Supplementary Material

I. Structure Generating Process for Inhibitor 1

Before GrowMol begins the process of generating molecules, a three dimensional structure provided by X-ray crystallography or a NMR structure of the enzyme is needed. We began with the crystal structure of R. *chinensis* pepsin *vida infra*. The enzyme was reduced in size to only the residues within six or seven angstroms of the known inhibitor pepstatin bound in the active site. From this enzyme "shell" a decision must be made where to initiate growth of the new molecule. Several atoms in the active site were selected and colored allowing the QXP program (*vida infra*) to use those atoms as vertices and fill the binding site with connected triangles called a "tessel." This defines the volume that GrowMol uses to create new molecules and must be optimized by trial and error until the desired area of the binding site is enclosed by the tessel as determined by visual inspection. This contained volume is then marked off as a three dimensional grid with points spaced 0.25 Å apart. Each grid point contains information about its distance and complementarity (such as hydrophobicity or hydrogen bonding capacity) to the nearest enzyme atoms. We then defined a "root". This is a growth point atom which is linearly connected to at least three other non-hydrogen atoms. All molecules generated will begin their growth from this point. To help set the initial growth points, the hydroxyl of the bound inhibitor pepstatin, the transition state isostere, was selected and used as the root. This is justified since the experimentally determined geometry observed in the crystal structure of the inhibitor-enzyme complex should provide a realistic starting point for growth. We then adjusted the probability that GrowMol would add a certain atom type to the growth point. This information is contained in the probability table (see Table 1) which contains a list of probabilities for adding new atoms to exisiting ones. The number of molecules (1,000-4,000 per run) and maximum number of atoms each molecule (20) was also inputted before we started the molecular generation. The program then creates the requisite number of molecules and we viewed the cyclic structures and visually evaluated them for their structural interest and synthetic feasibility.

II. Table 1

Probabilities for the Different Atom Types

	General	Aromatic	C=O	NH (sp2)	Oxygen	Ar2	Root
Carbon	0.50-0.65	0.60	0.80	0.60	0.65	0.75	1.00
Benzyl	0.20	0.30	0.20	0.25	0.35	0.05	0.00
C=O	0.00	0.00	0.00	0.00	0.00	0.05	0.00
NH (sp^2)	0.00	0.00	0.00	0.00	0.00	0.05	0.00
N (sp^3)	0.00-0.15	0.00	0.00	0.00	0.00	0.05	0.00
Oxygen	0.00	0.00	0.00	0.00	0.00	0.05	0.00
O minus	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hydrogen	0.00	0.00	0.00	0.15	0.00	0.00	0.00
Five	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FiveT	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6 Fused	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5 Fused	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sulfur	0.00-0.15	0.10	0.00	0.00	0.00	0.00	0.00

Aromatic2: the newly grown ring is already attached to another aromatic ring
Root: the probability is always 100% that GrowMol will start growth only at a carbon atom
6 Fused: adding another phenyl ring to an existing one
5 Fused: adding a cyclopentadiene ring to an existing phenyl ring

The top of the table lists the atom types of the most recently grown atom in the GrowMol growth algorithm. On the left side of the table are listed atoms or groups that can be added to these newly grown atoms. The numbers listed in the columns represent the probabilities that a given atom or group will be added to that growth point. For example, the probability of adding a benzyl group to a NH(sp^2) atom is 25%. This is the probability table that was used for the generation of the 1000 cyclic structures grown in the R. *chinensis* active site.

III. Experimental Details for Compounds 8ab-12

*N-Acetyl-3[2-oxocyclohexyl)thio]alanine (**9**)*

A solution of Ac-Cys-OH (4.58g, 20.1 mmol, 1.0 eq.) in absolute ethanol (10 mL) was cooled to -5ºC in an acetone-ice bath. To this solution was added 3N NaOH (11.7 mL, 35.1 mmol, 1.25 eq.) and the resulting mixture was stirred for 10 min. A solution of 2-chlorocyclohexanone (4.84 g, 3.65 mmol, 1.3 eq.) in ethanol (3 mL) was added and the mixture stirred for another 5 minutes. A phosphate (KH_2PO_4:Na_2HPO_4, pH 7.41) buffer (10 mL) was added in two portions over 10 minutes until a neutral pH was obtained. As necessary more 1N NaOH was added to neutralize the solution. The mixture was extracted with hexanes (6 x 30 mL) and the aqueous layer was concentrated and then allowed to stand for three days at room temperature. The mother liquor was filtered and the solid was collected to afford 2.92 g (41%) of **9** as flat white plates: mp 119-122ºC; ^{1}H NMR (DMSO) δ 12.8 (s, 1H), 8.23 (d, 1H), 4.37 (m, 1H), 3.60 (m, 1H), 2.77 (m, 2H), 2.24 (m, 2H), 1.87 (s, 3H), 1.79 (m, 6H); MS (FAB, 3-NBA matrix) $C_{11}H_{17}NO_4S$ $[M+H]^+$ 260.1.

*Z-Lys(Boc)-CH_2OH (**11**)*

To a 100 mL round bottom was added Z-Lys(Boc)-OH (4.49 g, 11.8 mmol, 1.0 eq.) followed by 25 mL freshly distilled THF. The solution was cooled to -10ºC in an acetone-ice bath. A solution of 1M BH_3-THF (26 mL, 26.0 mmol, 2.2 eq.) was added dropwise over 20 minutes. The resulting solution was stirred for 80 minutes below 0ºC and then warmed to room temperature for two hours. The reaction was quenched by careful addition of methanol (20 mL) and then concentrated *in vacuo*. The product was purified by flash chromatography (1:1 EtOAc : hexanes) to give 2.98 g (69%) of an amorphous white solid **11**: TLC R_f=0.24 (95:5:2 EtOAc : MeOH : H_2O); $[\alpha]^{24}_D$= -9.31º (c=1.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7-2.7-7.37 (m, 5H), 5.09 (bs, 2H), 3.61 (m, 3H), 3.10 (m, 2H), 1.34-1.59 (m, 6H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 156.79, 156.42, 136. 47, 128.52, 128.12, 128.09, 79.28, 66.77, 64.82, 52.98, 39.73, 29.89, 28.41, 22.66; MS (FAB, 3-NBA matrix) $C_{19}H_{30}N_2O_5$ $[M+H]^+$ 367.2.

H-Lys(Boc)-CH_2OH

A 50 mM solution of Z-Lys(Boc)-CH_2OH (**11**) (812 mgs, 2.2 mmol) in MeOH was evacuated and stirred under an atmosphere of H_2 for 12 hours. The solution was then filtered through a 0.2 μm Gellman Sciences CR PTFE acrodisc or filter paper, washed with methanl and concentrated *in vacuo*. to afford 513 mgs (99%) of a colorless oil. This oil was taken on to the next coupling step without further purification.

*Fmoc-Val-Lys(Boc)-CH_2OH (**10**)*

H-Lys(Boc)-CH_2OH (513 mgs, 2.22 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (14 mL) and cooled to -10ºC in an acetone-ice bath. To this solution was added together, all at once, Fmoc-Val-OH (827 mgs, 2.44 mmol, 1.1 eq.), HOBt (390 mgs, 2.55 mmol, 1.15 eq.) and EDCI (488 mgs, 2.55 mmol, 1.15 eq.). Flash chromatography (gradient 97:3 to 96:4 $CHCl_3$: MeOH) afforded 851 mgs (69%) of a white solid **10**: mp 160-161ºC; TLC R_f=0.12 (96:4 $CHCl_3$: MeOH); $[\alpha]^{24}_D$= -21.94º (c=0.98, $CHCl_3$); ^{1}H NMR (DMSO) δ 7.89 (d, 2H), 7.76 (m, 2H), 7.58 (d, 1H), 7.25-7.48 (m, 5H), 6.70 (t, 1H), 4.62 (t, 1H), 4.18-4.34 (m, 3H), 3.82 (t, 1H), 3.67 (t, 1H), 3.17-3.40 (m, 2H), 2.85 (m, 2H), 1.92 (m, 1H), 1.39 (s, 9H), 0.93 (m, 6H); ^{13}C NMR (DMSO) δ 170.77, 156.01, 155.50, 143.90, 143.75, 140.69, 127.60, 127.01, 125.33, 120.06, 77.24, 65.62, 63.14, 60.36, 50.55, 46.68, 30.32, 29.47, 28.22, 22.82, 19.22, 18.30; MS (FAB, 3-NBA matrix) $C_{31}H_{43}N_3O_6$ $[M+H]^+$ 554.3.

H-Val-Lys(Boc)-CH_2OH

Fmoc-Val-Lys(Boc)-CH_2OH **10** (542 mgs, 0.98 mmol) and Et_2NH (15 mL) were dissolved in DMF (15 mL). After 30 minutes, no starting material was seen by TLC visualization and the reaction was then concentrated *in vacuo* to a clear oil and taken on to the next step without further purification.

*N-Acetyl-3[2-oxocyclohexyl)thio]alanine-Val-Lys(Boc)-CH_2OH (**12**)*

A solution of H-Val-Lys(Boc)-CH_2OH (599 mgs, 1.81 mmol, 1.0 eq) in CH_2Cl_2 (18 mL) was cooled to -5ºC in an acetone-ice bath. N-Acetyl-3[2-oxocyclohexyl)thio]alanine **9** (515 mgs, 1.99 mmol, 1.1 eq.) and PyBOP (976 mgs, 1.81 mmol, 1.0 eq.) were added and the resulting mixture stirred for 24 hours while warming to room temperature. The reaction was poured into EtOAc (100 mL) and washed with 5% $KHSO_4$ (60 mL), 1N

$NaHCO_3$ (60 mL) and brine (30 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. Flash chromatography (95:5:1 EtOAc : MeOH : H_2O) afforded 604 mgs (58%) of white solid **12** as a mixture of two diastereomers: mp 165-168°C; TLC R_f= 0.15 (95:5:1 EtOAc : MeOH : H_2O); ^1H NMR (DMSO) δ 8.18 (d, 1H), 7.78 (dd, 1H), 7.51 (t, 1H), 6.73 (t, 1H), 4.58 (t, 1H), 4.44 (m, 1H), 4.08 (m, 1H), 3.62 (m, 2H), 3.18 (m, 1H), 2.87 (m, 2H), 2.75 (dd, 1H), 2.61 (m, 2H), 2.22 (m, 2H), 1.48-2.00 (m, 11H), 1.85 (2s, 1H, diasteromeric methyls from Ac), 1.13-1.43 (m, 6H), 1.37 (s, 9H); MS (FAB, 3-NBA matrix) $C_{27}H_{48}N_4O_7S$ $[M+H]^+$ 573.3.

*N-(10-hydroxymethyl-13-isopropyl-12,15-dioxo-octadecahydro-18-thia-5,11,14-triaza-benzocyclohexadecen-16-yl)-actetamide (**8ab**)*

Deprotected tripeptide (48 mgs, 0.082 mmol, 1.0 eq.) was dissolved in anhydrous EtOH (50 mL). Solid $NaHCO_3$ was then added until a pH of 6 was obtained. Then $NaBH_3CN$ (5 mgs, 0.082 mmol, 1.0 eq.) was added and the resulting mixture stirred for three days at room temperature. The final pH of the reaction mixture was 9. The reaction was filtered through an acrodisc and partially purified by column chromatography (5:4:1 $CHCl_3$: MeOH : H_2O). This crude material was further purified by HPLC to yield 10.3mgs (22%) of a early eluting diasteromeric mixture **8a** and 16.0 mgs (34%) of a later eluting diastereomeric mixture **8b**:

8a: ^1H (D_2O) δ [diastereomeric αHs: 4.53 (d, 1H), 4.43 (d, 1H)], 4.06 (m, 1H), [diastereomers 3.99 (d, 1H), 3.91 (d, 1H)], 3.49-3.60 (m, 2H), 3.45 (m, 2H), [mixture of diastereomeric Hs: 2.96-3.23 (m, 8H)], 2.89 (dt, 1H), 2.81 (t, 1H), 1.27- 2.26 (m, 18H), [diastereomeric acetyls: 2.00 (s, 3H), 1.99 (s, 3H)], 0.96 (pair d, 6H); MS (FAB, 3-NBA matrix) $C_{22}H_{40}N_4O_4S$ $[M+H]^+$ 457.2.

8b: ^1H (CD_3OD) δ [diastereomeric proton pairs: 4.77 (dd, 1H) and 4.51 (dd, 1H), 4.06 (m, 2H), 3.92 (d, 1H) and 3.90 (d, 1H)], 3.55 (m, 1H), 3.48 (m, 2H), 3.40 (td, 1H), 3.21 (dt, 1H), 3.06-3.17 (m, 2H), 2.90- 3.01 (m, 1H), 2.86 (dt, 1H), 2.79 (dd, 1H), 2.19 (bd, 1H), 1.28-2.03 (m, 17H), [diastereomeric acetyls: 2.00 (s, 3H), 1.96 (s, 3H)], 1.01 (pair d, 6H); ^{13}C (D_2O) δ 177.58, 177.27, 176.97, 176.64, 175.02, 174.94, 67.61, 66.12, 64.21, 63.98, 62.15, 61.68, 58.00, 55.38, 55.26, 53.90, 53.19, 48.26, 48.12, 46.73 35.79, 35.40, 33.97, 33.40, 33.03, 32.76, 32.31,

31.86, 30.50, 28.75, 28.57, 27.40, 27.03, 25.99, 25.26, 25.19, 22.77, 22.01, 21.94, 21.89, 21.80, 21.34, 19.87, 15.76; MS (FAB, 3-NBA matrix) $C_{22}H_{40}N_4O_4S$ $[M+H]^+$ 457.2.