Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 2477–2494

BIOORGANIC & MEDICINAL CHEMISTRY

Structure-Based Design, Synthesis and Evaluation of Conformationally Constrained Cysteine Protease Inhibitors

Karl A. Scheidt,[a,b] William R. Roush,[a,b,*] James H. McKerrow,[c] Paul M. Selzer,[c] Elizabeth Hansell,[c] Philip J. Rosenthal[d]

[a]*Department of Chemistry, Indiana University, Bloomington, IN 47405, USA*
[b]*Department of Chemistry, University of Michigan, Ann Arbor, MI 48109, USA*
[c]*Department of Pathology, University of California, San Francisco, CA 94121, USA*
[d]*Department of Medicine, University of California, San Francisco, CA 94143, USA*

Received 2 June 1998; accepted 4 September 1998

Abstract—The inhibition of cysteine proteases is being studied as a strategy to combat parasitic diseases such as Chagas' disease, leishmaniasis, and malaria. Cruzain is the major cysteine protease of *Trypanosoma cruzi*, the etiologic agent of Chagas' disease. A crystal structure of cruzain, covalently inactivated by fluoromethyl ketone inhibitor **1** (Cbz-Phe-Ala-FMK), was used as a template to design potential inhibitors. Conformationally constrained γ-lactams containing electrophilic aldehyde (**12**, **17**, **18**, **25**, **26**, and **29**) or vinyl sulfone (**43**, **44**, and **46**) units were synthesized. Constrained lactam **26** had IC_{50} values of ca. 20 nM against the *Leishmania major* protease and ca. 50 nM versus falcipain, an important cysteine protease isolated from *Plasmodium falciparum*. However, all of the conformationally constrained inhibitors were weak inhibitors of cruzain, compared to unconstrained peptide aldehyde (e.g. **5**) and vinyl sulfone inhibitors (e.g. **48**, which proved to be an excellent inhibitor of cruzain with an apparent second order inhibition rate constant (k_{inact}/K_i) of 634,000 $s^{-1}M^{-1}$). A significant reduction in activity was also observed with acyclic inhibitors **30** and **51** containing α-methyl phenylalanine residues at the P_2 position. These data indicate that the pyrrolidinone ring, especially the quarternary center at P_2, interferes with the normal substrate binding mode with cruzain, but not with falcipain or the leishmania protease.

Introduction

Cysteine proteases constitute an important class of enzymes involved in the hydrolysis of peptide bonds. Cysteine proteases play vital roles in mammalian cellular turnover[1] and apoptosis,[2,3] and are also very important in the life cycle of many parasites.[4] A possible strategy for combating parasitic infections is to inhibit cysteine proteases that are crucial to parasitic metabolism and reproductive function.[5,6]

Many parasites use cysteine proteases for important biological tasks. One such parasite is *Trypanosoma cruzi*, the etiologic agent of Chagas' disease. Chagas' disease is a permanent infection that affects more than 25 million people annually in South America and causes more than 45,000 deaths per year.[7] *Trypanosoma cruzi* is transferred to humans through the bite of the triatomid beetle, or kissing bug, during a blood meal. Chagas' disease may result in intestinal or esophageal obstruction and fatal heart damage.[8] Treatment of this disease has been limited to the use of nifurtimox and benznidazole.[9] However, these drug are not effective for treating the chronic stages of the infection, and have serious, undesirable side effects.[10] *Trypanosoma cruzi* has a major cysteine protease called cruzain that was identified by Cazzulo and co-workers.[11] The protease, classified as a member of the papain family, is necessary for intracellular replication and differentiation and has been identified as a chemotherapeutic target.[12–14] Recently, the UCSF group has demonstrated that *T. cruzi* infections in mice can be cured by using cysteine protease inhibitors specifically targeting cruzain,[15] and still other

Key words: cysteine protease inhibitors; conformationally constrained peptidyl aldehydes; peptidyl vinyl sulfones.
*Corresponding author. The Department of Chemistry, University of Michigan, Ann Arbor, MI 48109, USA; Tel: 734-647-9278; Fax: 734-647-9279; E-mail: roush@unmich.edu

0968-0896/98/$ - see front matter
PII: S0968-0896(98)00189-8

studies suggest that the protease inhibitors function by preventing the normal autocatalytic processing and trafficking of cruzain within the Golgi apparatus.[16] Two other therapeutic targets currently under development for treatment of Chagas' disease are trypanothione reductase[17,18] and sterol C-14α demethylase.[8]

Two other globally important parasites are *Leishmania major*,[19] one of the organisms that causes leishmaniasis, and *Plasmodium falciparum*, the most dangerous of the malarial parasites.[20] Leishmaniasis is a spectrum of infections by related species of parasites that can cause local, often disfiguring skin lesions, or disseminated, fatal disease if not treated. Malaria continues to be one of the most important diseases in the world, with over 120 million cases and an estimated 1 million deaths each year. Unfortunately, resistance of *P. falciparum* to established chemotherapeutic agents is increasing.[21] Falcipain, the major cysteine protease of the *P. falciparum* trophozoite,[22,23] has been identified as a potential therapeutic target, and reports on the development of inhibitors of this enzyme have appeared.[20,23,24] Similarly, the major cathepsin B-like protease of *L. major* is also of interest as a therapeutic target.[25] Both falcipain and the leishmania protease are members of the papain family, and are highly homologous to cruzain.

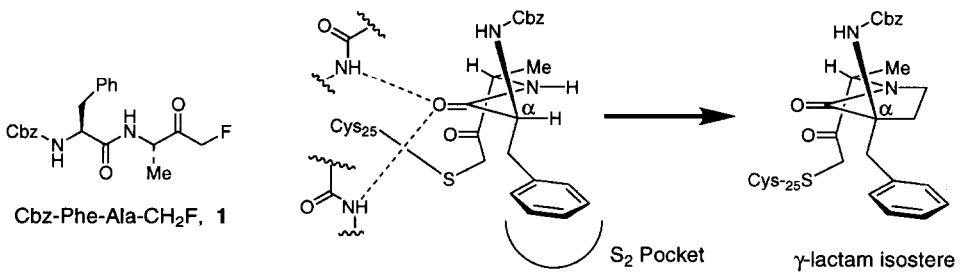
Cysteine protease inhibitors have been reviewed.[26] Peptide aldehydes,[27] diamino ketones,[28] and peptide nitriles[29] are examples of irreversible covalent inhibitors that form hemithioacetals, thioketals, and thioimidates, respectively, with the thiol of the active site cysteine residue. These mimic the initial covalent adduct in normal proteolytic turnover.[29] Epoxysuccinyl derivatives,[30] peptide Michael acceptors,[31–34] (acyloxy)-methyl ketones,[35] and halomethyl ketones[12] are examples of irreversible inhibitors, which form a hydrolytically stable covalent bond with the thiol of the active site cysteine.

Kenyon and co-workers have recently reported a rationally designed noncovalent inhibitor of cruzain with an IC_{50} value of 600 nM,[36] while the SmithKline group has reported a series of nonpeptidic bis hydrazides that are potent reversible inhibitors of cathepsin K.[37] Several other strategies for inhibition of cruzain have been reported.[8,38]

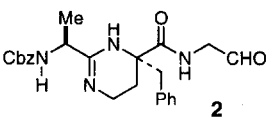
Several X-ray crystal structures of cruzain with several covalently bound inhibitors have been reported.[13,39] We were interested in using these structures to design conformationally constrained inhibitors. Peptides and unconstrained peptide inhibitors generally exist as a mixture of conformers in solution. Limiting the number of conformations of a molecule can improve binding energies by lowering the entropic contribution to the binding of particular conformation.[40,41]

Examination of the X-ray structure of cruzain containing the covalently bound fluoromethyl ketone Cbz-Phe-Ala-FMK (**1**) reveals that Phe-α-H and Ala N-H are eclipsed.[13,39] We speculated that fixing the conformation of as a γ-lactam would rigidify the bioactive conformation of **1**, and also provide a template for the development of selective cruzain inhibitors. The γ-lactam, or pyrrolidinone, isostere has proven to be an effective conformational constraint for inhibitors of other classes of proteases,[42–47] although in at least one instance it has been reported that use of this isostere resulted in a loss in potency when compared to the corresponding unconstrained compounds.[48] Using different design criteria deriving from X-ray structural data,[49] Jones has synthesized the conformationally constrained peptide aldehyde **2** (IC_{50} = 790 nM) as an inhibitor of papain.[50]

We report herein the synthesis and evaluation of a series of conformationally constrained peptide aldehydes and vinyl sulfones targeted towards cruzain.



Conformation of **1** in cruzain active site



2

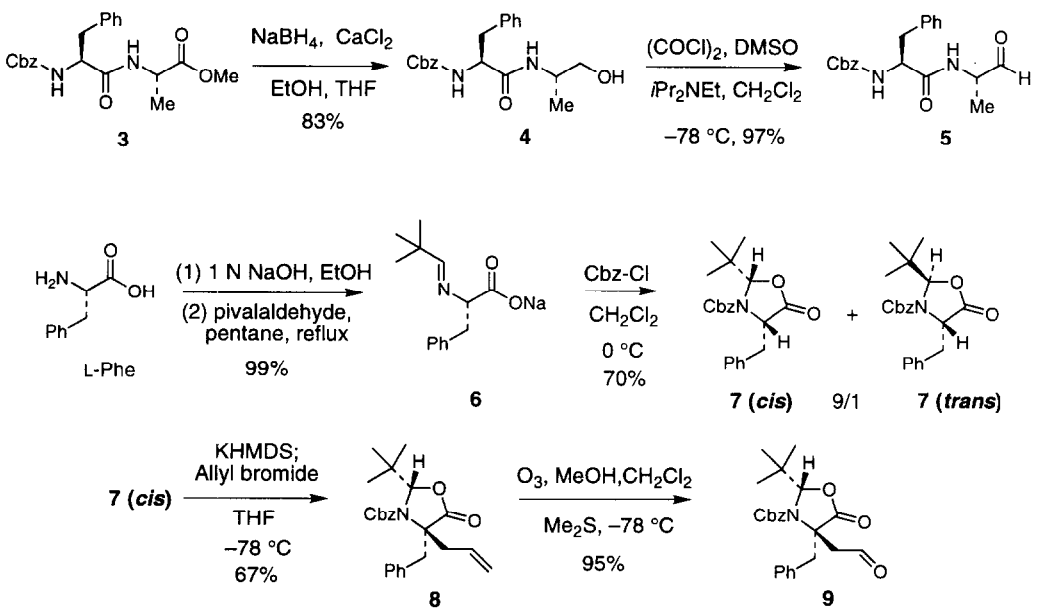
Results and discussion

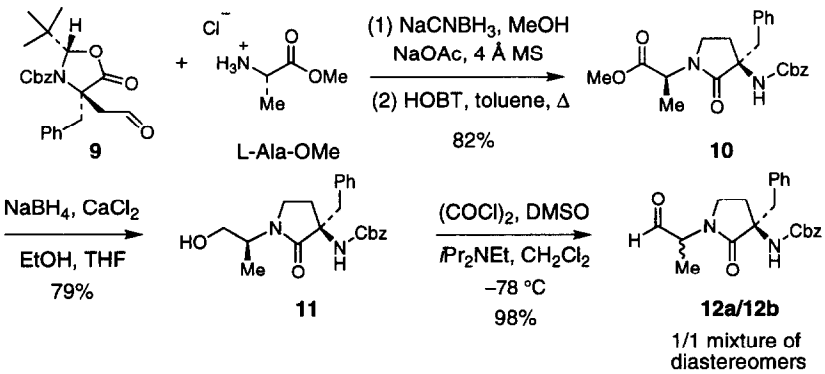
Conformationally constrained peptide aldehyde inhibitors. Peptide aldehydes are potent in vitro inhibitors of cysteine proteases. For example, *N*-Ac-Phe-Gly-H has a K_d of 40 nM with papain.[27,29,49] Although aldehydes can be unstable in vivo because of oxidation to the corresponding carboxylic acid and the potential for epimerization at the α-carbon, we elected to use the electrophilic aldehyde strategy in the present work in order to probe the activity of conformationally constrained pyrrolidinone inhibitors.

We began our studies by minimizing Cbz-phenylalanyl-alanal **5**, the aldehyde analogue of **1**, in the active site of cruzain. The computer-minimized[51] structure of aldehyde **5** bound in the active site of cruzain was superimposed with the crystal structure of **1** and cruzain. Not surprisingly, there was little deviation of the aldehyde from the original inhibitor backbone.[52] Aldehyde **5** was prepared starting from the known dipeptide **3**.[53] Reduction of **3** with $Ca(BH_4)_2$ in ethanol and THF afforded **4**,[54] which was then oxidized using Swern conditions[55,56] to afford aldehyde **5** in 81% yield for the two steps.

The targeted pyrrolidinone-containing aldehyde inhibitors were synthesized by employing the strategy introduced by Dellaria.[47] The key aldehyde intermediate **9**, which was used in the syntheses of all of the conformationally constrained inhibitors reported in this manuscript, was prepared from L-phenylalanine using chemistry introduced by Seebach.[57,58] Accordingly, L-phenylalanine was converted to the sodium salt and then condensed with pivalaldehyde in refluxing pentane using a Dean–Stark apparatus to afford **6** in 99% yield. Imine **6** was then treated with benzyl chloroformate in CH_2Cl_2 over 4 days to generate a 9/1 mixture of oxazolidinones **7** (*cis*) and **7** (*trans*) (70% yield), which were separated chromatographically. 1H nuclear Overhauser enhancement experiments (NOE)[59] verified the stereochemistry of **7** (*cis*). Oxazolidinone **7** (*cis*) was treated with potassium hexamethyldisilazide (KHMDS) in THF at −78 °C followed by allyl bromide to afford **8** with >95/5 diastereoselectivity in 67% yield. The stereochemistry of **8** was also verified by NOE experiments.[60] Aldehyde **9** was then obtained in 95% yield by ozonolysis of **8**.

Reductive amination of **9** with the HCl salt of L-alanine methyl ester and sodium cyanoborohydride[61] in MeOH afforded the corresponding amine, which upon heating in toluene in the presence of 1-hydroxybenzotriazole (HOBt), afforded pyrrolidinone **10** in 82% yield.[47] Reduction of lactam **10** with $Ca(BH_4)_2$ and subsequent Swern oxidation of alcohol **11** produced pyrrolidinone aldehydes **12a**/**12b** in 77% yield as a mixture of diastereomers at the alanine α-carbon. ^1H NMR analysis of the crude aldehyde obtained following the Swern oxidation usually displayed a 95/5 mixture of diastereomers. However, attempts to purify **12** resulted in epimerization at the α-carbon. Because the **12a**/**12b** mixture could not be separated, this mixture was used as such in the enzyme assays. Interestingly, other aldehydes (such as **17**) did not epimerize when they were purified using flash silica gel chromatography.

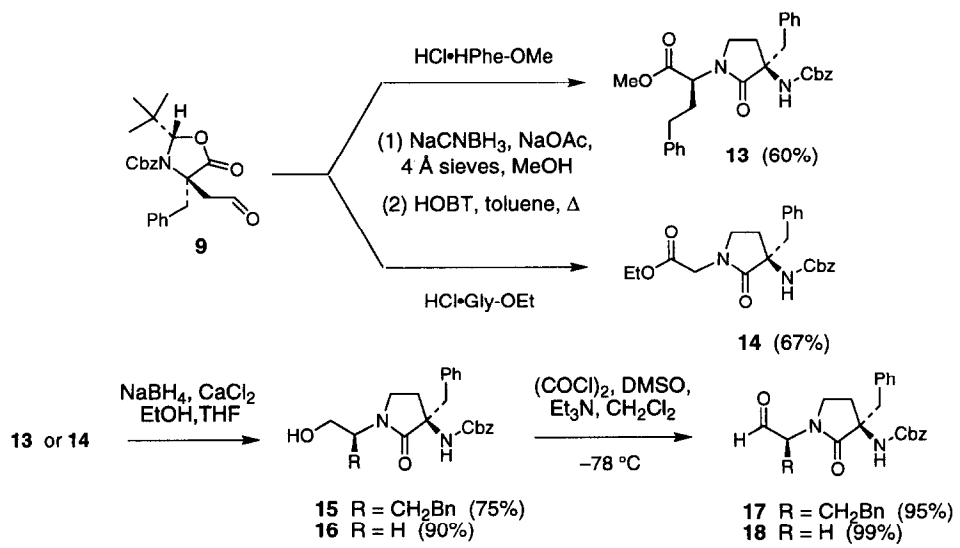


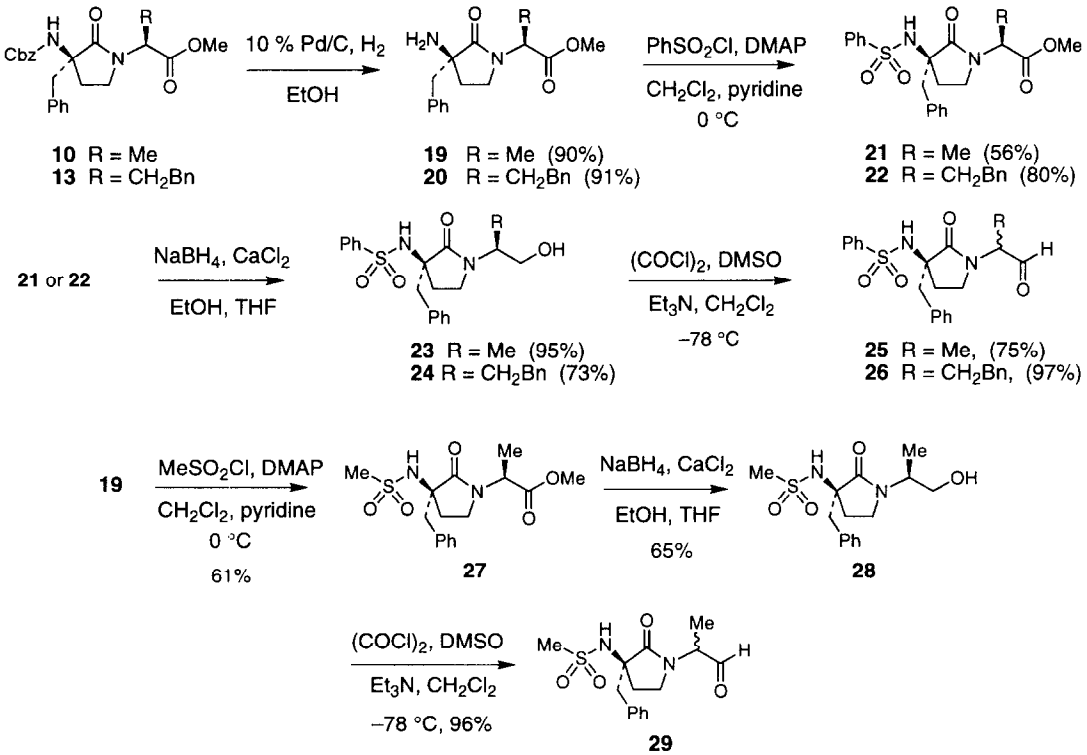


Additional pyrrolidinone aldehydes were synthesized in an analogous manner. The homophenylalanine derivative **17** was prepared to increase the hydrophobic interactions with the S_1 binding site, and the glycine analogue **18** to determine if a substituent at this position was necessary for activity.[33]

Several constrained pyrrolidinone inhibitors were synthesized by replacing the benzyloxycarbonyl group (Cbz) in order to acquire additional binding interactions with the cruzain S_3 site. Although the cruzain·**1** crystal structure displays few van der Waals contacts between cruzain and the Cbz group of **1**,[13,62] there is a hydrogen bond between the Phe N-H and the carbonyl of residue Gly66. Perrin has suggested that a hydrogen bond is strongest when the hydrogen atom involved in bonding is equally shared between the donor and the acceptor.[63] Matching the acidity of the hydrogen bond donor with the basicity of the acceptor is a requirement for a strong interaction. While there is not a direct correlation between pK_a values of donors and acceptors and binding energies, Drueckhammer has shown that unusually strong binding occurs between a ligand and a receptor when a hydrogen bond is modified by altering the pK_a of the hydrogen bond donor.[64] On this basis, we hoped we could enhance binding and ultimately activity of the pyrrolidinone aldehydes by modifying the pK_a of the carbamate N-H, which is approximately 15–17.[65] We chose to synthesize sulfonamides because this functional group is more acidic (N-H pK_a value approximately 12) than carbamates.[65,66]

The Cbz groups of **10** and **13** were removed by hydrogenolysis to afford amines **19** and **20**. Solutions of these amines in CH_2Cl_2 and pyridine were treated with benzenesulfonyl chloride or methanesulfonyl chloride and 4-dimethylaminopyridine (DMAP). The phenylsulfonamide esters **21** and **22** were then reduced with $Ca(BH_4)_2$ and the primary alcohols oxidized using Swern conditions[55,56] to afford aldehydes **25** and **26** in 70–71% yield for the two steps. In the same manner, methanesulfonamide **29** was obtained in 62% yield from ester **27**.



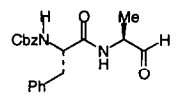
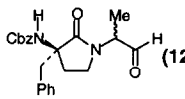
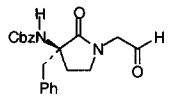
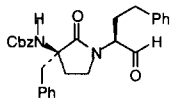


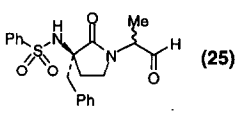
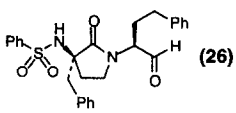
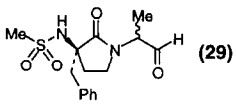
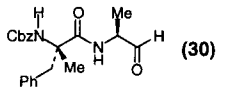
Results of enzyme assays are summarized in Table 1. It should be noted that all of the peptide aldehydes were treated as tight binding reversible inhibitors, and that the best of the constrained inhibitors were screened against papain, cathepsin B (a lysosomal cysteine protease necessary for normal protein processing)[67,68] and the leishmania cysteine protease. The parent peptide aldehyde, Cbz-Phe-Ala-H (**5**) had an IC_{50} value against cruzain of approximately 10 nM. This result correlated well with data for other peptide aldehydes against papain.[27] However, all the pyrrolidinone aldehydes showed substantially reduced activity against cruzain. The best cruzain inhibitor of this set of inhibitors was **17**, containing a homoPhe residue at P_1, which displayed an IC_{50} of $0.60 > \mu M$. As shown by these data, introduction of the P_3 sulfonamide did not lead to improved activity.

Although aldehydes **12a/b**, **17**, **25**, **26**, and **29** showed reduced activity against cruzain compared to **5**, several of these compounds exhibited notable activity against the *L. major* cysteine protease. In particular, the alanine analogue **12** (a 1/1 mixture of diastereomers) gave an IC_{50} of 100 nM versus the *L. major* cysteine protease. The homophenylalanine analogue **17** had an IC_{50} of ca. 30 nM versus the leishmania protease, the *N*-phenyl sulfonamide **26** was a ca. 20 nM inhibitor of this enzyme. Interestingly, the latter compound also exhibited excellent potency (50 nM) versus falcipain. These observations will be further developed in future studies of falcipain and the leishmania protease.

The data obtained for **12**, **17**, **18**, **25**, **26**, and **29** clearly indicate that the pyrrolidinone unit diminishes the ability of these inhibitors to interact with the cruzain active site. One potential reason for the decreased inhibitory activity is that the replacement of the P_1 N-H proton by the ethylene bridge disrupts a favorable hydrogen bonding system that facilitates binding of the natural peptide substrates (although such hydrogen bonds are not apparent in the cruzain•**1** crystal structure) (Table 1).[13] A second possibility is that the introduction of the ethylene bridge introduces interactions with a residue in the active site. While we do not have data to address the first possibility conclusively, it is clear from studies of (*S*)-Cbz-α-(methyl)phenylalanyl-alanal, **30** (which is virtually inactive against cruzain) that a quarternary center in the P_2 residue leads to substantial loss in potency. Results of a molecular modeling study suggest that Asp 158 and the β′ methylene of the pyrrolidinone unit approach within 2.5 Å in certain conformations, suggesting that this may be the detrimental interaction that causes the pyrrolidinone inhibitors to have substantially reduced potency versus cruzain. However, because X-ray structures of falcipain and the leishmania cathepsin B-like protease are not yet

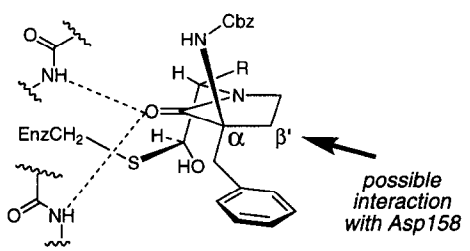
Table 1. Inhibition of cysteine proteases by pyrrolidinone aldehydes (IC_{50})

	Enzyme	IC_{50} (μM)
 (5)	cruzain[a]	0.010
	cathepsin B[b]	0.05
 (12a/b)	cruzain	10
	cathepsin B	>10
	leishmania cpB[c]	0.1
 (18)	cruzain	10
	cathepsin B	10
	leishmania cpB	0.6
 (17)	cruzain	0.6
	cathepsin B	1.0
	leishmania cpB	0.03

	Enzyme	IC_{50} (μM)
 (25)	cruzain	>10
	cathepsin B[b]	1
 (26)	cruzain	2
	cathepsin B	1
	leishmania cpB	0.02
	falcipain[d]	0.05
 (29)	cruzain	>10
 (30)	cruzain	10

[a]Cruzain: purified recombinant protein lacking the C terminal domain.[69]
[b]Cathepsin B.[68,70] bovine spleen, EC 3.4.22.1, Sigma.
[c]Leishmania cpB: the major cathepsin B-like cysteine protease from *L. major*.[25]
[d]Falcipain: the major cysteine protease from *P. falciparum* trophozoites.[20,23]

available,[23,25] it is not entirely clear why this structural modification is not detrimental towards these enzymes.

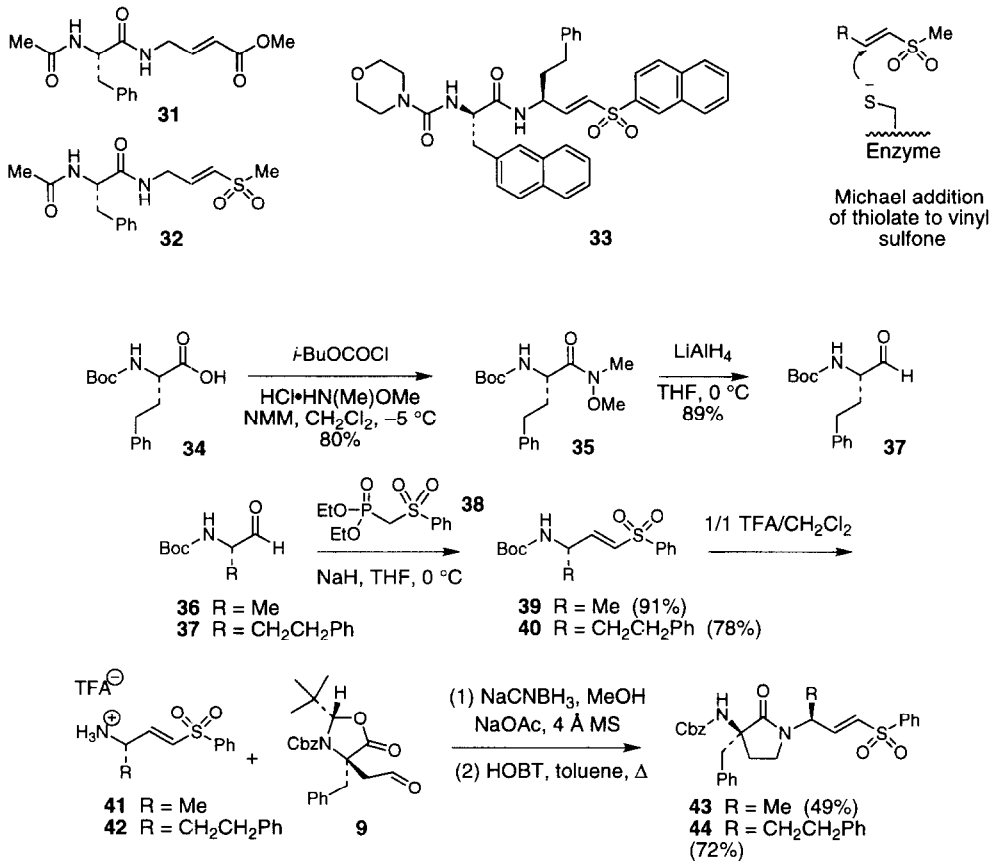


Conformationally constrained peptide vinyl sulfone inhibitors. In 1984, Hanzlik reported that vinylogous amino acid esters are good inhibitors of papain.[31] More recently, Hanzlik reported a series of Michael acceptors that are specific, irreversible inhibitors of papain.[32] Vinyl sulfone **32** was second only to α,β-unsaturated ester **31** in its ability to inhibit papain. In 1995–96, a group at Khepri Pharmaceuticals published an extensive study of vinyl sulfones as potent cysteine protease inhibitors.[33,34] During this work, naphthyl vinyl sulfone **33** was shown to inhibit cruzain with a second order rate constant (k_{inact}/K_i) of $1.04\times10^6\,s^{-1}\,M^{-1}$.

We hoped that the combination of an electrophilic vinyl sulfone and a P_1/P_2 pyrrolidinone unit would lead to a family of irreversible inhibitors that would be more potent than the pyrrolidinone aldehydes. Accordingly, we targeted the synthesis of the conformationally restricted vinyl sulfones **43**, **44**, and **46**. We elected to use phenyl vinyl sulfones in these studies because work at UCSF has revealed that the naphthyl vinyl sulfones (e.g. **33**) are toxic to mammalian cells.[71]

The pyrrolidinone-containing vinyl sulfones **43** and **44** were synthesized by reductive amination of aldehyde **9** with ammonium salts **41** and **42**, which were synthesized from L-alanine and L-homophenylalanine by using the general method described by Palmer.[33] The key step of the synthesis of **41** and **42** involved the Horner–Wadsworth–Emmons reaction of Boc-protected amino aldehydes **36**[72] and **37** with phosphonate **38**,[73] which provided the vinyl sulfones **39** and **40** in good yield. The Boc groups were removed by treatment with 1/1 TFA/CH_2Cl_2 and the crude salts **41** and **42** were subjected to the standard reductive amination protocol with **9** to give the targeted pyrrolidinone vinyl sulfones **43** and **44**. *N*-Boc-homophenylalanal **37** required for this work was prepared by conversion of Boc-homophenylalanine to the Weinreb amide[74] **35** followed by reduction with $LiAlH_4$.[72]

Vinyl sulfone **46**, containing the *N*-phenylsulfonyl blocking group, was synthesized starting from **44**. Removal of the Cbz group was accomplished by addition

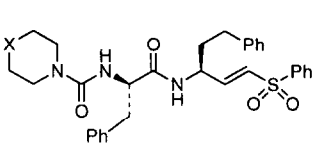


of TMS-I to a solution of **44** in a 2/3 mixture of CH_3CN and CH_2Cl_2.[75] Treatment of the resulting amine **45** with phenylsulfonyl chloride afforded vinyl sulfone **46** in 56% yield for the two steps.

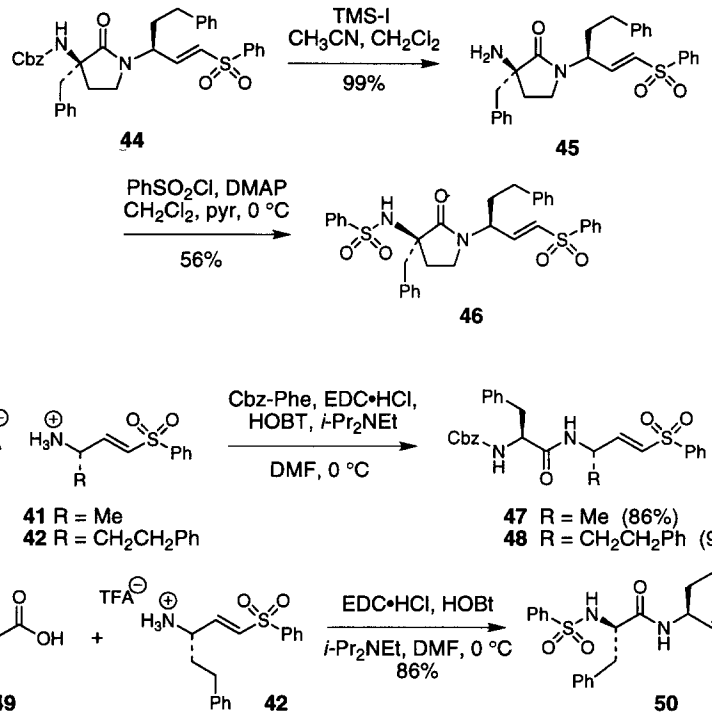
For comparative purposes we also synthesized vinyl sulfones **47**, **48**, **50**, and **51**. Vinyl sulfones **47** and **48** were prepared by standard peptide coupling of salts **41** and **42** with Z-Phe-OH.[76] Similarly, **50** was prepared by coupling **47** and the known *N*-phenylalanyl benzenesulfonamide **49**.[77] Finally, vinyl sulfone **51**, containing an α-Me phenylalanine residue, was synthesized **47** and L-α-Me Phe using EDC and 1-hydroxyazabenzotriazole (HOAt) as the coupling agent.[78]

A preliminary assessment of the relative potency of the vinyl sulfone inhibitors was achieved by rough IC_{50} determinations (see Table 2). It was readily apparent from these experiments that the conformationally constrained vinyl sulfones **43**, **44**, and **46** are significantly less active as inhibitors of cruzain when compared to the unconstrained analogues **47**, **48**, and **50**. Vinyl sulfone **48** displayed the best activity against cruzain of all compounds tested from this group, and exhibited an IC_{50} of less than 10 nM according to this analysis (see Experimental section). The significantly reduced activity of the conformationally constrained inhibitors **43**, **44**, and **46** and the α-Me phenylalanyl vinyl sulfone **51** is consistent with the decreased activity of the pyrrolidinone aldehydes **12**, **17**, **18**, **25**, **26**, and **29** described in the first part of this manuscript, and again points to the fact that the added ethylene unit of the pyrrolidinone ring impose steric problems that prevents optimal binding of these inhibitors in the cruzain active site.

More precise assessment of the potency of these inhibitors was obtained by a full kinetic analysis, which reveals that vinyl sulfones **47**, **48**, and **50** are time dependent inhibitors of cruzain and other cysteine proteases (Table 3). Compound **48** is an excellent inhibitor of cruzain with an apparent second order inhibition rate constant (k_{inact}/K_i) of 634,000 $s^{-1}M^{-1}$.[79] That **48** is an excellent cruzain inhibitor is not surprising, since the related inhibitors **52** and **53** have proven to be



52, X = O
53, X = NMe



excellent inhibitors of *T. cruzi* in in vivo experiments.[15] However, comparison of the kinetic data for **48** and **50** indicates that the N-sulfonyl blocking group does not lead to enhanced activity, as we had anticipated by the discussion presented earlier. It is also interesting to note that vinyl sulfones **47** and **48** are much more potent inhibitors of cruzain than of bovine cathepsin B (Tables 2 and 3).

However, attempted kinetic analysis the conformationally constrained inhibitors **44** and **46** indicated that these compounds are not time dependent inhibitors of cruzain. Evidently, the pyrrolidinone unit causes these inhibitors to bind in such a way that the active site cysteine residue cannot interact optimally with the vinyl sulfone moiety. Inhibitors **44** and **46** thus appear to be weak, reversible inhibitors of this enzyme.

Conclusion

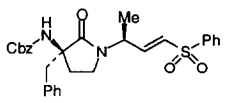
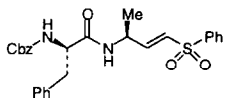
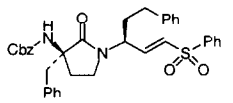
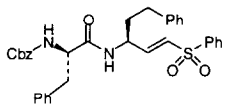
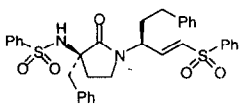
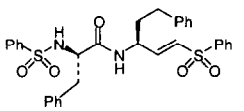
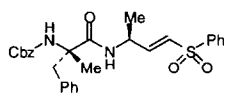
Two different series of conformationally constrained cysteine protease inhibitors have been developed: pyrrolidinone containing peptide aldehydes (**12**, **17**, **18**, **25**, **26**, and **29**) and vinyl sulfones (**43**, **44**, and **46**). Unfortunately, activity against cruzain was substantially reduced for all of these inhibitors, compared to unconstrained peptide aldehyde (e.g. **5**) and vinyl sulfone inhibitors (e.g. **48** and **50**). A significant reduction in activity is also observed with acyclic inhibitors **30** and **51** containing α-methyl phenylalanine residues at the P_2 position. These data indicate that the pyrrolidinone ring—especially the quarternary center at P_2—interferes with the normal substrate binding mode of cruzain. However, the conformationally constrained inhibitors **12** and especially **17** and **26** were very effective inhibitors of the *Leishmania major* cathepsin B-like cysteine protease; compound **26** displayed an IC_{50} of 20 nM versus the *L. major* protease, and a ca. 50 nM IC_{50} versus falcipain, deriving from the *P. falciparum* trophozoite. Inhibitors containing homophenylalanine residues at P_1 were consistently more potent than the corresponding alanine derivatives (compare, for example, **47** and **48**). Unconstrained vinyl sulfones **48** and especially **50** are excellent inhibitors of cruzain, and are selective for cruzain with respect to mammalian cathepsin B.

Studies to develop more potent and selective inhibitors of cruzain and the cysteine proteases from *Plasmodium falciparum* and *Leishmania major*, are in progress and will be reported in due course.

Experimental

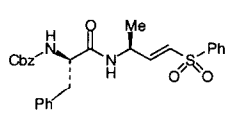
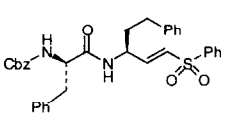
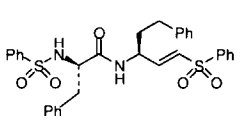
General. All reactions were conducted in flame-dried or oven-dried glassware under an atmosphere of dry nitrogen or argon. All solvents except dimethyl formamide and absolute ethanol were purified before use. Diethyl

Table 2. Inhibition of cruzain by vinyl sulfones (IC_{50} values)

	IC_{50} (μM)		IC_{50} (μM)
 (43)	> 10	 (47)	0.03
 (44)	2.0	 (48)	0.001
 (46)	3.0	 (50)	0.02
		 (51)	> 10

ether and tetrahydrofuran were distilled from sodium benzophenone ketyl; triethylamine, diisopropylethylamine and pyridine were distilled from CaH_2; toluene and dichloromethane were distilled from CaH_2; and methanol was distilled from magnesium turnings or CaH_2. Reactions were dried over anhydrous Na_2SO_4 unless otherwise noted. ^1H NMR spectra were measured at 400 MHz on a Varian VXR-400 instrument or at 500 MHz on a Varian Inova 500 instrument. Chemical shifts are reported in δ units to 0.01 ppm precision with coupling constants reported to 0.1 Hz precision. Residual chloroform (δ 7.26 ppm) and methanol (δ 4.87 ppm) were used as internal references for spectra measured in these solvents. ^{13}C NMR spectra were measured at 100 MHz; residual chloroform (δ 77.0 ppm) was used as internal reference for spectra measured in this solvent. High-resolution mass spectra were measured at 70 eV on a Kratos GC/MS 80 RFA mass spectrometer at the Indiana University Mass Spectrometry Laboratory. Optical rotations were measured on a Rudolph Autopol III polarimeter using a quartz cell with 1 mL capacity and a 10 cm path length. Elemental analyses were performed by Robertson Microlit Laboratories of Madison, NJ. Analytical thin-layer chromatography (TLC) was performed using plates coated with a 0.25 mm thickness of silica gel containing PF254 indicator (Analtech), and compounds were visualized with UV light, iodine, *p*-anisaldehyde stain, ceric ammonium molybdate stain, or phosphomolybdic acid in EtOH. Flash chromatography was performed as described by Still80 using Kieselgel 60 (230–400 mesh).

Table 3. Second order rate constants for inhibition of cruzain and other cysteine proteases with vinyl sulfones **47**, **48**, and **50**

	Enzyme	Second order inactivation rates ($s^{-1}M^{-1}$)
 (47)	cruzain	93,800
	papain	14,600
	cathepsin B	500
	leishmania	4700
 (48)	cruzain	634,000 ± 133,000
	papain	7000 ± 1100
	cathepsin B	< 2000
 (50)	cruzain	125,000

Recombinant cruzain lacking the C terminal domain was purified to a single species by SDS-PAGE chromatography according to the method reported by Eakin, et al.[69] The absence of the C-terminal domain does not effect the catalytic properties of this enzyme.[81] Commercial samples of papain (EC 3.4.22.2, Sigma) and cathepsin B (bovine spleen, EC 3.4.22.1, Sigma) were purified to a single species by SDS-PAGE, as was the leishmania B protease isolated from *L. major*.[25] A single amino terminal sequence was confirmed for the latter enzyme.

(2*S*,4*S*)-4-Benzyl-2-*tert*-butyl-5-oxo-oxazolidine-3-carboxylic acid benzyl ester (7(*cis*)). To a 23 °C solution of 1 N aq NaOH (70 mL) was added L-phenylalanine (11.5 g, 69.8 mmol). The mixture was stirred for 1 h after

the reaction became homogeneous and was then concentrated in vacuo. The resulting solid was dried overnight under high vacuum. To this solid was added pentane (100 mL) and pivalaldehyde (12 mL, 110 mmol) and the mixture was heated at reflux overnight with a Dean–Stark apparatus. A total of approximately 5 mL of water was collected. The solvents were removed in vacuo and the crude material was co-evaporated with benzene twice to yield imine **6**. To a 0 °C mixture of **6** in CH_2Cl_2 (150 mL) was added benzyl chloroformate (11.5 mL, 78.5 mmol). The mixture was allowed to slowly warm to 25 °C and then stirred for 4 days. The mixture was then partitioned between CH_2Cl_2 (100 mL) and brine (100 mL). The layers were separated and the aq layer extracted with CH_2Cl_2 (4×50 mL). The combined organic extracts were washed with saturated aq $NaHCO_3$ (3×100 mL) and brine, then dried, filtered, and concentrated in vacuo. Purification of the crude product by flash column chromatography using a 15% EtOAc/hexanes afforded **7** (17.6 g, 69% yield) as a 9/1 mixture of diastereomers. This mixture was separated by careful flash chromatography on 600 g of silica gel using a 5–15% EtOAc/hexanes gradient, giving 11.3 g (44%) of diastereomerically pure **7** (*cis*): $[\alpha]_D^{26}$ −11.3° (*c* 1.7, CH_2Cl_2); IR (neat) 3100, 3020, 2980, 2880, 1790, 1720, 1600, 1590, 1450, 1400, 1300, 1230, 1200, 1180, 890, 780, 750, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40–7.20 (m, 10H), 5.56 (s, 1H), 5.14 and 4.92 (AB system, J_{AB} = 11.7 Hz, 2H) 3.22 (dd, *J* = 7.6, 13.8 Hz, 1H), 3.11 (dd, *J* = 5.4, 13.8 Hz, 1H), 4.48 (dd, *J* = 5.7, 7.6 Hz, 1H), 1.00 (s, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 155.8, 136.8, 135.1, 129.4, 128.6, 128.57, 128.53, 128.4, 126.9, 96.2, 68.3, 58.9, 39.3, 37.1, 24.9; HRMS calcd for $C_{22}H_{25}NO_4$ $(M+1)^+$ 368.1861, found 368.1877 *m/z*. Anal. calcd for $C_{22}H_{25}NO_4$: C, 71.91; H, 6.86; N, 3.81. Found: C, 71.67; H, 6.81; N, 3.73.

(2*S*,4*S*)-Allyl-4-benzyl-2-*tert*-butyl-5-oxo-oxazolidine-3-carboxylic acid benzyl ester (8). To a −78 °C solution of **7** (*cis*) (17.8 g, 48.4 mmol) in THF (200 mL) was slowly added potassium hexamethyldisilazide (0.5 M in toluene, 106 mL, 53 mmol). The solution was stirred for 30 min at −78 °C, then allyl bromide (4.6 mL, 53 mmol) was added dropwise via syringe. The solution was allowed to warm to 25 °C and stirred for 16 h. Saturated aq ammonium chloride (100 mL) was added to quench the reaction. The biphasic mixture was partitioned between EtOAc (100 mL) and brine (100 mL). The layers were separated and the aq layer extracted with EtOAc (2×50 mL). The combined organic extracts were washed with brine, then dried, filtered, and concentrated in vacuo to yield 18.9 g of a crude yellow oil. Purification of the crude product by flash column chromatography using 2.5–8% EtOAc/hexanes afforded **8** (15.1 g, 77% yield) as a > 95/5 mixture of diastereomers: $[\alpha]_D^{26}$ −34.1° (*c* 1.5, CH_2Cl_2); IR (neat) 3100, 3060, 2970, 1790, 1715, 1500, 1490, 1400, 1320, 1215, 1200, 1050, 1000, 930, 800 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 (m, 5H), 7.21 (m, 5H), 5.50 (m, 1H), 5.36 (br s, 1H), 5.20 (m, 3H), 4.96 (m, 1H), 3.30 (br s, 2H), 3.00 (br s, 1H), 2.43 (dd, *J* = 13.8, 6.3 Hz, 1H), 0.56 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 135.5, 135.0, 131.0, 130.0, 129.0, 128.7, 128.6, 128.2, 127.2, 121.7, 95.2, 68.6, 67.9, 42.1, 37.6, 29.6, 28.9, 25.0; HRMS calcd for $C_{25}H_{29}NO_4$ $(M+1)^+$ 408.2175, found 408.2168 *m/z*. Anal. calcd for $C_{25}H_{29}NO_4$: C, 73.69; H, 7.17; N, 3.44. Found: C, 73.67; H, 7.12; N, 3.26.

(2*S*,4*R*)-4-Benzyl-2-*tert*-butyl-5-oxo-4-(2-oxo-ethyl)-oxazolidine-3-carboxylic acid (9). Ozone was bubbled through a −78 °C suspension of **8** (1.58 g, 3.9 mmol) and solid $NaHCO_3$ (340 mg, 4.0 mmol) in 50 mL of CH_2Cl_2/MeOH (4/1) until the solution turned a light-blue color. The suspension was flushed with oxygen until the vented gas was negative by KI/starch paper. To the suspension was added $Me2_S$ (3 mL) and the reaction mixture was allowed to warm to 25 °C then stirred overnight. The suspension was filtered and then concentrated in vacuo to yield 2.09 g of a crude light-yellow oil. Purification of the crude product by flash column chromatography using 17% EtOAc/hexanes afforded **9** (1.48 g, 95% yield) as a clear oil. $[\alpha]_D^{26}$ −20.5° (*c* 2.48, CH_2Cl_2); IR (neat) 3030, 2960, 2870, 2740, 1780, 1725, 1400, 1320, 1190, 1040 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.23 (s, 1H), 7.28–7.25 (m, 10H), 5.72 (s, 1H), 5.10 (d, *J* = 11.6 Hz, 2H), 4.85 (br s, 1H), 3.23 and 3.12 (AB system, J_{AB} = 13.8 Hz, 2H) 2.80 (d, *J* = 18.9 Hz, 1H), 0.93 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.9, 173.1, 155.0, 135.0, 134.5, 128.8, 128.7, 128.6 (2×), 128.3, 127.5, 96.2, 67.7, 62.7, 43.4, 37.9, 37.8, 25.8; HRMS calcd for $C_{24}H_{27}NO_5$ $(M+1)^+$ 410.1967, found 410.1979 *m/z*. Anal. calcd for $C_{24}H_{27}NO_5$: C, 70.40; H, 6.65; N, 3.42. Found: C, 70.34; H, 6.77; N, 3.37.

(2*S*,3*S*)-2-(3-Benzyl-3-benzyloxycarbonylamino-2-oxo-pyrrolidin-1-yl)-propionic acid methyl ester (10). Compound **9** (725 mg, 1.80 mmol) was cyclized with the hydrochloride salt of alanine methyl ester (467 mg, 3.30 mmol) using the conditions described for the preparation of **13** to afford **10** (610 mg, 82% yield) as a clear oil: $[\alpha]_D^{26}$ −10.6° (*c* 0.8, CH_2Cl_2); IR (neat) 3400–3280 (broad), 3075, 3040, 3000, 2960, 1750, 1735, 1700, 1500, 1455, 1435, 1300–1200 (broad), 1070, 1030, 920, 740, 710; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35–7.23 (m, 10H), 5.39 (br s, 1H), 5.10 and 5.04 (AB system, J_{AB} = 12.3 Hz, 2H), 4.88 (q, *J* = 7.2 Hz, 1H), 3.70 (s, 3H), 3.35, (t, *J* = 8.9 Hz, 1H), 3.12 (m, 1H) 3.10 and 3.06 (AB system, J_{AB} = 13.2 Hz, 2H), 2.58 (m, 1H), 2.41 (m, 1H), 1.43 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 171.3, 154.9, 136.2, 134.8, 130.3, 128.4, 128.2, 127.0, 66.5, 61.3, 52.5, 49.6, 40.4, 40.1, 31.1, 14.4; HRMS calcd for $C_{23}H_{26}N_2O_5$ $(M+1)^+$ 411.1920, found

411.1918 *m/z*. Anal. calcd for $C_{23}H_{26}N_2O_5$: C, 67.30; H, 6.38; N, 6.82. Found: C, 67.15; H, 6.21; N, 6.75.

(2*S*,3*S*)-[3-Benzyl-1-(2-hydroxy-1-methyl-ethyl)-2-oxo-pyrrolidin-3-yl]-carbamic acid benzyl ester (11). Compound **10** (150 mg, 0.37 mmol) was reduced using the procedure described for the preparation of **15** to afford **11** (113 mg, 79% yield) as a white foam: $[\alpha]_D^{26}$ +20.8° (*c* 1.5, CH_2Cl_2); IR (CH_2Cl_2) 3400, 3040, 3020, 2980, 2970, 1725, 1695, 1500, 1450, 1300, 1220, 1210, 750, 710; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.22 (m, 10H), 5.51 (br s, 1H), 5.11 and 5.04 (AB system, J_{AB} = 12.3 Hz, 2H), 3.70, (m, 1H), 3.62 (m, 1H), 3.51 (m, 1H) 3.14 (m, 1H), 3.09 and 3.00 (AB system, J_{AB} = 12.9 Hz, 2H), 2.68 (br s, 1H), 2.50 (m, 3H), 1.08 (d, J = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 155.0, 136.2, 134.7, 130.2, 128.5, 128.3, 128.1, 128.0, 127.3, 66.6, 63.9, 62.3, 52.3, 42.8, 42.0, 30.5, 13.4; HRMS calcd for $C_{22}H_{26}N_2O_4$ $(M+1)^+$ 383.1970, found 383.1961 *m/z*. Anal. calcd for $C_{22}H_{26}N_2O_4$: C, 69.09; H, 6.85; N, 7.32. Found: C, 69.34; H, 6.45; N, 7.15.

(3*S*)-[3-Benzyl-1-(1-methyl-2-oxo-ethyl)-2-oxo-pyrrolidin-3-yl]-carbamic acid benzyl ester (12a/12b). Compound **11** (65 mg, 0.17 mmol) was oxidized using the procedure described for the preparation of **17** to afford a 1/1 mixture of diastereomeric aldehydes **12a/12b** (63 mg, 98% yield) as a clear oil: IR (neat) 3400, 3090, 3000, 2980, 2920, 2790, 1750–1700 (broad), 1490, 1420, 1360, 1220, 1100, 900, 700; ^{1}H NMR before epimerization by chromatography (400 MHz, $CDCl_3$) δ 9.22 (s, 1H), 7.37–7.21 (m 10H), 5.42 (br s, 1H) 5.12 and 5.05 (AB system, J_{AB} = 12.1 Hz, 2H), 3.20 (m, 1H), 3.13 (s, 2H), 2.65 (m, 1H), 2.50 (m 1H), 1.28 (d, J = 6.6 Hz, 3H); ^{13}C NMR of diastereomers (100 MHz, $CDCl_3$) δ 198.9, 174.6, 154.9, 136.2, 134.6, 130.3, 130.1, 128.5 (2x), 128.1, 127.4, 66.7, 61.5 (2×), 56.7, 42.6, 42.1, 30.5, 29.7, 10.8, 10.4; HRMS calcd for $C_{22}H_{24}N_2O_4$ $(M+1)^+$ 381.1814 found, 381.1810 *m/z*.

(2*S*,3*S*)-(3-Benzyl-benzyloxycarbonylamino-2-oxo-pyrrolidin-1-yl)-4-phenyl-butyric acid methyl ester (13). To suspension of **9** (953 mg, 2.33 mmol), NaOAc (400 mg, 4.88 mmol) and 4 Å molecular sieves (2.60 g) in MeOH (50 mL) was added the hydrochloride salt of homophenylalanine methyl ester (615 mg, 2.68 mmol). After 15 min, $NaCNBH_3$ (305 mg, 4.85 mmol) was added and the reaction mixture was stirred at 25 °C overnight. The suspension was then filtered and the filtrate was acidified to pH 1 with 1 N aq HCl and stirred for 20 min. The solution was then basified to pH 10 with saturated aq $NaHCO_3$ and then extracted with EtOAc (4×50 mL). The combined organic extracts were washed with brine, then dried, filtered, and concentrated in vacuo to yield a yellow oil. This oil was dissolved in toluene (30 mL) to which HOBt (310 mg, 2.30 mmol) was added. The mixture was then heated at reflux for 16 h. The mixture was cooled and the toluene was removed in vacuo. The residue was taken up in EtOAc (75 mL) and washed with saturated aq $NaHCO_3$ (3×50 mL) and brine, then dried, filtered, and concentrated to dryness in vacuo to yield a crude yellow oil. Purification of the crude product by flash column chromatography using 25% EtOAc/hexanes afforded lactam **13** (697 mg, 60% yield) as a clear oil: $[\alpha]_D^{26}$ +18.4° (*c* 1.8, CH_2Cl_2); IR (neat) 3420, 3220, 3090, 3025, 2990, 1750–1680 (broad), 1600, 1500, 1450, 1440, 1250, 1150, 750, 700; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.13 (m, 15H), 5.37 (br s, 1H), 5.13 and 5.05 (AB system, J_{AB} = 12.3 Hz, 2H), 4.81 (dd, J = 10.7, 4.7 Hz, 1H), 3.68 (s, 3H), 3.27 (m, 1H), 3.10 (m, 3H), 2.65 (m, 3H), 2.15 (m, 2H), 2.07 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 170.6, 154.9, 140.4, 134.9, 130.3, 129.9, 128.8, 128.5 (2×), 128.4, 128.3, 128.1, 127.0, 126.2, 66.6, 61.4, 53.8, 52.2, 40.7, 40.3, 32.1, 31.0, 29.9; HRMS calcd for $C_{30}H_{32}N_2O_5$ (M^+) 500.2311, found 500.2312 *m/z*. Anal. calcd for $C_{30}H_{32}N_2O_5$: C, 71.98; H, 6.44; N, 5.60. Found: C, 71.93; H, 6.39; N, 5.48.

(2*S*,3*S*)-[3-Benzyl-1-(1-hydroxymethyl-3-phenylpropyl)-2-oxo-pyrrolidin-3-yl]-carbamic acid benzyl ester (15). To a −5 °C solution of **13** (325 mg, 0.65 mmol) in EtOH (3 mL) was added a suspension of $CaCl_2$ (152 mg, 1.37 mmol) in THF (2 mL). After 5 min, $NaBH_4$ (99 mg, 2.61 mmol) was added in one portion. The reaction was allowed to warm to 25 °C overnight. The mixture was poured carefully into an ice-cold 1 N citric acid solution (20 mL) then extracted with EtOAc (3×30 mL). The combined organic extracts were washed with saturated aq $NaHCO_3$ (3×30 mL) and brine, then dried, filtered, and concentrated to dryness in vacuo to yield a crude oil. Purification of the crude product by flash column chromatography using 1/1 EtOAc/hexanes afforded alcohol **15** (223 mg, 75% yield) as a clear oil: $[\alpha]_D^{26}$ +48.3° (*c* 0.22, CH_2Cl_2); IR (neat) 3450 (broad), 3080, 3000, 1730, 1700, 1500, 1460, 1420, 1280, 750, 710; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.12 (m, 15H), 5.48 (br s, 1H), 5.12 and 5.07 (AB system, J_{AB} = 12.3 Hz, 2H), 3.71 (s, 2H), 3.51 (m, 1H), 3.17 (t, J = 9.2, 1H), 3.08 and 3.01 (AB system, J_{AB} = 12.9 Hz, 2H), 2.63 (q, J = 8.6 Hz, 1H), 2.52 (m, 4H), 1.83 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 155.0, 141.2, 136.2, 134.8, 128.4, 127.4, 126.0, 66.8, 62.8, 62.3, 56.5, 43.3, 42.2, 32.2, 30.4, 29.3; HRMS calcd for $C_{29}H_{32}N_2O_4$ $(M+1)^+$ 473.2440, found 473.2432 *m/z*.

(2*S*,3*S*)-[3-Benzyl-1-(1-formyl-3-phenyl-propyl)-2-oxo-pyrrolidin-3-yl]-carbamic acid benzyl ester (17). To a −78 °C solution of $(COCl)_2$ (0.075 mL, 0.85 mmol) in CH_2Cl_2 (2 mL) was added DMSO (0.096 mL, 1.35 mmol). After 10 min, a solution of **15** (129 mg, 0.27 mmol) in CH_2Cl_2 (5 mL) was added via cannula to the reaction. Et_3N (0.200 mL, 1.43 mmol) was added

and the reaction was slowly warmed to 0 °C. The reaction was diluted with EtOAc (15 mL) then poured into saturated aq $NaHCO_3$ (15 mL). The layers were separated and the organic layer was washed with 1 N HCl (2×15 mL), saturated aq $NaHCO_3$ (15 mL) and brine then dried, filtered, and concentrated to dryness in vacuo to yield a crude oil. Purification of the crude product by flash column chromatography using 35% EtOAc/hexanes afforded aldehyde **17** (121 mg, 95% yield) as a clear oil: $[\alpha]_D^{26}$ +40.3° (*c* 0.3, CH_2Cl_2); IR (neat) 3300 (broad), 3020, 3010, 2950, 2920, 2820, 1735, 1705, 1500, 1450, 1270, 1200, 1060, 1030, 750, 700; ^1H NMR (400 MHz, $CDCl_3$) δ 9.09 (s, 1H), 7.36–7.23 (m, 15H), 5.42 (br s, 1H), 5.11 and 5.07 (AB system, J_{AB} = 12.3 Hz, 2H), 4.50 (dd, *J* = 10.9, 4.2 Hz, 1H), 3.10 (m, 3H), 2.72 (m, 1H), 2.60 (m, 3H), 2.36 (m, 1H), 2.20 (m 1H), 1.84 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.7, 174.0, 154.9, 140.3, 136.2, 134.6, 130.2, 128.5 (2×), 128.2, 128.1, 127.4, 126.3, 66.7, 61.7, 60.5, 42.2, 41.0, 31.7, 30.4, 26.6; HRMS calcd for $C_{29}H_{30}N_2O_4$ $(M+1)^+$ 471.2283, found 471.2291 *m/z*.

(2*S*,3*S*)-2-(3-Amino-3-benzyl-2-oxo-pyrrolidin-1-yl)-propionic acid methyl ester (19). To a solution of **10** (955 mg, 2.35 mmol) in EtOH (20 mL) was added 10% Pd/C (100 mg). After thoroughly flushing the flask with Ar, a hydrogen atmosphere was introduced with a balloon. After 17 h, the reaction was filtered through Celite then concentrated in vacuo to yield a light-yellow oil. Amine **19** (590 mg, 90% yield) was used without further purification: $[\alpha]_D^{26}$ +10.6° (*c* 2.0, CH_2Cl_2); IR (neat) 3480, 3400, 3060, 2995, 2980, 1745, 1700, 1500, 1460, 1440, 1270, 1210, 1080, 900, 750, 710; ^1H NMR (400 MHz, $CDCl_3$) δ 7.28–7.20 (m, 5H), 4.82 (q, *J* = 7.2 Hz, 1H), 3.68 (s, 3H), 3.29 (ddd, *J* = 9.1, 9.1, 3.2 Hz, 1H), 3.11 (m, 1H), 2.93 and 2.83 (AB system, J_{AB} = 13.2 Hz, 2H), 2.50 (br s, 2H), 2.24 (ddd, *J* = 12.9, 7.2, 31. Hz, 1H), 1.85 (ddd, *J* = 16.7, 8.5, 8.1 Hz, 1H), 1.40 (d, 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.8, 171.5, 135.8, 130.5, 128.3, 126.9, 60.4, 52.3, 49.6, 43.0, 39.8, 31.5, 14.6; HRMS calcd for $C_{15}H_{20}N_2O_3$ $(M+1)^+$ 277.1552, found 277.1562 *m/z*.

(2*S*,3*S*)-2-(3-Benzenesulfonylamino-3-benzyl-2-oxo-pyrrolidin-1-yl)-propionic acid methyl ester (21). To a 0 °C solution of amine **19** (156 mg, 0.69 mmol) and a catalytic amount of DMAP (10 mg) in CH_2Cl_2 (2 mL) and pyridine (1 mL) was added phenylsulfonyl chloride (0.260 mL, 2.03 mmol). The mixture was allowed to warm to 25 °C overnight. The reaction was carefully quenched with saturated aq $NaHCO_3$ (1 mL), diluted with EtOAc (15 mL), then poured into H_2O (10 mL). The layers were separated and the aq layer was extracted with EtOAc (2×5 mL). The combined organic extracts were washed with 1 M HCl (3×20 mL) and brine, then dried, filtered, and concentrated in vacuo to yield a crude yellow oil. Purification of the crude product by flash column chromatography using 40% EtOAc/hexanes afforded sulfonamide **21** (160 mg, 56% yield) as a clear oil: $[\alpha]_D^{26}$ −19.6° (*c* 0.9, CH_2Cl_2); IR ($CDCl_3$) 3430, 3080, 3030, 3000, 2980, 1750, 1700, 1500, 1450, 1440, 1400, 1350, 1300, 1210, 1165, 900, 700; ^1H NMR (400 MHz, $CDCl_3$) δ 7.88–7.20 (m, 10H), 5.57 (br s, 1H), 4.70 (q, *J* = 7.2 Hz, 1H), 3.68 (s, 3H), 3.17 (m, 1H), 3.09 and 3.03 (AB system, J_{AB} = 13.5 Hz, 2H), 2.97 (m, 1H), 2.35 (m, 2H), 1.29 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 171.0, 142.5, 134.4, 132.4, 130.6, 128.8, 128.2, 127.0, 126.7, 64.1, 52.2, 49.6, 42.7, 40.2, 29.3, 14.3; HRMS calcd for $C_{21}H_{24}N_2O_5S$ $(M+1)^+$ 417.1484, found 417.1484 *m/z*. Anal. calcd for $C_{21}H_{24}N_2O_5S$: C, 60.56; H, 5.81; N, 6.73. Found: C, 60.86; H, 6.00; N, 6.35.

(2*S*,3*S*)-*N*-(3-Benzenesulfonylamino-3-benzyl-2-oxo-pyrrolidin-1-yl)-4-phenylbutyric acid methyl ester (22). Compound **20** (287 mg, 0.78 mmol) was sulfonylated using the same conditions as **19** to afford sulfonamide **22** (318 mg, 80% yield) as a clear oil: $[\alpha]_D^{25}$ −1.0° (*c* 4.6, CH_2Cl_2); IR (neat) 3200, 3075, 3020, 2980, 2975, 1745, 1690, 1600, 1450, 1320, 1285, 1200, 1160, 1100, 900, 750, 720, 700; ^1H NMR (400 MHz, $CDCl_3$) δ 7.90–7.86 (m, 2H), 7.51–7.08 (m, 13H), 5.19 (br s, 1H), 4.67 (dd, *J* = 10.4, 5.3 Hz, 1H), 3.64 (s, 3H), 3.13 (m, 1H), 3.06 (d, *J* = 2.8 Hz, 2H), 2.97 (m, 1H), 2.42 (m, 2H), 2.38 (m, 2H), 2.17 (m, 1H), 1.89 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 170.3, 142.8, 140.1, 134.4, 132.5, 128.9, 128.4, 128.34, 128.32, 127.2, 126.2, 64.3, 53.8, 52.2, 43.0, 40.5, 32.0, 30.1, 29.3; HRMS calcd for $C_{28}H_{30}N_2O_5S$ $(M+1)^+$ 507.1953, found 507.1960 *m/z*. Anal. calcd for $C_{28}H_{30}N_2O_5S$: C, 66.38; H, 5.97; N, 5.53. Found: C, 66.24; H, 5.89; N, 5.50.

(2*S*,3*S*)-*N*-[3-Benzyl-1-(2-hydroxyl-1-methyl-ethyl)-2-oxo-pyrrolidin-3-yl]-benzene-sulfonamide (23). Compound **21** (130 mg, 0.32 mmol) was reduced using the same conditions as **15** to afford alcohol **23** (116 mg, 95% yield) as a clear oil: $[\alpha]_D^{25}$ +12.6° (*c* 1.8, CH_2Cl_2); IR ($CDCl_3$) 3500–3100 (broad), 3430, 3035, 3020, 2990, 2980, 2900, 1735, 1700, 1600, 1580, 1550, 1450, 1400, 1300, 1265, 1100, 1050, 910, 720; ^1H NMR (400 MHz, $CDCl_3$) δ 7.91–7.22 (m, 10H), 5.95 (br s, 1H). 3.81 (m, 1H), 3.45 (m, 1H), 3.04 (m, 3H), 2.62 (m, 1H), 2.50 (m, 2H), 2.35 (m, 1H), 0.98 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 142.6, 134.6, 132.5, 130.4, 128.9, 128.3, 127.3, 126.7, 65.1, 63.9, 51.6, 43.8, 42.0, 29.9, 13.4; HRMS calcd for $C_{20}H_{24}N_2O_4S$ $(M+1)^+$ 389.1534 found 389.1550 *m/z*. Anal. calcd for $C_{20}H_{24}N_2O_4S$: C, 61.84; H, 6.23; N, 7.21. Found: C, 61.24; H, 6.33; N, 7.17.

(2*S*,3*S*)-*N*-[3-Benzyl-1-(1-hydroxymethyl-3-phenylpropyl)-2-oxo-pyrrolidin-1-yl]-benzenesulfonamide (24). Compound **22** (268 mg, 0.53 mmol) was reduced using the

same conditions as **15** to afford alcohol **24** (185 mg, 73% yield) as 1/1 mixture of diastereomers: $[\alpha]_D^{26}$ +13.5° (*c* 0.8, CH_2Cl_2); IR (neat) 3500–3100 (broad), 3060, 2950, 1680, 1500, 1450, 1320, 1290, 1160, 1100, 760, 700; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93–7.11 (m, 2H), 7.53–7.47 (m, 3H), 7.28–7.09 (m, 10H), 5.74 (br s, 1H), 3.78 (m, 1H), 3.54 (m, 2H), 3.10 and 3.02 (AB system, J_{AB} = 12.3 Hz, 2H), 3.05 (m, 1H), 2.61 (ddd, *J* = 8.0 Hz, 1H), 2.49 (m, 4H), 2.30 (br s, 1H), 1.80 (m, 1H), 1.68 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 142.8, 141.0, 134.6, 132.5, 130.4, 129.1, 129.0, 128.4, 128.3, 127.4, 126.6, 126.0, 65.2, 62.7, 55.4, 43.7, 42.0, 32.1, 29.1, 29.0; HRMS calcd for $C_{27}H_{30}N_2O_4S$ $(M+1)^+$ 479.2004, found 479.1998 *m/z*. Anal. calcd for $C_{27}H_{30}N_2O_4S$: C, 67.76; H,6.32; N, 5.85. Found: C, 67.36; H, 6.56 N, 5.58.

(2*S*,3*S*)-*N*-[3-Benzyl-1-(1-methyl-2-oxoethyl)-2-oxo-pyrrolidin-3-yl]-benzenesulfon-amide (25). Compound **23** (64 mg, 0.18 mmol) was oxidized using the same conditions used for the preparation of **17** to afford aldehyde **25** (45 mg, 75% yield) as 1/1 mixture of diastereomers: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.35, 9.10 (s, 1H), 7.94–7.90 (m, 2H), 7.58–7.50 (m, 3H), 7.30–7.20 (m, 5H), 5.45 (br s, 1H), 4.45, 4.36 (q, *J* = 7.5, 7.2 Hz, 1H), 3.10 (m, 3H), 2.85, 2.15 (m, 1H), 2.54 (m, 2H), 1.18, 0.92 (d, *J* = 7.2, 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.3, 172.9, 171.8, 142.7, 134.4, 132.7, 130.2, 129.0, 128.5, 127.6, 126.8, 64.5, 56.7, 56.0, 44.8, 44.2, 41.3, 29.6, 107, 10.4; HRMS calcd for $C_{20}H_{22}N_2O_4S$ $(M+1)^+$ 387.1378, found 387.1369 *m/z*.

(2*S*,3*S*)-*N*-[3-Benzyl-1-(1-formyl-3-phenyl-propyl)-2-oxo-pyrrolidin-3-yl]-benzene-sulfonamide (26). Compound **24** (115 mg, 0.24 mmol) was oxidized using the same conditions as described for the preparation of **17** to afford aldehyde **26** (95 mg, 84% yield) as a 1/1 mixture of diastereomers: IR (neat) 3360, 3020, 3015, 2995, 2910, 2810, 1740, 1700, 1500, 1450, 1410, 1350, 1260, 1165, 900, 700; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.38, 9.03 (s, 1H), 7.95–7.91 (m, 2H), 7.56–7.46 (m, 2H), 7.28–7.05 (m, 11H), 5.58, 5.51 (br s, 1H), 4.42, 4.33 (dd, *J* = 10.5, 4.5 Hz, dd, *J* = 9.4, 5.3 Hz, 2H), 3.10 (m, 3H), 2.40 (m, 5H), 2.28, 2.18 (m, 1H), 1.76, 1.57 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.2, 197.7, 173.3, 173.27, 142.6, 142.5, 140.1, 140.0, 134.4, 134.3, 132.7, 130.3, 129.1, 128.6, 128.4, 128.3, 127.6, 126.7, 126.4, 64.75, 64.73, 60.8, 60.4, 44.3, 44.1, 42.0, 41.2, 32.1, 31.8, 29.6, 29.3, 27.4, 26.8; HRMS calcd for $C_{27}H_{28}N_2O_4S$ $(M+1)^+$ 477.1848, found 477.1838 *m/z*.

(2*S*,3*S*)-2-(3-Benzyl-3-methylsulfonylamino-2-oxo-pyrrolidin-1-yl)-propionic acid methyl ester (27). Amine **19** (127 mg, 0.46 mmol) was sulfonylated with methanesulfonyl chloride using the same conditions used to generate **21** to afford sulfonamide **27** (100 mg, 61% yield) as a clear oil: $[\alpha]_D^{26}$ −25.9° (*c* 1.2, CH_2Cl_2); IR (CH_2Cl_2) 3440, 3060, 2990, 1750, 1705, 1500, 1430, 1340, 1280, 1210, 1150, 980, 900, 700; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (m, 5H), 4.89 (br s, 1H), 3.68 (s, 3H), 3.38 (ddd, *J* = 9.1, 9.1, 3.1 Hz, 1H), 3.10 (m, 4H), 3.05 (m, 3H), 2.30 (m, 2H), 1.42 (s, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 171.2, 134.0, 130.5, 128.6, 127.5, 64.6, 52.2, 49.9, 44.2, 42.7, 39.9, 30.8, 14.3; HRMS calcd for $C_{16}H_{22}N_2O_4S$ $(M+1)^+$ 355.1327, found 355.1340 *m/z*.

(2*S*,3*S*)-*N*-[3-Benzyl-1-(2-hydroxyl-1-methyl-ethyl)-2-oxo-pyrrolidin-3-yl]-methane-sulfonamide (28). Compound **27** (54 mg, 0.15 mmol) was reduced using the same conditions as described for the preparation of **15** to afford alcohol **28** (32 mg, 65% yield) as a clear oil: $[\alpha]_D^{26}$ +18.9° (*c* 0.3 in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.26 (m, 5H), 5.20 (br s, 1H), 3.83 (m, 1H), 3.60 (m, 2H), 3.21 (m, 1H), 3.16 (s, 3H), 3.14 and 3.02 (AB system, J_{AB} = 12.9 Hz, 2H), 2.52 (m, 4H), 1.09 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 134.1, 130.4, 128.6, 127.7, 65.7, 63.9, 52.5, 44.3, 43.9, 42.4, 31.0, 13.3; HRMS calcd for $C_{15}H_{20}N_2O_4S$ $(M+1)^+$ 325.1222, found 325.1230 *m/z*.

(2*S*,3*S*)-*N*-[3-Benzyl-1-(1-methyl-2-oxo-ethyl)-2-oxo-pyrrolidin-3-yl]-methanesulfon-amide (29). Compound **28** (27 mg, 0.08 mmol) was oxidized using the same conditions as described for the preparation of **17** to afford aldehyde **29** (24 mg, 96% yield) as 1/1 mixture of diastereomers: IR (neat) 3400, 3250, 3080, 3050, 3020, 2965, 1735, 1690, 1450, 1320, 1290, 1150, 1120; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.47, 9.26 (s, 1H), 7.31–7.22 (m, 5H), 5.20, 5.04 (br s, 1H), 4.49, 4.44 (q, *J* = 7.5, 7.2 Hz, 1H), 3.15, 3.12 (s, 3H), 3.10, 3.05 (m, 1H), 3.12 (m, 2H), 2.70 (m, 1H), 2.55 (m, 2H), 1.29, 1.27 (d, *J* = 7.5, 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.7, 198.6, 172.6, 138.5, 133.9, 130.4, 130.2, 130.2, 128.6, 127.8, 64.8, 57.1, 56.4, 44.5, 44.4, 41.1, 31.1, 30.6, 10.8, 10.5; HRMS calcd for $C_{15}H_{22}N_2O_4S$ $(M+1)^+$ 326.1300, found 326.1305 *m/z*.

(3*S*)-*N*-(1-Benzenesulfonyl-butenyl)-carbamic acid *tert*-butyl ester (39). To a −10 °C solution of 3873 (450 mg, 1.54 mmol) was added NaH (40 mg, 1.67 mmol). After gas evolution had ceased (ca. 10 min), a −10 °C solution of 3672 (215 mg, 1.24 mmol) in THF (2 mL) was added via cannula. The reaction was allowed to warm to 25 °C and stirred for a period of 1 h. The reaction was diluted with Et_2O (15 mL) then poured into brine (10 mL). The layers were separated and the organic layer was dried, filtered, and concentrated in vacuo to yield a crude oil. Purification of the crude product by flash column chromatography using 40% EtOAc/hexanes afforded vinyl sulfone **39** (350 mg, 91% yield) as a clear oil: $[\alpha]_D^{26}$ −18.4° (*c* 1.8, CH_2Cl_2); IR (neat) 3360, 3060, 2980, 2940, 1715, 1630, 1520, 1450, 1370, 1300, 1250, 1150,

1090, 850, 750, 700; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82–7.46 (m, 5H), 6.85 (d, J=14.8 Hz, 1H), 6.36 (d, J=14.8 Hz, 1H), 4.84 (br s, 1H), 4.39 (s, 1H), 1.32 (s, 9H), 1.21 (d, J=6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.6, 147.5, 140.1, 133.3, 129.6, 129.1, 79.8, 46.5, 28.2, 28.1, 19.7; HRMS calcd for $C_{15}H_{21}NO_4S$ $(M+1)^+$ 312.1269, found 312.1269 m/z. Anal. calcd for $C_{15}H_{21}NO_4S$: C, 57.86; H, 6.80; N, 4.50. Found: C, 57.63; H, 6.89; N, 4.47.

(3*S*)-*N*-(1-Benzenesulfonyl-5-phenylpentenyl)carbamic acid *tert*-butyl ester (40). Aldehyde **37**[33] (710 mg, 2.6 mmol) was converted to vinyl sulfone **40** (750 mg, 78% yield, clear oil) using the conditions described for the preparation of **39**: $[\alpha]_D^{26}$ −1.0° (c 4.1, CH_2Cl_2); IR (neat) 3440, 3060, 3025, 2980, 2920, 2860, 1720, 1500, 1450, 1370, 1320, 1250, 1150, 1090, 1050, 1030, 850; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87–7.21 (m, 10H), 6.90 (dd, J=15.1, 4.9 Hz, 1H), 6.42 (d, J=15.1 Hz, 1H), 4.65 (s, 1H), 4.38 (s, 1H), 2.66 (m, 2H), 1.75 (m, 2H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.9, 140.3, 140.1, 133.5, 130.5, 129.2, 128.5, 128.3, 127.5, 126.2, 80.0, 50.6, 35.8, 31.8, 28.2; HRMS calcd for $C_{22}H_{27}NO_4S$ $(M)^+$ 401.1661, found 401.1655 m/z. Anal. calcd for $C_{22}H_{27}NO_4S$: C, 65.81; H, 6.78; N, 3.49. Found: C, 65.90; H, 6.94; N, 3.37.

(3*S*)-(1-Benzenesulfonyl-butenyl)-ammonium trifluoroacetate (41). To a solution of **39** (229 mg, 0.69 mmol) in CH_2Cl_2 (2 mL) was added 1/1 TFA/CH_2Cl_2 (2 mL). After 1 h, the reaction was concentrated in vacuo to yield the TFA salt **41** as a light brown oil (212 mg, 99%), which was used without further purification: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81–7.50 (m, 5H), 7.03 (dd, J=15.1, 5.6 Hz, 1H), 6.71 (d, J=15.1 Hz, 1H), 5.30 (br s, 3H), 4.20 (m, 1H), 1.47 (d, J=6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.8, 138.3, 134.3, 133.7, 129.6, 127.7, 47.8, 17.8.

(3*S*)-(1-Benzenesulfonyl-5-phenylpentenyl)-ammonium trifluoroacetate (42). To a solution of **40** (340 mg, 0.92 mmol) in CH_2Cl_2 (2 mL) was added 1/1 TFA/CH_2Cl_2 (2 mL). After 1 h, the reaction was concentrated in vacuo to yield the TFA salt **42** as a light-brown oil (380 mg, 99%). This salt was used without further purification: ^{1}H NMR (500 MHz, CD_3OD) δ 7.95–7.10 (m, 10H), 6.94 (d, J=15.2 Hz, 1H), 6.88 (dd, J=15.2, 7.4 Hz, 1H), 4.89 (br s, 3H), 3.97 (m, 1H), 2.59 (m, 2H), 2.10 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 140.9, 140.8, 140.5, 137.2, 135.3, 130.8, 129.8, 129.3, 129.0, 127.6, 52.1, 35.2, 32.1.

3(*S*)-Benzyl-3[(benzyloxycarbonyl)amino]-2-oxo-1-pyrrolidine-3′(*S*)-1-benzene-sulfonylbutene (43). To suspension of **9** (132 mg, 0.32 mmol), NaOAc (68 mg, 0.83 mmol) and 4 Å molecular sieves (310 mg) in MeOH (5 mL) was added **41** (110 mg, 2.68 mmol). After 15 min, $NaCNBH_3$ (55 mg, 0.87 mmol) was added and the reaction was stirred at 25 °C overnight. The suspension was then filtered and the filtrate was acidified to pH 1 with 1 N aq HCl and stirred for 20 min. The solution was then basified to pH 10 with saturated aq $NaHCO_3$ and extracted with EtOAc (4×20 mL). The combined organic extracts were washed with brine, dried, filtered, and concentrated to dryness in vacuo to yield a yellow oil. This oil was dissolved in toluene (3 mL) and DME (3 mL) to which HOBt (54 mg, 0.40 mmol) was added. The mixture was then heated at reflux for 24 h. The mixture was cooled and the solvents were removed in vacuo. The residue was taken up in EtOAc (30 mL) and washed with saturated aq $NaHCO_3$ (3×30 mL) and brine, then dried, filtered, and concentrated to dryness in vacuo to yield a crude yellow oil. Purification of the crude product by flash column chromatography using 35% EtOAc/hexanes afforded **43** (81 mg, 49% yield) as a clear oil: $[\alpha]_D^{26}$ −25.2° (c 1.2, CH_2Cl_2); IR (CH_2Cl_2) 3420, 3040, 2990, 2980, 1725, 1700, 1500, 1450, 1430, 1310, 1310, 1150, 1090, 1060, 970, 830, 690; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83–7.80 (m, 2H), 7.64–7.53 (m, 3H), 7.36–7.16 (m, 10H), 6.56 (dd, J=15.4, 4.7 Hz, 1H), 6.07 (dd, J=15.1, 1.9 Hz, 1H), 5.45 (s, 1H), 5.10 and 5.40 (AB system, J_{AB}=12.3 Hz, 2H), 4.91 (m, 1H), 3.07 (m, 3H), 2.50 (m, 1H), 2.40 (m, 1H), 2.21 (m, 1H), 1.24 (d, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 154.9, 143.8, 140.0, 136.0, 134.3, 133.5, 131.8, 130.0, 129.3, 128.6, 128.5, 128.1, 128.0, 127.8, 127.7, 66.7, 61.8, 47.2, 42.5, 39.6, 29.8, 16.0; HRMS calcd for $C_{29}H_{30}N_2O_5S$ $(M)^+$ 518.1875, found 518.1862 m/z. Anal. calcd for $C_{29}H_{30}N_2O_5S$: C, 67.16; H, 5.83; N, 5.40. Found: C, 67.17; H, 5.39; N, 5.40.

3(*S*)-Benzyl-3[(benzyloxycarbonyl)amino]-2-oxo-1-pyrrolidine-3′(*S*)-1-benzene-sulfonyl-5-phenylpentene (44). Amine salt **42** (300 mg, 0.73 mmol) was converted to pyrrolidinone **44** (322 mg, 72% yield) using the conditions described for the synthesis of **43**: mp 176–178 °C; $[\alpha]_D^{26}$ −6.4° (c 1.2, CH_2Cl_2); IR (neat) 3320, 3080, 3020, 2920, 1725, 1695, 1540, 1500, 1450, 1290, 1150, 1090, 1070, 750, 740, 700; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (m, 2H), 7.60 (m, 3H), 7.38–710 (m, 15H), 6.52 (dd, J=15.4, 5.8 Hz, 1H), 6.10 (d, J=15.4 Hz, 1H), 5.43 (s, 1H), 5.09 (s, 2H), 4.81 (m, 1H), 3.07 (m, 3H), 2.53 (m, 3H), 2.30 (m, 2H), 1.87 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 154.8, 142.3, 142.2, 140.4, 138.9, 136.2, 136.1, 134.3, 133.6, 132.4, 130.0, 129.4, 128.7, 128.5 (2x), 128.4, 128.2, 128.1, 127.8, 127.8, 126.7, 126.2, 66.7, 61.8, 51.2, 42.6, 39.6, 31.9, 31.6, 29.5.

3(*S*)-Benzyl-3-amino-2-oxo-1-pyrrolidine-3′(*S*)-1-benzene-sulfonyl-5-phenylpentene (45). To a 25 °C solution of **44** (68 mg, 0.11 mmol) in CH_2Cl_2 (1.5 mL) and CH_3CN (1.0 mL) was added trimethylsilyl iodide (0.100 mL,

0.69 mmol). After 30 min, MeOH (2 mL) was added to quench the reaction. The solvents were removed in vacuo to afford amine **45** (52 mg, 99% yield) as a brown crude oil that was used in the next step without further purification: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (s, 2H), 7.85 (m, 2H), 7.63–7.52 (m, 3H), 7.40–7.00 (m, 10H), 6.48 (dd, J = 15.2, 6.7 Hz, 1H), 6.38 (d, J = 15.2 Hz, 1H), 4.60 (m, 1H), 3.43 and 3.38 (AB system, J_{AB} = 13.0 Hz, 2H), 3.05 (m, 1H), 2.90 (m, 1H), 2.56 (m, 1H), 2.47 (m, 1H), 2.14 (m, 2H), 1.96 (m, 1H), 1.74 (m, 1H); HRMS calcd for $C_{28}H_{30}N_2O_3S$ $(M+1)^+$ 475.2055, found 475.2036 *m/z*.

3(*S*)-Benzyl-3[(*N*-phenylsulfonyl)amino]-2-oxo-1-pyrrolidine-3′(*S*)-1-benzene-sulfonyl-5-phenylpentene (46). Compound **45** (95 mg, 0.12 mmol) was sulfonylated using the conditions described for the synthesis of **22**, thereby affording sulfonamide **46** (40 mg, 56% yield) as a white oil: $[\alpha]_D^{26}$ −15.4° (*c* 1.3, CH_2Cl_2); IR (neat) 3250, 3060, 3030, 2960, 1690, 1500, 1450, 1320, 1290, 1150, 1100, 750, 710, 690; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (m, 2H), 7.81 (m, 3H), 7.65–7.48 (m, 5H), 7.28–7.04 (m, 10H), 6.46 (dd, J = 15.2, 6.0 Hz, 1H), 6.06 (d, J = 15.2 Hz, 1H), 5.42 (br s, 1H), 4.72 (dd, J = 13.8, 6.9 Hz, 1H), 3.04 (s, 2H), 2.93 (ddd, J = 9.4, 9.4, 2.8 Hz, 1H), 2.46 (m, 4H), 2.24 (m, 1H), 1.82 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 142.7, 141.6, 140.0, 139.5, 134.0, 133.7, 132.7, 132.6, 130.0, 129.4, 129.1, 128.8, 128.6, 128.2, 128.0, 127.7, 126.9, 126.4, 64.9, 51.3, 44.3, 39.8, 32.0, 31.7, 28.9; HRMS calcd for $C_{34}H_{34}N_2O_5S_2$ $(M+1)^+$ 615.1984, found 615.1990 *m/z*. Anal. calcd for $C_{34}H_{34}N_2O_5S_2$: C, 66.43; H,5.57; N, 4.56. Found: C, 66.45; H, 5.94; N, 4.21.

Phenyl (3*S*)-*N*-(*N*′-carbobenzyloxy-L-phenylalanyl)-3-amino-but-1-enyl sulfone (47). To a 0 °C solution of Cbz-Phe-OH (340 mg, 1.10 mmol), amine salt **41** (480 mg, 1.30 mmol), *i*-Pr_2NEt (0.500 mL, 2.87 mmol) and 1-hydroxybenzotriazole (HOBt) (184 mg, 1.40 mmol) in DMF (10 mL) was added 1-(3-diethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) (258 mg, 1.30 mmol). The reaction was allowed to warm to 25 °C and stirred overnight. The reaction was diluted with EtOAc (30 mL) and then poured into saturated aq $NaHCO_3$ (50 mL). The layers were separated and the aq layer was extracted with EtOAc (30 mL). The combined organic extracts were washed with saturated aq $NaHCO_3$ (2×50 mL), 1 N aq HCl (2×50 mL), and brine then dried, filtered, and concentrated in vacuo to yield a crude solid. Purification of the crude product by flash column chromatography using 40% EtOAc/hexanes afforded vinyl sulfone **47** (444 mg, 86% yield) as a white solid: mp 140 °C; $[\alpha]_D^{26}$ +3.6° (*c* 2.8, CH_2Cl_2); IR (neat) 3420, 3220, 3060, 2980, 2915, 1725, 1685, 1500, 1450, 1425, 1320, 1270, 1150, 1090, 900, 750; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83–7.80 (m, 2H), 7.51–7.20 (m, 3H), 7.17–7.09 (m, 10H), 6.74 (dd, J = 15.2, 4.5 Hz, 1H), 6.16 (d, J = 7.2 Hz, 1H), 6.03 (dd, J = 15.2, 1.9 Hz, 1H), 5.45 (d, J = 6.9 Hz, 1H), 5.03 and 4.98 (AB system, J_{AB} = 12.3 Hz, 2H), 4.65 (m, 1H), 4.32 (q, J = 6.9 Hz, 1H), 3.00 (dd, J = 13.5, 6.3 Hz, 1H), 2.93 (dd, J = 13.5, 8.2 Hz, 1H), 1.13 (d, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 155.9, 146.3, 136.0, 133.5, 130.1, 130.0, 129.3, 129.2, 128.8, 128.5, 128.2, 128.0, 127.6, 127.3, 67.2, 56.5, 45.1, 38.6, 19.6; HRMS calcd for $C_{27}H_{28}N_2O_5S$ $(M)^+$ 492.1719, found 492.1707 *m/z*. Anal. calcd for $C_{27}H_{28}N_2O_5S$: C, 65.84; H, 5.73; N, 5.69. Found: C, 65.63; H, 5.65; N, 5.52.

Phenyl (3*S*)-*N*-(*N*′-carbobenzyloxy-L-phenylalanyl)-3-amino-5-phenylpent-1-enyl sulfone (48). Amine salt **42** (35 mg, 0.12 mmol) was coupled with Z-Phe-OH using the same conditions as described for the preparation of **47** to give the dipeptide vinyl sulfone **48** (69 mg, 97% yield) as a clear oil: $[\alpha]_D^{26}$ +6.9° (*c* 0.9, CH_2Cl_2); IR (neat) 3320, 3060, 3030, 2910, 2830, 1660, 1550, 1450, 1300, 1150, 1100, 750, 730, 700; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (m, 2H), 7.62–7.53 (m, 3H) 7.32–7.03 (m, 15H), 6.76 (dd, J = 15.1, 4.9 Hz, 1H), 6.05 (dd, J = 15.1, 1.4 Hz, 1H) 5.97 (d, J = 7.4 Hz, 1H), 5.25 (d, J = 6.7 Hz, 1H), 5.05 (s, 2H), 4.64 (m, 1H), 4.30 (m, 1H), 3.02 (m, 2H), 2.95 (m, 2H), 1.86 (m, 1H), 1.72 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 155.9, 145.2, 140.2, 140.0, 136.0, 135.9, 133.5, 130.6, 129.3, 129.2, 128.8, 128.6 (3×), 128.3, 128.1, 127.6, 127.3, 67.3, 56.5, 49.2, 38.1, 35.4, 31.7; HRMS calcd for $C_{34}H_{34}N_3O_5S$ $(M+1)^+$ 583.2267, found 583.2246 *m/z*. Anal. calcd for $C_{34}H_{34}N_2O_5S$: C, 70.08; H, 5.88; N, 4.81. Found: C, 70.10; H, 6.07; N, 4.68.

Phenyl (3*S*)-*N*-(*N*′-phenylsulfonyl-L-phenylalanyl)-3-amino-5-phenylpent-1-enyl sulfone (50). Compound **49** (56 mg, 0.18 mmol) was combined with **42** using the conditions described for the synthesis of **47** to afford dipeptide **50** (85 mg, 86% yield) as a clear oil: $[\alpha]_D^{26}$ −64.0° (*c* 1.0, CH_2Cl_2); IR (neat) 3320, 3030, 3010, 2900, 2820, 1665, 1535, 1450, 1300, 1150, 1090, 750, 700; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (m, 2H), 7.54–7.50 (m, 3H), 7.38–7.07 (m, 13H), 6.87 (m, 3H), 6.55 (dd, J = 15.1, 1.4 Hz, 1H), 6.50 (d, J = 8.8 Hz, 1H), 4.90 (d, J = 6.0 Hz, 1H), 4.68 (m, 1H), 3.75 (m, 1H), 2.94 (dd, J = 13.8, 5.6 Hz, 1H), 2.76 (dd, J = 13.8, 8.1 Hz, 1H), 2.56 (m, 2H), 1.91 (m, 1H), 1.85 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 144.8, 140.3, 140.1, 138.0, 136.4, 134.8, 133.4, 133.1, 130.8, 129.3, 129.1, 128.9, 128.7, 128.3, 127.6, 127.5, 127.1, 126.3, 57.9, 49.5, 38.2, 35.2, 31.9; HRMS calcd for $C_{32}H_{32}N_2O_5S_2$ $(M+1)^+$ 541.1831, found 589.1863 *m/z*. Anal. calcd for $C_{32}H_{32}N_2O_5S_2$: C, 65.28; H, 5.48; N, 4.76. Found: C, 65.47; H, 5.54; N, 4.55.

IC_{50} determinations. Inhibitors were screened for effectiveness against the *Trypanosoma cruzi* cathepsin L-like

protease (cruzain) using purified recombinant protein lacking the C terminal domain.[69] Cruzain (4 nM) was incubated with 1 nM to 10,000 nM inhibitor in 100 mM sodium acetate buffer (pH 5.5) and 10 mM DTT (buffer A) for 5 min at room temperature. Z-Phe-Arg-AMC (Bachem) was added to 20 μM in a final volume of 200 μL, and the increase in fluorescence (excitation at 355 nm and emission at 460 nm) was followed with an automated microtiter plate spectrofluorometer (Labsystems Fluoroskan II). Inhibitor stock solutions were prepared at 20 mM in DMSO, and serial dilutions were made in DMSO.[82] Controls were performed using enzyme alone, enzyme with DMSO and enzyme with a previously known, highly effective irreversible inhibitor, (Mu-Phe-HPhe-(CH=CHSO$_2$Ph), Arris Pharmaceuticals Inc., South San Francisco, CA)[3334] with each assay set. Inhibitors which had IC_{50}s of less than 1 μM were further analyzed. IC_{50}s for other enzymes were determined similarly: papain (EC 3.4.22.2, Sigma) at 6 nM enzyme and 15 μM Z-Phe-Arg-AMC (K_m = 50 μM) in buffer B (buffer A with 1 mM EDTA and 0.1% Triton-X 100); cathepsin B (bovine spleen, EC 3.4.22.1, Sigma) at 10 nM enzyme and 10 μM Z-Phe-Arg-AMC (K_m = 110 μM) in buffer B; and leishmania B protease (*L. major*)[25] at 1.3 nM and 25 μM Z-Phe-Arg-AMC (K_m = 7 M) in buffer B; falcipain (in extracts of *P. falciparum* trophozoites) at ca. 30 nM and Z-Phe-Arg-AMC (50 μM) in buffer A (with the exception that falcipain incubation with inhibitor was for 30 min).[83]

Kinetic assays of irreversible inhibitors. Kinetic analyses of the irreversible cysteine protease inhibitors were performed as follows.[82,84] Cruzain (2 nM) in 100 μL of assay buffer was added to inhibitor dilutions in 100 μL of 5 μM Z-Phe-Arg-AMC (K_m = 1 μM) in buffer A. Progress curves were obtained for 5 min at room temperature (less than 5% of substrate consumed) with tenfold dilutions of inhibitor, starting at 10 μM. Inhibitor dilutions that gave simple exponential progress curves over a wide range of kobs were used to determine kinetic parameters. The value of k_{obs}, the rate constant for loss of enzyme activity, was determined from an equation for pseudo first order dynamics using UltraFit (Biosoft), when $r^2 > 0.95$. A double reciprocal plot of k_{obs} versus the inhibitor concentration then yielded the inhibitor K_i and k_{inact} as the slope and intersection of the x axis, respectively,[82] from which the apparent second order inhibition rate constants (k_{inact}/K_i) were calculated. The kinetic data reported in Table 3 for **47** and **50** were determined in this way (see section on IC_{50} determinations for details of the assay conditions for papain, cathepsin B, and the leishmania protease). The errors associated with the rate constants determined in this way are estimated to be on the order of 10–20%. Kinetic constants for inhibitor **48**, the most potent inhibitor of the three inhibitors examined, were determined by regression analysis.[82] When the relationship between k_{obs} and inhibitor concentration was hyperbolic, indicating stauration inhibition kinetics, k_{inact} and K_i were determined from an equation describing a two step irreversible inhibitor mechanism ($k_{obs} = k_{inact}[I]_o/([I]_o + K_i^*(1 + [S]_o/K_m))$) and nonlinear regression analysis using UltraFit. This treatment applied in the case of the **48**-cruzain pair. The value of k_{inact}/K_i for **48**-cruzain determined by the double recriprocal plot method was 667,000 $s^{-1} M^{-1}$, well within the error range for the value (634,000 ± 133,000 $s^{-1} M^{-1}$) determined by regression analysis. However, if k_{obs} varied linearly with inhibitor concentration (due to the range of inhibitor concentrations that were studied), the second order inhibition rate constant determined by regression analysis was treated as k_{ass}.[82] The data for the **48**-papain pair reported in Table 3 was determined in this way.

Acknowledgements

This work was supported by NIH Program Project Grant AI35707 (to J. M. C. and W. R. R.), and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (to P. J. R.). We thank Dr. Mark D. Pagel (Indiana University) for assistance with the molecular modeling, and Jed Olson for technical assistance.

References and Notes

1. Bond, J. S.; Butler, P. E. *Annu. Rev. Biochem.* **1987**, *56*, 333.
2. Nicholson, D. W.; Ali, A.; Thornberry, N. A.; Vaillancourt, J. P.; Ding, C. K.; Gallant, M.; Gareau, Y.; Griffin, P. R.; Labelle, M.; Lazebnik, Y. A.; Munday, N. A.; Raju, S. M.; Smulson, M. E.; Yamin, T.-T.; Lu, V. L.; Miller, D. K. *Nature (London)* **1995**, *376*, 37.
3. Thornberry, N. A.; Miller, D. K.; Nicholson, D. W. *Perspec. Drug Discov. Design* **1994**, *2*, 389.
4. Robertson, C. D.; Coombs, G. H.; North, M. J.; Mottram, J. C. In *Perspectives in Drug Discovery and Design*; Anderson, P. S.; Kenyon, G. L.; Marshall, G. R., Eds.; ESCOM Science: Leiden, 1996; Vol. 6, p 99.
5. Cohen, F. E.; Gregoret, L. M.; Amiri, P.; Aldape, K.; Railey, J.; McKerrow, J. H. *Biochem.* **1991**, *30*, 11221.
6. McKerrow, J. H. In *Perspec. Drug Discov. Design*; Anderson, P. S.; Kenyon G. L.; Marshall, G. R., Eds.; ESCOM Science: Leiden, 1994; Vol. 2, p 437.
7. http://www.who.ch/ctd/html/chag.html.
8. Urbina, J. A.; Payares, G.; Molina, J.; Sanoja, C.; Liendo, A.; Lazardi, K.; Piras, M.; Perez, N.; Wincker, P.; Ryley, J. F. *Science* **1996**, *273*, 969.
9. Docampo, R.; Moreno, S. N.; Stoppani, A. O.; Leon, W.; Cruz, F. S.; Villalta, F.; Muniz, R. F. A. *Biochem. Pharmacol.* **1981**, *30*, 1947.
10. Van den Bossche, H. *Nature (London)* **1978**, *273*, 626.

11. Cazzulo, J. J.; Couso, R.; Raimondi, A.; Wernstedt, C.; Hellman, U. *Mol. Biochem. Parasitol.* **1989**, *33*, 33.

12. Harth, G.; Andrews, N.; Mills, A. A.; Engel, J. C.; Smith, R.; McKerrow, J. H. *Mol. Biochem. Parasitol.* **1993**, *58*, 17.

13. McGrath, M. E.; Eakin, A. E.; Engel, J. C.; McKerrow, J. H.; Craik, C. S.; Fletterick, R. J. *J. Mol. Biol.* **1995**, *247*, 251.

14. Serveau, C.; Lalmanach, G.; Juliano, M. A.; Scharfstein, J.; Juliano, L.; Gauthier, F. *Biochem. J.* **1996**, *313*, 951.

15. Engel, J. C.; Doyle, P. S.; Hsieh, I.; McKerrow, J. H. *J. Exp. Med.* **1998**, in press.

16. Engel, J. C.; Doyle, P. S.; Palmer, J.; Hsieh, I.; Bainton, D. F.; McKerrow, J. H. *J. Cell Sci.* **1998**, *111*, 597.

17. Schirmer, R. H.; Müller, J. G.; Krauth-Siegel, R. L. *Angew. Chem. Int. Ed.* **1995**, *34*, 141.

18. Chan, C.; Yin, H.; Garforth, J.; McKie, J. H.; Jaouhari, R.; Speers, P.; Douglas, K. T.; Rock, P. J.; Yardley, V.; Croft, S. L.; Fairlamb, A. H. *J. Med. Chem.* **1998**, *41*, 148.

19. Markell, E. K.; Voge, M.; John D. T. *Medical Parasitology*, W. B. Saunders Co.: Philadelphia, 1992, 7th edn, for more information about leishmaniasis.

20. Rosenthal, P. J. *Emerg. Infect. Dis.* **1998**, *4*, 49.

21. http://www.who.ch/ctd/html/malaria.html.

22. Rosenthal, P. J.; McKerrow, J. H.; Aikawa, M.; Nagasawa, H.; Leech, J. H. *J. Clin. Invest.* **1988**, *82*, 1560.

23. Ring, C. S.; Sun, E.; McKerrow, J. H.; Lee, G. K.; Rosenthal, P. J.; Kuntz, I. D.; Cohen, F. E. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 3583.

24. Li, Z.; Chen, X.; Davidson, E.; Zwang, O.; Mendis, C.; Ring, C. S.; Roush, W. R.; Fegley, G.; Li, R.; Rosenthal, P. J.; Lee, G.; Kenyon, G. L.; Kuntz, I. D.; Cohen, F. E. *Chem. Biol.* **1994**, *1*, 31.

25. Selzer, P. M.; Chen, X.; Chan, V. J.; Cohen, F. E.; McKerrow, J. H. *Exp. Parasitol.* **1997**, *87*, 212.

26. Otto, H.-H.; Schirmeister, T. *Chem. Rev.* **1997**, *97*, 133.

27. Hanzlik, R. P.; Jacober, S. P.; Zygmunt, J. *Biochim. Biophys. Acta* **1991**, *1073*, 33.

28. Yamashita, D. S.; Smith, W. W.; Zhao, B.; Janson, C. A.; Tomaszek, T. A.; Bossard, M. J.; Levy, M. A.; Oh, H.-J.; Carr, T. J.; Thompson, S. K.; Ijames, C. F.; Carr, S. A.; McQueney, M.; D'Alessio, K. J.; Amegadzie, B. Y.; Hanning, C. R.; Abdel-Meguid, S.; DesJarlais, R. L.; Gleason, J. G.; Veber, D. F. *J. Am. Chem. Soc.* **1997**, *119*, 11351.

29. Dufour, E.; Storer, A. C.; Ménard, R. *Biochemistry* **1995**, *34*, 9136.

30. Hanada, K.; Tamai, M.; Yamagishi, M.; Ohmura, S.; Sawada, J.; Tanaka, I. *Agric. Biol. Chem.* **1978**, *42*, 523.

31. Hanzlik, R. P.; Thompson, S. A. *J. Med. Chem.* **1984**, *27*, 711.

32. Liu, S.; Hanzlik, R. P. *J. Med. Chem.* **1992**, *35*, 1067.

33. Palmer, J. T.; Rasnick, D.; Klaus, J. L.; Brömme, D. *J. Med. Chem.* **1995**, *38*, 3193.

34. Brömme, D.; Klaus, J. L.; Okamoto, K.; Rasnick, D.; Palmer, J. T. *Biochem. J.* **1996**, *315*, 85.

35. Krantz, A.; Copp, L. J.; Coles, P. J.; Smith, R. A.; Heard, S. B. *Biochemistry* **1991**, *30*, 4678.

36. Li, R.; Chen, X.; Gong, B.; Li, Z.; Selzer, P. M.; Davidson, E.; Kurzban, G.; Miller, R. E.; Nuzum, E. O.; McKerrow, J. H.; Fletterick, R. J.; Gillmor, S. A.; Craik, C. S.; Kuntz, I. D.; Cohen, F. E.; Kenyon, G. L. *Bioorg. Med. Chem.* **1996**, *4*, 1421.

37. Thompson, S. K.; Halbert, S. M.; Bossard, M. J.; Tomaszek, T. A.; Levy, M. A.; Zhao, B.; Smith, W. W.; Abdel-Meguid, S. S.; Janson, C. A.; D'Alessio, K. J.; McQueney, M. S.; Amegadzie, B. Y.; Hanning, C. R.; DesJarlais, R. L.; Briand, J.; Sarkar, S. K.; Huddleston, M. J.; Ijamaes, C. F.; Carr, S. A.; Garnes, K. T.; Shu, A.; Heys, J. R.; Bradbeer, J.; Zembryki, D.; Lee-Rykaczewski, L.; James, I. E.; Lark, M. W.; Drake, F. H.; Gowen, M.; Gleason, G. J.; Veber, D. F. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 14249.

38. Ribeiro-Rodrigues, R.; dos Santos, W. G.; Oliveira, A. B.; Snicckus, V.; Zani, C. L.; Romanha, A. J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1509.

39. McGrath, M. E.; Klaus, J. L.; Barnes, M. G.; Brömme, D. *Nature Structural Biol.* **1997**, *4*, 105.

40. Silverman, R. B. *The Organic Chemistry of Drug Design and Action*; Academic: London, 1992; p 83.

41. Liskamp, R. M. J. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 1.

42. Thaisrivongs, S.; Pals, D. T.; Turner, S. R.; Kroll, L. T. *J. Med. Chem.* **1988**, *31*, 1369.

43. Baldwin, J. E.; Lee, V.; Schofield, C. J. *Heterocycles* **1992**, *34*, 903.

44. Freidinger, R. M. *J. Org. Chem.* **1985**, *50*, 3631.

45. Freidinger, R. M.; Perlow, D. S.; Veber, D. F. *J. Org. Chem.* **1982**, *47*, 104.

46. Freidinger, R. M.; Veber, D. F.; Perlow, D. S.; Brooks, J. R.; Saperstein, R. *Science* **1980**, *210*, 656.

47. Zydowsky, T. M.; Dellaria, J. F., Jr.; Nellans, H. N. *J. Org. Chem.* **1988**, *53*, 5607.

48. Vacca, J. P.; Fitzgerald, P. M. D.; Holloway, M. K.; Hungate, R. W.; Starbuck, K. E.; Chen, L. J.; Darke, P. L.; Anderson, P. S.; Huff, J. R. *Bioorg. Med. Chem. Lett* **1994**, *4*, 499.

49. Westerik, J. O.; Wolfenden, R. *J. Biol. Chem.* **1972**, *247*, 8195.

50. Cheng, H.; Keitz, P.; Jones, J. B. *J. Org. Chem.* **1994**, *59*, 7671.

51. The modeling and energy minimizations were done with the InsightII® software package (Molecular Simulations Inc.) on a Silicon Graphics Inc. Indy® workstation. The minimizations were done with the Discover® platform within the InsightII® program with a CVFF (continual valence) forcefield (RMS energy derivative value of 0.0001). For information about the CVFF force field, see Dauber-Osguthorpe, P; Roberts, V. A.; Osguthorpe, D. J.; Wolff, J.; Genest, M.; Hagler, A. T. *Proteins, Structure, Function and Genetics* **1989**, *4*, 31. The algorithims utilized for minimizations were typically the conjugent gradient or VAO9A options. The dialectric field was set to a value of 4 and was chosen to be distance dependent. The covalently bound ligand was minimized while the enzyme (cruzain) was kept rigid using the Discover® option 'constraint'.

52. The RMS value determined after superimposition of cruzain backbone in cruzain-**1** crystal structure with the backbone of the minimized structure of **3** bound to cruzain was 0.817.

53. Matsuda, F.; Itoh, S.; Hattori, N. *Tetrahedron* **1985**, *41*, 3625.

54. Luly, J. R.; Dellaria, J. F.; Plattner, J. J.; Soderquist, J. L.; Yi, N. *J. Org. Chem.* **1987**, *52*, 1487.

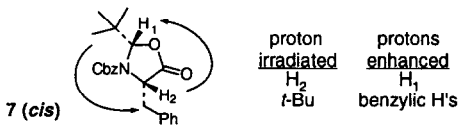
55. Mancuso, A. J.; Swern, D. *Synthesis* **1981**, 165.

56. Tidwell, T. T. *Synthesis* **1990**, 857.

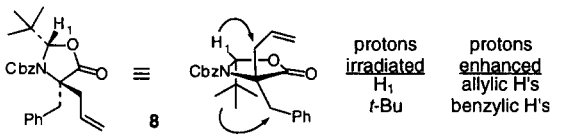
57. Seebach, D.; Fadel, A. *Helv. Chim. Acta* **1985**, *68*, 1243.

58. Fadel, A.; Salaün, J. *Tetrahedron Lett.* **1987**, *28*, 2243.

59. The nuclear Overhauser data for **7** (*cis*) were determined to be as shown below:



60. The nuclear Overhauser data for **8** were determined to be as shown below:



61. Borch, R. A.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897.

62. McGrath, M. E., personal communication.

63. Perrin, C. L. *Science* **1994**, *266*, 1665.

64. Schwartz, B.; Drueckhammer, D. G.; Usher, K. C.; Remington, S. *J. Biochem.* **1995**, *34*, 15459.

65. Koppel, I.; Koppel, J.; Leito, I.; Pihl, V.; Grehn, L.; Ragnarsson, U. *J. Chem. Res. (M)* **1994**, 1173.

66. Nyasse, B.; Grehn, L.; Ragnarsson, U.; Maia, H. L. S.; Monteiro, L. S.; Leito, I.; Koppel, I.; Koppel, J. *J. Chem. Soc., Perkin Trans. 1* **1995**, *1*, 2025.

67. Chagas, J. R.; Ferrerdimartino, M.; Gauthier, F.; Lalmanach, G. *FEBS Lett.* **1996**, *392*, 233.

68. Wagner, B. M.; Smith, R. A.; Coles, P. J.; J., C. L.; Ernest, M. J.; Krantz, A. *J. Med. Chem.* **1994**, *37*, 1833.

69. Eakin, A. E.; McGrath, M. E.; McKerrow, J. H.; Fletterick, R. J.; Craik, C. S. *J. Biol. Chem.* **1993**, *268*, 6115.

70. Rasnick, D. *Anal. Biochem.* **1985**, *149*, 461.

71. McKerrow, J. H.; Engel, J., unpublished research results.

72. Fehrentz, J.-A.; Castro, B. *Synthesis* **1983**, 676.

73. de Jong, B. E.; de Koning, H.; Huisman, H. O. *Recl. J. Neth. Chem. Soc.* **1981**, *100*, 410.

74. Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 2815.

75. Lott, R. S.; Chauhan, V. S.; Stammer, C. H. *J. Chem. Soc., Chem. Commun.* **1979**, 495.

76. Bodanszky, M. *Principles of Peptide Synthesis*; Verlag: New York, 1993; pp 9–61.

77. Milne, H. B.; Peng, C. *J. Am. Chem. Soc.* **1957**, *79*, 639.

78. Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.

79. A preliminary report containing kinetic data for **48** in comparison to a novel series of vinyl sulfonate ester and vinyl sulfonamide inhibitors of cysteine proteases has appeared: Roush, W. R.; Gwaltney, S. L., II; Cheng, J.; Scheidt, K. A.; McKerrow, J. H.; Hansell, E. *J. Am. Chem. Soc.* **1998**, *120*, in press.

80. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

81. Eakin, A. E.; Mills, A. A.; Harth, G.; McKerrow, J. H.; Craik, C. S. *J. Biol. Chem.* **1992**, *267*, 7411.

82. Beith, J. G. *Methods in Enzymology* **1995**, *248*, 59.

83. Rosenthal, P. J.; Olson, J. E.; Lee, G. K.; Palmer, J. T.; Klaus, J. L.; Rasnick, D. *Antimicrob. Agents Chemother.* **1996**, *40*, 1600.

84. Tian, W.-X.; Tsou, C.-L. *Biochemistry* **1982**, *21*, 1028.