, MH+ = 2065.3

Peptide **17**: Prepared by above procedure using boc-(S)-statine , MH+ = 1781.9

Peptide **18**: Prepared by above procedure using boc-(S)-statine, MH+ = 1668.7

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Peptide **19**: Prepared by above procedure using boc-(R)-statine, MH+ = 1666.1

Peptide **20**: Prepared by above procedure using boc (R,S)-O-acetyl-statine, MH+ = 1709.4

The two isomers were separated by HPLC. The faster eluting peak was assigned

as the R-isomer based on retention time with peptide 19 on treatment with 1N NaOH.

Peptide **21**: Prepared by above procedure using boc-statine, MH+ = 1624.1

Peptide **22**: Prepared by above procedure using boc-stine, MH+ = 1651.8

Peptide **23**: Prepared by above procedure using boc-statine, MH+ = 964.4

Peptide **24**: Prepared by above procedure using boc-statine, MH+ = 922.4

Peptide **25**: Prepared by above procedure using boc-statine, MH+ = 835.6

Peptide **26**: Prepared by above procedure using boc-statine, MH+ = 752.6

Peptide **27**: Prepared by above procedure using boc-statine and capping with acetyl-valine (purchased from Bachem), MH+ = 917.3 (sodium adduct)

Peptide **28**: Prepared similar to peptide 27, MH+ = 917.3 (sodium adduct)

Peptide **29**: Prepared as above using boc-statine, MH+ = 887.6

Peptide **30**: Prepared as above using boc-statine, MH+ = 836.8

Peptide **31**: Prepared as above using boc-statine, MH+ = 818.5

Peptide **32**: Prepared as above using boc-statine, MH+ = 820.5

Peptide **33**: Prepared as above using boc-statine, MH+ = 932.3 (sodium adduct)

Peptide **34**: Prepared as above using boc-statine, MH+ = 963.6

Peptide **35**: Prepared as above using boc-statine, MH+ = 896.6

Peptide **36**: Prepared as above using boc-statine, MH+ = 892.6

Peptide **37**: Prepared as above using boc-statine, MH+ = 770.2

Peptide **38**: Prepared as above using boc-statine, MH+ = 639.4 (sodium adduct)

Peptide **39**: Prepared as above using boc-statine, MH+ = 568.6 (sodium adduct)

Peptide **40**: Prepared as above using boc-'AHPPA'[4(*S*)-amino-3-hydroxy-5-phenylpentanoic acid] purchased from Neosystems, MH+ = 952.5 (sodium adduct)

Peptide **41**: Prepared as above using boc-'ACPHA' [4 (*S*)-amino-5-phenylpentanoic acid] purchased from Neosystems, MH+ = 958.5 (sodium adduct)

Peptide **42**: Prepared as above using the TBDMS protected hydroxyethylene **49**, MH+ = 852.4

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BACE enzyme assay[7]

-Cleavage assays were carried out in 200 mM sodium acetate, pH 4.8, 0.06% Triton X-100, with 10ug/ml MBPC125Swe substrate. Reaction mixtures were incubated at 37°C for 1-2h , and the quenched reaction mixtures were then loaded onto 96-well plates coated with a polyclonal antibody raised to the maltose binding protein. Generated -cleaved product was detected using biotinylated antibody (Sw192) or biotinyated antibody (Wt192) as specific reporter antibodies and quantitated against the appropriate MBP-C26 standard.

Molecular Modeling.

The modeling data was generated on a SGI octane system using the mosaic-modeling program developed at

Pharmacia.

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