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Design, Synthesis and Testing of Hepatitis A Virus 3C Proteinase Inhibitors

by

Yeeman Koomar Ramtohul

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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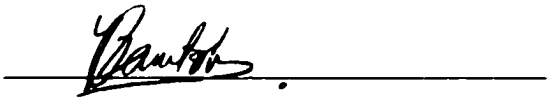

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To my brother, Sudhir, sister, Reshma, mother, Namantee and late father, Iswar

ABSTRACT

The design and synthesis of eight types of hepatitis A virus (HAV) 3C cysteine proteinase inhibitors are described. This enzyme is crucial for picornaviral replication and inhibitors of HAV 3C proteinase may lead to the development of therapeutic agents. Keto-glutamine (4*S*)-*N,N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-(4-phenyl-butyrylamino)hexanamide (**22**), analogous to nanomolar inhibitors of cathepsin K, was synthesized. In addition, (2*S*)-2-(*N,N*-dimethylpropanamid-3-yl)-1-(*tert*-butyloxycarbonyl)-azetidin-3-one (**35**) incorporating a 3-azetidinone scaffold into the glutamine fragment was prepared. Both of these compounds exhibited only modest levels of inhibition (IC_{50}>100 μM). However, introduction of a phthalhydrazido group alpha to the ketone moiety in ((4*S*)-*N,N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-(2,3-dihydrophthalazin-1,4-dione)hexanamide (**28**), gave a significantly better inhibitor with an IC_{50} value of 89 μM, presumably due to the effect of intramolecular hydrogen bonding to the ketone. A related tetrapeptide phthalhydrazide namely (4*S*)-*N,N*-dimethyl-4-(Ac-Leu-Ala-Ala)-amino-5-oxo-6-(2,3-dihydro-phthalazin-1,4-dione)hexanamide (**34**) functioned as a competitive reversible inhibitor (K_i = 9×10^{-6} M) and was also found to display no loss of inhibitory potency in the presence of dithiothreitol. Macrocyclic peptides having the P_1' and P_2 residues linked through a disulfide bridge, Ac-Leu-Ser-*S*-*S*-cyclo[Hcys-Gln-Hcys]-NH_2 (**43**) or an ethylene linker, Ac-Leu-Ser-cyclo-[*O*-allyl-Ser-Gln-*O*-allyl-Ser]-NH_2 (**45**), were prepared but displayed no inhibition of the HAV 3C proteinase. Recent studies have shown that *N*-Cbz-serine and threonine β-lactones are potent inhibitors of HAV 3C proteinase. To determine the importance of the α-nitrogen

functionality, several β-lactone analogues lacking the α-nitrogen functionality such as (3*R*,*S*)-phenyl-oxetan-2-one (**47**), (2*R*,3*R*)-3-(*p*-methoxybenzyl)-4-methyl-oxetan-2-one (**52a**) and (3*RS*,4*RS*)-3-benzoyl-4-methyl-oxetan-2-one (**53**) were prepared. The poor inhibition obtained indicates that the α-nitrogen functionality is required for successful inhibition of the enzyme. Next, Monophenyl pseudoxazolones *E*-(**60a**) and *Z*-(**60b**)-2-benzylidene-*2H*-oxazol-5-one, were prepared and found to display potent time-dependent inhibition of HAV 3C proteinase with IC_{50} values of 6 and 4 μM, respectively. In addition, a new methodology for the preparation of *E*-(**64a**) and *Z*-(**64b**)-2-(4-fluoro-benzylidene)-*2H*-oxazol-5-one and *E*-(**65a**) and *Z*-(**65b**)-2-(4-methoxy-benzylidene)-*2H*-oxazol-5-one was developed starting from glyoxylic acid and they inhibited the HAV 3C proteinase with IC_{50} values in the range of 6 to 20 μM. Inhibition studies with HRV 3C cysteine proteinase, indicate that the pseudoxazolones exhibited similar potency as with HAV 3C. Finally, mass spectrometry and gHMQC NMR spectroscopy experiments with ^{13}C labeled pseudoxazolone **60b(α-^{13}C)** demonstrate that the enzyme forms a covalent adduct with the inhibitor *via* a thioether bond. While promising, the low stability of the pseudoxazolones in phosphate buffer at pH 7.5 indicates a limitation of these derivatives.

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LIST OF ABBREVIATIONS

[α]	specific rotation
abs	absorbance units
Ac	acetyl
ACE	angiotensin converting enzyme
Acm	acetamidomethyl
AcOH	acetic acid
AIDS	acquired immune deficiency syndrome
Anal.	analysis
APT	attached proton test
Asp	aspartic acid
aq	aqueous
Arg	arginine
Ar	aryl
atm	atmosphere
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
BOP	benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate
br	broad
BSA	bovine serum albumin
tert-	tertiary
n-BuLi	*n*-butyl lithium

c	concentration
calcd	calculated
Cbz	benzyloxycarbonyl
Cbz-Cl	benzyl chloroformate
CDI	1,1'-carbonyldiimidazole
CI	chemical ionization
Conc.	concentrated
COSY	correlated spectroscopy
Cys	cysteine
δ	chemical shift in parts per million downfield from TMS
d	doublet
Dabcyl	4-(4-dimethylaminophenylazo)benzoyl
DCC	1,3-dicyclohexylcarbodiimide
DEAD	diethyl azodicarboxylate
DIPEA	diiopropylethyl amine
DMAD	dimethyl azodicarboxylate
DMAP	4-*N,N*-dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
DTT	dithiothreitol
Edans	5-[(2-aminoethyl)amino]-naphthalene-1-sulfonic acid

EDCI	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
EDTA	ethylenediaminetetraacetic acid
EI	electron impact ionization
Enz	enzyme
ES	electrospray ionization
Et_2O	diethyl ether
EtOH	ethanol
FMDV	foot and mouth disease virus
FMK	fluoromethyl ketone
Fmoc	9-fluorenylmethoxycarbonyl
Gln	glutamine
Gly	glycine
gHMQC	gradient heteronuclear multiple quantum coherence
HAV	hepatitis A virus
HBTU	*N*-[(1*H*-benzotriazol-1-yl)-dimethylamino)-methylene]-*N*-methylanaminium hexafluorophosphate *N*-oxide
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIV	human immunodeficiency virus
His	histidine
HMBC	heteronuclear multiple bond coherence
HMG-CoA	β-hydroxy-β-methylglutaryl coenzymeA
HMQC	heteronuclear multiple quantum coherence

HPLC	high performance liquid chromatography
HPV	human polio virus
HRMS	high resolution mass spectrometry
HRV	human rhinovirus
IC_{50}	concentration causing 50% inhibition
ICE	interleukin-1β converting enzyme
IR	infrared
J	coupling constant
k_{cat}	catalytic rate constant
K_I	dissociation constant of mechanism-based inactivator
K_i	dissociation constant of enzyme-reversible inhibitor complex
K_i^*	overall dissociation constant of enzyme-reversible inhibitor tight complex EI^*
$K_{i,app}$	apparent dissociation constant of enzyme-reversible inhibitor complex
k_{inact}	rate of enzyme inactivation
k_{obs}	first order rate constant for the enzyme inactivation
K_m	Michaelis-Menten constant
kDa	kiloDalton
LDA	lithium diisopropylamine
Leu	leucine

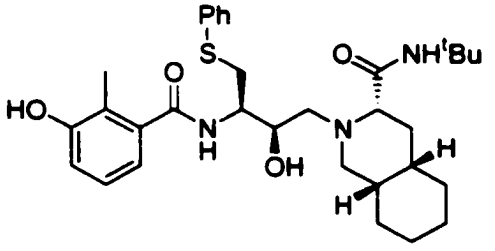
MALDI	matrix assisted laser desorption/ionization
Me	methyl
m	multiplet
MeCN	acetonitrile
MeOH	methanol
m/z	mass to charge ratio
MHz	megahertz
min	minute(s)
mp	melting point
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MW	molecular weight
NMR	nuclear magnetic resonance
nm	nanometers
NOE	nuclear Overhauser effect
Nu	nucleophile
Ph	phenyl
Phe	phenylalanine
PPh_3	triphenylphosphine
ppm	parts per million
Pht	phthalimido
pNA	para-nitroanilide
psi	pound per square inch

q	quartet
RCM	ring closing metathesis
R_f	retention factor
RNA	ribonucleic acid
RP	reverse phase
rt	room temperature
s	singlet
Ser	serine
t	triplet
$t_{1/2}$	half life
TES	triethylsilane
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TLC	thin layer chromatography
Thr	threonine
TMS	trimethylsilyl
p-TsOH	para-toluenesulfonic acid
UV	ultraviolet
Val	valine

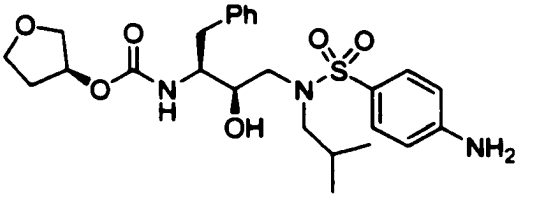
INTRODUCTION

For the last 30 years, considerable effort has been devoted towards the study of proteinases because of their roles in many diseases such as cancer, arthritis, osteoporosis, and viral infections.[1] Thus, the development of specific and potent inhibitors targeting these proteolytic enzymes could potentially lead to clinically effective chemotherapeutic agents. In particular, the emergence of drug-resistant viral strains warrants the development of new antiviral agents,[2,3] including those which target virus-encoded proteinases. Certain viral proteinases are absolutely required for widespread viral infections because they process the initial viral-encoded proteins to give fragments necessary for assembly of viral particles. Notable examples of therapeutically useful proteinase inhibitors are nelfinavir **1** and amprenavir **2** (Figure 1) which target the aspartyl proteinase of human immunodeficiency virus (HIV) and are used for the treatment of acquired immune deficiency syndrome (AIDS).[1]

Figure 1 Proteinase inhibitors of HIV used as therapeutic drugs



Nelfinavir **1**



Amprenavir **2**

1.0 The Picornavirus Family

The picornavirus ('pico' meaning small, 'rna' for ribonucleic acid) family are small icosahedral positive-sense single stranded RNA viruses that cause a wide variety of diseases in humans and animals.[4] More than 200 members are known which are classified into six genera, namely Entero, Rhino, Aphtho, Cardio, Hepato and Parecho. Picornaviruses include important pathogens such as the human poliovirus (HPV)[5] (Figure 2), human rhinovirus (HRV), foot and mouth disease virus (FMDV)[6] (Figure 2), encephalomyocarditis virus (EMCV), hepatitis A virus (HAV) and Parechovirus.[7] The diseases associated with these viruses range from symthomatically mild and wide-spread infections, in the case of the common cold and hepatitis A, to permanent and life threatening conditions, as in the case of polio.

Figure 2 3-Dimensional X-ray structure of HPV 1[5] (left) and FMDV[6] (right) (http://www.virology.net)

The hepatitis A virus is the only known member of the genus hepatovirus and causes an acute form of infectious hepatitis.[4] The majority of hepatitis A infection cases are sporadic, although miniepidemics still occur periodically in the third world and are usually foodborne.[8] The most recent large HAV epidemic (mollusk-linked) occurred in

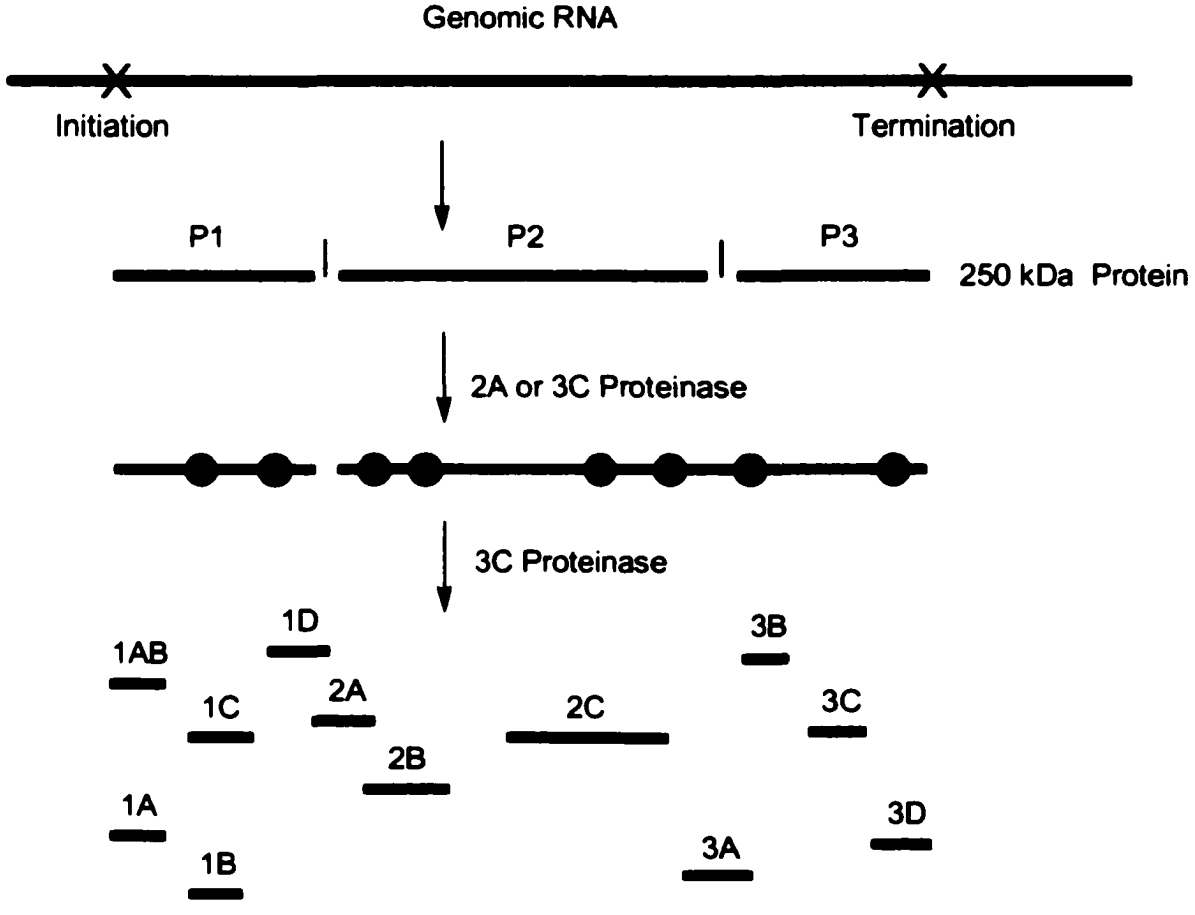
Shanghai, China in 1988, where ~300,000 people were infected with the virus.[9] Although acute HAV infections are in most cases relatively harmless, co-infection of patients with chronic hepatitis is often more dangerous.[10]

Although safe and effective vaccines against HAV[11] and HPV[12] have recently become available, development of vaccines for many picornaviruses is impractical due the high mutation rate of the virus capsid proteins (for example, >100 serotypes are known for HRV). This rapid diversification of the virion protein coat can lead to the formation of escape mutants capable of evading the human host's immune system and leading to infections in previously vaccinated individuals.[13] Complementary therapeutic route, is the development of therapeutic or prophylactic agents for picornaviruses. The picornaviral proteinases (picornains) produced by the viral RNA genome, appear quite invariant in a number of serotypes and are therefore potentially effective targets for the inhibition of viral growth. Hence, picornains inhibition has been the focus for novel antiviral agents to combat picornaviral diseases.[14,15]

Members of the picornavirus family share common features in their life cycle, including proteolytic processing of the viral polyprotein by specific proteinases.[7a] The life cycle of the picornaviruses is initiated by the attachment of the virus to a specific cell surface receptor. The positive-sense single stranded RNA genome is then released into the cytosol where it functions as mRNA to produce a single ~250 kDa polyprotein precursor (Figure 3).[15] Within this polyprotein are found the 2A and/or 3C proteinases. This polyprotein may undergo a co-translational cleavage by the 2A proteinase to yield a capsid (P1) and a non-structural protein precursor (P2-P3). The P1 and P2-P3 fragments are then cleaved by the 3C proteinase, resulting in mature capsid and viral components.[15]

In the case of entero- and rhinoviruses, the first cleavage is mediated by the 2A proteinase and further processing is performed by the 3C proteinase.[16] However, in HAV the 3C proteinase is the sole viral enzyme responsible for the primary as well as all the secondary proteolytic cleavages.[7]

Figure 3 Schematic representation of the polyprotein translation and cleavage in the picornaviruses



Studies have demonstrated that the activity of the 3C proteinase is critical for viral maturation and infectivity, and interruption of the 3C proteolytic processing prevents the formation of new virions.[15] There is little sequence similarity between the picomains and

the mammalian enzymes. Hence, potent and selective inhibitors of the 2A and/or 3C proteinases could serve as effective drug candidates for the treatment of picornaviral diseases.

2.0 The Picornains 3C: Serine Proteinase Fold with a Cysteine Nucleophile

All picornains 3C are cysteine proteinases. The wild type HAV 3C enzyme spans 219 residues exhibiting a molecular weight of 24 kDa and functions as an active monomer.[17] The HRV 3C enzyme of serotype 14 is smaller with 182 residues and a molecular weight of 20 kDa.[18] Preliminary attempts to crystallize the wild type HAV 3C for X-ray structure determination were unsuccessful due to the presence and reactivity of two cysteines in the molecule, one on the protein surface at position 24 and one found in the active site at position 172. These cysteines tend to oxidize and/or form intermolecular disulfide bridges that lead to heterogeneous species. To overcome these problems, site-specific mutagenesis was used to generate an inactive C24S-C172A double mutant which crystallized and whose structure was determined to 2.3 Å resolution by the James group (Biochemistry Department, University of Alberta).[17] More recently, a refined crystal structure of an active HAV 3C C24S mutant, where only the external cysteine-24 has been replaced by serine, was determined to 2.0 Å resolution (Figure 4).[19] X-ray crystal structures of HRV[18] and HPV[20] 3C proteinases have also been reported. Although these 3C enzymes are cysteine proteinases, they are structurally distinct from the papain family, and more closely resemble the chymotrypsin-like serine proteinases.[17] The overall architecture consists of two distinct domains, each having an antiparallel β-barrel topology (shown in blue and purple in Figure 4).[17] The active site cleft resides between

the two domains and residues from both domains contribute to the catalytic surface and play critical roles in the proteolytic mechanism the recognition and binding of substrates.[7] The active site cysteine (Cys-172 in HAV) acts as the nucleophile during peptide bond attack and this process is assisted by a histidine (His-44 in HAV) serving as a general acid-base catalyst (Figure 5).

Figure 4 Ribbon secondary structure of HAV 3C (C24S) mutant proteinase (blue spheres represent water molecules, courtesy of Dr. Ernst M. Bergmann)

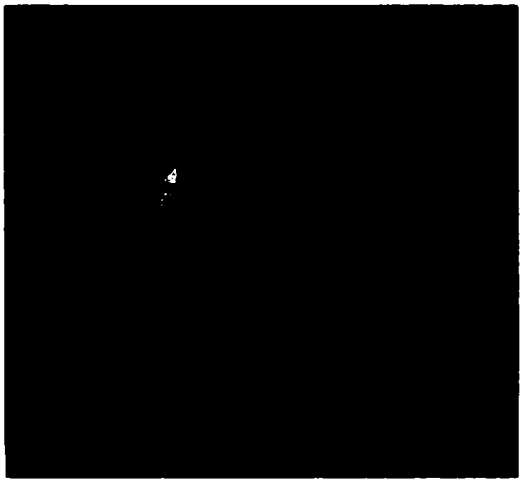
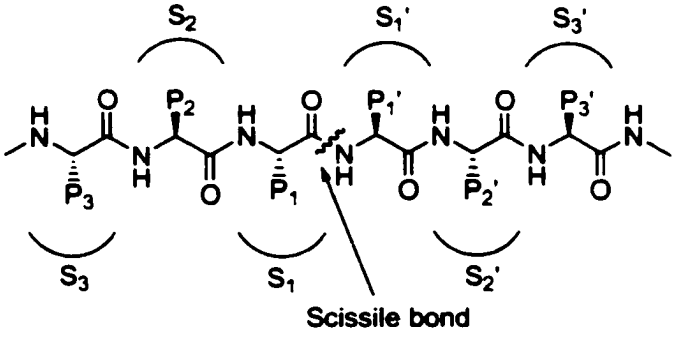


Figure 5 Active site of HAV 3C (C24S) mutant proteinase (blue spheres represent water molecules, courtesy of Dr. Ernst M. Bergmann)

The existence of a true catalytic triad (Cys, His, and Asp or Glu) in the 3C proteinases remains a point of debate. An aspartate or glutamate residue, corresponding topologically to the third member of the catalytic triad in chymotrypsin-like serine proteinases, is conserved throughout the picornains 3C. However, it has been proposed that the carboxylate of the Asp or Glu residue is not necessary for proteolytic activity .[15] In HAV 3C the corresponding residue, Asp-84, is pointing away from the active site imidazole of His-44, and thus may not directly assist in catalysis (Figure 5). A water molecule occupies the position of the third member of the catalytic triad (Figure 5, shown in blue circle).[7a,21]

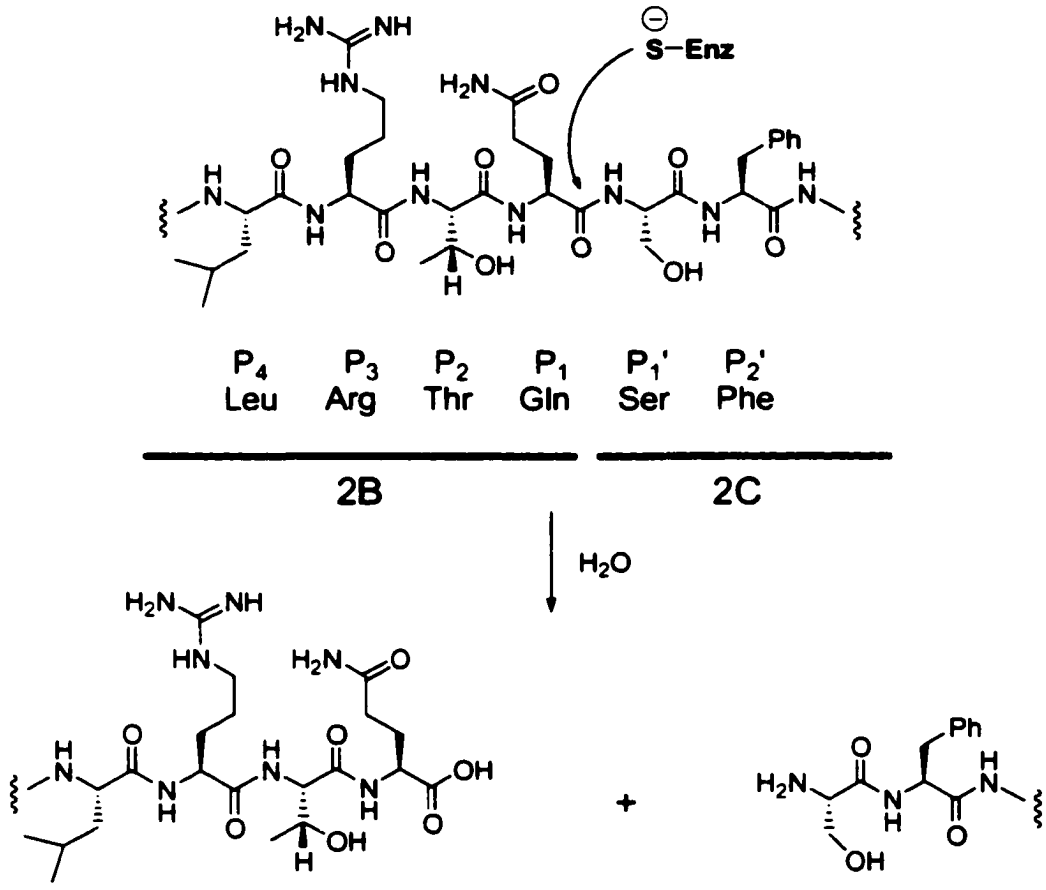
The standard nomenclature used for describing substrate or inhibitor residues are denoted by P_3, P_2, P_1, P_1', P_2', P_3' (notation of Schechter & Berger).[22] Enzymes cleave their substrates at the scissile amide bond between the P_1-P_1' junction. These residues bind to the corresponding enzyme subsites which are referred to as S_3, S_2, S_1, S_1', S_2', S_3' as shown in Figure 6.

Figure 6 Standard nomenclature for substrate residues and their corresponding binding sites



All picornains 3C cleave after a glutamine residue at the P_1 site.[15] Detailed examination of the HAV 3C substrate specificity using synthetic peptides that correspond to the 2B/2C and 2C/3A junctions of the large precursor protein has shown that P_4 to P_2' residues are necessary for optimal cleavage efficiency (Figure 7).[23] For an octapeptide substrate (Ac-ELRTQSFS-NH_2) mimicking the 2B/2C junction with glutamine at the P_1 position (Figure 7), the k_{cat} is 1.8 s^{-1} with a K_m of 2.1 mM at pH 7.5.[23] The S_1 subsite of HAV 3C is a shallow hydrophobic pocket, in which resides a histidine residue (His-191) that is believed to be involved in hydrogen bond formation with the substrate's distal glutamine carbonyl at P_1. Substrates containing *N,N*-dimethylglutamine at P_1 bind equally well to the enzyme. However, alterations of the side chain length or introduction of charged residues at P_1 drastically lowers the recognition of the HAV 3C proteinase.[23]

Figure 7 A preferred cleavage site (2B/2C) of HAV 3C proteinase

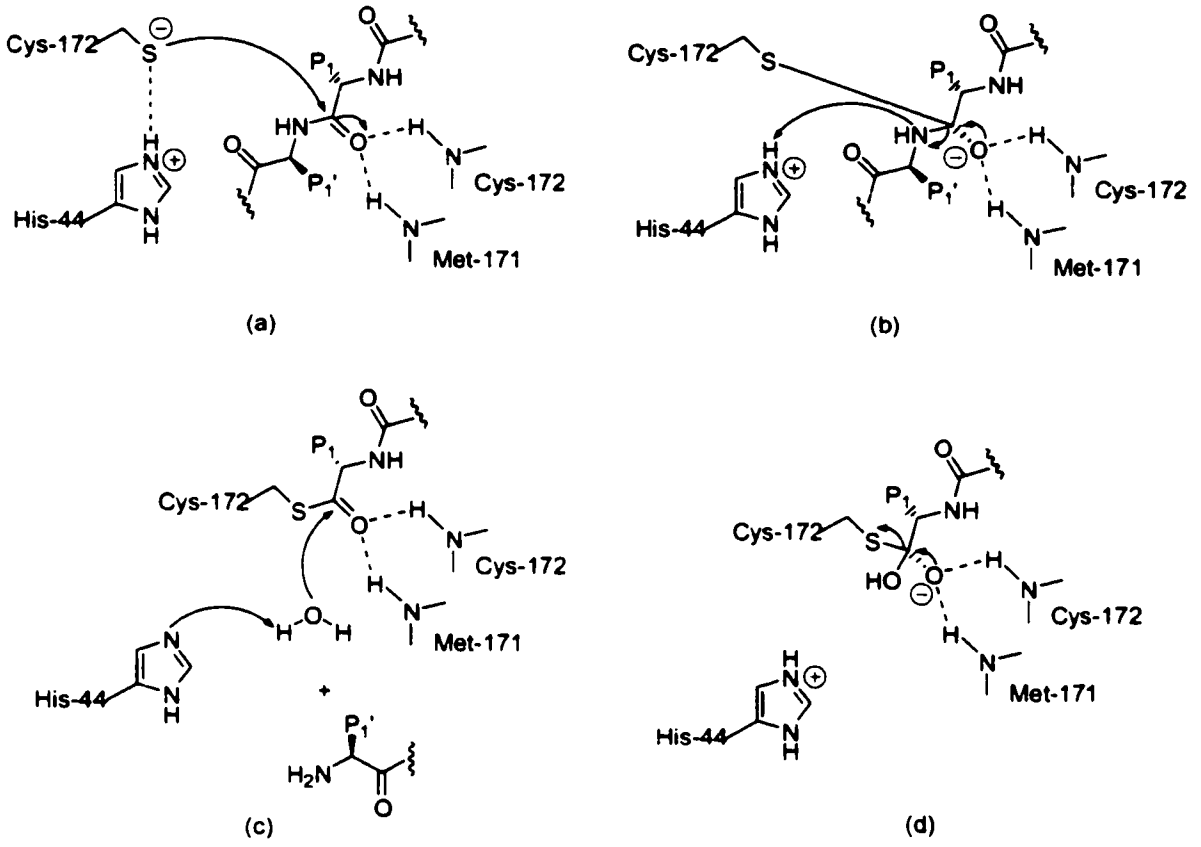


In the case of entero- and rhinoviruses, experiments with synthetic peptide substrates demonstrate that the glutamine residue should be followed by glycine and proline at the P_1' and P_2' positions, respectively, to ensure efficient recognition and cleavage. Studies carried out on HAV 3C show that it is less discriminating and effectively cleaves peptides in which small amino acid residues such as glycine, alanine, or serine reside at P_1' and virtually any amino acid residue is in the P_2' position with the exception of proline and arginine.[15] At the P_4 position, polio- and rhinovirus have preference for small side chains such as alanine, whereas in HAV 3C, large side chains such as branched aliphatic or aromatic residues including, leucine, isoleucine, tryptophan, are preferred. The S_4 subsite in HAV 3C appears to be a rather open hydrophobic cleft,

which may account for the enzyme's preference for various large hydrophobic residues at P_4. In contrast, the HRV 3C enzyme appears to maintain a shallow pocket, and consequently prefers smaller residues such as alanine and valine.[15] The side chains at positions P_3 and P_2 do not contribute significantly for recognition of substrates in picornains 3C.[23]

A generalized catalytic mechanism for HAV 3C cysteine proteinase is shown in Figure 8. After binding of the substrate to the enzyme active site, the anionic thiolate nucleophile is generated (Cys-172) using general base catalysis provided by the imidazole side chain of histidine (His-44) attacks the carbonyl carbon of the scissile amide bond (Figure 8a). This leads to the formation of a tetrahedral intermediate stabilized by hydrogen bonding to the backbone NH of Cys-172 and Met-171, which form part of the oxyanion hole. Proton transfer from the imidazolium cation of His-44 to the amine of the tetrahedral intermediate facilitates elimination of peptide amino group (Figure 8b). The remaining covalent acyl-enzyme complex is attacked by a nucleophilic hydroxide anion generated from an active site water and His-44 mediated general base catalysis to generate a new tetrahedral intermediate (Figure 8c), which subsequently breaks down via acid-assisted catalysis by His-44 to form the carboxyl fragment of the cleaved substrate and regeneration of the free enzyme.[1a,18]

Figure 8 Generalized catalytic mechanism for HAV 3C cysteine proteinase



3.0 Proteinase Inhibitors as Antiviral Drugs

Viral proteinases, which are essential for the production of infectious virus, have long been considered viable targets for the development of antiviral chemotherapeutic agents. Research on inhibitors directed against these targets has been aided by advances in high-throughput screening of compound libraries and by structure-based rational drug design strategies using experimental protein structures determined by X-ray crystallography and computer aided molecular modelling.[1] To be effective, proteinase inhibitors must exhibit both high potency and selective proteinase binding. In addition,

successful drug candidates must possess favorable pharmacokinetic and pharmacodynamic profiles. Peptidic inhibitors of proteinases often give important mechanistic and structural clues for further rounds of drug design. However, their use as drugs is usually compromised by their instability, low bioavailability, and poor pharmacological properties.[24] To be effective drugs, proteinase inhibitors should ideally have minimal peptide character, high stability against nonselective proteolytic degradation, good membrane permeability, bioavailability and reasonable life times in both the bloodstream and in cells.[24]

4.0 Inhibitors of HAV and HRV 3C Proteinases

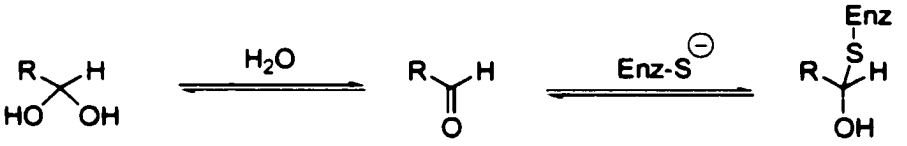
Several classes of inhibitors have been reported for HAV and HRV 3C proteinases, including peptide aldehydes,[25,26] halomethyl ketones,[27,28] iodoacetamides,[29] azapeptides,[30,31] azodicarboxamides,[32] β-lactones,[33] α-β-unsaturated esters and sulfones,[34-36] isatins,[37] homophthalimides,[38] and benzamides.[39] These inhibitors were designed based on the substrate specificity of the enzymes. Most of them possess functionalities that mimic the important P_1 glutamine residue, and have a thiol reactive group ("warhead") which usually results in covalent modification of the active site cysteine residue. These compounds can be broadly classified into peptidic (for example, peptide aldehyde) and non-peptidic (for example, β-lactones and benzamides) chemical agents. Although peptide based inhibitors are not suitable candidates for drug development because of their poor bioavailability, insights gained from crystallographic studies of these molecules bound in the enzyme active site should provide valuable clues

regarding the catalytic mechanism and molecular recognition. This would assist the design of second-generation non-peptidic inhibitors.

4.1 Peptide Aldehyde

Peptide aldehydes are well known and effective reversible inhibitors of thiol-containing proteinases.[40] Their mode of inhibition is primarily due to the formation of reversible covalent adducts (thiohemiacetals) that mimic the initial tetrahedral transition state as the proteinase cleaves the scissile amide bond (Figure 9).

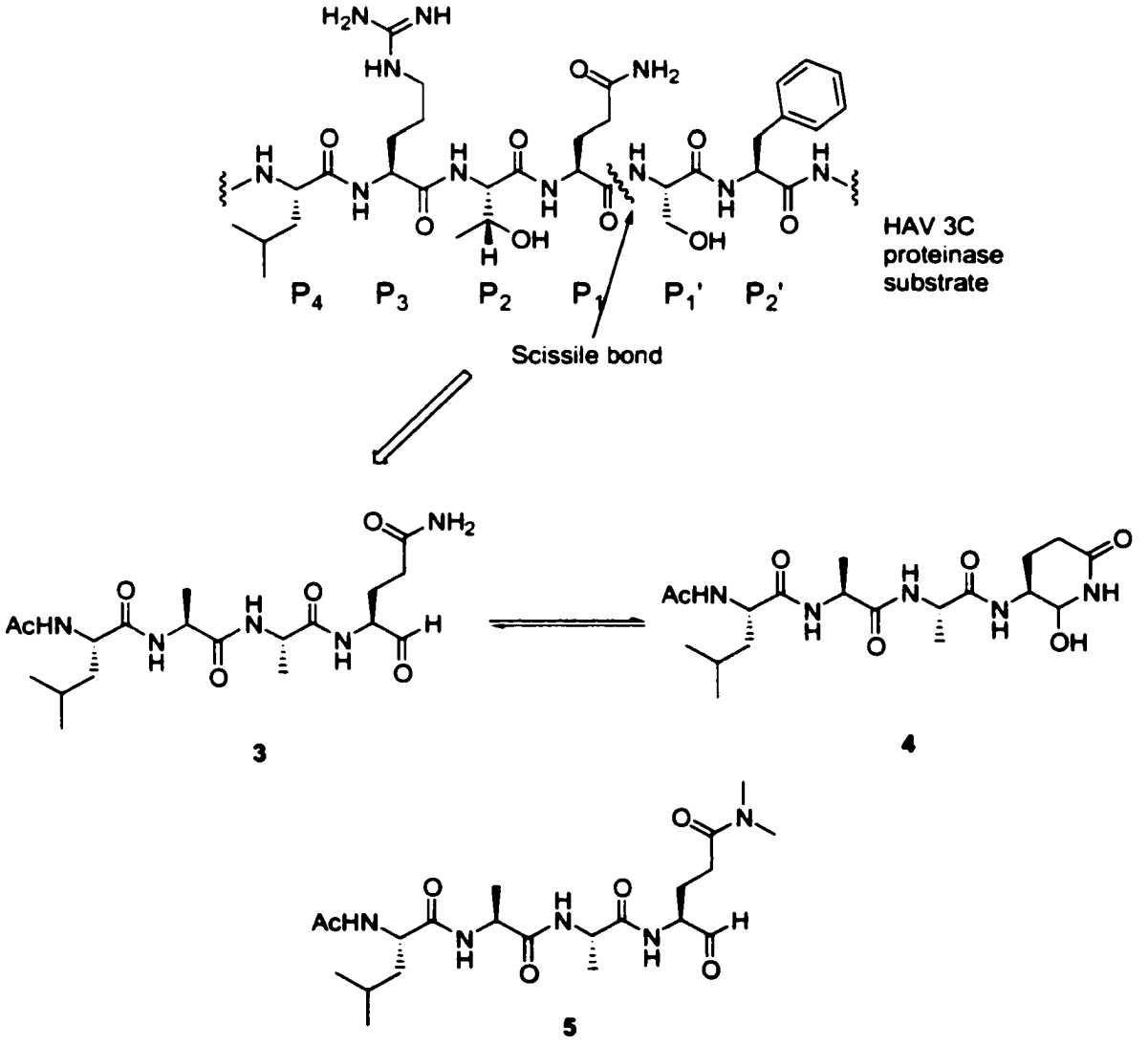
Figure 9 Reaction of an aldehyde inhibitor with a cysteine enzyme in aqueous solution



Most of the reported peptide aldehydes present the aldehyde moiety ("warhead") to the target proteinase while tethered to a peptide portion that corresponds to the known substrate preference of the enzyme. For example, the peptide aldehyde **3** is a likely inhibitor of HAV 3C based on the enzyme's substrate (Figure 10).[25] However, one limitation for the combination of the P_1 glutamine and an aldehyde functionality is the high propensity of the primary amide to cyclize on the aldehyde forming the glutaminal **4** which might reduce the activity of the inhibitor. Since the enzyme recognizes the carbonyl moiety and not the NH group of the side chain primary amide, this inherent reactivity can be overcome by alkylating the amide functionality such as in **5** which

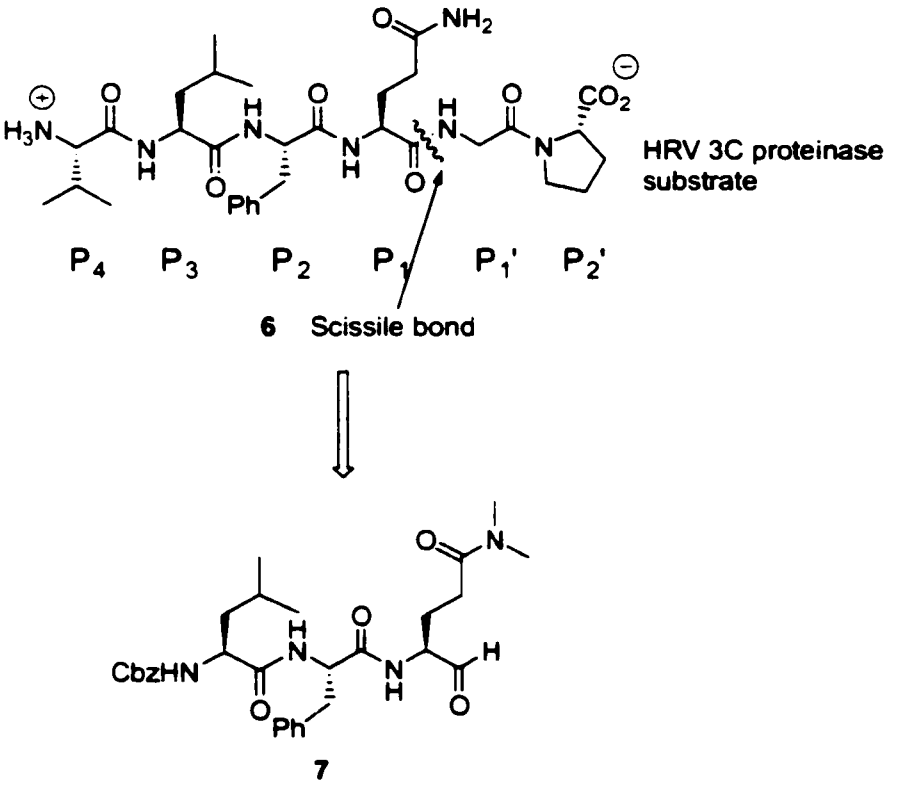
displayed a K_i^* of 42 nM (overall dissociation constant for the tight enzyme-inhibitor complex, EI*, slow binding).[25] For inhibitor **5**, Ac-Leu-Ala-Ala is a reasonable substitute for the Leu-Arg-Thr sequence in substrate analogues as it has been demonstrated that neither arginine nor threonine side chains contribute significantly to binding.[23] The inhibitor **5** shows 50 fold less activity against the highly homologous HRV 3C proteinase whose peptide substrate is slightly different, indicating some degree of selectivity.[25]

Figure 10 Peptide aldehyde inhibitor of HAV 3C enzyme



A similar strategy was later applied for HRV 3C proteinase inhibitors and peptide aldehyde **7** was found to have a K_i of 0.01 μM (Figure 11).[26a]

Figure 11 Peptide aldehyde inhibitor of HRV 3C enzyme

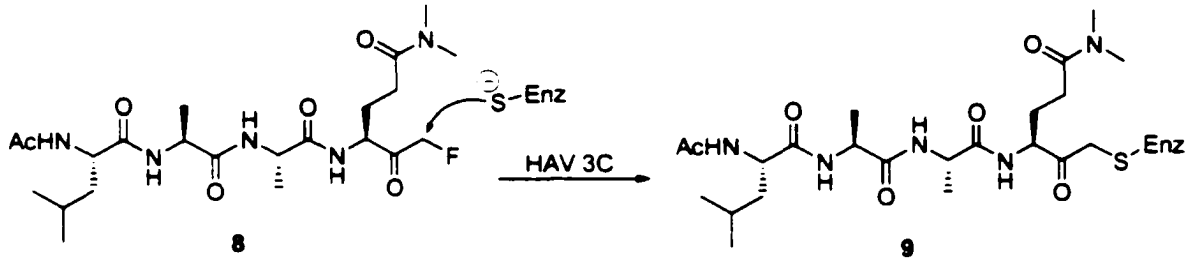


4.2 Peptidyl Fluoromethyl Ketone

Fluoromethyl ketone (FMK) inhibitors are used as affinity labels to inactivate cysteine proteinases through alkylation of the active site thiol. Peptidyl FMKs have been found to be highly effective against cysteine proteinases, but only marginally active against the mammalian serine proteinase counterparts.[41] By varying the peptidyl side chain for maximal discriminatory activity, this selective activity can be employed

advantageously to target specific cysteine proteinases in the biological system. The tetrapeptide FMK **8** (Figure 12) was prepared in our group based on the substrate specificity for the HAV 3C substrate. This compound was found to be a potent irreversible inactivator of HAV 3C proteinase with a second order rate constant of $K_{inact}/K_I = 330\ M^{-1}s^{-1}$ ([E] = 0.07 μM, [I] = 1.0 μM).[27]

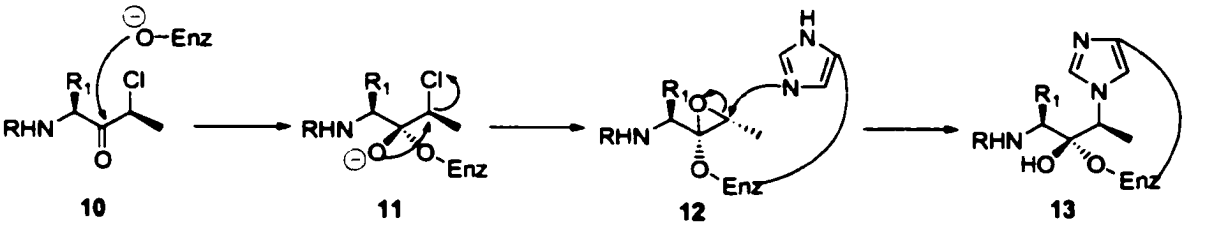
Figure 12 Inactivation of HAV 3C proteinase by a peptidyl FMK



8 **9**

Previous crystallographic studies of the papain-like cysteine proteinase cruzain[42] modified and inactivated by a fluoromethyl ketone, together with NMR studies of the cysteine proteinase papain[43] demonstrate that the halogen has been displaced by the cysteine thiol to form an α-keto sulfide such as **9** (Figure 12). However, mechanistic studies conducted on the serine proteinase chymotrypsin, using an α-chloroethyl ketone supports a different mechanistic pathway (Figure 13).[44] Nucleophilic attack of the serine hydroxyl group on the ketone carbonyl carbon **10** followed by intramolecular displacement of chloride **11** generates an epoxide **12**. The epoxide ring is then opened by nucleophilic attack by the histidine of the catalytic triad to generate the N-alkylated acetal enzyme-inhibitor complex **13**.

Figure 13 Mode of inhibition of serine proteinase by an α-chloroethyl ketone

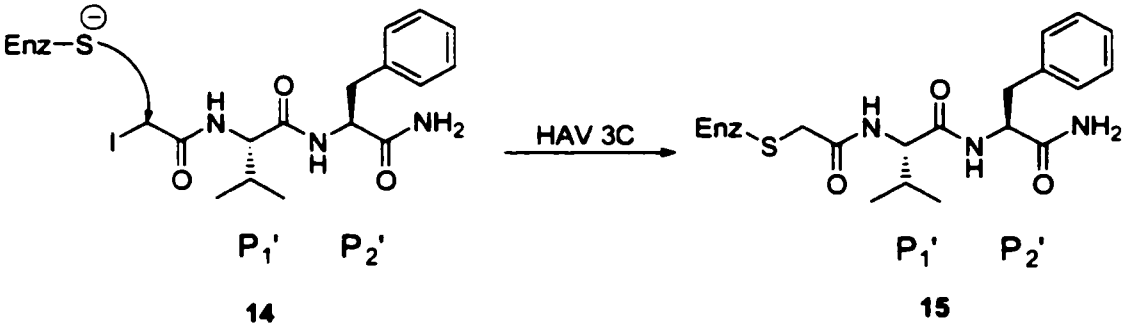


Since the HAV 3C geometry more closely resembles serine proteinases rather than the papain-like cysteine proteinases, an inhibitory mechanism like that depicted in Figure 13 may be involved in HAV 3C proteinase inactivation by FMKs. To further test this hypothesis, the HAV 3C proteinase was inactivated using peptidyl FMK labeled with ^{13}C at the α-halo methylene position. ^{13}C and ^{19}F NMR indicated that no N-alkylation by the active site histidine took place, and that the mode of inhibition was through simple displacement of fluoride by the active site cysteine thiol as shown in Figure 12.[27] *Ex vivo* studies, to test for antiviral activity, demonstrated a 25-fold reduction in viral progeny production as a result of treatment with 5 μM inhibitor **8** after 24 h post infection.[27]

4.3 Peptidyl Iodoacetamides

Peptide aldehydes and fluoromethyl ketones are inhibitors that possess the P side chain recognition sites. However, inhibitors that bind to the S' site of the enzyme can also be designed by attaching a reactive group such as iodoacetamide to P' peptide fragments (Figure 14).

Figure 14 Peptidyl iodoacetamide inhibitor of HAV 3C proteinase



Iodoacetamide **14** was prepared in our group and displayed potent irreversible inhibition of HAV 3C proteinase, with a second order rate of inactivation, k_{inact}/K_I = 200 $M^{-1}s^{-1}$ ([E] = 0.07 μM, [I] = 1.0 μM).[29] The fact that this inhibitor inactivates the HAV 3C proteinase by specifically alkylating the enzyme's cysteine thiol forming the covalent enzyme/inhibitor complex **15**, was recently demonstrated by elucidation of its X-ray co-crystal structure by the James group.[45] The C24S, F82A double mutant of HAV 3C proteinase was used along with **15** during crystallization trials. The resultant crystals of the enzyme/inhibitor complex were used to determine the refined crystal structure to 1.9 Å resolution.[45] The complex has the *N*-acetyl-Val-Phe-amide group covalently attached to the sulfur atom of the active site Cys-172, with the dipeptide side chains bound in their appropriate S_1' and S_2' subsites (Figure 15).

Figure 15 Active site of HAV 3C (C24S, F82A) proteinase residues (light grey) bound to acetyl-Val-Phe-amide (dark grey) (courtesy of Dr. Ernst M. Bergmann)

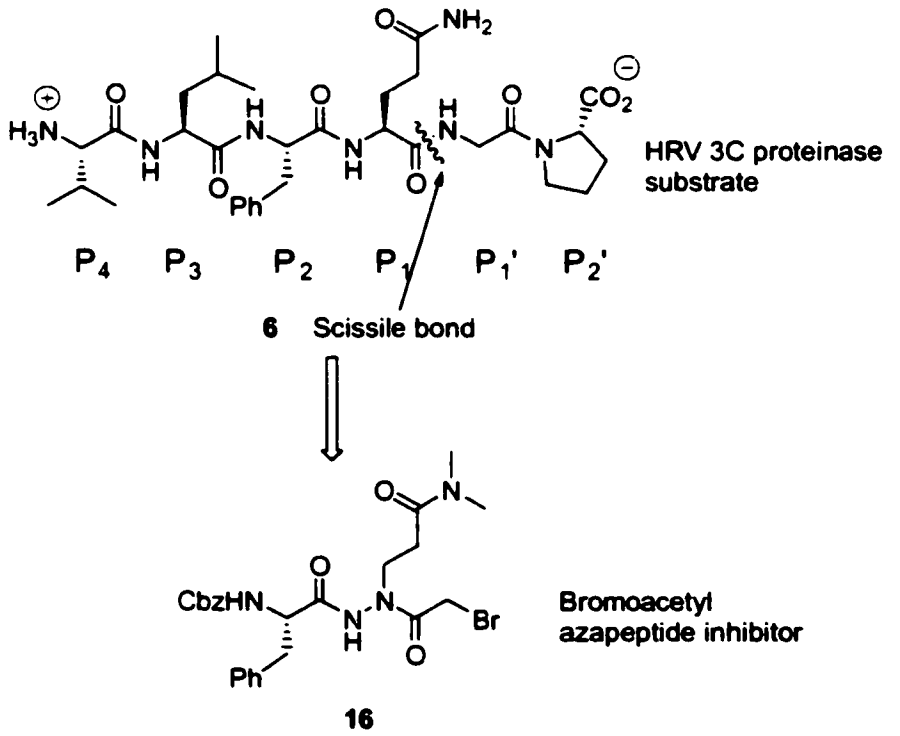
This is the first example of any structure for a chymotrypsin-like serine proteinase a small molecule bound in the S' sites. The architecture of this novel complex maintains a well-defined S_2' specificity pocket and that the P_2' phenylalanine residue is a likely determinant for the selection of the primary cleavage site in HAV 3C proteinase polyprotein substrates.

4.4 Azapeptides and Azodicarboxamides

Azapeptides are a class of backbone-modified peptides that have become important in the pharmaceutical industry.[46] Incorporation of hydrazine functionality into the peptide backbone is a well-established strategy for the generation of proteinase inhibitors.[47] In these analogues, the α-CH group of one or more amino acid residues in a

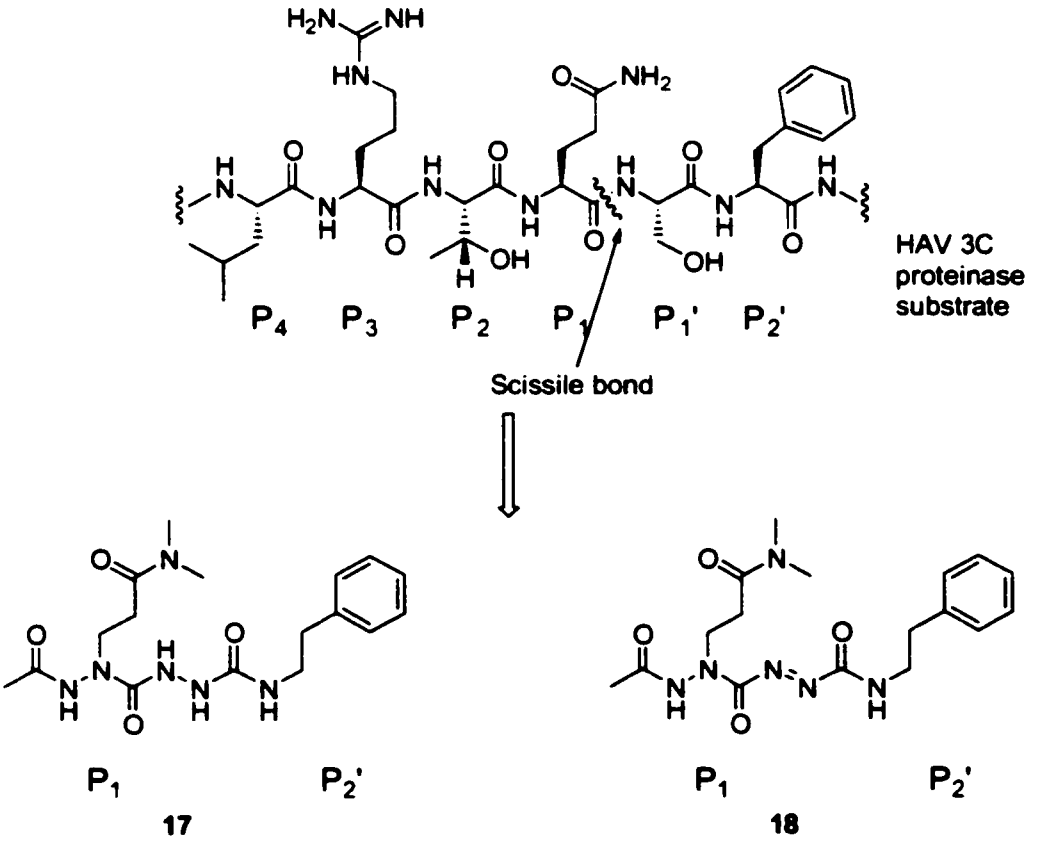
peptide chain is replaced by a nitrogen atom with retention of the original side chain (Figure 16). Recently, Sham *et al.*[48] showed that the bromoacetyl azapeptide analogue **16** is a potent inhibitor of the HRV 3C proteinase. This inhibitor, based on backbone replacement at a common cleavage site (between glutamine and glycine) of HRV 3C proteinase, is a time-dependent irreversible inhibitor with a $k_{inact}/K_I > 2500\ M^{-1}s^{-1}$ (Figure 16). Although the potency of compound **16** is primarily due to the reactive bromoketone functionality, this work demonstrates that an azapeptide motif may substitute for a peptide residue in the design of peptidomimetic inhibitors.

Figure 16 Bromoacetyl azapeptide inhibitor of HRV 3C proteinase



More recently, azapeptide inhibitors of HAV 3C were reported by our group.[30,32] Compound **17** (Figure 17) displayed poor inhibitory activity against HAV and HRV 3C proteinases. Noticeably increased potency was achieved through oxidation to the more reactive azodicarboxamide **18** resulting in significantly enhanced inhibition.[32] Inhibitor **18** was found to have IC_{50} values of 10 and 12 μM against HAV and HRV 3C proteinases, respectively. These inhibitors were designed based on the substrate specificity of the HAV 3C substrate and contain the P_1 and P_2' residues important for enzyme recognition. In the case of compound **18**, it is believed that the active site cysteine thiolate adds to the azo nitrogen in a Michael type fashion thereby forming a covalent adduct.[32]

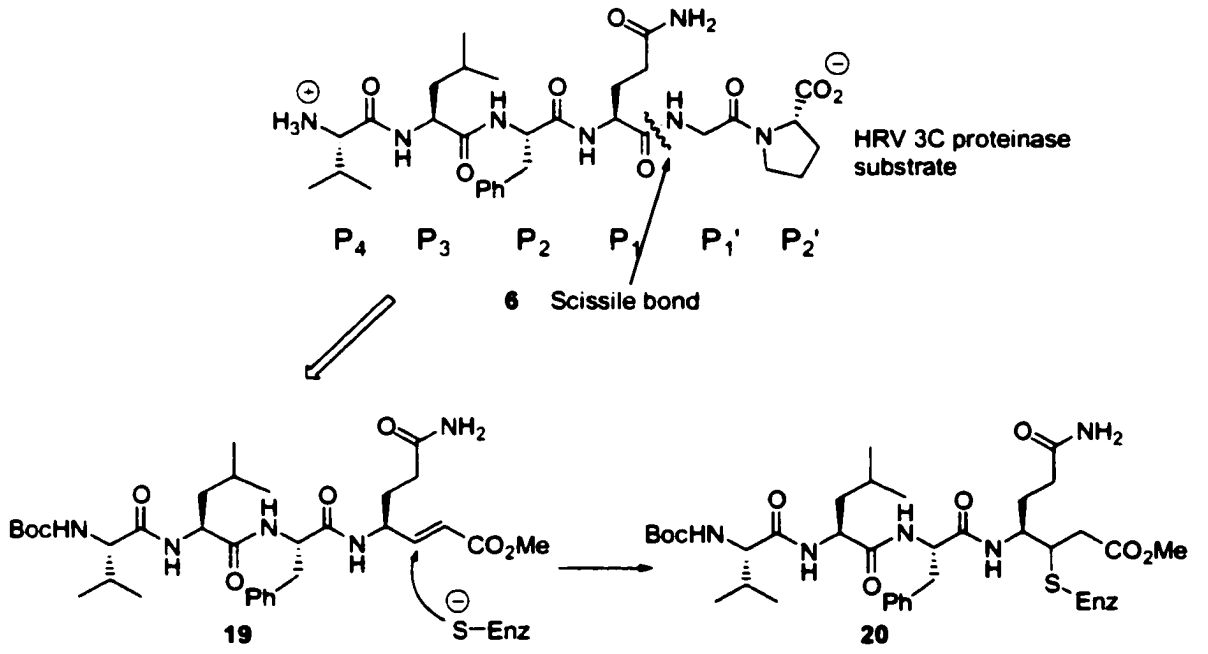
Figure 17 Azapeptide and azodicarboxamide inhibitors of HAV and HRV 3C proteinases



4.5 Peptidyl Michael Acceptors

These inhibitors consist of a peptide-derived section which provides affinity for the target proteinase and a Michael acceptor moiety (α,β-unsaturated carbonyl or sulfone) which can react with the active site nucleophile (for example, **19** and **20**, Figure 18). Compound **19** was prepared by Kong *et al.*[25] and found to be a potent inhibitor of HRV 3C with an IC_{50} of 0.25 μM. At low micromolar concentrations this compound also reduces HRV replication by 50% in cell culture.

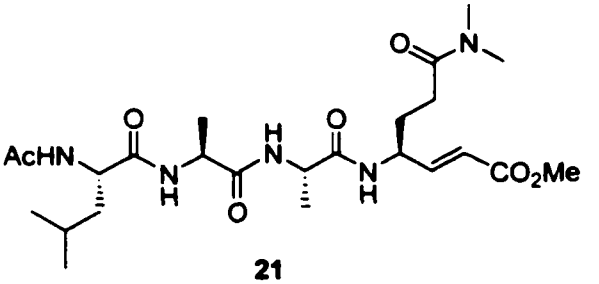
Figure 18 Peptidyl Michael acceptor inhibitor of HRV 3C proteinase with rationale for inhibition



A related analogue **21** (Figure 19) was independently designed and prepared for testing against HAV 3C by a previous graduate student in our group, Dr. Manjinder S.

Lall.[49] However, it showed only modest inhibition with a second order rate constant k_{inact}/K_I = 137 $M^{-1}s^{-1}$ ([E] = 0.07 μM, [I] = 10.0 μM) (unpublished).

Figure 19 Peptidyl Michael acceptor inhibitor of HAV 3C proteinase



21

5.0 Project Goals: Design, Synthesis and Testing of HAV 3C Proteinase Inhibitors

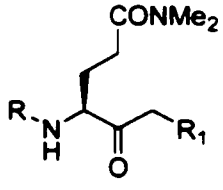
The objective of this work is to design, synthesize, and examine the interaction of several potential and novel inhibitors against the HAV 3C proteinase. Since the HAV 3C proteinase has structural features common to all members of the picornain family, it is likely that insights gained during the study of putative HAV 3C inhibitors will help in the development of specific inhibitors for other picronains 3C. Eight classes of compounds (Targets **A-H**, Figure 20-23) were prepared as potential inhibitors for the HAV 3C proteinase.

Targets **A-C** possess the essential dimethyl glutamine that is required for enzyme recognition at the P_1 position and a ketone functionality in place of the scissile peptide bond as the "warhead". Compounds containing a ketone moiety analogous to target **A** are known inhibitors of other cysteine proteinases such as cathepsin K,[50,51] cathepsin B,[52] papain,[52] and interleukin-1β converting enzyme (ICE)[53]. The target **B** class of compounds

which incorporate a 3-azetidinone backbone for presentation of the dimethylglutamine side chain constitute a novel class of compounds that have not been previously reported as inhibitors of cysteine proteinases. On the other hand, the α-ketoamides similar to target **C** are inhibitors of the calpain cysteine proteinase.[54,55] The HAV 3C proteinase is expected to react with the ketone functionality of targets **A-C** in a covalent fashion forming a hemithioketal intermediate.

Figure 20 Targets **A-C**

Target **A**: Keto-glutamine



22 R = Boc, R_1 = $NHCO(CH_2)_3Ph$

23 R = Boc, R_1 = $NHCO(CH_2)_2Ph$

24 R = Boc, R_1 = $NHCO_2CH_2Ph$

25 R = Boc, R_1 = Cyano

26 R = Boc, R_1 = Phthalimido

27 R = Boc, R_1 = Phenoxy

28 R = Boc, R_1 = Phthalhydrazido

29 R = Boc, R_1 = *N*-Methylphthalhydrazido

30 R = Boc, R_1 = Tetrafluorophthalhydrazido

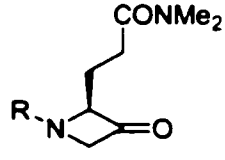
31 R = Boc, R_1 = Nitro-phthalhydrazido

32 R = Boc, R_1 = Pyridazindione

33 R = Boc, R_1 = Dimethyl hydrazodicarboxylate

34 R = Ac-Leu-Ala-Ala, R_1 = Phthalhydrazido

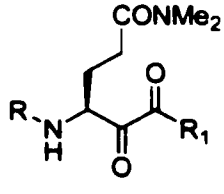
Target **B**: 3-Azetidinone



35 R = Boc

36 R = Ac-Leu-Ala-Ala

Target **C**: α- and α,β-Ketoamides



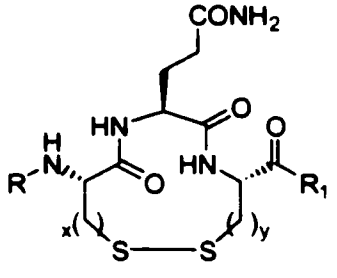
37 R = Boc, R_1 = NHMe

38 R = Boc, R_1 = CONHMe

Targets **D** and **E** are macrocyclic peptides that possess the HAV 3C proteianse's substrate residues connected by either disulfide or ethylene linkers, respectively (Figure 21). Restrictions of a given peptide's or mimetic's conformational space often result in increased affinity of the inhibitor for the target enzyme's active site due to the reduced entropic penalty paid during formation of the high affinity binding complex.[56,57]

Figure 21 Targets **D**, **E**

Target **D**: Macrocyclic peptide - disulfide linker



39 R = H, R_1 = NH_2
x = 2, y = 1

40 R = Fmoc, R_1 = OH
x = 2, y = 1

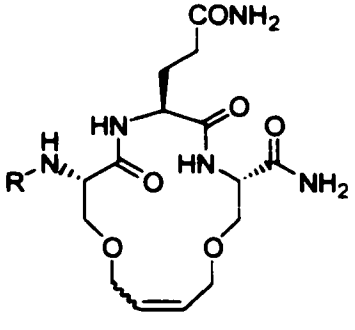
41 R = Fmoc, R_1 = Phe-OH
x = 2, y = 1

42 R = Ac-Leu-Ala-, R_1 = NH_2
x = 2, y = 2

43 R = Ac-Leu-Ser-, R_1 = NH_2
x = 2, y = 2

44 R = Ac-Leu-Ser-, R_1 = NH_2
x = 1, y = 2

Target **E**: Macrocyclic peptide - ethylene linker

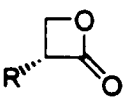


45 R = Ac-Leu-Ser-

N-Cbz serine β-lactones are potent inhibitors of HAV 3C proteinase.[33] In an attempt to synthesize more potent and stable inhibitors, the β-lactone analogues (target **F**, Figure 22) were prepared. Analogous 5-membered ring *N*-Cbz serine γ-lactones are poor inhibitors of the HAV 3C proteinase. To determine the importance of the 4-membered ring functionality in the *N*-Cbz serine β-lactone system, several 4-membered ring derivatives (target **G**, Figure 22) possessing a *N*-Cbz side chain **54-57** were prepared and tested.

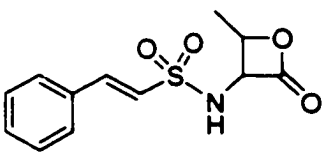
Figure 22 Targets **F**, **G**

Target **F**: β-Lactone analogues

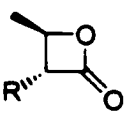


46 R = Phthalimido

47 R = *rac*-Ph



48 Unsaturated sulfonamide β-lactones



49 R = $PhCH_2SO_2NH$

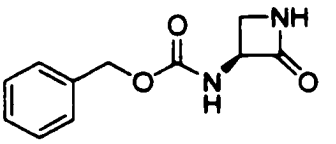
50 R = $PhSO_2NH$

51 R = CbzNH

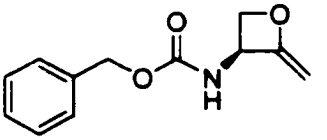
52a R = *p*-MeOBn

53 R = *rac*-PhCO

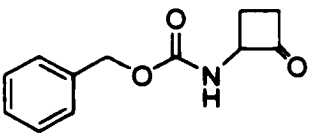
Target **G**: 4-membered ring analogues



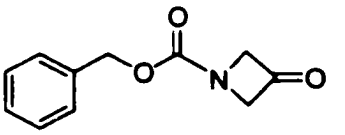
54 *N*-Cbz-L-β-lactam



55 *N*-Cbz-2-methylene oxetane



56 *N*-Cbz-cyclobutanone

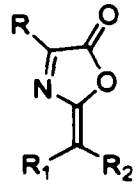


57 *N*-Cbz-3-azetidinone

Monophenyl pseudoxazolones are known to react with thiols at the imine position.[58] Such compounds (targets **H**, Figure 23) may irreversibly alkylate the active site cysteine thiol of the HAV 3C proteinase. To investigate the effectiveness of the pseudoxazolones as inhibitors, several diphenyl (**58, 59**) and monophenyl (**60, 61**) pseudoxazolones were prepared. In addition, monophenyl pseudoxazolones with an essential dimethyl amide side chain (for example, **62** and **63**) and also with a different substituent on the phenyl ring (for example, electron withdrawing (fluoro) **64** and electron donating (methoxy) **65**) were synthesized and tested against the HAV 3C proteinase.

Figure 23 Target **H**

Target **H**: Pseudoxazolone analogues



58 R = H, $R_1 = R_2$ = Ph

59 R = Me, $R_1 = R_2$ = Ph

60a R = H, R_1 = Ph, R_2 = H

60b R = H, R_1 = H, R_2 = Ph

61a R = Me, R_1 = Ph, R_2 = H

61b R = Me, R_1 = H, R_2 = Ph

62a R = $(CH_2)_3CONH_2$, R_1 = Ph, R_2 = H

62b R = $(CH_2)_3CONH_2$, R_1 = H, R_2 = Ph

63a R = $(CH_2)_2CONH_2$, R_1 = Ph, R_2 = H

63b R = $(CH_2)_2CONH_2$, R_1 = H, R_2 = Ph

64a R = H, R_1 = *p*-F-C_6H_4, R_2 = H

64b R = H, R_1 = H, R_2 = *p*-F-C_6H_4

65a R = H, R_1 = *p*-MeO-C_6H_4, R_2 = H

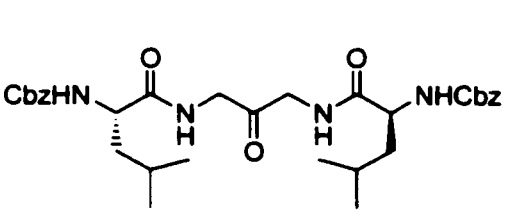
65b R = H, R_1 = H, R_2 = *p*-MeO-C_6H_4

RESULTS AND DISCUSSION

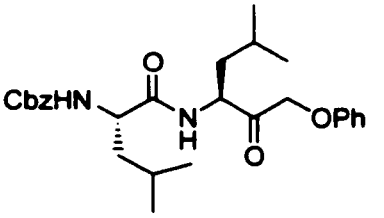
1.0 Keto-Glutamine Analogues as Inhibitors of HAV 3C Proteinase - Target A

Although aldehyde-based inhibitors often provide high potency, their therapeutic utility is limited by the reactive aldehyde functionality. Hence, it is unlikely that aldehydes can serve as viable therapeutic agents. Substitution of the aldehyde with a ketone moiety is a reasonable approach to attenuate the reactivity of the carbonyl moiety.[59] Recently, Veber and coworkers at SmithKline Beecham Pharmaceuticals reported 1,3-diamino ketone **66**[60] and α-alkoxy ketone **67**[61] (Figure 24) as potent and selective inhibitors of cathepsin K, a cysteine proteinase of the papain family involved in the process of bone resorption (osteoporosis). Both **66** and **67** are nanomolar reversible inhibitors with $K_{i,app}$ of 22 nM and IC_{50} of 3.7 nM, respectively. Analysis of the X-ray crystal structures of enzyme inhibitor adducts indicates that a hemi-thioketal is formed between the ketone carbonyl moiety and the active site cysteine thiol.[60,61]

Figure 24 Ketone-based inhibitors of cathepsin K



66

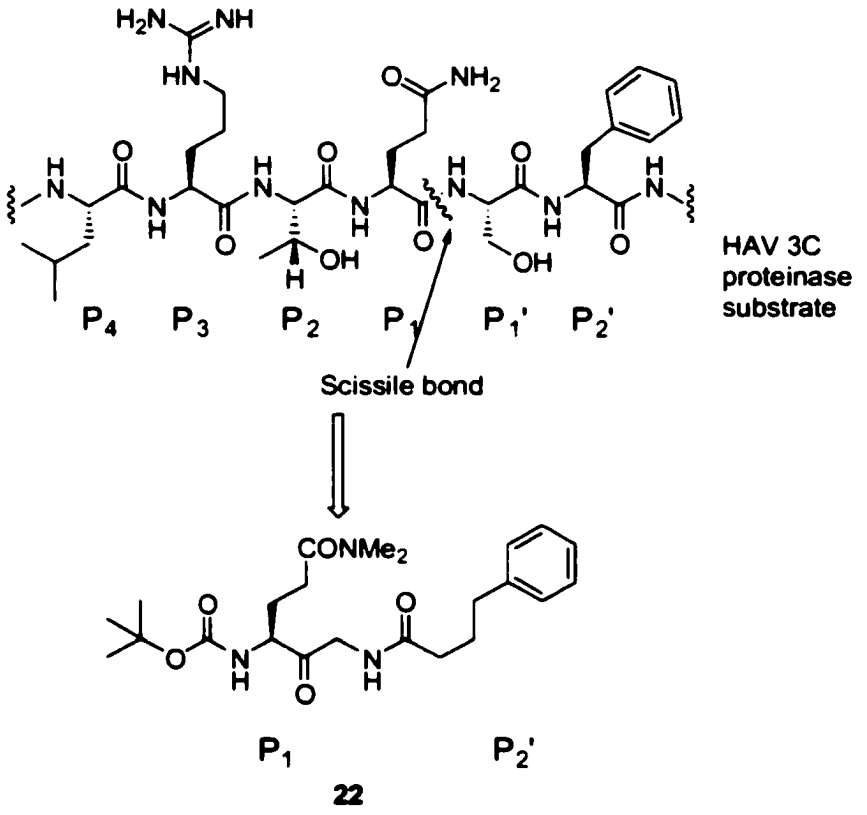


67

Based on these observations, a series of dimethyl glutamine analogues containing a ketone functionality were prepared and evaluated as potential inhibitors of HAV 3C

proteinase. The initial targets chosen are amino-ketones **22-24** (Figure 20). In addition to the dimethyl glutamine at P_1, these compounds also possess a phenyl group at P_2', designed to mimic the P_2' phenylalanine of the natural substrate (Figure 25).

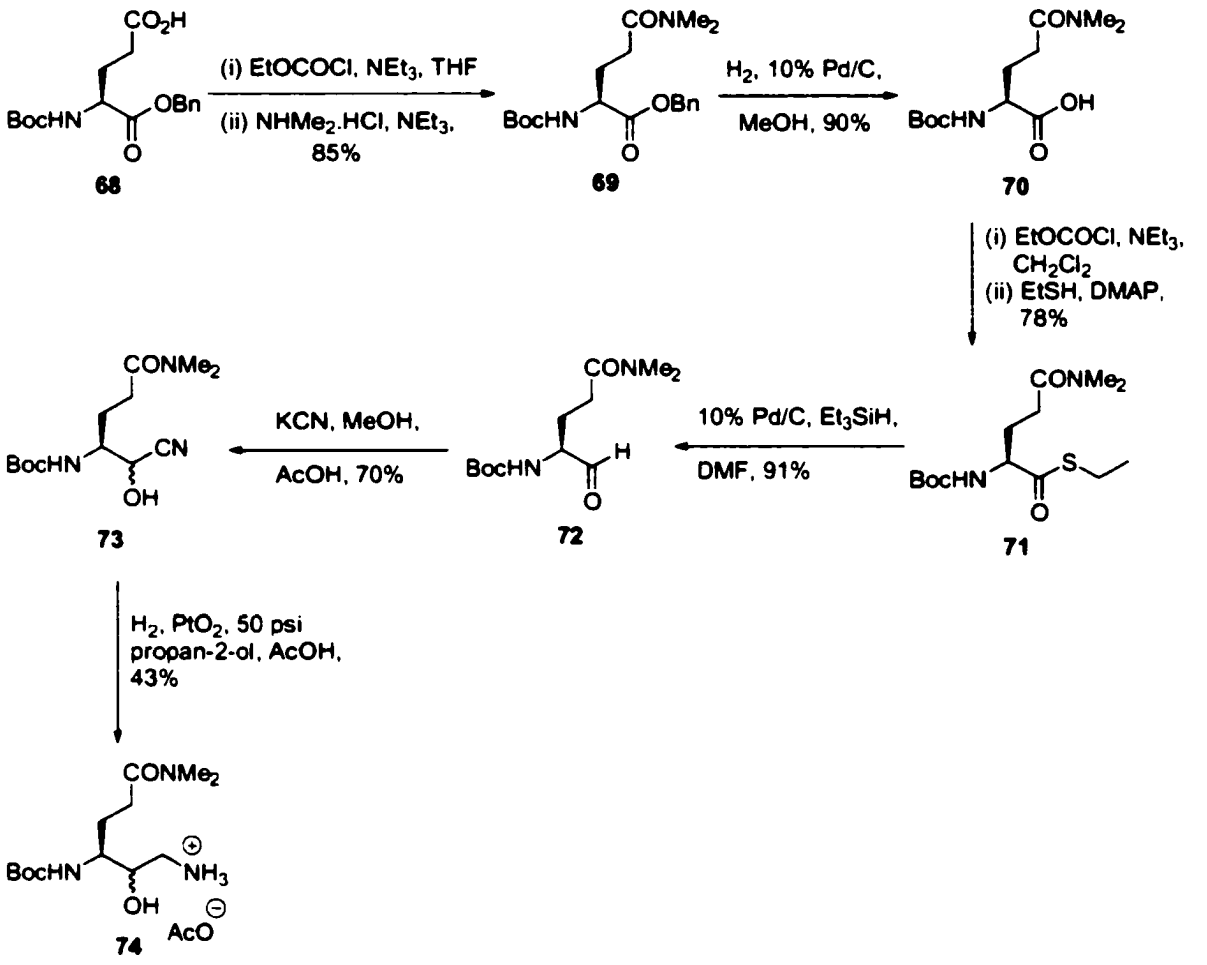
Figure 25 Amino-ketone inhibitor of HAV 3C



The synthesis of compound **22** is outlined in Scheme 1. Coupling of the commercially available Boc protected glutamic acid α-benzyl ester **68** with dimethylamine using ethyl chloroformate gives **69** in 85% yield.[27] Removal of the benzyl group by catalytic hydrogenation over palladium on carbon affords Boc-*N,N*-dimethyl glutamine **70** which can be subsequently converted to the thioester **71** using a procedure

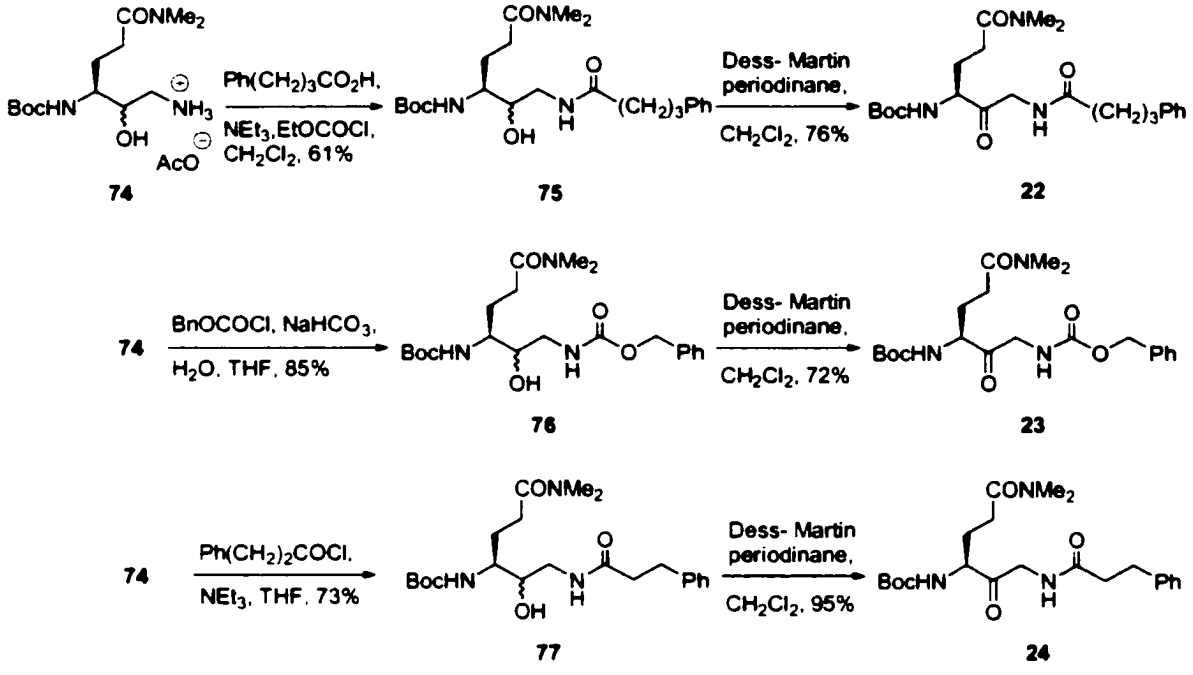
similar to that described by Fukuyama *et al.*[62] Activation of the acid **70** with ethyl chloroformate followed by reaction with DMAP and ethyl thiol gives the thioester **71**. Conversion of the thioester **71** to the aldehyde **72** proceeds in good yield by reduction with triethylsilane in the presence of a catalytic amount of palladium on carbon.[62] This methodology developed by Fukuyama *et al.*[62] is a mild and efficient procedure for the conversion of optically active amino acid thioesters to their corresponding aldehyde with no epimerization of the α-center. Reaction of aldehyde **72** with potassium cyanide generates cyanohydrin **73** in a 1:1 diastereomeric mixture.[63] Initial attempts to reduce the cyano group of **73** over PtO_2 at 50 psi using acetic acid as a solvent as described by Greenlee *et al.*[63] for the reduction of *N*-Boc-cyanohydrins, resulted in loss of the Boc group. A combination of propan-2-ol as solvent and excess acetic acid was also unsuccessful. However, the use of only 2 equivalents of acetic acid in propan-2-ol was found to be the appropriate conditions to afford the amino alcohol **74** in 43% yield.

Scheme 1



With the amino alcohol **74** available, targets **22-24** can be accessed by coupling to the appropriate phenyl-containing side chain followed by oxidation of the alcohol to ketone. Thus, amino alcohol **74** is conveniently coupled to 4-phenylbutyric acid, benzyl chloroformate or hydrocinnamoyl chloride to yield the corresponding hydroxy adducts **75-77**. These alcohols are subsequently oxidized with Dess-Martin periodinane[64] to generate the desired amino ketones **22-24** respectively (Scheme 2).

Scheme 2

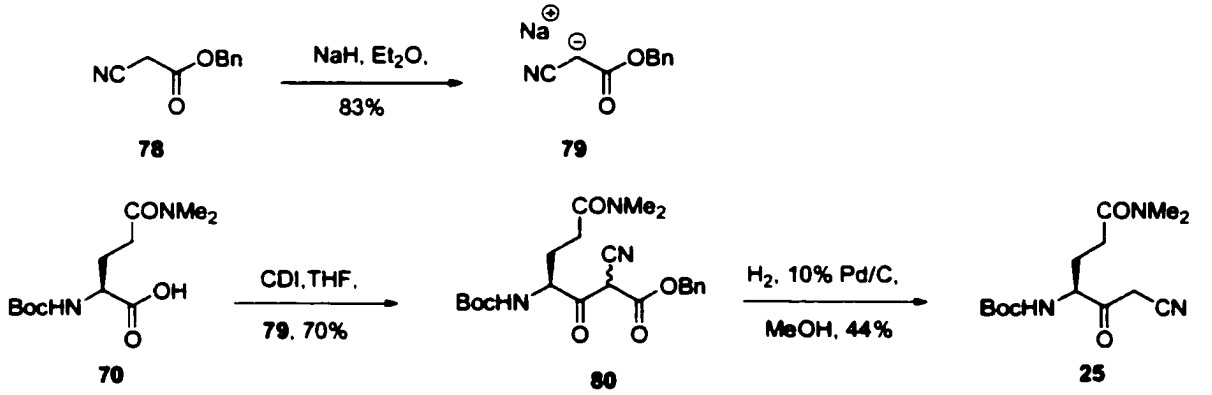


Inhibition assays of compounds **22-24** against HAV 3C proteinase employ an overexpressed C24S mutant in which the non-essential surface cysteine is replaced with serine, and which displays catalytic parameters indistinguishable from the wild type proteinase.[65] The enzyme activity is monitored using a fluorometric recovery assay at an enzyme concentration of approximately 0.1 μM using Dabcyl-GLRTQSFS-Edans (~10 μM) as the substrate.[66,67] The enzyme is incubated with the appropriate inhibitor for 15 min and the subsequent proteolytic reaction is initiated by the addition of the initially quenched substrate as described in the experimental section. Disappointingly, compounds **22** and **24** display no inhibition and **23** displays only poor inhibitory activity (20% diminuition of unreacted activity) at a concentration of 100 μM. This poor inhibitory activity contrasts sharply with the nanomolar inhibition achieved with the structurally

related 1,3-diamino ketone **66**[60] against the mammalian cysteine proteinase, cathepsin K. Although the exact reasons for the lack of HAV 3C proteinase inhibition are unknown, it is possible that the carbonyl moiety is not sufficiently electrophilic to react with the cysteine thiol of the enzyme.

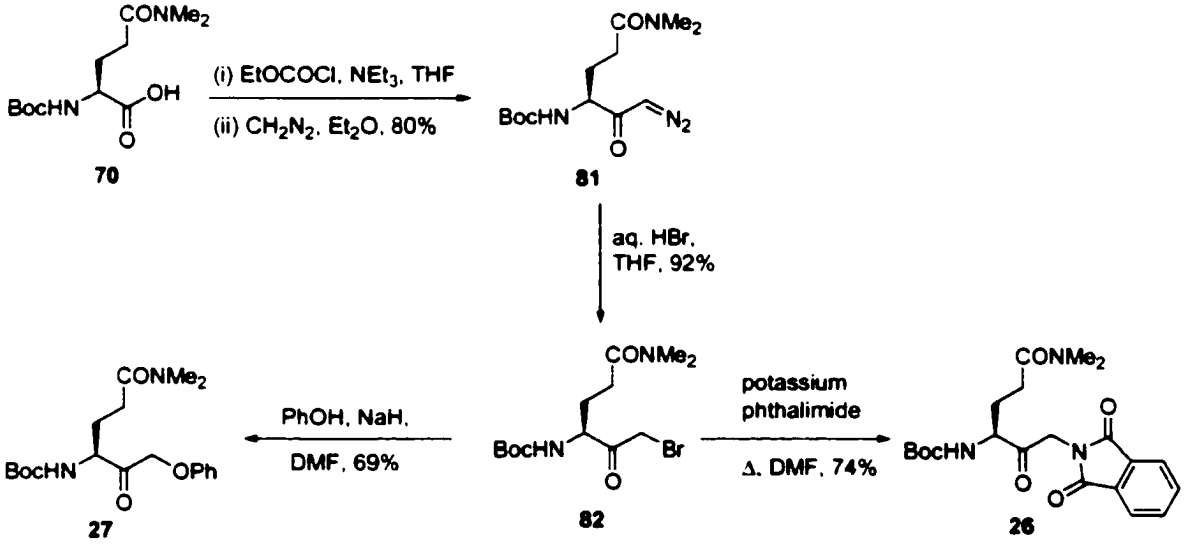
Since the more electrophilic Ac-Leu-Ala-Ala-(*N,N*-dimethyl)glutaminal **5** is a very potent slow-binding inhibitor of HAV 3C proteinase (K_i^* of 42 nM),[25] adjustment of the electrophilicity of the ketone carbonyl could potentially enhance inhibition of the target enzyme while providing metabolic stability. Thus, the α-cyano **25**, α-phthalimido **26** and α-phenoxy **27** derivatives (Figure 20) analogous to the cathepsin K inhibitor **67** were next synthesized and examined. The benzyl cyanoacetate monosodium salt **79** is prepared by the reaction of one equivalent sodium hydride with benzyl cyanoacetate **78** (Scheme 3). Synthesis of **25** starts from the previously prepared Boc-*N,N*-dimethyl glutamine **70** (cf. Scheme 3). Activation of the acid **70** with CDI followed by coupling with benzyl cyanoacetate monosodium salt **79** generates the keto-benzylester **80**. The benzyl group is hydrogenolyzed over palladium on carbon to give the corresponding acid which decarboxylates *in situ* to afford the α-cyano derivative **25.**

Scheme 3



Analogues **26** and **27** could be prepared as presented in Scheme 4. Following the literature procedure of Morris *et al.*,[27] acid **70** is converted to the α-diazoketone **81** by formation of the mixed anhydride with ethyl chloroformate followed by trapping with diazomethane. Treatment of **81** with aqueous HBr provides the bromomethyl ketone **82**, which is subsequently converted to the phthalimidomethyl ketone **26** or phenoxymethyl ketone **27** by nucleophilic displacement with potassium phthalimide or sodium phenoxide, respectively. However, testing with the HAV 3C enzyme indicates poor inhibition for **25** (13% inhibition), **27** (20% inhibition) at a concentration of 100 μM and **26** (IC_{50} > 500 μM).

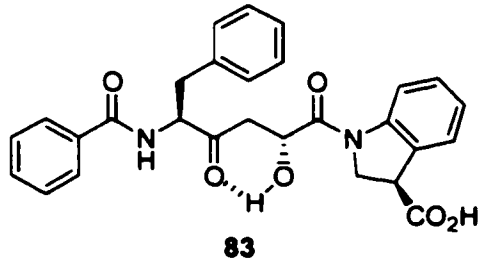
Scheme 4



Another approach for enhancement of the electrophilicity of the target carbonyl group in an inhibitor involves creation of a potential intramolecular hydrogen bonding arrangement to the carbonyl oxygen. An example of this chemical architecture is seen in

compound **28** (Scheme 5), which positions the phthalhydrazido N-H proton for such an intramolecular hydrogen bond forming in a six-membered chelate. In fact, activation of the amide scissile carbonyl by hydrogen bonding occurs in the enzyme active site during proteolytic cleavage of the substrate (Figure 8a). Another advantage of employing an intramolecular hydrogen bond is that it can further restrict the conformational freedom of the target molecule, thereby potentially reducing the entropic cost during high affinity binding to the target protein.[56] This concept was envisioned in the design of α-hydroxy ketomethylene dipeptide **83** (Figure 26), which is a potent inhibitor of the metalloproteinase (zinc-dependent) angiotensin converting enzyme (ACE) with an IC_{50} of 90 nM.[68] Although the α-hydroxyl group of **83** is recognized at the S_2' subsite of the enzyme, Kim *et al.*[68] suggest that it might also be hydrogen bonding with the ketone carbonyl moiety, thereby reducing the flexibility of the molecule.

Figure 26 α-Hydroxy ketomethylene inhibitor of angiotensin converting enzyme

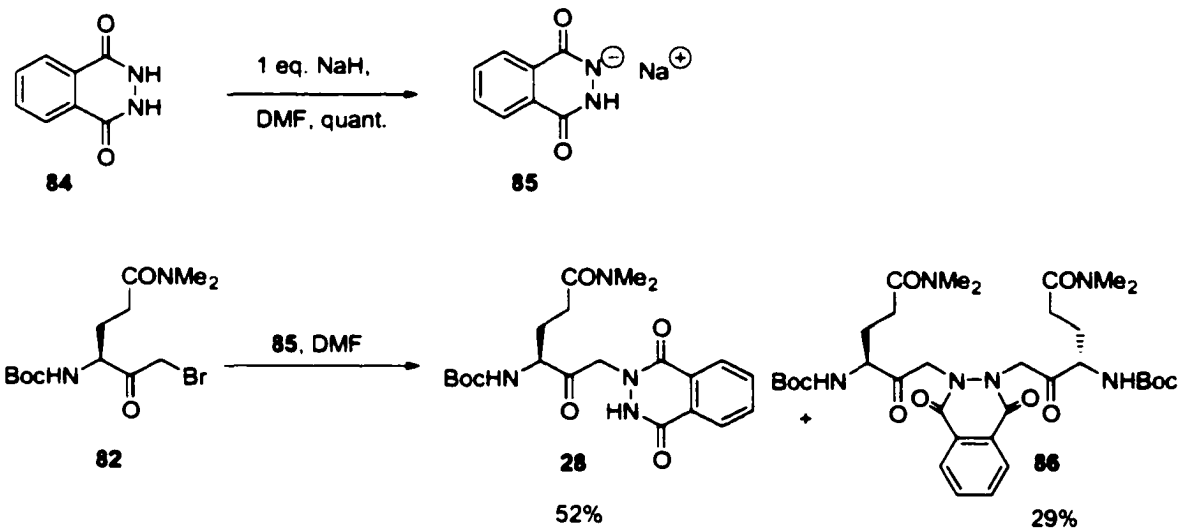


83

For preparation of **28** sodium phthalhydrazide **85** is generated by the reaction of 1 equivalent of sodium hydride with phthalhydrazide **84** (Scheme 5). Nucleophilic displacement of **85** on bromomethyl ketone **82** under standard conditions affords the

desired compound **28** as well as the dimer **86** in a ~1:2 ratio. This is presumably due to the low solubility of salt **85** in DMF or DMSO. Attempts to solubilize the salt by the use of crown ethers (e.g. 10-crown-5) were unsuccessful. However, slow portionwise addition of the salt **85** over 1 h to the bromomethyl ketone **82** gives **28** as the major product along with the dimer **86** in ~2:1 ratio (Scheme 5).

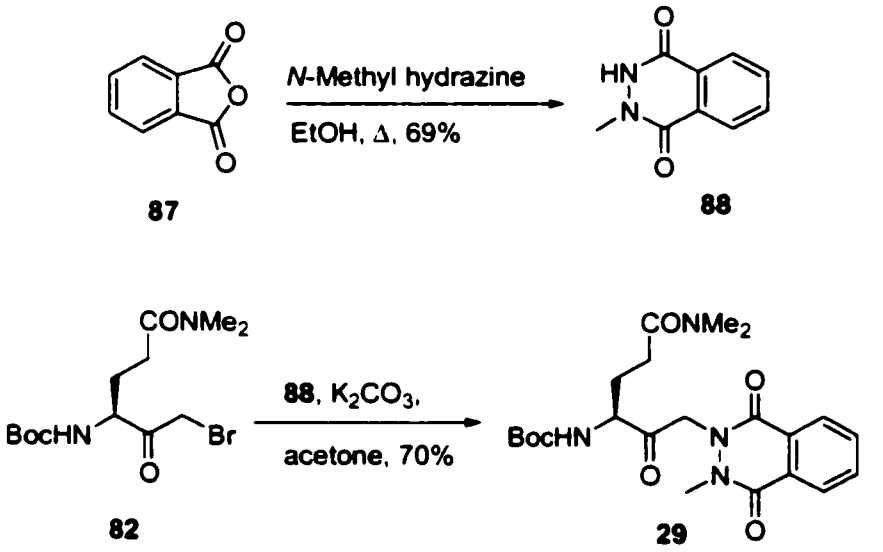
Scheme 5



Compound **28** exhibits reasonable reversible inhibition against HAV 3C proteinase and gives good reversible inhibition with an IC_{50} of 89 μM. This contrasts sharply with the poor inhibition shown by **26** (IC_{50} > 500 μM), which has related functionality but lacks the analogous internal hydrogen bonding arrangement. To examine the effect of the intramolecular hydrogen bond on the observed inhibitory activity, compound **29** was prepared wherein the N-H proton is replaced by a methyl moiety. *N*-Methyl phthalhydrazide **88** is generated by the reaction of *N*-methyl hydrazine

with phthalic anhydride **87** (Scheme 6).[69] Using a modified procedure described previously, this hydrazide is treated with potassium carbonate in acetone followed by addition of the bromoketone **82** to afford the desired compound **29**. As anticipated, testing of **29** against HAV 3C proteinase is weakly inhibitory with 21% of the untreated proteianse activity at a concentration of 100 μM.

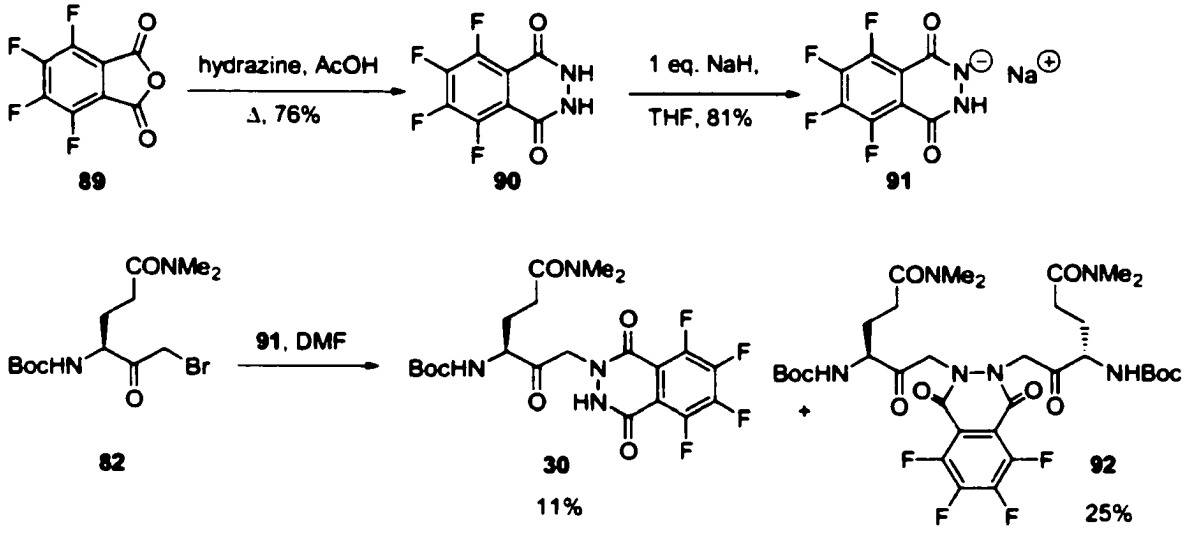
Scheme 6



In light of these results, analogues containing electron withdrawing groups on the phenyl ring should hypothetically increase the acidity of the N-H proton, and thereby enhance the electrophilicity of the ketone moiety.[70] Towards this end, tetrafluorophthalhydrazide **30** and nitrophthalhydrazide **31** (Figure 20) were selected as synthetic targets. Reaction of tetrafluorophthalic anhydride **89** with hydrazine in ethanol as described for **88** (Scheme 6) proved problematic due to ring opening of the anhydride

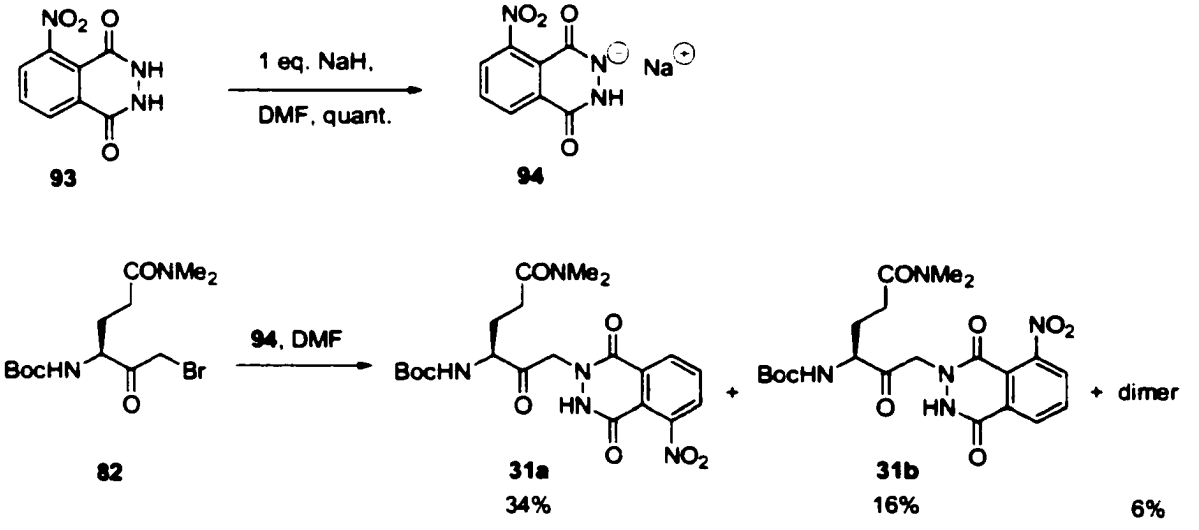
by ethanol. However, analogous reaction in acetic acid as the solvent affords tetrafluorophthalhydrazide **90** in 76% yield.[71] Moreover, reaction of **90** with potassium carbonate in acetone followed by addition of the bromoketone **82**, as described for compound **29** (Scheme 6), gives exclusively the dimer **92**, possibly due to the double deprotonation of the tetrafluorophthalhydrazide **90** to form a dianion. To circumvent this problem, the monosodium salt **91** was prepared by reaction of tetrafluorophthalhydrazide **90** with 1 equivalent of sodium hydride. Displacement of the bromide of **82** by the sodium salt **91** affords the desired compound **30**, albeit as the minor product in addition to the dimer **92**. However, enzymatic testing of **30** against HAV 3C proteinase gives only a slight increase in inhibitory activity with an IC_{50} of 77 μM when compared to **28** (IC_{50} of 89 μM).

Scheme 7



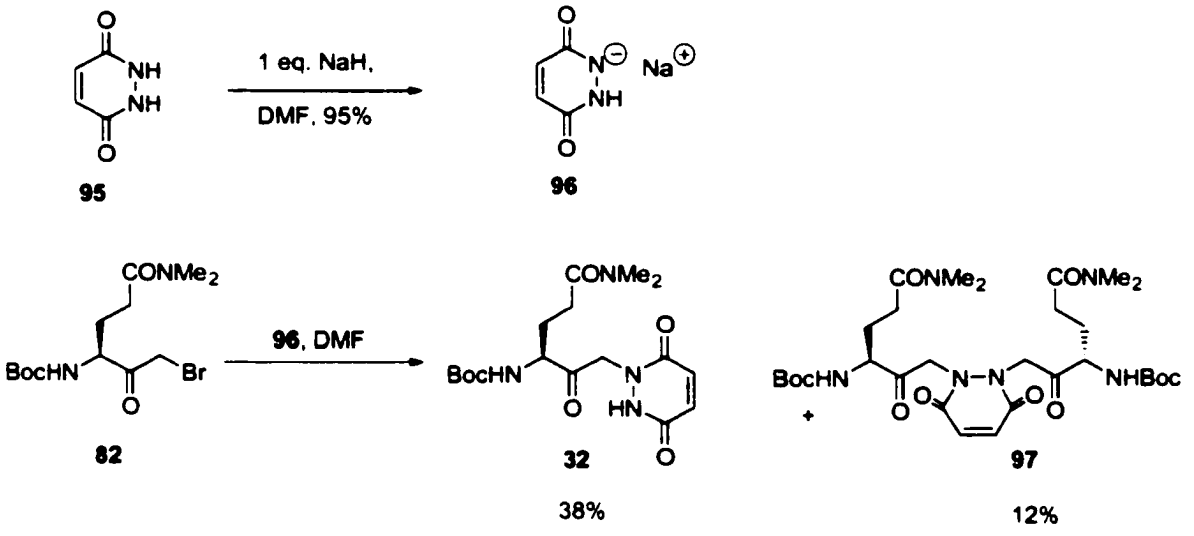
The nitrophthalhydrazido derivative **31** is prepared as delineated in Scheme 8. Reaction of the commercially available 3-nitrophthalhydrazide **93** with 1 equivalent of sodium hydride affords the sodium 3-nitrophthalhydrazide salt **94** (Scheme 8). Nucleophilic displacement by **94** on the bromomethyl ketone **82** gives the isomeric compounds **31a** and **31b** as the major products. These compounds are separable by flash column chromatography and could be characterized using HMQC and HMBC NMR spectroscopy. However, incubation of **31a** and **31b** with the HAV 3C proteinase does not show improved inhibition and gives IC_{50} values of 146 and 164 μM, respectively. One possible reason for this poor inhibitory activity may be due to the nitro group having unfavorable steric or electronic interactions in the region of the enzyme active site that accommodates small amino acids (Gly, Ala, Val, or Ser) at the S_1' pocket.

Scheme 8



To provide greater structural diversity and to examine the phthalhydrazido motif further, the pyridazinedione **32** and dimethyl hydrazodicarboxylate derivatives **33** were constructed as both maintain the hydrazido N-H for intramolecular hydrogen bond formation, but each lacks the phenyl ring system of **28**, **30** and **31**. Thus, reaction of the pyridazinedione **95** with 1 equivalent of sodium hydride yields the sodium pyridazinedione salt **96** (Scheme 9). Treatment of the salt **96** with bromomethyl ketone **82** gives the desired compound **32** and the dimer **97**.

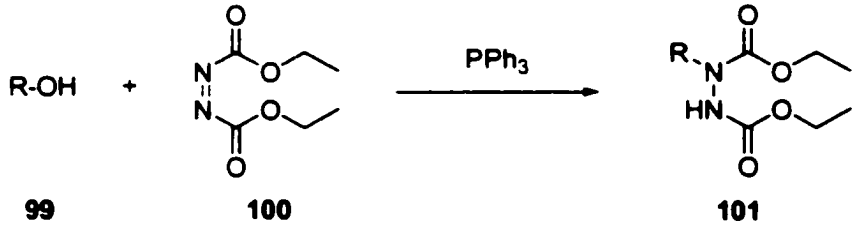
Scheme 9



Attempts to prepare the dimethyl hydrazodicarboxylate derivative **33** by reaction of sodium dimethyl hydrazodicarboxylate salt with the bromoketone **82** as described previously were unsuccessful and the bromoketone **82** was recovered. Therefore, another route which involves a Mitsunobu-type protocol was explored. Dow and coworkers[72] reported that when a primary or secondary alcohol **99** (Figure 27) is treated with triphenyl

phosphine and diethyl azodicarboxylate (DEAD) **100**, in the absence of a nucleophile[73], the activated hydroxyl group is displaced and adduct **101** is obtained.[72]

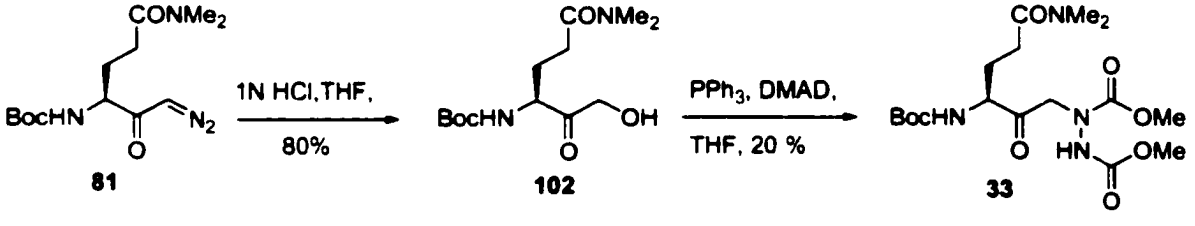
Figure 27 Mitsunobu-type reaction where the nucleophile is excluded



99 **100** **101**

Precursor **102** for the modified Mitsunobu procedure can be accessed by conversion of diazoketone **81** to the hydroxymethyl ketone **102** by treatment with 1 N HCl (Scheme 10).[74] This is then coupled to the dimethyl hydrazodicarboxylate (DMAD) *via* the Mitsunobu-type[72] reaction to give **33** in low yield (20%).

Scheme 10



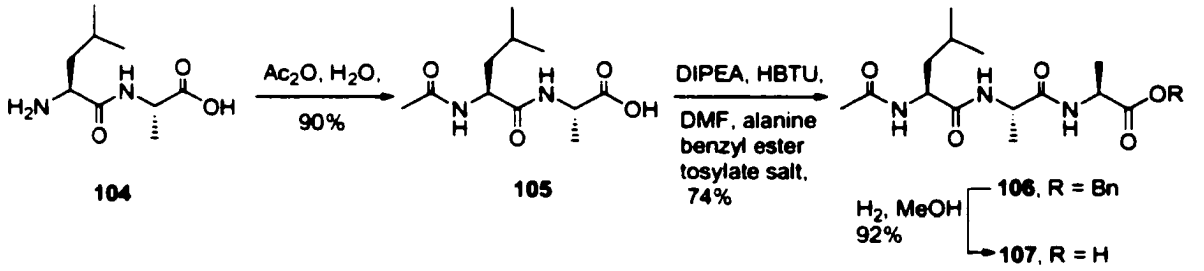
81 **102** **33**

Although the compounds **32** and **33** have the required N-H functionality predisposed to form an intramolecular hydrogen bond with the ketone moiety, assay of

these compounds against HAV 3C proteinase yields gives poor inhibitory activity of 20% and 15% respectively, at a concentration of 100 μM. Thus, removal of the aromatic ring coupled with increased flexibility and alteration of the preferred conformation of the hydrazido functionality[75] drastically reduces binding to the enzyme. In addition, the phenyl ring in **28**, **30**, and **31** may result in unfavorable steric clashes at the S_1' of the enzyme since this site accommodates small amino acid side chains (Gly, Ala, Val or Ser).[15,45]

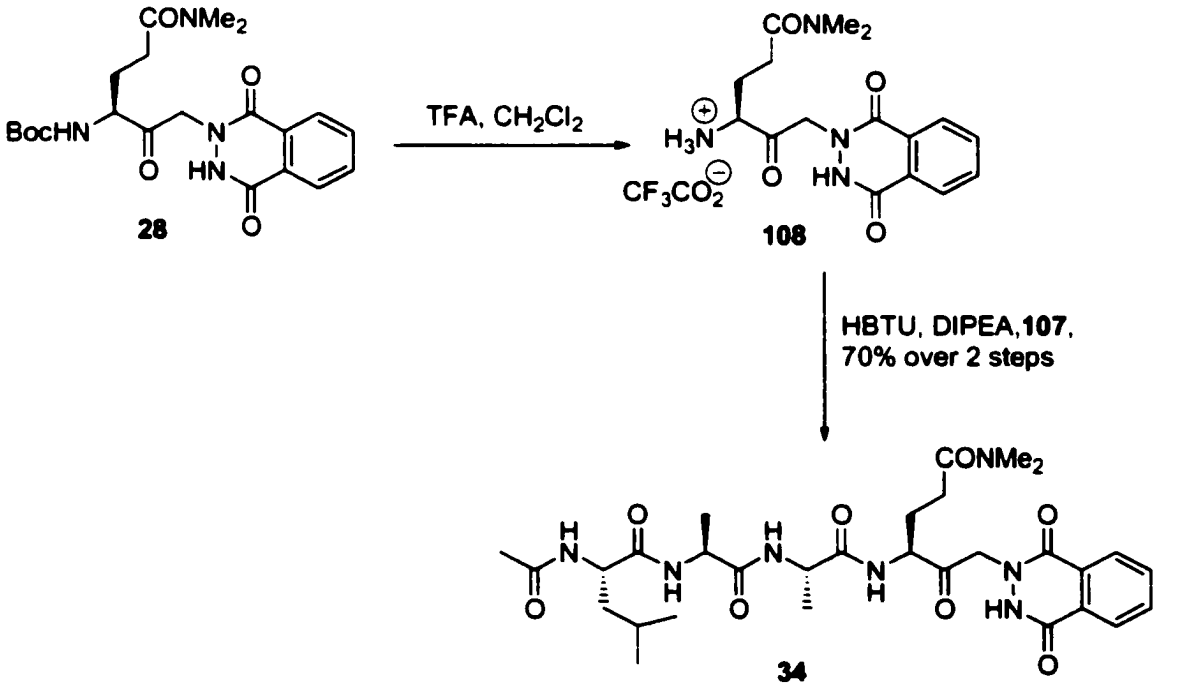
Another approach for enhancing inhibitory activity of compound **28** is to incorporate additional HAV 3C proteinase preferred residues into the target molecules. Previous work in our group by Dr. C. Lowe, has shown that *N*-acetyl-(*N,N*-dimethyl)glutaminal has a K_i of 2.5 mM against the HAV 3C proteinase, but attachment of the tripeptide (*N*-Ac-Leu-Ala-Ala) to the N-terminus generates inhibitor **5** that displays almost 9000 times more inhibitory activity than the simple *N*-acetyl derivative.[25] Hence, the next approach involved coupling of inhibitor **28** to the Ac-Leu-Ala-Ala-OH (**107**) to generate the tetrapeptide analogue **34**. The tripeptide **107** was prepared as depicted in Scheme 11. Reaction of the commercially available dipeptide Leu-Ala-OH (**104**) with acetic anhydride proceeds smoothly to afford Ac-Leu-Ala-OH (**105**). Coupling of **105** with alanine benzyl ester in the presence of DIPEA and HBTU as the coupling agent gives the benzyl tripeptide **106**.[76] The benzyl group of **106** is then hydrogenolyzed over palladium on carbon to yield the desired tripeptide **107**.

Scheme 11



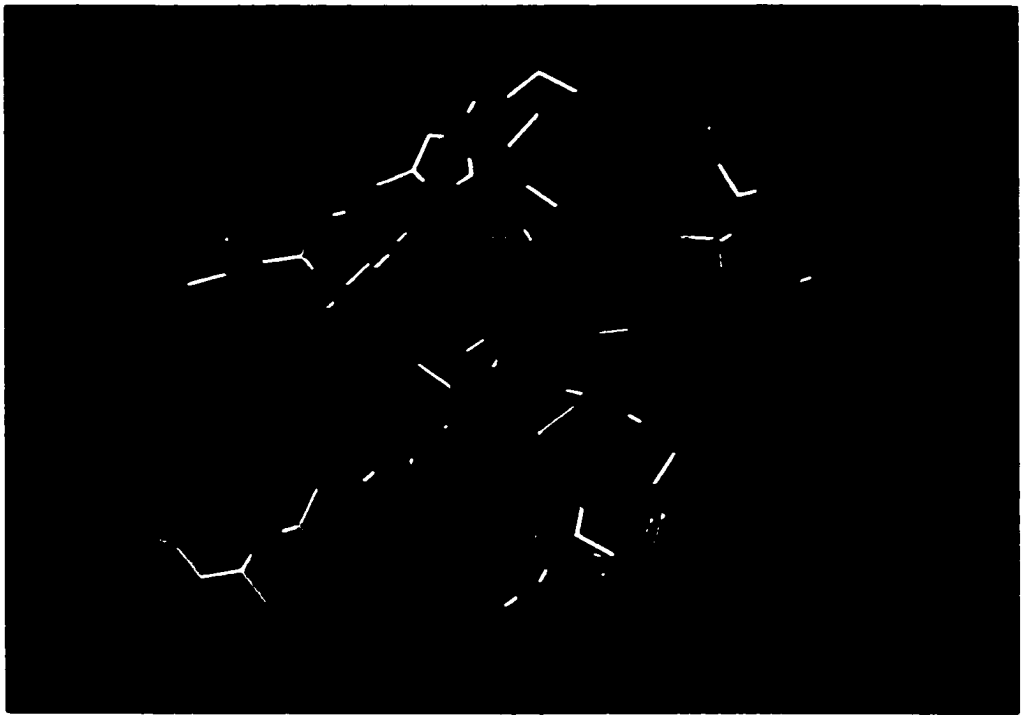
With the tripeptide **107** available, the next step is to remove the Boc group of **28**. This is accomplished by treatment of **28** with trifluoroacetic acid (Scheme 12) to afford the trifluoroacetate salt **108**. Coupling of **108** with Ac-Leu-Ala-Ala-OH (**107**) using HBTU and DIPEA proceeds readily to yield the desired tetrapeptide **34**.

Scheme 12



The HAV 3C proteinase assays indicate that the tetrapeptide **34** acts a competitive and reversible inhibitor of the proteinase with an IC_{50} of 13 μM and a K_i of 9 μM.[77] Furthermore, the inhibitory activity of **34** is not altered in the presence of a 100-fold molar excess of dithiothreitol (DTT), indicating that this type of keto-phthalhydrazido system may be a highly specific physiological inhibitor of the HAV 3C proteinase since **34** does not react inadvertently with reactive thiols such as DTT or ubiquitous thiols found in cells. Although the peptide residues in **34** do enhance HAV 3C proteinase inhibition nearly 7-fold to **28**, the effect is not nearly so dramatic as with the *N*-acetyl-(*N,N*-dimethyl)glutaminal. Thus, **28** represents an interesting lead structure that could be further modified to generate drug candidates for picornaviral cysteine proteinases. Modeling of the tetrapeptide **34** in the active site of HAV 3C proteinase by Dr. Jonathan C. Parrish[78,79] (Figure 28) shows that the phenyl ring of the phthalhydrazido group is pointing towards the S_1' pocket (Asn 148, Met 29, Val 28) of the enzyme. This pocket normally accommodates small amino acids (Gly, Ala, Val or Ser).[15,45] As seen with the previous compounds, in addition to the intramolecular hydrogen bonding needed to activate the ketone carbonyl, a phenyl ring system such as the phthalhydrazido group is also a determinant factor for successful inhibition.

Figure 28 Modeling of tetrapeptide **34** (green) in the active site of HAV 3C proteinase (courtesy of Dr. Jonathan C. Parrish)

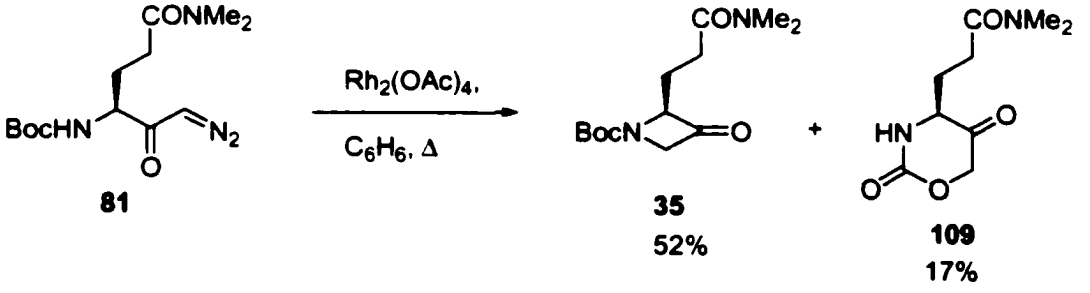


1.1 3-Azetidinone Analogues - Target B

In the previous section we have seen that both the presence of appropriate residues for recognition and the reactivity of the "warhead" are important factors for successful inhibition of HAV 3C proteinase. Another approach for enhancing the electrophilicity and reactivity of α-amino ketones is to introduce a rigid 3-azetidinone scaffold at the P_1 site (for example, **35**). Amino acid derivatives containing 3-azetidinone units have been reported previously, however, there have been no reports published when using such motifs in enzyme studies.[80,81] Therefore, the *N,N*-dimethyl glutamine with a 3-azetidinone scaffold **35** is an interesting new scaffold to explore for HAV 3C proteinase inhibitors.

The synthesis of compound **35** is shown in Scheme 13. Reaction of the previously prepared α-diazoketone **81** with a catalytic amount of rhodium (II) acetate dimer in refluxing benzene[82,83] generates a carbenoid which inserts intramolecularly into the N-H bond to afford the desired compound **35** along with the six membered ring urethane **109** in a ratio of ~3:1. Urethane **110** presumably forms by addition of the carbonyl oxygen atom of the Boc protecting group to the carbene followed by loss of the *t*-butyl group. Both compounds **35** and **109** were tested against the HAV 3C enzyme and disappointingly only **35** gave moderate inhibition with an IC_{50} of 358 μM, while the urethane **109** displayed 20% inhibition at a concentration of 100 μM.

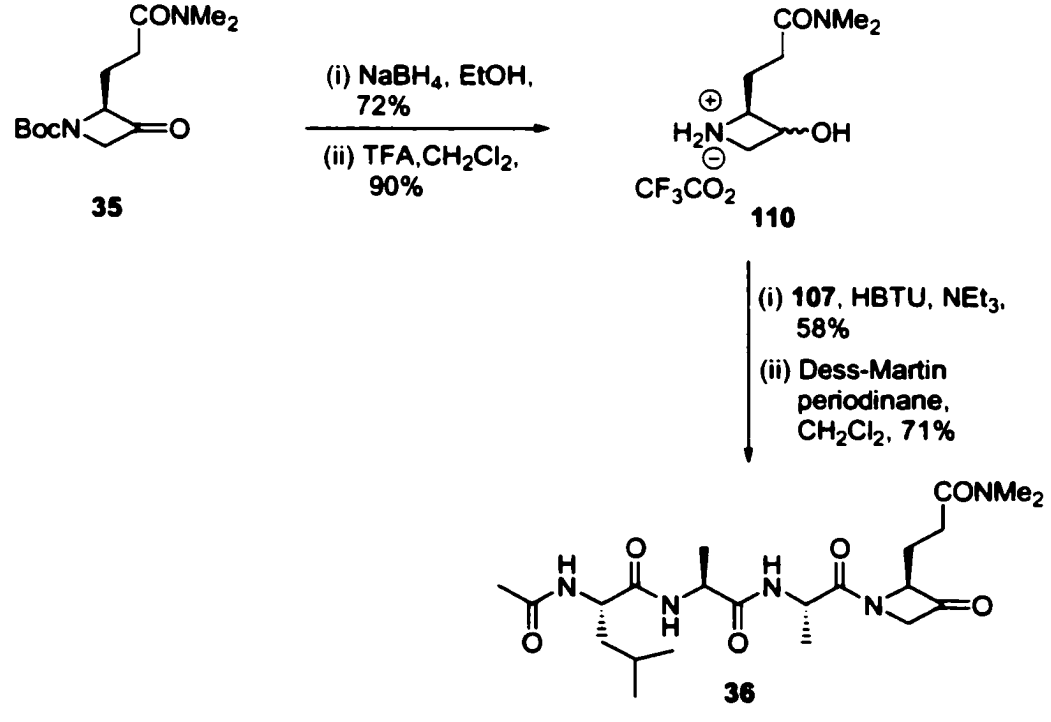
Scheme 13



Compound **35** is a less effective inhibitor compared to the hydrogen-bonded phthalhydrazide **28**. To examine the influence of additional peptidic residues with this four-membered ring system, the tripeptide Ac-Leu-Ala-Ala was attached to **35**. To prevent unwanted side reactions, the ketone functionality of **35** is reduced prior to coupling. Thus reduction of **35** with sodium borohydride followed by Boc deprotection with trifluoroacetic acid affords the amino alcohol **110** (Scheme 14). This is subsequently

coupled to Ac-Leu-Ala-Ala-OH (**107**) in the presence of triethylamine and HBTU as the coupling agent. The hydroxyl group is then oxidized with Dess-Martin periodinane[64] to afford the target **36** in 71% yield. Incubation of **36** with the HAV 3C proteinase displays only a slight enhancement of reversible inhibition (IC_{50} = 258 μM) relative to **35** despite the presence of the P_4-P_1 peptide backbone. Although the reversible binding of the 3-azetidinones to the target enzyme is significantly greater than that of an optimized octapeptide substrate (K_m of 2.1 mM),[23] this cyclic ketone motif appears to be less effective for HAV 3C proteinase inhibitor development than the phthalhydrazido system. Nevertheless, it may prove promising for other types of cysteine proteinases of therapeutic interest.

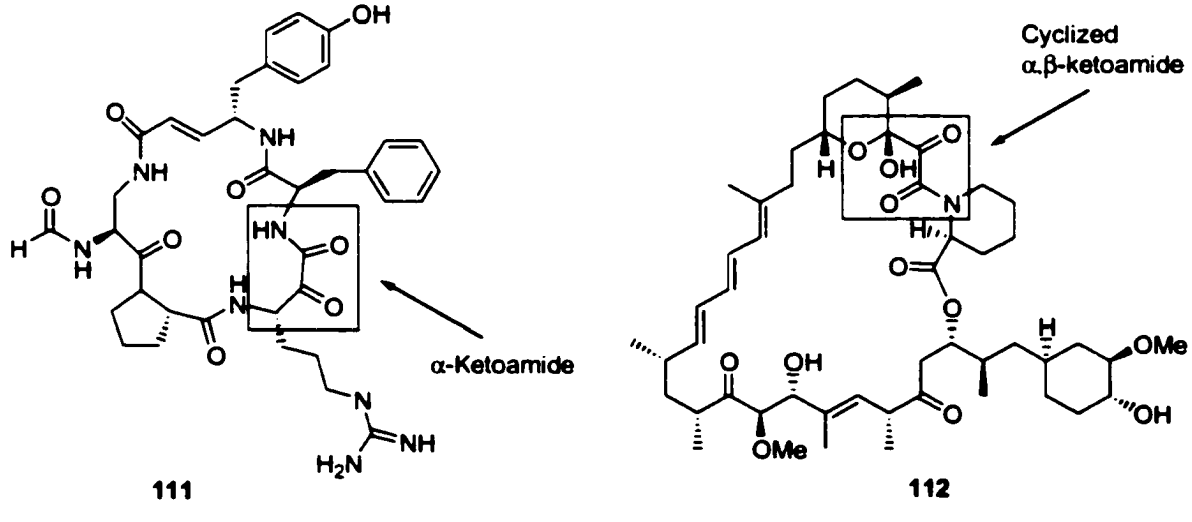
Scheme 14



1.2 α-Ketoamides and α,β-Ketoamides - Target C

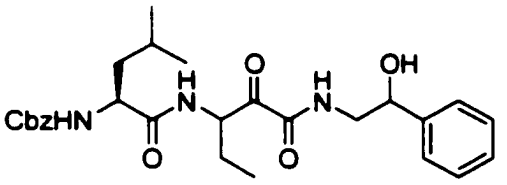
α-Ketoamides and α,β-ketoamides occur naturally in a variety of cyclic peptides and many possess important biological activities.[84] For example, cyclotheonamide A (Figure 29) **111** which incorporates an α-ketoamide functionality is a potent inhibitor of the serine proteinase thrombin with a K_i of 1.0 nM and in a physiological settings also inhibits the aggregation of human platelets.[85] The tricarbonyl system of α,β-ketoamide is present in the immunosuppressant agent rapamycin **112** which is a potent inhibitor of serine/threonine kinases.[86] The activity of these inhibitors has been attributed to the presence of the electron deficient α-keto carbonyl group. NMR studies have indicated that α-ketoamides are readily hydrated in the presence of water, suggesting that inhibitors containing these functionalities either form tetrahedral covalent adducts with the active site nucleophile or exist as *gem*-diols that mimic the tetrahedral intermediate (Figure 8) during proteolytic cleavage of amide bonds.[87]

Figure 29 Cyclotheonamide A **111** and rapamycin **112**



Peptidyl α-ketoamide inhibitors of the cysteine proteinase calpains and cathepsins have also been reported.[54,55] For example the α-ketoamide **113** (Figure 30) is a potent inhibitor of calpain II with a K_i of 15 nM. These aforementioned studies demonstrate that the α-ketoamide functionality potentially inhibits thiol containing enzymes.

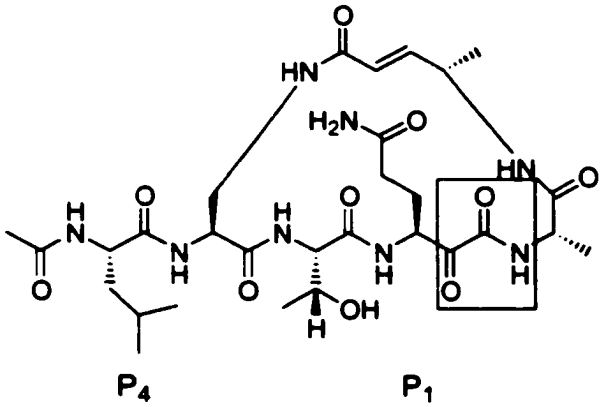
Figure 30 α-Ketoamide inhibitor of calpain



113

Modeling studies[78] suggest that the α-ketoamide cyclic peptide **114** (Figure 31), analogous to cyclotheonamide A **111**, would bind favorably in the active site of HAV 3C proteinase. This macrocyclic peptide contains the essential glutamine at P_1 position and a leucine residue at P_4 position, both of which contribute to peptide recognition.

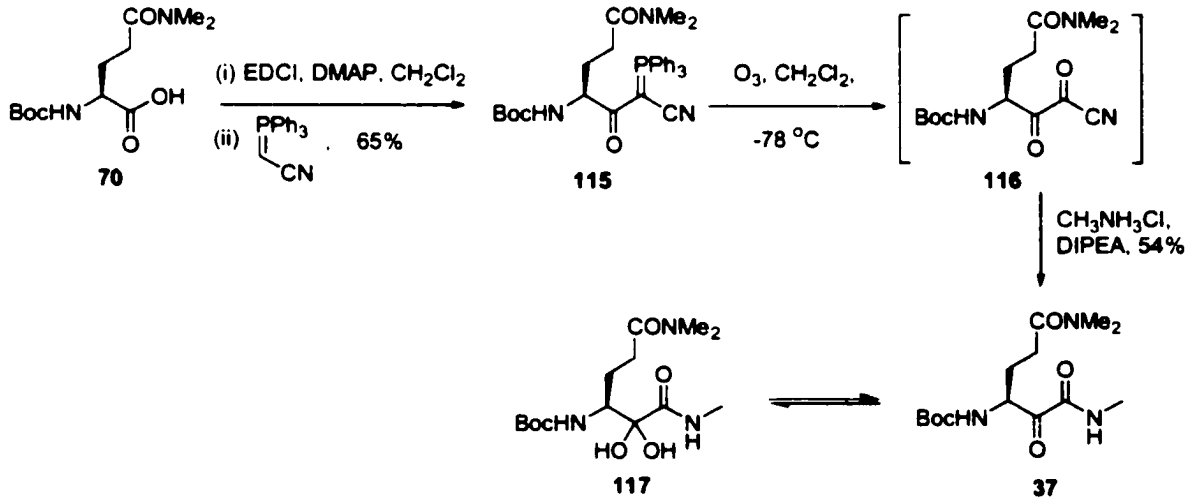
Figure 31 α-Ketoamide cyclic peptide as potential inhibitor of HAV 3C proteinase



114

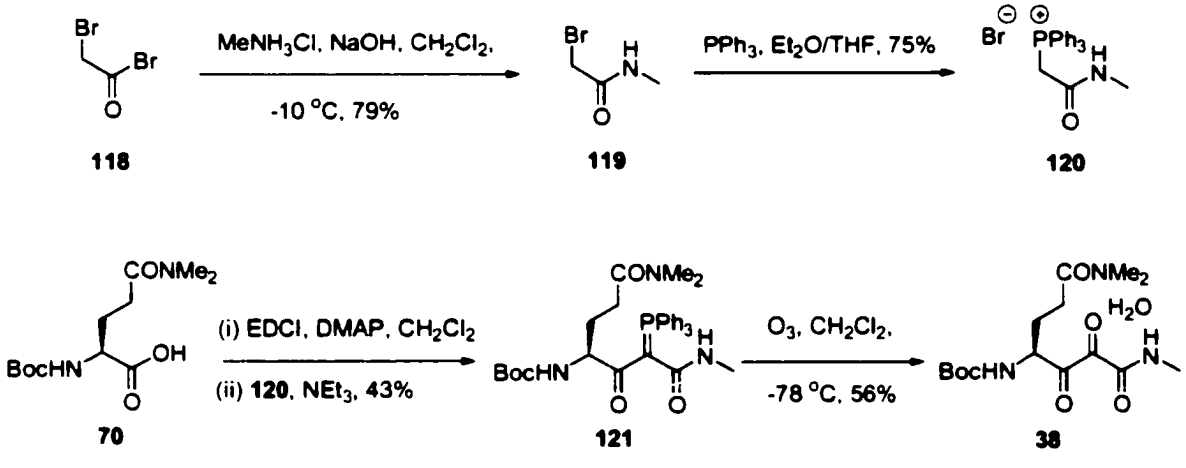
However, before embarking on a lengthy synthesis of molecule **114**, the α-ketoamide model compound **37** and the α,β-ketoamide **38** were prepared to evaluate their potential as "warheads" for HAV 3C proteinase. The synthesis involves the chemistry developed by Wasserman[88] and is described in Scheme 15. The key step is the formation of the cyanomethylene phosphorane ylide **115**, which upon ozonolysis generates an acyl cyanide, which could subsequently be reacted with a variety of nucleophiles. The previously prepared Boc-*N,N*-dimethyl glutamine (**70**) was activated with EDCI and then reacted with cyanomethylene phosphorane to afford the adduct **115**. Cleavage of the triphenylphosphine moiety by ozonolysis proceeds *via* the acyl cyanide intermediate **116** which is trapped by methyl amine to furnish the desired α-ketoamide **37**. This compound was found to be in equilibrium with a 1:1 mixture of its hydrate **117** by ^{1}H NMR.

Scheme 15



The synthesis of α,β-ketoamide **38** is depicted in Scheme 16. Treatment of bromoacetyl bromide (**118**) with methylamine affords the *N*-methyl α-bromoacetamide (**119**).[89] This, upon reaction with triphenyl phosphine yields the triphenyl phosphonium salt **120**. Activation of Boc-*N*,*N*-dimethyl glutamine (**70**) with EDCI followed by reaction with the triphenyl phosphonium salt **120** in the presence of triethylamine generates the adduct **121** which contains the highly electrophilic internal carbonyl of the α,β-ketoamide system masked as the phosphorus double bond.[90] Oxidative cleavage of the carbon-phosphorus double bond by ozonolysis gives the desired α,β-ketoamide **38** which is isolated as its monohydrate.

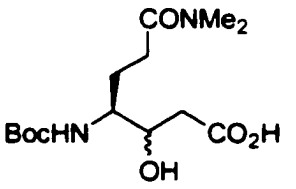
Scheme 16



Compounds **37** and **38** were incubated with HAV 3C proteinase. Disappointingly, **37** gave no inhibition and **38** displayed only a weak 15% inhibition at a concentration of 100 μM. Presumably the hydrated nature of the carbonyl moiety which exists as a *gem*-

diol attenuates the "warhead" reactivity towards nucleophilic attack by the enzyme thiolate. It would be reasonable to propose that a hydroxyl group at the scissile amide bond would be a suitable mimic of the tetrahedral intermediate formed during proteolytic cleavage (Figure 8), and moreover, such a hydoxyl bearing compound should effectively bind to the enzyme active site (for example, inhibitors **1** and **2**). However, previous studies carried out by Dr. Manjinder S. Lall[33] in our group have shown that transition state mimics such as **122** (Figure 32) are poor inhibitors of HAV 3C proteinase. This may explain why the α- and α,β-ketoamides are not effective "warhead" motifs for HAV 3C proteinase inhibition.

Figure 32 β-Hydroxy acid tested as a transition state mimic for HAV 3C proteinase



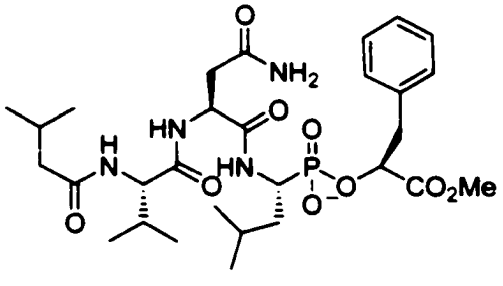
122

2.0 Macrocyclic Peptide with a Disulfide Linker - Target D

Conformationally restricted peptides have assumed a prominent role in drug design and development.[57] Because of the inherent flexibility of linear peptide molecules, the desired biologically active conformation(s) constitute a small proportion of the energetically accessible conformations within a population of thousands of inactive conformers. In order to energetically optimize the kenetic and thermodynamic interaction between the peptide ligand and the receptor, it is desirable to maintain the active site

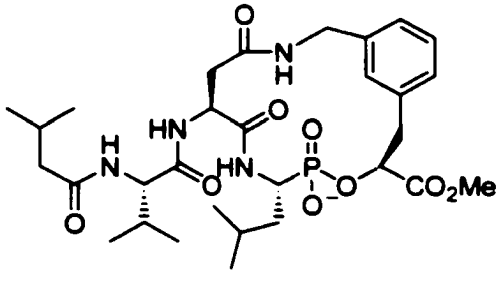
conformation of the peptide through rigidification.[57] A common approach for restricting the number of peptide conformers involves the introduction of cyclic substructure into the overall peptide framework leading to restrictions in the conformational space sampled by the rigidified molecule. Examples of this approach include the formation of disulfide bridges between cysteine residues,[91] lactam bridges between glutamic/aspartic acid and lysine residues,[92] ethylene linkers between allyl groups,[93,94] and other types of chemical cross links.[56] Recent studies have shown that cyclic peptide phosphonate analogues with restricted degrees of freedom are significantly better binders to the aspartyl proteinase, penicillopepsin, than acyclic counterparts (Figure 33).[56c] Hence, we decided to apply a similar strategy for the inhibition of HAV 3C proteinase through connection of the P_2 and P_1' residues' side chains.

Figure 33 Acylic (left) and cyclic inhibitor phosphonate inhibitor (right) of penicillopepsin



K_i = 42 nM

Acylic phosphonate inhibitor

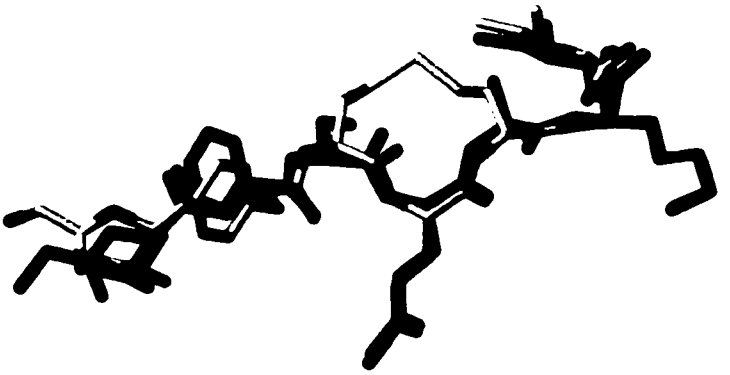


K_i = 0.1 nM

Cyclic phosphonate inhibitor

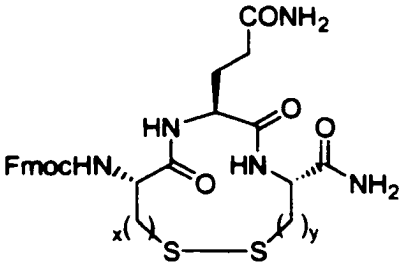
Modeling studies[78,79] carried out by Dr. Jonathan C. Parrish[78,79] using the structure of the substrate and macrocyclic peptide suitably oriented in the HAV 3C proteinase active site (Figure 34) indicate that systems bearing a homocysteine residue at the P_1' position and a cysteine at the P_2 position show good overlap with the native substrate's residues. Hence, such a constrained macrocyclic peptide could bind to the active site of the HAV 3C proteinase with minimal steric clashes and therefore exhibit improved inhibitory properties.

Figure 34 Overlay of macrocyclic peptide (grey) and substrate (green) bound to the HAV 3C proteinase active site (courtesy of Dr. Jonathan C. Parrish)



All three combinations of homocysteine and cysteine residues at the P_1' and P_2 positions, namely **123**, **124**, and **125** (Figure 35) were chosen as the initial synthetic targets to evaluate their binding in the HAV 3C proteinase active site. These compounds were prepared by a previous postdoctoral fellow in our group, Dr. David Brown.[95] To access these analogues by solid phase peptide chemistry, an efficient route to *N*-Fmoc *S*-Acm homocysteine was required.

Figure 35 Initial macrocyclic targets for HAV 3C proteinase



123 x = 1, y = 2

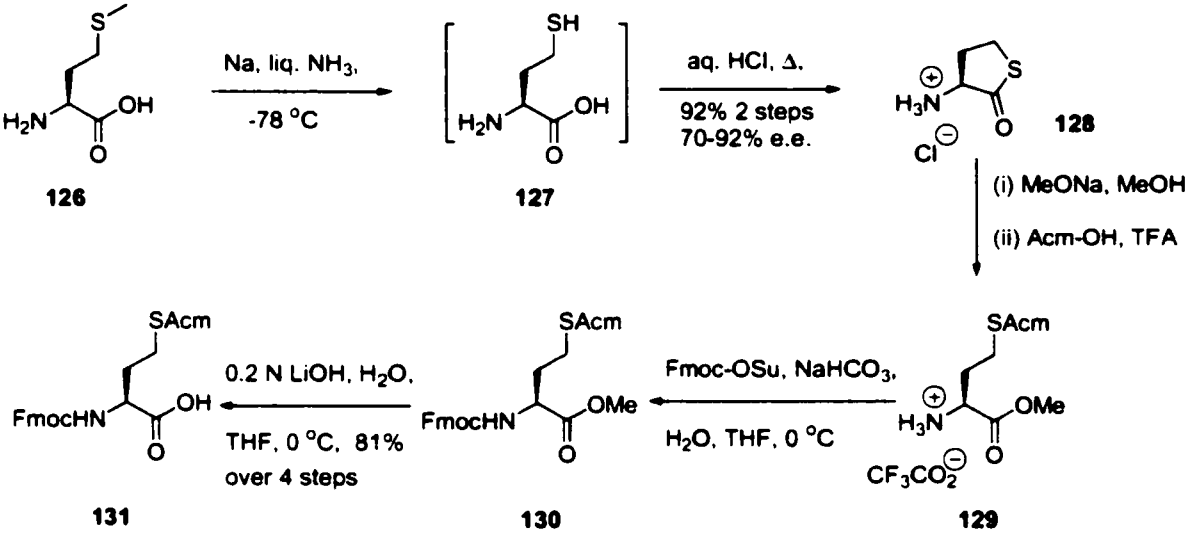
124 x = 2, y = 1

125 x = 2, y = 2

Although a literature procedure is known for the synthesis of *N*-Fmoc *S*-Acm homocysteine[96] (**131**) a new alternative approach was investigated which involves the formation of a γ-thiolactone **128** (Scheme 17). With this γ-thiolactone **128** available, a variety of N-protected homocysteine analogues could be accessed. The synthesis was done by Dr. David Brown as depicted in Scheme 17. Demethylation of L-methionine (**126**) with sodium in liquid ammonia gives the homocysteine intermediate **127**, which upon heating to reflux in aqueous HCl generates the thiolactone **128**. However, the thiolactone **128** was not enantiomerically pure (70-92% e.e., determined by ^{1}H NMR) and attempts to optimize the procedure by using different temperatures and concentrations of HCl were unsuccessful. This is presumably due to the acidic α-proton of **128** which is susceptible to epimerization. The next step involves ring opening of the thiolactone **128** with sodium methoxide followed by protection of the free thiol with an acetamidomethyl (Acm) group to furnish the homocysteine methyl ester **129**. The amino group of this compound was then protected with Fmoc by treatment with Fmoc-

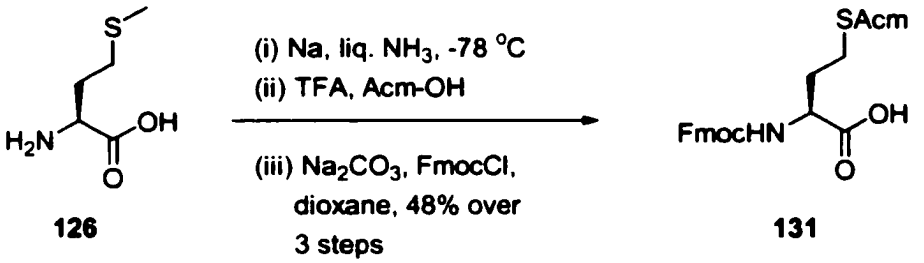
succinimide to yield compound **130**. Finally the methyl ester was hydrolyzed with lithium hydroxide to afford the desired *N*-Fmoc, *S*-Acm homocysteine (**131**).

Scheme 17



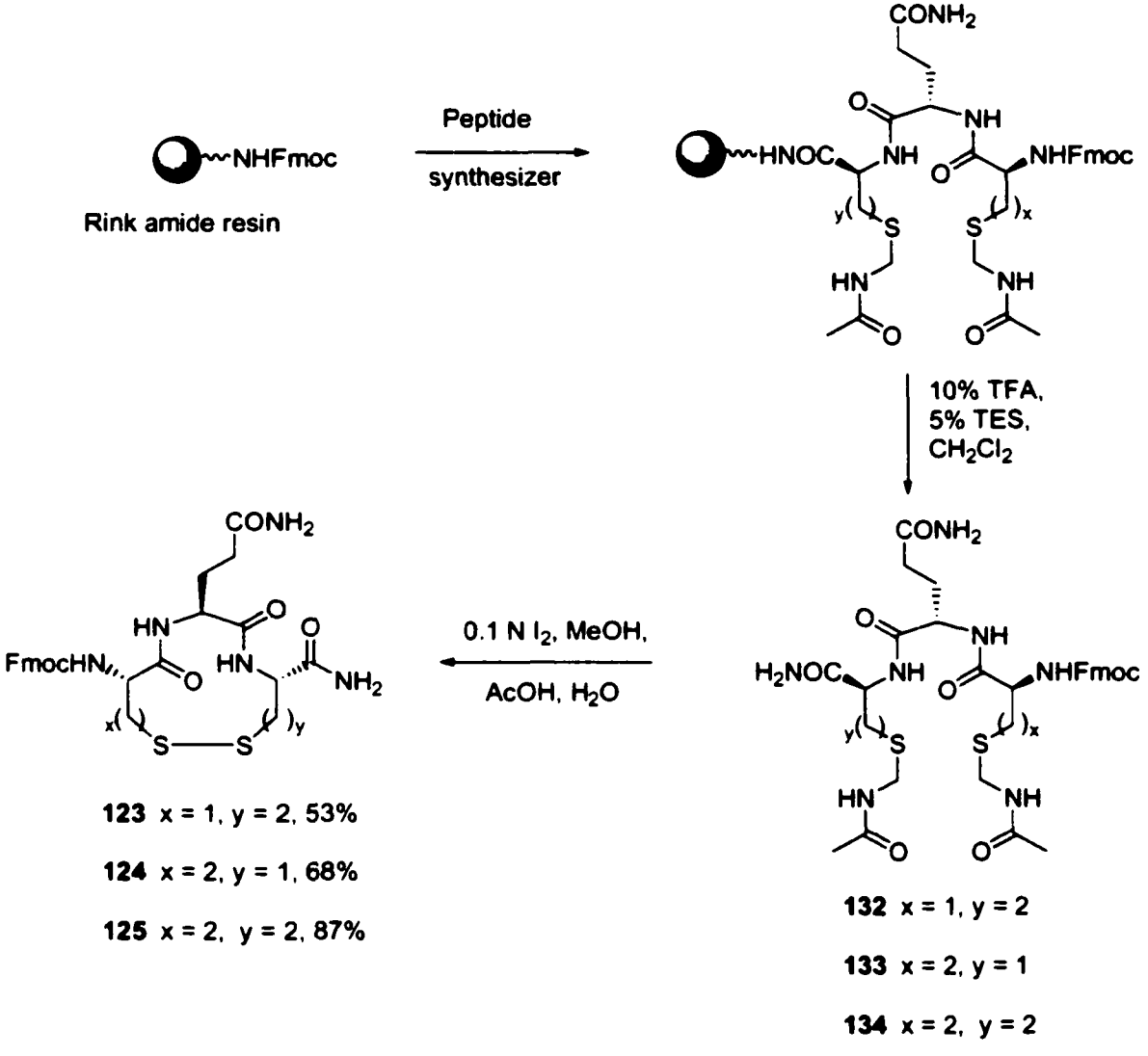
However, because compound **131** was not enantiomerically pure, we decided to prepare this compound following the known literature[96] procedure (Scheme 18). Thus, L-methionine is demethylated with sodium in liquid ammonia. Protection of the thiol by treatment with Acm-OH in trifluoroacetic acid followed by protection of the amino group with Fmoc-Cl gives the *N*-Fmoc *S*-Acm homocysteine (**131**) in enantiomerically pure form.

Scheme 18



The tripeptides **132-134** were individually prepared by solid phase peptide synthesis using standard Fmoc[97] chemistry on Rink amide resin[98] using HBTU[99] as the coupling reagent (Scheme 19). Cleavage of each of the linear peptides **132-134** from the resin is accomplished by using a solution of 10% TFA and 5% triethylsilane in dichloromethane. These peptides are then treated with a solution of 0.1 N iodine solution to effect removal of the Acm group followed by *in situ* cyclization to afford the desired cyclic peptides **123-125**, respectively. These cyclic peptides **123-125** proved to be quite insoluble in most common solvents, but were soluble in DMSO with prolonged warming and sonication. Enzyme assays against HAV 3C proteinase display only modest inhibition for **124** and **125**, with 51% and 55% inhibitory activity at concentrations of 100 μM for each compound. However, macrocyclic peptide **123**, which may adopt a similar conformation as the substrate, exhibits no inhibition of HAV 3C proteinase at the same concentration. This may indicate that **123** serves as a substrate of the enzyme, but because of solubility problems this was not investigated further.

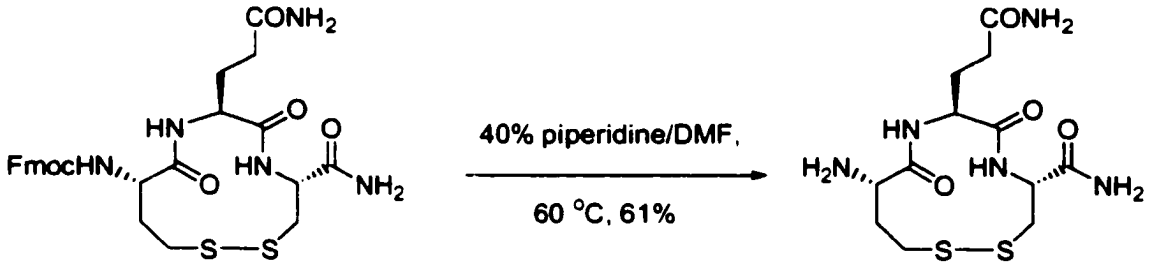
Scheme 19



With a view to improving solubility and to optimizing inhibition, we decided to remove the Fmoc protecting group (for example, **39**, Scheme 20), substitute the primary amide of the cysteine residue by a carboxylic group (for example, **40**, Scheme 21), and also add the P_2' phenylalanine residue (for example, **41**) for better recognition. Thus,

removal of the Fmoc group by treatment of compound **124** with a solution of piperidine in refluxing DMF affords the cyclic peptide **39** with the N-terminal amino group.

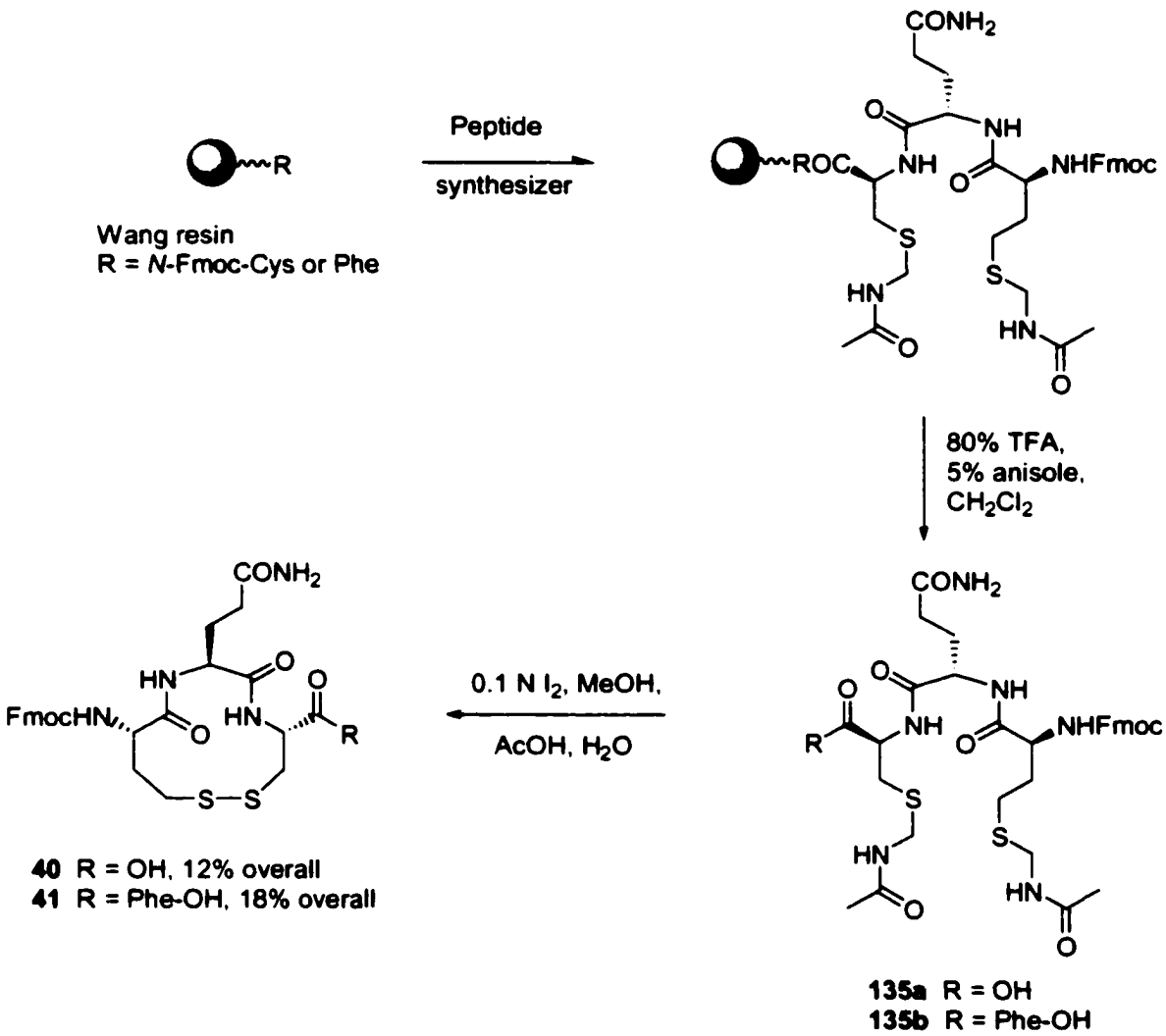
Scheme 20



124 **39**

The cyclic peptides **40** and **41** were prepared from cysteine and phenylalanine preloaded Wang resins,[100] respectively, using a peptide synthesizer as described previously (Scheme 21). Cleavage of the free linear peptide in this case from the resin is performed with 80% TFA and 5% anisole in dichloromethane.[100] Finally cyclization of the peptides **135a** and **135b** is effected by treatment with 0.1 N iodine[101] solution to afford the desired cyclic peptides **40** and **41**, respectively.

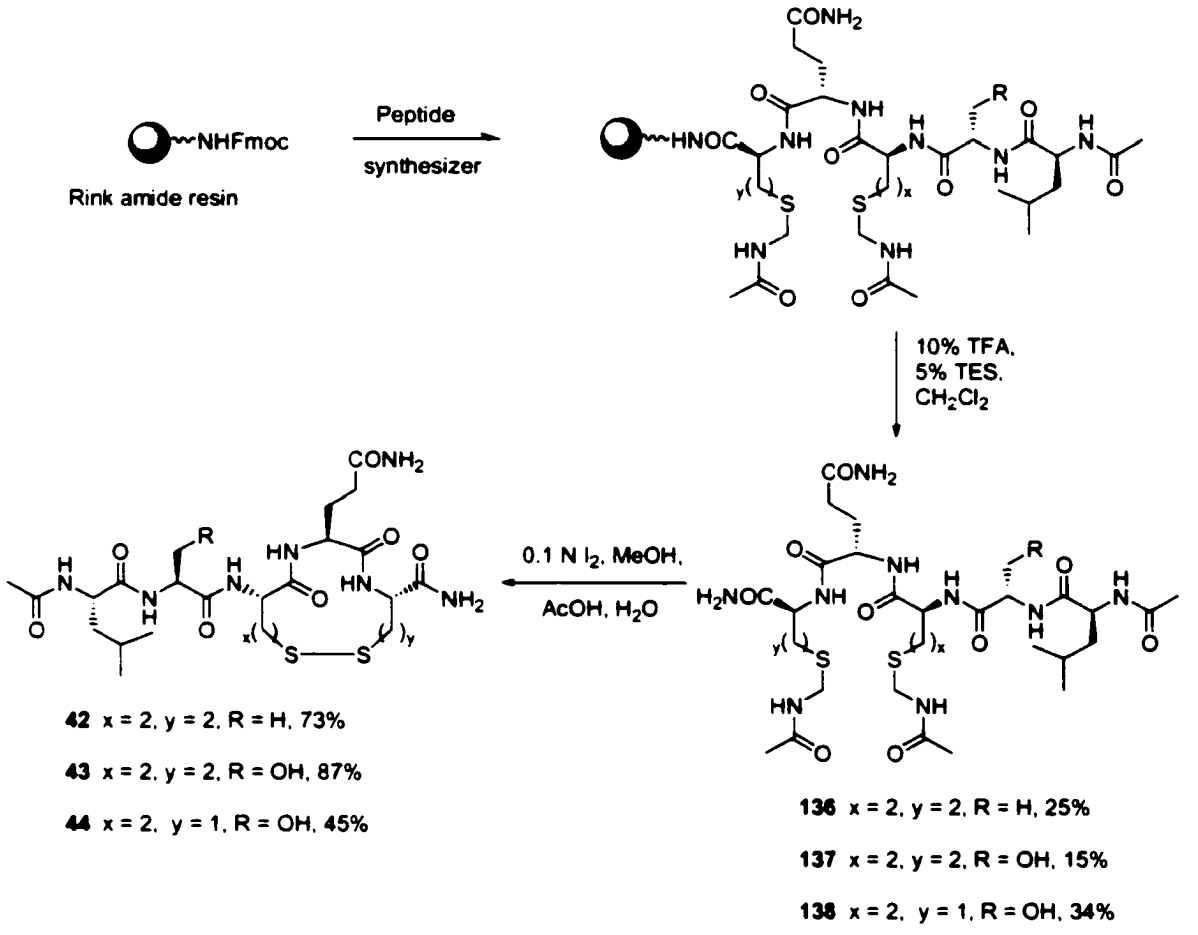
Scheme 21



Compounds **39**, **40**, and **41** were assayed against HAV 3C proteinase. Although these compounds showed enhanced solubility in DMF and DMSO, solvents that are compatible with the enzyme assay, disappointingly these compounds showed no significant inhibition of HAV 3C proteinase mediated cleavage of the Dabcyl quenched of fluorescent substrate, Dabcyl-GLRTQSFS-Edans, at a concentration of 100 μM.

Since cyclic peptide **41**, which possesses the important P_2' phenylalanine residue, did not display any significant inhibition of HAV 3C proteinase, we decided to investigate the role of the P_4 leucine residue on the inhibitory activity of these cyclic peptides. The cyclic peptide **42**, which has a leucine at the P_4 position and an alanine residue at the P_3 position, was chosen as the target. The synthesis is described in Scheme 22.

Scheme 22



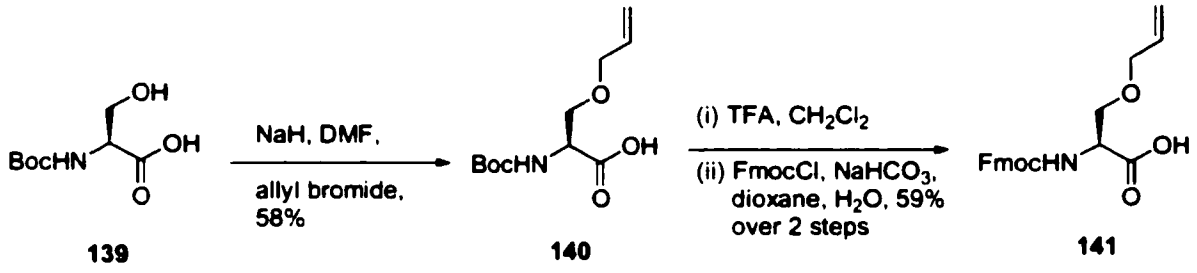
The linear pentapetide **136** was prepared on Rink amide resin as described previously. Cyclization of **136** proceeds smoothly with 0.1 N iodine solution to afford the cyclic peptide **42**. However, once this peptide has been purified by HPLC, it proved very difficult to dissolve in DMF and DMSO, possibly because of the hydrophobic cyclic disulfide in the peptidic ring system. With a view to increasing the solubility of this macrocyclic peptide, we decided to incorporate a serine residue at the P_3 position, since residues at this position do not contribute significantly to recognition.[23] The polar hydroxyl functionality of the serine residue is expected to form hydrogen bonds with the solvent and help in solvation. Toward this end, cyclic peptide **43** wherein the P_3 alanine is replaced by serine, and also **44** with a cysteine at P_2', were prepared using a similar procedure as that described for **42**. Each displayed enhanced solubility in DMF or DMSO. However, inhibition assays using compounds **43** and **44** against HAV 3C proteinase disappointingly gave no significant inhibition of the enzyme at a concentration of 100 μM. The failure to display some inhibition of the fluorescent enhancement accompanying cleavage of the substrate Dabcyl-GLRTQSFS-Edans indicates that binding of these peptides to HAV 3C proteinase is weak even when acting as substrates.

2.1 Macrocyclic Peptide with an Ethylene Linker - Target E

To introduce structural diversity and to investigate whether a macrocyclic peptide with an ethylene linker would display inhibition of the HAV 3C proteinase, we decided to synthesize compound **45** (Figure 21). In order to generate this by solid phase chemistry, *N*-Fmoc *O*-allyl serine (**141**) was synthesized. This material was prepared in three steps starting from *N*-Boc-serine (**139**) as depicted in Scheme 23. Following the

literature procedure of Itoh,[102] *N*-Boc-serine (**139**) is treated with 2 equivalents of sodium hydride followed by allyl bromide to give *N*-Boc *O*-allyl serine (**140**). The Boc protecting group of **140** is then cleaved by reaction with TFA and the resulting salt is treated with FmocCl in aqueous sodium bicarbonate solution to afford the desired *N*-Fmoc *O*-allyl serine (**141**).[103]

Scheme 23

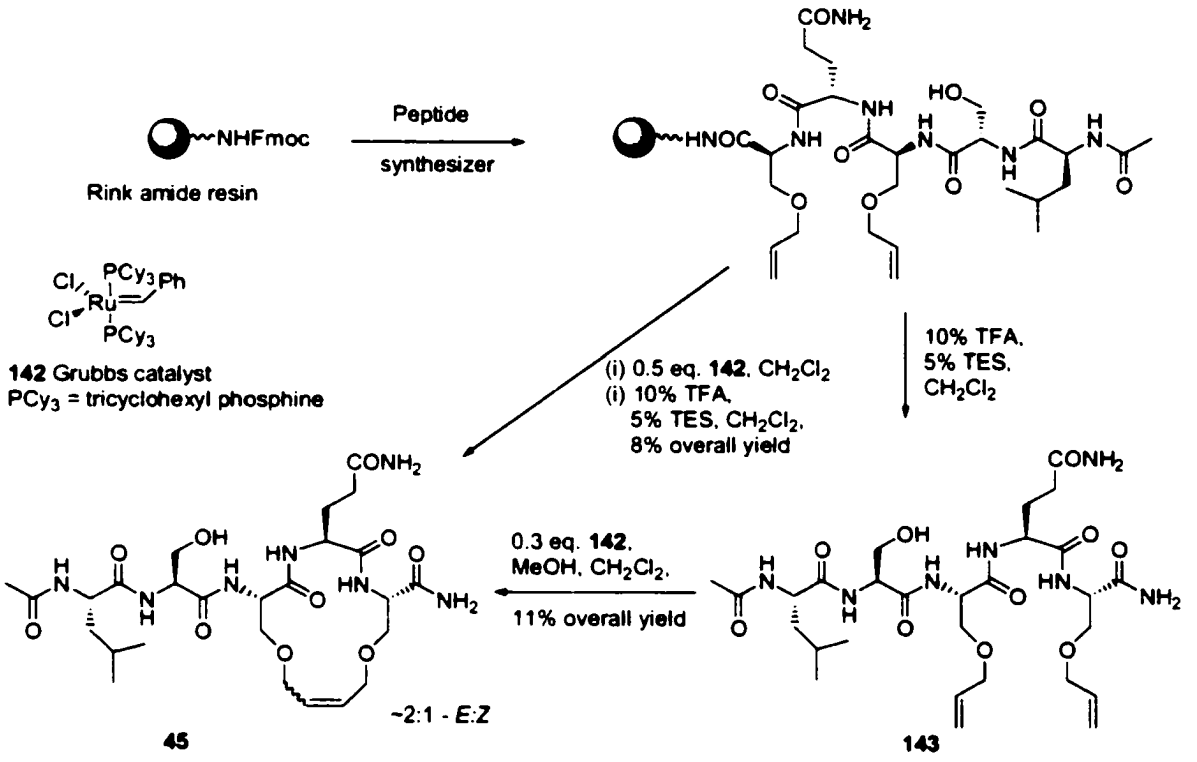


139 **140** **141**

With the *N*-Fmoc *O*-allyl serine (**141**) available, the synthesis of the pentapeptide precursor **143** proceeds as shown in Scheme 24. It employs the same Rink amide resin as that described in the previous section. A serine residue is incorporated at the P_3 site to enhance solubility of the target molecule **45**. After cleavage from the resin with 10% TFA and 5% triethylsilane in dichloromethane, cyclization of **143** *via* olefin metathesis was attempted using the ruthenium Grubbs catalyst **142**.[104] However, the linear peptide **143** is quite insoluble in most non-polar solvents (e.g. toluene, hexane, dichloromethane) wherein the ring closing metathesis (RCM) reaction is most effective. Initial attempts to cyclize **143** using non-polar solvents (e.g. dichloromethane) did not give the desired product.[104] However, a combination of methanol and dichloromethane furnishes the

cyclized product **45** in a low overall yield of 11%. The RCM reaction could also be applied successfully to the peptide attached on the solid support resin[106] to afford the cyclized product **45** in 8% overall yield, after cleavage from the resin.

Scheme 24



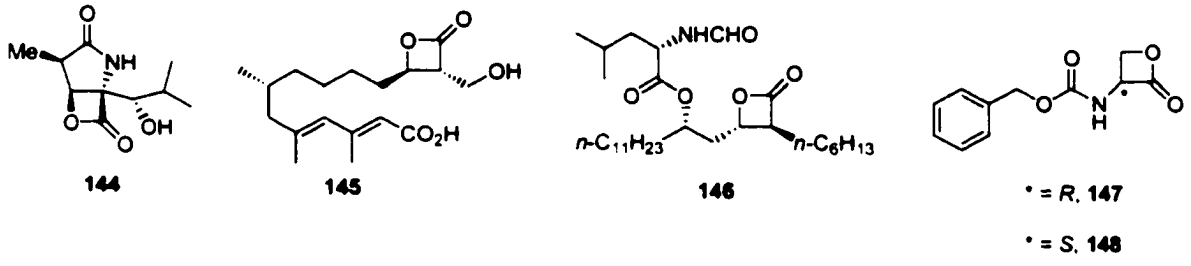
Unfortunately, the macrocyclic peptide **45** displays no inhibition of the HAV 3C proteinase at a concentration of 100 μM. Nevertheless, this study demonstrates that small cyclic peptides with a disulfide or an ethylene bridge can readily be synthesized using this methodology. This synthetic procedure could therefore be used to construct other peptide inhibitors targeting other proteinases.

3.0 β-Lactone Analogues - Target F

Over the last twenty years, much attention has been focused on β-lactones, because of their occurrence in many biologically active natural products.[107] The most widely studied β-lactones are, the *clasto*-lactacystin β-lactone (Omuralide) **144** (Figure 36), a potent inhibitor of the threonine proteinase 20S proteasome,[108] the antibiotic F-244 (1233 A) **145**, an inhibitor of HMG-CoA synthase,[109] and the antiobesity drug tetrahydrolipstatin (Orlistat) **146**, an inhibitor of the serine pancreatic lipase.[110] Studies done by Dr. Manjinder S. Lall[49] in our group have shown that *N*-Cbz-serine β-lactones **147** and **148** are potent inhibitors of the HAV 3C proteinase.[33] The D-enantiomer **147** is a competitive reversible inhibitor of the HAV 3C proteinase with a K_i of 1.50 x 10^{-6} M. Surprisingly, the L-enantiomer **148** displays a different mode of inhibition under the same conditions and acts as a time-dependent irreversible inhibitor (k_{inact} = 0.70 min^{-1}, K_I = 1.84 x 10^{-4} M and k_{inact}/K_I = 3800 M^{-1} min^{-1}).[33] The factors governing this behaviour are unclear, although one possible explanation postulates the occurrence of distinct orientations of the oxetanone ring towards the enzyme cysteine thiolate. The phenyl group is believed to mimic the P_2' phenylalanine of the natural substrate. HMQC NMR studies carried out on the enzyme/inhibitor complex using **148** with ^{13}C labeled at β-position have shown that inhibition occurs by nucleophilic attack the enzyme active site thiolate at the C-3 position of the β-lactone. In short, alkylation occurs as opposed to acylation by nucleophilic attack on the carbonyl. However, one limitation of **148** is its low stability in phosphate buffer at pH 7.5. Utilizing IR spectroscopy, the rate of β-lactone hydrolysis in the enzyme buffer solution was determined by following the disappearance of the unique β-lactone carbonyl stretch (~1830 cm^{-1}), as described in the

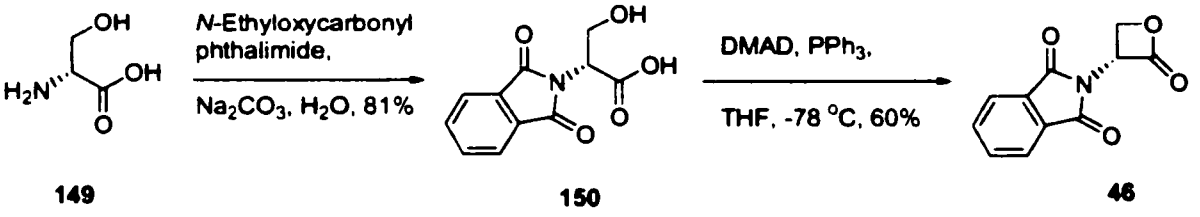
experimental section. The half-life for hydrolysis of **148** in phosphate buffer at pH 7.5 is 76 min. While too fast for an effective physiological inhibitor, the half-life is sufficiently long to carry out enzyme inhibition studies.[33]

Figure 36 Some important β-lactones and *N*-Cbz-serine β-lactone inhibitors of HAV 3C proteinase



To find more stable β-lactones and to provide structural diversity, a number of analogues were prepared and tested against HAV 3C proteinase. Since the D-*N*-Cbz-serine β-lactone (**147**) is more potent than its L-enantiomer **148**, the D-*N*-phthalimido-serine β-lactone (**46**) was prepared (Scheme 25). Treatment of D-serine (**149**) with *N*-ethyloxycarbonyl phthalimide[111] in aqueous sodium carbonate generates the D-*N*-phthalimido serine (**150**). Cyclization of the hydroxy-acid **150** using the preformed adduct of triphenyl phosphine and dimethyl azodicarboxylate under Mitsunobu conditions[112,73] gives the desired D-*N*-pthalimido-serine β-lactone (**46**).

Scheme 25



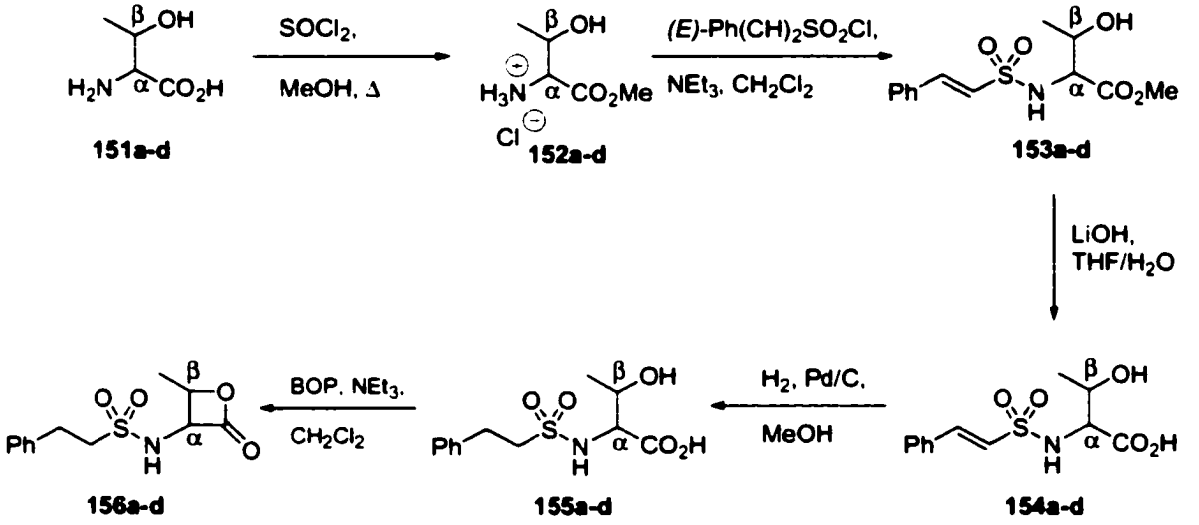
149 **150** **46**

β-Lactone **46** was tested against HAV 3C proteinase without pre-incubation with the enzyme, and **46** displayed time independent (reversible) inhibition with an IC_{50} of 27 μM. However, aqueous stability was not improved as the β-lactone **46** exhibits a half-life of 67 min at pH 7.5 as determined by IR studies.

The presence of a methyl substituent at the β-position of threonine β-lactones is expected to improve the hydrolytic stability in basic media.[112] Hence, all four stereoisomers of *N*-sulfonamide threonine **156a,b** and *allo*-threonine β-lactones **156c,d** derived from enantiomerically pure threonine **151a,b** and *allo*-threonine **151c,d** were prepared by Dr. Manjinder S. Lall[49] as outlined in Scheme 26. The free carboxyl group of D and L threonine and *allo*-threonine (**151a-d**) are protected as their methyl esters by treatment with thionyl chloride in methanol to afford the methyl ester salts **152a-d**. These salts are then coupled to *trans* β–styrenesulfonyl chloride to furnish the corresponding sulfonamides **153a-d**. Hydrolysis of sulfonamides **153a-d** with lithium hydroxide followed by reduction of the side chain double bond yield the β–hydroxy acids **155a-d**. The β–hydroxy acids **155a-d** are cyclized using BOP[113] to afford the desired *N*-sulfonamide threonine and *allo*-threonine β-lactones **156a-d**. In contrast to the serine analogues, cyclization of β-alkyl substituted β-hydroxy-α-amino acids **155a-d** requires

carboxyl activation as opposed to hydroxyl group activation in order to avoid decarboxylative elimination to form the corresponding enamines.[112b]

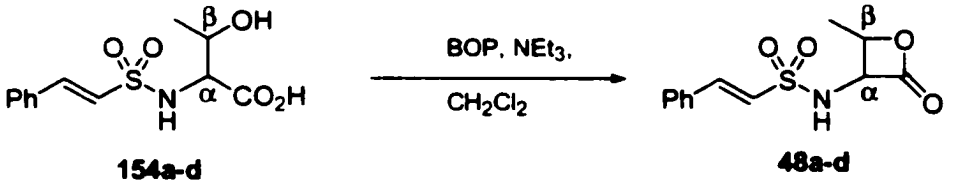
Scheme 26



a α = *S*, β = *R*; **b** α = *R*, β = *S*; **c** α = *S*, β = *S*; **d** α = *R*, β = *R*

All the four stereoisomers of unsaturated *N*-sulfonamide threonine and *allo*-threonine β-lactones **48a-d** were also prepared (Scheme 27). The presence of a double bond on the side chain is expected to confer rigidity to the β-lactone molecules and possibly influence the inhibitory potential of each compound. The hydroxy acids **154a-d** are cyclized using BOP[113] as described previously to afford desired unsaturated *N*-sulfonamide threonine and *allo*-threonine β-lactones **48a-d**.

Scheme 27

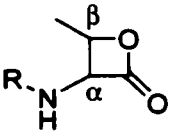


154a-d **48a-d**

a α = *S*, β = *R*; **b** α = *R*, β = *S*; **c** α = *S*, β = *S*; **d** α = *R*, β = *R*

The *N*-sulfonamide threonine and *allo*-threonine β-lactones were tested against HAV 3C proteinase by Dr. Manjinder S. Lall. The IC_{50} values and the hydrolysis half-life in phosphate buffer at pH 7.5 are given in Table 1. β-lactones **156a-d** and **48a-d** display time dependent inhibition with IC_{50} values in the range of 5 to 168 μM. The *trans* β-lactones **156c**, **48c**, **156d** and **48d** are generally more potent (IC_{50} 5-32 μM) than the *cis* β-lactones **156a**, **48a**, **156b** and **48b** (IC_{50} 86-168 μM). The *trans* β-lactones derived from the D *allo*-threonine **156d** and **148d** (IC_{50} 12, 5 μM) are in turn more potent than the L *allo*-threonine derivatives **156c** and **48c** (IC_{50} 32, 20 μM). As expected, these β-methyl substituted β-lactones show enhanced stability in basic aqueous media ($t_{1/2}$ 135-595 min) compared to the serine β-lactones **147**, **148** and **46**.

Table 1 HAV 3C inhibition and half-life of threonine and *allo*-threonine β-lactones



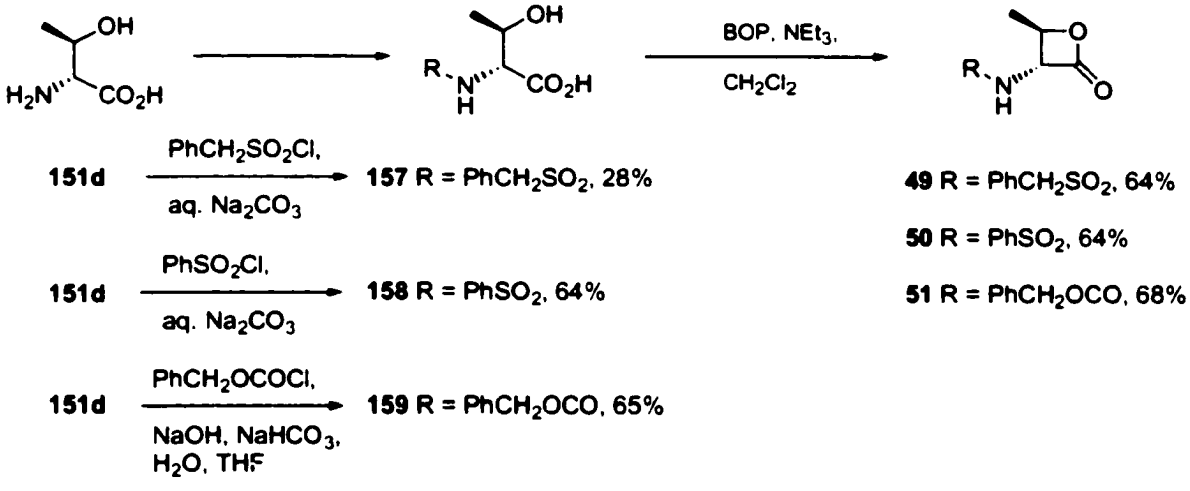
Compounds	R	Config. α	Config. β	HAV 3C $IC_{50}(\mu M)^a$	Aqueous Hydrolysis $t_{1/2}(min)^b$
156a	$R = SO_2(CH_2)_2Ph$	*S*	*R*	168	358
156b	$R = SO_2(CH_2)_2Ph$	*R*	*S*	136	358
156c	$R = SO_2(CH_2)_2Ph$	*S*	*S*	32	136
156d	$R = SO_2(CH_2)_2Ph$	*R*	*R*	12	136
48a	R = *(E)*-$SO_2(CH)_2Ph$	*S*	*R*	131	595
48b	R = *(E)*-$SO_2(CH)_2Ph$	*R*	*S*	86	595
48c	R= *(E)*-$SO_2(CH)_2Ph$	*S*	*S*	20	135
48d	R = *(E)*-$SO_2(CH)_2Ph$	*R*	*R*	5	135
49	$R = SO_2CH_2Ph$	*R*	*R*	6	96
50	$R = SO_2Ph$	*R*	*R*	25	135
51	$R = PhCH_2OCO$	*R*	*R*	13	647

[a]IC_{50} Conditions: 0.1 μM HAV 3C C24S, 10 μM Dabcyl-GLRTQSFS-Edans, 2 mM EDTA, 0.1 mg/mL BSA, 100 mM KH_2PO_4/K_2HPO_4 at pH 7.5, 1% DMF. [b]β-Lactone hydrolysis half-life in phosphate buffer pH 7.5.

Since the *trans*-substituted D-*allo*-threonine β-lactones **156d** and **48d** (IC_{50} 12, 5 μM) are the most potent inhibitors in this series, we decided to prepare *trans*-substituted D-*allo*-threonine β-lactones **49** and **50** with varying *N*-sulfonamide side chain length and also **51** with a Cbz side chain. In contrast to the β-hydroxy acids **155**, the *N*-sulfonamide β-hydroxy acids of **20** and **21** were prepared by direct coupling of D-*allo*-threonine **151d** to the corresponding sulfonyl chloride. Thus, reaction of D-*allo*-threonine **151d** with α-

toluene sulfonyl chloride or phenylsulfonyl chloride in aqueous sodium carbonate gives the corresponding β-hydroxy acids of **157** and **158**, respectively.[114] The *N*-Cbz side chain derivative **159** is prepared by reaction of D-*allo*-threonine **151d** with benzyl carbamate in the presence of sodium hydroxide and sodium carbonate. All three β-hydroxy acids **157**, **158** and **159** are smoothly cyclized using BOP[113] in the presence of triethylamine to give the corresponding β-lactones **49**, **50** and **51**, respectively.

Scheme 28

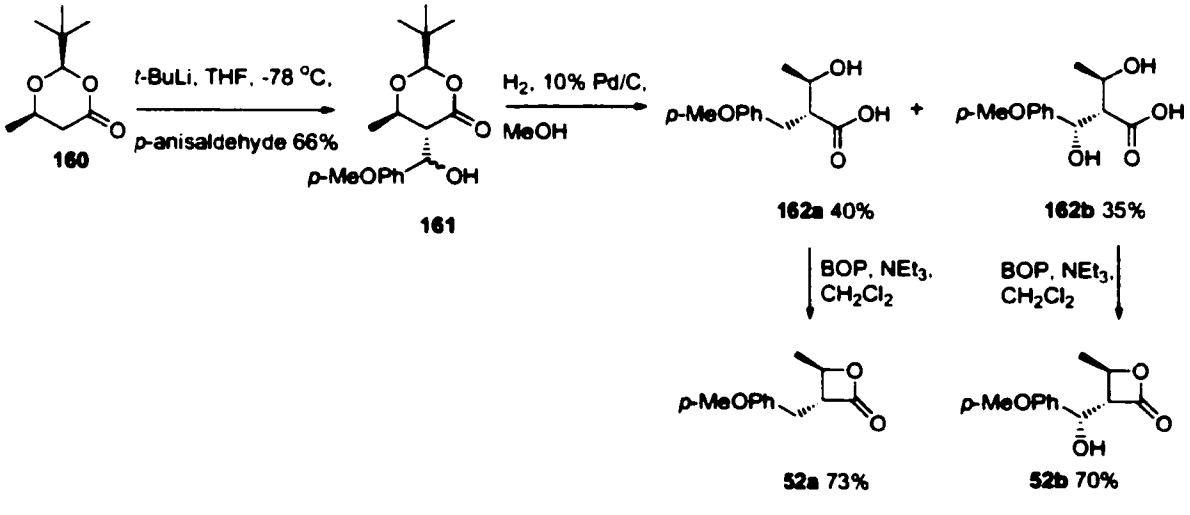


Compounds **49-51** exhibit potent, time-dependent inhibition with IC_{50} values in the range of 6-25 μM (Table 1). Although the effects are not dramatic, shortening of the side chain by *one* carbon (cf. **156d** and **49**) results in a two-fold increase in inhibitory activity. However, reducing the same side chain by *two* carbons (cf. **156d** and **50**) leads to a two-fold decrease in inhibition. One notable feature is that the aqueous half-life of the β-lactones (except for **51**) seems to decrease with increasing potency for enzyme

inactivation, presumably because enhanced reactivity of the ring system to enzyme nucleophiles parallels susceptibility to attack by water.

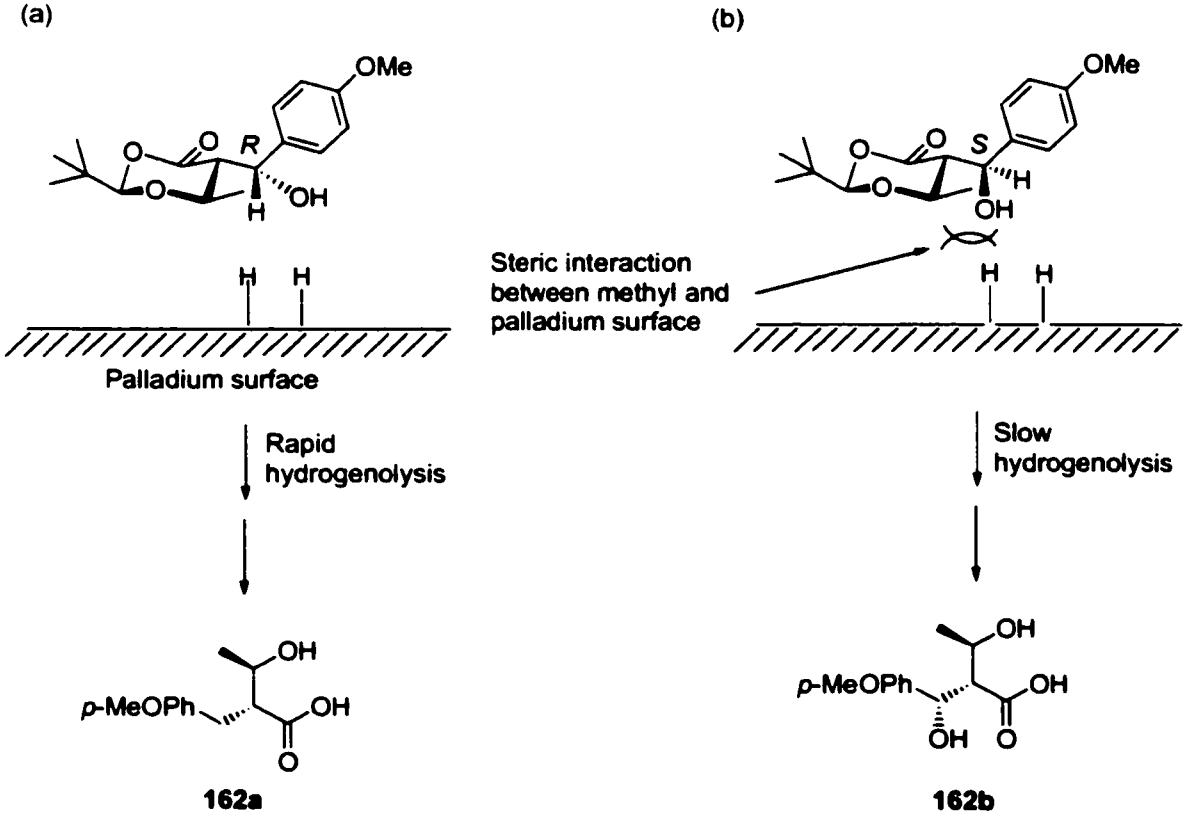
The next goal was to examine whether the α-nitrogen functionality in the amino-acid derived β-lactones influenced inhibitory activity. Toward this end, analogues **52a**, **52b** and **47** which contain a methylene and **53** which has a carbonyl group at the α-position were synthesized. Using a procedure of Seebach,[115] the dioxanone **160** was treated with *tert*-butyl lithium at -78 °C (Scheme 29) followed by trapping of the enolate with benzyl bromide, but this approach did not give the alkylation product. Since the benzyl bromide may not be sufficiently reactive, a more electrophilic alkylating agent was used, *p*-anisaldehyde. Thus, trapping of the enolate with *p*-anisaldehyde affords a 1:1 mixture of diastereoisomers **161**. Hydrogenolysis of the benzylic hydroxyl group using palladium on carbon results in preferential cleavage of the *R* hydroxyl with concomitant removal of the pivaloyl group to generate **162a** and **162b** in a ~1:1 ratio. Both the β-hydroxy acids **162a** and **162b** are readily cyclized with BOP to furnish the β-lactones **52a** and **52b**, respectively.

Scheme 29



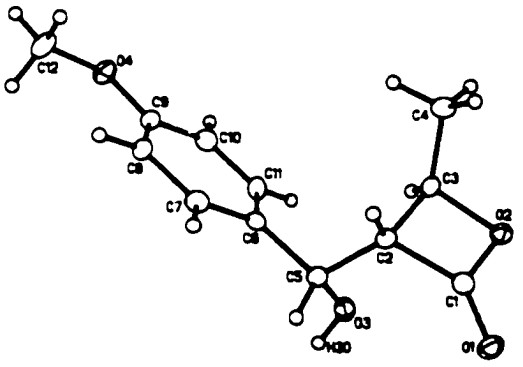
Previous studies on the hydrogenolysis of benzylic alkoxy substituents indicate that adsorption of the phenyl ring and the benzylic alkoxy group on the catalyst surface is important for successful hydrogenolysis.[116a] The preferential cleavage of the *R* benzylic alcohol of **161** may result from the presence of the contiguous chiral center. The *R* hydroxyl moiety (Figure 37a) is pointing away from the methyl group and therefore experiences no steric hindrance with the methyl group and the palladium surface. However, the *S* hydroxyl moiety (Figure 37b) is pointing towards the methyl group and presumably prevents approach to the palladium catalyst surface for hydrogenolysis.[116]

Figure 37 Rationale for the selective cleavage of *R* benzylic alcohol of **161**



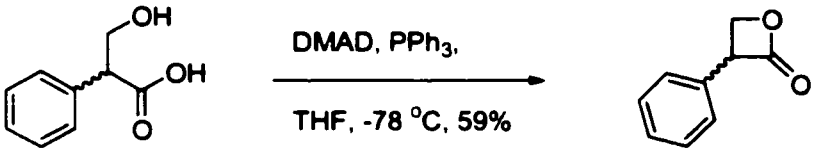
The relative stereochemistry of β-lactone **52a** can be determined by the vicinal coupling constants of the two *trans* ring protons. Mulzer[117] reported that *cis* and *trans* 3,4-disubstituted β-lactones can be unambiguously identified from vicinal proton coupling constants. The *cis* coupling constant is $^3J_{cis} \approx 6.5$ Hz and that of *trans* is $^3J_{trans} \approx$ 4-4.5 Hz. The β-lactone **52a** has a coupling constant of 4.1 Hz, which is consistent with a *trans* stereochemistry. For conformation, the relative stereochemistry of β-lactone **52b** was determined from its X-ray crystal structure (Figure 38).

Figure 38 X-ray crystal structure of β-lactone **52b**



The α-phenyl β-lactone **47** which lacks the carbamate functionality could be prepared (Scheme 30) from the commercially available D,L tropic acid **163** by cyclization using the Mitsunobu protocol.[112,73]

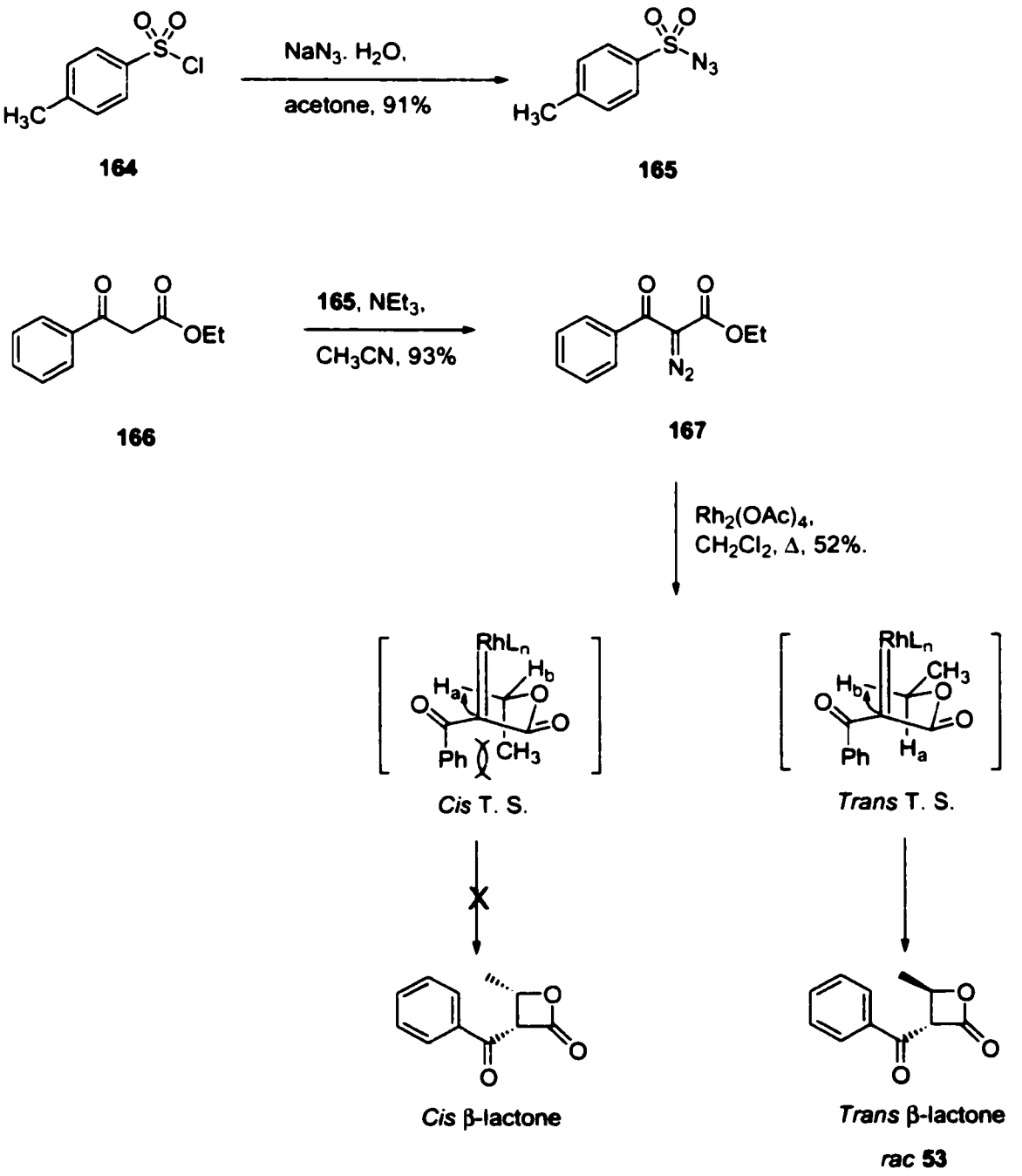
Scheme 30



163 **47**

The known β-lactone **53** is available using a modified literature procedure (Scheme 31).[118] Treatment of tosyl chloride **164** with sodium azide generates the tosyl azide **165**.[119] Reaction of the β-keto ester **166** with tosyl azide **165** in the presence of triethylamine affords the diazoester **167**,[120] which on treatment with a refluxing solution of rhodium (II) acetate dimer[82,83] yields the racemic *trans* β-lactone **53**. The reaction proceeds through insertion of the intermediate carbenoid species into the carbon-hydrogen bond of the methylene group of the ethyl ester. Two transition states for this reaction are shown in Scheme 31. The *cis* β-lactone product is not observed, presumably because the *cis* transition state experiences steric interaction between the axial phenyl residue and the axial methyl substituent. However, in the *trans* transition state, the methyl group is in the equatorial position and this unfavorable interaction is absent.[118b]

Scheme 31



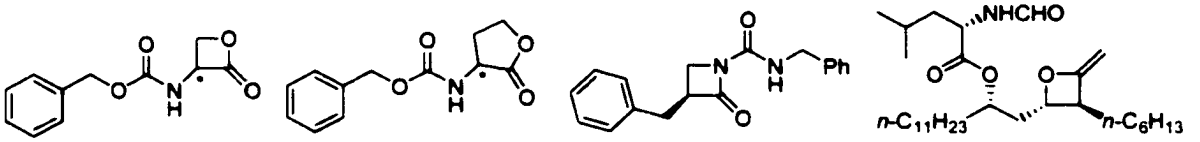
Testing of **52a, 52b, 47** and **53** against HAV 3C proteinase shows poor inhibition by these compounds at a concentration of 100 μM (IC_{50}'s >>100 μM). Although the β-

lactone ring and a phenyl side chain are necessary for effective inhibition, the results indicate that the α-nitrogen functionality may also play an important role in enzyme recognition.

3.1 4-Membered Ring Analogues - Target G

In the previous section it was demonstrated that 4-membered ring β-lactones with the correct side chains (e.g. *N*-Cbz-serine β-lactones **147** and **148)** are potent inhibitors of HAV 3C proteinase. Previous studies within the Vederas group have shown that analogous 5-membered ring *N*-Cbz-homoserine γ-lactones[33] (**168** and **169**, Figure 39) are poor inhibitors (IC_{50} >> 100 μM) of the same enzyme. In order to probe the importance of the 4-membered ring system of the β-lactones, we decided to prepare 4-membered lactam, enol ether, and ketone ring systems with a *N*-Cbz side chain. The targets chosen are *N*-Cbz-L-β-lactam **54**, *N*-Cbz-L-2-methylene oxetane **55**, *N*-Cbz-cyclobutanone **56** and *N*-Cbz-3-azetidinone **57** (Figure 22). Monocyclic β-lactams e.g. **170** (Figure 39) are known inhibitors of the serine human cytomegalovirus (HMCV) proteinase (IC_{50} of 10 μM).[121] The 2-methylene oxetane derivative of tetrahydrolipstatin **171** is an inhibitor of the porcine pancreatic lipase (a serine proteinase) with an IC_{50} of 1.7 μg/mL.[122] Hence, **54** and **55** are attractive structures to investigate for binding to HAV 3C proteinase. Targets **55**, **56**, and **57** are new compounds, previously unreported at the time of this study.

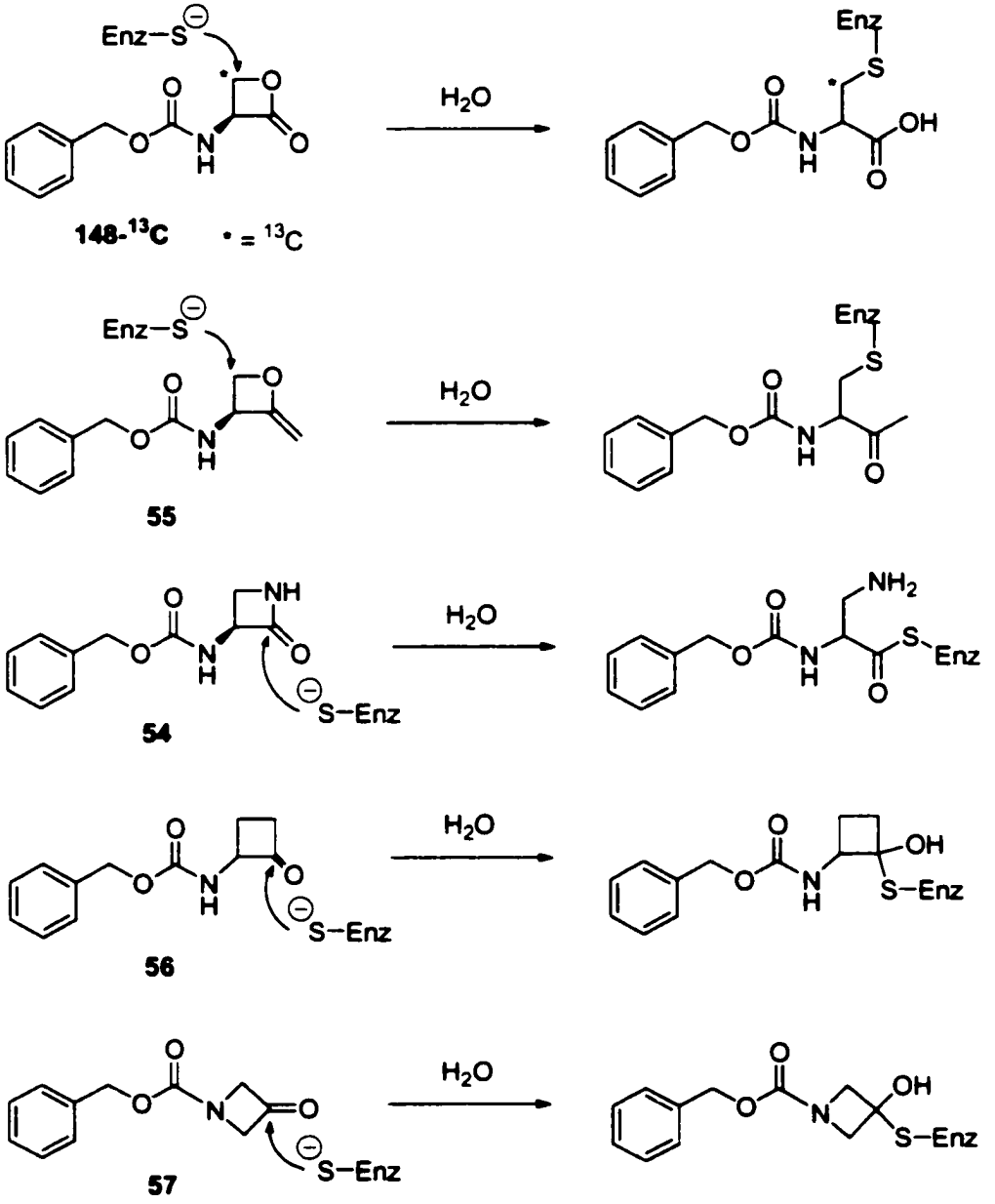
Figure 39 4- and 5-membered ring inhibitors of proteinases



* = *R*, **147** * = *S*, **148** * = *R*, **168** * = *S*, **169** **170** **171**

Previous studies carried out by Dr. Manjinder S. Lall in our laboratory, using L-*N*-Cbz-serine β-lactone (**148**) labeled with ^{13}C, show that the HAV 3C enzyme is inactivated through capture at the β-position of the β-lactone ring by the active site cysteine thiolate (Figure 40). Based on this observation, it seemed that 2-methylene oxetane **55** could react analogously (Figure 40). Alternatively, the site of attack on β-lactam **54**, cyclobutanone **56**, and 3-azetidinone **57** is expected to be the carbonyl functionality.

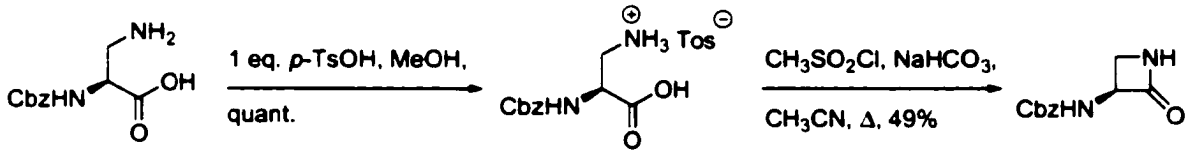
Figure 40 Mode of inhibition for β-lactones **148** and rationale for targets **54**, **55**, **56** and **57**



Attempts to effect cyclization of amino acid **172** using methane sulfonyl chloride or DCC in DMF or acetonitrile[121a] were unsuccessful as a result of the poor solubility of

the starting material. To improve the solubility of **172**, it was converted to its tosylate salt by treatment with 1 equivalent of *para*-toluene sulfonic acid (Scheme 32). This approach proved successful, and cyclization of the tosylate salt **173** using a refluxing solution of methane sulfonyl chloride affords the desired β-lactam **54** in 49% yield.

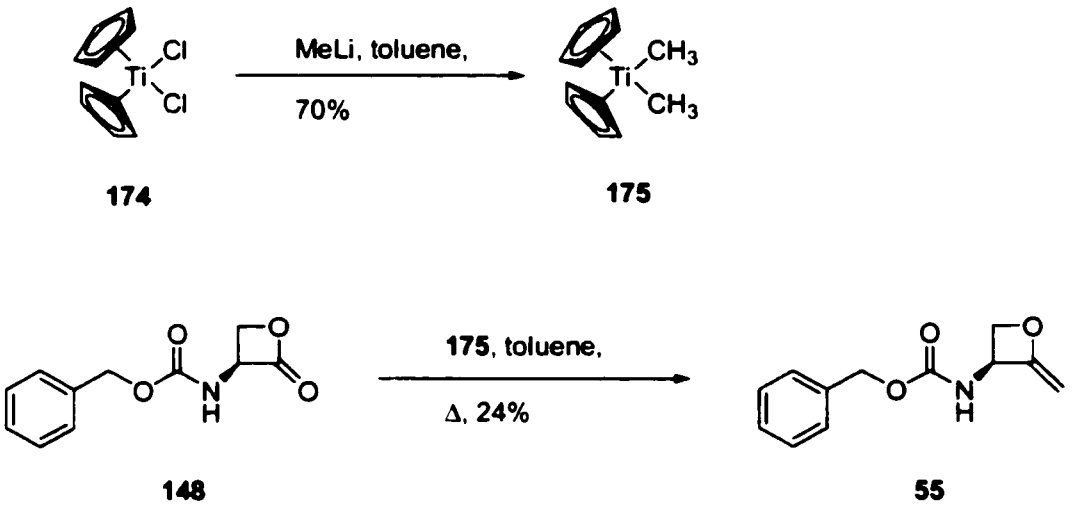
Scheme 32



172 **173** **54**

The known dimethyltitanocene **175**[122] is reported to work under mild conditions and selectively methylenate lactones and esters in the presence of other carbonyl functionalities such as urethanes.[122b] Thus treatment of bis(cyclopentadienyl) titanium dichloride **174** with methyl lithium affords the dimethyltitanocene **175** as reported previously (Scheme 33).[122] Reaction of β-lactone **148** with **175** in refluxing toluene generates the desired 2-methylene oxetane **55**, albeit in low yield, 24%. Extensive attempts to improve the yield of this process were not successful.

Scheme 33

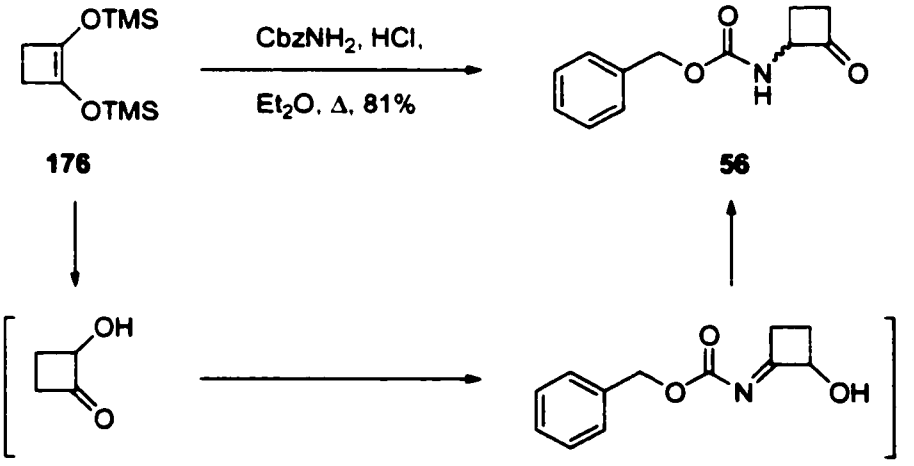


174 175

148 55

The cyclobutanone **56** is accessible by reaction of commercially available 1,2-bis(trimethylsiloxy)cyclobutene (**176**) with benzyl carbamate in a presaturated solution of hydrogen chloride in dry ether (Scheme 34).[123] The reaction may proceed through the removal of TMS protecting groups to form an α-hydroxy ketone, followed by condensation of benzyl carbamate with the carbonyl and subsequent tautomerization.

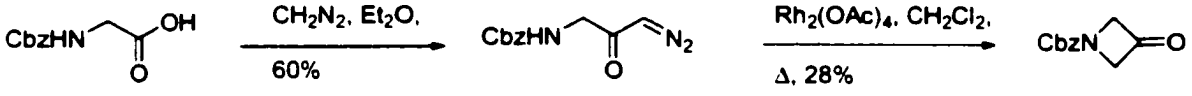
Scheme 34



176 56

The *N*-Cbz-3-azetidinone (**57**) could be made analogously to the *N,N*-dimethyl glutamine 3-azetidinone analogue **35**. Thus activation of *N*-Cbz-glycine with ethyl chloroformate followed by trapping of the mixed anhydride with diazomethane gives the α-diazoketone **178**.[27] Treatment of **178** with rhodium (II) acetate dimer[82] affords *N*-Cbz-3-azetidinone **57** in 28% yield.[124] Recently, this compound was also reported by Wang[124] in 32% yield using copper (II) acetylacetonate instead of rhodium (II) acetate dimer as the catalyst to generate the intermediate carbene.

Scheme 35



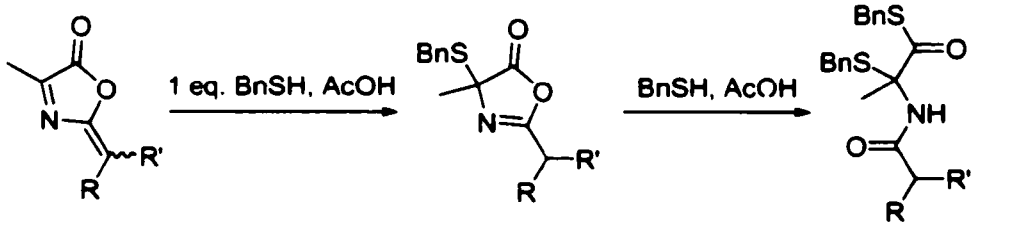
177 **178** **57**

Testing of **54**, **55**, **56** and **57** against HAV 3C proteinase disappointingly gives no significant inhibition of the enzyme at a concentration of 100 μM. Although, these molecules possess the required phenyl side chain and the 4 membered ring system, the results clearly demonstrate the importance of the β-lactone functionality for inhibitory activity of 4-membered ring analogues, either in the reversible mode (for example, **148**) or as irreversible inactivators (for example, **147**).

4.0 The Pseudoxazolones - Target H

The 3-oxazolin-5-one system, referred to as pseudoxazolone has been cited in the literature since the 1930's, but no inhibition studies against proteinases have been reported.[125,126] Dichloro **179a** and monophenyl **179b** pseudoxazolones (Figure 41) are known to add one thiol at the imine position to give adduct **180a** and **180b**, with further addition of a second thiol at the carbonyl position of **180a** leading to the thioester **181a**.[58] The ability of pseudoxazolones to react with thiols led us to propose that these compounds could be used to inhibit thiol containing enzymes.

Figure 41 Reaction of pseudoxazolones with α-toluenethiol



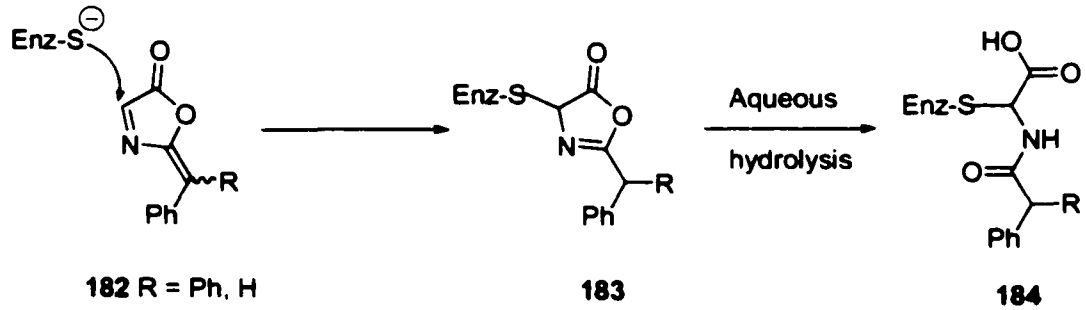
179a R = R' = Cl
179b R = Ph, R' = H

180a R = R' = Cl
180b R = Ph, R' = H

181a R = R' = Cl

In the case of HAV 3C proteinase, the enzyme active site thiolate is expected to react with the pseudoxazolone, for example, **182** at the imine position (Figure 42) forming the covalent adduct **183**. Under aqueous conditions, hydrolysis could occur to generate the covalently modified enzyme-inhibitor complex **184**.

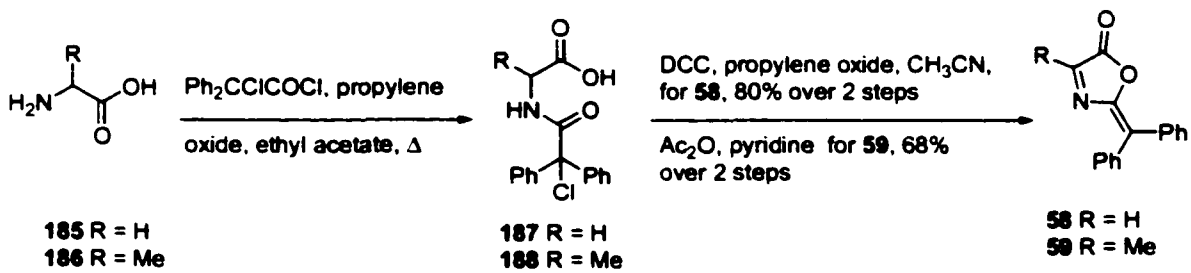
Figure 42 Rationale for inhibition of HAV 3C proteinase by pseudoxazolones



182 R = Ph, H **183** **184**

The initial targets, diphenyl pseudoxazolones derivatives **58** and **59** were prepared by Nathaniel I. Martin[127] (graduate student) and Lara Silkin[128] (previous summer student) in our group as part of a program to generate enantiomerically pure amino acids from racemic precursors by asymmetric reduction. The synthesis begins from glycine (**185**) or D,L alanine (**186**) using a modified literature procedure of King *et al.*[129a] (Scheme 36). Reaction with 2-chloro-2,2-diphenylacetyl chloride gives the corresponding chloro adducts **187** and **188**, respectively. These compounds cyclize with either acetic anhydride/pyridine or DCC with *in situ* elimination of HCl to afford diphenyl pseudoxazolones **58** and **59**, respectively.

Scheme 36



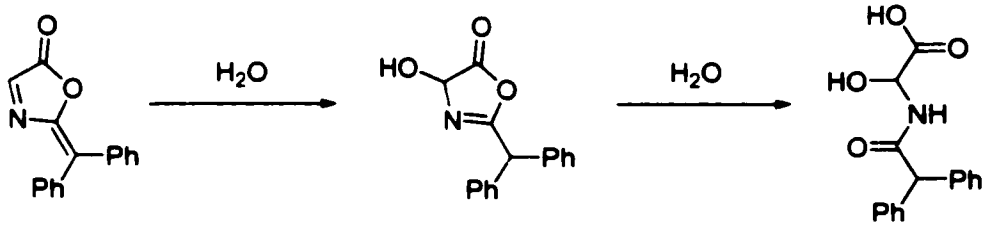
185 R = H
186 R = Me

187 R = H
188 R = Me

58 R = H
59 R = Me

The diphenyl pseudoxazolones **58** and **59** were tested against HAV 3C proteinase. The glycine derivative **58** is a time dependent inhibitor with an IC_{50} of 33 μM. The alanine derivative **59** is less active (IC_{50} >100 μM), possibly due to steric interaction between the methyl group in **59** and the nucleophilic thiolate of the enzyme. However, one limitation is the low stability of these compounds in aqueous phosphate buffer at pH 7.5. They are presumably hydrolyzed at the imine position to form an intermediate azalactone (for example, **189**) which further reacts with water at the carbonyl to generate the hydroxy acid **190** (Figure 43).[125,126] The half lives of these pseudoxazolones can be determined by monitoring the disappearance of the characteristic imine absorption band (~350 nM) using a UV spectrometer, as described in the experimental section. The diphenyl pseudoxazolones **58** and **59** have a half-life of 15 and 40 mins, respectively, which is long enough to carried out enzyme inhibition studies.

Figure 43 Hydrolytic pathway of pseudoxazolone in aqueous media

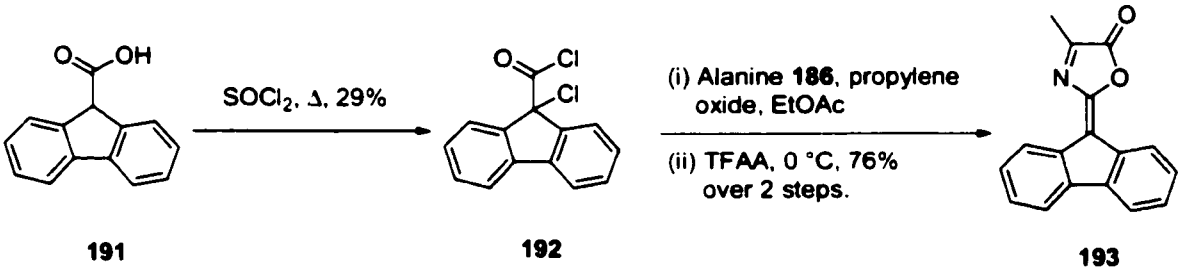


58 **189** **190**

Our next goal was to prepare the fluorenone pseudoxazolone analogue **193**, since the planarity of the fluorenone system would be expected to alter chemical behaviour, and perhaps improve inhibition and aqueous stability. The alanine derivative **193** was

synthesized by Nathaniel I. Martin[127] as depicted in Scheme 37. Reaction of 9-fluorenecarboxylic acid **191** with thionyl chloride gives the α-chloro acyl chloride **192**,[130] which upon reaction with alanine (**186**) followed by cyclization with TFAA affords the desired compound **193**. The glycine derivative was also prepared analogously but was found to be unstable. Although better stability ($t_{1/2}$ = 68 min) is observed for **193** in phosphate buffer, testing against HAV 3C proteinase gives only modest improvement in inhibition with an IC_{50} of 68 μM.

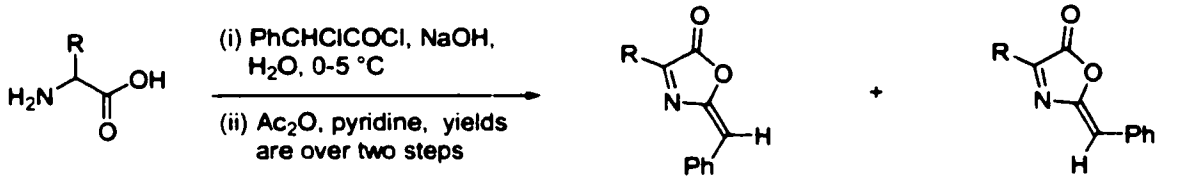
Scheme 37



191 **192** **193**

To examine the structural influences and to potentially identify better inhibitors, the monophenyl pseudoxazolones of glycine **60a,b** and alanine **61a,b** were prepared by Lara Silkin[127] and Nathaniel I. Martin[128] as shown in Scheme 38. Condensation of glycine (**185**) and alanine (**186**) with DL-2-chloro-2-phenylacetyl chloride gives the corresponding α-chloroacid, which upon cyclization with acetic anhydride and pyridine affords the monophenyl pseudoxazolones **60a,b** and **61a,b**, respectively.

Scheme 38



185 R = H

186 R = Me

60a R = H, 3%

61a R = Me, 38%

60b R = H, 14%

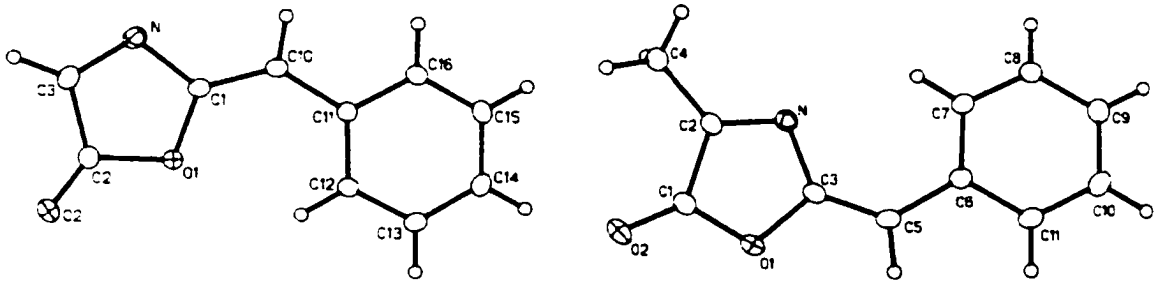
61b R = Me, 38%

Although these compounds have been reported previously, no attempts had been made to separate the geometric *E* and *Z* isomers.[126,129] We were able to separate the two geometric isomers by flash column chromatography for full characterization. Assignment of the *E* and *Z* isomers is based on the proton (^{1}H) chemical shift of the olefinic hydrogen. The less polar *E* isomer on TLC (e.g. **60a**, Figure 44) has its olefinic proton aligned with the oxazolone ring oxygen and is therefore more deshielded (6.72 ppm), whereas the more polar *Z* isomer (e.g. **60b**) has its olefinic proton aligned with the imine nitrogen and is consequently less deshielded (6.61 ppm). Although isomerization of the double bond could potentially occur under prolonged exposure to light, these compounds were stored in the dark and no such effect was observed using ^{1}H NMR analysis of the samples.

Figure 44 ^{1}H Chemical shift of the glycine pseudoxazolone olefinic proton in acetone-d_6 (300 MHz)

Further support for stereochemical assignments comes from two X-ray crystal structures of **60b** and **61a**, which confirmed the geometry of the double bond (Figure 45). Analogous application of ^{1}H NMR spectroscopy can be used to assign the *E* and *Z* configuration of other monophenyl pseudoxazolones unambiguously.

Figure 45 Crystal structures of **60b** (left) and **61a** (right)

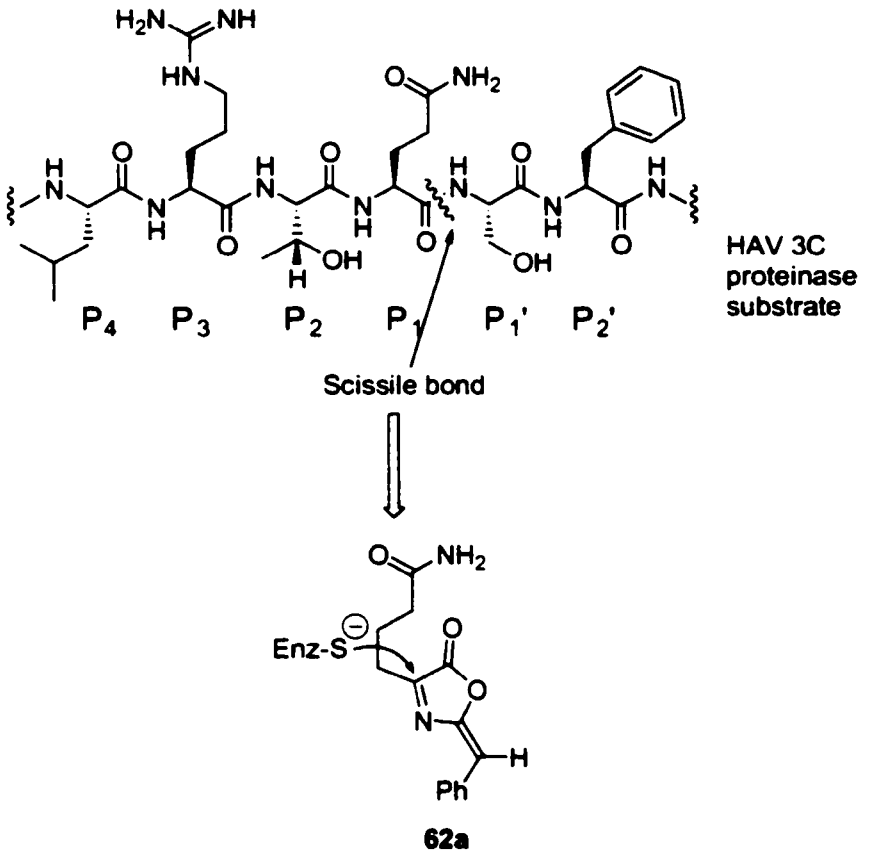


Enzymatic testing of **60a** and **60b** against HAV 3C proteinase shows good time dependent inhibition with IC_{50}'s of 6 μM and 4 μM, respectively. However, the alanine pseudoxazolones **61a** and **61b** displayed different levels of inhibition. The *E* isomer **61a**

is a time dependent inhibitor with an IC_{50} of 26 μM, whereas the *Z* isomer **61b** is a much weaker inhibitor with IC_{50} >100 μM. The enzyme presumably prefers the *E* over the *Z* isomer when there is substitution at the imine carbon. Although better inhibition is achieved with pseudoxazolones **60a** and **60b**, there is no improvement of aqueous stability. Pseudoxazolones **60a,b** and **61a,b** have half-lives of 28, 16 and 17, 14 mins, respectively, in phosphate buffer (pH 7.5).

Although substitution at the imine carbon reduces the potency of the pseudoxazolones, further evaluation of the effect of additional recognition elements in these molecules was carried out. Since the monophenyl pseudoxazolones are more potent than the diphenyl pseudoxazolones, (cf. **58** and **60**), the monophenyl pseudoxazolone **62a** with a primary amide side chain that mimics the P_1 glutamine residue of the natural substrate appeared as an interesting target (Figure 46).

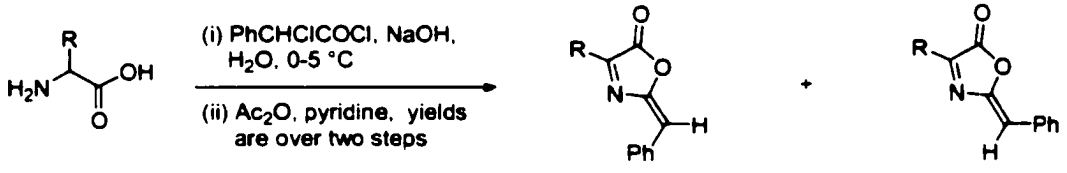
Figure 46 Rationale for design of monophenyl pseudoxazolone with a glutamine side chain



Both the homoglutamine **62a,b** (4 carbon side chain) and glutamine **63a,b** (3 carbon side chain) monophenyl pseudoxazolones were prepared (Scheme 39). Condensation of homoglutamine (**194**) and glutamine (**195**) with DL-2-chloro-2-phenylacetyl chloride gives the corresponding α-chloroacids, which are cyclized with acetic anhydride and pyridine to generate the monophenyl pseudoxazolones **62a,b** and **63a,b**, respectively. Disappointingly, these compounds show no improved inhibition against HAV 3C proteinase with IC_{50} values of 43, >100, 47, >100 μM for **62a,b** and

63a,b respectively. The effect of geometric isomers follows the same trend as observed previously, with the *E* isomers **62a** and **63a** being more potent inhibitors than the *Z* pseudoxazolones **62b** and **63b**. Moreover, compounds **62a,b** and **63a,b** did not display any enhanced aqueous stability, with half-lives of 29, 13, 24 and 12 mins, respectively.

Scheme 39



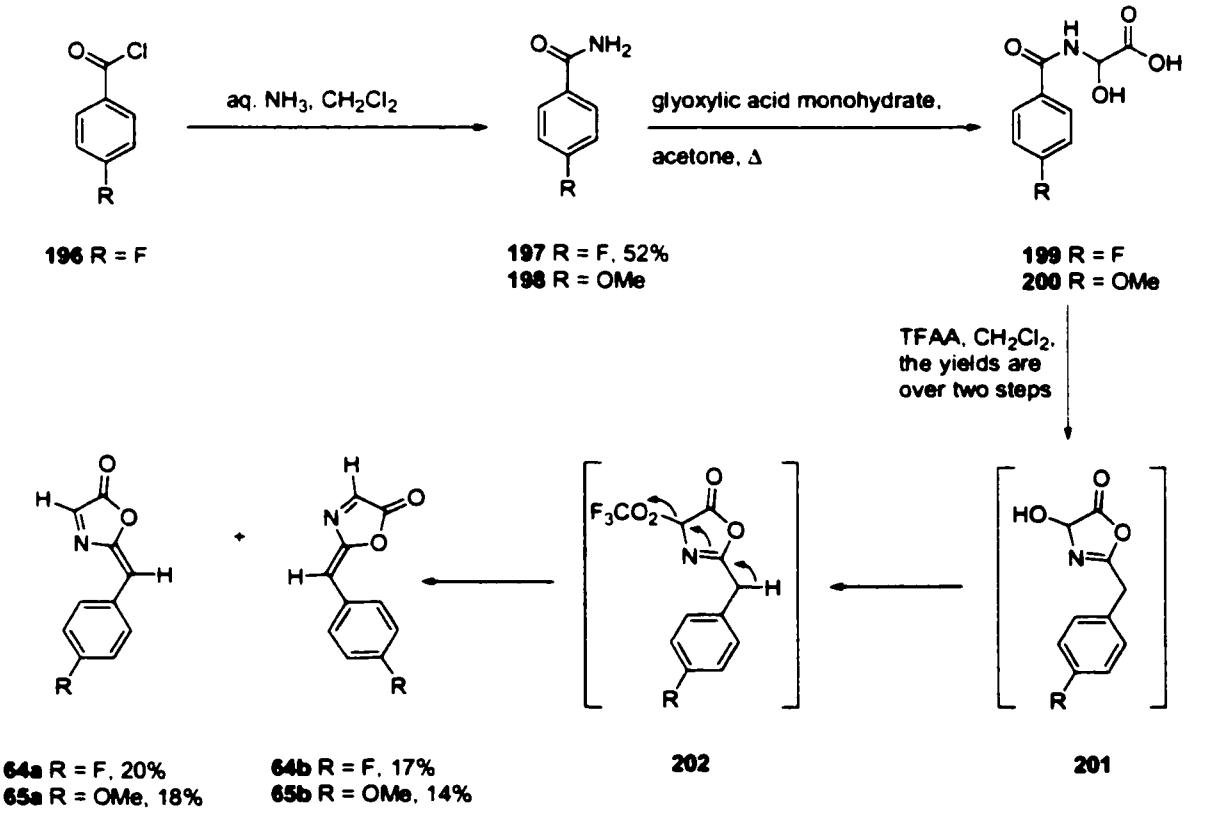
194 R = $(CH_2)_3CONH_2$
195 R = $(CH_2)_2CONH_2$

62a R = $(CH_2)_3CONH_2$, 7% **62b** R = $(CH_2)_3CONH_2$, 11%
63a R = $(CH_2)_2CONH_2$, 9% **63b** R = $(CH_2)_2CONH_2$, 10%

The results demonstrate that the monophenyl glycine pseudoxazolones are the most potent in this series. Next, the effect of different substituents on the phenyl ring e.g. electron withdrawing (fluoro) **64a,b** and electron donating (methoxy) **65a,b** was evaluated. It appeared that systems with electron demand would influence inhibition and hydrolytic stability in basic aqueous media. For instance, the monophenyl glycine pseudoxazolones **65a,b** with a methoxy group conjugated to the imine system would be expected to possess decreased reactivity to external nucleophiles (including water), and hence enhance stability in aqueous media. DL-2-chloro-2-*para*-methoxyphenylacetyl chloride is expected to be unstable because the electron rich benzene would enhance loss of the chlorine. Because of this limitation, a new synthetic pathway for the pseudoxazolones **64a,b** and **65a,b** was devised. It appeared that a hydroxyl group at the

α-position of the glycine (for example, **199** and **200**, Scheme 40) could be converted to a leaving group, which after cyclization would eliminate to produce the conjugated imine system. Thus, treatment of 4-fluorophenylacetyl chloride (**196**) with an aqueous solution of ammonia generates the 4-fluorophenylacetamide (**197**) (Scheme 40). This amide or the commercially available 4-methoxyphenylacetamide (**198**) can then react with glyoxylic acid[131] in refluxing acetone to give the corresponding hydroxy acids **199** and **200**. Cyclization with TFAA presumably proceeds through the intermediate **201**, followed by elimination of the activated α-hydroxyl group in **202**, to furnish the desired *p*-fluoro **64a**, **64b** and the *p*-methoxy **65a**, **65b** derivatives, respectively.

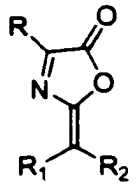
Scheme 40



Incubations of these compounds with HAV 3C proteinase display acceptable time-dependent inhibition with IC_{50} values of 3 and 4 μM for the *p*-fluoro compounds **64a** and **64b**, respectively. The *p*-methoxy derivatives **65a** and **65b** are slightly less potent with IC_{50} values of 20 and 10 μM, respectively, possibly due to the decreased reactivity of the imine system because of the greater electron density. The *p*-methoxy derivatives **65a** and **65b** show marginal improvement in hydrolytic stability ($t_{1/2}$ 21 and 39 min) compared to the *p*-fluoro derivatives **64a** and **64b** ($t_{1/2}$ 17 and 19 min). However, this does not represent a very significant increase in stability as compared to the parent monophenyl glycine pseudoxazolones **60a** and **60b** ($t_{1/2}$ 28 and 16 min).

All the pseudoxazolones were also tested against HRV 3C proteinase from the serotype 14. The enzyme (~0.4 μM) activity is conveniently monitored using a continuous UV assay in the presence of EALFQ-pNA (~250 μM) as the substrate.[67] The results are presented in Table 2. The same trend of inhibition is observed for both HAV and HRV 3C proteinases. Although the pseudoxazolones appear to show better inhibition against HAV rather than HRV 3C proteinase, this may be due to the higher concentration of enzyme used in the latter case (~0.4 μM) compared to the former (~0.1 μM). Higher concentrations of enzyme were needed because of the limitation of the continuous UV assay which measures the *p*-nitroanilide released from the substrate upon enzymatic cleavage. The *p*-methoxy pseudoxazolones **65a** and **65b** were not tested against HRV because of their strong absorption at 405 nm, the wavelength used to detect the formation of *p*-nitroanilide.

Table 2 Inhibition data for HAV 3C, HRV 3C and half-life of the pseudoxazolones in phosphate buffer at pH 7.5



Compounds	Substituents	HAV 3C IC_{50} (μM)[a]	HRV 3C IC_{50} (μM)[b]	Aqueous Hydrolysis $t_{1/2}$(min)[c]
58	R = H, R_1 = R_2 = Ph	33	38	40
59	R = Me, R_1 = R_2 = Ph	>100	>100	15
193	R = Me, R_1 = R_2 = Fluorenone	68	>100	68
60a	R = H, R_1 = Ph, R_2 = H	6	16	28
60b	R = H, R_1 = H, R_2 = Ph	4	17	16
61a	R = Me, R_1 = Ph, R_2 = H	26	43	17
61b	R = Me, R_1 = H, R_2 = Ph	>100	>100	14
62a	R = $(CH_2)_3CONH_2$, R_1 = Ph, R_2 = H	43	>100	29
62b	R = $(CH_2)_3CONH_2$, R_1 = H, R_2 = Ph	>100	>100	13
63a	R = $(CH_2)_2CONH_2$, R_1 = Ph, R_2 = H	47	>100	24
63b	R = $(CH_2)_2CONH_2$, R_1 = H, R_2 = Ph	>100	>100	12
64a	R = H, R_1 = *p*F-Ph, R_2 = H	3	11	17
64b	R = H, R_1 = H, R_2 = *p*F-Ph	4	13	19
65a	R = H, R_1 = *p*MeO-Ph, R_2 = H	20	n.d.	21
65b	R = H, R_1 = H, R_2 = *p*MeO-Ph	10	n.d.	39

[a]*Fluorometric assay conditions*: 0.1 μM HAV 3C, 10 μM Dabcyl-GLRTQSFS-Edans, 2 mM EDTA, 0.1 mg/mL BSA, 100 mM KH_2PO_4/K_2HPO_4, pH 7.5, 1% DMF, 5 min pre-incubation of the enzyme with inhibitor. [b]*Continuous UV assay conditions*: 0.4 μM HRV 3C, 250 μM EALFQ-pNA, 50 mM Hepes, 150 mM NaCl, 1 mM EDTA, pH 7.5, 1% DMF, 5 min pre-incubation of the enzyme with inhibitor. [c]Pseudoxazolone hydrolysis half-life in phosphate buffer at pH 7.5. n.d. = not determined.

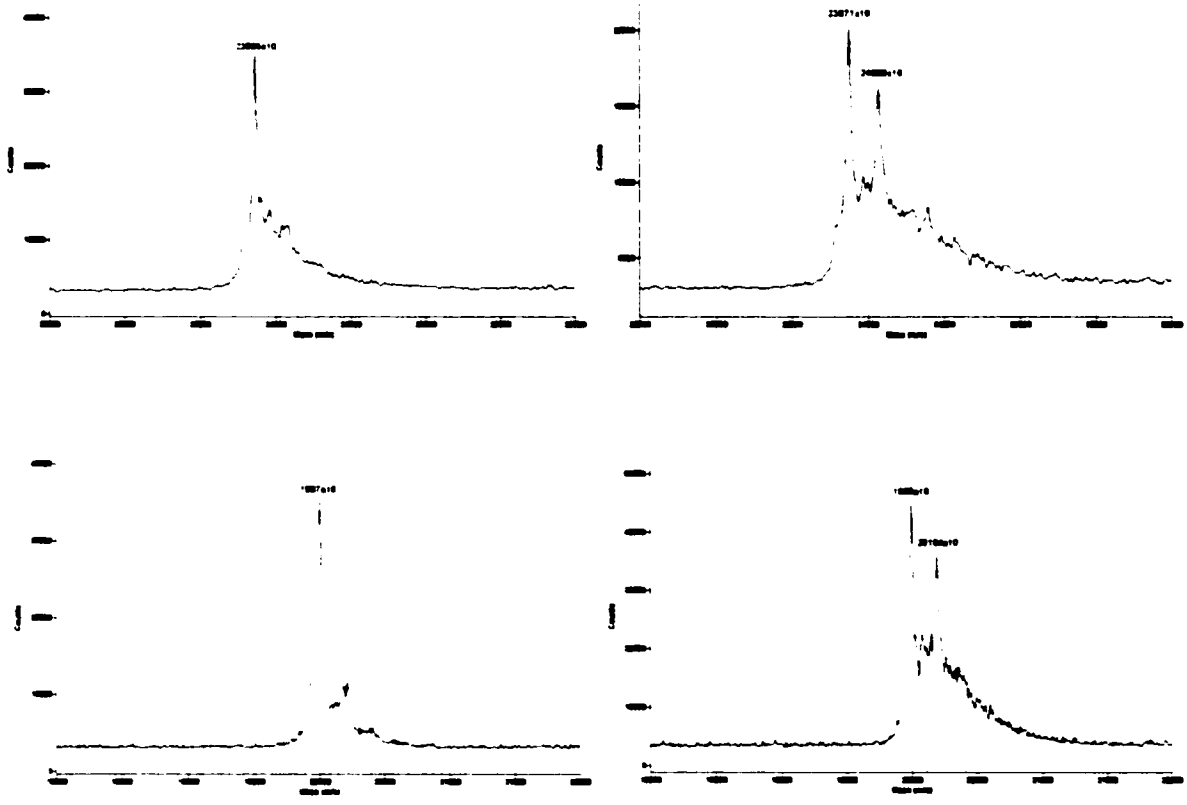
The results indicate that the glycine derived pseudoxazolones (**58, 60a,b**) are better inhibitors than the pseudoxazolones with substitution at the imine carbon (**59, 61a,b, 62a,b, 63a,b**). The monophenyl pseudoxazolones (**60a, 61a,b 62a,b 63a,b**) are more potent than the diphenyl pseudoxazolones (**58, 59**). The *E* monophenyl derivatives (**61a, 62a, 63a**) are generally better inhibitors than the *Z* monophenyl pseudoxazolones (**61b, 62b, 63b**) when there is substitution at the imine carbon. Although no significant increase in stability is observed for the compounds **64a,b** and **65a,b**, these results demonstrate that additional functionality on the phenyl ring can be tolerated with no drastic loss of inhibition. Such systems with appropriate functional groups as recognition sites can potentially be applied to other cysteine proteinases.

4.1 Mode of Inhibition of the Pseudoxazolones

As seen in the previous section, the time-dependent inhibition displayed by the pseudoxazolones is presumably due to an irreversible modification of the enzyme likely by addition of the enzyme's cysteine thiolate to the imine of the pseudoxazolone. This is supported by the observation that **60a** and **60b** show complete loss of inhibitory activity for HAV 3C proteinase in the presence of a 10 fold excess of dithiothreitol (DTT). This suggests that the added thiol (in DTT) reacts rapidly with the pseudoxazolones. Further insight as to the nature the covalent adduct could be obtained by subjecting the enzyme-inhibitor complex to analysis by mass spectrometry. Both HAV and HRV 3C proteinases were incubated with a 10-fold excess of **60b** in DMF for 1 h. The mixtures were dialysed against deionised water (to remove any excess inhibitor and DMF) before analysis by MALDI mass spectrometry. The HAV 3C mass spectrum shows peaks at 23871(±10) for

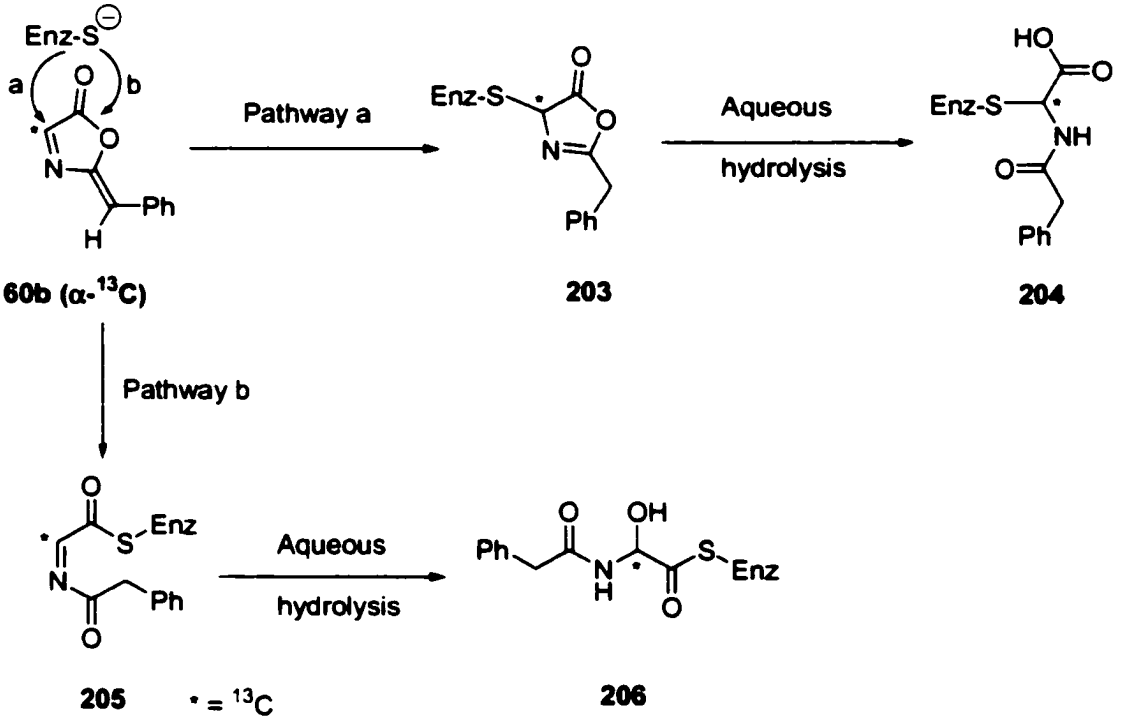
the enzyme alone and 24066(±10) for the enzyme-inhibitor complex (Figure 47). This mass difference of ~195 represents the incorporation of the inhibitor **60b** (MW 173) plus water (MW 18). In a control experiment, the HAV 3C proteinase was incubated with DMF containing no inhibitor and after dialysis as described before, the mass spectrum shows a peak at 23865(±10) for the uninhibited enzyme (Figure 47). Similarly, the HRV 3C proteinase gives a mass of 19995(±10) for the enzyme alone and 20184(±10) for the enzyme-inhibitor complex with a mass difference of ~189 corresponding to the addition of inhibitor **60b** plus water (Figure 47). These results are consistent with the formation of a covalent adduct with the enzyme and the inhibitor **60b**.

Figure 47 MALDI mass spectra of HAV 3C + DMF (top-left), HAV 3C + **60b** in DMF (top-right), HRV 3C + DMF (bottom-left), and HRV 3C + **60b** in DMF (bottom-right)



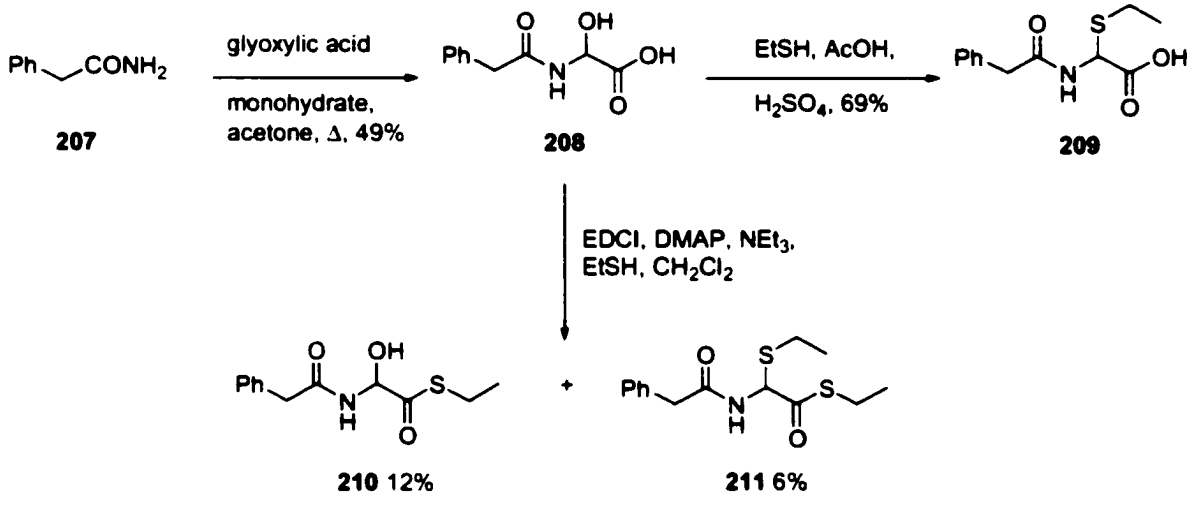
Possible modes of inactivation of cysteine proteinases are shown in Figure 48. Addition of the enzyme thiolate at the imine position of the inhibitor **60b** (pathway a) would form a thioether-lactone species **203** which could add water to generate the thioether acid **204**. An alternative is nucleophilic addition of the enzyme thiolate at the carbonyl of the oxazolone ring system which is also a reactive site. This reaction would produce the thioester **205** (pathway b) which could subsequently add water at the imine position to form the hydroxy-thioester **206**. Both pathways would fit the observed mass of the enzyme/inhibitor complex.

Figure 48 Possible modes of inactivation of α ^{13}C-labelled pseudoxazolone **60b** by HAV or HRV 3C proteinases



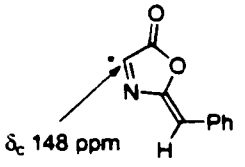
To determine the mode of inhibition and the type of enzyme-inhibitor adduct, pseudoxazolone **60b(α-^{13}C)** was prepared from glycine-2-^{13}C using a similar procedure as described in Scheme 38. The enzyme-inhibitor complex was subsequently subjected to gHMQC NMR spectroscopy to establish whether the adduct is a thioether (i.e. **204**) or a thioester (i.e. **206**). In addition, model compounds such as the hydroxyacid **208**, thioether **209**, and thioester **210** were prepared (Scheme 41) to provide models for chemical shift analysis. Reaction of phenylacetamide (**207**) with glyoxylic acid[131] gives the hydroxyacid **208**, which upon reaction with ethyl thiol under acidic conditions affords the thioether **209**.[132] Alternatively, activation of the hydroxyacid **208** with EDCI followed by coupling with ethyl thiol, produces the hydroxythioester **210** and the thioester **211** in low yield.

Scheme 41

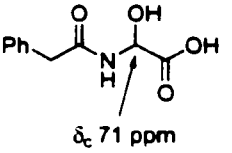


The chemical shifts for the α-carbon of **60b(α-^{13}C)**, **208**, **209** and **210** (Figure 49) were determined by gHMQC in deuterated DMF-d_7 solvent and phosphate buffer Na_3PO_4/D_2O at pD = 7.5 at 600 MHz.

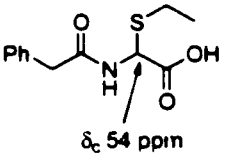
Figure 49 α-Carbon chemical shifts for model compounds in DMF-d_7 and Na_3PO_4/D_2O at pD = 7.5. gHMQC spectra were acquired on a 600 MHz Varian Inova spectrometer



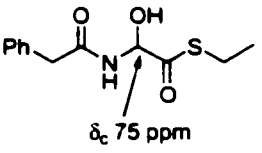
60b (α-^{13}C)



208



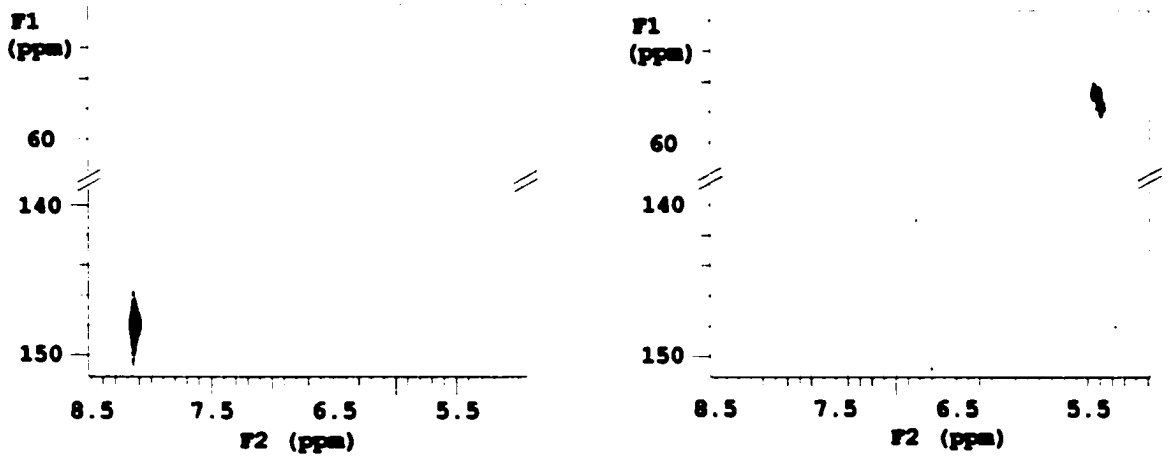
209



210

The gHMQC spectrum of the pseudoxazolone **60b(α-^{13}C)** alone shows a cross peak at δ_c 148 ppm for the α-labelled carbon (Figure 50). Upon formation of the enzyme-inhibitor [HAV3C-**60b(α-^{13}C)**] complex the gHMQC spectrum shows a new cross peak at δ_c 57 ppm for the α-carbon. This corresponds to the formation of a thioether, as opposed to a thioester, based on the α-carbon chemical shift observed for the thioether **209**, (δ_c 54 ppm) under identical conditions. These results suggest the hypothesis that the enzyme thiolate reacts with the pseudoxazolones at the imine position rather than the carbonyl group (pathway a in Figure 48).

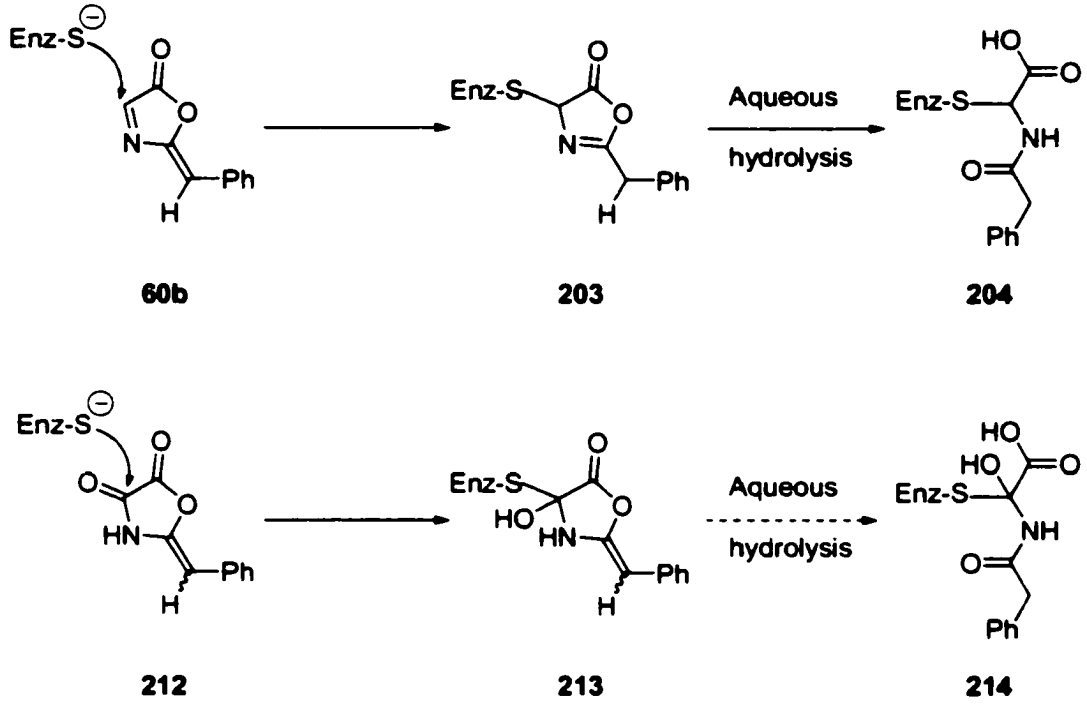
Figure 50 Expansions of the gHMQC spectra of the inhibitor **60b(α-^{13}C)** alone (left) and the enzyme-inhibitor complex [HAV3C-**60b(α-^{13}C)**] (right) in DMF-d_7 and Na_3PO_4/D_2O, pD = 7.5 at 600 MHz



4.2 Oxazolidine-4,5-dione - Target I

The monophenyl oxazolidine-4,5-dione **212** (Figure 51) is structurally similar to the corresponding monophenyl pseudoxazolone **60b** except that the former has a carbonyl functionality instead of an imine. As seen in the previous section, the HAV 3C proteinase thiolate adds to the imine carbon of the oxazolone ring followed by aqueous hydrolysis to generate the enzyme-inhibitor complex **214**. Based on these observations, it seemed that oxazolidine-4,5-dione e.g. **212** might inhibit the enzyme *via* a similar mechanistic pathway (Figure 51). The enzyme thiolate is expected to react at the carbonyl carbonyl of the oxazolidine-4,5-dione to form a tetrahedral intermediate **213**, which could presumably be hydrolyzed under aqueous conditions to generate an enzyme-inhibitor complex **214**.

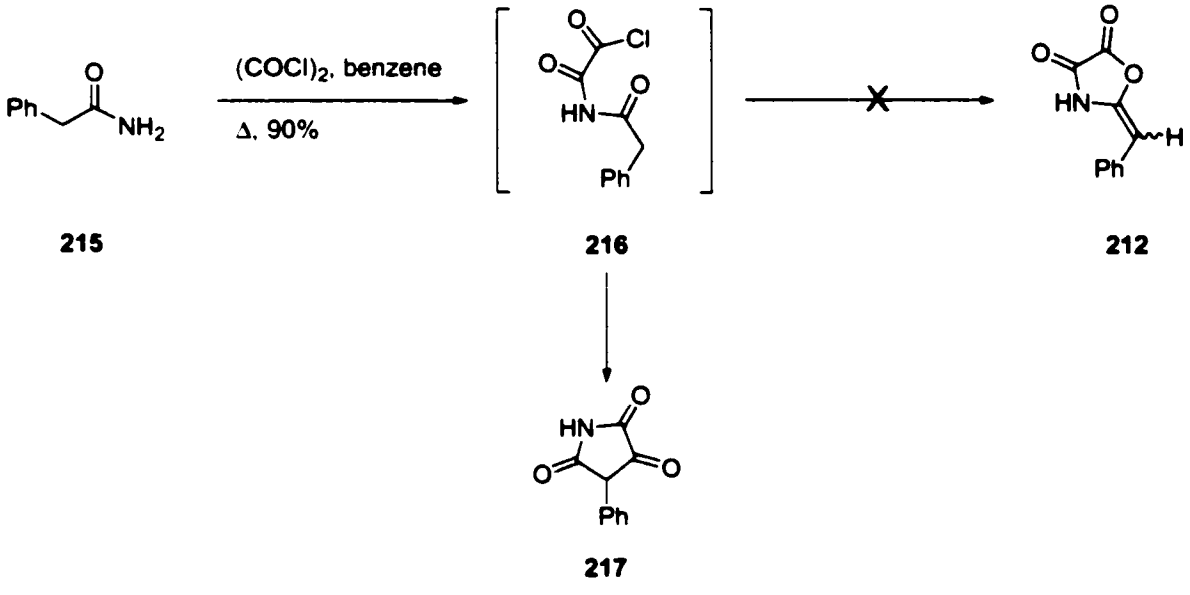
Figure 51 Comparison and rationale for inhibition of HAV 3C proteinase by pseudoxazolones and oxazolidine-4,5-dione



Although **212** has been reported in the literature,[133] no biological investigation has been performed with such a system. Following the procedure of Skinner,[133] phenylacetamide (**215**) is treated with oxalyl chloride in refluxing benzene (Scheme 42). However, the desired product **212** was not obtained and instead the pyrrolidinetrione **217** was isolated. This compound is probably formed by enolization of the benzylic proton of intermediate **216** followed by addition of the enolate to the acyl chloride. Skinner[133] reported that the pyrrolidinetrione **217** can be prepared from **212** by heating to reflux in ethanol. Several attempts at different temperatures (rt to 60 °C) and in various solvents

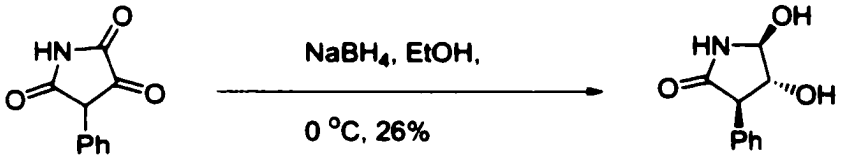
(anhydrous benzene and toluene) with **215** did not generate compound **212**. The melting point of pyrrolidinetrione **217** (215-216 °C) is close to its reported literature[133] melting point (217 °C).

Scheme 42



Since these studies were done in the early 1950's with insufficient spectroscopic data to conclusively prove the structure of **217**, we decided to subject the pyrrolidinetrione **217** to X-ray crystallography, but the crystals failed to diffract well. In order to determine the structure unequivocally, **217** was reduced to the racemic alcohol **218** with sodium borohydride (Scheme 43).

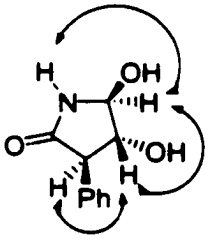
Scheme 43



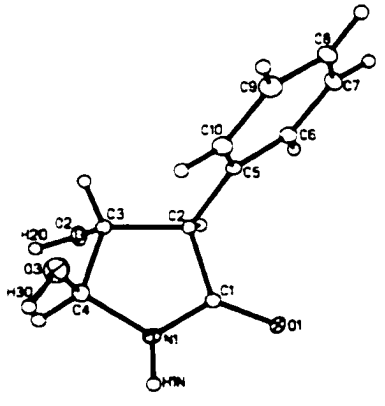
217 *rac* **218**

The structure of alcohol **218** was confirmed both by ^{1}H-^{1}H COSY NMR and X-ray crystallography (Figure 52). A key feature in the ^{1}H-^{1}H COSY spectrum of **218** is the correlation between the adjacent ring vicinal protons (Figure 52).

Figure 52 Key correlations for ^{1}H-^{1}H COSY of alcohol **218** and its X-ray crystal structure

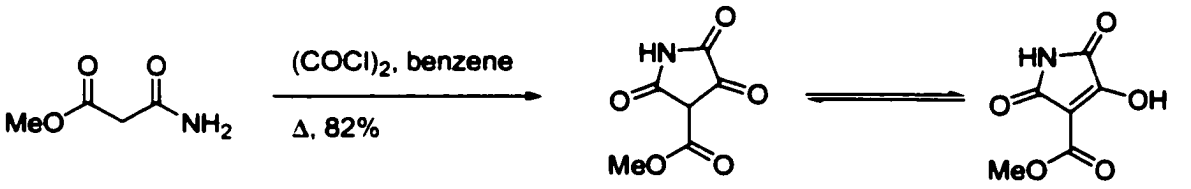


rac **218**



To examine the effect of other structural variants on the inhibition of HAV 3C proteinase, the malonamide methyl ester **219** was reacted with oxalyl chloride (Scheme 44), and as observed in the previous case, the pyrrolidinetrione methyl ester **220** was obtained which, presumably exists in equilibrium with its tautomer **221**.

Scheme 44



219 **220** **221**

Enzymatic testing of pyrrolidinetrione **217** and **220** at concentrations of 100 μM against HAV 3C proteinase displayed modest inhibition of 52% and no inhibition, respectively. Although **217** is not a useful inhibitor of this enzyme, such a system may potentially inhibit other proteinases.

Summary and Future work

Several inhibitors have been synthesized and tested against HAV 3C proteinase to identify potential therapeutic leads against picornaviral infections and to gain additional knowledge concerning picornaviral proteinase inhibition. A series of ketone-derived *N,N*-dimethyl glutamine analogues **22-27** were prepared based on the substrate specificity of HAV 3C proteinase at P_1 (Gln) and/or P_2' (Phe). Although these compounds possess the essential *N,N*-dimethyl glutamine at P_1 and a ketone "warhead" mimicking the scissile amide carbonyl, they display poor inhibitory activity against HAV 3C proteinase. Increasing ketone electrophilicity *via* intramolecular hydrogen bonding together with restricted conformational mobility (e.g. phthalhydrazido analogue **28**) significantly improves reversible inhibition (IC_{50} of 89 μM). In addition, **34**, which incorporates the P_4 leucine, displays additional seven-fold increase in reversible inhibition (K_i = 9 μM). Furthermore, analogues lacking the phenyl ring namely **32, 33** or the N-H functionality **26, 27** of the phthalhydrazido **28** display poor inhibition of HAV 3C proteinase, indicating that a combination of these structural features is important for successful binding. Other analogues with a reactive ketone functionality such as the 3-azetidinone **35**, α- **37**, and α, β-ketoamides **38**, proved to be poor inhibitors.

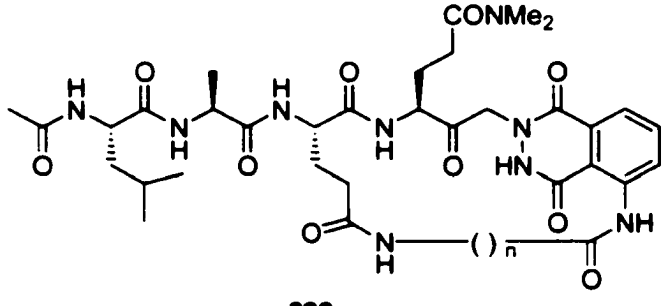
Macrocyclic peptides having the P_1' and P_2 residues linked through a disulfide **39-44**, or linked via an ethylene bridge, **45**, were prepared, but each displayed poor solubility properties in aqueous solution. Introduction of a serine residue at P_3 or a free C-terminal group improved the solubility, but these compounds remained poor inhibitors of the HAV 3C proteinase. Moreover, incorporation of additional recognition residues (P_4 leucine and P_2' phenylalanine), failed to improve inhibition of the HAV 3C proteinase.

β-Lactone analogues **47**, **52a,b**, and **53** show no significant inhibition of the HAV 3C proteinase. This indicates that the α-nitrogen functionality is important for binding. Several 4-membered ring analogues with a *N*-Cbz side chain **54-57** were prepared to probe the importance of the β-lactone ring, but these compounds display no significant inhibition of the HAV 3C proteinase.

The pseudoxazolones have been identified as a new class of cysteine proteinase inhibitors. Several monophenyl, **60**, **61**, and diphenyl, **58**, **59**, pseudoxazolones derived from glycine or alanine were found to display potent time-dependent inhibition in the micromolar range (IC_{50} 4-38 μM). The monophenyl pseudoxazolone derivatives were more potent than the diphenyl analogues and the pseudoxazolones derived from glycine are, in turn, more potent than those based on alanine. Elucidation of the X-ray crystal structures of the monophenyl pseudoxazolones **60b** and **61a** has enabled us to unambiguously identify the *E* and *Z* geometrical isomers of the monophenyl pseudoxazolones and to assign their olefinic proton (^1H) chemical shift. The *E* isomers are generally more potent inhibitors than the *Z* isomers. Incorporation of a glutamine side chain on the pseudoxazolones **62, 63** that potentially mimic the P_1 glutamine of the substrate failed to improve inhibition. A new pathway to access substituted phenyl derivatives of the pseudoxazolones **64, 65** was devised. Inhibition studies indicate that the pseudoxazolones have similar potency for both HAV 3C and HRV 3C proteinases. Further studies using mass spectrometry, and gHMQC spectroscopy with ^{13}C labeled **60b(α-^{13}C)** pseudoxazolone demonstrate that the enzyme forms a covalent thioether adduct with the inhibitor. However, a significant limitation of the pseudoxazolones as *in vivo* inhibitors is their low stability in phosphate buffer at pH 7.5 ($t_{1/2}$ = 12 to 68 min).

Since the phthalhydrazido tetrapeptide inhibitor **34** displays potent reversible inhibition of HAV 3C proteinase (K_i = 9 μM), a cyclic analogue such as **222** would be expected to display even better inhibition due to its restricted conformational mobility. Modeling studies of analogue **222** in the active site of HAV 3C should determine the appropriate ring size of this phthalhydrazido macrocyclic peptide.

Figure 53 Potential phthalhydrazido macrocyclic peptide inhibitor of the HAV 3C proteinase



222

EXPERIMENTAL PROCEDURES

General Procedures

All non-aqueous reactions involving air or moisture sensitive reactants were performed 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.[134] Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled over sodium under an argon atmosphere. Acetonitrile, dichloromethane, triethylamine and pyridine were distilled over calcium hydride. *N,N*-Dimethylformamide (DMF) was distilled *in vacuo* over calcium hydride. 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 HCl, $NaHCO_3$, KOH and NaOH refer to aqueous solutions. Solvent evaporation was performed under reduced pressure below 40 °C using a Büchi rotary evaporator, followed by evacuation (<0.1 torr) to constant sample weight. Isotopically labeled glycine-2-^{13}C (99%) was purchased from Cambridge Isotope Laboratories (Andover MA) and was used directly without further purification.

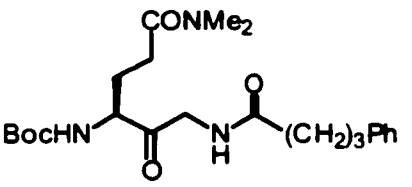
Reactions and fractions from column chromatography were monitored and analyzed by thin-layer chromatography (TLC) using glass plates with a UV fluorescent indicator (silica gel, Merck 60 F_{254}; Merck RP-8 and Merck RP-18 F_{254}). One or more of the following methods were used for visualization: UV fluorescence, iodine staining, phosphomolybdic acid/ceric sulfate/sulfuric acid (10 g:1.25 g:8%, 250 mL) spray for

general hydrocarbons, ninhydrin/methanol (1 g:100 mL) spray for amines; bromocresol green/ethanol/sodium hydroxide (0.04 g:100 mL:0.1 N added until the blue color appears) spray for carboxylic acids. Flash column chromatography was performed by the method of Still[135] using 230-400 mesh silica (Merck, silica gel). HPLC separations were performed on either a Rainin Dynamax instrument equipped with a variable wavelength model UV-1 detector, a solvent delivery system model SD-200, and a Rheodyne injector or the Beckman System instrument equipped with a 166 variable wavelength UV detector and an Altex 210A injector. HPLC separations were monitored at a wavelength of 219 nm. The columns used were Waters C_{18} Resolve 10 μm, Bondpak C_8 or Bondpak C_{18}. Sample solutions were filtered through a 2 μm filter before injection. HPLC grade acetonitrile (190 nm UV cutoff) were obtained from Fisher (Fair Lawn, NJ). All HPLC solvents were filtered with a Millipore vacuum filtration system before use.

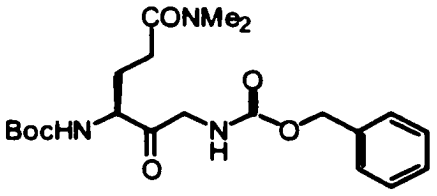
Melting points were determined on a Thomas-Hoover oil immersion apparatus using open capillary tubes and are uncorrected. Optical rotations were measured on a Perkin Elmer 241 polarimeter with a microcell (10.00 cm, 0.9 mL) at ambient temperature and are reported in units of 10^{-1} deg cm^2 g^{-1}. All specific rotations reported were referenced against air and were measured at the sodium D line. Infrared spectra (IR) were recorded on a Nicolet Magna 750 FT-IR spectrometer as either cast or microscope. Cast refers to the evaporation of a solution on a NaCl plate. Mass spectra (MS) were recorded on a Kratos AEIMS-50 high resolution mass spectrometer (HRMS), electron impact ionization (EI), MS-12 chemical ionization ((CI), NH_3), MS-9 fast atom bombardment ((FAB), argon), Voyager Elite MALDI-TOF instrument, Applied Biosystems (sinapinic acid as the matrix) and Micromass ZabSpec Hybrid Sector-TOF

positive mode electrospray ionization ((ES), 0.5% solution of formic acid in acetonitrile: H_2O (1:1)) instruments. Cleland matrix was used in 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 Bruker AM-300 and Inova Varian 300, 400, 500 and 600 MHz instruments. ^{1}H NMR chemical shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS) using the residual solvent resonance as the reference: $CDCl_3$, δ 7.24; CD_2Cl_2, δ 5.32; CD_3OD, δ 3.30; *N,N*-$(CD_3)_2NCDO$, δ 2.74; $(CD_3)_2SO$, δ 2.49; $(CD_3)_2CO$, δ 2.04; CD_3CN, δ 1.93 and C_4D_8O, δ 1.73. The coupling constants reported are within an error range of 0.2-0.4 Hz. ^{13}C NMR shifts are reported relative to: $CDCl_3$, δ 77.0; C_4D_8O, δ 67.4; CD_2Cl_2, δ 53.8; CD_3OD, δ 49.0; $(CD_3)_2CO$, δ 39.5; *N,N*-$(CD_3)_2NCDO$, δ 30.1; $(CD_3)_2CO$, δ 29.8 and CD_3CN, δ 1.3. Selective homonuclear decoupling, attached proton test (APT), ^{1}H-^{1}H, ^{1}H-^{13}C, nuclear Overhauser effect (NOE) correlations, gradient heteronuclear multiple quantum coherence (gHMQC) and gradient heteronuclear multiple bond coherence (gHMBC) experiments were occasionally used for signal assignments. ^{1}H NMR data are reported 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. Where appropriate, the multiplicity is preceded by br, indicating that the signal was broad. All literature compounds had IR, ^{1}H NMR and mass spectra consistent with the reported data. Unless stated otherwise, peptides were prepared using a double coupling procedure with standard Fmoc[97] chemistry on Rink[98] amide or Wang[100] resin, with HBTU[99] as the coupling reagent

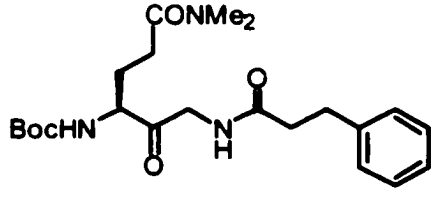
(Advanced ChemTech; SA5030) on a Rainin peptide synthesizer (Protein Technologies PS3).



(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-(4-phenyl-butyrylamino)hexanamide (22). To a solution of alcohol **75** (80 mg, 18.5 mmol) in CH_2Cl_2 (5 mL) at rt was added Dess-Martin periodinane[64] (199 mg, 46.2 mmol). The resulting mixture was stirred for 4 h after which it was quenched with 1.3 N NaOH (5 mL) and stirred for a further 15 min. The mixture was diluted with H_2O (10 mL), extracted with EtOAc (3 x 5 mL) and the combined organic layers dried over $MgSO_4$. The solvent was removed *in vacuo* and the residual oil was purified by column chromatography (SiO_2, 5% MeOH/EtOAc) to provide the title compound as a colorless oil (61 mg, 76%); $[\alpha]_D^{26}$ +2.1° (*c* 1.4, $CHCl_3$); IR ($CHCl_3$ cast) 3312, 2927, 1701, 1636, 1497 cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2) δ 1.40 (s, 9H, $(C\underline{H}_3)_3$), 1.80-2.00 (m, 3H, $CHC\underline{H}_2$, $CH_2C\underline{H}_2$), 2.00-2.18 (m, 1H, $CHC\underline{H}_2$), 2.22 (t, *J* = 8.2 Hz, 2H, $C\underline{H}_2Ph$), 2.28-2.50 (m, 2H, $C\underline{H}_2CO_2$), 2.85 (t, *J* = 7.3 Hz, 2H, $COC\underline{H}_2$), 2.90 (s, 3H, $N(C\underline{H}_3)_2$), 2.98 (s, 3H, $N(C\underline{H}_3)_2$), 4.18-4.32 (m, 3H, $C\underline{H}$, $C\underline{H}_2NH$), 5.94-5.98 (m, 1H, $N\underline{H}$), 6.38-6.44 (m, 1H, $N\underline{H}$), 7.10-7.35 (m, 5H, $\underline{Ph}$); ^{13}C NMR (75 MHz, CD_2Cl_2)(mixture of rotamers) δ 26.5, 27.6, 28.4, 29.2, 35.5, 35.7, 35.8, 37.4, 47.2, 58.8, 80.1, 126.2, 128.7, 128.8, 142.2, 149.6, 156.1, 172.5, 173.3, 206.1; HRMS (ES) Calcd for $C_{23}H_{35}N_3O_5Na$ (MNa^+) 456.2474, found 456.2472.

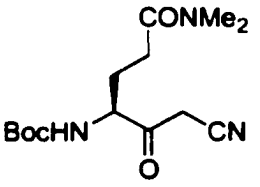


(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-6-(benzyloxycarbonyl-amino)-5-oxo-hexanamide (23). A solution of alcohol **76** (68 mg, 0.16 mmol) in CH_2Cl_2 (4 mL) was treated with Dess-Martin periodinane[64] (103 mg, 0.24 mmol) and the reaction mixture was stirred for 2 h. Purification by column chromatography (SiO_2, 5% MeOH/EtOAc) gave the title product as a colorless oil (48.8 mg, 72%); $[\alpha]_D^{25}$ -2.6° (*c* 1.3, $CHCl_3$); IR ($CHCl_3$ cast) 3318, 1712, 1635, 1507 cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2) δ 1.41 (s, 9H, $(C\underline{H}_3)_3$), 1.82-1.98 (m, 1H, $CHC\underline{H}_2$), 2.01-2.20 (m, 1H, $CHC\underline{H}_2$), 2.20-2.50 (m, 2H, $C\underline{H}_2CO$), 2.92 (s, 3H, $N(C\underline{H}_3)_2$), 2.98 (s, 3H, $N(C\underline{H}_3)_2$), 4.15-4.40 (m, 3H, $C\underline{H}$, $C\underline{H}_2NH$), 5.11 (s, 2H, $C\underline{H}_2Ph$), 5.54 (br s, 1H, $N\underline{H}$), 5.90 (br s, 1H, $N\underline{H}$), 7.20-7.40 (m, 5H, $\underline{Ph}$); ^{13}C NMR (75 MHz, CD_2Cl_2)(mixture of rotamers) δ 26.4, 28.4, 29.2, 35.2, 48.7, 58.3, 67.1, 80.1, 128.3, 128.4, 128.8, 129.0, 129.1, 137.2, 156.1, 156.6, 172.5, 206.1; HRMS (ES) Calcd for $C_{21}H_{31}N_3O_6Na$ (MNa^+) 444.2110, found 444.2114.



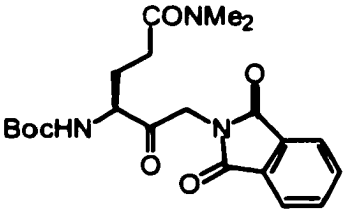
(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-6-(3-propionylamino)-5-oxo-hexanamide (24). A solution of alcohol **77** (37.2 mg, 0.08 mmol) in CH_2Cl_2 (4 mL) was treated with Dess-Martin periodinane[64] (56.9 mg, 0.13 mmol) and the reaction mixture was stirred 4 h. Purification by column chromatography (SiO_2, 100% EtOAc)

gave the title product as a colorless oil (35.1 mg, 95%); $[\alpha]_D^{26}$ -2.3° (*c* 0.7, $CHCl_3$); IR ($CHCl_3$ cast) 3301, 2926, 1709, 1682, 1520, 1471 cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2) δ 1.41 (s, 9H, $(C\underline{H}_3)_3$), 1.80-2.20 (m, 2H, $CHC\underline{H}_2$), 2.31-2.52 (m, 2H, $C\underline{H}_2CO$), 2.55 (t, *J* = 8.2 Hz, 2H, $C\underline{H}_2Ph$), 2.88 (s, 3H, $N(C\underline{H}_3)_2$), 2.94 (s, 3H, $N(C\underline{H}_3)_2$), 2.92-3.20 (m, 2H, $COC\underline{H}_2$), 4.12-4.32 (m, 3H, $C\underline{H}$, $C\underline{H}_2NH$), 5.84 (br s, 1H, $N\underline{H}$), 6.35 (br s, 1H, $N\underline{H}$), 7.14-7.40 (m, 5H, $\underline{Ph}$); ^{13}C NMR (125 MHz, CD_2Cl_2) δ 26.5, 30.3, 39.2, 31.7, 35.7, 37.4, 38.1, 58.3, 80.1, 129.5, 126.8, 128.7, 128.9, 141.5, 156.2, 172.5, 189.8, 206.1; LRMS (ES) for $C_{22}H_{33}O_5N_3$ *m/z* (relative intensity) 420.2 (MH^+, 100%).

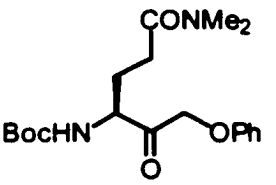


(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-cyano-hexanamide (25). The benzyl ester **80** (690 mg, 1.61 mmol) was dissolved in MeOH (60 mL) in the presence of 10% Pd/C (70 mg, 10% w/w). The suspension was stirred under a hydrogen atmosphere until the uptake of hydrogen ceased (approximately 4 h). Filtration through celite, followed by removal of the solvent *in vacuo*, afforded the crude product which was purified by column chromatography (SiO_2, 10% MeOH/EtOAc) to give the title compound as an oil (211 mg, 44%); $[\alpha]_D^{26}$ -0.7° (*c* 3.0, $CHCl_3$); IR (CH_2Cl_2 cast) ^1H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H, $(C\underline{H}_3)_3$), 1.95-2.18 (m, 2H, $CHC\underline{H}_2$), 2.22-2.44 (m, 2H, $C\underline{H}_2CO$), 2.88 (s, 3H, $N(C\underline{H}_3)_2$), 2.95 (s, 3H, $N(C\underline{H}_3)_2$), 3.74 (s, 2H, $C\underline{H}_2CN$), 4.18-4.22 (m, 1H, $C\underline{H}$), 5.98 (d, *J* = 5.9 Hz, 1H, $N\underline{H}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.5,

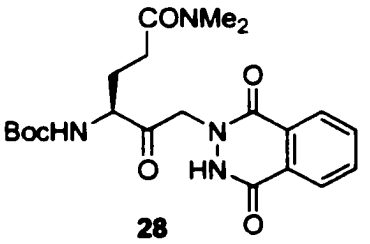
28.3, 28.7, 29.5, 35.6, 37.2, 59.2, 80.5, 113.8, 155.9, 172.0, 198.2; HRMS (EI) Calcd for $C_{14}H_{23}N_3O_4$ (M^+) 297.1689, found 297.1699.



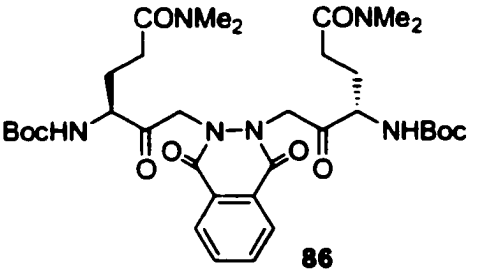
(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-(*N*-phthalimido)-hexanamide (26). To a solution of bromoketone **82** (400 mg, 1.14 mmol) in DMF (10 mL) was added potassium phthalimide (253 mg, 1.37 mmol). The reaction mixture was heated at 60 °C for 6 h after which the solvent was removed *in vacuo* and the residue diluted with H_2O (20 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over $MgSO_4$ and the solvent evaporated *in vacuo*. Purification of the crude product by column chromatography (SiO_2, 5% MeOH/EtOAc) followed by recrystallization from CH_2Cl_2/Et_2O gave **26** as a white solid (351 mg, 74%); mp 127-128 °C; $[\alpha]_D^{26}$ −5.0° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3289, 2930, 1745, 1720, 1623, 1524 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.42 (s, 9H, $(C\underline{H}_3)_3$), 1.92-2.05 (m, 1H, $CHC\underline{H}_2$), 2.21-2.30 (m, 1H, $CHC\underline{H}_2$), 2.36 (ddd, J = 16.5, 7.0, 6.1 Hz, 1H, $C\underline{H}_2CO$), 2.51 (ddd, J = 16.4, 7.0, 6.1 Hz, $C\underline{H}_2CO$), 2.94 (s, 3H, $N(C\underline{H}_3)_2$), 2.98 (s, 3H, $N(C\underline{H}_3)_2$), 4.39-4.48 (m, 1H, $C\underline{H}$), 4.65 (d, J = 18.4 Hz, 1H, $C\underline{H}_2N$), 4.77 (d, J = 18.1 Hz, 1H, $C\underline{H}_2N$), 5.72-5.82 (m, 1H, $N\underline{H}$), 7.64-7.72 (m, 2H, Ph), 7.79-7.85 (m, 2H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.6, 28.3, 28.8, 35.7, 44.3, 44.6, 57.7, 80.1, 123.5, 132.2, 134.1, 155.8, 167.6, 172.2, 202.4; HRMS (ES) Calcd for $C_{21}H_{27}N_3O_6Na$ (MNa^+) 440.1798 found, 440.1794; Anal. Calcd for $C_{21}H_{27}N_3O_6$: C, 60.42 H, 6.52, N, 10.07. Found C, 60.68, H, 6.56, N, 10.08.



(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-phenoxy-hexanamide (27). A solution of phenol (147 mg, 0.16 mmol) in DMF (5 mL) was treated with NaH (3.9 mg, 0.16 mmol) at rt. After 15 min, the phenoxide solution was cooled to 0 °C and the bromoketone **82** (50 mg, 0.14 mmol) added in a single portion. The reaction mixture was stirred for a further 1 h at 0 °C and then allowed to warm up to rt over 3 h. The reaction mixture was quenched with saturated aq. NH_4Cl (5 mL) and the solvent removed *in vacuo*. The residue obtained was diluted with H_2O (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over $MgSO_4$ and evaporation of the solvent *in vacuo* followed by purification by column chromatography (SiO_2, 30% hexane/EtOAc) afforded the title product as a colorless oil (361 mg, 69%); $[\alpha]_D^{25}$ +24.2° (*c* 0.6, $CHCl_3$); IR ($CHCl_3$ cast) 3304, 1745, 1703, 1634, 1599 cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2) δ 1.42 (s, 9H, $(C\underline{H}_3)_3$), 1.98 (m, 1H, $CHC\underline{H}_2$), 2.12-2.25 (m, 1H, $CHC\underline{H}_2$), 2.40 (ddd, *J* = 16.9, 7.5, 5.9 Hz, 1H, $C\underline{H}_2CO$), 2.49 (ddd, *J* = 16.7, 7.1, 6.1 Hz, 1H, $C\underline{H}_2CO$), 2.90 (s, 3H, $N(C\underline{H}_3)_2$), 2.98 (s, 3H, $N(C\underline{H}_3)_2$), 4.38-4.44 (1H, m, $C\underline{H}$), 4.85 (d, *J* = 17.2 Hz, 1H, $C\underline{H}_2O$), 4.92 (d, *J* = 17.1 Hz, 1H, $C\underline{H}_2O$, $N\underline{H}$), 5.95 (br d, *J* = 5.3 Hz, 1H), 6.90 (d, *J* = 6.6 Hz, 1H, Ph), 7.00 (t, *J* = 7.3 Hz, 2H, Ph), 7.30 (t, *J* = 7.5 Hz, 2H, Ph); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 26.2, 28.2, 29.2, 35.5, 37.2, 57.4, 71.2, 80.0, 114.8, 121.7, 129.7, 132.7, 158.1, 172.3, 205.7; HRMS (ES) Calcd for $C_{19}H_{29}N_2O_5$ (MH^+) 365.2076, found 365.2075.



28



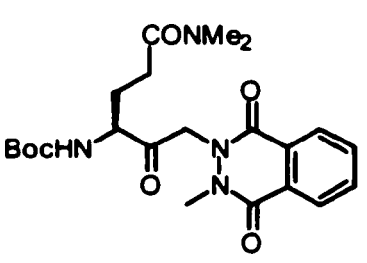
86

((4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-(2,3-dihydrophthalazin-1,4-dione)hexanamide (28) and 2,3-Bis[(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-methylhexanamide]-2,3-dihydrophthalazin-1,4-dione (86). A solution of phthalhydrazide **84** (2.0 g, 12.3 mmol) in DMF (60 mL) was treated with NaH (0.34 g, 13.6 mmol) at rt. After 2 h of stirring, the mixture was filtered and washed with anhydrous Et_2O to yield sodium phthalhydrazide **85** as a white solid (2.37 g, quantitative). To a suspension of sodium phthalhydrazide **85** (58 mg, 0.31 mmol) in DMF (10 mL) was added the bromoketone **82** (100 mg, 0.28 mmol) in small portions over 1 h. After stirring for 5 h at rt, the solvent was removed *in vacuo* and the residue diluted with H_2O (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over $MgSO_4$ and the solvent removed *in vacuo*. Purification by column chromatography (SiO_2, with a gradient elution of 20% EtOAc/hexane to 100% EtOAc) yielded **28** as a white powder (42 mg, 52%) after recrystallization from CH_2Cl_2/Et_2O, and **86** as an oil (59 mg, 29%).

Data for **28**: mp 153-154 °C; $[\alpha]_D^{26}$ −8.2° (*c* 1.0, MeOH); IR (μscope) 3273, 1735, 1702, 1650, 1618 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H, $(C\underline{H}_3)_3$), 2.00-2.40 (m, 2H, $CHC\underline{H}_2$), 2.42-2.58 (m, 2H, $C\underline{H}_2CO$), 2.92 (s, 3H, $N(C\underline{H}_3)_2$), 2.98 (s, 3H, $N(C\underline{H}_3)_2$), 4.42-4.57 (m, 1H, C<u>H</u>), 5.08 (d, *J* = 16.7 Hz, 1H, $C\underline{H}_2N$), 5.18 (d, *J* = 16.7 Hz, 1H, $C\underline{H}_2N$), 5.80 (d, *J* = 5.8 Hz, 1H, N<u>H</u>), 7.70-7.85 (m, 2H, <u>Ph</u>), 8.05 (d, *J* = 7.3 Hz, 1H, <u>Ph</u>), 8.40

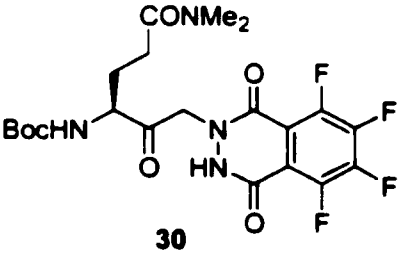
(d, J = 6.7 Hz, 1H, Ph), 10.70 (br s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.7, 28.4, 28.7, 35.8, 37.4, 57.1, 68.6, 80.2, 123.9, 124.7, 127.0, 129.0, 132.2, 133.5, 149.6, 155.8, 159.9, 172.4, 203.8; HRMS (ES) Calcd for $C_{21}H_{29}N_4O_6$ (MH^+) 433.2087, found 433.2083; Anal. Calcd for $C_{21}H_{28}N_4O_6$: C, 58.32 H, 6.53, N, 12.96. Found C, 58.03, H, 6.49, N, 12.69.

Data for **86**: $[\alpha]_D^{26}$ −20.8° (*c* 1.8, $CHCl_3$); IR ($CHCl_3$ cast) 3288, 1740, 1706, 1632, 1592 cm^{-1}; ^{1}H NMR (300 MHz, CD_2Cl_2) δ 1.42 (s, 18H, 2x(CH_3)$_3$), 1.82-2.02 (m, 2H, 2xCHCH_2), 2.20-2.38 (m, 2H, 2xCHCH_2), 2.40-2.55 (m, 4H, 2xCH_2CO), 2.96 (s, 6H, 2xN(CH_3)$_2$), 3.00 (s, 6H, 2xN(CH_3)$_2$), 4.20-4.41 (m, 2H, 2xCH), 4.92-5.21(m, 4H, 2xCH_2N), 6.28 (br d, J = 2.2 Hz, 1H, NH), 6.47 (br d, J = 1.9 Hz, 1H, NH), 7.82 (dt, J = 14.2, 6.6 Hz, 2H, Ph), 8.06 (d, J = 7.2 Hz, 1H, Ph), 8.32 (d, J = 7.2 Hz, 1H, Ph); ^{13}C NMR (75 MHz, CD_2Cl_2)(mixture of rotamers) δ 26.6, 27.2, 28.4, 29.3, 29.8, 35.8, 35.9, 37.4, 37.5, 57.2, 58.1, 58.6, 68.7, 80.0, 80.1, 124.0, 124.8, 127.4, 129.1, 132.7, 133.6, 149.2, 156.3, 159.2, 172.8, 204.2, 204.4; HRMS (ES) Calcd for $C_{34}H_{51}N_6O_{10}$ (MH^+) 703.3666, found 703.3663.

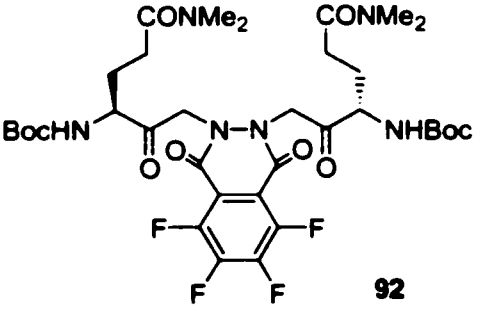


((4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-(3-methyl-2,3-dihydrophthalazin-1,4-dione)hexanamide (29). To a suspension of hydrazide **88** (150 mg, 0.85 mmol) in acetone (15 mL) was added K_2CO_3 (118 mg, 0.85 mmol) followed by

the bromoketone **82** (150 mg, 0.43 mmol) in 4 portions over 1 h. The reaction mixture was stirred for 12 h at rt, the solvent was removed *in vacuo* and the residue diluted with H_2O (10 mL), extracted with EtOAc (3 x 5 mL) and dried over $MgSO_4$. Evaporation of the solvent followed by purification by column chromatography (SiO_2, 5% MeOH/EtOAc) yielded **29** as a white foam (130 mg, 70%); $[\alpha]_D^{25}$ +1.0° (*c* 1.0, $CHCl_3$); IR (μscope) 3292, 2976, 2932, 1740, 1706, 1647, 1591, 1491 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9H, $(C\underline{H}_3)_3$), 2.92-2.12 (m, 1H, $CHC\underline{H}_2$), 2.48-2.99 (m, 2H, 1HxCHC<u>H</u>, 1HxC<u>H</u>$_2$CO), 2.50-2.58 (m, 1H, 1HxC<u>H</u>$_2$CO), 2.94 (s, 3H, $N(C\underline{H}_3)_2$), 2.99 (s, 3H, $N(C\underline{H}_3)_2$), 3.62 (s, 3H, $C\underline{H}_3$), 4.49-4.58 (m, 1H, C<u>H</u>), 5.10 (d, *J* = 17.0 Hz, 1H, $C\underline{H}_2N$), 5.18 (d, *J* = 16.9 Hz, 1H, $C\underline{H}_2N$), 5.64 (br s, 1H, N<u>H</u>), 7.72-7.82 (m, 2H, <u>Ph</u>), 8.10-8.08 (m, 1H, <u>Ph</u>), 8.36-8.40 (m, 1H, <u>Ph</u>); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.8, 28.5, 29.1, 35.7, 37.2, 38.7, 57.2, 68.9, 80.2, 123.6, 124.3, 127.1, 129.2, 132.1, 132.7, 148.3, 158.8, 171.8, 203.6; HRMS (ES) Calcd for $C_{22}H_{30}N_4O_6Na$ (MNa^+) 469.2063, found 469.2069.



30



92

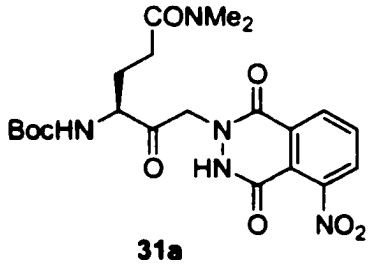
((4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-(5,6,7,8-tetrafluoro-2,3-dihydrophthalazin-1,4-dione)hexanamide (30) and 2,3-Bis[(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-methylhexanamide]-5,6,7,8-

tetrafluoro-2,3-dihydrophthalazin-1,4-dione (92). Sodium tetrafluorophthalhydrazide **91** was prepared by the reaction tetrafluorophthalhydrazide **90** (0.4 g, 1.72 mmol) and NaH (41.4 mg, 1.72 mmol) in anhydrous THF (15 mL). After 3 h of stirring, the solvent was evaporated *in vacuo* and the residue taken up in anhydrous Et_2O (30 mL), filtered and washed successively with more anhydrous Et_2O to yield **91** as a solid (0.35 g, 81%). To a solution of the sodium salt **91** (292 mg, 1.14 mmol) in DMF (10 mL) was added the bromoketone **82** (200 mg, 0.57 mmol) in small portions over 2 h. After stirring for an additional 2 h at rt, the solvent was removed *in vacuo* and the residue diluted with H_2O (15 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over $MgSO_4$ and evaporation of the solvent followed by purification by column chromatography (SiO_2, 80% EtOAc/hexane) yielded **30** (30 mg, 11%) and **92** (112 mg, 25%) as foams.

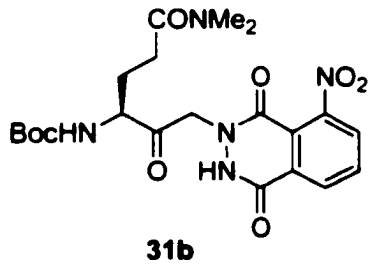
Data for **30**: $[\alpha]_D^{26}$ +15.5° (*c* 1.3, $CHCl_3$); IR (μscope) 3208, 2979, 2934, 1739, 1681, 1621, 1516, 1403 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.41 (s, 9H, $(C\underline{H}_3)_3$), 2.00-2.12 (m, 1H, $CHC\underline{H}_2$), 2.32-2.40 (m, 2H, 1xH $CHC\underline{H}_2$, 1xH $C\underline{H}_2CO$), 2.46-2. (m, 1H, $C\underline{H}_2CO$), 2.96 (s, 3H, $N(C\underline{H}_3)_2$), 3.04 (s, 3H, $N(C\underline{H}_3)_2$), 4.48-4.52 (m, 1H, $C\underline{H}$), 5.01 (d, J = 16.6 Hz, 1H, $C\underline{H}_2N$), 5.15 (d, J = 16.8 Hz, 1H, $C\underline{H}_2N$), 5.58-5.60 (m, 1H, $N\underline{H}$), 10.68 (br s, 1H, $N\underline{H}$); ^{19}F NMR (376 MHz, $CDCl_3$) δ -147.1, -145.1, -137.1, -134.9; ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.9, 28.4, 28.8, 30.9, 36.1, 37.7, 56.9, 68.9, 80.6, 110.8, 114.2, 141.9, 143.0, 145.6, 146.3 (m), 155.4, 155.7, 172.9, 202.8; HRMS (ES) Calcd for $C_{21}H_{24}N_4O_6F_4Na$ (MNa^+) 527.1529, found 527.1527.

Data for **92**: $[\alpha]_D^{26}$ −7.7° (*c* 1.4, $CHCl_3$); IR ($CHCl_3$ cast) 3291, 3029, 2974, 2931, 1733, 1658, 1624, 1517, 1495 cm^{-1}; ^1H NMR (300 MHz, CD_2Cl_2) δ 1.40 (s, 18H, 2x$(C\underline{H}_3)_3$),

1.80-2.00 (m, 2H, 2xCHC$\underline{H}_2$), 2.20-2.38 (m, 2H, 2xCHC$\underline{H}_2$), 2.40-2.62 (m, 4H, 2xC$\underline{H}_2$CO), 2.98 (s, 6H, 2xN(C$\underline{H}_3$)$_2$), 3.06 (s, 6H, 2xN(C$\underline{H}_3$)$_2$), 4.32-4.40 (m, 2H, 2xC$\underline{H}$), 4.84-5.08 (m, 4H, 2xC$\underline{H}_2$N), 5.78-5.80 (m, 1H, N$\underline{H}$), 6.18 (m, 1H, N$\underline{H}$); ^{13}C NMR (75 MHz, CD_2Cl_2)(mixture of rotamers) δ 26.5, 26.8, 27.6, 28.3, 28.9, 29.3, 35.9, 36.0, 37.4, 37.6, 56.8, 57.1, 57.3, 57.8, 69.0, 80.2, 80.4, 110.9, 114.0, 141.8, 142.8, 144.3, 145.5, 154.0, 156.1, 172.9, 173.4, 202.4, 203.1; HRMS (ES) Calcd for $C_{34}H_{46}N_6O_{10}F_4Na$ (MNa^+) 797.3109, found 797.3099.



31a

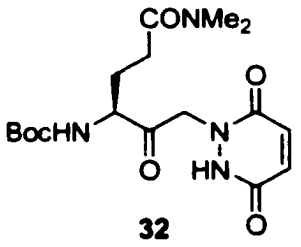


31b

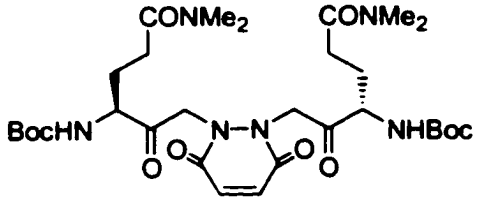
(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-(5-nitro-2,3-dihydro-phthalazin-1,4-dione)hexanamide (31a) and (4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-(8-nitro-2,3-dihydrophthalazin-1,4-dione)hexanamide (31b). A similar procedure was employed as that described for the preparation of **28**. Reaction of 3-nitrophthalhydrazide **93** (1.5 g, 7.24 mmol) and NaH (174 mg, 6.89 mmol) gave sodium 3-nitrophthalhydrazide **94** as a light yellow salt (1.49 g, 90%). The salt (72 mg, 0.31mmol) was added to the bromo-ketone **82** (100 mg, 0.28 mmol) in DMF (10 mL) over 1 h and allowed to stir for 5 h. Purification by column chromatography (SiO_2, with a gradient of 20% EtOAc/hexane to 100% EtOAc) furnished compounds **31a** (46 mg, 34%) and **231b** (22 mg, 16%) as light yellow foams.

Data for isomer **31a**: $[\alpha]_D^{26}$ +2.1° (*c* 2.8, $CHCl_3$); IR (μscope) 3224, 1739, 1671, 1623, 1601 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.42 (s, 9H, $(C\underline{H}_3)_3$), 1.98-2.12 (m, 1H, $CHC\underline{H}_2$), 2.24-2.60 (m, 3H, $CHC\underline{H}_2$, $C\underline{H}_2CO$), 2.92 (s, 3H, $N(C\underline{H}_3)_2$), 2.98 (s, 3H, $N(C\underline{H}_3)_2$), 4.40-4.51 (m, 1H, C<u>H</u>), 5.09 (d, *J* = 16.8 Hz, 1H, $C\underline{H}_2N$), 5.23 (d, *J* = 16.6 Hz, 1H, $C\underline{H}_2N$), 5.78-5.80 (m, 1H, N<u>H</u>), 7.72 (d, *J* = 7.0 Hz, 1H, <u>Ph</u>), 7.92 (dd, *J* = 7.9, 7.9 Hz, 1H, <u>Ph</u>), 8.23 (d, *J* = 7.3 Hz, 1H, <u>Ph</u>), 11.04 (br s, 1H, N<u>H</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.4, 28.3, 28.7, 35.9, 37.5, 57.2, 69.0, 80.3, 119.6, 125.7, 126.0, 126.7, 134.1, 148.6, 148.9, 156.0, 156.4, 173.0, 203.4; HRMS (ES) Calcd for $C_{21}H_{27}N_5O_8Na$ (MNa^+) 500.1757, found 500.1752.

Data for isomer **31b**: $[\alpha]_D^{26}$ +158.8° (*c* 0.6, $CHCl_3$); IR ($CHCl_3$ cast) 3183, 1741, 1672, 1630, 1599 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.42 (s, 9H, $(C\underline{H}_3)_3$), 1.98-2.17 (m, 1H, $CHC\underline{H}_2$), 2.22-2.38 (m, 2H, $CHC\underline{H}_2$, $C\underline{H}_2CO$), 2.42-2.60 (m, 1H, $C\underline{H}_2CO$), 2.92 (s, 3H, $N(C\underline{H}_3)_2$), 2.98 (s, 3H, $N(C\underline{H}_3)_2$), 4.40-4.52 (m, 1H, C<u>H</u>), 4.92 (d, *J* = 16.5 Hz, 1H, $C\underline{H}_2N$), 5.12 (d, *J* = 16.7 Hz, 1H, $C\underline{H}_2N$), 5.61 (d, *J* = 4.6 Hz, 1H, N<u>H</u>), 7.81-7.92 (m, 2H, <u>Ph</u>), 8.58 (dd, *J* = 14.2, 1.9 Hz, 1H, <u>Ph</u>), 11.04 (br s, 1H, N<u>H</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.8, 28.3, 28.7, 35.9, 37.5, 56.9, 69.1, 80.3, 115.9, 127.4, 130.0, 130.5, 132.5, 146.0, 146.3, 155.7, 158.1, 173.0, 202.5; HRMS (ES) Calcd for $C_{21}H_{27}N_5O_8Na$ (MNa^+) 500.1757, found 500.1763.



32

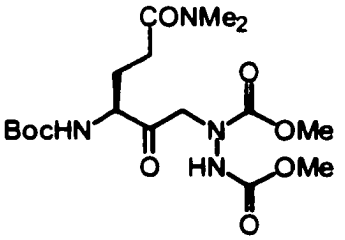


97

((4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-(3,6-dioxo-3,6-dihydro-2*H*-pyridazin-1-yL)hexanamide (32) and 2,3-bis[(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-methylhexanamide]-(3,6-dioxo-3,6-dihydro-2*H*-pyridazin-1-yL) (97). The title compounds were prepared using the same procedure as that described for **28**. Reaction of pyridazine **95** (3.0 g, 26.8 mmol) and NaH (677 mg, 26.8 mmol) gave the pyridazine salt **96** as a white solid (3.4 g, 95%). Reaction of the sodium pyridazine salt **96** (84 mg, 0.63 mmol) and bromoketone **15** (200 mg, 0.28 mmol) followed by purification by column chromatography (SiO_2, with a gradient elution of 100% EtOAc to 95% EtOAc/MeOH) furnished **32** (82 mg, 38%) and **97** (46 mg, 12%) as colorless oils.

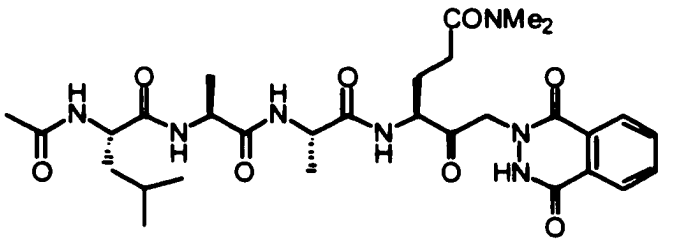
Data for **32**: $[\alpha]_D^{25}$ +2.3° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 2978, 1740, 1681, 1599, 1504, 1453, 1406, 1367, 1290, 1166, 1053 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.42 (s, 9H, $(C\underline{H}_3)_3$), 1.80-1.99 (m, 1H, $CHC\underline{H}_2$), 2.20-2.00 (m, 1H, $CHC\underline{H}_2$), 2.32-2.56 (m, 2H, $C\underline{H}_2CO$), 2.94 (s, 3H, $N(C\underline{H}_3)_2$), 3.02 (s, 3H, $N(C\underline{H}_3)_2$), 4.44 (br s, 1H, C<u>H</u>), 4.92 (d, *J* = 16.9 Hz, 1H, $C\underline{H}_2N$), 5.12 (d, *J* = 16.9 Hz, 1H, $C\underline{H}_2N$), 5.70 (br s, N<u>H</u>), 7.08 (d, *J* = 9.9 Hz, 1H, C<u>H</u>=CH), 7.44 (d, *J* = 9.9 Hz, 1H, C<u>H</u>=CH), 12.62 (br s, 1H, N<u>H</u>); ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.5, 27.5, 28.2, 28.7, 35.9, 37.5, 56.9, 68.9, 80.4, 128.5, 132.4, 153.5, 155.8, 172.9, 203.1; HRMS (ES) Calcd for $C_{17}H_{27}N_4O_6$ (MH^+) 383.1925, found 383.1927.

Data for **97**: $[\alpha]_D^{26}$ −25.0° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3285, 2977, 2933, 1738, 1706, 1673, 1632, 1597, 1505, 1450 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.42 (s, 18H, 2x($C\underline{H}_3$)$_3$), 1.72-1.98 (m, 2H, 2xCHC$\underline{H}_2$), 2.19-2.32 (m, 2H, 2xCHC$\underline{H}_2$), 2.38-2.52 (m, 4H, 2xC$\underline{H}_2$CO), 2.97 (s, 6H, 2xN($C\underline{H}_3$)$_2$), 3.02 (s, 6H, 2xN($C\underline{H}_3$)$_2$), 4.25-4.41 (m, 2H, 2xC$\underline{H}$), 4.85-5.18 (m, 4H, 2xC$\underline{H}_2$N), 5.82 (br d, *J* = 6.4 Hz, 1H, N$\underline{H}$), 6.47 (br d, *J* = 3.5 Hz, 1H, N$\underline{H}$), 7.22 (d, *J* = 9.9 Hz, 1H, C$\underline{H}$=CH), 7.12 (d, *J* = 9.7 Hz, 1H, C$\underline{H}$=CH); ^{13}C NMR (75 MHz, CD_2Cl_2)(mixture of rotamers) δ 26.4, 26.8, 28.3, 28.9, 29.2, 35.9, 36.0, 37.3, 37.5, 57.1, 57.9, 58.1, 68.7, 80.1, 80.3, 127.4, 132.4, 152.1, 155.9, 159.7, 172.7, 173.3, 202.0, 203.3; HRMS (ES) Calcd for $C_{30}H_{49}N_6O_{10}$ (MH^+) 653.3505, found 653.3498.



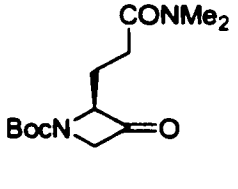
(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-(1,2-dimethoxycarbonyl-hydrazino)hexanamide (33). To a solution of triphenyl phosphine (182 mg, 0.69 mmol) in THF at −78 °C was added dimethyl azodicarboxylate (95 μL, 0.86 mmol) over 5 min. After 30 min of stirring at −78 °C, hydroxyketone **102** (100 mg, 0.35 mmol) was added and the reaction mixture was slowly allowed to warm to rt overnight.[72] The solvent was removed *in vacuo* and the residue purified by column chromatography (SiO_2, gradient elution of 75% to 50% hexane/EtOAc) to afford **33** as a pale yellow oil (29.6 mg, 20%); $[\alpha]_D^{26}$ +3.8° (*c* 0.5, $CHCl_3$); IR (CH_2Cl_2) 3280, 2958,

1740, 1712, 1631, 1506 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (6:1 mixture of rotamers) δ 1.40 (s, 9H, $(C\underline{H}_3)_3$), 1.89-2.01 (m, 1H, $CHC\underline{H}_2$), 2.05-2.20 (m, 1H, $CHC\underline{H}_2$), 2.35 (ddd, J = 16.5, 7.1, 6.3 Hz, 1H, $C\underline{H}_2CO$), 2.49 (ddd, J = 16.5, 7.2, 6.6 Hz, 1H, $C\underline{H}_2CO$), 2.92 (s, 3H, $N(C\underline{H}_3)_2$), 2.98 (s, 3H, $N(C\underline{H}_3)_2$), 3.72-3.90 (m, 6H, $2xOC\underline{H}_3$), 4.28-4.37 (br m, 1H, $C\underline{H}$), 4.86 (d, J = 17.3 Hz, 1H, $C\underline{H}_2N$), 4.95 (d, J = 17.3 Hz, 1H, $C\underline{H}_2N$), 5.75 (d, J = 3.8 Hz, 1H, $N\underline{H}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.3, 28.9, 29.4, 29.7, 35.8, 37.3, 55.3, 54.9, 56.9, 69.3, 155.4, 155.9, 171.9, 172.3, 203.0; HRMS (ES) Calcd for $C_{17}H_{30}N_4O_8Na(MNa^+)$ 441.1961, found 441.1953.



(4*S*)-*N*,*N*-dimethyl-4-(acetyl-L-leucyl-L-alanyl-L-alanyl)-amino-5-oxo-6-(2,3-dihydro-phthalazin-1,4-dione)hexanamide (34). Trifluoroacetic acid (2 mL) was added to a solution of **28** (65 mg, 0.12 mmol) in CH_2Cl_2 (2 mL) at 0 °C. After 1.5 h, the reaction mixture was concentrated *in vacuo* and the residue triturated with Et_2O to yield the trifluoroacetate salt **108** as a light brown foam (60 mg, quantitative). The salt obtained was used in the next step without any further purification. To a solution of Ac-Leu-Ala-Ala-OH **107** (64 mg, 0.15 mmol) in DMF (8 mL) at rt was added DIPEA (51 μM, 0.29 mmol) followed by HBTU[76] (59 mg, 0.15 mmol). The mixture was stirred for 30 min after which it was treated with a solution of the trifluoroacetate salt **108** (60 mg, 0.13 mmol) in DMF (2 mL). After 6 h of stirring, the solvent was removed *in vacuo* and the crude product purified by HPLC (Waters C18 Bondpak; 100 x 40 mm, linear gradient

elution over 20 min of 0 to 40% acetonitrile in 0.1% TFA/H_2O, t_R = 16.6 min) to afford the title product as a white powder after lyophilization (59 mg, 70%); m.p 88-90 °C; $[\alpha]_D^{26}$ –60.0° (*c* 0.3, $CHCl_3$); IR ($CHCl_3$, cast) 3279, 2958, 1740, 1653, 1600, 1540 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) (1:1 mixture of rotamers) δ 0.94 (d, *J* = 5.8 Hz, 3H, $C\underline{H}_3$(Leu)), 0.95 (d, *J* = 6.4 Hz, 3H, $C\underline{H}_3$(Leu)), 1.28-1.44 (m, 6H, 2x$C\underline{H}_3$(Ala)), 1.45-1.70 (m, 3H, C<u>H</u>, $C\underline{H}_2$(Leu)), 1.85-2.08 (m, 4H, $C\underline{H}_3CO$, $CHC\underline{H}_2$(Gln)), 2.24-2.40 (m, 1H, $CHC\underline{H}_2$(Gln)), 2.42-2.58 (m, 2H, $C\underline{H}_2CO$(Gln)), 2.92 (s, 3H, $N(C\underline{H}_3)_2$), 3.05 (s, 3H, $N(C\underline{H}_3)_2$), 4.18-4.40 (m, 3H, 3x<u>CH</u>), 4.58-4.69 (m, 1H, <u>CH</u>) 5.15 (d, *J* = 24.6 Hz, 1H, , $C\underline{H}_2N$), 5.22 (d, *J* = 24.3 Hz, 1H, $C\underline{H}_2N$), 7.84-7.96 (m, 2H, 2x<u>NH</u>), 8.12 (d, *J* = 6.0 Hz, 1H, <u>NH</u>), 8.32 (d, *J* = 7.5 Hz, 1H, <u>NH</u>); ^{13}C NMR (125 MHz, CD_3OD)(mixture of rotamers) δ 17.2, 17.3, 17.7, 21.9, 22.1, 22.3, 22.5, 23.1, 23.2, 25.9, 26.6, 26.7, 26.9, 35.9, 37.8, 41.7, 41.8, 41.9, 50.7, 50.8, 51.1, 53.4, 53.7, 54.0, 56.7, 57.2, 70.0, 70.1, 125.0, 126.1, 127.4, 130.0, 133.5, 134.9, 151.4, 164.7, 174.5, 175.1, 175.4, 204.5; HRMS (ES) Calcd for $C_{30}H_{43}N_7O_8Na$ (MNa^+) 652.3071, found 652.3068.



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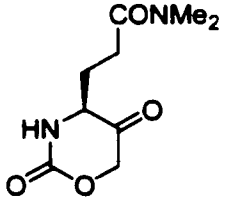


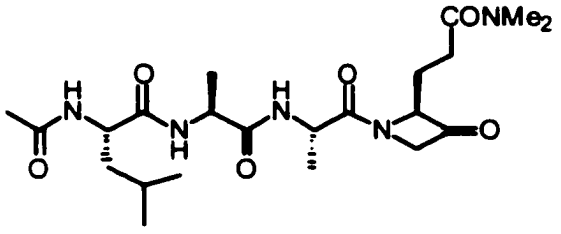
109

(2*S*)-2-(*N*,*N*-dimethylpropanamid-3-yl)-1-(*tert*-butyloxycarbonyl)-azetidin-3-one (35) and (4*S*)-4-(*N*,*N*-dimethylpropanamid-3-yl)-[1,3]-oxazinane-2,5-dione (109). A solution of diazoketone **81** (800 mg, 2.7 mmol) in benzene (50 mL) was added dropwise over 2 h to a refluxing solution of rhodium (II) acetate dimer (2.22 mg,

27 μmol) in benzene (50 mL).[82,83] The mixture was heated under reflux for a further 1 h at which time the color changed from green to pink. The solvent was evaporated and the residue diluted with EtOAc (30 mL) and filtered through a pad of celite. Concentration of the filtrate followed by purification of the crude product by column chromatography (SiO_2, 5% MeOH/EtOAc) afforded **35** (378 mg, 52%) and **109** (99 mg, 17%) as pale yellow oils.

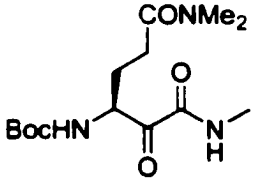
Data for **35:** $[\alpha]_D^{25}$ +28.7° (*c* 1.2, $CHCl_3$); IR ($CHCl_3$ cast) 2927, 1822, 1701, 1640, 1456 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H, $(C\underline{H}_3)_3$), 2.08-2.18 (m, 2H, $CHC\underline{H}_2$), 2.34-2.55 (m, 2H, $C\underline{H}_2CO$), 2.90 (s, 3H, $N(C\underline{H}_3)_2$), 2.96 (s, 3H, $N(C\underline{H}_3)_2$), 4.50 (dd, *J* = 16.8, 4.2 Hz, 1H, $NC\underline{H}_2$), 4.65 (d, *J* = 16.6 Hz, 1H, $NC\underline{H}_2$), 4.95 (ddd, *J* = 11.0, 6.7, 4.2 Hz, 1H, $C\underline{H}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.9, 28.2, 28.4, 35.4, 37.1, 69.1, 80.9, 82.1, 156.5, 171.5, 200.1; HRMS (EI) Calcd for $C_{13}H_{22}N_2O_4$ (M^+) 270.1579, found 270.1586.

Data for **109:** $[\alpha]_D^{25}$ -2.4° (*c* 0.4, MeOH); IR ($CHCl_3$ cast) 3268, 2927, 1715, 1625, 1503 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.98-2.11 (m, 1H, $CHC\underline{H}_2$), 2.18-2.32 (m, 1H, $CHC\underline{H}_2$), 2.46-2.52 (m, 2H, $C\underline{H}_2CO$), 2.92 (s, 3H, $N(C\underline{H}_3)_2$), 2.98 (s, 3H, $N(C\underline{H}_3)_2$), 3.92 (dd, *J* = 5.2, 0.9 Hz, 1H, $C\underline{H}$), 4.55 (d, *J* = 17.5 Hz, 1H, $C\underline{H}_2O$), 4.61 (d, *J* = 17.5 Hz, 1H, $C\underline{H}_2O$), 6.95 (br s, 1H, $N\underline{H}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.2, 29.4, 35.8, 37.8, 59.2, 71.6, 154.7, 172.0, 203.4; HRMS (ES) Calcd for $C_9H_{14}N_2O_4Na$ (MNa^+) 237.0851, found 237.0854.



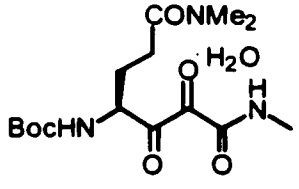
(2*S*)-2-(*N*,*N*-dimethyl-propanamid-3-yl)-1-(acetyl-L-leucyl-L-alanyl-L-alanyl)-azetidin-3-one (36). Ac-Leu-Ala-Ala-OH **107** (95 mg, 0.30 mmol) in DMF (8 mL) was treated with DIPEA (126 μM, 0.72 mmol) followed by HBTU (120 mg, 0.32 mmol) at rt. The trifluoroacetate salt **110** (95 mg, 0.33 mmol) in DMF (2 mL) was added. After 6 h of stirring, the solvent was removed *in vacuo* and the crude product purified by HPLC (Waters C18 Bondpak; 100 x 40 mm, linear gradient elution over 30 min of 5 to 50% acetonitrile in 0.1% TFA/H_2O, t_R = 13.9 min.) to afford the peptidyl alcohol as an oil (87 mg, 58%); IR ($CHCl_3$, cast) 3286, 2986, 2852, 1632, 1537 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) (3:2 mixture of diastereomers) δ 0.92 (d, J = 4.0 Hz, 3H, C$\underline{H}_3$(Leu)), 0.95 (d, J = 6.5 Hz, 3H, C$\underline{H}_3$(Leu)), 1.21-1.29 (m, 6H, 2xC$\underline{H}_3$(Ala)), 1.52-1.60 (m, 2H, C$\underline{H}_2$(Leu)), 1.61-1.78 (m, 1H C$\underline{H}$(Leu)), 1.98 (s, 3H), 2.10 (dt, J = 14.7, 7.3 Hz, 1H, CHC$\underline{H}_2$(Gln)), 2.22-2.40 (m, 1H CHC$\underline{H}_2$(Gln)), 2.48-2.60 (m, 2H, C$\underline{H}_2$CO(Gln)), 2.94 (s, 3H, N(C$\underline{H}_3$)$_2$), 3.05 (s, 3H, N(C$\underline{H}_3$)$_2$), 3.65 (dd, J = 10.6, 3.5 Hz, 1H, NC$\underline{H}_2$), 3.98-4.10 (m, 1H, NC$\underline{H}_2$), 4.22-4.42 (m, 3H, 3xC$\underline{H}$), 4.52-4.70 (m, 2H, C$\underline{H}$, C$\underline{H}$OH); HRMS (ES) Calcd for $C_{22}H_{39}N_5O_6Na$ (MNa^+) 492.2798, found 492.2791. Dess-Martin periodinane[64] (206 mg, 0.48 mmol) was added to a solution of the alcohol (75 mg, 0.16 mmol) in DMF (5 mL). After stirring for 6 h at rt, the solvent was removed *in vacuo* and the crude product purified by HPLC (Waters C18 Bondpak; 100 x 40 mm, linear gradient elution over 30 min of 5 to 50% acetonitrile in 0.1% TFA/H_2O, t_R 16.9 min) to afford the title

product as a white powder after lyophilization (53 mg, 71%); m.p 56-58 °C; $[\alpha]_D^{25}$ -29.5° (*c* 0.2, $CHCl_3$); IR ($CHCl_3$, cast) 3286, 2958, 1826, 1779, 1641, 1539 cm^{-1}; ^1H NMR (300 MHz, CD_3CN) (1:1 mixture of rotamers) δ 0.87 (d, *J* = 6.5 Hz, 3H, CH_3(Leu)), 0.92 (d, *J* = 6.5 Hz, 3H, CH_3(Leu)), 1.20-1.32 (m, 6H, 2xCH_3(Ala)), 1.50 (dd, *J* = 7.0, 7.0 Hz, 2H, CH_2(Leu)), 1.58-1.72 (m, 1H, CH(Leu)), 1.98 (s, 3H, CH_3CO), 2.05-2.12 (m, 2H, $CHCH_2$(Gln)), 2.45-2.62 (m, 2H, $CHCH_2$(Gln)), 2.88 (s, 3H, $N(CH_3)_2$), 2.98 (s, 3H, $N(CH_3)_2$), 4.15-4.44 (m, 3H, 3xCH_α), 4.65-5.12 (m, 3H, CH, NCH_2), 7.05-7.25 (m, 3H, NH); ^{13}C NMR (75 MHz, CD_3CN)(1:1 mixture of rotamers) δ 17.9, 21.9, 22.1, 22.8, 23.0, 23.3, 25.5, 25.9, 29.3, 35.7, 37.5, 40.9, 41.3, 48.0, 49.8, 53.7, 71.1, 82.7, 173.2, 173.7, 200.6; HRMS (ES) Calcd for $C_{22}H_{37}N_5O_6Na$ (MNa^+) 490.2642, found 490.2642.



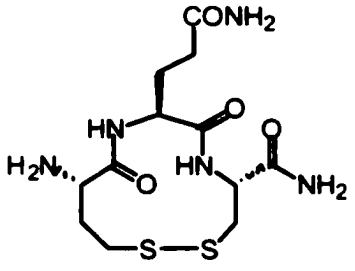
(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxalyl-6-methylamino-hexanamide (37). This compound was prepared by a modified procedure of Wasserman.[88] A solution of the cyanophosphorane **115** (0.5 g, 0.89 mmol) in CH_2Cl_2 (15 mL) was ozonized at −78 °C for 10 min. The blue green reaction mixture was purged with O_2 and Ar for 5 min and 8 min, respectively. To the resulting solution of was added methylamine HCl salt (57.5 mg, 0.85 mmol) followed by DIPEA (0.45 mL, 0.85 mmol). The resulting yellow solution was stirred for a further 1 h at −78 °C. The reaction mixture was concentrated *in vacuo* and purified directly by column chromatography (SiO_2, gradient elution, 100% EtOAc to 5% MeOH/EtOAc) to afford the title compound as an

oil (146 mg, 54%); $[\alpha]_D^{26}$ +6.0° (*c* 1.3, $CHCl_3$); IR ($CHCl_3$ cast) 3310, 2978, 2937, 1773, 1684, 1634, 1456, 1367, 1054; ^1H NMR (300 MHz, $CDCl_3$) (1:1 mixture hydrate **117** and non-hydrate **37**) δ 1.40 (s, 18H, $(C\underline{H}_3)_3$), 1.81-2.31 (m, 4H, 2xCHC$\underline{H}_2$), 2.31-2.62 (m, 4H, 2xC$\underline{H}_2$CO), 2.79 (d, *J* = 4.5 Hz, 3H, NHC$\underline{H}_3$), 2.84 (d, *J* = 4.5 Hz, 3H, NHC$\underline{H}_3$), 2.90 (s, 3H, N$(C\underline{H}_3)_2$), 2.94 (s, 3H, N$(C\underline{H}_3)_2$), 2.98 (s, 3H, N$(C\underline{H}_3)_2$), 3.0 (s, 3H, N$(C\underline{H}_3)_2$), 4.15 (br s, 1H, C$\underline{H}$C$(OH)_2$), 5.0 (br s, 1H, C$\underline{H}$CO), 5.61 (br s, 1H, BocN$\underline{H}$), 5.82 (br s, 1H, BocN$\underline{H}$), 7.10 (br s, 2H, 2xN$\underline{H}$$CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$)(1:1 mixture hydrate **117** and non-hydrate **37**) δ 25.9, 26.8, 28.3, 29.5, 35.9, 37.4, 44.8, 55.3, 155.7, 160.3, 173.4, 195.9; HRMS (ES) Calcd for $C_{14}H_{25}N_3O_5Na$ (MNa^+) 338.1692, found 338.1690 and Calcd for $C_{14}H_{27}N_3O_6Na$ ($M+H_2O+Na^+$) 356.1798, found 356.1793.

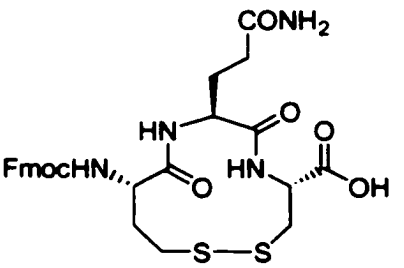


(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5,6-oxo-7-methylcarbamoyl -heptanamide hydrate (38). This compound was prepared by a modified procedure of Wasserman.[90] A solution of ylide **121** (282 mg, 0.48 mmol) in CH_2Cl_2 (8 mL) was ozonized at −78 °C for 5 min. The blue green reaction mixture was purged with O_2 and Ar for 5 min and 8 min, respectively. The solvent was evaporated and the crude product purified directly by column chromatography (SiO_2, 5% MeOH/EtOAc) to afford the title compound as an oil (44 mg, 56%); $[\alpha]_D^{26}$ +2.5° (*c* 0.7, $CHCl_3$); IR ($CHCl_3$ cast) 3307, 2977, 2932, 1685, 1633, 1511, 1454, 1367, 1057; ^1H NMR (300 MHz, $CDCl_3$) δ 1.42 (br s, 9H, $(C\underline{H}_3)_3$), 2.11-2.31 (m, 2H, CHC$\underline{H}_2$), 2.32-2.56 (m, 2H, C$\underline{H}_2$CO), 2.79-3.21 (m,

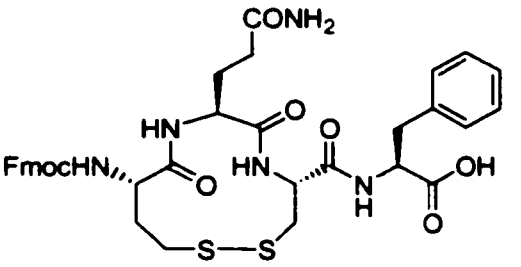
9H, NHC<u>H</u>$_3$, N(C<u>H</u>$_3$)$_2$), 4.82 (dd, J = 10.3, 5.5 Hz, 1H, C<u>H</u>), 5.65 (br s, 1H, BocN<u>H</u>), 7.52 (br s, 1H, N<u>H</u>CH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.7, 26.5, 28.0, 28.3, 29.7, 35.6, 37.2, 55.8, 80.5, 94.1, 161.6, 172.9, 206.3; HRMS (ES) Calcd for $C_{15}H_{27}N_3O_7Na$ (MNa^+) 384.1747, found 384.1738.



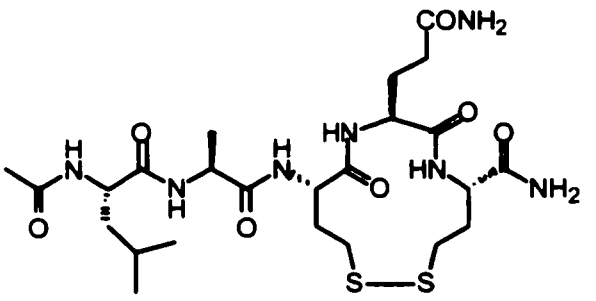
***S-S*-cyclo[-L-homocysteinyl-L-glutamyl-L-cysteinamide] (39).** A mixture of macrocyclic peptide **124** (90 mg, 0.15 mmol) in DMF (8 mL) was treated with piperidine (40% in DMF, 0.5 mL) and warmed to 60 °C for 2 h. The solvent was removed *in vacuo* and the crude product purified directly by HPLC (Waters C18 Bondpak; 100 x 40 mm; linear gradient elution over 15 min of 0 to 40% acetonitrile in 0.1% TFA/H_2O, t_R 5.0 min) to afford the title product as a white solid (34 mg, 61%); $[\alpha]_D^{25}$ -71.7° (c 0.3, MeOH); IR (μscope) 3272, 3074, 2474, 2410, 1666, 1420, 1201, 1138, 838, 800; ^{1}H NMR (500 MHz, CD_3OD) δ 1.95-2.00 (m, 1H, CHC<u>H</u>$_2$(Gln)), 2.15-2.19 (m, 1H, CHC<u>H</u>$_2$(Gln)), 2.22-2.40 (m, 4H, C<u>H</u>$_2$CO(Gln), C<u>H</u>$_2$S(Hcy), 2.49-2.65 (m, 2H, C<u>H</u>$_2$S(Hcy)), 3.95-4.02 (m, 1H, α-C<u>H</u>(Hcy)), 4.48-4.54 (m, 1H, α-C<u>H</u>(Cys)) 4.68 (t, 1H, J = 6.9 Hz, α-C<u>H</u>(Gln)); ^{13}C NMR (125 MHz, CD_3OD)(mixture of rotamers) δ 23.9, 26.5, 26.6, 26.7, 30.9, 32.5, 32.6, 32.7, 52.4, 53.2, 53.3, 53.4, 53.5, 53.6, 53.7, 53.7, 172.7, 172.8, 173.8, 177.6, 177.7; HRMS (ES) Calcd for $C_{12}H_{22}N_5O_4S_2$ (MH^+) 364.1108, found 364.1103.



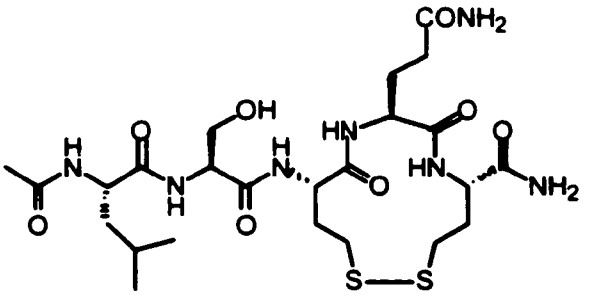
***N*-Fmoc-*S*-*S*-cyclo[-L-homocysteinyl-L-glutamyl-L-cysteine] (40).** This compound was prepared as that described for **123**. Fmoc-Hcy(Acm)-Gln-Cys(Acm)-OH **134** (60 mg, 83 μmol) in 1:1 AcOH:H_2O (60 mL) at rt and 0.1 M I_2 in MeOH (3.3 mL, 330 μmol) gave the title product as a white solid (18 mg, 37%) after purification by HPLC (Waters C18 Bondpak; 100 x 40 mm; linear gradient elution over 20 min of 20 to 80% acetonitrile in 0.1% TFA/H_2O, t_R 10.6 min); $[\alpha]_D^{25}$ -32.6° (*c* 0.5, DMSO); IR (μscope) 3451, 3300, 3065, 2924, 1721, 1634, 1534, 1450, 1305, 1233, 1136; ^{1}H NMR (500 MHz, CD_3OD) δ 1.60-1.79 (m, 1H, CHC$\underline{H}_2$(Gln)), 1.90-2.12 (m, 5H, CHC$\underline{H}_2$(Gln), CHC$\underline{H}_2$(Hcy), C$\underline{H}_2$S(Hcy)), 2.48-2.70 (m, 2H, C$\underline{H}_2$S(Cys)), 4.01-4.45 (m, 6H, 3xα-C$\underline{H}$, C$\underline{H}$, C$\underline{H}_2$(Fmoc)), 7.50 (dd, 2H, *J* = 7.3, 7.3 Hz, $\underline{Ph}$), 7.40 (dd, *J* = 7.5, 7.5 Hz, 2H, $\underline{Ph}$), 7.52 (dd, *J* = 7.5, 7.5 Hz, 2H, $\underline{Ph}$), 7.88 (d, *J* = 7.6 Hz, 2H, $\underline{Ph}$); ^{13}C NMR (125 MHz, CD_3OD) δ 31.0, 40.0, 46.6, 52.2, 65.6, 88.2, 120.1, 125.3, 127.1, 127.6, 140.7, 143.8, 143.9, 155.5, 160.8, 170.2, 173.5; HRMS (ES) Calcd for $C_{27}H_{31}N_4O_8S_2Na$ (MNa^+) 587.1629, found 587.1634.



***N*-Fmoc-*S*-*S*-cyclo[-L-homocysteinyl-L-glutamyl-L-cysteinyl]-L-phenylalanine (41).** This compound was prepared as described for **123**. Fmoc-Hcy(Acm)-Gln-Cys(Acm)-Phe-OH **135b** (200 mg, 340 μmol) in 1:1 AcOH:H_2O (300 mL) at rt and 0.1 M I_2 in MeOH (13.7 mL, 137 μmol) gave the title product as a white solid (56 mg, 34%) after purification by HPLC (Waters C18 Bondpak; 100 x 40 mm; linear gradient elution over 20 min of 30 to 100% acetonitrile in 0.1% TFA/H_2O, t_R 10.6 min); $[\alpha]_D^6$ -60.2° (*c* 1.0, DMSO); IR (μscope) 3278, 3063, 2947, 2514, 2257, 2126, 1947, 1712, 1634, 1531, 1449, 1416; ^{1}H NMR (500 MHz, CD_3OD) δ 1.64-2.18 (m, 6H, CHC$\underline{H}_2$(Gln), C$\underline{H}_2$CO(Gln), CHC$\underline{H}_2$(Hcy)), 2.42-2.62 (m, 2H, C$\underline{H}_2$S(Hcy)), 2.88 (dd, 1H, *J* = 13.6, 9.0 Hz, C$\underline{H}_2$Ph), 3.40 (dd, 1H, *J* = 13.6, 9.4 Hz, C$\underline{H}_2$Ph), 4.10-4.42 (m, 7H, 4xα-C$\underline{H}$, C$\underline{H}$, C$\underline{H}_2$(Fmoc)), 6.75 (s, 1H, N$\underline{H}$), 7.18-7.34 (m, 7H, 5xC$\underline{H}$(Ph), 2xC$\underline{H}$(Fmoc)), 7.40 (t, *J* = 7.3 Hz, 2H, 2xC$\underline{H}$(Fmoc)), 7.60 (br s, 1H, N$\underline{H}$), 7.75 (dd, *J* = 7.6, 7.6 Hz, 2H, 2xC$\underline{H}$(Fmoc)), 7.85 (d, *J* = 7.3 Hz, 2H, 2xC$\underline{H}$(Fmoc)), 8.26 (d, 1H, *J* = 5.0 Hz, N$\underline{H}$), 8.65 (br s, 1H, N$\underline{H}$); ^{13}C NMR (125 MHz, CD_3OD)(mixture of rotamers) δ 28.9, 31.1, 31.2, 32.3, 32.4, 36.6, 36.7, 46.6, 53.1, 53.6, 65.6, 65.7, 65.7, 120.1, 125.3, 126.5, 127.1, 127.6, 127.7, 128.2, 128.3, 129.2, 129.3, 137.4, 139.4, 139.6, 139.8, 140.7, 143.8, 143.9, 155.6, 168.9, 170.9, 172.5, 172.6 173.6; HRMS (ES) Calcd for $C_{36}H_{39}N_5O_8S_2Na$ (MNa^+) 756.2138, found 756.2140.

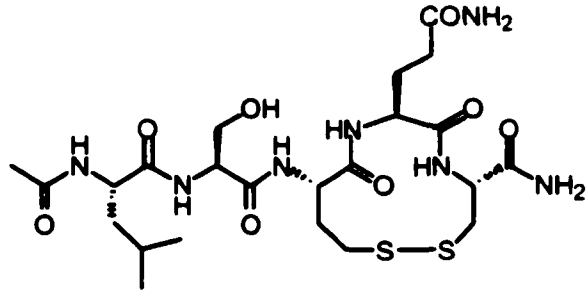


***N*-Acetyl-L-leucyl-L-alanyl-*S*-*S*-cyclo[-L-homocysteinyl-L-glutamyl-L-homocysteinamide] (42).** To a stirred solution of Ac-Leu-Ala-Hcy(Acm)-Gln-Hcy(Acm)-NH_2 **136** (20 mg, 27 μmol) in 1:1 AcOH:H_2O (20 mL) at rt was added 0.1 M I_2 in MeOH (1.1 mL, 107 μmol). After 3 h, the reaction was quenched by the addition of ascorbic acid. The solvents were removed *in vacuo* and the residue purified directly by HPLC (Waters C18 Bondpak; 100 x 40 mm; linear gradient elution over 20 min of 10 to 60% acetonitrile in 0.1% TFA/H_2O, t_R = 7.7 min) to give the desired product (11.8 mg, 73%) as a white solid; This material proved to be insoluble in most solvents and sparingly soluble in DMSO and DMF; HRMS (ES) Calcd for $C_{24}H_{42}N_7O_7S_2$ (MH^+) 604.2587, found 604.2596.



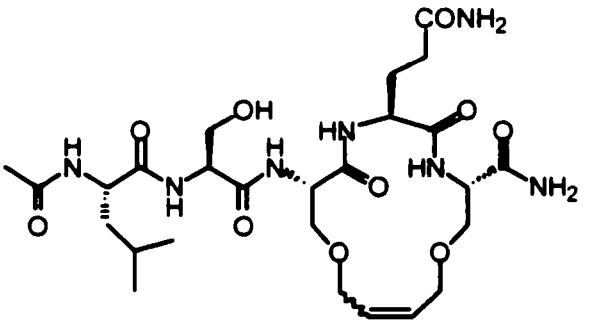
***N*-Acetyl-L-leucyl-L-serinyl-*S*-*S*-cyclo[-L-homocysteinyl-L-glutamyl-L-homocysteinamide] (43).** This compound was prepared as that described for **42**. Ac-Leu-Ser-Hcy(Acm)-Gln-Hcy(Acm)-NH_2 **137** (40 mg, 50 μmol) in 1:1 AcOH:H_2O (40 mL) at

rt and 0.1 M I_2 in MeOH (1.1 mL, 107 μmol) gave the title product as a white solid (27 mg, 87%) after purification by HPLC (Waters C18 Bondpak; 100 x 40 mm; linear gradient elution over 20 min of 0 to 50% acetonitrile in 0.1% TFA/H_2O, t_R = 11.0 min); $[\alpha]_D^{25}$ -56.7° (*c* 0.3, DMF); IR (μscope) 3278, 3076, 2929, 2871, 1625, 1537, 1433, 1285, 1203, 1179, 1132, 1048; ^{1}H NMR (500 MHz, DMSO-d_6, 1% D_2O) δ 0.78 (d, *J* = 6.4 Hz, 3H, C$\underline{H}_3$(Leu)), 0.84 (d, *J* = 6.6 Hz, 3H, C$\underline{H}_3$(Leu)), 1.40 (dd *J* = 6.7, 6.9 Hz, 2H, C$\underline{H}_2$(Leu)), 1.50-1.62 (m, 1H, C$\underline{H}$(Leu)), 1.69-2.19 (m, 9H, C$\underline{H}_3$CO, CHC$\underline{H}_2$(Hcy), β-C$\underline{H}_2$(Gln), C$\underline{H}_2$CO(Gln)), 2.58 (t, *J* = 7.3 Hz, 2H, C$\underline{H}_2$CO(Hcy)), 2.68-2.82 (m, 2H, β-C$\underline{H}_2$(Cys)), 3.52-3.62 (m, 2H, β-C$\underline{H}_2$(Ser)), 4.35-4.40 (m, 5H, 5xα-C$\underline{H}$); ^{13}C NMR (125 MHz, DMSO-d_6, 1% D_2O)(mixture of rotamers) δ 22.3, 23.1, 23.7, 24.9, 27.1, 30.1, 30.8, 32.0, 32.1, 33.4, 41.6, 51.2, 51.6, 52.1, 52.6, 55.5, 61.9, 169.9, 1750.1, 170.8, 171.1, 173.2, 173.7, 174.3; HRMS (ES) Calcd for $C_{24}H_{41}N_7O_8S_2Na$ (MNa^+) 642.2350, found 642.2359.



***N*-Acetyl-L-leucyl-L-serinyl-*S*-*S*-cyclo[-L-homocysteinyl-L-glutamyl-L-cysteinamide] (44).** This compound was prepared as that described for **42**. Ac-Leu-Ser-Hcy(Acm)-Gln-Hcy(Acm)-NH_2 **138** (70 mg, 93 μmol) in 1:1 AcOH:H_2O (70 mL) at rt and 0.1 M I_2 in MeOH (3.7 mL, 370 μmol) gave the title product as a white solid (25 mg,

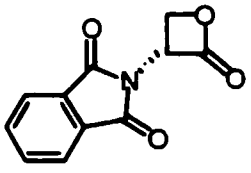
45%) after purification by HPLC (Waters C18 Bondpak; 100 x 40 mm; linear gradient elution over 20 min of 0 to 50% acetonitrile in 0.1% TFA/H_2O, t_R = 10.5 min); $[\alpha]_D^{26}$ -7.4° (*c* 0.3, DMSO); IR (μscope) 3289, 3075, 2956, 2468, 1626, 1544, 1420, 1284, 1244, 1204, 1142, 1062; ^{1}H NMR (500 MHz, CD_3OD) δ 0.84 (d, *J* = 6.5 Hz, 3H, C<u>H</u>$_3$(Leu)), 0.92 (d, *J* = 6.6 Hz, 3H, C<u>H</u>$_3$(Leu)), 1.54-1.73 (m, 3H, C<u>H</u>$_2$(Leu), C<u>H</u>(Leu)), 1.92-2.10 (m, 5H, C<u>H</u>$_3$CO, CHC<u>H</u>$_2$(Hcy)), 2.13-2.38 (m, 4H, CHC<u>H</u>$_2$(Gln), C<u>H</u>$_2$CO(Gln)), 2.42-2.64 (m, 2H, C<u>H</u>$_2$S(Cys)), 3.72-3.84 (m, 2H, C<u>H</u>$_2$O(Ser)), 4.28-4.64 (m, 5H, 5xα-C<u>H</u>); ^{13}C NMR (125 MHz, CD_3OD)(mixture of rotamers) δ 22.1, 22.8, 22.9, 23.4, 23.5, 24.9, 31.6, 31.7, 40.7, 41.1, 51.3, 51.4, 51.8, 52.2, 55.4, 61.7, 61.9, 170.3, 171.2, 171.3, 173.4, 175.3; HRMS (ES) Calcd for $C_{23}H_{39}N_7O_8S_2Na$ (MNa^+) 628.2194, found 628.2189.



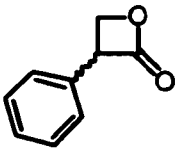
***N*-Acetyl-L-leucyl-L-serinyl-cyclo-[*O*-allyl-L-serinyl-L-glutamyl-*O*-allyl-L-serinamide] (45).** (Method 1) To a solution of the peptide **143** (44 mg, 68 μM) in MeOH/CH_2Cl_2 (16 mL, 1:3) was added Grubbs catalyst[104] **142** (16.7 mg, 0.02 mmol, 30 mol %) predissolved in CH_2Cl_2 (2 mL). The pink solution changed to brown after 10 min. The reaction mixture was stirred for a further 4 h at rt. HPLC analysis of a small portion showed complete consumption of the starting material. The reaction mixture was then quenched by the addition of triethylamine (0.5 mL) to deactivate any active catalyst. The

solvent was removed *in vacuo* and the dark green residue was purified directly by HPLC (Waters C18 Bondpak; 100 x 40 mm; linear gradient elution over 16 min of 5 to 60% acetonitrile in 0.1% TFA/H_2O, t_R = 8.7 min) to give the title product as a white solid (19.5 mg, 47%). The title product was also prepared by direct cyclization on the resin.[106] (Method 2) The linear peptide **143** attached to the Rink amide resin (0.6 g, 0.36 mmol) was solvated in CH_2Cl_2 (45 mL) for 0.5 h. To this solution was then added a solution of the Grubbs catalyst **142** (0.15 g, 0.18 mmol) in CH_2Cl_2 (5 mL). The solution turned from pink to orange brown over 4 h. the mixture was then warmed at 40 °C for 24 h. The beads were filtered, rinsed subsequently with CH_2Cl_2, DMF and MeOH and dried under high vacuum. The resin placed on a fine sintered glass filter, and the cleavage cocktail (85:10:5 CH_2CL_2:TFA:TES; 10 mL) was added. The solvent was allowed to slowly percolate through the resin over 15 min. The resin was treated with further portions of the cleavage cocktail (3 x 5 mL), and the combined filtrates were concentrated *in vacuo*. Purification by HPLC (Waters C18 Bondpak; 100 x 40 mm; linear gradient elution over 16 min of 5 to 60% acetonitrile in 0.1% TFA/H_2O, t_R = 8.7 min) gave the title product as a white solid (18 mg, 8%, overall yield). IR (μscope) 3278, 2953, 2866, 2413, 1662, 1625, 1544, 1454, 1285, 1205, 1139, 1057; ^{1}H NMR (300 MHz, CD_3OD) (2:1, *E:Z* isomers) δ 0.87 (d, *J* = 6.3 Hz, 3H, C$\underline{H}_3$(Leu)), 0.91 (d, *J* = 7.0 Hz, 3H, C$\underline{H}_3$(Leu)), 1.51-1.64 (m, 3H, C$\underline{H}$, C$\underline{H}_2$(Leu)), 1.82-2.32 (m, 5H, C$\underline{H}_3$CO, β-C$\underline{H}_2$(Gln)), 2.34 (t, *J* = 7.5 Hz, 2H, γ-C$\underline{H}_2$(Gln)), 3.69-4.09 (m, 10H, 3xβ-C$\underline{H}_2$(Ser), 2xOC$\underline{H}_2$(Ser)), 4.32-4.61 (m, 5H, 5xα-C$\underline{H}$), 5.60-5.64 (m, 1H, C$\underline{H}$=), 5.82 (br s, 1H, C$\underline{H}$=); ^{13}C NMR (75 MHz, CD_3OD)(mixture of rotamers) δ 21.1, 21.8, 22.6, 23.1, 23.2, 25.5, 26.9, 28.6, 28.7, 32.0, 32.1, 32.2, 36.9, 41.0, 41.1, 41.2, 45.5, 53.4, 53.5, 53.6, 53.8, 53.9, 54.5, 62.2, 62.3, 69.8,

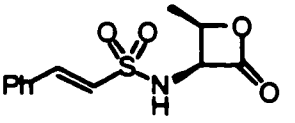
70.2, 70.6, 70.7, 78.0 129.1, 130.1, 130.2, 172.2, 172.3, 173.2, 174.8; HRMS (ES) Calcd for $C_{26}H_{43}N_7O_{10}Na$ (MNa^+) 636.2952, found 636.2964.



***N*-(Phthalimido)-D-serine-β-lactone (46).** A solution of triphenylphosphine (0.49 g, 1.87 mmol) in THF (25 mL) was cooled to -78 °C. Dimethyl azodicarboxylate (0.22 mL, 2.04 mmol) was added dropwise with a syringe over 10 min.[112,73] The resulting pale yellow solution was stirred at -78 °C for 10 min at which point a milky white slurry was obtained. *N*-(phthalimido)-D-serine **149** (0.40 g, 1.70 mmol) in THF (5 mL) was then added dropwise to the mixture over 30 min. After completion of the addition, the mixture was stirred at -78 °C for 20 min, the cooling bath was removed and the mixture was slowly warmed with stirring to rt over 2.5 h. The solvent was removed *in vacuo*, the residual pale yellow syrup was suspended in hexane-EtOAc (4:1) and filtered through a pad of celite. Upon concentration of the filtrate, white crystals precipitated. The crystals were filtered and washed with Et_2O. Recrystallization from CH_3CN/Et_2O gave the title compound as white crystalline solid (0.22 g, 60%): mp 240-241 °C; $[\alpha]_D^{25}$ +17.1° (*c* 0.1, CH_3CN); IR (μscope) 3345, 2917, 1823, 1775, 1712, 1611 cm^{-1}; ^1H NMR (300 MHz, CD_3CN) δ 4.62-4.53 (m, 2H, C<u>H</u>$_2$O), 5.88 (dd, J = 7.0, 5.0 Hz, 1H, C<u>H</u>N), 7.92-7.76 (m, 4H, <u>Ph</u>); ^{13}C NMR (75 MHz, CD_3CN) δ 56.2, 65.6, 124.6, 132.6, 135.8, 167.6, 168.5; HRMS (EI) Calcd for $C_{11}H_7NO_4$ (M^+) 217.0375, found 217.0371; Anal. Calcd for $C_{11}H_7NO_4$ C, 60.83; H, 3.23; N, 6.59. Found: C, 60.76; H, 3.24; N, 6.59.

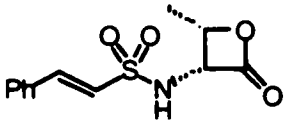


(3*R,S*)-Phenyl-oxetan-2-one (47). Cyclization of D,L-tropic acid **163** (0.40 g, 2.41 mmol) by Mitsunobu procedure,[112,73] dimethyl azodicarboxylate (0.32 mL, 2.89 mmol) and triphenyl phosphine (0.69 g, 2.65 mmol) as described for **46**, purification of the crude product by flash column chromatography (SiO_2, 66% hexane/Et_2O) gave the title β-lactone **47** as a colorless oil (0.21 g, 59%); IR (μscope) 3031, 2985, 1821, 1498, 1454, 1312, 1108 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.32 (dd, J = 4.9, 6.6 Hz, 1H, C$\underline{H}_2$O), 4.63 (dd, J = 6.8, 5.1 Hz, 1H, C$\underline{H}_2$O), 4.90 (dd, J = 6.8, 4.9 Hz, 1H, C$\underline{H}$CO), 7.22-7.41 (m, 5H, $\underline{Ph}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.8, 66.3, 127.1, 128.3, 129.1, 132.6, 169.5; HRMS (EI) Calcd for $C_9H_8O_2$ (M^+) 148.0524, found 148.0527; Anal. Calcd for $C_9H_8O_2$ C, 72.96; H, 5.44; Found: C, 72.69; H, 5.41.

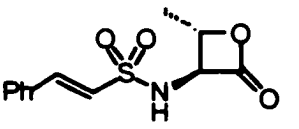


***N*-(*trans*-β-Styrenesulfonyl)-L-threonine-β-lactone (48a).** A suspension of *N*-(*trans*-β-phenethylsulfonyl)-L-threonine **154a** (0.5 g, 1.74 mmol) in CH_2Cl_2 (40 mL) was cooled to 0 °C and treated with triethylamine (0.72 mL, 5.22 mmol) followed by benzotriazole-1-yl-oxy-tri-(dimethylamino)-phosphonium hexafluorophosphate (BOP)[113] (0.92 g, 2.11 mmol). The cooling bath was removed and the reaction mixture was stirred at rt for 3 h. The solvent was removed *in vacuo* and the residue purified by flash column chromatography (SiO_2, 50% EtOAc/hexane), followed by recrystallization from

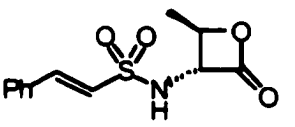
($CHCl_3$/hexane) to give β-lactone **48a** (0.29 g, 61%) as a white solid: mp 128-129 °C; $[\alpha]_D^{26}$ -19.5° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 2983, 1824, 1614, 1576, 1449 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.52 (d, *J* = 6.0 Hz, 3H, $CHC\underline{H}_3$), 4.88 (dq, *J* = 6.3, 6.2 Hz, 1H, $C\underline{H}CH_3$), 5.11 (d, *J* = 9.3, 6.0 Hz, 1H, NC<u>H</u>), 5.64-5.80 (m, 1H, N<u>H</u>), 6.83 (d, *J* = 15.4 Hz, 1H, PhCHC<u>H</u>), 7.32-7.58 (m, 6H, <u>Ph</u>, PhC<u>H</u>CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.4, 61.4, 75.0, 124.5, 128.6, 129.2, 131.4, 132.1, 142.9, 163.4; HRMS (EI) Calcd for $C_{12}H_{13}NO_4S$ (M^+) 267.0565, found 267.0559.



***N*-(*trans*-β-Styrenesulfonyl)-D-threonine-β-lactone (48b).** Cyclization of *N*-(*trans*-β-phenethylsulfonyl)-D-threonine **154b** (0.50 g, 1.74 mmol) using BOP (0.92 g, 2.11 mmol) and triethylamine (0.72 mL, 5.22 mmol) as described for **48a** gave β-lactone **154b** (0.33 g, 72%) as a white solid: mp 129-130 °C; $[\alpha]_D^{26}$ +16.0° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3266, 2982, 1823, 1615, 1576, 1386 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 1.52 (d, *J* = 6.0 Hz, 3H, $CHC\underline{H}_3$), 4.85 (dq, *J* = 6.3, 6.2 Hz, 1H, $C\underline{H}CH_3$), 5.10 (dd, *J* = 9.5, 6.0 Hz, 1H, NC<u>H</u>), 5.79 (d, *J* = 9.3 Hz, 1H, N<u>H</u>), 6.83 (d, *J* = 15.4 Hz, 1H, PhCHC<u>H</u>), 7.35-7.56 (m, 6H, <u>Ph</u>, PhC<u>H</u>CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.4, 61.4, 74.9, 124.4, 128.6, 129.2, 131.4, 132.1, 143.0, 168.3; HRMS (EI) Calcd for $C_{12}H_{13}NO_4S$ (M^+) 267.0565, found 267.0553.

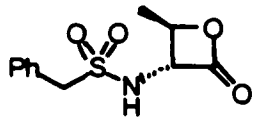


***N*-(*trans*-β-Styrenesulfonyl)-L-*allo*-threonine-β-lactone (48c).** Cyclization of *N*-(*trans*-β-phenethylsulfonyl)-L-*allo*-threonine **154c** (0.50 g, 1.74 mmol) using BOP (0.92 g, 2.11 mmol) and triethylamine (0.72 mL, 5.22 mmol) as described for **48a** gave β-lactone **48c** (0.39 g, 61%) as a white solid: mp 130-131 °C; $[\alpha]_D^{25}$ -56.0° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3278, 2977, 1831, 1614, 1495, 1449 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.78 (d, *J* = 6.0 Hz, 3H, $CHC\underline{H}_3$), 4.51 (dd, *J* = 8.2, 4.0 Hz, 1H, NC<u>H</u>), 4.70 (dq, *J* = 6.1, 4.4 Hz, 1H, C<u>H</u>CH_3), 5.94 (d, *J* = 8.2 Hz, 1H, N<u>H</u>), 6.85 (d, *J* = 15.4 Hz, 1H, PhCHC<u>H</u>), 7.40-7.55 (m, 6H, <u>Ph</u>, PhC<u>H</u>CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.7, 65.2, 78.3, 124.6, 128.6, 129.3, 131.4, 232.1, 143.0, 168.9; HRMS (EI) Calcd for $C_{12}H_{13}NO_4S$ (M^+) 267.0565, found 267.0557.

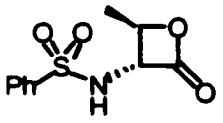


***N*-(*trans*-β-Styrenesulfonyl)-D-*allo*-threonine-β-lactone (48d).** Cyclization of *N*-(*trans*-β-phenethylsulfonyl)-D-*allo*-threonine **154d** (0.50 g, 1.74 mmol) using BOP (0.92 g, 2.11 mmol) and triethylamine (0.72 mL, 5.22 mmol) as described for **48a** gave β-lactone **48d** (0.29 g, 63%) as a white solid: mp 129-130 °C; $[\alpha]_D^{25}$ +51.7° (*c* 1.0, $CHCl_3$)); IR ($CHCl_3$ cast) 3319, 2977, 1827, 1615, 1448, 1425 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.60 (d, *J* = 6.0 Hz, 3H, $CHC\underline{H}_3$), 4.50 (d, *J* = 8.2, 4.1 Hz, 1H, NC<u>H</u>), 4.72 (dq, *J* = 6.2, 4.3 Hz, 1H, C<u>H</u>CH_3), 5.48 (d, *J* = 7.9 Hz, 1H, N<u>H</u>), 6.85 (d, *J* = 15.4

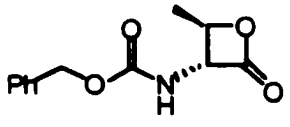
Hz, 1H, PhCHCH), 7.35-7.52 (m, 6H, Ph, PhCHCH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.7, 65.2, 78.3, 124.6, 128.6, 129.3, 131.4, 132.1, 143.0, 166.9; HRMS (EI) Calcd for $C_{12}H_{13}NO_4S$ (M^+) 267.0565, found 267.0559.



***N*-(Phenylmethanesulfonyl)-D-*allo*-threonine-β-lactone (49).** Cyclization of *N*-(phenylmethanesulfonyl)-D-*allo*-threonine **157** (100 mg, 0.37 mmol) using BOP (200 mg, 0.45 mmol) and triethylamine (0.16 mL, 1.12 mmol) as described for **48a** gave β-lactone **49** (60 mg, 64%) as a white solid after recrystallization from $CHCl_3$/hexane: mp 150-151 °C; $[\alpha]_D^{26}$ +47.5° (*c* 0.2, MeOH); IR ($CHCl_3$ cast) 3278, 2926, 1825, 1759, 1495, 1386, 1331 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (d, J = 6.3 Hz, 3H, CH(C<u>H</u>$_3$)), 4.30 (dd, J = 8.4, 4.2 Hz, 1H, C<u>H</u>NH), 4.40 (br d, J = 5.2 Hz, 2H, C<u>H</u>$_2$$SO_2$), 4.51 (dq, J = 6.3, 4.3, 1H, C<u>H</u>CH_3) 4.98 (d, J = 8.3 Hz, 1H, N<u>H</u>), 7.52-7.38 (m, 5H, <u>Ph</u>); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.5, 60.5, 65.6, 78.3, 128.4, 129.1, 129.3, 131.0, 166.9; HRMS (EI) Calcd for $C_{11}H_{13}NO_4S$ (M^+) 255.0572, found 255.0565; Anal. Calcd for $C_{11}H_{13}NO_4S$: C, 51.75; H, 5.13; N, 5.49. Found: C, 51.69; H, 5.07; N, 5.38.

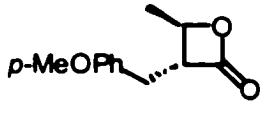


***N*-(Phenylsulfonyl)-D-*allo*-threonine-β-lactone (50).** Cyclization of *N*-(benzenesulfonyl)-D-*allo*-threonine **158** (0.44 g, 1.69 mmol) using BOP (0.89 g, 2.03 mmol) and triethylamine (0.71 mL, 5.07 mmol) as described for **48a** gave β-lactone **50** (0.26 g, 64%) as a white solid: mp 110-111 °C; $[\alpha]_D^{25}$ +9.2° (*c* 0.3, $CHCl_3$); IR ($CHCl_3$ cast) 3265, 3069, 2916, 1832, 1447, 1335 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.58 (d, *J* = 6.3 Hz, 3H, $CH(C\underline{H}_3)$), 4.42 (dd, *J* = 7.5, 4 Hz, 1H, C<u>H</u>NH), 4.68-4.59 (m, 1H, C<u>H</u>O), 5.50 (br d, 1H, N<u>H</u>), 7.64-7.52 (m, 3H, <u>Ph</u>), 7.85 (d, 2H, *J* = 10 Hz, <u>Ph</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.7, 65.2, 78.3, 127.2, 129.6, 133.6, 139.2, 166.1; HRMS (ES) Calcd for $C_{10}H_{11}NO_4SNa$ (MNa^+) 264.0307, found 264.0305; Anal. Calcd for $C_{10}H_{11}NO_4S$: C, 49.78; H, 4.60; N, 5.81. Found: C, 49.52; H, 4.41; N, 5.73.



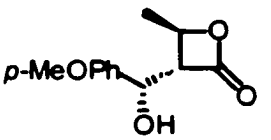
***N*-(Benzyloxycarbonyl)-D-*allo*-threonine-β-lactone (51).** D-*allo*-threonine **151d** (0.3 g, 2.52 mmol) was dissolved in H_2O (15 mL) and followed by addition of $NaHCO_3$ (0.42 g, 5.04 mmol) and 4 N NaOH (3 mL). To this solution was then added benzyl chloroformate (0.79 mL, 5.54 mmol) in THF (3 mL). The reaction mixture was stirred for 6 h after which it was basified with 4N NaOH and washed with EtOAc (2 x 15 mL). The aqueous layer was acidified with 4N HCl and extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with brine (15 mL) and dried over $MgSO_4$.

Evaporation of the solvent *in vacuo* followed by purification by flash column chromatography (SiO_2, 50% EtOAc/hexane, 1% AcOH) afforded *N*-Cbz-D-*allo*-threonine **159** as a colorless oil (0.41 g, 65%). Cyclization of *N*-Cbz-D-*allo*-threonine **159** (0.32 g, 1.27 mmol) using BOP (0.67 g, 1.52 mmol) and triethylamine (0.53 mL, 3.79 mmol) as described for **48a** gave β-lactone **51** (0.23 g, 68%) as a white solid: mp 136-137 °C; $[\alpha]_D^{26}$ +61.0° (*c* 0.4, MeOH); IR (μscope) 3312, 2972, 1830, 1535, 1382 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.60 (d, *J* = 6.3 Hz, 3H, CH(C<u>H</u>$_3$)), 4.65-4.58 (m, 1H, C<u>H</u>O), 4.80-4.68 (m, 1H, C<u>H</u>NH), 5.14 (br s, 2H, C<u>H</u>$_2$O), 5.40 (br s, 1H, N<u>H</u>), 7.40 (br s, 5H, <u>Ph</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.6, 64.5, 67.8, 76.7, 128.2, 128.4, 128.6, 135.6, 155.3, 167.5; HRMS (EI) Calcd for $C_{12}H_{13}NO_4$ (M^+) 235.0845, found 235.0835; Anal. Calcd for $C_{12}H_{13}NO_4$: C, 61.27; H, 5.57; N, 5.95. Found: C, 61.16; H, 5.73; N, 5.99.

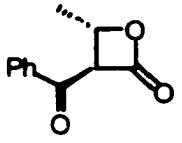


(2*R*,3*R*)-3-(*p*-Methoxybenzyl)-4-methyl-oxetan-2-one (52a). Cyclization of hydroxy acid **162a** (140 mg, 0.63 mmol) using BOP (330 mg, 0.75 mmol) and triethylamine (0.26 mL, 1.87 mmol) as described for **48a** gave β-lactone **52a** (90 mg, 73%) as a white solid after recrystallization from Et_2O/petroleum ether; mp 34-35 °C; $[\alpha]_D^{26}$ -28.7° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 2975, 2932, 1816, 1612, 1583, 1442, 1385 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.42 (d, *J* = 6.1 Hz, 3H, CH(C<u>H</u>$_3$)), 2.95 (dd, *J* = 14.6, 8.9 Hz, 1H, C<u>H</u>$_2$Ph), 3.04 (dd, *J* = 14.6, 5.9 Hz, 1H, C<u>H</u>$_2$Ph), 3.40 (ddd, *J* = 9.1, 5.9,

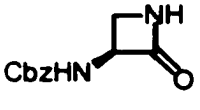
4.0, Hz, 1H, C<u>H</u>CO), 3.78 (s, 3H, C<u>H</u>$_3$O), 4.42 (dq, J = 6.1, 4.1 Hz, 1H, C<u>H</u>CH$_3$), 6.82 (d, J = 10 Hz, 2H, <u>Ph</u>), 7.18 (d, J = 10 Hz, 2H, <u>Ph</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.1, 32.6, 55.3, 58.7, 74.0, 114.3, 128.9, 129.6, 158.7, 170.7; HRMS (EI) Calcd for $C_{12}H_{14}O_3$ (M^+) 206.0943, found 206.0941.



(1'*S*,2*R*,3*R*)-2-(1'-Hydroxy-*p*-methoxybenzyl)-4-methyl-oxetan-2-one (52b). Cyclization of hydroxy acid **162b** (80 mg, 0.34 mmol) using BOP (190 mg, 0.43 mmol) and triethylamine (0.15 mL, 1.02 mmol) as described for **48a** gave β-lactone **52b** (50 mg, 70%) as a white solid after recrystallization from CH_2Cl_2/hexane: mp 88-89 °C; $[\alpha]_D^{26}$ -85.5° (*c* 0.3, $CHCl_3$); IR ($CHCl_3$ cast) 3411, 2984, 2960, 1807, 1612, 1514, 1448 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (d, J = 6.2 Hz, 3H, CH(C<u>H</u>$_3$)), 3.52 (dd, J = 4.4, 4.4 Hz, 1H, C<u>H</u>CO), 3.80 (s, 3H, C<u>H</u>$_3$O), 4.96-4.85 (m, 1H, C<u>H</u>CH$_3$), 5.18 (d, J = 4.4 Hz, 1H, C<u>H</u>OH), 6.90 (d, J = 11.3 Hz, 2H, <u>Ph</u>), 7.24 (d, J = 11.3 Hz, 2H, <u>Ph</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.1, 55.4, 64.8, 69.3, 70.6, 114.3, 126.7, 132.6, 159.6, 170.2; HRMS (EI) Calcd for $C_{12}H_{14}O_2$ (M^+) 222.0892, found 222.0887. Anal. Calcd for $C_{12}H_{14}O_2$: C, 64.85; H, 6.35; Found: C, 64.66; H, 6.34.

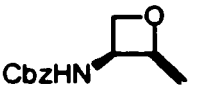


(3*RS*,4*RS*)-3-Benzoyl-4-methyl-oxetan-2-one (53). The procedure of Sengupta[82] was adapted for the synthesis of this known compound. A solution of diazo-ketoester **167** (0.8 g, 3.67 mmol) in CH_2Cl_2 (10 mL) was added dropwise over 2 h to a refluxing solution of rhodium (II) acetate dimer (8.0 mg, 0.04 mmol) in CH_2Cl_2 (40 mL). The reaction mixture was heated under reflux for a further 1 h and the solvent evaporated *in vacuo*. The residue was then diluted with Et_2O (30 mL) and filtered through a pad of celite. Concentration of the filtrate followed by purification of the crude product by column chromatography (SiO_2, 33% Et_2O/hexane) afforded **53** which was recrystallized from Et_2O/hexane to give a white solid (0.36 g, 52%); mp 64-65 °C; IR ($CHCl_3$ cast) 3024, 2977, 2916, 1817, 1681, 1597, 1449 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.75 (d, J = 6.3 Hz, 3H, $CH(C\underline{H}_3)$), 4.94 (d, J = 4.3 Hz, 1H, C<u>H</u>CO), 5.38 (dq, J = 4.2, 6.1 Hz, 1H, $CH_3C\underline{H}$), 7.65-7.50 (m, 3H, <u>Ph</u>), 8.10 (d, J = 11.3 Hz, 2H, <u>Ph</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.7, 66.5, 70.2, 129.0, 129.3, 134.5, 134.9, 163.6, 188.2; HRMS (ES) Calcd for $C_{11}H_{10}O_3Na$ (MNa^+) 213.0528, found 213.0527; Anal. Calcd for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30; Found: C, 69.36; H, 5.28.



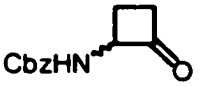
(3*S*)-3-Benzyloxycarbonylamino-2-azetidinone (54). A suspension of $NaHCO_3$ (0.33 g, 3.90 mmol) in CH_3CN (40 mL) was stirred and heated to gentle reflux.[121a] To this was added methanesulfonyl chloride (0.08 mL, 0.97 mmol) followed by portionwise

addition of the amino acid **173** (prepared by mixing the amino acid **172** with 1 equivalent of *p*-toluene sulfonic acid in MeOH followed by evaporation of the MeOH) (0.2 g, 0.84 mmol) over 4 h. The reaction mixture was heated under reflux overnight and the solid removed by filtration at 60 °C. Concentration of the filtrate *in vacuo* followed by purification of the crude product by column chromatography (SiO_2, 100 % EtOAc) afforded **54** which was recrystallized from CH_3Cl/hexane to give a white powder (0.05 g, 49%); mp 164-165 °C, lit.[121c] m.p 164-165 °C; IR ($CHCl_3$ cast) 3334, 1727, 1697, 1547, 1452, 1372 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.39 (br s, 1H, C<u>H</u>$_2$NH), 3.60 (dd, J = 10.6, 5.1 Hz, 1H, C<u>H</u>$_2$NH), 4.80-4.84 (m, 1H, C<u>H</u>), 5.10 (br s, 2H, C<u>H</u>$_2$O), 5.38 (d, J = 6.1 Hz, 1H, CbzN<u>H</u>), 5.80 (br s, 1H, CON<u>H</u>), 7.35 (br s, 5H, <u>Ph</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 44.8, 59.6, 67.8, 128.9, 129.1, 129.5, 138.0, 157.9, 171.4; HRMS (EI) Calcd for $C_{11}H_{12}O_3N_2$ (M^+) 220.0848, found 220.0826; Anal. Calcd for $C_{11}H_{12}O_3N_2$: C, 59.99; H, 5.49; N, 12.7; Found: C, 59.82; H, 5.44; N, 12.39.



(*S*)-*N*-(Benzyloxycarbonyl)-3-amino-2-methyleneoxetane (55). The procedure of Dollinger[122b] was empoloyed in this synthesis. Dimethyltitanocene (2.87 mL, 0.5 M in toluene, 1.44 mmol) and β-lactone **148** (0.15 g, 0.72 mmol) were stirred at 80 °C under an atmosphere of argon in the dark. The reaction was monitored by TLC and after the disappearance of the starting material (2 h), the reaction mixture was allowed to cool and treated with an equal volume of petroleum ether. The reaction mixture was stirred for a further 30 min and the yellow precipitate was filtered through a pad of celite and washed

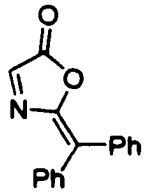
with more petroleum ether until the filtrate was colorless. The solvent was removed *in vacuo* and the residue purified by column chromatography (SiO_2, 20% EtOAc/hexane) to afford **55** which was recrystallized from Et_2O/pentane to give a white crystalline solid (0.04 g, 24%); mp 76-77 °C; $[\alpha]_D^{26}$ +30.6° (*c* 0.3, $CHCl_3$); IR ($CHCl_3$ cast) 3323, 3033, 2961, 1695, 1595, 1528, 1454 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.00 (d, *J* = 4.3 Hz, 1H, CC<u>H</u>$_2$), 4.22 (d, *J* = 4.2 Hz, 1H, CC<u>H</u>$_2$), 4.42 (dd, *J* = 5.2, 2.8 Hz, 1H, C<u>H</u>), 4.84-4.75 (m, 1H, C<u>H</u>$_2$O), 5.10 (s, 2H, C<u>H</u>$_2$O (Cbz)), 5.25 (br s, 1H, N<u>H</u>), 7.38 (br s, 5H, <u>Ph</u>); ^{13}C NMR (125 MHz, $CDCl_3$) δ 50.8, 67.3, 77.2, 81.2, 128.3, 128.4, 128.7, 135.9, 155.3, 166.5; HRMS (ES) Calcd for $C_{12}H_{13}O_3NNa$ (MNa^+) 242.0793, found 242.0793.



(*R,S*)-*N*-(Benzyloxycarbonyl)-cyclobutanone (56). A mixture of benzyl carbamate (0.24 g, 1.56 mmol) and HCl-saturated Et_2O (3 ml) was cooled to 0 °C and 1,2-bis(dimethylsilyoxy) cyclobutene **176** (0.3 g, 1.30 mmol) was added dropwise with stirring under argon.[123] After completion of the addition, the reaction mixture was heated to 80 °C for 4 h. The solvent was removed *in vacuo* and the residue purified by column chromatography (SiO_2, 50% Et_2O/hexane) to afford **56** as a colorless oil (0.23 g, 81%); IR ($CHCl_3$ cast) 3336, 3064, 2960, 1790, 1709, 1526 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.00 (dt, *J* = 19.2, 9.1 Hz, 1H, C<u>H</u>$_2$CH), 2.48-2.32 (m, 1H, C<u>H</u>$_2$CH), 2.94-2.79 (m, 2H, C<u>H</u>$_2$CO), 4.82 (q, *J* = 8.8 Hz, 1H, C<u>H</u>NH), 5.15 (br s, 2H, C<u>H</u>$_2$O), 5.40 (br s, 1H, N<u>H</u>), 7.35 (br s, 5H, <u>Ph</u>); ^{13}C NMR (75 MHz, $CDCl_3$)(mixture of rotamers) δ 19.8, 41.6, 65.2, 66.9, 67.2, 128.1, 128.2, 128.6, 136.0, 136.3, 156.4, 156.8, 205.3; HRMS (ES) Calcd for

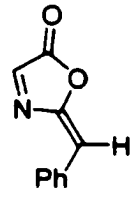
$C_{12}H_{13}O_3NNa$ (MNa^+) 242.0793, found 242.0792; Anal. Calcd for $C_{12}H_{13}O_3N$: C, 65.74; H, 5.98; N, 6.39; Found: C, 65.49; H, 6.02; N, 6.44.

***N*-(Benzyloxycarbonyl)-azetidinone-3-one (57).** This known compound was prepared from a modified procedure of Wang.[124] Reaction of the diazoketone **178** (0.3 g, 1.28 mmol) with rhodium (II) acetate dimer (3.0 mg, 0.01 mmol) as described for **53** afforded the 3-azetidinone **57** (0.08 g, 28%) as a colorless oil; IR ($CHCl_3$ cast) 2331, 1828, 1710, 1498, 1415, 1353, 1262 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.78 (d, J = 1.0 Hz, 4H, $(C\underline{H}_2)_2N$), 5.16 (d, J = 8.7 Hz, 1H, $C\underline{H}_2O$), 5.14 (d, J = 8.9 Hz, 1H, $C\underline{H}_2O$), 7.40 (br s, 5H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 67.8, 71.4, 128.3, 128.5, 128.7, 128.8, 136.0, 156.4, 195.5; LRMS (CI) for $C_{11}H_{11}O_3N$ *m/z* (relative intensity) 223.2 (MNH_4^+, 41.34%).

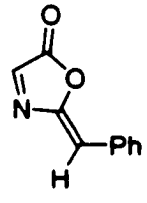


2,2-Dibenzylidene-*2H*-oxazol-5-one (58).[128] The title compound was prepared using a modified literature procedure of King.[129] 2-chloro-2,2-diphenylacetyl chloride (6.6 g, 25.0 mmol) was dissolved in anhydrous EtOAc (100 mL) under an argon atmosphere. Propylene oxide (1.75 mL, 25.0 mmol) was added, followed by glycine **185** (1.7 g, 22.5 mmol). The mixture was heated to reflux and stirred overnight. The reaction

mixture was cooled to rt, filtered and concentrated *in vacuo*. Recrystallization from EtOAc/petroleum ether gave the α-chloro-diphenylacetylglycine **187** as a white solid (6.48 g, 94%). A portion of **187** (1.0 g, 3.30 mmol) was dissolved in anhydrous MeCN (40 mL) under an argon atmosphere. 1,3-Dicyclohexylcarbodiimide (0.75 g, 3.6 mmol) was added, followed by propylene oxide (0.70 g, 9.9 mmol). The mixture was stirred overnight, filtered and concentrated *in vacuo*. Purification by flash chromatography (SiO_2, 75% petroleum ether/Et_2O) gave the title compound **58** (0.70 g, 85%) as a yellow solid: mp 111-113 °C; IR ($CHCl_3$ cast) 3057, 1792, 1770, 1099 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.36-7.55 (m, 10H, Ph), 7.74 (s, 1H, CH=N); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.7, 153.3, 146.9, 136.3, 136.1, 132.2, 131.7, 130.0, 129.7, 129.6, 128.4, 128.1; HRMS (EI) Calcd for $C_{16}H_{11}O_2N$ (M^+) 249.0790, found 249.0786; Anal. Calcd for $C_{16}H_{11}O_2N$: C, 77.10; H, 4.45; N, 5.62. Found: C, 76.70; H, 4.34; N, 5.46.



60a



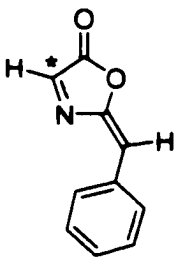
60b

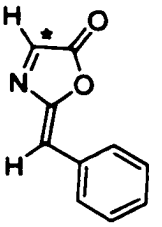
***E*-(60a) and *Z*-(60b)-2-benzylidene-*2H*-oxazol-5-one.**[128] The title compounds were prepared by a modification of the procedure of King.[129] To a solution of glycine **185** (0.93 g, 23.0 mmol) in H_2O (7.5 mL), THF (3 mL) and NaOH (1.09 g, 27.30 mmol) at 0-5 °C was added D,L-2-chloro,2-phenylacetyl chloride (2.0 mL, 13.0 mmol). The reaction mixture was stirred for 30 min then warmed to rt over 30 min. The mixture was basified with 1N NaOH and washed with EtOAc (2 x 15 mL). The aqueous layer was

acidified to pH 1 with 4 N HCl, extracted with EtOAc (3x15 mL) and dried over $MgSO_4$. Evaporation of the solvent *in vacuo* followed by recrystallization from MeOH/Et_2O/hexane) furnished the α-chloroacid as a white solid (0.84 g, 37%). A portion of the acid (0.5 g, 2.2 mmol) was dissolved in acetic anhydride (20 mL) and pyridine (5 mL). The mixture was stirred for 1 h after which it was evaporated *in vacuo*. The crude product was taken up in EtOAc (15 mL) and washed successively with 1N HCl (2 x 10 mL) and then saturated aq. $NaHCO_3$ (2 x 10 mL). The EtOAc extract was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2, 80% Et_2O/Hexane) to afford the title products which were recrystallized from hexane to give yellow crystalline solids.

Data for **60a**: (0.03 g, 9%, over 2 steps); mp 94-96 °C; IR ($CHCl_3$ cast) 3341, 3065, 2951, 1971, 1837, 1789, 1670, 1602, 1498, 1087 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 6.72 (d, J = 2.0 Hz, 1H, C<u>H</u>=C), 7.36-7.49 (m, 3H, <u>Ph</u>), 7.94-7.99 (m, 2H, <u>Ph</u>), 8.12 (d, J = 2.0 Hz, 1H, C<u>H</u>=N); ^{13}C NMR (125 MHz, acetone-d_6) δ 113.6, 126.7, 130.4, 132.0, 133.5, 151.4, 156.6, 163.5; HRMS (EI) Calcd for $C_{10}H_7O_2N$ (M^+) 173.0477, found 173.0476.

Data for **60b**: (0.14 g, 37%, over 2 steps); mp 92-94 °C; IR ($CHCl_3$ cast) 3036, 1838, 1780, 1672, 1508, 1451, 1361, 1284, 1088 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 6.62 (s, 1H, C<u>H</u>=C), 7.38-7.52 (m, 3H, <u>Ph</u>), 7.86-7.8 (m, 2H, <u>Ph</u>), 8.05 (d, J = 1.0 Hz, 1H, C<u>H</u>=N); ^{13}C NMR (125 MHz, acetone-d_6) δ 114.5, 129.9, 130.8, 131.8, 133.3, 148.7, 156.0, 164.3; HRMS (EI) Calcd for $C_{10}H_7O_2N$ (M^+) 173.0477, found 173.0479.



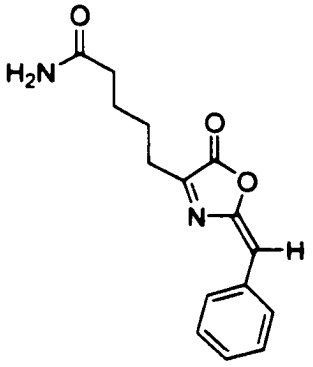


60a(α-13C) * = ^{13}C **60b(α-13C)**

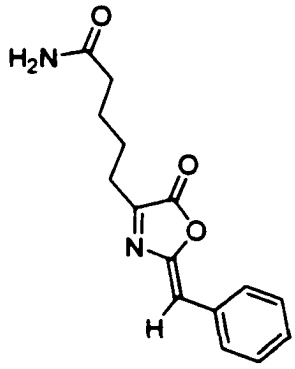
***E*-(60a(α-^{13}C)) and *Z*-(60b(α-^{13}C))-2-benzylidene-*2H*-oxazol-5-one.** These compounds were prepared as that described for **60a** and **60b**. Reaction of glycine-2-^{13}C (1.0 g, 13.2 mmol), NaOH (1.06 g, 26.30 mmol) and D,L-2-chloro, 2-phenylacetyl chloride (2.23 mL, 14.5 mmol) gave the corresponding chloroacid which was cyclized with acetic anhydride (30 mL) and pyridine (6 mL). Purification by column chromatography (SiO_2, Et_2O/Hexane - 4:1) followed by recrystallization from hexane gave the title products as yellow crystalline solids.

Data for **60a(α-^{13}C)**: (82.1 mg, 4%, over 2 steps); mp 93-94 °C; IR (μscope) 3326, 3061, 1788, 1768, 1650, 1552, 1529, 1499 cm^{-1}; ^{1}H NMR (400 MHz, CD_2Cl_2) δ 6.61 (d, J = 2.0 Hz, 1H, C<u>H</u>=C), 7.38-7.48 (m, 3H, <u>Ph</u>), 7.83 (dd, $^1J_{13C\text{-}H}$ = 204.4, 2.1 Hz, 1H, ^{13}C<u>H</u>=N) 7.86-7.90 (m, 2H, <u>Ph</u>); HRMS (EI) Calcd for for $^{13}CC_9H_7O_2N$ (M^+) 174.0510, found 174.0512.

Data for **60b(α-^{13}C)**: (0.38 g, 17%, over 2 steps); mp 94-95 °C; IR (μscope) 3523, 3080, 3061, 2987, 1816, 1788, 1680, 1650, 1572 cm^{-1}; ^{1}H NMR (400 MHz, CD_2Cl_2) δ 6.52 (s, C<u>H</u>=C), 7.37-7.49 (m, 3H, <u>Ph</u>), 7.76 (dd, $^1J_{13C\text{-}H}$ = 204.9, 1.0 Hz, 1H, ^{13}C<u>H</u>=N), 7.79-7.84 (m, 2H, <u>Ph</u>); HRMS (EI) Calcd for $^{13}CC_9H_7O_2N$ (M^+) 174.0510, found 174.0511.



62a



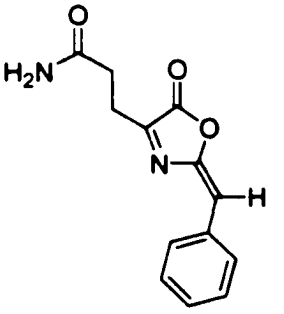
62b

***E*-(62a) and *Z*-(62b)-2-benzylidene-4-butanamide-*2H*-oxazol-5-one.** These compounds were prepared as that described for **60a** and **60b**. Reaction of L-homoglutamine **194** (210 mg, 1.31 mmol), NaOH (0.12 g, 2.89 mmol) and D,L-2-chloro, 2-phenylacetyl chloride (0.23 mL, 1.44 mmol) gave the corresponding chloroacid. This acid was cyclized with acetic anhydride (20 mL) and pyridine (5 mL). Purification by column chromatography (SiO_2, 66% EtOAc/Hexane) gave the title products, which were recrystallized from EtOAc/hexane to give yellow crystalline solids.

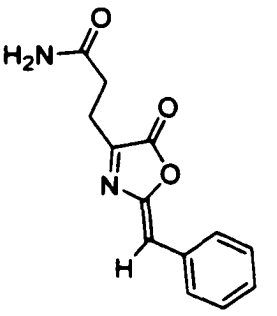
Data for **62a**: (25 mg, 7%, over 2 steps); mp 146-148 °C; IR (μscope) 3419, 3214, 3064, 2957, 1778, 1649, 1487, 1449, 1364, 1089 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 1.96 (quintet, J = 7.2 Hz, 2H, C$\underline{H_2}$CH$_2$), 2.20 (t, J = 7.2 Hz, 2H, C$\underline{H_2}$C=N), 2.69 (t, J = 7.6 Hz, 2H, C$\underline{H_2}$CO), 6.62 (s, 1H, C$\underline{H}$=C), 6.64 (br s, 1H, N$\underline{H_2}$), 7.22-7.48 (m, 4H, 1xH(N$\underline{H_2}$), 3xH(C$\underline{Ph}$)), 7.90 (d, J = 7.1 Hz, 2H, C$\underline{Ph}$); ^{13}C NMR (75 MHz, acetone-d_6) δ 22.1, 28.1, 34.8, 110.3, 128.9, 129.5, 129.7, 131.6, 133.9, 154.9, 163.5, 164.4, 174.5; HRMS (EI) Calcd for $C_{14}H_{14}O_3N_2$ (M^+) 258.1004, found 258.1002.

Data for **62b**: (37 mg, 11%, over 2 steps); mp 194-196 °C; IR (μscope) 3442, 3188, 1778, 1680, 1447, 1415, 1331, 1160, 1066 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.92 (quintet, J = 7.2 Hz, 2H, C$\underline{H_2}$CH$_2$), 2.16 (t, J = 7.2 Hz, 2H, C$\underline{H_2}$C=N), 2.65 (t, J = 7.3 Hz,

2H, C$\underline{H}_2$CO), 6.28 (br s, 1H, N$\underline{H}_2$), 6.54 (s, 1H, C$\underline{H}$=C), 6.74 (br s, 1H, N$\underline{H}_2$), 7.24-7.49 (m, 4H, 1xH(N$\underline{H}_2$), 3xH($\underline{Ph}$)), 7.78 (d, J = 7.5 Hz, 2H, $\underline{Ph}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.9, 26.7, 33.9, 109.9, 127.9, 128.9, 129.2, 130.2, 132.5, 152.9, 160.6, 163.3, 173.6; HRMS (EI) Calcd for $C_{14}H_{14}O_3N_2$ (M^+) 258.1004, found 258.1003.



63a

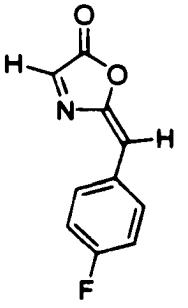


63b

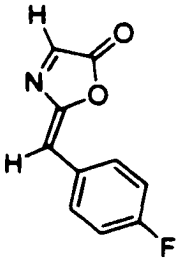
***E*-(63a) and *Z*-(63b)-2-benzylidene-4-propionamide-*2H*-oxazol-5-one.** These compounds were prepared as that described for **60a** and **60b**. Reaction of L-glutamine **195** (2.0 g, 13.69 mmol), NaOH (1.09 g, 27.30 mmol) and D,L-2-chloro,2-phenylacetyl chloride ((2.39 mL, 15.06 mmol) gave the corresponding chloroacid (1.68 g, 41%). A portion of this acid (0.3 g, 1.0 mmol) was cyclized with acetic anhydride (20 mL) and pyridine (5 mL). The crude product was purified by column chromatography (SiO_2, 66% EtOAc/Hexane) to afford the title products, which were recrystallized from CH_2Cl_2/Et_2O to give yellow crystalline solids.

Data for **63a**: (52 mg, 9%, over 2 steps); mp 160-161°C; IR (μscope) 3509, 3459, 3211, 2919, 2849, 1759, 1659, 1614, 1413, 1220 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.62 (t, J = 6.9 Hz, 2H, C$\underline{H}_2$C=N), 2.88 (t, J = 6.9 Hz, 2H, C$\underline{H}_2$CO), 6.62 (s, 1H, C$\underline{H}$=C), 6.86 (br s, 1H, N$\underline{H}_2$), 7.31-7.49 (m, 4H, 1xH(N$\underline{H}_2$), 3xH($\underline{Ph}$)), 7.91 (d, J = 8.1 Hz, 2H, $\underline{Ph}$); ^{13}C

NMR (75 MHz, DMSO-d_6) δ 23.6, 30.9, 109.2, 129.0, 129.1, 130.7, 132.9, 153.6, 162.7, 163.9, 172.8; HRMS (ES) Calcd for $C_{13}H_{12}O_3N_2Na$ (MNa^+) 267.0746, found 267.0748. Data for **63b**: (58 mg, 10%, over 2 steps); mp 160-161°C; IR (μscope) 3352, 3179, 3070, 3024, 2940, 2803, 1948, 1782, 1657, 1490, 1447, 1161 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 2.56 (t, J = 7.5 Hz, 2H, C<u>H</u>$_2$C=N), 2.83 (t, J = 7.1 Hz, 2H, C<u>H</u>$_2$CO), 6.52 (s, 1H, C<u>H</u>=C), 6.85 (br s, 1H, N<u>H</u>$_2$), 7.34-7.51 (m, 4H, 1xH(N<u>H</u>$_2$), 3xH(<u>Ph</u>)), 7.75 (d, J = 7.2 Hz, 2H, <u>Ph</u>); ^{13}C NMR (75 MHz, DMSO-d_6) δ 23.2, 30.9, 110.1, 129.3, 129.5, 130.5, 132.7, 153.0, 161.0, 163.5, 172.7; HRMS (ES) Calcd for $C_{13}H_{12}O_3N_2Na$ (MNa^+) 267.0746, found 267.0749; Anal. Calcd for $C_{13}H_{12}O_3N_2$: C, 63.93; H, 4.95; N, 11.47. Found: C, 63.56; H, 4.74; N, 11.20.



64a



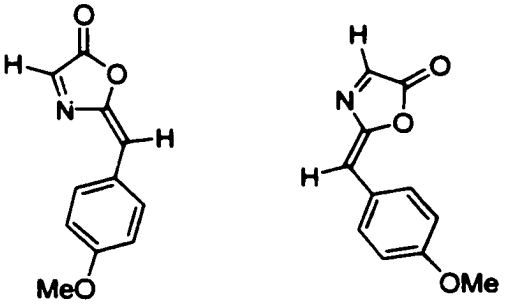
64b

***E*-(64a) and *Z*-(64b)-2-(4-fluro-benzylidene)-*2H*-oxazol-5-one.** A solution of 4-fluorophenyl acetamide **197** (0.33 g, 3.59 mmol) and glyoxylic acid monohydrate (0.33 g, 3.59 mmol) in acetone (15 mL) was refluxed at 65 °C for 4 h until a homogeneous solution was obtained.[131] The solvent was evaporated *in vacuo* and the residue diluted with H_2O (10 mL), extracted with EtOAc (3 x 10 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the white solid **199** obtained was dissolved in CH_2Cl_2 (15 mL) and trifluoroacetic anhydride added (0.92 mL, 6.54 mmol) to it. The reaction

mixture was stirred for 30 min at rt after which the yellow solution was concentrated *in vacuo* and the residue purified by column chromatography (SiO_2, 25% Et_2O/petroleum ether) to afford the title products which were recrystallized from Et_2O/hexane to give yellow crystalline solids.

Data for **64a**: (108 mg, 20%, over 2 steps); mp 200 °C (dec.); IR (μscope) 3077, 2945, 1802, 1666, 1605, 1099 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 6.74 (d, J = 1.7 Hz, 1H, C<u>H</u>=C), 7.18-7.26 (m, 2H, <u>Ph</u>), 8.02-8.08 (m, 2H, <u>Ph</u>), 8.14 (d, J = 2.0 Hz, 1H, C<u>H</u>=N); ^{13}C NMR (125 MHz, acetone-d_6) δ 112.4, 116.6 (d, $^2J_{C\text{-}F}$ = 22.5 Hz), 129.9, 134.1 (d, $^3J_{C\text{-}F}$ = 8.8 Hz), 151.2, 156.3, 163.2 (d, $^1J_{C\text{-}F}$ = 250.0 Hz), 163.4; HRMS (EI) Calcd for $C_{10}H_6O_2NF$ (M^+) 191.0383, found 191.0383.

Data for **64b**: (90 mg, 17%, over 2 steps); mp 128-129 °C; IR (μscope) 3109, 3069, 2986, 1826, 1765, 1654, 1607 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 6.62 (s, 1H, C<u>H</u>=C), 7.22-7.32 (m, 2H, <u>Ph</u>), 7.88-7.96 (m, 2H, <u>Ph</u>), 8.01 (s, 1H, C<u>H</u>=N); ^{13}C NMR (125 MHz, acetone-d_6) δ 113.2, 116.9 (d, $^2J_{C\text{-}F}$ = 22.5 Hz), 129.9, 134.0 (d, $^3J_{C\text{-}F}$ = 8.5 Hz), 151.2, 155.8, 163.2 (d, $^1J_{C\text{-}F}$ = 249.1 Hz), 164.2; HRMS (EI) Calcd for $C_{10}H_6O_2$ NF (M^+) 191.0383, found 191.0386.



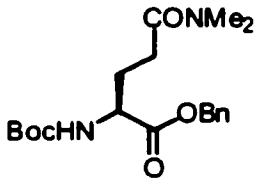
65a **65b**

***E*-(65a) and *Z*-(65b)-2-(4-methoxy-benzylidene)-*2H*-oxazol-5-one.** These compounds were prepared as that described for **64a** and **64b**. Reaction of glyoxylic acid monohydrate (0.21 g, 3.33 mmol) and 4-methoxy-phenylacetamide **198** (0.5 g, 3.03 mmol) gave the hydroxyacid **200** which was recrystallized from acetone/hexane to give a white solid (0.37 g, 51%). The hydroxyacid **200** was cyclized with TFAA (0.86 mL, 6.54 mmol) to give the title products after purification by column chromatography (SiO_2, 20% Et_2O/hexane). The products were recrystallized from Et_2O/hexane to give **65a** as an orange and **65b** as a yellow crystalline solid.

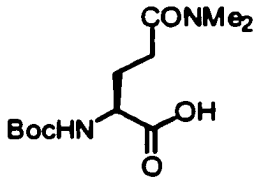
Data for **65a**: (110 mg, 18%, over 2 steps); mp 230 °C (dec.); IR (μscope) 3098, 3064, 2937, 2836, 2360, 1830, 1763, 1646, 1601 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 3.84 (s, 3H, $OC\underline{H}_3$), 6.67 (d, 1H, J = 2.0 Hz, $C\underline{H}$=C), 6.98-7.08 (m, 2H, Ph), 7.92-7.98 (m, 2H, Ph), 8.02 (d, 1H, J = 2.0 Hz, $C\underline{H}$=N); ^{13}C NMR (125 MHz, acetone-d_6) δ 55.8, 113.9, 115.4, 115.6, 126.1, 133.8, 155.4, 162.2, 163.7; HRMS (EI) Calcd for (M^+) $C_{11}H_9O_3N$ 203.0582, found 203.0582.

Data for **65b**: (83 mg, 14%, over 2 steps); mp 108-110 °C; IR (μscope) 2937, 2909, 2097, 2836, 1890, 1855, 1787, 1760, 1651, 1606, 1511 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 3.88 (s, 3H, $OC\underline{H}_3$), 6.58 (s, 1H, $C\underline{H}$=C), 7.02-7.11 (m, 2H, Ph), 7.80-7.84 (m, 2H, Ph), 7.85 (d, 1H, J = 1.0 Hz, $C\underline{H}$=N); ^{13}C NMR (125 MHz, acetone-d_6) δ 55.8, 114.9, 115.9,

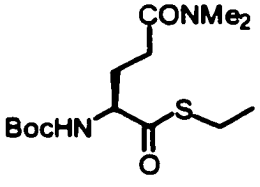
125.9, 151.1, 133.8, 154.9, 162.3, 164.6; HRMS (EI) Calcd for $C_{11}H_9O_3N$ (M^+) 203.0582, found 203.0583.



(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-pentanoic acid benzyl ester (69). This compound was prepared by a modified procedure of Matsoukas.[136] To a stirred solution of Boc-Glu-OBn **68** (5.00 g, 14.82 mmol) in anhydrous CH_2Cl_2 (70 mL) at 0 °C was added triethylamine (2.27 mL, 16.30 mmol) followed by ethyl chloroformate over a period of 5 min. After stirring at 0 °C for 30 min, solid dimethylamine hydrochloride (1.33 g, 16.83 mmol) and triethylamine (2.60 mL, 18.51 mmol) were added. The reaction mixture was stirred for a further 2 h at 0 °C and then allowed to stir overnight at rt. The solvent was removed *in vacuo* and the residue diluted with H_2O (60 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with 1N HCl (20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Recrystallization of the crude product from CH_2Cl_2/hexane gave white needles (4.61 g, 85%); mp 97-98 °C, lit.[136] mp 99-100 °C; $[\alpha]_D^{26}$ -25.8° (*c* 1.0, EtOH), lit.[136] $[\alpha]_D^{26}$ -27.5° (*c* 1.0, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H, $(C\underline{H}_3)_3$), 1.92-2.02 (m, 1H, $CHC\underline{H}_2$), 2.11-2.22 (m, 1H, $CHC\underline{H}_2$), 2.25-2.40 (m, 2H, $C\underline{H}_2CO$), 2.85 (s, 3H, $N(C\underline{H}_3)_2$), 2.88 (s, 3H, $N(C\underline{H}_3)_2$), 4.22-4.38 (m, 1H, C<u>H</u>), 5.12 (d, J = 13.2 Hz, 1H, $C\underline{H}_2Ph$), 5.18 (d, J = 12.9 Hz, 1H, $C\underline{H}_2Ph$), 5.42 (br d, J = 5.4 Hz, 1H, N<u>H</u>), 7.25-7.29 (m, 5H, <u>Ph</u>); HRMS (EI) Calcd for $C_{19}H_{28}N_2O_5$ (M^+) 364.1998, found 364.1999.

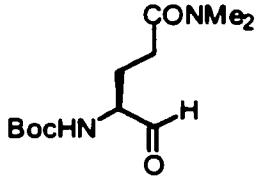


(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-pentanoic acid (70). Glutamine **69** (2.95 g, 8.10 mmol) was dissolved in MeOH (25 mL) in the presence of 10% Pd/C (300 mg, 10% w/w). The suspension was stirred under a hydrogen atmosphere until the uptake of hydrogen ceased (approximately 6 h). Filtration through celite, followed by removal of the solvent *in vacuo*, afforded the title compound which was recrystallized from CH_2Cl_2/hexane to give a white crystalline solid (2.22 g, 90%); mp 121-122 °C, lit.[137] mp 124-125 °C; $[\alpha]_D^{26}$ +3.5° (*c* 1.0, EtOH); lit.[137] $[\alpha]_D^{26}$ +2.2° (*c* 1.0, EtOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H, $(C\underline{H}_3)_3$), 1.95-2.04 (m, 1H, $CHC\underline{H}_2$), 2.21-2.29 (m, 1H, $CHC\underline{H}_2$), 2.42-2.82 (m, 1H, $C\underline{H}_2CO$), 2.70-2.84 (m, 1H, $C\underline{H}_2CO$), 2.95 (s, 3H, $N(C\underline{H}_3)_2$), 3.05 (s, 3H, $N(C\underline{H}_3)_2$), 4.15 (q, J = 5.6 Hz, 1H, $C\underline{H}$), 5.65 (d, J = 5.6 Hz, 1H, $N\underline{H}$); HRMS (EI) Calcd for $C_{12}H_{22}N_2O_5$ (M^+) 274.1529, found 274.1533.



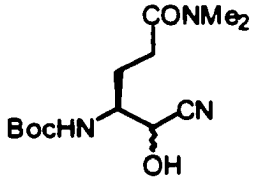
(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-4-ethansulfanylcarbonyl-butyramide (71). This compound was prepared by an adaptation of the Malcolm procedure.[25] A solution of acid **70** (5.00 g, 18.25 mmol) in anhydrous CH_2Cl_2 at 0 °C was treated with triethylamine (17.98 mL, 129.56 mmol) followed by ethyl chloroformate (6.29 mL, 65.69 mmol) over a period of 15 min. Ethanethiol (5.53 mL, 74.82 mmol) and

DMAP (22.3 mg, 1.82 mmol) were added and the reaction mixture was stirred for a further 2 h at 0 °, quenched by the addition of glacial acetic acid (5 mL) and then concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (50 mL) and washed subsequently with 5% aq. $NaHCO_3$ (25 mL), 5% aq. citric acid (25 mL) and brine (25 mL). The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. Recrystallization of the crude product from CH_2Cl_2/hexane furnished the desired product as an off-white crystalline solid (4.49 g, 78%); mp 122-123 °C; $[\alpha]_D^{25}$ -14.6° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3214, 2979, 1710, 1674, 1618, 1539 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.22 (t, *J* = 7.5 Hz, 3H, $C\underline{H}_3$), 1.42 (s, 9H, $(C\underline{H}_3)_3$), 1.95-2.00 (m, 2H, $CHC\underline{H}_2$), 2.30-2.50 (m, 2H, $C\underline{H}_2CO$), 2.84 (q, *J* = 7.5 Hz, 2H, $C\underline{H}_2CH_3$), 2.95 (s, 3H, $N(C\underline{H}_3)_2$), 3.00 (s, 3H, $N(C\underline{H}_3)_2$), 4.22-4.37 (m, 1H, $C\underline{H}$), 5.70 (d, *J* = 7.5 Hz, 1H, $N\underline{H}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5, 23.2, 27.5, 28.4, 29.4, 35.7, 37.2, 60.6, 80.0, 155.6, 172.1, 201.8; HRMS (EI) Calcd for $C_{14}H_{26}N_2SO_4$ (M^+) 318.1613, found 318.1604; Anal. Calcd for $C_{14}H_{26}N_2SO_4$: C, 52.81, H, 8.23, N, 8.79. Found C, 52.51, H, 8.37, N, 8.61.



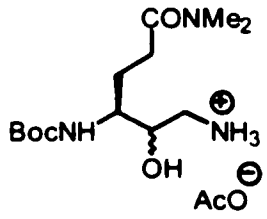
(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-pentanamide (72). To a solution of thioester **71** (4.5 g, 14.2 mmol) in anhydrous DMF (100 mL) at 0 °C was added triethylsilane (13.5 mL, 84.9 mmol) followed by 10% Pd/C (450 mg, 10% w/w). The reaction mixture was stirred for a further 2 h at 0 °C and then filtered through celite and washed successively with DMF, H_2O and acetone. The filtrate was evaporated *in*

vacuo and purified by column chromatography (SiO_2, 5% MeOH/EtOAc) to yield a colorless oil (3.31 g, 91%); $[\alpha]_D^{26}$+23.2° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3295, 2878, 1733, 1707, 1634, 1505 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H, $(C\underline{H}_3)_3$), 1.92-2.00 (m, 1H, $CHC\underline{H}_2$), 2.12-2.25 (m, 1H, , $CHC\underline{H}_2$), 2.30-2.51 (m, 2H, $C\underline{H}_2CO$), 2.92 (s, 3H, $N(C\underline{H}_3)_2$), 2.98 (s, 3H, $N(C\underline{H}_3)_2$), 4.10-4.19 (m, 1H, C<u>H</u>), 5.60 (br s, 1H, N<u>H</u>), 9.48 (s, 1H, C<u>H</u>O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.2, 28.3, 28.7, 35.6, 37.2, 59.5, 79.9, 157.7, 171.5, 200.0; HRMS (EI) Calcd for $C_{12}H_{22}N_2O_4$ (M^+) 258.1579, found 258.1578; Anal. Calcd for $C_{12}H_{22}N_2O_4$: C, 55.80, H, 8.58, N, 10.84. Found C, 55.87, H, 8.69, N, 10.89.



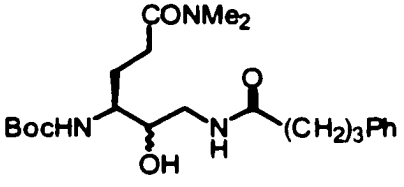
(4*S*,5*RS*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-cyano-5-hydroxypentanamide (73). A procedure of Greenlee[63] was used in this synthesis. Aldehyde **72** (1.32 g, 5.13 mmol) was dissolved in MeOH (60 mL) and to this solution was added AcOH (0.44 mL, 7.70 mmol) followed by potassium cyanide (0.5 g, 7.70 mmol). The reaction mixture was stirred at rt for 18 h and then concentrated *in vacuo*. The residue was slurried with EtOAc (20 mL) and filtered. The filtrate was concentrated and the crude product purified by column chromatography (SiO_2, 10% MeOH/EtOAc) to afford a white solid which was recrystallized from CH_2Cl_2/hexane (1.03 g, 70%); mp 114-115 °C; IR ($CHCl_3$ cast) 3307, 2350, 1709, 1627 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (1:1 mixture of diasteromers, data for one isomer) δ 1.42 (s, 9H, $(C\underline{H}_3)_3$), 1.94-2.10 (m,

2H, CHC$\underline{H}_2$), 2.36-2.52 (m, 2H, C$\underline{H}_2$CO), 2.92 (s, 3H, N(C$\underline{H}_3$)$_2$), 2.98 (s, 3H, N(C$\underline{H}_3$)$_2$), 3.79-3.95 (m, 1H, C$\underline{H}$), 4.54 (d, J = 9.0 Hz, 1H, C$\underline{H}$OH), 5.43 (d, J = 7.4 Hz, 0.5H, N$\underline{H}$), 5.55 (d, J = 7.5 Hz, 0.5H, N$\underline{H}$), 5.80-6.00 (m, 1H, CO$\underline{H}$); ^{13}C NMR (75 MHz, $CDCl_3$) (1:1 mixture of diasteromers) δ 25.0, 36.0, 37.3, 38.3, 39.3, 39.6, 53.7, 54.6, 64.2, 65.2, 80.4, 80.6, 118.5, 118.7, 154.0, 156.7, 172.8; HRMS (EI) Calcd for $C_{16}H_{17}N_2O_3$ (M^+) 285.1239, found 285.1231; Anal. Calcd for $C_{16}H_{17}N_2O_3$: C, 54.72, H, 8.12, N, 14.73. Found C, 54.44, H, 8.24, N, 14.42.



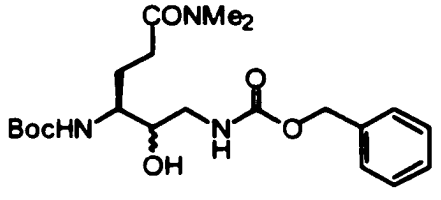
(4*S*,5*RS*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-6-amino-5-hydroxyhexanamide acetate salt (74). This compound was prepared by a modified procedure of Greenlee.[63] To a solution of cyanohydrin **73** (55 mg, 0.19 mmol) in propan-2-ol (10 mL) and AcOH (22 μL, 0.38 mmol) was added PtO_2 (10 mg, 50 μmol). The mixture was agitated in a Parr vessel (500 mL) under a hydrogen atmosphere at 50 psi for 24 h after which it was filtered through celite and washed through with propan-2-ol. Evaporation of the solvent *in vacuo* and purification of the crude product by column chromatography (SiO_2; $CHCl_3$/MeOH/AcOH 90:9:1) afforded the title compound as a foam (28.9 mg, 43%); IR ($CHCl_3$ cast) 3400, 2924, 1705, 1633 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) (1:1 mixture of diastereomers) δ 1.42 (s, 9H, (C$\underline{H}_3$)$_3$), 1.74-2.08 (m, 5H, CHC$\underline{H}_2$, C$\underline{H}_3$CO$_2$), 2.38-2.50 (m, 2H, C$\underline{H}_2$CO), 2.92 (s, 3H, N(C$\underline{H}_3$)$_2$), 2.98 (s, 3H, N(C$\underline{H}_3$)$_2$), 3.00-3.15 (m, 1H, C$\underline{H}$OH), 3.21-3.32 (m, 1H, C$\underline{H}_2$N), 3.42-3.68 (m, 1H,

C<u>H</u>$_2$N), 3.92-4.05 (m, 0.5H, C<u>H</u>), 4.08-4.19 (m, 0.5H, C<u>H</u>), 5.72 (d, J = 7.5 Hz, 0.5H, N<u>H</u>), 6.05 (d, J = 7.5 Hz, 0.5H, N<u>H</u>), 7.00-7.40 (m, 3H, N<u>H</u>$_3$); ^{13}C NMR (75 MHz, $CDCl_3$)(1:1 mixture of diasteromers δ 27.9, 28.5, 29.5, 29.7, 35.8, 37.4, 43.1, 51.9, 69.3, 70.3, 75.8, 79.3, 156.7, 173.3, 173.5; HRMS (ES) Calcd for $C_{13}H_{28}N_3O_4$ (M-$CH_3CO_2^+$) 290.2079, found 290.2080; Anal. Calcd for $C_{15}H_{31}N_3O_6$: C, 51.56, H, 8.94, N, 12.03. Found C, 51.22, H, 8.63, N, 12.21.



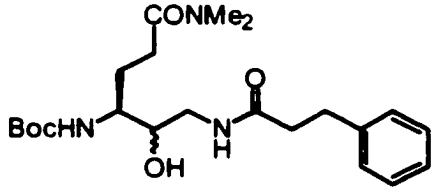
(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-hydroxy-6-(4-phenyl-butyrylamino)hexanamide (75). A solution of 4-phenyl butyric acid (56.8 mg, 0.35 mmol) in CH_2Cl_2 (10 mL) at 0 °C was treated with triethylamine (52 μL, 0.38 mmol) followed by dropwise addition of ethyl chloroformate (33.1 μL, 0.35 mmol). The mixture was stirred for a further 20 min and subsequently treated with the amino alcohol **74** (110 mg, 0.32 mmol) and triethylamine (52 μL, 0.38 mmol). The reaction mixture was allowed to warm to rt over 3 h and quenched with saturated aq. $NaHCO_3$ (10 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the crude product by column chromatography (SiO_2, 1% MeOH/EtOAc) afforded the title compound as a colorless oil (84.3 mg, 61%); IR ($CHCl_3$ cast) 3323, 2929, 1694, 1633, 1497 cm^{-1}; ^{1}H NMR (300 MHz, CD_2Cl_2) (2:1 mixture of diasteromers, data for major compound) δ 1.40 (s, 9H, (C<u>H</u>$_3$)$_3$), 1.65-2.10 (m, 4H, CHC<u>H</u>$_2$, CH$_2$C<u>H</u>$_2$), 2.15-2.50 (m, 4H, C<u>H</u>$_2$CO, C<u>H</u>$_2$Ph), 2.65 (m, 2H, COC<u>H</u>$_2$), 2.90

(s, 3H, N(C<u>H</u>$_3$)$_2$), 2.96 (s, 3H, N(C<u>H</u>$_3$)$_2$), 3.10-3.30 (m, 1H, C<u>H</u>OH), 4.40-4.65 (m, 3H, C<u>H</u>, C<u>H</u>$_2$NH), 4.55 (br s, 1H, O<u>H</u>), 5.15 (d, J = 9.1 Hz, 1H, N<u>H</u>), 6.58-6.02 (m, 1H, N<u>H</u>), 7.10-7.35 (m, 5H, <u>Ph</u>); ^{13}C NMR (75 MHz, CD_2Cl_2)(2:1 mixture of diasteromers δ 27.1, 27.6, 28.5, 29.7, 29.9, 35.6, 35.8, 36.1, 37.4, 42.9, 53.0, 71.4, 74.4, 79.5, 126.2, 128.6, 128.8, 142.3, 157.0, 173.3, 173.5, 173.7, 174.9; HRMS (ES) Calcd for $C_{23}H_{37}N_3O_5Na$ (MNa^+) 458.2631, found 458.2631.



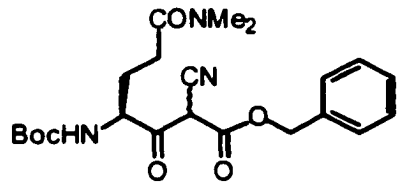
(4*S*,5*RS*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-6-(benzyloxycarbonyl-amino)-5-hydroxyhexanamide (76). A solution of amino alcohol **74** (76.5 mg, 0.22 mmol) in H_2O/THF (2:1, 6 mL) was treated with $NaHCO_3$ (64.4 mg, 0.77 mmol). After gas evolution ceased, benzyl chloroformate (34 μL, 0.24 mmol) was added and stirring was continued overnight at rt. The reaction mixture was diluted with H_2O (5 mL), extracted with EtOAc (3 x 5 mL) and the combined organic layers were dried over $MgSO_4$. Evaporation of the solvent followed by purification of the crude product by column chromatography (SiO_2, 10% MeOH/EtOAc) furnished the title compound as a colorless oil (79 mg, 85%); IR ($CHCl_3$ cast) 3332, 2932, 1706, 1651, 1632, 1575 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) (1:1 mixture of diasteromers) δ 1.41 (s, 9H, (C<u>H</u>$_3$)$_3$), 1.80-2.10 (m, 2H, CHC<u>H</u>$_2$), 2.20-2.50 (m, 2H, C<u>H</u>$_2$CO), 2.92 (s, 3H, N(C<u>H</u>$_3$)$_2$), 2.98 (s, 3H, N(C<u>H</u>$_3$)$_2$), 3.00-3.15 (m, 1H, C<u>H</u>OH), 3.35-3.40 (m, 1H, C<u>H</u>), 3.55-3.64 (m, 2H, C<u>H</u>$_2$NH), 5.05 (d, J = 8.1 Hz, 1H, C<u>H</u>$_2$Ph), 5.12 (d, J = 7.9 Hz, 1H, C<u>H</u>$_2$Ph), 5.28-5.30

(m, 1H, NH), 5.60-5.64 (m, 1H, NH), 5.92 (br s, 1H, OH), 7.25-7.40 (m, 5H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.2, 26.5, 28.4, 29.6, 33.8, 35.8, 37.3, 37.3, 43.7, 52.5, 53.2, 66.7, 66.9, 70.5, 74.0, 79.6, 128.1, 128.5, 136.6, 136.7, 156.6, 156.9, 173.2; HRMS (ES) Calcd for $C_{21}H_{33}N_3O_6Na$ (MNa^+) 446.2267, found 446.2263.



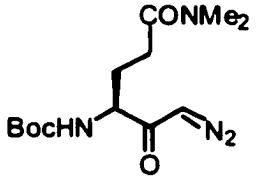
(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-hydroxy-6-(3-propionyl-amino)hexanamide (77). To a solution of amino alcohol **74** (63.3 mg, 0.18 mmol) in THF (4 mL) was added hydrocinnamoyl chloride (30 μL, 0.20 mmol) followed by triethylamine (38 μL, 0.27 mmol). The reaction mixture was stirred for 6 h after which it was diluted with saturated aq. $NaHCO_3$ (10 mL), extracted with EtOAc (3 x 5 mL) and the combined organic layers then dried over $MgSO_4$. Concentration *in vacuo* followed by purification of the crude product by column chromatography (SiO_2, 10% MeOH/EtOAc) afforded the title compound as a colorless oil (55.9 mg, 73%); IR ($CHCl_3$ cast) 3316, 2975, 1708, 1633, 1454, 1050 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (1:1 mixture of diasteromers, data for 1 isomer) δ 1.41 (s, 9H, $(CH_3)_3$), 1.60-2.10 (m, 2H, $CHCH_2$), 2.20-2.40 (m, 2H, CH_2CO), 2.92 (s, 3H, $N(CH_3)_2$), 3.0 (s, 3H, $N(CH_3)_2$), 3.0-3.2 (m, 1H, CHOH), 3.35-3.78 (m, 3H, CH, CH_2NH), 5.10 (d, J = 8.7 Hz, 1H, NH), 6.78 (d, J = 7.3 Hz, 1H, NHCO), 7.15-7.40 (m, 5H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$)(1:1 mixture of diasteromers)(1:1 mixture of diasteromers δ 25.1, 16.6, 29.5, 35.7, 37.3, 38.2, 42.3, 52.5,

70.5, 79.5, 126.1, 128.4, 128.5, 140.9, 156.7, 172.9, 173.3; LRMS (ES) for $C_{22}H_{35}O_5N_3$ *m/z* (relative intensity) 422.4 (MH^+, 100%).

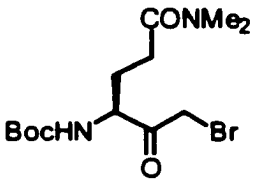


(4*S*,6*RS*)-)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-cyano-6-(oxo-hexanoic acid benzyl ester)hexanamide (80). To a solution of Benzyl cyanoacetate **78** (4.0 g, 22.8 mmol) in anhydrous Et_2O (40 mL) was added NaH (0.52 g, 21.8 mmol) at 0 °C. After 30 min of stirring, the mixture was warmed to rt and filtered and then washed with anhydrous Et_2O to give sodium benzyl cyanoacetate **79** as a white solid (3.74 g, 83%). To a solution of acid **70** (4.0 g, 14.5 mmol) in THF (60 mL) was added *1,1'*-carbonyl diimidazole (3.8 g, 23.3 mmol). The reaction mixture was stirred for 1 h after which benzyl cyanoacetate monosodium salt **79** (4.3 g, 21.8 mmol) was added and the reaction stirred for a further 12 h. The solvent was removed *in vacuo*, 1 M HCl (20 mL) was added to the residue and then extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the crude product by column chromatography (SiO_2, gradient elution, 75% $CHCl_3$/EtOAc to 50% $CHCl_3$/EtOAc) afforded the title compound as a foam (4.66 g, 70%); IR ($CHCl_3$ cast) 3410, 3299, 2976, 2194, 1712, 1647, 1455, 1403 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (1:1 mixture of diasteromers) δ 1.40 (s, 9H, $(C\underline{H}_3)_3$), 2.00-2.20 (m, 2H, $CHC\underline{H}_2$), 2.30-2.55 (m, 2H, $C\underline{H}_2CO$), 2.92 (s, 3H, $N(C\underline{H}_3)_2$), 2.98 (s, 3H, $N(C\underline{H}_3)_2$), 4.54-4.62 (m, 1H, C<u>H</u>), 5.23 (s, 1H, $C\underline{H}_2Ph$), 5.82 (br d, J = 3.7 Hz, 1H, N<u>H</u>), 7.22-7.40

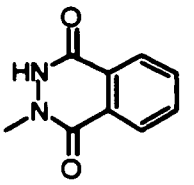
(m, 5H, Ph), 13.65 (br s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$)(1:1 mixture of diasteromers) δ 27.4, 28.0, 28.3, 29.8, 35.7, 37.2, 53.9, 68.0, 80.1, 80.2, 113.4, 127.8, 128.3, 128.7, 128.8, 134.3, 155.3, 170.0, 171.6, 190; HRMS (EI) Calcd for $C_{22}H_{29}N_3O_6$ (M^+) 431.2056, found 431.2053.



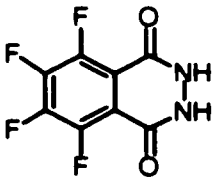
(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-diazohexanamide (81).[27] This known compound was prepared using the same literature procedure as that described by Morris.[27] To solution of acid **70** (5.0 g, 18.2 mmol) in THF (120 mL) at 0 °C was added triethylamine (2.79 mL, 20 mmol) followed by ethyl chloroformate (1.94 mL, 20 mmol). The reaction mixture was stirred for 30 min and the mixture was filtered into an ethereal solution of diazomethane (200 mL, ca. 91 mmol) at 0 °C. After stirring for a further 4 h the solvent was evaporated and the crude product recrystallized from CH_2Cl_2/hexane to give an off-white solid (4.35 g, 80%); mp 112-113 °C, lit.[27] mp 112-113 °C; $[\alpha]_D^{26}$ -1.3° (*c* 1.1, $CHCl_3$), lit.[27] $[\alpha]_D^{26}$ -1.7° (*c* 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H, $(CH_3)_3$), 1.75-1.90 (m, 1H, $CHCH_2$), 2.02-2.20 (m, 1H, $CHCH_2$), 2.38 (ddd, *J* = 16.5,7.1, 6.0 Hz, 1H, CH_2CO), 2.48 (ddd, *J* = 16.5,7.2, 6.0 Hz, 1H, CH_2CO), 2.90 (s, 3H, $N(CH_3)_2$), 2.98 (s, 3H, $N(CH_3)_2$), 4.11-4.19 (m, 1H, CH), 5.55 (br s, 1H, CHN_2), 5.40 (d, *J* = 5.8 Hz ,1H, NH); HRMS (ES) Calcd for $C_{13}H_{22}N_4O_4Na$ (MNa^+) 321.1539, found 321.1533.



(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-6-bromo-5-oxo-hexanamide (82).[27] This known compound was prepared by a modification of the Morris[27] procedure. A solution of the diazo-ketone **81** (4.09 g, 13.7 mmol) in THF (150 mL) at 0 °C was treated dropwise with aq. 48% HBr (2.43 mL, 14.4 mmol). The reaction mixture was stirred at 0 °C until the evolution of gas ceased, quenched with saturated aq. $NaHCO_3$ (20 mL) and the solvent removed *in vacuo*. The residue was diluted with H_2O (50 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over $MgSO_4$ and then concentrated *in vacuo*. Purification of the crude product by column chromatography (SiO_2, 50% $CHCl_3$/EtOAc) afforded the desired product which was recrystallized from CH_2Cl_2/hexane to give a light yellow solid (743 mg, 81%); mp 59-60 °C, lit.[27] mp 61 °C; $[\alpha]_D^{25}$ +21.4° (*c* 1.3, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H, ($C\underline{H}_3$)$_3$), 1.90-2.04 (m, 1H, CHC$\underline{H}_2$), 2.10-2.25 (m, 1H, CHC$\underline{H}_2$), 2.36 (ddd, *J* = 12.8, 7.1, 5.7 Hz, 1H, C$\underline{H}_2$CO), 2.49 (ddd, *J* = 13.0, 7.2, 5.9 Hz, 1H, C$\underline{H}_2$CO), 2.92 (s, 3H, N($C\underline{H}_3$)$_2$), 2.98 (s, 3H, N($C\underline{H}_3$)$_2$), 4.12 (d, *J* = 13.5 Hz, 1H, C$\underline{H}_2$Br), 4.19 (d, *J* = 13.5 Hz, 1H, C$\underline{H}_2$Br), 4.45-4.57 (m, 1H, C$\underline{H}$), 5.62-5.74 (m, 1H, N$\underline{H}$); HRMS (ES) Calcd for $C_{13}H_{24}N_2O_4{}^{79}Br$ (MH^+) 351.0914, found 351.0914.

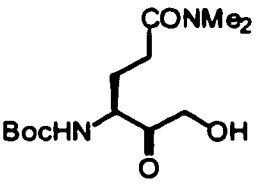


2-Methyl-2,3-dihydro-phthalazine-1,4-dione (88). [69a] This literature compound was prepared as that described by Watanabe.[69a] Methyl hydrazine (5.0 mL, excess) was added to a suspension of phthalic anhydride **87** in EtOH (30 mL), then heated under reflux for 1 h. Upon cooling, a white solid (2.45 g, 69%) formed which was filtered and washed with EtOH; mp 238-239 °C, lit.[69b] mp 236-237 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.52 (s, 3H, C<u>H</u>$_3$), 7.78-7.89 (m, 2H, <u>Ph</u>), 7.96 (d, J = 7.0 Hz, 1H, <u>Ph</u>), 8.16 (d, J = 7.0 Hz, 1H, <u>Ph</u>); HRMS (EI) Calcd for $C_9H_8N_2O_2$ (M^+) 176.0586, found 176.0589.

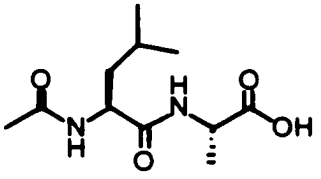


5,6,7,8-Tetrafluoro-2,3-dihydro-phthalazine-1,4-dione (90). The title product was prepared by a modified procedure of Gould.[71] To a solution of tetrafluorophthalic anhydride **89** (1.0 g, 4.55 mmol) in acetic acid was added hydrazine monohydrate (0.22 mL, 4.55 mol, 18% solution). The reaction mixture was heated at 80 °C for 1 h. Upon cooling a solid (0.81 g, 76%) precipitated which was filtered and washed with H_2O giving the title compound as an offwhite solid; mp 226-228 °C; IR (μscope) 3494, 3341, 3282, 3204, 2940, 2341, 1992, 1784, 1644, 1614 cm^{-1}; ^{19}F NMR (376 MHz, $CDCl_3$) δ -147.7, -140.7; ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.8, 144.1, 146.7, 159.2 (m); HRMS

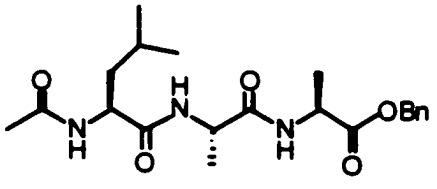
(EI) Calcd for $C_8H_2N_2O_2F_4$ (M^-) 234.0052, found 234.0058. Anal. Calcd for $C_8H_2N_2O_2F_4$: C, 41.04; H, 0.86; N, 11.97; Found: C, 41.06; H, 0.68; N, 11.84.



(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-6-hydroxy-5-oxohexanamide (102). Diazoketone **81** (2.5 g, 8.39 mmol) was dissolved in THF (50 mL) and cooled to 0 °C. After 5 min, 1N HCl (21 mL, 16.8 mmol) was added dropwise until evolution of gas ceased. The reaction mixture was stirred for 5 h at 0 °C after which it was carefully quenched with saturated aq. $NaHCO_3$ (10 mL). The solvent was removed *in vacuo* and the residue diluted with H_2O (30 mL) and extracted with EtOAc (3 x 15 mL). The combined organic layers were dried over $MgSO_4$ and evaporation of the solvent followed by purification by column chromatography (SiO_2, 5% MeOH/EtOAc) afforded **102** as a white solid (1.93 g, 80%) after recrystallization from CH_2Cl_2/hexane; mp 118-119 °C; $[\alpha]_D^{26}$ +21.0° (*c* 1.0, $CHCl_3$); IR (μscope) 3277, 3011, 1720, 1697, 1604, 1518 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H, $(C\underline{H}_3)_3$), 1.89-2.00 (m, 1H, $CHC\underline{H}_2$), 2.08-2.41 (m, 2H, $CHC\underline{H}_2$, O<u>H</u>), 2.28-2.54 (m, 3H $C\underline{H}_2CO$, O<u>H</u>), 2.92 (s, 3H, $N(C\underline{H}_3)_2$), 2.98 (s, 3H, $N(C\underline{H}_3)_2$), 4.30-4.39 (m, 1H, C<u>H</u>), 4.38 (d, J = 17.3 Hz, 1H, $C\underline{H}_2O$), 4.43 (d, J = 17.6 Hz, 1H, $C\underline{H}_2O$), 5.85 (br s, 1H, N<u>H</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.6, 28.3, 28.9, 35.8, 37.2, 56.8, 66.5, 80.1, 155.8, 172.2, 209.9; HRMS (EI) Calcd for $C_{13}H_{24}N_2O_5$ (M^-) 288.1685, found 288.1683.

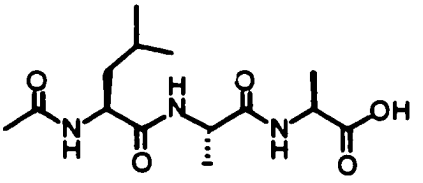


***N*-acetyl-L-leucyl-L-alanine (105).** L-Leucyl-L-alanine **104** (2.0 g, 10.5 mmol) was dissolved in H_2O (60 mL), after 15 min acetic anhydride (10 mL, excess) was added over a period of 20 min and the reaction mixture allowed to stir for 12 h. The solvent was the removed *in vacuo* and the crude solid was triturated with Et_2O (3x20 mL) and recrystallized from (CH_2Cl_2/hexane) to give a white crystalline solid (2.29 g, 90%); m.p. 185-186 °C; IR ($CHCl_3$, cast) 3310, 3105, 2951, 2872, 1738, 1673, 1457, 1385 cm^{-1}; $[\alpha]_D^{25}$ -51.2° (*c* 1.0, MeOH); ^1H NMR (300 MHz, CD_3OD) δ 0.92 (d, *J* = 6.5 Hz, 3H, C<u>H</u>$_3$(Leu)), 0.97 (d, *J* = 6.5 Hz, 3H, C<u>H</u>$_3$(Leu)), 1.38 (d, *J* = 6.0 Hz, 3H, C<u>H</u>$_3$(Ala)), 1.45-1.72 (m, 3H, C<u>H</u>, C<u>H</u>$_2$(Leu)), 1.98 (s, 3H, C<u>H</u>$_3$CO), 4.30-4.44 (m, 2H, 2xC<u>H</u>); ^{13}C NMR (125 MHz, CD_3OD) δ 20.1, 24.6, 24.9, 25.9, 28.4, 44.5, 51.5, 55.6, 175.8, 177.2, 178.3; LRMS (ES) for $C_{11}H_{20}O_4N_2Na$ *m/z* (relative intensity) 267.1 (MNa^+, 100%); Anal. Calcd for $C_{11}H_{20}O_4N_2$: C 54.08 H, 8.25, N, 11.47. Found C, 54.01, H, 8.42, N, 11.39.



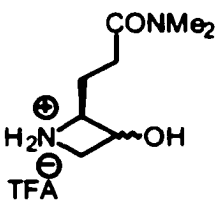
***N*-acetyl-L-leucyl-L-alanyl-L-alanine-benzyl ester (106).** To a solution of the dipeptide L-Leucyl-L-alanine **105** (1.95 g, 8.0 mmol) in DMF (100 mL) was added DIPEA (3.35 mL, 19.2 mmol). After 5 min of stirring, HBTU[76] (3.19 g, 8.4 mmol) was added and the reaction mixture was stirred for a further 30 min. To this mixture alanine

benzyl ester tosylate salt (3.09 g, 8.8 mmol) was added. After 6 h of stirring, the DMF was removed *in vacuo*, saturated aq. $NaHCO_3$ (50 mL) was added to the residue and then extracted with EtOAc (3x20 mL). The combined organic layers were washed with 5% citric acid (40 mL). Evaporation of the solvent *in vacuo* followed by recrystallizion from CH_2Cl_2/Et_2O gave **106** as a white crystalline product (2.38 g, 74%); $[\alpha]_D^{26}$ -4.5° (*c* 0.6, MeOH); IR (μscope) 3282, 3208, 2988, 1751, 1726, 1685, 1345, 1088; ^{1}H NMR (300 MHz, CD_3OD) δ 0.92 (d, *J* = 3.0 Hz, 3H, C<u>H</u>$_3$(Leu)), 0.94 (d, *J* = 3.9 Hz, 3H, C<u>H</u>$_3$(Leu)), 1.30 (d, *J* = 7.0 Hz, 3H, C<u>H</u>$_3$(Ala)), 1.38 (d, *J* = 7.3 Hz, 3H, C<u>H</u>$_3$(Ala)), 1.45-1.72 (m, 3H, C<u>H</u>, C<u>H</u>$_2$(Leu)), 1.94 (s, 3H, C<u>H</u>$_3$CO), 4.20-4.48 (m, 3H, 3xC<u>H</u>), 5.12 (d, *J* = 12.3 Hz, 1H, C<u>H</u>$_2$Ph), 5.18 (d, *J* = 12.4 Hz, 1H, C<u>H</u>$_2$Ph), 7.24-7.40 (m, 5H, <u>Ph</u>); ^{13}C NMR (75 MHz, CD_3OD)(mixture of rotamers) δ 17.2, 17.3, 17.8, 18.1, 21.9, 22.3, 23.2, 23.4, 25.9, 41.2, 41.8, 49.9, 50.0, 50.4, 53.3, 54.0, 129.2, 129.2, 139.3, 129.6, 137.4, 173.4, 173.6, 173.8, 173.9, 174.6; HRMS (ES) Calcd for $C_{21}H_{31}N_3O_5Na$ (MNa^+) 428.2161, found 428.2160.



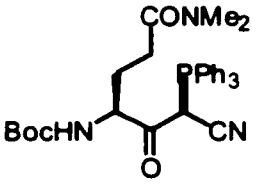
***N*-acetyl-L-leucyl-L-alanyl-L-alanine (107).** The tripeptide *N*-acetyl-L-leucyl-L-alanyl-L-alanine-benzyl ester **106** (3.88 g, 8.10 mmol) was dissolved in MeOH (80 mL) in the presence of 10% Pd/C (400 mg, 10% w/w). The suspension was stirred under an atmosphere of hydrogen until the uptake of hydrogen ceased (approximately 5 h). Filtration through celite, followed by removal of the solvent *in vacuo*, afforded the title

compound which was recrystallized from MeOH/EtOAc/Et_2O to give white solid (2.78 g, 92%);%); $[\alpha]_D^{25}$ -42.6° (*c* 1.0, MeOH); IR (μscope) 3284, 3207, 2958, 1726, 1640, 1387, 1453, 1387; ^{1}H NMR (300 MHz, CD_3OD)(mixture of rotamers) δ 0.92 (d, *J* = 6.1 Hz, 3H, C<u>H</u>$_3$(Leu)), 0.93 (d, *J* = 6.5 Hz, 3H, C<u>H</u>$_3$(Leu)), 1.32 (d, *J* = 6.7 Hz, 3H, C<u>H</u>$_3$(Ala)), 1.38 (d, *J* = 7.7 Hz, 3H, C<u>H</u>$_3$(Ala)), 1.48-1.74 (m, 3H, C<u>H</u>, C<u>H</u>$_2$(Leu)), 1.96 (s, 3H, C<u>H</u>$_3$CO), 4.20-4.44 (m, 3H, 3xC<u>H</u>); ^{13}C NMR (75 MHz, CD_3OD)(mixture of rotamers) δ 17.3, 17.4, 17.5, 17.7, 17.8, 18.0, 31.9, 22.2, 22.3, 33.4, 23.2, 23.7, 25.9, 41.3, 41.9, 50.1, 50.3, 52.7, 53.2, 53.9, 173.4, 173.6, 174.5, 174.7, 175.1, 175.7; HRMS (ES) Calcd for $C_{14}H_{25}N_3O_5Na$ (MNa^+) 338.1692, found 338.1689.

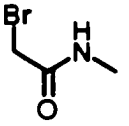


(2*S*)-2-(*N*,*N*-dimethylpropanamid-3-yl)-3-hydroxyazetidine trifluoroacetate salt (110). To a solution of ketone **35** (258 mg, 0.95 mmol) in EtOH (10 mL) at 0 °C was added $NaBH_4$ (180 mg, 4.77 mmol). The mixture was stirred for 4 h after which it was quenched with saturated aq. citric acid (2 mL). The solvent was evaporated and the residue obtained was diluted with H_2O (15 mL). The aqueous layer was extracted with EtOAc (3 x 5 mL) and the combined organic layers were dried over $MgSO_4$. Evaporation of the solvent followed by purification of the crude product by column chromatography (SiO_2, 5% MeOH/EtOAc) furnished the alcohol as a colorless oil (187 mg, 72%); IR ($CHCl_3$ cast) 3356, 2917, 1685, 1629, 1455 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) (1:1 mixture of diastereomers) δ 1.40 (s, 9H, (C<u>H</u>$_3$)$_3$), 1.94-2.05 (m, 1H, CHC<u>H</u>$_2$), 2.20-2.42

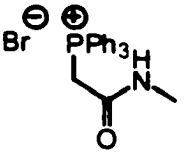
(m, 2H, CHC$\underline{H}_2$, C$\underline{H}_2$CO), 2.50-2.60 (m, 1H, C$\underline{H}_2$CO), 2.92 (s, 3H, N(C$\underline{H}_3$)$_2$), 3.00 (s, 3H, N(C$\underline{H}_3$)$_2$), 3.60 (dd, J = 10.4, 3.7 Hz, 1H, NC$\underline{H}_2$), 3.95-4.12 (m, 2H, C$\underline{H}$, NC$\underline{H}_2$), 4.38-4.48 (m, 1H C$\underline{H}$OH), 5.20 (br s, 1H, O$\underline{H}$); ^{13}C NMR (75 MHz, $CDCl_3$)(1:1 mixture of diastereomers) δ 21.6, 22.0, 28.1, 28.4, 30.5, 35.7, 37.3, 56.5, 62.9, 67.8, 79.3, 156.8, 173.5; HRMS (ES) Calcd for $C_{13}H_{24}N_2O_4Na$ (MNa^+) 295.1634, found 295.1633. The Boc protecting group was cleaved using the procedure as described for the preparation of **34**. Trifluoroacetic acid (2 mL) was added to a solution of the alcohol (127 mg, 0.47 mmol) in CH_2Cl_2 (2 mL) at 0 °C. After 1.5 h, the reaction mixture was concentrated *in vacuo* and the trifluoroacetate salt **110** was obtained as an oil (120 mg, 90%). This was used in the next step without any further purification. An analytical sample was purified by HPLC (Waters C18 Bondpak; 100 x 40 mm; linear gradient elution over 15 min. of 5 to 60% acetonitrile in 0.1% TFA/H_2O, t_R = 4.3 min.) IR ($CHCl_3$ cast) 3286, 2924, 1632, 1537, 1461 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) (2:1 mixture of diastereomers) δ 2.09 (m, 2H, CHC$\underline{H}_2$), 2.40-2.69 (m, 2H, C$\underline{H}_2$CO), 2.92 (s, 3H, N(C$\underline{H}_3$)$_2$), 3.02 (s, 3H, N(C$\underline{H}_3$)$_2$), 3.75 (dd, J = 11.3, 4.7 Hz, 1H, NC$\underline{H}_2$), 4.18 (dd, J = 11.0, 6.5 Hz, 1H, NC$\underline{H}_2$), 4.38-4.50 (m, 1H, C$\underline{H}$), 4.64 (ddd, J = 11.2, 6.6, 4.8 Hz, 1H, C$\underline{H}$OH); ^{13}C NMR (125 MHz, CD_3CN) δ 23.0, 29.9, 30.1, 35.9, 37.6, 54.2, 65.4, 68.3, 174.2; HRMS (ES) Calcd for $C_8H_{17}N_2O_2$ ($M-CF_3CO_2^+$) 173.1290, found 173.1286.



(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-(triphenylphosphoranylidene)-6-cyano-hexanamide (115). This compound was prepared by the procedure of Wasserman.[88] To a solution of the acid **70** (3.0 g, 10.94 mmol) in CH_2Cl_2 (75 mL) was added DMAP (134 mg, 1.09 mmol). After 1 min, triphenyl phosphoranylidene acetonitrile (3.46 g, 11.49 mmol) was added and the mixture stirred for 4 h at rt. The reaction mixture was quenched with H_2O (25 mL) and extracted with EtOAc (3x20 mL). The combined organic layers were dried over $MgSO_4$ and then evaporated *in vacuo*. Purification of the crude product by column chromatography (SiO_2, 100% EtOAc) furnished the title compound as a white foam (3.97 g, 65%); $[\alpha]_D^{26}$ +30.5° (*c* 0.6, $CHCl_3$); IR ($CHCl_3$ cast) 3315, 3058, 2976, 2930, 2170, 1705, 1640, 1595, 1438, 1365; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (br s, 9H, $(C\underline{H}_3)_3$), 1.95-2.14 (m, 2H, $CHC\underline{H}_2$), 2.12-2.40 (m, 2H, $C\underline{H}_2CO$), 2.90 (br s, 6H, $N(C\underline{H}_3)_2$), 4.80-4.86 (m, 1H, C<u>H</u>), 5.40 (br s, 1H, BocN<u>H</u>), 7.42-7.73 (m, 15H, $P\underline{Ph}_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.4, 29.2, 30.3, 35.5, 37.3, 56.2 (d, J = 4.3 Hz), 78.9, 119.6 (d, $J_{c\text{-}p}$ = 94.4 Hz), 120.9 (d, $J_{c\text{-}p}$ = 15.9 Hz), 122.2 (d, $J_{c\text{-}p}$ = 94.4 Hz), 123.5,128.5, 129.2, 129.9, 131.9, 132.1, 133.6, 134.2 (d, $J_{c\text{-}p}$ = 13.2 Hz), 155.9, 172.7, 194.4 (d, $J_{c\text{-}p}$ = 18.0 Hz); HRMS (EI) Calcd for $C_{32}H_{36}N_2O_4P$ (M^+) 557.2443, found 557.2450; Anal. Calcd for $C_{32}H_{36}N_2O_4P$: C, 68.93; H, 6.51; N, 7.54. Found: C, 68.58; H, 6.59; N 7.44.

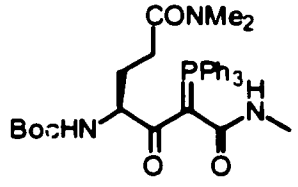


***N*-Methyl-2-bromo-acetamide (119).** Methyl amine HCl salt (1.67 g, 24.8 mmol) was added to a mixture of CH_2Cl_2 (40 mL) and sodium hydroxide (1.98 g, 49.5 mmol) in H_2O (10 mL). The mixture was cooled to –10 °C and bromoacetyl bromide **118** in CH_2Cl_2 (10 mL).[89] After the addition the reaction mixture was warmed up to rt over 30 min. The organic layer was separated and washed with brine (15 mL) and dried over $MgSO_4$. Evaporation of the solvent *in vacuo* followed by trituration with hexane gave a white solid (2.70 g, 79%); mp 43-44 °C, lit.[138] mp 44-45 °C; IR ($CHCl_3$ cast) 3282, 3031, 2974, 2903, 2802, 1573, 1427, 1320, 1135; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.86 (d, J = 1.7 Hz, 3H, C<u>H</u>$_3$), 3.88 (s, 2H, C<u>H</u>$_2$Br), 6.52 (br s, 1H, N<u>H</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.0, 29.2, 166.1; HRMS (EI) Calcd for $C_3H_6NO^{79}Br(M^+)$ 150.9633, found 150.9628.



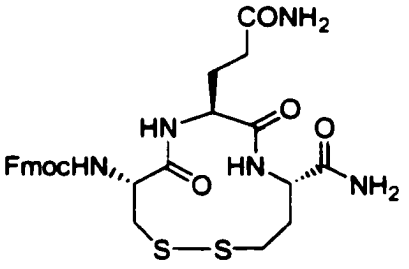
***N*-methyl-2-triphenylphosphonium-acetamido bromide (120).** This compound was prepared using the procedure of Wasserman.[90] The bromo derivative **119** (2.80 g, 18.4 mmol) was dissolved in THF/Et_2O (1:3, 30 mL) and to this was added triphenyl phosphine (9.67 g, 36.8 mmol). The reaction mixture was stirred for 24 h and the reaction monitored by TLC. The solvent was removed *in vacuo* and the thick white suspension was collected by filtration and washed several times with Et_2O to remove the excess triphenyl phosphine. This product was recrystallized from MeOH/Et_2O to give a white

solid (5.73 g, 75%); mp 264-266 °C; IR (μscope) 3207, 3056, 2961, 2939, 2848, 2763, 1668, 1585, 1484, 1408; ^{1}H NMR (300 MHz, CD_3OD) δ 2.64 (d, J = 4.2 Hz, 3H, C<u>H</u>$_3$), 4.72 (d, J = 14.6 Hz, 2H, C<u>H</u>$_2$P), 7.68-7.93 (m, 15H, P<u>Ph</u>$_3$), 8.25 (br s, 1H, N<u>H</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.7, 32.9 (d, J = 58.5 Hz), 119.5 (d, $J_{c\text{-}p}$ = 89.3 Hz), 131.19 (d, $J_{c\text{-}p}$ = 12.8 Hz), 135.0 (d, $J_{c\text{-}p}$ = 15.0 Hz), 136.3 (d, $J_{c\text{-}p}$ = 3.0 Hz), 165.0 (d, $J_{c\text{-}p}$ = 9.0 Hz); HRMS (EI) Calcd for $C_{21}H_{20}NOP$ (M-HBr$^+$) 333.1283, found 333.1269; Anal. Calcd for $C_{21}H_{21}NOPBr$: C, 60.88; H, 5.11; N, 3.38. Found: C, 60.55; H, 5.05; N, 3.39.

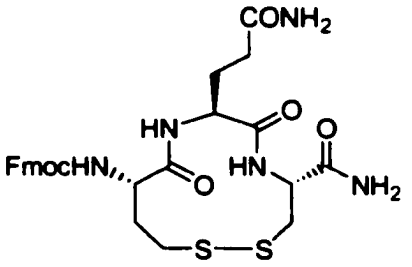


(4*S*)-*N*,*N*-dimethyl-4-*tert*-butyloxycarbonylamino-5-oxo-6-(triphenylphosphoranylidene)-7-methylcarbamoyl-heptanamide (121). This compound was prepared using the procedure of Wasserman.[90] To a solution of the ylide salt **120** (0.5 g, 1.21 mmol) in CH_2Cl_2 (10 mL) was added triethylamine (1.67 mL, 12.1 mmol) and the reaction mixture stirred for 1 h. In another flask, a solution of the acid **70** (0.5 g, 1.81 mmol) in CH_2Cl_2 (10 mL) was treated with EDCI (0.35 g, 1.81 mmol) followed by DMAP (22.1 mg, 0.18 mmol). The reaction mixture was stirred for 15 min after which the *in situ* generated ylide was added via cannula. The resulting mixture was stirred for a further 24 h at rt. H_2O (20 mL) was added and extracted with CH_2Cl_2 (3x10 mL), dried over $MgSO_4$ and the crude product purified by column chromatography (SiO_2, gradient elution, 100% EtOAc to 10% MeOH/EtOAc) to furnish the title compound as a white foam (319 mg, 43%); $[\alpha]_D^{26}$ -7.8° (*c* 2.3, $CHCl_3$); IR ($CHCl_3$ cast) 3288, 3063,

2980, 2937, 1772, 1678, 1636, 1505, 1439, 1412, 1322; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (br s, 9H, $(C\underline{H}_3)_3$), 1.84-1.95 (m, 2H, $CHC\underline{H}_2$), 2.10-2.38 (m, 2H, $C\underline{H}_2CO$), 2.90 (d, J = 5.0 Hz, 3H, $NHC\underline{H}_3$), 2.94 (s, 3H, $N(C\underline{H}_3)_2$), 2.98 (s, 3H, $N(C\underline{H}_3)_2$), 4.72-4.80 (m, 1H, (d, J = 5.0 Hz, 3H, $NHC\underline{H}_3$), 2.94 (s, 3H, $N(C\underline{H}_3)_2$), 2.98 (s, 3H, $N(C\underline{H}_3)_2$), 4.72-4.80 (m, MHz, $CDCl_3$) δ 28.4, 29.3, 32.3, 35.5, 37.1, 121.5, 128.6 (d, J = 12.5 Hz), 128.7, 131.9, 132.0 (d, $J_{c\text{-}p}$ = 10.0 Hz), 132.1, 133.05 (d, $J_{c\text{-}p}$ = 10.0 Hz), 133.1, 162.9, 134.84, 171.8, 172.59; HRMS (ES) Calcd for $C_{33}H_{41}N_3O_5P$ (MH^+) 590.2784, found 590.2782.

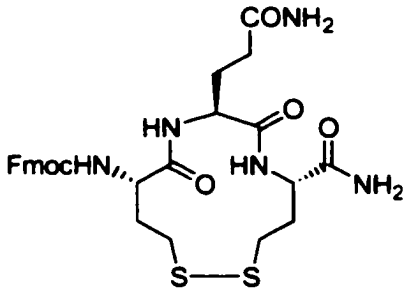


***S*-*S*-cyclo[*N*-Fmoc-L-cysteinyl-L-glutamyl-L-homocysteinamide] (123).** To a stirred solution of Fmoc-Cys(Acm)-Gln-Hcy(Acm)-NH_2 **132** (10 mg, 13 μmol) in 1:1 AcOH:H_2O (14 mL) at rt was added 0.1 M I_2 in MeOH (0.5 mL, 50 μmol). After 2 h, the reaction was quenched by the addition of ascorbic acid. The solvents were removed *in vacuo* and the residue purified directly by HPLC (Waters C18 Bondpak; 100 x 40 mm; 10-60 % MeCN/H_2O over 15 min, flow rate 15 mL/min, t_R = 12.2 min;) to give **123** (4 mg, 53%) as a white solid; the material proved to be quite insoluble even in DMSO once purified.

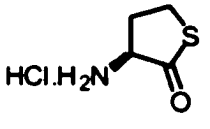


***S*-*S*-cyclo[*N*-Fmoc-L-homocysteinyl-L-glutamyl-L-cysteinamide] (124).**

Fmoc-Hcy(Acm)-Gln-Cys(Acm)-NH_2 **133** (15 mg, 20 μmol) in 1:1 AcOH:H_2O (20 mL) was treated with 0.1 M I_2 (0.8 mL, 80 μmol) in an analogous manner as that described for **123**. Purification by HPLC (Waters C18 Bondpak; 100 x 40 mm; 10-60 % MeCN/H_2O over 15 min, flow rate 15 mL/min, t_R = 12.0 min) gave starting material **133** (3 mg, 4 μmol, 20 %) and **124** (8 mg, 14 μmol, 68 %) as a white solid; $[\alpha]_D^{25}$ +40.0° (*c* 0.1, DMF); IR (μscope) 3271, 1643, 1201 cm^{-1}; ^1H NMR (500 MHz, DMF-d_7) δ 8.82 (1H, br s, NH), 7.93 (2H, d, *J* = 7.5 Hz, Ar-CH), 7.83-7.70 (4H, m, Ar-CH, 2 x NH), 7.44 (2H, dd, *J* = 7.0, 7.0 Hz, Ar-CH), 7.36-7.32 (3H, m, Ar-CH, NH), 6.76 (1H, br s, NH), 4.63-4.25 (6H, m, 3 x α-CH, Fmoc-CH, Fmoc-CH_2), 3.00-2.78 (4H, m, Cys CH_2S, Hcy CH_2S),] 2.34-1.85 (6H, m, Hcy and Gln $CHCH_2$, Gln CH_2CO); ^{13}C NMR (125 MHz, DMF-d_7)(mixture of rotamers) δ 174.6, 156.5, 145.0, 144.8, 141.8, 128.3, 127.8, 126.1, 120.7, 67.0, 54.3, 52.4, 51.8, 47.7, 45.8, 32.1; MS (ES) *m/z* 608.0 (MNa^+, 100%).

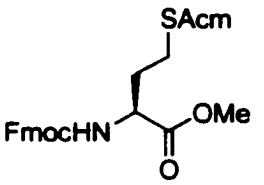


***S*-*S*-cyclo[*N*-Fmoc-L-homocysteinyl-L-glutamyl-L-homocysteinamide] (125).** Fmoc-Hcy(Acm)-Gln-Hcy(Acm)-NH_2 **134** (10 mg, 13 μmol) in 1:1 AcOH:H_2O (14 mL) was treated with 0.1 M I_2 (0.5 mL, 50 μmol) as described previously for **123**. Purification by HPLC (Waters C18 Bondpak; 100 x 40 mm; 10-60 % MeCN/H_2O over 15 min, flow rate 15 mL/min, t_R = 12.3 min) gave **125** (7 mg, 11 μmol, 87 %) as a white solid; $[\alpha]_D^{25}$ -61.0° (*c* 0.2, DMSO); ^{1}H NMR (360 MHz, DMF-d_7) δ 8.61 (1H, d, *J* = 8.5 Hz, NH), 7.92 (2H, d, *J* = 7.5 Hz, Ar-CH), 7.77 (3H, m, Ar-CH, NH), 7.60 (1H, br s, NH), 7.44 (2H, dd, *J* = 7.0, 7.0 Hz, Ar-CH), 7.36-7.32 (3H, m, Ar-CH, NH), 7.14 (1H, br s, NH), 6.76 (1H, br s, NH), 4.54-4.23 (6H, m, 3 x α-CH, Fmoc-CH, Fmoc-CH_2), 3.00-2.78 (4H, m, 2 x $CHCH_2$), 2.27-1.86 (8H, m, 4 x $CHCH_2$, CH_2CO).



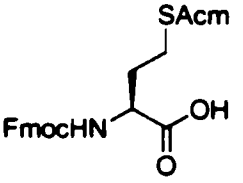
L-Homocysteine thiolactone hydrochloride (128). This compound was prepared by a modified procedure of Lutgring.[96] A 2 L, 3-necked round-bottomed flask equiped with a mechanical stirrer, an ammonia inlet, and a dry-ice condenser fitted with a KOH drying tube at -78 °C was purged with argon before being charged with ammonia (~1.0

L). To the liquid ammonia was first added L-methionine **126** (43.1 g, 0.30 mol) followed by sodium (26.4 g, 1.15 mol) over a period of 45 min. Stirring was continued for a further 2 h before quenching the reaction by the careful addition of anhydrous ammonium chloride. The reaction vessel was then transferred to a water bath maintained at 20-30 °C, and the ammonia was removed *in vacuo*. The residue was dissolved in H_2O (500 mL) and was acidified to pH 1 by the addition of conc. HCl. The solution was concentrated *in vacuo*, redissolved in 6N HCl (500 mL) and heated at 100 °C for 2 h, after which time ^{1}H NMR of an aliquot indicated complete conversion. The water was removed *in vacuo*, the residue treated with isopropyl alcohol (200 mL) and reconcentrated. The residue was then treated with ethanol (500 mL) and was heated under reflux for 1 h. Solid material was removed by filtration and was washed with further ethanol (250 mL). The crude product was then isolated as a pale yellow solid after removal of the solvent. This material was treated with acetone (500 mL) and the title compound (40.8 g, 0.27 mmol, 92 %) isolated as a white solid by filtration. A sample was recrystallized from ethanol for characterization: $[\alpha]_D^{25}$ +20.8° (*c* 1.0, H_2O), lit.[139] $[\alpha]_D^{25}$ +21.5° (*c* 1.0, H_2O); IR (MeOH cast) 3327, 2055, 1744, 1704 cm^{-1}; ^{1}H NMR (300 MHz, D_2O) δ 4.20 (1H, dd, J = 7.0, 13.0 Hz, α-C<u>H</u>), 3.44-3.31 (2H, m, C<u>H</u>$_2$S), 2.74-2.67 (1H, m, CHC<u>H</u>$_2$), 2.21-2.08 (1H, m, CHC<u>H</u>$_2$); ^{13}C NMR (75 MHz, D_2O; referenced to DMSO δ 39.5) δ 206.7, 58.9, 29.8, 28.6; MS (ES) *m/z* 118.1 ([M+H]$^+$, 100 %). Anal. Calcd. for C_4H_8ClNOS C, 31.27; H, 5.25; N, 9.12; Cl, 23.08. Found: C, 31.01; H, 5.24; N, 9.04; Cl, 23.33.



***S*-Acm-*N*-Fmoc-L-homocysteine methyl ester (130).** To a stirred solution of sodium methoxide in methanol (prepared *in situ* by the addition of sodium (8.55 g, 0.37 mol) to methanol (1.25 L)) at rt under argon was added solid HCl.H-cycloHcy **128** (25.9 g, 0.17 mol) and stirring was continued for 1 h. The reaction was quenched by the addition of TFA (50 mL) and the solvent was removed *in vacuo* to give the crude methyl ester; ^{1}H NMR (300 MHz, D_2O) δ 4.18 (1H, dd, J = 6.5, 6.5 Hz, α-C<u>H</u>), 3.70 (3H, s, $CO_2C\underline{H}_3$), 2.55 (2H, t, J = 7.0 Hz, $C\underline{H}_2S$), 2.21-1.96 (2H, m, $CHC\underline{H}_2$). This material was treated with TFA (175 mL) at rt and acetamidomethanol (15.0 g, 0.17 mol) was added. After stirring for 1 h, the TFA was removed *in vacuo* to give the crude Acm-protected amino acid **129**; ^{1}H NMR (360 MHz, D_2O) δ 4.21-4.12 (2H, AB q, J = 14.0 Hz, Acm-$C\underline{H}_2$), 4.12 (1H, dd, J = 6.5, 6.5 Hz, α-C<u>H</u>), 3.70 (3H, s, $CO_2C\underline{H}_3$), 2.62 (2H, t, J = 7.5 Hz, $C\underline{H}_2S$), 2.22-2.04 (2H, m, $CHC\underline{H}_2$), 1.87 (3H, s, Acm-$C\underline{H}_3$). To a solution of **129** in 1:1 THF:H_2O (1.0 L) at 0 °C was added $NaHCO_3$ (140.5 g, 1.67 mol) followed by Fmoc-OSu (54.2 g, 0.16 mol). The reaction mixture was allowed to warm to rt and stirring was continued overnight. The THF was then removed *in vacuo*, the aqueous mixture diluted with H_2O (500 mL) and extracted with EtOAc (2 x 500 mL). The combined extracts were washed with 1N HCl (500 mL) and brine (250 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the fully protected amino acid **130** as a pale yellow oil. A sample of which was purified by flash column chromatography (Et_2O) and recrystallized from EtOAc to give Fmoc-Hcy(Acm)-OMe **130** as a white solid; m.p. 116-117 °C; $[\alpha]_D^{25}$ -6.6° (*c* 1.0,

$CHCl_3$); IR ($CHCl_3$ cast) 3300, 1719, 1658, 1534, 1254, 1217, 758, 741 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 7.77 (2H, d, J = 7.5 Hz, Ar-C<u>H</u>), 7.65-7.59 (2H, m, Ar-C<u>H</u>), 7.41 (2H, dd, J = 7.5, 7.5 Hz, Ar-C<u>H</u>), 7.32 (2H, dd, J = 7.5, 7.5 Hz, Ar-C<u>H</u>), 6.22 (1H, br s, N<u>H</u>), 5.77 (1H, d, J = 7.5 Hz, N<u>H</u>), 4.50-4.31 (5H, m, Acm-C<u>H</u>$_2$, Fmoc-C<u>H</u>, Fmoc-C<u>H</u>$_2$), 4.24 (1H, dd, J = 7.0, 7.0 Hz, α-C<u>H</u>), 3.77 (3H, s, $CO_2C\underline{H}_3$), 2.71-2.58 (2H, m, C<u>H</u>$_2$S), 2.19-2.02 (2H, m, CHC<u>H</u>$_2$), 2.05 (3H, s, Acm-C<u>H</u>$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.6, 141.3, 127.7, 127.6, 127.1, 125.1, 124.7, 120.0, 77.2, 67.1, 52.8, 52.6, 47.2, 41.0, 32.3, 27.2, 23.0; MS (ES) *m/z* 465.1 (MNa^+, 75 %), 443.2 (MH^+, 45), 179.1 (100); Anal. Calcd for $C_{23}H_{26}N_2O_5S$: C, 62.42; H, 5.92; N, 6.33. Found: C, 62.44; H, 5.86; N, 6.28.

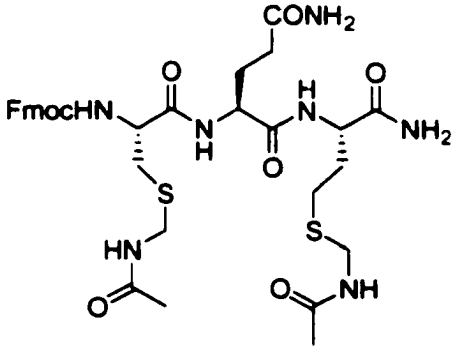


***S*-Acm-*N*-Fmoc-L-homocysteine (131).** (Method 1) To a stirred solution of the crude material **130** in THF (1.0 L) at 0 °C was added 0.2N LiOH (1.0 L). After stirring at 0 °C for 1.5 h,the THF was removed *in vacuo* and the aqueous solution washed with EtOAc (2x500 mL). The aqueous layer was acidified with 1N HCl (250 mL) and the free acid extracted with EtOAc (2 x 500 mL). The combined organic extracts were washed with 1N HCl (500 mL), brine (250 mL), then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as a solid. Recrystallization from EtOAc yielded **131** (59.0 g, 0.14 mol, 81 % over 4 steps) as a white solid; m.p. 128-131 °C; lit.[96] mp 134 °C; IR (μscope) 3320, 1711, 1536, 1253 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 7.83 (2H, d, J = 7.5 Hz, Ar-C<u>H</u>), 7.78 (1H, br, N<u>H</u>), 7.72 (2H, d, J = 7.5 Hz, Ar-C<u>H</u>), 7.39 (2H, dd, J =

7.5, 7.5 Hz, Ar-C<u>H</u>), 7.31 (2H, dd, *J* = 7.5, 7.5 Hz, Ar-C<u>H</u>), 6.81 (1H, d, *J* = 8.0 Hz, N<u>H</u>), 4.47-4.21 (6H, m, Acm-C<u>H</u>$_2$, Fmoc-C<u>H</u>, Fmoc-C<u>H</u>$_2$, α-C<u>H</u>), 2.80-2.64 (2H, m, C<u>H</u>$_2$S), 2.22-2.05 (2H, m, β-C<u>H</u>$_2$), 1.95 (3H, s, Acm-C<u>H</u>$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.6, 171.8, 156.5, 143.8, 141.3, 127.7, 127.1, 125.1, 120.0, 67.2, 52.9, 47.1, 40.9, 32.3, 26.9, 22.9; MS (ES) *m/z* 429.1 ([M+H]$^+$, 100 %); Anal. Calcd for $C_{22}H_{24}N_2O_5S$: C, 61.66; H, 5.65; N, 6.54; S, 7.48. Found: C, 59.67; H, 5.49; N, 6.04; S, 7.53. A sample was treated with 0.2N LiOH, and was recrystallized from MeOH to give the lithium salt of *S*-Acm-*N*-Fmoc-L-homocysteine as a colorless crystalline solid; m.p. 134-136 °C (dec.); $[\alpha]_D^{25}$ -15.8° (*c* 1.0, H_2O); IR (μscope) 3294, 1687, 1597, 1421 cm^{-1}; ^{1}H NMR (300 MHz, D_2O) δ 7.52-7.11 (8H, m, Ar-C<u>H</u>), 4.40-4.21 (4H, m, Acm-C<u>H</u>$_2$, Fmoc-C<u>H</u>, Fmoc-C<u>H</u>$_2$, α-C<u>H</u>), 3.95-4.11 (m, 2H, Acm-C<u>H</u>$_2$), 2.62-2.46 (2H, m,C<u>H</u>$_2$S), 2.15-1.87 (2H, m, CHC<u>H</u>$_2$), 2.02 (3H, s, Acm-C<u>H</u>$_3$); MS (ES) *m/z* 435.1 ([M+H]$^+$, 100%).

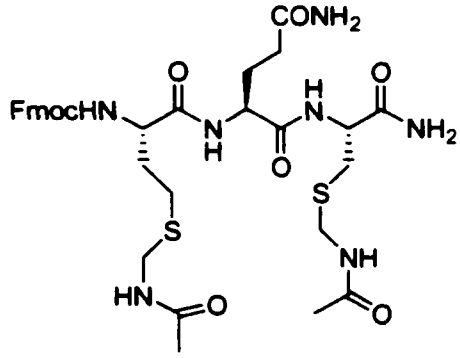
(Method 2) Compound **131** was prepared following the procedure of Lutgring.[96] L-Methionine **126** (7.00 g, 47 mmol) was dissolved in liquid ammonia (~250 mL) at -78 °C and sodium (3.24 g, 141 mmol) added slowly over 15 min until the reaction mixture remained blue. After stirring for 30 min, the reaction mixture was quenched by slow addition of ammonium acetate (5.79 g, 75.2 mmol) and the ammonia allowed to evaporate at rt. The residue was dissolved in TFA (150 mL) then treated with acetamido methanol at rt for 1 h. The TFA was removed *in vacuo* and the pH was adjusted to 8-9 with saturated aq. Na_2CO_3. The residue was dissolved in H_2O (250 mL) and lyophilized to dryness. The residue was dissolved in H_2O (300 mL), treated with Na_2CO_3 (20 g, 188 mmol) and dioxane (100 mL) and the solution cooled to 0 °C. FmocCl (13.37 g, 51.7 mmol) in dioxane (50 mL) was added and the reaction mixture was stirred for 4 h at 0 °C,

then at rt for 12 h. The reaction mixture was washed with EtOAc (2x100 mL) and the aqueous layer acidified to pH 2-3 with 4N HCl and extracted with EtOAc (3x100 mL). The EtOAc extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2, 94% CH_2Cl_2/ 5% MeOH/ 1% AcOH) to afford **131** which was recrystallized from MeOH/H_2O to give a white solid (9.64 g, 48%); mp 132-133 °C, lit.[96] mp 134 °C; $[\alpha]_D^{26}$ -15.0° (*c* 2.6, MeOH), lit.[96] $[\alpha]_D^{26}$ -17.3° (*c* 2.6, MeOH); IR (μscope) 3311, 3064, 2935, 1704, 1659, 1535, 1478, 1449, 1372, 1334 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 1.94-2.01 (m, 4H, $C\underline{H}_3CO$, $C\underline{H}_2S$), 2.03-2.22 (1H, $C\underline{H}_2S$), 2.52-2.78 (m, 2H, $C\underline{H}_2CH_2S$), 4.18-4.40 (m, 6H, $C\underline{H}_2NH$, $C\underline{H}NH$, $C\underline{H}_2O$, $C\underline{H}CH_2O$), 7.28 (dd, *J* = 7.4, 7.4 Hz, 2H, Ph), 7.36 (dd, *J* = 7.5, 7.5 Hz, 2H, Ph), 7.62-7.70 (m, 2H, Ph), 7.78 (d, *J* = 7.3 Hz, 2H, Ph); ^{13}C NMR (75 MHz, CD_3OD) δ 22.6, 28.4, 32.7, 41.4, 54.2, 67.9, 120.9, 126.3, 128.2, 128.8, 142.6, 145.2, 145.3, 158.8, 173.2, 175.6; HRMS (ES) Calcd for $C_{22}H_{25}O_5N_2S$ (MH^+) 429.1479, found 429.1481.



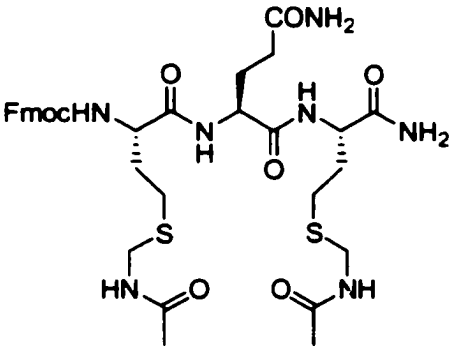
S-Acm-N-Fmoc-L-cysteinyl-L-glutamyl-S-Acm-homocysteinamide (132). This compound was prepared using standard Fmoc[97] chemistry on Rink amide resin[98] with HBTU[99] as the coupling reagent (Advanced ChemTech; SA5030) on a Rainin peptide synthesizer 'Protein Technologies PS3'. On completion of the synthesis, the resin

was washed with CH_2CL_2 (2 x 10 mL) and MeOH (2 x 10 mL), and dried *in vacuo*. The resin was placed on a fine sintered glass filter, and the cleavage cocktail (85:10:5 CH_2CL_2:TFA:TES; 5 mL) was added. The solvent was allowed to slowly percolate through the resin. The resin was treated with further portions of the cleavage cocktail (3 x 5 mL), and the combined filtrates were concentrated *in vacuo*. Purification by HPLC (Waters C18 Bondpak; 100 x 40 mm; 10-60 % MeCN/H_2O over 15 min, flow rate 15 mL/min, t_R = 10.1 min) gave the title compound (70 mg, 96 μmol) as a white solid; $[\alpha]_D^{25}$ -15.0° (*c* 0.1, DMF); IR (μscope) 3289, 1658, 1539 cm^{-1}; ^1H NMR (360 MHz, DMSO-d_6) δ 7.86 (2H, d, *J* = 7.5 Hz, Ar-C<u>H</u>), 7.71 (2H, d, *J* = 7 5 Hz, Ar-C<u>H</u>), 7.40 (2H, dd, *J* = 7.5, 7.5 Hz, Ar-C<u>H</u>), 7.31 (2H, t, *J* = 7.5 Hz, Ar-C<u>H</u>), 4.36-4.12 (10H, m, 3 x α-C<u>H</u>, Fmoc-C<u>H</u>, Fmoc-C<u>H</u>$_2$, 2 x Acm-C<u>H</u>$_2$), 2.90 (1H, dd, *J* = 4.5, 14.0 Hz, one of C<u>H</u>$_2$S), 2.67 (1H, dd, *J* = 14.0, 4.5 Hz, one of Cys C<u>H</u>$_2$S), 2.57-2.43 (2H, m, C<u>H</u>$_2$CO), 2.12 (2H, t, *J* = 8.0 Hz, C<u>H</u>$_2$S), 2.07-1.73 (4H, m, 2 x CHC<u>H</u>$_2$), 1.86, 1.83 (2 x 3H, 2 x s, 2 x Acm-C<u>H</u>$_3$), ^{13}C NMR (75 MHz, DMSO-d_6)(mixture of rotamers) δ 173.8, 171.0, 170.7, 170.3, 170.0, 169.5, 156.1, 143.8, 140.7, 127.6, 127.1, 125.3, 120.1, (103.9), 65.8, 54.5, 52.3, 51.7, 47.8, 47.6, 47.3, 46.6, 32.2, 31.3, 26.7, 22.5; MS (ES) *m/z* 752.3 (MNa^+, 100 %).



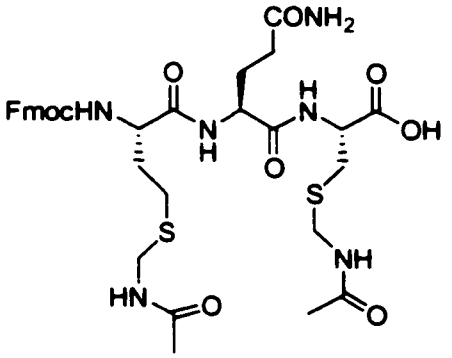
***S*-Acm-*N*-Fmoc-L-homocysteinyl-L-glutamyl-*S*-Acm-cysteinamide (133).**

This compound was prepared and purified as described for **132**, HPLC (Waters C18 Bondpak; 100 x 40 mm; 10-60 % MeCN:H_2O, 15min; 15mL/min, t_R = 10.9 min); $[\alpha]_D^{25}$ -26.7° (*c* 1.0, DMF); IR ($CHCl_3$ cast) 3287, 1637, 1541 cm^{-1}; ^1H NMR (360 MHz, DMF-d_7) δ 8.56-8.49 (3H, m, 2 x Ar-C<u>H</u>, N<u>H</u>), 8.14 (1H, d, *J* = 8.0 Hz, N<u>H</u>), 7.92 (2H, d, *J* = 7.5 Hz, 2 x Ar-C<u>H</u>), 7.77 (2H, dd, *J* = 8.5, 8.5 Hz, 2 x Ar-C<u>H</u>), 7.68 (1H, d, *J* = 7.0 Hz, N<u>H</u>), 7.46-7.33 (4H, m, 4 x Ar-C<u>H</u>), 7.24 (1H, br s, N<u>H</u>), 6.83 (1H, br s, N<u>H</u>), 4.60 (1H, dt, *J* = 4.5, 8.5 Hz, α-C<u>H</u>), 4.44-4.24 (9H, m, 2 x α-C<u>H</u>, 2 x Acm-C<u>H</u>$_2$, Fmoc-C<u>H</u>, Fmoc-C<u>H</u>$_2$), 3.12 (1H, dd, *J* = 4.5, 14.0 Hz, one of Cys C<u>H</u>$_2$S), 2.36 (2H, t, *J* = 7.5 Hz, C<u>H</u>$_2$S), 2.19-1.92 (4H, m, 2 x CHC<u>H</u>$_2$), 1.94, 1.93 (2 x 3H, 2 x s, Acm-C<u>H</u>$_3$); ^{13}C NMR (75 MHz, DMF-d_7)(mixture of rotamers) δ 175.1, 173.0, 172.9, 172.3, 170.6, 170.4, 162.9, 157.3, 145.0, 144.7, 141.8, 128.3, 127.8, 126.1, 120.6, 67.1, 55.4, 54.2, 53.7, 48.0, 41.4, 40.7, 33.6, 33.0, 32.1, 28.3, 27.5, 22.8; MS (ES) *m/z* 752.3 (MNa^+, 100 %).

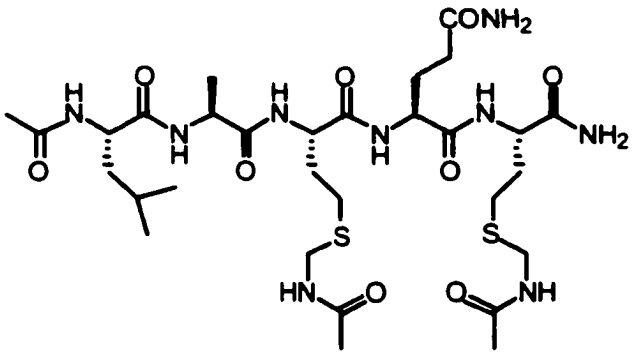


***S*-Acm-*N*-Fmoc-L-homocysteinyl-L-glutamyl-*S*-Acm-homocysteinamide**

(134). This compound was prepared and purified as described for **132**, HPLC (Waters C18 Bondpak; 100 x 40 mm; 10-60 % MeCN:H_2O, 15min; 15mL/min, t_R = 11.1 min); $[\alpha]_D^{25}$ -16.5° (*c* 0.2, DMF); IR (μscope) 3291s, 1640s, 1542s cm^{-1}; ^1H NMR (360 MHz, DMF-d_7) δ 8.54-8.48 (3H, m, NH, 2 x Ar-CH), 7.98 (1H, d, *J* = 8.0 Hz, NH), 7.92 (2H, d, *J* = 7.5Hz, 2 x Ar-CH), 7.77 (2H, dd, *J* = 7.0, 7.0 Hz, 2 x Ar-CH), 7.68 (1H, d, *J* = 7.0 Hz, NH), 7.46-7.33 (6H, m, 4 x Ar-CH, 2 x NH), 7.14 (1H, br s, NH), 6.83 (1H, br s, NH), 4.46-4.22 (10H, m, 3 x α-CH, 2 x Acm-CH_2, Fmoc-CH, Fmoc-CH_2), 2.76-2.56 (4H, m, 2 x CH_2S), 2.34-2.37 (m, 2H, CH_2CO), 2.20-1.92 (6H, m, 3 x $CHCH_2$), 1.94, 1.93 (2 x 3H, 2 x s, 2 x Acm-CH_3); ^{13}C NMR (75 MHz, DMF-d_7)(mixture of rotamers) δ 175.1, 174.1, 173.0, 172.3, 170.4, 157.3, 144.9, 144.8, 141.8, 128.3, 127.8, 126.1, 120.7, 67.1, 55.4, 54.3, 53.0, 47.7, 40.8, 40.6, 33.1, 33.0, 32.2, 28.1, 27.7, 27.4, 22.9; MS (ES) *m/z* 766.3 (MNa^+, 100 %).

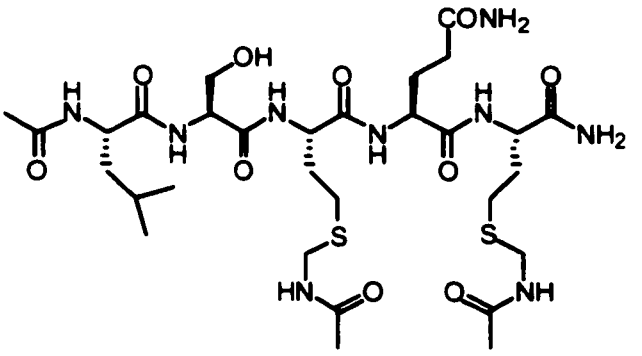


***S*-Acm-*N*-Fmoc-L-homocysteinyl-L-glutamyl-*S*-Acm-L-cysteine (135a).** This compound was prepared as described for **132** but in this case Wang resin[100] was used. Purification of the crude product by HPLC (Waters C18 Bondpak; 100 x 40 mm; linear gradient elution over 20 min of 10 to 80% acetonitrile in 0.1% TFA/H_2O, t_R 10.6 min) gave the title product as a white solid (0.097 g, 33%); $[\alpha]_D^{25}$ -36.0° (*c* 1.0, DMF); IR (μscope) 3282, 3065, 1686, 1638, 1536, 1445, 1371, 1253, 1086, 1044; ^{1}H NMR (500 MHz, CD_3OD) δ 1.90-2.01 (m, 8H, 2xC<u>H</u>$_3$CO(Acm), CHC<u>H</u>$_2$(Hcy)), 2.04-2.19 (m, 2H, CHC<u>H</u>$_2$(Gln)), 2.28-2.39 (m, 2H, C<u>H</u>$_2$CO(Gln)), 2.48-2.74 (m, 2H, C<u>H</u>$_2$S(Hcy)), 2.92 (dd, *J* = 12.5, 8.3 Hz, 1H, CHC<u>H</u>$_2$(Cys)), 3.14 (dd, *J* = 12.5, 8.3 Hz, 1H, CHC<u>H</u>$_2$(Cys)), 4.18-4.44 (m, 8H, 2xα-C<u>H</u>, 2xC<u>H</u>$_2$(Acm), C<u>H</u>, C<u>H</u>$_2$(Fmoc)), 4.62 (dd, *J* = 2.1, 2.1 Hz, 1H, α-C<u>H</u>(Gln)), 7.28 (dd, 2H, *J* = 7.3, 7.3 Hz, <u>Ph</u>), 7.39 (dd, *J* = 7.3, 7.3 Hz, 2H, <u>Ph</u>), 7.65-7.72 (m, 2H, <u>Ph</u>), 7.79 (d, *J* = 7.4 Hz, 2H, <u>Ph</u>); ^{13}C NMR (75 MHz, CD_3OD)(mixture of rotamers) δ 22.7, 22.8, 28.0, 28.9, 32.5, 32.3, 32.5, 41.4, 42.4, 54.1, 54.2, 55.7, 120.9, 126.3, 128.2, 128.8, 142.4, 157.9, 173.3, 173.4, 173.6, 174.4, 177.9; HRMS (ES) Calcd for $C_{33}H_{43}N_6O_9S_2Na$ (MNa^+) 731.2528, found 731.2528.

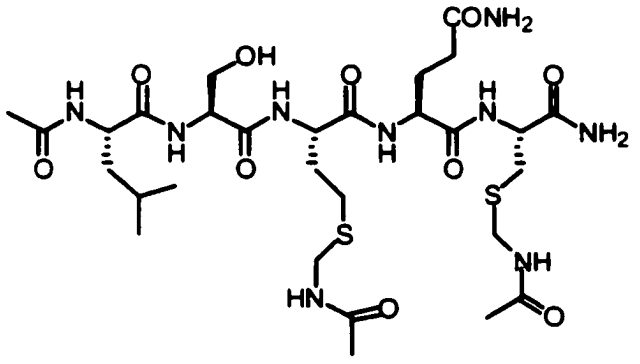


***N*-Acetyl-L-leucyl-L-alanyl-*S*-Acm-L-homocysteinyl-L-glutamyl-*S*-Acm-L-homocysteinamide (136).** This compound was prepared as described for **132**. On completion of the synthesis, the resin was washed with CH_2CL_2 (2 x 10 mL) and MeOH (2 x 10 mL), and dried. The resin was placed on a fine sintered glass filter, and the cleavage cocktail (85:10:5 CH_2CL_2:TFA:TES; 10 mL) was added. The solvent was allowed to slowly percolate through the resin over 15 min. The resin was treated with further portions of the cleavage cocktail (3 x 5 mL), and the combined filtrates were concentrated *in vacuo*. Purification by HPLC (Waters C18 Bondpak; 100 x 40 mm; linear gradient elution over 20 min of 10 to 60% acetonitrile in 0.1% TFA/H_2O, t_R 7.4 min) gave the title product as a white solid (0.104 g, 25%); $[\alpha]_D^{26}$ -8.5° (*c* 1.0, MeOH); IR (μscope) 3292, 3072, 2959. 1656, 1542, 1441, 1373, 1268; ^{1}H NMR (300 MHz, CD_3OD) δ 0.90-1.10 (m, 6H, ($C\underline{H}_3$)$_2$(Leu)), 1.35-1.42 (m, 3H, $C\underline{H}_3$(Ala)), 1.52-1.68 (m, 3H, $C\underline{H}$, $C\underline{H}_2$(Leu)), 1.88 (s, 3H, $C\underline{H}_3CO$), 1.92 (s, 3H, $C\underline{H}_3CO$(Acm)), 2.01 (s, 3H, $C\underline{H}_3CO$(Acm)), 2.02-2.21 (m, 6H, 2x$CHC\underline{H}_2$(Hcy), β-$C\underline{H}_2$(Gln)), 2.32-2.41 (m, 2H, $C\underline{H}_2CO$(Gln)), 2.52-2.74 (m, 4H, $C\underline{H}_2S$(Hcy)), 4.19-4.43 (m, 9H, 5xα-$C\underline{H}$, 2x$C\underline{H}_2$(Acm)); ^{13}C NMR (125 MHz, CD_3OD)(mixture of rotamers) δ 17.2, 17.3, 17.4, 21.9, 22.0, 22.3, 22.5, 22.6, 22.8, 23.3, 23.4, 23.6, 25.9, 28.1, 28.2, 28.3, 28.4, 32.0, 32.4, 32.7, 32.9, 33.1, 41.3, 41.5, 41.7, 50.9, 51.1, 51.5, 53.5, 53.9, 54.0, 54.3, 54.7, 54.9, 55.0,

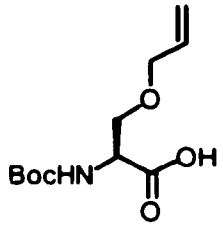
55.2, 55.2, 173.2, 173.9, 174.4, 174.9, 175.6, 175.8, 175.9, 176.6, 177.7; HRMS (ES) Calcd for $C_{30}H_{53}N_9O_9S_2Na$ (MNa^+) 770.3316, found 770.3305.



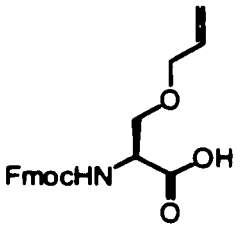
***N*-Acetyl-L-leucyl-L-serinyl-*S*-Acm-L-homocysteinyl-L-glutamyl-*S*-Acm-L-homocysteinamide (137).** This compound was prepared as described for **132**. Purification of the crude product by HPLC (Waters C18 Bondpak; 100 x 40 mm; linear gradient elution over 20 min of 0 to 50% acetonitrile in 0.1% TFA/H_2O, t_R = 9.9 min) gave the title product as a white solid (0.047 g, 15%); $[\alpha]_D^{26}$ -13.0° (*c* 0.8, MeOH); IR (μscope) 3286, 3071, 2857, 1656, 1536, 1439, 1371, 1566, 1201; ^{1}H NMR (300 MHz, CD_3OD) δ 0.88-1.10 (m, 6H, ($C\underline{H}_3$)$_2$(Leu)), 1.52-1.76 (m, 3H, C$\underline{H}$, C$\underline{H}_2$(Leu)), 1.98-2.22 (m, 15H, C$\underline{H}_3$CO, 2xC$\underline{H}_3$CO(Acm), 2xCHC$\underline{H}_2$(Hcy), CHC$\underline{H}_2$(Gln)), 2.30-2.42 (m, 2H, C$\underline{H}_2$CO(Gln)), 2.45-2.79 (m, 4H, 2xC$\underline{H}_2$CO(Hcy)), 3.75-3.99 (m, 2H, C$\underline{H}_2$O(Ser)), 4.19-4.49 (m, 9H, 5xα-C$\underline{H}$, 2xC$\underline{H}_2$(Acm)); ^{13}C NMR (125 MHz, CD_3OD)(mixture of rotamers) δ 22.0, 22.3, 22.5, 22.8, 23.2, 23.4, 25.9, 28.0, 28.1, 28.2, 28.3, 28.4, 32.3, 32.8, 33.0, 33.1, 41.3, 41.4, 41.5, 41.6, 41.7, 53.8, 53.9, 54.2, 54.6, 54.8, 55.1, 57.2, 57.4, 62.5, 62.6, 62.7, 173.4, 173.8, 173.9, 174.0, 174.3, 174.4, 175.6, 176.5, 177.8; HRMS (ES) Calcd for $C_{30}H_{53}N_9O_{10}S_2Na$ (MNa^+) 786.3249, found 786.3256.



***N*-Acetyl-L-leucyl-L-serinyl-*S*-Acm-L-homocysteinyl-L-glutamyl-*S*-Acm-L-cysteinamide (138).** This compound was prepared as described for **132**. Purification of the crude product by HPLC (Waters C18 Bondpak; 100 x 40 mm; linear gradient elution over 20 min of 0 to 50% acetonitrile in 0.1% TFA/H_2O, t_R = 9.7 min) gave the title product as a white solid (0.102 g, 34%); $[\alpha]_D^{26}$ -19.4° (*c* 2.6, MeOH); IR (μscope) 3276, 3073, 2958, 2419, 1657, 1536, 1416, 1374, 1271, 1202; ^{1}H NMR (300 MHz, CD_3OD) δ 0.88-1.00 (m, 6H, ($C\underline{H}_3$)$_2$(Leu)), 1.54-1.72 (m, 3H, C$\underline{H}$, C$\underline{H}_2$(Leu)), 1.98-2.22 (m, 13H, C$\underline{H}_3$CO, 2xC$\underline{H}_3$CO(Acm), CHC$\underline{H}_2$(Hcy), CHC$\underline{H}_2$(Gln)), 2.29-2.40 (m, 2H, CHC$\underline{H}_2$(Gln)), 2.44-2.72 (m, 2H, C$\underline{H}_2$S(Hcy)), 2.82-2.94 (m, 1H, C$\underline{H}_2$S(Cys)), 3.33-3.38 (m, 1H, C$\underline{H}_2$S(Cys)), 3.74-3.98 (m, 2H, C$\underline{H}_2$O(Ser)), 4.22-4.60 (m, 9H, 5xα-C$\underline{H}$, 2xC$\underline{H}_2$(Acm)); ^{13}C NMR (125 MHz, CD_3OD)(mixture of rotamers) δ 21.9, 22.1, 22.6, 22.9, 23.2, 25.7, 27.9, 28.1, 32.1, 32.3, 33.5, 33.6, 35.7, 41.1, 41.3, 41.5, 41.6, 53.8, 53.9, 54.14, 54.2, 54.3, 54.5, 54.8, 57.2, 62.4, 62.5, 173.1, 173.3, 173.8, 174.1, 174.3, 174.4, 175.1, 175.6, 175.9, 178.1; HRMS (ES) Calcd for $C_{29}H_{51}N_9O_{10}S_2Na$ (MNa^+) 772.3093, found 772.3079.

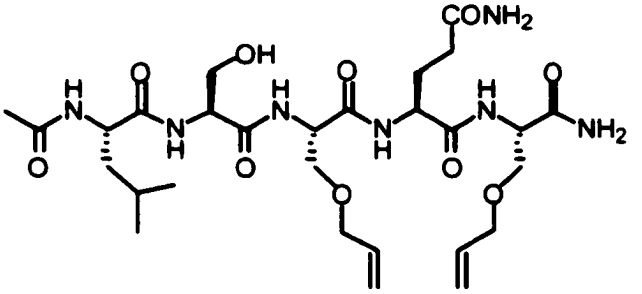


***N*-Boc-*O*-allylserine (140).** This compound was prepared using an adaptation of the procedure of Itoh.[102] *N*-Boc-Ser-OH **139** (1.0 g, 4.87 mmol) was dissolved in anhydrous DMF (25 mL) and cooled to 0 °C. NaH (0.25 g, 9.76 mmol) was then added and after 30 min of stirring, allyl bromide (freshly filtered through basic alumina) (0.45 mL, 5.37 mmol) was added then the reaction mixture allowed to warm to rt over 4 h. The solvent was removed *in vacuo* and the residue diluted with NaOH (1N, 10 mL) then washed with EtOAc (2x10 mL). After acidifying with 1N HCl the aqueous layer was extracted with EtOAc (3x15 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography (SiO_2, 1:1 EtOAc/hexane, 1% AcOH) to afford the title product as an oil (0.69 g, 58%); $[\alpha]_D^{26}$ +19.0° (*c* 1.7, $CHCl_3$), lit.[102] $[\alpha]_D^{26}$ −17.3° (*c* 2.6, MeOH); IR (μscope) 2979, 2932, 1717, 1511, 1455, 1394, 1368, 1248, 1163, 1109 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 1.42 (s, 9H, $(C\underline{H}_3)_3$), 3.72 (dd, *J* = 9.5, 3.6 Hz, 1H, $C\underline{H}_2O$), 3.78-3.92 (m, 1H, $C\underline{H}_2O$), 3.98 (d, 2H, *J* = 5.6 Hz, $C\underline{H}_2CH$=) 4.38-4.48 (m, 1H, $C\underline{H}NH$), 5.18 (dd, *J* = 10.0, 1.6 Hz, 1H, $C\underline{H}_2$=), 5.22 (dd, *J* = 15.7, 1.6 Hz, 1H, $C\underline{H}_2$=), 5.42 (d, *J* = 8.7 Hz, 1H, N$\underline{H}$), 5.78-5.84 (m, 1H, $C\underline{H}$=), 9.48 (br s, 1H, $CO_2\underline{H}$); ^{13}C NMR (75 MHz, CD_3OD) δ 28.3, 53.8, 69.6, 72.4, 80.4, 117.8, 133.9, 155.8, 175.1; HRMS (ES) Calcd for $C_{11}H_{19}O_5NNa$ (MNa^+) 268.1161, found 268.1154.

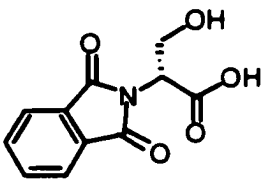


***N*-Fmoc-*O*-allylserine (141).** This compound was prepared by a modified procedure of Hasegawa.[103] To a solution of *N*-Boc-Ser(*O*-all)-OH **140** (0.59 g, 2.40 mmol) in CH_2Cl_2 (30 mL) was added TFA (5 mL, excess) at rt. The reaction was stirred for 2 h after which the solvent was removed *in vacuo* to give an oil, which was triturated with Et_2O. The TFA salt (0.59g, 2.28 mmol) was dissolved in H_2O (10 mL) and saturated aq. $NaHCO_3$ (0.77g, 9.11 mmol) was added at 0 °C. After 5 min, FmocCl (0.59 g, 2.50 mmol) in dioxane (20 mL) was added and the reaction mixture was stirred for 4 h. The reaction mixture was washed with EtOAc (3x10 mL), the aqueous layer acidified with 1N HCl, extracted with EtOAc (3x10 mL) and then dried over $MgSO_4$. The crude product was purified by column chromatography (SiO_2, 1:2 EtOAc/hexane, 1% AcOH) to afford the title product which was recrystallized from Et_2O/Hexane to give a white solid (0.49, 59%); mp 108-109 °C, $[\alpha]_D^{25}$ +23.0° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3065, 2949, 1721, 1519, 1477, 1450, 1420, 1336, 1216, 1105, 1085 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 3.70 (dd, *J* = 9.8, 4.0 Hz, 1H, C<u>H</u>$_2$O), 3.82 (dd, *J* = 9.6, 5.2 Hz, 1H, C<u>H</u>$_2$O (all)), 3.94-4.01 (m, 1H, C<u>H</u>$_2$CH=), 4.21 (t, *J* = 6.7 Hz, 1H, C<u>H</u>CH$_2$O), 4.32-4.40 (m, 3H, C<u>H</u>$_2$O (Fmoc), C<u>H</u>NH), 5.12 (dd, *J* = 10.5, 1.0 Hz, 1H, C<u>H</u>$_2$=), 5.24 (dd, *J* = 17.1, 1.0 Hz, 1H, C<u>H</u>$_2$=), 5.01-5.22 (m, 1H, C<u>H</u>=), 7.26 (dd, 2H, *J* = 7.3, 7.3 Hz, <u>Ph</u>), 7.34 (dd, *J* = 7.5, 7.5 Hz, 2H, <u>Ph</u>), 7.58-7.66 (m, 2H, <u>Ph</u>), 7.79 (d, *J* = 7.5 Hz, 2H, <u>Ph</u>); ^{13}C NMR (75 MHz, CD_3OD) δ 48.2, 55.8, 68.2, 70.7, 73.2, 117.5, 120.9, 126.3, 128.2, 128.9, 135.8, 142.6,

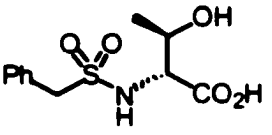
145.3, 158.6, 173.6; HRMS (EI) Calcd for $C_{21}H_{21}O_5N$ (M^+) 367.1419, found 367.1427. Anal. Calcd for $C_{21}H_{21}O_5N$: C, 68.65; H, 5.76; N, 3.81; Found: C, 68.40; H, 5.41; N, 3.75.



***N*-Acetyl-L-leucyl-L-serinyl-*O*-allyl-L-serinyl-L-glutamyl-*O*-allyl-L-serinamide (143).** This compound was prepared as described for **132**. Purification of the crude product by HPLC (Waters C18 Bondpak; 100 x 40 mm; linear gradient elution over 15 min of 15 to 80% acetonitrile in 0.1% TFA/H_2O, t_R 7.0 min) gave the title product as a white solid (0.62 g, 24%); $[\alpha]_D^{26}$ -0.7° (*c* 0.7, MeOH); IR (μscope) 3280, 2935, 2872, 2421, 1625, 1539, 1431, 1285, 1255, 1205, 1134; ^{1}H NMR (300 MHz, CD_3OD) δ 0.90 (d, *J* = 6.3 Hz, 3H, C<u>H</u>$_3$(Leu)), 0.95 (d, *J* = 6.3 Hz, 3H, C<u>H</u>$_3$(Leu)), 1.52-1.72 (m, 3H, C<u>H</u>, C<u>H</u>$_2$(Leu)), 1.92-2.21 (m, 5H, C<u>H</u>$_3$CO, CHC<u>H</u>$_2$(Gln)), 2.32 (t, *J* = 7.2 Hz, 2H, C<u>H</u>$_2$CO(Gln)), 3.78-4.44 (m, 10H, 3xC<u>H</u>$_2$O(Ser), 2xOC<u>H</u>$_2$(Ser)), 4.31-4.52 (m, 5H, 5xα-C<u>H</u>), 5.18 (dd, *J* = 12.1, 1.6 Hz, 2x1H, 2xC<u>H</u>$_2$=), 5.22 (dd, *J* = 17.3, 1.6 Hz, 2x1H, 2xC<u>H</u>$_2$=), 5.80-5.87 (m, 2x1H, 2xC<u>H</u>=CH$_2$); ^{13}C NMR (75 MHz, CD_3OD)(mixture of rotamers) δ 21.9, 23.2, 25.8, 28.2, 32.5, 41.5, 53.5, 54.7, 55.6, 69.6, 70.1, 73.1, 73.2, 118.4, 135.3, 172.7, 173.0, 173.7, 174.6, 178.1; HRMS (ES) Calcd for $C_{28}H_{47}N_7O_{10}Na$ (MNa^+) 664.3272, found 660.3277.

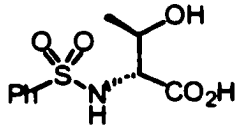


***N*-(Phthalimido)-D-serine (150).** To a solution of D-serine **149** (3.00 g, 28.6 mmol) in H_2O (60 mL) and Na_2CO_3 (3.33 g, 31.4 mmol) was added *N*-ethyloxycarbonyl phthalimide (6.26 g, 28.6 mmol).[111] The reaction mixture was stirred for 1 h until most of the solid dissolved. The mixture was filtered and the filtrate acidified to pH 1 with 6 N HCl, extracted with EtOAc (3x20 mL) and the combined organic layers dried over $MgSO_4$. Evaporation of the solvent *in vacuo* followed by purification of the crude product by column chromatography (SiO_2, 2:1 hexane/EtOAc and 1% AcOH) afforded **150** as a white solid (5.43 g, 81%) after recrystallization from MeOH/CH_2Cl_2/hexane; mp 128-129 °C; $[\alpha]_D^{26}$ +42.5° (*c* 0.2, MeOH); IR (μscope) 3512, 3296, 2986, 2946, 2922, 2598, 1751, 1692, 1604, 1340, 1293, 1191 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 4.18 (dd, *J* = 11.7, 5.5 Hz, 1H, C<u>H</u>$_2$O), 4.24 (dd, *J* = 11.6, 9.2 Hz, 1H, C<u>H</u>$_2$O), 4.98 (dd, *J* = 9.2, 5.5 Hz, 1H, C<u>H</u>N), 7.80-7.96 (m, 4H, <u>Ph</u>); ^{13}C NMR (75 MHz, CD_3OD) δ 55.6, 60.0, 124.3, 133.2, 135.6, 169.3, 170.7; HRMS (ES) Calcd for $C_{11}H_9NO_5Na$ (MNa^-) 258.0378, found 258.0372; Anal. Calcd for $C_{11}H_9NO_5$ C, 56.17; H, 3.86; N, 5.96. Found: C, 55.98; H, 3.51; N, 5.82.



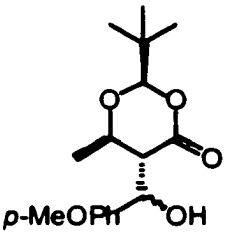
***N*-(Phenylmethanesulfonyl)-D-*allo*-threonine (157).** This compound was prepared using the procedure of Maurer.[114] D-*allo*–threonine **151d** (0.30 g, 2.52 mmol)

was dissolved in H_2O (15 mL) and treated with Na_2CO_3 (1.07 g, 10.1 mmol) followed by α-toluenesulfonyl chloride (0.28 g, 3.02 mmol). The mixture was stirred for 24 h, washed with Et_2O (2 x 10 mL), acidified to pH 2 with 1N HCl and then extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over $MgSO_4$ and evaporation of the volatiles *in vacuo* followed by recrystallization of the crude product from MeOH/Et_2O/hexane afforded a white solid (0.95 g, 28%); mp 138-139 °C; IR (μscope) 3489, 3207, 2974, 1732, 1696, 1456 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 1.18 (d, J = 6.2 Hz, 3H, CH(C<u>H</u>3)), 3.90 (d, J = 5 Hz, 1H, C<u>H</u>NH), 4.0 (dq, J = 6.4, 5.2 Hz, 1H, C<u>H</u>O), 4.34 (d, J = 13.7 Hz, 1H, C<u>H</u>$_2SO_2$), 4.38 (d, J = 13.7 Hz, 1H, C<u>H</u>SO_2), 7.44-7.32 (m, 5H, <u>Ph</u>); ^{13}C NMR (75 MHz, CD_3OD) δ 19.1, 60.5, 63.5, 69.3, 129.3, 129.4, 131.1, 132.1, 173.4; HRMS (EI) Calcd for $C_{11}H_{15}NO_5S$ (M^+) 273.0671, found 273.0658.



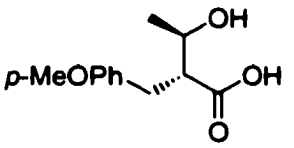
***N*-(Phenylsulfonyl)-D-*allo*-threonine (158).**[114] Reaction of D-*allo*–threonine **151d** (0.44 g, 3.69 mmol) with benzenesulfonyl chloride (0.56 g, 4.43 mmol) and Na_2CO_3 (1.57 g, 14.7 mmol) as described for **157** gave the title product as a white solid (0.46 g, 48.5%); mp 171-173 °C; $[\alpha]_D^{26}$ -17.2° (*c* 0.3, MeOH), Lit.[114] $[\alpha]_D^{26}$ -16.8° (*c* 1.6, MeOH); IR (μscope) 3450, 3330, 2983, 1727, 1448, 1390 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 1.38 (d, J = 6.3 Hz, 3H, CH(C<u>H</u>3), 3.78 (d, J = 5.9 Hz, 1H, C<u>H</u>NH), 3.85 (dq, J = 6.4, 5.6 Hz, 1H, C<u>H</u>OH), 7.60-7.44 (m, 3H, <u>Ph</u>); ^{13}C NMR (75 MHz, CD_3OD) δ 19.5, 63.2, 69.3, 128.9, 129.9, 136.6, 142.2, 172.9; HRMS (ES) Calcd for

$C_{10}H_{13}NO_4SNa$ (MNa^+) 282.0412, found 282.0405; Anal. Calcd for $C_{10}H_{13}NO_4S$: C, 46.32; H, 5.05; N, 5.40. Found: C, 46.19; H, 4.94; N, 5.25.

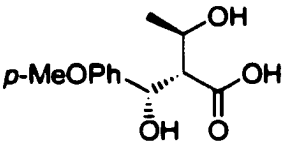


(1'*RS*,2*R*,5*R*,6*R*)-5-[α-*p*-Methoxybenzyl]-2-(*tert*-butyl)-6-methyl-1,3-dioxan-4-one (161). This material was prepared from a modified procedure of Gautschi.[115b] To a solution of *t*-BuLi (1.88 mL, 1.7 M in pentane, 3.19 mmol) in THF (10 mL) at -78 °C under an atmosphere of argon was added a precooled solution of dioxanone **160** (0.5 g, 2.90 mmol) in THF (3 mL) via cannula. The reaction mixture was stirred for 45 min after which it was treated dropwise with *p*-anisaldehyde (0.40 mL, 3.77 mmol). The mixture was stirred for a further 24 h at -78 °C after which it was quenched with saturated aq. NH_4Cl (10 mL), extracted with EtOAc (3 x 5 mL) and then dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the crude product purified by flash column chromatography (SiO_2, 75% hexane/Et_2O) and then recrystallized from (Et_2O/hexane) to give a white solid (0.59 g, 66%); mp 157-158 °C IR (μscope) 3408, 2960, 2918, 1795, 1720, 1611, 1511 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$)(1:1 mixture of diastereomers) δ 0.85 (d, J = 7.5 Hz, 3H, CH(C<u>H</u>3)), 0.98 (s, 9H, (C<u>H</u>3)3), 2.78 (dd, J = 9.2, 3.4 Hz, 1H, C<u>H</u>CO), 3.98-3.88 (m, 1H, $CH_3C\underline{H}O$), 4.83 (s, 1H, C<u>H</u>O2), 5.32 (d, J = 3.4 Hz, 1H, C<u>H</u>OH), 6.90 (d, J = 8.8 Hz, 2H, <u>Ph</u>), 7.25 (d, J = 8.9 Hz, 2H, <u>Ph</u>); ^{13}C NMR (75 MHz, $CDCl_3$)(1:1 mixture of diastereomers) δ 19.2, 21.1, 23.9, 24.4, 24.7, 34.9, 35.1, 46.9,

55.3, 55.4, 69.3, 71.2, 72.5, 74.2, 102.0, 107.7, 114.1, 114.5, 126.9, 127.8, 128.2, 128.8, 132.9, 159.4, 171.4, 171.9; HRMS (EI) Calcd for $C_{17}H_{24}O_5$ (M^+) 235.0845, found 235.0835; Anal. Calcd for $C_{17}H_{24}O_5$: C, 66.21; H, 7.84; Found: C, 65.99; H, 7.79.



162a

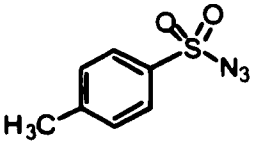


162b

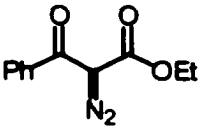
(2*R*,3*R*)-2-(*p*-Methoxybenzyl)-3-hydroxybutanoic acid (162a) and (1'*S*,2*R*,3*R*)-2-(1'-Hydroxy-*p*-methoxybenzyl)-3-hydroxybutanoic acid (162b). Reaction of dioxanone **161** (0.4 g, 1.95 mmol) and 10% palladium on carbon (0.04 g) for 24 h as described for *N*-(Phenethylsulfonyl)-D-serine gave **162a** (0.13 g, 40%) and **162b** (0.14 g, 35%) as white solids after purification by column chromatography (SiO_2, 66% EtOAc:hexane and 1% AcOH). These materials were recrystallized from ($CHCl_3$/hexane).

Data for **162a**: mp 107-108 °C; $[\alpha]_D^{26}$ -39.7° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3259, 3059, 2936, 2835, 1696, 1610, 1582, 1512 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.38 (d, *J* = 6.4 Hz, 3H, C<u>H</u>$_3$), 2.62 (ddd, *J* = 13.1, 7.0, 5.1 Hz, 1H, C<u>H</u>CO), 2.84-2.88 (m, PhC<u>H</u>$_2$), 3.75 (s, 3H, OC<u>H</u>$_3$), 3.85 (dq, *J* = 12.7, 6.4 Hz, 1H, C<u>H</u>OH), 6.79 (d, *J* = 8.6 Hz, 2H, <u>Ph</u>), 7.11 (d, *J* = 8.6 Hz, 2H, <u>Ph</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.85, 34.4, 54.3, 55.3, 67.3, 114.0, 129.9, 130.3, 158.4, 179.2; HRMS (EI) Calcd for $C_{12}H_{16}O_5$ (M^+) 240.0998, found 240.0997; Anal. Calcd for $C_{12}H_{16}O_5$: C, 64.85; H, 6.35; Found: C, 64.66; H, 6.34.

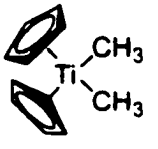
Data for **162b**: mp 128-129 °C; $[\alpha]_D^{26}$ -5.8° (*c* 0.9, $CHCl_3$); IR ($CHCl_3$ cast) 3387, 2934, 1708, 1612, 1586, 1456 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.24 (d, *J* = 6.6 Hz, 3H, $CH(C\underline{H}_3)$), 2.70 (dd, *J* = 6.0, 2.3 Hz, 1H, C<u>H</u>CO), 3.78 (s, 3H, $C\underline{H}_3O$), 4.12-4.21 (m, 1H, $CH_3C\underline{H}OH$), 5.18 (d, *J* = 6.0 Hz, 1H, C<u>H</u>OH), 6.84 (d, *J* = 11.3 Hz, 2H, <u>Ph</u>), 7.22 (d, *J* = 11.3 Hz, 2H, <u>Ph</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.4, 55.3, 57.2, 65.7, 172.9, 113.9, 127.3, 133.2, 159.2, 176.0; HRMS (EI) Calcd for $C_{12}H_{16}O_4$ (M^+) 224.1049, found 224.1053.



Tosyl azide (165).[119] This known compound was prepared by the method of Curphey.[119] To a solution of sodium hydrazide (3.75 g, 57.6 mmol) in H_2O/acetone (75 mL, 1:7) was added slowly tosyl chloride **164** and the reaction mixture stirred for 2 h. The solvent was removed *in vacuo* and the residue partitioned between H_2O (50 mL) and CH_2Cl_2 (20 mL), extracted with CH_2Cl_2 (3x20 mL) and dried over $MgSO_4$ to give a colorless oil (9.40 g, 91 %) which was pure enough to use for the next step as judged by ^1H NMR: IR ($CHCl_3$ cast) 3067, 2957, 2926, 2358, 2340, 2127, 1595, 1494, 1450, 1398; ^1H NMR (300 MHz, $CDCl_3$) δ 2.42 (s, 3H, $C\underline{H}_3$), 7.38 (d, *J* = 10.0 Hz, 2H, <u>Ph</u>), 7.80 (d, *J* = 10.0 Hz, 2H, <u>Ph</u>); ^{13}C NMR (125MHz, $CDCl_3$) δ 21.6, 127.4, 130.2, 135.4, 146.2; HRMS (EI) Calcd for $C_7H_7O_2N_3S$ (M^+) 197.0259, found 197.0253.

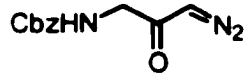


2-Diazo-3-oxo-3-phenyl-propionic acid ethyl ester (167).[120] To a solution of ethyl benzoyl acetate **166** (2.5 g, 13.0 mmol) and triethylamine (1.98 mL, 14.3 mmol) in acetonitrile (25 mL) was added tosyl azide **165** (2.56 g, 13.0 mmol). The reaction mixture was stirred for 16 h after which the solvent was removed *in vacuo* and the residue purified directly by column chromatography (SiO_2, 33% Et_2O/hexane) to give a light greenish oil (2.63 g, 93%); ^1H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, J = 11.3 Hz, 3H, C<u>H</u>3), 4.25 (q, J = 10.9 Hz, 2H, C<u>H</u>2O), 7.40-7.65 (m, 5H, <u>Ph</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 61.6, 76.2, 127.9, 128.4, 132.3, 137.2, 161.0, 189.9; HRMS (EI) Calcd for $C_{11}H_{10}O_3N_2$ (M^+) 218.0689, found 218.0691.

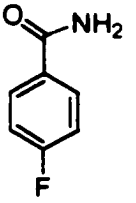


Dimethyltitanocene (175).[122] This compound was prepared by the procedure of Dollinger.[122] Methyl lithium (30.8 ml, 46.2 mmol, 1.5 M in Et_2O) was added dropwise to a stirred slurry of bis(cyclopentadienyl) titanium dichloride **174** (5.0 g, 20.1 mmol) in anhydrous toluene at –5 °C. The orange mixture was stirred at –5 °C for 1 h and then allowed to warm to rt over 1 h. The mixture was cooled to 0 °C and carefully quenched with ice-cold 6% aq. NH_4Cl (15 mL). After separation, the organic layer was washed with H_2O (20 mL), brine (20 mL), dried over $MgSO_4$ and then filtered to provide a red solution which was concentrated to one-third its volume. ^1H NMR assay indicated (2.93

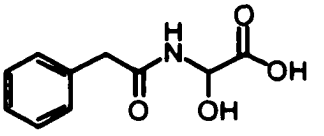
g, 70%) of the title product. This compound was stored in the freezer as a toluene solution; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.19 (s, 6H, 2xC<u>H</u>$_3$), 6.20 (s, 10H, <u>cp</u>); ^{13}C NMR (125 MHz, $CDCl_3$) δ 45.6, 113.0.



Diazo-(*N*-benzyloxycarbonyl-glycyl)-methane (178).[124] This known compound was prepared from a modified procedure of Wang.[124] To a cooled 0 °C solution of acid **177** (5.0 g, 23.9 mmol) in THF (125 mL) was added triethylamine (3.65 mL, 26.3 mmol) followed by ethyl chloroformate (2.52 mL, 26.3 mmol). The reaction mixture was stirred for 30 min and then filtered into an ethereal solution of diazomethane (200 mL, ca. 96 mmol) at 0 °C. After stirring for a further 4 h the solvent was carefully evaporated and the crude product purified by column chromatography (SiO_2, EtOAc) to afford **178** as an oil (3.32 g, 60%); IR (μscope) 3298, 2107, 1732, 1695, 1469, 1380 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.95 (br s, 1H, C<u>H</u>), 5.15 (br s, 2H, C<u>H</u>$_2$O), 5.35 (s, 1H, C<u>H</u>N$_2$), 5.55 (br s, 1H, N<u>H</u>), 7.25 (br s, 5H, <u>Ph</u>); ^{13}C NMR (75 MHz, $CDCl_3$) δ 48.1, 48.8, 67.2, 128.1, 128.3, 128.6, 136.2, 156.3, 190.0; HRMS (ES) Calcd for $C_{11}H_{11}O_3N_3Na$ (MNa^+) 256.0698, found 256.0704.

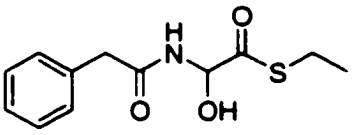


4-Fluoro-phenylacetamide (197).[140] To a solution of 4-fluoro-phenylacetyl chloride **196** (3.0 g, 17.4 mmol) in anhydrous CH_2Cl_2 (15 mL) at 0 °C was added aqueous ammonia (15 mL, 48%, excess). After stirring at 0 °C for 30 min, the solvent was evaporated *in vacuo*, the residue taken up in CH_2Cl_2/MeOH (2:1, 50 mL), washed successively with saturated aq. $NaHCO_3$ (30 mL) and then HCl (30 mL, 1N). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Recrystallization of the crude product from MeOH/CH_2Cl_2/hexane gave **197** as a white crystalline solid (1.39 g, 52%); mp 155.156 °C, lit.[140] mp 156-157 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.45 (s, 2H, C<u>H</u>$_2$), 5.32 (br s, 1H, N<u>H</u>$_2$), 5.70 (br s, 1H, N<u>H</u>$_2$), 6.94-7.08 (m, 2H, <u>Ph</u>), 7.21-7.29 (m, 2H, <u>Ph</u>); HRMS (EI) Calcd for C_8H_8ONF (M^+) 153.0589, found 153.0591.



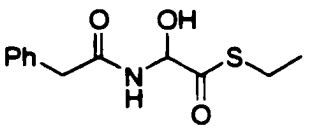
2-Hydroxy-phenylacetylamino-acetic acid (208).[131] This known literature compound was prepared using a modified procedure of Ben-Ishai.[131] A solution of phenyl acetamide **207** (5.0 g, 37.0 mmol) and glyoxylic acid monohydrate (3.8 g, 40.7 mmol) in acetone (100 mL) was refluxed at 65 °C for 4 h until a homogeneous solution was obtained. The solvent was evaporated *in vacuo* and the residue diluted with $NaHCO_3$ (50 mL) and washed with EtOAc (2 x 20 mL). The aqueous layer was acidified to pH 1 with

4N HCl, extracted with EtOAc (3 x 50 mL) and dried over $MgSO_4$. The white solid obtained after evaporation *in vacuo* was recrystallized from CH_2Cl_2/hexane to give a white crystalline solid (3.78 g, 49%); mp 91-92 °C; lit[131] mp 90-91 °C; ^1H NMR (400 MHz, CD_3OD) δ 3.58 (s, 2H, $C\underline{H}_2Ph$), 5.58 (s, 1H, $C\underline{H}OH$), 7.12-7.36 (m, 5H, Ph); HRMS (ES) Calcd for $C_{10}H_{11}O_4NNa$ (MNa^-) 132.0586, found 232.0583.

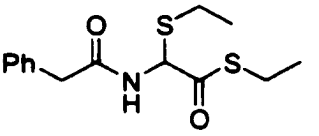


2-Ethylsulfanyl-phenylacetylamino-acetic acid (209). This compound was prepared using a similar procedure as described by Davies.[132] To a solution of the hydroxyacid **208** (0.5 g. 2.4 mmol) in glacial acetic acid (5 mL) was added conc. H_2SO_4 followed by EtSH (0.7 mL, 9.7 mmol). The mixture was stirred for 12 h after which H_2O (10 mL) was added and extracted with EtOAc (3 x 10 mL). The combined organic layers were extracted with saturated aq. Na_2CO_3 (20 mL) then washed with EtOAc (2 x 10 mL). The aqueous layer was acidified to pH 1 with 1N HCl, re-extracted with EtOAc (3 x10 mL) and dried over $MgSO_4$. The product obtained after evaporation *in vacuo* was recrystallized from Et_2O/hexane to give **209** as a solid (0.42 g, 69%); mp 118-120 °C; IR (μscope) 3321, 3028, 2968, 2930, 2565, 1917, 1713, 1594, 1530, 1495 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.20 (t, J = 7.5 Hz, 3H, $C\underline{H}_3$), 2.58 (q, J = 7.5 Hz, 2H, $SC\underline{H}_2$), 3.62 (s, 2H, $C\underline{H}_2Ph$), 5.49 (d, J = 8.3 Hz, 1H, $C\underline{H}OH$), 6.42 (d, J = 8.3 Hz, 1H, $N\underline{H}$), 7.20-7.38 (m, 5H, Ph), 9.58 (br s, 1H, $CO_2\underline{H}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.6, 25.5, 43.4, 53.5, 127.7, 129.1, 129.3, 133.6, 171.1, 171.8; HRMS (ES) Calcd for $C_{12}H_{15}O_3NSNa$

(MNa^+) 276.0670, found 276.0671. Anal. Calcd for $C_{12}H_{15}O_3NS$: C, 56.90; H, 5.97; N, 5.53; Found: C, 56.85; H, 5.89; N, 5.55.



210



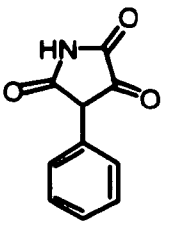
211

2-Hydroxy-phenylacetylamino-thioacetic acid *S*-ethyl ester (210) and 2-Ethylsulfanyl-phenylacetylamino-thioacetic acid *S*-ethyl ester (211). To a solution of hydroxyacid **208** (0.5 g, 2.39 mmol) in CH_2Cl_2 (25 mL) at 0 °C was added EDCI (0.51 g, 2.63 mmol), DMAP (29.2 mg, 0.24 mmol), EtSH (0.18 mL, 2.39 mmol) and NEt_3 (0.33 mL, 2.39 mmol) in sequential order. The reaction mixture was stirred at 0 °C for 2 h and then diluted with saturated aq. $NaHCO_3$ (20 mL). The CH_2Cl_2 layer was separated, washed with 1 N HCl (20 mL) and dried over $MgSO_4$. Purification of the crude product by column chromatography (SiO_2, 50% Et_2O/Hexane) furnished the title products as solids.

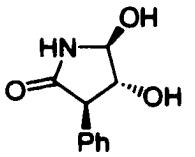
Data for **210**: (75.0 mg, 12%); IR (μscope) 3287, 3030, 2969, 2930, 1663, 1522, 1495, 1453 cm^{-1}; ^1H NMR (400 MHz, CD_2Cl_2) δ 1.72 (t, J = 7.5 Hz, 3H, C<u>H</u>$_3$), 2.89 (q, J = 7.5 Hz, 2H, SC<u>H</u>$_2$), 3.60 (s, 2H, C<u>H</u>$_2$Ph), 5.49 (d, J = 7.3 Hz, 1H, C<u>H</u>OH), 6.58 (br s, 1H, N<u>H</u>), 7.22-7.40 (m, 5H, <u>Ph</u>); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 14.6, 23.9, 43.7, 77.5, 127.7, 129.2, 129.8, 134.4, 172.3, 198.9; LRMS (CI) for $C_{12}H_{19}O_3N_2S$ *m/z* (relative intensity) 271.3 (MNH_4^+, 0.11%).

Data for **211**: (42 mg, 6%); mp 59-60 °C; IR (μscope) 3309, 3084, 3029, 2965, 2928, 2868, 1677, 1648, 1600, 1514, 1494 cm^{-1}; ^1H NMR (400 MHz, CD_2Cl_2) δ 1.28-1.34 (m,

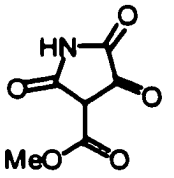
6H, 2xC<u>H</u>$_3$), 2.44-2.62 (m, 2H, SC<u>H</u>$_2$), 2.84 (q, J = 7.3 Hz, 2H, SC<u>H</u>$_2$), 3.60 (s, 2H, C<u>H</u>$_2$Ph), 5.49 (d, J = 7.3 Hz, 1H, C<u>H</u>S), 6.58 (br s, 1H, N<u>H</u>), 7.22-7.40 (m, 5H, <u>Ph</u>); ^{13}C NMR (125 MHz, CD_2Cl_2) δ 14.6, 14.7, 24.3, 25.2, 43.7, 60.7, 127.7, 129.3, 129.7, 134.9, 170.2, 195.7; HRMS (EI) Calcd for $C_{14}H_{19}O_2NS_2$ (M^+) 297.0857, found 297.0860.



(4*R*,*S*)-4-Phenyl-pyrrolidine-2,3,5-trione (217). To a mixture of phenyl acetamide (0.3 g. 2.22 mmol) in toluene (10 mL) 60 °C was added oxalyl chloride (0.23 mL, 2.67 mmol) over 20 min. The yellow colored solution was stirred for 3 h after which it was evaporated *in vacuo* and the solid recrystallized from THF to give a yellow crystalline solid (0.38 g, 90%); mp 215-216 °C, lit.[133] mp 217 °C. IR (μscope) 3070, 2911, 2879, 2851, 2784, 1858, 1814, 1773, 1712, 1362 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.32 (s, 1H, C<u>H</u>), 7.18 (dd, J = 7.0, 7.0 Hz, 1H, <u>Ph</u>), 7.35 (t, J = 7.6 Hz, 2H, <u>Ph</u>), 7.48 (d, J = 7.3 Hz, 1H, <u>Ph</u>), 12.78 (br s, 1H, <u>NH</u>); ^{13}C NMR (75 MHz, DMSO-d_6) δ 89.2, 126.4, 126.9, 128.0, 128.3, 128.6, 129.4, 132.9, 140.8, 151.7, 156.5; HRMS (EI) Calcd for $C_{10}H_7O_3N$ (M^+) 189.0426, found 189.0423.



***rac-(4R,5S)*-Dihydroxy-3-phenyl-pyrrolidin-2-one (218).** To a solution of the trione **217** (0.60 g. 3.17 mmol) in EtOH (5 mL) was added $NaBH_4$ (0.60 g, 15.8 mmol) at 0 °C. The yellow color immediately disappeared and the mixture was stirred for another 5 min after which it was quenched with saturated aq. $NaHCO_3$ (15 mL), extracted with EtOAc (3 x 5 mL) and the combined organic layers dried over $MgSO_4$. The product obtained after concentration *in vacuo* was recrystallized from MeOH/Et_2O/hexane to give a white crystalline solid (0.15 g, 26%); mp 168-169 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 3.43 (d, J = 5.6 Hz, 1H, C<u>H</u>Ph), 4.25 (dd, J = 5.7, 3.0 Hz, 1H, C<u>H</u>OH), 5.22 (d, J = 2.9 Hz, 1H, C<u>H</u>OH), (s, 1H, C<u>H</u>), 7.22-7.38 (m, 5H, <u>Ph</u>); ^{13}C NMR (75 MHz, CD_3OD) δ 58.7, 83.7, 86.0, 128.2, 129.6, 129.8, 138.8, 177.2; HRMS (ES) Calcd for $C_{10}H_{12}O_3N$ (MH^+) 194.0812, found 194.0814.



(4*R,S*)-4-Carboxylic acid methyl ester-pyrrolidine-2,3,5-trione (220). The title compound was prepared as that described for **217**. Reaction of amide **219** (0.30 g. 2.56 mmol) and oxalyl chloride (0.27 mL, 3.08 mmol) gave the title product which was recrystallized from acetone/hexane to give **220** as a white crystalline solid (0.36 g, 82%); mp 149-150 °C. IR (μscope) 3692, 3565, 3256, 3060, 1965, 1848, 1789, 1747, 1666, 1574 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.80 (s, 3H, OC<u>H</u>$_3$), 5.24 (s, 1H, C<u>H</u>), 10.82

(br s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 52.4, 81.0, 149.4, 152.2, 152.9, 167.6; gHMQC (500 MHz, $CDCl_3$) correlations (δ_H 5.24 (CH), δ_C 81.0 (CONH)), (δ_H 3.80 (CO_2CH_3), δ_C 52.4 (CO_2CH_3)); gHMBC (500 MHz, $CDCl_3$) key correlations ($^3J_{H\text{-}C}$, δ_H 10.82 (NH), δ_C 81.0 (CONH)), ($^2J_{H\text{-}C}$, δ_H 5.24 (CH), δ_C 167.6 (CONH)), ($^2J_{H\text{-}C}$, δ_H 5.24 (CH), δ_C 152.9 (CO_2CH_3)); HRMS (ES) Calcd for $C_6H_5O_5NNa$ (MNa^+) 194.0065, found 194.0069.

Crystallographic data for 52b, 60b, 61a and 218.

Data were acquired on a Bruker P4/RA/SMART 1000 CCD diffractometer. All intensity measurements were performed using graphite monochromated Mo-Kα radiation (λ = 0.71073 Å).

Crystal data for β-lactone ***52b***: $C_{12}H_{14}O_4$: M = 222.23, monoclinic, a = 7.7336(11) Å, b = 5.6977(8) Å, c = 13.2376(19) Å, β = 102.597(3)°, U = 569.26(14) Å^3, T = -80 °C, space group $P2_1$ (No. 4), Z = 2, μ(Mo-Kα) = 2 mm^{-1}, 2817 reflections measured, 1192 unique which were used in all least squares calculations, $R1(F)$ = 0.0498 (for 1192 reflections with $F_o^2 \geq 2\sigma(F_o^2)$), $wR(F^2)$ = 0.1139 (for all unique reflections).

Crystal data for pseudoxazolone ***60a***: $C_{10}H_7NO_2$: M = 173.17, triclinic, a = 5.5390(6) Å, b = 7.2645(8) Å, c = 10.4291(11) Å, α = 83.673(2)°, β = 83.789(2)°, γ = 80.624(8)°, U = 409.77(8) Å^3, T = 193 K, space group $P\bar{1}$ (No. 2), Z = 2, μ(Mo-Kα) = 0.100 mm^{-1}, 2179 reflections measured, 1641 unique (R_{int} = 0.0164) which were used in all least squares calculations, $R_1(F)$ = 0.0374 (for 1365 reflections with $F_o^2 \geq 2\sigma(F_o^2)$), $wR_2(F^2)$ = 0.1037 (for all unique reflections).

Crystal data for pseudoxazolone ***61b***: $C_{11}H_9NO_2$: M = 187.19, triclinic, a = 6.0008(8) Å, b = 7.2370(11) Å, c = 10.9282(16) Å, α = 95.403(2)°, β = 101.029(3)° , γ = 102.046(3)°, U = 451.10(11) Å^3, T = 193 K, space group $P\bar{1}$ (No. 2), Z = 2, μ(Mo-Kα) = 0.096 mm^{-1}, 2409 reflections measured, 1827 unique (R_{int} = 0.0422) which were used in all least squares calculations, $R1(F)$ = 0.0449 (for 1207 reflections with $F_o^2 \geq 2\sigma(F_o^2)$), $wR(F^2)$ = 0.1113 for all unique reflections).

Crystal data for pyrrolidin-2-one ***218***: $C_{10}H_{11}NO_3$: M = 193.20, monoclinic, a = 5.7041(12) Å, b = 6.3343(13) Å, c = 25.318(5) Å, β = 95.145(4)°, U = 911.1(3) Å^3, T = -80 °C, space group $P2_1/n$ (an alternate setting of $P2_1/c$, No. 14), Z = 4, μ(Mo-Kα) = 2 mm^{-1}, 5127 reflections measured, 1159 unique which were used in all least squares calculations, $R1(F)$ = 0.0532 (for 1159 reflections with $F_o^2 \geq 2\sigma(F_o^2)$), $wR(F^2)$ = 0.1250 for all unique reflections).

The structures were solved by direct methods *(SHELXS-86),*[141] and refined by full-matrix least-squares methods on F^2 *(SHELXL-93).*[142] Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Center.

Materials and methods for inhibition studies with HAV and HRV 3C proteinases.

Both HAV[65] and HRV 3C[143] proteinases were expressed and purified according to previously described procedures. The HAV 3C proteinase assays employed a C24S mutant in which the nonessential surface cysteine was replaced by serine and which displays catalytic parameters similar to the wild type enzyme.[27] Purity of the enzyme samples was greater than 90% as determined by SDS-PAGE analysis (data not

shown).[27,143] The HAV 3C proteinase concentration was determined spectrophotometrically ε_{280} = 1.2 mg/mL. Cleavage reactions (700 µl) were performed at 30 °C in a solution containing 100 mM KH_2PO_4/K_2HPO_4 at pH 7.5, 2 mM EDTA, 0.1 mg/mL bovine serum albumin (BSA), 10 µM fluorogenic substrate Dabcyl-GLRTQSFS-Edans (Bachem), 0.1 µM HAV 3C proteinase and 1% DMF.[27,66] The activity was monitored by a fluorometric assay similar to one described for HRV 3C proteinase.[67] Increase in fluorescence (λ_{ex} 336 nm, λ_{em} 472 nm) was continuously monitored using a Shimadzu RF5301 spectrofluorometer. In the case of HRV, the 3C proteinase from serotype 14 was used and the concentration was determined using the BSA assay. HRV 3C assays (1 mL) were performed at 30 °C in a solution containing 50 mM Hepes, 150 mM NaCl, 1 mM EDTA, pH 7.5, 1% DMF, 0.4 µM HRV 3C and 250 µM EALFQ-pNA (Bachem) as the substrate. The increase in absorbance at λ_{abs} 405 nm was measured using a GBC Cintra 40 UV spectrometer against a blank containing buffer and the appropriate inhibitor. In both cases, the proteinases were dialyzed against reaction buffer to remove DTT immediately prior to use. The enzymes were incubated with targets A-E for 15 min, targets F,G with no incubation, target H for 5 min, and the reaction initiated by addition of substrate. For proteinase inhibition studies, the initial 3 minutes of the reaction were used for calculation of initial rate. Inhibitor stock solutions were prepared at 10 mM in DMF except targets D and E where DMSO was used, and serial dilutions made in DMF or DMSO. At least five different inhibitor concentrations were examined along with a control sample containing no inhibitor under the conditions described above. The proteinase activity in the presence of the specified inhibitor was expressed as a percentage of that obtained from the respective control samples. For inhibitors displaying

dose-dependent inhibition of the proteinase activity, IC_{50} values were determined from plots of the relative proteinase activity versus the log of inhibitor concentration. IC_{50} values were not determined for compounds showing weak inhibition. The sensitivity of inhibitors **34**, **60a** and **60b** to dithiothreitol (DTT) was evaluated using reactions similar to those described above, but with the addition of up to 1.0 mM concentrations of DTT to the inhibitor-containing mixture followed by the addition of enzyme. The competitive inhibition constant (K_i) for compound **24** was determined from the Dixon plot.[77]

Rate of Hydrolysis of β-Lactones in Phosphate Buffer

Assuming pseudo-first order kinetics, the hydrolysis of β-lactone was followed by FT-IR with a Nicolet Magna 750 FT-IR instrument using a 0.1 mm IR-Trans 4 cell (Kodak, polycrystalline ZnS). A solution containing 100 mM KH_2PO_4/K_2HPO_4 pH 7.5, 2 mM EDTA and 20 mM of the appropriate β-lactone in 20% DMF was prepared, an aliquot was removed and placed in the IR cell at 22 °C and the disappearance of the β-lactone carbonyl stretch (1830 cm^{-1}) was monitored over a 1 h period (the $t_{1/2}$ data reported are within ±20% error).

Rate of Hydrolysis of Pseudoxazolones in Phosphate Buffer

Assuming pseudo-first order kinetics, the hydrolysis of the pseudoxazolone was followed using a Hewlett Packard 8452A diode array spectrometer. A solution containing 100 mM KH_2PO_4/K_2HPO_4 at pH 7.5, 2 mM EDTA and 100 μM of the appropriate inhibitor in DMF was prepared and placed in a 1 mL cell, and the disappearance of the

pseudoxazolone imine band (λ_{abs} ~350 nm) was monitored over a 1 h period (the $t_{1/2}$ data reported are within ±10% error).

Mass Spectrometry of HAV and HRV 3C-60b Inhibitor Complexes

The enzymes were dialyzed separately against a solution containing 2 mM EDTA and 100 mM KH_2PO_4/K_2HPO_4 at pH 7.5 to remove DTT using a Centriprep-10 (Amicon) centricon ultrafiltration unit. The dialyzed enzymes (~0.2 mM) were incubated with (10 equivalents) of **60b** and 1% DMF at 25 °C for 1 h with gentle shaking. The enzyme-inhibitor complexes were then dialyzed against deionized H_2O for 1 h to a volume of approximately 300 µL. In addition, a control parallel experiment was performed on the enzyme alone without inhibitor **60b**. Mass spectrometric analysis was performed on a Voyager Elite MALDI-TOF instrument (Applied Biosystems) and sinapinic acid was used as the matrix.

$^1H/^{13}C$ gHMQC NMR Spectroscopy of Model Compounds, 60b(α-^{13}C), 208-210, HAV 3C and HAV 3C-60b(α-^{13}C) Inhibitor Complex

Solutions of individual compounds **60b(α-^{13}C)** and **208-210** in 20% DMF-d_7 and 20 mM Na_3PO_4/D_2O at pD 7.5 were prepared to give a total volume of 700 µL each. Prior to use, DTT was removed from the enzyme preparation by dialysis with a Centriprep-10 (Amicon) centricon ultrafiltration unit with 20 mM Na_3PO_4/D_2O at pD 7.5. The resulting enzyme solution (0.5 mM) was incubated with the inhibitor **60b(α-^{13}C)** (5 mM) and 1% DMF-d_7 for 1 h with gentle shaking. The enzyme-inhibitor complex was dialyzed against Na_3PO_4/D_2O at pD 7.5 several times for 3 h, and concentrated to a

volume of approximately 700 µl. Model compounds **60b(α-^{13}C)** and **208-210**, HAV 3C alone and the HAV 3C-**60b(α-^{13}C)** enzyme inhibitor complex were analyzed by gHMQC NMR using a 600 Varian Inova instrument. The parameters for model compound **13b(α-^{13}C)**:- temperature: 27 °C, solvent: D_2O, number of transients: 1, number of increments: 512, number of data points: 3000, acquisition time: 0.250 sec, sweep width in F2: 6001 Hz, sweep width in F1: 29996 Hz. The same parameters as above were used for the HAV 3C-**60b(α-^{13}C)** enzyme inhibitor complex except the number of transients = 16 and ^{1}H, ^{13}C decoupling was applied.

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