Design and Synthesis of a Conformationally Restricted Cysteine Protease Inhibitor

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Using the X-ray analyses of papain and papain–chloromethyl ketone inhibitor complexes as representative cysteine protease structures, molecular graphics analyses were applied to design (4*R*,1′*S*)-2-[1′-[(benzyloxycarbon)amino]ethyl]-4-benzyl-4-[[(2-oxoethyl)amino]carbonyl]-3,4,5,6-tetrahydropyrimidine (**1**) as a conformationally restricted, competitive inhibitor of cysteine proteases. Two routes to this target inhibitor, which was found to be a good competitive inhibitor of papain with a K_I of 790 nM, are described.

Cysteine proteases are a class of enzymes whose proteolytic activity is dependent on the thiol group of a cysteine residue.[1] Because of the key roles played by cysteine proteases in promoting diseases such as muscular dystrophy[2] and myocardial infarction,[3] their inhibition has been extensively studied.[4] The best documented cysteine protease is papain, which is isolated from the latex of the *Carica papaya* fruit. The enzyme has been well characterized,[1,5] and its X-ray structure,[6] as well as those of chloromethyl ketone-inhibited papain complexes,[7] are also available. While papain is not itself involved in disease-mediation, it provides a good representative mechanistic[8] and structural[9] model for evaluating the design of cysteine protease inhibitors of potential therapeutic value.

We elected to evaluate aldehyde inhibitors that were also conformationally restricted. It is well established that peptidyl aldehydes are potent, reversible inhibitors that form transition state-like hemithioacetal complexes with cysteine proteases.[10] Conformationally restricted enzyme inhibitors[11] are of considerable interest because they impede degradation by the proteases in the living cells, while retaining the potential for high activity and selectivity.

Suitable conformational restrictions for incorporation into the structure of a target inhibitor were identified from the X-ray structures of papain covalently alkylated by good, irreversible, chloromethyl ketone inhibitors,[7] whose peptidyl groups **A**–**C** are shown in Figure 1. The X-ray data show that, with such inhibitors covalently bound at the active site, the peptidyl backbones of **A**–**C** are conformationally constrained such that the bonds linking atoms abcd, on either side of the phenylalanyl benzyl group that locates in the S_2-pocket,[12] subtend dihedral angles (Φ) in the range $-88.5°$ to $-82.3°$ as measured by graphics analysis. The key hydrogen bonding interactions[7,13] were identified in the same way. Heterocyclic functions have been identified as attractive, protease-stable, bioisosteric replacements for peptide moieties.[14] Accordingly, five- and six-membered heterocyclic ring structures mimicking the abcd-peptide moieties of the inhibitor structures **A**–**C** were examined to identify those that reproduced as closely as possible the Figure 1 dihedral angle patterns without disturbing any key hydrogen bonding interaction, while maintaining the dominant specificity-determining affinity of the S_2-site for the phenylalanine side chain. Of the heterocyclic combinations surveyed, that incorporating a six-membered amidine ring, with an estimated Φ angle of $\sim -81°$, matched the corresponding Figure 1 angles most closely and was therefore chosen as the conformation-restriction instrument for incorporation into the target inhibitor.

The target inhibitor structure selected was (4*R*,1′*S*)-2-[1′-[(benzyloxycarbonyl)amino]ethyl]-4-benzyl-4-[[(2-oxoethyl)amino]carbonyl]-3,4,5,6-tetrahydropyrimidine (**1**). Retrosynthetic analysis (Scheme 1) identified the activated thioimidate **2**, the diamino acid **3**,[14b,15] and the commercially available aminoacetaldehyde dimethyl acetal (**4**), as potential precursors of **1**.

The diamino acid **3** was prepared as outlined in Scheme 2. Application of the BF_3-mediated condensation of N-Cbz-phenylalanine with benzaldehyde dimethyl

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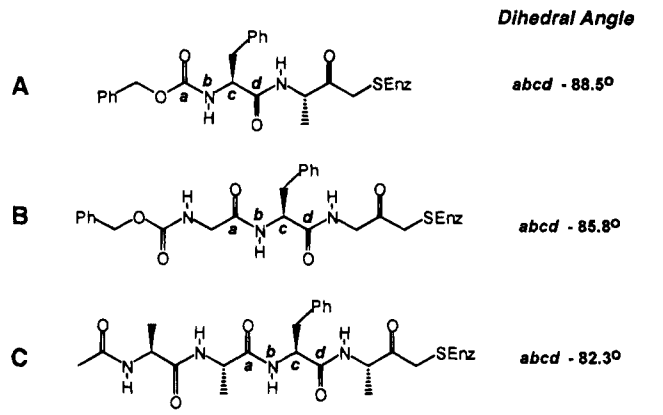
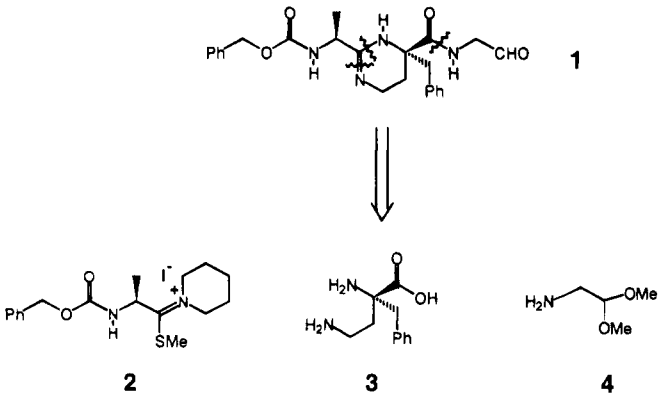


Figure 1. Dihedral angles subtended by the key abcd atom regions of papain–inhibitor complexes (from X-ray structures[7]).

Scheme 1

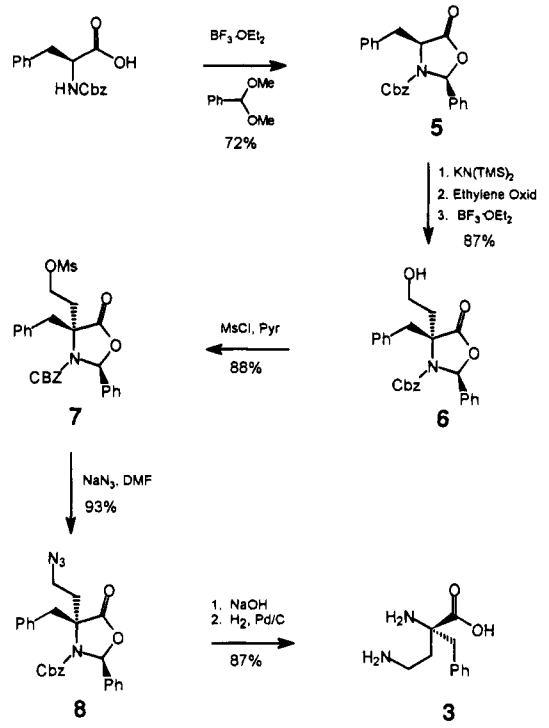


acetal was a modification of the method of Karady *et al.*[16] that provided a 2-fold improvement in the yield of the oxazolidinone **5**. Conversion of **5** to the desired synthon **3** was then accomplished by the **5** → **6** → **7** → **8** → **3** sequence of Scheme 2, each reaction of which proceeded with high efficiency, with the overall yield of **3** from N-Cbz-phenylalanine being 45%. The precursor **9** of the activated thioimidate **2** was prepared in 77% overall yield from N-Cbz-L-alanine as shown in Scheme 3.

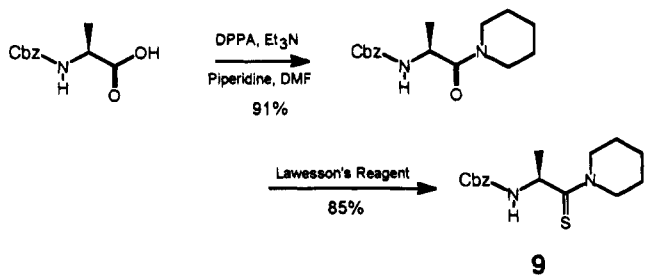
Two approaches to the formation of the key conformationally restricting tetrahydropyrimidine ring of the target inhibitor **1** were envisaged, one involving protection of the acid moiety of the diamino acid **3** prior to coupling with the activated thioimidate **2**, and the other entailing protection of the amino functions of **3**, followed by reaction with the aldehyde precursor **4** prior to the cyclization step.

The first approach is summarized in Scheme 4. Protection of the carboxylic acid group of **3** as the methyl ester was easily accomplished by Fischer esterification or with diazomethane. However, this ester was unstable and cyclized readily to its γ-lactam derivative. Accordingly, **3** was converted instead into its trimethylsilyl[17] ester **10**. The activated thioimidate **2** was prepared from **9** by treatment with MeI and then coupled immediately with **10** to generate the cyclized acid **11** in excellent yield. The remaining steps of coupling **11** with aminoacetaldehyde dimethyl acetal to give **12**, followed by unmasking

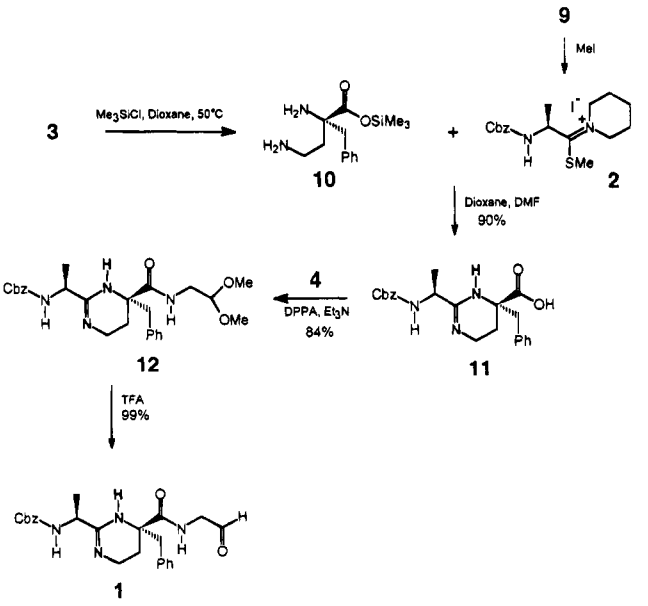
Scheme 2



Scheme 3



Scheme 4



of the protected aldehyde function, were achieved in excellent yields, with the target inhibitor **1** being obtained in 75% overall yield from the diamino acid **3**.

The second route explored is shown in Scheme 5. The amino groups of the acid **3** were protected, via the

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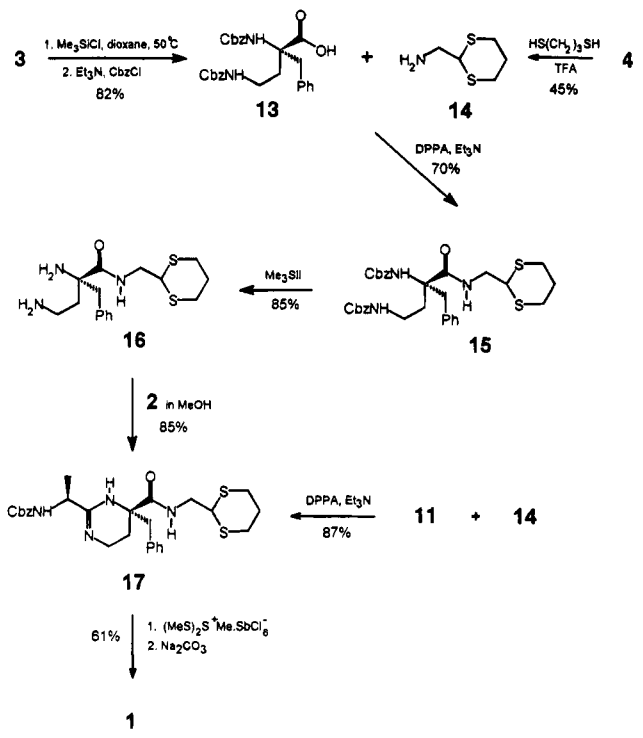
Scheme 5



trimethylsilyl ester **10**, as their Cbz-derivatives to generate the acid **13** in 82% yield.[18,19] Next, the dimethyl acetal protection of the aldehyde synthon **4** was exchanged[20] with 1,3-ethanediol to give the 1,3-dithiane **14**. The initial product of this acid-catalyzed exchange preparation of **14** contained an impurity that required Boc-protection, followed by purification and then deprotection, to remove it. As a result, the yield of pure **14** was reduced to 45%. Despite this yield penalty, removal of the impurity at this stage was an imperative since it was polymerized by the bases utilized in subsequent steps. Coupling of **14** with **13** to give **15** proceeded smoothly, as did the deprotection of **15** with trimethylsilyl iodide[21] to give the diaminodithiane **16**. Trimethylsilyl iodide-mediated deprotection of **15** was employed as a result of the problems encountered earlier in removal of Cbz groups by hydrogenation.[18] Reaction of **16** with the activated thioimidate **2** in methanol gave the cyclic product **17**, with the yield reaching 85% when an excess of **2** was used. The tetrahydropyrimidinyldithiane **17** was also obtained in good yield by coupling of **11** with **14**. Finally, treatment of **17**, as its hydrochloride salt, with methylbis[methylthio]sulfonium hexachloroantimonate[22] followed by aqueous sodium carbonate, removed the dithiane protecting group to generate the desired aldehyde inhibitor **1**. The overall yield of **1** from **13** by this route was 25%.

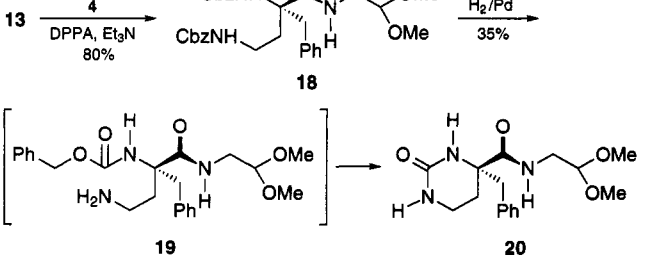
The validity of the inhibitor design strategy was confirmed by the high inhibition potency of **1**, which proved to be a competitive inhibitor of papain with a K_I value of 790 nM. This confirms the amidine group as a viable conformationally restricted replacement of a peptide moiety. While structure **1** represents a very encouraging starting point, the fact that its K_I is higher than that of its closest peptidyl analog, *N*-acetyl-Phe-GlyCHO (K_I 46 nM[10a]) shows that additional refinement is still needed. In this regard, it is noted that at the pH 6.5 conditions at which the kinetic assays were carried out, the tetrahydropyrimidine function of **1** is undoubtedly protonated to a considerable degree. Since only the unprotonated form of the inhibitor would be expected to bind well at papain's active site, the true K_I value of **1** will in reality be very much smaller. In any event, the present results establish that the tetrahydropyrimidine ring is a good bioisosteric peptidyl substitute that does not elicit any seriously adverse enzyme-binding interactions.

Experimental Section

Melting points were uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 80 spectrometer. Nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) spectra were recorded on a Gemini 200 (200 MHz, 50 MHz) spectrometer unless otherwise indicated. Mass spectra were measured on a Bell and Howell 21-490 (low resolution) or an AEI MS3074 instrument (high resolution). Elemental analyses were performed at Galbraith Laboratories, Inc. Knoxville, TN. All flash chromatographic separations were carried out on Merck silica gel 60 (60–200 mesh), and reactions were routinely performed under an inert atmosphere unless otherwise indicated. Solvents were reagent grade and dried prior to use. Commercial and organometallic reagents were obtained from Aldrich Chemical Co. unless otherwise specified. Amino acids and amino acid derivatives used as starting materials were obtained from Sigma Chemical Co. The chiral N-Cbz-L-phenylalanine starting material was enantiomerically pure. Within the limits of detection of the NMR method (±3%), the ee's and de's of the chiral compounds prepared were >97%. No NMR peaks attributable to a contaminating diastereomer were observed for any compound made.

(2*R*)-2-Benzyl-2,4-diaminobutyric Acid (3). In a modification of the method of Karady *et al.*,[16] dried N-Cbz-L-phenylalanine (30.0 g, 100 mmol) and benzaldehyde dimethyl acetyl (14.4 mL, 95.9 mmol) in Et_2O (500 mL) was cooled to −78 °C. BF_3·Et_2O (60.2 mL, 489 mmol) was then added, and the mixture was then allowed to warm to 20 °C and stirred for 4 days. The reaction mixture was worked up at 0 °C by adding saturated aqueous $NaHCO_3$, initially in 1 mL portions until strong bubbling ceased, and the mixture extracted with Et_2O (3 × 150 mL). The ether extracts were dried (Na_2SO_4) and rotary evaporated, and the crude product was purified by flash chromatography with Et_2O/EtOAc/hexanes (5:1:20) to give (2*S*,4*S*)-2-phenyl-3-(carbobenzyloxy)-4-benzyloxazolidinone (**5**, 24.5 g, 72%): mp 123–125 °C; IR (KBr) 3050, 1749, 1691, 1413, 1350 cm^{-1}; ^{1}H NMR (200 MHz, DMSO) δ 3.30 (m, 2H), 4.76 (d of d, J = 6.0 Hz, J = 3.0 Hz, 1 H), 5.10 (br s, 2 H),

(18) Logically, from the retrosynthetic analysis, the next step should have coupled **13** with aminoacetaldehyde dimethyl acetal (**4**). This was, in fact, readily accomplished employing the DPPA–Et_3N procedure[19] to give **18** in 72% yield. Disappointingly, however, deprotection of the amino groups of **15** by hydrogenation yielded the pyrimidinone **20**, presumably via the amino-Cbz intermediate **19**, for which intramolecular cyclization to **20** would be favored.



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6.37 (s, 1 H), 6.64 (br, 2 H), 7.09 (m, 5 H), 7.30 (m, 8 H). Anal. Calcd for $C_{24}H_{21}NO_4$: C, 74.40; H, 5.46. Found: C, 74.50; H, 5.48.

The dried oxazolidone **5** (1.81 g, 4.68 mmol) in dry THF (25 mL) was cooled to −78 °C and $KN(TMS)_2$ (10.3 mL, 5.15 mmol) then added. The reaction mixture was stirred at −78 °C for 15 min, and then condensed ethylene oxide (dried over CaH_2, 0.257 mL, 5.20 mmol) was added, followed by $BF_3{\cdot}Et_2O$ (0.634 mL, 5.15 mmol). After stirring at −78 °C for 1.5 h, the reaction mixture was warmed to 20 °C, poured into saturated aqueous $NaHCO_3$ (100 mL), and extracted with Et_2O (3 × 200 mL). The combined organic layers were washed successively with H_2O (40 mL), 1 M HCl (2 × 40 mL), and saturated aqueous NaCl (2 × 40 mL). The Et_2O solution was dried (Na_2SO_4) and rotary evaporated, and the crude product was purified by flash chromatography with Et_2O/benzene (1:3) to give (2*R*,4*S*)-2-phenyl-3-(carbobenzyloxy)-4-benzyl-4-(2-hydroxyethyl)oxazolidinone (**6**, 1.75 g, 87%): IR ($CHCl_3$) 3455, 3016, 1788, 1704, 1407 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.32, 2.75 (m of m, 2 H), 3.28, 3.63 (d of d, J = 13.5 Hz, J = 13.5 Hz, 2 H), 3.50–3.90 (m, 2 H), 4.80–5.35 (m, 2 H), 6.12 (d, J = 7.3 Hz, 1 H), 6.23 (s, 1 H), 6.70 (d, J = 8.1 Hz, 1 H), 6.92–7.46 (m, 13 H); FAB-MS 432.2 (MH^+), calcd for $C_{26}H_{25}NO_5$ 431.17.

To a stirred solution of the (hydroxyethyl)oxazolidinone **6** (5.95 g, 13.8 mmol) in CH_2Cl_2 at 0 °C was added pyridine (2.20 mL, 27.2 mmol), followed by methylsulfonyl chloride (2.13 mL, 27.2 mmol). The reaction mixture was then stirred at 20 °C for 2 days, the solvent rotary evaporated, and the residue dissolved in EtOAc (500 mL). The organic layer was washed with 1 M H_2SO_4 and then with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and rotary evaporated. The crude product was purified by flash chromatography using EtOAc/hexanes (1:3) to give (2*R*,4*S*)-2-phenyl-3-(carbobenzyloxy)-4-benzyl-4-[2-(methylsulfonyloxy)ethyl]oxazolidinone (**7**, 6.20 g, 88%): mp 100–102 °C; IR (KBr) 3033 (m), 1796, 1786, 1708, 1412, 1357, 1329, 1169 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.44, 2.91 (m of m, 2 H), 3.03 (s, 3 H), 3.28, 3.63 (d of d, J = 13.3 Hz, J = 13.3 Hz, 2 H), 4.15–4.43 (m, 2 H), 4.83, 5.12 (d of d, J = 12.2 Hz, J = 12.2 Hz, 2 H), 6.12 (d, J = 7.3 Hz), 6.30 (s, 1 H), 6.73 (d, J = 6.4 Hz, 1 H), 6.96–7.48 (m, 13 H); FAB-MS: 510.1 (MH^+). Calcd for $C_{27}H_{27}NO_7S$, 509.15.

The mesylate **7** (1.08 g, 2.12 mmol) and NaN_3 were dried under vacuum overnight, DMF (25 mL) was added, and the mixture was heated at 40 °C for 20 h.[23] The reaction mixture was then cooled to 20 °C, EtOAc (100 mL) and benzene (50 mL) were added, and the mixture was shaken with water (15 mL). The aqueous layer was extracted once more with EtOAc (50 mL) and benzene (25 mL). The combined organic layers were washed with saturated aqueous NaCl (20 mL) and then dried (Na_2SO_4). Rotary evaporation of the solvent followed by purification by flash chromatography with EtOAc/hexane (1:5) afforded (2*R*,4*S*)-2-phenyl-3-(carbobenzyloxy)-4-(2-azidoethyl)-4-benzyloxazolidinone (**8**, 900 mg, 93%): IR ($CHCl_3$) 3006, 2108, 1790, 1707, 1404, 1341 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.2–2.9 (m, 2 H), 3.27, 3.63 (d of d, J = 13.5 Hz, J = 13.5 Hz, 2 H), 3.41 (m, 2 H), 4.87, 5.09 (d of d, J = 12.1 Hz, J = 12.2 Hz, 2 H), 6.11 (d, J = 7.2 Hz, 1 H), 6.12 (s, 1 H), 6.73 (d, J = 6.9 Hz, 1 H), 6.95–7.45 (m, 13 H); FAB-MS 457.2 (MH^+), calcd for $C_{26}H_{24}N_4O_4$ 456.18. Anal. Calcd for $C_{19}H_{17}N_4O_4$: C, 68.41; H, 5.30. Found: C, 68.10; H, 5.38.

To the azidoethyl compound **8** (1.36 g, 2.98 mmol) in aqueous MeOH (1:1, 120 mL) was added 1 M NaOH (20 mL) and the mixture stirred at 20 °C for 2 days. The MeOH was removed by rotoevaporation and the aqueous solution acidified with concd HCl and extracted with EtOAc. The organic layer was dried (Na_2SO_4), the solvent rotoevaporated, the residue dissolved in EtOH (30 mL), and 10% Pd on carbon (250 mg) and 2 M HCl (10 mL) added. The mixture was shaken under H_2 (55 psi) at 20 °C for 2 h, filtered, and rotoevaporated, and the crude product was recrystallized from EtOH/dioxane to give (2*R*)-2-benzyl-2,4-diaminobutyric acid (**3**, 730 mg, 87%) as its bis-hydrochloride salt: mp 154 °C dec); IR (KBr) 3382, 2900, 1738, 1617, 1527, 1497 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 2.13–2.41 (m, 2 H), 3.01–3.32 (m, 2 H), 3.06, 3.39 (d of d, J = 14.4 Hz, J = 14.4 Hz, 2 H), 7.18–7.36 (m, 5 H); FAB-MS; 209.2 (MH^+). Calcd for $C_{11}H_{16}N_2O_2$, 208.12. Anal. Calcd for $C_{11}H_{16}N_2O_2{\cdot}2HCl$: C, 46.99; H, 6.45. Found: C, 46.93; H, 6.32.

N-Cbz-_l-Alanine Piperidine Thioamide (9). N-Cbz-L-Ala (6.70 g, 30.0 mmol) was dried over P_2O_5 under vacuum overnight. This was dissolved in DMF (50 mL) and cooled in an ice–water bath. Under stirring conditions were added piperidine (3.26 mL, 33.0 mmol), DPPA (7.09 mL, 33.0 mmol), and Et_3N (4.60 mL, 33.0 mmol). The reaction mixture was stirred at 0 °C for 6 h and then taken into EtOAc (300 mL) and benzene (150 mL). The organic layer was washed with 1 N HCl (2 × 50 mL), H_2O (50 mL), saturated $NaHCO_3$ (2 × 50 mL), H_2O (50 mL), and saturated NaCl (2 × 50 mL). The organic layer was then dried (Na_2SO_4) and solvent was removed *in vacuo* to generate the crude product which was then purified by flash chromatography using 40% EtOAc in hexanes to give N-Cbz-L-alanine piperidine amide (7.95 g, 91%): IR ($CHCl_3$) 3418, 2944, 1711, 1639, 1447 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.32 (d, J = 6.8 Hz, 3 H), 1.60 (m, 6 H), 3.40, 3.53 (m, m, 4 H), 4.66 (m, 1 H), 5.10 (s, 2 H), 5.90 (d, 1 H, J = 7.0 Hz), 7.36 (m, 5 H); HRMS 290.1617, calcd for $C_{16}H_{22}N_2O_3$ 290.1630.

The above piperidine amide (4.20 g, 14.5 mmol) and Lawesson's reagent[24] (2.95 g, 7.30 mmol) in benzene (50 mL) were heated at 80 °C for 1 h. The mixture was then cooled to 20 °C, the solvent rotoevaporated, and the residue purified by flash chromatography with EtOAc/hexanes (1:5) to give *N*-Cbz-L-alanine piperidine thioamide (**9**, 3.62 g, 85%): mp 77–79 °C; IR ($CHCl_3$) 3350, 2949, 1705, 1479, 1458, 1445 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.36 (d, J = 6.7 Hz, 3 H), 1.59 (br s, 6 H), 3.73 (m, 2 H), 4.15, 4.37 (m of m, 2 H), 4.93 (m, 1 H), 5.10 (d, J = 2.1 Hz, 2 H), 6.46 (d, J = 7.0 Hz, 1 H), 7.35 (m, 5 H). Anal. Calcd for $C_{16}H_{22}N_2O_2S$: C, 62.72; H, 7.24. Found: C, 62.83; H, 6.97.

Preparation of (4*R*,1′*S*)-2-[1′-[(Benzyloxycarbonyl)-amino]ethyl]-4-benzyl-4-[[(2-oxoethyl)amino]carbonyl]-3,4,5,6-tetrahydropyrimidine (1) by the Scheme 4 Route. The piperidine thioamide **9** (2.90 g, 9.48 mmol) in MeI (15 mL) was stirred at 20 °C for 2 days. The excess MeI was then removed *in vacuo* and the activated thioimidate product **2** was coupling directly to the trimethylsilyl ester **10**, prepared *in* situ from the bis-hydrochloride salt of diamino acid **3** (660 mg, 2.35 mmol, dried over P_2O_5 under vacuum) in dioxane (50 mL) to which Me_3SiCl (0.387 mL, 3.05 mmol) was added. This mixture was stirred at 55 °C for 4 h and then cooled to 20 °C. To this solution of **10** was added the above thioimidate salt **2** in dioxane/DMF (1:1, 100 mL), followed by Et_3N (1.38 mL, 9.87 mmol). The resulting solution was stirred at 20 °C for 4 h and then at 40 °C overnight. The reaction mixture was then quenched with MeOH (20 mL) and 1 M HCl (10 mL) and then diluted with H_2O (40 mL). The aqueous layer was washed with Et_2O (60 mL) and then lyophilized, and the crude product was purified by flash chromatography with MeOH/$CHCl_3$ (1:20 → 1:5 gradient). Contaminating Et_3N and piperidine hydrochloride salts were then removed by C-18 reverse phase chromatography (MPLC) with an H_2O → CH_3CN/H_2O (1:1) elution gradient to give (4*R*,1′*S*)-2-[1′-[(benzyloxycarbonyl)-amino]ethyl]-4-benzyl-4-carboxy-3,4,5,6-tetrahydropyrimidine (**11**, 836 mg, 90%): mp 145 °C dec; IR (KBr) 3215, 3029, 1713, 1653, 1612, 1378, 1261 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 1.43, 1.45 (d of d, J = 7.2 Hz, J = 7.2 Hz, 3 H), 1.99 (m, 2 H), 2.8–3.3 (m, 4 H), 4.43 (m, 1 H), 5.10 (m, 2 H), 7.3 (m, 10 H); FAB-MS 396 (MH^+) calcd for $C_{22}H_{25}N_3O_4$ 395.18. Anal. Calcd for $C_{22}H_{25}N_3O_4$: C, 66.82; H, 6.37. Found: C, 66.68; H, 6.76.

To the tetrahydropyrimidine **11** (170 mg, 0.394 mmol, dried over P_2O_5 under vacuum) in DMF (15 mL) at 0 °C with stirring were added aminoacetaldehyde dimethyl acetyl (**4**, 0.150 mL, 1.38 mmol), DPPA (0.102 mL, 0.473 mmol), and Et_3N (0.121 mL, 0.867 mmol). The mixture was stirred at 0 °C for 5 h and then at 20 °C overnight. DMF was removed under high vacuum and the residue subjected to flash chromatography

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(on silica gel pretreated with Et_3N) with $MeOH/CH_2Cl_2$ (1:30) to give (4*R*,1′*S*)-2-[1′-[(benzyloxycarbonyl)amino]ethyl]-4-benzyl-4-[[(2,2-dimethoxyethyl)amino]carbonyl]-3,4,5,6-tetrahydropyrimidine (**12**) as a colorless gum (169 mg, 84%): IR ($CHCl_3$) 3448, 3386, 2992, 2932, 1705, 1648, 1488, 1067 cm^{-1}; ^1H NMR (200 MHz, CD_2Cl_2) δ 1.34, 1.36 (d of d, J = 7.0 Hz, J = 7.0 Hz, 3 H), 1.71, 2.10 (br, 2 H), 2.72 (m, 1 H), 3.03–3.30 (m, 6 H), 3.20 (s, 3 H), 3.24 (s, 3 H), 4.01–4.14 (m, 2 H), 5.04–5.12 (m, 2 H), 5.69–5.84 (br, 1 H), 6.91 (br s, 1 H), 7.10–7.24 (m, 5 H), 7.30–7.40 (m, 5 H); HRMS 482.2518, calcd for $C_{26}H_{34}N_4O_5$ 482.2529.

The dimethyl acetal **12** (53 mg, 0.11 mmol) in neat TFA (8 mL) was stirred at 20 °C for 45 min. The TFA was then removed by prolonged lyophilization to give (4*R*,1′*S*)-2-[1′-[(benzyloxycarbonyl)amino]ethyl]-4-benzyl-4-[[(2-oxoethyl)amino]carbonyl]-3,4,5,6-tetrahydropyrimidine (**1**, 60 mg, quant), as its chromatographically pure (in 10% MeOH/$CHCl_3$ and 10% MeOH/EtOAc as two-dimensional TLC solvents) TFA salt, mp 85–7 °C (accompanied by slow sublimation); $[\alpha]^{22}_D$ −45.2° (*c* 1, $CHCl_3$); IR (KBr) 3289 (br), 3032 (m), 1671 (br), 1528, 1260, 1203, 1135 cm^{-1}; ^1H NMR (200 MHz, CD_3CN) δ 1.54 (d, J = 7.2 Hz, 3 H), 1.65 (m, 1 H), 2.45 (m, 1 H[25]), 3.20–3.60 (m, 5 H), 4.08 (m, 1 H), 4.56 (m, 1 H), 5.20 (m, 2 H), 7.44 (m, 10 H), 7.13–7.68 (br, 1 H), 8.32 (br s, 1 H), 9.24–9.38 (br, 1 H), 9.58 (s, 1 H), 10.02 (br s, 1 H); HRMS 436.2101, calcd for $C_{24}H_{28}N_4O_4$ 436.2111. Anal. Calcd for $C_{26}H_{29}F_3N_4O_6$: C, 56.72; H, 5.31. Found: C, 56.47; H, 5.47.

Preparation of (4*R*,1′*S*)-2-[1′-[(Benzyloxycarbonyl)amino]ethyl]-4-benzyl-4-[[(2-oxoethyl)amino]carbonyl]-3,4,5,6-tetrahydropyrimidine (1) by the Scheme 5 Route. Compound **3** (139 mg, 0.493 mmol) was dried under vacuum over P_2O_5 overnight and then dissolved in dioxane. Me_3SiCl (0.125 mL, 0.986 mmol) was added and the resulting solution was stirred at 50 °C for 2 h. The solution was then cooled to 0 °C, followed by the addition of Cbz-Cl (0.704 mL, 4.93 mmol) and Et_3N (0.412 mL, 2.96 mmol). After stirring at 35 °C overnight, the reaction mixture was quenched with 1 N HCl (4 mL) and MeOH (5 mL). After the organic solvent was removed *in vacuo*, the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried, and the solvent was removed to give the crude product. This was then dissolved in saturated $NaHCO_3$ (15 mL), washed with hexane (15 mL), and then acidified with 1 N HCl (20 mL). The aqueous layer was extracted with EtOAc (3 × 20 mL), the combined organic layers were dried, and solvent was removed *in vacuo* to give (2*R*)-2-benzyl-2,4-bis[(benzyloxycarbonyl)amino]butyric acid (**13**, 193 mg, 82%): IR ($CHCl_3$) 3407, 2975, 1702, 1490, 1190 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 2.10, 2.52 (m of m, 2 H), 3.10 (m, 4 H), 5.18 (m, 4 H), 7.02 (m, 5 H), 7.36 (m, 10 H); FAB-MS 476 (M^-), calcd for $C_{27}H_{28}N_2O_6$ 476.19.

The synthon **14** to be coupled with the above bis-Cbz-acid **13** was obtained by the following procedure. 1,3-Propanedithiol (2.76 mL, 27.5 mmol) and concd HCl (5 mL) were mixed at 0 °C and aminoacetaldehyde dimethyl acetyl (**4**, 2.63 g, 22.9 mmol) was added with stirring. The mixture was stirred at 0 °C for 1 h and then at 20 °C overnight. The solvent was rotary evaporated and the contaminated crude product purified by treatment with BOC-ON (5.63 g, 22.9 mmol) in H_2O/acetone (1:1, 100 mL). The mixture was heated to 40 °C and Et_3N (3.19 mL, 22.9 mmol) added. After stirring for 2 h, the acetone was evaporated and the aqueous layer extracted with EtOAc (3 × 100 mL). The combined organic layers were dried (Na_2SO_4) and rotoevaporated to give the BOC-protected product, which was purified by flash chromatography with EtOAc/hexane (1:10). To remove the BOC group, TFA (15 mL) was added and the mixture stirred at 20 °C for 30 min. Following rotoevaporation of the excess TFA, the residue was dissolved in water (100 mL) and the insoluble precipitate was filtered off. The aqueous solution was lyophilized to give 2-(2-aminomethyl)-1,3-dithiane trifluoroacetic acid salt (**14**, 2.71 g, 45%): mp 144–146 C; IR (KBr) 3000 (br), 1684, 1613, 1508 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 2.04 (m, 2 H), 2.70, 3.04 (m of m, 4 H), 3.44 (d, J = 7.9 Hz, 2 H), 3.98 (t, J = 7.9 Hz, 1 H); FAB-MS 150.1 (MH^+) calcd for $C_5H_{11}NS_2$ 149.10.

Compounds **13** (260 mg, 0.546 mmol) and **14** (574 mg, 2.19 mmol) were individually dried over P_2O_5 under vacuum overnight and then dissolved in DMF (20 mL). The resulting solution was cooled to 0 °C with stirring, and DPPA (0.141 mL, 0.655 mmol) and Et_3N (0.419 mL, 3.00 mmol) were added. Stirring was continued at 0 °C for 6 h and the reaction mixture then warmed to 20 °C, and EtOAc (200 mL) and benzene (100 mL) were added. The organic layer was washed successively with 1 M HCl (2 × 10 mL), H_2O (10 mL), saturated aqueous $NaHCO_3$ (2 × 10 mL), H_2O (10 mL), and saturated aqueous NaCl (2 × 10 mL). The organic layer was dried (Na_2SO_4) and rotoevaporated and the crude product purified by flash chromatography with EtOAc/hexane (1:3) to yield (2′*R*)-2-[[(2′-benzyl-2′,4′-bis[(benzyloxycarbonyl)amino]-1′-oxobutyl]amino]-methyl]-1,3-dithiane (**15**, 240 mg, 72%): IR ($CHCl_3$) 1724, 1669, 1483 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.0 (m, 3 H), 2.64–2.82 (m, 5 H), 3.07, 3.44 (d of d, J = 3.8 Hz, J = 3.8 Hz, 2 H), 3.22 (m, 2 H), 3.63 (m, 2 H), 3.99 (t, J = 7.2 Hz, 1 H), 5.08 (m, 4 H), 5.93 (br s, 1 H, N*H*), 6.98 (br s, 1 H), 7.10–7.44 (m, 16 H); FAB-MS 608.1 (MH^+), calcd for $C_{32}H_{37}N_3O_5S_2$ 607.27.

To a stirred solution of the dithiane **15** (26.0 mg, 0.0428 mmol) in CH_3CN (8 mL) was added Me_3SiI (80.0 μL, 0.562 mmol), with stirring being continued at 20 °C for 15 min. The solvent was then rotoevaporated, and 1 M HCl (2 mL) and H_2O (5 mL) were added to the residue. The aqueous layer was washed with Et_2O (2 × 5 mL) and then lyophilized, and the residue was purified by reverse-phase HPLC (Waters C_{18} Nova-Pak Radial-Pak, 8 mm × 10 cm, 6 μm cartridge; retention time 3.27 min at 2.00 mL/min flow rate, isocratic solvent system with 19% CH_3CN in water (0.15% TFA)) to generate (2′*R*)-2-[[(2′-benzyl-2′,4′-diamino-1′-oxobutyl)amino]-methyl]-1,3-dithiane (**16**, 19.3 mg, 54%) mp 98–100 °C; IR (KBr) 3430, 3065, 2930, 1617, 1538, 1205, 1138 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 1.88–2.14 (m, 2 H), 2.26, 2.48 (m of m, 2 H), 2.78, 2.98 (m of m, 4 H), 3.17–3.40 (m, 4 H), 3.57 (d of d, J = 13.9 Hz, J = 7.2 Hz, 1 H), 3.88 (d of d, J = 13.9 Hz, J = 7.7 Hz, 1 H), 4.18 (t, J = 7.3 Hz, 1 H), 7.32 (m, 5 H); FAB-MS 340.2 (MH^+), calcd for $C_{16}H_{25}N_3OS_2$ 339.14. The next intermediate, **17**, was prepared in two ways.

(a) To the tetrahydropyrimidinodithiane **16** (19.3 mg, 0.0340 mmol) and the thioimidate salt **2** (prepared from **9** (104 mg, 0.382 mmol) and MeI (3 mL) as described for the Scheme 4 reactions above) in MeOH (8 mL) was added Et_3N (150 μL, 1.08 mmol). The mixture was stirred at 20 °C overnight and the solvent rotoevaporated. The residue was purified by flash chromatography (on silica gel pretreated with Et_3N) with $MeOH/CH_2Cl_2$ (1:25) and the Et_3N and piperidine hydrochloride salts still present removed by dissolving this material in EtOAc (15 mL) and washing successively with 1 M HCl (2 mL), H_2O (2 mL), saturated aqueous $NaHCO_3$ solution (2 mL), H_2O (2 mL), and saturated aqueous NaCl solution (2 mL). The organic layer was dried ($MgSO_4$) and solvent was evaporated to generate (4*R*,2′*S*)-2-[2′-[(benzyloxycarbonyl)amino]ethyl]-4-benzyl-4-[[[2-(1,3-dithianyl)]methyl]amino]carbonyl]-3,4,5,6-tetrahydropyrimidine (**17**, 16.4 mg, 85%): mp 48 °C; IR (KBr) 3325, 2929, 1711, 1638, 1512, 1244 cm^{-1}; ^1H NMR (400 MHz, CD_2Cl_2) δ 1.38 (d, J = 7.0 Hz, 3 H), 1.67, 2.06 (m of m, 2 H), 1.80, 1.90 (m of m, 2 H), 2.55–2.88 (m, 4 H), 2.79 (d, J = 12.9 Hz, 2 H), 3.07–3.26 (m, 4 H), 3.32, 3.55 (m of m, 2 H), 3.69, 3.79 (m of m, 1 H), 4.15 (m, 1 H), 5.08 (d, J = 8 Hz, 2 H), 5.82–5.98 (br, 1 H), 7.17–7.40 (m, 10 H); HRMS 526.2086, calcd for $C_{27}H_{34}N_4O_3S_2$ 526.2072. For elemental analysis, some of **17** was converted into its HCl salt by dissolving **17** in EtOAc and washing the organic layer with 1 N HCl. Anal. Calcd for $C_{27}H_{34}N_4O_3S_2 \cdot HCl \cdot H_2O$: C, 55.80; H, 6.42. Found: C, 55.63; H, 6.16.

(b) Compound **17** was also prepared by coupling **11** and **14** using the DPPA–Et_3N procedure. The tetrahydropyrimidinyl acid **11** (191 mg, 0.442 mmol) and the amino dithiane **14** (140 mg, 0.531 mmol) were dried over P_2O_5 under vacuum overnight and then dissolved in DMF (20 mL). The mixture was cooled to 0 °C and DPPA (0.114 mL, 0.530 mmol) and Et_3N (0.246 mL, 1.77 mmol) were added with stirring. Stirring was

(25) This proton was masked to some extent by an H_2O peak from the traces of water tenaciously retained by the trifluoroacetate salt of **1**.

continued at 0 °C for 6 h, the DMF was removed under high vacuum, and the residue was purified as described above to afford **17** (202 mg, 87%), identical in every respect with the previous sample.

For the conversion of **17** to the target inhibitor **1**, methylbis-[methylthio]sulfonium hexachloroantimonate (42.8 mg, 0.0900 mmol, prepared from Me_2S_2 and $SbCl_5$ in 88% yield[22]) in CH_2Cl_2 (4 mL) was added dropwise, over 2 min, to a well-stirred solution of the hydrochloride salt of the tetrahydropyrimidinyl dithiane **17** (16.9 mg, 0.0300 mmol) in CH_2Cl_2 (8 mL) at −78 °C. Stirring was continued for 8 min and the mixture then poured into saturated aqueous Na_2CO_3 (6 mL). The organic layer was washed with H_2O (3 × 5 mL) and dried ($MgSO_4$) and one drop of TFA added to convert the product to its trifluoroacetate salt which was then purified by flash chromatography (on a microcolumn) with MeOH/$CHCl_3$ (1:10) to give the TFA salt of (4*R*,1′*S*)-2-[1′-[(benzyloxycarbonyl)amino]-ethyl]-4-benzyl-4-[[(2-oxoethyl)amino]carbonyl]-3,4,5,6-tetrahydropyrimidine (**1**, 10.1 mg, 61%), identical with the material isolatedpreviously from the Scheme 4 route.

Inhibition of Papain by 1.[26] Papain (from Sigma) was purified and activated as described previously.[27] Papain was assayed using CBZ-Phe-Arg-MCA (IAF Biochem International Inc., Laval, Quebec) as substrate, with substrate hydrolysis monitored on an SPEX Fluorolog-2 spectrofluorimeter set for excitation at 380 nm and emission at 440 nm. The kinetic assays were carried out at pH 6.5 in 50 mM phosphate buffer containing 200 mM NaCl, 5 mM EDTA, 1 mM DTT, and 10% CH_3CN. The assay temperature was kept constant at 25 °C, and substrate and inhibitor **1** concentrations were maintained in excess of the 0.05 nM enzyme concentration. The competitive inhibition constant for **1** was determined by measuring the initial rate of substrate hydrolysis at three different substrate concentrations (0.01, 0.04, and 0.15 mM where K_M = 0.09 mM) in the presence of inhibitor concentrations ranging between 0.1−5.0 mM. Linear regression analysis of the $1/v$ vs [I] Dixon-plot data confirmed competitive inhibition of papain by **1**, with K_I = 790 ± 72 nM.

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