Inhibitors of the Protease from Human Immunodeficiency Virus: Design and Modeling of a Compound Containing a Dihydroxyethylene Isostere Insert with High Binding Affinity and Effective Antiviral Activity

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The peptidomimetic template and the dihydroxyethylene isostere insert that were applied successfully to the design of renin inhibitors have been extended to the related protease from human immunodeficiency virus (HIV). The present report describes the structure–activity study leading to the identification of an inhibitor with a K_i of <1 nM for the HIV type-1 protease (compound II). This compound, containing a diol insert, is highly effective in blocking polyprotein processing in in vitro cell culture assays. Results obtained from kinetic analysis, studies of the stereochemistry of the insert, and modeling have led to insights as to the requisites involved in the active site–inhibitor interaction.

Introduction

The rapid spread of the acquired immunodeficiency syndrome (AIDS) epidemic and the difficulties encountered in the development of an efficacious vaccine have stimulated a world-wide quest for therapeutic agents to arrest the replication of the causative virus in AIDS, human immunodeficiency virus (HIV). The formidable task is to develop drugs that can interrupt the life cycle of HIV without harming the infected host. At present, the most widely used anti-AIDS drug is the nucleoside analogue, 3′-azido-3′-deoxythymidine (AZT).[1-3] AZT is a suicide substrate and its incorporation into viral DNA by the retroviral reverse transcriptase (RT) results in chain termination. AZT and other such nucleoside derivatives have shown beneficial effects in AIDS patients, but their prolonged use is often compromised by serious side effects, such as bone marrow suppression.[4] Moreover, it is generally the case that AZT-resistant strains of the virus can overcome the effectiveness of the drug.[5]

There is much interest currently in combination antiviral therapy with drugs directed toward different targets in the viral life cycle.[6] One such possibility is the use of inhibitors of the virally encoded protease responsible for viral maturation, possibly alone, or in combination with AZT. The protease represents another unique aspect of HIV biochemistry, in addition to reverse transcription, in that this enzyme is indispensable for generation of an infectious particle.[7,8] HIV protease processes the viral *gag* and *gag/pol* polyproteins in the final stages of viral maturation. It cleaves the p55 *gag* precursor into the four structural proteins forming the core of the virion, p17, p24, p8, and p7. It also processes the p160 *gag/pol* precursor to liberate the structural elements just mentioned, and the enzymes of the virus, i.e., the protease itself, RT, and the endonuclease, or integrase. Although the HIV protease has been characterized extensively,[9-13] and its three-dimensional structure has been determined both alone and in complexation with a variety of inhibitors,[14-19] details as to the mode, time, and microenvironment of its action, remain obscure. It is clear, however, that if the protease is catalytically defective,[7,8] or if it is inhibited,[20-23] viral maturation in HIV-infected cell culture is blocked and, consequently, infection is arrested.

The most effective inhibitors of the HIV protease reported thus far are pseudopeptide/peptidemimetic compounds containing transition-state inserts in place of the residues occupying the P_1- and P_1'-positions of the peptide.

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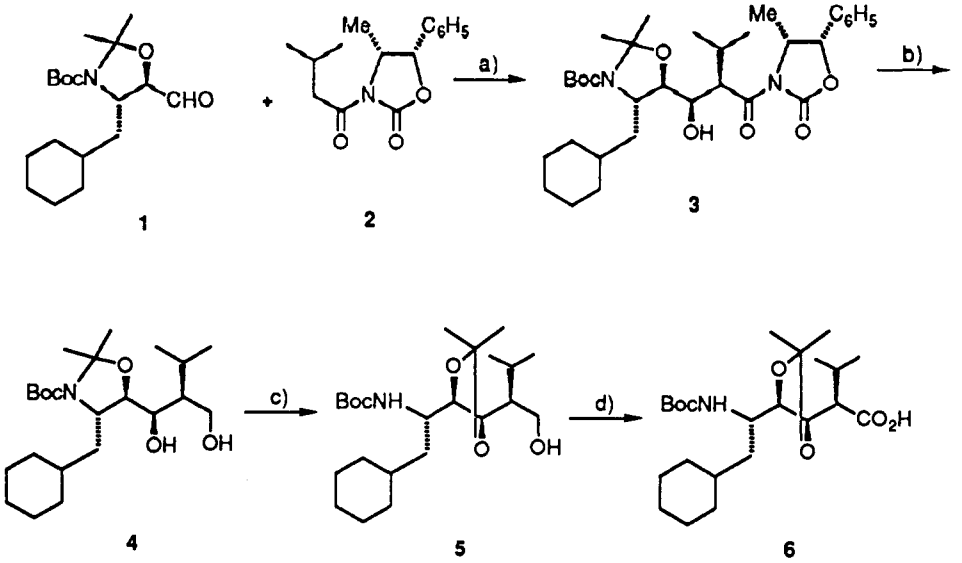
Scheme I. Synthesis of Acid 6[a]



[a] (a) *n*-Bu_2BOTf, *i*-Pr_2NEt, CH_2Cl_2; (b) *n*-Bu_2BOTf, *i*-Pr_2NEt, THF; $LiBH_4$; (c) camphorsulfonic acid, CH_2Cl_2; (d) $RuCl_3 \cdot H_2O$, H_5IO_6, $CH_3CN/CCl_4/H_2O$.

These inserts include reduced peptide bond (methyleneamino, CH_2NH), hydroxyethylene [$CH(OH)CH_2$], hydroxyethylamine [$CH(OH)CH_2N$], and dihydroxyethylene [$CH(OH)CH(OH)$] moieties.[24-29] Much of the development in this area has occurred as a result of work in the generation of renin inhibitors as antihypertensive agents. Indeed, knowledge on peptidomimetic inhibitors of renin, an enzyme closely related to the HIV protease, has served as a guide in the design of templates and inserts for HIV

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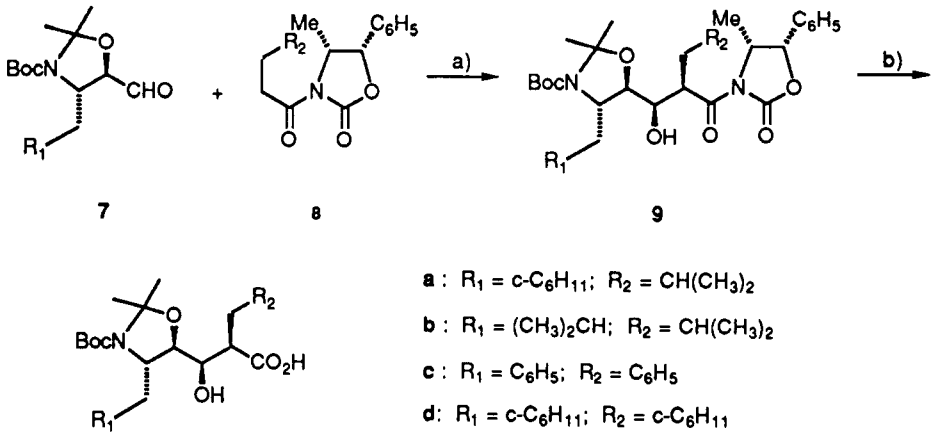
Scheme II. Synthesis of Acids 10a–d[a]



[a] (a) n-Bu_2BOTf, i-Pr_2NEt, CH_2Cl_2; (b) LiOH, H_2O_2, CH_3OH/H_2O.

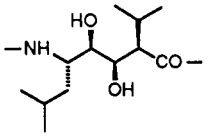
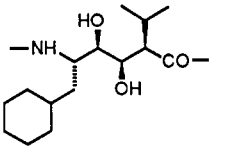
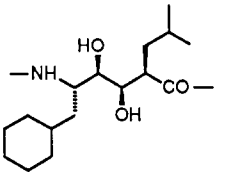
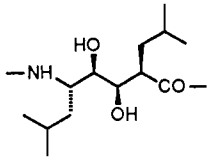
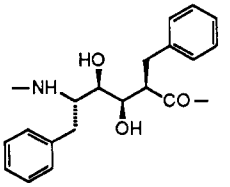
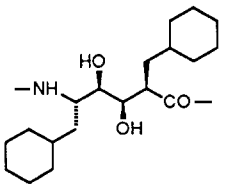
protease inhibitors. As an example, we have shown that the potent, metabolically stable pseudopeptide inhibitor of renin, Boc-Pro-Phe-N-MeHis-Leuψ[CH(OH)CH_2]Val-Ile-Amp (K_i = 70 pM) is also a good inhibitor of the HIV protease with a K_i of 10 nM.[28] However, it shows only weak antiviral activity in HIV-infected cell culture assays.

In this report, we describe how selective modifications of this template at both N- and C-termini and in the scissile bond insert have led to the identification of compound II, Noa-His-Calψ[CH(OH)CH(OH)]Val-Ile-Amp, a potent antiviral agent with high affinity for the HIV protease. In this study we have examined the stereochemistry of the dihydroxyethylene insert, and we have also modeled the structure within the active site cavity of the protease in order to gain some insight in the interaction of the ligand to the enzyme.

Chemistry

Syntheses of the Protected Dipeptide Isosteres. We have previously described[30] the synthesis of the dipeptide isostere Leuψ[CH(OH)CH(OH)]Val[31] in a protected form which was used in the preparation of peptidic renin inhibitors. The synthesis of the corresponding Calψ[CH(OH)CH(OH)]Val isostere in a suitably protected form is shown in Scheme I. The starting aldehyde 1 was prepared from phenylalanine by a synthetic route which was previously described for the analogous aldehyde from leucine.[30] A highly diastereoselective aldol addition[32] between chiral aldehyde 1 and oxazolidinone 2[30] gave adduct 3 in 90% yield. Removal of the chiral auxiliary by basic hydrolysis in the presence of hydrogen peroxide was not successful. For this substrate, the nucleophile added to the carbamate carbonyl due to steric congestion. As was demonstrated previously,[30] formation of a boron–ate complex with dibutylboron triflate activated the carbonyl group for a reaction with the reducing agent lithium bo-

Table I. Inhibitory Activity against HIV-1 Protease[a]

peptide	Noa—His—X—Ile—Amp: X	K_i, nM
I		5
II		<1
III		5
IV		7
V		12
VI		26

[a] Noa = 1-naphthoxyacetyl; Amp = 2-pyridylmethylamine.

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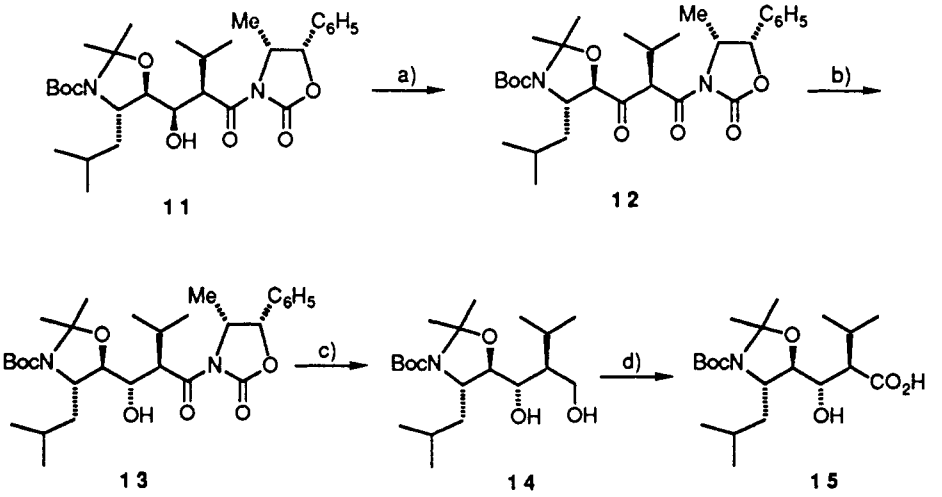
Scheme III. Synthesis of Acid 15[a]



[a] (a) $(COCl)_2$, DMSO, CH_2Cl_2; Et_3N; (b) $NaBH_4$, CH_3OH; (c) *n*-Bu_2BOTf, *i*-Pr_2NEt, THF; $LiBH_4$; (d) $RuCl_3 \cdot H_2O$, H_5IO_6, $CH_3CN/CCl_4/H_2O$.

rohydride,[33] and alcohol 4 was obtained in 85% yield. The two hydroxylic functions could be differentiated by an acid-catalyzed migration of the isopropylidene group, in 80% yield, from the *N,O*-acetal to the *O,O*-acetal, structures of which contain trans substitution for the five-membered rings. The remaining hydroxyl group in compound **5** was then oxidized to the desired acid **6** in 98% yield using ruthenium trichloride/periodic acid.[34]

The remaining four dipeptide isosteres shown in Table I were synthesized in the same manner as that illustrated in Scheme II. For the synthesis of acid **10a**, the aldol addition[32] between chiral aldehyde **7a** (aldehyde **1**) and oxazolidinone **8a** gave diastereoselectively adduct **9a** in 73% yield. In contrast to aldol adduct **3**, this analogous adduct **9a**, without a β-branched alkyl group, could be directly hydrolyzed with lithium hydroxide and hydrogen peroxide to give acid **10a** in 65% yield. Acids **10b–d** were prepared in the same manner.

The dipeptide isostere building block **15** used for the preparation of peptide VIII was obtained by a synthetic route as shown in Scheme III. Aldol adduct **11**, described previously,[30] was oxidized with dimethyl sulfoxide and oxalyl chloride[35] to give the corresponding ketone **12** in 90% yield. It has been previously established[36] that a 1,3-dicarbonyl structure of this type is resistant to epimerization at the 2-position due to steric factor in which the two carbonyl groups could not be coplanar. The ketone was then reduced nonstereoselectively to provide the desired alcohol **13** as the minor component in 15% yield and the starting alcohol **11** (70%) as the major component. The chiral auxiliary in compound **13** was reductively removed with dibutylboron triflate and lithium borohydride[33] in 70% yield to give alcohol **14**. The two hydroxylic functions in compound **4** had been differentiated by an acid-catalyzed isopropylidene migration. In the case of compound **14**, however, the corresponding *O,O*-acetal would be a cis-substituted five-membered ring and a mixture with the six-membered *O,O*-acetal would result. Alcohol **14**, however, could be oxidized with ruthenium trichloride/periodic acid[34] to give the corresponding acid **15** in a lower yield of 69%.

The dipeptide isostere building block **22** used for the preparation of peptide IX was obtained by a synthetic route as shown in Scheme IV. The previously described[30] aldehyde **16** was reacted with (carbethoxymethylene)triphenylphosphorane in 50% yield to give the α,β-unsaturated ester **17**. Reduction of the ester with diisobutylaluminum hydride gave the corresponding allylic alcohol **18** in 90% yield. Epoxidation of the olefinic function with *m*-chloroperbenzoic acid proceeded in a highly diastereoselective fashion and yielded epoxide **19** in 96% yield. Reaction with a copper-catalyzed Grignard reagent[37] afforded diol **20** in 50% yield. The *N,O*-acetal was rearranged to the *O,O*-acetal by acid catalysis to give alcohol **21** in 64% yield. The alcohol was then oxidized with ruthenium trichloride/periodic acid[34] to give the desired acid **22** in 86% yield.

Synthesis of the Representative Peptide IX. Peptides VII and X were reported previously.[30] The remaining peptides were prepared by the solution method, and the preparation of peptide IX as shown in Scheme V is illustrative for the syntheses of all the peptides. The dipeptide isostere building block **22** was condensed with *N*-L-isoleucyl-2-pyridylmethylamine (**23**)[38] by using diethylphosphoryl cyanide[39] to give product **24** in quantitative yield. The two acid-labile protecting groups were removed with hydrogen chloride in methanol to give amine **25** in 70% yield. Prolonged treatment with acid led to the formation of γ-lactone and loss of the *N*-L-isoleucyl-2-pyridylmethylamine group. Amine **25** was then coupled to *N*-(1-naphthoxyacetyl)-N^{im}-(*tert*-butyloxycarbonyl)-L-

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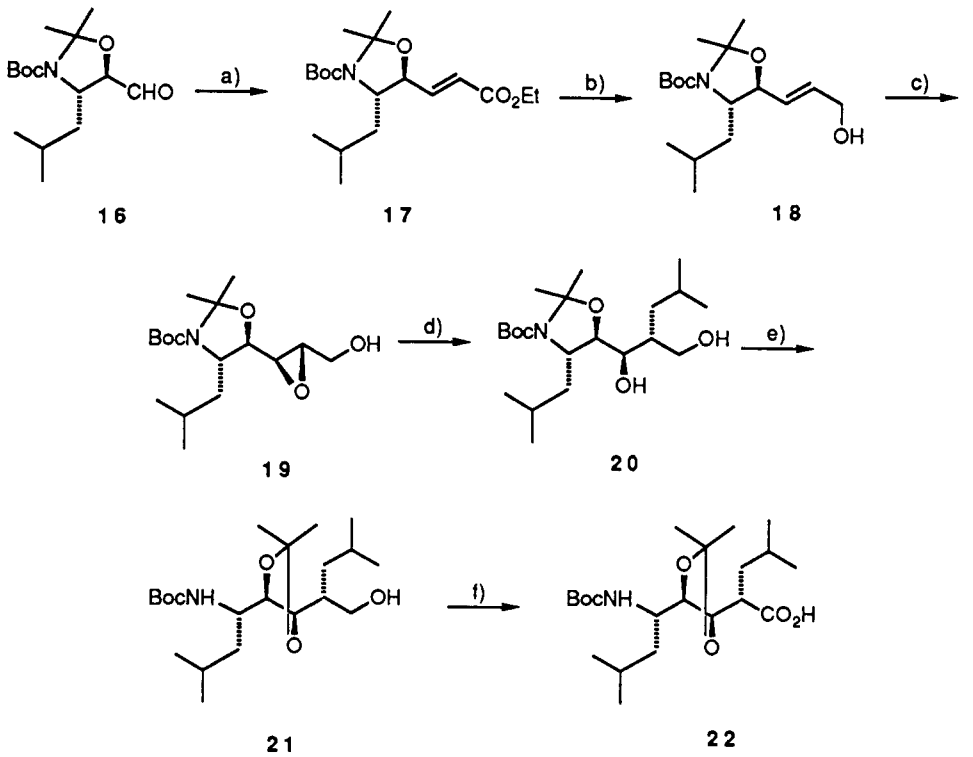
Scheme IV. Synthesis of Acid **22**[a]



[a] (a) $(C_6H_5)_3PCHCO_2Et$, toluene; (b) *i*-Bu_2AlH, ether/toluene; (c) *m*-$ClC_6H_4CO_3H$, $NaHCO_3$, CH_2Cl_2; (d) $(CH_3)_2CHCH_2MgBr$, CuI, ether/THF; (e) camphorsulfonic acid, CH_2Cl_2; (f) $RuCl_3 \cdot H_2O$, H_5IO_6, $CH_3CN/CCl_4/H_2O$.

histidine with BOP reagent[40] to give product **26** in 90% yield. The *tert*-butyloxycarbonyl protecting group was then removed with trifluoroacetic acid in 40% yield to give the desired peptide IX.

HIV-1 Protease Inhibitory Study with Peptide II

As shown in Figure 1, linear plots of velocity versus inhibitor concentration are obtained for three different concentrations of GSP substrate. The lines intersect the abscissa at a value of about 70 nM which corresponds exactly to the concentration of HIV protease employed in these experiments. Therefore, peptide II is an active site titrant of the protease and binds stoichiometrically to the enzyme. Results similar to these were also obtained in assays at pH 7 as well as at pH 5.5.

Discussion

Our previous work in the area of inhibitors of renin has led us to develop Boc-Pro-Phe-N^{α}-Me-His-Leuψ-[$CHOHCH_2$]Val-Ile-Amp as a potent, selective, and metabolically stable peptidic inhibitor which lowers blood pressure in a renin-dependent hypertensive animal model.[38] Our interest in HIV protease, which belongs to the same family of aspartyl proteases, has prompted us to assess whether this compound has any inhibitory effect against HIV protease. To our surprise, it showed high inhibitory activity against HIV-1 protease with a K_i value of 10 nM. This template became a viable starting point for our structure–activity relationships study for series of compounds that are designed to inhibit the HIV protease. We had also previously reported[30] that the dihydroxyethylene dipeptide isostere, such as Leuψ[CH(OH)CH-(OH)]Val, was an effective transition-state-analogue insert for the preparation of potent renin inhibitors such as the

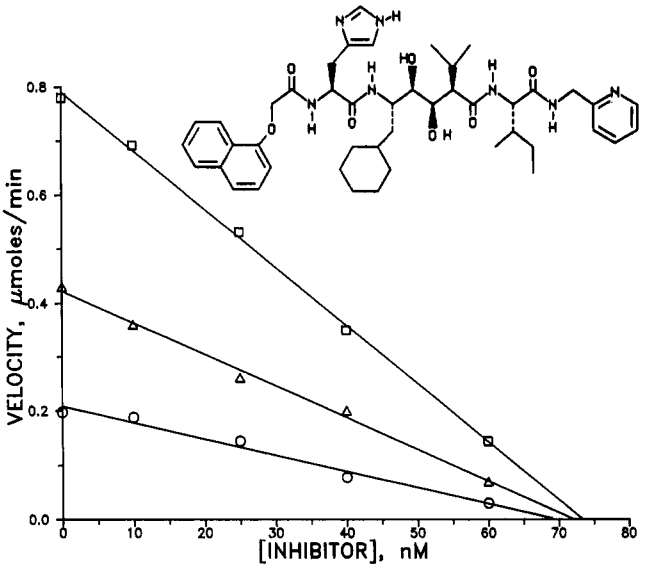


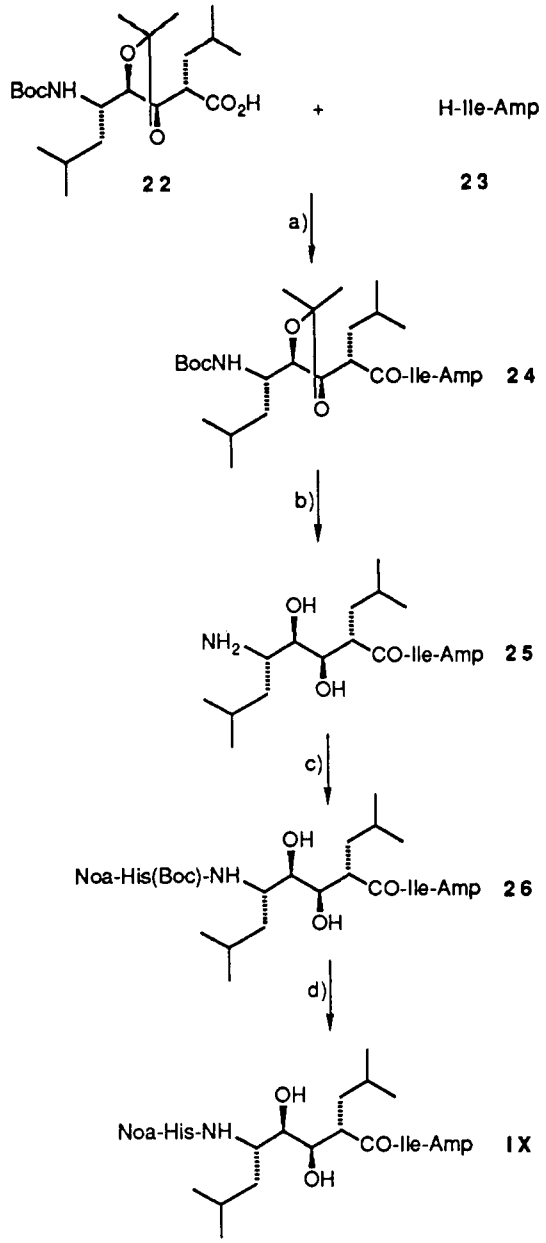
Figure 1. Active site titration of HIV protease with peptide II. Velocity was determined at three different concentrations of substrate, 2.5 (□), 1.0 (△), and 0.5 (○) mM. The experiment was carried out at pH 5.5 with the conditions described in the Experimental Section.

previously reported peptides VII and X. In this report, we have extended the use of this class of transition-state insert to the study of potent inhibitors of HIV protease.

We quickly established that the N-terminal portion of Boc-Pro-Phe-N^{α}-Me-His-Leuψ[$CH(OH)CH_2$]Val-Ile-Amp could be much simplified, and compounds in the template Noa-His-X-Ile-Amp as shown in Table I exhibited high HIV protease inhibitory activity. Peptide I with the Leu-Val dipeptide isostere is a potent inhibitor with a K_i value of 5 nM. Cyclohexylalanine at the P_1 site has been previously shown[41] to enhance renin inhibitory activity of

(40) Castro, B.; Dormay, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219–1222.

Scheme V. Synthesis of Peptide IX[a]



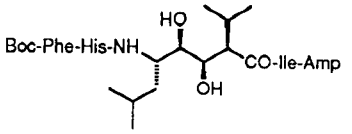
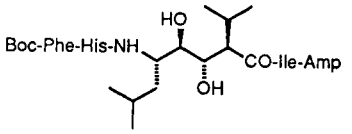
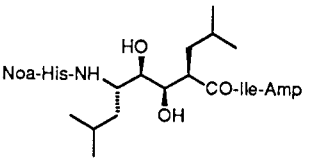
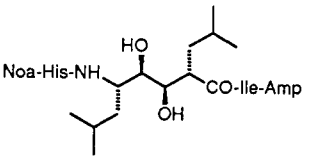
[a] (a) $(EtO)_2P(O)CN$, *i*-Pr_2NEt, CH_2Cl_2; (b) CH_3COCl, CH_3OH, $HS(CH_2)_2SH$; (c) Noa-His(Boc)-OH, BOP reagent, *i*-Pr_2NEt, CH_2Cl_2; (d) CF_3CO_2H, CH_2Cl_2.

the resulting peptide as compared to the leucine residue at P_1. Peptide II, with the cyclohexylmethyl side chain at the P_1 site, did exhibit improved HIV protease inhibitory activity. It is an active site titrant and a detailed inhibitory study is also reported here. Peptide III, with the isobutyl side chain in place of the isopropyl side chain at the P_1' site, showed a slight decrease in binding affinity when compared to peptide II. Since the HIV protease is a homodimer, we were interested in exploring a few transition-state inserts with identical side chains at the P_1 and P_1' sites. Cogeneric peptives IV–VI were prepared and assayed for HIV protease inhibitory activity. They did not show enhancement of activity over the previously prepared peptides I–III. The smaller P_1-P_1' side chains in peptide IV resulted in highest binding affinity of the resulting peptide. Increasing the size of the side chains to a pair of cyclohexylmethyl groups as in peptide VI resulted in a significant reduction in binding affinity.

We were also interested in probing some of the stereochemical requirements of the dipeptide isostere in this series of compounds. Compounds in Table II were prepared in which epimers at C-2 and C-3 of the transition-state insert were examined. The previously reported renin inhibitory peptide VII, with the same dipeptide isostere insert as in peptide I, showed reasonably good HIV protease inhibitory activity. However, peptide VIII, which is epimeric with peptide VII at the hydroxyl group at C-3, showed much reduced binding affinity to the enzyme. Peptide IX, which is epimeric with peptide IV at the alkyl group at C-2, also showed much reduced activity. Our investigation demonstrated that the absolute stereochemistry of these transition-state inserts is critical for biological activity.

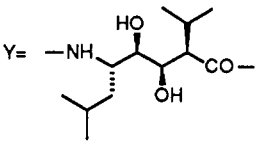
Interest in smaller peptides which could maintain high inhibitory activity led us to explore some C-terminal

Table II. Inhibitory Activity against HIV-1 Protease[a]

peptide	structure	K_i, nM
VII		28
VIII		500
IV		7
IX		220

[a] Noa = 1-naphthoxyacetyl; Amp = 2-pyridylmethylamine.

Table III. Inhibitory Activity against HIV-1 Protease[a]

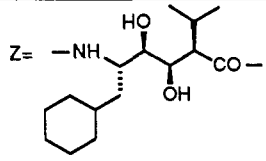


peptide	sequence	K_i, nM
VII	Boc-Phe-His-Y-Ile-Amp	28
X	Boc-Phe-His-Y-Mba	360
XI	Poa-His-Y-Ile-Amp	5
XII	Poa-His-Y-Mba	360

[a] Poa = phenoxyacetyl; Mba = 2(*S*)-methylbutylamine.

(41) Boger, J.; Payne, L. S.; Perlow, D. S.; Lohr, N. S.; Poe, M.; Blaine, E. H.; Ulm, E. H.; Schorn, T. W.; La-Mont, B. I.; Lin, T.-Y.; Kawai, M.; Rich, D.; Veber, D. F. Renin Inhibitors. Syntheses of Subnanomolar, Competitive, Transition-State Analogue Inhibitors Containing a Novel Analogue of Statine. *J. Med. Chem.* **1985**, *28*, 1779–1790.

Table IV. Inhibitory Activity against HIV-1 Protease and Antiviral Activity against Vaccinia Virus in CV-1 Monkey Cells[a]



peptide	sequence	K_i, nM	% CV-1 inhibn at 10 μM
XIII	Poa-His-Z-Ile-Amp	2	100
XIV	Hac-His-Z-Ile-Amp	4	0
XV	Gly-His-Z-Ile-Amp	11	0
XVI	Cha-Z-Ile-Amp	22	80

[a] Hac = 2-hydroxyacetyl; Cha = cyclohexylacetyl.

truncation of these peptides. As shown in Table III, the 2(*S*)-methylbutylamine group[30] was used to mimic the side chain of isoleucine at the P_2' site. Relative to peptide VII, the previously reported 2(*S*)-methylbutylamine-containing renin-inhibitory peptide X was much less effective. Peptide XI, with the (phenoxyacetyl)-L-histidine group, showed high binding affinity; however, the methylbutylamine-containing peptide XII showed significantly reduced activity. In this series of compounds illustrated, a C-terminal group larger than a simple alkyl group was shown to be necessary for significant binding affinity.

We have studied the processing of the HIV p55 and the maturation of the HIV-like particles in recombinant vaccinia virus (vVK-1) infected CV-1 cells.[20] They were engineered to express the HIV *gag/pol* genes and resulted in the synthesis and processing of *gag/pol* precursors. The initial prominent polypeptides synthesized are p55, p46, and p41, which are further processed to the mature viral proteins p17 and p24. The HIV-like particles are assembled and undergo maturation process that closely mimic the natural HIV-1 infection. We analyzed the vVK-1-infected CV-1 cell lysates for the processing of p55 to p24 by densitometric analyses of protein immunoblotting. We have shown[20] that HIV protease inhibitory compounds that are effective in this assay have arrested viral maturation and infectivity. We have found that antiviral activity in cell culture as defined by this CV-1 cell assay is associated with compounds that exhibit significant lipophilicity. This can be illustrated by the results shown in Table IV at doses which show no cytotoxicity. Peptide XIII is a potent inhibitor of the HIV-1 protease with a K_i value of 2 nM. It also possesses very good antiviral activity and shows complete inhibition of the *gag/pol* processing in the CV-1 cell assay at 10 μM. Replacing the lipophilic moiety, the phenoxyacetyl group, with the hydrophilic groups such as the hydroxy or amino containing acetyl groups gave peptides XIV and XV, which maintain very high binding affinity to the enzyme. In the cell culture assay, however, these compounds showed drastically reduced efficacy. Replacing these hydrophilic N-terminal groups with a simple alkyl group such as the cyclohexylacetyl group gave peptide XVI, which showed reasonable binding affinity and also good antiviral activity.

Among the peptides that we have studied thus far, peptide II exhibited highly potent binding affinity to the enzyme as an active site titrant of the HIV-1 protease. It also inhibited HIV-2 protease with a K_i value of 30 nM. Since the template for these HIV protease inhibitors was derived from renin inhibitors, some compounds retain renin inhibitory activity. Compound II also inhibited renin with an IC_{50} value of 2.3 nM. With the HIV protease inhibition, it showed very effective antiviral activity with an IC_{50} value of 0.1 μM against the vaccinia virus in the CV-1 cell and an IC_{50} value of 3 nM against HIV-1 in human peripheral blood lymphocytes, which is comparable to that of AZT in this assay. Peptide II was shown to have sustained antiviral activity with complete inhibition, at 1 μM concentration, of HIV-1_{LAV}/CEMX 174, HIV-2_{ST}/CEMX 174, and SIV_{MAC251}/HUT 78. Details of these findings and additional biological evaluation can be found elsewhere.[23]

Molecular Modeling of Peptide II/HIV-1 Protease Complex

Models of compound II in multiple bound conformations were derived from the X-ray crystallographic structure of the pseudopeptide inhibitor MVT-101 (Ac-Thr-Ile-Nleψ-[CH_2NH]Nle-Gln-Arg-NH_2) bound to synthetic HIV-1 protease.[17] Several putative conformations of the inhibitor bound to the protease are shown superimposed in Figure 2. Construction of the models involved application of molecular graphics techniques, specialized ligand modeling software, and molecular mechanics. Details of the model-building process are given in the Experimental Section. The models are consistent with the initial MVT-101/HIV-1 protease structure and with recently determined X-ray crystallographic structures of other peptidic inhibitors bound to HIV-1 protease.[19b] Figure 3 shows the overlay of the lowest-energy model of compound II with two X-ray crystallographic structures: MVT-101 (from which the modeling work was done) and U-85548E, Val-Ser-Gln-Asn-Leuψ[$CHOHCH_2$]Val-Ile-Val (with a hydroxyethylene insert). These structures and the constructed model show a roughly C-2 symmetric orientation of inhibitor side chains with respect to the enzyme's axis of symmetry. The model of compound II and the published X-ray crystallographic structures also show each inhibitor amide group between P_3 and P_3' positioned to make two hydrogen bonds to the protein backbone, except for the P_2 and P_1' carbonyl oxygen atoms, which are indirectly hydrogen bonded to the protein through a buried water molecule. There are two noteworthy features of the inhibitor models. First, the naphthoxyacetyl and the 2-pyridylmethylamide groups lie mainly outside of the active site cleft and appear to make no specific interactions with the enzyme, other than hydrophobic interactions with a number of large hydrophobic regions of the protein surface. Second, the two hydroxyl groups of the dihydroxyethylene insert appear to interact with the catalytic aspartic acid residues, but in an asymmetric fashion, in which one hydroxyl group can hydrogen bond to both carboxylic acids and the other forms a hydrogen bond to only one. Models of symmetrically substituted dihydroxyethylene groups, constructed and oriented to hydrogen bond symmetrically with the aspartate groups, reverted to the same asymmetric orientation upon energy minimization. This convergence to an asymmetric orientation was independent of the protonation state modeled for the aspartic acid groups.

The models of the bound inhibitor structure are consistent with the structure–activity data presented and support interpretation of some of the observed trends in the data. The low sensitivity of binding to the choice of residues at P_3 may be attributed to the apparent lack of specific interactions between this position and the protein. Inhibitor residues beyond the P_3-position in the N-terminal direction appear from the modeling to lie well outside the binding cleft and, as expected, do not show a significant contribution to the binding activity. Any truncation of inhibitors which would result in the loss of amide groups between P_3 and P_3' would be expected to cause a loss of binding affinity, due to the resultant loss of specific inhibitor/enzyme hydrogen bonds. The data seem to sup-

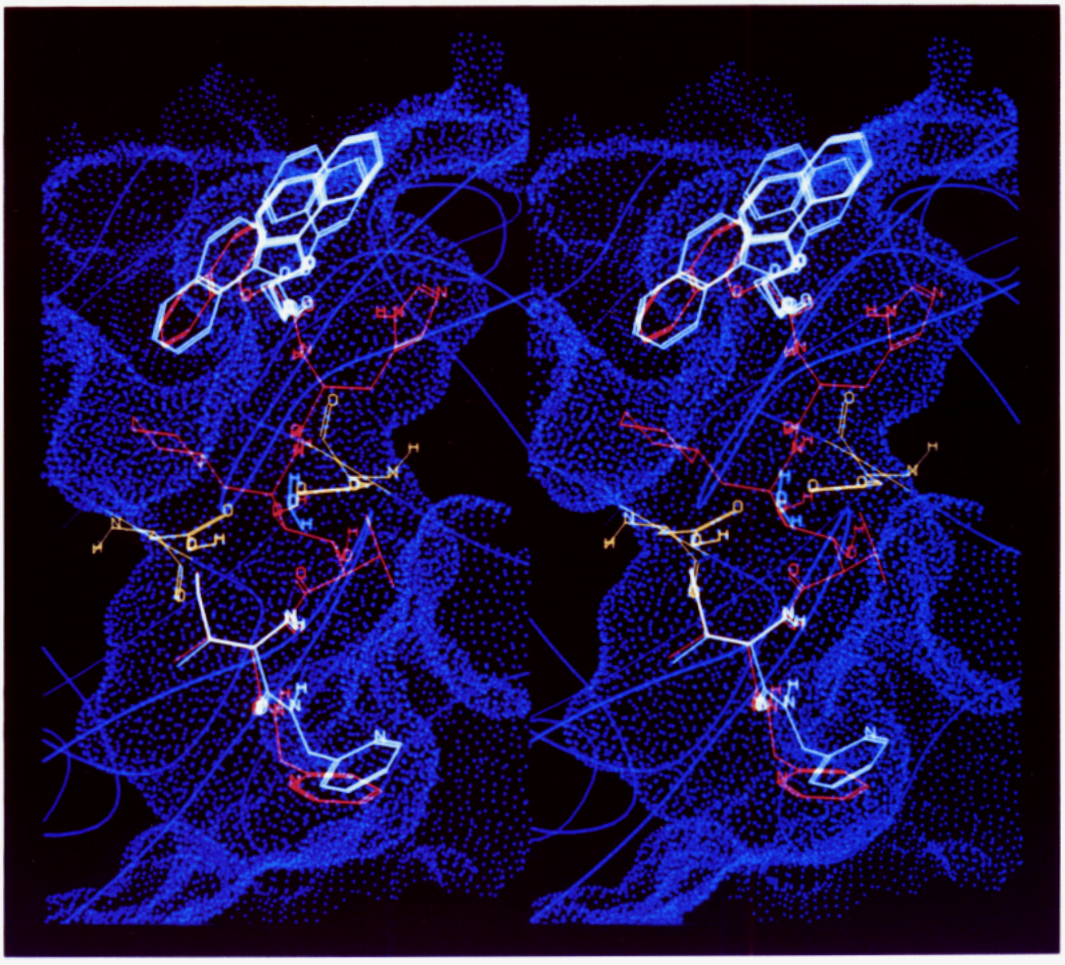


Figure 2. Modeled structures of peptide II bound to the HIV-1 protease active site, viewed through the enzyme's two flaps and along its C_2 axis. The most stable bound conformation found for the inhibitor is shown in red. Shown in white are alternate conformations for the Noa and Ile-Amp groups, suggested by the GROW ligand design software. The catalytic aspartic acid residues of the protease are in yellow, with the remaining protease backbone structure depicted with a simplified string representation. A molecular dot surface shows the shape of the active site cleft, which is fully covered by the flaps.

port this effect. Another interesting observation is that the data show a preference by the enzyme for "asymmetric" inserts, in which P_1 and P_1' side chain groups are different, over inserts with identical P_1 and P_1' side chains. On the basis of the symmetry of the enzyme's active site, one might expect the more highly symmetrical inserts to bind more tightly. However, asymmetric arrangement of the dihydroxyethylene groups relative to the catalytic aspartates, as suggested by the modeling, may favor binding of inhibitors whose P_1 and P_1' side chains can accommodate such an arrangement.

Summary

A previously prepared renin inhibitor exhibited reasonable binding affinity to the HIV-1 protease. With this template, we have investigated a series of peptides that contain the dihydroxyethylene isostere as potentially effective inhibitors of the HIV-1 protease. The effect of different side chains at P_1 and P_1' on the binding affinity of these peptides was assessed. The structure–activity relationship due to the stereochemistry of the substituents at the transition-state insert was examined. Additionally, the effect of modifications at the N- and C-terminal groups on the activity of the resulting peptides was also briefly investigated. A highly potent inhibitor of the HIV-1 protease was identified in peptide II. It was further shown to possess desirable antiviral activity in cell culture. A molecular modeling study of this compound as bound in the active site of the enzyme was conducted and gave insight into the structure–activity relationship of analogous peptides. This class of compounds may provide therapeutic agents for the treatment of HIV infection.

Experimental Section

Chemistry. Mass spectra, infrared spectra, optical rotations, and combustion analyses were obtained by the Physical and Analytical Chemistry Department of the Upjohn Laboratories. ^{1}H NMR spectra were recorded at 80 MHz with a Varian Model CFT-20 and at 300 MHz with a Bruker Model AM-300 spectrometer. Chemical shifts were reported as δ units relative to tetramethylsilane as internal standard. Thin-layer chromatography was conducted with Analtech 0.25-mm glass plates precoated with silica gel GF. Chromatography used E. Merck silica gel 60 (70–230 mesh for column chromatography and 230–400 mesh for flash chromatography). All solvents for chromatography were reagent grade.

Reagents were from commercial sources and used without further purification unless otherwise noted. Diethyl ether was Mallinckrodt anhydrous grade. Dichloromethane was dried over 4A molecular sieves. Diisopropylethylamine and triethylamine were distilled from calcium hydride. Diethylphosphoryl cyanide was distilled before use. Tetrahydrofuran was distilled under argon from sodium metal in the presence of benzophenone. Oxalyl chloride was distilled from calcium hydride. Dimethyl sulfoxide was distilled from calcium hydride. Dibutylboron triflate was freshly prepared and distilled.

3-[3(*R*)-[3-(*tert*-Butyloxycarbonyl)-4(*S*)-(cyclohexylmethyl)-2,2-dimethyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-isopropyl-1-oxopropyl]-4(*R*)-methyl-5(*S*)-phenyl-2-oxazolidinone (3). To a stirred solution of 460 mg (1.75 mmol) of

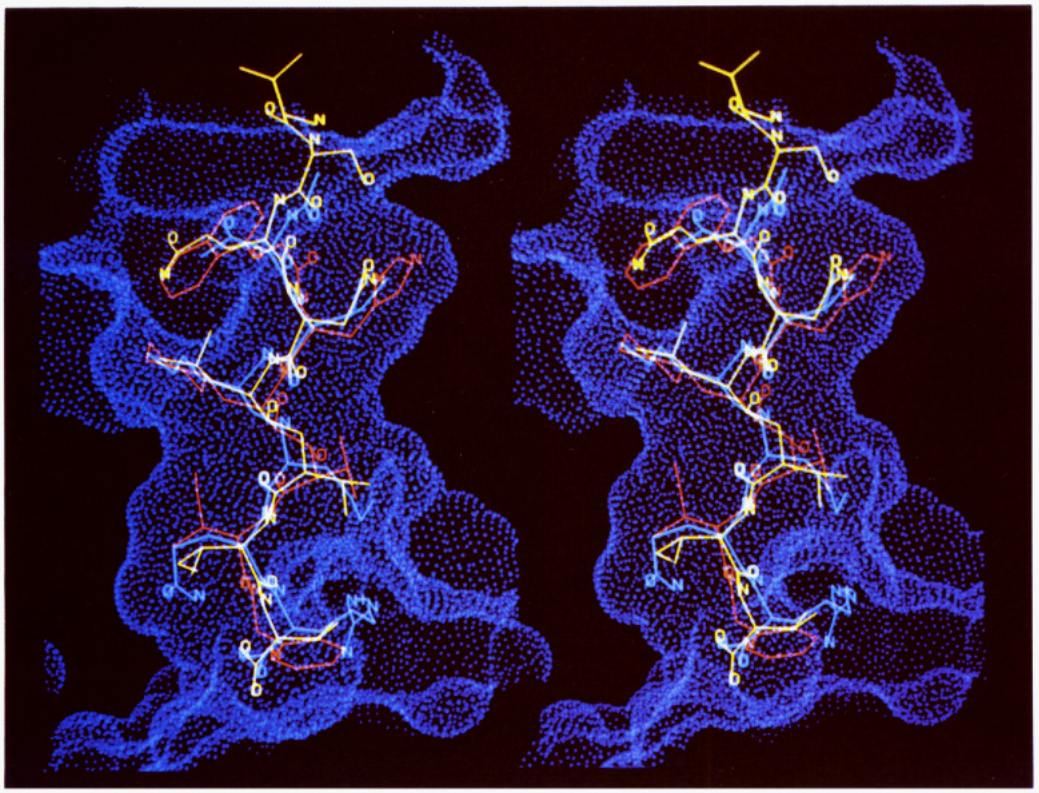


Figure 3. Modeled structure of compound II (red) overlaid with X-ray crystallographic structures of inhibitors MVT-101 (cyan) and U-85548E (yellow) in the HIV-1 protease active site. The molecular surface of the enzyme's active site is shown in blue. The viewpoint is approximately the same as in Figure 2.

4(*R*)-methyl-3-(1-oxo-3-methylbutyl)-5(*S*)-phenyl-2-oxazolidinone (**2**) in 1.8 mL of dichloromethane at 0 °C under argon was added dropwise 0.49 mL (1.92 mmol) of dibutylboron triflate followed by 0.36 mL (2.1 mmol) of diisopropylethylamine. After 30 min, the reaction mixture was cooled to −78 °C and 520 mg (1.59 mmol) of 3-(*tert*-butyloxycarbonyl)-4(*S*)-(cyclohexylmethyl)-2,2-dimethyl-5(*R*)-formyloxazolidine (**1**) in 1.5 mL of dichloromethane was added dropwise. After 30 min, the reaction mixture was allowed to warm to room temperature. After an additional 90 min, it was cooled in an ice bath and treated with 1.3 mL of methanol and 1.3 mL of 30% hydrogen peroxide in 2.6 mL of methanol. After 1 h, the reaction mixture was partitioned between dichloromethane and aqueous phosphate pH 7 buffer. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 15% ethyl acetate in hexane to give 860 mg (1.46 mmol, 90%) of 3-[3(*R*)-[3-(*tert*-butyloxycarbonyl)-4(*S*)-(cyclohexylmethyl)-2,2-dimethyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-isopropyl-1-oxopropyl]-4(*R*)-methyl-5(*S*)-phenyl-2-oxazolidinone (**3**): ^{1}H NMR ($CDCl_3$) δ 1.0 (m, 19 H), 1.5 (s, 9 H), 1.7 (s, 3 H), 4.0 (m, 4 H), 4.8 (m, 1 H), 5.6 (d, 1 H, J = 7 Hz), 7.4 (m, 5 H); IR (mull) 3470, 2868, 2853, 1789, 1692 cm^{-1}; $[\alpha]_D$ +18° (c = 1.0, $CHCl_3$); FAB HRMS m/z 587.3706 $(M + H)^+$ (calcd for $C_{33}H_{52}N_2O_7$ 587.3696). Anal. ($C_{33}H_{51}N_2O_7$) C, H, N.

1(*R*)-[3-(*tert*-Butyloxycarbonyl)-4(*S*)-(cyclohexylmethyl)-2,2-dimethyl-5(*R*)-oxazolidinyl]-2(*S*)-isopropyl-1,3-propanediol (4). To a stirred solution of 1.6 g (2.72 mmol) of 3-[3(*R*)-[3-(*tert*-butyloxycarbonyl)-4(*S*)-(cyclohexylmethyl)-2,2-dimethyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-isopropyl-1-oxopropyl]-4(*R*)-methyl-5(*S*)-phenyl-2-oxazolidinone (**3**) in 5.4 mL of tetrahydrofuran at 0 °C under argon was added dropwise 0.59 mL (3.4 mmol) of diisopropylethylamine, followed by 0.76 mL (3.0 mmol) of dibutylboron triflate. After 30 min, 3.4 mL (6.8 mmol) of a 2 M solution of lithium borohydride in tetrahydrofuran was added dropwise. After 90 min, the reaction mixture was allowed to warm to room temperature. After 30 min, it was cooled in an ice bath and treated with a solution containing 5.4 mL of methanol, 5.4 mL of aqueous phosphate pH 7 buffer, and 2.7 mL of 30% hydrogen peroxide. After stirring at room temperature for 14 h, the reaction mixture was partitioned between dichloromethane and aqueous phosphate pH 7 buffer. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 20% ethyl acetate in hexane to give 955 mg (2.31 mmol, 85%) of 1(*R*)-[3-(*tert*-butyloxycarbonyl)-4(*S*)-(cyclohexylmethyl)-2,2-dimethyl-5(*R*)-oxazolidinyl]-2(*S*)-isopropyl-1,3-propanediol (**4**): ^{1}H NMR ($CDCl_3$) δ 0.96 (2 × d, 6 H, J = 8 Hz), 1.47 (s, 9 H), 1.51 (s, 3 H), 1.65 (s, 3 H), 2.53 (m, 1 H), 3.76 (m, 1 H), 4.07 (m, 1 H); IR (neat) 3470, 2926, 1700, 1680 cm^{-1}; $[\alpha]_D$ +11° (c = 0.32, $CHCl_3$); FAB HRMS m/z 414 $(M + H)^+$.

2(*S*)-[5(*R*)-[1(*S*)-[(*tert*-Butyloxycarbonyl)amino]-2-cyclohexylethyl]-2,2-dimethyl-4(*R*)-dioxolanyl]-3-methylbutanol (5). A solution of 562 mg (1.36 mmol) of 1(*R*)-[3-(*tert*-butyloxycarbonyl)-4(*S*)-(cyclohexylmethyl)-2,2-dimethyl-5-(*R*)-oxazolidinyl]-2(*S*)-isopropyl-1,3-propanediol (**4**) in 5.5 mL of a 0.1 M solution of camphorsulfonic acid in dichloromethane was allowed to stir at room temperature for 45 min, and then 7.5 mL of acetone was added. After 14 h, the reaction mixture was treated with excess solid $NaHCO_3$. After 40 min, it was filtered through Celite and the filtrate concentrated. The residue was chromatographed on silica gel with 10% ethyl acetate in hexane to give 450 mg (1.08 mmol, 80%) of 2(*S*)-[5(*R*)-[1(*S*)-[(*tert*-butyloxycarbonyl)amino]-2-cyclohexylethyl]-2,2-dimethyl-4(*R*)-dioxolanyl]-3-methylbutanol (**5**): ^{1}H NMR ($CDCl_3$) δ 1.0–2.0 (m, 12 H), 1.36 (s, 3 H), 1.44 (s, 9 H), 2.90 (m, 1 H), 3.82 (m, 5 H), 4.75 (m, 1 H); IR (mull) 3397, 3267, 2954, 1671 cm^{-1}; $[\alpha]_D$ −26° (c = 0.89, $CHCl_3$); FAB HRMS m/z 414.3207 $(M + H)^+$ (calcd for $C_{23}H_{44}NO_5$ 414.3219). Anal. ($C_{23}H_{43}NO_5$) C, H. N.

2(*R*)-[5(*R*)-[1(*S*)-[(*tert*-Butyloxycarbonyl)amino]-2-cyclohexylethyl]-2,2-dimethyl-4(*R*)-dioxolanyl]-3-methylbutanoic Acid (6). To a stirred solution of 450 mg (1.08 mmol) of 2(*S*)-[5(*R*)-[1(*S*)-[(*tert*-butyloxycarbonyl)amino]-2-cyclohexylethyl]-2,2-dimethyl-4(*R*)-dioxolanyl]-3-methylbutanol (**5**) in 2 mL of acetonitrile, 2 mL of carbon tetrachloride, and 3 mL of water were added 993 mg (4.35 mmol) of periodic acid and 5.7 mg (0.02 mmol) of ruthenium trichloride hydrate. The resulting mixture was vigorously stirred for 2 h and then partitioned between dichloromethane and water. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 30% ethyl acetate in hexane to give

455 mg (1.06 mmol, 98%) of 2(*R*)-[5(*R*)-[1(*S*)-[(*tert*-butyloxycarbonyl)amino]-2-cyclohexylethyl]-2,2-dimethyl-4(*R*)-dioxolanyl]-3-methylbutanoic acid (**6**): ^{1}H NMR ($CDCl_3$) δ 1.0–2.0 (m, 11 H), 1.05 (3 × s, 9 H), 1.34 (s, 3 H), 1.44 (s, 9 H), 2.5 (m, 1 H), 4.02 (m, 4 H), 4.85 (m, 1 H), 9.25 (bs, 1 H); IR (mull) 3333, 2934, 1710, 1649 cm^{-1}; FAB HRMS *m/z* 428.2007 (M + H)$^+$ (calcd for $C_{23}H_{42}NO_6$ 428.3012). Anal. ($C_{23}H_{41}NO_6$) C, H, N.

3-[3(*R*)-[3-(*tert*-Butyloxycarbonyl)-4(*S*)-(cyclohexylmethyl)-2,2-dimethyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-isobutyl-1-oxopropyl]-4(*R*)-methyl-5(*S*)-phenyl-2-oxazolidinone (9a). By the same procedure as in the preparation of compound **3**, 193 mg (0.7 mmol) of 4(*R*)-methyl-3-(1-oxo-4-methylpentyl)-5(*S*)-phenyl-2-oxazolidinone (**8a**) in 0.3 mL of dichloromethane with 0.77 mL (0.77 mmol) of a 1 M solution of dibutylboron triflate in dichloromethane, 0.15 mL (0.86 mmol) of diisopropylethylamine, and 228 mg (0.7 mmol) of 3-(*tert*-butyloxycarbonyl)-4(*S*)-cyclohexylmethyl-2,2-dimethyl-5(*R*)-formyloxazolidine (**7a**) (same as aldehyde **1**), after chromatography on silica gel with 15–20% ethyl acetate in hexane, gave 307 mg (0.51 mmol, 73%) of 3-[3(*R*)-[3-(*tert*-butyloxycarbonyl)-4(*S*)-cyclohexylmethyl-2,2-dimethyl-5(*R*)-oxazolidinyl]-3-hydroxy-2-(*R*)-isobutyl-1-oxopropyl]-4(*R*)-methyl-5(*S*)-phenyl-2-oxazolidinone (**9a**): ^{1}H NMR ($CDCl_3$) δ 0.9 (m, 9 H), 1.49 (s, 9 H), 1.52 (s, 3 H), 1.65 (s, 3 H), 4.83 (m, 1 H), 5.65 (d, 1 H, *J* = 7 Hz), 7.2–7.5 (m, 5 H); FAB HRMS *m/z* 601.3882 (M + H)$^+$ (calcd for $C_{34}H_{53}N_2O_7$, 601.3853).

3-[3(*R*)-[3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-isobutyl-1-oxopropyl]-4(*R*)-methyl-5(*S*)-phenyl-2-oxazolidinone (9b): ^{1}H NMR ($CDCl_3$) δ 0.8–1.0 (m, 15 H), 1.48 (s, 9 H), 1.50 (s, 3 H), 1.64 (s, 3 H), 4.82 (m, 1 H), 5.64 (d, 1 H, *J* = 7 Hz), 7.2–7.5 (m, 5 H); FAB MS *m/z* 561 (M + H)$^+$ ($C_{31}H_{49}N_2O_7$).

3-[3(*R*)-[3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4(*S*)-phenylmethyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-phenylmethyl-1-oxopropyl]-4(*R*)-methyl-5(*S*)-phenyl-2-oxazolidinone (9c): ^{1}H NMR ($CDCl_3$) δ 1.57 (s, 9 H), 1.58 (s, 3 H), 1.66 (s, 3 H), 2.98 (d, 2 H, *J* = 13 Hz), 3.03 (d, 2 H, *J* = 11 Hz), 4.60 (m, 1 H), 7.0–7.4 (m, 15 H); FAB HRMS *m/z* 629.3219 (M + H)$^+$ (calcd for $C_{37}H_{45}N_2O_7$, 629.3227).

3-[3(*R*)-[3-(*tert*-Butyloxycarbonyl)-4(*S*)-(cyclohexylmethyl)-2,2-dimethyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-cyclohexylmethyl-1-oxopropyl]-4(*R*)-methyl-5(*S*)-phenyl-2-oxazolidinone (9d): ^{1}H NMR ($CDCl_3$) δ 0.88 (d, 3 H), 1.49 (s, 9 H), 1.53 (s, 3 H), 1.69 (s, 3 H), 4.83 (m, 1 H), 5.65 (d, 1 H, *J* = 7 Hz), 7.2–7.5 (m, 5 H); FAB HRMS *m/z* 641.4178 (M + H)$^+$ (calcd for $C_{37}H_{57}N_2O_7$, 641.4165).

3(*R*)-[3-(*tert*-Butyloxycarbonyl)-4(*S*)-(cyclohexylmethyl)-2,2-dimethyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-isobutylpropanoic Acid (10a). To a stirred solution of 800 mg (1.33 mmol) of 3-[3(*R*)-[3-(*tert*-butyloxycarbonyl)-4(*S*)-(cyclohexylmethyl)-2,2-dimethyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-isobutyl-1-oxopropyl]-4(*R*)-methyl-5(*S*)-phenyl-2-oxazolidinone (**9a**) in 13 mL of methanol at 0 °C was added 0.85 mL of 30% hydrogen peroxide, followed by a solution of 120 mg (2.86 mmol) of lithium hydroxide in 7 mL of cold water. The resulting mixture was allowed to warm to room temperature and stirred for 4 h. It was then partitioned between ether and water. The aqueous phase was acidified with aqueous hydrochloric acid and then extracted with dichloromethane. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 30% ethyl acetate in hexane to give 382 mg (0.87 mmol, 65%) of 3(*R*)-[3-(*tert*-butyloxycarbonyl)-4-(*S*)-(cyclohexylmethyl)-2,2-dimethyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-isobutylpropanoic acid (**10a**): ^{1}H NMR ($CDCl_3$) δ 0.93 (2 d, 6 H, *J* = 7 Hz), 1.48 (s, 9 H), 1.49 (s, 3 H), 1.63 (s, 3 H), 2.65 (m, 1 H), 3.71 (m, 1 H), 3.83 (m, 1 H); FAB HRMS *m/z* 442.3196 (M + H)$^+$ (calcd for $C_{24}H_{44}NO_6$, 442.3168).

3(*R*)-[3-(*tert*-Butyloxycarbonyl)-4(*S*)-isobutyl-2,2-dimethyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-isobutylpropanoic acid (10b): ^{1}H NMR ($CDCl_3$) δ 0.9–1.0 (m, 12 H), 1.47 (s, 9 H), 1.49 (s, 3 H), 1.63 (s, 3 H): FAB MS *m/z* 402 (M + H)$^+$ ($C_{21}H_{40}NO_6$).

3(*R*)-[3-(*tert*-Butyloxycarbonyl)-4(*S*)-phenylmethyl-2,2-dimethyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-(phenylmethyl)propanoic acid (10c): ^{1}H NMR ($CDCl_3$) δ 1.55 (s, 9 H), 7.1–7.3 (m, 10 H); FAB MS *m/z* 470 (M + H)$^+$ ($C_{27}H_{36}NO_6$).

3(*R*)-[3-(*tert*-Butyloxycarbonyl)-4(*S*)-(cyclohexylmethyl)-2,2-dimethyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-(cyclohexylmethyl)propanoic acid (10d): ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 9 H), 1.49 (s, 3 H), 1.62 (s, 3 H), 2.67 (m, 1 H), 3.72 (m, 1 H), 3.84 (m, 1 H); FAB HRMS *m/z* 482.3465 (M + H)$^+$ (calcd for $C_{27}H_{48}NO_6$, 482.3481).

3-[3-[3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*R*)-oxazolidinyl]-2(*R*)-isopropyl-1,3-dioxopropyl]-4(*R*)-methyl-5(*S*)-phenyl-2-oxazolidinone (12). To a stirred solution of 0.68 mL (7.7 mmol) of oxalyl chloride in 15 mL of dichloromethane at −78 °C under argon was added dropwise 1.1 mL (14.8 mmol) of dimethyl sulfoxide. After 10 min, a solution of 3.24 g (5.93 mmol) of 3-[3(*R*)-[3-(*tert*-butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-isopropyl-1-oxopropyl]-4(*R*)-methyl-5(*S*)-phenyl-2-oxazolidinone (**11**) in 12 mL of dichloromethane was added dropwise. After 20 min, the reaction mixture was added to 4.3 mL (30.8 mmol) of triethylamine and allowed to warm to room temperature. It was partitioned between dichloromethane and saturated aqueous $NaHCO_3$. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 10% ethyl acetate in hexane to give 2.94 g (5.4 mmol, 90%) of 3-[3-[3-(*tert*-butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5-(*R*)-oxazolidinyl]-2(*R*)-isopropyl-1,3-dioxopropyl]-4(*R*)-methyl-5(*S*)-phenyl-2-oxazolidinone (**12**): ^{1}H NMR ($CDCl_3$) δ 1.5 (s, 9 H), 1.6 (s, 6 H), 4.3 (bs, 1 H), 4.9 (m, 1 H), 5.2 (m, 1 H), 5.7 (m, 1 H), 7.4 (m, 5 H); IR (mull) 2957, 2934, 1791, 1705 cm^{-1}; $[\alpha]_D$ +43.7° (*c* = 0.96, $CHCl_3$); FAB HRMS *m/z* 545.3245 (M + H)$^+$ (calcd for $C_{30}H_{44}N_2O_7$ 545.3227).

3-[3(*S*)-[3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-isopropyl-1-oxopropyl]-4(*R*)-methyl-5(*S*)-phenyl-2-oxazolidinone (13). To a stirrred solution of 2.91 g (5.35 mmol) of 3-[3-[3-(*tert*-butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*R*)-oxazolidinyl]-2-(*R*)-isopropyl-1,3-dioxopropyl]-4(*R*)-methyl-5(*S*)-phenyl-2-oxoazolidinone (**12**) in 20 mL of methanol at 0 °C under argon was added dropwise a solution of 608 mg (16 mmol) of sodium borohydride in 5.3 mL of methanol. After 15 min, the reaction mixture was treated with saturated aqueous $NaHCO_3$ and methanol was removed under reduced pressure. The aqueous phase was extracted with dichloromethane. The organic phase was dried ($MgSO_4$) and the concentrated. The residue was chromatographed on silica gel with 10% ethyl acetate in hexane to give 1.75 g (3.2 mmol, 60%) of 3-[3(*R*)-[3-(*tert*-butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-isopropyl-1-oxopropyl]-4(*R*)-methyl-5(*S*)-phenyl-2-oxazolidinone (**11**) and 441 mg (0.806 mmol, 15%) of 3-[3-(*S*)-[3-(*tert*-butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-isopropyl-1-oxopropyl]-4(*R*)-methyl-5(*S*)-phenyl-2-oxazolidinone (**13**): ^{1}H NMR ($CDCl_3$) δ 1.0 (m, 6 H), 1.4 (s, 9 H), 1.5 (s, 6 H), 3.5 (m, 1 H), 4.1 (m, 1 H), 4.8 (m, 1 H), 5.7 (m, 1 H), 7.5 (m, 5 H); $[\alpha]_D$ +11° (*c* = 475, $CHCl_3$). Anal. ($C_{30}H_{46}N_2O_7$) C, H, N.

1(*S*)-[3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*R*)-oxazolidinyl]-2(*S*)-isopropyl-1,3-propanediol (14). By the same procedure as in the preparation of compound **4**, 408.5 mg (0.747 mmol) of (*tert*-butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-isopropyl-1-oxopropyl]-4(*R*)-methyl-5(*S*)-phenyl-2-oxazolidinone (**13**) in 1.5 mL of tetrahydrofuran with 0.16 mL (0.934 mmol) of diisopropylethylamine, 0.21 mL (0.822 mmol) of dibutylboron triflate, and 0.93 mL (1.87 mmol) of a 2 M solution of lithium borohydride in tetrahydrofuran, after chromatography on silica gel with 10% ethyl acetate in hexane, gave 196 mg (0.52 mmol, 70%) of 1-(*S*)-[3-(*tert*-butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*R*)-oxazolidinyl]-2(*S*)-isopropyl-1,3-propanediol (**14**): ^{1}H NMR ($CDCl_3$) δ 1.0 (m, 6 H), 1.45 (s, 9 H), 1.5 (s, 6 H), 2.0 (m, 1 H), 2.6 (m, 1 H), 3.5–4.4 (m, 4 H); IR ($CHCl_3$) 3450, 2930, 1770 cm^{-1}; $[\alpha]_D$ +16.7° (*c* = 0.88, $CHCl_3$); FAB HRMS *m/z* 374.2886 (M + H)$^+$ (calcd for $C_{20}H_{40}NO_5$ 374.2906).

3(*S*)-[3-(*tert*-Butyloxycarbonyl)-4(*S*)-isobutyl-2,2-dimethyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-isopropyl-propanoic Acid (15). By the same procedure as in the preparation of compound **6**, 139 mg (0.373 mmol) of 1(*S*)-[3-(*tert*-butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*R*)-oxazolidinyl]-2(*S*)-isopropyl-1,3-propanediol (**14**) in 0.68 mL of acetonitrile, 0.68 mL

of carbon tetrachloride, and 1.02 mL of water with 340 mg (1.49 mmol) of periodic acid and 1.95 mg (0.007 mmol) of ruthenium trichloride hydrate, after chromatography on silica gel with 60% ethyl acetate in hexane, gave 95.6 mg (0.256 mmol, 69%) of 3(*S*)-[3-(*tert*-butyloxycarbonyl)-4(*S*)-isobutyl-2,2-dimethyl-5-(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-isopropylpropanoic acid (**15**): ^{1}H NMR ($CDCl_3$) δ 1.0 (m, 6 H), 1.45 (s, 9 H), 1.5 (s, 6 H), 2.5 (m, 1 H), 3.5 (m, 1 H), 4.2 (m, 1 H); FAB HRMS *m/z* 388.2621 $(M + H)^+$ (calcd for $C_{20}H_{37}NO_6$ 388.2699).

Ethyl 3-[3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*S*)-oxazolidinyl]propenoate (17). To a stirred solution of 2.0 g (7.0 mmol) of 3-(*tert*-butyloxycarbonyl)-2,2-dimethyl-5(*R*)-formyl-4(*S*)-(2-methylpropyl)oxazolidine (**16**) in 14 mL of toluene was added 2.93 g (8.4 mmol) of (carbethoxymethylene)triphenylphosphorane. The resulting mixture was heated at 80 °C for 14 h. The cooled reaction mixture was diluted with ether and then filtered. The concentrated filtrate was triturated with ether and the ethereal extract was then concentrated. The residue was chromatographed on silica gel with 20% ether in hexane to give 1.12 g (3.5 mmol, 50%) of ethyl 3-[3-(*tert*-butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*S*)-oxazolidinyl]propenoate (**17**): ^{1}H NMR ($CDCl_3$) δ 0.94 (d, 3 H, J = 7 Hz), 0.95 (d, 3 H, J = 7 Hz), 1.30 (t, 3 H, J = 7 Hz), 1.48 (s, 9 H), 1.54 (s, 3 H), 1.62 (s, 3 H), 4.21 (q, 2 H, J = 7 Hz), 4.43 (m, 1 H), 6.05 (dd, 1 H, J = 1.5, 15.5 Hz), 6.96 (dd, 1 H, J = 6, 15.5 Hz); IR (neat) 2960, 1725, 1702, 1662 cm^{-1}. Anal. ($C_{19}H_{33}NO_5$) C, H, N.

3-[3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*S*)-oxazolidinyl]-2-propen-1-ol (18). To a stirred solution of 1.07 g (3.0 mmol) of ethyl 3-[3-(*tert*-butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*S*)-oxazolidinyl]propenoate (**17**) in 4 mL of ether at −78 °C under argon was added dropwise 9 mL (9 mmol) of a 1 M solution of diisobutylaluminum hydride in toluene. The resulting mixture was allowed to stir at −30 °C for 1 h. It was cooled to −78 °C and then treated with 1.5 mL of methanol. The mixture was allowed to warm to room temperature and a solution of 10 g of sodium potassium tartrate in 20 mL of water was added. After stirring for 2 h, the organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 40% ethyl acetate in hexane to give 0.856 g (2.73 mmol, 90%) of 3-[3-(*tert*-butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*S*)-oxazolidinyl]-2-propen-1-ol (**18**): ^{1}H NMR ($CDCl_3$) δ 0.96 (d, 3 H, J = 7 Hz), 0.97 (d, 3 H, J = 7 Hz), 1.47 (s, 9 H), 1.52 (s, 3 H), 1.64 (s, 3 H), 3.75 (bs, 1 H), 4.2 (m, 2 H), 4.33 (m, 1 H), 5.9 (m, 2 H): IR (neat) 3453, 2960, 1700, 1683 cm^{-1}; MS *m/z* 314 $(M + H)^+$. Anal. ($C_{17}H_{31}NO_4$) C, H, N.

(2*S*,3*R*)-3-[3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4-(*S*)-isobutyl-5(*R*)-oxazolidinyl]-2,3-epoxy-1-propanol (19). To a stirred solution of 818 mg (2.61 mmol) of 3-[3-(*tert*-butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*S*)-oxazolidinyl]-2-propen-1-ol (**18**) in 13 mL of dichloromethane at 0 °C was added 329 mg (3.92 mmol) of solid $NaHCO_3$, followed by 583 mg (2.9 mmol) of 85% *m*-chloroperbenzoic acid. The resulting mixture was allowed to warm to room temperature and stirred for 14 h, and then 1 g of solid Na_2SO_3 was added. After stirring for 1 h, the mixture was filtered and the filtrate concentrated. The residue was partitioned between ether and aqueous $NaHCO_3$. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 50% ethyl acetate in hexane to give 823 mg (2.5 mmol, 96%) of (2*S*,3*R*)-3-[3-(*tert*-butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*R*)-oxazolidinyl]-2,3-epoxy-1-propanol (**19**): ^{1}H NMR ($CDCl_3$) δ 0.95 (d, 3 H, J = 7 Hz), 0.96 (d, 3 H, J = 7 Hz), 1.49 (s, 9 H), 1.52 (s, 3 H), 1.62 (s, 3 H), 3.0–3.2 (m, 2 H), 3.6–4.1 (m, 4 H); IR (mull) 3480, 3212, 2924, 1708, 1678 cm^{-1}. Anal. ($C_{17}H_{31}NO_5$) C, H, N.

1(*R*)-[3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*R*)-oxazolidinyl]-2(*R*)-isobutyl-1,3-propanediol (20). To a stirred suspension of 67 mg (0.35 mmol) of copper(I) iodide and 329 mg (1.0 mmol) of (2*S*,3*R*)-3-[3-(*tert*-butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*R*)-oxazolidinyl]-2,3-epoxy-1-propanol (**19**) in 1 mL of tetrahydrofuran at −78 °C under argon was added dropwise 1.8 mL (3.6 mmol) of a 2 M solution of isobutylmagnesium bromide in ether. The resulting mixture was allowed to stir at −25 °C for 14 h, and then saturated aqueous NH_4Cl was added. The resulting mixture was allowed to warm to room temperature and then partitioned between ether and saturated aqueous NH_4Cl. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 40% ethyl acetate in hexane to give 188 mg (0.49 mmol, 49%), of 1(*R*)-[3-(*tert*-butyloxycarbonyl)-2,2-dimethyl-4-(*S*)-isobutyl-5(*R*)-oxazolidinyl]-2(*R*)-isobutyl-1,3-propanediol (**20**): ^{1}H NMR ($CDCl_3$) δ 0.9–1.0 (m, 12 H), 1.48 (s, 9 H), 1.53 (s, 3 H), 1.67 (s, 3 H), 3.5–4.0 (m, 5 H); IR (neat) 3456, 2957, 1702, 1684 cm^{-1}; MS *m/z* 388 $(M + H)^+$. Anal. ($C_{21}H_{41}NO_5$) C, H, N.

2(*R*)-[5(*R*)-[1(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-methylbutyl]-2,2-dimethyl-4(*R*)-dioxolanyl]-4-methylpentanol (21). By the same procedure as in the preparation of compound **5**, 1.46 g (3.77 mmol) of 1(*R*)-[3-(*tert*-butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*R*)-oxazolidinyl]-2(*R*)-isobutyl-1,3-propanediol (**20**) in 19 mL of 0.025 M solution of camphorsulfonic acid in dichloromethane, after chromatography on silica gel with 40–70% ethyl acetate in hexane, gave 0.92 g (2.4 mmol, 64%) of 2(*R*)-[5(*R*)-[1(*S*)-[(*tert*-butyloxycarbonyl)-amino]-3-methylbutyl]-2,2-dimethyl-4(*R*)-dioxolanyl]-4-methylpentanol (**21**): ^{1}H NMR ($CDCl_3$) δ 0.8–1.0 (m, 12 H), 1.40 (s, 3 H), 1.42 (s, 3 H), 1.45 (s, 9 H), 3.3–3.9 (m, 5 H); IR (neat) 3454, 2957, 1707 cm^{-1}; MS *m/z* 388 $(M + H)^+$. Anal. ($C_{21}H_{41}NO_5$) C, H, N.

2(*S*)-[5(*R*)-[1(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-methylbutyl]-2,2-dimethyl-4(*R*)-dioxolanyl]-4-methylpentanoic Acid (22). By the same procedure as in the preparation of compound **6**, 122 mg (0.315 mmol) of 2(*R*)-[5(*R*)-[1-(*S*)-[(*tert*-butyloxycarbonyl)amino]-3-methylbutyl]-2,2-dimethyl-4(*R*)-dioxolanyl]-4-methylpentanol (**21**) in 0.62 mL of carbon tetrachloride, 0.62 mL of acetonitrile, and 0.95 mL of water with 287 mg (1.3 mmol) of periodic acid and 2 mg of ruthenium trichloride hydrate, after chromatography on silica gel with 8% methanol in dichloromethane, gave 108 mg (0.27 mmol, 96%) of 2(*S*)-[5(*R*)-[1(*S*)-[(*tert*-butyloxycarbonyl)amino]-3-methylbutyl]-2,2-dimethyl-4(*R*)-dioxolanyl]-4-methylpentanoic acid (**22**): ^{1}H NMR ($CDCl_3$) δ 0.9–1.0 (m, 12 H), 1.37 (s, 3 H), 1.40 (s, 3 H), 1.46 (s, 9 H), 2.7 (m, 1 H), 3.7–3.9 (m, 3 H), 9.8 (bs, 1 H); MS *m/z* 402 $(M + H)^+$, 424 $(M + Na)^+$, 440 $(M + K)^+$.

***N*-[*N*-[2(*S*)-[5(*R*)-[1(*S*)-[(*tert*-Butyloxycarbonyl)-amino]-3-methylbutyl]-2,2-dimethyl-4(*R*)-dioxolanyl]-4-methylpentanoyl]-L-isoleucyl]-2-pyridylmethylamine (24).** To a stirred solution of 35.9 mg (89 μmol) of 2(*S*)-[(5*R*)-[1(*S*)-[(*tert*-butyloxycarbonyl)amino]-3-methylbutyl]-2,2-dimethyl-4-(*R*)-dioxolanyl]-4-methylpentanoic acid (**22**) and 22 mg (100 μmol) of *N*-L-isoleucyl-2-pyridylmethylamine (**23**) in 1 mL of dichloromethane was added 17 μL (100 μmol) of diisopropylethylamine and 15 μL (100 μmol) of diethylphosphoryl cyanide. The resulting solution was stirred at room temperature for 14 h. The concentrated mixture was chromatographed on silica gel with 3% methanol in ethyl acetate to give 54 mg (89 μmol, 100%) of *N*-[*N*-[2(*S*)-[5(*R*)-[1(*S*)-[(*tert*-butyloxycarbonyl)amino]-3-methylbutyl]-2,2-dimethyl-4(*R*)-dioxolanyl]-4-methylpentanoyl]-L-isoleucyl]-2-pyridylmethylamine (**24**): ^{1}H NMR ($CDCl_3$) δ 0.85–0.95 (m, 18 H), 1.39 (s, 3 H), 1.45 (s, 9 H), 1.52 (s, 3 H), 7.15 (dd, 1 H, J = 3, 7.5 Hz), 7.23 (d, 1 H, J = 7.5 Hz), 7.35 (t, 1 H, J = 7 Hz), 7.63 (t, 1 H, J = 7.5 Hz), 8.5 (d, 1 H, J = 3 Hz); FAB MS *m/z* 605 $(M + H)^+$ ($C_{33}H_{57}N_4O_6$).

***N*-[*N*-[5(*S*)-Amino-3(*R*),4(*R*)-dihydroxy-2(*S*)-isobutyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine (25).** A solution of 70 μL (1 mmol) of acetyl chloride in 1 mL of methanol was stirred for 15 min and then 54 mg (89 μmol) of *N*-[*N*-[2(*S*)-[5(*R*)-[1(*S*)-[(*tert*-butyloxycarbonyl)amino]-3-methylbutyl]-2,2-dimethyl-4(*R*)-dioxolanyl]-4-methylpentanoyl]-L-isoleucyl]-2-pyridylmethylamine (**24**) was dissolved in the resulting solution, and then 15 μL of ethanedithiol was added. After 5 h, excess solid $NaHCO_3$ was added and mixture stirred for 1 h. It was diluted with dichloromethane and filtered. The filtrate was then concentrated. The residue was triturated with dichloromethane and the concentrated material was chromatographed on silica gel with 5% methanol (saturated with ammonia) in dichloromethane to give 28.5 mg (61 μmol, 70%) of *N*-[*N*-[5(*S*)-amino-3(*R*),4(*R*)-dihydroxy-2(*S*)-isobutyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine (**25**): ^{1}H NMR ($CDCl_3$) δ 0.85–0.95 (m, 18 H), 1.1–1.8 (m, 9 H), 2.3 (m, 1 H), 2.62 (m, 1 H), 2.85 (m, 1 H), 4.4–4.6 (m, 3 H), 6.5 (d, 1 H, J = 9 Hz), 7.17 (dd, 1 H, J = 3, 7.5 Hz), 7.28 (d, 1 H, J = 7.5 Hz), 7.55 (t,

1 H, J = 7.5 Hz), 8.2 (t, 1 H, J = 7 Hz), 8.45 (d, 1 H, J = 3 Hz); FAB MS m/z 465 $(M + H)^+$ ($C_{25}H_{45}N_4O_4$).

N-[N-[N-[N-(1-Naphthoxyacetyl)-N^{im}-(*tert*-butyloxycarbonyl)-L-histidyl]-5(S)-amino-3(R),4(R)-dihydroxy-2(S)-isobutyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine (26). To a stirred solution of 28.5 mg (61 μmol) of N-[N-[5(S)-amino-3(R),4(R)-dihydroxy-2(S)-isobutyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine (**25**) and 32 mg (73 μmol) of N-(1-naphthoxyacetyl)-N^{im}-(*tert*-butyloxycarbonyl)-L-histidine in 0.6 mL of dichloromethane was added 13 μL (75 μmol) of diisopropylethylamine and 32 mg (72 μmol) of (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate. After stirring for 14 h, the concentrated reaction mixture was chromatographed on silica gel with 5% methanol (saturated with ammonia) in dichloromethane to give 49 mg (55 μmol, 90%) of N-[N-[N-[N-(1-naphthoxyacetyl)-N^{im}-(*tert*-butyloxycarbonyl)-L-histidyl]-5(S)-amino-3(R),4(R)-dihydroxy-2(S)-isobutyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine (**26**): ^{1}H NMR ($CDCl_3$) δ 0.85–0.95 (m, 18 H), 1.52 (s, 9 H); FAB MS m/z 886 $(M + H)^+$ ($C_{48}H_{68}N_7O_9$).

N-[N-[N-[N-(1-Naphthoxyacetyl)-L-histidyl]-5(S)-amino-3(R),4(R)-dihydroxy-2(S)-isobutyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine (IX). A solution of 49 mg (55 μmol) of N-[N-[N-[N-(1-naphthoxyacetyl)-N^{im}-(*tert*-butyloxycarbonyl)-L-histidyl]-5(S)-amino-3(R),4(R)-dihydroxy-2(S)-isobutyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine (**26**) in 0.5 mL of 50% trifluoroacetic acid in dichloromethane was stirred for 15 min. The reaction mixture was added to a stirred solution of saturated aqueous $NaHCO_3$ and the resulting mixture extracted with dichloromethane. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 6% methanol in dichloromethane to give 17.3 mg (22 μmol, 40%) of N-[N-[N-[N-(1-naphthoxyacetyl)-L-histidyl]-5(S)-amino-3(R),4(R)-dihydroxy-2(S)-isobutyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine (IX): ^{1}H NMR ($CDCl_3$) δ 0.85–0.95 (m, 18 H), 4.61 (bs, 1 H); FAB HRMS m/z 786.4579 $(M + H)^+$ (calcd for $C_{43}H_{60}N_7O_7$, 786.4554).

The following are FAB HRMS data for peptides I–XVI:

peptides	formula	calcd	found
I	$C_{42}H_{58}N_7O_7$	772.4397	772.4404
II	$C_{45}H_{62}N_7O_7$	812.4710	812.4689
III	$C_{49}H_{56}N_7O_7$	854.4241	854.4230
IV	$C_{43}H_{60}N_7O_7$	786.4554	786.4540
V	$C_{49}H_{56}N_7O_7$	854.4241	854.4230
VI	$C_{49}H_{68}N_7O_7$	866.5180	866.5189
VIII	$C_{44}H_{67}N_8O_8$	835.5082	835.5084
IX	$C_{43}N_{60}N_7O_7$	786.4554	786.4579
XI	$C_{38}H_{56}N_7O_7$	722.4241	722.4276
XII	$C_{31}H_{50}N_5O_6$	588.3761	588.3782
XIII	$C_{41}H_{60}N_7O_7$	762.4554	762.4574
XIV	$C_{35}H_{56}N_7O_7$	686.4241	686.4244
XV	$C_{35}H_{57}N_8O_6$	685.4401	685.4382
XVI	$C_{34}H_{56}N_4O_5$	601.4329	601.4331

HIV Protease Inhibition Assay. Recombinant HIV-1 protease was recovered and refolded from *Escherichia coli* inclusion bodies as described previously.[9] The enzyme was assayed against synthetic peptide H-Val-Ser-Gln-Asn-Tyr-Pro-Ile-Val-OH (GSP) as described previously.[9] The HIV protease activity was measured at 30 °C in 100 mM sodium acetate, pH 5.5, containing 10% glycerol, 5% ethylene glycol, and 0.1% Nonidet P-40 in a total volume of 50 μL. In experiments at pH 7, 100 mM MOPS was substituted for sodium acetate. The reaction was stopped after 30 min by the addition of 75 μL of 1% trifluoroacetic acid and samples subjected to HPLC analysis. For inhibitory study, the peptides were added to the incubation mixture before the addition of the HIV-1 protease. In these cases, five to seven inhibitor concentrations were examined with GSP at 2.5 mM concentration. Data were analyzed by Dixon plots.

Inhibition of p55 Processing in vVk-1 Infected CV-1 Cells.[20] CV-1 cells were seeded at 2×10^5 cells per well in 24-well Costar dishes and infected 4–6 h later with vVK-1 at five plaque-forming units (PFU) per cell. Peptides in Dulbecco's Modified Eagles medium (DMEM) containing 2.5% fetal bovine serum were added to duplicate wells at the indicated final concentration 2 h after virus addition. After 24 h, the culture medium was removed and the monolayer washed with 1 mL of PBS, and the cells lysed by the addition of 0.1 mL of loading buffer (62.5 mM Tris, pH 6.8, 2.3% SDS, 5% β-mercaptoethanol, and 10% glycerol). The cell lysates were collected individually and placed in boiling water for 3 min, and then 0.025 mL of each sample was subjected to electrophoresis in 12% SDS–polyacrylamide gels. The proteins were electroblotted onto nitrocellulose and analyzed by protein immunoblotting. The primary antibodies were sheep antibody to p24 (International Enzyme, Inc., Fallbrook, CA) and the secondary antibody was alkaline-phosphatase-conjugated rabbit antibody to sheep immunoglobulin G (Kirkegaard & Perry Laboratories, Gaithersburg, MD). The levels of immunoreactive proteins were quantified by densitometry (Bio-Rad, Model 260) with the accompanying 1-D Analyst Software. Inhibition refers to percent decrease in p24 level relative to non-drug-treated controls.

Molecular Modeling. A set of molecular models of compound II was constructed by modification of the crystallographic structure of the MVT-101/HIV-1 protease complex[17] obtained from A. Wlodawer. The 3-D coordinates of this complex have since been made available through the Brookhaven Protein Databank[42] and the Brookhaven entry name for the PR/MVT-101 complex is 4 HVP. Construction of the models was achieved with the Mosaic modeling system[43] in conjunction with the GROW ligand-design program.[44] BatchMin,[45] a program for molecular mechanics-based energy minimization, was used for structural optimizations of the models. The first step in this process involved changing the Nleψ[CH_2NH]Nle insert to the Calψ[CH(OH)CH(OH)]Val group of the target compound. The backbone atom positions were retained, and the Nle side-chain atoms beyond C_β were replaced by the appropriate groups. Side-chain torsion angles were interactively adjusted to optimize fit into the binding site. The structure was subjected to energy minimization using the BatchMin implementation of the AMBER force field with BatchMin's PRCG (Polak-Ribiere conjugate gradient) minimizer. Minimization was considered complete when an root mean square gradient of <0.1 kcal/Å was obtained. Positioning of the Noa-His and Ile-Amp groups was then performed with GROW program. In this method, sequences are algorithmically "grown" from a user-defined starting position in a binding site. Conformations of each residue which are calculated to interact most favorably with the binding site atoms are connected together to build one or more models of a bound ligand. The program found multiple conformations of the Noa-His and Ile-Amp sequences which could fit the binding cleft well. The His and Ile residues appeared to adopt single preferred conformations, while several feasible conformations were found for the Noa and Amp end groups. Models containing combinations of the different end group conformations were optimized in the presence of the binding site atoms, using BatchMin/AMBER minimization. Differences in interaction energies computed for the various models were not large enough to suggest a single correct conformation for the bound inhibitor. The models collectively form a hypothesis of the binding interactions between the inhibitor and enzyme.

Registry No. **1**, 117997-58-7; **2**, 107599-99-5; **3**, 112190-42-8; **4**, 134527-83-6; **5**, 134527-84-7; **6**, 112190-45-1; **7c**, 134458-64-3; **8a**, 134458-52-9; **8c**, 95841-14-8; **8d**, 134458-53-0; **9a**, 134458-65-4; **9b**, 134458-66-5; **9c**, 134458-67-6; **9d**, 134458-68-7; **10a**, 134458-69-8; **10b**, 134458-70-1; **10c**, 134458-71-2; **10d**, 134458-72-3; **11**,

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2-Chloro-1-(2,4-dichlorophenyl)-3-(1*H*-imidazol-1-yl)-2-phenylpropan-1-one Hydrochloride, a Novel, Nonmutagenic Antibacterial with Specific Activity against Anaerobic Bacteria

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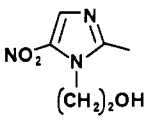
1-(2,4-Dichlorophenyl)-2-phenylpropen-1-one (**2**) is identified as a potent antibacterial agent. A compound, 2-chloro-1-(2,4-dichlorophenyl)-3-(1*H*-imidazol-1-yl)-2-phenylpropan-1-one (**5**) has been designed with the intention of its acting as a pro-drug, liberating the lethal species **2** specifically within the target anaerobic bacterial cell following bioreduction by bacterial ferredoxin or related electron transfer proteins. The synthesis and biological activity of **5** is described and compared with the activities of the analogous α-bromo ketone **6** and α-fluoro ketone **7**. Synthesis of **6**, **7**, and the corresponding α-hydroxy ketone **11** is also described.

Anaerobic bacteria are accepted as a major cause of infection and are particularly important in postoperative sepsis following surgery of either the gastrointestinal or genitourinary tracts. For the prophylaxis and therapy of anaerobic sepsis, nitroimidazoles such as metronidazole **1** have been shown to be of considerable benefit.[1-3]



(1)

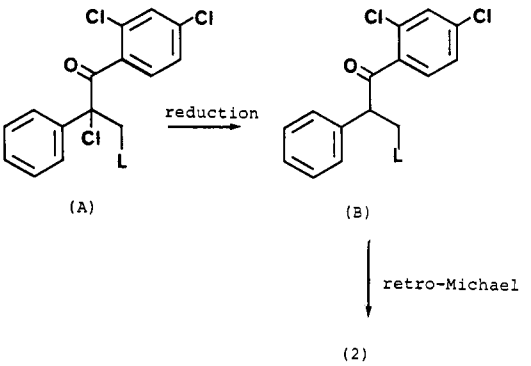
Anaerobic bacteria also play a significant role in periodontal disease. As in postoperative sepsis, nitroimidazoles have been shown to be effective,[4,5] but their usage has not yet gained as wide acceptance. One possible explanation is that metronidazole is reported to be mutagenic[6] as determined in the Ames test.[7]

It is therefore anticipated that a novel, nonmutagenic specific antianaerobe would be of considerable interest, not only in the treatment and prophylaxis of postoperative sepsis, but also in the treatment of periodontal disease.

The mode of action of metronidazole results in a remarkable specificity for anaerobic organisms. Metronidazole is a precursor of lethal intermediates (as yet unidentified) which are generated via specific bioreduction by highly reducing ferredoxins or electron-transfer proteins unique to anaerobic bacteria and some protozoa.[8-12] The absence of such reducing systems in aerobic bacteria and, more importantly, within the mammalian host ensures that such lethal species are generated specifically within the target anaerobe.

For the design of a novel agent with the same level of specificity it is desirable to utilize the uniqueness of these bacterial ferredoxins. Conceptually this involves the choice of a suitable lethal intermediate which is generated only under such reducing conditions. Other groups[13] have screened potentially bioreducible compounds in the search for specific antianaerobe activity but we have chosen to take a compound with demonstrable antibacterial activity and seek to enhance its specificity by ensuring that it is

Scheme I



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