Supporting Information

Aminoethylenes: A Tetrahedral Intermediate Isostere Yielding Potent Inhibitors of the Aspartyl Protease BACE-1

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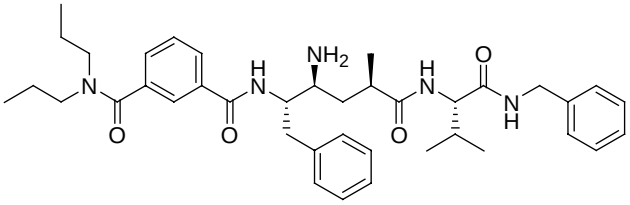
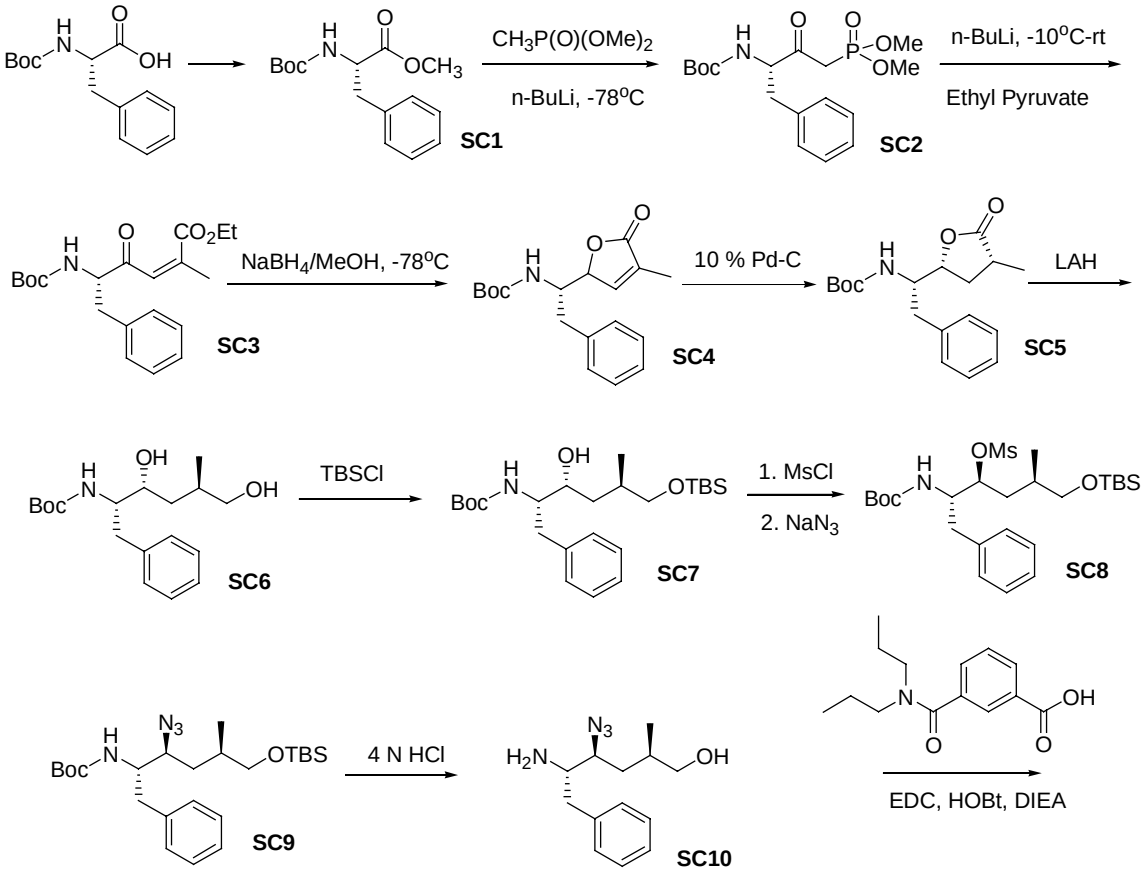
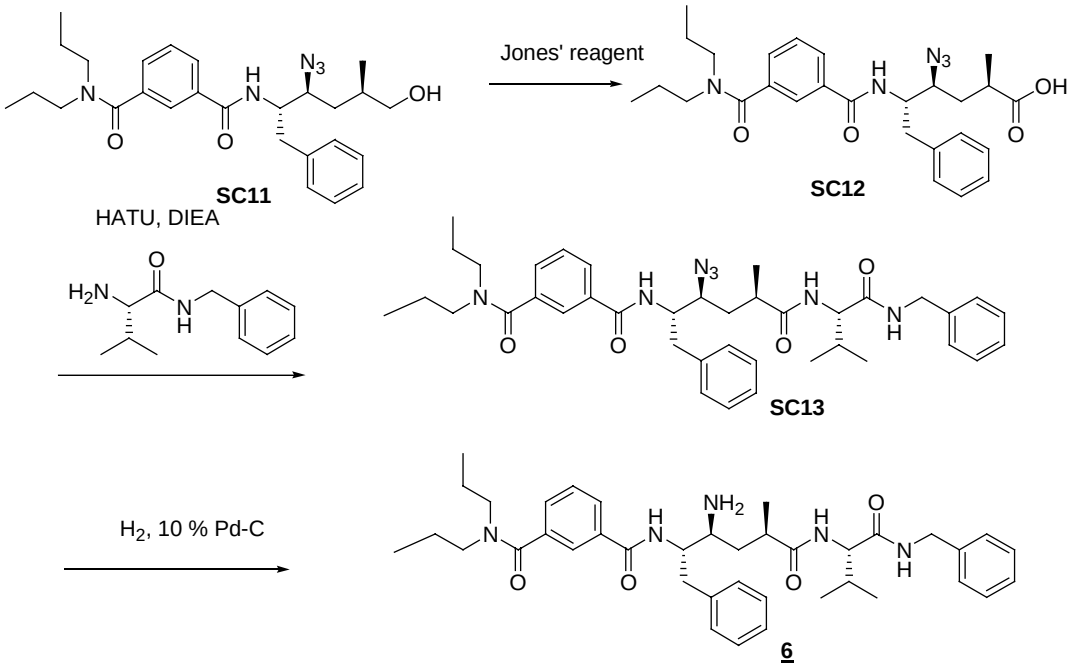
Chemistry

The following example describes the synthesis of compound **6**. Other compounds were synthesized in a similar fashion.



6





a) Preparation of **SC2**: A solution of dimethyl methylphosphonate **SC1** (34.1 g, 215.4 mmol) in THF (250 mL) in a nitrogen atmosphere was cooled to –78 °C and then was added 2.0 M solution of n-butyl lithium (107 mL, 215.4 mmol) via cannula in 20 min. The solution was stirred at –78 °C for 20 min, and a THF (150 mL) solution of *N*-Boc-L-phenylalanine methyl ester (10.0 g, 35.8 mmol) was slowly added via dropping funnel. The mixture was stirred at –78 °C for 1h. The reaction was then quenched with 10% AcOH (250 mL) and warmed to room temperature. The solution was extracted with EtOAc, and the combined organic extracts were washed with saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. Solvents and the excess dimethyl methylphosphonate were removed in vacuum at 90 °C water bath. The resulted oily product containing 5-10% dimethyl methylphosphonate (shown by ^{1}H NMR) was used without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.29 (m, 6 H) 1.35 (s, 9 H) 2.92 (dd, *J*=14.24, 8.39 Hz, 1 H) 3.03 (dd, *J*=22.38, 13.73 Hz, 1 H) 3.22 (m, 2 H) 4.10 (m, 4 H) 4.55 (d, *J*=5.59 Hz, 1 H) 5.38 (d, *J*=7.88 Hz, 1 H) 7.20 (m, 5 H); MS (m/z): 422 ($M+Na^+$)

b) Preparation of Ethyl (5*S*,2*Z*)-2-methyl-[5-(tert-butoxycarbonyl)amino]-4-oxo-6-phenylhex-2-enonate **SC3**: A solution of the crude phosphonate **SC2** above (16.8 g, ca. 35.8 mmol) in THF (100 mL) in a nitrogen atmosphere was cooled to 0 °C and then was added 1.6 M solution of n-butyl lithium (22.4 mL, 35.8 mmol) via syringe in 10 min. The solution was stirred at 0 °C for 30 min, and ethyl pyruvate (7.1 mL, 63.2 mmol) was added slowly. Stirring was continued at 0 °C for 1 h and at room temperature overnight. The reaction was quenched with saturated aqueous NH_4Cl (100 mL) and diluted with EtOAc (400 mL). The organic layer was separated, washed brine and dried over $MgSO_4$. Solvents were evaporated and the residue was purified by column chromatography (silica gel, Hexane-EtOAc 5:1) to give oily pure product **SC3** (9.0 g, 70% starting from **SC1**). ^{1}H NMR (400 MHz, acetone-D6) δ ppm 1.24 (t, *J*=7.12 Hz, 3 H) 1.33 (s, 9 H) 2.00 (d, *J*=1.27 Hz, 3 H) 2.86 (m, 1 H) 3.19 (dd, *J*=13.99, 5.09 Hz, 1 H) 4.18 (q, *J*=7.12 Hz, 2 H)

4.53 (m, 1 H) 6.13 (d, *J*=7.88 Hz, 1 H) 6.51 (d, *J*=1.27 Hz, 1 H) 7.22 (m, 5 H); MS (m/z): 384 ($M+Na^+$)

c) Preparation of (5*R*)-3-methyl-5- {(1*S*)-1-[(tert-butoxycarbonyl) amino]-2-phenylethyl}-2,5-dihydrofuran-2-one **SC4**: To a solution of **SC3** (8.4 g, 23.2 mmol) in MeOH (200 mL) cooled to –78 °C was added sodium borohydride (1.7 g, 46.4 mmol). The mixture was stirred and warmed to -15 °C in 3 h, and then kept at the same temperature overnight. 1N aqueous HCl (100 mL) was added to the cold reaction mixture, and the volatiles were removed by rotary evaporation. The resulting mixture was extracted with EtOAc. The combined extracts were washed with saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. After removal of solvents, the residue was purified by column chromatography (silica gel, Hexane-EtOAc 3:1) to give pure product (**SC4)** (4.3 g, 58%) and its 5-epimer (3.0 g, 41%) both as white solids. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.36 (s, 9 H) 1.88 (s, 3 H) 2.80 (d, *J*=6.61 Hz, 2 H) 4.05 (m, 1 H) 4.68 (d, *J*=8.39 Hz, 1 H) 4.92 (s, 1 H) 6.91 (s, 1 H) 7.23 (m, 5 H); MS (m/z): 340 ($M+Na^+$)

d) Preparation of (3*R*,5*R*)-3-methyl-5-{(1*S*)-1-[(tert-butoxycarbonyl)amino]-2-phenylethyl}-tetrahydrofuran-2-one **SC5**: Lactone **SC4** (3.5 g, 11.0 mmol) was dissolved in THF (300 mL), and to this solution 10% Pd/C (350 mg) was added. The mixture was stirred under an atmosphere of H_2 (balloon) for 5 h, followed by filtration and concentration in vacuo, to provide product **SC5** (3.5 g, 99%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.26 (d, *J*=7.12 Hz, 3 H) 1.35 (s, 9 H) 1.71 (m, 1 H) 2.42 (m, 1 H) 2.64 (m, 1 H) 2.87 (m, 1 H) 3.01 (m, 1 H) 3.92 (m, 1 H) 4.25 (m, 1 H) 4.46 (m, 1 H) 7.24 (m, 5 H); MS (m/z): 342 ($M+Na^+$)

e) Preparation of (2*S*,3*R*,5*R*)-2-tert-butoxycarbonylamino-1-phenyl-5-methylhexan-3,6-diol **SC6**: To a solution of **SC5** (3.5 g, 11.0 mmol) in THF (50 mL), cooled to 0 °C, was added 0.5 M $LiAlH_4$ in DME (22.0 mL, 11.0 mmol). The mixture was stirred at 0 °C for 0.5 h and warmed to room temperature. Stirring was continued until TLC indicated completion of the reduction. The mixture was cooled to 0 °C and 1N $NaHSO_4$ (30 mL) was added slowly. After stirring for 30 min at room temperature, the mixture was extracted with EtOAc. The combined extracts were washed with saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. After removal of solvents, the residue was purified by column chromatography (silica gel, Hexane-EtOAc 1:1) to yield pure product **SC6** (2.7 g, 76%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.82 (d, *J*=6.61 Hz, 3 H) 1.19 (s, 9 H) 1.26 (m, 1 H) 1.41 (m, 1 H) 1.75 (m, *J*=15.13, 5.72 Hz, 1 H) 2.46 (dd, *J*=13.61, 10.05 Hz, 1 H) 2.93 (dd, *J*=13.73, 3.05 Hz, 1 H) 3.30 (m, 2 H) 3.50 (m, 2 H) 7.10 (m, 5 H); MS (m/z): 324 (MH^+)

f) Preparation of [(1*S*,2*R*,4*R*)-1-Benzyl-5-(tert-butyl-dimethyl-silanyloxy)-2-hydroxy-4-methyl-pentyl]-carbamic acid tert-butyl ester **SC7**: To a solution of **SC6** (1.0 g, 3.10 mmol) in DCM (15 mL) was added imidazole (422 mg, 6.20 mmol) and TBSCl (578 mg, 3.72 mmol) sequentially at 0 °C. After stirring at 0 °C for 1 h, H_2O (10 mL) was added to quench the reaction. The organic layer was separated and the aqueous layer was extracted with DCM. The combined DCM solution was washed with H_2O and dried over $MgSO_4$. After removal of solvents, the residue was purified by column chromatography (silica

gel, Hexane-EtOAc 3:1) to afford pure product **SC7** (1.34 g, 98%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.08 (s, 6 H) 0.87 (d, *J*=6.87 Hz, 3 H) 0.91 (s, 9 H) 1.32 (s, 9 H) 1.49 (m, *J*=6.36 Hz, 2 H) 1.80 (m, 1 H) 2.77 (m, 1 H) 2.89 (m, 1 H) 3.40 (m, 1 H) 3.58 (dd, *J*=10.05, 4.20 Hz, 1 H) 3.68 (s, 1 H) 3.81 (s, 1 H) 4.18 (m, *J*=12.33, 4.96 Hz, 1 H) 4.78 (d, *J*=8.65 Hz, 1 H) 7.21 (m, 5 H); MS (m/z): 438 (MH^+), 460 (MNa^+)

g) Preparation of Methanesulfonic acid (1*R*,2*S*,4*R*)-1-(1-tert-butoxycarbonyl-amino-2-phenyl-ethyl)-4-(tert-butyl-dimethyl-silanyloxy)-3-methyl-butyl ester **SC8**: To a solution of **SC7** (1.2 g, 2.75 mmol) in chloroform (10 mL) was added triethylamine (0.8 mL, 5.5 mmol) and MsCl (0.32 mL, 4.12 mmol) sequentially at 0 °C. After stirring at 0 °C for 1 h, 1 N HCl (5 mL) was added to quench the reaction. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined CH_2Cl_2 solution was washed with H_2O and dried over $MgSO_4$. After removal of solvents, the residue was purified by column chromatography (silica gel, Hexane-EtOAc 3:1) to afford pure product **SC8** (1.34 g, 95%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.04 (s, 6 H) 0.88 (m, 12 H) 1.32 (s, 9 H) 1.37 (m, 1 H) 1.79 (m, 1 H) 1.95 (m, 1 H) 2.66 (dd, *J*=13.22, 10.68 Hz, 1 H) 2.90 (dd, *J*=14.24, 4.83 Hz, 1 H) 3.03 (s, 3 H) 3.40 (m, 1 H) 3.52 (m, 1 H) 4.16 (s, 1 H) 4.87 (d, *J*=8.65 Hz, 1 H) 4.96 (d, *J*=9.16 Hz, 1 H) 7.23 (m, 5 H); MS (m/z): 515 (MNa^+)

h) Preparation of [(1S,2S,4R)-2-Azido-1-benzyl-5-(tert-butyl-dimethyl-silanyloxy)-4-methyl-pentyl]-carbamic acid tert-butyl ester **SC9**: Mesylate **SC8** (1.34 g, 2.60 mmol) was dissolved in anhydrous DMF (300 mL). To this stirred solution was added NaN_3 (2.50g, 39.0 mmol). The mixture was heated to 60 °C with an oil bath and stirred at this temperature for 8 h. The solvent was evaporated in vacuo and then H_2O (20 mL) was added to dissolve the solid. The solution was extracted with ether, and the combined extracts were dried over $MgSO_4$. After removal of solvents, the residue was purified by column chromatography (silica gel, Hexane-EtOAc 10:1) to afford pure product **SC9** (0.72 g, 60%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm -0.05 (s, 3 H) -0.03 (s, 3 H) 0.74 (d, *J*=6.87 Hz, 3 H) 0.84 (s, 9 H) 1.39 (s, 9 H) 1.43 (m, 1 H) 1.63 (m, 1 H) 1.73 (m, 1 H) 2.75 (dd, *J*=13.61, 8.77 Hz, 1 H) 2.92 (m, 1 H) 3.37 (m, 2 H) 3.53 (t, *J*=7.12 Hz, 1 H) 3.91 (q, *J*=8.22 Hz, 1 H) 4.65 (d, *J*=9.66 Hz, 1 H) 7.25 (m, 5 H); MS (m/z): 485 (MNa^+)

i) N-[(1S,2S,4R)-2-Azido-1-benzyl-5-hydroxy-4-methyl-pentyl]-N',N'-dipropyl-isophthalamide (**SC11**): Azide **SC9** (109 mg, 0.24 mmol) was treated with 4N HCl in dioxane (5 mL). After stirring for 1 h, the mixture was concentrated in vacuo and the resulting crude product **SC10** was used for the next step without purification. To a solution of the crude compound **SC10** in DMF (1 mL) was added N,N-Dipropyl-isophthalamic acid (58 mg, 0.24 mmol), diisopropylethylamine (0.3 mL, 1.42 mmol), HOBt (54 mg, 0.35 mmol) and EDC (68 mg, 0.35 mmol) sequentially. The mixture was stirred at room temperature for 8 h. 1N HCl (2 mL) was added, followed by addition of EtOAc (80 mL). After stirring for 10 min, the organic layer was separated, washed with brine and dried over $MgSO_4$. After removal of solvents, the residue was purified by column chromatography (silica gel, DCM-MeOH 19:1) to afford pure product **SC11** (113 mg, 81% starting from **SC9**) as a colorless oil. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 0.60 (t, *J*=7.12 Hz, 3 H) 0.81 (d, *J*=6.61 Hz, 3 H) 0.89 (t, *J*=7.25 Hz, 3 H) 1.32 (m, 1 H) 1.43 (m, 2 H) 1.64 (m, 3 H) 1.75 (m, 1 H) 2.84 (m, 1 H) 2.94 (m, 1 H) 3.06 (m, 2 H) 3.32 (m,

4 H) 3.61 (m, 1 H) 4.41 (m, 1 H) 7.06 (m, 1 H) 7.16 (q, *J*=7.46 Hz, 4 H) 7.40 (m, 2 H) 7.55 (s, 1 H) 7.67 (d, *J*=7.12 Hz, 1 H); MS (m/z): 480 (MH^+)

j) Preparation of **SC12**: To a solution of **SC11** (57 mg, 0,12 mmol) in acetone (1 mL) was added Jones' reagent (2.7M, 88 μM, 0,24 mmol). After stirring at room temperature for 20 min, iPrOH (40 μL) was added. Stirring was continued for 10 min and the reaction mixture was diluted with acetone (20 mL), followed by filtration over celite. The solution was dried (Na_2SO_4) and concentrated. The resulted crude product **SC12** was used for next step without purification.

k) Preparation of N-{(1S,2S,4R)-2-Azido-1-benzyl-4-[(1S)-1-benzylcarbamoyl-2-methyl-propylcarbamoyl]-pentyl}-N',N'-dipropyl-isophthalamide **SC13**:To a DMF (0.5 mL) solution of the crude acid **SC12**, prepared from **SC11** (24 mg, 0.05 mmol) as described above, was added 2-Amino-N-benzyl-3-methyl-butyramide (20 mg, 0.075 mmol), diisopropylethylamine (0.1 mL, 0.45 mmol) and HATU (38 mg, 0.10 mmol) sequentially. The mixture was stirred at room temperature for 2 h, and then purified by HPLC to provide pure product **SC13** (26 mg, 76% starting from **SC11**) as a white solid. ^{1}H NMR (400 MHz, METHANOL-D) δ ppm 0.57 (t, *J*=7.25 Hz, 3 H) 0.76 (m, 6 H) 0.87 (t, *J*=7.25 Hz, 4 H) 0.98 (d, *J*=6.61 Hz, 3 H) 1.41 (m, 3 H) 1.59 (m, 2 H) 1.90 (m, 2 H) 2.66 (m, 1 H) 2.79 (dd, *J*=13.73, 9.66 Hz, 1 H) 2.94 (dd, *J*=13.99, 5.60 Hz, 1 H) 3.03 (m, 2 H) 3.22 (s, 1 H) 3.36 (m, 3 H) 3.99 (d, *J*=7.63 Hz, 1 H) 4.25 (s, 2 H) 4.40 (s, 1 H) 7.10 (m, 10 H) 7.38 (q, *J*=7.97 Hz, 2 H) 7.53 (s, 1 H) 7.65 (d, *J*=6.87 Hz, 1 H); MS (m/z): 682 (MH^+)

l) N-{(1S,2S,4R)-2-Amino-1-benzyl-4-[(1S)-1-benzylcarbamoyl-2-methyl-propylcarbamoyl]-pentyl}-N',N'-dipropyl-isophthalamide **6**: To a solution of Compound **SC13** (19 mg, 0.0279 mmol) in MeOH (5 mL) 10% Pd-C (6 mg) was added. The mixture was stirred under an atmosphere of H_2 for 30 min, followed by filtration and concentration. The resulting crude product was purified by preparative TLC to afford pure product **6** (16 mg, 88%) as a white solid. ^{1}H NMR (400 MHz, METHANOL-D) δ ppm 0.61 (t, *J*=7.25 Hz, 3 H) 0.76 (d, *J*=6.61 Hz, 6 H) 0.88 (m, 4 H) 0.98 (d, *J*=6.87 Hz, 3 H) 1.28 (m, 1 H) 1.42 (m, 2 H) 1.62 (m, 2 H) 1.79 (m, 1 H) 1.91 (m, 1 H) 2.65 (dd, *J*=14.62, 6.23 Hz, 1 H) 2.75 (m, 2 H) 2.91 (m, 1 H) 3.07 (m, 2 H) 3.37 (m, 2 H) 4.00 (d, *J*=7.38 Hz, 1 H) 4.27 (m, 2 H) 7.05 (t, *J*=7.12 Hz, 1 H) 7.16 (m, 9 H) 7.40 (m, 2 H) 7.58 (s, 1 H) 7.71 (d, *J*=7.38 Hz, 1 H); MS (m/z) : 656 (MH^+)

Analytical data for compounds 1-7

The compositions and purities of compounds **1-7** were characterized as follows: Elemental analyses (C,H,N) were performed by Roberson Microlit Laboratories, Madison, NJ, USA, on a Perkin Elmer 2400 CHN analyzer. ^{1}H NMR spectra were recorded at 400 MHz on a Bruker spectrometer with chemical shifts reported in units of parts per million (ppm). High resolution mass spectra (HRMS) were determined on a Applied Biosystems Qstar Pular-i. LC-MS was performed on an Agilent 1100 series using a Phenomenex column (Aqua 5u C18 125 A, 50 X 4.6 mm, 5 μm particle size), total time for method is 10.5 min 100 % 0.1 TFA/water for 1 min; 0% - 100% acetonitrile v/v over 8 min flushed at 100 % acetonitrile for 2.5 min.

Compound 1: colorless solid; mp: 138.0-139.0 °C; LC-MS r.t. 5.62 min m/z 623.5 (MH+); ^{1}H NMR (400 MHz, methanol-d4) δ 0.76 (t, *J* = 7.7 Hz, 3H), 0.90 (d, *J* = 6.8 Hz, 3H), 0.91 (d, *J* = 6.9 Hz, 3H), 0.96 (d, *J* = 6.4 Hz, 3H), 0.97 (d, *J* = 6.3 Hz, 3H), 1.02 (t, *J* = 7.4 Hz, 3H), 1.15 (d, *J* = 6.9 Hz, 3H), 1.37-1.51 (m, 2H), 1.53-1.77 (m, 7H), 1.87 (ddd, *J* = 13.8, 9.4, 3.7 Hz, 1H), 1.97-2.10 (m, 1H), 2.72-2.81 (m, 1H), 3.23 (t, *J* = 7.6 Hz, 2H), 3.50 (t, *J* = 7.6 Hz, 2H), 3.59-3.66 (m, 1H), 4.15 (d, *J* = 7.3 Hz, 1H), 4.18-4.26 (m, 2H), 4.40 (ABq, *J* =14.6, 5.4 Hz , 2H), 7.24-7.34 (m, 5H), 7.53-7.60 (m, 2H), 7.86 (S, 1H), 7.95 (d, *J* = 7.3 Hz, 1H); HRMS calcd for $C_{36}H_{55}N_4O_5$ (MH+) 623.4172, found 623.4186; Anal. (C36H54N4O5) C, H, N.

Compound 2: colorless solid as a hydrochloride salt; mp: 145.0-146.0 °C; LC-MS r.t. 5.39 min m/z 622.5 (MH+); ^{1}H NMR (400 MHz, methanol-d4) δ 0.76 (t, *J* = 7.4 Hz, 3H), 0.91 (d, *J* = 6.8 Hz, 3H), 0.92 (d, *J* = 6.8 Hz, 3H), 0.98 (t, *J* = 6.4 Hz, 3H), 0.98 (d, *J* = 6.3 Hz, 3H), 1.02 (d, *J* = 6.9 Hz, 3H), 1.23 (d, *J* = 6.8 Hz, 3H), 1.42-1.46 (m, 1H), 1.51-1.85 (m, 8H), 1.98-2.19 (m, 2H), 2.75-2.86 (m, 1H), 3.23 (t, *J* = 7.6 Hz, 2H), 3.50 (t, *J* = 7.6 Hz, 2H), 4.14 (d, *J* = 7.8 Hz, 1H), 4.25-4.36 (m, 1H), 4.40 (apparent td, *J* =14.6, 6.4 Hz, 2H), 7.22-7.36 (m, 5H), 7.57-7.63 (m, 2H), 7.90 (S, 1H), 7.95 (apparent td, *J* = 7.3, 1.0 Hz, 1H); HRMS calcd for $C_{36}H_{56}N_5O_4$ (MH+) 622.4332, found 622.4337; Anal. ($C_{36}H_{55}N_5O_4$·HCl) C, H, N.

Compound 3: colorless solid as a hydrochloride salt; mp: 143.0-144.0 °C; LC-MS r.t. 5.42 min m/z 622.5 (MH+); ^{1}H NMR (400 MHz, methanol-d4) δ 0.76 (t, *J* = 7.4 Hz, 3H), 0.96 (d, *J* = 7.8 Hz, 3H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.99 (d, *J* = 5.9 Hz, 3H), 1.02 (t, *J* = 6.8 Hz, 3H), 1.04 (d, *J* = 6.8 Hz, 3H), 1.23 (d, *J* = 6.9 Hz, 3H), 1.34-1.43 (m, 1H), 1.54-1.65 (m, 2H), 1.70-1.85 (m, 5H), 1.85-1.97 (m, 1H), 2.00-2.17 (m, 1H), 2.62-2.76 (m, 1H), 3.23 (t, *J* = 7.4 Hz, 2H), 3.51 (t, *J* = 7.6 Hz, 2H), 4.18 (d, *J* = 7.8 Hz, 1H), 4.35-4.44 (m, 2H), 4.46-4.55 (m, 1H), 7.21-7.36 (m, 5H), 7.58-7.65 (m, 2H), 7.89 (S, 1H), 8.00 (apparent d, *J* = 6.8 Hz, 1H); HRMS calcd for $C_{36}H_{56}N_5O_4$ (MH+) 622.4332, found 622.4332; Anal. ($C_{36}H_{55}N_5O_4$·HCl·1/2H_2O) C, H, N.

Compound 4: colorless solid; mp: 81.5-82.5 °C; LC-MS r.t. 5.71 min m/z 528.4 (MH+);^{1}H NMR (400 MHz, methanol-d4) δ 0.74 (t, *J* = 7.4 Hz, 3H), 0.98 (d, *J* = 5.9 Hz, 3H), 1.02 (t, *J* = 7.4 Hz, 3H), 1.24 (d, J = 6.8 Hz, 3H), 1.37-1.63 (m, 4H), 1.63-1.80 (m, 4H), 1.91-2.02 (m, 1H), 2.78-2.90 (m, 1H), 3.22 (t, *J* = 7.4 Hz, 2H), 3.50 (t, *J* = 7.6 Hz, 2H), 3.66 (dt, *J* = 9.3, 3.4 Hz, 1H), 4.22 (dt, *J* = 10.7, 3.4 Hz, 1H), 7.03 (t, *J* = 8.8 Hz, 2H), 7.52-7.62 (m, 4H), 7.85 (bs, 1H), 7.95 (apparent d, *J* = 7.4 Hz, 1H); HRMS calcd for $C_{30}H_{43}N_3O_4F$ (MH+) 528.3237, found 528.3253; Anal. ($C_{30}H_{42}N_3O_4F$) C, H, N.

Compound 5: colorless solid as a hydrochloride salt; mp: 130.5-131.5 °C; LC-MS r.t. 5.44 min m/z 527.4 (MH+); ^{1}H NMR (400 MHz, methanol-d4) δ 0.73 (t, *J* = 7.1 Hz, 3H), 0.99 (d, *J* = 6.3 Hz, 3H), 1.02 (t, *J* = 7.9 Hz, 3H), 1.03 (d, *J* = 6.9 Hz), 1.41-1.49 (m, 1H), 1.53-1.65 (m, 2H), 1.65-1.85 (m, 6H), 2.21 (ddd, J = 14.6, 8.4, 3.9 Hz, 1H), 2.82-2.95 (m, 1H), 3.22 (t, *J* = 7.6 Hz, 2H), 3.41 (dt, *J* = 8.3, 4.4 Hz, 1H), 3.47-3.54 (m, 2H), 4.28-4.45 (m, 1H), 7.03 (d, *J* = 8.8 Hz, 1H), 7.05 (d, J = 8.3 Hz, 1H), 7.54-7.64 (m, 4H), 7.88 (bs, 1H), 7.98 (apparent d, *J* = 6.9 Hz, 1H); HRMS calcd for $C_{30}H_{44}N_4O_3F$ (MH+) 527.3397, found 527.3399; Anal. ($C_{30}H_{43}N_4O_3F$·HCl·1/2H_2O) C, H, N.

Compound 6: colorless solid as a hydrochloride salt; mp: 149.0-150.0 °C; LC-MS r.t. 5.45 min m/z 656.5 (MH+); ^{1}H NMR (400 MHz, methanol-d4) δ 0.72 (t, *J* = 7.3 Hz, 3H), 0.91 (d, *J* = 6.4 Hz, 3H), 0.94 (d, *J* = 5.3 Hz, 3H), 1.01 (t, *J* = 7.1 Hz, 3H), 1.23 (d, *J* = 6.8 Hz, 3H), 1.48-1.58 (m, 2H), 1.69-1.79 (m, 3H), 2.00-2.10 (m, 1H), 2.15-2.24 (m, 1H), 2.86 (apparent dd, *J* = 7.3, 5.9 Hz, 1H), 3.04 (d, *J* = 11.3 Hz, 2H), 3.14-3.19 (m, 3H), 3.47-3.51 (m, 3H), 4.15 (d, *J* = 7.8 Hz, 1H), 4.39-4.47 (m, 3H), 7.22-7.34 (m, 10H), 7.54 (d, *J* = 4.9 Hz, 2H), 7.64 (bs, 1H), 7.77 (m, 1H);

HRMS calcd for $C_{39}H_{54}N_5O_4$ (MH+) 656.4175, found 656.4199; Anal. ($C_{39}H_{53}N_5O_4 \cdot HCl \cdot H_2O$) C, H, N.

Compound 7: colorless solid as a hydrochloride salt; mp: 137.5-138.5 °C; LC-MS r.t. 5.42 min m/z 561.4 (MH+); ^{1}H NMR (400 MHz, methanol-d4) δ 0.70 (t, *J* = 7.3 Hz, 3H), 1.02 (t, *J* = 7.4 Hz, 3H), 1.34 (t, *J* = 6.8 Hz, 3H), 1.47-1.58 (m, 2H), 1.67-1.86 (m, 3H), 2.31 (ddd, *J* = 12.7, 8.3, 4.4 Hz, 1H), 2.86-2.95 (m, 1H), 3.02 (apparent dd, *J* = 13.7, 11.3 Hz, 1H), 3.12-3.22 (m, 3H), 3.46-3.57 (m, 3H), 4.46 (dt, *J* = 11.3, 4.9 Hz, 1H), 7.06 (t, J = 8.8 Hz, 2H), 7.23 (d, J = 6.9 Hz, 1H), 7.27-7.33 (m, 4H), 7.53 (d, J = 5.4 Hz, 2H) 7.55-7.63 (m, 3H), 7.72-7.84 (m, 1H) ; HRMS calcd for $C_{33}H_{41}N_4O_3F$ (MH+) 561.3240, found 561.3262; Anal. ($C_{33}H_{41}N_4O_3F \cdot HCl \cdot 1/2H_2O$) C, H, N.

Table 1. HRMS data for Compounds **1-7**.

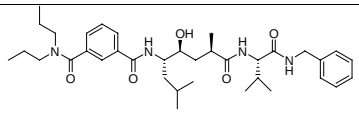
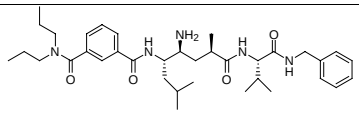
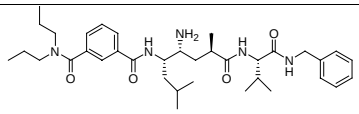
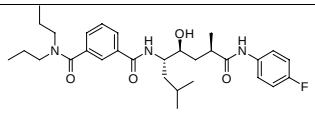
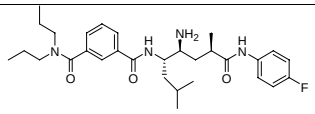
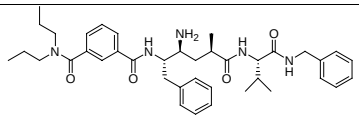
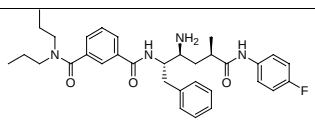
	Structure	Formula	m/z (MH+) Calcd.	m/z(MH+) Found
1		$C_{36}H_{54}N_4O_5$	623.4172	623.4186
2		$C_{36}H_{55}N_5O_4$	622.4332	622.4337
3		$C_{36}H_{55}N_5O_4$	622.4332	622.4332
4		$C_{30}H_{42}N_3O_4F$	528.3237	528.3253
5		$C_{30}H_{43}N_4O_3F$	527.3397	527.3399
6		$C_{39}H_{53}N_5O_4$	656.4175	656.4199
7		$C_{33}H_{41}N_4O_3F$	561.3240	561.3262

Table 2. Elemental Analysis Data (C, H, N) for Compounds **1**-**7**.

	Formula	Calculated				Found		
		C%	H%	N%		C%	H%	N%
1	$C_{36}H_{54}N_4O_5$	69.42	8.74	9.00		69.39	8.64	8.88
2	$C_{36}H_{55}N_5O_4 \cdot HCl$	65.68	8.57	10.64		65.50	8.41	10.42
3	$C_{36}H_{55}N_5O_4 \cdot HCl \cdot 1/2H_2O$	64.79	8.61	10.50		64.67	8.41	10.18
4	$C_{30}H_{42}N_3O_4F$	68.29	8.02	7.96		68.05	7.94	7.95
5	$C_{30}H_{43}N_4O_3F \cdot HCl \cdot 1/2H_2O$	62.97	7.93	9.79		63.09	7.70	9.53
6	$C_{39}H_{53}N_5O_4 \cdot HCl \cdot H_2O$	65.94	7.95	9.86		65.43	7.52	9.78
7	$C_{33}H_{41}N_4O_3F \cdot HCl \cdot 1/2H_2O$	65.38	7.15	9.24		65.21	6.53	9.04

Enzyme Inhibition Assay

C-terminally 6XHis-tagged human proBACE-1 (residues 22-454, numbering starting from the N-terminal methionine) was expressed in Hi5 cells using the baculovirus expression vector pFastbac1 (Invitrogen) and purified on Ni-NTA fast flow resin (Qiagen). Compounds were tested for their ability to inhibit BACE-1 hydrolysis of the internally quenched fluorescent substrate FS-2[1]:

FS-2: MOCAc-Ser-Glu-Val-Asn-Leu-↓-Asp-Ala-Glu-Phe-Lys(DNP)-Arg-Arg-COOH

Inhibitors solutions in DMSO were serially diluted and mixed with a solution of FS-2 in 100mM sodium acetate buffer (pH 4.5), yielding a final FS-2 concentration of 20μM and DMSO concentration of 10%. Reactions were initiated by the addition of BACE-1 (10nM final concentration of active sites; determined by titration against the tight-binding inhibitor OM003[2] using Equation 1 below) and brief mixing. The increase in fluorescence over time was monitored on a Gemini XS fluorometric plate reader using $\lambda_{excitation}$ = 328nm and $\lambda_{emission}$ = 405nm. Initial rates were fit to equation **1**[3] and K_i^{app}'s determined using Graphpad Prism software.

$$v = v_0 \bullet \frac{[E]_0 - [I]_0 - K_i^{app} + \sqrt{([E]_0 - [I]_0 - K_i^{app})^2 + 4[E]_0 K_i^{app}}}{2[E]_0} \qquad \textbf{(1)}$$

In equation **1**, v is the initial rate measured in the presence of $[I]_0$, the inhibitor concentration, using an enzyme concentration $[E]_0$. v_0 is the initial rate measured in the absence of inhibitor.

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(2) Ghosh, A. K.; Bilcer, G.; Harwood, C.; Kawahama, R.; Shin, D.; Hussain, K. A.; Hong, L.; Loy, J. A.; Nguyen, C.; Koelsch, G.; Ermolieff, J.; Tang, J. Structure-based design: potent inhibitors of human brain memapsin 2 (beta-secretase). *J. Med. Chem.* **2001**, *44*, 2865-2868.

(3) Williams, J. W.; Morrison, J. F. The Kinetics of Reversible Tight-Binding Inhibition. *Methods in Enzymology* **1979**, *63*, 437-467.