Nonpeptide Inhibitors of Cathepsin G. Optimization of a Novel β-Ketophosphonic Acid Lead by Structure-Based Drug Design

Michael N. Greco,† Michael J. Hawkins,† Eugene T. Powell,† Harold R. Almond, Jr.,† Thomas W. Corcoran,† Lawrence de Garavilla,† Jack A. Kauffman,† Rosario Recacha,‡ Debashish Chattopadhyay,‡ Patricia Andrade-Gordon,† and Bruce E. Maryanoff *,†

Johnson & Johnson Pharmaceutical Research & Development, Spring House, Pennsylvania 19477-0776

Center for Biophysical Sciences and Engineering, University of Alabama at Birmingham, Birmingham, Alabama 35294

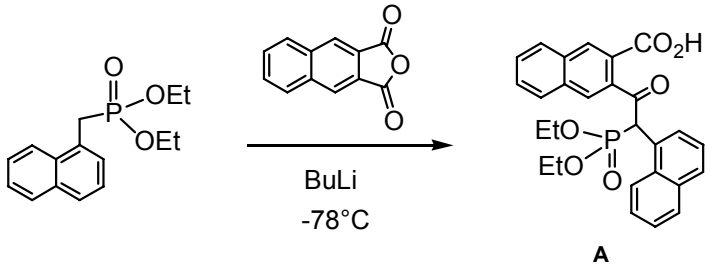
Supporting Information

(11 Pages)

† Johnson & Johnson Pharmaceutical Research & Development (formerly, The R. W. Johnson Pharmaceutical Research Institute).

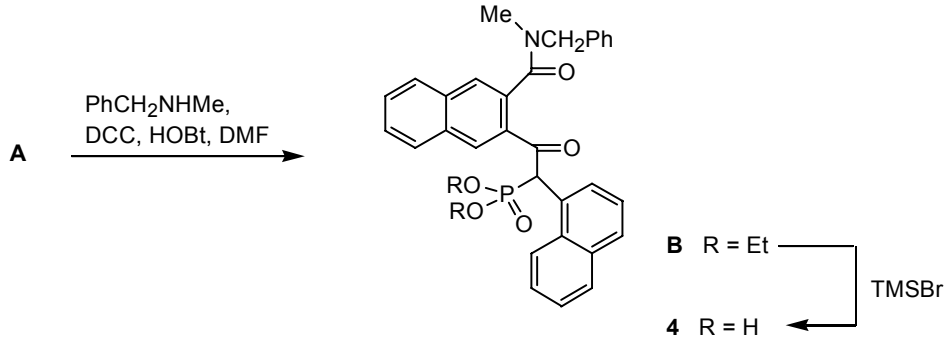
‡ University of Alabama at Birmingham.

General Information. NMR spectra were acquired on either a Bruker Avance 300-MHz or DMX 600-MHz spectrometer with Me_4Si as an internal standard. NMR abbreviations used: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. HPLC analysis was performed on a Hewlett Packard Series 1100 High-Performance Liquid Chromatograph, using a gradient elution: 10-90% MeCN/0.16% TFA in water/0.2% TFA over 4 min; 0.75 mL/min flow rate @ 32°C; Kromasil C18 (50 x 2 mm; 3.5 μm particle size) column. Signals were recorded simultaneously at 220 nm and 254 nm, with a diode array detector. Preparative thin-layer chromatography was conducted on Uniplate (Analtech) silica gel GF plates (1500 μm) with UV visualization. Column chromatography was performed on silica gel 60 (40-63 μm; EM Science). Electrospray (ES) mass spectra were run on a Micromass Platform LC single quadrupole mass spectrometer in the positive mode, unless indicated otherwise. Accurate mass spectra were run on a Micromass Autospec-OATOF double focusing mass spectrometer using fast atom bombardment ionization with thioglycerol as the sample matrix. Elemental analysis was provided by Robertson Microlit Laboratories, Inc., Madison, NJ. Chemical abbreviations: DIPEA, diisopropylethylamine; DCC, dicyclohexylcarbodiimide.

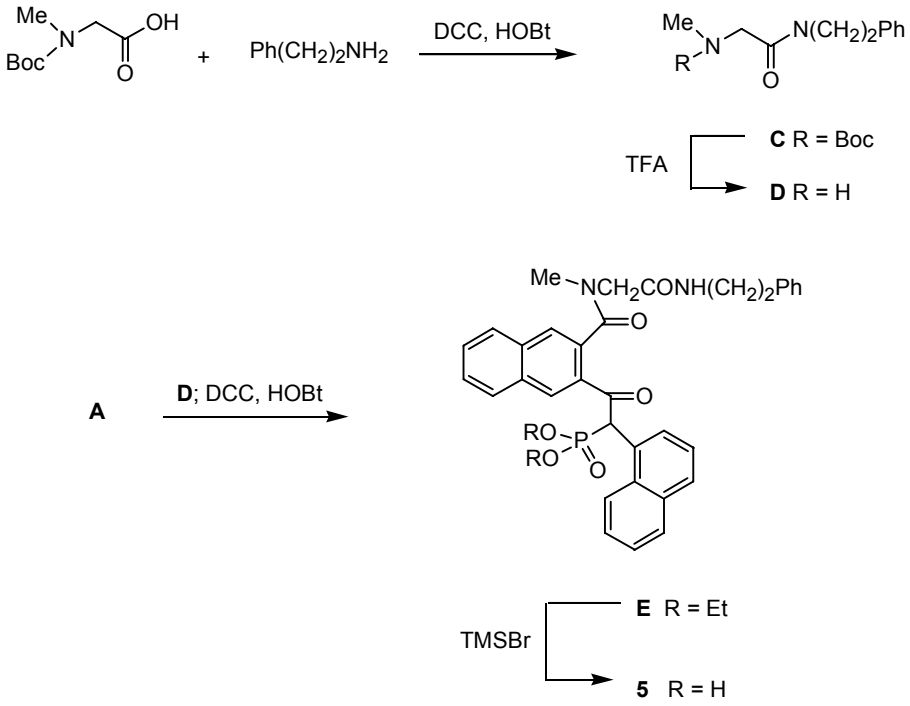


A

Preparation of Intermediate A. To a solution of 2.5 M BuLi in hexanes (40 mL, 0.1 mol) in 70 mL of THF at -78°C was added dropwise a solution of 1-naphthyldiethylphosphonate (Lancaster; 28 g, 0.1 mol) in 60 mL THF over 30 min. After stirring for an additional 30 min, 20 g (0.1 mol) of 2,3-naphthalenedicarboxylic anhydride (TCI America) was added portionwise via solid-addition funnel to the mixture over 20 min. After the addition was complete, the slurry was gradually warmed to 0°C where it was held for another 1.5 h. Excess satd. aq. NH_4Cl was added, and the mixture was filtered through a pad of Celite 545. The filtrate was extracted with 200 mL of EtOAc and the layers were separated. The organic phase was concentrated (without drying) under reduced pressure, and the residue was triturated four times with boiling ether. The residue was treated with 200 mL of EtOAc and adjusted to pH 3 with 2 N HCl while vigorously stirring. The layers were separated and the organic phase was washed once with water, dried (Na_2SO_4), and concentrated to afford 24 g of compound **A** as a white powder, which was used without further purification. MS m/*z* (ion, rel. intensity) 477 (MH^+, 70%), 499 (M + Na, 100%). HPLC R_t = 3.68 min, 86% purity (220 nm).

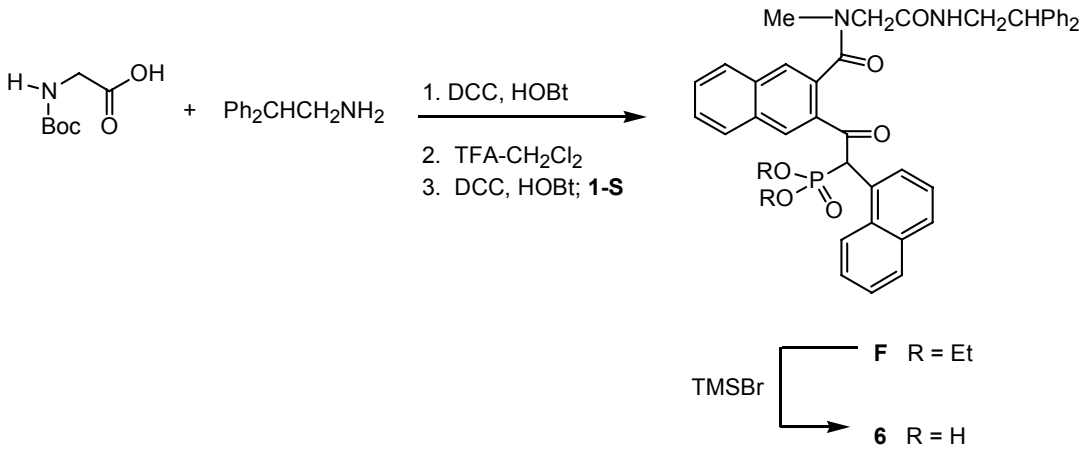


Preparation of 4. A solution of **A** (0.50 g, 1.05 mmol), *N*-methylbenzylamine (0.13 g, 1.1 mmol), and HOBt (0.14 g, 1.05 mmol) in 20 mL of DMF was treated with DCC (0.43 g, 2.1 mmol). After 3 h, the mixture was filtered through Celite, and the filtrate was diluted with EtOAc and washed with 10% aq. citric acid. The organic phase was dried (Na_2SO_4) and concentrated and the residue was purified by flash-column chromatography (Biotage 160 X 40 mm silica gel column; 3% MeOH-CH_2Cl_2 to 10% MeOH-CH_2Cl_2) to afford 0.30 g of **B** (40%): ES-MS m/*z* 578 (M^-). To a mixture of **B** (0.30 g, 0.52 mmol) in 5 mL of CH_2Cl_2 was added TMSBr (0.62 mL, 4.7 mmol) at 0°C under argon. The mixture was stirred for 4 h at 0°C, then concentrated under reduced pressure. A suspension of the residue was stirred in 5 mL of 1 N HCl for 1 h. The white solid was collected and triturated with MeCN to afford 172 mg (31% from **A**) of **4**. ^{1}H NMR (300 MHz, MeOH-d_4) δ 2.58 (s, 2/3 of 3H), 3.05 (1/3 of 3H), 4.13-4.19 (m, 2/3 of 2H), 4.61-4.66 (m, 1/3 of 2H), 6.48 (d, 1/3 of 1H, J_{PH} = 22 Hz), 6.50 (d, 2/3 of 1H, J_{PH} = 22 Hz), 7.11-8.64 (overlapping m, 20H, Ar*H* and $P(OH)_2$). Exact mass calcd for $C_{31}H_{26}NO_5P$ 524.1549 (MH^+), found 524.1651. HPLC R_t = 3.8 min.

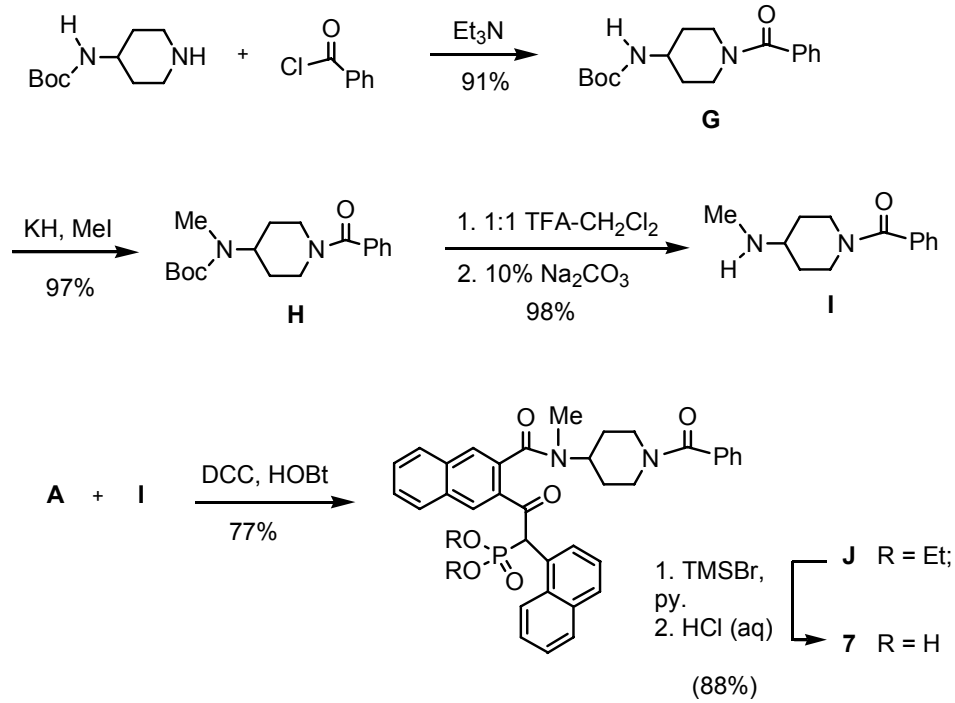


Preparation of 5. A solution of *N*-Boc-sarcosine (0.5 g, 2.6 mmol), phenethylamine (0.3 g, 2.6 mmol), and HOBt (0.4 g, 2.6 mmol) in 20 mL of CH_2Cl_2 was treated with DCC (0.5 g, 2.6 mmol). The mixture was stirred for 12 h, then filtered and concentrated under reduced pressure. The residue was taken up in MeCN, filtered and concentrated to give 0.9 g of **C** which was used in the following step without purification: HPLC R_t = 0.49 min; 73% (220 nm). A solution of 0.9 g of **C** was treated with a solution of 25 mL of 1:1 TFA-CH_2Cl_2 and stirred for 2 h. The solution was concentrated to afford 1.6 g of **D** (assumed quantitative conversion to **D** as a TFA salt containing a total of 9.4 mmol of TFA as a combined salt/solvate) which was used in the following step without purification. A solution of **D** (1.6 g; assumed 2.6 mmol), **A** (1.3 g, 2.6 mmol), and HOBt (0.4 g, 2.6 mmol) in 20 mL of CH_2Cl_2 was treated with 1.64 mL (9.41 mmol,) of DIPEA followed by 0.5 g (2.6 mmol) of DCC. After 3.5 h, another 1-mL portion of DIPEA was added and the mixture was stirred for 48 h. The mixture was filtered and the filtrate was washed sequentially with 1 N $KHSO_4$, satd. aq. $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The residue was purified by flash-column chromatography (silica gel; 0-4% MeOH-CH_2Cl_2) to afford 1.6 g (92%) of **E**: MS *m*/*z* 651 (MH^+); HPLC R_t = 3.88 min.

A solution of **E** (0.14 g, 0.22 mmol) in 1 mL of pyridine was treated with TMSBr (0.24 mL, 1.9 mmol) in 3 equal portions 15 min apart. After 2.5 h, the mixture was concentrated to dryness, and the residue was treated with 5 mL of 1 N HCl and stirred for 1 h. The solid product was collected and washed sequentially with water and ether to yield 0.11 g (86%) of **5**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.56-2.70 (m, 2H), 2.77-2.82 (m, 1H), 2.90-2.97 (m, 1H), 3.16-3.29 (m, 1H), 3.31-3.38 (m, 1H), 3.50-3.58 (m, 1/3 of 1H), 4.0 (s, 2/3 of 1H), 6.31 (d, 1/3 of 1H, J_{PH} = 23 Hz), 6.45 (d, 2/3 of 1H, J_{PH} = 23 Hz), 6.99-8.02 (overlapping m, 17H), 8.51-8.71 (m, 1H), 8.78 (s, 1/3 of 1H), 9.01 (s, 2/3 of 1H). MS m/*z* 595 (MH^+); HPLC R_t = 3.56 min. Exact mass calcd for $C_{34}H_{31}N_2O_6P$ 595.1920 (MH^+), found 595.1998.



Preparation of 6. A solution of *N*-Boc-sarcosine (Acros; 0.25 g, 1.32 mmol), 2,2-diphenyethylamine (0.26 g, 1.32 mmol), and HOBt (0.18 g, 1.32 mmol) in 20 mL of CH_2Cl_2 was treated with DCC (0.27 g, 1.3 mmol). The mixture was stirred for 12 h, filtered, and concentrated under reduced pressure. The residue was taken up in MeCN, filtered, and concentrated. The residue (R_t = 3.68 min) was dissolved in 20 mL of 1:1 CH_2Cl_2-TFA and stirred for 1.5 h. The solution was concentrated, and the residue (0.99 g, assumed quantitative conversion to the TFA salt containing total of 4.2 equiv of TFA as a combined TFA salt/solvate) was combined with **A** (0.63 g, 1.32 mmol), HOBt (0.18 g, 1.32 mmol), DIPEA (0.97 mL, 5.6 mmol) and 20 mL of CH_2Cl_2. After stirring for 12 h, 1.5 mL of DIPEA was added and the reaction was stirred an additional 24 h. The mixture was filtered, and the filtrate was washed sequentially with 1 N $KHSO_4$, satd. aq. $NaHCO_3$, and brine, then dried (Na_2SO_4) and concentrated. The residue was purified by flash-column chromatography (silica gel, MeOH-CH_2Cl_2, gradient from 0 to 4%) to afford 0.33 g (37% overall from *N*-Boc-sarcosine) of **F**: HPLC R_t = 4.18 min; MS m/*z* 670 (MH^+); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03 (t, 3H, *J* = 7 Hz), 1.17 (t, 3H, *J* = 7 Hz), 2.32 (s, 3H), 3.80-4.50 (overlapping m, 8H), 4.81 (t, 1H, *J* = 9 Hz), 6.33 (d, 1H, J_{PH} = 22 Hz), 7.11-7.93 (m, 21H), 8.29-8.48 (m, 2H). A solution of 0.11 g (0.15 mmol) of **F** in 1 mL of pyridine was treated with 0.18 mL (1.2 mmol) of TMSBr in three portions then stirred for 2.5 h. The mixture was concentrated under reduced pressure, and the residue was treated with 5 mL of 1 N HCl and stirred for 1 h. The solid product was collected and washed sequentially with water and ether to yield 83 mg of **6** (82%) as a powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.29 (s, 2/3 of 3H), 2.79 (s, 1/3 of 3H), 3.54-3.96 (overlapping m, 4H), 4.15 (t, 1/3 of 1H, *J* = 8 Hz), 4.51 (t, 2/3 of 1H, *J* = 8 Hz), 6.34 (d, 1/3 of 1H, J_{PH} = 23 Hz), 6.53 (d, 2/3 of 1H, J_{PH}= 23 Hz), 7.11-8.10 (overlapping m, 21 H), 8.56-8.61 (m, 1H), 8.81 (s, 1/3 of 1H), 9.07 (s, 2/3 of 1H). HPLC R_t = 3.96 min. Exact mass calcd for $C_{40}H_{35}N_2O_6P$ 671.2233 (MH^+), found 671.2311.

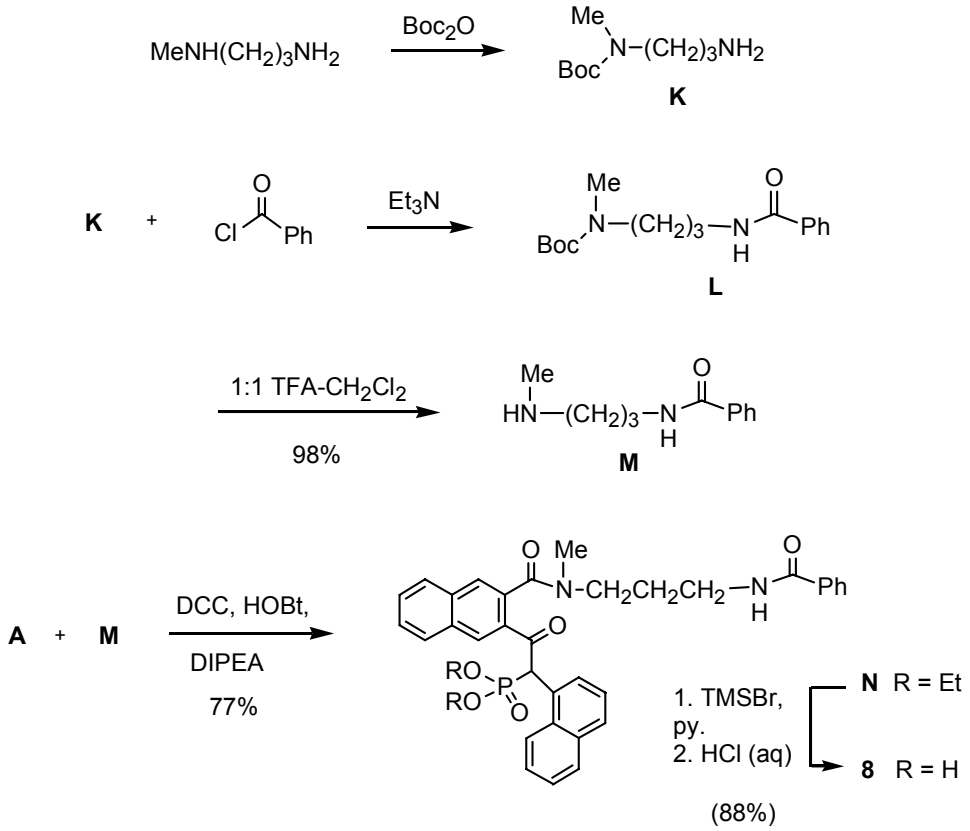


Preparation of 7. To a solution of 4-Boc-amino piperidine (Astatech; 0.4 g, 1.7 mmol) containing 0.3 mL of triethylamine (1.8 mmol) in 10 mL of CH_2Cl_2 was added benzoyl chloride (0.2 mL, 1.8 mmol). After stirring for 18 h, the mixture was washed sequentially with water, Na_2CO_3 (10%, aq.), water, 1 N $KHSO_4$, and water. The organic phase was dried (Na_2SO_4) and concentrated to afford 0.5 g of **G** as a white solid, which was used without further purification: MS m/*z* 305 (MH^+); HPLC R_t = 3.04 min.

Potassium hydride (0.8 g of a 35% oil dispersion; 3.6 mmol) was washed with hexanes, then treated with 20 mL of THF and cooled to 0 °C. To the suspension was added dropwise a solution of **G** (0.5 g, 1.8 mmol) in 5 mL of THF. The mixture was stirred at 0°C for 0.5 h, then stirred an additional 0.5 h at room temperature. The mixture was cooled to 0°C and iodomethane (1.7 g, 10.8 mmol) was added dropwise. The mixture was warmed to room temperature gradually and stirred an additional 2 h. Excess 10% Na_2CO_3 (aq.) was added slowly at 0°C, and the volatiles removed under reduced pressure. The aqueous layer was extracted three times with EtOAc and once with Et_2O, and the combined extracts were dried (Na_2SO_4) and concentrated to yield 0.6 g of **H** as yellow semisolid, which was used without purification in the next step: HPLC R_t= 3.76 min, 100% (254 nm); MS m/*z* 319 (MH^+). A solution of **H** (0.6 g, 1.9 mmol) was dissolved in 10 mL of a 1:1 solution of TFA-CH_2Cl_2 and stirred for 1 h. Volatiles were removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 and treated with excess 10% Na_2CO_3 (aq.). The layers were separated, and the aqueous phase was extracted three times with CH_2Cl_2. The organic extracts were combined, dried (Na_2SO_4) and concentrated to afford 0.42 g of **I** as a viscous oil which was used without further purification in the following step: HPLC R_t = 0.35 min, MS m/*z* 219 (MH^+). A solution of **A** (0.25 g, 0.52 mmol), **I** (0.14 g, 0.64 mmol), and HOBt (0.11 g, 0.79 mmol) in 10 mL MeCN was treated with a solution of DCC (0.13 g, 0.63 mmol) in 2 mL of MeCN. After stirring for 12 h, 2 mL of DIPEA was added and the reaction was stirred for an additional 48 h. The mixture was filtered and concentrated, and the residue was purified by flash-column chromatography (silica, CH_2Cl_2-MeOH 99:1 to 95:5) to yield 0.29 g (80%) of **J**: HPLC R_t = 4.3 min; MS m/*z* 677 (MH^+).

To a solution of **J** (0.28 g, 0.42 mmol) in 4 mL of pyridine was added bromotrimethylsilane (0.7 mL, 5.3 mmol). The mixture was stirred for 45 min, then concentrated under reduced pressure. The residue was treated with excess 3 N HCl, then stirred for 2 h. The white precipitate was collected and rinsed with water, then triturated with MeCN to afford 0.18 g of **7**. HPLC R_t = 3.51 min; MS m/*z* 621 (MH^+); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.50-1.90 (overlapping m, 4H), 2.42 (s, 2/3 of 3H), 2.90 (s, 1/3 of 3 H), 3.18-3.67

(overlapping m, 4H), 4.50-4.90 (m, 1H), 6.41 (d, 1/3 of 1H, J_{PH} = 23 Hz), 6.45 (d, 2/3 of 1H, J_{PH}= 23 Hz), 7.31-8.97 (overlapping m, 20H, ArH + $P(OH)_2$). Anal. Calcd for $C_{36}H_{33}N_2O_6$•1.0 H_2O C, 67.70; H, 5.52; N, 4.39; H_2O, 2.82. Found: C, 67.87; H, 5.36; N, 4.20; H_2O, 2.53.



Preparation of 8. To a solution of *N*-methyl-1,3-diaminopropane (Acros; 4.2 g, 47.9 mmol) in 100 mL of THF was added dropwise a solution of di-*t*-butyl-dicarbonate (3.1 g, 14.4 mmol) in 30 mL of THF. After stirring for 18 h, the mixture was filtered and the filtrate was concentrated. The residue was taken up in EtOAc, washed with water, dried (Na_2SO_4) and concentrated to afford 2.5 g of an oil that consisted of ca. 70% *N*-methyl-*N*-Boc-1,3-diaminopropane (**K**) and 30% *N*-methyl-*N'*-Boc-1,3-diaminopropane, as determined by ^{1}H NMR (300 MHz, DMSO-d_6): *N*-Me δ = 2.83 and 2.41 ppm, respectively. A solution of this mixture (0.5 g, 2.8 mmol) and DIPEA (0.4 g, 2.8 mmol) in 10 mL of CH_2Cl_2 was treated with benzoyl chloride (4 g, 2.8 mmol). After 30 min, the mixture was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by flash-column chromatography (silica gel; CH_2Cl_2 to 96:4 CH_2Cl_2-MeOH) to afford 0.2 g (28%) of **L** as an oil: HPLC R_t = 3.17 min, 93% (220 nm); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37 (s, 9H), 1.71-1.74 (m, 2H), 2.78 (s, 3H), 3.19-3.32 (overlapping m, 4H), 7.43-7.52 (overlapping m, 3H), 7.81-7.84 (overlapping m, 2H), 8.44 (br s, 1H); further proof of structure was obtained by 2D NOESY NMR (600 MHz, DMSO-d_6).

A solution of **L** (0.23 g, 0.77 mmol) in 1.0 mL of TFA was stirred for 30 min. The mixture was concentrated under reduced pressure to give 0.43 g (2.5 mmol, TFA = 3.2 equiv) of **M**: MS m/*z* 193 (MH^+). A solution of **M** (0.43 g 2.5 mmol) was combined with **A** (0.37 g, 0.77 mmol), HOBt (0.11 g, 0.77 mmol), DIPEA (0.32 g, 2.5 mmol) and DCC (0.16 g, 0.77 mmol) in 25 mL of CH_2Cl_2. After 3 h, 1.5 mL of DIPEA was added and the reaction was stirred for 24 h. The reaction filtrate was washed sequentially with 1 N $KHSO_4$ and brine, then dried (Na_2SO_4) and concentrated. The residue was purified by flash-column chromatography (silica gel; CH_2Cl_2 to 97:3 CH_2Cl_2-MeOH) followed by preparative TLC (silica gel, 9:1 hexanes-EtOAc) to yield 0.2 g of **N** (40% from **M**): MS *m/z* 652 (MH^+): HPLC: R_t = 3.99 min.

A solution of **N** (0.20 g, 0.31 mmol) in 2.5 mL of pyridine was treated with TMSBr (0.32 mL, 2.5 mmol). After 1.5 h, the mixture was concentrated under reduced pressure, and the residue was suspended in 2 mL of 1 N HCl. The white solid product was collected and triturated with acetonitrile to afford 0.095 g (51%) of **8** as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.70-1.91 (m, 2H), 2.06 (s, 2/3 of 3H), 2.61 (s, 1/3 of 3H), 3.43-3.54 (m, 2H), 6.29 (d, 1/3 of 1H, J_{PH} = 23 Hz), 6.39 (d, 2/3 of 1H, J_{PH} = 23 Hz), 7.28-8.91 (overlapping m, 18H, ArH). Exact mass calcd for $C_{34}H_{31}N_2O_6P$ 595.191975 (MH^+), found 595.202208.

Enzyme Assays. Enzyme catalyzed hydrolysis rates were measured spectrophotometrically using the appropriate enzyme and chromogenic substrate. For human neutrophil cathepsin G (Athens Research and Technology #16-14-030107), the assay was conducted in an aqueous buffer (100 mM Hepes, 500 mM NaCl, pH 7.4) using the chromogenic substrate *N*-Suc-Ala-Ala-Pro-Phe-pNa (Bachem #L1400; K_m cathepsin G = 3.0 ± 0.6 mM) and a microplate reader (Molecular Devices). IC_{50} experiments were conducted by fixing the enzyme and substrate concentrations (70 nM enzyme, 5 μM) and varying the inhibitor concentration. Changes in absorbance at 405 nm were monitored using the software program Softmax (Molecular Devices), upon addition of enzyme, with and without inhibitor present at 37°C for 30 min. Percent inhibition was calculated by comparing the initial reaction slopes of samples without inhibitor to those with inhibitor. IC_{50} determination was made using a four parameter fit logistics model. Inhibition constants (K_i values) were determined under conditions for the analysis of Michaelis-Menten kinetics (70 nM cathepsin G and 0.3–10 mM substrate). Changes in absorbance at 405 nm were monitored (37°C for 30 min) using the software program Softmax (Molecular Devices), on addition of enzyme, with and without inhibitor present. Initial reaction slopes of samples were analyzed using the program K-Cat (BioMetallics).

Enzyme-catalyzed hydrolysis rates were measured spectrophotometrically as described for cathepsin G for the following enzymes: chymotrypsin, thrombin, factor Xa, factor IXa, plasmin, trypsin, tryptase, proteinase 3, and elastase. The enzyme sources, substrates/K_m, buffer, IC_{50} assay concentrations [E]/[S], and K_i assay concentrations [E]/[S], were as follows: for bovine pancreas **α-chymotrypsin** (Sigma C-7762), *N*-Suc-Ala-Ala-Pro-Phe-pNa (Bachem L-1400)/K_m 91 µM, buffer A (10mM Tris, 10 mM Hepes, 150 mM NaCl, 0.1% PEG 8000, pH 7.4), 0.1 µg/ml [E]/250 µM [S], 0.1 µg/ml [E]/10-300 µM [S]; for human **α-thrombin** (American Diagnostica #470HT), H-D-HHT-Ala-Arg-pNa (American Diagnostica #238)/K_m 24 µM, buffer A, 0.9 nM [E]/50 µM [S], 0.9 nM [E]/5-100 µM [S]; for human plasma coagulation **factor Xa** (American Diagnostica #526), MeO-CO-CHG-Gly-Arg-pNA (American Diagnostica #222)/K_m 430 µM, buffer A, 0.28 U/ml [E]/500 µM [S], 0.28 U/ml [E]/50-700 µM [S]; for for human plasma coagulation **factor IXa** (Calbiochem #233290), MeO-CO-CHG-Gly-Arg-pNA (American Diagnostica #222)/K_m 700 µM, Buffer B (50 mM Tris, 100 mM NaCl, 5 mM $CaCl_2$, 33% ethylene glycol, pH 7.4), 65 nM [E]/500 µM [S], 65 nM [E]/100-700 µM [S]; for human **plasmin** (American Diagnostica #421), H-D-Nle-HHT-Lys-pNa (American Diagnostica #251)/K_m 42 µM, buffer A, 1.4 µg/ml [E]/200 µM [S], 1.4 µg/ml [E]/20-500 µM [S]; for human pancreas **trypsin** (Calbiochem #650275), *N*-α-Cbo-D-Arg-Gly-Arg-pNa (Diapharma #S-2765)/K_m 35 µM, buffer C (50 mM Hepes, 200 mM NaCl, 0.05% *n*-octyl glucoside, pH 7.4), 0.9 nM [E]/60 µM [S], 0.9 nM [E]/3-60 µM [S]; for human lung **tryptase** (Cortex Biochem #CP3033), H-D-HHT-Ala-Arg-pNa (American Diagnostica #238)/K_m 580 µM, buffer A, 5 nM [E]/715 µM [S], 1 nM [E]/30-700 µM [S]; for human neutrophil **proteinase 3** (Athens Research and Technology #16-14-161820), MeOSuc-Ala-Ala-Pro-Val-pNA (Bachem #L-1335)/K_m 880 µM, buffer D (100mM Tris, 50 mM, pH 7.5), 110 nM [E]/7900 µM [S], 55 nM [E]/300-10000 µM [S]; for human leukocyte **elastase** (ICN#191337), MeOSuc-Ala-Ala-Pro-Val-pNA (Bachem #L-1335)/K_m 780 µM, buffer E (50 mM Tris, 0.1% Tween 80, pH 8.4), 27 nM [E]/2000 µM [S], 27 nM [E]/100-2000 µM [S].

Molecular Modeling Study with 7. Simulated annealing was used to obtain a low energy structure of the ligand **7** docked into the active site of Cat G. Explicit water was placed about the active site of Cat G; the α-carbons of the amino acid backbone of Cat G were restrained; the phosphorus atom of **7** was restrained at the position found in the X-ray crystal structure of **1**▪Cat G.[7] The system was heated over 3 ps to 600 K and held at that temperature with AMBER-5[a] by using "belly"-type dynamics. A sample was taken at each 1 ps and it was slowly cooled and equilibrated over 12 ps. The lowest energy structure is shown in Figure 4.

a. Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Gould, I. R.; Merz, K. M., Jr.; Ferguson, D. M.; Spellmeyer, D. C.; Fox, T.; Caldwell, W. J.; Kollman, P. A. *J. Am. Chem. Soc*. **1995**, 117, 5179-5197.

Figure 4. View of **7** (gray with heteroatoms colored) within the active site of cathepsin G (green electron-density surface), derived from simulated annealing studies. The complex is oriented so as to peer directly into the S1 pocket.

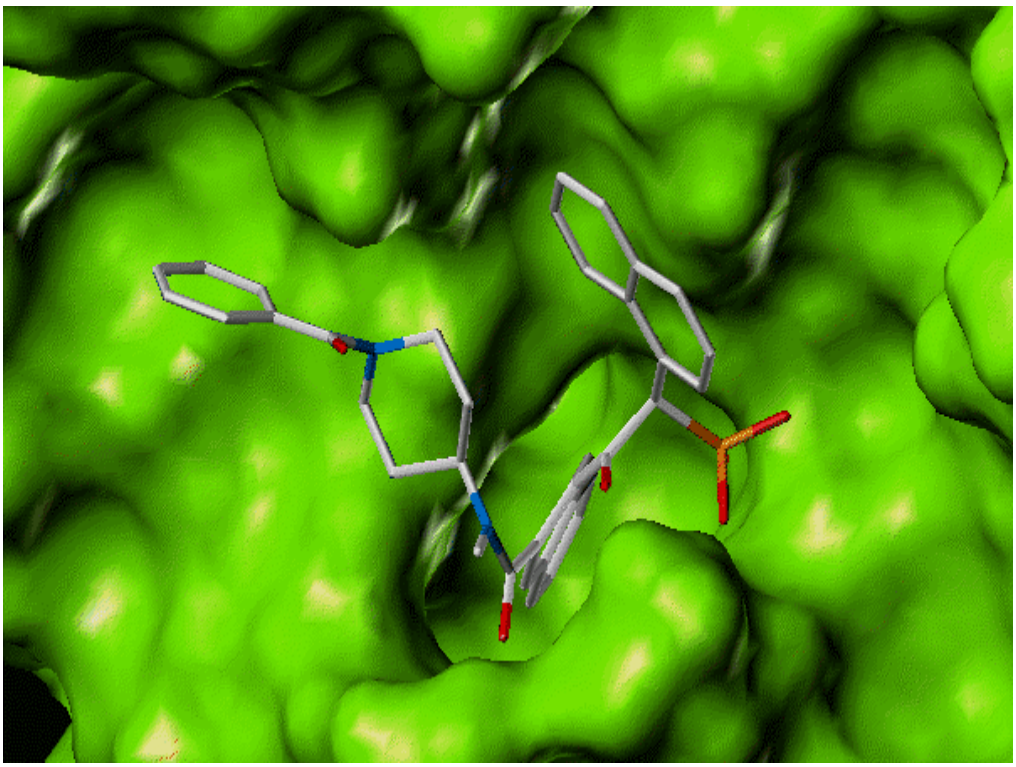


Figure A. Schematic of the interactions of **1** with Cat G and our design rationale for enhancing the potency of **1** by using the S3 pocket.

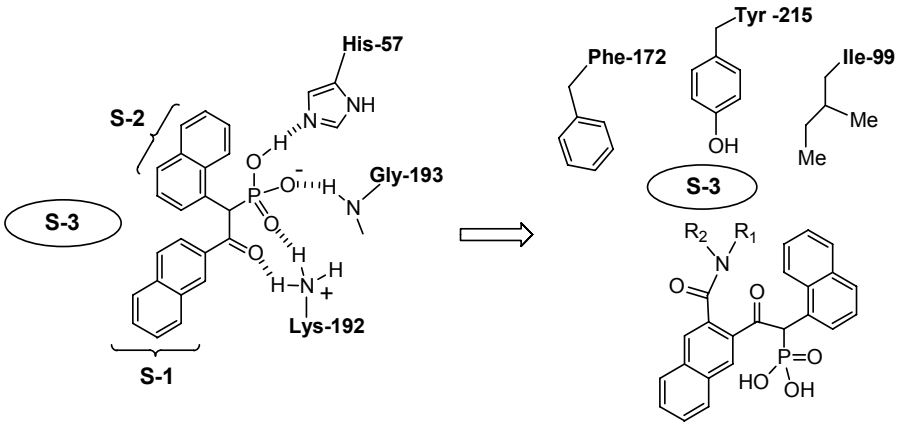


Figure B. Stereoview of Figure 1 in the paper.

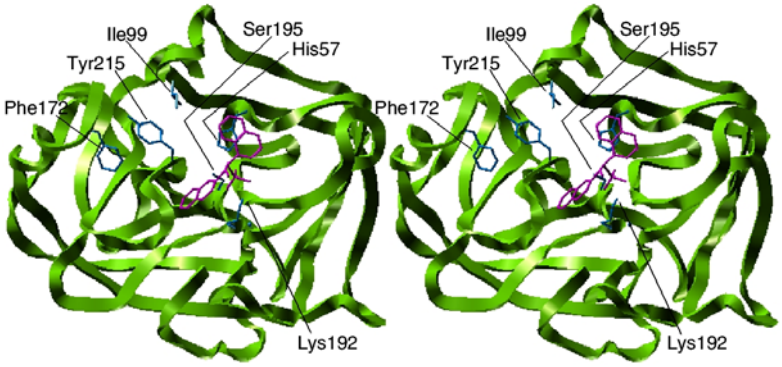
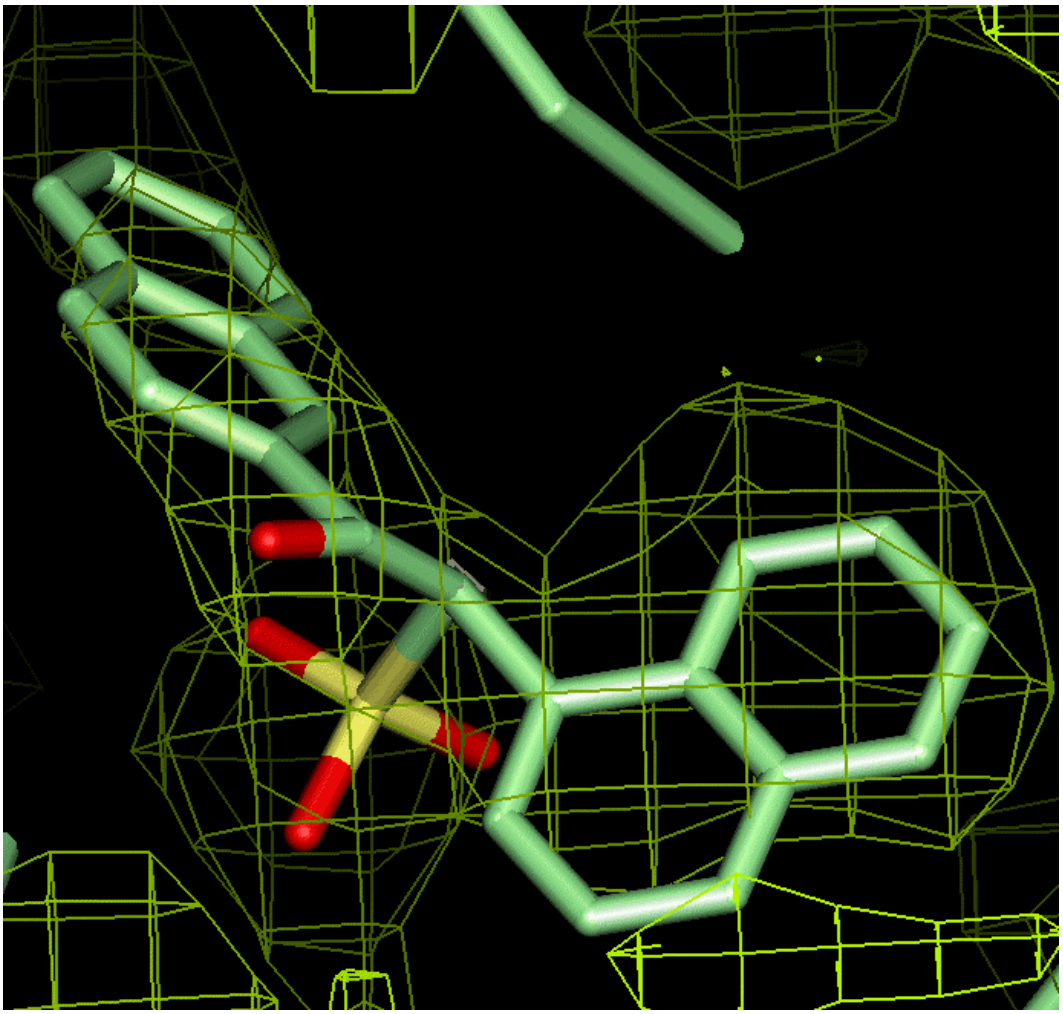
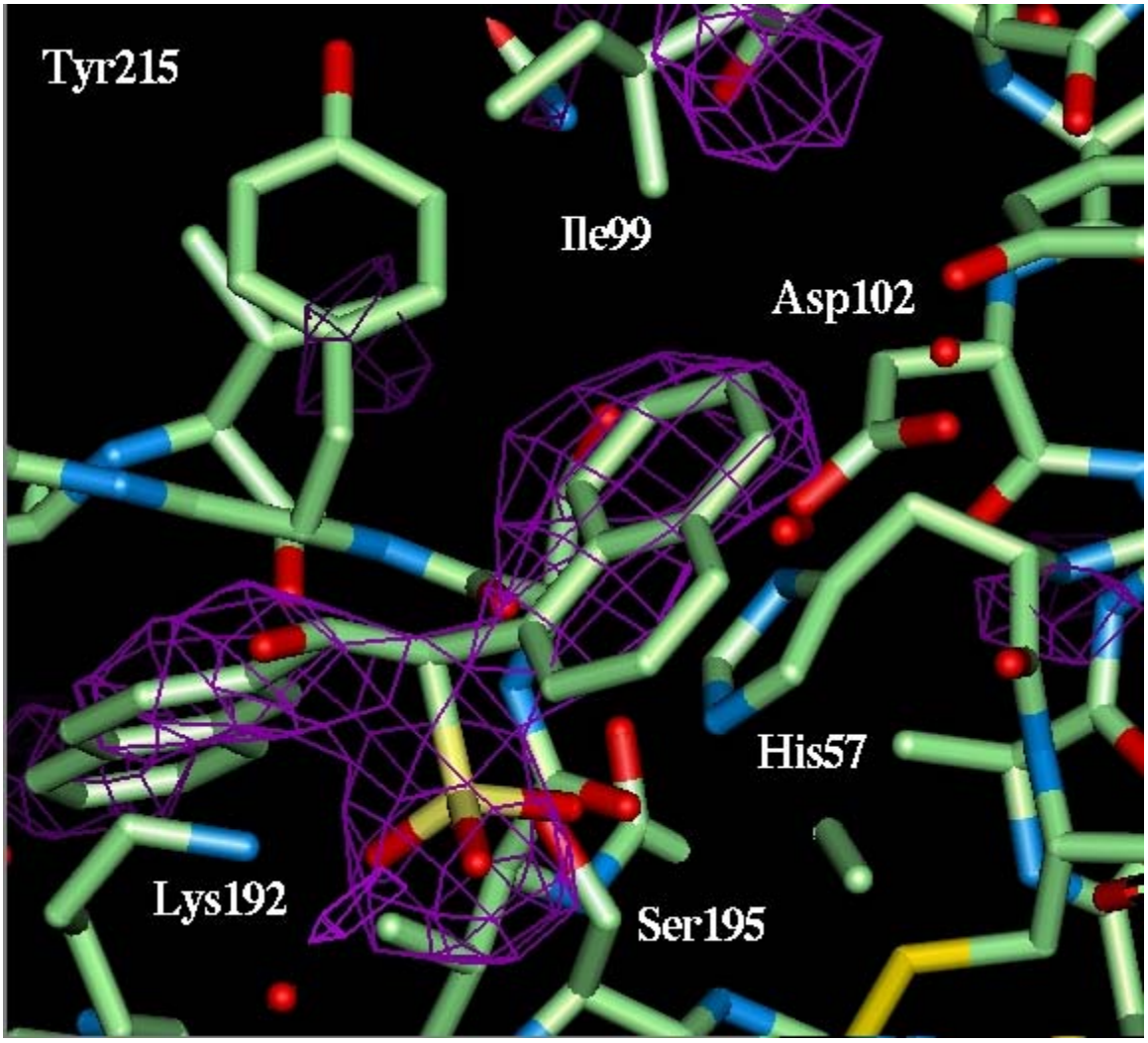


Figure C. Two views of the electron-density map (*F*o-*F*c), contoured at 2σ, showing **1** within the active site of cathepsin G.




Tyr215
Ile99
Asp102
His57
Lys192
Ser195

Filename: ja017506h-4_s1_word2000
Directory: C:\Documents and Settings\mnd96\Desktop
Template: C:\Documents and Settings\mnd96\Application Data\Microsoft\Templates\Normal.dot
Title: Preparation of 1-S
Subject:
Author: J&J User
Keywords:
Comments:
Creation Date: 1/3/2002 11:13 AM
Change Number: 8
Last Saved On: 1/31/2002 5:17 PM
Last Saved By: bmaryano
Total Editing Time: 31 Minutes
Last Printed On: 2/1/2002 6:14 PM
As of Last Complete Printing
Number of Pages: 11
Number of Words: 2,891 (approx.)
Number of Characters: 16,479 (approx.)