Potent HIV Protease Inhibitors: The Development of Tetrahydrofuranylglycines as Novel P_2-Ligands and Pyrazine Amides as P_3-Ligands

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Received April 5, 1993

A series of protease inhibitors bearing constrained unnatural amino acids at the P_2-position and novel heterocycles at the P_3-position of compound 1 (Ro 31-8959) were synthesized, and their in vitro enzyme inhibitory and antiviral activities were evaluated. Replacement of P_2-asparagine of compound 1 with (2*S*,3′*R*)-tetrahydrofuranylglycine resulted in improvement in enzyme inhibitory as well as antiviral potencies (compound **23**). Interestingly, incorporation of (2*S*,3′*S*)-tetrahydrofuranylglycine at the P_2-position proved to be less effective. The resulting compound **24** was 100-fold less potent than the 2*S*,3*R*-isomer (compound **23**). This stereochemical preference indicated a hydrogen-bonding interaction between the tetrahydrofuranyl oxygen and the residues of the S_2-region of the enzyme active site. Furthermore, replacement of P_3-quinolinoyl ligand of 1 with various novel heterocycles resulted in potent inhibitors of HIV proteases. Of particular interest, compound **2** with (2*S*,3′*R*)-tetrahydrofuranylglycine at P_2 and pyrazine derivative at P_3 is one of the most potent inhibitors of HIV-1 (IC_{50} value 0.07 nM) and HIV-2 (IC_{50} value 0.18 nM) proteases. Another important result in this series is the identification of compound **27** in which the P_2–P_3-amide carbonyl has been removed. The resulting compound **27** has exhibited improvement in antiviral potency while retaining the enzyme inhibitory potency similar to compound 1.

The inhibition of the enzyme HIV-1 protease, which cleaves the *gag* and *gag-pol* polyproteins into the functional proteins of infectious virions, continues to be a major therapeutic target for the treatment of AIDS and related ailments.[1] Of the numerous potent protease inhibitors that have been reported recently,[2] the present clinical candidate (2*S*,4a*S*,8a*S*,2′*R*,3′*S*)-*N*-*tert*-butyl-2-(2′-hydroxy-4′-phenyl-3′-((*N*-(2-quinolinylcarbonyl)-L-asparaginyl)-amino)butyl)decahydroisoquinoline-3-carboxamide (1) (Ro 31-8959) is particularly unique because of its effectiveness against both the enzymes HIV-1 and HIV-2 proteases.[3] Since these are the genetically most divergent strains of HIV known to exist to date, a protease inhibitor with this property may result in reduced susceptibility to clinical resistance. Subsequently, we have investigated the effect of incorporation of conformationally constrained unnatural amino acids in place of asparagine at the P_2 subsite of compound 1. As reported in a recent paper,[4] the replacement of asparagine with (2*S*,3′*R*)-tetrahydrofuranylglycine not only increased the enzyme affinities for HIV-1 and HIV-2 proteases but led to significant enhancement of antiviral potencies compared to 1. We now report the synthesis, enzyme inhibition, and antiviral potencies of a structurally novel class of protease inhibitors in which various constrained unnatural amino acids and novel heterocyclic derivatives were incorporated at the P_2- and P_3-positions of the present clinical candidate 1 (Ro 31-8959). This study has resulted in protease inhibitors with improved enzyme inhibitory and antiviral potencies. Particularly noteworthy is the inhibitor **2** which is very potent against HIV-1 (IC_{50} = 0.07 nM) and HIV-2 (IC_{50} = 0.18 nM).

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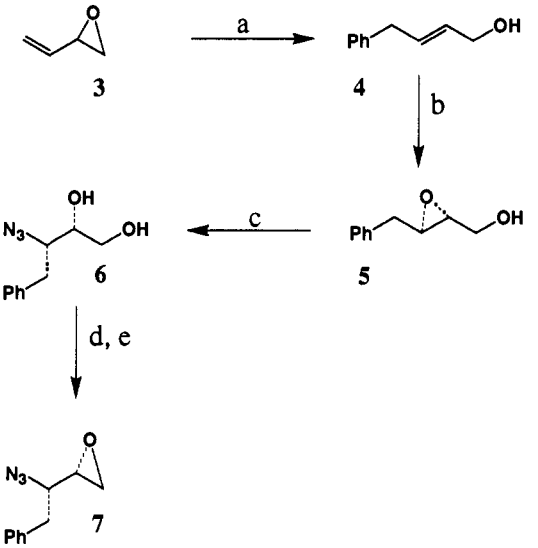
Scheme I. Synthesis of Azido Epoxide[a]



[a] Key: (a) PhMgBr, CuCN, THF, −78 °C; (b) tBuOOH, (−)-DET, $Ti(OiPr)_4$, CH_2Cl_2, −22 °C; (c) $Ti(OiPr)_2(N_3)_2$, PhH, 80 °C; (d) $AcOCMe_2COCl$, $CHCl_3$, 23 °C; (e) NaOMe, THF, 23 °C.

Chemistry

The desired (2*R*,3*S*)-azido epoxide **7** was prepared as shown in Scheme I. The commercially available butadiene monoxide **3** was converted to allylic alcohol **4** by reaction with phenylmagnesium bromide in the presence of a catalytic amount of cuprous iodide. The allylic alcohol **4** was then subjected to the Sharpless epoxidation[5] condition with (−)-diethyl D-tartrate to furnish the epoxide **5**. Regioselective ring opening of epoxide **5** with diisopropoxytitanium diazide in benzene at 75 °C, as described by Sharpless and co-workers,[6] afforded the azidodiol **6** in very good yield. Azidodiol **6** was then converted efficiently to the desired azidoepoxide **7** by treatment with 2-acetox-

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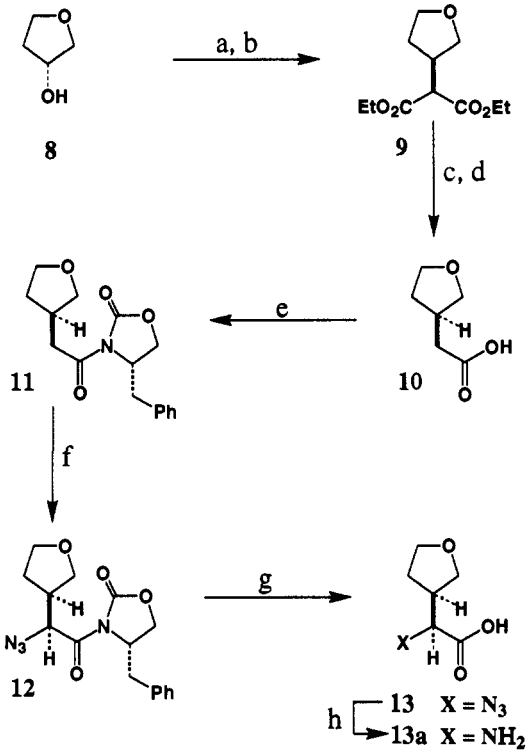
Scheme II. Synthesis of Amino Acid[a]

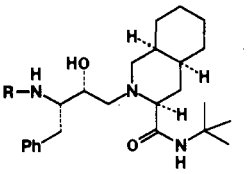


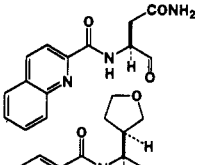
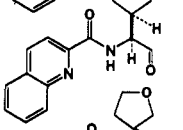
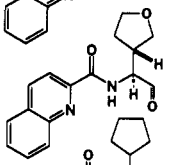
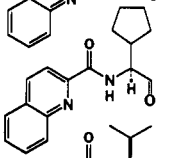
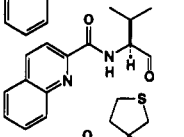
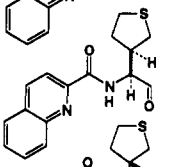
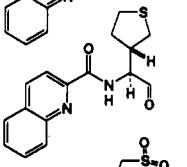
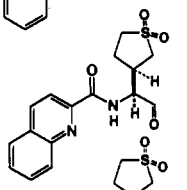
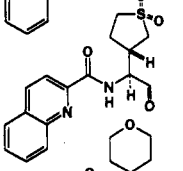
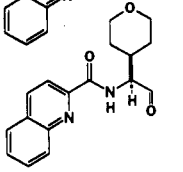
[a] Key: (a) MsCl, Et_3N, CH_2Cl_2, −10 °C; (b) NaH, $CH_2(CO_2Et)_2$, DMF; (c) 1 N NaOH then H_3O^+; (d) Cu_2O (cat), CH_3CN, 80 °C; (e) Me_3COCl, Et_3N, THF, −78 °C then *N*-lithio-(*S*)-(−)-4-benzyloxazolidinone; (f) $KN(TMS)_2$, THF, −78 °C, 30 min, then trisyl azide, −78 °C, 2 min, then AcOH, 30 °C, 1 h; (g) LiOH, THF–H_2O; (h) 5% Pd–C, EtOH–H_2O.

yisobutyryl chloride in chloroform at 23 °C followed by exposure of the resulting chloroacetate derivative to an excess of sodium methoxide in tetrahydrofuran at 23 °C for 3 h.[7]

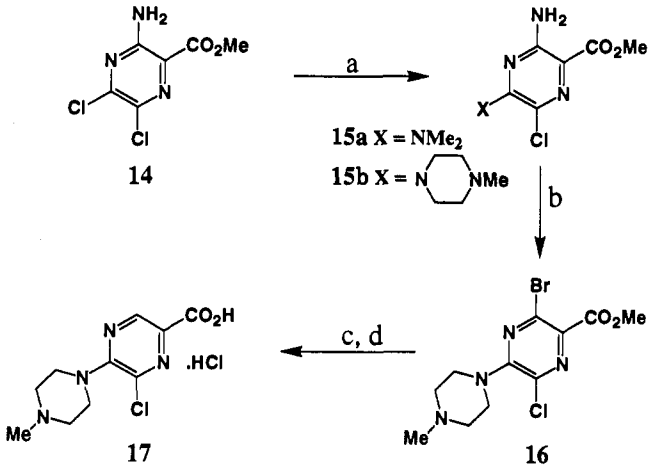
The synthetic route leading to the (2*S*,3′*R*)-tetrahydrofuranylglycine equivalent is described in Scheme II. Readily prepared[8] enantiomerically pure (*S*)-(+)-3-hydroxytetrahydrofuran (**8**) was mesylated with mesyl chloride and triethylamine in methylene chloride at 0 °C for 20 min. Displacement[9] of the resulting mesylate with the sodium salt of diethyl malonate in DMF at 100 °C furnished the malonate derivative **9**. Ester hydrolysis followed by copper ion promoted decarboxylation[10] furnished the (*R*)-tetrahydrofuranylacetic acid (**10**) in very good yield. The highly diastereoselective azidation protocol developed by Evans[11] was then employed to introduce the α-amine functionality. Thus, deprotonation of the (*S*)-(−)-4-benzyloxazolidinone with n-BuLi followed by acylation with the mixed anhydride resulting from the reaction of acid **10** with pivaloyl chloride in the presence of triethylamine provided the carboximide **11** after silica gel chromatography. Treatment of this carboximide with potassium hexamethyldisilazide in tetrahydrofuran at −78 °C for 30 min provided the potassium enolate which was reacted with trisyl azide at −78 °C for 2 min and then quenched with glacial acetic acid and warmed to 30 °C. The α-azido carboximide thus obtained was purified by silica gel chromatography to furnish the azide **12** as a single diastereomer by HPLC and ^1H-NMR (400-MHz) analysis. Removal of the chiral auxiliary was effected by exposure to lithium hydroxide in aqueous tetrahydrofuran to provide the desired acid **13**. The resulting azido acid was hydrogenated over 5% palladium on charcoal in a mixture of ethanol and water (2:1) to furnish the amino acid **13a**. The corresponding (2*S*,3′*S*)-tetrahydrofuranylglycine equivalent was obtained utilizing (*R*)-(−)-3-hydroxytetrahydrofuran as the starting material. Similarly, diastereomeric azido acid **28** and other cyclic amino acids utilized in Table I were prepared following a similar course of reaction as described in Scheme II. Various pyrazine derivatives were synthesized as shown in Scheme III. The known[12] dichloropyrazine derivative **14** was heated with dimethylamine or 1-methyl piperazine in 2-propanol at 85 °C for 12 h to furnish the pyrazine derivative **15a** or **15b**. Hydrolysis of the corresponding methyl ester with aqueous NaOH in ethanol and subsequent acidification provided the acid derivative. For the preparation of pyrazine derivative **17**, pyrazine **15b** was first converted to bromide

Table I. Structure and Inhibitory Potencies of Various Constrained P_2-Ligands



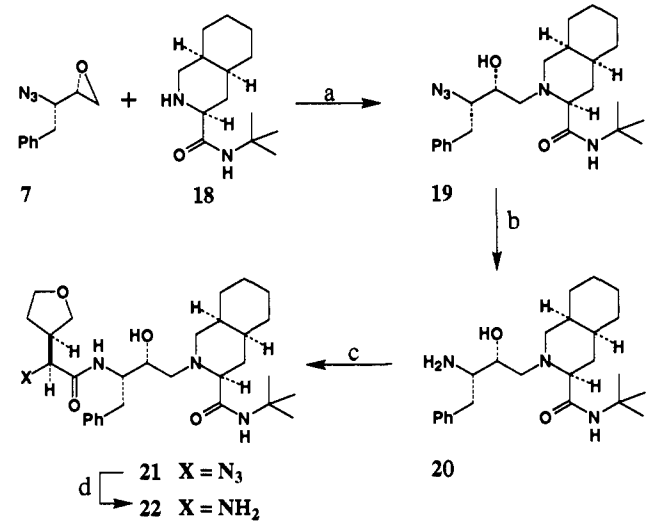
Compd	R	IC_{50} (nM)	CIC_{95} (nM)
1.		0.23±0.1 (n=3)	22±7 (n=10)
23.		0.054±0.027 (n=4)	8±4 (n=8)
24.		5.4	100
25.		2.6	---
26.		0.7	100
31.		0.6	50
32.		3.3	>200
33.		0.5	---
34.		39	---
35.		67.9	---

Scheme III. Synthesis of Pyrazine Derivatives[a]



[a] Key: (a) dimethylamine or 1-methyl piperazine, iPrOH, 85 °C; (b) $NaNO_2$, Br_2, HBr, AcOH; (c) H_2, 5% Pd–C, THF; (d) 1 N NaOH, EtOH then H_3O^+.

Scheme IV[a]

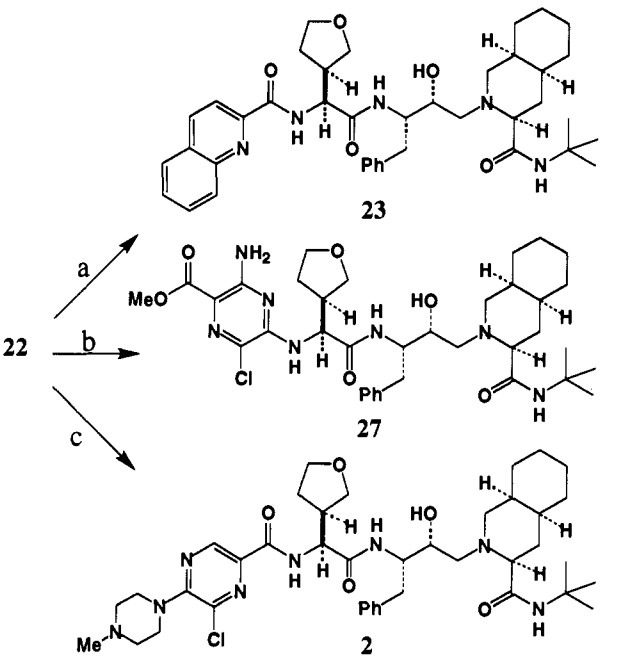


[a] Key: (a) iPrOH, 80 °C, 12 h; (b) H_2, 10% Pd–C, AcOH, MeOH–THF, 12 h; (c) azido acid 13, EDC, HOBt, Et_3N, DMF; (d) H_2, 5% Pd–C, AcOH, MeOH–THF, 12 h.

16 by reaction with bromine and sodium nitrite in a mixture of glacial acetic acid and 48% hydrobromic acid. Bromo ester 16 was then debrominated upon hydrogenation over 10% palladium on charcoal, and the resulting ester was saponified to furnish acid 17.

Various hydroxyethylamine isosteres containing tetrahydrofuranylglycine at the P_2 position were prepared following the general synthetic route outlined in Scheme IV. Azido epoxide 7 and decahydroisoquinoline[13,14] derivative 18 were heated at reflux in 2-propanol for 12 h to furnish the azido alcohol 19 in 83% yield after silica gel chromatography. Catalytic hydrogenation of azide 19 with 10% palladium on charcoal in a mixture of tetrahydrofuran and methanol (4:1) in the presence of acetic acid afforded the amine 20 in excellent yield. Using a standard peptide coupling procedure,[15] amine 20 was reacted with the azido acid 13 in the presence of *N*-ethyl-*N'*-(3-(dimethylamino)-propyl)carbodiimide hydrochloride, triethylamine, and 1-hydroxybenzotriazole hydrate in DMF to furnish the azide 21 which was hydrogenated over 5% palladium on charcoal to provide amine 22. The amine 22 was then converted to various protease inhibitors by standard coupling reaction with the corresponding acid (Scheme V). For example, reaction of quinaldic acid with diphenylphosphinic chloride and triethylamine in tetrahydrofuran at −10 °C for 1 h followed by addition of amine 22 afforded the inhibitor 23 in excellent yield.[16] Similarly, reaction of acid 17 with amine 22 resulted in the inhibitor 2. The *N*-pyrazinyl compound 27 has been prepared by reaction of pyrazine derivative 14 and the amine 22 in refluxing 2-propanol for 12 h. Various inhibitors with tetrahydrothiophene derivatives or sulfolanes at the P_2 position were synthesized according to Scheme VI. Azido acids 28 were prepared as a mixture of diastereomers following Evans' asymmetric azidation protocol described in Scheme II. The coupling of acid 28 with amine 20 under standard conditions afforded the coupling products 29 and 30. The isomers were easily separated by column chromatography over silica gel, and the stereochemistry at the 3-position of the tetrahydrothiophene ring was assigned by comparison of ^1H NMR (300 MHz) spectra of the azide 21 and the diastereomer of 21 that contains the 2(*S*)-azido-3(*S*)-tetrahydrofuranyl moiety. The azide derivatives 29 and 30 were then converted to inhibitors 31 and 32 following a similar course of reaction as described for inhibitor 23. Further assignments of tetrahydrothiophene ring stereochemistry of compound 31 and 32 were made based on comparison of ^1H NMR of the corresponding tetrahydrofuranyl derived inhibitors 23 and 24. Selective oxidation[17] of the ring sulfur of inhibitors 31 and 32 with a catalytic amount of osmium tetraoxide and an excess of *N*-methylmorpholine *N*-oxide in a mixture (3:1) of acetone and water furnished the sulfolane derivatives 33 and 34 in good yield. Various inhibitors in Tables I and II have been synthesized by following a similar course as shown in Schemes V and VI.

Scheme V[a]

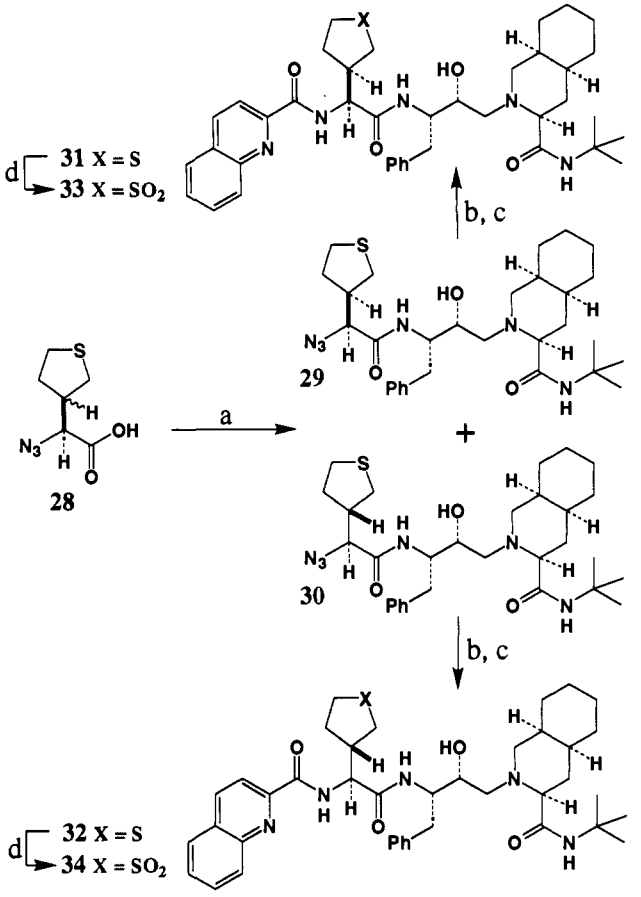


[a] Key: (a) quinaldic acid, Ph_2POCl, Et_3N, THF, −10 °C, 1 h, then 23 °C, 3 h; (b) pyrazine 14, iPrOH, 84 °C, 12 h; (c) acid 17, Ph_2POCl, Et_3N, THF, −10 °C, 2 h, then 23 °C, 3 h.

Results and Discussion

The X-ray crystal structure of the enzyme–inhibitor complex of L-689,502 bound to HIV-1 protease (2.25 Å

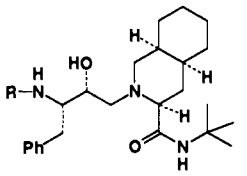
Scheme VI[a]

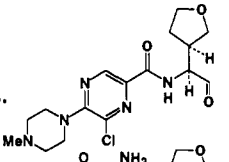
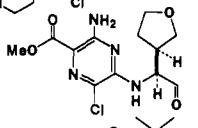
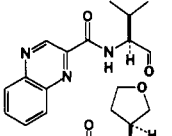
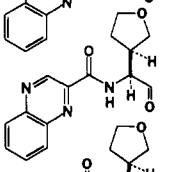
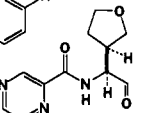
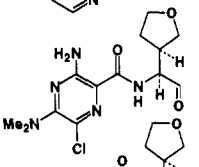
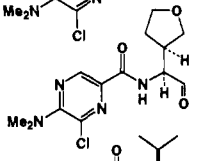
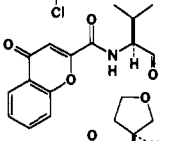
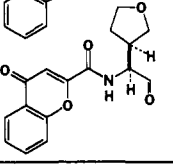


[a] Key: (a) amine **20**, EDC, HOBt, Et_3N, DMF; (b) H_2, 10% Pd–C, MeOH–EtOAc; (c) quinaldic acid, Ph_2POCl, Et_3N, THF, −10 °C, 1 h, then 23 °C, 3 h; (d) OsO_4, 4-methylmorpholine *N*-oxide, acetone–water.

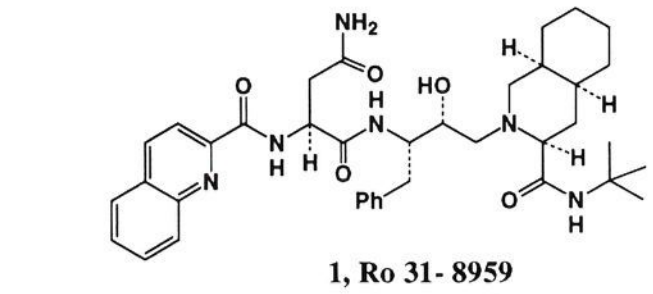
resolution)[18] was utilized to construct the modeled structure of compound **1** (Ro 31-8959). The structure was then energy minimized in the active site using the Merck molecular force field, OPTIMOL,[19] which is a variant of the MM2 program.[20] An examination of the modeled structure of inhibitor **1** revealed that the carbonyl oxygen of the asparagine is within hydrogen bonding distance to the asp 29 and asp 30 NH present in the S_2 binding domain of the HIV-1 protease. On the basis of this possible insight, we hypothesize that a conformationally constrained cyclic ether oxygen might donate its loan pairs for hydrogen bonding to the appropriate residues in the S_2 region of the enzyme active site. Such a design may effectively replace the P_2-asparagine, providing an inhibitor with improved *in vitro* potencies and pharmacokinetic properties. Indeed, as can be seen from Table I, the replacement of the asparagine of compound **1**[21] with (2*S*,3′*R*)-tetrahydrofuranylglycine (compound **23**) resulted in an over 4-fold increase in its inhibitory potency.[22] Interestingly, (2*S*,3′*S*)-tetrahydrofuranylglycine (3′(*S*)-Thfg) containing inhibitor (compound **24**) showed a 16-fold loss in potency compared to **1**. Furthermore, examination of the cyclopentyl derivative **25** established that the ring oxygen is essential for potency enhancement. Also, as expected, the inhibitory potency of the corresponding acyclic analog **26** is over 17-fold less potent than the compound **23**. The fact that the 3′(*R*)-Thfg-derived inhibitor **23** is more potent than the 3′(*S*)-Thfg-derived inhibitor **24** suggested that 3′(*R*)-Thfg is successfully mimicking the enzyme-bound conformation of the asparagine side chain of compound **1**. Accordingly,

Table II. Structure and Inhibitory Potencies of Various Constrained P_3-Ligands



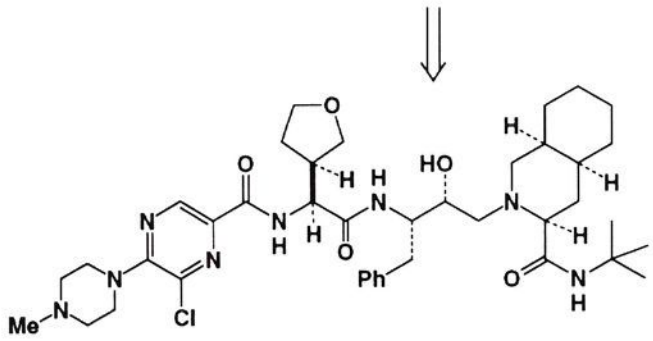
Compd	R	IC_{50} (nM)	CIC_{95} (nM)
2.		0.071±0.003 (n=2)	12
27.		0.16	3
36.		0.39	---
37.		0.12	12
38.		2.83	---
39.		0.24	25
40.		0.06	12
41.		0.15	25
42.		0.11	25

the active model for the inhibitor **23** was created and based on the superimposition on the model structure of **1** (Figure 2); it appeared that the tetrahydrofuran oxygen of **23** is in close proximity to the carbonyl oxygen of the asparagine moiety.[23] Thus, the position of the oxygen in 3′(*R*)-Thfg seems to set up for hydrogen-bonding interaction with the asp 29 and asp 30 of the HIV-1 protease. The antiviral potencies of the inhibitors **23** and **24** are consistent with their enzyme inhibitory potencies. As is evident in Table I, 3′(*R*)-Thfg-derived inhibitor **23** has prevented the spread of HIV-1 in MT4 human T-lymphoid cells infected with IIIb isolate[18] at an average concentration of 8 nM (CIC_{95}), a 3-fold potency enhancement over inhibitor **1**. In contrast, 3′(*S*)-Thfg derived inhibitor **24** has exhibited an antiviral potency of 100 nM. The synthesis of compounds **31**–**35** was undertaken to evaluate the influence of other heteroatoms and heterocycles on the enzyme inhibitory and antiviral potencies. Tetrahydrothiofuranylglycine-derived inhibitor **31** with a 3′*R* configuration is significantly less



1, Ro 31-8959

IC_{50} [HIV-1] = 0.4 nM
[HIV-2] = 0.5 nM



2 IC_{50} [HIV-1] = 0.07 nM
[HIV-2] = 0.18 nM

Figure 1.

potent (IC_{50} 0.6 nM) than the corresponding tetrahydrofuran derivative **23**. Since the sulfur atom in the tetrahydrothiophene ring is a poor hydrogen bond acceptor,[24] a weak hydrogen-bonding interaction may account for this loss of potency. The stereochemical preference for 3′*R*-configuration by this binding region, however, remained consistent with our earlier observation with the 3′-Thfg-derived inhibitors. Next, in order to examine the effect of sulfone oxygens in this binding pocket, the corresponding sulfolane derivatives were prepared. The sulfolane derivative with a 3′*R*-configuration is equipotent to the corresponding sulfide (compound **33**, IC_{50} 0.5 nM). On the other hand, the sulfone derivative with a 3′*S*-configuration turned out to be 10-fold less potent than the sulfide **32**. Furthermore, the replacement of the 3-tetrahydrofuran ring in **23** with 4-tetrahydropyran (compound **35**) was examined. This substitution has resulted in a dramatic loss in enzyme inhibitory potency (compound **35**, IC_{50} 68 nM). The reason for this loss of potency is probably due to the increase in ring size as well as the position of the oxygen, which impedes the access to the appropriate residues for specific interaction in the S_2 subsite of HIV-1 protease. Thus, from these studies, it is quite apparent that 3′(*R*)-Thfg is a highly effective asparagine surrogate for the S_2 region of the HIV-1 substrate binding site.

In an attempt to further improve the in vitro potencies and possibly the pharmacokinetic properties of 3′(*R*)-Thfg-derived inhibitor **23**, we have incorporated various pyrazine derivatives and heterocycles at the P_3 position. As shown in Table II, substitution of the 2-quinolinoyl moiety in **26** with the quinoxalinoyl group resulted in (compound **36**) a slight improvement in potency. However, incorporation of the 2-quinoxalinoyl moiety in **23** afforded the inhibitor **37** with an IC_{50} value of 0.12 nM, a 2-fold loss in potency compared to **23**. The removal of the second aromatic ring of the 2-quinoxalinoyl moiety in **37** provided unsubstituted pyrazine-derived inhibitor **38** (IC_{50} 2.8 nM) with a more than 20-fold loss in potency. Next, we have examined a number of pyrazine derivatives with various functionalities in order to determine whether or not substitution on the pyrazine ring would have any significant effect on potency. Indeed, compound **39** with 2-amino-5-(dimethylamino)-6-chloropyrazinoyl moiety at the P_3 position resulted in a greater than 10-fold potency enhancement (IC_{50} 0.24 nM) compared to unsubstituted pyrazine derived compound **38**. The removal of the 3-amino functionality from the pyrazine ring of compound **39** has significant potency enhancing effect. As shown, compound **40** has displayed an enzyme inhibitory potency of 0.06 nM, a 4-fold improvement over **39**. Further attachment of a basic amine functionality (compound **2**) appears to have no effect on IC_{50} value (0.07 nM) compared to compound **40**. The marked enhancement of enzyme inhibitory potency of compounds **40** and **2** was translated into their antiviral potencies. Both compounds consistently exhibited an antiviral potency of 12 nM in cell culture assay.

With the goal of minimizing peptide-like character while retaining comparable enzyme inhibitory and antiviral activity, we then explored the possibility of removal of the P_2–P_3 amide carbonyl by attaching the 3′(*R*)-Thfg-derived amine directly to the pyrazine ring. This turned out to be a rewarding endeavor. Compound **27** thus obtained

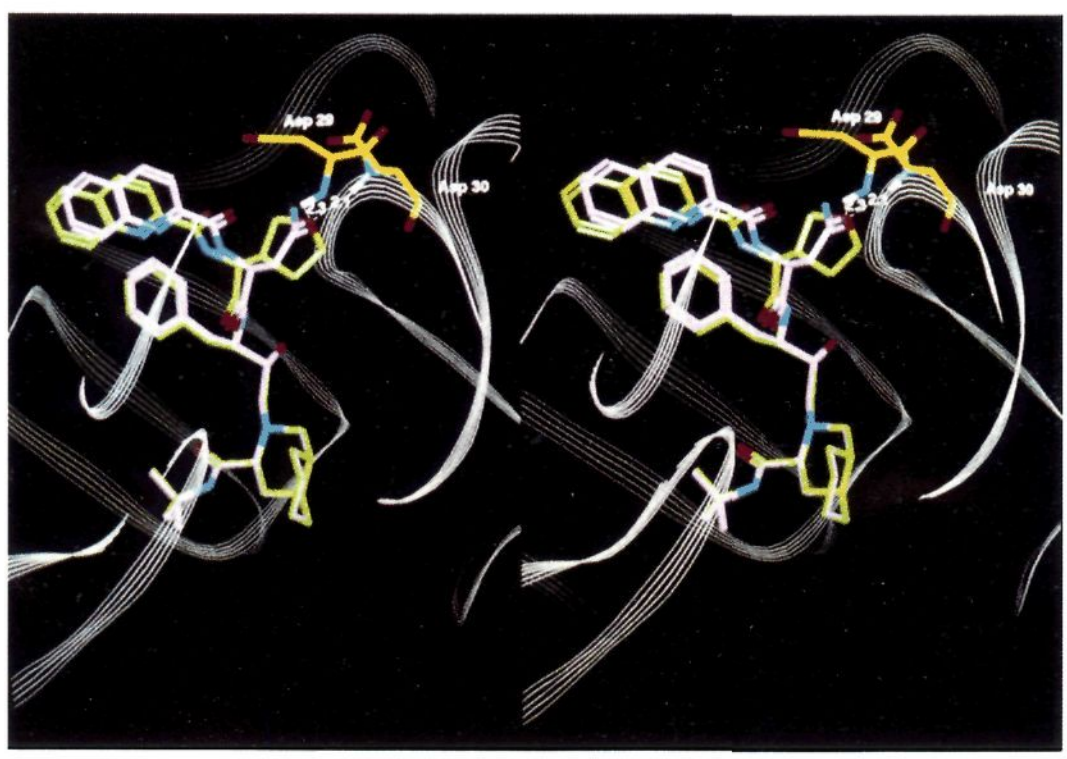


Figure 2. Stereoview of the optimized bound conformations of compound 1 (Ro 31-8959) (pink) and the inhibitor **23** (green) superimposed in the L-689,502 inhibited HIV-1 protease active site.[18]

Table III. Inhibitory Potencies (HIV-2) of Selected Compounds

compd	IC_{50}, nM (HIV-2)[25]	compd	IC_{50}, nM (HIV-2)[25]
1	0.5	26	3.2
2	0.18	27	24.9
23	0.24		

showed a similar level of enzyme inhibitory activity (IC_{50} 0.16 nM) as the inhibitor 1. More importantly, there is a dramatic improvement in antiviral potency (CIC_{95} 3 nM)[25] of compound 27 compared to 1. Presumably, various functional groups present in the pyrazine ring of compound 27 are consequential to binding; however, the actual role of the pyrazine substituents has not been specifically examined. A thorough understanding of the contributions of the various functional groups in this binding region should await a detailed structure–activity relationship investigation. Intriguing changes in activity were observed upon introduction of 2-chromone carboxamide as the P_3 ligand. Replacement of the 2-quinolinoyl group with a 2-chromone derivative (compound 41) resulted in 4-fold enhancement of both enzyme inhibitory and antiviral potencies compared to compound 26. Thus, simple replacement of P_2-asparagine and P_3-2-quinolinoyl units with valine and 2-chromonoyl moieties afforded an inhibitor (compound 41) with comparable in vitro potencies to compound 1. Interestingly, substitution of valine in 41 with 3′(*R*)-Thfg has no effect on potency.

Selected inhibitors from this present series have been evaluated for their ability to inhibit HIV-2 protease.[26] As shown in Table III, inhibitor 23, derived from 3′(*R*)-Thfg (IC_{50} 0.24 nM), is 2-fold more potent than compound 1 (IC_{50} 0.5 nM). In contrast, replacement of asparagine in 1 with valine (compound 26) resulted in over 6-fold loss of potency. Furthermore, the *N*-pyrazinyl compound 27 showed an inhibitory potency of 24.9 nM. Compound 2 with 3′(*R*)-Thfg at the P_2 and substituted pyrazinoyl moiety at P_3 is the most potent inhibitor (HIV-2) in this series. This compound has exhibited an IC_{50} value of 0.18 nM (HIV-2), nearly a 3-fold improvement over compound 1 (Ro 31-8959).

Conclusion

Replacement of the P_2-asparagine and P_3-2-quinolinoyl ligand of present clinical candidate 1 with 3′(*R*)-Thfg and substituted pyrazine derivatives has led to a novel series of potent inhibitors of HIV-1 and HIV-2 proteases. Interestingly, the inhibitor containing tetrahydrofuranylglycine with the 2*S*,3*R*-configuration has exhibited nearly 100-fold potency enhancement over the 2*S*,3*S*-derived inhibitor. This marked difference in activity and strong stereochemical preference has suggested some specific hydrogen-bonding interaction with the residues in the S_2-binding domain of HIV-1 protease. Also, it has been demonstrated that substituted pyrazinoyl derivatives are effective as P_3-ligands, leading to potent protease inhibitors. Of particular interest, compound 2, with 3′-(*R*)-Thfg at P_2 and pyrazine derivative at P_3, is one of the most potent inhibitors of HIV-1 and HIV-2 proteases. Since these are the genetically most divergent strains of HIV known to exist to date, the protease inhibitors which are potent against HIV-1 and HIV-2 strains may have a beneficial effect in terms of reducing susceptibility to clinical resistance. Another intriguing result in this series is the identification of compound 27 in which the P_2–P_3 amide carbonyl is removed. This has resulted in substantial improvement in antiviral potency while retaining the enzyme inhibitory potency at a level similar to that of compound 1. Further design of compounds from the study of ligand binding site interaction may lead to structurally novel inhibitors with less peptide-like character. Investigations along this line are in progress.

Experimental Section

All melting points were recorded on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton magnetic resonance spectra were recorded on a Varian XL-300 spectrometer using tetramethylsilane as internal standard. Significant ^1H NMR data for representative compounds are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons, coupling constant(s) in Hz. FAB mass spectra were recorded on a VG Model 7070 mass spectrometer, and relevant data are tabulated as *m/z*. Elemental analyses were performed by the analytical department, Merck Research Laboratories, West Point, PA, and were within ±0.4% of the theoretical values. Anhydrous solvents were obtained as follows: methylene chloride, distillation from P_4O_{10}; tetrahydrofuran, distillation from sodium/benzophenone; dimethylformamide and pyridine, distillation from CaH_2. All other solvents were HPLC grade. The abbreviations DME, DMF, THF, HOBT and EDC refer to 1,2-dimethoxyethane, *N*,*N*-dimethylformamide, tetrahydrofuran, 1-hydroxybenzotriazole hydrate, and 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride. Column chromatography was performed with E. Merck 240–400 mesh silica gel under low pressure of 5–10 psi. Thin-layer chromatography (TLC) was carried out with E. Merck silica gel 60 F-254 plates.

***trans*-4-Phenyl-2-buten-1-ol (4).** A mixture of CuCN (2.43 g, 27 mmol) was added to a solution of butadiene monooxide (19 g, 270 mmol) in 500 mL of anhydrous tetrahydrofuran, and the mixture was cooled to −78 °C. Phenylmagnesium bromide solution in ether (32 mmol) was added dropwise to this mixture. The reaction mixture was warmed to 0 °C and was stirred until the reaction mixture became homogeneous. The reaction mixture was cooled to −78 °C, and 0.29 mol of phenylmagnesium bromide solution in ether was added dropwise over 30 min. The reaction mixture was allowed to warm to room temperature with stirring and then quenched by slow addition of saturated NH_4Cl (50 mL) followed by NH_4OH (30 mL), saturated NH_4Cl (200 mL), and H_2O (100 mL). The aqueous layer was extracted with two 200-mL portions of ethyl acetate. The combined organic layers were dried and concentrated. The residue was distilled under vacuum (0.1 Torr) at 100 °C to give *trans*-4-phenyl-2-buten-1-ol (4, 38.9 g).

2(*R*),3(*R*)-Epoxy-4-phenylbuten-1-ol (5). A mixture of powdered 4-Å molecular sieves (3 g), titanium tetraisopropoxide (1.5 mL), and diethyl D-tartrate (1.1 mL) in anhydrous methylene chloride (350 mL) was cooled to −22 °C, and *tert*-butyl hydroperoxide solution in isooctane (210 mmol) was added slowly with stirring. After 30 min at −22 °C a solution of 4 (15.3 g, 100 mmol) in anhydrous methylene chloride (50 mL) was added dropwise for 20 min at −22 °C. The reaction mixture was aged at −22 °C in a freezer for 20 h. Water (40 mL) was added to the reaction mixture, and after 30 min at 0 °C, 30% NaOH in brine (6 mL) was added. The resulting mixture was stirred for 1 h at room temperature. The organic phase was separated, and the aqueous layer was extracted with two 30-mL portions of methylene chloride. Combined organic layers were dried over Na_2SO_4, diluted with toluene (300 mL), and concentrated. Chromatography on silica gel with 40% ethyl acetate in hexane gave (2*R*,3*R*)-epoxy-4-phenylbutan-1-ol (5, 10.3 g).

(2*S*,3*S*)-2-Hydroxy-3-azido-4-phenylbutan-1-ol (6). A solution of titanium tetraisopropoxide (5.6 mL) and azidotrimethylsilane (5.0 mL) in anhydrous benzene (100 mL) was refluxed for 5 h. To this refluxing mixture was added a solution of 2.6 g of 2(*R*),3(*R*)-epoxy-4-phenylbutan-1-ol (5) in anhydrous benzene (10 mL). The reaction mixture was refluxed for 15 min, cooled

to room temperature, and quenched by addition of 5% H_2SO_4 (150 mL). After the resulting biphasic mixture was stirred for 1 h, the organic layer was separated and the aqueous layer was extracted with two 20-mL portions of ethyl acetate. Combined organic layers were washed with saturated $NaHCO_3$ (50 mL), dried over $MgSO_4$, and concentrated to give the azido diol 6 (2.7 g) as a white solid: mp 80–82 °C (lit.[6] mp 80.5–82 °C).

3(*S*)-Azido-1,2(*R*)-epoxy-4-phenylbutane (7). The azido diol 6 (2.7 g, 13 mmol) was dissolved in chloroform (30 mL), and 2-acetoxyisobutyryl chloride (2.5 mL) was added. After the solution was stirred for 5 h at room temperature, saturated sodium bicarbonate (50 mL) was added, and the resulting biphasic mixture was stirred for 10 min. The aqueous layer was extracted with two 30-mL portions of chloroform. Combined organic layers were dried over Na_2SO_4 and concentrated. The residue was dissolved in anhydrous THF (10 mL), and solid NaOMe (1 g) was added. After the mixture was stirred for 3 h at room temperature, saturated NH_4Cl (20 mL) was added and the mixture extracted with two 20-mL portions of ethyl acetate. Combined organic layers were dried over $MgSO_4$ and concentrated. Chromatography on silica gel with 8% ethyl acetate in hexanes gave 3(*S*)-azido-1,2(*R*)-epoxy-4-phenylbutane (7, 1.75 g) as an oil: $[\alpha]^{25}_D$ = +14° (*c* = 1.5, MeOH); ^{1}H NMR (300 MHz) $CDCl_3$ δ 7.4–7.2 (m, 5H), 3.6 (m, 1H), 3.1 (m, 1H), 2.95 (dd, 1H, *J* = 4.6, 13.9 Hz), 2.8 (m, 3H).

Diethyl (3(*R*)-Tetrahydrofuranyl)malonate (9). To a stirred solution of 13 g (130 mmol) of 3(*R*)-hydroxytetrahydrofuran in 200 mL of methylene chloride cooled to −10 °C was added 31 mL of triethylamine and 13 mL of methanesulfonyl chloride, keeping the temperature below 5 °C. After 20 min at 0 °C, 50 mL of water was added and stirring continued for 10 min. The mixture was diluted with 500 mL of methylene chloride, and the layers were separated. The organic layer was washed with 20 mL of 6 N HCl, and the combined aqueous layers were extracted with two 50-mL portions of methylene chloride. The combined organic extracts were washed with 25 mL of saturated sodium bicarbonate, dried ($MgSO_4$), and concentrated. After the extracts were dried further under vacuum (1 mm) at room temperature for 30 min, 32.2 g of the mesylate was obtained. The mesylate was added to a solution of 5 molar equiv of diethyl sodiomalonate in dimethylformamide prepared by adding 110 mL of diethyl malonate to an ice-cold suspension of 29 g of sodium hydride 60% oil dispersion (washed with three 200-mL portions of hexanes) in 300 mL of dimethylformamide over 1 h. The resulting mixture was heated to 95 ± 5 °C for 16 h and then cooled and quenched by addition of 120 mL of glacial acetic acid. The mixture was diluted with 1 L of ether and 2 L of water and separated. The aqueous layer was extracted with two 200-mL portions of ether. Combined ether extracts were washed with 50 mL of saturated sodium bicarbonate, dried ($MgSO_4$), and concentrated. After removal of excess diethyl malonate by distillation through a 20-cm Vigreux column at 5–10 mm (bp 85 °C), the product was distilled through a short path at 1.5 mm, bp 115–20 °C. There was obtained 27.7 g of product as a colorless liquid: $[\alpha]^{23}_D$ = −21.2° (*c* = 1.0, MeOH). ^{1}H-NMR ($CDCl_3$) δ 4.2 (m, 4H) 4.0 (dd, 1H, *J* = 7, 8 Hz), 3.88 (m, 1H), 3.75 (dd, 1H, *J* = 7, 15 Hz), 3.5 (dd, 1H, *J* = 7, 8 Hz), 3.3 (d, 1H, *J* = 8 Hz), 2.9 (m, 1H), 2.1 (m, 1H), 1.68 (m, 1H), 1.3 (m, 6H). Anal. ($C_{11}H_{18}O_5$) C, H, N.

Diethyl (3(*S*)-Tetrahydrofuranyl)malonate. From 17 g (170 mmol) of 3(*S*)-hydroxytetrahydrofuran there was obtained 33 g of product as a colorless liquid: $[\alpha]^{23}_D$ = +21.4° (*c* = 1.0, MeOH). Anal. ($C_{11}H_{18}O_5$) C, H, N.

(3(*R*)-Tetrahydrofuranyl)acetic Acid (10). A mixture of 27.7 g (120 mmol) of diethyl (3(*R*)-tetrahydrofuranyl)malonate, 400 mL of 1 N sodium hydroxide, and 200 mL of ethanol was stirred at room temperature for 48 h. After the ethanol was removed by concentration under reduced pressure, the aqueous solution was acidified to pH 1 with 6 N HCl and concentrated to dryness. The solid residue was extracted with 500 mL of ethyl acetate, filtered, and concentrated to dryness. The resulting thick oil (diacid) and 0.9 g of Cu_2O were dissolved in 250 mL of anhydrous acetonitrile and heated to 80 °C for 6 h. The mixture was concentrated to dryness and the residue stirred with 40 mL of 6 N HCl for 5 min and then extracted with three 250-mL portions of ethyl acetate. The organic extracts were dried ($MgSO_4$) and concentrated and the crude product (17 g) purified by evaporative distillation at 1 mm (oven temperature 120–150 °C). There was obtained 13.6 g of product as a colorless liquid: $[\alpha]^{23}_D$ = −25° (*c* = 1.40, MeOH); ^{1}H-NMR ($CDCl_3$) δ 3.98 (dd, 1H, *J* = 7, 8 Hz), 3.88 (m, 1H), 3.8 (m, 2H), 3.45 (dd, 1H, *J* = 7, 8 Hz), 2.62 (m, 1H), 2.45 (d, 2H, *J* = 8 Hz), 2.15 (m, 1H), 1.6 (m, 1H). Anal. ($C_6H_{10}O_3$) C, H, N.

(3(*S*)-Tetrahydrofuranyl)acetic Acid. From 30 g (130 mmol) of diethyl (3(*S*)-tetrahydrofuranyl)malonate was obtained 16.85 g of product as a colorless liquid: $[\alpha]^{23}_D$ = +25° (*c* = 1.44, MeOH). Anal. ($C_6H_{10}O_3$) C, H, N.

3-(2-(3(*R*)-Tetrahydrofuranyl)-1-oxoethyl)-4(*S*)-(phenylmethyl)-2-oxazolidinone (11). To a stirred solution of 2.6 g (20 mmol) of optically pure (3(*R*)-tetrahydrofuranyl)acetic acid in 20 mL of dry tetrahydrofuran was added 3.6 mL of triethylamine and then 2.7 mL of trimethylacetyl chloride. After 15 min at −15 °C, the reaction slurry was warmed to 0 °C over 20 min and then recooled to −78 °C. In a separate flask, 6.36 g of 4(*S*)-(phenylmethyl)-2-oxazolidinone was dissolved in 100 mL of dry tetrahydrofuran and cooled to −60 °C. To this solution was added 23 mL of 1.6 M n-butyllithium in hexanes over 15 min. This cold solution was taken up in a syringe and added to the white slurry prepared as described above. After the mixture was stirred for 1 h at −78 °C, 20 mL of 1 N sodium bisulfate was added. The tetrahydrofuran was removed under reduced pressure, and the remaining aqueous mixture was extracted three times with 100-mL portions of methylene chloride. The combined extracts were washed with 20 mL of saturated sodium bicarbonate, dried ($MgSO_4$), and concentrated to dryness. Purification by chromatography on silica gel with 35% ethyl acetate in hexanes gave 5.4 g of product as a thick oil. Anal. ($C_{16}H_{19}NO_4$) C, H, N.

3-(2-(3(*S*)-Tetrahydrofuranyl)-1-oxoethyl)-4(*S*)-(phenylmethyl)-2-oxazolidinone. From 2.6 g (20 mmol) of optically pure 3(*S*)-tetrahydrofuranylacetic acid was obtained 5.4 g of product as a white crystalline solid: mp 76–77 °C. Anal. ($C_{16}H_{19}NO_4$) C, H, N.

3-(2(*S*)-Azido-2-(3(*R*)-tetrahydrofuranyl)-1-oxoethyl)-4-(*S*)-(phenylmethyl)-2-oxazolidinone (12). A cooled (−78 °C) solution of 5.4 g of stereochemically pure 11 in 66 mL of dry tetrahydrofuran was added to a cooled (−78 °C) solution of 30 mL of 0.69 M potassium bis(trimethylsilyl)amide in toluene diluted with 66 mL of dry tetrahydrofuran, keeping the temperature below −60 °C. After 30 min at −78 °C, a precooled solution of 7.34 g of 2,4,6-triisopropylbenzenesulfonyl azide in 45 mL of dry THF was added. After 2 min at −75 °C, 5.4 mL of glacial acetic acid was added and the mixture warmed immediately to 30 °C for 1 h. The solution was partitioned between 200 mL of methylene chloride and 100 mL of dilute brine. The aqueous phase was extracted with three 75-mL portions of methylene chloride. Combined organic extracts were washed with 100 mL of saturated sodium bicarbonate, dried ($MgSO_4$), and concentrated. Chromatography on silica gel with 1:9 ethyl acetate–methylene chloride gave 5.3 g of product as a thick oil.

3-(2(*S*)-Azido-2-(3(*S*)-tetrahydrofuranyl)-1-oxoethyl)-4-(*S*)-(phenylmethyl)-2-oxazolidinone: mp 108–109.5 °C (softens at 95 °C).

2(*S*)-Azido-2-(3(*R*)-tetrahydrofuranyl)acetic Acid (13). To a stirred solution of 5.2 g of 12 in 110 mL of THF was added 37 mL of water and 0.78 g of LiOH. After 45 min at room temperature 2.77 g of $NaHCO_3$ was added and the mixture concentrated to remove THF. The aqueous solution was diluted to 80 mL and extracted with two 50-mL portions of methylene chloride. The aqueous layer was made acidic with 5 mL of concd HCl and extracted with four 50-mL portions of ethyl acetate. Combined ethyl acetate extracts were dried ($MgSO_4$) and concentrated. After drying there was obtained 2.9 g of product as a white crystalline solid. Sublimation at 0.1 mm, 80–90 °C, gave 2.78 g of pure 13: mp 83–5 °C; $[\alpha]^{23}_D$ = −92.9° (*c* = 1.75, MeOH); ^{1}H-NMR ($CDCl_3$) δ 9.5 (br s, 1H), 3.8 (m, 3H), 2.75 (m, 1H, *J* = 8 Hz), 2.15 (m, 1H), 1.85 (m, 1H). Anal. ($C_6H_9N_3O_3$) C, H, N.

(2*S*,3′*R*)-3′-Tetrahydrofuranylglycine (13a). A solution of 400 mg of (2*S*,3′*R*)-2-azido-2-(3′-tetrahydrofuranyl)acetic acid in 20 mL of ethanol and 10 mL of H_2O was stirred under an atmosphere of hydrogen over 50 mg of 5% Pd/C for 24 h. Removal of the catalyst by filtration and concentration under reduced

pressure gave, after drying, 355 mg of product as a white solid: $[\alpha]^{23}_D$ = −17.7° (c = 1.29, H_2O); ^{1}H-NMR ($CDCl_3$) δ 3.7–4 (m, 5H), 2.75 (m, 1H), 2.15 (m, 1H), 1.9 (m, 1H). Anal. ($C_6H_{11}NO_3$·0.2EtOH) C, H, N.

2(*S*)-Azido-2-(3(*S*)-tetrahydrofuranyl)acetic Acid. The product was a thick oil: $[\alpha]^{23}_D$ = −107° (c = 1.75, MeOH); ^{1}H-NMR ($CDCl_3$) δ 8.3 (br s, 1H), 3.9 (m, 3H), 3.8 (dd, 1H, J = 8, 15 Hz), 3.72 (dd, 1H, J = 7, 8 Hz), 2.75 (m, 1H, J = 8Hz), 2.15 (m, 1H), 1.92 (m, 1H). Anal. ($C_6H_9N_3O_3$) C, H, N.

(2*S*,3′*S*)-3′-Tetrahydrofuranylglycine. A solution of 300 mg of (2*S*,3′*S*)-2-azido-2-(3′-tetrahydrofuranyl)acetic acid in 20 mL of ethanol and 10 mL of H_2O was stirred under an atmosphere of hydrogen over 50 mg of 5% Pd/C for 24 h. Removal of the catalyst by filtration and concentration under reduced pressure gave, after drying, 270 mg of product as a white solid: $[\alpha]^{23}_D$ = +10.3° (c = 1.125, H_2O); ^{1}H-NMR ($CDCl_3$) δ 3.6–4 (m, 5H), 2.72 (m, 1H), 2.2 (m, 1H), 1.85 (m, 1H). Anal. ($C_6H_{11}NO_3$·0.2EtOH) C, H, N.

Methyl 3-Bromo-5-(4-methylpiperazinyl)-6-chloro-2-pyrazinecarboxylate (16). To a stirred suspension of 11.3 g of methyl 3-amino-5-(4-methylpiperazinyl)-6-chloro-2-pyrazinoate[12] (**15b**) in 75 mL of 48% HBr and 120 mL of glacial AcOH cooled in an ice bath to 5 °C was added a solution of 6 mL of bromine in 10 mL of AcOH over 45 min with efficient stirring. To this mixture was added a solution of 8.3 g of sodium nitrite in 18 mL of water, maintaining the temperature below 8 °C. After addition was complete the mixture was stirred for 30 min, and then excess bromine was destroyed by dropwise addition of 100 mL of 30% $NaHSO_3$. The solution was neutralized to pH 8 by dropwise addition of 20% NaOH (ca. 200 mL) and then extracted with 5 × 100 mL of CH_2Cl_2. The organic extracts were washed with 50 mL of dilute NH_4OH, dried over $MgSO_4$, and concentrated to dryness. After drying under vacuum, there was obtained 6.3 g (47%) of the bromide as a yellow crystalline solid: mp 113–4 °C. Anal. ($C_{11}H_{14}BrClN_4O_2$) C, H, N.

5-(4-Methylpiperazinyl)-6-chloro-2-pyrazinecarboxylic Acid Hydrochloride (17). A solution of 2.6 g of methyl 3-bromo-5-(4-methylpiperazinyl)-6-chloro-2-pyrazinecarboxylate (**16**) in 100 mL of THF was stirred under a balloon filled with hydrogen over 0.26 g of 5% palladium on carbon for 2 days. The catalyst was removed by filtration, and the residue after concentration was partitioned between saturated $NaHCO_3$ and ethyl acetate. The organic extracts were dried over $MgSO_4$ and concentrated. Chromatography of the residue on silica gel eluting with 5% MeOH in CH_2Cl_2 gave 1.42 g (70%) of a yellow solid: mp 96–7 °C. Anal. ($C_{11}H_{15}ClN_4O_2$) C, H, N.

A solution of 0.5 g of methyl 5-(4-methylpiperazinyl)-6-chloro-2-pyrazinecarboxylate and 6 mL of 1 N NaOH in 20 mL of EtOH was allowed to stir at room temperature overnight. The mixture was acidified to pH < 2 with 6 N HCl and concentrated to dryness. The solids were stirred with 200 mL of 10% EtOH in $CHCl_3$ and filtered, and the filtrate was concentrated to dryness. The off-white solid product weighed 0.52 g (96%) and was homogeneous by HPLC.

***N*-*tert*-Butyl-2-(2(*R*)-hydroxy-4-phenyl-3(*S*)-azidobutyl)-decahydro(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide (19).** A mixture of 6.46 g of *N*-*tert*-butyldecahydro-(4a*R*,8a*S*)-isoquinoline-3(*S*)-carboxamide (18) ($[\alpha]^{23}_D$ = −70 °C (c = 1.0, MeOH)) and 10.3 g of 3(*S*)-azido-1,2(*R*)-epoxy-4-phenylbutane (**7**) in 200 mL of 2-propanol was heated to 80 °C overnight and then concentrated to dryness under reduced pressure. Recrystallization from ethyl acetate–hexanes gave 9.63 g of product: mp 149–50 °C; $[\alpha]^{23}_D$ = −97.8° (c = 1.1, MeOH). Anal. ($C_{24}H_{37}N_5O_2$) C, H, N.

***N*-*tert*-Butyldecahydro-2-(2(*R*)-hydroxy-4-phenyl-3(*S*)-aminobutyl)(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide (20).** A solution of 5.0 g of **19** in THF (200 mL), MeOH (50 mL), and acetic acid (2 mL) was shaken with 1 g of 10% palladium on carbon catalyst under an atmosphere of hydrogen for 12 h. Removal of the catalyst by filtration and concentration under reduced pressure gave 4.68 g of product as a white solid: mp 165–166 °C; $[\alpha]^{23}_D$ = −108° (c = 1.0, MeOH). Anal. ($C_{24}H_{39}N_3O_2$) C, H, N.

***N*-*tert*-Butyl-2-[2(*R*)-hydroxy-4-phenyl-3(*S*)-((2(*S*)-amino-2-(3(*R*)-tetrahydrofuranyl)acetyl)butyl)decahydro-(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide (22).** To a stirred mixture of 0.050 g of 2(*S*)-azido-(3(*R*)-tetrahydrofuranyl)acetic acid (**13**), 0.10 g of the amine **20**, 0.034 g of 1-hydroxybenzotriazole hydrate, and 5 mL of dimethylformamide was added 0.055 g of 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride and 0.027 mL of 4-methylmorpholine. After being stirred for 4 h, the mixture was concentrated to dryness under reduced pressure. The residue was partitioned between 50 mL of ethyl acetate and 20 mL of water. The organic layer was washed with 20 mL of saturated $NaHCO_3$, and the combined aqueous layers were extracted with 20 mL of ethyl acetate. Combined organic extracts were dried ($MgSO_4$) and concentrated. Chromatography on silica gel eluting with ethyl acetate gave 0.150 g of azido amide **21** as a thick oil: ^{1}H-NMR ($CDCl_3$) δ 7.1–7.4 (m, 5H), 6.7 (d, 1H, J = 8.8 Hz), 5.8 (brs, 1H), 4.35 (m, 1H), 3.9 (m, 1H), 3.8 (brd, 2H, J = 5.6 Hz), 3.5–3.7 (m, 2H), 3.34 (dd, 1H, J = 6.6, 6.1 Hz), 2.9–3.05 (m, 4H), 2.5–2.7 (m, 2H), 2.5–2.25 (m, 3H), 1.2–2.1 (m, 15H), 1.35 (s, 9H).

The azido amide **21** was dissolved in 20 mL of THF and 5 mL of methanol, and 50 mg of 5% palladium on carbon was suspended in the mixture. The resulting mixture was stirred under an atmosphere of hydrogen for 12 h. Removal of the catalyst by filtration and the solvents by concentration under reduced pressure gave the amine **22** (0.13 g).

***N*-*tert*-Butyl-2-(2(*R*)-hydroxy-4-phenyl-3(*S*)-((*N*-(2-quinolylcarbonyl)-2(*S*)-(3(*R*)-tetrahydrofuranyl)glycinyl)-amino(butyl)decahydro-(4a*S*,8a*S*), isoquinoline-3(*S*)-carboxamide (23).** A solution of 0.056 g of diphenylphosphinic chloride in 2 mL of dry THF was added to a precooled (−10 °C) solution of quinaldic acid (60 mg) and triethylamine (0.042 mL) in 10 mL of dry THF. After the mixture was stirred for 1 h at −10 °C, a solution of 0.130 g of the amine **22** in 5 mL of THF was added and the mixture allowed to warm to room temperature and stir for 3 h. The solvents were removed under reduced pressure and the residue partitioned between 50 mL of ethyl acetate and 10 mL of dilute $NaHCO_3$. The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. Chromatography on silica gel eluting with 4% methanol in ethyl acetate gave after trituration with ether–hexanes 0.160 g of product as a white amorphous solid. A sample crystallized from ethyl acetate as colorless prisms (hemihydrate): mp 206–6°C; ^{1}H-NMR ($CDCl_3$) δ 8.7 (d, 1H, J = 8.3 Hz), 8.35 (d, 1H, J = 8.4 Hz), 8.25 (d, 1H, J = 8.4 Hz), 8.15 (d, 1H, J = 8.4 Hz), 7.9 (d, 1H, J = 8.5 Hz), 7.8 (t, 1H, J = 6.8 Hz), 7.65 (t, 1H, J = 6.8 Hz), 6.95–7.1 (m, 4H), 6.9 (t, 1H, J = 7.6 Hz), 6.7 (br d, 1H, J = 8.8 Hz), 5.97 (brs, 1H), 4.5 (dd, 1H, J = 7.2, 7.8 Hz), 4.3 (m, 1H), 3.9 (m, 2H), 3.5–3.7 (m, 4H), 2.6–3.1 (m, 4H), 2.3 (m, 2H), 1.7–2.1 (m, 6H), 1.2–1.6 (m, 9 H), 1.3 (s, 9H). Anal. ($C_{40}H_{53}N_5O_5$·0.5H_2O) C, H, N.

***N*-*tert*-Butyl-2-[2(*R*)-hydroxy-4-phenyl-3(*S*)-((2(*S*)-amino-2-(3(*S*)-tetrahydrofuranyl)acetyl)butyl)decahydro-(4a*S*,-8a*S*)-3(*S*)-carboxamide.** Following the procedure for compound **22**, 0.050 g of 2(*S*)-azido-2-(3(*S*)-tetrahydrofuranyl)acetic acid was coupled to 0.10 g of the amine **20** to provide 0.132 g of the diastereoisomer of **21** that contains the 2(*S*)-azido-3(*S*)-tetrahydrofuranyl moiety: ^{1}H-NMR ($CDCl_3$) δ 7.15–7.4 (m, 5H), 6.7 (d, 1H, J = 8.7 Hz), 5.8 (brs, 1H), 4.3 (m, 1H), 3.9 (m, 1H), 3.3–3.8 (m, 5H), 2.9–3.02 (m, 3H), 2.6 (m, 2H), 2.45 (m, 1H), 2.3 (m, 2H), 1.2–2.1 (m, 15H), 1.35 (s, 9H).

The above azido amide was hydrogenated over 5% palladium on carbon to provide the titled amine (0.12 g).

***N*-*tert*-Butyl-2-(2(*S*)-hydroxy-4-phenyl-3(*S*)-((*N*-(2-quinolylcarbonyl)-2(*S*)-(3(*S*)-tetrahydrofuranyl)glycinyl)-amino)butyl)decahydro-(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide (24).** A sample crystallized from ethyl acetate (hemihydrate): mp 206–6 °C; ^{1}H-NMR ($CDCl_3$) δ 8.5 (d, 1H, J = 8.8 Hz), 8.35 (d, 1H, J = 8.3 Hz), 8.25 (d, 1H, J = 8.4 Hz), 8.1 (d, 1H, J = 8.4 Hz), 7.9 (d, 1H, J = 8.5 Hz), 7.8 (t, 1H, J = 7.2 Hz), 7.65 (t, 1H, J = 7.2 Hz), 6.85–7.1 (m, 4H), 6.7 (m, 2H), 6.0 (brs, 1H), 4.5 (t, 1H, J = 8.8 Hz), 4.25 (m, 1H), 3.5–3.9 (m, 5H), 2.6–3.1 (m, 4H), 2.3 (m, 2H), 1.3–1.9 (m, 16H), 1.3 (s, 9H). Anal. ($C_{40}H_{53}N_5O_5$·0.5H_2O) C, H, N.

***N*-*tert*-Butyl-2-(2(*R*)-hydroxy-4-phenyl-3(*S*)-((*N*-((5-(4-methylpiperazinyl)-6-chloro-2-pyrazinyl)carbonyl)-2(*S*)-(3(*R*)-tetrahydrofuranyl)glycinyl)amino)butyl)decahydro-(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide (2).** A mixture of 80 mg of 5-(4-methylpiperazinyl)-6-chloro-2-pyrazinecarboxylic acid hydrochloride (**17**), 52 μL of diphenylphosphinic chloride,

and 80 μL of Et_3N in 5 mL of THF was stirred at −10 °C for 2 h. To this mixture was added 120 mg of amine **22** in 5 mL of THF. After being stirred overnight at room temperature, the mixture was diluted with 50 mL of ethyl acetate, washed with 10 mL of saturated $NaHCO_3$ and 10 mL of dilute aqueous NH_3, and concentrated to dryness. Preparative thin-layer chromatography on silica gel eluting with 15% MeOH in $CHCl_3$ gave 80 mg of an off-white solid: mp 115–8 °C; ^{1}H-NMR ($CDCl_3$) δ 8.76 (m, 1H), 7.78 (m, 1H), 7.1–7.3 (m, 5H), 6.7 (m, 1H), 4.4 (m, 1H), 4.3 (m, 1H), 3.5–3.9 (m, 11H), 2.7–2.9 (m, 12H), 2.5 (m, 3H), 1.3–2.0 (m, 14 H), 1.3 (s, 9H). Anal. ($C_{40}H_{59}ClN_8O_5$) C, H, N.

***N-tert*-Butyl-2-(2(*R*)-hydroxy-4-phenyl-3(*S*)-((*N*-((2-methoxycarbonyl)-3-amino-6-chloro-5-pyrazinyl)-2(*S*)-(3(*R*)-tetrahydrofuranyl)glycinyl)amino)butyl)decahydro-(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide (27).** A mixture of 0.10 g of *N-tert*-butyl-2-(2(*R*)-hydroxy-4-phenyl-3(*S*)-((2(*S*)-(3(*R*)-tetrahydrofuranyl)glycinyl)amino)butyl)decahydro-(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide and 0.10 g of methyl 2-amino-4,5-dichloropyrazinoate in 10 mL of 2-propanol was heated to reflux overnight. After removal of solvents, the residue was purified by preparative thin-layer chromatography (5% methanol in ethyl acetate). Trituration with ether gave 23 mg of an amorphous solid: mp 265 °C dec; ^{1}H-NMR ($CDCl_3$) δ 8.5 (m, 1H), 7.1–7.3 (m, 6H), 6.3 (m, 1H), 5.8 (m, 1H), 5.7 (m, 1H), 4.5 (m, 1H), 4.3 (m, 2H), 3.8–3.9 (m, 5H), 3.5–3.7 (m, 5H), 2.5–3.1 (m, 4H), 2.0–2.2 (m, 2H), 1.2–1.9 (m, 14H), 1.3 (s, 9H). Anal. ($C_{36}H_{52}ClN_7O_6$·0.2$CHCl_3$) C, H, N.

2(*S*)-Azido-2-(3(*SR*)-tetrahydrofuranyl)acetic Acid (28). A suspension of NaH was prepared by washing 60% NaH (1.9 g, 48 mmol) with hexane (2 × 10 mL), and toluene (40 mL) was added to the residue. To this was added triethyl phosphonoacetate (8.7 g, 39 mmol) dropwise at 24 °C via an addition funnel. The resulting mixture was stirred for 30 min and then cooled to 20 °C, and a solution of thiofuran-3-one (4.0 g, 39 mmol) in toluene (15 mL) was added dropwise, keeping the internal temperature between 24 and 26 °C throughout the addition. The mixture was allowed to stand for 10 min after the complete addition and then quenched with water (100 mL), and the layers were separated. The aqueous layer was extracted with ether, and the combined organic layers were dried over anhydrous Na_2SO_4. Filtration and concentration under reduced pressure afforded crude product which was chromatographed over silica gel to afford the unsaturated ester (5 g) as an oil.

To a solution of the unsaturated ester (2.5 g, 14.5 mmol) in 3:1 ethyl acetate/methanol (100 ml) was added 10% palladium on charcoal (0.6 g), and the resulting mixture was hydrogenated at 50 psi for 12 h. After this period, the catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The resulting residue was chromatographed over silica gel to provide the saturated ester (0.66 g) and recovered unsaturated ester (1.5 g).

To a solution of saturated ester (0.66 g, 3.8 mmol) in DME (10 mL) was added 1 M LiOH (10 mL), and the resulting mixture was stirred for 12 h at 24 °C. The mixture was then acidified with concd HCl and extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were dried over Na_2SO_4. Filtration and concentration provided the (3(*SR*)-Tetrahydrothiofuranyl)acetic acid (0.6 g): ^{1}H NMR ($CDCl_3$) δ 3.0–3.1 (m, 1H), 2.9 (m, 2H), 2.5–2.6 (m, 2H), 2.5 (m, 2H), 2.2 (m, 1H), 1.7 (m, 1H).

From 0.6 g of (3(*SR*)-tetrahydrothiofuranyl)acetic acid was obtained 0.54 g of the title azido acid **28** following asymmetric azidation and hydrolysis procedures as mentioned above (compounds **12** and **13**): ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 3.9 (m, 1H), 3.65 (m, 1H), 2.9 (m, 2H), 2.8 (m, 1H), 2.6 (m, 1H), 2.2 (m, 1H), 1.9 (m, 1H).

***N-tert*-Butyl-2-(2(*R*)-hydroxy-4-phenyl-3(*S*)-((2(*S*)-azido-2-(3(*R*)-tetrahydrothiofuranyl)acetyl)amino)butyl)-decahydro-(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide (29) and *N-tert*-Butyl-2-(2(*R*)-hydroxy-4-phenyl-3(*S*)-((2(*S*)-azido-2-(3(*S*)-tetrahydrothiofuranyl)acetyl)amino)butyl)-decahydro-(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide (30).** To a stirred mixture of 0.150 g of 2(*R*)-azido-2-(3(*SR*)-tetrahydrothiofuranyl)acetic acid (**28**), 0.270 g of the amine **20**, 0.135 g of 1-hydroxybenzotriazole hydrate, and 5 mL of dimethylformamide was added 0.191 g of 1-ethyl-3-(3-(dimethylamino)-propyl)carbodiimide hydrochloride and 0.5 mL of triethylamine. After being stirred 12 h at 23 °C, the mixture was concentrated to dryness under reduced pressure. The residue was partitioned between 100 mL of ethyl acetate and 40 mL of water. The organic layer was washed with 30 mL of saturated $NaHCO_3$, and the combined aqueous layers were extracted with 50 mL of ethyl acetate. Combined organic extracts were dried ($MgSO_4$) and concentrated. Chromatography on silica gel eluting with 50% ethyl acetate in hexane provided 0.126 g of azido amide **29** (R_f = 0.2) and 0.105 g of azido amide **30** (R_f = 0.43) as a thick oil. Compound **29**: ^{1}H-NMR ($CDCl_3$) δ 7.15–7.35 (m, 5H), 6.7 (brd, 1H, J = 8.6 Hz), 5.8 (brs, 1H), 4.35 (brs, 1H), 3.9 (m, 2H), 2.9–3.1 (m, 4H), 2.4–2.7 (m, 6H), 2.2–2.4 (m, 5H), 1.4–1.95 (m, 12 H), 1.35 (s, 9H). Compound **30**: ^{1}H-NMR ($CDCl_3$) δ; 7.2–7.4 (m, 5H), 6.7 (brd, 1H, J = 8.5 Hz), 5.85 (brs, 1H), 4.3 (brs, 1H), 3.9 (brs, 1H), 3.85 (brd, 1H, J = 6.2 Hz), 3.4 (brs, 1H), 2.8–3.05 (m, 4H), 2.55–2.8 (m, 5H), 2.5 (m, 1H), 2.3 (m, 4H), 1.4–1.9 (m, 12H), 1.35 (s, 9H).

***N-tert*-Butyl-2-(2(*R*)-hydroxy-4-phenyl-3(*S*)-((*N*-(2-quinolylcarbonyl)-2(*S*)-(3(*R*)-tetrahydrothiofuranyl)glycinyl)amino)butyl)decahydro-(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide (31).** The azide derivatives **29** and **30** described above were converted into **31** and **32** using the procedures described above for compound **23**. The isomers were separated by silica gel chromatography (90% ethyl acetate in hexane), and the more polar isomer (R_f 0.72) was obtained as a crystalline solid (hemihydrate): mp 129–31 °C; ^{1}H-NMR ($CDCl_3$) δ 8.7 (d, 1H, J = 8.1 Hz), 8.35 (d, 1H, J = 8.3 Hz), 8.25 (d, 1H, J = 8.3 Hz), 8.15 (d, 1H, J = 8.5 Hz), 7.9 (d, 1H, J = 8.6 Hz), 7.82 (t, 1H, J = 6.6 Hz), 7.67 (t, 1H, J = 6.8 Hz), 6.95–7.2 (m, 4H), 6.8 (br d, 1H, J = 8.7 Hz), 6.0 (brs, 1H), 4.6 (t, 1H, J = 7.5 Hz), 4.3 (m, 1H), 3.9 (m, 1H), 2.45–3.1 (m, 6H), 2.1–2.4 (m, 4H), 1.7–2.1 (m, 7H), 1.2–1.6 (m, 10 H), 1.3 (s, 9H). Anal. ($C_{40}H_{53}N_5O_4S$·0.15$CHCl_3$) C, H, N.

***N-tert*-Butyl-2-(2(*R*)-hydroxy-4-phenyl-3(*S*)-((*N*-(2-quinolylcarbonyl)-2(*S*)-(3(*S*)-tetrahydrothiofuranyl)glycinyl)amino)butyl)decahydro-(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide (32).** The less polar isomer (R_f 0.81) was crystallized from ethyl acetate (hemihydrate): mp 108–11 °C; ^{1}H-NMR ($CDCl_3$) δ 8.5 (d, 1H, J = 8.7 Hz), 8.35 (d, 1H, J = 8.4 Hz), 8.25 (d, 1H, J = 8.4 Hz), 8.15 (d, 1H, J = 8.3 Hz), 7.9 (d, 1H, J = 8.3 Hz), 7.8 (m, 1H), 7.65 (t, 1H, J = 7.4 Hz), 6.95–7.15 (m, 4H), 6.7 (m, 2H, J = 7.0 Hz), 6.0 (brs, 1H), 4.6 (t, 1H, J = 8.6 Hz), 4.3 (m, 1H), 3.9 (m, 1H), 2.5–3.1 (m, 6H), 2.3 (m, 2H), 1.3–2.1 (m, 18H), 1.3 (s, 9H). Anal. ($C_{40}H_{53}N_5O_4S$·0.45EtOAc) C, H, N.

***N-tert*-Butyl-2-(2(*R*)-hydroxy-4-phenyl-3(*S*)-((*N*-(2-quinolylcarbonyl)-2(*S*)-(3(*R*)-dioxotetrahydrothiofuranyl)-glycinyl)amino)butyl)decahydro-(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide (33).** To a stirred solution of compound **31** (32.4 mg) and 4-methylmorpholine *N*-oxide (16.2 mg) in acetone (2 mL) and water (1 mL) at 0 °C was added a solution of osmium tetraoxide (2.5%) in 2-methyl-2-propanol (0.1 mL). The resulting mixture was stirred at 24 °C for 24 h and then concentrated under reduced pressure. The residue was partitioned between ethyl acetate (50 mL) and water (20 mL), and the layers were separated. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. Filtration and evaporation of the solvent under reduced pressure gave a residue which was purified by silica gel chromatography (90% ethyl acetate in hexane) to afford 19 mg of compound **33** as a white solid: mp 133–34 °C. Anal. ($C_{40}H_{53}N_5O_6S$·0.75MeOH) C, H, N.

***N-tert*-Butyl-2-(2(*R*)-hydroxy-4-phenyl-3(*S*)-((*N*-(2-quinolylcarbonyl)-2(*S*)-(3(*S*)-dioxotetrahydrothiofuranyl)-glycinyl)amino)butyl)decahydro-(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide (34).** Following the above oxidation procedure, 16.4 mg of compound **32** was converted to sulfone derivative **34** (10.4 mg). The product was obtained as a crystalline solid (hemihydrate): mp 128–30 °C. Anal. ($C_{40}H_{53}N_5O_6S$·0.15$CHCl_3$, 0.5 MeOH) C, H, N.

4-Tetrahydropyranylacetic Acid. To a stirred, cooled suspension of 0.9 g of sodium hydride (60% oil dispersion) in 5 mL of benzene was added 5.32 g of triethyl phosphonoacetate in 3 mL of benzene, keeping the temperature below 30 °C. After 1 h at 25 °C, a solution of 2 g of tetrahydro-4*H*-pyran-4-one in 3 mL of benzene was added slowly. After 1 h at 25 °C, 20 mL of water and 50 mL of ether were added. The organic layer was dried over $MgSO_4$ and concentrated to dryness. Evaporative

distillation at 5 mm, oven temperature 130 °C, gave 1.8 g of product as a fragrant liquid. Hydrogenation in 20 mL of ethanol with 0.5 g of 10% palladium on carbon under 1 atm of hydrogen for 10 days gave 1.8 g of saturated product after filtration and concentration. Saponification using 20 mL of ethanol and 10 mL of 10% NaOH for 4 h followed by acidification (H_2SO_4) and ether extraction gave 1.5 g of product. Evaporative distillation at 0.1 mm, oven temperature 160–8 °C, gave 1.1 g of product as a crystalline solid: mp 61–2 °C. Anal. ($C_7H_{12}O_3$) C, H, N.

***N*-*tert*-Butyl-2-(2(*S*)-hydroxy-4-phenyl-3(*S*)-((*N*-(2-quinolylcarbonyl)-2(*S*)-(4-tetrahydropyranyl)glycinyl)-amino)butyl)decahydro-(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide (35).** The 4-tetrahydropyranylacetic acid described above was converted into the 2(*S*)-azido-2-(tetrahydropyranyl)-acetic acid and **35** using the procedures described above for compounds **13** and **23**. The product was obtained as a crystalline solid (hemihydrate): mp 122–4 °C. Anal. ($C_{41}H_{55}N_5O_5 \cdot 0.55H_2O$) C, H, N.

***N*-*tert*-Butyl-2-(2(*R*)-hydroxy-4-phenyl-3(*S*)-((*N*-(2-quinolylcarbonyl)-2(*S*)-(3(*R*)-tetrahydrofuranyl)glycinyl)-amino)butyl)decahydro-(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide (37).** From quinoxaline-2-carboxylic acid and **22**, using the procedures described above for **2**, was obtained a white solid: mp 207–8 °C. Anal. ($C_{39}H_{52}N_6O_5 \cdot 0.5EtOAc$) C, H, N.

***N*-*tert*-Butyl-2-(2(*R*)-hydroxy-4-phenyl-3(*S*)-((*N*-(2-pyrazinylcarbonyl)-2(*S*)-(3(*R*)-tetrahydrofuranyl)glycinyl)amino)butyl)decahydro-(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide (38).** From pyrazine-2-carboxylic acid and **22**, using the procedures described above for **2**, there was obtained a white solid: mp 105–9 °C. Anal. ($C_{35}H_{50}N_6O_5 \cdot 0.6CHCl_3$) C, H, N.

***N*-*tert*-Butyl-2-(2(*R*)-hydroxy-4-phenyl-3(*S*)-((*N*-((5-(dimethylamino)-6-chloro-2-pyrazinyl)carbonyl)-2(*S*)-(3(*R*)-tetrahydrofuranyl)glycinyl)amino)butyl)decahydro-(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide (39).** From methyl 3-bromo-5-(dimethylamino)-6-chloro-2-pyrazinecarboxylate[12] and **22**, using the procedures described above for **17** and **2**, was obtained a white solid: mp 101–3 °C. Anal. ($C_{37}H_{55}ClN_8O_5 \cdot 0.5EtOH$) C, H, N.

***N*-*tert*-Butyl-2-(2(*R*)-hydroxy-4-phenyl-3(*S*)-((*N*-((5-(dimethylamino)-6-chloro-2-pyrazinyl)carbonyl)-2(*S*)-(3(*R*)-tetrahydrofuranyl)glycinyl)amino)butyl)decahydro-(4a*S*,8a*S*)-isoquinoline-3(*S*)-carboxamide (40).** From methyl 3-amino-5-(dimethylamino)-6-chloro-2-pyrazinecarboxylate[12] and **22**, using the procedures described above for **17** and **2**, was obtained a white solid (60%) after recrystallization from ethanol-water: mp 115–7 °C. Anal. ($C_{37}H_{54}ClN_7O_5 \cdot 2.0AcOH$) C, H, N.

Biology. Inhibition of HIV-1 Protease. The IC_{50} values were determined using purified HIV-1 protease.[22] Inhibition of the cleavage of the peptide H-Val-Ser-Gln-Asn-(L-b-naphthylalanine)-Pro-Ile-Val-OH was assessed at 30 °C, pH = 5.5 with [Enz] = 30 pM and BSA along with the inhibitor. The reaction was quenched with H_3PO_4, and the products were analyzed by using HPLC with UV detection (225 nm) for quantification of the products. For IC_{50} determination, a substrate concentration of 0.4 mg/mL was used, and the data were fit to a four-parameter sigmoidal equation. The results of these measurements are shown in Tables I and II.

Inhibition of HIV-1 Viral Spread in Cell Culture. Selected compounds were dissolved in dimethyl sulfoxide and serially diluted into cell culture medium to achieve the test concentrations. Cells were infected and grown in a medium of RPMI-1640 (Whittaker BioProducts), 10% inactivated fetal bovine serum, 4 mM glutamine (Gibco Labs), and 1:100 penicillin/streptomycin (Gibco Labs). Cells were treated with the compound for 24 h prior to HIV-1 infection. Cells were infected at day 0 at a concentration of 250 000 per mL with a 1:2000 dilution of HIV-1 variant. The multiplicity of infection was 0.01 in all cases. Fresh compound was added at the time of infection and every 2–3 days thereafter. Incubations were performed at 37 °C in a CO_2 atmosphere. H9 and MT-4 human T-lymphoid cells are described by Popovic et al.[27] and Miyoshi et al.,[28] respectively. Primary peripheral blood lymphocytes and primary monocytes/macrophages were obtained from fresh human plasmaphoresis residues. The monocytes/macrophages were separated from the lymphocytes by adherence to plastic and were maintained in GM-CSF-containing medium (1000 mg/mL, Amgen). The lymphocytes were activated with phytohemaglutinin (5 mg/mL, Sigma) prior to virus infection and were maintained in medium containing IL-2 (1000 mg/mL, Dupont). The measurement of viral expression was performed by fixed cell immunofluorescence using anti-HIV-1 human serum.

Measurement of Viral Spread. The measurement of viral expression was performed by either fixed cell immunofluorescence using anti-HIV-1 human serum or p24 ELISA. In the immunofluorescence assay the lowest concentration of compound which completely prevented the spread of the virus from initially infected cells at 7–24 days post infection was defined as the CIC (cell culture minimal inhibitory concentration).

In the p24 ELISA assay, the cell culture inhibitory concentration (CIC) is defined as the concentration which inhibited by greater than 95% the spread of infection, as assessed by a greater than 95% reduction in p24 antigen production relative to untreated controls. Using a multichannel pipettor, the settled cells were resuspended, and a 125-mL aliquot was harvested into a separate microtiter plate. After the settling of the cells, the plates were frozen subsequent to assay of the supernatant for HIV p24 antigen. The concentration of p24 antigen was measured by an enzyme immunoassay, described as follows. Aliquots of p24 antigen to be measured were added to microwells coated with a monoclonal antibody specific for HIV core antigen. The microwells were washed at this point and at other appropriate steps that follow. Biotinylated HIV-specific antibody was then added, followed by conjugated streptavirudin–horseradish peroxidase. A color reaction occurs from the added hydrogen peroxide and tetramethylbenzidine substrate. Color intensity is proportional to the concentration of HIV p24 antigen.

Molecular Modeling. All modeled structures were built using the Merck molecular modeling program AMF (Advanced Modeling Facility)[29] and energy minimized using the Merck molecular force field, OPTIMOL, which is a variant of the MM2 program. Due to the low pH optimum of the HIV-1 protease,[26,30] all titratable residues were charged in the calculations with the exception of Tyr59 and one of the pair of catalytic aspartic acids, Asp25.[31] During energy minimizations the enzyme-active site was held fixed at the X-ray geometry.

Graphics visualization and molecular surface calculations were performed using Quanta.[32] All molecular surfaces were generated using a neutral 0.95-Å radius probe with contouring performed at an interaction energy of 0 kcal/mol.

Acknowledgment. The authors gratefully acknowledge the assistance of Dr. H. Ramjit for mass spectra, J. P. Moreau for elemental analysis, and Ms. Jean Kaysen for help in manuscript preparation.

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