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UNIVERSITY OF ALBERTA

Design and Synthesis of Potential Inhibitors of HAV-3C Proteinase

by

Yanting Huang

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

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Dr. Jeffrey M. Stryker

Dr. George Kotovych

Dr. Peter Sporns

DATED: December 18, 1996

To my parents; husband, Rulin; and son, Sijia

ABSTRACT

Three types of compounds were designed and synthesized as potential substrate analogues and inhibitors of hepatitis A virus (HAV) 3C proteinase. Since this enzyme is essential for viral replication, successful inhibitors may be lead compounds for antiviral therapeutic agents. The target compounds are:

1) **Four ketosulfonamides 17-20 and two hydroxysulfonamides 21-22:** (*3S*)-{N^3-(Benzyloxycarbonyl)-N^1-[3'-(*N,N*-dimethylamino)-3'-oxopropyl]-N^1-(methylsulfonyl)}-1,3-diaminobutan-2-one (**17**) and the *p*-methylphenylsulfonyl analogue **18** are synthesized through the key intermediates, α-bromomethyl ketone **89** and sulfonamides **90** and **91** in six steps in 8.0% and 8.4% overall yield, respectively. The syntheses of (*3S*)-{N^3-(acetyl-L-leucyl-L-alanyl)-N^1-[3'-(*N,N*-dimethylamino)-3'-oxopropyl]-N^1-(methylsulfonyl)}-1,3-diaminobutan-2-one (**19**) and the *p*-methylphenylsulfonyl analogue **20** are based on the deprotection of **17** and **18** by hydrogenation, followed by coupling with dipeptide acetyl-leucyl-alanine *via* a mixed anhydride, in eight steps in 4.0% and 4.7% overall yield, respectively. (*2RS, 3R*)-{N^3-(benzyloxycarbonyl)-N^1-[3'-(*N,N*-dimethyl amino)-3'-oxopropyl]-N^1-(methylsulfonyl)}-1,3-diaminobutan-2-ol (**21**) and the *p*-methylphenylsulfonyl analogue **22** are prepared by $NaBH_4$ reduction of sulfonamides **17** and **18** in seven steps in 7.1% and 7.3% overall yield, respectively. Enzyme inhibition studies show compound **17** to be a competitive inhibitor of HAV-3C with an IC_{50} of about 75 μM, which is significantly lower than the K_m for the ideal hexapeptide substrate (2.1 mM). The other compounds show no significant inhibition of HAV-3C proteinase at 100 μM.

2) **Five sulfenamides 23-27:** 3-[N^1-(*o*-Nitrophenylsulfenyl)-N^2-(acetyl-L-leucyl-L-alanyl-L-alanyl)hydrazino]-*N,N*-(dimethyl)propanamide (**23**) is synthesized *via* the key intermediate Cbz-hydrazino derivative **105** in five steps in 14% overall yield. The synthesis of 3-[N^1-(acetyl-D-alanyl)-N^2-(*o*-nitrophenylsulfenyl)hydrazino]-*N,N*-(dimethyl)

propanamide (**24**), and the L-isomer **25** involves generating intermediate 3-[(N^1-*t*-Boc)-(N^2-Cbz)hydrazino]-*N,N*-(dimethyl)propanamide (**126**) in seven steps in 30% and 26% overall yield, respectively. Tetrapeptides 3-[N^1-(acetyl-L-leucyl-L-alanyl-ß-alanyl)-N^2-(*o*-nitrophenylsulfenyl)hydrazino]-*N,N*-(dimethyl)propanamide (**26**) and 3-[N^1-(acetyl-L-leucyl-L-alanyl-L-alanyl)-N^2-(*o*-nitrophenylsulfenyl)hydrazino]-*N,N*-(dimethyl) propanamide (**27**) are synthesized by a similar approach to **24** in nine steps in 11% and 13% overall yield, respectively. Inhibition studies showed that they are weak inhibitors of HAV-3C proteinase, with IC_{50} values from 100 µM to 500 µM when preincubated with the enzyme. Electrospray mass spectrometry provides evidence for the formation of an enzyme-nitrophenyl disulfide covalent adduct for compound **26**. The results show that these types of modification on the substrate peptide backbone are far from ideal for the HAV-3C enzyme. A probable difficulty is the bulk of the aryl group on the sulfur. Nevertheless, such compounds may be effective against other cysteine proteinases, including other picornaviral 3C proteinases.

3) **Two hydrazo haloacetyl tetrapeptides 28 and 29**: 3-[N^1-(Chloroacetyl)-N^2-(acetyl-L-leucyl-L-alanyl-L-alanyl)hydrazino]-*N,N*-(dimethyl)propanamide (**28**) and the bromoacetyl analogue **29** have been synthesized. The synthetic strategy is based on that developed for azapeptide **103**. Modified acylation of azapeptide **103** with haloacetyl halides (**134**, **135**) gives the corresponding hydrazo haloacetyl tetrapeptides (**28**, **29**) in five steps in 10% and 9% overall yield, respectively. Inhibition studies have shown these compounds to be potent, irreversible inhibitors of HAV-3C proteinase, with second order rate constants ($k_{obs}/[I]$) of 684 (Y = Cl) and 866 (Y = Br) $M^{-1}s^{-1}$. Electrospray mass spectrometry further confirmed the formation of a covalent adduct [(M^+-Y (Y = Cl, Br)].

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LIST OF ABBREVIATIONS

[α]	specific rotation
Ac	acetyl
Ala	alanyl
anhyd	anhydrous
APT	attached proton test
Ar	aryl
Boc	butyloxycarbonyl
BOP	benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate
bp	boiling point
br	broad
Bu	butyl
n-BuLi	*n*-butyl lithium
calcd	calculated
Cbz	benzyloxycarbonyl
CDI	carbonyl diimidazole
CI	chemical ionization
concd	concentrated
δ	chemical shift in parts per million downfield from TMS
d	doublet
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
EMCV	encephalomyocarditis
EI	electron impact ionization
Enz	enzyme

Et	ethyl
FAB	fast atom bombardment
HAV	hepatitis A virus
HOBT	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
HRV	human rhinovirus
IBCF	*iso*-butyl chloroformate
IR	infrared
J	coupling constant
Leu	leucyl
m	multiplet
m/z	mass to charge ratio
Me	methyl
MHz	megahertz
mp	melting point
MS	mass spectrometry
NMM	*N*-methyl morpholine
NMR	nuclear magnetic resonance
Nu	nucleophile
Ph	phenyl
ppm	parts per million
Pr	propyl
PV	poliovirus
Pyr	pyridine
q	quartet
Rf	retention factor
RNA	ribonucleic acid

RP	reverse phase
rt	room temperature
s	singlet
t	triplet
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
Ts	*p*-toluenesulfonyl
UV	ultraviolet

INTRODUCTION

The picornaviral family contains many important human pathogens including poliovirus (PV), human rhinovirus (HRV, the common cold), encephalomyocarditis (EMCV) and hepatitis A virus (HAV, infectious hepatitis).[1] The clinical syndromes associated with the various members of this family range from transient and benign (colds), to permanent and life threatening (polio). Likewise, picornavirus associated morbidity in North America runs from sporadic outbreaks (polio) to endemic pools (colds). Poliovirus (PV) and hepatitis A virus (HAV) are still epidemic in many parts of the third world. The largest mollusk-linked HAV epidemic in recent years occurred in Shanghai in 1988, involving ~ 300,000 people.[2] Although the vaccines for PV have been very effective, importation and subsequent outbreak in industrialized nations, as recently occurred in Finland, is still of major concern to epidemiologists and public health officials.[3,4] There are as yet no effective antiviral agents which can be used for either the treatment or prophylaxis of any picornavirus. Thus, it is important to develop antiviral agents to combat such diseases. Apart from the medicinal use of antiviral agents, such compounds play a pivotal role as tools in molecular virology, and are very useful for elucidating numerous viral processes.[5] For example, they provide tools that aid in the dissection of the complicated molecular steps of viral genome replication.

Potential inhibitors can be classified into five groups, according to the different steps they block in the picornavirus replication cycle. They may interfere with: the early steps of infection; translation of viral RNA; picornavirus genome replication; viral RNA synthesis; or alternatively, unknown targets.[5] Picornavirus proteinases, produced by translation of viral RNA, have been suggested as potentially effective targets for the inhibition of viral growth,[6,7] and are the focus of this thesis.

Vaccination is the major alternative to the development of therapeutic or prophylactic agents for picornaviruses. Vaccine strategies have been successful for polio

virus and hepatitis A virus.[8,9] However, for many picornaviruses the development of effective vaccines is impractical due to the high mutation rate of the virus capsid proteins, which leads to the generation of escape mutants capable of infecting previously vaccinated individuals.[10] In contrast to the high mutation rate observed in capsid proteins, the processing proteinases appear relatively invariant,[11] and thus provide unique and highly susceptible targets for therapeutic intervention. Specific inhibitors of these proteinases can potentially be used as both prophylactic and therapeutic reagents (alone or in combination therapies) to control the spread and limit the severity and duration of viral infection.

There are features common to all members of the picornavirus family, although the various genera show subtle differences in genome organization, processing and morphology.[1] All picornaviruses possess a small, positive strand RNA genome that functions as an extended messenger RNA. The resulting single 250 kDa polyprotein is cotranslationally cleaved by the 2A proteinase in most picornaviruses to yield a structural (P1) and a non-structural (P2/P3) precursor protein (Figure 1). The P1 and P2/P3 precursors then undergo multiple proteolytic cleavages, resulting in mature capsid and viral proteins. These changes are mediated by the 3C proteinase, an activity found in all picornaviruses.[12,13]

The picornaviral 3C processing enzymes are cysteine proteinases, which catalyze peptide-bond cleavage through nucleophilic attack by the sulfur atom of the active-site cysteine residue upon the substrate carbonyl carbon atom of the scissile bond, to form a covalent tetrahedral intermediate. The HAV-3C enzyme is typical of the picornaviral family of 3C proteinases and there is high specificity in the P_1-P_4 and P_1'-P_2' subsites (Figure 2). The wild type HAV-3C enzyme is a cysteine proteinase of 219 amino acids with a molecular weight of 24 kilodaltons and exists as an active monomer. For ideal peptide substrates mimicking the 2B/2C junction, the k_{cat} is typically about 1.8 sec^{-1} with an approximate K_m of 2.1 mM at a pH of 7.5. More recently, the crystal structures of active HAV-3C mutants wherein only the external cysteine-24 has been replaced (James, M.

unpublished) and HRV-3C proteinase show that these enzymes are structurally distinct from the papain family and represent a new class of cysteine proteinases whose fold is similar to the chymotrypsin family of serine proteinases.[14,15]

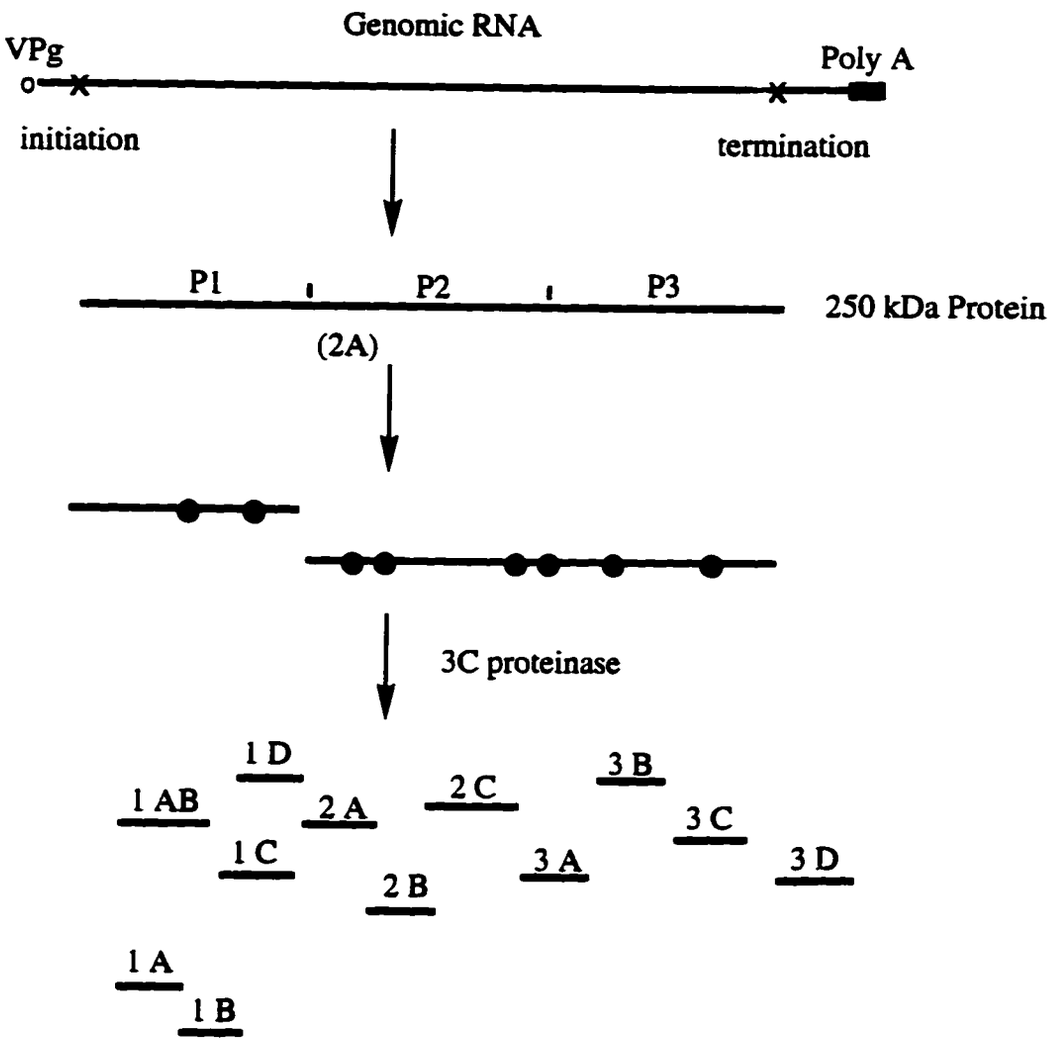


Figure 1 Generation of picornaviral proteins from a 250 kDa precursor[13]

The cleavage points between the functional components of the picornavirus 3C proteinases predominantly consist of glutamine followed by a small amino acid.[16] In the case of the enteroviruses and rhinoviruses, synthetic peptide studies have shown that the

glutamine residue should be followed by a glycine and proline residue in the P_1' and P_2' subsites, respectively, to ensure efficient cleavage.[17] In PV, -Gln*Gly- is the only site at which cleavage occurs.[18-19] Studies on HAV-3C show that it is less discriminating, and effectively cleaves peptides in which a small amino acid (glycine, alanine, serine) is in the P_1' position and virtually any amino acid is in the P_2' position with the exception of proline and arginine.[20] In addition to these determinants, peptide studies have also identified discrimination on the part of 3C enzymes for residues in the P_4 subsite. In the cases of poliovirus and rhinovirus, preferences for small side chains such as alanine are evident,[21] whereas in the case of HAV-3C, large side chains such as the branched aliphatic or aromatic side chains of leucine, isoleucine, or trytophan are preferred (Figure 2).[22]

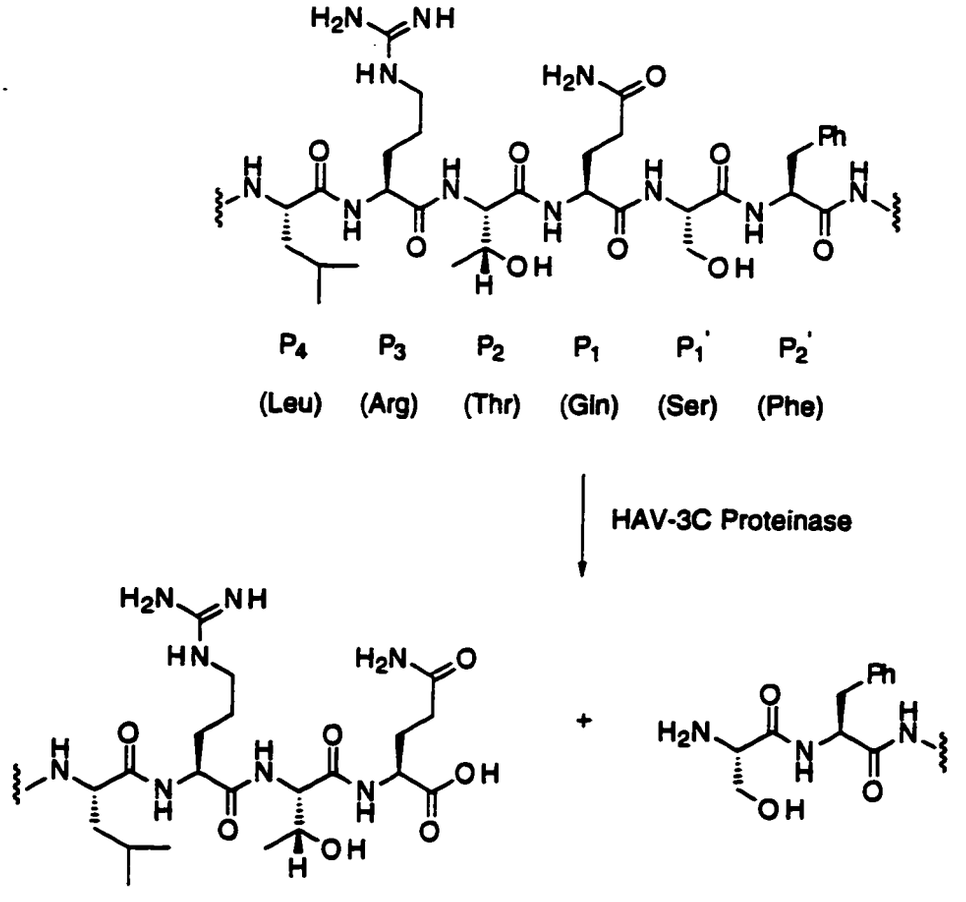


Figure 2 A preferred cleavage site of HAV-3C proteinase

Modeling of enzyme-substrate complexes[15,23] helps to rationalize the substrate preferences of the 3C enzymes (Figure 3). Studies with peptide substrates containing glutamine analogues[22,24] and the recently obtained crystal structures of HAV and HRV 3C proteinases[14,15] suggest that an uncharged δ–carbonyl oxygen is required in the P_1 position of the substrate for efficient recognition by the picornaviral enzymes. The P_4 subsite in HAV-3C appears to be a rather open hydrophobic cleft, which easily rationalizes the enzyme's preference for a variety of large hydrophobic residues in the S_4 position.[18] In contrast, the HRV-3C enzyme appears to have a shallow pocket and consequently prefers smaller residues such as valine and alanine at this site.

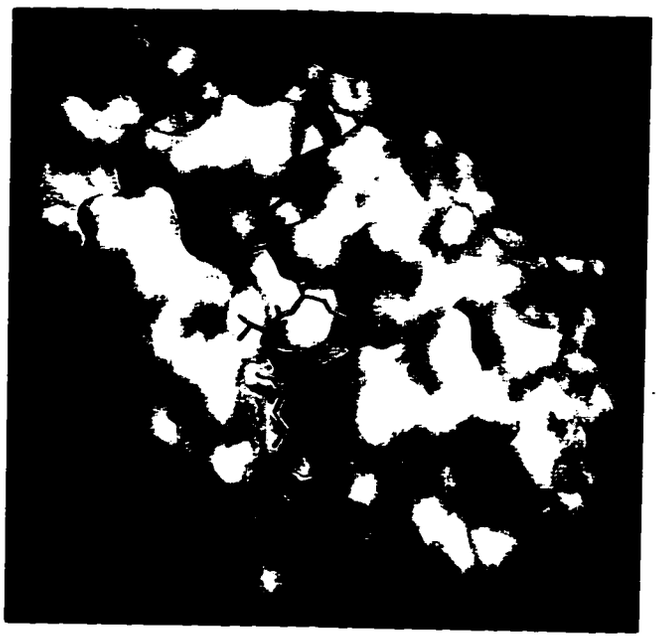


Figure 3 GRASP (Nicholls *et al.*, 1991)[25] model showing a peptide substrate (LATQGA) modeled into the active site of HAV-3C proteinase. Modeling of the hexapeptide substrate was based on coordinates from the high-resolution structure of turkey ovomucoid-α-chymotrypsin complex (Fujinaga *et al.*, 1987).[26] Superposition of the HAV-3C proteinase and chymotrypsin- ovomucoid complex yielded initial coordinates for the hexapeptide. Appropriate amino acid substitutions were made into the P_4-P_2' positions of the active site loop. The structure was subsequently refined using Discover (Biosym, San Diego, California). Yellow indicates the nucleophilic sulfur; blue, the histidine at the base of the P_1 subsite; pink, the postulated hydrophobic P_4 subsite. The oxy-anion hole of the mutant HAV-3C was "repaired" using chymotrypsin prior to modeling of the peptide substrate (taken from ref. 16).

As discussed below, a number of agents have been described as inhibitors of picornaviral 3C processing enzymes. These agents were employed to identify picornavirus precursors and to block the formation of mature proteins.[5] They can be broadly classified into three categories: (1) protein inhibitors (cystatin); (2) non-peptide inhibitors; and (3) peptide-based inhibitors. Since peptide-based inhibitors mimic natural substrates, an attractive feature of these compounds is that the selectivity and specificity can be designed to avoid cross reactivity with essential human proteinases.

(1) Protein inhibitors (cystatin)

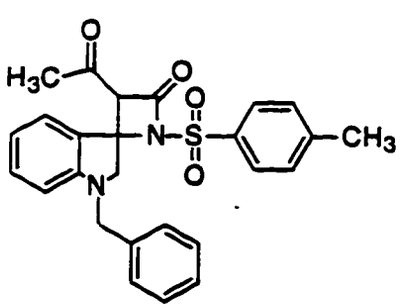
The activities of cysteine proteinases are controlled by a set of protein inhibitors that constitute the cystatin superfamily of proteins.[5] Cystatins are small proteins (100-120 amino acids) that bind and selectively inhibit cysteine proteinases. Chicken cystatin, isolated from egg white, bound and inhibited poliovirus 3C cysteine proteinase (45% inhibition at 10 μM) and blocked the replication of poliovirus in cultured cells.[27]

(2) Non-peptide inhibitors

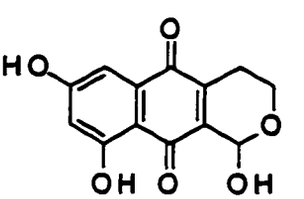
Treatment of the viral 3C proteinases with inhibitors of cysteine proteinases which act by modifying the sulfur atom of the active cysteine residue (e.g., iodoacetic acid, *N*-ethylmaleimide) (Table 1) all led to total loss of activity.[28-30] Recently, three novel inhibitors (**3-5**) (Figure 4) of HRV-3C proteinase were identified using a fluorescence-based assay.[31] The IC_{50} values for these three compounds were: citrinin hydrate (**3**), 75 μg/mL; radicinin (**4**), 118 μg/mL; kalafungin (**5**), 0.93 μg/mL. The selectivity of this kind of inhibitor was the major problem for these cysteine proteinases. For example, spiro indolinone ß-lactam (**1**) (Figure 4) inhibited not only poliovirus and HRV 3C proteinases (IC_{50} = 20 μg/mL) but also other cellular proteinases such as human leukocyte elastase (HLE) (IC_{50} = 0.4 μg/mL) and cathepsin G (IC_{50} = 4.0 μg/mL).[28]

Inhibitors	Inhibition (%)	
	Polio-M	HRV14
p-Chloromercuriphenylsulfonic acid/mercuric acetate	100	100
Iodoacetate/amide	96	100
N-Ethylmaleimide	100	100
Leupeptin	2	85
Spiro indolinone ß-lactam (**1**)	50	50
Thysanone (**2**)	n.d.	50

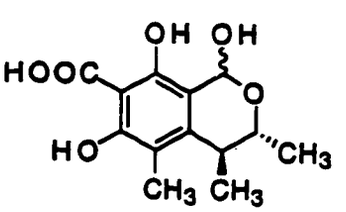
Table 1 Effect of inhibitors on the activity of picornavirus 3C proteinase. Concentrations of each inhibitor in the range of 10-100 µg/mL were used. Spiro indolinone ß-lactam(**1**) and Thysanone (**2**) are IC_{50} values. n.d., not determined. (Taken from refs. 28-30).



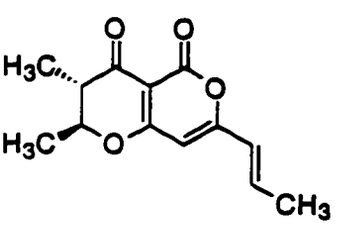
Spiro indolinone ß-lactam (**1**)



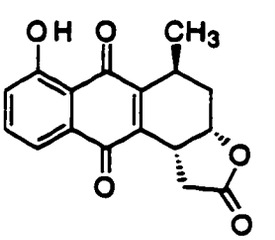
Thysanone (**2**)



Citrinin hydrate (**3**)



Radicinin (**4**)



Kalafungin (**5**)

Figure 4 Chemical structures of non-peptide inhibitors

(3) Peptide-based inhibitors

As previously stated, the picornavirus 3C enzymes are cysteine proteinases with a high degree of specificity in the P_1-P_4 and P_1'-P_2' subsites. Some specific picornavirus 3C proteinase inhibitors have been developed on the basis of selective affinity labeling of the active site cysteine residue. These inhibitors were made by attaching a thiol-reactive group to a peptide portion corresponding to the known substrate specificity of the enzyme; this results in covalent modification of the active site cysteine residue.[32] Groups that are chemically reactive toward thiols include aldehyde, methyl ketone, halomethyl ketone, diazomethyl ketone, nitrile, epoxysuccinyl moiety, iodoacetate/amide, and haloacetyl, among others. Several examples of these have recently been examined for 3C proteinase inhibition by our group as well as by others, and are described below.

(i) Peptidyl aldehyde

It is known that peptide aldehydes are powerful reversible inhibitors of cysteine proteinases.[33,34] The inhibition is due to the formation of covalent adducts (thiohemiacetals) that mimic the tetrahedral transition states on the enzyme reaction pathway (Figure 5).[35-38]

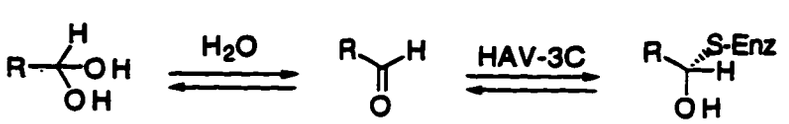


Figure 5 Reaction of peptidyl aldehyde with HAV-3C enzyme in aqueous solution

Recently, the peptide aldehyde Ac-Leu-Ala-Ala-(*N,N*-dimethylglutaminal) (**6**) (Figure 6) was generated, and found by our group to be an effective and reversible, slow-binding[39] inhibitor of HAV-3C proteinase, with a K_i^* of 42 nM, where K_i^* is the overall dissociation constant of the tight enzyme-inhibitor complex EI*.[40] This inhibitor shows 50 fold less activity against the highly homologous HRV-14 3C proteinase whose peptide

substrate specificity is slightly different, suggesting a high degree of selectivity. NMR spectrometry of the adduct of the ^{13}C-labeled inhibitor with the HAV-3C proteinase indicates that a thiohemiacetal is formed.[40] Tetrapeptide aldehyde Boc-Val-Leu-Phe-Gln-H (7), designed to mimic the P_1-P_4 position of the minimum sequence required for cleavage of the HRV-3C substrate, was subsequently reported by an Eli Lilly group to be a good inhibitor for HRV-14 3C proteinase.[41]

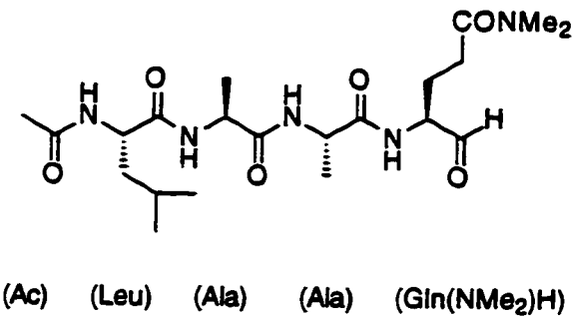


Figure 6 Peptidyl aldehyde **6**

(ii) Peptidyl halomethyl ketone

Affinity labeling of cysteine proteinases by peptidyl halomethyl ketones blocks the essential thiol group by alkylation.[42] Recently, Ringe and Abeles[43] reported the mechanism of interaction between chymotrypsin (a serine proteinase) and an α-chloroethyl ketone (Figure 7). They propose attack of the serine hydroxyl on the haloketone carbonyl, internal displacement of halide to form an epoxide, followed by oxirane ring opening at the less hindered position *via* attack of the histidine nitrogen. This results in an *N*-alkylated acetal as shown in Figure 7. However, crystallographic studies of the papain-like proteinase cruzain[44] inhibited by a fluoromethyl ketone and NMR studies of papain inactivated by a chloromethyl ketone[45] clearly demonstrate that these cysteine proteinases have the active site sulfhydryl replacing the halogen to form an α-keto sulfide (Figure 7). This could in principle occur by a mechanism similar to that proposed for chymotrypsin and chloroethyl

α-ketone,[43] namely attack of sulfhydryl on the carbonyl and generation of an epoxide, which would then have to be followed by sulfur migration. However, direct halogen displacement by the thiolate, which is much more nucleophilic than the serine hydroxyl, is likely.

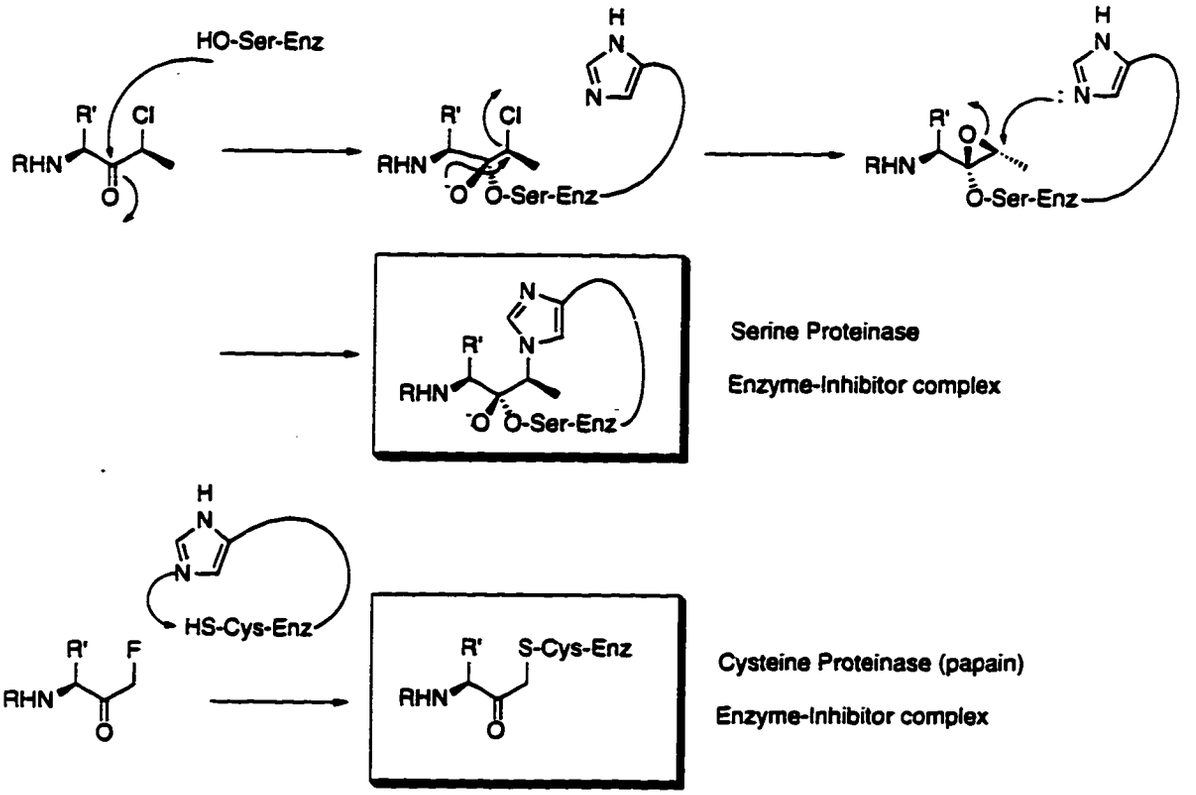


Figure 7 Modes of inhibition of serine and cysteine proteinases by haloalkyl ketones (taken from ref. 13)

Although selectivity can be achieved by varying the peptidyl residue, the high chemical reactivity of the halomethyl moiety towards general nucleophilic attack causes severe toxic effects in cellular systems.[46] Attempts were made to overcome these nonspecific side-reactions by using fluorine as the halogen, since the rate of alkylation of glutathione by fluoromethyl ketone is reduced to 0.2% of that observed with a chloromethyl ketone.[47] In spite of this, peptidyl fluoromethyl ketones retain a high degree of reactivity towards the cysteine proteinases, whereas the serine proteinases drop

considerably in susceptibility.[48] In Table 2 the affinity and rate of covalent bond formation in the reaction of cathepsin B with two fluoromethyl ketones (**8**, **9**) and a chloromethyl ketone (**10**) are compared.[49] Although the rate, k_i, of alkylation within the enzyme inhibitor complex is somewhat slower for both fluoromethyl ketones, this is offset by somewhat tighter binding (equilibrium constant K_i) such that the second-order rates of inactivation for the fluoromethyl ketones can even exceed that of the chloromethyl ketone.

Inhibitors	K_i (M)	k_i (s^{-1})	k_i/K_i ($M^{-1}s^{-1}$)
Cbz-Phe-Phe-CH_2F (**8**)	1.4×10^{-5}	0.055	3900
Cbz-Phe-Ala-CH_2F (**9**)	5.5×10^{-7}	0.030	54500
Cbz-Phe-Phe-CH_2Cl (**10**)	2.3×10^{-5}	0.208	9000

Table 2 Kinetic properties of peptidyl fluoromethyl and chloromethyl ketones inactivating cathepsin B.[49]

The peptide fluoromethyl ketone Ac-Leu-Ala-Ala-Gln(NMe_2)CH_2F (**11**) (Figure 8), recently synthesized in our group, was found to be a potent time-dependent, irreversible inhibitor of HAV-3C proteinase, with a second order rate constant ($k_{obs}/[I]$) = 330 $M^{-1}s^{-1}$ ([E] = 0.07μM, [I] = 1.0 μM) (unpublished results). The inhibitor reacts at the active site cysteine to form a covalent bond with the thiol group, as shown by NMR experiments.

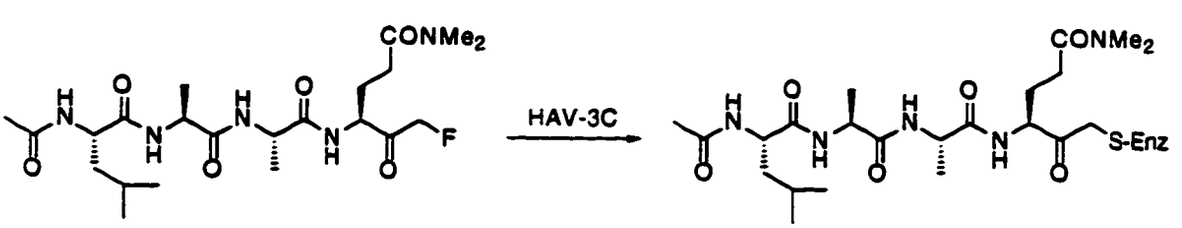


Figure 8 Reaction of peptidyl fluoromethyl ketone **11** with HAV-3C enzyme

(iii) Bromoacetyl azapeptide

Azapeptides are a class of backbone-modified peptides that have become important in pharmaceutical chemistry.[50] In these peptide analogues the α-CH group of one or more amino acid residues in a peptide chain is replaced by a nitrogen atom (Figure 9) with

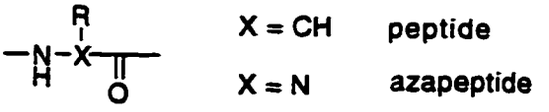


Figure 9 Azapeptides formed by the exchange of an α-CH group in a peptide chain by a N atom

retention of the original side chain.[50] Sham *et al.*[51] recently showed that the bromoacetyl azapeptide analogue (**12**) (Figure 10) is a potent inhibitor of the HRV-3C proteinase (IC_{50} = 48 nM). This inhibitor, based on backbone replacement at a common cleavage site (between glutamine and glycine) of HRV-3C proteinase, is a time-dependent irreversible inhibitor with a k_{inact}/K_i value of > 2500 $M^{-1}s^{-1}$.

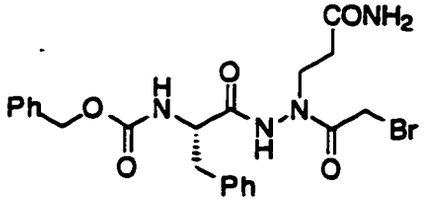
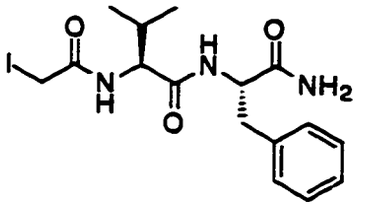


Figure 10 Bromoacetyl azapeptide (**12**)

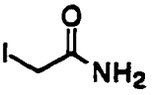
(iv) Peptidyl iodoacetamide (P' inhibitor)

Peptides that mimic the 3C cleavage site from the P' side also provide the potential for the development of new inhibitors. Peptidyl *N*-iodoacetamides, recently synthesized in our group by Dr. Sven Frormann (unpublished results), are P' inhibitors with modified N-termini. Iodoacetamide itself is a cysteine proteinase inhibitor, and also shows inhibition

against poliovirus 3C and HRV-3C proteinases (Table 1). Attachment of peptides that mimic the P_1'-P_2' subsite can increase selectivity. For example, ICH_2CO-Val-Phe-NH_2 (**13**) was shown to be a potent inhibitor of HAV-3C with a second order inactivation rate constant 45 times [$(k_{obs}/[I]) = 1.4 \times 10^2\ M^{-1}s^{-1}$ ([E] = 0.07μM, [I] = 1.0 μM)] that of iodoacetamide (**14**) [$(k_{obs}/[I]) = 3.1\ M^{-1}s^{-1}$] (Figure 11).



(**13**)



(**14**)

Figure 11 P' inhibitors

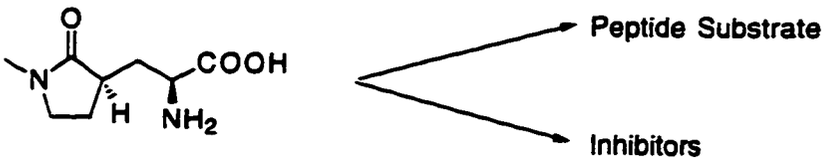
Project Aims: Design and synthesis of potential inhibitors of HAV-3C proteinase

The goal of this study is to investigate new types of potential warheads for selective HAV-3C proteinase inhibition. Since there are structural features common to all members of the picornavirus family, it is likely that insights gained into the mechanism and specificity of HAV-3C proteinase will aid in the development of specific inhibitors of other human picornaviruses. Five types of substrate analogues for HAV-3C were designed as illustrated in Figures 12 and 13 (**A-E**).

Target **A** (Figure 12) is a modified glutamine which can be incorporated as the P_1 residue (X) in a substrate analogue or inhibitor, for example, Glu-Leu-Arg-Thr-X-Ser-Phe-Ser-NH_2. Molecular modeling studies done by Dr. Marc Allarie using the mutant HAV-3C

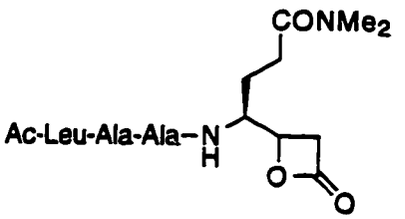
crystallographic structure suggest that such a cyclic rigid glutamine analogue **15** may bind especially effectively in the active site. Target **B** (Figure 12) is a modification of the P_1 residue bearing an adjacent ß-lactone. Since ß-lactones are readily opened by thiols[52] through attack at the ß-position, such a peptide (ie **16**) could irreversibly alkylate the active site cysteine-172 of HAV-3C.

Target **A**. Glutamine analogue



15

Target **B**. Peptidyl ß-lactone



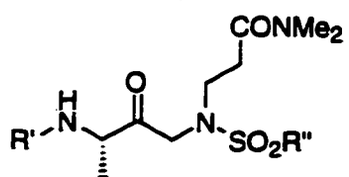
16

Figure 12 Targets **A** and **B**

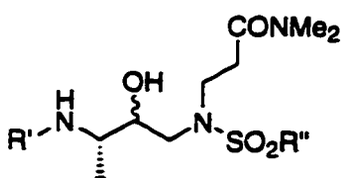
Three types (**C-E**) (Figure 13) of modification of the P_1 residue have the α-carbon replaced by a nitrogen with an adjacent sulfonamide, sulfenamide or haloacetyl moiety. The rationale for these targets will be discussed in more detail in the following section. However, with a few exceptions,[53] the potential of such nitrogen-sulfur bond replacements for proteinase inhibition has not been explored in detail previously. Thus, keto sulfonamides **17-20**, hydroxysulfonamides **21-22**, and two types of hydrazosulfenamides **23-27** are interesting substrate analogues for enzyme inhibition studies. The haloacetyl derivatives **28** and **29** are probable inhibitors based on recent

studies by Sham.[51] In the following sections, the design and results of both synthetic studies and biological assays of these targets are described.

Target C. Sulfonamides

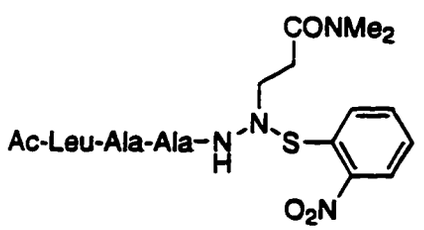


17 R' = Cbz R" = CH_3
18 R' = Cbz R" = *p*-CH_3Ph
19 R' = Ac-Leu-Ala R" = CH_3
20 R' = Ac-Leu-Ala R" = *p*-CH_3Ph

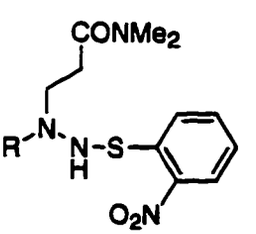


21 R' = Cbz R" = CH_3
22 R' = Cbz R" = *p*-CH_3Ph

Target D. Sulfenamides

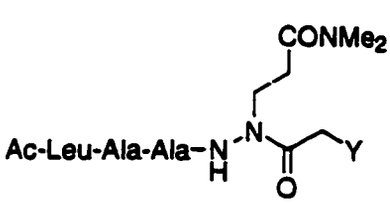


23



24 R = Ac-D-Ala
25 R = Ac-L-Ala
26 R = Ac-Leu-Ala-Ala
27 R = Ac-Leu-Ala-ß-Ala

Target E. Hydrazo haloacetyl tetrapeptides



28 Y = Cl
29 Y = Br

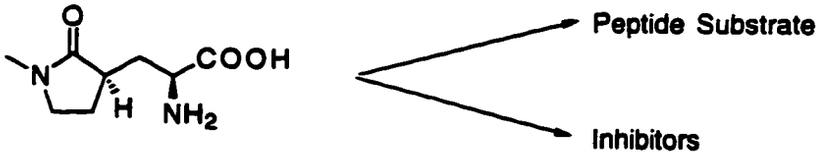
Figure 13 Targets C-E

RESULTS AND DISCUSSION

Part 1. Possible substrates and inhibitors of HAV-3C with *N*-methyl-2-pyrrolidinon-3-yl-alanine (15) (target A) as a glutamine analogue

1.1 Design

As indicated previously, the 3C proteinases require L-glutamine (or a suitable analogue) at the P_1 site for recognition and effective binding. This poses many problems for chemical synthesis and stability of substrate analogues or inhibitors. Molecular modeling of peptide substrates in the active site using the crystallographic coordinates of the mutant HAV-3C proteinase suggests that peptides having [(*3R*)-*N*-methyl-2-pyrrolidinon-3-yl]-L-alanine (**15**) (target A) (Figure 14) as a glutamine replacement may bind especially well. To test this, this compound was designed to be incorporated as the P_1 residue (X) in the octapeptide substrate analogue Glu-Leu-Arg-Thr-X-Ser-Phe-Ser-NH_2. If this were to prove to be either a good substrate or inhibitor of HAV-3C proteinase, it could be modified to generate additional inhibitors.



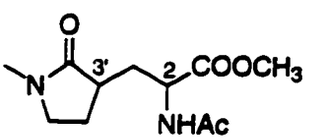
15

Figure 14 Target A.

1.2 Synthetic studies towards [(*3R*)-*N*-methyl-2-pyrrolidinon-3-yl]-L-alanine (15)

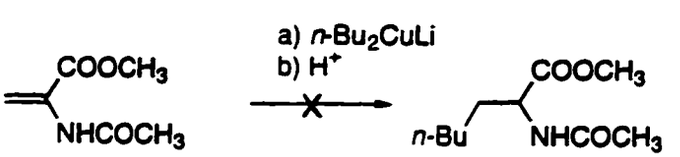
1.2.1. 1,4-Addition route

Our approach towards target **A** began with attempts to prepare [(*N*-methyl-2-pyrrolidinon-3-yl)-*N*-acetyl]alanine methyl ester (**30**) without extensive effort to control stereochemistry. It seemed likely that two diastereomeric pairs (e.g. 2*R*3'*S* + 2*S*3'*R* from 2*S*3'*S* + 2*R*3'*R*) of the four possible isomers could be separated by HPLC. The two sets of racemates could then be resolved by deacetylation with Acylase I.[54] This would generate four pure isomers for incorporation into the peptide and enzymatic testing.



30

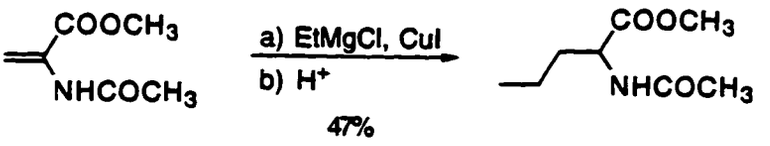
In principle, α-amino acid derivatives can be prepared by conjugate addition of a suitable organometallic reagent onto an α,ß-unsaturated ester such as methyl 2-acetamidoacrylate (**31**). It has been reported that no 1,4-addition product is obtained from the reaction between *N*-acylaminoacrylic acids[55] or related methyl esters[56] with lithium diorganocuprates. For example, reaction of methyl 2-acetamidoacrylate (**31**) with lithium diorganocuprate *n*-Bu_2CuLi does not provide any 1,4-addition product **32** (Scheme 1).



31 **32**

Scheme 1

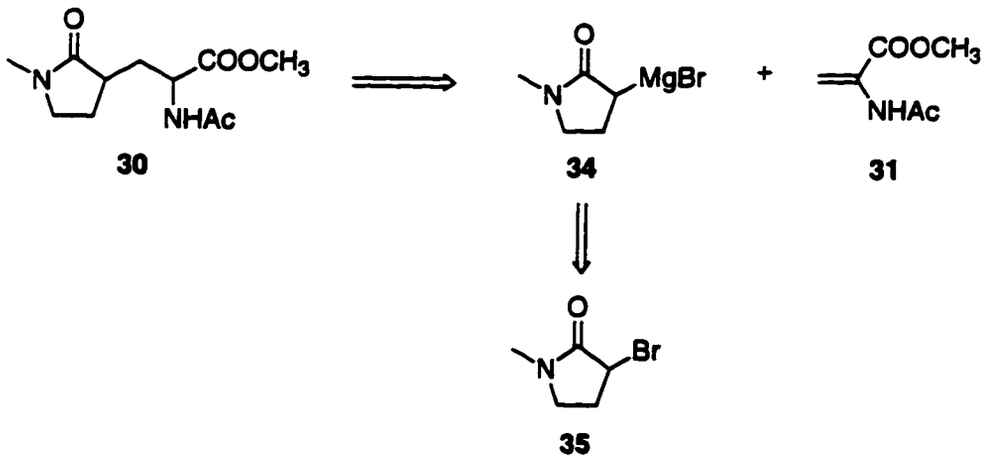
However, the reaction can be performed using a copper (I) catalyzed conjugate addition of a Grignard reagent onto the *N*-acylaminoacrylic acid or ester.[57] For example, reaction of methyl 2-acetamidoacrylate (**31**) with ethyl magnesium chloride in the presence of cuprous iodide (CuI) yields the 1,4-adduct methyl (2*RS*)-2-acetylaminopentanoate (**33**) (Scheme 2).



31 **33**

Scheme 2

In the hope of obtaining [(*N*-methyl-2-pyrrolidinon-3-yl)-*N*-acetyl]alanine methyl ester (**30**) directly, we applied this methodology starting from *N*-methyl-3-bromo-2-pyrrolidinone (**35**) and methyl 2-acetamidoacrylate (**31**) (Scheme 3).



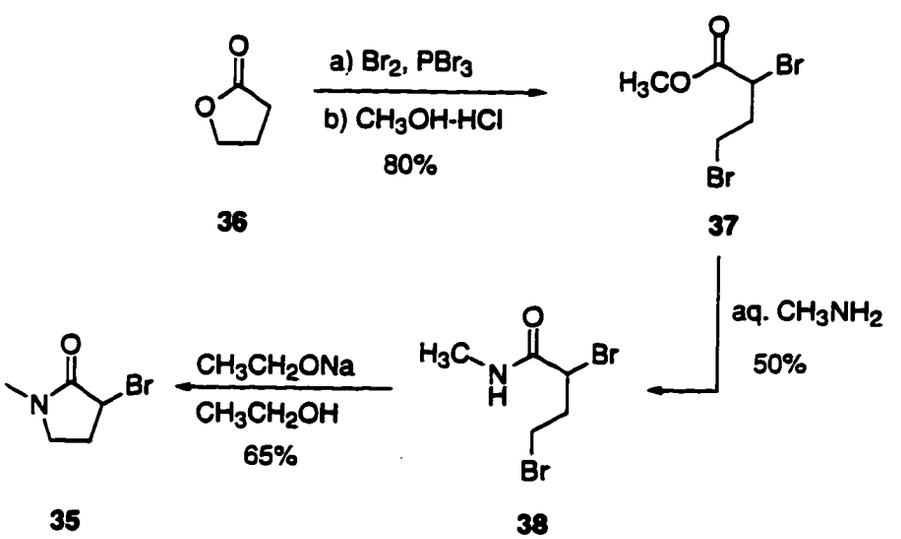
30 **34** **31**

35

Scheme 3

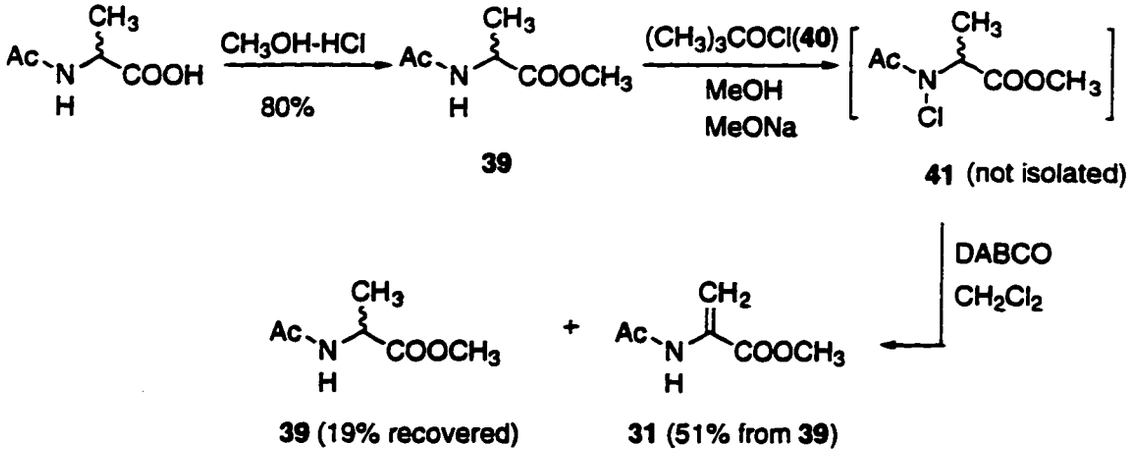
N-methyl-3-bromo-2-pyrrolidinone (**35**) can be synthesized by the literature procedure (Scheme 4).[58,59] Reaction of γ-butyrolactone (**36**) and bromine in the presence

of phosphorus tribromide provides 2,4-dibromobutyric acid. In order to avoid formation of α-bromo-γ-butyrolactone by elimination of hydrogen bromide, this crude acid is treated with a methanolic solution of hydrogen chloride, to generate methyl ester **37**. Subsequent reaction of **37** with aqueous CH_3NH_2 (40%) affords *N*-methyl-2,4-dibromobutanamide (**38**), which reacts with sodium ethoxide in absolute ethanol to yield *N*-methyl-3-bromo-2-pyrrolidinone (**35**)



Scheme 4

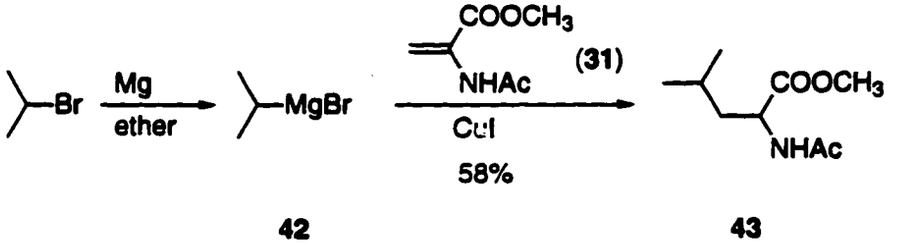
Methyl 2-acetamidoacrylate (**31**) is also available by a literature procedure (Scheme 5).[60] Treatment of *N*-acetyl alanine with methanol saturated with hydrogen chloride generates *N*-acetyl alanine methyl ester (**39**). The *N*-chlorination of ester **39** with *t*-butyl hypochlorite (**40**) in methanol with catalytic sodium methoxide provides the *N*-chloro derivative **41**. An elimination-isomerization process on the *N*-chloro compound **41** in the presence of 1,4-diazabicyclo[2,2,2]octane (DABCO) yields a separable 4 : 1 mixture of the acrylate **31** and the alanine ester **39**.



Scheme 5

Treatment of *N*-methyl-3-bromo-2-pyrrolidinone (**35**) with fresh magnesium turnings, following the literature procedure,[61] failed to give the desired Grignard reagent. Only starting material **35** could be recovered. All attempts to drive the reaction by varying conditions were unsuccessful.

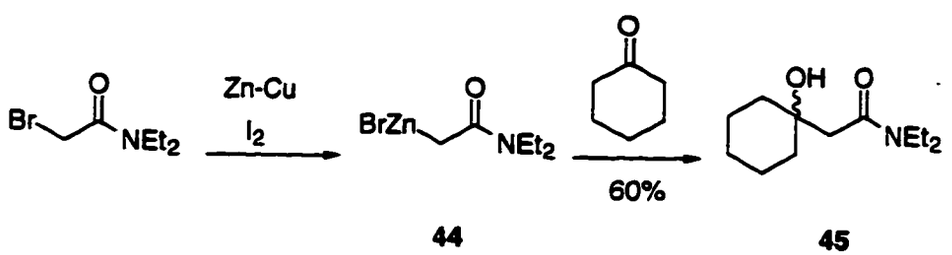
As a model reaction to test the feasibility of this process, a solution of isopropyl bromide and fresh magnesium turnings in THF readily affords Grignard reagent isopropyl magnesium bromide (**42**) (Scheme 6). Subsequent 1,4-addition of Grignard reagent **42** onto methyl 2-acetamidoacrylate (**31**) in the presence of CuI provides the desired 1,4-adduct, *N*-acetyl-DL-leucine methyl ester (**43**), in 58% yield. The yield is comparable to that (60 %) reported by Cardellicchio *et al.*[57] for a similar reaction using isopropyl magnesium chloride and compound **31**.



42 **43**

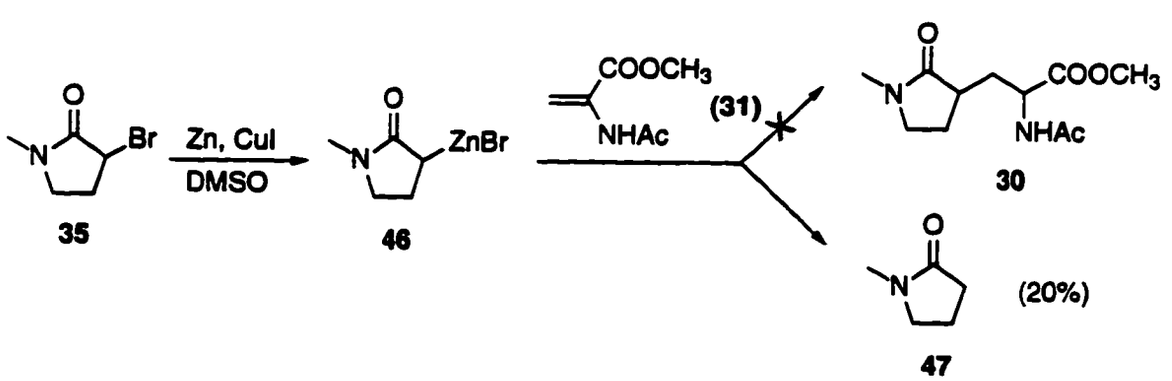
Scheme 6

Drake *et al.* reported a useful application of the Reformatsky reaction involving *N,N*-dialkyl-α-haloamides.[62] They demonstrated that *N,N*-dialkyl-α-haloamides can be substituted for α-bromoesters in the Reformatsky reaction with little reduction in yield. For example, *N,N*-diethyl-α-bromoacetamide reacts with zinc-copper alloy in the presence of iodine to generate the bromozinc compound **44**, which reacts with cyclohexanone to generate *N,N*-diethyl-α-[1-hydroxycyclohexyl]acetamide (**45**) in 60% yield (Scheme 7).



Scheme 7

Therefore, in an attempt to synthesize *N*-methyl-2-pyrrolidinon-3-yl-*N*-acetylalanine methyl ester (**30**), an analogous reaction was attempted with methyl 2-acetamidoacrylate (**31**) and the Reformatsky reagent **46**, generated by reacting *N*-methyl-3-bromo-2-pyrrolidinone (**35**) with zinc dust in the presence of CuI (Scheme 8). Disappointingly, treatment of the Reformatsky reagent **46** with the methyl



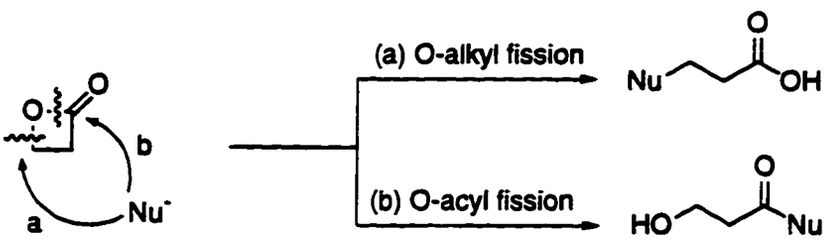
Scheme 8

2-acetamidoacrylate (**31**) in the presence of CuI does not yield any of the desired 1,4-addition product. The reaction produces *N*-methyl-2-pyrrolidinone (**47**) as a by-product, which may originate from 3-bromozinc-*N*-methyl-2-pyrrolidinone (**46**). Although not confirmed, possible problems with such reactions of organometallic derivatives of **45** may include protonation by the acidic amide NH of **31** as well as 1,2-addition.

Based on these results, it seems that the 1,4-addition route to *N*-methyl-2 pyrrolidinon-3-yl-*N*-acetylalanine methyl ester (**30**) is problematic, and this prompted us to explore an alternative method of synthesis.

1.2.2. ß-Lactone route

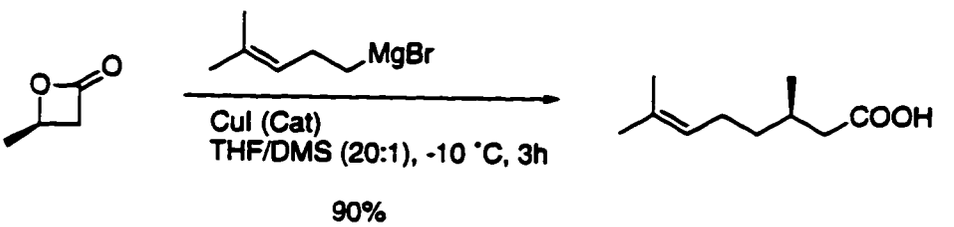
Over the last ten years, much attention has focused on the chemistry of ß-lactones.[63] This is because this moiety is present in many biologically active natural products[63b] and the inherent strain in the 4-membered ring can be exploited to perform useful synthetic reactions not normally associated with esters or lactones. Just as ß-lactones can be formed by oxygen-alkyl or oxygen-acyl bond formation,[64] in the presence of nucleophiles they also undergo cleavage by attack at the carbonyl or the ß-carbon (Scheme 9). ß-Lactones are therefore ambident electrophiles.[65]



Scheme 9

The reaction of organomagnesium and organolithium reagents with ß-lactones has been known for many years.[66-68] In the absence of Cu (I) salts, these reagents attack ß-lactones at the carbonyl group, leading to oxygen-acyl cleavage. However, Fujisawa *et*

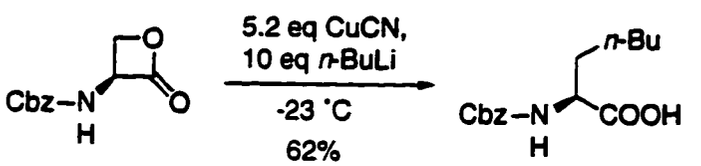
al.[69,70] and Normant *et al.*[71] independently showed that organocuprates react with ß-lactones *via* oxygen-alkyl cleavage, leading to a useful 3-carbon homologation. The reaction has been applied successfully to 2-oxetanone,[71] 4-methyl-2-oxetanone,[72] polymethyl-substituted 2-oxetanones[69,70] and serine ß-lactones[73] with a wide variety of organocuprates including alkyl-, allyl-, vinyl- and arylcuprates. Fujisawa and coworkers[74] also showed that ring opening of (+)-(*R*)-4-methyl-2-oxetanone (**48**) proceeds with clean inversion of configuration and thus obtained pure (*R*)-citronellic acid (**49**) when using 4-methyl-3-pentenylmagnesium bromide and copper (I) iodide as catalyst (Scheme 10).



48 **49**

Scheme 10

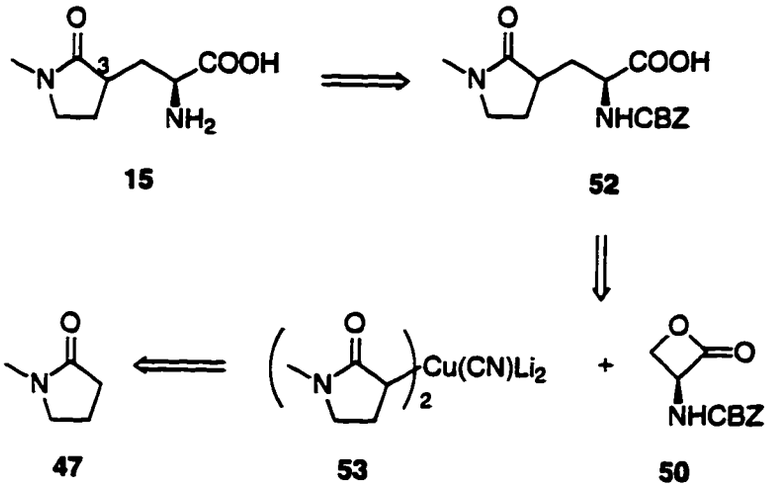
The following example reported by Vederas and coworkers[73] illustrates this process. Reaction of mono- and di-*N*-protected α-amino-β-lactones with organolithium-derived cuprate reagents (R_2CuLi or $R_2Cu(CN)Li_2$) at low temperature produces *N*-protected α-amino acids by attack at the β-methylene group. For example, the reaction of *N*-(benzyloxycarbonyl)-L-serine-β-lactone (**50**) with the cuprate *n*-$Bu_2Cu(CN)Li_2$, which is formed from CuCN in THF and *n*-BuLi in hexane, provides the corresponding 2-(benzyloxycarbonyl)aminoheptanoic acid (**51**) (Scheme 11).



50 **51**

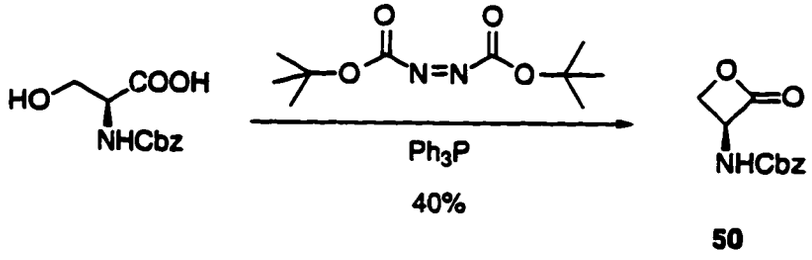
Scheme 11

Scheme 12 shows how this methodology could potentially be applied to the synthesis of target **A** (**15**). Although the stereochemistry at C-3 of the pyrrolidinone ring may not be controlled, possible resulting diastereomers should be separable.



Scheme 12

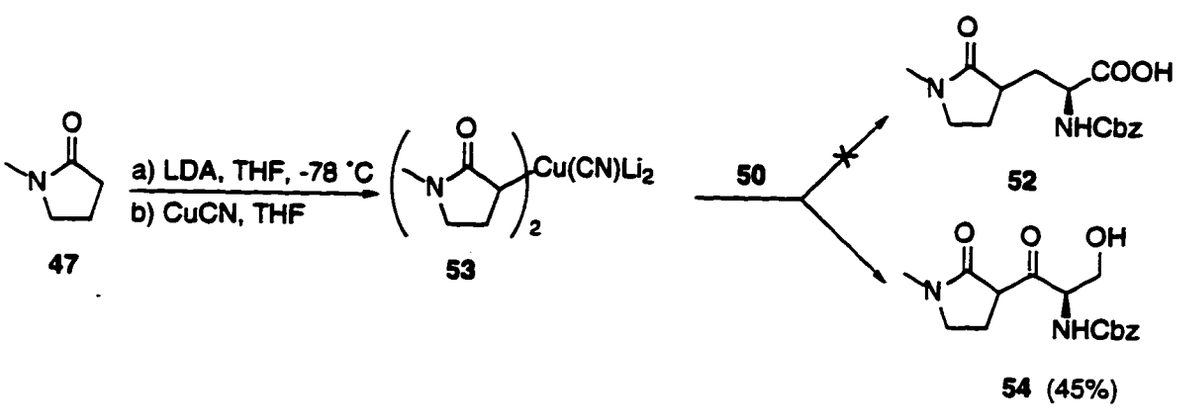
Reagent **50** is readily prepared following the literature procedure (Scheme 13).[75] Cyclization of the *N*-benzyloxycarbonyl-L-serine under modified Mitsunobu conditions using the preformed *N*-phosphonium adduct of Ph_3P and di-*tert*-butyl azodicarboxylate gives ß-lactone **50** without loss of optical purity.



Scheme 13

In an attempt to prepare *N*-Cbz-(*N*-methyl-2-pyrrolidinon-3-yl)alanine (**52**), an analogous reaction to that used in the synthesis of 2-(benzyloxycarbonyl)aminoheptanoic

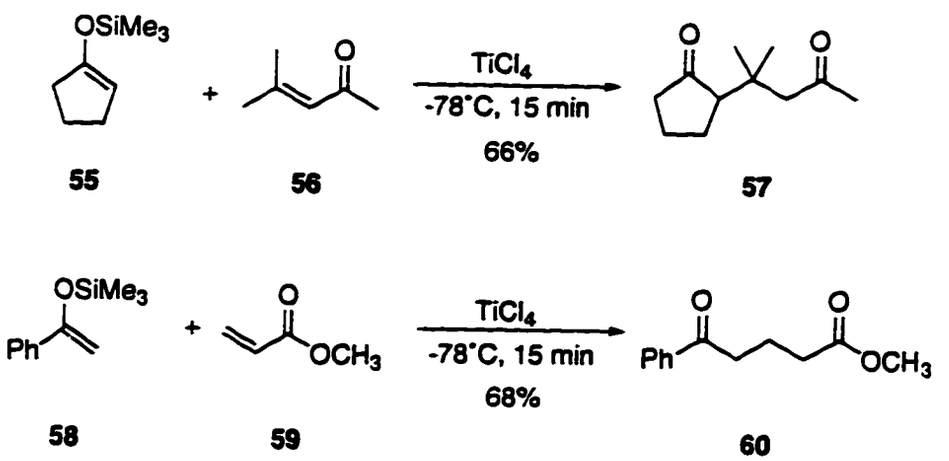
acid **51** (Scheme 11)[73] was attempted with β-lactone **50** and the cuprate reagent **53**. The latter is generated by treating *N*-methyl-2-pyrrolidinone (**47**) with lithium diisopropylamide (LDA), and then with CuCN (Scheme 14). Disappointingly, treatment of the β-lactone **50** with the cuprate reagent **53** does not provide the desired product *N*-Cbz-(*N*-methyl-2 pyrrolidinon-3-yl)alanine (**52**) by attack at the β-methylene group. The only product generated is the corresponding ketone (**54**) resulting from attack at the carbonyl group of the lactone with oxygen-acyl cleavage (Scheme 14).



Scheme 14

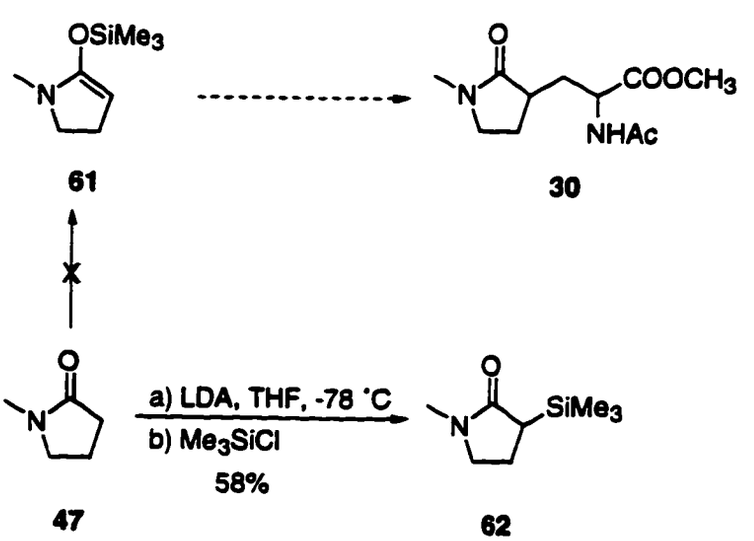
1.2.3. Silyl enol ether route

The Lewis acid-promoted reaction of α,β-unsaturated ketones and esters with silyl enol ethers is a successful Michael addition route for the preparation of 1,5-dicarbonyl compounds. For example, the reaction of 1-(trimethylsilyloxy)cyclopentene (**55**) with mesityl oxide (**56**) in the presence of $TiCl_4$ at -78 °C provides the corresponding Michael adduct **57** (Scheme 15).[76] Similarly, α-trimethylsilyloxystyrene (**58**) reacts with methyl acrylate (**59**) in the presence of $TiCl_4$ to generate the corresponding Michael adduct **60**.[76]



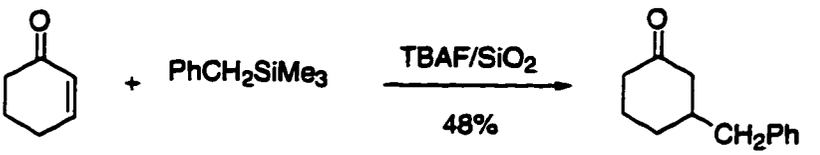
Scheme 15

In an attempt to prepare silyl enol ether **61**, which could hopefully be used in the synthesis of the [(*N*-methyl-2-pyrrolidinon-3-yl)-*N*-acetyl]alanine methyl ester **30**, *N*-methyl-2-pyrrolidinone (**47**) was treated with lithium diisopropylamide (LDA), followed by chlorotrimethylsilane (Scheme 16).[77] Interestingly, the reaction generates only (*N*-methyl-2-pyrrolidinon-3-yl)trimethylsilane (**62**), and none of the desired silyl enol ether **61**.



Scheme 16

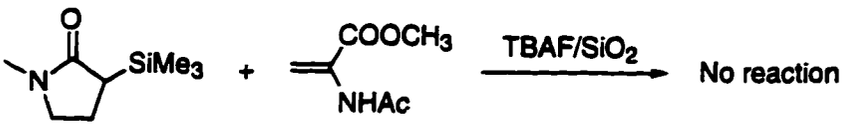
Ricci *et al.* reported fluoride ion induced Michael reactions.[78] Treatment of organosilanes containing a C-SiR_3 bond with fluoride ions in the presence of either saturated lactones or α,β-enones gave the corresponding 1,2-adducts and Michael products, respectively. For example, the reaction of benzyltrimethylsilane **63** with cyclohexen-2-one in the presence of TBAF/SiO_2 provides the Michael adduct **64** (Scheme 17).



63 **64**

Scheme 17

In an attempt to introduce the *N*-methyl-2-pyrrolidinon-3-yl moiety in a similar manner, an analogous reaction was attempted with *N*-methyl-2-pyrrolidinon-3-yl-trimethylsilane (**62**) (Scheme 18). Unfortunately, treatment of the organosilane **62** with methyl 2-acetamidoacrylate (**31**) in the presence of TBAF/SiO_2 does not yield any desired Michael adduct **30**, and a significant amount (ca. 60 %) of silane **62** is recovered. Neither heating the reaction mixture nor the addition of CsF to assist the desilylation is successful in effecting the Michael addition.



62 **31**

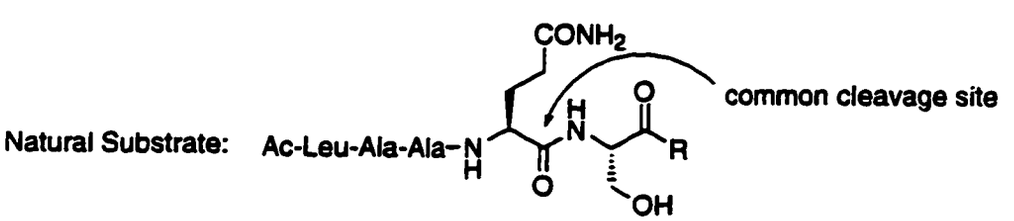
Scheme 18

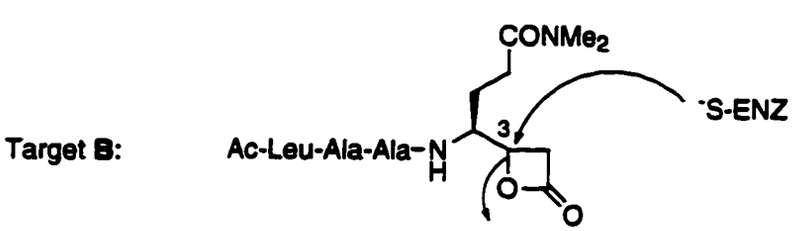
Based on the above observations, it appears that the low reactivity of organosilane **62** with methyl 2-acetamidoacrylate **31** might be responsible for the failure of this process. The lack of success from these approaches combined with progress toward the other targets caused us to abandon the synthesis of target **A**.

Part 2. Potential inhibitors bearing a ß-lactone at the C-terminus (Target B)

2.1 Design

N-Acyl-α-amino-ß-lactones are useful synthetic intermediates and also occur naturally;[63b] they are produced by many microorganisms as antibiotics.[75,79-81] The ß-lactone functionality is generally quite stable below pH 7.5. However, thiolate reacts at the ß-position to form sulfides under neutral or somewhat acidic (pH 5.5) aqueous conditions, or at the carbonyl to form thiol esters in non-aqueous environments.[82] A wide variety of carbon, nitrogen, phosphorus and oxygen nucleophiles are also known to attack the methylene group of serine ß-lactone.[75,83] It is likely that such functionality may react irreversibly with the HAV-3C proteinase at the active site. Hence, target **B** (**16**) (Figure 15) incorporates the tetrapeptide analogue, Ac-Leu-Ala-Ala-Gln(NMe_2), as a mimic of the substrate bearing a ß-lactone functionality for specific recognition and binding to the HAV 3C proteinase. Both stereoisomers at C-3 are interesting targets since either could in principle form a covalent bond with the active site thiol. Molecular modeling also suggests that such tetrapeptide ß-lactones could be potential inhibitors of the enzyme.[84]



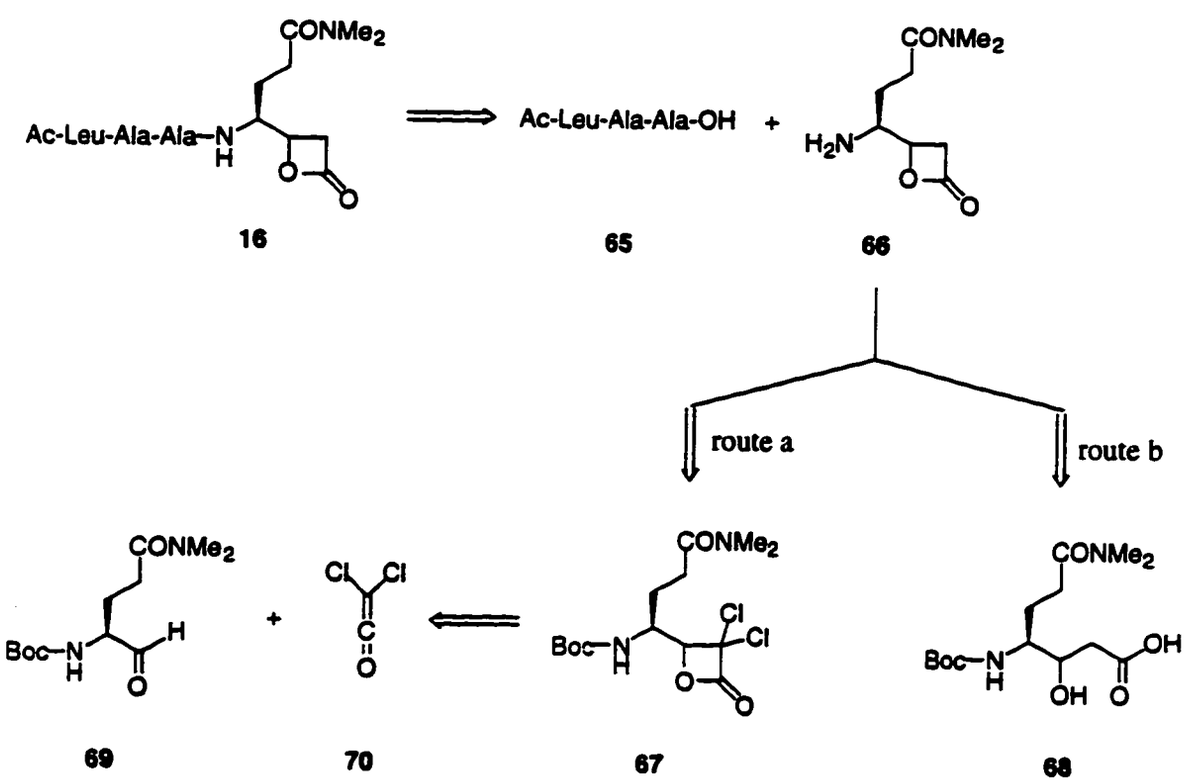


16

Figure 15 Target B

2.2 Synthetic studies towards peptidyl ß-lactone (target B)

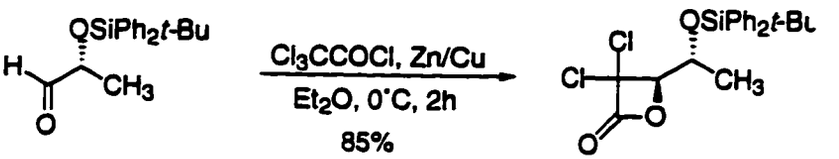
The strategy for the construction of target **B** is based on the retrosynthetic analysis outlined in Scheme 19. The target molecule **16** could be derived from a tripeptide Ac-Leu-Ala-Ala-OH (**65**) and a Gln(NMe_2)(ß-lactone) **66**. Tripeptide **65** is readily prepared on a Rainin PS-3 solid-phase peptide synthesizer using standard Fmoc chemistry on Wang Resin. The key ß-lactone **66** could in principle be synthesized by [2 + 2] cycloaddition of the aldehyde **69** with dichloroketene **70** (route a) to generate dichloro-β-lactone **67**, which could be dehalogenated using Pd/C catalyzed hydrogenation. Alternatively, cyclization of ß-hydroxy carboxylic acid **68** could lead to the desired ß-lactone **66** (route b).



Scheme 19

2.2.1. [2 + 2] Cycloaddition route (route a)

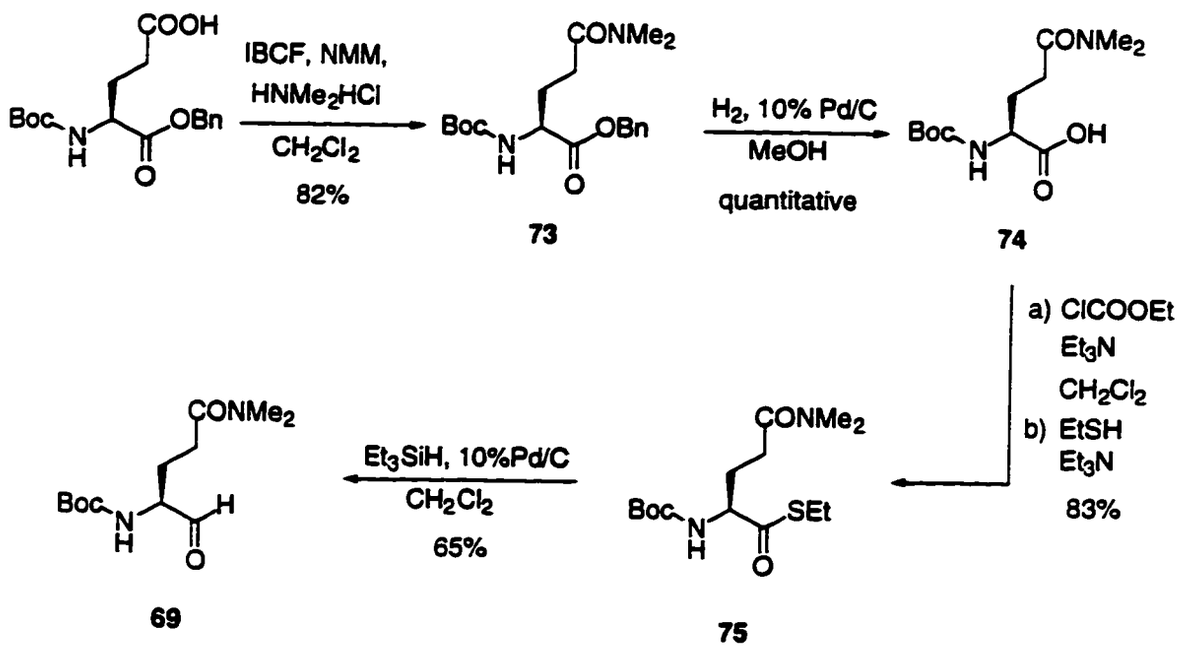
The cycloaddition of ketenes, particularly halogenated ketenes,[85] with carbonyl compounds is a powerful approach to ß-lactones.[86] The following example reported by Palomo *et al.* illustrates this process.[87] Reaction of dichloroketene, generated from trichloroacetyl chloride and Zn/Cu,[88] with the *O*-(*tert*-butyldiphenylsilyl)lactaldehyde (**71**) affords ß-lactone **72** in 85% yield (Scheme 20).[87]



71 **72**

Scheme 20

In the hope of obtaining monomer ß-lactone **66** in a similar manner, we applied this methodology to precursor aldehyde **69**. The aldehyde **69** can be synthesized by the following procedure (Scheme 21). *N*-*t*-Boc-glutamic acid α-benzyl ester reacts with *iso*-butyl chloroformate (IBCF) in the presence of *N*-methyl morpholine (NMM) to generate a mixed anhydride which condenses with dimethylamine hydrochloride, to provide *N*-*t*-Boc-δ-dimethylglutamine α-benzyl ester (**73**).[89] Subsequent hydrogenation of **73** in the presence of 10% palladium on charcoal in MeOH affords acid **74**,[90] which is readily converted to thioester **75** by reaction with ethyl chloroformate and ethanethiol in the presence of triethylamine.[91] Reduction of thioester **75** with triethylsilane and a catalytic amount of 10% palladium on charcoal generates the aldehyde **69**.[92]

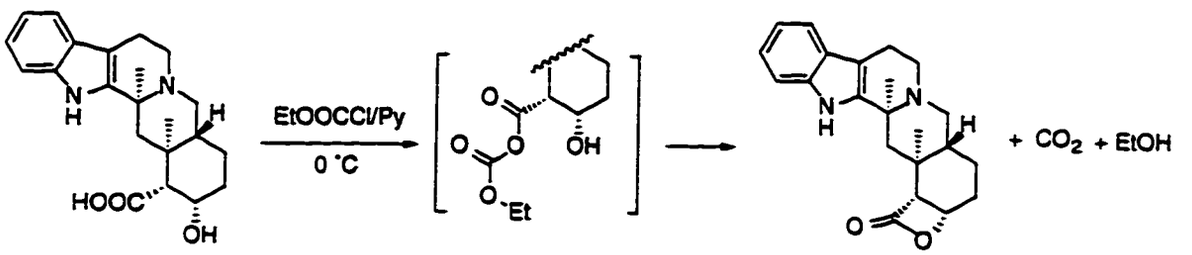


Scheme 21

With the successful synthesis of precursor aldehyde **69** complete, attention focused on the preparation of ß-lactone **66**. Treatment of aldehyde **69** with dichloroketene, generated from trichloroacetyl chloride and Zn/Cu[88] in ether, following the literature procedure,[87] fails to give the desired product, possibly due to the sensitivity of the Boc group and the harsh reaction conditions. A second method for the preparation of dichloroketene involves reaction of dichloroacetyl chloride and triethylamine in CH_2Cl_2.[87] This procedure also fails to give the desired product, and hence this approach was abandoned.

2.2.2. Cyclization of ß-hydroxy carboxylic acid 68 (route b)

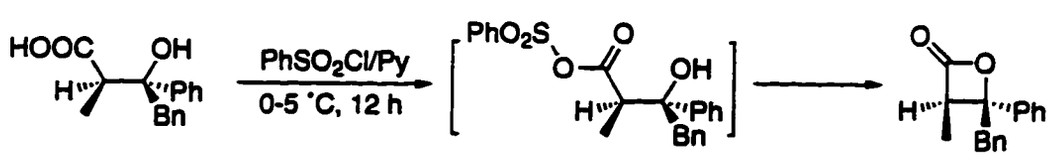
It has been about 40 years since Diassi synthesized yohimbic acid lactone (**77**), in which the ß-lactone moiety was obtained *via* direct cyclization of yohimbic acid (**76**) with ethyl chloroformate in pyridine (Scheme 22).[93] Such cyclizations of ß-hydroxy carboxylic



76 **77**

Scheme 22

acids have also been observed with carbodiimide reagents.[94,95] A major improvement occurred with the introduction, by Adam *et al.*,[96] of the use of benzenesulfonyl chloride/pyridine as the lactonization reagent. The conversion of ß-hydroxy acids to ß-lactones proceeds *via* a mechanism involving the formation of a mixed anhydride intermediate and its attack by the hydroxy group. For example, ß-lactone **80** is prepared in excellent yield from acid **78** *via* mixed anhydride **79** (Scheme 23).[96] Benzenesulfonyl chloride/pyridine is presently the most commonly used reagent for the preparation of ß-lactones,[97-100] including α-methylene-ß-lactones.[101]

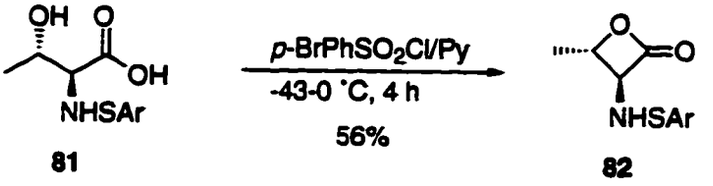


78 **79** **80**

Scheme 23

Other sulfonyl chlorides have also been successfully used, for example, tosyl chloride/pyridine[102] and *p*-bromobenzenesulfonyl chloride/pyridine.[80] Vederas and coworkers observed that the best cyclization conditions for ß-hydroxy amino acid derivatives which bear a ß-alkyl substitutent are *p*-bromobenzenesulfonyl chloride/pyridine at -43 °C to 0 °C.[80a] Thus, optically pure ß-hydroxy amino acid derivative **81** (Ar=

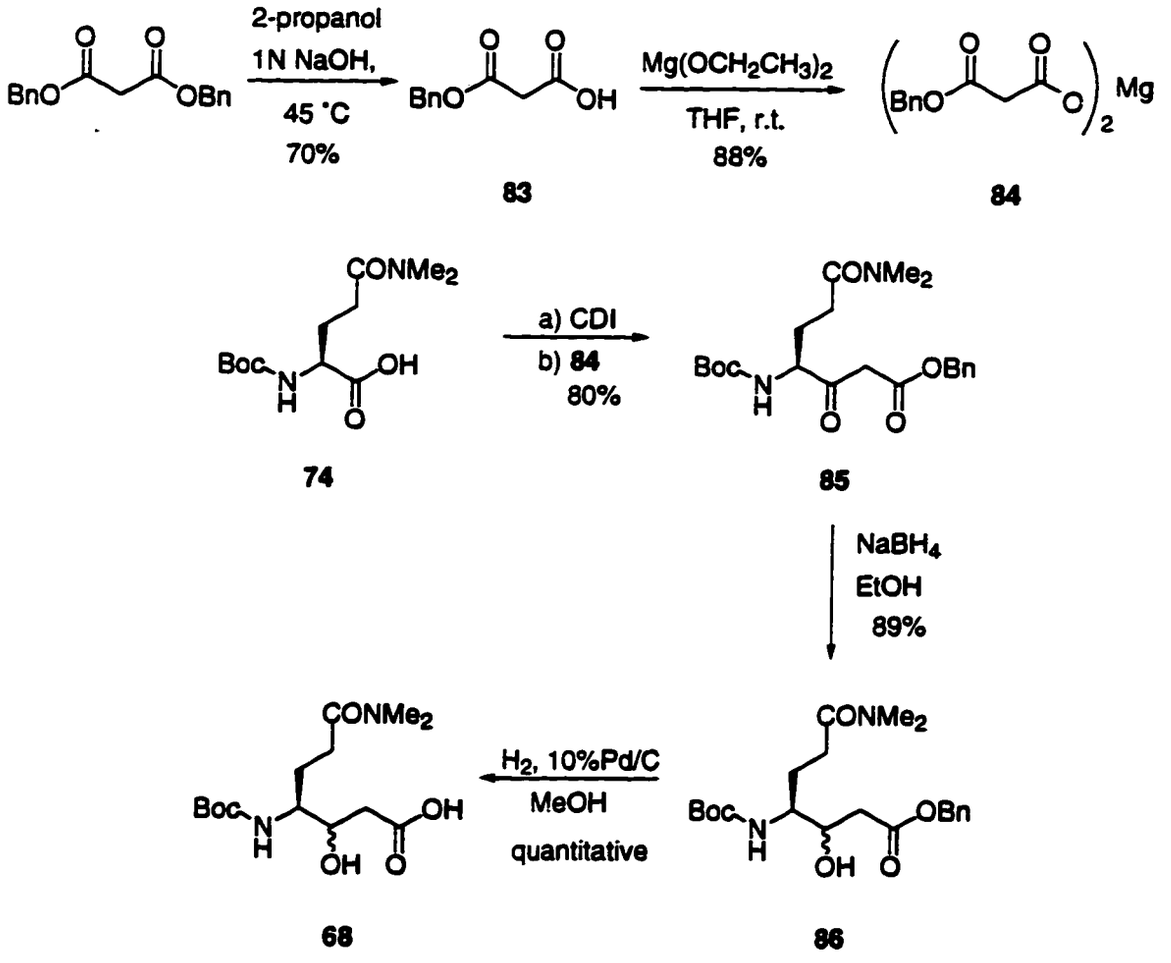
o-nitrophenyl) cyclizes to the corresponding ß-lactone **82** on treatment with *p*-bromobenzenesulfonyl chloride/pyridine, in 56% yield (Scheme 24).



81 **82**

Scheme 24

To adapt this procedure for the preparation of ß-lactone **66**, the precursor ß-hydroxy amino acid derivative **68** was synthesized as shown in Scheme 25.[89] Treatment



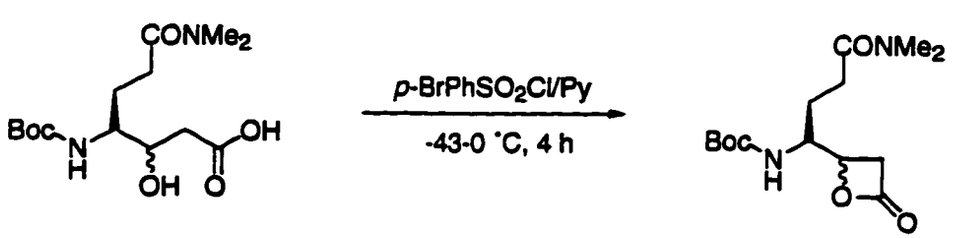
74 **85**

68 **86**

Scheme 25

of dibenzyl malonate with aqueous 1N NaOH in 2-propanol, at 45 °C, affords the monobenzyl malonate **83**, which is then readily converted to the magnesium salt **84** by reaction with magnesium ethoxide. The acid **74** is activated with carbonyl-1,1-diimidazole (CDI), then condensed with magnesium salt **84**, and then decarboxylated by acid work-up to provide β-keto ester **85**. Reduction of **85** with $NaBH_4$ in ethanol generates β-hydroxy ester **86**.[103] Hydrogenation of benzyl ester **86** using 10% palladium on charcoal in MeOH provides the desired ß-hydroxy acid **68**.[90]

Monitoring of the cyclization reaction of ß-hydroxy acid **68** in the presence of *p*-bromobenzenesulfonyl chloride/pyridine by TLC suggests formation of the ß-lactone **87** (Scheme 26). Generally, the ß-lactones appear as a yellow spot on a blue background after heating when developed with alkaline bromocresol green spray.[104] This is observed for the reaction of ß-hydroxy acid **68** with *p*-bromobenzenesulfonyl chloride/pyridine before work-up. However, after acidic work-up, the intensity of the yellow spot decreases, and the IR spectrum shows only a very weak peak around 1840 cm^{-1}. After purification by flash chromatography, no ß-lactone is present by TLC. This observation suggests that *N-t*-Boc-Gln(NMe_2)(ß-lactone) **87** decomposes readily under these work-up conditions.



68 **87**

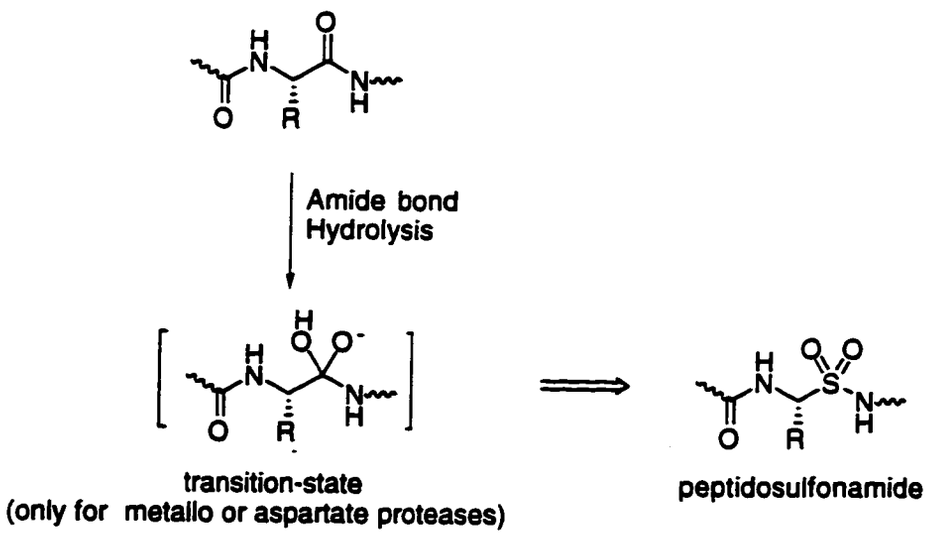
Scheme 26

From ß-hydroxy acid **68**, only three more steps are required to reach the target **B**. Accumulation of sufficient ß-hydroxy acid **68** to complete the subsequent reactions and investigation of conditions under which the cyclization may be improved, using different coupling strategies, are necessary before synthesis of target **B** can be achieved.

Part 3. Peptidosulfonamides as potential inhibitors of HAV-3C proteinase (Target C)

3.1 Design

Transition state isosteres that mimic intermediates in hydrolysis of the amide bond are important proteinase inhibitors used to develop therapeutic agents and are used for the generation of catalytic antibodies.[105] Peptidosulfonamides, in which the amide bond in a peptide or peptidomimetic is replaced by a sulfonamide unit, have been employed as such transition state isosteres (Scheme 27).[106]



Scheme 27 Metal or aspartate protease

For example, VX-478, a *N,N*-disubstituted(hydroxyethyl)amino sulfonamide (Figure 16), has been found to be a potent, low molecular weight, orally bioavailable competitive inhibitor of HIV-1 and HIV-2 proteinases with K_i values of 0.60 nM and 19 nM, respectively.[106] The HIV (human immunodeficiency virus) proteinases belong to the aspartate proteinase family,[107] and employ enzyme-bound water as the nucleophile to cleave the peptide bond. The inhibitor, VX-478, reduces HIV-IIIB viral load by greater than 90% in CEM cells at a concentration of 40 nM.

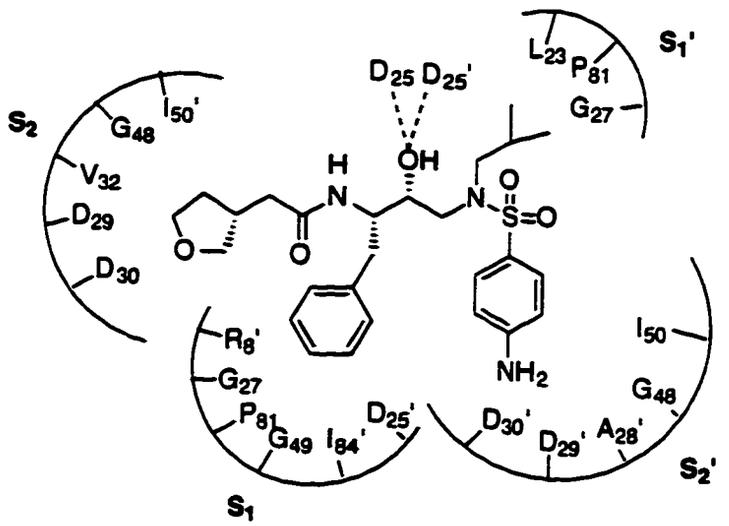
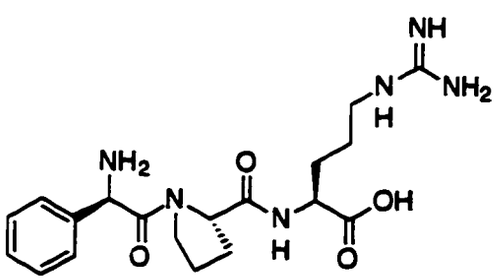
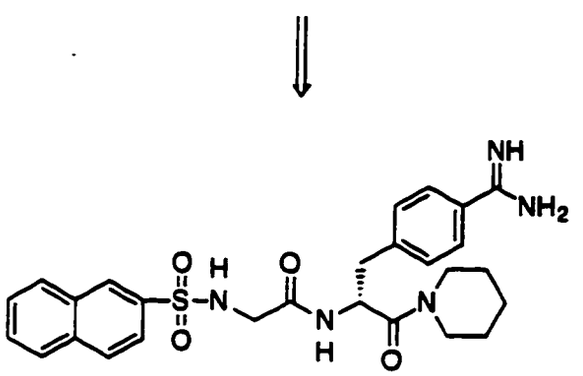


Figure 16 Chemical structure of VX-478, with binding pockets

Sulfonamides are also potent and highly selective inhibitors of thrombin, which is a trypsin-like serine proteinase.[108] For example, peptidosulfonamide ([*R*]-NAPAP) **88** (Scheme 28), which is based on the substrate analogue D-Phe-Pro-Arg, has been found to



(D-Phe-Pro-Arg)

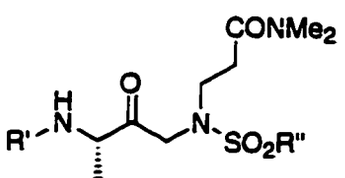


88

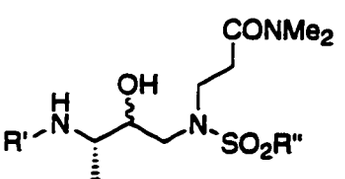
Scheme 28

be a potent and highly selective thrombin inhibitor with K_i = 4 nM and the ratio of K_i trypsin/ K_i thrombin = 30.

Although the above examples show that peptidosulfonamide transition state isosteres can inhibit both aspartate and serine proteinases, they have never been reported as cysteine proteinase inhibitors. In order to test this use with HAV-3C, several peptidosulfonamides (Figure 17) were designed as potential inhibitors. Since a L-glutamine (or suitable analogue) at the P_1 site is required for recognition and efficient binding, a series of sulfonamide compounds (**17-22**) was designed, as shown in Figure 17. The compounds **17**, **18**, **21** and **22** were chosen as sulfonamide dipeptide analogues to compare specific recognition and binding to tetrapeptide analogues such as **19** and **20**.



17 R' = Cbz R" = CH_3
18 R' = Cbz R" = *p*-CH_3Ph
19 R' = Ac-Leu-Ala R" = CH_3
20 R' = Ac-Leu-Ala R" = *p*-CH_3Ph

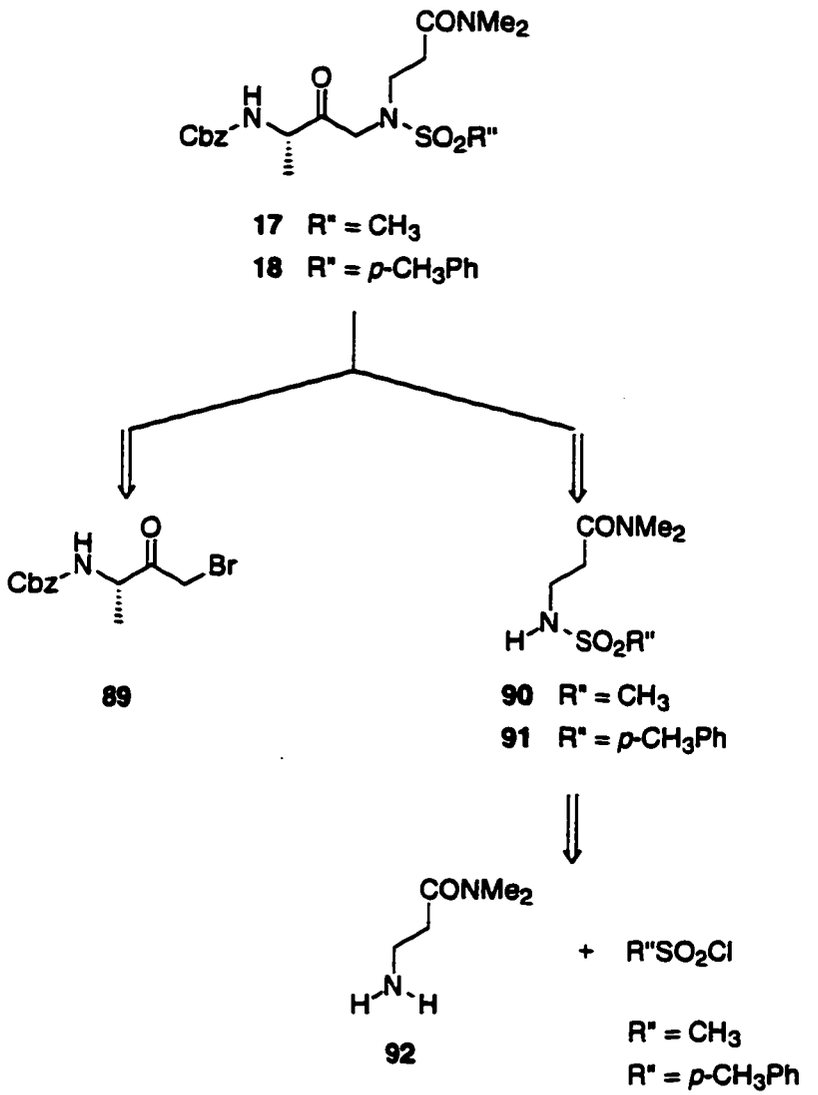


21 R' = Cbz R" = CH_3
22 R' = Cbz R" = *p*-CH_3Ph

Figure 17 Target C Peptidosulfonamides

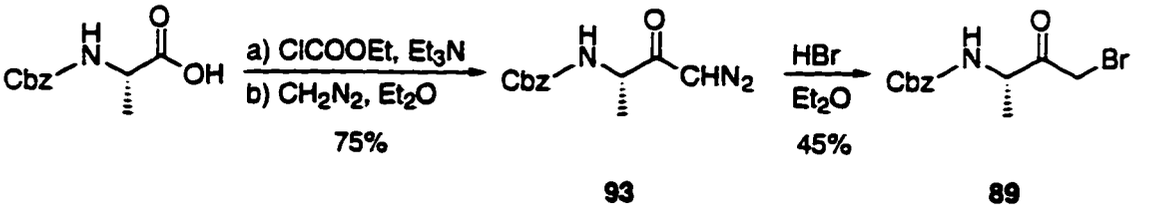
3.2 Synthetic studies towards peptidosulfonamides (target C)

A retrosynthetic analysis (Scheme 29) of compounds **17** and **18** indicates that key intermediates would be α-bromomethyl ketone **89** and sulfonamides **90** and **91**. A possible route for the construction of the sulfonamides (**90**, **91**) would involve sulfonylation of amine **92**.



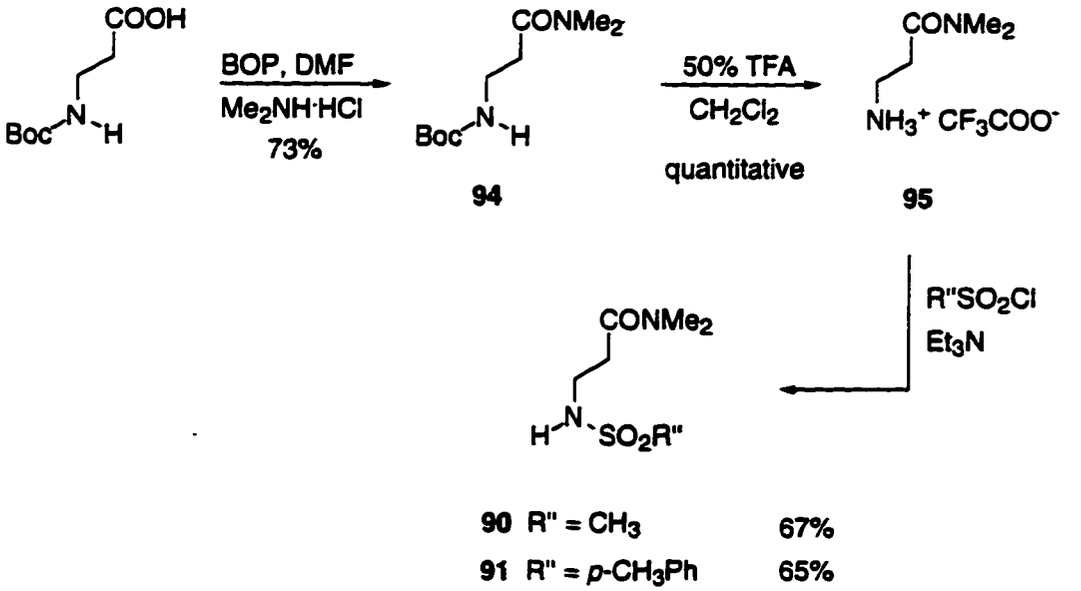
Scheme 29

α-Bromomethyl ketone **89** was synthesized from Cbz-alanine following a literature procedure (Scheme 30).[109] Cbz-Alanine reacts with ethyl chloroformate in the presence of triethylamine to produce the expected mixed anhydride, which upon treatment with ethereal diazomethane affords α-diazomethyl ketone **93**. Subsequent bromination of **93** with HBr provides α-bromomethyl ketone **89**.



Scheme 30

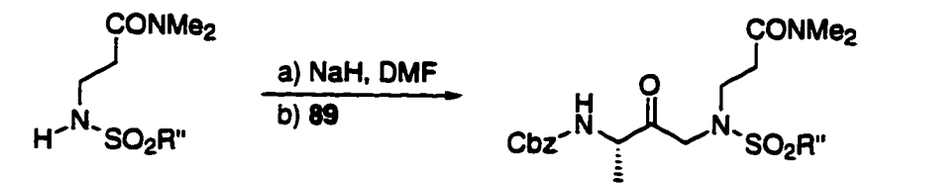
The key intermediate sulfonamides (**90**, **91**) can be prepared by sulfonylation of amine salt **95** in the presence of triethylamine. The salt **95** is synthesized by coupling *N-t*-Boc-ß-alanine with dimethylamine hydrochloride in the presence of benzotriazolyl-*N*-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) to generate *N-t*-Boc-ß-Ala(NMe_2) (**94**), which is then deprotected with 50% trifluoroacetic acid in methylene chloride (Scheme 31).



Scheme 31

Jones reported the *N*-alkylation of sulfonamides with α-halo ketones in the presence of NaH. His procedure generates *N,N*-disubstituted sulfonamides in very high yields.[110] This methodology is ideal for preparation of target compounds **17** and **18**.

Reaction of sulfonamides **90** and **91** with α-bromomethyl ketone **89** in the presence of NaH gives the corresponding *N*-alkylated sulfonamides **17** and **18** (Scheme 32). With the successful preparation of the target compounds **17** and **18**, attention focused on the other target compounds **19-22**.

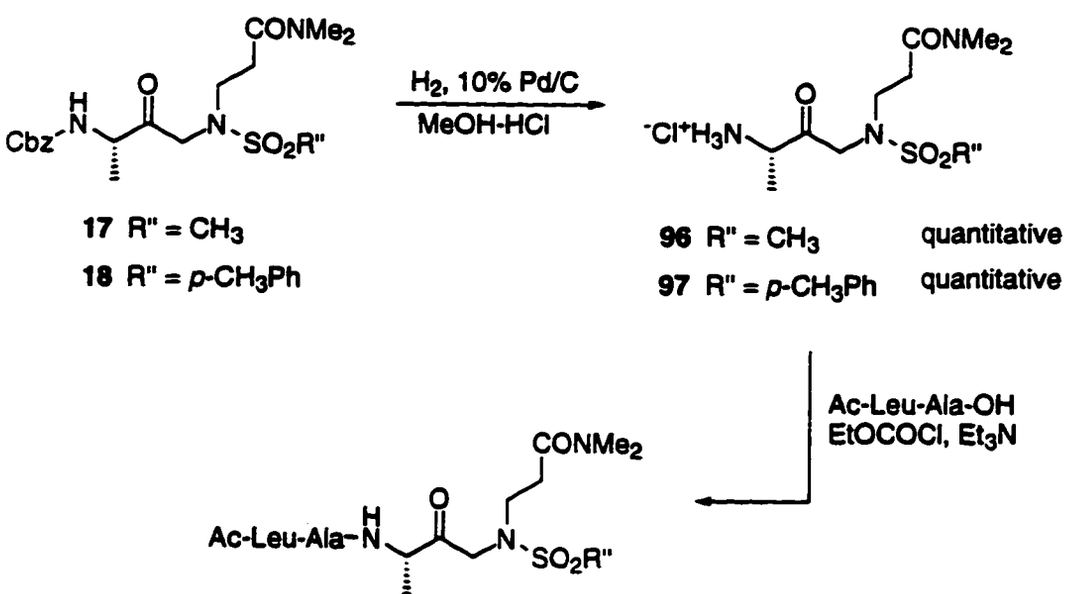


90 R" = CH_3
91 R" = *p*-CH_3Ph

17 R" = CH_3 48%
18 R" = *p*-CH_3Ph 52%

Scheme 32

Compounds **19** and **20** can be approached by the routes outlined in Scheme 33. Hydrogenation of **17** and **18** in the presence of 10% Pd/C catalyst in MeOH-HCl



17 R" = CH_3
18 R" = *p*-CH_3Ph

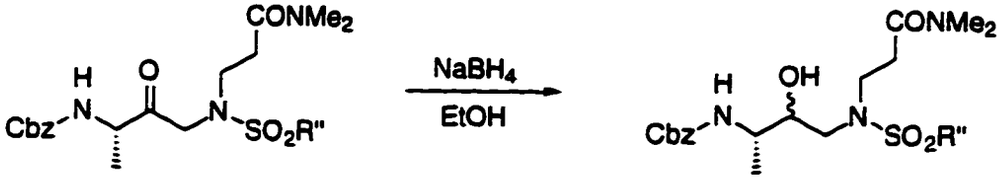
96 R" = CH_3 quantitative
97 R" = *p*-CH_3Ph quantitative

19 R" = CH_3 50%
20 R" = *p*-CH_3Ph 56%

Scheme 33

provides hydrochloride salts **96** and **97**, respectively. Subsequent coupling with dipeptide Ac-Leu-Ala-OH *via* a mixed anhydride methodology affords **19** and **20**, respectively. Acidic conditions are used for the hydrogenation in order to trap the free β-keto amine, which otherwise might form an imine by intermolecular condensation.

Compounds **21** and **22** can be generated by $NaBH_4$ reduction of **17** and **18** respectively (Scheme 34).[103]



17 R" = CH_3
18 R" = *p*-CH_3Ph

21 R" = CH_3 89%
22 R" = *p*-CH_3Ph 87%

Scheme 34

3.3 Results of inhibition studies with HAV-3C proteinase

Target **C** compounds (**17-22**) were assayed by the standard methods,[39] which are described in the experimental section. These enzyme inhibition studies were done by Shirley Shechosky (Department of Biochemistry, University of Alberta).[39b] Only compound **17** proved to be a competitive inhibitor of HAV-3C with an IC_{50} of about 75 μM, which is significantly lower than the K_m for an ideal hexapeptide substrate (2.1 mM), but not potent enough to warrant extensive study. The other target **C** compounds (**18-22**) did not show any significant inhibition of HAV-3C proteinase at 100 μM.[39b]

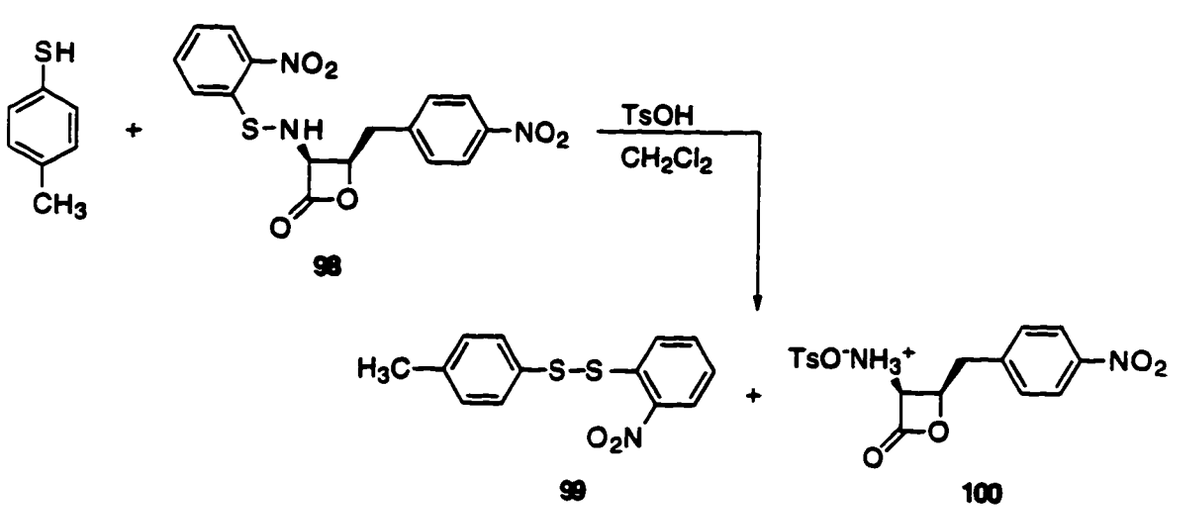
In summary, target **C** compounds (**17-22**), were designed as potential inhibitors of HAV-3C proteinase. These were synthesized through the key intermediates, α-bromomethyl ketone **89** and sulfonamides **90** and **91**. Although the exact reasons for the failure of tetrapeptide analogues **19** and **20** to inhibit HAV-3C remain unknown, it may be that the geometry of the sulfonamide moiety cannot be accommodated in the active site.

In particular, the difference in bond lengths (sulfur vs carbon) and presence of an additional oxygen on the sulfur ("carbonyl isostere") may be problematic. Nevertheless, such compounds could still be tested with other cysteine proteinases[111] for development of medicinal agents.

Part 4. Hydrazo peptidyl sulfenamides as potential inhibitors of HAV-3C proteinase (Target D)

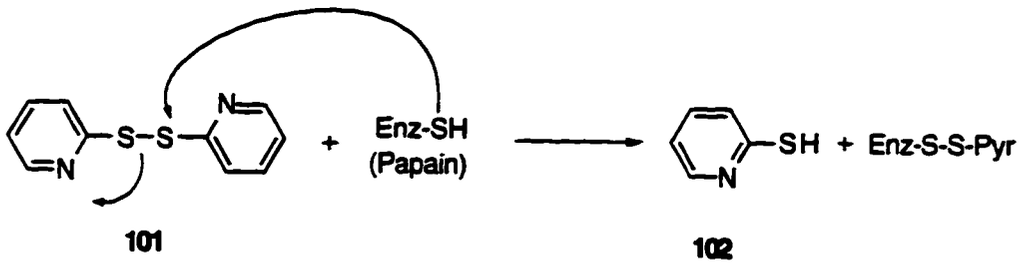
4.1 Design

Sulfenamides have been of interest because of their industrial applications, their utility as synthetic reagents and their interesting stereochemical properties.[112] They are readily cleaved by acid hydrolysis or by nucleophiles such as thiols. In reactions with nucleophiles, the sulfenamide bond is usually attacked at the sulfur. For example, treatment of *N*-[(2-nitrophenyl)sulfenyl]-(*2S, 3R*)-2-amino-3-(4-nitrobenzyl)-oxetanone (**98**) with 4-thiocresol and 4-toluenesulfonic acid generates the unsymmetrical disulfide **99** and the tosylate salt of the parent oxetanone (**100**) (Scheme 35).[80] This suggests that replacement of the P_1 carbonyl with the sulfur of a sulfenamide could generate an irreversible



Scheme 35

inhibitor of a cysteine proteinase. A precedent for this exists in the reaction of papain (a cysteine proteinase) with 2,2'-dipyridyl disulfide (**101**) (Scheme 36) which results in the stoichiometric release of the thiol fragment 2-mercaptopyridine (**102**).[113] As described in



Scheme 36

the introduction, azapeptides are a class of backbone-modified peptides that has become important in pharmaceutical chemistry. Hence, compound **23**, which combines an aza analogue of *N,N*-dimethylglutamine with a peptidyl sulfenamide, was chosen as a target (Figure 18). The structural isomer **26**, wherein the hydrazo nitrogen is placed at the nominal position of the P_1 carbonyl and the sulfur occupies the P_1' nitrogen site, and

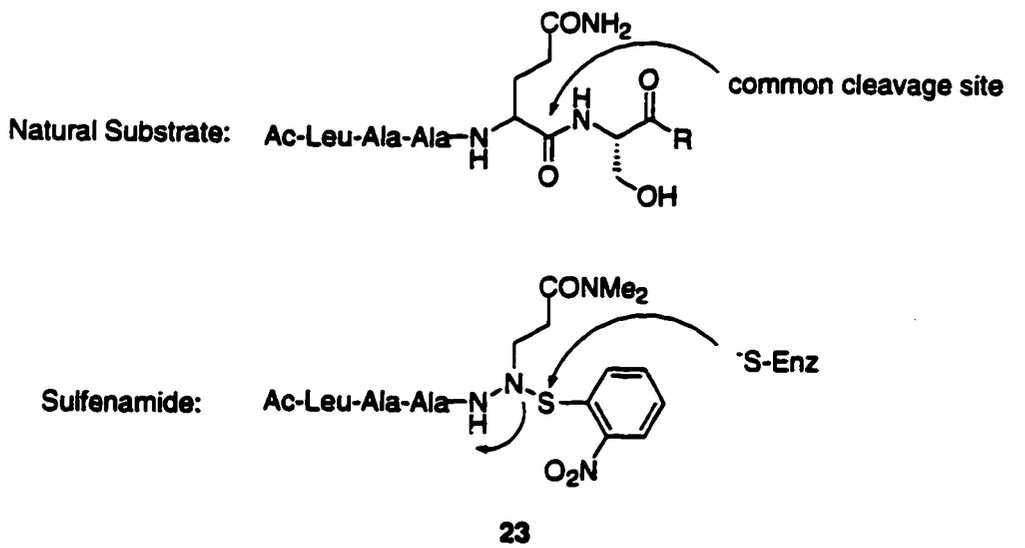


Figure 18 Target **D** compound

analogues (**24**, **25** and **27**) were also designed as target **D** compounds (Figure 19).

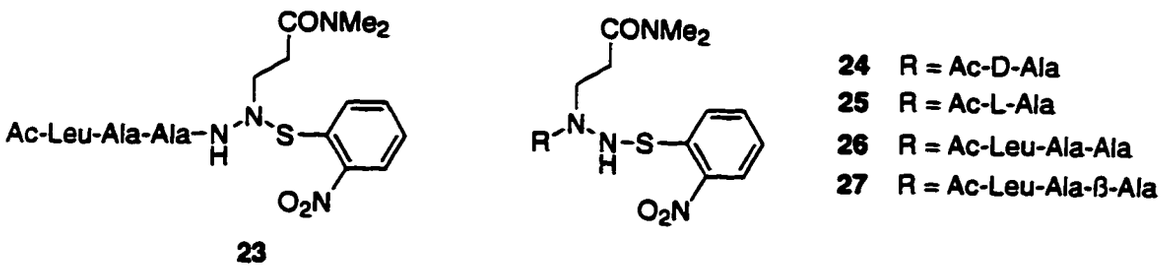
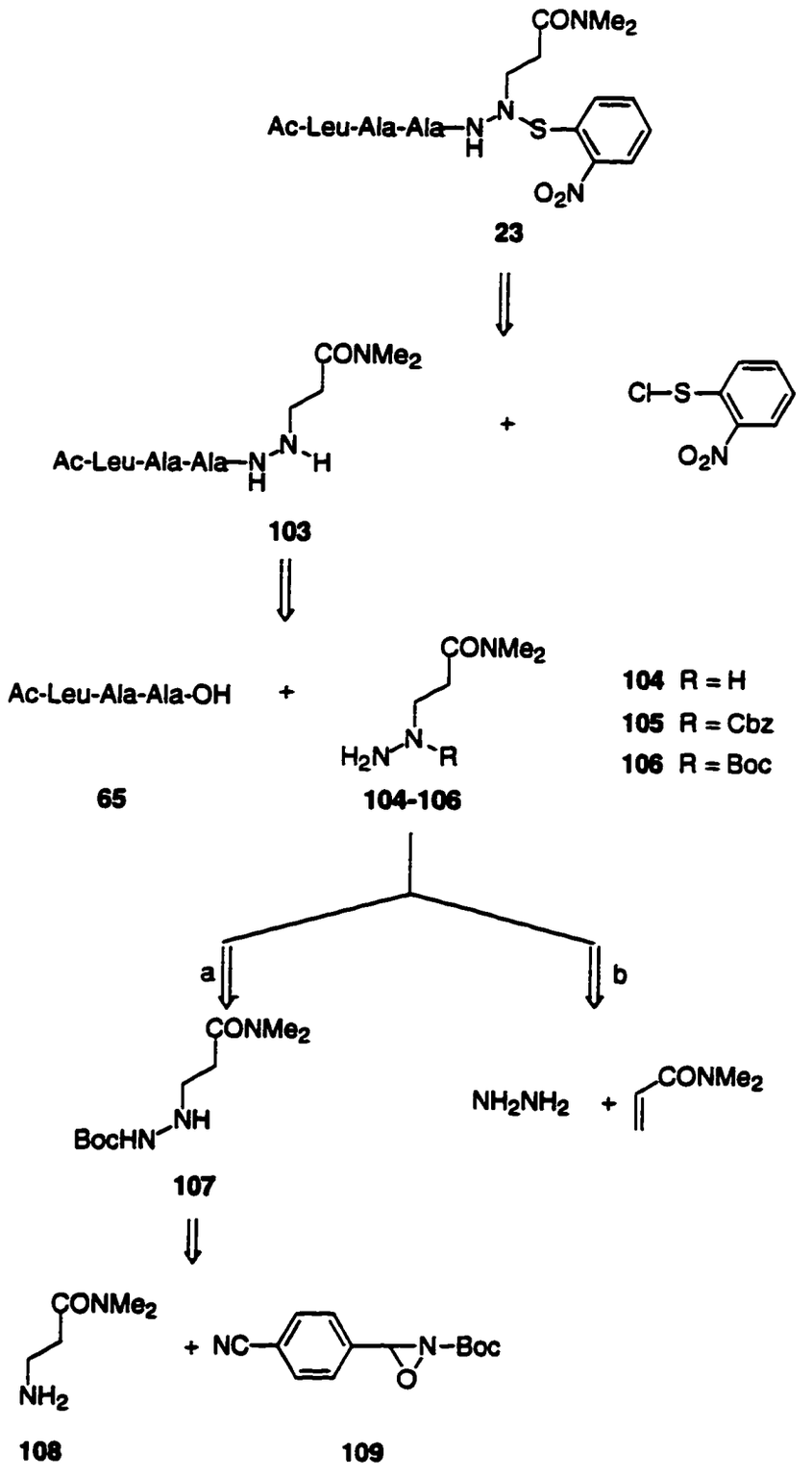


Figure 19 Target **D** compounds

4.2 Synthetic studies towards hydrazo peptidyl sulfenamides (target D)

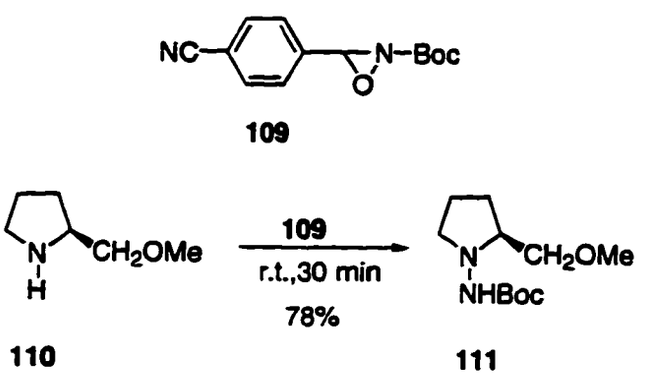
Retrosynthetic analysis (Scheme 37) of the target compound **23** indicates that the key intermediate would be 3-hydrazino-*N,N*-(dimethyl)propanamide (**104**). This could be coupled with tripeptide **65**, to produce the azapeptide **103**. Further reaction with *o*-nitrophenylsulfenyl chloride would afford **23**. Two possible routes were envisaged for the construction of **104**. One is the electrophilic amination of 3-amino-*N,N*-(dimethyl)propanamide (**108**) by *N*-Boc-3-(4-cyanophenyl)oxaziridine (**109**), a reagent that transfers a *N*-Boc group to nitrogen nucleophiles to form hydrazine derivatives (route a),[114] to provide 3-[(N^2-*t*-Boc)hydrazino]-*N,N*-(dimethyl)propanamide (**107**), which could be deprotected by trifluoroacetic acid to afford **104**. The second possibility is 1,4-addition of hydrazine derivatives to *N,N*-dimethylacrylamide (route b).


CONMe2
Ac-Leu-Ala-Ala-N
N
S
H
O2N
23
CONMe2
Ac-Leu-Ala-Ala-N
N
H
H
103
+
Cl-S
O2N
Ac-Leu-Ala-Ala-OH
+
CONMe2
H2N
N
R
65
104-106
104 R = H
105 R = Cbz
106 R = Boc
a
b
CONMe2
BocHN
NH
107
NH2NH2
+
CONMe2
CONMe2
NH2
108
+ NC
N-Boc
O
109

Scheme 37

4.2.1 Electrophilic amination by *N*-Boc-3-(4-cyanophenyl)oxaziridine (route a)

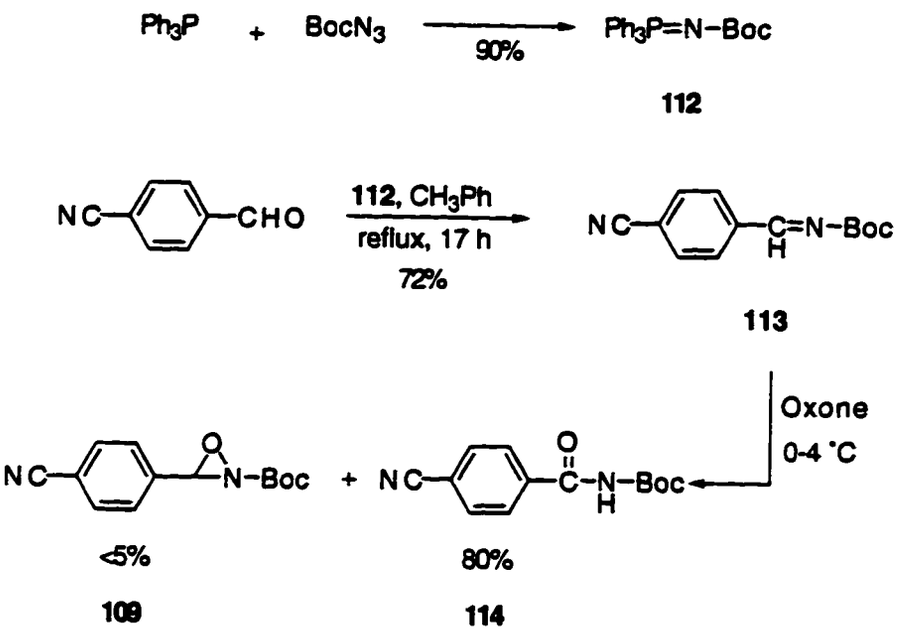
Electrophilic amination is an important synthetic process,[114] and Collet and coworkers reported that *N*-Boc-3-(4-cyanophenyl)oxaziridine (**109**) transfers its *N*-Boc group to various N- and C-nucleophiles under very mild conditions.[115] For example, as shown in Scheme 38, oxaziridine **109** converts (*S*)-2-(methoxymethyl)pyrrolidine (**110**) to (*S*)-*N*-(Boc-amino)-2-(methoxymethyl)pyrrolidine (**111**) in 30 min at room temperature (78% yield), thus providing simple access to this synthetically useful chiral hydrazine.[115]



Scheme 38

This methodology appeared ideal for preparation of the hydrazine derivative **107**. Synthesis of oxaziridine **109** is reported in the literature to proceed in 30% overall yield.[114] Following that procedure, treatment of $BocN_3$ with triphenylphosphine generates iminophosphorane **112** (Scheme 39). Aza-Wittig reaction between the iminophosphorane **112** and 4-cyanobenzaldehyde affords *N*-Boc-imine **113**. Oxidation of *N*-Boc-imine **113** using buffered potassium peroxymonosulfate (Oxone) under biphasic conditions at 0-4 °C, disappointingly, in our hands, provides the desired oxaziridine **109** in less than 5% yield, with the amide **114** as the major product (80%). Attempts to improve this oxidation procedure by using new oxone and recycling the aqueous phase several times in order to

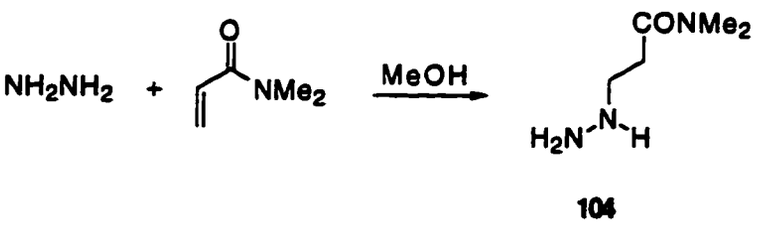
regenerate the oxidizing reagent all proved fruitless. This approach was therefore abandoned.



Scheme 39

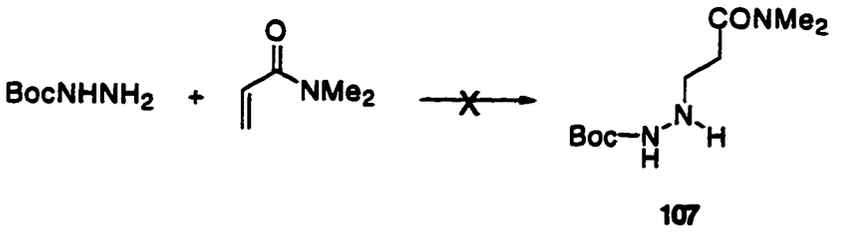
4.2.2 1,4-Addition (route b)

The key intermediate **104** could be synthesized from hydrazine monohydrate and *N,N*-dimethylacrylamide by a literature procedure (Scheme 40).[116] However, the purification of **104** is problematic. Compound **104** decomposes on distillation under high vacuum, or on flash chromatography using silica gel or Florisil. In order to



Scheme 40

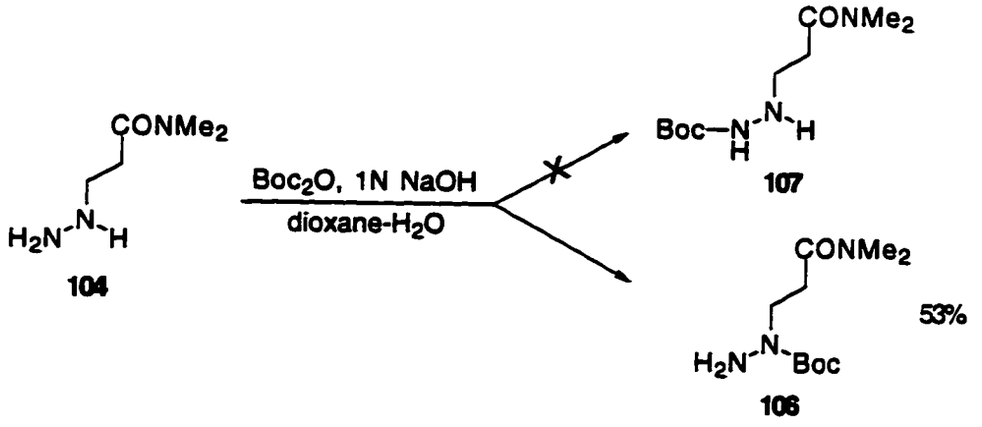
circumvent this difficulty, a 1,4-addition reaction was attempted using *tert*-butyl carbazate and *N,N*-dimethylacrylamide (Scheme 41). Unfortunately, none of the desired 1,4-adduct **107** was detected, and virtually all of the *tert*-butyl carbazate starting material was recovered.



Scheme 41

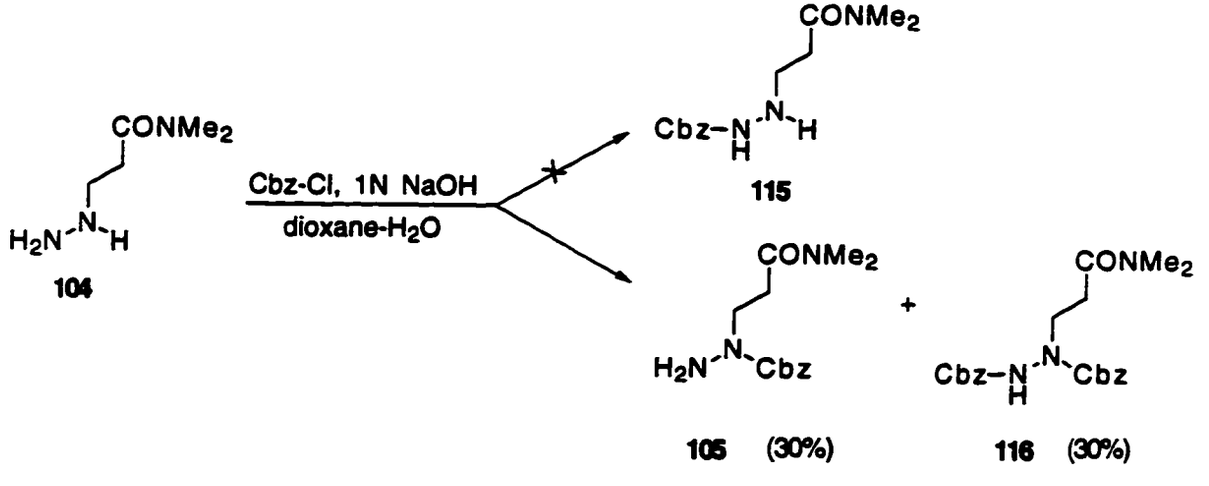
Even though the crude 3-hydrazino-*N,N*-(dimethyl)propanamide (**104**) is not stable, it seemed possible to couple **104** directly with peptide Ac-Leu-Ala-Ala-OH (**65**) without further purification. It was hoped that once azapeptide formed, it would be more stable than hydrazine **104**. Disappointingly, reaction of compound **104** with tripeptide **65** in the presence of BOP coupling reagent generated azapeptide **103** in less than 5% yield. In an attempt to optimize this approach, Ac-DL-alanine was chosen as the peptide reagent. Treatment of Ac-DL-alanine with **104** in the presence of BOP gave the coupled product in only 20% yield. However, it seemed that hydrazine **104** could possibly be trapped as a carbazate using benzyl chloroformate or di-*tert*-butyl dicarbonate.

Treatment of hydrazine **104** with Boc_2O in the presence of aqueous 1N NaOH affords 3-[(N^1-*t*-Boc)hydrazino]-*N,N*-(dimethyl)propanamide (**106**) in 53% yield, with none of the isomeric 3-[(N^2-*t*-Boc)hydrazino]-*N,N*-(dimethyl)propanamide (**107**) being isolated (Scheme 42). Although it is not completely certain why **106** is formed in preference to compound **107**, it may be that the electron-donating effect of the alkyl group attached to the secondary nitrogen makes it more nucleophilic.



Scheme 42

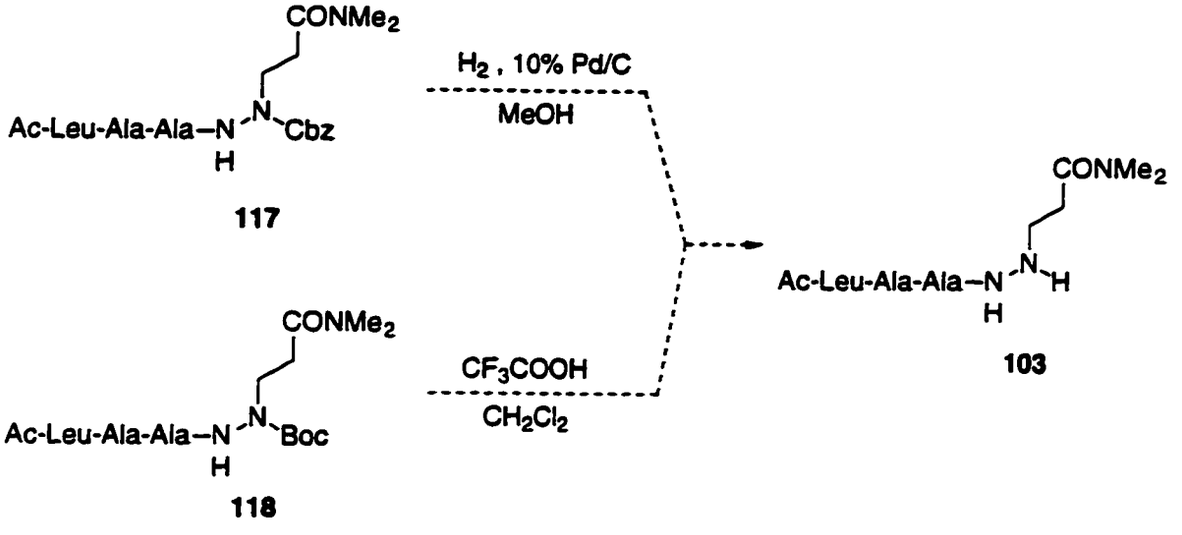
Reaction of Cbz-Cl with **104** in the presence of aqueous 1N NaOH provides the analogous product, 3-[(N^1-Cbz)hydrazino]-*N,N*-(dimethyl)propanamide (**105**), in 30% yield. In this case, the low yield is due to the formation of 3-[(N^1,N^2-bis-Cbz)hydrazino]-*N,N*-(dimethyl)propanamide (**116**) in 30% yield (Scheme 43).



Scheme 43

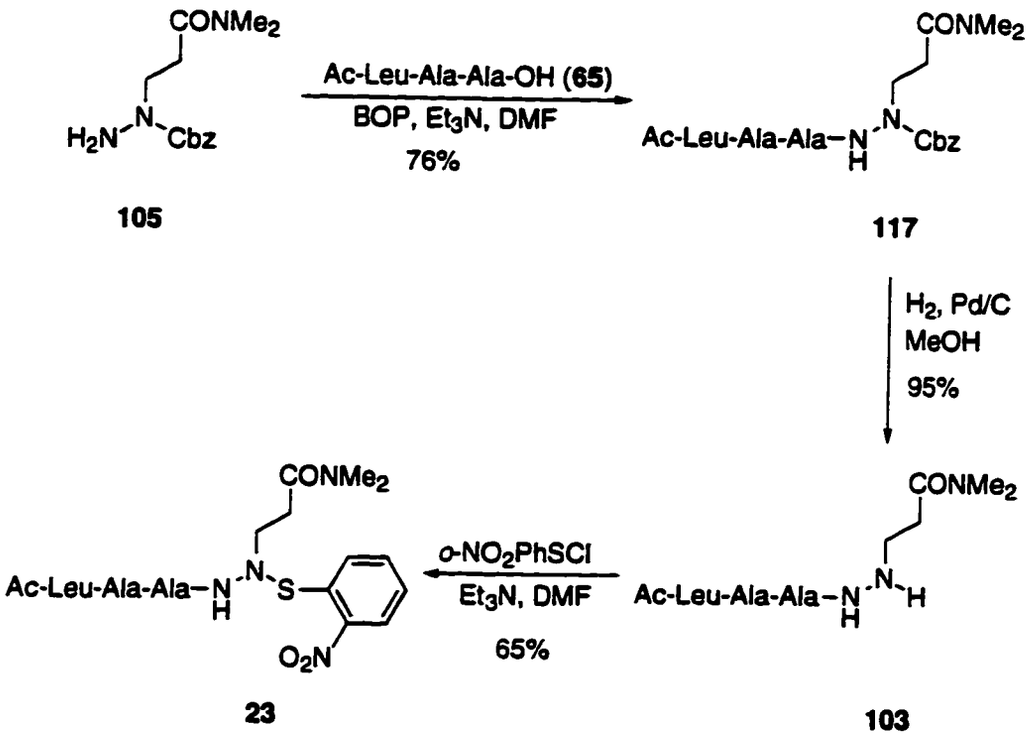
Consideration of the synthesis of azapeptide **103** suggested that hydrogenolytic deprotection of the Cbz group would be milder than removal of the Boc group with trifluoroacetic acid (Scheme 44). In addition, there should be no solubility problem for the hydrogenolytic deprotection of compound **117** because methanol could be used as a

solvent. Hence, Cbz-protected hydrazino derivative **105** was chosen as the key intermediate for synthesis of **23**.



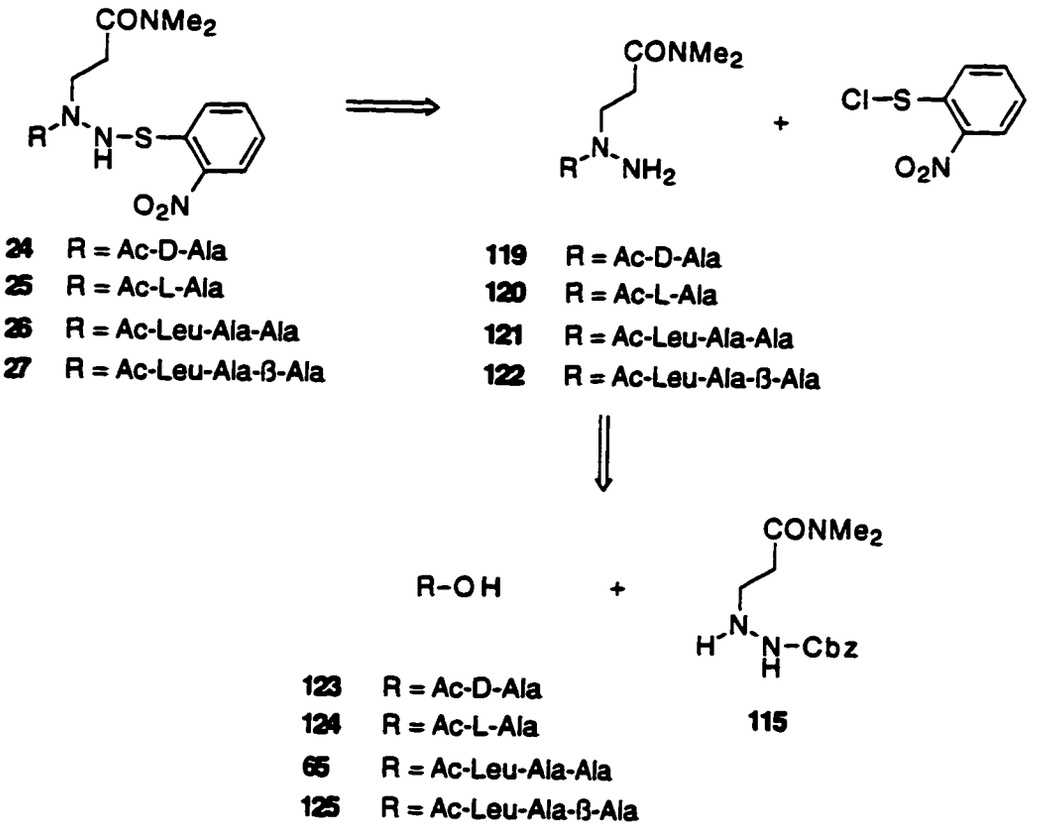
Scheme 44

Coupling of tripeptide Ac-Leu-Ala-Ala-OH (**65**) with **105** in the presence of BOP and triethylamine, in anhydrous DMF, at room temperature gives compound **117** in 76% yield (Scheme 45). The Cbz group is removed by hydrogenation using 10% Pd/C catalyst to form the azatetrapeptide **103**, which is treated with *o*-nitrophenylsulfenyl chloride in the presence of triethylamine to form target compound **23**.[117]



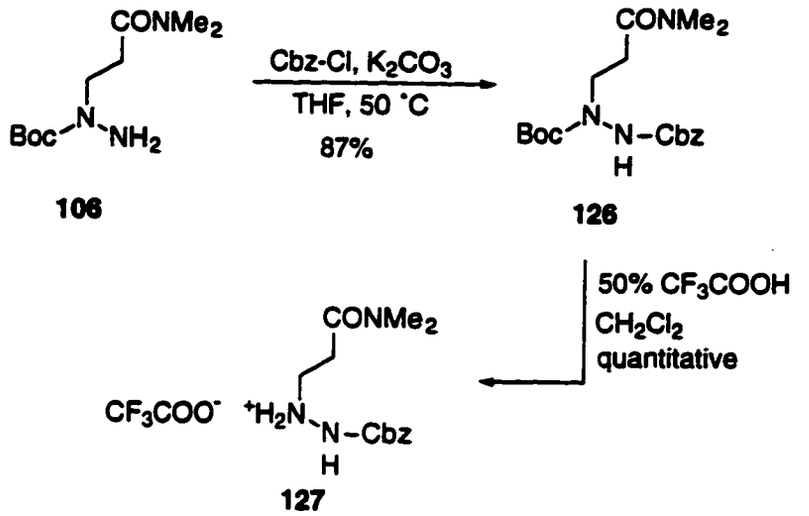
Scheme 45

The next objective was the synthesis of target compounds **24-27**. According to experience with the synthesis **23**, protected hydrazino derivatives should couple with peptides in good yields. Hence, the synthesis of the sulfenamides (**24-27**) was designed as follows (Scheme 46). The key intermediate would be 3-[(N^2-Cbz)hydrazino]-*N,N*-(dimethyl)propanamide (**115**). This would be coupled with the relevant amino acids or peptides, and then deprotected and treated with *o*-nitrophenylsulfenyl chloride to afford the targets.



Scheme 46

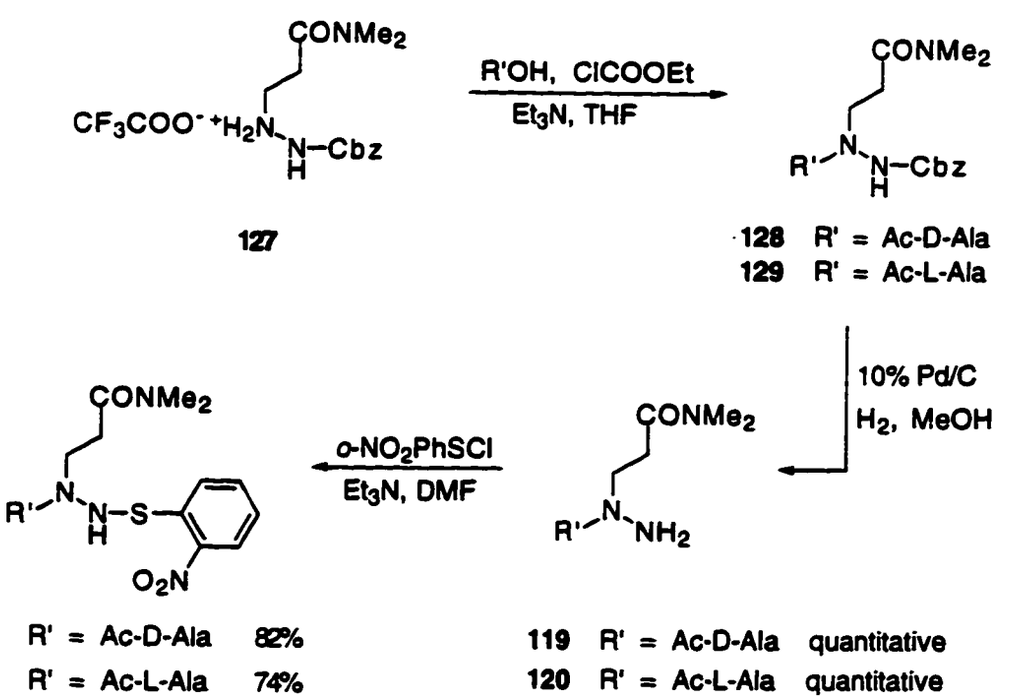
The TFA salt **127** of the key intermediate 3-[(N^2-Cbz)hydrazino]-*N*,*N*-(dimethyl)propanamide (**115**) can be synthesized as shown in Scheme 47. Treatment of



Scheme 47

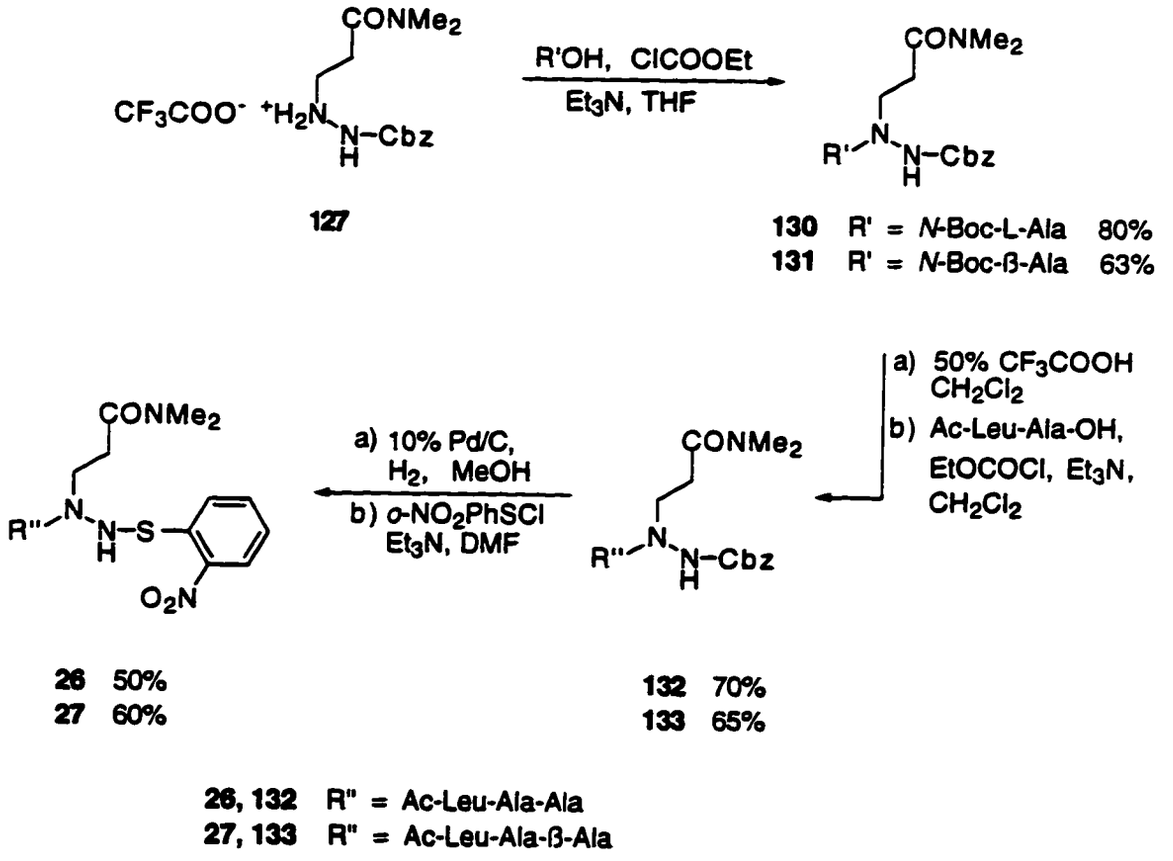
3-[(N^1-*t*-Boc)hydrazino]-*N,N*-(dimethyl)propanamide (**106**) with benzyl chloroformate in the presence of anhydrous K_2CO_3 provides 3-[(N^1-*t*-Boc-N^2-Cbz)hydrazino]-*N,N*-(dimethyl)propanamide (**126**). The Boc protecting group of **126** is removed with 50% TFA in CH_2Cl_2 to afford **115** as its TFA salt **127**.

Treatment of *N*-acetyl-D-alanine (**123**) or the corresponding L-isomer (**124**) with ethyl chloroformate generates the expected mixed anhydride, which reacts with **127** in the presence of triethylamine to generate **128** and **129**, respectively (Scheme 48).[91] The Cbz group is removed by hydrogenation, using 10% palladium on charcoal catalyst, to generate 3-[(N^1-acetyl-D-alanyl)hydrazino]-*N,N*-(dimethyl)propanamide (**119**) and its L-isomer **120**, respectively.[90] These are treated with *o*-nitrophenylsulfenyl chloride to form **24** and **25**.[117] These compounds both show complex ^{1}H NMR spectra due to conformational isomers at the amide bond(s). To confirm this, ^{1}H NMR spectra of **24** were acquired in DMF-d_7 at high temperature (100 °C) and room temperature. At 100 °C sharp signals are observed due to rapid isomer interconversion.



Scheme 48

The tripeptidic hydrazo sulfenamides **26** and **27** (Scheme 49) could be prepared by a similar procedure to the monomers **24** and **25**. Attempts to directly couple the tripeptide (Ac-Leu-Ala-Ala-OH or Ac-Leu-Ala-ß-Ala-OH) with trifluoroacetate salt **127** using BOP and triethylamine in anhydrous DMF, give less than 20% yields of **132** and **133**. Therefore, the tripeptides are made in three steps, proceeding *via* the coupling of a dipeptide Ac-Leu-Ala-OH to the protected monomers **130** and **131**, which are synthesized from trifluoroacetate salt **127** by coupling with *N-t*-Boc-L-alanine and *N-t*-Boc-ß-alanine, respectively.



Scheme 49

Compounds (**23**, **24**, **25**, **26** and **27**) could be efficiently generated for inhibition studies. The most effective approach for the synthesis of **23** involves trapping unstable

1,4-adduct **104** with Cbz-Cl to provide Cbz-protected hydrazino derivative **105**; coupling **105** with tripeptide **65** to afford peptide derivative **117**; deprotection of **117** and then nucleophilic substitution with *o*-nitrophenylsulfenyl chloride to give compound **23** in five steps in 14% overall yield. The synthesis of compounds **24** and **25** involves generating the TFA salt **127** of key intermediate **115** by treatment of Boc-protected hydrazino derivative **106** with Cbz-Cl and then deprotection with trifluoroacetic acid; coupling *N*-acetyl-D-alanine (**123**) or the corresponding L-isomer (**124**) with **127** *via* mixed anhydride, deprotection by hydrogenation and nucleophilic substitution with *o*-nitrophenylsulfenyl chloride to give compounds **24** and **25** in seven steps in 30% and 26% overall yield, respectively. Tetrapeptide derivatives **26** and **27** are synthesized by a similar approach in nine steps in 11% and 13% overall yield, respectively.

4.3 Results of inhibition studies with HAV-3C proteinase

Target **D** compounds **23-27** were assayed as described under Materials and Methods in the experimental section.[39c] The IC_{50} values were measured after preincubation with the enzyme for 60 minutes ([E] = 0.7 μM, [I] = 100-500 μM) (Table 3). Compound

Compounds	IC_{50} (μM)
23	500
24	500
25	500
26	100
27	400

Table 3 Results of inhibition studies of compounds **23-27**

23 shows no significant inhibition of HAV-3C proteinase despite the presence of the P_4-P_1 backbone. Apparently the aromatic moiety is too bulky for the enzyme site which binds to the P_1' residue. The structural isomer **26** of compound **23** does inhibit HAV-3C with an IC_{50} of 100 μM and eventual formation of an enzyme-nitrophenyl disulfide, as demonstrated by electrospray mass spectrometry (Figure 20). The difference in mass between the complex and free enzyme, 154 Da, is in agreement with the calculated mass of the fragment (-S-*o*-NO_2Ph). The relatively weak inhibition by **26** may again be due to unfavorable interactions of the aromatic portion with the P_1' binding pocket in the enzyme active site. Monomers **24** and **25** and peptide **27**, wherein the backbone is modified as 3-[(*N*^{l}-acetyl-L-leucyl-L-alanyl-ß-alanyl)hydrazino]-*N*,*N*-(dimethyl)propanamide show no significant inhibition of HAV-3C proteinase.

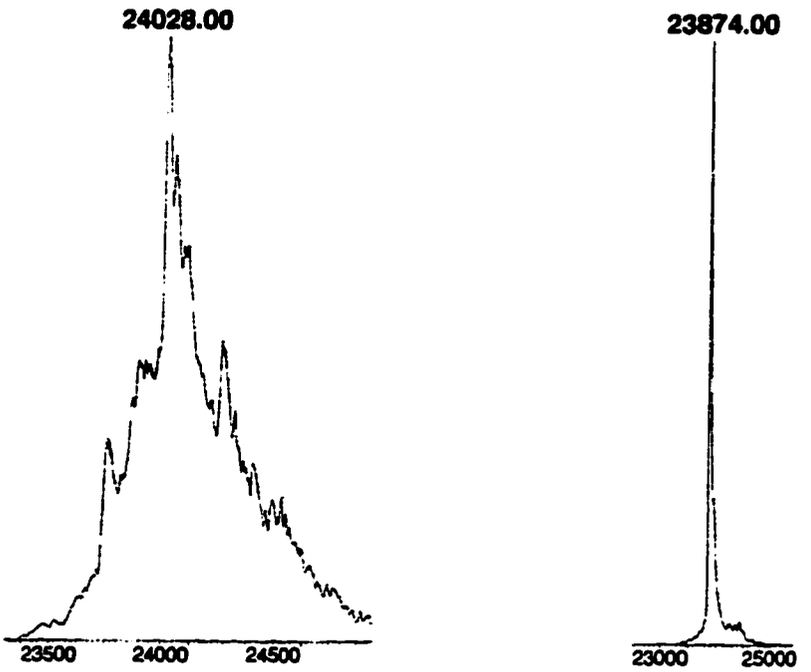


Figure 20 Mass spectra of C24S HAV-3C (panel 2) and C24S HAV-3C in complex with compound **26** (panel 1). Mass analysis of the protein and complex were performed on a Fisons VG Quattro triple-Quadrupole mass spectrometer (Manchester, England) fitted with an electrospray ionization source operating in positive ion mode.

In summary, target **D** compounds **23-27** were synthesized. Inhibition studies showed that they are weak inhibitors of HAV-3C proteinase, with IC_{50} values from 100 μM to 500 μM when preincubated with the enzyme. Electrospray mass spectrometry provides evidence for the formation of an enzyme-nitrophenyl disulfide covalent adduct for compound **26**. The results show that these types of modification on the substrate peptide backbone are far from ideal for the HAV-3C enzyme. A probable difficulty is the bulk of the aryl group on the sulfur, but preliminary studies suggest that corresponding alkyl (e.g. methyl, trifluoroethyl) sulfenamides are not stable under aqueous conditions. Although compound **26** inhibits in an irreversible fashion, it displays poor potency compared to the tetrapeptide aldehyde **6** (K_i^* = 42 nM) (where K_i^* is the overall dissociation constant of the tight enzyme-inhibitor complex EI*) or fluoromethyl ketone **11** [second order rate constant $k_{obs}/[I] = 330\ M^{-1}s^{-1}$ ([E] = 0.07 μM, [I] = 1 μM)]. Nevertheless, such compounds may be effective against other cysteine proteinases, including other picornaviral 3C proteinases.

Part 5. Hydrazo haloacetyl tetrapeptides as potential inhibitors of HAV-3C proteinase (Target E)

5.1 Design

As previously stated, bromoacetyl azapeptide analogue (**12**) (Figure 10) is a potent inhibitor of the HRV-3C proteinase (IC_{50} = 48 nM). It was found to be a time-dependent irreversible inhibitor with a k_{inact}/K_i value of > 2500 $M^{-1}s^{-1}$. An analogous compound incorporating the HAV-3C substrate analogue, 3-[(*N*²-acetyl-L-leucyl-L-alanyl-L-alanyl)hydrazino]-*N*,*N*-(dimethyl)propanamide may similarly inhibit HAV-3C proteinase. Hence, target **E** compounds, 3-[(*N*¹-haloacetyl-*N*²-acetyl-L-leucyl-L-alanyl-L-alanyl)hydrazino]-*N*,*N*-(dimethyl)propanamide (**28**, **29**), were designed for specific recognition and binding to the HAV-3C proteinase (Y = Cl, Br) (Figure 21).

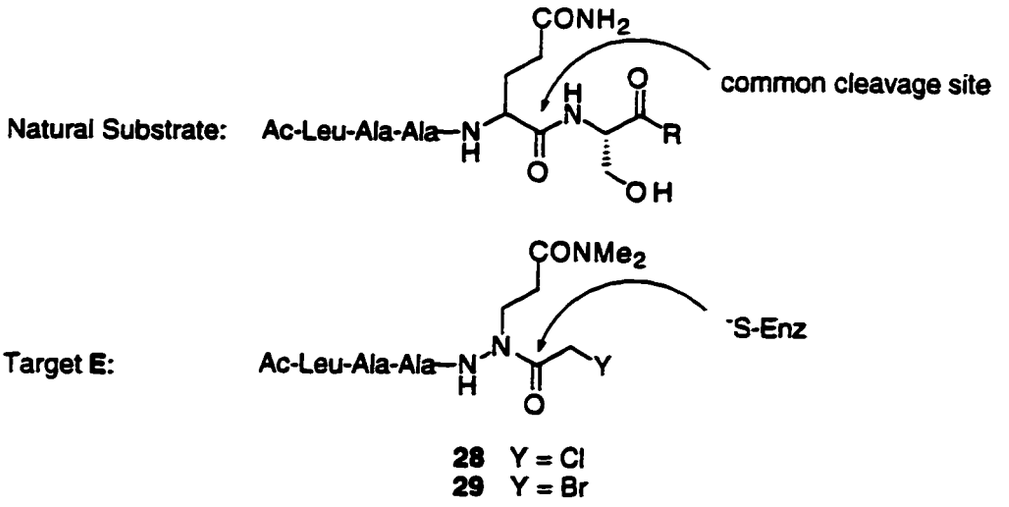
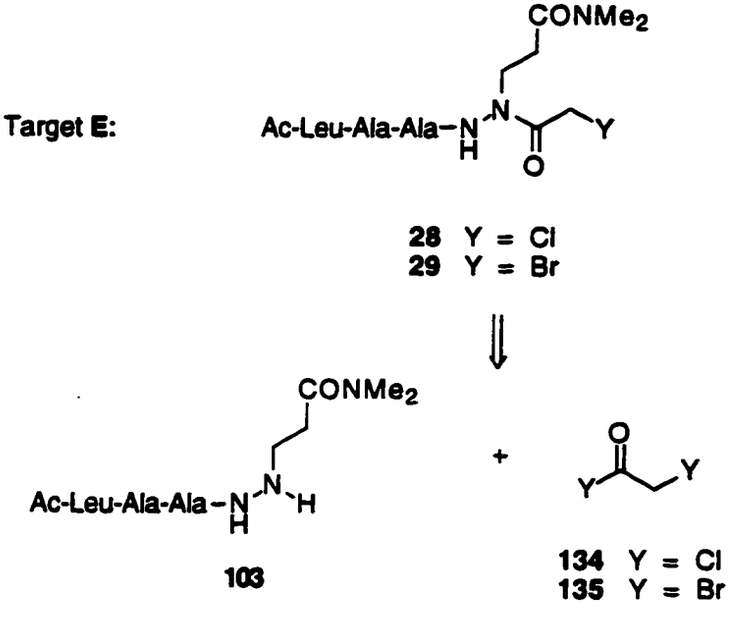


Figure 21 Hydrazo haloacetyl tetrapeptides

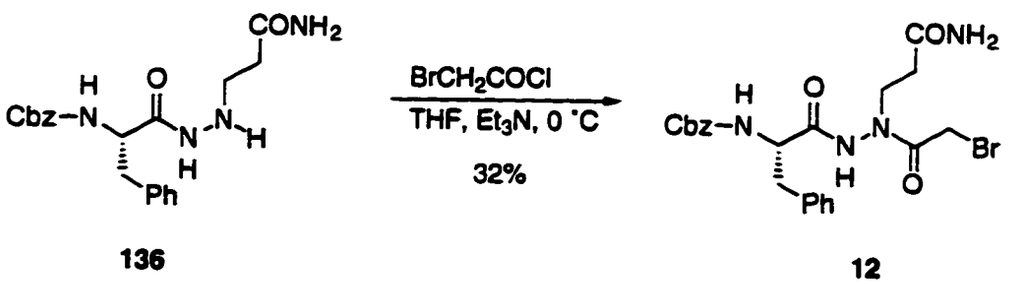
5.2 Synthetic studies on hydrazo haloacetyl tetrapeptides (target E)

The synthetic strategy for the construction of target **E** is based on the retrosynthetic analysis outlined in Scheme 50. The target compounds (**28**, **29**) could be envisaged to be derived from the azapeptide **103** and haloacetyl halides **134** (Y=Cl) and **135** (Y=Br). Preparation of azapeptide **103** was described above. Haloacetyl halides **134** (Y=Cl) and **135** (Y=Br) are commercially available.



Scheme 50

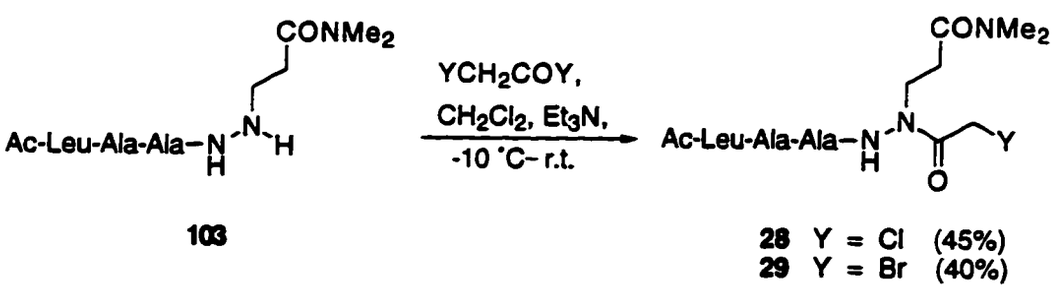
During our studies, Sham and coworkers reported the synthesis of bromoacetyl azapeptide **12** by acylation of azapeptide **136** by a similar basic approach (Scheme 51).[51]



136 **12**

Scheme 51

Acylation of azapeptide **103** with chloroacetyl chloride (**134**) or bromoacetyl bromide (**135**) in the presence of triethylamine produces compounds **28** and **29**, respectively (Scheme 52).



103

28 Y = Cl (45%)
29 Y = Br (40%)

Scheme 52

5.3 Results of inhibition studies of HAV-3C proteinase by 28 and 29

Target **E** compounds **28** and **29** were assayed as described under Materials and Methods in the experimental section.[39c] They are both potent, time-dependent irreversible inhibitors of HAV-3C enzyme. Figures 22 and 23 show the time-dependent inhibition curves of HAV-3C proteinase by chloroacetyl peptide **28** and bromoacetyl peptide **29** respectively. Electrospray mass spectrometry further confirmed the formation of a covalent

adduct. The spectra of the uncomplexed enzyme and the HAV-3C-haloacetyl peptide (**28** and **29**) complexes (Figure 24) show the differences in mass between the complex and free enzyme are 466 Da (for compound **28**) and 470 Da (for compound **29**). These are in good agreement with the calculated mass (470 Da) of the fragment (M^+-Y when Y = Cl, Br). The second order inhibition rate constants of **28** and **29** [k_{obs}/[I] $M^{-1}s^{-1}$ ([E] = 0.07 μM, [I] = 1 μM)] are shown in Table 4, these were calculated from k_{obs} (k_{obs} = coefficient c) by Quasi-Newton's fitting function.

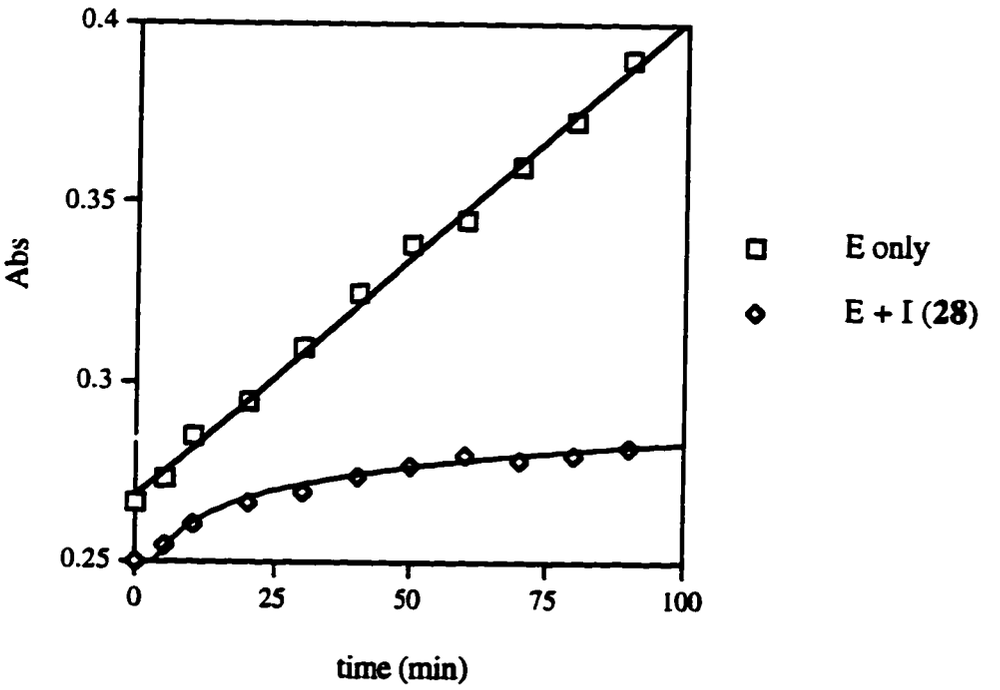


Figure 22 Time-dependent inhibition of HAV-3C proteinase by compound **28**

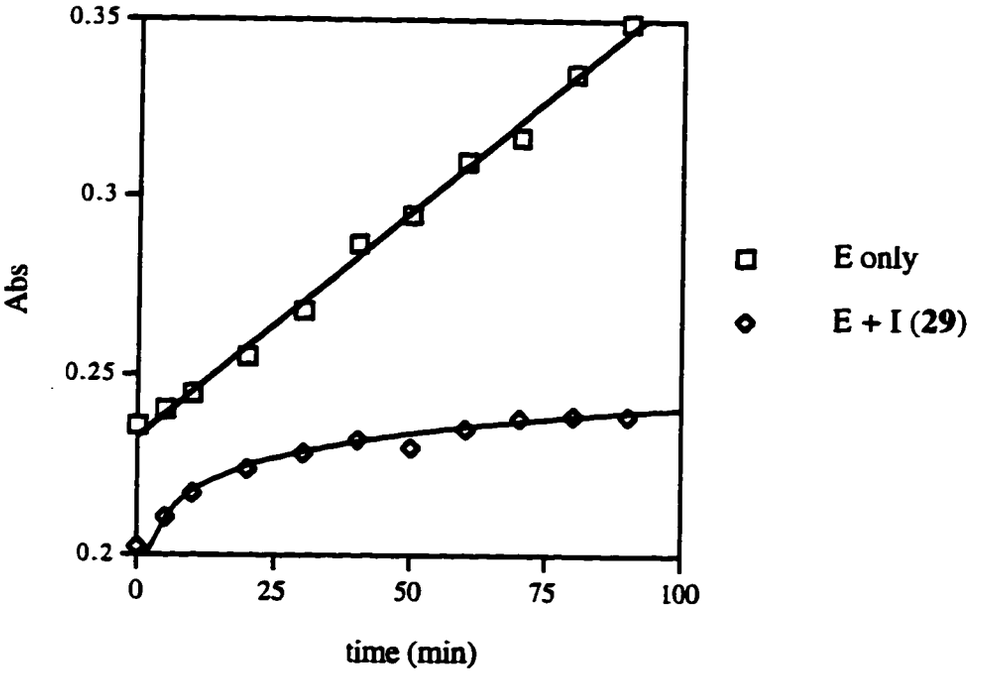


Figure 23 Time-dependent inhibition of HAV-3C proteinase by compound **29**

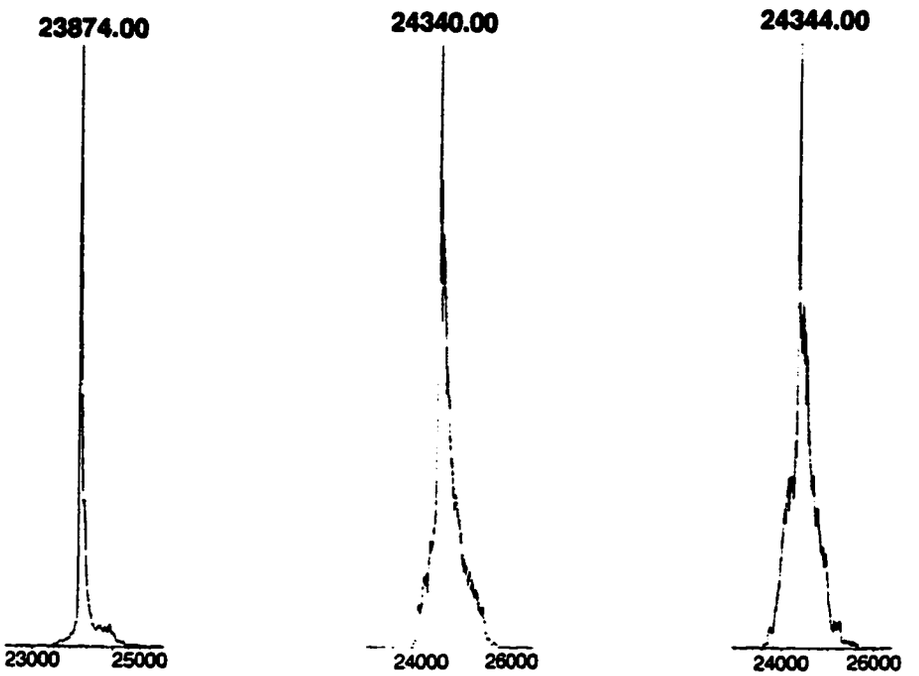


Figure 24 Mass spectra of C24S HAV-3C (panel 1), C24S HAV-3C in complex with compound **28** (panel 2) and C24S HAV-3C in complex with compound **29** (panel 3).

Compounds	$(k_{obs}/[I])$ $(M^{-1}s^{-1})$
28	684
29	866

Table 4 Results of inhibition studies of HAV-3C with **28** and **29**

In summary, hydrazo haloacetyl tetrapeptides (**28, 29**) have been synthesized. The synthetic strategy was based on that developed in part 4 for azapeptide **103**. Modified acylation of azapeptide **103** with haloacetyl halides (**134, 135**) gave the corresponding hydrazo haloacetyl tetrapeptides (**28, 29**) in five steps in 10% and 9% overall yield, respectively. Inhibition studies have shown these compounds to be potent, irreversible inhibitors of HAV-3C proteinase, with second order rate constants $(k_{obs}/[I])$ of 684 (Y = Cl) and 866 (Y = Br) $M^{-1}s^{-1}$.

EXPERIMENTAL PROCEDURES

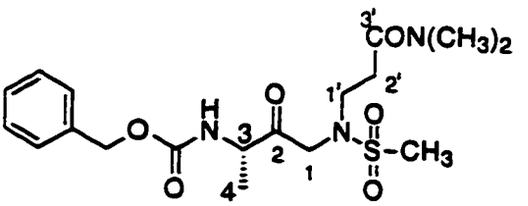
General Methods:

All processes involving air or moisture sensitive reactants were done under an atmosphere of dry argon using oven-dried glassware. Reagents and solvents were reagent grade and used as supplied unless otherwise stated. Solvents for anhydrous reactions were dried according to Perrin *et al.*[118] Tetrahydrofuran (THF) and diethyl ether were distilled over sodium and benzophenone under an argon atmosphere. Toluene was distilled from sodium under an argon atmosphere. Acetonitrile, dichloromethane, triethylamine and pyridine were distilled over calcium hydride. *N,N*-Dimethylformamide (DMF) was distilled under reduced pressure from calcium hydride, and nitromethane was fractionated from P_2O_5. Methanol and ethanol were distilled over magnesium turnings and a catalytic amount of iodine. Dimethyl sulfoxide (DMSO) was distilled over calcium hydride and stored over CaH_2. Water was obtained from a Milli-Q reagent water system. "Brine" refers to a saturated aqueous solution of NaCl. Unless otherwise specified, solutions of NH_4Cl, $NaHCO_3$, KOH and NaOH refer to aqueous solutions. Solvent evaporation was performed under reduced pressure below 40 °C using a Buchi rotary evaporator, followed by evacuation (<0.1 torr) to constant sample weight. Reactions involving triethyl phosphite and other phosphate reagents were performed in a fumehood.

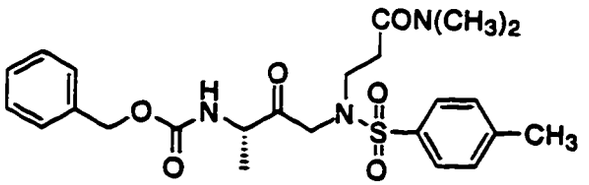
Reactions and fractions from column chromatography were monitored and analyzed by thin-layer chromatography (TLC) using glass plates with a UV fluorescent indicator (silica gel, Merck 60 F_{254}; reverse phase, Merck RP-8 and RP-18 F_{254}). One or more of the following methods were used for visualization: UV absorption by fluorescence quenching; iodine staining; phosphomolybdic acid/ceric sulfate/sulfuric acid (10 g : 1.25 g : 8% 250 mL) spray; Ninhydrin/methanol (1 g : 100 mL) spray. Flash column chromatography was performed by the method of Still[119] using 230-400 mesh silica (Merck, silica gel).

Melting points were determined on a Thomas-Hoover or Buchi oil immersion apparatus using open capillary tubes and are uncorrected. Infrared spectra (IR) were recorded on a Nicolet 7199 FT-IR spectrometer. Cast refers to the evaporation of a solution on a NaCl plate. Mass spectra (MS) were recorded on Kratos AEIMS-50 high resolution mass spectrometry (HRMS), electron impact ionization (EI), MS-12 chemical ionization ((CI), NH_3), and MS-9 fast atom bombardment ((FAB), argon) instruments. Cleland matrix was used in all FAB experiments and refers to a 5 : 1 mixture of dithiothreitol and dithioerythritol. Microanalyses were obtained on Perkin Elmer 240 or Carlo Erba 1180 elemental analyzers. Nuclear magnetic resonance (NMR) spectra were obtained on Brucker WH-200, AM-300, WM-360 and WH-400 instruments. ^1H NMR chemical shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS) using the solvent resonance as the reference: $CDCl_3$, δ 7.24; CD_2Cl_2, δ 5.32; D_2O, δ 4.72; CD_3OD, δ 3.30; and $(CD_3)_2SO$, δ 2.49. ^{13}C NMR shifts are reported relative to: $CDCl_3$, δ 77.0; CD_2Cl_2, δ 53.8; CD_3OD, δ 49.0; and $(CD_3)_2SO$, δ 39.5. Selective homonuclear decoupling, attached proton test (APT), and ^1H-^{13}C correlation experiments were occasionally used for signal assignments. ^1H NMR data are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet), number of protons, coupling constant(s) in Hertz (Hz), and assignment. When appropriate, the multiplicity is preceded by br, indicating that the signal was broad. All literature compounds had IR, ^1H NMR, and mass spectra consistent with the reported data.

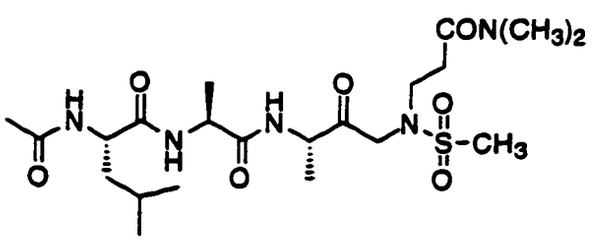
The peptides Ac-Leu-Ala-Ala-OH and Ac-Leu-Ala-OH were prepared on a Rainin PS-3 solid-phase peptide synthesizer using standard Fmoc chemistry on Wang Resin and were purified by HPLC (C-18 reverse phase) before use. Target compounds were purified by HPLC (Waters, Resolve C-18 reverse phase, 25 mm x 100 mm, 10 μm, flow rate 15.0 mL/min).



(*3S*)-{N^3-(Benzyloxycarbonyl)-N^1-[3'-(*N*,*N*-dimethylamino)-3'-oxopropyl]-N^1-(methylsulfonyl)}-1,3-diaminobutan-2-one (17). To a stirred solution of sulfonamide **90** (0.82 g, 4.2 mmol) in anhydrous DMF (10 mL) under argon at 0 °C, was added NaH (60% dispersion in mineral oil) (0.237 g, 5.9 mmol) in small portions, and the mixture was stirred at 0 °C for an additional 30 min. The resulting yellow anion solution was added dropwise over a 30 min period to a stirred solution of bromo compound **89** (1.51 g, 5.0 mmol) in DMF (5 mL). After 1 h at room temperature, the solvent was removed *in vacuo*, and the residue was dissolved in CH_2Cl_2, and then washed with 1% HCl (10 mL), 5% $NaHCO_3$ (10 mL) and brine (10 mL). The organic phase was dried (Na_2SO_4) and concentrated *in vacuo* to give an oil. Purification by flash chromatography (ethyl acetate : petroleum spirit, 1:1) gave the sulfonamide **17** (0.83 g, 48%) as an oil : IR (CH_2Cl_2 cast) 3315, 3032, 2935, 1715, 1637, 1522, 1454, 1328, 1248, 1146 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.45-7.29 (m, 5 H, ArH), 5.61-5.45 (brs, 1 H, CONH), 5.12 (s, 2 H, OCH_2), 4.47 (s, 2 H, CH_2), 4.40-4.25 (m, 1 H, CH), 3.60-3.40 (m, 2 H, CH_2N), 2.97 (s, 3 H, NCH_3), 2.94 (s, 3 H, SO_2CH_3), 2.88 (s, 3 H, NCH_3), 2.70-2.50 (m, 2 H, $COCH_2$), 1.35 (d, 3 H, J = 7.1 Hz, CH_3); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 206.43 (C=O), 170.98 (CON), 156.19 (OCON), 136.94 (C, ArH), 128.75 (CH, ArH), 128.38 (CH, ArH), 128.25 (CH, ArH), 67.08 (OCH_2), 55.16 (CH_2), 53.96 (CH), 44.95 (NCH_2), 39.42 (SO_2CH_3), 37.16 (NCH_3), 35.34 (NCH_3), 34.06 ($COCH_2$), 16.92 (CH_3); HRMS calcd for $C_{18}H_{27}N_3O_6S$ 413.1621, found 413.1632.

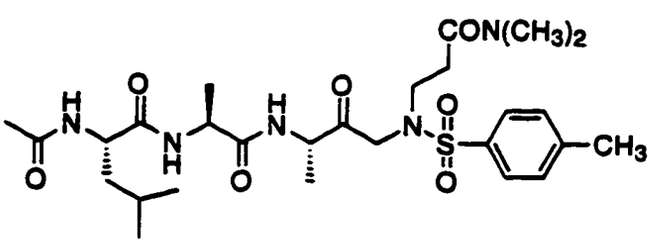


(3*S*)-{N^3-(Benzyloxycarbonyl)-N^1-[3'-(*N*,*N*-dimethylamino)-3'-oxopropyl]-N^1-(*p*-methylphenylsulfonyl)}-1,3-diaminobutan-2-one (18). The procedure used for the preparation of **17**, was employed to condense sulfonamide **91** (1.00 g, 3.7 mmol) and bromomethyl ketone **89** (1.33 g, 4.44 mmol) to give **18** (0.94 g, 52%) as a waxy solid: IR (CH_2Cl_2 cast) 3295, 3032, 2935, 1714, 1638, 1519, 1497, 1401, 1336, 1248, 1156, 1093 cm^{-1}; ^1H NMR (360 MHz, CD_2Cl_2) δ 7.67 (d, 2 H, J = 8.2 Hz, ArH), 7.41-7.20 (m, 7 H, ArH), 5.65 (d, 1 H, J= 6.9 Hz, CONH), 5.09 (s, 2 H, OCH_2), 4.46-4.28 (m, 3 H, CH and $COCH_2N$), 3.41 (t, 2 H, J = 6.3 Hz, CH_2N), 2.91 (s, 3 H, NCH_3), 2.82 (s, 3 H, NCH_3), 2.67-2.50 (m, 2 H, $COCH_2$), 2.42 (s, 3 H, $ArCH_3$), 1.32 (d, 3 H, J = 7.1 Hz, CH_3); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 204.93 (C=O), 171.05 (CON), 156.13 (OCON), 144.04 (C, ArH), 137.09 (C, ArH), 130.00 (CH, ArH), 128.86 (CH, ArH), 128.44 (CH, ArH), 128.32 (CH, ArH), 127.59 (CH, ArH), 115.93 (C, ArH), 67.11 (OCH_2), 55.86 ($COCH_2N$), 53.81 (CH), 45.69 (NCH_2), 37.19 (NCH_3), 35.37 (NCH_3), 33.96 ($COCH_2$), 21.62 (Ar CH_3), 17.30 (CH_3); HRMS calcd for $C_{24}H_{32}N_3O_6S$ 490.2012, found 490.2001.



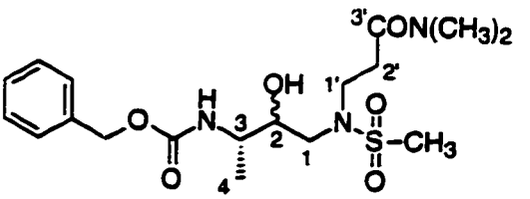
(3*S*)-{N^3-(Acetyl-L-leucyl-L-alanyl)-N^1-[3'-(*N*,*N*-dimethylamino)-3'-oxopropyl]-N^1-(methylsulfonyl)}-1,3-diaminobutan-2-one (19). A solution of the dipeptide Ac-Leu-Ala-OH (0.216 g, 0.89 mmol) in dry THF (10 mL) under argon at 0 °C was treated with ethyl chloroformate (90 μL, 0.89 mmol) and triethylamine

(0.13 mL, 0.91 mmol) and stirred for 20 min. A solution of sulfonamido amine hydrochloride **96** (0.280 g, 0.89 mmol) in THF (5 mL), and triethylamine (0.13 mL, 0.91 mmol) were added, and the reaction mixture was stirred for 30 min. It was then allowed to warm to room temperature and stirred overnight. To the mixture was added EtOAc (40 mL), and the organic phase was washed with saturated aqueous $NaHCO_3$ (20mL) and brine (20 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give the crude product **19**. Purification by HPLC (linear gradient elution over 25 min of acetonitrile and water, from 20% to 35%, t_R 10.4 min) gave **19** (0.224 g, 50%) as a white solid: mp 122-124 °C; IR (MeOH cast) 3287, 2935, 1731, 1650, 1643, 1547, 1327, 1202, 1147 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 4.45-4.15 (m, 5 H, α-CH Leu, 2 α-CH Ala, and $COCH_2N$), 3.46 (t, 2 H, J = 7.0 Hz, NCH_2), 3.06 (s, 3 H, SO_2CH_3), 2.96 (s, 3 H, NCH_3), 2.89 (s, 3 H, NCH_3), 2.69 (d, 3 H, J = 7.0 Hz, $COCH_2$), 1.97 (s, 3 H, $COCH_3$), 1.72-1.59 (m, 1 H, CH Leu), 1.58-1.50 (m, 2 H, CH_2 Leu), 1.37 (d, 3 H, J = 7.2 Hz, CH_3 Ala), 1.33 (d, 3 H, J = 7.2 Hz, CH_3 Ala), 0.96 (d, 3 H, J = 6.5 Hz, CH_3 Leu), 0.92 (d, 3 H, J = 6.5 Hz, CH_3 Leu); ^{13}C NMR (100 MHz, CD_3OD) δ 207.20, 175.37, 175.32, 173.83, 173.28, 55.22, 54.12, 54.02, 50.65, 46.19, 40.14, 39.49, 38.86, 36.38, 34.12, 25.67, 22.80, 22.39, 21.97, 19.39, 15.76; MS (FAB) 506.3 (80) (MH^+).



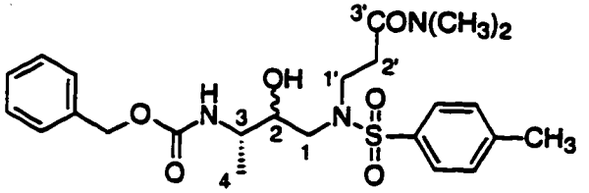
(*3S*)-{N^3-(Acetyl-L-leucyl-L-alanyl)-N^1-[3'-(*N*,*N*-dimethylamino)-3'-oxopropyl]-N^1-(*p*-methylphenylsulfonyl)}-1,3-diaminobutan-2-one (20). The procedure used for the preparation of **19**, was used to condense dipeptide Ac-Leu-Ala-OH (0.156 g, 0.64 mmol) and sulfonamido amine hydrochloride **97** (0.250 g, 0.64 mmol) in the presence of ethyl chloroformate (62 μL, 0.64 mmol) and triethylamine (0.178 mL,

1.28 mmol). Purification by HPLC (linear gradient elution over 20 min of acetonitrile and water, from 30% to 50%, t_R 15.8 min) gave the title compound **20** (0.208 g, 56%) as a solid. Spectral characterization was performed on a mixture of conformational isomers (isomer A : isomer B, 3:1): mp 145-147 °C; IR (MeOH cast) 3381, 2957, 1734, 1651, 1644, 1575, 1538, 1455, 1335, 1202, 1157 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) (isomer A) δ 7.77-7.69 (m, 2 H, ArH), 7.37 (d, 2 H, J = 8.4 Hz, ArH), 4.41-4.15 (m, 5 H, α-CH Leu, 2 α-CH Ala, and $COCH_2N$), 3.50-3.30 (m, 2 H, NCH_2), 2.99 (s, 3 H, NCH_3), 2.87 (s, 3 H, NCH_3), 2.65 (d, 3 H, J = 7.3 Hz, $COCH_2$), 2.42 (s, 3 H, $ArCH_3$), 1.94 (s, 3 H, $COCH_3$), 1.70-1.58 (m, 1 H, CH Leu), 1.58-1.50 (m, 2 H, CH_2 Leu), 1.37 (d, 3 H, J = 7.3 Hz, CH_3 Ala), 1.32 (d, 3 H, J = 7.2 Hz, CH_3 Ala), 0.97 (d, 3 H, J = 6.5 Hz, CH_3 Leu), 0.93 (d, 3 H, J = 6.5 Hz, CH_3 Leu); (isomer B) δ 7.77-7.69 (m, 2 H, ArH), 7.37 (d, 2 H, J = 8.4 Hz, ArH), 4.41-4.15 (m, 5 H, α-CH Leu, 2 α-CH Ala, and $COCH_2N$), 3.50-3.30 (m, 2 H, NCH_2), 2.99 (s, 3 H, NCH_3), 2.87 (s, 3 H, NCH_3), 2.65 (d, 3 H, J = 7.3 Hz, $COCH_2$), 2.42 (s, 3 H, $ArCH_3$), 1.94 (s, 3 H, $COCH_3$), 1.70-1.58 (m, 1 H, CH Leu), 1.58-1.50 (m, 2 H, CH_2 Leu), 1.36 (d, 3 H, J = 7.3 Hz, CH_3 Ala), 1.29 (d, 3 H, J = 7.2 Hz, CH_3 Ala), 0.96 (d, 3 H, J = 6.5 Hz, CH_3 Leu), 0.92 (d, 3 H, J = 6.5 Hz, CH_3 Leu); ^{13}C NMR (100 MHz, CD_3OD) (isomer A) δ 206.04, 175.17, 173.74, 173.56, 173.10, 145.00, 138.13, 130.75, 128.53, 55.91, 54.01, 53.93, 50.57, 46.54, 41.36, 37.64, 35.64, 33.84, 25.88, 23.18, 22.46, 22.30, 21.46, 17.50, 15.80; (isomer B) δ 206.13, 175.17, 174.97, 173.74, 173.10, 145.00, 138.13, 130.75, 128.53, 55.91, 54.01, 53.93, 50.57, 46.54, 41.36, 37.64, 35.64, 33.97, 25.88, 23.18, 22.46, 22.30, 21.46, 17.50, 15.90; MS (FAB) 582.2 (52) (MH^+).



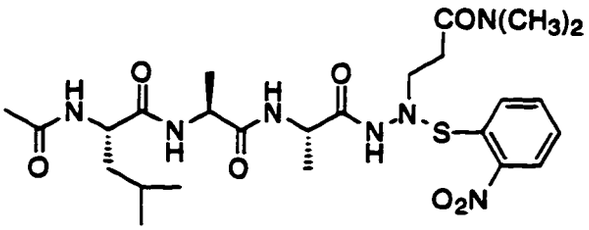
***(2RS, 3R)*-{N^3-(Benzyloxycarbonyl)-N^1-[3'-(*N*,*N*-dimethylamino)-3'-oxopropyl]-N^1-(methylsulfonyl)}-1,3-diaminobutan-2-ol (21).** To a stirred solution of sulfonamido ketone **17** (102.3 mg, 0.25 mmol) in absolute ethanol (2 mL) under argon at 0 °C was added dropwise $NaBH_4$ solution (0.1 M in EtOH, 2.5 mL). The reaction mixture was stirred at 0 °C for 10 min. The solvent was evaporated, and the residue was dissolved in EtOAc (20 mL). The organic phase was washed with water (10 mL) and brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo* to yield sulfonamido alcohol **21** (92 mg, 89%) as a glassy solid. Spectral characterization was performed on a mixture of diastereoisomers (isomer A : isomer B = 3:1): IR (CH_2Cl_2 cast) 3352, 2934, 1713, 1626, 1528, 1454, 1403, 1327, 1240, 1145, 1049 cm^{-1}; ^1H NMR (400 MHz, CD_2Cl_2) (isomer A) δ 7.40-7.23 (m, 5 H, ArH), 5.56-5.45 (d, 1 H, J = 8.4 Hz, CONH), 5.08 (s, 2 H, OCH_2), 4.77 (d, 1 H, J = 4.2 Hz, OH), 3.88-3.74 (m, 1 H, H-2), 3.72-3.65 (m, 1 H, H-3), 3.60-3.37 (m, 2 H, H-1), 3.34-3.22 (m, 2 H, CH_2N), 2.97 (s, 3 H, SO_2CH_3), 2.88 (s, 3 H, NCH_3), 2.82 (s, 3 H, NCH_3), 2.73-2.60 (m, 2 H, $COCH_2$), 1.15 (d, 3 H, J = 6.7 Hz, CH_3); (isomer B) δ 7.40-7.23 (m, 5 H, ArH), 5.44-5.37 (d, 1 H, J = 8.4 Hz, CONH), 5.08 (s, 2 H, OCH_2), 4.92 (d, 1 H, J = 4.2 Hz, OH), 3.88-3.74 (m, 1 H, H-2), 3.72-3.65 (m, 1 H, H-3), 3.60-3.37 (m, 2 H, H-1), 3.15-3.04 (m, 2 H, CH_2N), 2.97 (s, 3 H, SO_2CH_3), 2.89 (s, 3 H, NCH_3), 2.81 (s, 3 H, NCH_3), 2.80-2.73 (m, 2 H, $COCH_2$), 1.22 (d, 3 H, J = 6.7 Hz, CH_3); ^{13}C NMR (75 MHz, CD_2Cl_2) (isomer A) δ 171.59 (CON), 156.27 (OCON), 137.34 (C, ArH), 128.77 (CH, ArH), 128.26 (CH, ArH), 128.20 (CH, ArH), 72.89 (OCH), 66.72 (OCH_2), 53.80 (CH_2), 49.88 (CH), 46.24 (NCH_2), 37.44 (SO_2CH_3), 37.10 (NCH_3), 35.55 (NCH_3), 33.82 ($COCH_2$), 15.30 (CH_3); (isomer B) δ 171.59 (CON), 156.56 (OCON), 137.13 (C, ArH), 128.13 (CH,

ArH), 127.58 (CH, ArH), 127.16 (CH, ArH), 72.79 (OCH), 66.72 (OCH_2), 53.89 (CH_2), 49.48 (CH), 46.60 (NCH_2), 36.82 (SO_2CH_3), 37.10 (NCH_3), 35.55 (NCH_3), 33.69 ($COCH_2$), 18.74 (CH_3); MS (EI) *m/z* (rel inten) 415.18 (0.27) (M^+); HRMS calcd for $C_{18}H_{29}N_3O_6S$ 415.1777, found 415.1768.



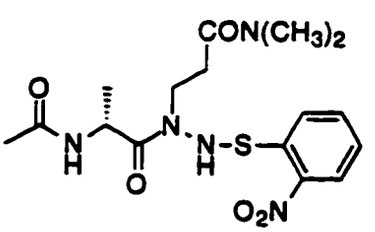
(*2RS, 3R*)-{N^3-(Benzyloxycarbonyl)-N^1-[3'-(*N,N*-dimethylamino)-3'-oxopropyl]-N^1-(*p*-methylphenylsulfonyl)}-1,3-diaminobutan-2-ol (22). The procedure used for the preparation of **21**, was used to reduce sulfonamido ketone **18** (97.9 mg, 0.2 mmol) with $NaBH_4$ solution (0.1 M in EtOH, 2 mL) to give **22** (85 mg, 87%) as a glassy solid. Spectral characterization was performed on a mixture of diastereoisomers (isomer A : isomer B, 2:1): IR (CH_2Cl_2 cast) 3331, 2935, 1715, 1626, 1525, 1498, 1401, 1336, 1237, 1158, 1090 cm^{-1}; ^1H NMR (360 MHz, CD_2Cl_2) (isomer A) δ 7.65 (d, 2 H, J = 8.2 Hz, ArH), 7.38-7.23 (m, 7 H, ArH), 5.38 (d, 1 H, J = 8.2 Hz, CONH), 5.15-5.00 (m, 3 H, OCH_2 and OH), 3.90-3.78 (m, 1 H, H-2), 3.75-3.63 (m, 1 H, H-3), 3.48-3.35 (m, 1 H, H-1), 3.34-3.20 (m, 2 H, NCH_2), 2.98 (s, 3 H, NCH_3), 2.89 (s, 3 H, NCH_3), 2.87-2.77 (m, 2 H, $COCH_2$), 2.76-2.60 (m, 1 H, NCH_2), 2.42 (s, 3 H, $ArCH_3$), 1.14 (d, 3 H, J = 6.7 Hz, H-4); (isomer B) δ 7.64 (d, 2 H, J = 8.2 Hz, ArH), 7.38-7.23 (m, 7 H, ArH), 5.22 (d, 1 H, J = 8.2 Hz, CONH), 5.15-5.00 (m, 3 H, OCH_2 and OH), 3.90-3.78 (m, 1 H, H-2), 3.75-3.63 (m, 1 H, H-3), 3.48-3.35 (m, 1 H, H-1), 3.34-3.20 (m, 2 H, NCH_2), 2.98 (s, 3 H, NCH_3), 2.89 (s, 3 H, NCH_3), 2.87-2.77 (m, 2 H, $COCH_2$), 2.76-2.60 (m, 1 H, NCH_2), 2.42 (s, 3 H, $ArCH_3$), 1.24 (d, 3 H, J = 6.7 Hz, CH_3); ^{13}C NMR (100 MHz, CD_2Cl_2) (isomer A) δ 171.72 (CON), 156.27 (OCON), 144.33 (C, ArH), 137.46 (C, ArH), 135.23 (C, ArH), 130.19 (CH, ArH), 128.81 (CH, ArH), 128.28 (CH, ArH), 128.09 (CH, ArH), 127.78 (CH, ArH), 73.48

(OCH), 66.73 (OCH_2), 55.62 (CH_2), 47.22 (NCH_2), 37.51 (NCH_3), 35.69 (NCH_3), 33.80 ($COCH_2$), 21.61 ($ArCH_3$), 17.30 (CH_3); (isomer B) δ 171.72 (CON), 156.27 (OCON), 144.33 (C, ArH), 137.46 (C, ArH), 135.18 (C, ArH), 130.10 (CH, ArH), 128.81 (CH, ArH), 128.18 (CH, ArH), 128.09 (CH, ArH), 127.78 (CH, ArH), 73.05 (OCH), 66.73 (OCH_2), 55.62 (CH_2), 47.22 (NCH_2), 37.51 (NCH_3), 35.69 (NCH_3), 33.66 ($COCH_2$), 21.61 ($ArCH_3$), 18.50 (CH_3); MS (FAB) 492.1 (46) (MH^+).



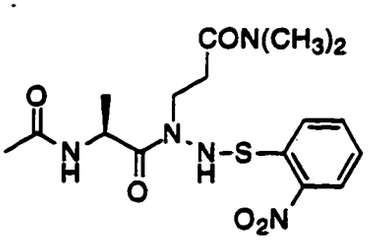
3-[N^1-(*o*-Nitrophenylsulfenyl)-N^2-(acetyl-L-leucyl-L-alanyl-L-alanyl)hydrazino]-*N,N*-(dimethyl)propanamide (23). To a solution of Cbz-hydrazino derivative **117** (56.2 mg, 0.10 mmol) in methanol (10 mL) under argon was added 10% palladium on charcoal catalyst (10 mg). The mixture was stirred under an atmosphere of hydrogen until gas absorption ceased. The catalyst was removed by filtration through a column of Celite, and the filtrate was concentrated *in vacuo* to give deprotected hydrazine **103** (42.8 mg, quantitative). To a stirred solution of **103** (42.8 mg, 0.10 mmol) and *o*-nitrophenylsulfenyl chloride (18.9 mg, 0.10 mmol) in DMF (1 mL) under argon, at -10 °C, was added triethylamine (14 μL, 0.10 mmol). After 30 min at -10 °C, the reaction mixture was allowed to warm to room temperature and was stirred for 1 h. The solution was then concentrated *in vacuo*. Purification by HPLC (linear gradient elution over 20 min of acetonitrile and water, from 25% to 40%, t_R 10.6 min) gave the title compound **23** (37.8 mg, 65%) as a yellow powder. Spectral characterization was performed on a mixture of conformational isomers (isomer A : isomer B, 5 : 6): mp 133-145 °C (decomposed); IR (μscope) 3278, 3268, 3070, 2956, 2935, 2872, 1651, 1628, 1593, 1512, 1450, 1335 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) (isomer A) δ 8.52 (d, 1 H, J = 8.5 Hz, ArH), 8.26 (dd, 1

H, J = 8.5, 1.0 Hz, ArH), 7.76-7.65 (m, 1 H, ArH), 7.40-7.30 (m, 1 H, ArH), 4.38-4.13 (m, 3 H, α-CH Leu and 2 α-CH Ala), 3.76-3.60 (m, 2 H, NCH_2), 2.98 (s, 3 H, NCH_3), 2.89 (s, 3 H, NCH_3), 2.77-2.58 (m, 2 H, $COCH_2$), 1.98 (s, 3 H, $COCH_3$), 1.76-1.62 (m, 1 H, CH Leu), 1.62-1.50 (m, 2 H, CH_2 Leu), 1.41-1.24 (m, 6 H, CH_3 Leu), 1.00-0.82 (m, 6 H, 2 CH_3 Ala); (isomer B) δ 8.43 (d, 1 H, J = 8.5 Hz, ArH), 8.26 (dd, 1 H, J = 8.5, 1.0 Hz, ArH), 7.76-7.65 (m, 1 H, ArH), 7.40-7.30 (m, 1 H, ArH), 4.38-4.13 (m, 3 H, α-CH Leu and 2 α-CH Ala), 3.76-3.60 (m, 2 H, NCH_2), 2.98 (s, 3 H, NCH_3), 2.89 (s, 3 H, NCH_3), 2.77-2.58 (m, 2 H, $COCH_2$), 1.97 (s, 3 H, $COCH_3$), 1.76-1.62 (m, 1 H, CH Leu), 1.62-1.50 (m, 2 H, CH_2 Leu), 1.41-1.24 (m, 6 H, 2 CH_3 Leu), 1.00-0.82 (m, 6 H, 2 CH_3 Ala); ^{13}C NMR (75 MHz, CD_3OD) (isomer A) δ 175.10, 174.65, 173.68, 173.57, 173.13, 143.91, 143.10, 135.34, 127.26, 126.51, 126.37, 57.89, 53.57, 50.55, 49.60, 41.68, 37.84, 35.82, 32.74, 25.90, 23.44, 22.45, 22.02, 17.65, 17.28; (isomer B) δ 175.13, 174.77, 173.76, 173.68, 173.13, 143.91, 143.10, 135.34, 127.06, 126.51, 126.37, 57.89, 53.46, 50.78, 49.60, 41.68, 37.84, 35.82, 32.74, 25.90, 23.40, 22.53, 21.92, 17.56, 17.51; MS (FAB) 582.1 (25) (MH^+).



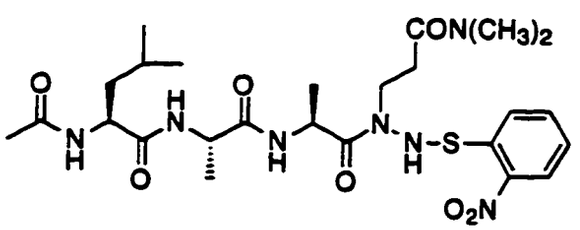
3-[N^1-(Acetyl-D-alanyl)-N^2-(*o*-nitrophenylsulfenyl)hydrazino] -*N*,*N*-(dimethyl)propanamide (24). To a stirred solution of **119** (81.1mg, 0.33 mmol) and *o*-nitrophenylsulfenyl chloride (62.9 mg, 0.33 mmol) in DMF (2 mL) under argon, at -10 °C, was added triethylamine (50 μL, 0.33 mmol). After 30 min at -10 °C, the reaction mixture was allowed to warm to room temperature, and was then stirred for 1 h. The solution was concentrated *in vacuo*. Purification by HPLC (linear gradient elution over 20 min of acetonitrile and water, from 25% to 45%, flow rate 15.0 mL/min, t_R 11.6 min) gave the title compound **24** (0.108 g, 82%) as a yellow powder. Spectral characterization

was performed on a mixture of conformational isomers (isomer A : isomer B, 2:1): mp 52-63 °C (decomposed); IR (CH_2Cl_2 cast) 3283, 3209, 2934, 1641, 1592, 1565, 1510, 1337, 735 cm^{-1}; ^1H NMR (360 MHz, CD_2Cl_2) (isomer A) δ 8.33-8.20 (m, 3 H, N-NH, ArH), 7.80 (ddd, 1 H, J = 7.7, 7.1, 1.3 Hz, ArH), 7.38-7.28 (m, 1 H, ArH), 6.41 (d, 1 H, J = 6.5 Hz, CONH), 5.15-5.05 (m, 1 H, CH), 3.88-3.77 (m, 2 H, NCH_2), 2.95 (s, 3 H, NCH_3), 2.92 (s, 3 H, NCH_3), 2.75-2.67 (m, 2 H, CH_2CO), 1.88 (s, 3 H, $COCH_3$), 1.17 (d, 3 H, J = 6.8 Hz, CH_3 Ala); (isomer B) δ 8.33-8.20 (m, 3 H, N-NH, ArH), 7.69 (ddd, 1 H, J = 7.7, 7.1, 1.3 Hz, ArH), 7.38-7.28 (m, 1 H ArH), 6.58 (d, 1 H, J = 6.5 Hz, CONH), 4.97-4.87 (m, 1 H, CH), 3.88-3.77 (m, 2 H, NCH_2), 2.88 (s, 3 H, NCH_3), 2.85 (s, 3 H, NCH_3), 2.82-2.74 (m, 2 H, CH_2CO), 1.95 (s, 3 H, $COCH_3$), 1.30 (d, 3 H, J = 6.8 Hz, CH_3 Ala); ^{13}C NMR (75 MHz, CD_3OD) (isomer A) δ 174.40, 172.29, 169.37, 143.05, 141.31, 134.74, 126.51, 125.96, 125.82, 45.94, 45.79, 37.54, 35.59, 32.43, 23.18, 18.69; (isomer B) δ 173.09, 170.63, 169.58, 142.54, 141.31, 134.50, 125.92, 125.82, 125.46, 45.66, 45.19, 37.15, 35.29, 31.70, 23.18, 18.69; MS (FAB) 398.0 (21) (MH^+). Anal Calcd. for $C_{16}H_{23}N_5O_5S$: C, 48.35; H, 5.83; N, 17.62. Found: C, 48.11; H, 5.56; N, 17.32.



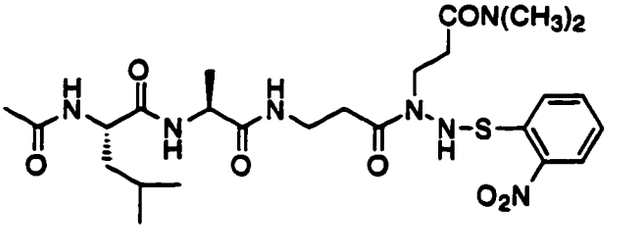
3-[N^1-(Acetyl-L-alanyl)-N^2-(o-nitrophenylsulfenyl)hydrazino]-N,N-(dimethyl)propanamide (25). The procedure used for the preparation of **24** was employed with hydrazine derivative **120** (0.100 g, 0.41 mmol), o-nitrophenylsulfenyl chloride (77.7 mg, 0.41 mmol) and triethylamine (57.2 μL, 0.41 mmol) to give the crude product. This was purified by HPLC (linear gradient elution over 20 min of acetonitrile and water, from 25% to 45%, t_R 10.1 min) to give the title compound **25** (0.120 g, 74%) as a yellow powder. Spectral characterization was performed on a mixture of conformational

isomers (isomer A : isomer B, 2:1): mp 58-70 °C (decomposed); IR (CH_2Cl_2 cast) 3284, 3209, 2934, 1642, 1592, 1565, 1510, 1337, 735 cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2) (isomer A) δ 8.34-8.20 (m, 3 H, N-NH, ArH), 7.78 (ddd, 1 H, J = 7.7, 7.2, 1.3 Hz, ArH), 7.40-7.28 (m, 1 H, ArH), 6.34 (d, 1 H, J = 7.30 Hz, CONH), 5.15-5.00 (m, 1 H, CH), 3.90-3.77 (m, 2 H, NCH_2), 2.95 (s, 3 H, NCH_3), 2.92 (s, 3 H, NCH_3), 2.71 (t, 2 H, J = 5.5 Hz, CH_2CO), 1.88 (s, 3 H, $COCH_3$), 1.16 (d, 3 H, J = 6.8 Hz, CH_3); (isomer B) δ 8.34-8.20 (m, 3 H, N-NH, ArH), 7.69 (ddd, 1 H, J = 7.7, 7.2, 1.3 Hz, ArH), 7.40-7.28 (m, 1 H, ArH), 6.48 (d, 1 H, J = 7.10 Hz, CONH), 4.98-4.85 (m, 1 H, CH), 3.90-3.77 (m, 2 H, NCH_2), 2.88 (s, 3 H, NCH_3), 2.86 (s, 3 H, NCH_3), 2.80 (t, 2 H, J = 5.5 Hz, CH_2CO), 1.95 (s, 3 H, $COCH_3$), 1.30 (d, 3 H, J = 6.8 Hz, CH_3); ^{13}C NMR (75 MHz, CD_3OD) (isomer A) δ 174.36, 172.33, 169.40, 143.05, 141.28, 134.75, 126.51, 125.97, 125.83, 45.94, 45.84, 37.57, 35.61, 32.46, 23.20, 18.69; (isomer B) δ 173.05, 170.07, 169.62, 143.05, 142.50, 134.50, 125.97, 125.83, 125.44, 45.68, 45.14, 37.16, 35.31, 31.69, 23.20, 18.69; MS (FAB) 398.2 (17) (MH^+).



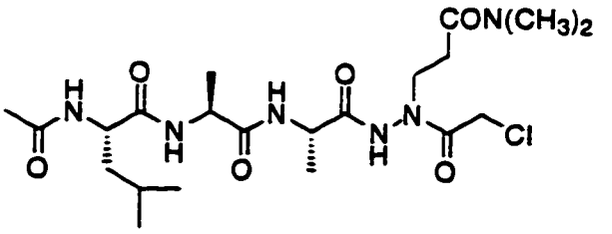
3-[N^1-(Acetyl-L-leucyl-L-alanyl-L-alanyl)-N^2-(*o*-nitrophenylsulfenyl)hydrazino]-*N*,*N*-(dimethyl)propanamide (26). The procedure used for the preparation of **23**, with Cbz-hydrazino derivative **132** (58.2 mg, 0.10 mmol) and 10% palladium on charcoal catalyst (10 mg) in methanol (10 mL), followed by *o*-nitrophenylsulfenyl chloride (18.9 mg, 0.10 mmol) gave the crude product **26**. Purification by HPLC (linear gradient elution over 20 min of acetonitrile and water, from 25% to 40%, t_R 15.6 min) gave the title compound **26** (29.1 mg, 50%) as a yellow powder. Spectral characterization was performed on a mixture of conformational isomers (isomer A : isomer B, 4 : 5): mp 94-105 °C (decomposed); IR (CH_2Cl_2 cast) 3284, 3071,

2956, 2934, 1637, 1592, 1512, 1449, 1338, 1305 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) (isomer A) δ 8.39 (d, 1 H, *J* = 8.0 Hz, ArH), 8.25 (d, 1 H, *J* = 8.4 Hz, ArH), 7.85-7.70 (m, 1 H, ArH), 7.42-7.28 (m, 1 H, ArH), 5.11-5.00 (m, 1 H, NCHCON-N), 4.43-4.28 (m, 2 H, α-CH Leu and α-CH Ala), 3.90-3.68 (m, 2 H, NCH_2), 2.96 (s, 3 H, NCH_3), 2.88 (s, 3 H, NCH_3), 2.90-2.80 (m, 2 H, $COCH_2$), 1.98 (s, 3 H, $COCH_3$), 1.76-1.58 (m, 1 H, CH Leu), 1.58-1.46 (m, 2 H, CH_2 Leu), 1.37-1.18 (m, 6 H, 2 CH_3 Ala), 0.95 (d, 3 H, *J* = 6.5 Hz, CH_3 Leu), 0.91 (d, 3 H, *J* = 6.5 Hz, CH_3 Leu); (isomer B) δ 8.25 (d, 2 H, *J* = 8.4 Hz, ArH), 7.85-7.70 (m, 1 H, ArH), 7.42-7.28 (m, 1 H, ArH), 4.86-4.76 (m, 1 H, NCHCON-N), 4.43-4.28 (m, 2 H, α-CH Leu and α-CH Ala), 3.90-3.68 (m, 2 H, NCH_2), 3.01 (s, 3 H, NCH_3), 2.91 (s, 3 H, NCH_3), 2.80-2.70 (m, 2 H, $COCH_2$), 1.98 (s, 3 H, $COCH_3$), 1.76-1.58 (m, 1 H, CH Leu), 1.58-1.46 (m, 2 H, CH_2 Leu), 1.37-1.18 (m, 6 H, 2 CH_3 Ala), 0.95 (d, 3 H, *J* = 6.5 Hz, CH_3 Leu), 0.91 (d, 3 H, *J* = 6.5 Hz, CH_3 Leu); ^{13}C NMR (75 MHz, CD_3OD) (isomer A) δ 175.91, 174.66, 174.15, 173.35, 172.65, 143.98, 142.13, 135.55, 135.34, 127.17, 126.55, 53.46, 50.03, 46.89, 46.73, 41.67, 37.65, 35.70, 32.50, 25.91, 23.28, 22.43, 22.15, 17.97, 17.78; (isomer B) δ 175.91, 174.66, 174.15, 173.35, 173.25, 144.35, 142.13, 135.55, 135.34, 127.17, 126.55, 53.18, 50.03, 46.89, 46.51, 41.86, 37.76, 35.70, 32.26, 25.91, 23.44, 22.41, 21.96, 17.95, 17.62; MS (FAB) 582.3 (4) (MH^+).



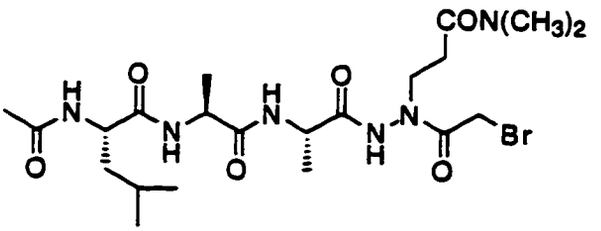
3-[*N*^{1}-(Acetyl-L-leucyl-L-alanyl-ß-alanyl)-*N*^{2}-(*o*-nitrophenylsulfenyl)hydrazino]-*N*,*N*-(dimethyl)propanamide (27). The procedure used for the preparation of **23**, was employed with Cbz-hydrazino derivative **133** (58.2 mg, 0.10 mmol) and 10% palladium on charcoal catalyst (10 mg) in methanol (10 mL), followed by

o-nitrophenylsulfenyl chloride (18.9 mg, 0.10 mmol) to give the crude product **27**. Purification by HPLC (linear gradient elution over 20 min of acetonitrile and water, from 25% to 40%, t_R 17.5 min) gave the title compound **27** (35 mg, 60%) as a yellow powder. Spectral characterization was performed on a mixture of conformational isomers (isomer A : isomer B, 2:1): mp 66-78 °C (decomposed); IR (CH_2Cl_2 cast) 3288, 3064, 2955, 2935, 1646, 1592, 1541, 1511, 1448, 1337 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) (isomer A) δ 8.38 (dd, 1 H, *J* = 8.3, 1.1 Hz, ArH), 8.26 (dd, 1 H, *J* = 8.4, 1.3 Hz, ArH), 7.86-7.72 (m, 1 H, ArH), 7.45-7.30 (m, 1 H, ArH), 4.35-4.17 (m, 2 H, α-CH Leu and α-CH Ala), 3.86-3.70 (m, 2 H, N-NCH_2), 3.54-3.37 (m, 2 H, $CONCH_2$), 3.00 (s, 3 H, NCH_3), 2.89 (s, 3 H, NCH_3), 2.82-2.65 (m, 4 H, 2 $COCH_2$), 1.98 (s, 3 H, $COCH_3$), 1.72-1.58 (m, 1 H, CH Leu), 1.58-1.50 (m, 2 H, CH_2 Leu), 1.33 (d, 3 H, *J* = 7.3 Hz, CH_3 Ala), 0.96 (d, 3 H, *J* = 6.4 Hz, CH_3 Leu), 0.92 (d, 3 H, *J* = 6.4 Hz, CH_3 Leu); (isomer B) δ 8.28 (dd, 1 H, *J* = 8.3 Hz, 1.1 Hz, ArH), 8.26 (dd, 1 H, *J* = 8.4 Hz, 1.3 Hz, ArH), 7.86-7.72 (m, 1 H, ArH), 7.45-7.30 (m, 1 H, ArH), 4.35-4.17 (m, 2 H, α-CH Leu and α-CH Ala), 3.86-3.70 (m, 2 H, N-NCH_2), 3.54-3.37 (m, 2 H, $CONCH_2$), 2.90 (s, 3 H, NCH_3), 2.86 (s, 3 H, NCH_3), 2.82-2.65 (m, 4 H, 2 $COCH_2$), 1.96 (s, 3 H, $COCH_3$), 1.72-1.58 (m, 1 H, CH Leu), 1.58-1.50 (m, 2 H, CH_2 Leu), 1.33 (d, 3 H, *J* = 7.3 Hz, CH_3 Ala), 0.96 (d, 3 H, *J* = 6.4 Hz, CH_3 Leu), 0.92 (d, 3 H, *J* = 6.4 Hz, CH_3 Leu); ^{13}C NMR (75 MHz, CD_3OD) (isomer A) δ 175.23, 174.96, 174.83, 173.72, 172.94, 144.32, 142.55, 135.54, 127.11, 126.56, 126.49, 53.90, 50.49, 46.17, 41.44, 37.76, 36.77, 35.68, 33.78, 32.25, 25.91, 23.22, 22.47, 22.22, 17.81; (isomer B) δ 175.23, 175.10, 174.83, 173.38, 172.61, 144.22, 143.97, 135.34, 127.11, 126.56, 126.49, 54.02, 50.49, 46.54, 41.44, 37.53, 36.84, 35.68, 33.13, 32.43, 25.91, 23.22, 22.47, 22.22, 17.81; MS (FAB) 582.3 (22) (MH^+). Anal Calcd. for $C_{25}H_{39}N_7O_7S$: C, 51.62; H, 6.76; N, 16.86. Found: C, 51.25; H, 6.83; N, 16.51.



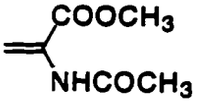
3-[N^1-(Chloroacetyl)-N^2-(acetyl-L-leucyl-L-alanyl-L-alanyl) hydrazino]-*N,N*-(dimethyl)propanamide (28). To a solution of Cbz-hydrazino derivative **117** (56.2 mg, 0.10 mmol) in methanol (10 mL) under argon was added 10% palladium on charcoal catalyst (10 mg). The mixture was stirred under an atmosphere of hydrogen until gas absorption ceased. The catalyst was removed by filtration through a column of Celite and the filtrate was concentrated *in vacuo* to give hydrazino derivative **103** (42.8 mg, quantitative). To a solution of **103** (42.8 mg, 0.1 mmol) in CH_2Cl_2 (5 mL) at -10 °C was added triethylamine (30.1 μL, 0.2 mmol) and chloroacetyl chloride (12.5 μL, 0.15 mmol). After removal of the cooling bath, the solution was stirred at room temperature for 1 h and then concentrated *in vacuo*. The crude product was purified by HPLC (linear gradient elution over 20 min of 0.1% TFA in acetonitrile and 0.1% TFA in water, from 20% to 40%, t_R 9.2 min) to give the title compound **28** (22.7 mg, 45%) as a white powder. Spectral characterization was performed on a mixture of conformational isomers (isomer A : isomer B, 3:1): mp 133-143 °C (decomposed); IR (μscope) 3282, 2956, 2937, 2871, 1641, 1631, 1529, 1447, 1402, 1369 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) (isomer A) δ 4.40-4.00 (m, 5 H, α-CH Leu, 2 α-CH Ala and $COCH_2Cl$), 4.00-3.50 (brs, 2 H, NCH_2), 3.03 (s, 3 H, NCH_3), 2.90 (s, 3 H, NCH_3), 2.67 (t, 2 H, J = 7.3 Hz, $COCH_2$), 1.97 (s, 3 H, $COCH_3$), 1.75-1.60 (m, 1 H, CH Leu), 1.60-1.50 (m, 2 H, CH_2 Leu), 1.42 (d, 3 H, J = 7.2 Hz, CH_3 Ala), 1.36 (d, 3 H, J = 7.2 Hz, CH_3 Ala), 0.96 (d, 3 H, J = 6.5 Hz, CH_3 Leu), 0.92 (d, 3 H, J = 6.5 Hz, CH_3 Leu); (isomer B) δ 4.40-4.00 (m, 5 H, α-CH Leu, 2 α-CH Ala, and CH_2Cl), 4.00-3.50 (brs, 2 H, NCH_2), 3.03 (s, 3 H, NCH_3), 2.90 (s, 3 H, NCH_3), 2.67 (t, 2 H, J = 7.3 Hz, $COCH_2$), 1.96 (s, 3 H, $COCH_3$), 1.75-1.60 (m, 1 H, CH Leu), 1.60-1.50 (m, 2 H, CH_2 Leu), 1.43 (d, 3 H, J =

7.2 Hz, CH_3 Ala), 1.35 (d, 3 H, J = 7.2 Hz, CH_3 Ala), 0.96 (d, 3 H, J = 6.5 Hz, CH_3 Leu), 0.92 (d, 3 H, J = 6.5 Hz, CH_3 Leu); ^{13}C NMR (75 MHz, CD_3OD) (isomer A) δ 175.20, 175.02, 174.40, 173.66, 172.87, 170.32, 53.67, 50.43, 49.71, 46.51, 42.66, 41.69, 37.68, 35.69, 31.79, 25.90, 23.39, 22.44, 21.97, 17.65, 16.89; (isomer B) δ 175.20, 175.02, 174.40, 173.66, 172.87, 170.32, 53.34, 50.70, 50.03, 46.57, 42.70, 41.75, 37.68, 35.69, 31.72, 25.90, 23.48, 22.44, 21.86, 17.32, 16.89; MS (FAB) 505.2 (47) (MH^+).

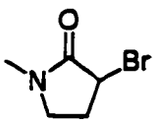


3-[N^1-(Bromoacetyl)-N^2-(acetyl-L-leucyl-L-alanyl-L-alanyl) hydrazino]-*N*,*N*-(dimethyl)propanamide (29). The procedure used for the preparation of **28**, with Cbz-hydrazino derivative **117** (58.2 mg, 0.10 mmol) and 10% palladium on charcoal catalyst (10 mg) in methanol (10 mL), followed by triethylamine (30.1 μL, 0.2 mmol) and bromoacetyl bromide (13 μL, 0.15 mmol) in CH_2Cl_2 (5 mL) gave the crude product **29**. Purification by HPLC (linear gradient elution over 20 min of 0.1% TFA in acetonitrile and 0.1% TFA in water, from 20% to 40%, t_R 9.8 min) gave pure **29** (21.9 mg, 40%) as a white powder. Spectral characterization was performed on a mixture of conformational isomers (isomer A : isomer B, 3 : 1): mp 81-90 °C (decomposed); IR (μscope) 3283, 2956, 2935, 2871, 1645, 1537, 1448, 1402, 1370 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) (isomer A) δ 4.40-4.20 (m, 3 H, α-CH Leu and 2 α-CH Ala), 4.20-3.50 (m, 4 H, $COCH_2Br$ and NCH_2), 3.04 (s, 3 H, NCH_3), 2.86 (s, 3 H, NCH_3), 2.75-2.60 (brs, 2 H, $COCH_2$), 1.97 (s, 3 H, $COCH_3$), 1.76-1.60 (m, 1 H, CH Leu), 1.60-1.48 (m, 2 H, CH_2 Leu), 1.42 (d, 3 H, J = 7.2 Hz, CH_3 Ala), 4.2), 1.36 (d, 3 H, J = 7.2 Hz, CH_3 Ala), 4.2), 0.96 (d, 3 H, J = 6.5 Hz, CH_3 Leu), 0.92 (d, 3 H, J =

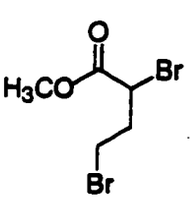
6.5 Hz, CH_3 Leu); (isomer B) δ 4.40-4.20 (m, 3 H, α-CH Leu and 2 α-CH Ala), 4.20-3.50 (m, 4 H, CH_2Br and NCH_2), 3.04 (s, 3 H, NCH_3), 2.86 (s, 3 H, NCH_3), 2.75-2.60 (brs, 2 H, $COCH_2$), 1.98 (s, 3 H, $COCH_3$), 1.76-1.6 (m, 1 H, CH Leu), 1.60-1.48 (m, 2 H, CH_2 Leu), 1.41 (d, 3 H, J = 7.2 Hz, CH_3 Ala), 1.37 (d, 3 H, J = 7.2 Hz, CH_3 Ala), 0.96 (d, 3 H, J = 6.5 Hz, CH_3 Leu), 0.92 (d, 3 H, J = 6.5 Hz, CH_3 Leu); ^{13}C NMR (75 MHz, CD_3OD) (isomer A) δ 175.14, 174.98, 174.36, 173.63, 172.86, 170.52, 53.64, 50.38, 49.97, 46.52, 42.68, 41.71, 37.72, 35.72, 31.73, 25.91, 23.41, 22.46, 21.99, 17.71, 16.96; (isomer B) δ 175.14, 174.98, 174.36, 173.63, 172.86, 170.52, 53.40, 50.63, 49.86, 46.58, 42.68, 41.71, 37.72, 35.72, 31.66, 25.91, 23.50, 22.46, 21.88, 17.45, 16.96; MS (FAB) 549.0 (27) (MH^+).



Methyl 2-Acetamidoacrylate (31).[60] To a solution of the *N*-chloro derivative **41** (59 mL, 0.101 mol) in CH_2Cl_2 (200 mL) was added 1,4-diazobicyclo[2,2,2]octane (DABCO) (11.34 g, 0.101 mmol) at a rate such that a gentle reflux was maintained. The reaction mixture was stirred for an additional 10-20 min until reflux had subsided and then heated at 40-50 °C in a water bath for 10-20 min with vigorous stirring. The reaction was cooled in an ice bath and the hydrochloride salt was removed by filtration. The organic phase was washed with water (40 mL), 1N HCl (40 mL), saturated $NaHCO_3$ (40 mL), and dried ($MgSO_4$). After drying the organic phase, a trace of hydroquinone was added and the solvent was removed *in vacuo* to give the crude product as a colorless oil. Crystallization from CH_2Cl_2-Hex of the crude oil gave compound **31** (5.80 g, 51%) as a white solid: mp 51-53 °C (Lit.[60], 50-52 °C); ^1H NMR (200 MHz, $CDCl_3$) δ 7.85-7.50 (brs, 1 H, NH), 6.56 (s, 1 H, $=CH_2$), 5.86 (s, 1 H, $=CH_2$), 3.82 (s, 3 H, OCH_3), 2.16 (s, 3 H, $CH_3C=O$).

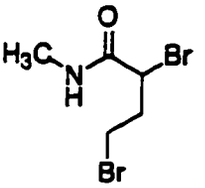


***N*-Methyl-3-bromo-2-pyrrolidinone (35).**[59] To a solution of *N*-methyl 2,4-dibromobutanamide (**38**) (8.0 g, 31 mmol) in absolute ethanol (20 mL) at room temperature was added dropwise sodium ethoxide solution (2 M in EtOH, 15.5 mL). The reaction mixture was stirred for 3 h, and the precipitate (NaBr) was removed by filtration. The filtrate was concentrated *in vacuo*. The residue was dissolved in $CHCl_3$ (100 mL), and the solution was washed with water (60 mL) and brine (60 mL). The organic phase was dried (Na_2SO_4) and concentrated *in vacuo*. Purification by flash chromatography (EtOAc : Hex, 1 : 1) gave pyrrolidinone **35** (3.76 g, 65%) as an oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.38 (dd, 1 H, J = 7.3, 2.7 Hz, COCHBr), 3.64-3.47 (m, 1 H, CH_2N), 3.37-3.24 (m, 1 H, CH_2N), 2.89 (s, 3 H, NCH_3), 2.71-2.49 (m, 1 H, CH_2), 2.40-2.24 (m, 1 H, CH_2); HRMS calcd for C_5H_8NOBr 178.9769, found 178.9773.

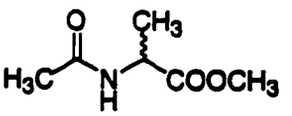


Methyl 2,4-Dibromobutanoate (37).[58] Phosphorous tribromide (1 mL) was added to γ–butyrolactone (49.5 g, 0.575 mol) and the reaction mixture was heated to 100 °C. The mixture was stirred while bromine (28 mL, 0.55 mol) was added dropwise beneath the surface of the liquid. The temperature was maintained at 110-115 °C by the rate of bromine addition. When the rate of bromine uptake decreased, phosphorous tribromide (0.25 mL) was added and heat was applied to maintain reaction temperature. The addition of bromine was continued until hydrogen bromide evolution was evident. Then the product was stirred and cooled to room temperature and finally cooled in an ice bath. To this crude product was added absolute methanol (240 mL) and the resulting solution was saturated with dry hydrochloric acid. The reaction mixture was allowed to stand at room temperature

for 22 h, and the methanol was removed *in vacuo*. The residue was extracted with ether. The ether extract was washed with 3% sodium bicarbonate solution to remove unreacted acid and then dried over anhydrous sodium sulfate. The solvent was removed and the residue distilled under reduced pressure. A fraction was collected boiling at 63-65 °C/1.2 mm Hg(Lit.[58], 68-70 °C/2 mm Hg) to yield **37** (119.7 g, 80%). ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.48 (t, 1 H, J = 6.8 Hz, CH), 3.78 (s, 3 H, OCH_3), 3.51 (t, 2 H, J = 6.3 Hz, CH_2Br), 2.60-2.48 (m, 2H, CH_2).

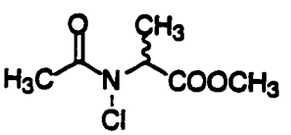


***N*-Methyl-2,4-dibromobutanamide (38).**[59] To stirred methyl 2,4-dibromobutanoate (**37**) (26 g, 0.1 mmol) in an ice-bath was added 30% aqueous solution of CH_3NH_3 (110 mL). The reaction mixture was stirred at room temperature for 16 h, and then extracted with $CHCl_3$ (2 x 150 mL). The organic phase was washed with brine (200 mL) and dried (Na_2SO_4). The solution was concentrated *in vacuo*. A fraction was collected boiling at 103-105°C/0.1 mmHg to give amide **38** (13.0 g, 50%) as a solid: mp 45-47°C (Lit.[59] 46-47°C); ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.70-6.20 (brs, 1 H, CONH), 4.52 (dd, 1 H, J = 8.8, 4.8 Hz, COCHBr), 3.62-3.45 (m, 2 H, CH_2Br), 2.88 (d, 3 H, J = 4.8 Hz, NCH_3), 2.75-2.32 (m, 2 H, CH_2); HRMS calcd for $C_5H_9NOBr_2$ 260.9010, found 260.9004.

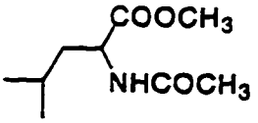


***N*-Acetyl-DL-alanine Methyl Ester (39).** To a stirred solution of *N*-acetyl-DL-alanine (2.62 g, 20 mmol) in anhydrous MeOH (30 mL) in an ice bath was added slowly HCl gas. The adddition of HCl gas was continued until HCl evolution was evident.

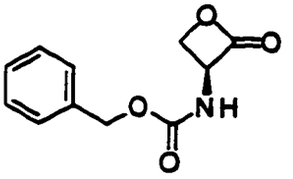
Then the reaction mixture was stirred in an ice bath for another 1 h and allowed to warm to room temperature overnight. The solvent was removed *in vacuo*, and the residual hydrogen chloride was neutralized by the addition of saturated hydrogen carbonate. The aqueous phase was extracted with CH_2Cl_2 (2 x 100 mL). The organic phase was washed with brine (50 mL), and again extracted with CH_2Cl_2 (2 x 30 mL). The combined CH_2Cl_2 extracts were dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. Purification by distillation gave methyl ester **39** (2.30 g, 80%) as an oil: bp 90-93 °C/2 mmHg (Lit.[60], 78-85 °C/0.2 mmHg); ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.55-6.30 (brs, 1 H, CONH), 4.54 (q, 1 H, J = 7.3 Hz, CH), 3.70 (s, 3 H, OCH_3), 1.97 (s, 3 H, CH_3C=O), 1.35 (d, 3 H, J = 7.3 Hz, CH_3).



***N*-Acetyl-*N*-chloro-DL-alanine Methyl Ester (41).** *N*-Acetyl-DL-alanine methyl ester (**39**) (14.68 g, 0.101 mol) was dissolved in anhydrous methanol (20 mL) containing a trace of hydroquinone. The resulting solution was stirred at 10-20 °C in a cold water bath. *t*-Butyl hypochlorite (**40**) (15 mL, 0.125 mol) was added in one portion followed by the addition of 0.3 mL of a 1% solution of sodium in methanol. The reaction mixture was stirred at 10-20 °C for 2 h. Alternatively, the reaction flask was wrapped with aluminum foil and stored overnight in a refrigerator at - 8 °C. The excess *t*-butyl hypochlorite and methanol were removed *in vacuo* maintaining the water bath below 40 °C. The resulting oil was taken up in CH_2Cl_2 (60 mL), washed once with saturated NaCl (15 mL), and dried ($MgSO_4$) while being cooled in an ice bath. The filtrate containing the *N*-chloro compound was stored in a freezer until required. A sample of the *N*-chloro derivative **41** was isolated by removal of the solvent *in vacuo*: ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.30 (m, 1 H, CH), 3.70 (s, 3 H, OCH_3), 2.25 (s, 3 H, CH_3C=O), 1.45 (d, 3 H, J = 7.2 Hz, CH_3).

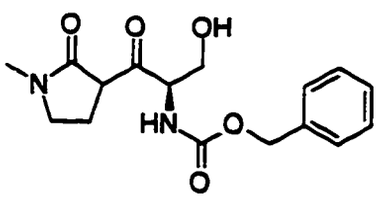


***N*-Acetyl-DL-leucine Methyl Ester (43).** A slurry of CuI (0.27 g, 1.4 mmol) in ether (10 mL) was stirred and cooled to 0 °C in an ice bath under argon. A solution of isopropylmagnesium chloride (35 mL of 0.80 M, 28 mmol) in ether/benzene (3:1) was added and, after 10 min, a solution of methyl 2-acetamidoacrylate (2.0 g, 14 mmol) in ether (15 ml) was added dropwise. Stirring was continued for an additional 30 min at 0 °C. The reaction mixture was then quenched with saturated aqueous NH_4Cl solution and the aqueous layer extracted with ether (3 x 60 mL). After drying over anhydrous sodium sulfate, the combined organic extracts were concentrated *in vacuo* and the residue was crystallized from petroleum ether to give **43** (1.52 g, 58%) as a solid: mp 77-79 °C (lit.[57] 76.5-77 °C); ^1H NMR (200 MHz, $CDCl_3$) δ 6.60-6.25 (brs, 1 H, CONH), 4.30-4.21 (m, 1 H, NCHC=O), 3.80 (s, 3 H, OCH_3), 1.98 (s, 3 H, CH_3C=O), 1.72-1.59 (m, 1 H, CH), 1.58-1.49 (m, 2 H, CH_2), 0.96 (d, 3 H, J = 6.4 Hz, CH_3), 0.92 (d, 3 H, J = 6.4 Hz, CH_3).



***N*-(Benzyloxycarbonyl)-L-serine-ß-lactone (50).**[75] To a solution of triphenylphosphine (2.62 g, 10.0 mmol) in THF (40 mL) under argon, cooled to -78 °C, was added dropwise over 5 min, a solution of di-*tert*-butyl azodicarboxylate (2.30 g, 10.0 mmol) in THF (10 mL). The resulting pale yellow slurry was stirred at -78 °C for 10 min, and a solution of Cbz-L-serine (2.39 g, 10.0 mmol) in THF (15 mL) was added dropwise to the mixture over 15 min. After completion of the addition, the reaction mixture was stirred at -75 °C to -78 °C for 20 min, then the cooling bath was removed. The reaction

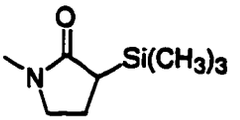
mixture was slowly warmed with stirring to room temperature over 2.5 h. The solvent was removed on a rotary evaporator at 35 °C. The residue was dried briefly under high vacuum. Purification by column chromatography (EtOAc : Hex, 1 : 4) gave **50** (0.82 g, 40%) as a white solid: mp 134-135 °C (lit.[75] mp 133-134 °C); ^{1}H NMR (200 MHz, CD_2Cl_2) δ 7.41-7.30 (m, 5 H, ArH), 5.60-5.40 (brs, 1 H, CONH), 5.15 (s, 2 H, OCH_2Ar), 5.11-4.98 (m, 1 H, NCH), 4.50-4.37 (m, 2 H, OCH_2C).



***(2S, 3'RS)*-2-(Benzyloxycarbonylamino)-1-(*N*-methyl-2'-pyrrolidinon-3'-yl)-3-hydroxypropanone (54).** To a solution of LDA (6.8 mmol) in THF (6.8 mL) under argon, cooled to -78 °C, was added *N*-methyl-2-pyrrolidinone (2 M solution in THF, 3.40 mL). The reaction mixture was stirred at -78 °C for 1 h to form lithium salt **A**.

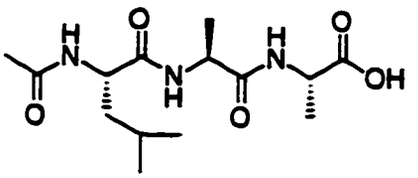
In a 100 mL dry three-necked flask was placed CuCN (311.5 mg, 3.48 mmol). The vessel was flushed with argon and then evacuated under high vacuum. The process was repeated three times leaving the CuCN under argon. Dry THF (7 mL) was added and the slurry was cooled to -78 °C. To this slowly stirring suspension was added dropwise the lithium salt **A**. The heterogeneous mixture was allowed to warm gradually until complete dissolution occurred and was then recooled to -15 °C. A solution of *N*-Cbz ß-lactone **50** (150 mg, 0.68 mmol) in THF (2.5 mL) was added dropwise over 5 min. The reaction mixture was stirred at -15 °C for 2 h, and the reaction was quenched by addition of degassed 0.5 N HCl (10 mL). The mixture was stirred for 20 min, then the precipitate was filtered. The filtrate was extracted with CH_2Cl_2 (2 x 30 mL) and the organic phase was washed with saturated aqueous $NaHCO_3$ (25 mL), water (25 mL) and brine (25 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give crude **54**. Purification by flash

chromatography (EtOAc : Hex, 1 : 2) gave alcohol **54** (97 mg, 45 %) as an oil. Spectral characterization was performed on a mixture of diastereomers (isomer A : isomer B, 4 :6): IR (CH_2Cl_2 cast) 3319, 2954, 2931, 1713, 1670, 1500, 1538, 1455, 1304, 1260, 1067 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) (isomer A) δ 7.50-7.30 (m, 5 H, ArH), 6.70-6.45 (brs, 1 H, CONH), 5.19 (s, 2 H, OCH_2Ar), 4.62-4.50 (m, 1 H, OH), 4.20-4.05 (m, 1 H, NCHC=O), 4.02-3.75 (m, 1 H, COCHCO), 3.60-3.28 (m, 2 H, OCH_2) 3.20-2.90 (m, 2 H, NCH_2), 2.83 (s, 3 H, NCH_3), 2.60-2.38 (m, 1 H, CH_2), 2.10-1.86 (m, 1 H, CH_2) ; (isomer B) δ 7.50-7.30 (m, 5 H, ArH), 5.90-5.75 (brs, 1 H, CONH), 5.15 (s, 2 H, OCH_2Ar), 4.48-4.34 (m, 1 H, OH), 4.20-4.05 (m, 1 H, NCHC=O), 4.02-3.75 (m, 1 H, COCHCO), 3.60-3.28 (m, 2 H, OCH_2) 3.20-2.90 (m, 2 H, NCH_2), 2.87 (s, 3 H, NCH_3), 2.60-2.38 (m, 1 H, CH_2), 2.31-2.10 (m, 1 H, CH_2); HRMS calcd for $C_{16}H_{20}N_2O_5$ 320.1372, found 320.1370.

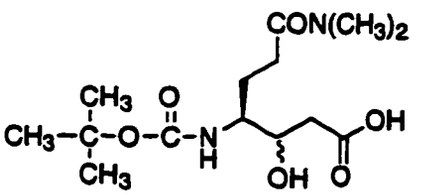


(*N*-Methyl-2-pyrrolidinon-3-yl)trimethylsilane (62). To a solution of LDA (1 M, 60 mmol) in THF (60 mL) at -78 °C under argon was added dropwise a solution of *N*-methylpyrrolidinone (4.7 mL, 50 mmol) in THF (20 mL). The temperature was allowed to rise to 0 °C, and chlorotrimethylsilane (9.5 mL, 75 mmol) was then added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 4 h. The reaction mixture was poured into pentane (150 mL) and the precipitate was filtered. The filtrate was concentrated *in vacuo*, and the residue was treated again with pentane (60 mL). The filtrate was concentrated *in vacuo* and the residue distilled under reduced pressure. A fraction was collected boiling at 51-52 °C/1.2 mm Hg to yield **62** (4.95 g, 58%) as a colorless liquid: IR (neat film) 2953, 2897, 1675, 1500, 1538, 1457, 1428, 1398, 1248, 1110, 1025, 843 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 3.40-3.20 (m, 2 H, NCH_2), 2.75 (s, 3 H, NCH_3), 2.25-2.06 (m, 1 H, CHC=O), 1.97-1.78 (m, 2 H, CH_2),

0.40 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 176.98 (CONH), 49.10 (NCH_2), 32.41 (NCH_3), 29.34 (CHC=O), 20.02 (CH_2), -2.08 ($Si(CH_3)_3$); HRMS calcd for $C_8H_{17}NOSi$ 171.1068, found 171.1079.

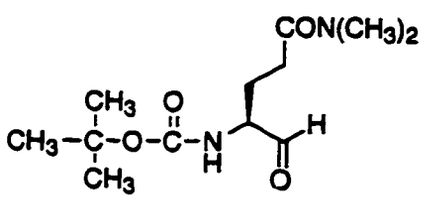


Acetyl-L-leucyl-L-alanyl-L-alanine (65).[40] The peptide **65** was prepared on a Rainin PS-3 solid-phase peptide synthesizer using standard Fmoc chemistry on Wang resin. The N-terminus was blocked using acetic anhydride. Purification by HPLC (gradient elution over 20 min of 0.1 % TFA in acetonitrile and 0.1 % TFA in water, from 10% to 20%, t_R 12.1 min) give peptide **65** (75%) as a white solid: ^1H NMR (360 MHz, CD_3OD) δ 4.82-4.60 (m, 3 H, α-H Leu and 2 α-H Ala), 1.97 (s, 3 H, CH_3C=O), 1.76-1.63 (m, 1 H, CH Leu), 1.60-1.52 (m, 2 H, CH_2 Leu), 1.38 (d, 3 H, J = 7.2 Hz, CH_3 Ala), 1.32 (d, 3 H, J = 7.2 Hz, CH_3 Ala), 0.96 (d, 3 H, J = 6.4 Hz, CH_3 Leu), 0.92 (d, 3 H, J = 6.4 Hz, CH_3 Leu); MS (FAB) 216.0 (54) (MH^+).



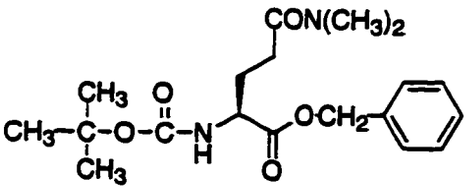
***(3RS, 4S)*-4-(*tert*-Butyloxycarbonylamino)-7-(*N,N*-dimethylamino)-3-hydroxy-7-oxoheptanoic Acid (68).** To a solution of hydroxy ester **86** (0.205 g, 0.5 mmol) in methanol (50 mL) under argon was added 10% palladium on charcoal catalyst (20.5 mg). The mixture was stirred under an atmosphere of hydrogen until gas absorption ceased. The catalyst was removed by filtration through a column of Celite and the filtrate was concentrated *in vacuo* to give acid **68** (0.159 g, quantitative): Spectral characterization was performed on a mixture of diastereoisomers (isomer A : isomer B, 10 : 3): IR (CH_2Cl_2

cast) 3328 (brs), 2977, 2933, 1708, 1625, 1521, 1402, 1366, 1249, 1169 cm^{-1}; ^1H NMR (360 MHz, CD_2Cl_2) (isomer A) δ 13.00-11.00 (brs, 1 H, COOH), 5.53-5.40 (d, 1 H, J = 8.2 Hz, CONH), 4.02-3.89 (m, 1 H, H-5), 3.60-3.44 (m, 1 H, H-4), 2.98 (s, 3 H, NCH_3), 2.93 (s, 3 H, NCH_3), 2.62-2.30 (m, 5 H, H-2, H-6 and OH), 2.02-1.86 (m, 1 H, H-3), 1.84-1.68 (m, 1 H, H-3), 1.42 (s, 9 H, $C(CH_3)_3$); (isomer B) δ 13.00-11.00 (brs, 1 H, COOH), 5.53-5.40 (d, 1 H, J = 8.2 Hz, CONH), 4.02-3.89 (m, 1 H, H-5), 3.60-3.44 (m, 1 H, H-4), 2.97 (s, 3 H, NCH_3), 2.92 (s, 3 H, NCH_3), 2.62-2.30 (m, 5 H, H-2, H-6 and OH), 2.02-1.86 (m, 1 H, H-3), 1.84-1.68 (m, 1 H, H-3), 1.42 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (75 MHz, CD_2Cl_2) (isomer A) δ 173.96, 173.68, 156.81, 79.47, 71.35, 55.03, 39.00, 37.59, 35.86, 29.90, 28.49, 25.58; (isomer B) δ 173.96, 172.90, 156.47, 79.47, 71.35, 55.20, 39.23, 37.59, 35.86, 29.90, 28.49, 25.45; MS (FAB) 318.9 (61) (MH^+).

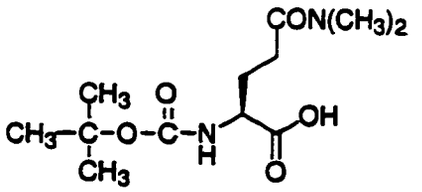


***N*-(*tert*-Butyloxycarbonyl)-γ-(*N,N*-dimethyl)-L-glutaminal (69).** To a stirred suspension of thioester **75** (1.59 g, 5 mmol) and 10% Pd on charcoal (80 mg) in dry CH_2Cl_2 was added triethylsilane (3.99 mL, 25 mmol) at 0 °C under argon. The reaction mixture was stirred at 0 °C for 50 min. The catalyst was removed by filtration through Celite and washed with CH_2Cl_2. The solvent was removed *in vacuo* to give a colorless oil. Purification by flash chromatography (ethyl acetate) gave aldehyde **69** (0.84 g, 65%) as an oil: IR (CH_2Cl_2 cast) 3302, 2976, 2932, 1733, 1707, 1635, 1511, 1366, 1252, 1167 cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2) δ 9.52 (s, 1 H, HC=O), 5.93-5.78 (brs, 1 H, CONH), 4.13-3.98 (m, 1 H, CH), 2.96 (s, 3 H, NCH_3), 2.89 (s, 3 H, NCH_3), 2.52-2.27 (m, 2 H, $COCH_2$), 2.20-2.04 (m, 1 H, CH_2), 1.97-1.79 (m, 1 H, CH_2), 1.42

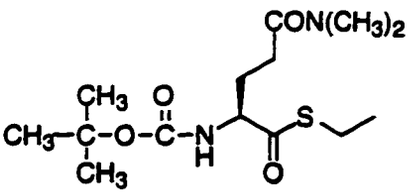
(s, 9 H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 200.77, 172.27, 156.19, 79.99, 60.08, 37.30, 35.59, 29.13, 28.38, 24.34; MS (FAB) 259.0 (22) (MH^+).



***N*-(*tert*-Butyloxycarbonyl)-γ-(*N*,*N*-dimethyl)-L-glutamine Benzyl Ester (73).** *N*-*t*-Boc-L-glutamic acid, α-benzyl ester (6.74 g, 20 mmol) was dissolved in dry THF (45 mL), with cooling to -20 °C under an argon atmosphere. *N*-Methylmorpholine (2.42 mL, 22 mmol) was added, followed by isobutyl chloroformate (2.85 mL, 22 mmol). The mixture was stirred for 20 min at -20 °C, and dimethylamine hydrochloride (1.80 g, 22 mmol) and *N*-methylmorpholine (2.42 mL, 22 mmol) were added. After 30 min at -20 °C, the reaction mixture was allowed to warm to room temperature and stirred for 5 h. The mixture was partitioned between ethyl acetate (60 mL) and water (50 mL). The aqueous phase was extracted with ethyl acetate (2 x 30 mL) and the combined organic extracts were washed with saturated aqueous $NaHCO_3$ (15 mL) and brine (50 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give a white solid. Purification by recrystallization (CH_2Cl_2-hexane) gave the title compound **73** (6.43 g, 82%) as a white solid: mp 93-95 °C; IR (CH_2Cl_2 cast) 2975, 2932, 1744, 1710, 1638, 1499, 1455, 1365, 1251, 1165 cm^{-1}; ^1H NMR (400 MHz, CD_2Cl_2) δ 7.40-7.28 (m, 5 H, ArH), 6.00 (d, 1 H, J = 7.2 Hz, CONH), 5.15 (dd, 2 H, J = 29.7, 12.5 Hz, CH_2O), 4.32-4.22 (m, 1 H, CH), 2.87 (s, 3 H, NCH_3), 2.85 (s, 3 H, NCH_3), 2.40-2.24 (m, 2 H, CH_2CO), 2.20-1.93 (m, 2 H, CH_2), 1.42 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 172.84, 172.09, 155.97, 136.32, 128.89, 128.60, 128.54, 79.59, 67.05, 54.12, 37.18, 35.55, 29.59, 28.48, 27.38; MS (FAB) 365.2 (69) (MH^+).

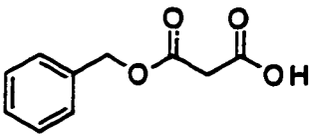


***N*-(*tert*-Butyloxycarbonyl)-γ-(*N*,*N*-dimethyl)-L-glutamine (74).** To a solution of benzyl ester **73** (3.64 g, 10 mmol) in methanol (50 mL) under argon was added 10% palladium on charcoal catalyst (0.364 g). The mixture was stirred under an atmosphere of hydrogen until gas absorption ceased. The catalyst was removed by filtration through a column of Celite and the filtrate was concentrated *in vacuo* to give **74** (2.73 g, quantitative) as a glassy solid: IR (CH_2Cl_2 cast) 3317, 2977, 2934, 1711, 1610, 1509, 1366, 1250, 1165 cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2) δ 5.82-5.64 (brs, 1 H, CONH), 4.16-4.04 (m, 1 H, CH), 3.04 (s, 3 H, NCH_3), 2.97 (s, 3 H, NCH_3), 2.96-2.78 (m, 1 H, CH_2CO), 2.61-2.40 (m, 1 H, CH_2CO), 2.26-2.08 (m, 1 H, CH_2), 1.97-1.83 (m, 1 H, CH_2), 1.42 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 174.09, 173.96, 156.01, 79.92, 53.62, 37.70, 35.98, 30.08, 28.77, 28.40; MS (FAB) 275.0 (93) (MH^+).

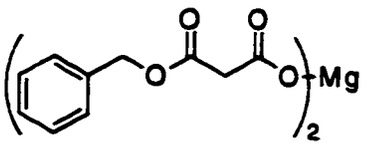


***N*-(*tert*-Butyloxycarbonyl)-γ-(*N*,*N*-dimethyl)-L-glutamine Thioethyl Ester (75).** A solution of of *N*-*t*-Boc-L-glutamic acid, γ-dimethylamide **74** (2.33 g, 8.50 mmol) in dry CH_2Cl_2 (40 mL) under argon at 0 °C was treated with ethyl chloroformate (0.90 mL, 9.35 mmol) and triethylamine (1.41 mL, 9.35 mmol) and stirred for 20 min. Ethanethiol (0.69 mL, 9.35 mmol) and triethylamine (1.41 mL, 9.35 mmol) were added, and the reaction mixture was stirred for a further 30 min. It was then allowed to warm to room temperature and stir overnight. To the mixture was added CH_2Cl_2 (60 mL), and the organic phase was then washed with 0.5 N HCl (2 x 20 mL), saturated aqueous $NaHCO_3$ (20mL) and brine (50 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give a white

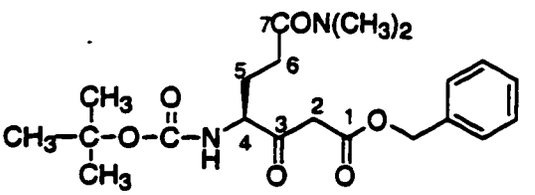
solid. Recrystallization (CH_2Cl_2-hexane) gave the thioester **75** (2.25 g, 83%) as a white solid: mp 145-146 °C; IR (CH_2Cl_2 cast) 3025, 2931, 1710, 1674, 1617, 1541, 1457, 1401, 1289, 1253, 1163 cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2) δ 6.01 (d, 1 H, J = 7.2 Hz, CONH), 4.28-4.15 (m, 1 H, CH), 2.95 (s, 3 H, NCH_3), 2.91 (s, 3 H, NCH_3), 2.83 (q, 2 H, J = 7.4 Hz, S-CH_2), 2.52-2.28 (m, 2 H, CH_2CO), 2.16-1.88 (m, 2 H, CH_2), 2.05-1.93 (m, 1 H, CH_2), 1.42 (s, 9 H, $C(CH_3)_3$), 1.21 (t, 3 H, J = 7.4 Hz, CH_3); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 202.36, 172.36, 155.80, 79.98, 61.15, 37.34, 35.68, 29.60, 28.47, 27.53, 23.42, 14.74; MS (FAB) 319.1 (100) (MH^+).



Monobenzyl Malonate (83).[89] A solution of dibenzyl malonate (8.22 g, 28.9 mmol) in isopropanol (48 mL) was heated with stirring to 45 °C. Over a 1 h period, 1 N NaOH (30.3 mL, 30.3 mmol) was added dropwise. After an additional 10 min, the solution was concentrated *in vacuo* to approximately 20 mL. Water was added to a total volume of 30 mL. The pH of the solution was adjusted to 8.6 using saturated aqueous $NaHCO_3$. The mixture was washed with CH_2Cl_2 (2 x 10 mL) to remove benzyl alcohol. The pH was adjusted to 2.0 with 6 N HCl, and the mixture was extracted with ethyl acetate (20 mL). The pH of the aqueous layer was adjusted to 2.0 with 1 N HCl, and a second 20 mL ethyl acetate extraction was performed. The combined extracts were washed with brine (15 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The oily residue was triturated with hexane (50 mL) overnight, and then filtered to give monobenzyl malonate (**83**) (3.90 g, 70%) as a white solid: mp 58-59 °C (lit.[89] mp 58-60 °C); IR (CH_2Cl_2 cast) 3091, 2951, 1746, 1455, 1411, 1381, 1323, 1214, 1155 cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2) δ 11.10-10.70 (brs, 1 H, COOH), 7.47-7.28 (m, 5 H, ArH), 5.22 (s, 2 H, OCH_2), 3.51 (s, 2 H, CH_2); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 171.70, 167.06, 135.62, 128.96, 128.86, 128.66, 67.91, 41.17; MS (FAB) 195.1 (100) (MH^+).

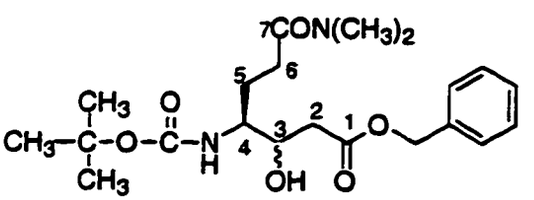


Magnesium Benzyl Malonate (84).[89] To a solution of monobenzyl malonate (**83**) (1.94 g, 10 mmol) in THF (20 mL), was added magnesium ethoxide (0.572 g, 5 mmol). The mixture was stirred vigorously at room temperature under argon for 2 h, and then filtered through a pad of Celite, and the solids were washed with THF (2 x 4 mL). The clear solution was added dropwise into hexane (100 mL) with vigorous stirring. The white precipitate was immediately filtered, washed with hexane (2 x 10 mL), and the precipitate was pumped dry overnight to yield magnesium salt **84** (1.80 g, 88%) as a white fine powder: IR (μscope) 3035, 2951, 1717, 1652, 1601, 1419, 1398, 1337, 1226, 1144 cm^{-1}; ^1H NMR (360 MHz, D_2O) δ 7.47-7.24 (m, 5 H, ArH), 5.10 (s, 2 H, OCH_2), 3.24 (s, 2 H, CH_2); ^{13}C NMR (100 MHz, D_2O-CD_3COCD_3) δ 174.11, 171.36, 135.83, 129.08, 128.83, 128.44, 67.47, 44.83; HRMS calcd for $C_{20}H_{18}O_8Mg$ 410.0852, found 410.0699.



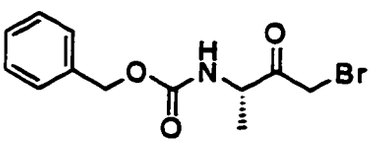
Benzyl (*4S*)-4-(*tert*-Butyloxycarbonylamino)-7-(*N,N*-dimethylamino) -3,7-dioxoheptanoate (85). To a solution of *N*-*t*-Boc-γ-(*N,N*-dimethyl) glutamine (**74**) (0.823 g, 3.00 mmol) in THF (10 mL) was added 1,1'-carbonyl diimidazole (0.486 g, 3.00 mmol). The clear solution was stirred for 1 h at room temperature under argon. Magnesium benzyl malonate (**84**) (1.33 g, 3.00 mmol) was added. The mixture was stirred overnight at room temperature. The pH was adjusted to 2 with 0.5 N HCl, and the product was extracted with ethyl acetate (2 x 50 mL). The combined extracts were washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated *in vacuo*.

Recrystallization (CH_2Cl_2-hexane) gave benzyl ester **85** (0.85 g, 80%) as a white solid: mp 93-95 °C; IR (CH_2Cl_2 cast) 2976, 1711, 1635, 1500, 1402, 1366, 1252, 1165 cm^{-1}; ^1H NMR (400 MHz, CD_2Cl_2) δ 7.39-7.26 (m, 5 H, ArH), 5.90-5.78 (brs, 1 H, CONH), 5.15 (s, 2 H, CH_2O), 4.30-4.18 (m, 1 H, CH), 3.68 (d, 1 H, J = 16.2 Hz, H-2), 3.62 (d, 1 H, J = 16.2 Hz, H-2), 2.93 (s, 3 H, NCH_3), 2.90 (s, 3 H, NCH_3), 2.50-2.37 (m, 1 H, H-6), 2.36-2.25 (m, 1 H, H-6), 2.18-2.04 (m, 1 H, H-5), 1.95-1.80 (m, 1 H, H-5), 1.42 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 203.10, 172.32, 167.35, 156.10, 136.11, 128.88, 128.63, 128.58, 80.06, 67.29, 60.31, 46.24, 37.28, 35.62, 29.36, 28.40, 25.88; MS (FAB) 407.0 (31) (MH^+).



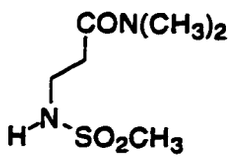
Benzyl (*3RS, 4S*)-4-(*tert*-Butyloxycarbonylamino)-7-(*N,N*-dimethyl amino)-3-hydroxy-7-oxoheptanoate (86). To a stirred solution of ß-keto ester **85** (0.406 g, 1.00 mmol) in CH_2Cl_2-EtOH (1:1) (10 mL) under argon at 0 °C, was added dropwise a solution of $NaBH_4$ (0.1 M, 10 mL) in absolute ethanol. The reaction mixture was stirred at 0 °C for 30 min. The solvent was evaporated, and the residue was dissolved in EtOAc (20 mL), and then washed with water (30 mL) and brine (30 mL), dried (Na_2SO_4) and concentrated *in vacuo* to yield crude alcohol **86**. Purification by flash chromatography (ethyl acetate) gave ß-hydroxy ester **86** (0.363 g, 89%) as a glassy solid. Spectral characterization was performed on a mixture of diastereomers (isomer A : isomer B, 5:6): IR (CH_2Cl_2 cast) 3357, 2975, 2931, 1708, 1630, 1500, 1454, 1365, 1248, 1167 cm^{-1}; ^1H NMR (360 MHz, CD_2Cl_2) (isomer A) δ 7.42-7.26 (m, 5 H, ArH), 5.23 (d, 1 H, J = 8.2 Hz, CONH), 5.13 (s, 2 H, CH_2O), 4.10 (d, 1 H, J = 4.2 Hz, OH), 4.06-3.90 (m, 1 H, H-3), 3.65-3.44 (m, 1 H, H-4), 2.96 (s, 3 H, NCH_3), 2.90 (s, 3 H, NCH_3), 2.65-

2.45 (m, 2 H, H-2), 2.40-2.27 (m, 2 H, H-6), 1.97-1.67 (m, 2 H, H-5), 1.42 (s, 9 H, $(CH_3)_3$); (isomer B) δ 7.42-7.26 (m, 5 H, ArH), 5.13 (s, 2 H, CH_2O), 5.06 (d, 1 H, J = 8.2 Hz, CONH), 4.06-3.90 (m, 1 H, H-3), 3.86 (d, 1 H, J = 4.2 Hz, OH), 3.65-3.44 (m,1 H, H-4), 2.96 (s, 3 H, NCH_3), 2.90 (s, 3 H, NCH_3), 2.65-2.45 (m, 2 H, H-2), 2.40-2.27 (m, 2 H, H-6), 1.97-1.67 (m, 2 H, H-5), 1.42 (s, 9 H, $(CH_3)_3$); ^{13}C NMR (75 MHz, CD_2Cl_2) (isomer A) δ 172.71, 172.51, 156.39, 136.46, 128.85, 128.48, 128.42, 79.50, 71.41, 66.61, 55.39, 38.95, 37.36, 35.72, 29.82, 28.47, 27.28; (isomer B) δ 173.27, 173.15, 156.68, 136.50, 128.85, 128.44, 128.42, 79.21, 68.87, 66.69, 54.59, 39.17, 37.36, 35.67, 29.82, 28.47, 25.33; MS (FAB) 409.3 (100) (MH^+).

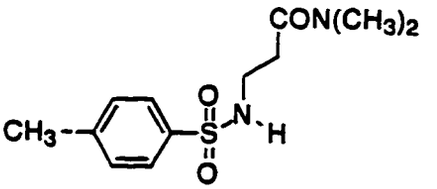


(*3S*)-3-(*N*-Benzyloxycarbonylamino)-1-bromobutan-2-one (89). A solution of Cbz-L-alanine (3.36 g, 15.0 mmol) in dry THF (25 mL) was cooled to -10 °C under argon. Triethylamine (2.25 mL, 15.0 mmol) and ethyl chloroformate (1.50 mL, 15.0 mmol) were added. The mixture was stirred for 20 min at -10 °C and then added to an excess of cold ethereal diazomethane in ether (45 mL of 0.5 N diazomethane). [Diazomethane was prepared from Diazald (Aldrich) using 2-ethoxyethanol and KOH, following the manufacturer's instructions on the bottle.] After 30 min at 0 °C, the reaction mixture was allowed to warm to room temperature and was then washed with 0.1 N CH_3COOH (22 mL), saturated aq. $NaHCO_3$ (22 mL) and brine (22 mL). The ethereal layer was dried ($MgSO_4$), treated with ethereal 5 M HBr (10 mL) at -78 °C for 10 min and then immediately extracted with 1 N HCl, saturated aq. $NaHCO_3$ (22 mL) and brine (22 mL), and dried ($MgSO_4$). The solvent was removed *in vacuo* to give a solid. Recrystallization (dichloromethane-hexane, 1:1) gave the protected bromomethyl ketone **89** (2.01 g, 45 %) as white needles: mp 132-133 °C; IR (CH_2Cl_2 cast) 3296, 1736, 1693, 1536, 1499, 1454, 1256, 1072 cm^{-1}; ^1H NMR (360 MHz, CD_2Cl_2) δ 7.42-7.26 (m, 5 H, ArH), 5.41-5.35

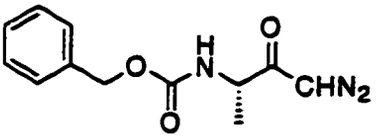
(brs, 1 H, CONH), 5.09 (s, 2 H, OCH_2), 4.64-4.55 (m, 1 H, CH), 4.10 (s, 1 H, CH_2Br), 1.38 (d, 3 H, J = 7.3 Hz, CH_3); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 201.32, 156.04, 136.81, 128.84, 128.50, 128.31, 127.15, 67.30, 54.34, 32.46, 17.73; MS (FAB) 299.8 (32) (MH^+). Anal. Calcd for $C_{12}H_{14}NO_3Br$: C, 48.02; H, 4.70, N, 4.67. Found: C, 48.21; H, 4.55; N, 4.62.



3-(Methylsulfonylamino)-*N,N*-(dimethyl)propanamide (90). Triethylamine (0.90 mL, 6.00 mmol) was added dropwise to a stirred solution of amine salt **95** (1.15 g, 5.00 mmol) in dry CH_2Cl_2 (20 mL) under argon at 0 °C. The mixture was stirred at 0 °C for 5 min, and then methanesulfonyl chloride (0.39 mL, 5.00 mmol) and triethylamine (0.90 mL, 6.00 mmol) were added dropwise. The reaction mixture was stirred at 0 °C for 2 h, and then concentrated *in vacuo*. EtOAc (150 mL) was added to the residue, and the precipitate was removed by filtration. The filtrate was washed with water (20 mL), saturated aq. $NaHCO_3$ (20 mL) and brine (50 mL), and dried (Na_2SO_4). The solvent was removed *in vacuo* to give an oil. Purification by flash chromatography (ethyl acetate) gave the sulfonamide **90** (0.65 g, 67%) as an oil: IR (CH_2Cl_2 cast) 3200, 2932, 1632, 1403, 1316, 1146 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 5.52-5.37 (brs, 1 H, CONH), 3.40-3.27 (m, 2 H, CH_2N), 2.97 (s, 3 H, NCH_3), 2.92 (s, 3 H, SO_2CH_3), 2.91 (s, 3 H, NCH_3), 2.59 (t, 2 H, J = 5.1 Hz, $COCH_2$); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 172.95 (CON), 39.72 (SO_2CH_3), 39.06 (CH_2N), 36.87 (NCH_3), 35.12 (NCH_3), 33.40 ($COCH_2$); HRMS calcd for $C_6H_{14}N_2O_3S$ 194.0803, found 194.0782.

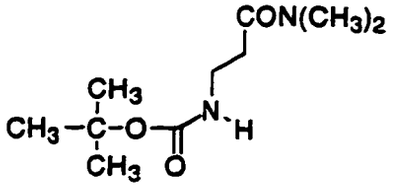


3-(*p*-Methylphenylsulfonylamino)-*N,N*-(dimethyl)propanamide (91). The procedure for preparation of **90** was employed to react amine salt **95** (1.15 g, 5.00 mmol), triethylamine (1.8 mL, 12.00 mmol) and *p*-toluenesulfonyl chloride (0.95 g, 5.00 mmol) to give the sulfonamide **91** (0.88 g, 65%) as an oil: IR (CH_2Cl_2 cast) 3180, 2927, 1631, 1495, 1401, 1326, 1158, 1093 cm^{-1}; ^1H NMR (360 MHz, CD_2Cl_2) δ 7.75-7.70 (m, 2 H, ArH), 7.36-7.31 (m, 2 H, ArH), 5.80-5.65 (brs, 1 H, CONH), 3.20-3.09 (m, 2 H, CH_2N), 2.87 (s, 3 H, NCH_3), 2.86 (s, 3 H, NCH_3), 2.46 (t, 2 H, J = 5.5 Hz, $COCH_2$), 2.42 (s, 3 H, CH_3); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 171.25 (CON), 143.67 (C, ArH), 137.74 (C, ArH), 129.96 (CH, ArH), 127.18 (CH, ArH), 39.33 (NCH_2), 37.03 (NCH_3), 35.20 (NCH_3), 32.92 ($COCH_2$), 21.50 (CH_3); HRMS calcd for $C_{12}H_{18}N_2O_3S$ 270.1038, found 270.1034.

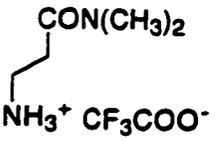


(*3S*)-3-(Benzyloxycarbonylamino)-1-diazobutan-2-one (93). A solution of Cbz-L-alanine (1.12 g, 5.00 mmol) in dry THF (15 mL) was cooled to -10 °C under argon. Triethylamine (0.75 mL, 5.00 mmol) and ethyl chloroformate (0.48 mL, 5.00 mmol) were added. The mixture was left for 20 min at -10 °C. To this solution was added an excess of cold ethereal diazomethane (40 mL of 0.5 M diazomethane). [Diazomethane was prepared from Diazald (Aldrich) using 2-ethoxyethanol and KOH, following the manufacturer's instructions on the bottle.] After 30 min at 0 °C, the reaction mixture was allowed to warm to room temperature and left overnight. The solution was washed with 0.1 N CH_3COOH (11 mL), saturated aq. $NaHCO_3$ (11 mL) and brine (11 mL), dried

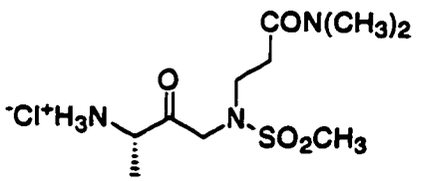
($MgSO_4$) and concentrated *in vacuo* to give an oil. Purification by flash chromatography (dichloromethane : petroleum spirit : ether, 2:2:1) gave the α-diazomethyl ketone **93** (0.925 g, 75%): IR (CH_2Cl_2 cast) 3321, 3091, 2108, 1716, 1640, 1526, 1454, 1361, 1246, 1055 cm^{-1}; ^1H NMR (360 MHz, CD_2Cl_2) δ 7.50-7.26 (m, 5 H, ArH), 5.52-5.35 (brs, 2 H, CONH and CHN_2), 5.09 (s, 2 H, OCH_2), 4.32-4.17 (brs, 1 H, CHCO), 1.33 (d, 3 H, J = 7.3 Hz, CH_3); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 194.55, 156.02, 137.04, 128.70, 128.40, 128.24, 127.15, 67.05, 54.35, 18.40; MS (FAB) 247.9 (8) (MH^+). Anal. Calcd for $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.30; N, 16.99. Found: C, 58.25; H, 5.34; N, 16.75.



3-(*tert*-Butyloxycarbonylamino)-(*N,N*-dimethyl)propanamide (94). To a solution of N-*t*-Boc-ß-alanine (3.78 g, 20.0 mmol), benzotriazolyl-*N*-oxytris(dimethylamino)phosphonium hexafluorophosphate (8.85 g, 20.0 mmol) and triethylamine (2.79 mL, 20.0 mmol) in CH_2Cl_2 (20 mL) under argon, was added dimethylamine hydrochloride (3.26 g, 40.0 mmol) and triethylamine (5.58 mL, 40 mmol). The mixture was stirred at room temperature for 6 h, and then concentrated *in vacuo*. The residue was dissolved in EtOAc (200 mL), washed with 10 % $NaHCO_3$ (2 x 120 mL), 10 % citric acid (2 x 120 mL) and brine (50 mL), and dried (Na_2SO_4). The solvent was removed *in vacuo* to give an oil. Purification by flash chromatography (ethyl acetate : hexane, 1:1) gave the title compound **94** (3.37 g, 73%) as a colorless oil: IR (CH_2Cl_2 cast) 3434, 2977, 1696, 1636, 1509, 1404, 1366, 1280, 1168, 845 cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2) δ 5.31-5.16 (brs, 1 H, CONH), 3.40-3.27 (m, 2 H, CH_2N), 2.96 (s, 3 H, NCH_3), 2.88 (s, 3 H, NCH_3), 2.45 (t. 2 H, J = 7.0 Hz, $COCH_2$), 1.42 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 172.08, 156.50, 79.25, 37.30, 36.64, 35.35, 33.82, 28.48; HRMS calcd for $C_{10}H_{20}N_2O_3$ 216.1474, found 216.1474.

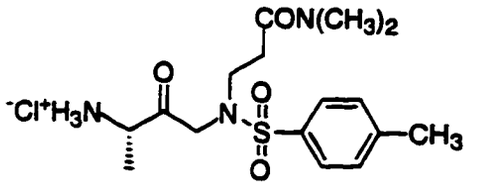


3-Amino-(*N,N*-dimethyl)propanamide Trifluoroacetate Salt (95). Trifluoroacetic acid (11.6 mL, 150 mmol) was added dropwise to a stirred solution of *N-t*-Boc-ß-Ala-NMe_2 (**93**) (2.16 g, 10 mmol) in dry distilled CH_2Cl_2 (11.6 mL) under argon at room temperature. The reaction mixture was stirred at room temperature for 2 h. The solvent was removed *in vacuo* and and the residue was dried overnight under high vacuum to yield the title compound **95** (2.30 g, quantitative): IR (CH_2Cl_2 cast) 2948, 1679, 1639, 1503, 1408, 1203, 1174 cm^{-1}; ^1H NMR (360 MHz, CD_2Cl_2) δ 7.90-7.60 (brs, 3 H, NH_3^+), 3.35-3.25 (m, 2 H, CH_2N), 2.98 (s, 3 H, NCH_3), 2.91 (s, 3 H, NCH_3), 2.74 (t, 2 H, J = 5.4 Hz, $COCH_2$); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 171.57 (CON), 161.3 (q, COO^-), 116.3 (q, CF_3), 37.21 (NCH_3), 36.90 ($CH_2NH_3^+$), 35.50 (NCH_3), 29.57 (CH_2); MS (FAB) 116.1 (100) (MH^+-CF_3COO^-).

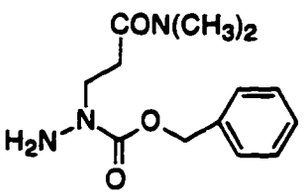


(*3S*)-{N^1-[3'-(*N,N*-Dimethylamino)-3'-oxopropyl]-N^1-(methylsulfonyl)}-1,3-diaminobutan-2-one Hydrochloride (96). To a solution of *N*-Cbz sulfonamide **17** (0.580 g, 1.40 mmol) in methanol-HCl (40 mL) under argon was added 10% palladium on charcoal catalyst (58 mg). The mixture was stirred under an atmosphere of hydrogen until gas absorption ceased. The catalyst was removed by filtration through a column of Celite, and the filtrate was concentrated *in vacuo* to give deprotected sulfonamide **96** (0.443 g, quantitative) as a glassy solid: IR (MeOH cast) 3440, 2921, 1737, 1619, 1325, 1145 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 4.55 (d, 1 H, J = 19.3 Hz, CH_2), 4.41 (d, 1 H, J = 19.3 Hz, CH_2), 4.25 (q, 1 H, J = 7.2 Hz, CH), 3.53 (t, 2 H, J =

6.4 Hz, CH_2), 3.01 (s, 3 H, SO_2CH_3), 2.94 (s, 3 H, NCH_3), 2.87 (s, 3 H, NCH_3), 2.74 (d, 3H, J = 6.4 Hz, $COCH_2$), 1.53 (d, 3H, J = 7.2 Hz, CH_3); ^{13}C NMR (75 MHz, CD_3OD) δ 204.14, 173.57, 55.35, 54.04, 46.17, 39.28, 38.32, 36.38, 34.12, 15.76; MS (FAB) 279.9 (33) (MH^+-HCl).



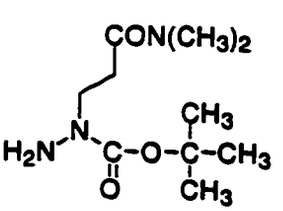
(3*S*)-{N^1-[3'-(*N*,*N*-Dimethylamino)-3'-oxopropyl]-N^1-(*p*-methylphenylsulfonyl)}-1,3-diaminobutan-2-one Hydrochloride (97). The procedure used for the preparation of **96**, was adapted to convert *N*-Cbz sulfonamide **18** (0.500 g, 1.02 mmol) with 10% palladium on charcoal (50 mg) in MeOH-HCl (40 mL) to the title compound **97** (0.400 g, quantitative) as a glassy solid: IR (MeOH cast) 3440, 2927, 1742, 1621, 1598, 1494, 1334, 1156 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.69 (d, 1 H, J = 8.3 Hz, ArH), 7.35 (d, 1 H, J = 8.3 Hz, ArH), 4.36 (s, 2 H, $COCH_2N$), 4.25 (q, 1 H, J = 7.3 Hz, CH), 3.38 (t, 2 H, J = 6.4 Hz, NCH_2), 2.92 (s, 3 H, NCH_3), 2.82 (s, 3 H, NCH_3), 2.67-2.58 (m, 2 H, $COCH_2$), 2.37 (s, 3 H, $ArCH_3$), 1.48 (d, 3 H, J = 7.3 Hz, CH_3); ^{13}C NMR (75 MHz, CD_3OD) δ 204.25, 173.54, 143.55, 137.64, 129.89, 127.28, 55.27, 54.01, 45.96, 37.24, 35.27, 32.92, 21.58, 16.20; MS (FAB) 356.0 (97) (MH^+-HCl).



3-[N^1-(Benzyloxycarbonyl)hydrazino]-*N*,*N*-(dimethyl)propanamide (105). *N*,*N*-Dimethylacrylamide (4.96 g, 50 mmol) was added dropwise to a solution of hydrazine monohydrate (2.50 g, 50 mmol) in MeOH (30 mL). The reaction mixture was

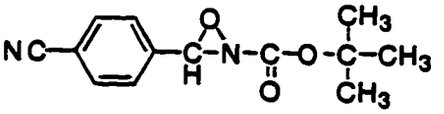
stirred at room temperature for 2 h, diluted with MeOH (30 mL), dried (Na_2SO_4), and then concentrated *in vacuo* to give crude 3-hydrazino-*N,N*-(dimethyl)propanamide **104** (6.5 g).

A stirred solution of crude 3-hydrazino-*N,N*-(dimethyl)propanamide **104** (2.62 g, 20 mmol) in a mixture of diethyl ether (30 mL), water (15 mL) and 1 N NaOH (20 mL) was cooled in an ice-bath. Benzyl chloroformate (2.8 mL, 20 mmol) was added and stirring was continued in the ice-bath for 1 h. The precipitate was filtered, the filtrate was concentrated *in vacuo* to 20 mL, and then extracted with ethyl acetate (3 x 80 mL). The combined organic extracts were washed with brine (20 mL), dried (Na_2SO_4) and the solvent was concentrated *in vacuo* to give an oil. Purification by flash chromatography (ethyl acetate-methanol, 95:5) gave propanamide **105** (1.59 g, 30%) as a white solid: mp 48-50 °C; IR (CH_2Cl_2 cast) 3334, 3215, 3031, 2941, 1699, 1643, 1586, 1497, 1411, 1200, 1109 cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2) δ 7.40-7.24 (m, 5 H, ArH), 5.12 (s, 2 H, CH_2O), 4.22-4.07 (brs, 2 H, NH_2), 3.69 (t, 2 H, J = 7.2 Hz, NCH_2), 2.94 (s, 3 H, NCH_3), 2.86 (s, 3 H, NCH_3), 2.60 (t, 2 H, J = 7.2 Hz, CH_2CO); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 171.05, 155.11, 137.14, 128.69, 128.25, 128.15, 67.57, 47.36, 37.17, 35.07, 31.70; HRMS calcd for $C_{13}H_{19}N_3O_3$ 265.1426, found 265.1432. Anal. Calcd for $C_{13}H_{19}N_3O_3$: C, 58.85; H, 7.22; N, 15.84. Found: C, 58.89; H, 7.01; N, 15.73.



3-[N^1-(*tert*-butyloxycarbonyl)hydrazino]-*N,N*-(dimethyl) propanamide (106). A stirred solution of the crude 3-hydrazino-*N,N*-(dimethyl) propanamide **104** (2.62 g, 20 mmol), which was obtained from the above procedure for preparation of propanamide **105**, in a mixture of dioxane (30 mL), water (15 mL) and 1 N NaOH (20 mL) was cooled in an ice-bath. Di-*tert*-butyl pyrocarbonate (4.8 g, 22 mmol) was added and stirring was continued at room temperature for 1 h. The precipitate was

filtered, the filtrate was concentrated *in vacuo* to 25 mL, and then extracted with ethyl acetate (3 x 80 mL). The combined organic extracts were washed with brine (20 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give an oil. Purification by flash chromatography (ethyl acetate-methanol, 95:5) gave propanamide **106** (2.25 g, 53%) as a colorless oil: IR (CH_2Cl_2 cast) 3334, 3217, 2975, 2932, 1694, 1645, 1397, 1365, 1168 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 4.38-4.20 (brs, 2 H, NH_2), 3.66 (t, 2 H, J = 7.0 Hz, NCH_2), 2.98 (s, 3 H, NCH_3), 2.89 (s, 3 H, NCH_3), 2.60 (t, 2 H, J = 7.0 Hz, $COCH_2$), 1.42 (s, 9 H, $(CH_3)_3C$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.54, 155.80, 79.62, 46.20, 36.60, 34.49, 30.99, 27.73; HRMS calcd for $C_{10}H_{21}N_3O_3$ 231.1583, found 231.1582.

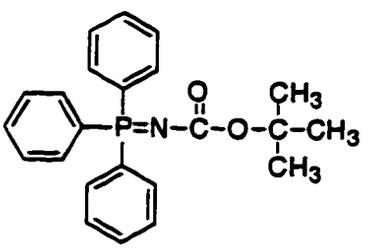


***N*-(*tert*-Butyloxycarbonyl)-3-(4-cyanophenyl)oxaziridine (109).** In a 2-L three-necked flask cooled in an ice-water bath and equipped with a very efficient stirrer was placed a solution of **113** (17.49 g, 76 mmol) in $CHCl_3$ (245 mL) and a chilled solution of K_2CO_3 (54.38 g, 388 mmol) in water (420 mL). To this vigorously stirred mixture was added over 10 min a cold solution of Oxone (83.96 g, 137 mmol) in water (860 mL). After 50 min (0-4°C), the water phase was discarded, and the organic phase was submitted to the same treatment as described above using new solutions of K_2CO_3 and Oxone. This process was repeated twice more. Finally, the chloroform layer was washed with 5% aqueous $KHSO_4$ and 5% aqueous $KHCO_3$, dried over $MgSO_4$, and concentrated *in vacuo* (bath temperature < 30 °C). The residue was purified by flash chromatography (Et_2O : pentane, 1 : 3) to give oxaziridine **109** (0.75 g, 4.1%), followed by *N*-(*tert*-butyloxycarbonyl)-4-cyanobenzamide **114** (15.0 g, 80 %).

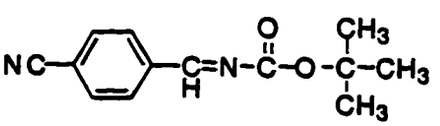
Data for **109**: mp 60-62 °C (lit.[114] mp 61-63 °C); ^1H NMR (200 MHz, $CDCl_3$) δ 7.70-7.57 (m, 4 H, ArH), 5.33 (s, 0.12 H, CH, cis), 5.04 (s, 0.88 H, CH, trans), 1.53

(s, 7.9 H, $C(CH_3)_3$, trans isomer, 88%), 1.14 (s, 1.1 H, $C(CH_3)_3$, cis isomer, 12%); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.5, 139.3, 132.5, 129.9, 119.1, 115.6, 86.6, 77.3, 27.9.

Data for **114**: mp 142-144 °C (lit.[114] mp 143-145 °C); ^1H NMR (200 MHz, $CDCl_3$) δ 7.66–7.50 (m, 4 H, ArH), 6.05 (s, 1 H, NHC=O), 1.35 (s, 9 H, $C(CH_3)_3$).

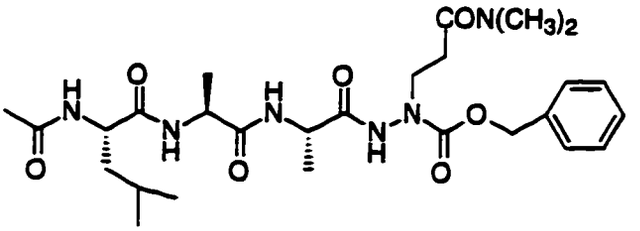


***N*-(*tert*-Butyloxycarbonyl)triphenyliminophosphorane (112).**[114] To a solution of triphenylphosphine (44.8 g, 0.171 mol) in ether (200 mL) at room temperature was added dropwise an ethereal solution of $BocN_3$ (2 M, 85.5 mL). On the formation of a white solid, ether (100 mL) was added. The reaction mixture was stirred at room temperature overnight. The precipitate was filtered and washed with ether to give **112** (57.8 g, 90%) as a white solid: ^1H NMR (200 MHz, $CDCl_3$) δ 7.80-7.60 (m, 6 H, ArH), 7.60-7.35 (m, 9 H, ArH), 1.35 (s, 9 H, $C(CH_3)_3$). ^{13}C NMR (100 MHz, CD_2Cl_2) δ 161.06, 133.21, 132.49, 129.84, 128.93, 77.50, 28.41.

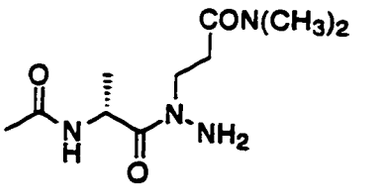


***N*-(*tert*-Butyloxycarbonyl)-4-cyanophenylimine (113).**[114] A mixture of **112** (38.26 g, 0.101 mol) and 4-cyanobenzaldehyde (13.28 g, 0.101 mol) in anhydrous toluene (50 mL) was refluxed under argon for 17 h. After cooling and addition of dry hexane (50 mL), most of the Ph_3PO precipitated from solution and was removed by suction filtration. The concentrated filtrate was purified rapidly (less than 15 min) by column chromatography (Et_2O : hexane, 2 : 1) to give imine **113** (16.8 g, 72%) as a white solid: mp 86-94 °C (decomposed) (lit.[114] mp 87 °C decomposed); ^1H NMR (200 MHz,

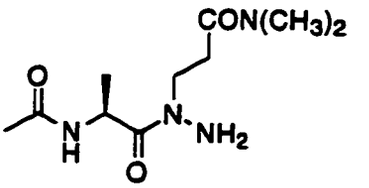
$CDCl_3$) δ 8.80 (s, 1 H, CH=N), 7.99 (d, 2 H, J = 8.2 Hz, ArH), 7.74 (d, 2 H, J = 8.2 Hz, ArH), 1.57 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 166.71, 161.75, 137.80, 132.53, 130.12, 116.43, 117.90, 83.15, 27.92.



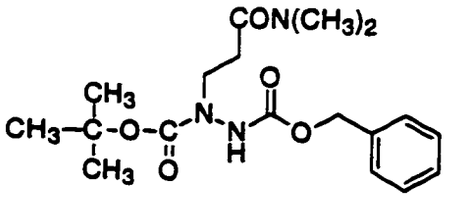
3-[N^1-(Benzyloxycarbonyl)-N^2-(acetyl-L-leucyl-L-alanyl-L-alanyl)hydrazino]-*N*,*N*-(dimethyl)propanamide (117). To a solution of tripeptide Ac-Leu-Ala-Ala-OH (**65**) (0.315 g, 1 mmol), Cbz hydrazino derivative **105** (0.265 g, 1 mmol) and benzotriazolyl-*N*-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) (0.442 g, 1 mmol) in DMF (10 mL) was added triethylamine (0.139 mL, 1 mmol). The mixture was stirred at room temperature for 6 h, and then concentrated *in vacuo* to give an oil. Purification by HPLC (linear gradient elution over 20 min of acetonitrile and water, from 20% to 40%, t_R 13.8 min) gave **117** (0.428 g, 76%) as a white powder: mp 141-142 °C; IR (CH_2Cl_2 cast) 3267, 3038, 2955, 2931, 1721, 1628, 1500, 1449, 1116 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) δ 7.40-7.20 (brs, 5 H, ArH), 5.12 (s, 2 H, OCH_2), 4.40-4.20 (m, 3 H, CH), 3.86-3.65 (m, 2 H, NCH_2), 3.04 (s, 3 H, NCH_3), 2.86 (s, 3 H, NCH_3), 2.67 (t, 2 H, J = 7.3 Hz, $COCH_2$), 1.95 (s, 3 H, CH_3), 1.72-1.58 (m, 1 H, CH), 1.57-1.50 (m, 2 H, CH_2), 1.48-1.20 (m, 6 H, CH_3), 0.96 (d, 3 H, J = 6.5 Hz, CH_3), 0.92 (d, 3 H, J = 6.5 Hz, CH_3); ^{13}C NMR (75 MHz, CD_3OD) δ 175.21, 174.71, 174.11, 173.46, 173.06, 157.01, 137.48, 129.51, 129.22, 129.05, 69.08, 53.84, 50.56, 49.84, 47.37, 41.51, 37.77, 35.64, 32.16, 25.91, 23.22, 22.39, 22.24, 17.54, 17.38; MS (FAB) 563.4 (34) (MH^+).



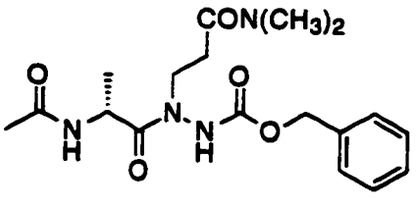
3-[*N'*-(Acetyl-D-alanyl)hydrazino]-*N,N*-(dimethyl)propanamide (119). To a solution of Cbz-hydrazino derivative **128** (0.378 g, 1 mmol) in methanol (20 mL) under argon was added 10% palladium on charcoal catalyst (38 mg). The mixture was stirred under an atmosphere of hydrogen until absorption of gas ceased. The catalyst was removed by filtration through a column of Celite and the filtrate was concentrated *in vacuo* to give **119** (0.244 g, quantitative) as a colorless oil: IR (CH_2Cl_2 cast) 3314, 3214, 2934, 1636, 1542 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) δ 5.24 (q, 1 H, J = 7.0 Hz, CH), 3.86-3.60 (m, 2 H, NCH_2), 3.04 (s, 3 H, NCH_3), 2.91 (s, 3 H, NCH_3), 2.72 (t, 2 H, J = 6.9 Hz, CH_2CO), 1.95 (s, 3 H, CH_3), 1.27 (d, 3 H, J = 7.0 Hz, CH_3); ^{13}C NMR (75 MHz, CD_3OD) δ 176.60, 173.55, 172.70, 48.38, 47.18, 37.73, 35.67, 31.74, 22.42, 17.54; MS (FAB) 245.3 (100) (MH^+). Anal Calcd. for $C_{10}H_{20}N_4O_3$: C, 49.17; H, 8.25; N, 22.93. Found: C, 48.86; H, 8.52; N, 22.62.



3-[*N'*-(Acetyl-L-alanyl)hydrazino]-*N,N*-(dimethyl)propanamide (120). The above procedure for the preparation of **119** was used with Cbz-hydrazino derivative **129** (0.378 g, 1 mmol) to give **120** (0.243 g, quantitative) as a colorless oil: IR (CH_2Cl_2 cast) 3312, 3213, 2933, 1633, 1541 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 5.24 (q, 1 H, J = 7.0 Hz, CH), 3.80-3.60 (m, 2 H, NCH_2), 3.04 (s, 3 H, NCH_3), 2.91 (s, 3 H, NCH_3), 2.72 (t, 2 H, J = 6.9 Hz, CH_2CO), 1.94 (s, 3 H, CH_3), 1.27 (d, 3 H, J = 7.0 Hz, CH_3); ^{13}C NMR (75 MHz, CD_3OD) δ 176.59, 173.55, 172.69, 48.15, 47.18, 37.74, 35.67, 31.74, 22.43, 17.54; MS (FAB) 245.2 (100) (MH^+).



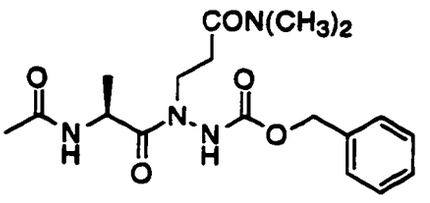
3-[N^1-(*tert*-Butyloxycarbonyl)-N^2-(benzyloxycarbonyl)hydrazino]-*N,N*-(dimethyl)propanamide (126). To a suspension of Boc-propanamide **106** (2.31 g, 10 mmol) and anhydrous potassium carbonate (6.91 g, 50 mmol) in dry THF (20 mL), was added benzyl chloroformate (1.88 g, 11 mmol) with vigorous stirring. The mixture was heated to 40-50 °C for 2 h. The precipitate of $KHCO_3$ and K_2CO_3 was removed by filtration. The filtrate was concentrated *in vacuo*. The residue was dissolved in ethyl acetate (100 mL), washed with 1 N $NaHCO_3$ (20 mL), water (20 mL) and brine (20 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give an oil. Purification by flash chromatography (ethyl acetate) gave **126** (3.2 g, 87%) as a colorless oil: IR (CH_2Cl_2 cast) 3251, 2976, 2934, 1741, 1709, 1632, 1497, 1400, 1366, 1257, 1209, 1159 cm^{-1}; ^1H NMR (360 MHz, CD_2Cl_2) δ 7.38-7.30 (m, 5 H, ArH), 7.22-7.05 (brs, 1 H, CONH), 5.14 (s, 2 H, CH_2O), 3.70 (t, 2 H, J = 6.7 Hz, NCH_2), 2.96 (s, 3 H, NCH_3), 2.87 (s, 3 H, NCH_3), 2.61 (t, 2 H, J = 6.7 Hz, CH_2CO), 1.40 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 171.33, 156.54, 155.11, 136.65, 128.84, 128.53, 128.38, 81.50, 67.60, 47.62, 37.36, 35.30, 31.99, 28.27; MS (FAB) 366.0 (28) (MH^+).



3-[N^1-(Acetyl-D-alanyl)-N^2-(benzyloxycarbonyl)hydrazino]-*N,N*-(dimethyl)propanamide (128). To a stirred solution of propanamide **126** (1.10 g, 3 mmol) in dry CH_2Cl_2 (10 mL) under argon, was added trifluoroacetic acid (10 mL). The solution was stirred at room temperature for 2 h and was then concentrated *in vacuo*.

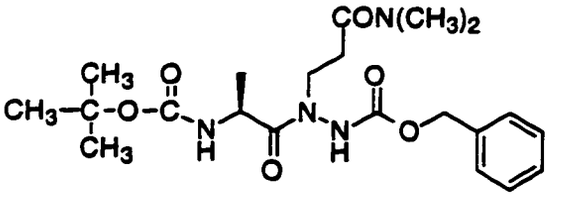
Toluene (10 mL) was added, and the mixture was concentrated *in vacuo*. The residue was dried *in vacuo* overnight to give the crude product **127**. The crude product was dissolved in dry THF (3 mL), and triethylamine (1.57 mL, 10.5 mmol) was added to form the amine solution **A**.

A solution of *N*-acetyl-D-alanine (0.393 g, 3.0 mmol) in dry THF (10 mL) was cooled to -5 °C with stirring under argon. Triethylamine (0.45 mL, 3.0 mmol) and ethyl chloroformate (0.29 mL, 3.0 mmol) were added. The mixture was stirred for 20 min at -5 °C, then the amine solution **A** was added. After 30 min at -5 °C, the reaction mixture was allowed to warm to room temperature and stirred for 2 h. The precipitate was filtered and the filtrate was concentrated *in vacuo*. The residue was dissolved in ethyl acetate (100 mL), washed with satd. NH_4Cl (15 mL), 1 N $NaHCO_3$ (15mL) and brine (15 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give an oil. Purification by flash chromatography (ethyl acetate-methanol, 95 : 5) gave **128** (0.91 g, 80%) as a colorless oil: IR (CH_2Cl_2 cast) 3282, 2936, 1738, 1645, 1500, 1455, 1259, 1189 cm^{-1}; ^1H NMR (360 MHz, CD_3OD) δ 7.42-7.25 (m, 5 H, ArH), 5.18 (s, 2 H, CH_2O), 4.80-4.70 (m, 1 H, CH), 4.10-3.90 (brs, 1 H, NCH_2), 3.70-3.40 (brs, 1 H, NCH_2), 2.99 (s, 3 H, NCH_3), 2.88 (s, 3 H, NCH_3), 2.80-2.40 (brs, 2 H, CH_2CO), 1.90 (s, 3 H, CH_3), 1.30-1.00 (brs, 3 H, CH_3); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 175.08, 171.84, 169.38, 156.82, 136.17, 128.91, 128.70, 128.50, 68.21, 46.18, 45.85, 37.44, 35.47, 31.62, 23.25, 18.52; MS (FAB) 379.0 (28) (MH^+).

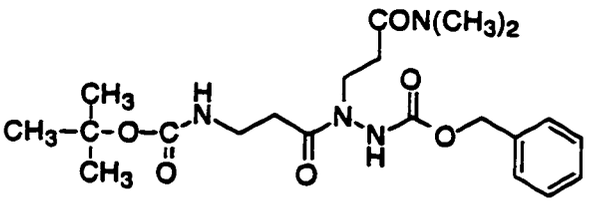


3-[N^1-(Acetyl-L-alanyl)-N^2-(benzyloxycarbonyl)hydrazino]-*N*,*N*-(dimethyl)propanamide (129). The procedure used for the preparation of the compound **128** was adapted to convert propanamide trifluoroacetate salt **127** (1.14 g, 3

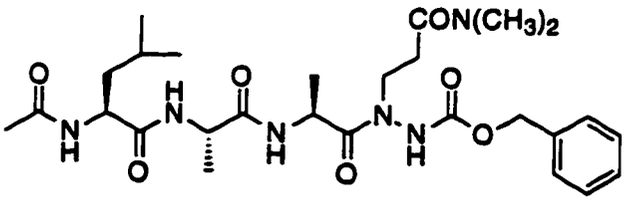
mmol) and *N*-acetyl-L-alanine (0.393 g, 3 mmol) to **129** (0.86 g, 76%) as a gum: IR (CH_2Cl_2 cast) 3284, 2937, 1738, 1646, 1500, 1455, 1259, 1189 cm^{-1}; ^1H NMR (360 MHz, CD_2Cl_2) δ 8.64-8.48 (brs, 1 H, N-NH), 7.47-7.28 (m, 5 H, ArH), 6.50-6.30 (brs, 1 H, CONH), 5.18 (s, 2 H, CH_2O), 4.92-4.78 (s, 1 H, CH), 4.25-4.00 (brs, 1 H, NCH_2), 3.50-3.10 (brs, 1 H, NCH_2), 2.95 (s, 3 H, NCH_3), 2.87 (s, 3 H, NCH_3), 2.80-2.40 (brs, 2 H, CH_2CO), 1.90 (s, 3 H, CH_3), 1.21 (d, 3 H, J = 6.6 Hz, CH_3); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 175.05, 171.89, 169.36, 156.86, 136.16, 128.92, 128.71, 128.50, 68.23, 46.23, 45.86, 37.45 35.48, 31.66, 23.29, 18.60; MS (FAB) 379.2 (65) (MH^+).



3-[N^1-(*tert*-Butyloxycarbonyl-L-alanyl)-N^2-(benzyloxycarbonyl) hydrazino]-*N*,*N*-(dimethyl)propanamide (130). The above procedure for the preparation of **128**, was adapted to convert propanamide trifluoroacetate salt **127** (0.376 g, 1 mmol) and *N*-*t*-Boc-L-alanine (0.131 g, 1 mmol) to **130** (0.350 g, 80%) as a colorless oil: IR (CH_2Cl_2 cast) 3242, 2976, 2934, 1742, 1712, 1670, 1650, 1499, 1454, 1246, 1167 cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2) δ 8.55-8.25 (s, 1 H, N-NH), 7.45-7.25 (m, 5 H, ArH), 5.30-5.10 (m, 3 H, CH_2O, CONH), 4.70-4.50 (s, 1 H, CH), 4.35-4.00 (s, 1 H, NCH_2), 3.40-3.00 (s, 1 H, NCH_2), 2.93 (s, 3 H, NCH_3), 2.88 (s, 3 H, NCH_3), 2.86-2.30 (m, 2 H, CH_2CO), 1.40 (s, 9 H, $C(CH_3)_3$), 1.19 (d, 3 H, J = 6.8 Hz, CH_3); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 175.31, 172.09, 156.93, 155.21, 136.23, 128.94, 128.72, 128.50, 79.50, 68.22, 46.85, 46.31, 37.49, 35.51, 31.77, 28.45, 18.81; MS (FAB) 437.1 (59) (MH^+).

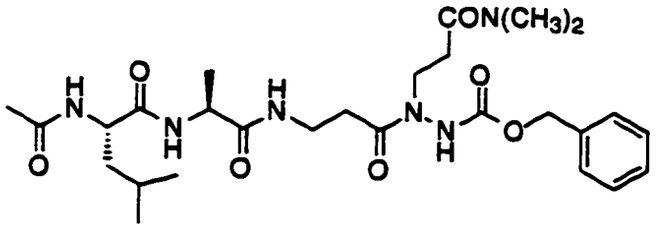


3-[N^1-(*tert*-Butyloxycarbonyl-ß-alanyl)-N^2-(benzyloxycarbonyl) hydrazino]-*N,N*-(dimethyl)propanamide (131). The procedure used for the preparation of **128**, was adapted to convert propanamide trifluoroacetate salt **127** (2.93 g, 7.8 mmol) and *N*-*t*-Boc-ß-alanine (1.47 g, 7.8 mmol) to **131** (2.14 g, 63%) as an oil: IR (CH_2Cl_2 cast) 3248, 2975, 2933, 1738, 1711, 1670, 1651, 1635, 1500, 1402, 1247, 1172 cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2) δ 8.50-8.30 (s, 1 H, N-NH), 7.50-7.25 (m, 5 H, ArH), 5.30-5.10 (s, 3 H, CH_2O, CONH), 4.30-4.05 (brs, 1 H, NCH_2), 3.40-3.10 (m, 3 H, NCH_2, NCH_2), 2.95 (s, 3 H, NCH_3), 2.88 (s, 3 H, NCH_3), 2.86-2.34 (m, 4 H, 2 CH_2CO), 1.40 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 174.32, 171.94, 156.45, 156.08, 136.27, 128.87, 128.63, 128.40, 78.96, 67.91, 45.77, 37.36, 36.33, 35.40, 33.05, 31.91, 28.45; MS (FAB) 437.2 (9) (MH^+).



3-[N^1-(Acetyl-L-leucyl-L-alanyl-L-alanyl)-N^2-(benzyloxycarbonyl) hydrazino]-*N,N*-(dimethyl)propanamide (132). The above procedure for the preparation of **128**, was adapted to convert **130** (0.130 g, 0.3 mmol) and Ac-Leu-Ala-OH (73.2 mg, 0.3 mmol) to the crude product. This was purified by HPLC (linear gradient elution over 20 min of acetonitrile and water, from 20% to 40%, t_R 12.8 min) to give **132** (0.118 g, 70%) as a white solid: mp 183-184 °C; IR (CH_2Cl_2 cast) 3280, 2955, 2934, 1741, 1634, 1537, 1455, 1412, 1243 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.43-7.25 (m, 5 H, ArH), 5.15 (s, 2 H, CH_2O), 4.80-4.70 (m, 1 H, CH), 4.40-4.20 (m, 2 H, CH),

4.10-3.80 (brs, 1 H, NCH_2), 3.70-3.40 (brs, 1 H, NCH_2), 2.97 (s, 3 H, NCH_3), 2.88 (s, 3 H, NCH_3), 2.70-2.50 (brs, 2 H, CH_2CO), 1.96 (s, 3 H, CH_3), 1.72-1.57 (m, 1 H, CH), 1.57-1.45 (m, 2 H, CH_2), 1.30-1.10 (m, 6 H, 2 CH_3), 0.95 (d, 3 H, J = 6.3 Hz, CH_3), 0.91 (d, 3 H, J = 6.3 Hz, CH_3); ^{13}C NMR (75 MHz, CD_3OD) δ 176.22, 174.71, 174.05, 173.31, 172.96, 157.61, 137.44, 129.60, 129.36, 129.16, 68.77, 53.16, 50.10, 46.73, 46.44, 41.90, 37.64, 35.67, 31.80, 25.91, 23.42, 22.40, 21.98, 17.91; MS (FAB) 563.1 (16) (MH^+).



3-[N^1-(Acetyl-L-leucyl-L-alanyl-ß-alanyl)-N^2-(benzyloxycarbonyl) hydrazino]-*N*,*N*-(dimethyl)propanamide (133). The above procedure for the preparation of **128**, was adapted to convert **131** (0.130 g, 0.3 mmol) and Ac-Leu-Ala-OH (73.2 mg, 0.3 mmol) to the crude product. This was purified by HPLC (linear gradient elution over 20 min of acetonitrile and water, from 20% to 40%, t_R 14.2 min) to give **133** (0.110 g, 65%) as a white solid: mp 156-158 °C; IR (CH_2Cl_2 cast) 3289, 2956, 2934, 1738, 1644, 1538, 1451, 1412, 1243 cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2) δ 8.85-8.50 (brs, 1 H, N-NH), 7.43-7.30 (m, 5 H, ArH), 7.30-7.15 (brs, 1 H, CONH), 7.15-7.00 (s, 1 H, CONH), 6.85-6.50 (brs, 1 H, CONH), 5.16 (s, 2 H, CH_2O), 4.55-4.30 (m, 2 H, CH), 4.30-4.00 (s, 1 H, NCH_2), 3.60-3.20 (m, 3 H, 2 NCH_2), 2.93 (s, 3 H, NCH_3), 2.86 (s, 3 H, NCH_3), 2.86-2.00 (m, 4 H, 2 CH_2CO), 1.95 (s, 3 H, CH_3), 1.75-1.40 (m, 3 H, CH and CH_2), 1.35-1.25 (d, 3 H, J = 7.0 Hz, CH_3), 0.91 (d, 3 H, J = 6.3 Hz, CH_3), 0.89 (d, 3 H, J = 6.3 Hz, CH_3); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 174.25, 172.72, 172.40, 172.00, 170.80, 156.66, 136.33, 128.95, 128.71, 128.43, 68.03, 52.47, 49.40,

45.64, 41.67, 37.47, 35.69, 35.49, 32.31, 31.86, 25.16, 23.18, 23.14, 22.06, 18.51; MS (FAB) 563.2 (38) (MH^+).

Materials and methods for inhibition studies with HAV-3C proteinase

Proteinase Production and Purification. Recombinant C24S HAV 3C proteinase (a mutant in which the nonessential surface cysteine has been replaced with serine and which exhibits identical catalytic parameters to wild-type enzyme, unpublished results) was expressed in *E. coli* and purified.[120] Purity of the enzyme samples was greater than 90% as determined by SDS-PAGE analysis. Proteinase concentrations were determined spectrophotometrically ε = 1.2 mg/ml.

Peptide Substrates. The peptide substrates were synthesized using solid phase Fmoc chemistry on Rink resin as previously described[40] All peptides were purified by reverse-phase HPLC (C-18, 5 X 25 cm, Vydac, 2%/minute linear gradient of 0.1% TFA/water adding 0.1% TFA/acetonitrile). Peptide structures were verified by NMR and mass spectrometry.

Proteinase Assays. Peptide proteolysis was monitored using the trinitrobenzene sulfonate (TNBS) assay as previously described[120] Reaction mixtures were incubated in reaction buffer at 20 °C. Aliquots (10 µL) were removed from the reaction mixture at timed intervals and peptide hydrolysis was quenched with 50 µL of 0.25 M sodium borate, pH 10. A solution (12.5 µL) of freshly prepared 0.14 M TNBS (Johnson-Matthey, Ward Hill, MA) in 0.25 M sodium borate solution was added to the quenched reaction mixture and incubated for 10 minutes at 20 °C. The color was stabilized by adding 200 µL of 3.5 mM Na_2SO_3, 0.2 M KH_2PO_4. The concentration of free amine generated during peptide hydrolysis was determined by measuring the absorbance at 405 nm using a microtitre plate reader (Biorad, Richmond, CA).

Progress Curve Analysis. Proteinase inactivation was quantitatively evaluated by progress curve analysis[121] as previously described.[40] The extent of peptide proteolysis (release of α-amino groups) was monitored using the TNBS assay as described above (see *Proteinase Assays*). The concentrations of targets **C-E** were varied from 0.5 to 500 μM; substrate (Ac-ELRTQSFS-amide) concentration was 2mM and HAV-3C proteinase (C24S mutant) concentration was 0.07 μM. Enzyme was dialyzed against reaction buffer to remove DTT immediately prior to use. Reactions were initiated with enzyme and absorbances were converted into μ moles of product using a glycine standard curve. All determinations were performed in triplicate with different enzyme and inhibitor preparations.

Progress curves were fitted using least squares non-linear regression analysis using Mac Curve Fit 1.0.7, (K. Raner) to:

$$P = \frac{v_o(1-e^{-kt})}{k}$$

where v_o is the initial velocity and k is the apparent first order rate constant (k_{obs}) for the inactivation process. Parameter estimates from individual experiments (weighted by standard error) were averaged to obtain the final value.

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