Azodicarboxamides: A New Class of Cysteine Proteinase Inhibitor for Hepatitis A Virus and Human Rhinovirus 3C Enzymes

Richard D. Hill and John C. Vederas*

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

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Hepatitis A virus (HAV) 3C and human rhinovirus (HRV) 3C cysteine proteinases have been shown to be essential for viral maturation and infectivity through the cleavage of a viral polyprotein precursor. A number of bis-hydrazides (e.g., **11**−**14**), analogous to nanomolar inhibitors of cathepsin K, were synthesized and tested for effectiveness against HAV 3C and HRV 3C proteinases, but these compounds showed no significant inhibition of the viral enzymes. However, oxidation of the bis-hydrazides to the corresponding azodicarboxamides gave potent, irreversible inhibitors with IC_{50}'s in the low micromolar range. These compounds probably act by adding the active site thiol to the azo moiety in a Michael fashion to give a covalent complex, which was detected by electrospray mass spectrometry. Azodicarboxamide **16** was shown to have a rate constant (k_{inact}/k_I) of 35 644 M^{-1} min^{-1}.

Introduction

The role of cysteine proteinases in a variety of diseases and physiological disorders makes them an attractive target for the development of therapeutic agents. Inhibitors of cysteine proteinases are currently being sought as potential agents against viral and parasitic infections, cancer, arthritis, and osteoporosis.[1−14] Hepatitis A virus (HAV) and human rhinovirus (HRV) belong to the Picornaviridae family, which is a large family of positive-sense, single-stranded RNA viruses with more than 200 known members.[2] In addition to HAV and HRV, picornaviruses are the causative agents of poliomyeltis, foot and mouth disease, and viral meningitis and are also implicated in triggering various autoimmune diseases.[2] Hepatitis A virus is the only known member of the genus hepatovirus and causes an acute form of infectious hepatitis.[3] Hepatitis A is still encountered in parts of the world with restricted access to safe drinking water supplies and sporadic outbreaks still occur in the developed world.[4] Human rhinovirus is the major cause of the common cold in humans and comprises more than 100 serotypes, thereby making vaccine development difficult. Picornaviral 3C cysteine proteinases are responsible for co- and posttranslational processing of an initially produced polyprotein and are known to be essential for viral infectivity and maturation.[5] They have a unique active site geometry,[2] which suggests that specific and selective inhibitors can be designed as therapeutic agents.

Although the picornaviral 3C enzymes are cysteine proteinases, they are structurally similar to the chymotrypsin-like serine proteinases. The active site cysteine (Cys 172 in HAV 3C) is in close proximity to a histidine residue (His 44 in HAV 3C), which acts as a general base to form the thiolate; an ordered water molecule is thought

* Corresponding author. Tel (780)-492-5475, fax (780)-492-8231, e-mail: john.vederas@ualberta.ca.

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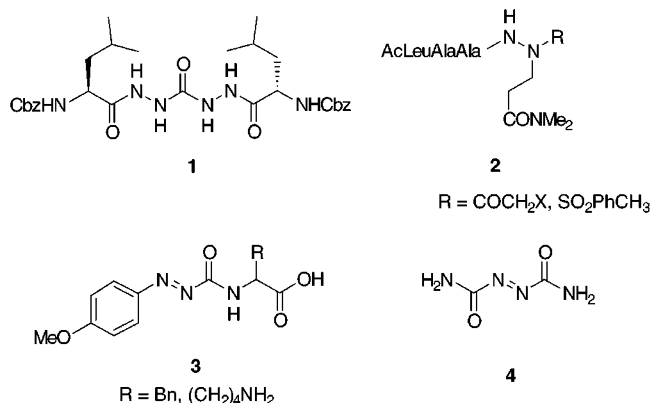

Figure 1.

to complete the catalytic triad.[2] Key substrate recognition is partly provided by another histidine residue (His 191 in HAV 3C) which hydrogen bonds to the side chain carbonyl of the P_1[6] residue, which is glutamine in the majority of the picornaviral 3C proteinases.

Various classes of inhibitors have been reported for HAV 3C/HRV 3C proteinases. These include peptide aldehydes,[7] azapeptides,[8] homophthalimides,[9] halomethyl ketones,[10] iodoacetamides,[11] β-lactones,[12] and α,β-unsaturated compounds.[13] Recently, a novel symmetric bis-hydrazide **1** was reported[14] as a potent and selective inhibitor of cathepsin K, a cysteine proteinase of the papain superfamily implicated in the process of bone resorption. This compound is able to span the enzyme active site and acts by adding the cysteine thiol to the central urea carbonyl. Bis-hydrazide **1** showed a K_i of 0.7 nM against cathepsin K.[14] We therefore chose to investigate this type of functionality for potential inhibitors of HAV 3C and HRV 3C proteinases.

Although HAV 3C proteinase is known to show a binding preference for a glutamine residue at P_1, replacement of the glutamine side chain with *N,N*-dimethylglutamine does not greatly reduce inhibition but simplifies synthetic strategies.[15] Hence, we targeted synthesis of nonsymmetrical bis-hydrazides in which one of the hydrazide moieties bears a *N,N*-dimethylpropanamide side chain to mimic the glutamine required at P_1, whereas the other bears a phenethylaminocarbonyl side chain to mimic the phenylalanine at P_2'. Incorporation of hydrazine moieties into peptide backbones is well established for the generation of proteinase inhibitors, and we have recently reported the synthesis and testing of azaglutamine haloacetyl derivatives such as **2** as inhibitors of HAV 3C proteinase.[16] Other substituted hydrazides as glutamine mimics have also been reported as HRV 3C proteinase inhibitors.[17]

In the present study, a number of bis-hydrazides are synthesized and tested for inhibition of HAV 3C and HRV 3C proteinases but show disappointing activity. However, oxidation of these compounds to the corresponding azodicarboxamides leads to potent irreversible inhibitors of these enzymes. To the best of our knowledge, azodicarboxamides have not previously been investigated as cysteine proteinase inhibitors, although certain arylazoformates (e.g., **3**) have been examined as cleavable substrates for metalloproteinases and serine proteinases.[18] Related compounds bearing electron-withdrawing groups have recently been shown to react with cysteine and thiol-containing enzymes.[19] The parent azodicarboxamide, azodicarbonamide **4,** inhibits HIV-1 replication and is currently in clinical trials in Europe for the treatment of advanced AIDS.[20] Azodicarbonamide is believed to interact with the zinc finger motifs of the nucleocapsid protein and cause extrusion of zinc.

Results and Discussion

Synthesis of Inhibitors. The initial target, bis-hydrazide **13**, is readily synthesized by the method outlined in Scheme 1. Michael addition of hydrazine to *N,N*-dimethylacrylamide using the procedure of Huang,[16] followed by diprotection using benzyl chloroformate gives **5** in 59% yield. Further protection of the terminal nitrogen with a *tert*-butoxycarbonyl group affords **6**, and removal of the Cbz groups by catalytic hydrogenation provides the selectively protected hydrazide **8**. Reaction of **8** with diphenyl hydrazodicarboxylate **10**[21] generates bis-hydrazide **11** in moderate (43%) yield. Substitution of the phenoxy group with phenethylamine in the presence of triethylamine provides the initial target **13**.

Since bis-hydrazide **13** and its precursor **11** show no inhibition of HAV 3C proteinase at 100 μM (see below), both compounds were oxidized to their azo derivatives. Oxidation of **13** using *N*-bromosuccinimide and pyridine[21] leads to azodicarboxamide **15** in 71% yield. This compound displays good inhibition of HAV 3C proteinase with an approximate IC_{50} of 23 μM. Following these results, a small number of analogues of **15** were prepared in which the Boc protecting group, dimethylpropanamide side chain and phenethyl moiety are individually altered. Cleavage of the Boc group with trifluoroacetic acid followed by attempted reprotection with an acetyl group is troublesome, possibly due to the sensitivity of unprotected hydrazines to aerobic oxidation.[22] The acetyl group is therefore introduced earlier in the synthesis (**5** $\rightarrow$ **7**), and the remainder of the transformations to azodicarboxamide **16** are done in a manner directly analogous to that outlined above (Scheme 1).

HRV 3C proteinase may not tolerate dimethylglutamine analogues at P_1 as well as HAV 3C does, but a sulfone moiety is accepted as a glutamine mimic.[7a] Therefore the sulfone azodicarboxamide **23** appeared an attractive target. The selectively protected sulfone **20** is available by a process similar to that used for **8**; Michael addition of hydrazine to vinyl methyl sulfone followed by Cbz

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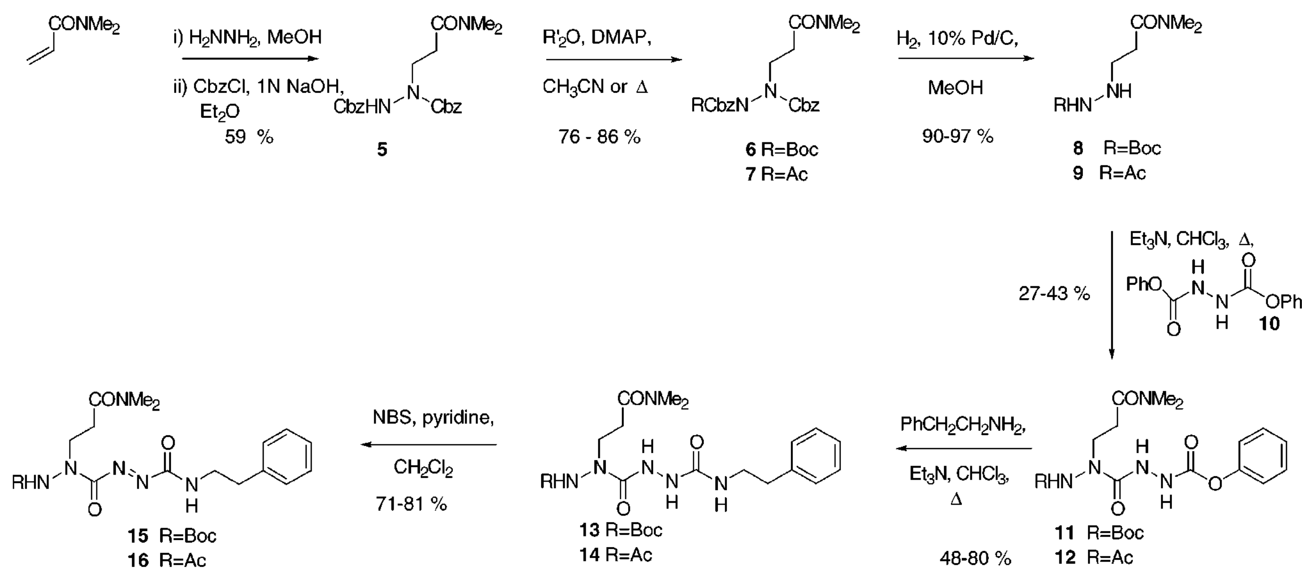
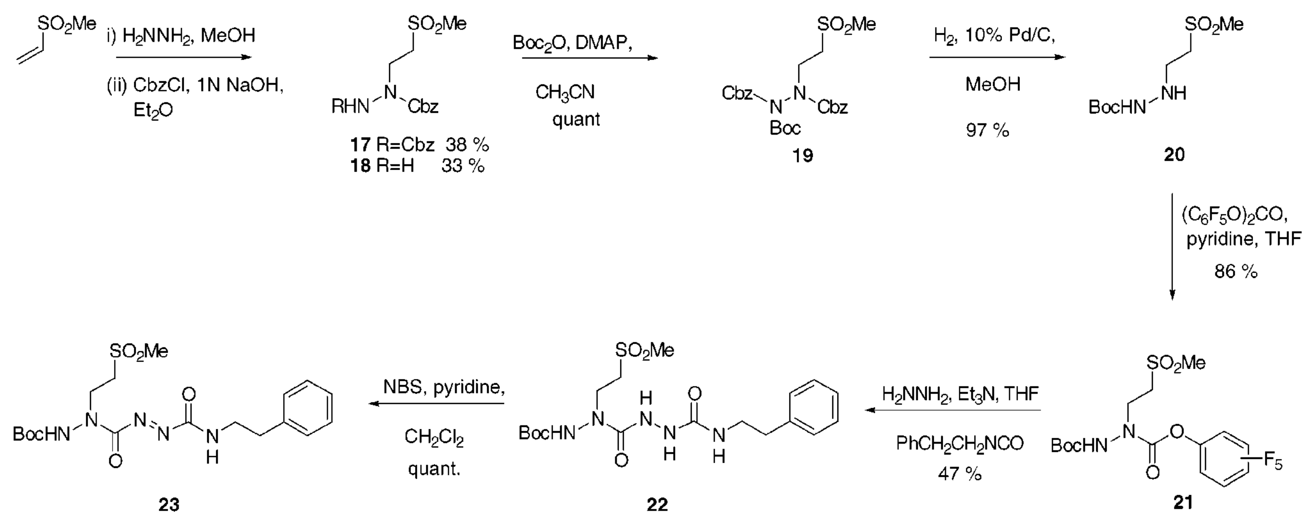
Scheme 1



Scheme 2



protection gives bis-Cbz protected sulfone **17** along with some of the monoprotected compound **18** (Scheme 2). Boc protection of **17** followed by cleavage of the Cbz groups generates compound **20** in 97% yield. Reaction of **20** with diphenyl hydrazodicarboxylate **10**, as described for the previous series of compounds, did not yield any of the desired product. However, reaction with bis-pentafluorophenyl carbonate[21] affords **21** in good yield. Substitution of the pentafluorophenoxy group with hydrazine followed by subsequent reaction with phenethyl isocyanate gives the desired bis-hydrazide **22**. Oxidation to azodicarboxamide **23** proceeds quantitatively using *N*-bromosuccinimide and pyridine.

It was also decided to alter the phenethylamine side chain on the P′ side, which was present as a phenylalanine surrogate. It seemed that the introduction of a phenylalanamide (i.e., insertion of $CONH_2$) at this position might reduce hydrophobicity and enhance recognition. Attempted reaction of bis-hydrazide **11** with either phenylalanine or phenylalanamide using similar conditions to those described earlier fails to generate the desired product, as does variation of the base and solvent employed. Treatment of **11** with benzyl phenylalaninate in chloroform in the presence of triethylamine gives the desired benzyl ester **24** albeit in poor yield (16%). The major product of this reaction is the hydantoin **25**, formed by intramolecular cyclization (Scheme 3). However, it is possible to convert hydantoin **25** into the desired primary amide **26** by treatment with concentrated ammonium hydroxide in 2-propanol. This can be oxidized to the azodicarboxamide **27** using *N*-bromosuccinimide and pyridine, but in considerably lower yield.

Enzyme Inhibition. Bis-hydrazides (**11**−**14**, **22**, and **26**) and azodicarboxamides (**15**−**16**, **23**, and **27**) were tested for inhibition of cloned and overexpressed HAV 3C and HRV 3C proteinases. The HAV 3C proteinase assays (enzyme concentration approximately 0.1 μM) employ a C24S mutant in which the nonessential surface cysteine is replaced with serine and which displays catalytic parameters indistinguishable from those of the wild-type enzyme.[23] HRV assays use the 3C proteinase from serotype 14 and are done using an enzyme concentration of approximately 0.4 μM. For HAV 3C proteinase, enzyme activity is conveniently monitored using a fluorometric assay similar to one described for HRV 3C

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Scheme 3

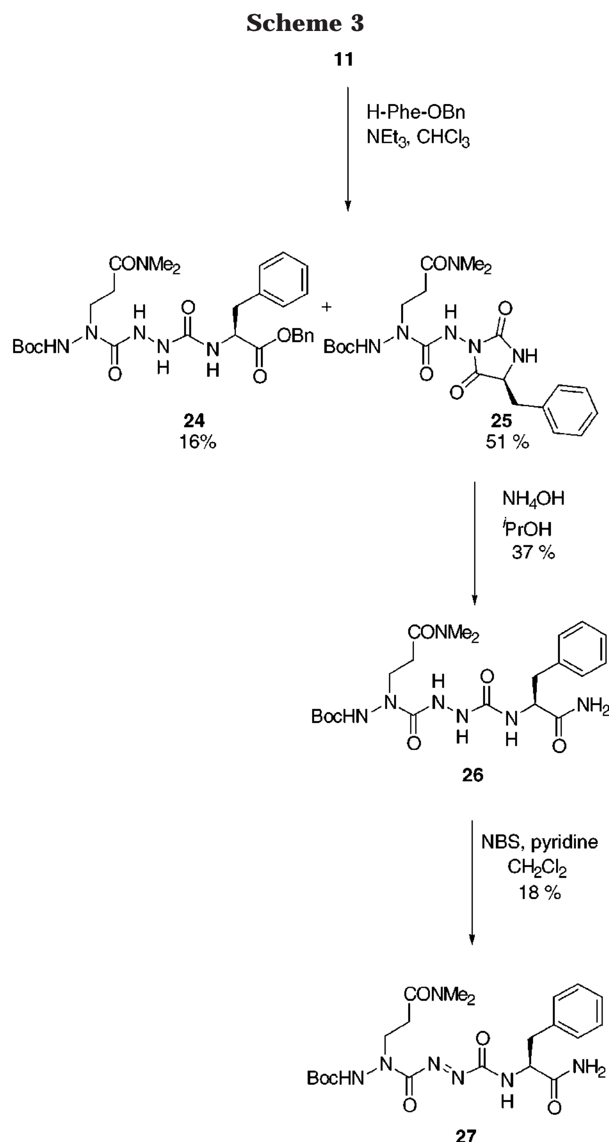

Table 1.[25]

compound	approximate IC_{50} values,[a] μM	
	HAV 3C[a]	HRV 3C[b]
15	23	10
16	10	12
23	32	4
27	62	nd

[a] 0.1 μM HAV 3C, 10 μM Dabsyl-GLRTQSFS−Edans, 2 mM EDTA, 0.1 mg/mL BSA, 100 mM K_3PO_4, 1% DMF, pH 7.5; [b] 0.4 μM HRV 3C, 10 μM EALFn-pNA, 50 mM HEPES, 150 mM NaCl, 1 mM, EDTA, 1% DMF, pH 7.5. nd = not determined.

proteinase.[24] The HRV 3C proteinase activity could be readily assayed in the presence of EALFQ-pNA as a chromogenic substrate. Assays are initiated by the addition of enzyme and no preincubation time is used. The inhibition results for the azodicarboxamides are summarized in Table 1. All of the bis-hydrazides show no inhibition of HAV 3C or HRV 3C at concentrations of 100 μM with the exception of **13** and **22** which give 19 and 43% inhibition, respectively, against HRV 3C proteinase. This contrasts the nanomolar inhibition constants achieved by structurally related hydrazides against mammalian cysteine proteinase, cathepsin K.[14] Representative examples of these bis-hydrazides were retested against HAV 3C with preincubation times of 15 min but this had no effect on enzyme activity. The corresponding azodicarboxamides display much stronger inhibition and have IC_{50}'s in the range 4−62 μM.[25] Table 1 shows that the activity of each azo compound is comparable for both enzymes, with the biggest difference being for sulfone **23**, which displays an 8-fold difference in inhibition of HRV 3C proteinase over HAV 3C proteinase. For the compound which shows the best inhibition of HAV 3C, azodicarboxamide **16**, a rate constant (k_{inact}/K_I) of 35 644 $M^{-1}min^{-1}$ was calculated using the method of Kitz and Wilson[26] at six different inhibitor concentrations. Azodicarboxamide **15** was also tested for inhibition of HAV 3C proteinase in the presence of additional thiols. When *N*-acetylcysteine or dithiothreitol (DTT) is added to the assay mixtures in excess or stoichiometric quantities, no enzyme inhibition by **15** is observed. This suggests that added thiol reacts rapidly with the azodicarboxamide moiety in a fashion analogous to that seen with azodicarboxylates,[27] namely Michael addition on the azo nitrogen. Model experiments with inhibitor **15** and *N*-acetylcysteamine in the absence of enzyme show that the azo chromophore disappears instantly upon exposure to thiol and that *N*-acetylcysteamine disulfide as well as thiol-azo adducts[27] can be seen by mass spectrometry. However, following inactivation of HAV 3C with azodicarboxamide **15**, it was not possible to regenerate enzyme activity by the addition of up to a 4-fold excess of *N*-acetylcysteamine. Since disulfide is readily formed in the model reaction and the hydrazo compound **13** which is produced as a product is a weak competitive inhibitor of HAV 3C, this suggests that the structural framework of the protein hinders attack by extraneous thiol (*N*-acetylcysteamine) on the enzyme−azodicarboxamide adduct. Although the reactivity of azodicarboxamides with extraneous thiols would initially seem to preclude the in vivo use of such molecules as drugs, it is important to note that the parent compound, azodicarbonamide **4**, is being clinically tested for the treatment of AIDS.[20] Thus, despite apparent reactivity to thiols, inhibitors such as **15** are attractive leads for the development of antiviral agents.

Mechanism of Inhibition. To confirm the hypothesis that enzyme inhibition is due to covalent modification of the active site thiol via Michael addition onto the azo moiety following the formation of an initial enzyme−inhibitor complex, the C24S HAV 3C mutant enzyme was incubated with a solution of azodicarboxamide **16** in DMSO for 15 min at 4 °C. Dialysis of the mixture followed by electrospray mass spectrometry shows peaks at 23881 (±12) for the enzyme alone and 24252 (±41) for the enzyme inhibitor complex (increase of ca. 371 mass units), which corresponds to the addition of azodicarboxamide **16** (MW 376) (Figure 2a). In a control experiment in which DMSO containing no inhibitor is added, a peak for the enzyme is observed at 23881 (±34) (Figure

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(25) IC_{50} values were all determined using at least five different inhibitor concentrations. In each case assays were initiated by the addition of enzyme, and the first 3 min of data were used for calculation purposes.

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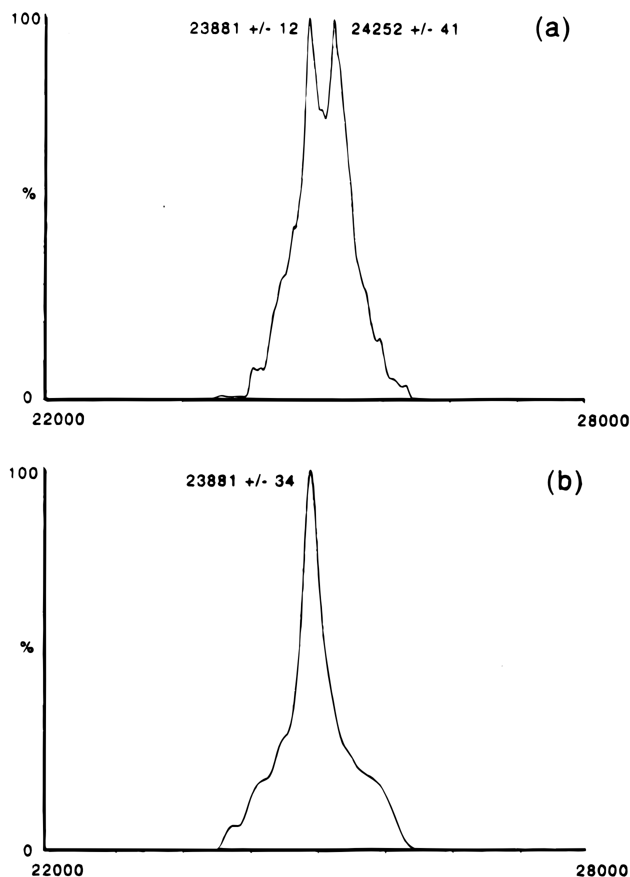

Figure 2. (a) Mass spectrum of HAV 3C C24S mutant + azodicarboxamide **16** in DMSO. (b) Mass spectrum of HAV 3C C24S mutant + DMSO. Enzyme was incubated for 15 min at 4 °C with a solution of **16** in DMSO or DMSO alone.

2b). This supports the proposal that the mode of inhibition for the azodicarboxamides involves the formation of a covalent complex. Figure 3 shows compound **16** modeled[28] into the active site of HAV 3C based upon a cocrystal structure of HAV 3C with a covalent addition of acetyl-Val-Phe-amide to Cys 172.[29] The carbonyl which hydrogen-bonds to the backbone nitrogen of residue 170 does not fall into the oxyanion hole canonically and does not appear to hydrogen-bond to the nitrogen of Cys 172. Furthermore, owing to the rigidity of the inhibitor relative to a peptide, the phenyl ring is not apparently able to fully enter the S_2' pocket.

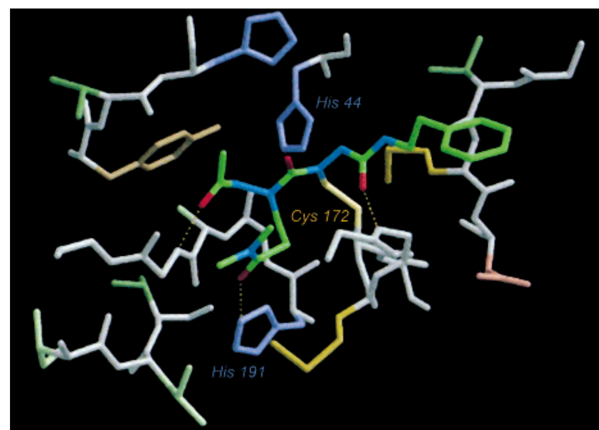

Figure 3.[28,29] Molecular model of azodicarboxamide **16** following Michael addition with Cys 172 in the active site of HAV 3C proteinase. For the inhibitor, carbon atoms are shown green, nitrogen atoms blue, and oxygen atoms red. The protein side chains are colored by type and the backbone is gray. Key hydrogen-bonds predicted between the inhibitor and the protein are shown as dotted yellow lines.

Summary

The present work describes the syntheses of a number of unsymmetrical bis-hydrazides and azodicarboxamides. In contrast to the cysteine proteinase cathepsin K, HAV 3C and HRV 3C proteinases are not effectively inhibited by bis-hydrazides. However, oxidation of these compounds to the corresponding azodicarboxamides results in compounds which are potent, irreversible inactivators of the target cysteine proteinases. Molecular modeling studies suggest that these compounds are potentially useful tools for cocrystallization studies since recognition elements can be built onto either side of the azo moiety, thereby spanning both the P and P′ recognition sites of the active site. Presently most cysteine proteinase inhibitors utilize recognition on only one side of the active site. Additional studies to improve recognition and specificity of inhibition are in progress.

Experimental Section

General Methods and Enzyme Assays. Most general procedures and instrumentation have been previously described.[30] Inhibitors were screened for effectiveness against HAV 3C proteinase and HRV 3C proteinase. Both enzymes were expressed and purified according to previously described procedures.[7c,31] HAV 3C proteinase assays employed a C24S mutant in which the nonessential surface cysteine was replaced with serine and which displays catalytic parameters indistinguishable from those of the wild-type enzyme.[23] HRV 3C assays used the 3C proteinase from serotype 14. Purity of the enzyme samples was greater than 90% as determined by SDS–PAGE analysis. Proteinase concentrations were determined spectrophotometrically using ϵ_{280} = 1.2 mg/mL. Enzymes were dialyzed against reaction buffer to remove DTT immediately prior to use.

In the case of HAV 3C proteinase, enzyme activity was monitored using a fluorometric assay similar to one described for HRV 3C proteinase.[24] Assays were done at pH 7.5 (100 mM KH_2PO_4/K_2HPO_4, 20 mM, EDTA) at 25 °C. Bovine serum albumin (BSA) was added to give a concentration of 0.1 mg/mL. Inhibitor stock solutions were prepared at 10 mM in DMF and serial dilutions made in DMF. Inhibitor concentrations were varied from 100 μM to 1 μM. Dabcyl-GLRTQSFS-Edans (Bachem) was prepared as a stock solution in DMF and a final concentration of 10 μM used in each assay. Assays were initiated by the addition of enzyme (0.1 μM), and the increase in fluorescence (λ_{ex} 336 nm, λ_{em} 472 nm) was monitored with a Shimadzu RF5301 spectrofluorophotometer. In the case of

(28) Model created by Dr. Jonathan Parrish, Biochemistry Department, University of Alberta, using InsightII (MSI corporation, San Diego, CA) and X-plor (Brunger, A. T. *X-PLOR: A System for X-ray Crystallography and NMR*; Yale University Press: New Haven, 1992). The figure created using BOBscript (Bacon, D. J.; Anderson, W. F. *J. Mol. Graphics* **1988**, *6*, 219–220, and Merritt, E. A.; Murphy, M. E. P. *Acta Crystallogr.* **1994**, *D50*, 869–873).

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HRV 3C proteinase, EALFQ-pNA (Bachem) (10 μM) was incubated at 30 °C with inhibitor (100 μM to 1 μM in DMSO) at pH 7.5 (HEPES 50 mM, NaCl 150 mM, EDTA 1 mM). HRV 3C proteinase (0.4 μM) was added and the increase at λ_{max} 405 nm monitored using a GBC Cintra 40 UV spectrometer.

In both cases the data for the initial 3 min of the assay were used for calculation of inhibition. In all cases control assays were performed using enzyme and solvent alone. IC_{50} values were calculated for compounds which showed a value <100 μM (as determined by an initial assay at 100 μM) and were determined using at least five different inhibitor concentrations. The rate of inactivation for azodicarboxamide **16** was determined using the method of Kitz and Wilson,[26] using six different inhibitor concentrations (1−50 μM) and six incubation times at each concentration. To see if the addition of a low molecular weight thiol could regenerate enzyme activity following enzyme inactivation by an azodicarboxamide, HAV 3C was inactivated by **15** (50 or 100 μM) as described above. A solution of *N*-acetylcysteamine in DMF (100−200 μM) was then added, and the fluorescence spectrum was monitored over 15−30 min. No recovery of enzyme activity was observed during any of the experiments.

3-[*N*^{1},*N*^{2}-Bis(carbobenzyloxy)hydrazino]-*N,N*-dimethylpropanamide (5). This was prepared by a modification of the procedure of Huang.[16] To a solution of hydrazine monohydrate (1.20 mL, 25.0 mmol) in MeOH (20 mL) at room temperature was added *N,N*-dimethylacrylamide (2.50 mL, 25.0 mmol) over a 10 min period. The reaction mixture was stirred for 2 h, dried ($MgSO_4$), and concentrated in vacuo. The residual oil was dissolved in diethyl ether (60 mL) and cooled to 0 °C. To this solution was added 1 N NaOH (50 mL) and water (15 mL), followed after 5 min by benzyl chloroformate (7.15 mL, 50 mmol). The reaction mixture was stirred for 1 h and diluted with EtOAc (100 mL), and the aqueous layer was washed with EtOAc (3 × 100 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated to give a yellow oil which crystallized on standing. Purification by column chromatography on silica gel eluting with 5% MeOH in EtOAc gave the desired product as a white crystalline solid (5.91 g, 59%). A sample was recrystallized from EtOAc for analysis. mp 106−108 °C; IR ($CHCl_3$ cast) 3228, 2949, 1715, and 1630 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.66 (2 H, br s), 2.68 (6 H, br s), 3.80 (2 H, t, J = 6.3 Hz), 5.11 (4 H, br s), and 7.29 (10 H, br s); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 31.66, 35.34, 37.22, 47.52, 67.62, 128.13, 128.31, 128.56, 129.15, 135.77, 136.02, 155.76, and 155.99; MS (+ve ES) 422.2 (MNa^+, 100%) and 400.2 (MH^+, 44%). Anal. Calcd for $C_{21}H_{25}N_3O_5$: C, 63.15; H, 6.31; N, 10.52%. Found: C, 63.26; H, 6.40; N, 10.48%.

3-[*N*^{1}-(Carbobenzyloxy)- *N*^{2}-(carbobenzyloxy,*tert*-butoxycarbonyl)hydrazino]-*N,N*-dimethylpropanamide (6). To a solution of hydrazide (**5**) (5.36 g, 13.43 mmol) in acetonitrile (75 mL) at room temperature, under an argon atmosphere, was added (dimethylamino)pyridine (DMAP) (164 mg, 1.34 mmol) followed, 5 min later, by a solution of di-*tert*-butyl dicarbonate (3.22 g, 14.8 mmol) in acetonitrile (50 mL). The reaction mixture was stirred overnight at room temperature under argon. The solvent was removed in vacuo, and the residual oil was purified by column chromatography on silica gel eluting with (2:1) EtOAc/hexane to give the desired compound as a clear colorless oil (5.07 g, 76%); IR ($CHCl_3$ cast) 1804, 1766, 1725, and 1651 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6, 120 °C) δ 1.40 (9 H, s), 2.58 (2 H, t, J = 7.3 Hz), 2.93 (6 H, s), 3.76 (2 H, m), 5.12 (2 H, br s), 5.20 (2 H, s) and 7.35 (10 H, br s); MS (+ve ES) 522.2 (MNa^+, 76%), 500.2 (MH^+, 7%) and 422.1 (M^+ − Boc, 100%). Anal. Calcd for $C_{26}H_{33}N_3O_7$: C, 62.51; H, 6.66; N, 8.41%. Found: C, 62.50; H, 6.77; N, 8.45%.

3-[*N*^{1}-(Carbobenzyloxy)-*N*^{2}-(carbobenzyloxy)-*N*^{2}-acetylhydrazino]-*N,N*-dimethylpropanamide (7). Hydrazide (**5**) (200 mg, 0.50 mmol) and DMAP (12 mg, 0.10 mmol) were suspended in acetic anhydride (5 mL) and heated at 80 °C for 4 h. The reaction was cooled to room temperature and purified by column chromatography on silica gel eluting with EtOAc. The product was obtained as a colorless oil (191 mg, 86%); IR ($CHCl_3$ cast) 1754, 1721, 1647, and 1402 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6, 120 °C) 2.42 (3 H, s), 2.55 (2 H, t, J = 7.1 Hz), 2.78 (6 H, br s), 3.66 (1 H, ddd, J = 7.1, 7.1, 14.7 Hz), 3.82 (1 H, ddd, J = 7.1, 7.1, 14.4 Hz), 5.05 (2 H, br s), 5.22 (2 H, s) and 7.35 (10 H, br s); MS (+ve ES) 905.3 (M_2Na^+, 13%), 464.1 (MNa^+, 21%) and 442.3 (MH^+, 100%). Anal. Calcd for $C_{23}H_{27}N_3O_6$: C, 62.57, H, 6.16, N, 9.52%. Found: C, 62.46; H, 6.17; N, 9.41%.

3-[*N*^{2}-(*tert*-Butoxycarbonyl)hydrazino]-*N,N*-dimethylpropanamide (8). Hydrazide (**6**) (4.51 g, 9.03 mmol) was dissolved in MeOH (100 mL) in the presence of 10% Pd/C (450 mg, 10% w/w) under an argon atmosphere. The suspension was stirred under a hydrogen atmosphere until the uptake of hydrogen ceased (approximately 4 h). Filtration through Celite, followed by removal of the solvent in vacuo, gave the title compound as a colorless oil (2.02 g, 97%); IR ($CHCl_3$ cast) 3295, 2976, 2933, 1709, and 1641 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (9 H, s), 2.46 (2 H, t, J = 6.5 Hz), 2.92 (3 H, s), 2.93 (3 H, s), 3.10 (2 H, t, J = 6.5 Hz), 4.11 (1 H, br s), and 6.13 (1 H, br s); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 28.33, 31.59, 35.32, 37.16, 47.91, 80.40, 156.67, and 171.45; MS (+ve ES) 254.1 (MNa^+, 100%). Anal. Calcd for $C_{10}H_{21}N_3O_3$: C, 51.93; H, 9.15; N, 18.17%. Found: C, 51.97; H, 8.98; N, 17.94%.

3-(*N*^{2}- Acetylhydrazino)-*N,N*-dimethylpropanamide (9). This was prepared using the same method as that described for the preparation of **8**. A solution of hydrazide (**7**) (157 mg, 0.36 mmol) in MeOH (10 mL) was stirred under a hydrogen atmosphere in the presence of 10% Pd/C (15 mg) until the uptake of hydrogen gas ceased (20 min) and then for a further 2 h. The suspension was filtered through Celite and the solvent removed in vacuo to yield a white solid which was recrystallized from EtOAc to give a white solid (56 mg, 90%); mp 112−113 °C; IR ($CHCl_3$ cast) 3264, 2933, and 1635 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.90 (3 H, s), 2.47 (2 H, t, J = 6.4 Hz), 2.91 (3 H, s), 2.97 (3 H, s), 3.12 (2 H, t, J = 6.4 Hz), 4.48 (1 H, br s), and 7.95 (1 H, br s); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 19.38, 21.11, 30.80, 32.04, 35.41, 37.12, 37.25, 47.65, 48.31, 169.21, and 171.61; MS (+ve ES) 196.1 (MNa^+, 17%), 174.1 (MH^+, 94%), and 130.1 (M^+ − CH_3CO, 100%). Anal. Calcd for $C_7H_{15}N_3O_2$: C, 48.54; H, 8.73; N, 24.26%. Found: C, 48.53; H, 8.74; N, 24.02%.

3-[*N*^{1}-Phenoxycarbonylhydrazido][*N*^{2}-(*tert*-butoxycarbonyl)hydrazino]-*N,N*-dimethylpropanamide (11). To a solution of Boc-hydrazide (**8**) (750 mg, 3.24 mmol) in chloroform (35 mL) at room temperature, was added diphenyl hydrazodicarboxylate (**10**)[21] (971 mg, 3.57 mmol) and the resulting suspension was stirred for 5 min. Triethylamine (0.45 mL, 3.24 mmol) was added and the suspension was heated to reflux at which point all of the solid dissolved. Heating was maintained for 3 h, the solution was cooled to room temperature and the solvent was removed in vacuo. Purification by column chromatography on silica gel eluting with 5% MeOH in EtOAc gave the desired product as a white solid which was recrystallized from EtOAc/hexane (567 mg, 43%); mp 149−151 °C; IR (microscope) 3337, 3226, 3003, 2979, 1770, 1727, 1664, and 1637 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (9 H, s), 2.71 (2 H, br s), 3.01 (6 H, 2 × s), 3.72 (2 H, br s), 6.87 (1 H, s), 7.12−7.35 (5 H, m), and 7.63 (2 H, br s); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 28.20, 32.38, 35.59, 46.70, 82.29, 121.48, 125.66, 129.35, 150.81, 155.19, 155.92, 157.35 and 172.13; MS (+ve ES) 432.2 (MNa^+, 100%), 410.2 (MH^+, 25%), 354.2 (M^+ − C_4H_9, 29%), 332.2 (M^+ − C_6H_5, 47%), and 310.2 (M^+ − Boc). Anal. Calcd for $C_{18}H_{27}N_5O_6$: C, 52.80; H, 6.65; N, 17.10%. Found: C, 52.82; H, 6.67; N, 16.87%.

3-[*N*^{1}-Phenoxycarbonylhydrazido][*N*^{2}-acetylhydrazino]-*N,N*-dimethylpropanamide (12). This was prepared using the same method as that described for the preparation of **11**. Hydrazide (**9**) (350 mg, 2.02 mmol) in chloroform (15 mL) was treated with triethylamine (0.42 mL, 3.04 mmol) and diphenyl hydrazodicarboxylate (**10**) (825 mg, 3.04 mmol) at reflux for 6 h. Purification by column chromatography on silica gel eluting with 5% MeOH in CH_2Cl_2 gave **12** as a white solid (192 mg, 27%); mp 139−141 °C; IR ($CHCl_3$ cast) 3243, 3008, 1959, 1771, 1750, 1733, 1683, 1675, and 1652 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.04 (3 H, s), 2.70 (2 H, br s), 2.91 (3 H, s), 2.96 (3 H, s), 3.75 (2 H, br s), 6.90 (<1 H, br s), 7.11−7.29 (5 H, m), 7.60 (<1 H, br s) and 8.82 (<1 H, br s); ^{13}C NMR (75.5 MHz, CD_3-

OD) δ 20.89, 32.72, 35.69, 37.71, 47.01, 61.53, 122.65, 126.61, 130.35, 152.46, 157.59, 159.38, 172.75 and 173.38; MS (+ve ES) 374.1 (MNa^+, 38%), 352.1 (MH^+, 100%); MS (+ve ES) Found 352.162389. $C_{15}H_{22}N_5O_5$ (MH^+) requires 352.162094.

3-[*N*¹-Phenethylamidohydrazido][(*N*²-*tert*-butoxycarbonyl)hydrazino]-*N,N*-dimethylpropanamide (13). To a solution of phenoxy bis-hydrazide (**11**) (389 mg, 0.95 mmol) in chloroform (25 mL) was added phenethylamine (0.126 mL, 1.0 mmol) followed by triethylamine (0.139 mL, 1.0 mmol) and the solution heated at reflux for 7 h. Removal of the solvent in vacuo gave a white solid which was purified by column chromatography on silica gel eluting with 5% MeOH in EtOAc. The title compound was collected as a white solid which was recrystallized from MeOH/EtOAc (331 mg, 80%); mp 179−180 °C; IR (microscope) 3357, 3305, 3269, 2981, 2922, 1706, and 1626 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.44 (9 H, s), 2.70 (2 H, br s), 2.75 (2 H, t, $J = 7.6$ Hz), 2.93 (3 H, s), 3.00 (3 H, s), 3.43 (2 H, t, $J = 7.6$ Hz), 3.80 (2 H, br s), 7.13−7.29 (5 H, m), 7.50 (1 H, br s, exchanges with D_2O), and 8.04 (1 H, br s, exchanges with D_2O); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 28.22, 32.42, 35.61, 36.31, 37.41, 41.55, 46.55, 82.50, 126.26, 128.48, 128.86, 139.26, 156.06, 157.31, 159.18, and 172.20; MS (+ve ES) 475.3 (MK^+), 459.3 (MNa^+, 100%), 437.3 (MH^+, 64%), 359.2 ($MNa^+ - Boc$, 37%) and 337.2 ($MH^+ - Boc$, 20%). Anal. Calcd for $C_{20}H_{32}N_6O_5$: C, 55.03; H, 7.39; N, 19.25%. Found: C, 55.08; H, 7.50; N, 19.39%.

3-[*N*¹-(Phenethylamidohydrazido)-*N*²-acetylhydrazino]-*N,N*-dimethylpropanamide (14). This was prepared using the same method as that described for the preparation of **13**. A solution of phenoxy bis-hydrazide (**12**) (192 mg, 0.55 mmol) in chloroform (15 mL) was treated with triethylamine (0.083 mL, 0.60 mmol) and phenethylamine (0.075 mL, 0.60 mmol) at reflux for 6.5 h. Purification by column chromatography eluting with 5% MeOH in CH_2Cl_2 gave the desired compound as a white solid (98 mg, 48%); mp 112−115 °C; IR ($CHCl_3$ cast) 3257, 2934, 1665, and 1547 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.02 (3 H, s), 2.63 (2 H, br s), 2.79 (2 H, t, $J = 7.2$ Hz), 2.88 (3 H, s), 2.95 (3 H, s), 3.37 (2 H, dt, $J = 6.0, 6.0$ Hz), 3.77 (2 H, br s), 6.06 (1 H, br s), 6.86 (1 H, br s), 7.12−7.27 (5 H, m), 8.06 (1 H, br s), and 9.55 (1 H, br s); ^{13}C NMR (75.5 MHz, CD_3OD) δ 20.95, 32.57, 35.70, 37.29, 37.70, 42.62, 46.97, 127.22, 129.45, 129.85, 140.67, 159.39, 161.73, 173.19, and 173.33; MS (+ve ES) 757.4 (M_2H^+, 56%), 401.2 (MNa^+, 63%), and 379.2 (MH^+, 100%); MS (+ve ES) Found: 401.191141. $C_{17}H_{26}N_6O_4Na$ (MNa^+) requires 401.191323.

Azo-*N*¹-(phenethylformamide)- *N*¹′-[*N,N*-dimethylpropdimethylpropanamide-*tert*-butoxycarbonyl hydrazinoformamide] (15). This was prepared by an adaptation of the method of Harris.[21] To a solution of bis-hydrazide (**13**) (48 mg, 0.11 mmol) in CH_2Cl_2 (5 mL) at room temperature were added pyridine (0.012 mL, 0.12 mmol) and *N*-bromosuccinimide (19 mg, 0.11 mmol). The resulting orange solution was stirred at room-temperature overnight, diluted with CH_2Cl_2 (20 mL), and washed with water and brine. The organic layer was dried ($MgSO_4$), and the solvent was removed in vacuo. The title compound was purified by column chromatography on silica gel eluting with EtOAc to give an orange solid (34 mg, 71%); mp 125−126 °C; IR ($CHCl_3$ cast) 3252, 2933, 1738, 1732, 1715, and 1651 cm^{-1}; UV (CH_3CN) 273 (ϵ 1777) and 414 (ϵ 38) nm; ^1H NMR (300 MHz, $CDCl_3$) δ 1.36 (9 H, s), 2.81 (2 H, s), 2.90−2.94 (5 H, m), 2.99 (3 H, s), 3.70 (2 H, dt, $J = 6.7, 6.8$ Hz, becomes t in D_2O), 3.92 (2 H, br s), 6.52 (1 H, br s, exchanges in D_2O), 7.27 (5 H, m) and 7.73 (1 H, br s, exchanges in D_2O); MS (+ve ES) 457.1 (MNa^+, 43%), 435.2 (MH^+, 45%) and 379.1 ($M^+ - C_4H_8$, 100%); MS (+ve ES) Found: 457.216799. $C_{20}H_{30}N_5O_5Na$ (MNa^+) requires 457.217538. Anal. Calcd for $C_{20}H_{30}N_6O_5$: C, 55.29; H, 6.96; N, 19.34%. Found: C, 55.09; H, 7.00; N, 19.00%.

Azo-*N*¹-(phenethylformamide)-*N*¹′-[*N,N*-dimethylpropanamide-acetyl hydrazinoformamide] (16). This was prepared using a method similar to that described for the preparation of **15**. Bis-hydrazide (**14**) (26 mg, 0.07 mmol) in CH_2Cl_2 (2 mL) was treated with pyridine (8 μL, 0.08 mmol) and *N*-bromosuccinimide (13 mg, 0.08 mmol) at room temperature for 5 h. The solvent was removed in vacuo, and the residue was purified by column chromatography on silica gel eluting with 5% MeOH in EtOAc to give the title compound which was recrystallized from EtOAc to give yellow needles (21 mg, 81%); mp 137−138 °C; IR ($CHCl_3$ cast) 3243, 3025, 1728, 1630, and 1498 cm^{-1}; UV (CH_3CN) 239 (ϵ 1879) and 410 (ϵ 47) nm; ^1H NMR (300 MHz, $CDCl_3$) δ 1.89 (3 H, s), 2.83 (2 H, br s), 2.94 (5 H, m), 3.00 (3 H, s), 3.73 (2 H, dt, $J = 6.7, 6.8$ Hz), 3.88 (2 H, br s), 6.59 (1 H, br t), 7.26 (5 H, m), and 8.91 (1 H, s); MS (+ve ES) 775.2 (M_2Na^+, 49%), 399.1 (MNa^+, 46%) and 377.2 (MH^+, 62%). Anal. Calcd for $C_{17}H_{24}N_6O_4$: C, 54.25; H, 6.43; N, 22.33%. Found: C, 53.94; H, 6.40; N, 21.91%.

2-[(*N*¹,*N*²-Bis(carbobenzyloxy)hydrazino]ethyl Methyl Sulfone (17) and 2-[*N*¹ -(Carbobenzyloxy)hydrazino]-ethyl Methyl Sulfone (18). This was prepared by a method similar to that described for the preparation of **5**. A solution of hydrazine monohydrate (0.49 mL, 10.0 mmol) in MeOH (10 mL) was treated with vinyl methyl sulfone (0.88 mL, 10.0 mmol) in MeOH (5 mL), followed by 1 N NaOH (22 mL) and benzyl chloroformate (2.86 mL, 20.0 mmol). After aqueous workup, purification by column chromatography on silica gel eluting with EtOAc gave bis-Cbz sulfone (**17**) as a white solid which was recrystallized from EtOAc/hexane to give white needles (1.558 g, 38%). The monoprotected sulfone (**18**) was also isolated as a white solid (884 mg, 33%).

Data for bis-Cbz sulfone (17): IR ($CHCl_3$ cast) 3300, 3031, 2930, 1718, and 1302 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.87 (3 H, br s), 3.32 (2 H, br s), 4.02 (2 H, br s), 5.12 (4 H, br s) and 7.25 (10 H, br s); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 41.12, 44.75, 52.03, 68.08, 68.62, 128.25, 128.49, 128.57, 128.63, 128.67, 135.33, 135.45, and 155.50; MS (+ve ES) 429.2 (MNa^+, 37%), 407.4 (MH^+, 52%), and 363.4 ($M^+ - CO_2$, 100%). Anal. Calcd for $C_{19}H_{22}N_2O_6S$: C, 56.15; H, 5.46; N, 6.89%. Found: C, 56.34; H, 5.27; N, 6.73%.

Data for mono-Cbz sulfone (18): mp 88−90 °C; IR ($CHCl_3$ cast) 3345, 2927, 1694, 1497, and 1290 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3/D_2O$) δ 2.86 (3 H, s), 3.28 (2 H, t, $J = 6.5$ Hz), 3.90 (2 H, t, $J = 6.5$ Hz), 5.13 (2 H, s), and 7.33 (5 H, m); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 40.74, 44.56, 51.79, 68.11, 128.18, 128.38, 128.58, 128.65, 135.86, and 157.00; MS (+ve ES) 295.4 (MNa^+, 23%) and 273.5 (MH^+, 100%). Anal. Calcd for $C_{11}H_{16}N_2O_4S$: C, 48.52; H, 5.92; N, 10.29%. Found: C, 48.42; H, 6.02; N, 10.16%.

2-[*N*¹-(Carbobenzyloxy)-*N*²-(carbobenzyloxy)-*N*²-(*tert*-butoxycarbonyl)hydrazino]ethyl Methyl Sulfone (19). This was prepared by a method similar to that described for the preparation of **6**. To a solution of bis-Cbz sulfone (**17**) (1.40 g, 3.45 mmol) in acetonitrile (25 mL) were added DMAP (42 mg, 0.35 mmol) and di-*tert*-butyl dicarbonate (828 mg, 3.79 mmol), and the reaction mixture was stirred overnight. Purification by column chromatography on silica gel, eluting with (1:1) EtOAc/hexane, gave the desired product as a colorless oil (1.75 g, quantitative); IR ($CHCl_3$ cast) 1805, 1766, 1727, and 1309 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6, 120 °C) δ 1.39 (9 H, s), 2.95 (3 H, s), 3.34 and 4.00 (2 H, 2 × t, $J = 7.5$ Hz), 3.92 (2 H, m), 5.13 (2 H, m), 5.25 (2 H, m), and 7.35 (10 H, m); MS (-ve ES) 405.6 ($M - Boc^-$, 34%) and 371.7 ($M - CO_2CH_2Ph^-$, 100%); MS (+ve ES) 529.3 (MNa^+, 11%). Anal. Calcd for $C_{24}H_{36}N_2O_5S$: C, 56.91; H, 5.97; N, 5.53%. Found: C, 57.18; H, 6.03; N, 5.47%.

2-[*N*²-(*tert*-Butoxycarbonyl)hydrazino]ethyl Methyl Sulfone (20). This was prepared by a method similar to that described for the preparation of **8**. A solution of sulfone (**19**) (1.677 g, 3.31 mmol) in MeOH (50 mL) was stirred under a hydrogen atmosphere in the presence of 10% Pd/C (160 mg) until the uptake of hydrogen gas ceased (20 min) and then for a further 1 h. The suspension was filtered through Celite and the solvent removed in vacuo to yield a white solid (769 mg, 97%). A sample was recrystallized from EtOAc/hexane for analysis. mp 99−100 °C; IR ($CHCl_3$ cast) 3334, 3321, 3308, 2928, 1687, and 1287 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.43 (9 H, s), 2.98 (3 H, s), 3.17 (2 H, dt, $J = 0.6, 6.3$ Hz), 3.36 (2 H, t, $J = 6.3$ Hz), 4.25 (<1 H, br t, $J = 0.6$ Hz, exchanges with D_2O), and 6.12 (<1 H, br s, exchanges with D_2O); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 28.33, 41.98, 45.69, 45.96, 75.81, and 156.88; MS (+ve ES) 261.5 (MNa^+, 52%), 183.6 ($M^+ - C_4H_9$,

100%) and 139.6 (M^+ − Boc, 3%). Anal. Calcd for $C_8H_{18}N_2O_4S$: C, 40.32; H, 7.61; N, 11.76%. Found: C, 40.37; H, 7.80; N, 11.63%.

2-[*N*1-(Pentafluorophenoxycarbonyl)-*N*2-(*tert*-butoxycarbonyl)hydrazino]ethyl Methyl Sulfone (21). To a solution of hydrazide (**20**) (332 mg, 1.40 mmol) in THF (10 mL) at room temperature under an argon atmosphere was added pyridine (0.138 mL, 1.40 mmol). After stirring for 2 min, bis-pentafluorophenyl carbonate[21] (500 mg, 1.27 mmol) was added and the stirring continued overnight. The solvent was removed in vacuo and the residue purified by column chromatography on silica gel eluting with a gradient of (2:1) hexane/EtOAc to (1:1) hexane/EtOAc. The product was collected as a white solid which was recrystallized from hexane/EtOAc (490 mg, 86%); mp 132−133 °C; IR ($CHCl_3$ cast) 3316, 2983, 2935, 1758, and 1523 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.48 (9 H, s), 2.98 (3 H, s), 3.41 and 3.47 (2 H, br t and br s, J = 6.0 Hz), 4.10 and 4.23 (2 H, br s and br t, J = 6.0 Hz), and 6.98 (<1 H, br s, exchanges with D_2O); MS (+ve ES) 471.0 (MH^+, 100%), 393.0 (M^+ − C_4H_7, 36%), 349.0 (M^+ − Boc, 30%). Anal. Calcd for $C_{15}H_{17}F_5N_2O_6S$: C, 40.18; H, 3.82; N, 6.25%. Found: C, 40.11; H, 3.71; N, 6.16%.

2-[*N*1-Phenethylamidohydrazido] 2-[*N*2-(*tert*-butoxycarbonyl)hydrazino]ethyl Methyl Sulfone (22). To a solution of pentafluorophenoxy hydrazide (**21**) (162 mg, 0.36 mmol) in THF (25 mL) at room temperature under an argon atmosphere was added hydrazine monohydrate (0.053 mL, 1.09 mmol) followed by triethylamine (0.151 mL, 1.09 mmol). After 2.5 h, phenethyl isocyanate (0.243 mL, 1.81 mmol) was added followed by a further portion of triethylamine (0.151 mL, 1.09 mmol), and a white suspension formed. After stirring at room-temperature overnight, the solvent was removed in vacuo, and the residue was diluted with EtOAc (50 mL) and filtered. The filtrate was concentrated in vacuo and purified by column chromatography on silica gel eluting with 5% MeOH in EtOAc to yield a white solid (75 mg, 47%); mp 79−82 °C; IR ($CHCl_3$ cast) 3320, 2979, 2931, 1717, 1671, 1546, and 1291 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.43 (9 H, s), 2.77 (2 H, t, J = 7.6 Hz), 2.92 (3 H, s), 3.37 (4 H, br d, J = 6.3 Hz), 4.00−4.50 (<2 H, br s), 6.00 (<1 H, br s), 6.83 (<1 H, br s), 7.13−7.27 (5 H, m), 8.35 (1 H, s), and 8.39 (1 H, s); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 28.25, 36.27, 40.88, 41.62, 43.42, 51.84, 82.59, 126.37, 128.53, 128.82, 138.97, 155.04, 158.39, and 159.76; MS (+ve ES) 887.5 (M_2H^+, 14%), 466.1 (MNa^+, 100%), and 444.1 (MH^+, 8%); MS (+ve ES) Found: 466.173494. $C_{18}H_{29}N_5O_6SNa$ (MNa^+) requires 466.173626.

Azo-*N*1-(phenethylformamide)-*N*1′-[2-(*N*2-*tert*-butoxycarbonyl)hydrazino]ethyl Methyl Sulfone Formamide (23). This was prepared using a method similar to that described for **15**. Bis-hydrazide (**22**) (50 mg, 0.11 mmol) in CH_2Cl_2 (5 mL) was treated with pyridine (0.012 mL, 0.12 mmol) and *N*-bromosuccinimide (22 mg, 0.12 mmol) under an argon atmosphere for 2 h. Removal of the solvent in vacuo followed by purification by column chromatography on silca gel, eluting with EtOAc, yielded **23** as a yellow solid (50 mg, quantitative). A sample was recrystallized from EtOAc/hexane; mp 111−112 °C; IR ($CHCl_3$ cast) 3300, 2981, 2933, 1732, and 1369 cm^{-1}; UV (CH_3CN) 250 (ε 2326) and 419 (ε 50) nm; ^1H NMR (300 MHz, $CDCl_3$) δ 1.38 (9 H, s), 2.93 (2 H, t, J = 7.1 Hz), 3.00 (3 H, s), 3.45 (2 H, t, J = 6.4 Hz), 3.73 (2 H, dt, J = 6.3, 13.3 Hz), 4.16 (2 H, m), 6.68 (1 H, br s), and 7.25 (6 H, m); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 27.96, 35.24, 41.34, 42.22, 44.43, 51.52, 126.84, 128.68, 128.84; MS (+ve ES) 480.2 (MK^+, 20%), 464.2 (MNa^+, 100%) and 459.2 (MNH_4^+, 23%); MS (+ve ES) Found: 464.157347. $C_{18}H_{27}N_5O_6SNa$ (MNa^+) requires 464.157975. Anal. Calcd for $C_{18}H_{27}N_5O_6S$: C, 48.97; H, 6.16; N, 15.86%. Found: C, 49.12; H, 6.40; N, 14.72%.

***tert*-Butoxycarbonyl-*N,N*-(dimethyl)azaglutamine-azaglycine-(2S)-phenylalanine Benzyl Ester (24) and *N*-(4*S*-Benzyl-2,5-dioxoimidazolidin-1-yl)-*N*-(*tert*-butoxycarbonylhydrazino)-*N,N*-dimethylpropanamide Urea (25).** To a solution of L-phenylalanine benzyl ester (264 mg, 1.04 mmol) in chloroform (10 mL) at room temperature was added triethylamine (0.26 mL, 1.86 mmol) followed by phenoxy bishydrazide (**11**) (339 mg, 0.83 mmol). The resulting solution was heated at reflux for 6 h, cooled, and stirred at room-temperature overnight. Removal of the solvent in vacuo followed by purification by column chromatography on silica gel, eluting with 5% MeOH in EtOAc, yielded benzyl ester (**24**) as an oil (77 mg, 16%) and hydantoin (**25**) as a white solid (197 mg, 51%).

Data for ester (24): $[\alpha]^{26}_D$ −5.8° (*c* 1, MeOH); IR ($CHCl_3$ cast) 2977, 2938, 1736, 1679, and 1497 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.45 (9 H, s), 2.65 (2 H, br s), 2.68 (3 H, s), 2.95 (3 H, s), 3.07 (2 H, d, J = 6.4 Hz), 3.65 (2 H, br s), 4.75 (1 H, dd, J = 6.4, 14.0 Hz), 5.10 (2 H, AB_q, J = 12.4 Hz), 6.06 (1 H, d, J = 7.3 Hz), 6.21 (1 H, s), 7.05−7.61 (10 H, m), and 7.64 (1 H, br s); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 28.18, 32.23, 35.53, 37.30, 38.26, 46.53, 54.19, 66.87, 82.27, 126.75, 128.24, 128.35, 128.40, 128.48, 128.93, 129.23, 129.45, 135.40, 136.27, 155.82, 157.58, 158.42, 171.96, and 172.04; MS (+ve ES) 593.3 (MNa^+, 75%), 571.3 (MH^+, 100%), 515.2 (M − C_4H_7, 16%), and 417.2 (M − Boc, 16%); MS (+ve ES) Found: 593.269838. $C_{28}H_{38}N_6O_7Na$ (MNa^+) requires 593.269968.

Data for hydantoin (25): mp 89−93 °C; $[\alpha]^{26}_D$ −53.3° (*c* 1.06, $CHCl_3$); IR ($CHCl_3$ cast) 3274, 1741, 1629, and 1498 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.46 (9 H, s), 2.65 (2 H, br s), 2.84 (1 H, dd, J = 9.9, 13.9 Hz), 2.90 (3 H, s), 2.95 (3 H, s), 3.32 (1 H, br d, J = 13.9 Hz), 3.50−4.00 (<2 H, br s), 4.27 (1 H, br d, J = 6.6 Hz), 5.86 (1 H, br s, exchanges with D_2O), 7.18−7.32 (5 H, m), and 7.56 (1 H, br s, exchanges with D_2O); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 28.10, 32.01, 35.61, 37.29, 38.15, 46.68, 57.28, 82.35, 127.40, 129.00, 129.24, 135.59, 154.79, 170.83, and 171.86; MS (+ve ES) 947.5 (M_2Na^+, 13%), 485.2 (MNa^+, 27%), 463.2 (MH^+, 100%), and 407.1 (M^+ −C_4H_7, 20%); MS (+ve ES) Found: 485.212846. $C_{21}H_{30}N_6O_6Na$ (MNa^+) requires 485.212453.

***tert*-Butoxycarbonyl-*N,N*-dimethylazaglutamine-azaglycine-(2*S*)-phenylalanamide (26).** To a solution of hydantoin (**25**) (47 mg, 0.10 mmol) in 2-propanol (1 mL) at room temperature was added concentrated ammonium hydroxide (0.25 mL) and the mixture stirred overnight. TLC analysis indicated that unreacted starting material remained, a further portion of NH_4OH (0.25 mL) was added, and stirring was continued. After 4 h a final portion of NH_4OH (0.40 mL) was added and the reaction stirred overnight. Removal of the solvents in vacuo followed by purification by column chromatography eluting with 5−10% MeOH in EtOAc gave **26** which was recrystallized from MeOH/EtOAc (18 mg, 37%); IR (microscope) 3449, 3334, 3283, 3213, 3024, 2980, 1709, 1681, 1642, 1608, and 1553 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 1.49 (9 H, s), 2.68 (2 H, t, J = 7.0 Hz), 2.92 (4 H, m), 3.04 (3 H, s), 3.22 (1 H, dd, J = 4.4, 14.1 Hz), 3.71 (2 H, br s), 4.45 (1 H, dd, J = 4.8, 9.4 Hz), and 7.23 (5 H, m); MS (+ve ES) 502.2 (MNa^+, 100%), 480.3 (MH^+, 65%), and 380.2 (M^+ − Boc, 20%); MS (+ve ES) Found: 502.239497. $C_{21}H_{33}N_7O_6Na$ (MNa^+) requires 502.239002.

Azo-*N*1-(carboxy-(2*S*)-phenylalanamide)- *N*1′-[*N,N*-dimethylpropanamide-*tert*-butoxycarbonyl hydrazinoformamide] (27). This was prepared using a method similar to that described for the preparation of **15**. Amide (**26**) (14 mg, 0.03 mmol) in CH_2Cl_2 (5 mL) was treated with pyridine (3 μL, 0.03 mmol) and *N*-bromosuccinimide (6 mg, 0.03 mmol) at room temperature for 2.5 h during which time a red/orange color developed. The solvent was removed in vacuo and the residue purified by column chromatography on silica gel eluting with 5% MeOH in CH_2Cl_2 to give **26** as a yellow oil (2.5 mg, 18%); ^1H NMR (300 MHz, $CDCl_3$) showed the presence of a 4:1 mixture of conformers, data for major compound: δ 1.36 (9 H, s), 2.81 (2 H, br s), 2.95 (3 H, s), 3.00 (3 H, s), 3.09 (1 H, dd, J = 8.2, 13.8 Hz), 3.26 (1 H, dd, J = 5.8, 13.8, Hz), 3.92 (2 H, br s), 4.65 (1 H, ddd, J = 5.7, 8.0, 10.1 Hz), 5.39 (1 H, br s), 5.50 (1 H, br s), 7.25 (5 H, m), and 7.75 (1 H, br s); MS (+ve ES) 500.2 (MNa^+, 100%) and 478.2 (MH^+, 15%); MS (+ve ES) Found: 500.222980. $C_{21}H_{31}N_7O_6Na$ (MNa^+) requires 500.223352.

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Supporting Information Available: Supporting Information Available: ^{1}H and ^{13}C NMR spectra of selected hydrazides and azodicarboxamides. This material is available free of charge via the Internet at http://pubs.acs.org.

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