Tripeptide Aldehyde Inhibitors of Human Rhinovirus 3C Protease: Design, Synthesis, Biological Evaluation, and Cocrystal Structure Solution of P_1 Glutamine Isosteric Replacements

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The investigation of tripeptide aldehydes as reversible covalent inhibitors of human rhinovirus (HRV) 3C protease (3CP) is reported. Molecular models based on the apo crystal structure of HRV-14 3CP and other trypsin-like serine proteases were constructed to approximate the binding of peptide substrates, generate transition state models of P_1-P_1' amide cleavage, and propose novel tripeptide aldehydes. Glutaminal derivatives have limitations since they exist predominantly in the cyclic hemiaminal form. Therefore, several isosteric replacements for the P_1 carboxamide side chain were designed and incorporated into the tripeptide aldehydes. These compounds were found to be potent inhibitors of purified HRV-14 3CP with K_is ranging from 0.005 to 0.64 μM. Several have low micromolar antiviral activity when tested against HRV-14-infected H1-HeLa cells. The *N*-acetyl derivative **3** was also shown to be active against HRV serotypes 2, 16, and 89. High-resolution cocrystal structures of HRV-2 3CP, covalently bound to compounds **3**, **15**, and **16**, were solved. These cocrystal structures were analyzed and compared with our original HRV-14 3CP−substrate and inhibitor models.

Introduction

HRVs are small picornaviruses primarily responsible for the common cold in the United States and Western Europe.[1] Enzymatic and structural proteins required for viral replication are generated by the virally encoded 2A and 3C proteases' co- and posttranslational processing of the 230 kDa polyprotein.[2] The HRV 3CPs are specific cysteine proteases most often responsible for the catalytic cleavage of P_1-P_1' glutamine−glycine peptide bonds.[3] Structurally, these 3CPs closely resemble trypsin-like serine proteases.[4]

Selective inhibition of the HRV 3CPs may be an effective approach toward antirhinoviral therapy since little homology exists between HRV 3CPs and prevailing mammalian enzymes. Several examples of HRV 3CP inhibitors have been described in the literature.[5] In particular, tetrapeptide aldehydes have been reported to be fairly potent reversible inhibitors of this protease.[5b−d] Early on in our structure-based HRV drug design program, the apo X-ray crystal structure of 3CP-serotype 14 was solved.[4a] We became interested in utilizing peptidic aldehyde inhibitors for the purpose of obtaining cocrystal structures. It was reasoned that a reversible, covalently bound molecule of this type would serve to validate our models and provide valuable clues regarding the catalytic mechanism and key atom interactions. The ultimate goal was to use this information as a guide for the discovery of more novel inhibitors.

Initial Models

Examination of the 2.3 Å apo X-ray crystal structure of HRV-14 3CP revealed a polypeptide backbone fold similar to the double β-barrel motif found for trypsin-like pancreatic and bacterial serine proteases, as opposed to cysteine proteases such as papain.[4a,6] For these trypsin-like serine proteases, substrate binding occurs within a long shallow groove between the β-barrel domains. Peptide substrates bind in a partially extended conformation making a series of antiparallel β-sheet type hydrogen bonds with the exposed edge of a protein β-strand. The catalytic triad for HRV-14 3CP is comprised of Cys-146, His-40, and Glu-71. Most HRV protease cleavage sites within the virally encoded polyprotein occur between Gln−Gly peptide bonds such that the S_1 subsite of this protease has specific recognition elements for a Gln side chain.[3] Peptide substrates, such as Glu-Thr-Leu-Phe-Gln-Gly-Pro-Val, representing the 2C−3A viral cleavage site, were initially positioned into the regions of the apo X-ray structure expected to bind P_5-P_3'. The initial alignment of the substrates was based upon the known binding mode of peptides in the active sites of trypsin-like enzymes, as observed in the cocrystal structures of the serine protease *Streptomyces griseus* B−ovomucoid inhibitor OMTKY3, trypsin−Bowman−Burk inhibitor, and trypsin−pancreatic trypsin inhibitor.[6c−e] While part of the S_1 pocket of HRV-14 3CP (residues 139−145) is highly flexible,[4a] a probable conformation for this segment was constructed on the basis of the best density among the eight copies in the crystallographic asymmetric unit combined with allowed backbone geometry. The model HRV-14 3CP complex, created for the entire active site and substrate, was refined through energy minimization and molecular dynamics simulations.[7]

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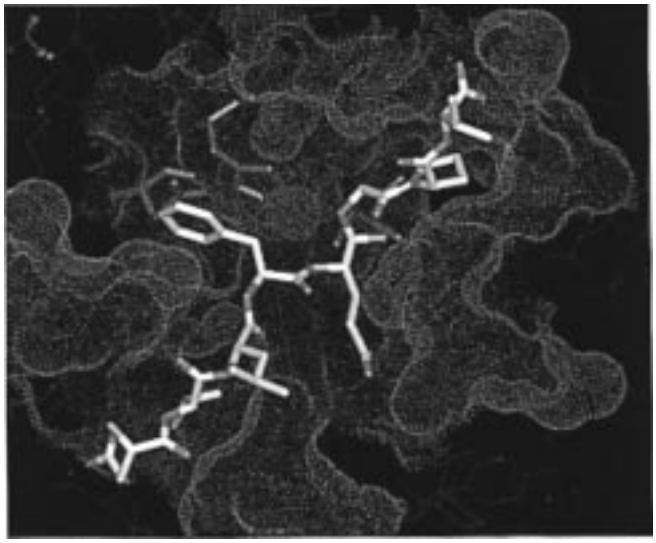

Figure 1. Model of an octameric peptide segment of the native 2C-3A cleavage sequence in the active site of HRV-14 3CP. The active site catalytic residues are displayed as purple licorice bonds while the substrate is depicted as an anionic tetrahedral intermediate (covalent bond omitted). The molecular surface was calculated from the van der Waals radii of the protein atoms.

It is generally assumed that the mechanism of hydrolysis in trypsin-like proteases involves formation of a tetrahedral intermediate in which the scissle peptide carbonyl oxygen is stabilized by an "oxyanion hole".[8] The tetrahedral geometry from an *ab initio* quantum mechanics calculation on a small model system was incorporated into the peptide substrate geometry and further optimized in the protein complex through several cycles of energy minimization and molecular dynamics simulation.[7] The resultant structure is shown in Figure 1. This native substrate analysis also allowed us to develop reasonable models for peptidic aldehyde inhibitors based on similarities in binding and the formation of a tetrahedral adduct. The results from this study will be summarized in the Crystallographic Analysis section.

Aldehyde Inhibitors and P_1 Isotere Design

An important objective set forth was to obtain a cocrystal structure with an inhibitor that would possibly mimic the cleavage of a natural substrate and provide atomic information regarding the catalytic mechanism. Peptide aldehydes were the most logical candidates since these molecules have been successfully used as inhibitors of cysteine and serine proteases and were shown to form reversible covalent adducts.[5b−d,9,10] Our first choice was peptidic P_1 glutaminals, since these structures should closely resemble the substrates, and were previously reported to be inhibitors of HRV 3CP and the related hepatitis A 3CP.[5b−d] The designs were limited to modified tripeptide aldehydes for three basic reasons. First, we predicted that spanning P_1 through P_3 would provide enough molecular recognition for binding. (The P_2 and P_3 specific amino acids, Phe and Leu, respectively, were used.) Second, the synthesis would be kept relatively simple. Third and foremost, the probability of obtaining cocrystals is often related to the solubility of the inhibitors; therefore we decided to keep the molecular weight as low as possible.

Following a slightly modified procedure published by the Lilly group, we successfully prepared CBZ-Leu-Phe-glutaminal **1**.[5b] The K_i for **1** versus HRV-14 3CP was measured to be 3.6 μM. Unfortunately, all efforts to obtain a cocrystal structure of this compound with HRV serotypes 2, 14, 16, or 89 failed. It is known that an equilibrium between the aldehyde and cyclic amidal exists for these molecules (Figure 2).[5b,11] Proton NMR of **1** in DMSO-d_6 revealed the presence of only the hemiglutaminal species. We first speculated that under various assay and/or crystallization conditions, the chemical equilibrium would be shifted predominantly toward the cyclic amidal form, which then may have a tendency to eliminate water to form a dihydropyridone via an acyliminium species. This was postulated since we had observed the formation of **19** in our unsuccessful attempts to synthesize **1** from **17** as shown in Scheme 1.[11] However, we were not convinced that this phenomenum had occurred and were able to disprove this theory experimentally. HPLC analyses, conducted on material recovered from the inhibition assays with **1** after a prolonged exposure time of 24 h, at either standard or high protease concentrations, were compared with that of pure inhibitor **1** and dihydropyridone **19**.[12] The retention time for the single compound recovered was identical to that of **1**. Therefore, it was reasoned that formation of the cyclic form of **1** competes effectively with formation of the thiohemiacetal at the enzyme active site.

To circumvent the problems associated with glutaminal type inhibitors, we decided to use our three-dimensional models to design isosteric replacements for the γ-carboxamide. One preliminary idea was to "invert" the primary amide bond, thereby replacing the γ-carbon of the Gln side chain with nitrogen, and substituting the NH_2 with carbon. The first P_1 β-amino alaninal prepared, the *N*-formyl derivative **2**, was found to be a 73 nM inhibitor of HRV-14 3CP. Compound **3** possesses the *N*-acetyl group, the closest isosteric replacement for the $-CH_2C(O)NH_2$ of Gln in this series. It is a 6 nM inhibitor of HRV-14 3CP. Suitable cocrystals for diffraction experiments were eventually

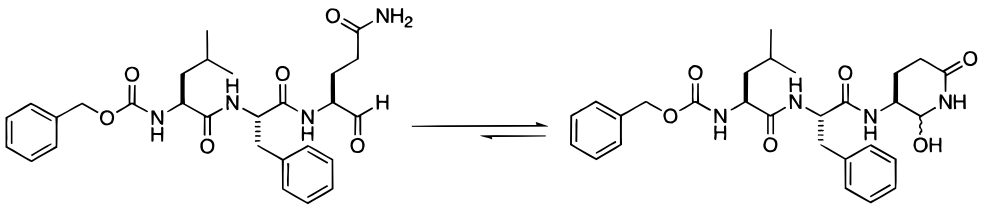

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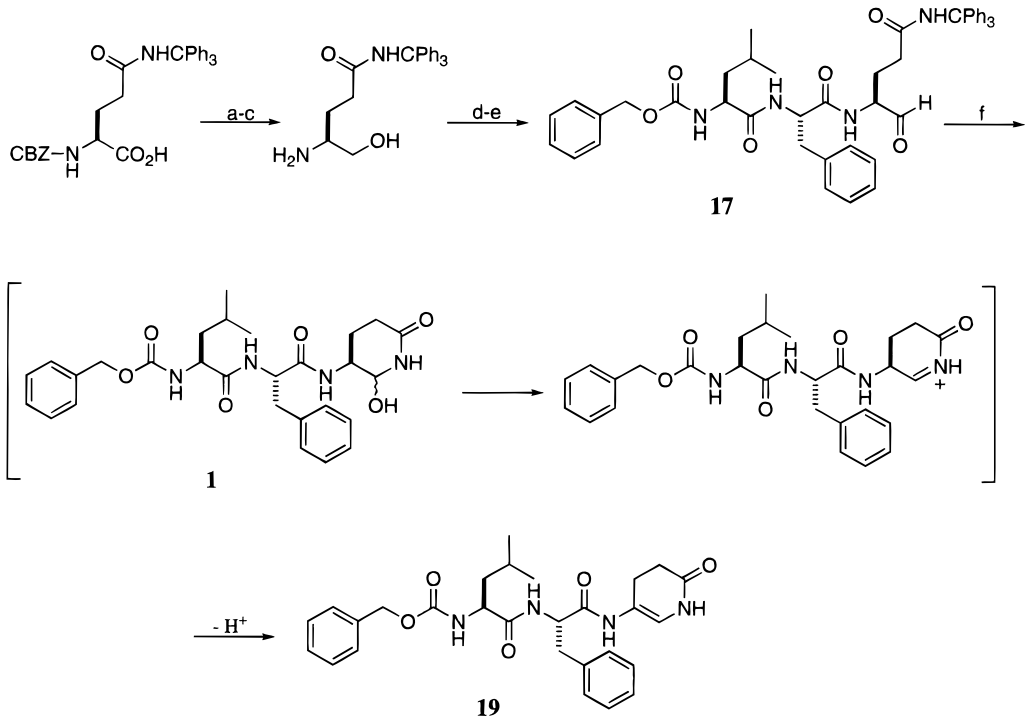
Figure 2. Glutaminal equilibrium.

Scheme 1. Formation of **19**[a]



[a] (a) AcCl, MeOH; (b) $NaBH_4$, LiCl, THF, EtOH; (c) 10% Pd/C, H_2, MeOH; (d) CBZ-Leu-Phe, *N*-hydroxysuccinimide, DCC, CH_2Cl_2, DMF; (e) IBX, DMSO; (f) TFA, CH_2Cl_2, with or without iPr$_3$SiH.

grown with this aldehyde and HRV-2 3CP. Encouraged by these results, several P_1 variations were envisaged with or without the aid of our models. Seven additional amides were prepared and tested. The inhibitory data is listed in Table 1. We were quite surprised to discover that the S_1 subsite of HRV-14 3CP could tolerate larger substituted alkyl and aryl amides. Simple alkyl substitutions on the amide nitrogen appear to be detrimental toward inhibition. The N-methylated compound **8** was 75 times less active than our benchmark **3**, against HRV-14 3CP. However, the more constrained cyclic derivative **9** was only about 9 times less active than **3**.

Other potential P_1 Gln isosteres were also investigated. Interestingly, the bulky *tert*-butyl carbamate **12** was twice as potent as an HRV-3CP inhibitor when compared to the corresponding methyl carbamate **11**. The methyl sulfonamide **13** was the poorest enzyme inhibitor of the P_1 β-amino alaninal series. The *N*,*N*-dimethylurea tripeptide aldehyde **14** and the analogous γ-*N*,*N*-dimethyl glutaminal **15**, were found to be 10 and 5 nM inhibitors of HRV-14 3CP, respectively. Previous structure–activity relationship (SAR) studies on the 2,3-dioxindole inhibitors revealed that the *N*,*N*-dimethylamide group was unfavorable in the S_1 subsite, most likely due to the loss of H-bonding and increased steric clash.[5a] The S_1 SAR for tripeptide aldehydes is apparently very different. To better understand these results, several cocrystal structures were solved and analyzed. Structural details for the complexes between HRV-2 3CP and inhibitors **3**, **15**, and **16** are elaborated in the Crystallographic Analysis section.

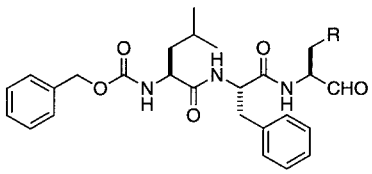
Chemistry

In general, the final products were generated by the oxidation of tripeptide alcohols using *o*-iodoxybenzoic acid (IBX)[13] or sulfur trioxide–pyridine[14] complex. To isolate the pure tripeptide aldehydes, it was best to avoid protic solvents. Usually, a mixture of an aldehyde and its corresponding methyl hemiacetal was formed if the product was exposed to methanol during workup or column chromatography. The pure hemiacetals were found to be as equally active as the parent aldehydes under standard assay conditions, indicating their rapid reversal back to the reactive carbonyl species.

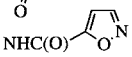
The penultimate intermediates for **1**–**18** were prepared from CBZ-Leu-Phe and modified amino acid esters or alcohols using standard peptide coupling methodology. Our first plan to synthesize **1** was via the trityl-protected glutamine derivative **17**.[15] Exposure of **17** to trifluoroacetic acid (TFA), either with or without the cation scavenger triisopropylsilane,[16] produced only a minute trace of the desired product as observed by mass spectral analysis. The major product isolated was identified as dihydropyridone **19**. As shown in Scheme 1, this compound would arise by the transient formation of cyclic amidal **1**, followed by the dehydration and quenching of the acyliminium ion. Aldehyde **1** was eventually synthesized by the partial reduction of glutarimide intermediate **20** as shown in Scheme 2.[5b]

We designated the "inverted amide" Gln isostere derivatives as "amino-alaninals". In general, the different N-substituents can be synthesized in a variety of ways starting with N_α-CBZ-L-2,3-diaminopropionic acid[17] as highlighted in Scheme 3. Route A is by far the most efficient method when applicable. Amino-alaninals derivatives **8**–**10** were prepared by modifications of this general procedure. We found that the best way to synthesize the *N*-methyl tripeptide aldehyde **8** was via the bicyclic intermediate **21**, using the Diels–Alder methodology described by Greico and Bahsas shown in Scheme 4.[18] The pyrrolidinone ring of ana-

Table 1. Tripeptide Aldehydes

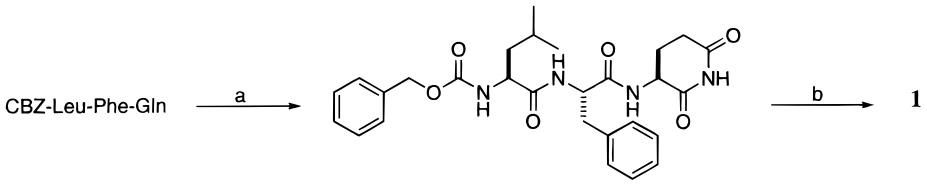


1-18

#	R	formula[a]	K_i (μM)[b]	EC_{50} (μM)[c,d]	TC_{50}(μM)[c,e]	TI[c,f]
1	CH_2CONH_2	$C_{28}H_{36}N_4O_6 \cdot 0.7\ H_2O$	3.6	66	398	6
2	NHCHO[g]	$C_{28}H_{38}N_4O_7 \cdot 0.5\ H_2O$	0.073	1.6	206	129
3	$NHCOCH_3$	$C_{28}H_{36}N_4O_6 \cdot 0.5\ H_2O$	0.006	2.4	316	132
4	$NHCOCH_2CH_3$	$C_{29}H_{38}N_4O_6 \cdot 1.0\ H_2O$	0.007	1.3	>100	>77
5	$NHCOCH(CH_3)_2$	$C_{30}H_{40}N_4O_6 \cdot 1.1\ H_2O$	0.018	4.0	18	5
6	$NHCOCF_3$	$C_{28}H_{33}N_4O_6F_3$	0.146	1.8	25	14
7	NHCOPh	$C_{33}H_{38}N_4O_6 \cdot 1.0\ H_2O$	0.012	1.0	51.2	51
8	$N(CH_3)COCH_3$	$C_{29}H_{38}N_4O_6 \cdot 0.5\ H_2O$	0.469	5.2	100	19
9		$C_{30}H_{38}N_4O_6 \cdot 1.4\ H_2O$	0.052	4.0	>100	>25
10		$C_{30}H_{35}N_5O_7 \cdot 0.6$ MeOH	0.005	1.5	59.5	40
11	$NHCO_2CH_3$	$C_{28}H_{36}N_4O_7 \cdot 1.2\ H_2O$	0.132	5.0	56.2	11
12	$NHCO_2C(CH_3)_3$	$C_{31}H_{42}N_4O_7 \cdot 0.5\ H_2O$	0.066	2.0	56.2	28
13	$NHSO_2CH_3$	$C_{27}H_{36}N_4O_7S \cdot 0.6\ H_2O$	0.64	20	63	3
14	$NHCON(CH_3)_2$	$C_{29}H_{39}N_5O_6 \cdot 0.75\ H_2O$	0.010	2.1	99	47
15	$CH_2CON(CH_3)_2$	$C_{30}H_{40}N_4O_6 \cdot 0.75\ H_2O$	0.005	1.3	63	48
16	CH_2SOCH_3	$C_{28}H_{37}N_3O_6S \cdot 0.25\ H_2O$	0.005	4.5	>100	>22
17	$CH_2CONHCPh_3$	$C_{47}H_{50}N_4O_6$	0.040	>15.8	15.8	
18	CH_2CN	$C_{28}H_{34}N_4O_5 \cdot 1.3\ H_2O$	0.19	8.0	31	4

[a] All compounds were analyzed for C, H, N; the results agreed to within ± 0.4% of the theoretical values. [b] K_i data was measured against HRV-14 3CP. Standard deviation = ±10%; See ref. 5a for methods and conditions. [c] HRV-14 infected H1-HeLa cell protection assay. See ref. 5a for methods and conditions. [d] 50% effective concentration. [e] 50% toxic concentration. [f] Therapeutic index. [g] Compound isolated and analyzed as methyl hemiacetal.

Scheme 2. Synthesis of **1**[a]



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[a] (a) *N*-Hydroxysuccinimide, DCC, DMF, 80 °C; (b) $NaBH_4$, MeOH.

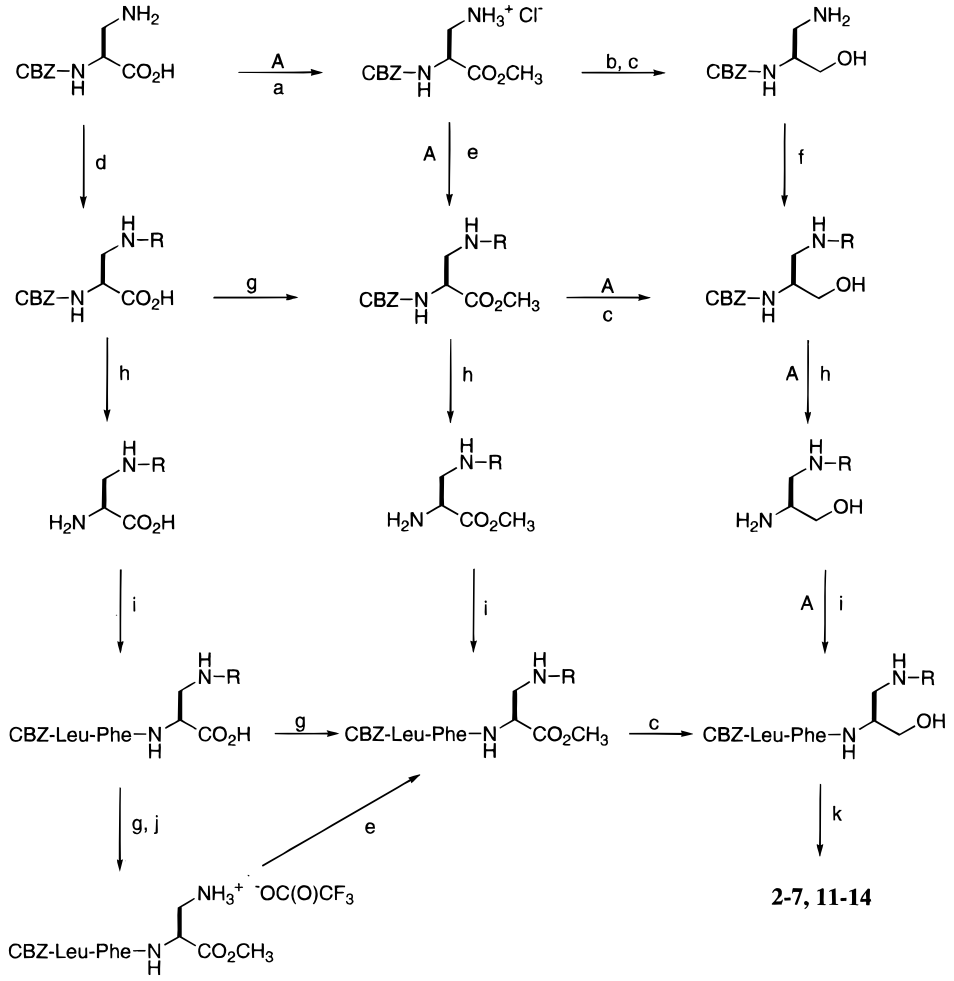
logue **9** was generated using an intramolecular cyclization as shown in Scheme 5. For the preparation of **10** we had to revise our synthetic strategy since any of the practical routes employed in Scheme 3 failed due to the lability of the isoxazole amide toward $LiBH_4$ reduction. The aldehyde function was successfully prepared, albeit in low yield, by the low-temperature diisobutylaluminum hydride (DIBAL) reduction of the corresponding *N*-methoxy-*N*-methylamide shown in Scheme 6.

The synthesis of the γ-*N*,*N*-dimethyl glutaminal derivative **15** was accomplished in six steps in high yield as illustrated in Scheme 7. The isosteric analogue **16** was prepared as a mixture of sulfoxide diastereomers in three steps from commercially available (S)-(−)-methioninol (Scheme 8), whereas the nitrile of **18** arose from dehydration of the primary carboxamide of the *tert*-butyloxycarbonyl (BOC)-protected glutamine methyl ester (Scheme 9).

Enzyme Inhibition and Antiviral Assessment

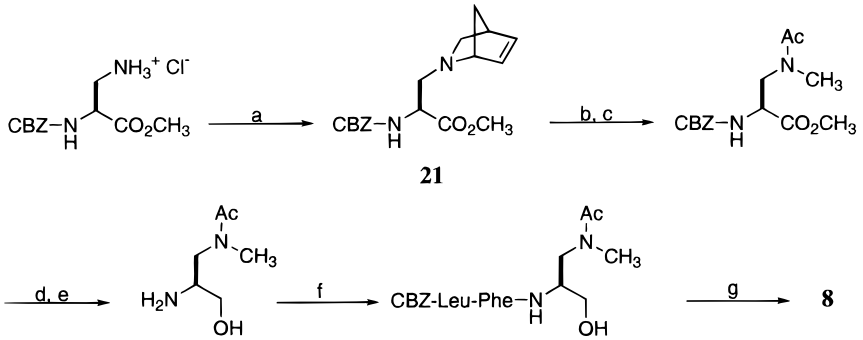
The HRV 3CP inhibition constants for compounds **1**−**18** were measured using a continuous fluorescence resonance energy transfer assay.[19] In contrast to the 2,3-dioxindole inhibitors, no aldehyde inactivation was observed upon the addition of 5 mM dithiothreitol to the assay.[5a] A cell protection assay was utilized to measure antirhinoviral activity. Using the known technique of measuring XTT dye reduction, the aldehydes were examined for their ability to protect H1-HeLa cells against the cytopathic effects of HRV infection.[20] The K_i and EC_{50} data are reported in Table 1. Also, a comparison of the K_is and EC_{50}s for tripeptide aldehyde **3** versus HRV-2, -14, -16, and -89 3CPs is listed in Table 2. These data imply that the *N*-acetylamino and other Gln isosteres can provide a wide range of activity against several rhinovirus serotypes.

Scheme 3. General Methods for the Preparation of Amino-Alaninal Derivatives[a,b]



[a] See the Experimental Section for R groups and details. [b] Route A is preferred. (a) HCl, MeOH, 0 °C; (b) $NaHCO_3$; (c) $NaBH_4$, LiCl, THF, EtOH; (d) $RC(O)OC(O)R$, H_2O or $(tBuO_2C)_2O$, H_2O, tBuOH, NaOH; (e) MeOCHO, Et_3N or acid chloride, CH_2Cl_2, Et_3N or pyr.; (f) CF_3CO_2Et; (g) CH_2N_2 or $TMSCHN_2$; (h) 10% Pd/C, H_2, MeOH; (i) CBZ-Leu-Phe, *N*-hydroxysuccinimide, DCC, CH_2Cl_2, DMF; (j) TFA, CH_2Cl_2, 0 °C to room temperature; (k) IBX, DMSO or SO_3 pyr., DMSO, CH_2Cl_2, Et_3N, 0 °C to room temperature.

Scheme 4. Synthesis of **8**[a]

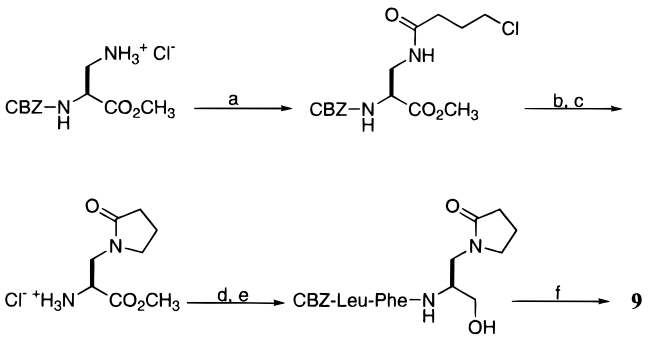


[a] (a) Cyclopentadiene, CH_2O, H_2O; (b) TFA, $CHCl_3$, Et_3SiH; (c) AcOAc, pyr.; (d) $NaBH_4$, LiCl, THF, EtOH; (e) 10% Pd/C, H_2, MeOH; (f) CBZ-Leu-Phe, *N*-hydroxysuccinimide, DCC, CH_2Cl_2, DMF; (g) IBX, DMSO.

Crystallographic Analysis

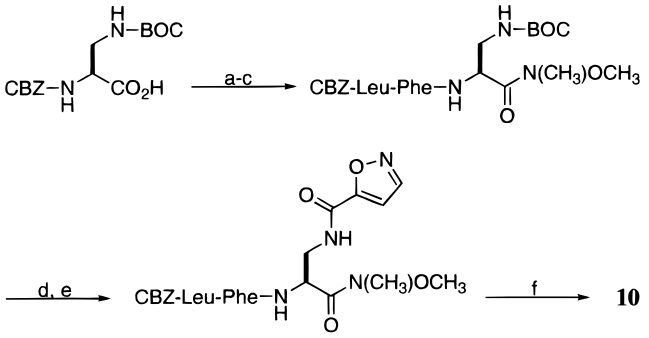
High-resolution cocrystal structures are reported for tripeptide aldehydes **3**, **15**, and **16** bound to HRV-2 3CP. As indicated in Figure 3, the *N*-acetyl oxygen of the bound inhibitor **3** accepts H-bonds from the side chains of Thr-142 and His-161. The P_1 backbone NH donates a weak H-bond (3.1 Å) to the carbonyl oxygen of Val-162. Hydrogen bonded water molecule 614 bridges the *N*-acetyl NH and the P_2 Phe carbonyl oxygen of **3**. The P_2 Phe side chain resides in a narrow pocket sandwiched between His-40 and the protein segment Leu-127−Ser-128. Both the side chain hydroxyl and backbone NH of Ser-128 form H-bonds with the inhibitor NH of P_2 Phe and the carbonyl oxygen of the terminal CBZ group, respectively. Two main chain hydrogen bonds tether the inhibitor's P_3 Leu to backbone atoms of Gly-164 while the isobutyl side chain is solvent exposed. The benzyl portion of the CBZ group packs into a shallow hydrophobic pocket that probably accommodates a substrate's P_4 side chain.

Scheme 5. Synthesis of **9**[a]



[a] (a) 4-Chlorobutyryl chloride, CH_2Cl_2, Et_3N, 0 °C to room temperature; (b) NaH, DMF, 0 °C to room temperature; (c) 10% Pd/C, H_2, MeOH, HCl; (d) CBZ-Leu-Phe, *N*-hydroxysuccinimide, DCC, CH_2Cl_2, DMF, Et_3N; (e) $NaBH_4$, LiCl, THF, EtOH; (f) SO_3-pyr., DMSO, CH_2Cl_2, Et_3N, 0 °C to room temperature.

Scheme 6. Synthesis of **10**[a]



[a] (a) MeO(Me)NH·HCl, CH_2Cl_2, EDCI, 4-methylmorpholine; (b) 10% Pd/C, H_2, MeOH; (c) CBZ-Leu-Phe, *N*-hydroxysuccinimide, DCC, CH_2Cl_2, DMF; (d) TFA, CH_2Cl_2, 0 °C to room temperature; (e) isoxazole-5-carboxylic acid, *N*-hydroxysuccinimide, DCC, CH_2Cl_2, DMF, Et_3N; (f) DIBAL, THF, −78 °C.

Peptidic aldehydes bind to cysteine and serine proteases as reversible adducts in which the nucleophilic Cys (or Ser) forms a covalent bond with the carbonyl carbon of the aldehyde.[5b−d,9,10] The HRV-2 3CP−**3** bound complex would have an *R* or *S* configuration at the thiohemiacetal carbon depending on which face of the incoming aldehyde is presented to Cys-147 for nucleophilic attack. X-ray and NMR studies of aldehydes bound to various cysteine and serine proteases provide examples of each configuration, and in at least the three instances of *apopain*, *Streptomyces griseus* A protease, and *interleukin-1β* converting enzyme, it was concluded that one or both anomeric configurations were present.[10] In the case of HRV-2 3CP, we showed previously that a 2,3-dioxindole inhibitor undergoes nucleophilic addition by Cys-147 to give a tetrahedral adduct in which the oxygen attached to the reactive carbon resides in the "oxyanion hole" and is stabilized by H-bonding interactions with backbone NH groups of Gly-145 and Cys-147.[5a] The situation for covalently bound peptidic aldehyde inhibitors is different. The thiohemiacetal oxygen appears to be protonated, and instead of binding into the "oxyanion hole" is pointed toward the His-40 side chain where it forms a H-bond with Nϵ2. This implies that in HRV-2 3CP only the *re* face of an aldehyde inhibitor is susceptible to attack by Cys-147. Compared to the 2,3-dioxindole complex, the peptide aldehyde cocrystal structures discussed here also differ in the main chain conformation for protein residues Ser-144 and Gly-145. The peptide bond joining segments 144 and 145 flips so that the NH of Gly-145 is now directed out toward the solvent where it H-bonds with an ordered water molecule instead of pointing into the oxyanion hole. Either orientation falls within the allowed range of protein backbone torsional angles.

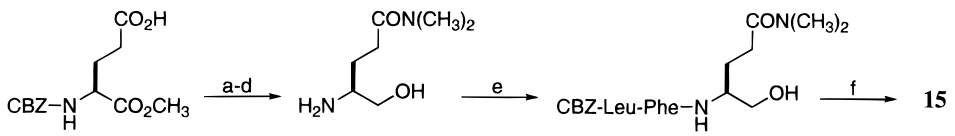
In general, the model of the aldehyde **3** complexed to HRV-14 3CP agrees well with the crystallographic structure in both the overall positioning of the inhibitor and in the nature of the detailed interactions between protein and ligand. The significant differences occur in the orientation of the peptide backbone catalytic region, the tetrahedral adduct, and the side chain of Thr-141. A superposition of the predicted structure of the HRV-14 3CP-**3**, and the experimentally determined structure of HRV-2 3CP-**3** is shown in Figure 4.

In contrast to what is assumed for a peptide substrate, the oxygen of the covalently bound thiohemiacetal is pointed toward solvent and 2.9 Å from Nϵ2 of His-40. The position of the carbonyl C−O bond vector is nearly coincident with the position of the Gln−Gly amide bond vector in our substrate model. Both the oxygen of the aldehyde and nitrogen from the peptide are in position to receive a proton from Nϵ2 of His-40. For amide bond cleavage this protonation would make the nitrogen a good leaving group for collapse of the tetrahedral oxyanion to the acyl−enzyme intermediate. The position of the oxygen nearest to the Nϵ2 of His-40 is consistent with the notion that nucleophilic attack of the sulfur and subsequent protonation could occur in rapid succession, perhaps even in a concerted fashion. With regard to the orientation of the side chain of Thr-141, the N−Cα−Cβ−Oγ dihedral angle is 44° in our models, compared to −164° in the crystal structure.

When the HRV-2 3CP complexes of **3** and **15** are examined together, very small differences, relating mostly to the P_1 side chains of the inhibitors, are noticed. Compared with the *N*-acetyl methyl of **3**, the bulkier *N*,*N*-dimethyl group of **15** also fits well into the S_1 subsite. An observed water molecule in this region may move slightly to accommodate the change.

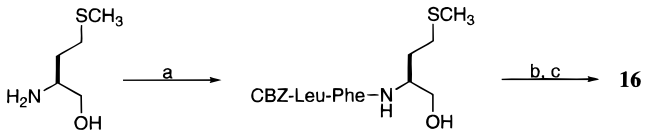
Apart from minor differences between the P_1 *N*-acetyl group of **3** and the methyl sulfoxide moiety of **16**, these two inhibitors bind almost identically to the protease. The sulfoxide oxygen is nearly coincident with the position occupied by the corresponding *N*-acetyl oxygen of **3** and forms an analogous pair of H-bonds with the

Scheme 7. Synthesis of **15**[a]



[a] (a) ClC(O)C(O)Cl, CH_2Cl_2, cat. DMF, 0 °C to room temperature; (b) Me_2NH, THF; (c) $NaBH_4$, LiCl, THF, EtOH; (d) 10% Pd/C, H_2, MeOH; (e) CBZ-Leu-Phe, *N*-hydroxysuccinimide, DCC, CH_2Cl_2, DMF; (f) IBX, DMSO.

Scheme 8. Synthesis of **16**[a]



[a] (a) CBZ-Leu-Phe, *N*-hydroxysuccinimide, DCC, CH_2Cl_2, DMF; (b) *m*-CPBA, CH_2Cl_2; (c) IBX, DMSO.

Table 2. Tripeptide Aldehyde **3** versus Different Serotypes

serotype	K_i (μM)[a]	EC_{50} (μM)[b,c]	TC_{50} (μM)[b,d]	TI[b,e]
2	0.16	3.1	>100	>32
14	0.006	2.4	316	132
16	0.069	2.1	>100	>48
89	0.036	2.2	>100	>45

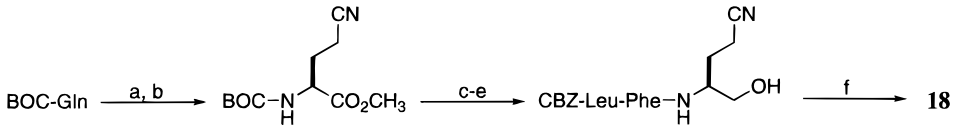
[a] K_i data was measured against 3CP. Standard deviation = ±10%; see ref 5a for methods and conditions. [b] HRV-infected H1-HeLa cell protection assay. See ref 5a for methods and conditions. [c] 50% effective concentration. [d] 50% toxic concentration. [e] Therapeutic index.

side chains of Thr-142 and His-161. Although **16** was cocrystallized as a diasteromeric mixture, electron density for this inhibitor indicates that only the *R* configuration (relative to sulfur) selectively binds to the HRV-2 3CP. Model building with the other anomer suggests that if the sulfoxide oxygen is constrained by H-bonds to Thr-142 and His-161, unacceptable steric interactions would occur between the sulfur and Gly-164, and between the S-methyl group and protein residues on the opposite side of the S_1 subsite, namely Thr-142 and Lys-143.[21] The electron density maps for the crystal structures of HRV-2 3CP bound with **3**, **15**, and **16** are displayed in stereo in Figure 5.

Conclusions

Protein crystal structures and molecular models of covalently bound substrates and inhibitors were utilized in the preliminary design of tripeptide aldehydes. CBZ-Leu-Phe-Gln-CHO, **1**, was prepared since P_1 recognition for most 3CPs is Gln. Compound **1** exists as the cyclic hemiaminal as shown by proton NMR. No cocrystal structures of HRV 3CPs and **1** could be obtained. This prompted us to find isosteric replacements for the P_1 γ-carboxamide. A representative group of tripeptide aldehydes derived from unnatural amino acids or alcohols in P_1 were prepared and analyzed. For these peptidic molecules, a primary amide (Gln-γ-$CONH_2$) was not required to achieve potent inhibition of HRV-14 3CP. High-resolution X-ray structures of HRV-2 3CP covalently bound with compounds **3** and **15** revealed that the carbonyl oxygen making H-bonds to Nϵ2 of His-161 and Oγ of Thr-142 is important for potency. As noted previously in our structural analysis of HRV-2 3CP-**3**, and as shown in Figure 3, H_2O-614 appears to intramolecularly orient the P_1 side chain of inhibitor **3** into a favorable binding conformation. This may partially explain why the corresponding N-methylated derivative **8** is a much weaker inhibitor. The loss of H-bonding and the reorganization of H_2O-614 into an altered environment must be considered, as well as the steric and less conformationally restricted effects of the added methyl group. HRV-2 3CP was also found to

Scheme 9. Synthesis of **18**[a]



[a] (a) CH_2N_2, MeOH, EtOAc, 0 °C; (b) $POCl_3$, pyr., 0 °C to room temperature; (c) TFA, CH_2Cl_2, 0 °C to room temperature; (d) CBZ-Leu-Phe, pyBOP, *i*Pr_2NEt, DMF, 0 °C to room temperature; (e) $NaBH_4$, LiCl, THF, EtOH; (f) SO_3–pyr., DMSO, CH_2Cl_2, Et_3N, 0 °C to room temperature.

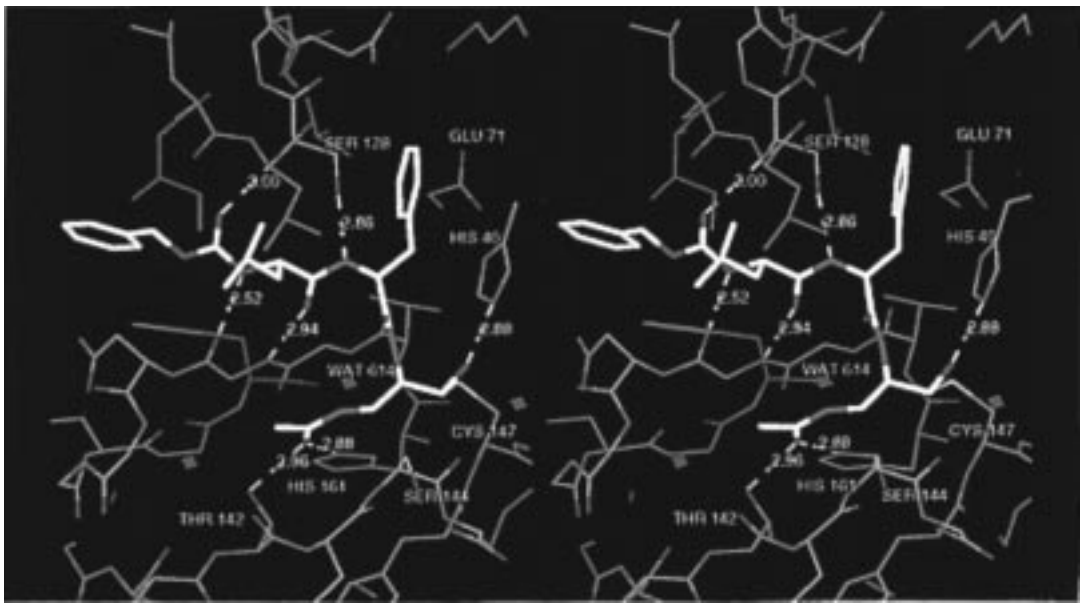


Figure 3. Stereoview of the 2.2 Å cocrystal structure of compound **3** (yellow) covalently bound to HRV-2 3CP (green). Several protein residues are identified along with ordered water molecule 614. Key H-bonds between the inhibitor and the protease are denoted.

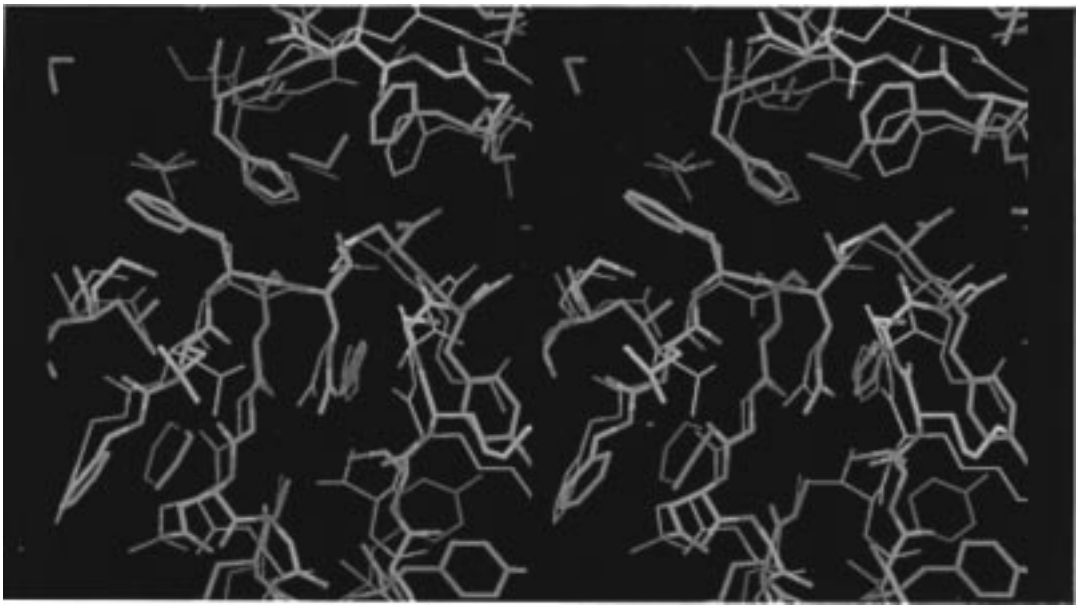


Figure 4. Stereoview of superposition of the predicted complex of compound **3** and HRV-14 3CP (purple) with the crystallographically determined HRV-2 3CP−**3** structure (green). Notable is how the experimental hemiacetal oxygen is pointed toward His-40 rather than in the oxyanion hole as expected. In addition, the amide bond of Ser-144 is reoriented 180° from that modeled.

selectively cocrystallize with the (*R*)-sulfoxide diastereomer of **16**. The polarized sulfoxide oxygen forms strong H-bonds to Nϵ2 of His-161 and Oγ of Thr-142. Taken together, the crystallographic and limited SAR data for peptidic aldehydes suggests that the S_1 subsite permits larger substituents beyond the P_1 δ-position. Even the extremely bulky BOC group of **12** and the trityl group of **17** are accommodated without a huge loss in activity against HRV-14 3CP. These results are not entirely clear, especially since the less sterically demanding methyl carbamate derivative **11** is 2-fold less potent than **12**.

Other important interactions between the protein and inhibitors were identified from the cocrystal structures. Our models of substrates and inhibitors covalently bound to HRV-14 3CP correspond well with the experimental X-ray data. The most notable difference was the position of the hemithioacetal oxygen in the active site. We originally predicted a deprotonated species buried in a classical oxyanion hole, whereas the oxygen actually appears to be protonated and H-bonded to Nϵ2 of His-40. The altered orientation of the hemithioacetal adduct gave us new insight regarding the mechanism of inhibition and, in particular, the conformation of the aldehydic carbonyl during the nucleophilic attack by the cysteine thiol.

The unnatural P_1 tripeptide aldehydes were more active against HRV-14 3CP than glutaminal **1**. Several possess low nanomolar potency. In addition, compound **3** was screened against four viral serotypes, demonstrating that an incorporated P_1 Gln isostere may be effective for inhibitors designed against the wide range of naturally occurring HRV variants. The tripeptide aldehydes prepared have modest antiviral activity. Although peptide aldehydes are not considered to have any therapeutic value as antirhinoviral agents, they nonetheless serve as good models for structure-based drug design.

Experimental Section

Proton magnetic resonance spectra (NMR) were determined using a Tech-Mag spectrometer operating at a field strength of 300 MHz. Chemical shifts are reported in parts per million (δ) with references set such that in $CDCl_3$ the $CHCl_3$ is at 7.26 ppm, in acetone-d_6 the acetone is at 2.02 ppm, and in DMSO-d_6 the DMSO is at 2.49 ppm. Standard and peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; bs, broad singlet; m, multiplet; etc. Mass spectra were determined at the Scripps Research Institute, La Jolla, CA, Mass Spectrometry Facilities. Infrared absorption (IR) spectra were taken on a MIDAC Corp. FTIR. Elemental microanalyses were performed by Atlantic Microlab Inc. Norcross, GA, and gave results for the elements stated with ±0.4% of the theoretical values. Flash column chromatography was performed using silica gel 60 (Merck Art 9385). Thin layer chromatographs were performed on precoated sheets of silica 60 F_{254} (Merck Art 5719). Analytical HPLC was performed using a Hewlett-Packard model 1050 equipped with UV diode array detector set at 210 and 280 nm with a Shandon Hypersil ODS C_{18} column (150 mm × 4.6 mm; 5 μm). An isocratic mobile phase of 25 mM aqueous Na_2HPO_4, 2 mM NEt_3 in CH_3CN was used. Flow rate = 1.0 mL/min; injection volume = 10 μL. Melting points were determined on a Mel-Temp apparatus and are uncorrected. N_α-CBZ-L-2,3-diaminopropionic acid was purchased from Fluka Chemical Co. or was prepared according to the literature.[17] CBZ-L-Leu-L-Phe was purchased from Bachem Chemical Co. (*S*)-(−)-Methioninol was purchased from Aldrich Chemical Co. Isoxazole-5-carboxylic acid was purchased from Maybridge Chemical Co. Anhydrous *N*,*N*-dimethylformamide (DMF), dichloromethane (CH_2Cl_2), and dimethyl sulfoxide (DMSO) were used as is. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under nitrogen. Et_2O refers to diethyl ether. Pet. ether refers to petroleum ether of bp 36−53 °C. Products and intermediates were named according to the conventional amino acid/peptide nomenclature system with modifications to the C-terminal residue when unnatural or protected amino acid or alcohols were used.

Inhibition Assays. All strains of human rhinovirus (HRV) were purchased from American Type Culture Collection (ATCC) except for HRV serotype 14 which was obtained from the supernatant of HeLa cells transfected with an RNA

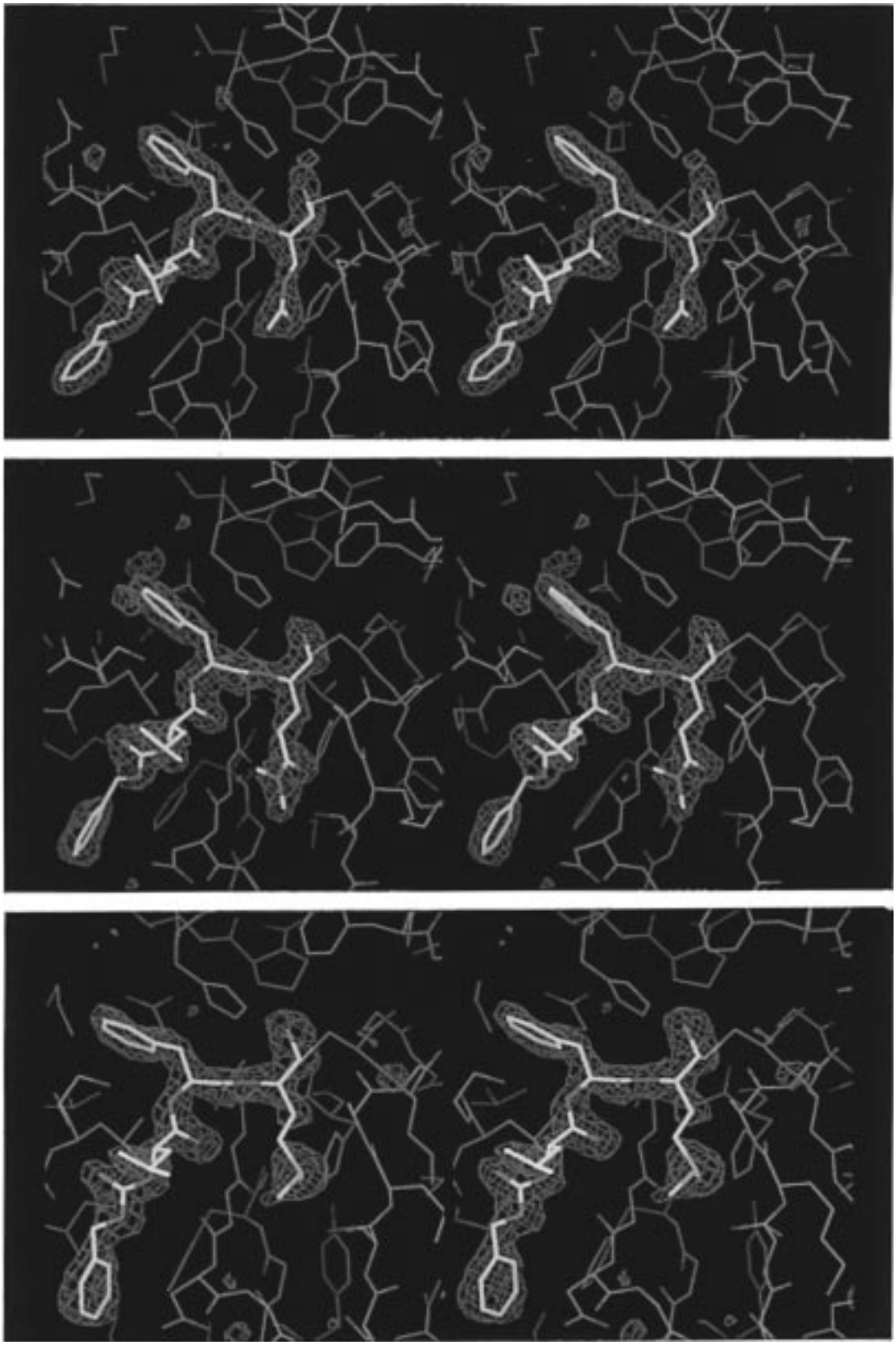


Figure 5. Stereoviews of the electron density maps for the crystal structures of compounds **3**, **15**, and **16** complexed with HRV-2 3CP. The maps were computed with coefficients $F_{obs} - F_{calc}$ using phases from the protein structure alone (without inhibitor) and are contoured at 3σ: (Top) map for **3** at 2.2 Å resolution, (middle) map for **15** at 2.0 Å resolution, (bottom) map for **16** at 1.8 Å resolution.

transcript derived from a cDNA clone constructed and supplied to us by Drs. R. Rueckert and W.-M. Lee at the Institute for Molecular Virology, University of Wisconsin, Madison, WI.[22] HRV 3CPs were expressed and purified as described previously.[5a] The HRV 3CP activity was measured by a continuous fluorescence resonance energy transfer assay as described previously.[5a,19a,23]

Antiviral Assays. HRV stocks were propagated in H1-HeLa cells purchased from ATCC, and antiviral assays were performed as previously reported.[5a]

Molecular Models and Calculations. Molecular mechanics calculations were performed with the CHARMM22[24] program using the MSI force field supplied in the Quanta[25] molecular modeling package. The initial modeling of the noncovalent octapeptide substrate/protein complex was done through molecular dynamics (MD) simulations of the full protein surrounded by a truncated octahedron[26] of TIPS3P water[27] (characteristic size parameter; $R = 29$ Å) at 300 K with periodic boundary conditions. For the dynamics simulations, the following parameters were set: nonbonded cutoffs were at 10 Å, SHAKE[28] constraints were applied on bonds to hydrogen, 2 fs time step, 10 ps heating, 40 ps equilibration. After equilibration, an average structure from 40 ps sampling was produced from coordinates that were saved every 50 time steps. This 9138 atom system was too large to do refining adjustments to the model complex; therefore the Stochastic Boundary Molecular Dynamics (SBMD) method was used.[29] In this calculation, a spherical active site region with a radius = 18 Å centered at Cys-147 was extracted from the HRV-14 3CP protein. The protein atoms near the edge of the sphere were restricted to their crystallographic positions using weak harmonic constraints, while the central region was free to move. The nonbonded cutoffs, SHAKE constraints, and time step were the same as described above. The SBMD methods permitted protein simulations with explicit solvent on a much smaller system (2807 atoms) with less computer time. In these simulations, the model was adjusted to a covalent tetrahedral intermediate, and side chain dihedral angles were adjusted to other local minimum using dihedral constraints with the aim of improving the overall fit and hydrogen bonding between the protein and substrate. During the early equilibration phase (10−20 ps) of these molecular dynamics simulations, flat potential well distance constraints (NOE type) were applied to maintain key intermolecular hydrogen bonding distances in the S_1 subsite and along the β-sheet−ligand interface. The tetrahedral adduct geometry used in the simulations was compared with *ab initio* calculations performed with the GAUSSIAN 92 program.[30] The geometry from an HF/6-31G* optimization of the neutral tetrahedral adduct of methyl sulfide with formamide agreed well with the MSI force field derived geometry, so the MSI parameters were used along with charges (CHELP[31]) from a single-point HF/6-31G* calculation on the anionic tetrahedral geometry.

Cocrystal Structure Determinations: HRV-2 3CP-3 Complex. HRV-2 3CP was incubated with a 3-fold molar excess of **3** and the complex concentrated to a final protein concentration of 13 mg/mL. Inhibitor binding was verified to be >99% as judged by peptide cleavage monitored by HPLC.[19a] The protein−ligand complex was then passed though a 0.45 μM Centrex centrifugal filter and set up for crystallization using the hanging drop vapor diffusion technique. Crystals were grown by mixing 3 μL of the protein complex on a plastic coverslip with an equal volume of a precipitating reservoir solution of 0.2 M NaOAc, 0.1 M TRIS, pH 8.5, 15% PEG 4000 (w/v). Drops were sealed over individual wells containing the above reservoir solution and allowed to equilibrate for 5 days at 21 °C. A crystal, grown as a large plate measuring 0.4 × 0.3 × 0.05 mm (space group $P2_12_12_1$; $a = 34.5$, $b = 66.5$, $c = 78.5$ Å) was mounted in a glass capillary for diffraction measurements. Cu Kα X-ray diffraction data were collected at 4 °C using a 9 kW rotating anode source and two Xuong-Hamlin multiwire area detector systems. These data are 88% complete to a resolution of 2.2 Å with R(sym) = 4.3%. The initial protein model was obtained by molecular replacement techniques (as implemented in X-PLOR[32]) using a search model based on our original structure of HRV-14 3CP.[4a] After manual readjustment and appropriate side chain replacement, refinement was initiated using the rigid body option in X-PLOR followed by simulated annealing and conjugate gradient minimization protocols. Atomic coordinates for **3** were then fit to the difference electron density maps, and the protein−ligand model was refined. This was followed by sequential addition of ordered solvent in several cycles involving stereochemically reasonable placement of candidate waters into difference maps and subsequent refinement of the combined model. The final R factor (using all data with $I > 1.0\ \sigma(I)$ between 10 and 2.2 Å) is 20.2% with rms deviations from ideal bond lengths and angles of 0.016 Å and 2.8°, respectively. The model includes all atoms for residues 1−180 of RVP, all inhibitor atoms, and 76 waters.

HRV-2 3CP−15 Complex. A complex of aldehyde **15** with HRV-2 3CP was prepared as described above, concentrated to a final protein concentration of 12 mg/mL, and passed through a 0.45 μM Centrex centrifugal filter. Crystals were grown as described for complex **3** except that the precipitating reservoir solution was 0.1 M $MgCl_2$, 0.1 M ADA pH 6.5, 30% PEG 8000 (w/w). The drops were sealed over the wells containing the precipitation solution and incubated at 13 °C. A crystal measuring 0.25 × 0.15 × 0.03 mm (space group $P2_12_12_1$; $a = 33.73$, $b = 65.97$, $c = 77.53$ Å) was prepared for low-temperature diffraction measurements by serial transfer to artficial mother liquor solutions of increasing glycerol concentration. When fully equilibrated against an artificial mother liquor containing 24% glycerol, the crystal was flash frozen. X-ray diffraction data were collected at −170 °C using a MAR imaging plate and processed with DENZO.[33] The data are 98.9% complete to a resolution of 2.0 Å with R(sym) = 6.4% Starting coordinates for the protein portion of the HRV-2 3CP−**15** complex were taken from the isomorphous HRV-2 3CP−**3** structure and subjected to rigid body refinement using X-PLOR. Placement of the inhibitor, addition of ordered solvent, and crystallographic refinement proceeded as described above. The final R factor is 21.6% (11 428 reflections with $F > 1\sigma(F)$ in the resolution range 10 to 2.0 Å). The root-mean-square (rms) deviations from ideal bond lengths and angles are 0.018 Å and 2.9 degrees, respectively. The model consists of all atoms for residues 1−180, all inhibitor atoms, plus 139 water molecules.

HRV-2 3CP−16 Complex. A complex of aldehyde **16** with HRV-2 3CP was prepared as described above, concentrated to a final protein concentration of 10 mg/mL, and passed through a 0.45 μM Centrex centrifugal filter. Crystals were grown as described for complex **3** except that the precipitating reservoir solution was 0.2 M NH_4OAc, 0.1 M TRIS pH 8.5, 1:1 1.5 M K_2HPO_4:NaH_2PO_4, and 1% 2-methyl-2,4-pentanediol. The drops were sealed over the wells containing the precipitation solution, equilibrated for 5 days at 21 °C, and seeded with microcrystals grown under similar conditions. A crystal measuring 0.4 × 0.1 × 0.04 mm (space group $P2_12_12$; $a = 62.28$, $b = 77.63$, $c = 34.10$ Å) was obtained. Procedures for X-ray data collection (99.6% complete to 1.8 Å, R(sym) = 5.4%) were similar to those for the HRV-2 3CP−**15** complex. Rotation and translation search procedures implemented in X-PLOR were used to orient and position a search model based on the HRV-2 3CP−**3** complex in the new unit cell. Placement of the inhibitor, addition of ordered solvent, and crystallographic refinement proceeded as described above. The final R factor is 20.5% (13 104 reflections with $F > 2\sigma(F)$ in the resolution range 10 to 1.8 Å). The rms deviations from ideal bond lengths and angles are 0.015 Å and 2.7°, respectively. The model consists of all atoms for residues 1−180 (excluding side chains for residues 12, 55, and 65), all inhibitor atoms, plus 132 water molecules.

Synthesis: CBZ-L-(Tr-Gln).[15] CBZ-L-Gln (28.03 g, 100 mmol) was dissolved in 300 mL of glacial acetic acid. To this solution was added triphenylmethanol (26.83 g, 100 mmol), acetic anhydride (18.87 mL, 200 mmol), and 0.5 mL of sulfuric acid. The reaction was heated to 55 °C, stirring for 1 h. After being cooled to room temperature, the mixture was concentrated under reduced pressure to one-third the original volume. Ice water was added, and the product was extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated. The crude product was recrystallized from CH_2Cl_2/hexane, and the resulting crystals were washed with Et_2O, yielding 37.27 g (71%) as a white solid: IR (KBr) 3418, 3295, 3059, 3032, 2949, 2515, 1699, 1628, 1539, 1504, 1447, 1418, 1341, 1242, 1209, 1061, 748, 696 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.71 (m, 1 H), 1.88 (m, 1 H), 2.38 (m, 2

H), 3.97 (m, 1 H), 5.04 (s, 2 H), 7.14−7.35 (m, 20H), 7.52 (d, 1 H, J = 7.7 Hz), 8.60 (s, 1 H).

CBZ-L-(Tr-Gln)OMe. CBZ-L-(Tr-Gln) (2.6 g, 5.0 mmol) was added to a stirred solution of 2.5 mL of acetyl chloride in 50 mL of MeOH. Stirring was continued at room temperature for 1 h at which time the solvent was removed in vacuo. The residue was dissolved in 300 mL of CH_2Cl_2, and the organic layer was washed with water, saturated $NaHCO_3$, and brine followed by drying over Na_2SO_4. The crude product was purified on a short flash silica gel column, eluting with 20% EtOAc/hexane. The product (2.3 g, 84%) was obtained as a white solid: IR (KBr) 3405, 3277, 3057, 3034, 2953, 1724, 1643, 1532, 1493, 1447, 1207, 1042, 750, 698 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.16 (t, 1 H, J = 7.0 Hz), 1.77 (m, 1 H), 1.97 (m, 1H), 3.61 (s, 3H), 4.99 (m, 1H), 5.03 (s, 2H), 7.02−7.55 (m, 20H), 7.69 (d, 1H, J = 7.7 Hz), 8.59 (s, 1H). Anal. ($C_{33}H_{32}N_2O_5$) C, H, N.

CBZ-L-(Tr-glutaminol). CBZ-L-(Tr-Gln)OMe (1.50 g, 2.79 mmol) was dissolved in 20 mL of THF and 10 mL of EtOH. LiCl (0.24 g, 5.6 mmol) was added, and the mixture was stirred for 10 min until all solids had dissolved. $NaBH_4$ (0.21 g, 5.6 mmol) was added, and the reaction was stirred overnight at room temperature. The solvents were removed in vacuo, the residue taken up in water, and the pH was adjusted to 2−3 with 10% HCl. The product was extracted with EtOAc, and the organic layer was washed with water and brine before drying over $MgSO_4$. The crude product was purified on a short flash silica gel column, eluting with an increasing gradient of EtOAc/benzene, yielding 1.02 g (72%) of a white glassy solid: IR (KBr) 3408, 3318, 3057, 3032, 2947, 1699, 1674, 1516, 1447, 1240, 1059, 752, 698 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.40 (m, 1H), 1.72 (m, 1H), 2.26 (m, 2H), 3.17−3.50 (m, 3H), 4.64 (t, 1H, J = 5.0 Hz), 5.00 (s, 2H), 7.00−7.40 (m, 20H), 6.96 (d, 1H, J = 8.5 Hz), 8.54 (s, 1H). Anal. ($C_{32}H_{32}N_2O_4$) C, H, N.

L-(Tr-glutaminol). To a stirred solution of CBZ-L-(Tr-glutaminol) (1.02 g, 2.0 mmol) in 25 mL of MeOH, under an argon atmosphere was cautiously added 10% Pd on carbon (60.0 mg). The reaction vessel was evacuated under vacuum and then put under an atmosphere of hydrogen using a balloon. The mixture was stirred for 2 h. At this time the hydrogen gas was evacuated and the catalyst was removed by filtration. The solvent was removed under vacuum giving an analytically pure white solid in quantitative yield: IR (KBr) 3255, 3057, 3016, 2916, 1642, 1527, 1491, 1446, 1057, 1036, 750, 700, 636 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.29 (m, 1H), 1.53 (m, 1H), 2.29 (m, 2H), 3.08 (m, 1H), 3.18 (m, 2H), 3.38 (bs, 2H), 4.43 (bs, 1H), 7.14−7.28 (m, 15H), 8.62 (s, 1H). Anal. ($C_{24}H_{26}N_2O_2$) C, H, N.

CBZ-L-Leu-L-Phe-L-(Tr-glutaminol). CBZ-Leu-Phe (0.825 g, 2.0 mmol) was dissolved in 8 mL of CH_2Cl_2 and 2 mL of DMF. To this stirred solution was added *N*-hydroxysuccinimide (0.23 g, 2.0 mmol). Once dissolved, *N*,*N*′-dicyclohexylcarbodiimide (DCC) (0.413 g, 2.0 mmol) was added, and the reaction was stirred at room temperature for 3 h. At this time the mixture was filtered directly into a separate flask containing L-(Tr-glutaminol) (0.75 g, 2.0 mmol) dissolved in 4 mL of 1:1 DMF:CH_2Cl_2, removing most the *N*,*N*′-dicyclohexylurea precipitate. The reaction mixture was allowed to stir overnight at room temperature. The solvents were removed under vacuum, and the resulting crude product was purified by flash chromatography (anhydrous NH_3/MeOH/$CHCl_3$, 0.5:4.5:9.5) on silica gel to give 1.33 g (86% yield) of a white solid: IR (KBr) 3302, 3057, 3032, 2951, 1954, 1885, 1657, 1520, 1238, 1045, 746, 698 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.79 (t, 6H, J = 7.0 Hz), 1.30 (m, 2H), 1.44 (m, 2H), 1.75 (m, 1H), 2.22 (m, 2H), 2.82 (m, 1H), 2.97 (m, 1H), 3.14 (m, 1H), 3.25 (m, 1H), 3.63 (m, 1H), 3.95 (m, 1H), 4.48 (m, 1H), 4.65 (t, 1H, J = 5.0 Hz), 4.96 (d, 1H, J = 13.0 Hz), 5.02 (d, 1H, J = 13.0 Hz), 7.07−7.33 (m, 25H), 7.42 (d, 1H, J = 8.0 Hz), 7.66 (d, 1H, J = 8.5 Hz), 7.86 (d, 1H, J = 8.0 Hz), 8.52 (s, 1H). Anal. ($C_{47}H_{52}N_4O_6 \cdot 0.5H_2O$) C, H, N.

CBZ-L-Leu-L-Phe-L-(Tr-glutaminal) (17). CBZ-L-Leu-L-Phe-L-(Tr-glutaminol) (0.723 g, 0.94 mmol) was dissolved in 14 mL of anhydrous DMSO and *o*-iodoxybenzoic acid (IBX)[13] (0.79 g, 2.82 mmol) was added. The clear reaction mixture becomes homogeneous after a few minutes and stirring at room temperature was continued for 3 h. The DMSO was removed under reduced pressure, and the residue was twice diluted with CH_2Cl_2 and reconcentrated to remove any residual DMSO. About 50 mL of EtOAc was added to the residue, and the resulting white precipitate was filtered off. The filtrate was diluted with 200 mL of EtOAc and washed sequentially with a 10% $Na_2S_2O_3$/10% $NaHCO_3$ solution, water, and brine before drying over Na_2SO_4. Removal of the organic solvent yielded 0.67 g (93%) of a white glassy solid after drying under vacuum at room temperature. No further purification was necessary: ^1H NMR (DMSO-d_6) δ 0.79 (t, 6H, J = 7.0 Hz), 1.00−1.98 (m, 5H), 2.27 (m, 2H), 2.84 (m, 1H), 3.02 (m, 1H), 3.98 (m, 2H), 4.58 (m, 1H), 4.99 (s, 2H), 7.14−7.32 (m, 25H), 7.39 (d, 1H, J = 8.0 Hz), 7.97 (d, 1H, J = 8.5 Hz), 8.38 (d, 1H, J = 8.0 Hz), 8.60 (s, 1H), 9.20 (s, 1H); HRMS calcd for $C_{47}H_{50}N_4O_6$ + H 767.3809 (M + H), found 767.3781. Anal. ($C_{47}H_{50}N_4O_6$) C, H, N.

CBZ-L-Leu-L-Phe-*N*-(6-oxo-1,4,5,6-tetrahydropyridin-3-yl)amide (19). {Attempts to prepare CBZ-L-Leu-L-Phe-L-glutaminal by the deprotection of CBZ-L-Leu-L-Phe-L-(Tr-glutaminal)}. To a stirred solution of aldehyde **17** (0.115 g, 0.15 mmol) dissolved in 4.0 mL of CH_2Cl_2 at 0 °C was added 0.3 mL of trifluoroacetic acid TFA. The mixture was allowed to warm to room temperature, and the progress of the reaction was followed by TLC (10% MeOH/$CHCl_3$). Upon disappearance of the starting material, the mixture was concentrated and the residue was dissolved in 100 mL of EtOAc. This solution was washed with 5% aqueous $NaHCO_3$, H_2O, and saturated aqueous NaCl and dried over Na_2SO_4. Filtration and concentration of the solution gave a oil which was triturated with Et_2O. The resultant precipitate was filtered and washed with 1:1 Et_2O:pet. ether and dried to give 40 mg of a white solid. ^1H NMR, FAB MS, and HPLC analysis indicated that the major product was the dehydrated dihydropyridone **19** contaminated with ∼18% of glutaminal **1**. This experiment was also repeated with an excess of the cation scavenger triisopropylsilane[16] in attempts to thwart acyliminium ion formation. However, similar results were observed: HPLC t_R = 6.7 min (**1**), t_R = 7.9 min (**19**); ^1H NMR (DMSO-d_6) δ 0.78 (d, 3H, J = 6.6 Hz), 0.81 (d, 3H, J = 6.6 Hz), 1.27 (m, 2H), 1.48 (m, 1H), 2.32 (m, 4H), 2.81 (dd, 1H, J = 14.0, 9.2 Hz), 2.95 (dd, 1H, J = 13.6, 5.5 Hz), 3.96 (m, 1H), 4.54 (m, 1H), 5.00 (s, 2H), 6.80 (d, 1H, J = 5.2 Hz), 7.21 (m, 5H), 7.34 (m, 5H), 7.39 (d, 1H, J = 8.5 Hz), 8.02 (d, 1H, J = 8.8 Hz), 8.76 (d, 1H, J = 5.2 Hz), 9.10 (s, 1H); MS calcd for (**19**), $C_{28}H_{34}N_4O_5$+Na 529, found 529; MS calcd for (**1**), $C_{28}H_{36}N_4O_6$+Na 547, found 547. Anal. ($C_{28}H_{34}N_4O_5$) C, H, N.

CBZ-L-Leu-L-Phe-L-Gln. The dipeptide CBZ-L-Leu-L-Phe (2.0 g, 4.85 mmol) was dissolved in 50 mL of CH_2Cl_2 and 2 mL of DMF. *N*-Hydroxysuccinimide (0.56 g, 4.85 mmol) was added followed by DCC (1.0 g, 4.85 mmol). After approximately 2 h of stirring at room temperature, the mixture was filtered directly into a solution of glutamine−sodium salt made by dissolving Gln (0.71 g, 4.85 mmol) in 20 mL of DMF and adding aqueous NaOH (0.194 g, 4.85 mmol, 2.0 mL H_2O) at 0 °C. The reaction mixture was stirred for 12 h at room temperature, and the solvents were removed under high vacuum. The residue was partitioned between 150 mL of 1 N HCl and 500 mL of EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give a residue that was purified by column chromatography (0.01% AcOH/5% MeOH/$CHCl_3$), yielding 66% of the tripeptide: ^1H NMR (DMSO-d_6) δ 0.78 (d, 3H, J = 6.3 Hz), 0.81 (d, 3H, J = 6.6 Hz), 1.29 (m, 2H), 1.46 (m, 1H), 1.80 (m, 1H), 1.99 (m, 1H), 2.15 (m, 2H), 2.78 (dd, 1H, J = 13.8, 9.4 Hz), 3.04 (dd, 1H, J = 14.0, 4.1 Hz), 3.98 (m, 1H), 4.17 (m, 1H), 4.55 (m, 1H), 5.00 (m, 2H), 6.78 (s, 1H), 7.13−7.35 (m, 11H), 7.39 (d, 1H, J = 8.5 Hz), 7.87 (d, 1H, J = 8.5 Hz), 8.26 (d, 1H, J = 7.7 Hz), 12.65 (bs, 1H); MS calcd for $C_{28}H_{36}N_4O_7$ + Na 563, found 563. Anal. ($C_{28}H_{36}N_4O_7$) C, H, N.

CBZ-L-Leu-L-Phe-L-glutaminimide (20). To CBZ-L-Leu-L-Phe-L-Gln (0.8 g, 1.48 mmol) dissolved in 3 mL of DMF was

added *N*-hydroxysuccinimide (0.18 g, 1.56 mmol), followed by DCC (0.32 g, 1.55 mmol). The reaction mixture was stirred at 80 °C for 3 h. Concentration of the solution under high vacuum and purification by column chromatography (5% MeOH/$CHCl_3$) gave the pure cyclized product in 91% yield as a white solid: IR (KBr) 3300, 3092, 3034, 2959, 2872, 1954, 1709, 1649, 1534, 1244, 1198, 1045, 740, 698 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.78 (d, 3H, J = 6.6 Hz), 0.82 (d, 3H, J = 6.6 Hz), 1.30 (m, 2H), 1.47 (m, 1H), 1.87 (m, 2H), 2.51 (m, 2H), 2.72 (q, 1H, J = 8.7 Hz), 2.85 (dd, 1H, J = 13.8, 9.0 Hz), 3.07 (dd, 1H, J = 13.6, 4.0 Hz), 3.96 (m, 1H), 4.56 (m, 2H), 4.97 (m, 2H), 7.15−7.35 (m, 10H), 7.40 (d, 1H, J = 8.5 Hz), 7.89 (d, 1H, J = 8.5 Hz), 8.36 (d, 1H, J = 8.5 Hz), 10.84 (s, 1H); MS calcd for $C_{28}H_{34}N_4O_6$ + Na 545 found 545. Anal. ($C_{28}H_{34}N_4O_6$) C, H, N.

CBZ-L-Leu-L-Phe-L-glutaminal-hemiaminal (1). To a solution of CBZ-L-Leu-L-Phe-L-glutaminimide, **20** (0.35 g, 0.67 mmol), in 10 mL of MeOH was added $NaBH_4$ (19 mg, 0.5 mmol). The reaction mixture was stirred at room temperature for 30 min and then poured into 100 mL of EtOAc and extracted with 25 mL of H_2O. The reaction was halted prematurely due to the appearance of more polar material by TLC (8% MeOH/$CHCl_3$), presumably alcohol from over-reduction. The EtOAc layer was dried over Na_2SO_4, concentrated, and purified by column chromatography (5% MeOH/$CHCl_3$) to give a white solid in 55% yield: ^1H NMR (DMSO-d_6) δ 0.78 (d, 3H, J = 6.3 Hz), 0.82 (d, 3H, J = 6.6 Hz), 1.35 (m, 2H), 1.51 (m, 2H), 1.98−2.18 (m, 3H), 2.76 (m, 1H), 2.98 (m, 1H), 3.86 (m, 2H), 4.54 (m, 2H), 5.01 (m, 2H), 5.88 (ddd, 1H, J = 10.7, 9.5, 5.9 Hz), 7.20 (m, 5H), 7.34 (m, 5H), 7.39 (d, 1H, J = 9.0 Hz), 7.86 (m, 2H), 8.04 (s, 1H); HRMS calcd for $C_{28}H_{36}N_4O_6$ + Na 547.2533, found 547.2544. Anal. ($C_{28}H_{36}N_4O_6 \cdot 0.7H_2O$) C, H, N.

Methyl N_α-CBZ-L-2,3-diaminopropionate Hydrochloride. In 100 mL of anhydrous MeOH, a stirred suspension of N_α-CBZ-L-2,3-diaminopropionic acid[17] (5.0 g, 21.0 mmol) was cooled to 0 °C. HCl gas was bubbled into the flask over a 5 min period, at which time the starting material dissolves. The reaction mixture was stirred for an additional 12 h at room temperature and then evaporated to dryness under vacuum. The resulting oil was triturated with MeOH, and the off-white solid which formed was collected by filtration and dried under vacuum to yield 6.0 g (99%) of a pure hygroscopic product: IR (KBr) 3312, 3038, 2953, 2876, 1736, 1692, 1599, 1541, 1308, 1265, 129, 1061, 1015, 754, 727, 700, 698 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.07 (dd, 1H, J = 13.1, 9.4 Hz), 3.18 (dd, 1H, J = 11.8, 7.4 Hz), 3.67 (s, 3H), 4.43 (m, 1H), 5.06 (s, 2H), 7.36 (m, 5H), 7.93 (d, 1H, J = 8.5 Hz), 8.28 (bs, 3H). Anal. ($C_{12}H_{17}N_2O_4Cl$) C, H, N, Cl.

CBZ-L-(*N*-formylamino-Ala)-OMe. Under anhydrous conditions, NEt_3 (3.7 g, 36.6 mmol) was added dropwise to a stirred solution of methyl N_α-CBZ-L-2,3-diaminopropionate hydrochloride (4.8 g, 16.6 mmol) in 150 mL of methyl formate. The mixture was stirred at room temperature for 12 h and then filtered. The filtrate was concentrated and purified by column chromatography (2% MeOH/$CHCl_3$) to afford the product in 78% yield: ^1H NMR ($CDCl_3$) δ 3.75 (m, 2H), 3.78 (s, 3H), 4.46 (m, 1H), 5.12 (s, 2H), 5.75 (m, 1H), 5.99 (m, 1H), 7.36 (m, 5H), 8.17 (s, 1H).

L-(*N*-Formylamino-Ala)-OMe: oil (99%); ^1H NMR (CD_3OD) δ 3.68 (dd, 1H, J = 14.7, 5.5 Hz), 3.85 (dd, 1H, J = 14.5, 4.60 Hz), 3.86 (s, 3H), 4.23 (t, 1H, J = 5.0 Hz), 8.15 (s, 1H); MS calcd for $C_5H_{10}N_2O_3$ + H 147, found 147.

CBZ-L-Leu-L-Phe-L-(*N*-formylamino-Ala)-OMe: white solid (62%) (2% MeOH/$CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (d, 3H, J = 6.3 Hz), 0.90 (d, 3H, J = 6.6 Hz), 1.35 (m, 1H), 1.53 (m, 2H), 3.03 (dd, 1H, J = 14.0, 8.1 Hz), 3.17 (dd, 1H, J = 14.0, 5.2 Hz), 3.66 (m, 1H), 3.75 (s, 3H), 4.07 (m, 1H), 4.59 (m, 1H), 4.64 (m, 1H), 5.08 (m, 4H), 6.59 (m, 2H), 7.11−7.36 (m, 11H), 8.05 (s, 1H); MS calcd for $C_{28}H_{36}N_4O_7$ + H 541, found 541.

CBZ-L-Leu-L-Phe-L-(*N*-formylamino-alaninol): white solid (75%) (3% MeOH/$CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (t, 6H, J = 7.4 Hz), 1.46 (m, 2H), 1.59 (m, 1H), 3.03 (m, 2H), 3.32 (m, 2H), 3.42 (m, 1H), 3.55 (m, 1H), 3.86 (m, 1H), 4.32 (m, 1H), 4.90 (m, 1H), 5.08 (m, 2H), 5.92 (m, 2H), 7.10−7.38 (m, 11H), 7.69 (m, 1H), 8.05 (s, 1H); MS calcd for $C_{27}H_{36}N_4O_6$ + H 513, found 513.

CBZ-L-Leu-L-Phe-L-(*N*-formylamino-alaninal) (2). CBZ-L-Leu-L-Phe-L-(*N*-formylamino-alaninol) (0.39 g, 0.76 mmol) in 2 mL of 1:1 DMSO:CH_2Cl_2 was treated with Et_3N (0.24 g, 2.3 mmol) and cooled to 0 °C. A solution of SO_3-pyridine[13] (0.37 g, 2.3 mmol) in 2 mL of 1:1 DMSO:CH_2Cl_2 (1:1) was added dropwise, and the mixture was stirred for 2 h after it was allowed to reach room temperature. The reaction mixture was then diluted with CH_2Cl_2, extracted with 10% citric acid and then saturated NaCl, dried over Na_2SO_4, and concentrated to give a residue that was purified by column chromatography (2% MeOH/$CHCl_3$). The solid white product was isolated predominantly as the stable methyl hemiacetal in 70% yield: IR (KBr) 3320, 2970, 1653, 1540, 1266, 1051, 704 cm^{-1}; ^1H NMR (CD_3OD) δ 0.90 (m, 6H), 1.37 (m, 2H), 1.54 (m, 1H), 2.96 (m, 1H), 3.22 (m, 2H), 3.34 (s, 3H), 3.62 (m, 2H), 4.03 (m, 3H), 4.91 (m, 2H), 4.59 (m, 2H), 5.11 (m, 2H), 7.18−7.39 (m, 10H), 8.02 (s, 1H); HRMS calcd for $C_{27}H_{34}N_4O_6$ + H 511.2556, found 511.2554. Anal. of methyl hemiacetal ($C_{28}H_{38}N_4O_7 \cdot 0.5\ H_2O$) C, H, N.

CBZ-L-(*N*-Ac-amino-Ala). In 50 mL of H_2O, N_α-CBZ-L-2,3-diaminopropionic acid[17] (1.5 g, 6.3 mmol) was suspended with stirring. To this suspension acetic anhydride (5.0 mL) was added slowly over a 30 min period, at which time the starting material dissolves. The reaction mixture was stirred for an additional 1 h at room temperature and then evaporated to dryness under vacuum. The resulting oil was dissolved in 30 mL of $CHCl_3$ and left for 12 h. The solid which forms was collected by filtration, washed with 30 mL of $CHCl_3$, and dried, yielding 1.29 g (73%) of product as a white solid: IR (KBr) 3271, 3125, 3065, 1734, 1703, 1614, 1545, 1289, 1244, 1053, 727 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.84 (s, 3H), 3.20−3.55 (m, 2H), 4.13 (m, 1H), 5.08 (s, 2H), 7.41 (m, 5H), 7.54 (d, 1H, J = 8.1 Hz), 8.02 (bt, 1H, J = 5.5 Hz), 12.78 (bs, 1H). Anal. ($C_{13}H_{16}N_2O_5$) C, H, N.

CBZ-L-(*N*-Ac-amino-Ala)-OMe. HCl gas was slowly bubbled into a stirred suspension of CBZ-L-*N*-Ac-amino-Ala (1.21 g, 4.3 mmol) in MeOH (43 mL) at 0 °C until the solids were dissolved. Stirring was continued for 30 min at 0 °C whereupon the methanol HCl was carefully evaporated to dryness. The methyl ester was formed as a white solid in quantitative yield and used without further purification: IR (KBr) 3324, 3285, 3094, 2957, 1755, 1736, 1686, 1651, 1531, 1277, 1057, 736, 600 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.78 (s, 3H), 3.22−3.47 (m, 2H), 3.61 (s, 3H), 4.15 (m, 1H), 5.02 (s, 2H), 7.35 (m, 5H), 7.64 (d, 1H, J = 7.7 Hz), 7.97 (bt, 1H, J = 6.3 Hz). Anal. ($C_{14}H_{18}N_2O_5$) C, H, N.

CBZ-L-(*N*-Ac-amino-alaninol). To a solution of CBZ-L-(*N*-Ac-amino)-Ala-OMe (1.8 g, 6.12 mmol) in 50 mL of anhydrous THF/EtOH (2:1) was added LiCl (0.52 g, 12.24 mmol). Upon dissolution, $NaBH_4$ (0.46 g, 12.24 mmol) was added, and the mixture was stirred at room temperature for 12 h. The reaction mixture was evaporated to near dryness, and then 45 mL of H_2O was added. The pH of this mixture was adjusted to 2−3 using concentrated HCl, followed by extraction with EtOAc (300 mL). The organic layer was washed with H_2O (50 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (10% MeOH/$CHCl_3$) to give 1.38 g (85%) of a white solid: IR (KBr) 3303, 3082, 2951, 2926, 1689, 1645, 1547, 1284, 1061, 1046, 756, 698 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.78 (s, 3H), 3.03 (m, 1H), 3.16−3.28 (m, 3H), 3.49 (m, 1H), 4.67 (t, 1H, J = 5.7 Hz), 5.00 (s, 2H), 6.95 (d, 1H, J = 8.1 Hz), 7.29−7.38 (m, 5H), 7.83 (bt, 1H, J = 5.5 Hz). Anal. ($C_{13}H_{18}N_2O_4$) C, H, N.

L-(*N*-Ac-amino-alaninol). To a solution of CBZ-L-(*N*-Ac-amino)-alaninol (1.36 g, 5.11 mmol) in 40 mL of MeOH, with stirring and under an argon atmosphere, was added 10% Pd on carbon (0.15 g). The reaction vessel was evacuated under vacuum and then put under an atmosphere of hydrogen using a balloon. The mixture was stirred for 2 h. At this time the hydrogen gas was evacuated and the catalyst was removed by filtration. The solvent was removed under vacuum. Ad-

dition of EtOAc and reconcentration gave a white hygroscopic solid in quantitative yield which was used without further purification: mp 80−82 °C; ^{1}H NMR (DMSO-d_6) δ 1.79 (s, 3H), 2.66 (m, 1H), 2.86 (m, 1H), 3.06 (m, 1H), 3.21 (2H, m), 3.4 (bs, 2H), 4.55 (bs, 1H), 7.76 (bs, 1H). Anal. ($C_5H_{12}N_2O_2$) C, H, N.

CBZ-L-Leu-L-Phe-L-(*N*-Ac-amino-alaninol). This compound was prepared from CBZ-L-Leu-L-Phe and L-(*N*-Ac-amino)-alaninol as described previously. The compound was purified by column chromatography (7% MeOH/$CHCl_3$) to give a white solid (81%): IR (KBr) 3302, 2955, 1694, 1651, 1539, 1454, 1236, 1047, 698 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.79 (d, 3H, J = 6.6 Hz), 0.82 (d, 3H, J = 6.6 Hz), 1.32 (m, 2H), 1.47 (m, 1H), 1.79 (s, 3H), 2.81 (m, 1H), 2.97 (m, 2H), 3.14−3.25 (m, 3H), 3.71 (m, 1H), 3.95 (m, 1H), 4.42 (m, 1H), 4.67 (t, 1H, J = 5.5 Hz), 5.00 (m, 2H), 7.16−7.34 (m, 10H), 7.45 (d, 1H, J = 8.1 Hz), 7.70 (m, 2H), 7.88 (d, 1H, J = 8.1 Hz). Anal. ($C_{28}H_{38}N_4O_6$) C, H, N.

CBZ-L-Leu-L-Phe-L-(*N*-Ac-amino-alaninal) (3). This compound was prepared in 73% yield as a white solid by the oxidation of CBZ-L-Leu-L-Phe-L-(*N*-Ac-amino)-alaninol with IBX as described previously. The product exist as a mixture of aldehyde and aldehyde hydrate: IR (KBr) 3294, 2957, 1695, 1649, 1539, 1263, 698 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.81 (dd, 6H, J = 8.8, 6.2 Hz), 1.31 (m, 2H), 1.50 (m, 1H), 1.78 (s, 3H), 2.83 (m, 1H), 3.00 (m, 1H), 3.35 (m, 1H), 3.97 (m, 2H), 4.16 (m, 1H), 4.54 (m, 1H), 5.01 (s, 2H), 6.28 (d, J = 7.0 Hz, hydrate), 6.41 (d, J = 6.6 Hz, hydrate), 7.12−7.50 (m, 10H), 7.63 (t, 1H, J = 7.9 Hz), 7.87 (m, 1H), 7.98 (d, 1H, J = 8.1 Hz), 8.40 (d, 1H, J = 7.0 Hz), 9.26 (s, 1H); HRMS calcd for $C_{28}H_{36}N_4O_6$ + H 523.2713, found 523.2725. Anal. ($C_{28}H_{36}N_4O_6 \cdot 0.5H_2O$) C, H, N.

CBZ-L-(*N*-propionylamino-Ala). Using the procedure to prepare CBZ-L-(*N*-Ac-amino-Ala), this intermediate was prepared as a white solid in 98% yield from N_α-CBZ-L-2,3-diaminopropionic acid and propionic anhydride: ^{1}H NMR ($CDOD_3$) δ 1.07 (t, 3H, J = 7.5 Hz), 2.15 (q, 2H, J = 7.7 Hz), 3.48 (dd, 1H, J = 13.8, 7.2 Hz), 3.60 (dd, 1H, J = 13.6, 4.4 Hz), 4.36 (t, 1H, J = 6.1 Hz), 5.04 (d, 1H, J = 12.5 Hz), 5.09 (d, 1H, J = 12.5 Hz), 7.32 (m, 5H), 8.01 (m, 1H).

L-(*N*-Propionylamino-Ala)·AcOH. CBZ-L-(*N*-propionylamino-Ala) was exposed to hydrogenation conditions as described previously except that a 6:3:1 AcOH:MeOH:H_2O solvent system was used instead of MeOH. The hydroscopic white solid was isolated in 75% yield: ^{1}H NMR ($CDOD_3$) δ 1.12 (t, 3H, J = 7.5 Hz), 1.95 (s, 3H), 2.25 (q, 2H, J = 7.6 Hz), 3.57 (m, 1H), 3.72 (m, 1H).

CBZ-L-Leu-L-Phe-L-(*N*-propionylamino-Ala). This intermediate was prepared from CBZ-L-Leu-L-Phe and L-(*N*-propionylamino-Ala)·AcOH using the procedure described to prepared CBZ-L-Leu-L-Phe-L-Gln except 2.0 equiv of NaOH was used: white solid (93%); ^{1}H NMR ($CDOD_3$) δ 0.84 (d, 3H, J = 6.3 Hz), 0.88 (d, 3H, J = 6.6 Hz), 1.08 (t, 3H, J = 7.7 Hz), 1.39 (m, 2H), 1.58 (m, 1H), 2.18 (q, 2H, J = 7.6 Hz), 2.95 (dd, 1H, J = 14.0, 9.5 Hz), 3.20 (dd, 1H, J = 13.8, 5.0 Hz), 3.45 (dd, 1H, J = 13.8, 7.9 Hz), 3.66 (dd, 1H, J = 13.8, 4.6 Hz), 4.07 (m, 1H), 4.48 (dd, 1H, J = 7.4, 4.4 Hz), 4.57 (dd, 1H, J = 9.2, 5.1 Hz), 5.07 (m, 2H), 7.12−7.34 (m, 12H), 7.80 (m, 1H), 8.12 (d, 1H, J = 7.7 Hz); MS calcd for $C_{29}H_{38}N_4O_7$ + H 555, found 555.

CBZ-L-Leu-L-Phe-L-(*N*-propionylamino-Ala)-OMe. To a solution of CBZ-L-Leu-L-Phe-L-(*N*-propionylamino-Ala) (0.93 g, 1.68 mmol) in 10 mL of benzene and 20 mL of MeOH at 0 °C was added (trimethylsilyl)diazomethane (2.0 mL, 2.0 M solution in hexanes). The stirred solution was allowed to warm to room temperature and then concentrated after 15 min. The residue was purified by column chromatography (5% MeOH/$CHCl_3$) to give a 97% yield of the solid white ester: ^{1}H NMR ($CDCl_3$) δ 0.83 (d, 3H, J = 6.6 Hz), 0.85 (d, 3H, J = 6.6 Hz), 1.09 (t, 3H, J = 7.5 Hz), 1.39 (m, 1H), 1.54 (m, 2H), 2.15 (q, 2H, J = 7.6 Hz), 2.95 (dd, 1H, J = 14.0, 8.1 Hz), 3.16 (dd, 1H, J = 14.0, 5.9 Hz), 3.51 (dt, 1H, J = 13.8, 5.0 Hz), 3.66 (m, 1H), 3.70 (s, 3H), 4.11 (m, 1H), 4.52 (m, 1H), 4.61 (m, 1H), 5.04 (d, 1H, J = 11.8 Hz), 5.09 (d, 1H, J = 11.8 Hz), 5.33 (m, 1H), 6.47 (m, 1H), 6.82 (bt, 1H, J = 6.4 Hz), 7.14−7.38 (m, 11H).

CBZ-L-Leu-L-Phe-L-(*N*-propionylamino-alaninol). Using the general $LiBH_4$ reduction procedure, CBZ-L-Leu-L-Phe-L-(*N*-propionylamino-Ala)-OMe was converted to the solid white alcohol intermediate in 52% yield (5% MeOH/$CHCl_3$): ^{1}H NMR ($CDOD_3$) δ 0.85 (d, 3H, J = 6.6 Hz), 0.89 (d, 3H, J = 6.6 Hz), 1.10 (t, 3H, J = 7.7 Hz), 1.41 (m, 2H), 1.57 (m, 1H), 2.19 (q, 2H, J = 7.6 Hz), 2.96 (dd, 1H, J = 13.8, 9.0 Hz), 3.16 (m, 2H), 3.34 (m, 2H), 3.42 (dd, 1H, J = 13.6, 4.8 Hz), 3.93 (m, 1H), 4.06 (dd, 1H, J = 5.0, 4.2 Hz), 4.48 (dd, 1H, J = 8.8, 6.4 Hz), 5.06 (d, 1H, J = 12.5 Hz), 5.11 (d, 1H, J = 12.1 Hz), 7.15−7.35 (m, 10H); MS calcd for $C_{29}H_{40}N_4O_6$ + H 541 found 541.

CBZ-L-Leu-L-Phe-L-(*N*-propionylamino-alaninal) (4). Following the general SO_3−pyridine procedure to oxidize CBZ-L-Leu-L-Phe-L-(*N*-propionylamino-alaninol), the white solid aldehyde was isolated in 53% yield (2% MeOH/$CHCl_3$): IR (KBr) 3308, 2961, 1653, 1541 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.87 (d, 3H, J = 6.3 Hz), 0.89 (d, 3H, J = 6.3 Hz), 1.10 (t, 3H, J = 7.7 Hz), 1.40 (m, 2H), 1.53 (m, 1H), 2.15 (q, 2H, J = 7.6 Hz), 3.02 (dd, 1H, J = 14.0, 8.1 Hz), 3.15 (dd, 1H, J = 13.6, 7.0 Hz), 3.51 (dt, 1H, J = 14.3, 5.1 Hz), 3.72 (m, 1H), 4.09 (m, 1H), 4.34 (m, 1H), 4.65 (m, 1H), 5.08 (m, 2H), 5.31 (d, 1H, J = 4.8 Hz), 6.55 (m, 1H), 6.85 (d, 1H, J = 7.7 Hz), 7.16−7.35 (m, 11H), 9.48 (s, 1H); HRMS calcd for $C_{29}H_{38}N_4O_6$ + H 539.2870, found 539.2896. Anal. ($C_{29}H_{38}N_4O_6 \cdot 1.0H_2O$) C, H, N.

CBZ-L-(*N*-isobutyrylamino-Ala). Using the procedure to prepare CBZ-L-(*N*-Ac-amino-Ala), this intermediate was prepared as a white solid in 95% yield from N_α-CBZ-L-2,3-diaminopropionic acid and isobutyric anhydride: ^{1}H NMR ($CDOD_3$) δ 1.04 (d, 6H, J = 7.0 Hz), 2.35 (sept, 1H, J = 7.0 Hz), 3.42 (dd, 1H, J = 13.5, 7.2 Hz), 3.58 (dd, 1H, J = 13.6, 4.5 Hz), 4.34 (dd, 1H, J = 7.5, 4.5 Hz), 5.02 (d, 1H, J = 12.5 Hz), 5.07 (d, 1H, J = 12.5 Hz), 7.34 (m, 5H).

L-(*N*-Isobutyrylamino-Ala)·AcOH. CBZ-L-(*N*-isobutyrylamino-Ala) was exposed to hydrogenation conditions as describe previously using a 6:3:1 AcOH:MeOH:H_2O solvent system instead of MeOH. The hydroscopic white solid was isolated in 95% yield: ^{1}H NMR ($CDOD_3$) δ 0.80 (d, 6H, J = 7.0 Hz), 1.70 (s, 3H), 2.15 (sept, 1H, J = 7.0 Hz), 3.18 (m, 1H), 3.35 (m, 2H).

CBZ-L-Leu-L-Phe-L-(*N*-isobutyrylamino-Ala). This intermediate was prepared from CBZ-L-Leu-L-Phe and L-(*N*-isobutyrylamino-Ala)·AcOH using the procedure described to prepared CBZ-L-Leu-L-Phe-L-Gln except 2.0 equiv of NaOH was used: white solid (94%); ^{1}H NMR ($CDOD_3$) δ 0.84 (d, 3H, J = 6.3 Hz), 0.88 (d, 3H, J = 6.6 Hz), 1.04 (d, 6H, J = 7.0 Hz), 1.36 (m, 2H), 1.55 (m, 1H), 2.37 (sept, 1H, J = 7.0 Hz), 2.90 (dd, 1H, J = 13.8, 8.5 Hz), 3.18 (dd, 1H, J = 13.4, 4.7 Hz), 3.40 (m, 1H), 3.61 (m, 1H), 4.03 (m, 1H), 4.44 (m, 1H), 4.52 (m, 1H), 5.00 (m, 2H), 7.02−7.32 (m, 11H), 7.75 (m, 1H), 8.06 (m, 2H).

CBZ-L-Leu-L-Phe-L-(*N*-isobutyrylamino-Ala)-OMe. The methyl ester was generated from CBZ-L-Leu-L-Phe-L-(*N*-isobutyrylamino-Ala) and (trimethylsilyl)diazomethane as described for CBZ-L-Leu-L-Phe-L-(*N*-propionylamino-Ala)-OMe in 90% yield as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 6H, J = 6.3 Hz), 1.00 (d, 6H, J = 6.3 Hz), 1.39 (m, 1H), 1.56 (m, 2H), 2.34 (sept, 1H, J = 7.0 Hz), 3.04 (dd, 1H, J = 13.8, 8.1 Hz), 3.54 (m, 1H), 3.70 (m, 1H), 3.73 (s, 3H), 4.09 (m, 1H), 4.52 (m, 1H), 4.57 (m, 1H), 5.06 (m, 2H), 6.32 (m, 1H), 6.53 (d, 1H, J = 6.6 Hz), 7.07 (d, 1H, J = 5.5 Hz), 7.19 (t, 1H, J = 7.4 Hz), 7.23−7.36 (m, 10H).

CBZ-L-Leu-L-Phe-L-(*N*-isobutyrylamino-alaninol). Using the general $LiBH_4$ reduction procedure, CBZ-L-Leu-L-Phe-L-(*N*-isobutyrylamino-Ala)-OMe was converted to the solid white alcohol intermediate in 86% yield (5% MeOH/$CHCl_3$): ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, J = 6.8 Hz), 1.14 (d, 6H, J = 6.6 Hz), 1.40−1.65 (m, 3H), 2.39 (sept, 1H, J = 6.6 Hz), 3.05 (m, 2H), 3.18 (m, 1H), 3.26 (m, 1H), 3.40 (m, 1H), 3.51 (m, 1H), 3.85 (m, 1H), 4.14 (m, 2H), 4.68 (m, 1H), 5.07 (m, 2H), 5.37 (d, 1H, J = 6.3 Hz), 6.67 (d, 1H, J = 8.1 Hz), 6.75 (m, 1H), 7.08 (d, 1H, J = 6.6 Hz), 7.14−7.38 (m, 10H).

CBZ-L-Leu-L-Phe-L-(*N*-isobutyrylamino-alaninal) (5). Following the IBX oxidation procedure, the solid white aldehyde product was generated in 80% yield from

CBZ-L-Leu-L-Phe-L-(*N*-isobutyrylamino-alaninol): IR (KBr) 3297, 3069, 2963, 1696, 1647, 1541, 1264, 1238, 1047, 698 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.79 (t, 6H, J = 6.3 Hz), 0.92 (d, 6H, J = 6.6 Hz), 1.28 (m, 2H), 1.49 (m, 1H), 2.25 (sept, 1H, J = 6.6 Hz), 2.80 (m, 1H), 2.95 (m, 2H), 3.18 (m, 1H), 3.80 (m, 1H), 3.95 (m, 1H), 4.42 (m, 1H), 4.99 (m, 2H), 6.25 (d, J = 7.0 Hz, hydrate), 6.38 (d, J = 6.6 Hz, hydrate), 7.16−7.38 (m, 10H), 7.42 (d, 1H, J = 6.5 Hz), 7.60 (m, 2H), 7.84 (t, 1H, J = 6.0 Hz), 9.18 (s, 1H); MS calcd for $C_{30}H_{40}N_4O_6$ + H 553 found 553. Anal. ($C_{30}H_{40}N_4O_6 \cdot 1.1H_2O$) C, H, N.

CBZ-L-(*N*-(trifluoroacetyl)amino-alaninol). Using the general procedure, methyl N_α-CBZ-L-2,3-diaminopropionate (2.0 g, 7.94 mmol; generated from the HCl salt by liberation of the free base with 10% aqueous $NaHCO_3$ and $CHCl_3$ extraction) was reduced with $LiBH_4$. A modified workup using neutral conditions (pH = 7.5), $CHCl_3$ (3 × 200 mL) extractions, drying (Na_2SO_4), and concentration gave 1.69 g of crude N_α-CBZ-L-2,3-diaminopropanol. The solid was dissolved in 50 mL of EtOH, and NEt_3 (0.77 g, 7.61 mmol) followed by ethyl trifluoroacetate (6.0 g, 42.2 mmol) was added. The stirred reaction mixture was heated to 60 °C for 2 h. The mixture was concentrated under vacuum, and the residue was purified by column chromatography (5% MeOH/$CHCl_3$) to give a white solid in 68% (two steps): IR (KBr) 3337, 3304, 3108, 2961, 1690, 1541, 1287, 1182, 1065, 731, 696 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.23 (m, 1H), 3.38 (m, 3H), 3.68 (m, 1H), 4.79 (t, 1H, J = 5.5 Hz), 5.00 (m, 2H), 7.05 (d, 1H, J = 8.8 Hz), 7.33 (m, 5H), 9.37 (m, 1H). Anal. ($C_{13}H_{15}F_3N_2O_4$) C, H, N.

CBZ-L-Leu-L-Phe-(*N*-(trifluoroacetyl)amino-alaninol): white solid, 82%; (5% MeOH/$CHCl_3$); IR (KBr) 3291, 3067, 2957, 1707, 1647, 1541, 1454, 1215, 1046, 698 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.78 (d, 3H, J = 6.6 Hz), 0.82 (d, 3H, J = 6.6 Hz), 1.31 (m, 2H), 1.48 (m, 1H), 2.80 (d, 1H, J = 14.0, 8.5 Hz), 2.97 (d, 1H, J = 13.6, 5.5 Hz), 3.20 (m, 1H), 3.31 (m, 3H), 3.91 (m, 2H), 4.45 (m, 1H), 4.80 (t, 1H, J = 5.5 Hz), 4.97 (d, 1H, J = 12.9 Hz), 5.03 (d, 1H, J = 12.5 Hz), 7.18 (m, 5H), 7.34 (m, 5H), 7.44 (d, 1H, J = 8.5 Hz), 7.80 (d, 1H, J = 8.5 Hz), 7.86 (d, 1H, J = 8.1 Hz), 9.26 (t, 1H, J = 6.3 Hz). Anal. ($C_{28}H_{35}F_3N_4O_6$) C, H, N.

CBZ-L-Leu-L-Phe-(*N*-trifluoroacetyl)amino-alaninal) (6): white solid, 69%; ^1H NMR (DMSO-d_6) δ 0.80 (m, 6H), 1.29 (m, 2H), 1.47 (m, 1H), 2.81 (m, 1H), 2.99 (m, 1H), 3.42 (m, 2H), 3.98 (m, 2H), 4.56 (m, 1H), 5.00 (m, 2H), 7.21 (m, 5H), 7.34 (m, 5H), 7.98 (m, 2H), 8.66 (d, 1H, J = 7.4 Hz), 9.35 (s, 1H), 9.41 (m, 1H). HRMS calcd for $C_{28}H_{33}F_3N_4O_6$ + Cs 711.1407, found 711.1375. Anal. ($C_{28}H_{33}F_3N_4O_6$) C, H, N.

CBZ-L-(*N*-benzoylamino-Ala)-OMe: white solid, 88%; IR (KBr) 3286, 3061, 2946, 1748, 1696, 1640, 1536, 1267, 1026, 698 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.55 (m, 2H), 3.61 (s, 3H), 4.32 (m, 1H), 5.00 (d, 1H, J = 12.5 Hz), 5.05 (d, 1H, J = 12.5 Hz), 7.33 (m, 5H), 7.49 (m, 3H), 7.79 (d, 2H, J = 7.0 Hz), 8.55 (t, 1H, J = 5.7 Hz). Anal. ($C_{19}H_{20}N_2O_5$) C, H, N.

CBZ-L-(*N*-benzoylamino-alaninol): white solid, 97%; IR (KBr) 3290, 3074, 3028, 2957, 2930, 1688, 1640, 1541, 1329, 1256, 1062, 1040, 696 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.27−3.44 (m, 4H), 3.73 (m, 2H), 4.96 (d, 1H, J = 12.9 Hz), 5.02 (d, 1H, J = 12.9 Hz), 7.03 (d, 1H, J = 8.5 Hz), 7.30 (m, 5H), 7.39−7.54 (m, 3H), 7.81 (d, 2H, J = 7.0 Hz), 8.43 (t, 1H, J = 5.3 Hz). Anal. ($C_{18}H_{20}N_2O_4$) C, H, N.

L-(*N*-Benzoylamino-alaninol): white solid, 98%; IR (KBr) 3355, 3297, 3178, 3088, 3028, 2926, 2845, 1632, 1543, 1487, 1318, 1065, 966, 698, 677 cm^{-1}; ^1H NMR (DMSO-d_6/D_2O) δ 2.82 (quin, 1H, J = 5.9 Hz), 3.12 (dd, 1H, J = 13.1, 6.8 Hz), 3.24 (dd, 1H, J = 10.7, 5.9 Hz), 3.31 (m, 2H), 7.41−7.56 (m, 3H), 7.82 (d, 2H, J = 7.0 Hz). Anal. ($C_{10}H_{14}N_2O_2$) C, H, N.

CBZ-L-Leu-L-Phe-L-(*N*-benzoylamino-alaninol): white solid, 60%; IR (KBr) 3277, 3171, 3065, 2955, 1690, 1640, 1539, 1231, 1036, 746, 698 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.78 (t, 6H, J = 7.2 Hz), 1.31 (m, 2H), 1.48 (m, 1H), 2.85 (dd, 1H, J = 13.2, 4.8 Hz), 2.98 (dd, 1H, J = 13.6, 5.2 Hz), 3.21−3.38 (m, 4H), 3.46 (m, 1H), 3.94 (m, 2H), 4.24 (m, 1H), 4.97 (d, 1H, J = 12.9 Hz), 5.03 (d, 1H, J = 12.9 Hz), 7.18 (m, 5H), 7.33 (m, 5H), 7.48 (m, 4H), 7.79 (m, 3H), 7.93 (d, 1H, J = 8.1 Hz), 8.32 (t, 1H, J = 6.3 Hz). Anal. ($C_{33}H_{40}N_4O_6$) C, H, N.

CBZ-L-Leu-L-Phe-L-(*N*-benzoylamino-alaninal) (7): white solid, 88%; IR (KBr) 3202, 3057, 2957, 2928, 1709, 1690, 1645, 1535, 1235, 1128, 698 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.79 (t, 6H, J = 7.4 Hz), 1.31 (m, 2H), 1.49 (m, 1H), 2.86 (dd, 1H, J = 13.8, 9.0 Hz), 3.02 (dd, 1H, J = 14.0, 5.5 Hz), 3.60 (m, 2H), 3.99 (m, 1H), 4.31 (m, 1H), 4.55 (m, 1H), 5.00 (s, 2H), 7.19 (m, 5H), 7.33 (m, 5H), 7.38−7.55 (m, 4H), 7.80 (d, 2H, J = 8.0 Hz), 8.01 (d, 1H, J = 8.1 Hz), 8.51 (t, 1H, J = 5.7 Hz), 8.55 (d, 1H, J = 6.6 Hz), 9.33 (s, 1H); HRMS calcd for $C_{33}H_{38}N_4O_6$ + Na 609.2689, found 609.2667. Anal. ($C_{33}H_{38}N_4O_6 \cdot 1.0H_2O$) C, H, N.

CBZ-L-[*N*-(Me)Ac-amino-Ala]-OMe. By applying the procedure of Grieco,[18] methyl N_α-CBZ-L-2,3-diaminopropionate hydrochloride (2.5 g, 8.67 mmol) was dissolved in 100 mL of H_2O, followed by 3.6 mL of 37% aqueous formaldehyde and freshly prepared cyclopentadiene (2.87 g, 43.4 mmol). The reaction mixture was stirred at room temperature for 12 h and then extracted with 300 mL of EtOAc. The organic phase was washed with 10% aqueous $NaHCO_3$, dried over $MgSO_4$, concentrated, and purified by column chromatography (5% MeOH/$CHCl_3$) to give a 3:2 diastereomeric mixture of the azanorbornene intermediate **21** in 74% yield. ^1H NMR (DMSO-d_6) δ 1.25 (m, 1H), 1.37 (m, 2H), 2.23 (m, 1H), 2.69, 2.80 (2 dd, 1H, J = 12.3, 9.0 Hz, J = 12.1, 6.6 Hz), 2.87 (bs, 1H), 3.05 (m, 1H), 3.60 (s, 3H), 3.76 (bs, 1H), 4.06 (m, 1H), 5.02 (s, 2H), 6.00 (m, 1H), 6.30 (m, 1H), 7.35 (m, 5H), 7.55, 7.60 (2 d, 1H, J = 8.5 Hz, J = 7.4 Hz).

Intermediate **21** (1.5 g, 4.55 mmol) was dissolved in 100 mL of 1:1 TFA:$CHCl_3$. To this stirred solution was added Et_3SiH (1.59 g, 13.7 mmol). After 2 h of stirring at room temperature, the solution was concentrated under vacuum and the residue was taken up in 200 mL of $CHCl_3$ and washed with 100 mL of 10% aqueous $NaHCO_3$. The organic layer was dried over $NaSO_4$, filtered, and concentrated to give 1.09 g (95%) of the crude CBZ-L-[*N*-Me-amino-Ala]-OMe which was used immediately. The amine was dissolved in 30 mL of 1:1 pyridine:AcOAc and stirred at room temperature for 12 h. At this time the mixture was pour into 100 mL of 1 N HCl and then extracted with 200 mL of EtOAc. The organic phase was washed with 100 mL of 10% aqueous $NaHCO_3$ and 100 mL of H_2O and dried over Na_2SO_4. Filtration, concentration, and purification of the residue by column chromatography (3% MeOH/$CHCl_3$) afforded the product as a white solid in 89% yield: ^1H NMR (DMSO-d_6) δ 1.92 (s, 3H), 2.75, 2.93 (2s, 3H, rotomers), 3.26 (dd, 1H, J = 13.4, 8.3 Hz), 3.61, 3.65 (2s, 3H, rotomers), 3.81 (dd, 1H, J = 13.4, 6.4 Hz), 4.33 (m, 1H), 5.03 (m, 2H), 7.34 (m, 5H), 7.73, 7.92 (2 d, 1H, rotomers, J = 8.1 Hz, J = 8.5 Hz). Anal. ($C_{15}H_{20}N_2O_5 \cdot 0.3H_2O$) C, H, N.

CBZ-L-[*N*-(Me)Ac-amino-alaninol]: white solid, 78%; (5% MeOH/$CHCl_3$); ^1H NMR (DMSO-d_6) δ 1.91, 1.97 (2s, 3H, rotomers), 2.76, 2.93 (2s, 3H, rotomers), 3.19−3.48 (m, 4H), 3.69 (m, 1H), 4.68, 4.85 (2t, 1H, rotomers, J = 5.7 Hz, J = 5.5 Hz), 4.99 (m, 2H), 6.94, 7.20 (2d, 1H, rotomers, J = 8.5 Hz, J = 9.2 Hz), 7.33 (m, 5H). Anal. ($C_{14}H_{20}N_2O_4 \cdot 0.5H_2O$) C, H, N.

CBZ-L-Leu-L-Phe-[*N*-(Me)Ac-amino-alaninol]: white solid, 48%; (5% MeOH/$CHCl_3$); ^1H NMR (DMSO-d_6) δ 0.79 (d, 3H, J = 6.6 Hz), 0.82 (d, 3H, J = 6.6 Hz), 1.28 (m, 2H), 1.46 (m, 1H), 1.90, 1.97 (2s, 3H, rotomers), 2.75, 2.90 (2s, 3H, rotomers), 2.80−2.98 (m, 2H), 3.20 (m, 4H), 3.44 (dd, 1H, J = 13.2, 5.5 Hz), 3.94 (m, 2H), 4.44 (m, 1H), 4.65, 4.88 (2t, 1H, rotomers, J = 5.5 Hz, J = 5.0 Hz), 5.01 (s, 2H), 7.19 (m, 5H), 7.34 (m, 5H), 7.41 (d, 1H, J = 8.5 Hz), 7.74, 7.97 (2d, 1H, rotomers, J = 8.8 Hz, J = 8.1 Hz), 7.86 (m, 1H). Anal. ($C_{29}H_{40}N_4O_6 \cdot 0.5 H_2O$) C, H, N.

CBZ-L-Leu-L-Phe-[*N*-(Me)Ac-amino-alaninal] (8): white solid, 29%; (5% MeOH/EtOAc); ^1H NMR (DMSO-d_6) δ 0.80 (m, 6H), 1.27 (m, 2H), 1.57 (m, 1H), 1.91, 1.97 (2bs, 3H, rotomers), 2.73, 2.98 (2bs, 3H, rotomers), 2.85 (m, 2H), 3.75 (m, 1H), 3.94 (m, 2H), 4.54, 4.81 (2m, 1H, rotomers), 5.00 (s, 2H), 5.94 (m, hydrate), 7.20 (m, 5H), 7.34 (m, 6H), 7.80−8.15 (m, 2H, rotomers), 8.48 (d, 1H, J = 7.4 Hz), 9.25 (bs, 1H). HRMS calcd for $C_{29}H_{38}N_4O_6$ + H 539.2870, found 539.2891. Anal. ($C_{29}H_{38}N_4O_6 \cdot 0.5H_2O$) C, H, N.

CBZ-L-[*N*-(4-chlorobutyryl-amino)-Ala]-OMe. Methyl N_α-CBZ-L-2,3-diaminopropionate hydrochloride (13.5 g, 46.8 mmol) was taken up in 200 mL of CH_2Cl_2 and cooled to 0 °C. To this was added Et_3N (10.6 g, 0.105 mol), followed by 4-chlorobutyryl chloride (7.1 g, 50.4 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 4 h. At this time the mixture was added to a saturated NaCl solution. The organic layer was extracted, washed with 1 N HCl, saturated NaCl, dried over $MgSO_4$, and concentrated, yielding 19 g of crude material. The material was purified by column chromatography (50% EtOAc/hexanes), giving an 87% yield of product: ^{1}H NMR ($CDCl_3$) δ 2.07 (m, 2H), 2.35 (t, 2H, J = 7.0 Hz), 3.57 (t, 2H, J = 6.3 Hz), 3.67 (t, 2H, J = 5.9 Hz), 3.77 (s, 3H), 4.45 (m, 1H), 5.12 (s, 2H), 5.84 (d, 1H, J = 6.3 Hz), 6.00 (bs, 1H), 7.37 (s, 5H).

CBZ-L-[*N*-(2-pyrrolidinone)-Ala]-OMe. A solution of CBZ-L-[*N*-(4-chlorobutyryl)-amino]-Ala-OMe (14.6 g, 39.0 mmol) in DMF (400 mL) was cooled to 0 °C. To the solution was added NaH (1.87 g − 60% dispersion in oil, 46.8 mmol), and the mixture was stirred at room temperature for 4 h. The DMF was removed under high vacuum, and the residue was taken up in EtOAc, washed with 1 N HCl, saturated aqueous $NaHCO_3$, and saturated NaCl, dried over $MgSO_4$, and concentrated. The material was purified by column chromatography (EtOAc), giving 7.0 g (56%) of the cyclized product: ^{1}H NMR δ ($CDCl_3$) 1.97 (m, 2H), 2.35 (m, 2H), 3.36 (m, 1H), 3.40−3.60 (m, 3H), 3.77 (s, 3H), 4.52 (m, 1H), 5.13 (d, 2H, J = 5.6 Hz), 5.83 (d, 1H, J = 6.3 Hz), 7.37 (m, 5H).

L-[*N*-(2-Pyrrolidinone)-Ala]-OMe Hydrochloride. This compound was prepared in quantitative yield from CBZ-L-[*N*-(2-pyrrolidinone)]-Ala-OMe by the general catalytic hydrogenation procedure, except methanolic HCl was used in order to isolate the product as the HCl salt: ^{1}H NMR ($CDCl_3$), δ 2.03 (m, 2H), 2.39 (m, 2H), 3.14 (bs, 2H), 3.40−3.70 (m, 5H), 3.75 (s, 3H).

CBZ-L-Leu-L-Phe-L-[*N*-(2-pyrrolidinone)-Ala]-OMe. This compound was prepared in 70% yield from CBZ-L-Leu-L-Phe and L-[*N*-(2-pyrrolidinone)]-Ala-OMe·HCl using the general peptide coupling procedure, except 1.2 equiv of Et_3N was added to liberate the free base of the amine·HCl salt. ^{1}H NMR ($CDCl_3$) δ 0.89 (m, 6H), 1.36 (m, 2H), 1.56 (m, 1H), 1.61 (m, 2H), 2.04 (m, 3H), 2.31 (m, 2H), 3.07−3.70 (m, 6H), 3.75 (s, 3H), 4.11 (m, 1H), 4.71 (m, 1H), 5.13 (bs, 1H), 5.18 (bs, 1H), 6.76, 6.88 (m, 1H, rotomer), 7.10−7.45 (m, 10H).

CBZ-L-Leu-L-Phe-L-[*N*-(2-pyrrolidinone)-Alaninol]. This compound was prepared in 60% yield by the reduction of CBZ-L-Leu-L-Phe-L-[*N*-(2-pyrrolidinone)]-Ala-OMe with $NaBH_4$ and LiCl using the procedure for CBZ-L-(*N*-Ac-amino)-alaninol as described: ^{1}H NMR ($CDCl_3$) δ 0.85 (d, 3H, J = 5.5 Hz), 0.89 (d, 3H, J = 5.5 Hz), 1.24−1.61 (m, 5H), 1.99 (m, 3H), 2.36 (t, 2H, J = 8.0 Hz), 3.00−3.60 (m, 7H), 4.03 (m, 1H), 4.12 (m, 1H), 4.72 (m, 1H), 5.10 (m, 2H), 5.38 (d, J = 6.4 Hz, rotomer), 5.46 (d, J = 6.4 Hz, rotomer), 6.92 (m, 1H), 7.15−7.40 (m, 10H); HRMS calcd for $C_{30}H_{40}N_4O_6$ + Na 575.2845, found 575.2828.

CBZ-L-Leu-L-Phe-L-[*N*-(2-pyrrolidinone)-alaninal] (9). This compound was prepared from CBZ-L-Leu-L-Phe-L-[*N*-(2-pyrrolidinone)]-alaninol using the SO_3−pyridine procedure as described (58% yield): ^{1}H NMR ($CDCl_3$) δ 0.87 (m, 6H), 1.20−2.20 (m, 5H), 2.27 (m, 2H), 2.85−3.55 (m, 3H), 3.70 (m, 1H), 4.10 (m, 2H), 4.48 (m, 1H), 4.80−5.00 (m, 2H), 5.09 (m, 2H), 5.79 (d, J = 7.3 Hz, rotomer), 5.85 (d, J = 7.3 Hz, rotomer), 6.60 (t, J = 8.1 Hz, rotomer), 6.77 (d, J = 8.5 Hz, rotomer), 7.06−7.38 (m, 10H), 7.58 (m, rotomer), 7.85 (m, rotomer), 9.48 (d, 1H, J = 4.4 Hz); HRMS calcd for $C_{30}H_{38}N_4O_6$ + Cs 683.1846 found 683.1860. Anal. ($C_{30}H_{38}N_4O_6$·1.4H_2O) C, H, N.

CBZ-L-(*N*-BOC-amino-Ala). To a stirred solution of NaOH (1.23 g, 30.76 mmol) in 36 mL of H_2O and 24 mL of *tert*-butyl alcohol was added N_α-CBZ-L-2,3-diaminopropionic acid[17] (7.15 g, 30 mmol). To this solution was added di-*tert*-butyl dicarbonate (6.88 g, 31.5 mmol). Stirring was continued at room temperature for 12 h at which time the solution was washed with pet. ether (2 × 150 mL). The organic layers were washed with aqueous saturated $NaHCO_3$ (3 × 20 mL), and the aqueous layers were combined and acidified at 0 °C with 25% aqueous $KHSO_4$ to pH 2−3. This milky white mixture was then extracted with a large excess of Et_2O, dried over anhydrous Na_2SO_4, and concentrated to yield 9.13 g (90%) of product as a white solid which was used without further purification: IR (film) 3424, 3366, 3067, 2982, 1726, 1684, 1530, 1306, 1167 1047, 740, 696 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.35 (s, 9H), 3.21 (m, 2H), 4.05 (m, 1H), 5.02 (s, 2H), 6.83 (bt, 1H, J = 6.6 Hz), 7.34 (m, 5H), 7.41 (d, 1H, J = 8.1 Hz), 12.65 (bs, 1H). Anal. ($C_{16}H_{22}N_2O_6$) C, H, N.

CBZ-L-(*N*-BOC-amino-Ala)-*N*-(Me)OMe. To a solution of CBZ-L-(*N*-BOC-amino-Ala) (5.0 g, 14.8 mmol) dissolved in 40 mL of CH_2Cl_2 was added *N*,*O*-dimethylhydroxylamine hydrochloride (1.44 g, 14.8 mmol), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDCI) (2.82 g, 14.8 mmol), and 4-methylmorpholine (1.6 g, 15.8 mmol). The reaction mixture was stirred for 12 h at room temperature, diluted with 200 mL of $CHCl_3$, and washed with 50 mL of 1 N HCl and 50 mL of H_2O. The organic layer was dried over Na_2SO_4, filtered, concentrated, and purified by column chromatography (50% EtOAc/hexanes) to give a colorless oil in 89% yield: ^{1}H NMR (DMSO-d_6) δ 1.34 (s, 9H), 3.07 (s, 3H), 3.14 (m, 2H), 3.70 (s, 3H), 4.56 (m, 1H), 5.01 (s, 2H), 6.76 (m, 1H), 7.34 (m, 5H). Anal. ($C_{18}H_{27}N_3O_6$) C, H, N.

L-(*N*-BOC-amino-Ala)-*N*-(Me)OMe. L-(*N*-BOC-amino-Ala)-*N*-(Me)OMe was prepared in quantitative yield from CBZ-L-(*N*-BOC-amino-Ala)-*N*-(Me)OMe using the general hydrogenation procedure described. The product was isolated as a colorless oil, which upon standing slowly crystallized: IR (KBr) 3306, 3102, 2984, 2942, 2872, 1709, 1711, 1597, 1524, 1252, 1173 cm^{-1}; ^{1}H NMR (DMSO-d_6) (rotomers) δ 1.35, 1.37 (2s, 9H), 3.00 (m, 1H), 3.08 (bs, 3H), 3.11−3.36 (m, 1H), 3.65 (s, 3H), 3.75 (m, 1H), 6.17 (bs, 2H), 6.80 (m, 1H); MS calcd for $C_{10}H_{21}N_3O_4$ + H, 248 found 248.

CBZ-L-Leu-L-Phe-L-(*N*-BOC-amino-Ala)-*N*-(Me)OMe. This compound was prepared in 81% yield from L-(*N*-BOC-amino-Ala)-*N*-(Me)OMe and CBZ-L-Leu-L-Phe using the general peptide coupling procedure: IR (KBr) 3302, 2962, 2932, 1715, 1644, 1526, 1252, 1173 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.79 (d, 3H, J = 6.3 Hz), 0.82 (d, 3H, J = 6.6 Hz), 1.30 (m, 2H), 1.35 (s, 9H), 1.47 (m, 1H), 2.79 (m, 1H), 3.01 (m, 1H), 3.09 (s, 3H), 3.23 (m, 2H), 3.68 (s, 3H), 3.95 (m, 1H), 4.52 (m, 1H), 4.84 (m, 1H), 5.03 (m, 2H), 6.77 (bt, 1H, J = 5.9 Hz), 7.18 (m, 5H), 7.34 (m, 5H), 7.43 (d, 1H, J = 8.5 Hz), 7.86 (d, 1H, J = 8.1 Hz), 8.09 (bd, 1H, J = 6.0 Hz). Anal. ($C_{33}H_{47}N_5O_8$) C, H, N.

CBZ-L-Leu-L-Phe-L-(amino-Ala)-*N*-(Me)OMe·TFA. To a solution of CBZ-L-Leu-L-Phe-L-(*N*-BOC-amino-Ala)-*N*-(Me)OMe (1.7 g, 2.65 mmol) in 30 mL of CH_2Cl_2, cooled to 0 °C with stirring, was added 3.5 mL of TFA. The reaction mixture was allowed to stir at room temperature for 12 h, at which time it was concentrated under vacuum. The residue was triturated with Et_2O and filtered to give the TFA salt in quantitative yield which was used without further purification: ^{1}H NMR (DMSO-d_6) δ 0.79 (d, 3H, J = 6.6 Hz), 0.82 (d, 3H, J = 6.6 Hz), 1.30 (m, 2H), 1.48 (m, 1H), 2.90 (m, 2H), 3.05 (m, 2H), 3.12 (s, 3H), 3.67 (s, 3H), 3.95 (m, 1H), 4.49 (m, 1H), 4.97 (d, 1H, J = 12.9 Hz), 5.04 (d, 1H, J = 12.5 Hz), 5.15 (m, 1H), 7.19 (m, 5H), 7.34 (m, 5H), 7.46 (d, 1H, J = 7.7 Hz), 7.91 (bm, 4H), 8.53 (bd, 1H, J = 7.7 Hz).

CBZ-L-Leu-L-Phe-L-[*N*-(isoxazole-5-carbonyl)-amino-Ala]-*N*-(Me)OMe. This intermediate was prepared in 42% yield from CBZ-L-Leu-L-Phe-L-(amino-Ala)-*N*-(Me)OMe·TFA and isoxazole-5-carboxylic acid using the general peptide coupling procedure, except 1.2 equiv of Et_3N was added to liberate the free base of the amine·TFA salt: IR (KBr) 3302, 3067, 2955, 2932, 1651, 1532, 1454, 1276, 699 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.77 (d, 3H, J = 6.6 Hz), 0.80 (d, 3H, J = 6.6 Hz), 1.28 (m, 2H), 1.47 (m, 1H), 2.78 (dd, 1H, J = 14.2, 9.4 Hz), 2.98 (dd, 1H, J = 13.8, 4.2 Hz), 3.09 (s, 3H), 3.46 (m, 2H), 3.68 (s, 3H), 3.95 (m, 1H), 4.53 (m, 1H), 4.98 (m, 2H), 5.05 (m, 1H), 7.05 (d, 1H, J = 0.7 Hz), 7.18 (m, 5H), 7.33 (m, 5H), 7.40 (d, 1H, J = 8.1 Hz), 7.89 (d, 1H, J = 8.1 Hz), 8.29 (m, 1H), 8.91 (t, 1H, J = 5.7 Hz). Anal. ($C_{32}H_{40}N_6O_8$) C, H, N.

CBZ-L-Leu-L-Phe-L-[*N*-(isoxazole-5-carbonyl)-amino-alaninal] (10). A stirred solution of CBZ-L-Leu-L-Phe-L-[*N*-(isoxazole-5-carbonyl)-amino-Ala]-*N*-(Me)OMe (0.1 g, 0.16 mmol) in 5 mL of anhydrous THF was cooled to −78 °C, and diisobutylaluminum hydride in THF (1.0 M, 0.82 mL, 0.82 mmol) was added dropwise. The mixture was allowed to warm to 0 °C over 1 h and then quenched by pouring into 100 mL EtOAc. The mixture was washed with 30 mL of 1 N HCl, 30 mL of H_2O, and 30 mL of saturated NaCl. The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum, affording a residue that was purified by column chromatography (3% MeOH/$CHCl_3$). The white solid, characterized as a mixture of 40% aldehyde and 60% methyl hemiacetal by elemental analysis, was isolated in 12% yield. ^{1}H NMR (DMSO-d_6) δ 0.78 (d, 3H, J = 6.3 Hz), 0.81 (d, 3H, J = 6.6 Hz), 1.28 (m, 2H), 1.48 (m, 1H), 2.82 (m, 1H), 2.99 (m, 1H), 3.16 (d, hemiacetal, J = 5.5 Hz), 3.24−3.65 (m, 3H), 3.99 (m, 1H), 4.25 (m, hemiacetal), 4.44 (m, hemiacetal), 4.60 (m, 1H), 5.00 (m, 2H), 7.05 (d, 1H, J = 1.8 Hz), 7.19 (m, 5H), 7.33 (m, 5H), 7.39 (d, 1H, J = 8.5 Hz), 7.98 (bt, 1H, J = 6.4 Hz), 8.59 (m, 1H), 8.73 (m, 1H), 8.95 (m, 1H), 9.31 (s, 1H); HRMS calcd for $C_{30}H_{35}N_5O_7$ + Cs (aldehyde) 710.1591, found 710.1616; MS calcd for $C_{31}H_{39}N_5O_8$ + Cs (methyl hemiacetal) 742, found 742. Anal. ($C_{30}H_{35}N_5O_7$·0.6CH_3OH) C, H, N.

L-(*N*-BOC-amino-Ala). Prepared in quantitative yield from CBZ-L-(*N*-BOC-amino-Ala) using the general hydrogenation procedure: ^{1}H NMR (CD_3OD) δ 1.44 (s, 9H), 3.43 (dd, 1H, J = 14.3, 7.0 Hz), 3.61 (m, 2H).

CBZ-L-Leu-L-Phe-(*N*-BOC-amino-Ala): white solid, 95%; ^{1}H NMR (CD_3OD) δ 0.85 (d, 3H, J = 6.6 Hz), 0.88 (d, 3H, J = 6.6 Hz), 1.39 (m, 2H), 1.41 (s, 9H), 1.55 (m, 1H), 2.96 (dd, 1H, J = 13.6, 9.2 Hz), 3.20 (dd, 1H, J = 13.6, 4.5 Hz), 3.37 (dd, 1H, J = 14.0, 7.7 Hz), 3.48 (dd, 1H, J = 14.7, 4.0 Hz), 4.07 (m, 1H), 4.46 (m, 1H), 4.63 (m, 1H), 5.07 (m, 2H), 7.13−7.35 (m, 12H), 8.04 (d, 1H, J = 7.7 Hz), 8.16 (d, 1H, J = 6.0 Hz).

CBZ-L-Leu-L-Phe-(*N*-BOC-amino-Ala)-OMe: white solid, 88%; ^{1}H NMR ($CDCl_3$) δ 0.88 (d, 6H, J = 6.3 Hz), 1.39 (m, 9H), 1.40−1.48 (m, 3H), 3.05 (m, 2H), 3.45 (m, 2H), 3.70 (s, 3H), 4.20 (m, 1H), 4.51 (m, 1H), 4.68 (m, 1H), 5.03 (d, 1H, J = 12.1 Hz), 5.11 (d, 1H, J = 11.8 Hz), 5.35 (bs, 1H), 5.48 (bs, 1H), 6.85 (d, 1H, J = 7.7 Hz), 7.05 (m, 1H), 7.13−7.33 (m, 10H).

CBZ-L-Leu-L-Phe-(amino-Ala)-OMe·TFA: white solid, 84%; ^{1}H NMR (CD_3OD) δ 0.86 (d, 3H, J = 6.6 Hz), 0.89 (d, 3H, J = 6.6 Hz), 1.40 (m, 2H), 1.58 (m, 1H), 3.02 (dd, 1H, J = 14.0, 8.8 Hz), 3.11 (dd, 1H, J = 12.0, 7.5 Hz), 3.18 (dd, 1H, J = 12.3, 5.0 Hz), 3.41 (dd, 1H, J = 13.2, 5.1 Hz), 3.74 (s, 3H), 4.06 (dd, 1H, J = 9.2, 5.9 Hz), 4.47 (dd, 1H, J = 8.5, 6.6 Hz), 4.77 (dd, 1H, J = 8.7, 5.0 Hz), 5.04 (d, 1H, J = 12.5 Hz), 5.09 (d, 1H, J = 12.5 Hz), 7.18−7.35 (m, 10H).

CBZ-L-Leu-L-Phe-(*N*-carbomethoxyamino-Ala)-OMe: white solid, 93%; ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 6H, J = 6.3 Hz), 1.41 (m, 1H), 1.55 (m, 2H), 3.04 (m, 1H), 3.12 (m, 1H), 3.53 (m, 2H), 3.59 (s, 3H), 3.72 (s, 3H), 4.14 (m, 1H), 4.57 (m, 1H), 4.66 (m, 1H), 5.01 (d, 1H, J = 11.4 Hz), 5.10 (d, 1H, J = 11.4 Hz), 5.32 (d, 1H, J = 6.3 Hz), 5.58 (m, 1H), 6.80 (d, 1H J = 7.4 Hz), 7.14−7.38 (m, 11H).

CBZ-L-Leu-L-Phe-(*N*-carbomethoxyamino-alaninol): white solid, 57%; ^{1}H NMR ($CDOD_3$) δ 0.87 (t, 6H, J = 6.3 Hz), 1.41 (m, 1H), 1.55 (m, 2H), 3.04 (m, 1H), 3.12 (m, 1H), 3.53 (m, 2H), 3.59 (s, 3H), 3.72 (s, 3H), 4.14 (m, 1H), 4.57 (m, 1H), 4.66 (m, 1H), 5.01 (d, 1H, J = 11.4 Hz), 5.10 (d, 1H, J = 11.4 Hz), 5.32 (d, 1H, J = 6.3 Hz), 5.58 (m, 1H), 6.80 (d, 1H J = 7.4 Hz), 7.14−7.38 (m, 11H).

CBZ-L-Leu-L-Phe-(*N*-carbomethoxyamino-alaninal) (11): white solid, 82%; IR (KBr) 3308, 2957, 2934, 1707, 1653, 1539, 1265, 1044, 698 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, J = 6.0 Hz), 1.49 (m, 2H), 1.59 (m, 1H), 3.02 (m, 2H), 3.52 (m, 2H), 3.57 (s, 3H), 4.32 (m, 1H), 4.72 (m, 2H), 5.01 (d, 1H, J = 12.5 Hz), 5.10 (d, 1H, J = 10.7 Hz), 5.68 (m, 2H), 7.12−7.32 (m, 11H), 7.50 (m, 1H), 9.46 (s, 1H); HRMS calcd for $C_{28}H_{36}N_4O_7$ + H 541.2662, found 541.2671. Anal. ($C_{28}H_{36}N_4O_7$·1.2H_2O) C, H, N.

CBZ-L-(*N*-BOC-amino-Ala)-OMe. An ethereal solution of diazomethane, generated from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (7.7 g, 36.0 mmol), 70 mL of Et_2O, 16 mL of EtOH, 12 mL of H_2O, and KOH (7.65 g, 13.6 mmol) was carefully distilled into a stirred solution of CBZ-L-(*N*-BOC-amino-Ala) (7.8 g, 23.0 mmol) in 50 mL of Et_2O and 10 mL of EtOH at 0 °C. The yellow solution was stirred for 30 min. The cold solution was then brought to room temperature, and argon was bubbled into the reaction flask to remove any excess diazomethane. After the solution turns clear it is concentrated to give the methyl ester as a white solid in quantitative yield: mp 72−74 °C; IR (KBr) 3418, 3331, 3005, 2955, 1753, 1724, 1676, 1552, 1525, 1298, 1045, 699 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H), 3.55 (m, 2H), 3.76 (s, 3H), 4.40 (m, 2H), 4.82 (m, 1H), 5.11 (s, 2H), 5.77 (m, 1H), 7.35 (m, 5H). Anal. ($C_{17}H_{24}N_2O_6$) C, H, N.

CBZ-L-(*N*-BOC-amino-alaninol). Using the general lithium borohydride reduction procedure, CBZ-L-(*N*-BOC-amino-Ala)-OMe was converted to the alcohol and isolated in 96% yield without column chromatography purification: mp = 116−119 °C; IR (KBr) 3327, 3277, 3065, 2976, 1699, 1682, 1543, 1315, 1250, 1062, 1001, 696 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.35 (s, 9H), 2.90−3.10 (m, 4H), 3.55 (m 1H), 4.60 (bt, 1H, J = 5.5 Hz), 4.99 (s, 2H), 6.72 (bt, 1H, J = 5.5 Hz), 6.86 (d, 1H, J = 8.1 Hz), 7.34 (m, 5H). Anal. ($C_{16}H_{24}N_2O_5$) C, H, N.

L-(*N*-BOC-amino-alaninol). Using the general hydrogenation procedure, the CBZ group was removed to give the amino alcohol in 98% yield: mp 61−64 °C; IR (KBr) 3362, 2980, 2935, 1680, 1534, 1370, 1287, 1175, 1059, 642 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.36 (s, 9H), 2.64 (m, 1H), 2.72 (m, 1H), 2.93 (m, 1H), 3.13 (m, 1H), 3.32 (m, 2H), 4.45 (bs, 1H), 6.67 (bs, 1H). Anal. ($C_8H_{18}N_2O_3$) C, H, N.

CBZ-L-Leu-L-Phe-L-(*N*-BOC-amino-alaninol). This compound was prepared from L-(*N*-BOC-amino-alaninol) and CBZ-L-Leu-L-Phe using the general peptide coupling procedure described above. The reaction mixture was purified by column chromatography (5% saturated anhydrous NH_3 in MeOH/CH_2Cl_2) to give a white solid in 90% yield: IR (KBr) 3420, 3327, 3289, 3032, 2953, 1694, 1643, 1535, 1284, 1036, 696 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.80 (dd, 6H, J = 11.2, 6.4 Hz), 1.35 (s, 9H), 1.55 (m 2H), 1.72 (m, 1H), 2.89 (m, 2H), 3.19 (m, 2H), 3.78 (m, 1H), 3.92 (m, 1H), 4.44 (m, 1H), 4.62 (t, 1H, J = 5.5 Hz), 5.01 (d, 2H, J = 5.9 Hz), 6.63 (bt, 1H, J = 5.5 Hz), 7.18 (m, 5H), 7.34 (m, 5H), 7.45 (d, 1H, J = 8.1 Hz), 7.60 (d, 1H, J = 7.7 Hz), 7.85 (d, 1H, J = 8.1 Hz). Anal. ($C_{31}H_{44}N_4O_7$) C, H, N.

CBZ-L-Leu-L-Phe-L-(*N*-BOC-amino-alaninal) (12). This compound was prepared in 90% yield as a white solid from CBZ-L-Leu-L-Phe-L-(*N*-BOC-amino-alaninol) using the general IBX oxidation procedure. The product was used immediately without further purification. The product exist as a mixture of aldehyde and aldehyde hydrate: IR (KBr) 3299, 3067, 2959, 2934, 1696, 1647, 1535, 1254, 1171, 747, 698 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.78 (d, 6H, J = 6.8 Hz), 0.81 (d, 6H, J = 6.8 Hz), 1.35 (s, 9H), 1.41 (m, 2H), 1.69 (m, 1H), 2.80−3.01 (m, 2H), 3.29 (m, 2H), 3.97 (m, 1H), 4.10 (m, 1H), 4.60 (m, 1H), 5.00 (s, 2H), 5.56 (d, J = 7.4 Hz, hydrate), 6.78 (t, 1H, J = 6.3 Hz), 7.20 (m, 5H), 7.33 (m, 5H), 7.40 (d, 1H, J = 8.1 Hz), 7.97 (d, 1H, J = 8.1 Hz), 8.39 (d, 1H, J = 6.6 Hz), 9.26 (s, 1H); HRMS calcd for $C_{31}H_{42}N_4O_7$ + Cs 715.2108, found 715.2133. Anal. ($C_{31}H_{42}N_4O_7$·0.5H_2O) C, H, N.

CBZ-L-(*N*-(methylsulfonyl)amino-Ala)-OMe. Under anhydrous conditions, methane sulfonyl chloride (1.75 g, 15.3 mmol) was added dropwise to a stirred solution of methyl N_α-CBZ-L-2,3-diaminopropionate hydrochloride (2.2 g, 7.63 mmol) in 50 mL of CH_2Cl_2 and pyridine (1.8 g, 22.9 mmol). The mixture was stirred at room temperature for 4 h and then diluted with 250 mL of CH_2Cl_2. The solution was washed with 25 mL of 1 N HCl, 25 mL of 10% aqueous $NaHCO_3$, and 25 mL of H_2O and dried over Na_2SO_4. The filtrate was concentrated and the purified by column chromatography (3% MeOH/$CHCl_3$) to afford the solid sulfonamide in 68% yield: ^{1}H NMR (DMSO-d_6) δ 2.88 (s, 3H), 3.33 (m, 2H), 3.64 (s, 3H), 4.22 (m, 1H), 5.04 (s, 2H), 7.19 (t, 1H, J = 6.1 Hz), 7.35 (m, 5H), 7.66 (d, 1H, J = 8.5 Hz). Anal. ($C_{13}H_{18}N_2O_6S$) C, H, N, S.

CBZ-L-(*N*-(methylsulfonyl)amino-alaninol): white solid, 92%; (5% MeOH/$CHCl_3$); IR (KBr) 3532, 3322, 3273, 2922, 1686, 1645, 1535, 1319, 1154, 1057, 972, 764 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.86 (s, 3H), 2.94 (m, 1H), 3.08 (m, 1H), 3.37 (m, 2H), 3.54 (m, 1H), 4.72 (t, 1H, J = 5.5 Hz), 5.01 (s, 2H), 7.00 (m, 2H), 7.35 (m, 5H). Anal. ($C_{12}H_{18}N_2O_5S$) C, H, N, S.

CBZ-L-Leu-L-Phe-(*N*-(methylsulfonyl)amino alaninol): white solid, 82%; (5% MeOH/$CHCl_3$); IR (KBr) 3302, 2957, 1701, 1647, 1540, 1454, 1319, 1150, 1046, 698 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.78 (d, 3H, J = 6.6 Hz), 0.82 (d, 3H, J = 6.6 Hz), 1.29 (m, 2H), 1.49 (m, 1H), 1.82 (m, 1H), 2.81 (m, 1H), 2.87 (s, 3H), 2.99 (m, 2H), 3.13 (m, 1H), 3.24 (m, 2H), 3.79 (m, 1H), 3.94 (m, 1H), 4.49 (m, 1H), 4.75 (t, 1H, J = 5.0 Hz), 4.98 (d, 1H, J = 12.5 Hz), 5.05 (d, 1H, J = 12.9 Hz), 6.88 (t, 1H, J = 5.7 Hz), 7.19 (m, 5H), 7.34 (m, 5H), 7.47 (d, 1H, J = 8.1 Hz), 7.73 (d, 1H, J = 8.1 Hz), 7.88 (d, 1H, J = 8.1 Hz). Anal. ($C_{27}H_{38}N_4O_7S\cdot0.3H_2O$) C, H, N.

CBZ-L-Leu-L-Phe-(*N*-(methylsulfonyl)amino-alaninal) (13): white solid, 55%; ^1H NMR (DMSO-d_6) δ 0.80 (m, 6H), 1.27 (m, 2H), 1.46 (m, 1H), 2.78 (m, 1H), 2.86 (s, 3H), 2.97 (m, 2H), 3.15 (m, 1H), 3.88 (m, 2H), 4.43 (m, 1H), 5.01 (m, 2H), 5.94 (t, hydrate, J = 5.5 Hz), 6.00 (t, hydrate, J = 5.1 Hz), 6.77 (t, 1H, J = 5.7 Hz), 7.20 (m, 5H), 7.34 (m, 6H), 7.38 (m, 2H), 9.36 (s, 1H); HRMS calcd for $C_{27}H_{36}N_4O_7S$ + H 561.2383, found 561.2375. Anal. ($C_{27}H_{36}N_4O_7S\cdot0.6H_2O$) C, H, N.

CBZ-L-[*N*-(*N*′,*N*′-dimethylcarbamoyl)amino-Ala]-OMe. Under anhydrous conditions, dimethylcarbamyl chloride (1.12 g, 10.4 mmol) was added dropwise to a stirred solution of methyl N_α-CBZ-L-2,3-diaminopropionate hydrochloride (3.0 g, 10.4 mmol) in 60 mL of CH_2Cl_2 and Et_3N (3.1 g, 30.6 mmol) at 0 °C. The mixture was stirred at room temperature for 12 h and then diluted with 150 mL of CH_2Cl_2. The solution was washed with 30 mL of 1 N HCl, 30 mL of 10% aqueous $NaHCO_3$, and 30 mL H_2O, saturated NaCl, and dried over Na_2SO_4. The filtrate was concentrated and purified by column chromatography (2% MeOH/$CHCl_3$) to afford the solid urea in 98% yield: ^1H NMR ($CDCl_3$) δ 2.84 (s, 6H), 3.57−3.71 (m, 2H), 3.74 (s, 3H), 4.36 (m, 1H), 5.10 (m, 2H), 6.50 (d, 1H, J = 6.6 Hz), 7.35 (m, 6H).

CBZ-L-[*N*-(*N*′,*N*′-dimethylcarbamoyl)amino-alaninol]: oil, 89%; (5% MeOH/$CHCl_3$); ^1H NMR (CD_3OD) δ 2.61 (s, 6H), 3.07 (m, 2H), 3.11 (m, 2H), 3.47 (m, 1H), 4.80 (d, 1H, J = 12.9 Hz), 4.86 (d, 1H, J = 11.8 Hz), 7.10 (m, 5H).

L-[*N*-(*N*′,*N*′-Dimethylcarbamoyl)amino-alaninol]: oil, 95%; ^1H NMR (CD_3OD) δ 2.85 (m, 1H), 2.89 (s, 6H), 3.13 (dd, 1H, J = 13.7, 6.2 Hz), 3.24 (dd, 1H, J = 13.9, 6.1 Hz), 3.43 (dd, 1H, J = 11.2, 5.6 Hz), 3.50 (dd, 1H, J = 11.2, 5.0 Hz).

CBZ-L-Leu-L-Phe-L-[*N*-(*N*′,*N*′-dimethylcarbamoyl)amino-alaninol]: white solid, 95%; (4% MeOH/$CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (t, 6H, J = 6.6 Hz), 1.41−1.53 (m, 3H), 2.87 (s, 6H), 3.04 (m, 2H), 3.24 (m, 2H), 3.48 (m, 1H), 3.62 (m, 1H), 3.85 (bs, 1H), 4.16 (m, 1H), 4.71 (m, 2H), 5.02 (d, 1H, J = 12.5 Hz), 5.09 (d, 1H, J = 12.1 Hz), 5.45 (m, 2H), 6.84 (d, 1H, J = 7.4 Hz), 7.14−7.36 (m, 11H). Anal. ($C_{29}H_{41}N_5O_6\cdot0.5H_2O$) C, H, N.

CBZ-L-Leu-L-Phe-L-[*N*-(*N*′,*N*′-dimethylcarbamoyl)amino-alaninal] (14): white solid, 70%; IR (KBr) 3308, 2955, 1707, 1647, 1541, 1454, 1262, 1238, 1049, 698 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (d, 3H, J = 6.6 Hz), 0.89 (d, 3H, J = 6.6 Hz), 1.40 (m, 1H), 1.55 (m, 2H), 2.84 (s, 3H), 2.86 (s, 3H), 3.07 (m, 2H), 3.60 (m, 1H), 3.80 (m, 1H), 4.12 (m, 1H), 4.35 (m, 1H), 4.60 (m, 1H), 5.09 (m, 3H), 6.67 (m, 2H), 7.16−7.39 (m, 10H), 7.55 (m, 1H), 9.48 (s, 1H); MS calcd for $C_{29}H_{39}N_5O_6$ + H 554, found 554. Anal. ($C_{29}H_{39}N_5O_6\cdot0.75H_2O$) C, H, N.

CBZ-L-(*N*,*N*-diMe-Gln)OMe. To a stirred, ice cold solution of CBZ-L-Glu-OMe (2.95 g, 10.0 mmol) in CH_2Cl_2 (40 mL) was added one drop of DMF followed by the slow addition of oxalyl chloride (1.4 g, 11.0 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 3 h. The mixture was concentrated and redissolved in 20 mL of CH_2Cl_2. The solution was cooled to 0 °C, and dimethylamine (0.9 g, 20.0 mmol) dissolved in anhydrous THF (25 mL) was added dropwise. The solution was brought to room temperature, stirred for 12 h, and then concentrated under vacuum to give an analytically pure pale yellow solid in 94% yield: IR (KBr) 3245, 3057, 2959, 2901, 1721, 1624, 1553, 1291, 1265, 1238, 1049, 992, 763 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.04 (m, 1H), 2.21 (m, 1H), 2.40 (m, 2H), 2.92 (s, 3H), 2.93 (s, 3H), 3.73 (s, 3H), 4.35 (m, 1H), 5.06 (d, 1H, J = 12.5 Hz), 5.13 (d, 1H, J = 12.1 Hz), 5.87 (d, 1H, J = 6.6 Hz), 7.34 (m, 5H); MS calcd for $C_{16}H_{22}N_2O_5$ + H 323, found 323. Anal. ($C_{16}H_{22}N_2O_5$) C, H, N.

CBZ-L-(*N*,*N*-diMe-glutaminol): viscous oil (80%); IR (film) 3340, 3065, 3036, 2949, 2895, 1713, 1634, 1537, 1260, 1067, 742, 698 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.87 (m, 1H), 1.98 (m, 1H), 2.41 (m, 2H), 2.96 (m, 8H), 3.58 (m, 2H), 4.05 (bs, 1H), 5.09 (m, 2H), 7.34 (m, 5H); MS calcd for $C_{15}H_{22}N_2O_4$ + H 295, found 295.

L-(*N*,*N*-diMe-glutaminol): oil (92%); IR (film) 3378, 2949, 2922, 2887, 1626, 1504, 1404, 1264, 1153, 1057 cm^{-1}; ^1H NMR (DMSO-d_6, D_2O) δ 1.24−1.36 (m, 1H), 1.53−1.64 (m, 1H), 2.18−2.45 (m, 2H), 2.57 (m, 1H), 2.80 (s, 3H), 2.96 (s, 3H), 3.14 (dd, 1H, J = 10.7, 6.6 Hz), 3.27 (dd, 1H, J = 10.5, 5.0 Hz); MS calcd for $C_7H_{16}N_2O_2$ + H 161, found 161.

CBZ-L-Leu-L-Phe-L-(*N*,*N*-diMe-Glutaminol): white solid, 84% (4% saturated NH_3−MeOH/$CHCl_3$); IR (KBr) 3308, 3065, 2951, 2870, 1692, 1644, 1547, 1244, 1049, 744, 698 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.78 (d, 3H, J = 6.6 Hz), 0.81 (d, 3H, J = 6.6 Hz), 1.32 (m, 2H), 1.47 (m, 2H), 1.77 (m, 1H), 2.18 (m, 2H), 2.76 (s, 3H), 2.81 (m, 1H), 2.87 (s, 3H), 2.96 (dd, 1H, J = 13.8, 5.3 Hz), 3.11 (dd, 1H, J = 10.7, 6.3 Hz), 3.24 (dd, 1H, J = 10.5, 5.0 Hz), 3.64 (m, 1H), 3.95 (m, 1H), 4.43 (m, 1H), 4.62 (t, 1H, J = 5.5 Hz), 4.97 (d, 1H, J = 12.9 Hz), 5.02 (d, 1H, J = 12.9 Hz), 7.20 (m, 5H), 7.34 (m, 5H), 7.42 (d, 1H, J = 8.1 Hz), 7.61 (d, 1H, J = 8.5 Hz), 7.93 (d, 1H, J = 7.7 Hz). MS calcd for $C_{30}H_{42}N_4O_6$ + Cs 687, found 687. Anal. ($C_{30}H_{42}N_4O_6$) C, H, N.

CBZ-L-Leu-L-Phe-L-(*N*,*N*-diMe-glutaminal) (15): white solid (90%); IR (KBr) 3291, 3065, 3041, 2951, 2874, 1723, 1645, 1537, 1264, 1046, 745, 698 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.81 (t, 6H, J = 7.4 Hz), 1.28 (m, 2H), 1.56 (m, 2H), 1.94 (m, 1H), 2.21 (t, 2H, J = 7.2 Hz), 2.78 (s, 3H), 2.85 (m, 1H), 2.87 (s, 3H), 3.00 (dd, 1H, J = 13.8, 6.1 Hz), 4.00 (m, 2H), 4.52 (m, 1H), 5.00 (m, 2H), 7.22 (m, 5H), 7.34 (m, 5H), 7.40 (d, 1H, J = 8.5 Hz), 8.06 (d, 1H, J = 7.7 Hz), 8.38 (d, 1H, J = 7.0 Hz), 9.21 (s, 1H); HRMS calcd for $C_{30}H_{40}N_4O_6$ + CH_3OH + Cs 717.2264, found 717.2285. Anal. ($C_{30}H_{40}N_4O_6\cdot0.75H_2O$) C, H, N.

CBZ-L-Leu-L-Phe-L-methioninol. CBZ-L-Leu-L-Phe (3.02 g, 7.3 mmol) was dissolved in 75 mL of CH_2Cl_2. To this solution were added *N*-hydroxysuccinimide (0.91 g, 7.7 mmol) and 2 mL of DMF, and stirring was continued until all solids had gone into solution. DCC (1.60 g, 7.7 mmol) was added to the reaction mixture, and the reaction was stirred at room temperature for 1 h. The mixture was then filtered into a separate flask containing *S*-(−)-methioninol (1.06 g, 7.7 mmol) dissolved in a minimum of DMF, removing the *N*,*N*′-dicyclohexylurea precipitate. The reaction mixture was stirred for 12 h at room temperature. The solvents were removed under vacuum, and the resulting crude product was purified by column chromatography (anhydrous NH_3/MeOH/$CHCl_3$, 0.5:4.5:9.5) on silica gel to give 3.72 g (96%) of white solid: IR (KBr) 3293, 3065, 2955, 1696, 1645, 1539, 1236, 698 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.79 (d, 3H, J = 6.3 Hz), 0.82 (d, 3H, J = 6.6 Hz), 1.31 (m, 2H), 1.51 (m, 2H), 1.82 (m, 1H), 2.00 (s, 3H), 2.43 (m, 2H), 2.90 (m, 1H), 3.11 (m, 1H), 3.24 (m, 1H), 3.30 (m, 1H), 3.72 (m, 1H), 3.97 (m, 1H), 4.45 (m, 1H), 4.66 (t, 1H, J = 5.5 Hz), 5.01 (m, 2H), 7.20 (m, 5H), 7.34 (m, 5H), 7.43 (d, 1H, J = 8.1 Hz), 7.62 (d, 1H, J = 8.5 Hz), 7.95 (d, 1H, J = 8.1 Hz). Anal. ($C_{28}H_{39}N_3O_5S$) C, H, N.

CBZ-L-Leu-L-Phe-L-[methioninol (±)-sulfoxide]. The sulfide CBZ-L-Leu-L-Phe-L-methioninol (1.50 g, 2.80 mmol) was dissolved in 50 mL of CH_2Cl_2. A total amount of 0.61 g of *m*-CPBA (57−86%) was added portionwise over a period of 5 h during which the reaction mixture was stirred at room temperature. After an additional hour of stirring, the mixture was poured into saturated aqueous $NaHCO_3$/CH_2Cl_2. The

organic layer was separated, washed with brine, and dried over Na_2SO_4. Filtration and concentration afforded a crude residue that was purified by column chromatography (5% MeOH/$CHCl_3$). The product was obtained as a white glassy solid in 90% yield: IR (KBr) 3295, 3063, 2955, 1694, 1644, 1541, 1263, 1234, 1043, 698 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.80 (d, 3H, J = 6.3 Hz), 0.83 (d, 3H, J = 6.6 Hz), 1.32 (m, 2H), 1.55 (m, 2H), 1.93 (m, 1H), 2.47 (s, 3 H), 2.65 (m, 2H), 2.83 (dd, 1H, J = 13.8, 8.6 Hz), 3.04 (m, 1H), 3.18 (m, 1H), 3.27 (m, 1H), 3.75 (m, 1H), 3.97 (m, 1H), 4.42 (m, 1H), 4.75 (t, 1H, J = 5.5 Hz), 4.99 (d, 1H, J = 12.9 Hz), 5.04 (d, 1H, J = 12.5 Hz), 7.21 (m, 5H), 7.35 (m, 5H), 7.44 (d, 1H, J = 7.7 Hz), 7.73 (d, 1H, J = 8.8 Hz), 7.98 (m, 1H). Anal. ($C_{28}H_{39}N_3O_6S$) C, H, N, S.

CBZ-L-Leu-L-Phe-L-[methioninal (±)-sulfoxide] (16). This compound was prepared in 71% yield as a white solid from CBZ-L-Leu-L-Phe-L-[methioninol (±)-sulfoxide] using the general IBX oxidation procedure: ^1H NMR (DMSO-d_6) δ 0.81 (m, 6H), 1.30 (m, 2H), 1.50 (m, 1H), 1.97 (m, 1H), 2.48 (s, 3H), 2.55−3.27 (m, 5H), 3.70 (m, 1H), 4.47 (m, 1H), 4.71 (m, 1H), 5.00 (s, 2H), 7.20−7.40 (m, 10H), 7.93 (m, 1H), 8.08 (m, 1H), 8.51 (m, 1H), 9.22 (s, 1H); HRMS calcd for $C_{28}H_{37}N_3O_6S$ + Cs 676.1457, found 676.1473. Anal. ($C_{28}H_{37}N_3O_6S \cdot 0.25H_2O$) C, H, N.

BOC-L-Gln-OMe. To a solution of BOC-L-Gln (20 g, 81 mmol) in 50 mL of EtOAc and MeOH at 0 °C was added diazomethane (prepared from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide; 42.8 g, 0.2 mol) in 250 mL of Et_2O with stirring. The resulting yellow solution was stirred at 0 °C for 5 min, warmed to room temperature, and stirred for an additional 20 min. Argon gas was then bubbled through the yellow reaction mixture to remove excess diazomethane. The crude product was concentrated and purified by crystallization (*t*-BuOMe) to afford a quantitative yield of the methyl ester: ^1H NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.96 (m, 1H), 2.21 (m, 1H), 2.36 (m, 2H), 3.76 (s, 3H,), 4.34 (m, 1H), 5.32 (m, 1H), 5.44 (bs, 1H), 6.16 (bs, 1H). Anal. ($C_{11}H_{20}N_2O_5$) C, H, N.

BOC-L-(cyanomethyl-Ala)-OMe. To a solution of BOC-L-Gln-OMe (10 g, 38 mmol) in 100 mL of pyridine at 0 °C was added $POCl_3$ (5.83 g, 38 mmol) dropwise. The reaction mixture was warmed to room temperature and stirred for 12 h. At this time the mixture was diluted with 100 mL of EtOAc and washed with 1 N HCl (2 × 50 mL). The organic phases were combined, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (25% EtOAc/hexane) to give the dehydration product in 67% yield: ^1H NMR ($CDCl_3$) δ 1.45 (s, 9H), 2.03 (m, 1H), 2.27 (m, 1H), 2.46 (m, 2H), 3.80 (s, 3H), 4.38 (m, 1H), 5.20 (m, 1H).

CBZ-L-Leu-L-Phe-L-(cyanomethyl-Ala)-OMe. To a solution of BOC-L-(cyanomethyl-Ala)-OMe (0.89 g, 3.7 mmol) in 35 mL of CH_2Cl_2 was added 10 mL of TFA at 0 °C. The reaction mixture was warmed to room temperature over 1 h and then concentrated. Benzene (25 mL) was added and evaporated repeatedly (3×) to remove residual TFA. The residue was taken up in 10 mL of DMF and cooled to 0 °C, and CBZ-Leu-Phe (1.51 g, 3.7 mmol), PyBOP (2.48 g, 4.8 mmol), and diisopropylethylamine (1.42 g, 11 mmol) were added. The mixture was stirred for 12 h at room temperature, diluted with EtOAc, washed with saturated aqueous $NaHCO_3$, 10% citric acid, and saturated NaCl, dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by column chromatography (50% hexanes/EtOAc) to afford a solid that was taken up in ca. 5 mL of $CHCl_3$ and precipitated by the slow addition to a solution of ether−hexane. The resulting amorphous white solid was filtered and dried to give a 66% of pure product: IR (KBr) 3289, 2959, 2247, 1749, 1690, 1645, 1537 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (m, 6H), 1.41 (m, 1H), 1.56 (m, 2H), 1.98 (m, 2H), 2.20 (m, 1H), 2.28 (m, 2H), 3.00−3.25 (m, 2H), 3.72 (s, 3H), 4.14 (m, 1H), 4.58 (m, 1H), 4.75 (m, 1H), 5.02 (d, 1H, J = 12.1 Hz), 5.11 (d, 1H, J = 12.1 Hz), 5.32 (bd, 1H, J = 6.3 Hz), 6.83 (bd, 1H, J = 7.7 Hz), 7.10−7.40 (m, 10H).

CBZ-L-Leu-L-Phe-L-(cyanomethyl-alaninol): amorphous white solid, 89% (2:1 EtOAc:hexanes); ^1H NMR ($CDCl_3$) δ 0.88 (d, 3H, J = 6.2 Hz), 0.85 (d, 3H, J = 6.2 Hz), 1.25−1.70 (m, 3H), 1.91 (m, 2H), 2.33 (m, 2H), 2.68 (m, 1H), 3.10 (dd, 1H, J = 14.0, 8.1 Hz), 3.27 (dd, 1H, J = 5.5, 1.7 Hz), 3.55 (m, 1H), 3.65 (m, 1H), 3.97 (m, 2H), 4.66 (q, 1H, J = 6.0 Hz), 5.00 (d, 1H, J = 12.0 Hz), 5.03 (bs, 1H), 5.10 (d, 1H, J = 12.0 Hz), 6.54 (d, 1H, J = 7.6 Hz), 6.63 (d, 1H, J = 9.0 Hz), 7.17−7.42 (10H, m). Anal. ($C_{28}H_{36}N_4O_5 \cdot 0.5H_2O$) C, H, N.

CBZ-L-Leu-L-Phe-L-(cyanomethyl-alaninal) (18). Using the general pyridine−SO_3 oxidation procedure, CBZ-L-Leu-L-Phe-L-(cyanomethyl-alaninal was prepared as a white solid in 75% yield after precipitation from EtOAc−CH_2Cl_2−ether at 0 °C. IR (KBr) 3298, 3061, 2957, 2247, 1688, 1645, 1539 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (m, 6H), 1.90 (m, 1H), 2.29 (m, 4H), 3.20 (m, 4H), 3.98 (m, 1H), 4.35 (m, 1H), 4.72 (m, 1H), 5.05 (m, 4H), 6.40 (d, J = 7.6 Hz, hydrate), 6.52 (d, J = 7.6 Hz, hydrate), 7.15−7.42 (m, 10H), 9.45 (d, J = 5.6 Hz). Anal. ($C_{28}H_{34}N_4O_5 \cdot 1.3H_2O$) C, H, N.

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