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Articles

Synthesis and Evaluation of Keto-Glutamine Analogues as Inhibitors of Hepatitis A Virus 3C Proteinase

Yeeman K. Ramtohul,[†] Michael N. G. James,[‡,||] and John C. Vederas*[,†,§]

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2, and Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

john.vederas@ualberta.ca

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Hepatitis A virus (HAV) 3C enzyme is a picornaviral cysteine proteinase involved in the processing of the initially synthesized viral polyprotein and is therefore important for viral maturation and infectivity. Although it is a cysteine proteinase, this enzyme has a topology similar to those of the chymotrypsin-like serine proteinases. Since the enzyme recognizes peptide substrates with a glutamine residue at the P_1 site, a number of ketone-containing glutamine compounds analogous to nanomolar inhibitors of cathepsin K were synthesized and tested for inhibition against HAV 3C proteinase. In addition, a 3-azetidinone scaffold was incorporated into the glutamine fragment but gave only modest inhibition. However, introduction of a phthalhydrazido group α to the ketone moiety gave significantly better inhibitors with IC_{50} values ranging from 13 to 164 μM, presumably due to the effect of intramolecular hydrogen bonding to the ketone. In addition, the tetrapeptide phthalhydrazide **24** was found to be a competitive reversible inhibitor ($K_i = 9 \times 10^{-6}$ M) and also showed no loss of inhibitory potency in the presence of dithiothreitol.

Introduction

Cysteine proteinases are among the most attractive targets for chemotherapy because of their role in the pathogenesis of many diseases, including osteoporosis, arthritis, cancer, viral, and parasitic infections.[1] The hepatitis A virus (HAV) belongs to the picornavirus family, which contains more than 200 known members including other pathogens such as the human rhinovirus (HRV), foot and mouth disease virus, poliovirus (PV), and encephalomyocarditis (EMCV).[2] HAV is the only known member of the genus hepatovirus and causes an acute form of infectious hepatitis.[2] Occasional outbreaks of hepatitis A still occur in the developed world, and these are usually attributed to contaminated food and drinking water.[3] Picornaviruses possess a small positive single-stranded RNA genome whose translation in the host cells produces a single ~250 kDa polyprotein. In HAV, the 3C

* To whom correspondence should be addressed. Tel (780) 492 5475. FAX (780) 492 8231.
[†] Department of Chemistry, University of Alberta.
[‡] Department of Biochemistry, University of Alberta.
[§] Canada Research Chair in Bioorganic and Medicinal Chemistry.
[||] Canada Research Chair in Protein Structure and Function.

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proteinase is responsible for the co- and posttranslational processing of this viral polyprotein into structural and nonstructural components and is therefore essential for viral maturation and infectivity.[4,5] In addition, 3C proteinases have a unique active site geometry[4a] which makes these enzymes highly specific in their choice of substrate. The cleavage sites of the 3C proteinases, which are absent in noninfected mammalian cells, have a high degree of conserved residues[6,7] and therefore a specific inhibitor of the 3C enzyme could potentially inhibit a large number of serotypes.

Crystal structures of HAV 3C,[8] HRV 3C,[9] and PV 3C[10] proteinases have been reported, and structurally these enzymes are distinct from the papain family in that they have a two-domain β-barrel fold that is characteristic of the chymotrypsin-like serine proteinases.[4,6] An active site cysteine (Cys-172) of the HAV 3C proteinase acts as the nucleophile with assistance from a histidine residue (His-44), which behaves as a general acid−base catalyst, with the formation of the tetrahedral intermediate being promoted by an electrophilic oxyanion hole.[4] The enzyme typically binds four to five residues (P_5 to P_1, Figure 1) preceding the scissile peptide bond and two to three residues (P_1' to P_3') following the site of cleavage.[11] In addition, hydrogen bonding between the carbonyl oxygen side chain of the P_1 glutamine of the substrate (or peptidic inhibitor) and His-191 at the S_1 subsite is one of the key recognition events.[4,6] For an octapeptide substrate (Ac-ELRTQSFS-NH_2) mimicking the 2B/2C junction of the viral polyprotein precursor with glutamine at the P_1 position (Figure 1), the k_{cat} is about 1.8 s^{-1} with a K_m of 2.1 mM at pH 7.5.[6] In search of potential therapeutic leads, enzyme inhibition studies on HAV 3C proteinase have employed a variety of compounds, including peptide aldehydes,[12] peptide fluoromethyl ketones,[13] iodoacetamides,[14] azapeptides,[15] azodicarboxamides,[16] and β-lactones.[17]

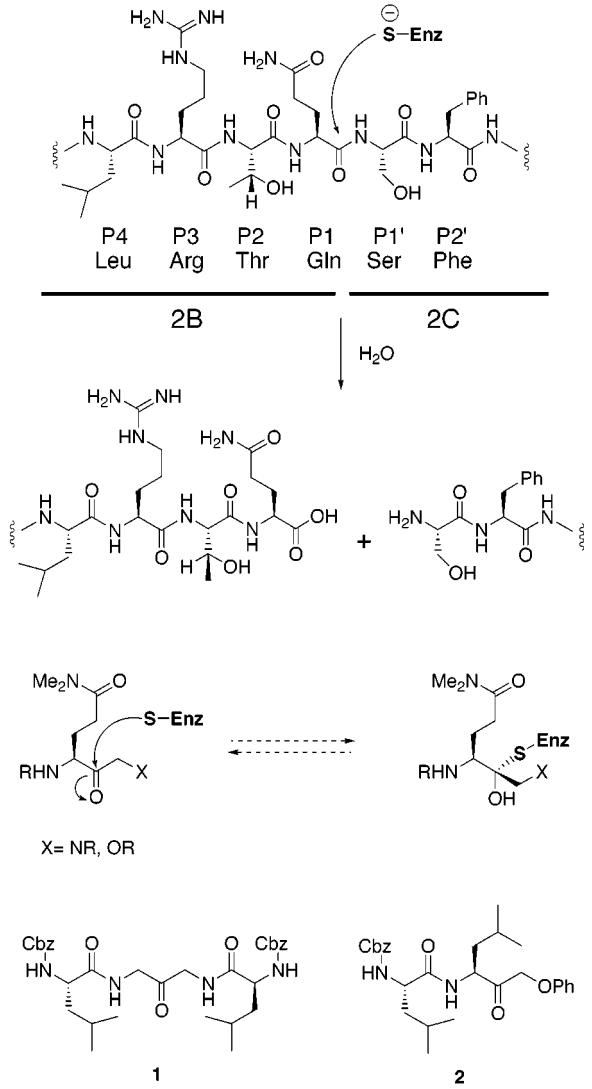

Figure 1. A preferred cleavage site of HAV 3C proteinase showing the sequence at the 2B−2C junction of the viral 250 kDa precursor protein with rationale for inhibition by ketoglutamine derivatives and structures of cathepsin K inhibitors **1** and **2**.

Recently, 1,3 diamino ketone **1**[18] and α-alkoxy ketone **2**[19] were reported as potent and selective inhibitors of cathepsin K, a cysteine proteinase of the papain superfamily involved in the process of bone resorption (Figure

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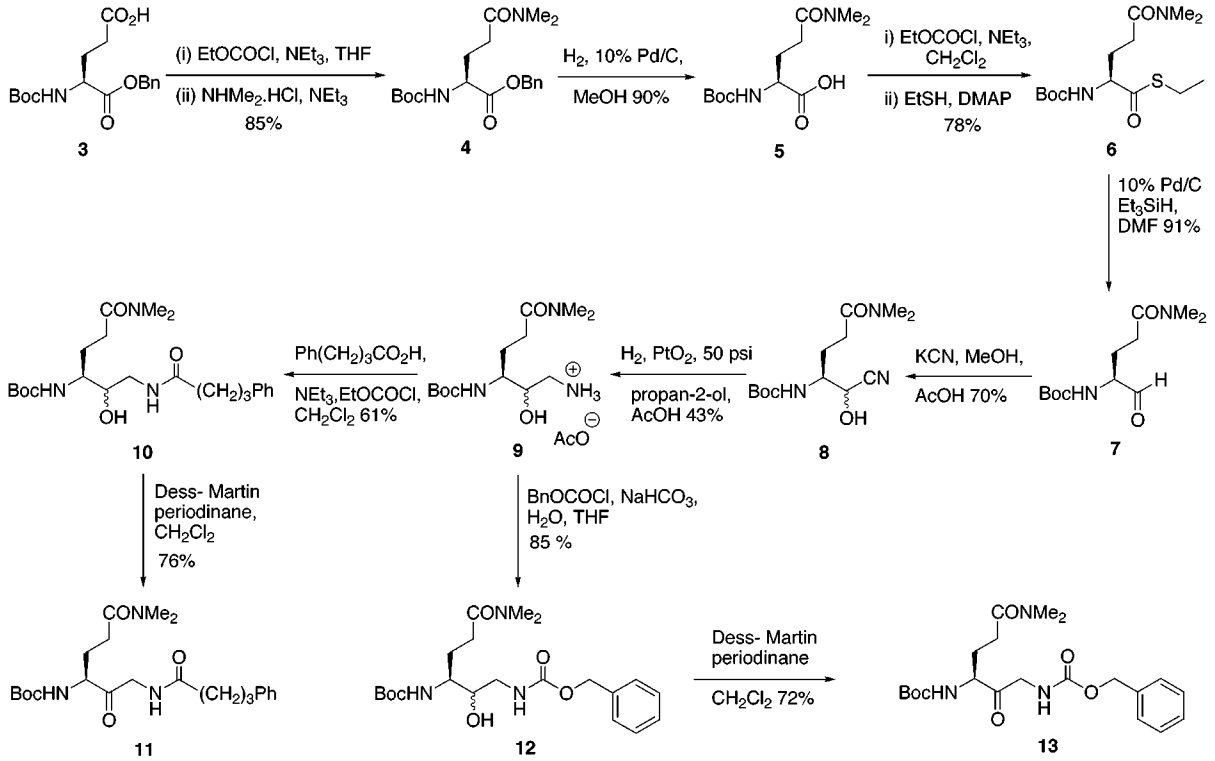
Scheme 1



1). Both ketone **1** and **2** are nanomolar inhibitors with $K_{i,app}$ of 22 nM and IC_{50} of 3.7 nM, respectively, and were found to form a hemi-thioketal bond between the ketone carbonyl and the active site cysteine.[18,19] In addition, these compounds contain a ketone moiety which has a reduced electrophilicity compared to their aldehyde counterparts and can therefore react more specifically with the proteinase as opposed to a variety of thiols present in the mamalian cells. We therefore chose to investigate a series of analogues containing this ketone functionality as potential inhibitors of HAV 3C proteinase. Although HAV 3C proteinase has a preference for a glutamine residue at the P_1 site, it has been shown that replacement of the glutamine with *N*,*N*-dimethylglutamine has no significant effect on substrate recognition or cleavage.[20] Hence, for the ease of synthesis and to hinder any interaction of the side-chain glutamine primary amide with the ketone moiety, we chose to synthesize 1,3-diamino ketone (e.g., **11**), α-heteroatom ketone (e.g., **17**), and cyclic ketone (e.g., **25**) analogues bearing the *N*,*N*-dimethylglutamine motif at the P_1 site and a side-chain phenyl moiety mimicking the P_2' phenylalanine. Although these compounds lack the functionality that mimics the P_1' amino acid, which is normally a serine residue, previous studies show that this residue does not contribute significantly to recognition of the substrate as compared to P_4 (Leu), P_1 (Gln), and P_2' (Phe).[6]

Results and Discussion

Compounds **11** and **13** were selected as initial targets based on their structural similarity to **1** and **2**, potent inhibitors of cathepsin K.[18,19] They were synthesized as outlined in Scheme 1. Coupling of the Boc-protected glutamic acid α-benzyl ester **3** with dimethylamine using ethyl chloroformate gives **4** in 85% yield.[13] Removal of the benzyl group by catalytic hydrogenation affords Boc-*N*,*N*-dimethylglutamine **5** which can be subsequently converted to the thioester **6** using a procedure similar to that described by Fukuyama et al.[21] Conversion of thioester **6** to the aldehyde **7** proceeds in good yield by reduction with triethylsilane in the presence of a catalytic amount of palladium on carbon.[21] Reaction of **7** with potassium cyanide generates cyanohydrin **8** in a 1:1 diastereomeric mixture.[22] Reduction of **8** over PtO_2 at 50 psi is sluggish and affords the amino alcohol **9** in 43% yield. Coupling of **9** to 4-phenylbutyric acid or benzyl chloroformate followed by Dess−Martin periodinane[23] oxidation proceeds readily to generate the desired compounds **11** and **13**, respectively. Although the α-center bearing the glutamine side chain could potentially be prone to epimerization, this was not observed with **11**, **13**, or other aminoketones described below.

Assay of **11** and **13** for inhibition against HAV 3C proteinase employed an overexpressed C24S mutant in which the nonessential surface cysteine is replaced with serine and which displays catalytic parameters indistinguishable from the wild-type proteinase.[24a] The enzyme activity is monitored using a fluorometric assay at an enzyme concentration of approximately 0.1 μM and

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Table 1

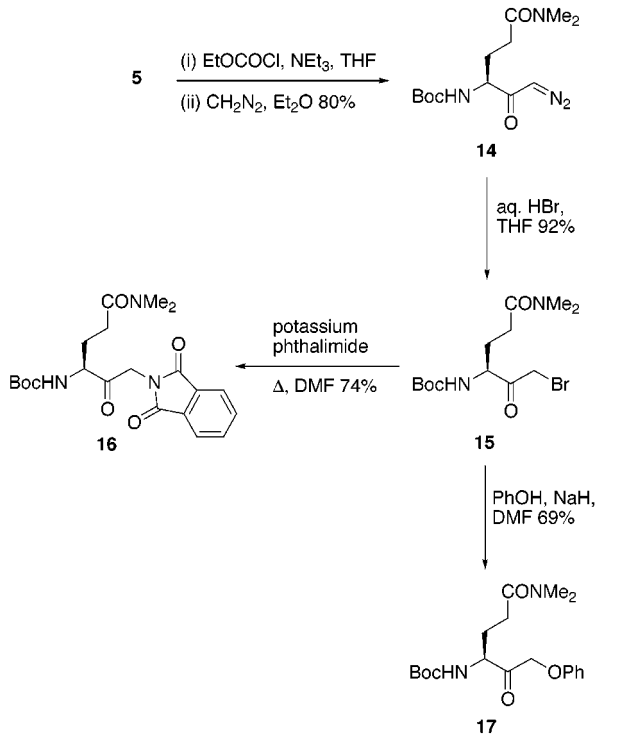
compounds	IC_{50} values,[a] μM	% inhibition[a,b]
11		no inhibition
13		20 ± 1
16	>500	
17		20 ± 2
18	89	
20	146	
21	164	
23		15 ± 7
24	13	
25	358	
26		20 ± 5
28	258	

[a] 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 at pH 7.5, 15 min preincubation of the enzyme with inhibitor. The IC_{50} values reported are within ±10% error. [b]Inhibition at [I] = 100 μM.

Dabcyl-GLRTQSFS-Edans as the substrate. The enzyme is incubated with the appropriate inhibitor for 15 min, and reaction is initiated by the addition of substrate. Disappointingly, compound **11** shows no inhibition, and **13** gives poor inhibition of 20 ± 1% at a concentration of 100 μM (Table 1). This contrasts the nanomolar inhibition achieved by the structurally related 1,3-diamino ketone **1** against mammalian cysteine proteinase, cathepsin K.[18,19] Although it might initially seem that the Boc group in **11** and **13** is a poor mimic of the peptide chain in substrates and interferes with enzyme recognition, the work described below shows that this is not the primary cause of weak binding.

The marked contrast in potency of **11** and **13** with HAV 3C proteinase, as compared to **1** and **2** with cathepsin K,[18,19] indicates the importance of factors beyond simple accommodation of the side chain(s) in the active site and electrophilicity of the keto group.[25] Although certain more electrophilic N-acylated α-amino aldehydes ("peptide aldehydes") such as Ac-Leu-Ala-Ala-(*N*,*N*-dimethyl)-glutaminal are very potent slow-binding inhibitors of HAV 3C proteinase (K_i^* of 42 nM),[12] their potential use as therapeutic agents is limited by poor bioavailability due to the reactive nature of the aldehyde moiety. The corresponding *N*-acetyl-(*N*,*N*-dimethyl)glutaminal, which is missing the P_4-P_2 residues, is a weak competitive inhibitor with K_i = 2.5 mM.[12] Hence, in addition to replacement of peptidic residues, the goals of the current work included adjustment of the electrophilicity of the reactive carbonyl to enhance metabolic stability and maintain selective inhibition of the target enzyme. To further examine the effect of the α-heteroatom substituent and its electron-withdrawing capability, compounds **16** and **17** were synthesized and tested (Scheme 2). Following the literature procedure of Morris et al.,[13] acid **5** can be converted to the α-diazoketone **14** by formation of the mixed anhydride with ethyl chloroformate followed by trapping with diazomethane. Treatment of **14** with aqueous HBr provides the bromomethyl ketone **15** which is subsequently converted to the phthalimidomethyl ketone **16** or phenoxymethyl ketone **17** by nucleophilic displacement with potassium phthalimide or sodium phenoxide, respectively. However, testing with the HAV 3C enzyme indicates only weak inhibition, with IC_{50} > 500 μM for **16** and 20 ± 2% inhibition at a concentration of 100 μM for **17** (Table 1).

Scheme 2



Another approach to enhancing the electrophilicity of the target carbonyl group in an inhibitor involves intramolecular hydrogen bonding to the doubly bonded oxygen, such as in compound **18**, which has the phthalhydrazido N−H proton available for such an interaction in a six-membered chelate. Such an idea has been successfully applied to the design of α-hydroxy ketomethylene dipeptide inhibitors of angiotensin converting enzyme.[26] This should also increase the conformational rigidity of the molecule, thereby reducing the entropic cost for tight binding to the target protein.[27] Nucleophilic displacement of sodium phthalhydrazide on bromomethyl ketone **15** affords the desired compound **18** as well as the dimer **19** in a 2:1 ratio (Scheme 3). Testing of **18** against HAV 3C proteinase gives reversible inhibition with an IC_{50} of 89 μM. This contrasts strongly with the poor inhibition shown by **16** (IC_{50} > 500 μM), which has related functionality but lacks the analogous hydrogen bond.[28] Following this result, it seemed that analogues of **18** with a nitro group on the phenyl ring could increase the acidity of the N−H proton and thereby enhance the electrophilicity of the ketone moiety. Reaction of bromomethyl ketone **15** with sodium 3-nitrophthalhydrazide affords **20** and **21** (Scheme 3).[29] However, incubation of

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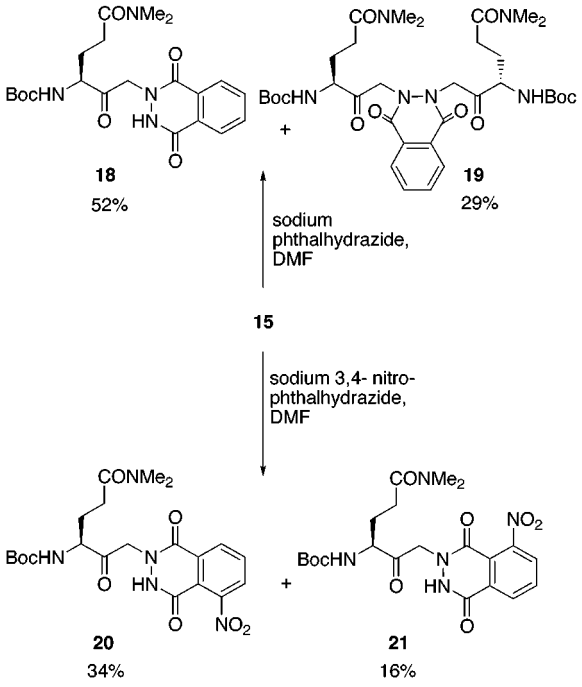
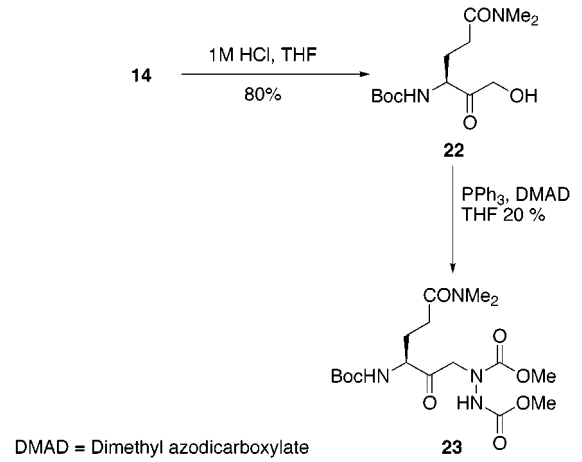
(28) Examination of the ketone carbonyl IR absorption band of compounds **16** (1745 cm^{-1}) and **18** (1735 cm^{-1}) indicates a lower absorption frequency for **18** possibly due to the effect of intramolecular hydrogen bonding to the ketone moiety, see: (a) Silverstein, R. M.; Webster, F. X. in *Spectrometric Identification of Organic Compounds*, 6th ed.; John Wiley & Sons: New York, 1998; p 96. (b) Pimentel, G. C.; McClellan, A. L. *The Hydrogen Bond*; W. H. Freeman and Co.: San Francisco, 1960; Chapter 5.

Scheme 3

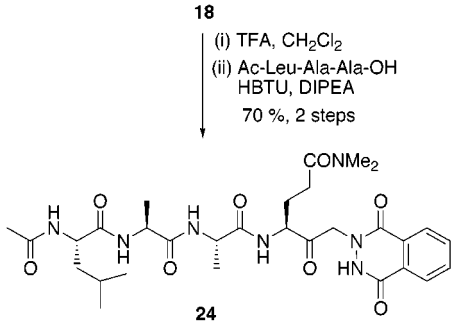


Scheme 4



20 and **21** with the HAV 3C proteinase does not show improved inhibition and gives IC_{50} values of 146 and 164 μM, respectively (Table 1). Possibly the nitro group has unfavorable steric or electronic interactions in the region of the enzyme active site which binds the P_2' phenylalanine residue in substrate analogues.[8b] To examine the motif further, compound **23** was prepared as it has the hydrazido N−H for intramolecular hydrogen bond formation but is missing the ring system of **18**, **20**, and **21** (Scheme 4). Conversion of **14** to the hydroxymethyl ketone **22** proceeds by treatment with 1 N HCl.[30] This is then coupled to the dimethyl hydrazodicarboxylate group via a Mitsunobu-type[31] reaction to give **23** in low yield (20%). Enzymatic testing of **23** shows weak reversible

Scheme 5



HBTU = 2-(1 H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
DIPEA = *N,N*- Diisopropylethylamine

inhibition of HAV 3C (15 ± 7% at 100 μM) (Table 1). Thus, removal of the aromatic ring coupled with increased flexibility and change of the preferred conformation of the hydrazido functionality[32] drastically reduces binding to the enzyme.

Although a major objective of this work was removal of peptidic linkages from potential inhibitors of the 3C cysteine proteinase, it is desirable to estimate the relative contribution to binding of such residues by attachment of the successful "warhead" motifs. In the case of (*N*,*N*-dimethyl)glutaminal, an analogue with an appropriate tripeptide (*N*-Ac-Leu-Ala-Ala) attached to the N-terminus is about 9000 times more effective than the simple *N*-acetyl derivative.[12] Hence **18** was coupled to the tripeptide Ac-Leu-Ala-Ala-OH to test whether this would drastically influence molecular recognition of the inhibitor and whether the Boc group is detrimental to binding. The tripeptide fragment Ac-Leu-Ala-Ala is a reasonable substitute for the Leu-Arg-Thr sequence in substrate analogues as it has been shown that neither arginine nor threonine side chains contribute significantly to binding.[20] This peptide sequence also simplifies the synthesis by eliminating side chain deprotection steps. Cleavage of the Boc protecting group of **18** with trifluoroacetic acid followed by coupling with Ac-Leu-Ala-Ala-OH using HBTU proceeds smoothly to afford the desired tetrapeptide **24** in 70% yield over two steps (Scheme 5). Compound **24** is a competitive reversible inhibitor of the HAV 3C enzyme with a K_i of 9 μM.[33] Furthermore, the inhibitory activity of **24** is not affected in the presence of a 100-fold excess of dithiothreitol (DTT), indicating that this type of phthalhydrazido system could be specific inhibitor of the HAV 3C proteinase which would not react inadvertently with ubiquitous thiols (e.g., glutathione). Although the peptide residues in **24** do enhance the inhibition by about a factor of 7 relative to **18**, the effect is not nearly so dramatic as with the *N*-acylated α-amino aldehyde, (*N*,*N*-dimethyl)glutaminal. Thus, **18** represents an interesting lead structure that could be further modified to generate drug candidates for picornaviral cysteine proteinases.

Another approach to enhancing electrophilicity and reactivity of α-amino ketones is the introduction of a rigid 3-azetidinone scaffold at the P_1 site. Thus reaction of α-diazoketone **14** with a catalytic amount of rhodium(II)

(29) Isomeric compounds **20** and **21** were characterized by using HMQC and HMBC NMR techniques, e.g., the key correlation in the HMBC spectrum of **20** is between the phthalhydrazido N−H proton (δ_H 11.04 ppm) and the aromatic C−NO_2 (δ_C 156 ppm) quaternary carbon.

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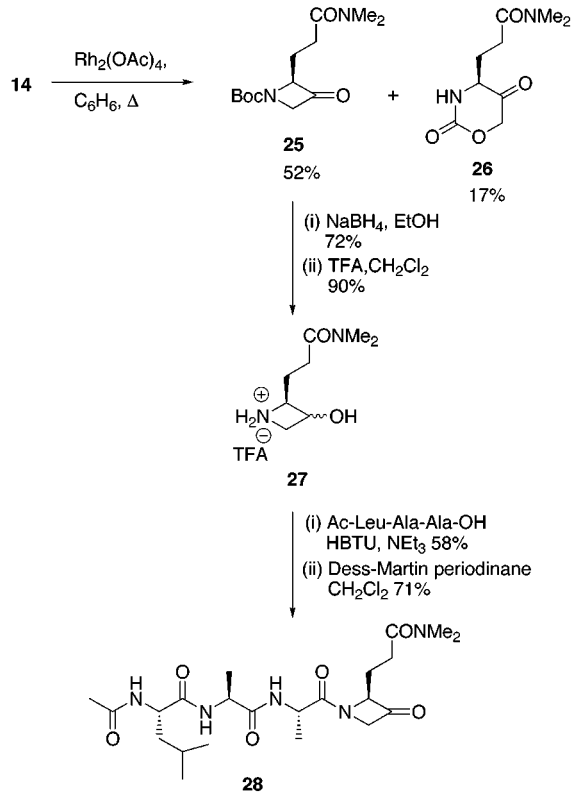
Scheme 6



acetate dimer[34] generates a carbenoid which inserts intramolecularly into the N−H bond to afford **25** along with **26** as a side product in a ratio of 3:1 (Scheme 6). Urethane **26** presumably forms by addition of the carbonyl oxygen atom of the Boc protecting group to the carbene followed by loss of the *tert*-butyl group. Testing of **25** with the HAV 3C enzyme gives moderate inhibition with an IC_{50} of 358 μM, and the urethane **26** displays 20 ± 5% inhibition at a concentration of 100 μM (Table 1). Thus the azetidinone motif in **25** appears inherently less effective compared to the hydrogen-bonding phthalhydrazide motif in **18**. To examine the influence of additional peptidic residues with this four-membered ring system, the tripeptide Ac-Leu-Ala-Ala was attached to **25**. To prevent unwanted side reactions, the ketone functionality of **25** is reduced prior to coupling. Thus reaction of **25** with sodium borohydride followed by treatment with trifluoroacetic acid affords the amino alcohol **27**. This is coupled to Ac-Leu-Ala-Ala-OH as described previously and subsequently oxidized with Dess−Martin periodinane[23] to afford the target **28** in 71% yield. Incubation of **28** with the HAV 3C proteinase displays only a slight enhancement of reversible inhibition (IC_{50} = 258 μM) relative to **25** despite the presence of the P_4−P_1 peptide backbone. Although the reversible binding of the azetidinones to the target enzyme is significantly greater than that of an optimized octapeptide substrate (K_m of 2.1 mM), this cyclic ketone motif appears to be less promising for HAV 3C inhibitor development than the phthalhydrazido system. Nevertheless, it may prove promising for other types of cysteine proteinases of therapeutic interest.

(34) (a) Sengupta, S.; Das, D. *Synth. Commun.* **1998**, *3*, 403−408. (b) For a review on organic synthesis with α-diazocarbonyl compounds see: Ye, T.; McKervey, M. A. *Chem. Rev.* **1994**, *94*, 1091−1160.

Summary

In conclusion, the present work provides access to several electrophilic keto-glutamine analogues which may allow development of potent inhibitors of 3C cysteine proteinases as therapeutic agents for picornaviral infections. Interestingly, analogues of inhibitors of the mammalian cysteine proteinase, cathepsin K, display relatively weak inhibition with HAV 3C proteinase. However, phthalhydrazido analogues **18** and **24** show good inhibition, possibly due to the effect of intramolecular hydrogen bonding to the ketone moiety and restricted conformational mobility. The absence of peptidic bonds in **18** makes it an especially promising structure for further modification. It is also likely that such keto-glutamine derivatives may be potent inhibitors of other picornaviral cysteine proteinases, such as HRV 3C, as most of these enzymes require a glutamine residue at the P_1 site in the substrate. Additional studies with modified analogues and other 3C proteinases will be reported in the future.

Experimental Section

General Methods and Enzyme Assays. Most general procedures and instrumentation have been previously described.[35] Peptide fragment Ac-Leu-Ala-Ala-OH was prepared using standard solution-phase chemistry with 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) as the coupling reagent.[36] HPLC purification was performed on a Rainin HPLC system (Waters C18 Bondapak, 25 × 100 mm, 15 mL/min flow rate, linear gradient of acetonitrile in 0.1% TFA/H_2O). Recombinant C24S HAV 3C proteinase was expressed in *Escherichia coli* and purified as reported previously.[24a] Purity of the enzyme was greater than 90% as determined by SDS−PAGE analysis.[24a] Proteinase concentrations were determined spectrophotometrically ϵ_{280} = 1.2 mg/mL. Enzyme was dialyzed against reaction buffer to remove dithiothreitol (DTT) immediately prior to use and its activity monitored by a fluorometric assay similar to one described for HRV 3C proteinase.[24b] Assays were done 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. Enzyme was incubated with the appropriate inhibitor for 15 min and reaction initiated by the addition of substrate. Increase in fluorescence (λ_{ex} 336 nm, λ_{em} 472 nm) was continuously monitored using a Shimadzu RF5301 spectrofluorometer. For proteinase inhibition studies, the initial 3 min of the reaction were used for calculation purposes. Inhibitor stock solutions were prepared at 10 mM in DMF and serial dilutions made in DMF. At least five different inhibitor concentrations were examined along with a control sample containing no inhibitor under the conditions described above. The HAV 3C 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 competitive inhibition constant (K_i) for compound **24** was determined from the Dixon plot.[33] The sensitivity of inhibitor **24** to DTT was evaluated using reactions similar to those described above, but with the addition of up to 5 mM molar concentrations of DTT to the inhibitor-containing mixture followed by the addition of enzyme.

(35) (a) Harris, J. M.; Bolessa, E. A.; Mendonca, A. J.; Feng, S.-C.; Vederas, J. C. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1945−1950. (b) Merkler, D. J.; Glufke, U.; Ritenour-Rodgers, K. J.; Baumgart, L. E.; DeBlassio, J. L.; Merkler, K. A.; Vederas, J. C. *J. Am. Chem. Soc.* **1999**, *121*, 4904−4905.

(36) Bodansky, M. *Peptide Chemistry*; Springer, New York, 1988, pp 55−146, and references therein.

(4*S*)-*N,N*-Dimethyl-4-(*tert*-butyloxycarbonylamino)-pentanoic Acid Benzyl Ester (4). This compound was prepared from a modified procedure of Matsoukas et al.[37] To a stirred solution of Boc-Glu-OBn (5.00 g, 14.82 mmol) in dry dichloromethane (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 more triethylamine (2.60 mL, 18.51 mmol) were added. The reaction mixture was stirred at 0 °C for a further 2 h and then allowed to stir overnight at room temperature. The solvent was removed in vacuo and the residue diluted with water (60 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with 1 N HCl (20 mL), dried over $MgSO_4$, and concentrated under reduced pressure. Recrystallization of the crude product from dichloromethane/hexane gave white needles (4.61 g, 85%); mp 97−98 °C, lit.[37] mp 99−100 °C; $[\alpha]^{26}_D$ −25.8° (*c* 1.0, EtOH), lit.[37] $[\alpha]^{20}_D$ −27.5° (*c* 1.0, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H), 1.92−2.02 (m, 1H), 2.11−2.22 (m, 1H), 2.25−2.40 (m, 2H), 2.85 (s, 3H), 2.88 (s, 3H), 4.22−4.38 (m, 1H), 5.12 (d, J = 13.2 Hz, 1H), 5.18 (d, J = 12.9 Hz, 1H), 5.42 (br d, J = 5.4 Hz, 1H), 7.25−7.29 (m, 5H); 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 (5). Glutamine **4** (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 dichloromethane/hexane to give white crystalline solid (2.22 g, 90%); mp 121−122 °C, lit.[38] mp 124−125 °C; $[\alpha]^{26}_D$ +3.5° (*c* 1.0, EtOH); lit.[38] $[\alpha]^{20}_D$ +2.2° (*c* 1.0, EtOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H), 1.95−2.04 (m, 1H), 2.21−2.29 (m, 1H), 2.42−2.82 (m, 1H), 2.70−2.84 (m, 1H), 2.95 (s, 3H), 3.05 (s, 3H), 4.15 (q, J = 5.6 Hz, 1H), 5.65 (d, J = 5.6 Hz, 1H); 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-(ethanesulfanylcarbonyl)butyramide (6). This was prepared by an adaptation of the Malcolm et al. procedure.[12] A solution of acid **5** (5.00 g, 18.25 mmol) in dry 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 °C. The reaction was quenched by the addition of glacial acetic acid (5 mL), and the reaction mixture was concentrated under reduced pressure. The residue was dissolved in dichloromethane (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 dichloromethane/hexane furnished the desired product as an off-white crystalline solid (4.49 g, 78%); mp 122−123 °C; $[\alpha]^{26}_D$ −14.6° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3214, 2979, 1710, 1674, 1618, 1539 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (t, J = 7.5 Hz, 3H), 1.42 (s, 9H), 1.95−2.00 (m, 2H), 2.30−2.50 (m, 2H), 2.84 (q, J = 7.5 Hz, 2H), 2.95 (s, 3H), 3.00 (s, 3H), 4.22−4.37 (m, 1H), 5.70 (d, J = 7.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 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-oxopentanamide (7). To a solution of thioester **6** (4.49 g, 14.15 mmol) in dry DMF (100 mL) at 0 °C was added triethylsilane (13.53 mL, 84.90 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 more DMF, water, 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%); $[\alpha]^{26}_D$ +23.2° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3295, 2878, 1733, 1707, 1634, 1505 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H), 1.92−2.00 (m, 1H), 2.12−2.25 (m, 1H), 2.30−2.51 (m, 2H), 2.92 (s, 3H), 2.98 (s, 3H), 4.10−4.19 (m, 1H), 5.60 (br s, 1H), 9.48 (s, 1H); ^{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 (8). Aldehyde **7** (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 room temperature 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 dichloromethane/hexane (1.03 g, 70%); mp 114−115 °C; IR ($CHCl_3$ cast) 3307, 2350, 1709, 1627 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) (1:1 mixture of diasteromers, data for one compound) δ 1.42 (s, 9H), 1.94−2.10 (m, 2H), 2.36−2.52 (m, 2H), 2.92 (s, 3H), 2.98 (s, 3H), 3.79−3.95 (m, 1H), 4.54 (d, J = 9.0 Hz, 1H), 5.43 (d, J = 7.4 Hz, 1H), 5.80−6.00 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 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 (9). To a solution of cyanohydrin **8** (55 mg, 0.19 mmol) in propan-2-ol (10 mL) and AcOH (22 μL, 0.38 mmol) was added PtO_2 (10 mg, 0.05 mmol). The mixture was agitated under a hydrogen atmosphere at 50 psi for 24 h after which it was filtered through Celite and washed through with more 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) provided the title compound as a light brown 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, data for one compound) δ 1.42 (s, 9H), 1.74−2.08 (m, 5H), 2.38−2.50 (m, 2H), 2.92 (s, 3H), 2.98 (s, 3H), 3.00−3.15 (m, 0.5H), 3.21−3.32 (m, 0.5H), 3.42−3.68 (m, 1H), 3.92−4.05 (m, 0.5H), 4.08−4.19 (m, 0.5H), 5.72 (d, J = 7.5 Hz, 1H), 6.05 (d, J = 7.5 Hz, 1H), 7.00−7.40 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 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 (10). A solution of 4-phenylbutyric acid (56.8 mg, 0.35 mmol) in dichloromethane (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 **9** (110 mg, 0.32 mmol) and triethylamine (52 μL, 0.38 mmol). The reaction mixture was allowed to warm to room temperature over 3 h and quenched with saturated aqueous $NaHCO_3$ (10 mL) and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over $MgSO_4$, and evaporation of the solvent in vacuo followed by purification of the crude product by column chromatography (SiO_2, 1% MeOH/EtOAc) afforded the desired 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), 1.65−2.10 (m, 4H), 2.15−2.50 (m, 4H), 2.65 (m, 2H), 2.90 (s, 3H),

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(38) Stahl, G. L.; Smith, C. W.; Walter, R.; Tsgenidis, T.; Stavropoulos, G. *J. Med. Chem.* **1980**, *2*, 213-217.

2.96 (s, 3H), 3.10−3.30 (m, 1H), 4.40−4.65 (m, 3H), 4.35 (br s, 0.3H), 4.55 (br s, 0.7H), 5.15 (d, J = 9.1 Hz, 0.7H), 5.50 (d, J = 7.2 Hz, 0.3H), 6.58−6.02 (m, 0.7H), 6.84−6.87 (m, 0.3H), 7.10−7.35 (m, 5H); ^{13}C NMR (75 MHz, CD_2Cl_2) 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*)-*N*,*N*-Dimethyl-4-(*tert*-butyloxycarbonylamino)-5-oxo-6-(4-phenylbutyrylamino)hexanamide (11). To a solution of alcohol **10** (80 mg, 18.5 mmol) in dichloromethane (5 mL) at room temperature was added Dess−Martin periodinane (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 water (10 mL) and extracted with EtOAc (3 × 5 mL), and the organic layers were 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%); $[\alpha]^{26}_D$ +2.1° (*c* 1.4, $CHCl_3$); IR ($CHCl_3$ cast) 3312, 2927, 1701, 1636, 1497 cm^{-1}; ^{1}H NMR (300 MHz, CD_2Cl_2) δ 1.40 (s, 9H), 1.80−2.00 (m, 3H), 2.00−2.18 (m, 1H), 2.22 (t, J = 8.2 Hz, 2H), 2.28−2.50 (m, 2H), 2.85 (t, J = 7.3 Hz, 2H), 2.90 (s, 3H), 2.98 (s, 3H), 4.18−4.32 (m, 3H), 5.94−5.98 (m, 1H), 6.38−6.44 (m, 1H), 7.10−7.35 (m, 5H); ^{13}C NMR (75 MHz, CD_2Cl_2) 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*,5*RS*)-*N*,*N*-Dimethyl-4-(*tert*-butyloxycarbonylamino)-6-(benzyloxycarbonylamino)-5-hydroxyhexanamide (12). A solution of amino alcohol **9** (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 room temperature. The reaction mixture was diluted with water (5 mL) and extracted with EtOAc (3 × 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), 1.80−2.10 (m, 2H), 2.20−2.50 (m, 2H), 2.92 (s, 3H), 2.98 (s, 3H), 3.00−3.15 (m, 1H), 3.35−3.40 (m, 1H), 3.55−3.64 (m, 2H), 5.05 (d, J = 8.1 Hz, 1H), 5.12 (d, J = 7.3 Hz, 1H), 5.28−5.30 (m, 1H), 5.60−5.64 (m, 1H), 5.92 (br s, 1H), 7.25−7.40 (m, 5H); ^{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)-6-(benzyloxycarbonylamino)-5-oxohexanamide (13). This was prepared by employing the same procedure as that described for the preparation of compound **11**. A solution of alcohol **12** (68 mg, 0.16 mmol) in dichloromethane (4 mL) was treated with Dess−Martin periodinane (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%); $[\alpha]^{26}_D$ −2.6° (*c* 1.3, $CHCl_3$); IR ($CHCl_3$ cast) 3318, 1712, 1635, 1507 cm^{-1}; ^{1}H NMR (300 MHz, CD_2Cl_2) δ 1.41 (s, 9H), 1.82−1.98 (m, 1H), 2.01−2.20 (m, 1H), 2.20−2.50 (m, 2H), 2.92 (s, 3H), 2.98 (s, 3H), 4.15−4.40 (m, 3H), 5.11 (s, 2H), 5.54 (br s, 1H), 5.90 (br s, 1H), 7.20−7.40 (m, 5H); ^{13}C NMR (75 MHz, CD_2Cl_2) 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)-5-oxo-6-diazohexanamide (14). This known compound was prepared using the same literature procedure as that described by Morris et al.[13] To a cooled 0 °C solution of acid **5** (5.0 g, 18.2 mmol) in THF (120 mL) 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 dichloromethane/hexane to give an off-white solid (4.35 g, 80%); mp 112−113 °C, lit.[13] mp 112−113 °C; $[\alpha]^{26}_D$ −1.3° (*c* 1.1, $CHCl_3$), lit.[13] $[\alpha]^{26}_D$ −1.7° (*c* 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H), 1.75−1.90 (m, 1H), 2.02−2.20 (m, 1H), 2.38 (dt, J = 16.5, 6.0 Hz, 1H), 2.48 (dt, J = 16.5, 6.0 Hz, 1H), 2.90 (s, 3H), 2.98 (s, 3H), 4.11−4.19 (m, 1H), 5.55 (br s, 1H), 5.40 (d, J = 5.8 Hz,1H); 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-oxohexanamide (15). This known compound was prepared by a modification of the Morris[13] procedure. A solution of the diazo-ketone **14** (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 then stirred at 0 °C until the evolution of gas ceased and quenched with saturated aqueous $NaHCO_3$ (20 mL) and the solvent removed in vacuo. The residue was diluted with H_2O (50 mL) and extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over $MgSO_4$ and then concentrated under reduced pressure. Purification of the crude product by column chromatography (SiO_2, 1:1 $CHCl_3$/EtOAc) afforded the desired product which was recrystallized from dichloromethane/hexane to give a light yellow solid (743 mg, 81%); mp 59−60 °C, lit.[13] mp 61 °C; $[\alpha]^{26}_D$ +21.4° (*c* 1.3, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H), 1.90−2.04 (m, 1H), 2.10−2.25 (m, 1H), 2.36 (dt, J = 18.1, 5.7 Hz, 1H), 2.49 (dt, J = 10.4, 5.9 Hz, 1H), 2.92 (s, 3H), 2.98 (s, 3H), 4.12 (d, J = 13.5 Hz, 1H), 4.19 (d, J = 13.5 Hz, 1H), 4.45−4.57 (m, 1H), 5.62−5.74 (m, 1H); HRMS (ES) calcd for $C_{13}H_{24}N_2O_4{}^{79}Br$ (MH^+) 351.0914, found 351.0914.

(4*S*)-*N*,*N*-Dimethyl-4-(*tert*-butyloxycarbonylamino)-5-oxo-6-(*N*-phthalimido)hexanamide (16). To a solution of bromoketone **15** (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 × 10 mL). The combined organic layers were dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. Purification of the crude product by column chromatography (SiO_2, 5% MeOH/EtOAc) furnished the desired product which was recrystallized from dichloromethane/diethyl ether to give a white powder (351 mg, 74%); mp 127−128 °C; $[\alpha]^{26}_D$ −5.0° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$ cast) 3289, 2930, 1745, 1720, 1623, 1524 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (s, 9H), 1.92−2.05 (m, 1H), 2.21−2.30 (m, 1H), 2.36 (dt, J = 16.5, 7.0 Hz, 1H), 2.51 (dt, J = 15.7, 7.0 Hz), 2.94 (s, 3H), 2.98 (s, 3H), 4.39−4.48 (m, 1H), 4.65 (d, J = 18.4 Hz, 1H), 4.77 (d, J = 18.1 Hz, 1H), 5.72−5.82 (m, 1H), 7.64−7.72 (m, 2H), 7.79−7.85 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.6, 28.3, 28.8, 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-phenoxyhexanamide (17). A solution of phenol (147 mg, 0.16 mmol) in DMF (5 mL) was treated with sodium hydride (3.9 mg, 0.16 mmol) at room temperature. After 15 min, the phenoxide solution was cooled to 0 °C, and the bromoketone **15** (50 mg, 0.14 mmol) was added in one portion. The reaction mixture was stirred for a further 1 h at 0 °C and then allowed to warm to room temperature over 3 h. The reaction mixture was quenched with saturated aqueous 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 × 10 mL). The combined organic layers were dried over $MgSO_4$, and evaporation of the solvent followed by purification by column chromatography (SiO_2, 30% hexane/EtOAc) afforded the desired product as a colorless oil (361 mg, 69%); $[\alpha]^{26}_D$ +24.2° (*c* 0.6, $CHCl_3$); IR ($CHCl_3$ cast) 3304, 1745, 1703, 1634, 1599 cm^{-1}; ^{1}H NMR (300 MHz, CD_2Cl_2) δ 1.42 (s, 9H), 1.98 (m, 1H), 2.12−2.25 (m, 1H), 2.40 (dt, J = 6.9, 5.9

Hz, 1H), 2.49 (dt, J = 16.7, 6.1 Hz, 1H), 2.90 (s, 3H), 2.98 (s, 3H), 4.38−4.44 (1H, m), 4.85 (d, J = 17.2 Hz, 1H), 4.92 (d, J = 17.1 Hz, 1H), 5.95 (br d, J = 5.3 Hz, 1H), 6.90 (d, J = 6.6 Hz, 1H), 7.00 (t, J = 7.3 Hz, 2H), 7.30 (t, J = 7.5 Hz, 2H); ^{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, 158.1, 172.3, 205.7; HRMS (ES) cacld for $C_{19}H_{29}N_2O_5$ (MH^+) 365.2076, found 365.2075.

(4*S*)-*N*,*N*-Dimethyl-4-(*tert*-butyloxycarbonylamino)-5-oxo-6-(2,3-dihydrophthalazine-1,4-dione)hexanamide (18) and 2,3-Bis[(4*S*)-*N*,*N*-dimethyl-4-(*tert*-butyloxycarbonylamino)-5-oxo-6-methylhexanamide]-2,3-dihydrophthalazine-1,4-dione (19). To a suspension of sodium phthalhydrazide (58 mg, 0.31 mmol) in DMF was added the bromoketone **15** (100 mg, 0.28 mmol) in small portions over 1 h. After stirring for 5 h at room temperature, the solvent was removed in vacuo and the residue diluted with H_2O (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over $MgSO_4$, and evaporation of the solvent followed by purification by column chromatography (SiO_2, with a gradient elution of 20% EtOAc/hexane to 100% EtOAc) yielded **18** as a white powder (42 mg, 52%) after recrystallization from dichloromethane/diethyl ether and dimer **19** as an oil (59 mg, 29%).

Data for 18: mp 153−154 °C; $[\alpha]^{26}_D$ −8.2° (*c* 1.0, MeOH); IR (microscope) 3273, 1735, 1702, 1650, 1618 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H), 2.00−2.40 (m, 2H), 2.42−2.58 (m, 2H), 2.92 (s, 3H), 2.98 (s, 3H), 4.42−4.57 (m, 1H), 5.08 (d, J = 16.7 Hz, 1H), 5.18 (d, J = 16.7 Hz, 1H), 5.80 (d, J = 5.8 Hz, 1H), 7.70−7.85 (m, 2H), 8.05 (d, J = 7.3 Hz, 1H), 8.40 (d, J = 6.7 Hz, 1H), 10.70 (br s, 1H); ^{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 dimer 19: $[\alpha]^{26}_D$ −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), 1.82−2.02 (m, 2H), 2.20−2.38 (m, 2H), 2.40−2.55 (m, 4H), 2.96 (s, 6H), 3.00 (s, 6H), 4.20−4.41 (m, 2H), 4.92−5.21(m, 4H), 6.28 (br d, J = 2.2 Hz, 1H), 6.47 (br d, J = 1.9 Hz, 1H), 7.82 (dt, J = 14.2, 6.6 Hz, 2H), 8.06 (d, J = 7.2 Hz, 1H), 8.32 (d, J = 7.2 Hz, 1H); ^{13}C NMR (75 MHz, CD_2Cl_2) 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-(5-nitro-2,3-dihydro-phthalazine-1,4-dione)hexanamide (20) and (4*S*)-*N*,*N*-Dimethyl-4-(*tert*-butyloxycarbonylamino)-5-oxo-6-(8-nitro-2,3-dihydrophthalazine-1,4-dione)hexanamide (21). A similar procedure was employed as that described for the preparation of **18**. Sodium 3-nitrophthalhydrazide (72 mg, 0.31 mmol) was added to the bromo-ketone **15** (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 **20** (46 mg, 34%) and **21** (22 mg, 16%) as light yellow foams.

Data for isomer 20: $[\alpha]^{26}_D$ +2.1° (*c* 2.8, $CHCl_3$); IR (microscope) 3224, 1739, 1671, 1623, 1601 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (s, 9H), 1.98−2.12 (m, 1H), 2.24−2.60 (m, 3H), 2.92 (s, 3H), 2.98 (s, 3H), 4.40−4.51 (m, 1H), 5.09 (d, J = 16.8 Hz, 1H), 5.23 (d, J = 16.6 Hz, 1H), 5.78−5.80 (m, 1H), 7.72 (d, J = 7.0 Hz, 1H), 7.92 (t, J = 7.9 Hz, 1H), 8.23 (d, J = 7.3 Hz, 1H), 11.04 (br s, 1H); ^{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 21: $[\alpha]^{26}_D$ +158.8° (*c* 0.6, $CHCl_3$); IR ($CHCl_3$ cast) 3183, 1741, 1672, 1630, 1599 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (s, 9H), 1.98−2.17 (m, 1H), 2.22−2.38 (m, 2H), 2.42−2.60 (m, 1H), 2.92 (s, 3H), 2.98 (s, 3H), 4.40−4.52 (m, 1H), 4.92 (d, J = 16.5 Hz, 1H), 5.12 (d, J = 16.7 Hz, 1H), 5.61 (d, J = 4.6 Hz, 1H), 7.81−7.92 (m, 2H), 8.58 (dd, J = 14.2, 1.9 Hz, 1H), 11.04 (br s, 1H); ^{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.

(4*S*)-*N*,*N*-Dimethyl-4-(*tert*-butyloxycarbonylamino)-6-hydroxy-5-oxohexanamide (22). Diazoketone **14** (2.5 g, 8.39 mmol) was dissolved in THF (50 mL) and cooled to 0 °C. After 5 min, 1 N 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 aqueous $NaHCO_3$ (10 mL). The solvent was removed in vacuo and the residue diluted with H_2O (30 mL) and extracted with EtOAc (3 × 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) furnished the desired product as a white solid (1.93 g, 80%) after recrystallization from dichloromethane/hexane; mp 118−119 °C; $[\alpha]^{26}_D$ +21.0° (*c* 1.0, $CHCl_3$); IR (microscope) 3277, 3011, 1720, 1697, 1604, 1518 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9H), 1.89−2.00 (m, 1H), 2.08−2.41 (m, 2H), 2.28−2.54 (m, 3H), 2.92 (s, 3H), 2.98 (s, 3H), 4.30−4.39 (m, 1H), 4.38 (d, J = 17.3 Hz, 1H), 4.43 (d, J = 19.1 Hz, 1H), 5.85 (br s, 1H); ^{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.

(4*S*)-*N*,*N*-Dimethyl-4-(*tert*-butyloxycarbonylamino)-5-oxo-6-(1,2-dimethoxycarbonylhydrazino)hexanamide (23). To a solution of triphenylphosphine (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 **22** (100 mg, 0.35 mmol) was added, and the reaction mixture was slowly allowed to warm to room-temperature overnight. The solvent was removed in vacuo and the residue purified by column chromatography (SiO_2, gradient elution of 3:1 hexane/EtOAc to 1:1 hexane/EtOAc) to afford **23** as a pale yellow oil (29.6 mg, 20%); $[\alpha]^{26}_D$ +3.8° (*c* 0.5, $CHCl_3$); IR (CH_2Cl_2) 3280, 2958, 1740, 1712, 1631, 1506 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) (6:1 mixture of conformers) δ 1.40 (s, 9H), 1.89−2.01 (m, 1H), 2.05−2.20 (m, 1H), 2.35 (dt, J = 16.5, 6.3 Hz, 1H), 2.49 (dt, J = 16.5, 6.6 Hz, 1H), 2.92 (s, 3H), 2.98 (s, 3H), 3.72−3.90 (m, 6H), 4.28−4.37 (br m, 1H), 4.86 (d, J = 17.3 Hz, 1H), 4.95 (d, J = 17.3 Hz, 1H), 5.75 (d, J = 17.3 Hz, 1H);^{13}C NMR (75 MHz, $CDCl_3$) δ 26.4, 28.3, 28.9, 35.8, 37.3, 55.3, 59.9, 69.3, 155.4, 162.4, 172.4, 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-phthalazine-1,4-dione)hexanamide (24). Trifluroacetic acid (2 mL) was added to a solution of **18** (65 mg, 0.12 mmol) in dichloromethane (2 mL) at 0 °C. After 1.5 h, the reaction mixture was concentrated in vacuo and the residue triturated with diethyl ether to yield the trifuoroacetate salt 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 (64 mg, 0.15 mmol) in DMF (8 mL) at room temperature was added DIPEA (51 μM, 0.29 mmol) followed by HBTU (59 mg, 0.15 mmol). The mixture was stirred for 30 min after which it was treated with a solution of the trifluoroacetate salt (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 (linear gradient elution over 20 min of 0 to 40% of 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; $[\alpha]^{26}_D$ −60.0° (*c* 0.3, $CHCl_3$); IR ($CHCl_3$, cast) 3279, 2958, 1740, 1653, 1600, 1540 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) (1:1 mixture of conformers) δ 0.88 (d, J = 5.7 Hz, 3H), 0.92 (d, J = 6.4 Hz, 3H) 1.28−1.44 (m, 6H), 1.45−1.70 (m, 3H), 1.85−2.08 (m, 4H), 2.24−2.58 (m, 3H), 2.92 (s, 3H), 3.05 (s, 3H), 3.10−3.20 (q, J = 7.2 Hz, 1H), 3.20−4.40 (m, 2H), 4.60 (dt, J = 9.6, 4.9 Hz, 1H), 5.15 (d, J = 16.9 Hz, 1H), 5.23 (d, J = 12.2 Hz, 1H), 7.93 (dt, J = 14.9, 7.5 Hz, 2H), 8.12 (d, J = 7.8 Hz, 1H), 8.32 (d, J = 6.7 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 17.5, 17.7, 21.8, 22.0, 23.3, 23.4, 25.9, 26.6, 26.8, 30.1, 35.8, 37.7, 41.7, 50.7, 50.9, 51.2, 53.3, 54.0, 57.2, 69.9, 125.0, 126.0, 127.4, 129.9, 133.5, 134.9, 151.4, 161.7, 174.6,

174.9, 175.3, 175.5, 204.5; HRMS (ES) calcd for $C_{30}H_{43}N_7O_8$-Na (MNa^+) 652.3071, found 652.3068.

(2*S*)-2-(*N*,*N*-Dimethylpropanamid-3-yl)-1-(*tert*-butyloxycarbonyl)azetidin-3-one (25) and (4*S*)-4-(*N*,*N*-Dimethylpropanamid-3-yl)-[1,3]-oxazinane-2,5-dione (26). A solution of diazoketone **14** (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, 0.027 mmol) in benzene (50 mL). 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 **25** (378 mg, 52%) and **26** (99 mg, 17%) as pale yellow oils.

Data for 25: $[\alpha]^{26}_D$ +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), 2.13 (q, J = 7.1 Hz, 2H), 2.34−2.55 (m, 2H), 2.90 (s, 3H), 2.96 (s, 3H), 4.50 (dd, J = 16.8, 4.2 Hz, 1H), 4.65 (d, J = 16.6 Hz, 1H), 4.95 (dt, J = 9.3, 4.2 Hz, 1H); ^{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 26: $[\alpha]^{26}_D$ −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), 2.18−2.32 (m, 1H), 2.46−2.52 (m, 2H), 2.92 (s, 3H), 2.98 (s, 3H), 3.92 (dd, J = 5.2, 0.9 Hz, 1H), 4.55 (d, J = 17.5 Hz, 1H), 4.61 (d, J = 17.5 Hz, 1H), 6.95 (br s, 1H); ^{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*-Dimethylpropanamid-3-yl)-3-hydroxyazetidine Trifluoroacetate salt (27). To a solution of ketone **25** (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 aqueous citric acid (2 mL). The solvent was evaporated and the residue obtained was diluted with water (15 mL). The aqueous layer was extracted with EtOAc (3 × 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}; ^1H NMR (300 MHz, $CDCl_3$) (1:1 mixture of diastereomers) δ 1.40 (s, 9H), 1.94−2.05 (m, 1H), 2.20−2.42 (m, 2H), 2.50−2.60 (m, 1H), 2.92 (s, 3H), 3.00 (s, 3H), 3.60 (dd, J = 10.4, 3.7 Hz, 1H), 3.95−4.12 (m, 2H), 4.38−4.48 (m, 1H), 5.20 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 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 a similar procedure as that described for the preparation of **24**. Trifluroacetic acid (2 mL) was added to a solution of the alcohol (127 mg, 0.47 mmol) in dichloromethane (2 mL) at 0 °C. After 1.5 h, the reaction mixture was concentrated in vacuo, and the trifluoroacetate salt **27** 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 (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}; ^1H NMR (300 MHz, CD_3OD) (2:1 mixture of diastereomers) δ 2.09 (m, 2H), 2.40−2.69 (m, 2H), 2.92 (s, 3H), 3.02 (s, 3H), 3.75 (dd, J = 11.3, 4.7 Hz, 1H), 4.18 (dd, J = 11.0, 6.5 Hz, 1H), 4.43 (dd, J = 13.0, 6.0 Hz, 1H), 4.64 (ddd, J = 11.2, 6.6, 4.8 Hz, 1H);^{13}C NMR (125 MHz, CD_3-CN) δ 23.0, 29.9, 30.1, 35.9, 37.6, 54.2, 65.4, 68.3, 174.2; HRMS (ES) calcd for $C_8H_{17}N_2O_2$ ($M\text{-}CF_3CO_2^+$) 173.1290, found 173.1286.

(2*S*)-2-(*N*,*N*-Dimethyl-propanamid-3-yl)-1-(acetyl-L-leucyl-L-alanyl-L-alanyl)azetidin-3-one (28). This compound was prepared using the same coupling procedure as that described for the preparation of **24**. Ac-Leu-Ala-Ala-OH (95 mg, 0.30 mmol) in DMF (8 mL) at room temperature was treated with DIPEA (126 μM, 0.72 mmol) followed by HBTU (120 mg, 0.32 mmol). The trifluoroacetate salt **27** (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 (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 and conformers) δ 0.92 (d, J = 4.0 Hz, 3H), 0.95 (d, J = 6.5 Hz, 3H), 1.21−1.29 (m, 6H), 1.52−1.60 (m, 2H), 1.61−1.78 (m, 2H), 1.98 (s, 3H), 2.00 (s, 3H), 2.10 (dt, J = 14.7, 7.3 Hz, 1H), 2.22−2.40 (m, 1H), 2.48−2.60 (m, 2H), 2.94 (s, 3H), 3.05 (s, 3H), 3.65 (ddd, J = 25.9, 10.5, 4.4 Hz, 1H), 3.98−4.10 (m, 1H), 4.22−4.42 (m, 4H), 4.52−4.70 (m, 2H); HRMS (ES) calcd for $C_{22}H_{39}N_5O_6Na$ (MNa^+) 492.2798, found 492.2791. The alcohol was oxidized using a similar procedure as that described for the preparation of **11**. Dess−Martin periodinane (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 room temperature, the solvent was removed in vacuo and the crude product purified by HPLC (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; $[\alpha]^{26}_D$ −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 conformers) δ 0.87 (d, J = 6.5 Hz, 3H), 0.92 (d, J = 6.5 Hz, 3H), 1.20−1.32 (m, 6H), 1.50 (t, J = 7.0 Hz, 2H), 1.58−1.72 (dq, J = 8.2, 5.5 Hz, 1H), 1.98 (s, 3H), 2.05−2.12 (m, 2H), 2.45−2.62 (m, 2H), 2.88 (s, 3H), 2.98 (s, 3H), 4.15−4.44 (m, 3H), 4.65−5.12 (m, 3H), 7.05−7.25 (m, 3H); ^{13}C NMR (75 MHz, CD_3CN) 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.

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

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