

Synthesis and Antiviral Activity of a Series of HIV-1 Protease Inhibitors with Functionality Tethered to the P₁ or P_{1'} Phenyl Substituents: X-ray Crystal Structure Assisted Design

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By tethering of a polar hydrophilic group to the P₁ or P_{1'} substituent of a Phe-based hydroxyethylene isostere, the antiviral potency of a series of HIV protease inhibitors was improved. The optimum enhancement of anti-HIV activity was observed with the 4-morpholinylethoxy substituent. The substituent effect is consistent with a model derived from inhibitor docked in the crystal structure of the native enzyme. An X-ray crystal structure of the inhibited enzyme determined to 2.25 Å verifies the modeling predictions.

Introduction

Human immunodeficiency virus (HIV) is the causative agent of AIDS. Recognition of the molecular events critical to virus replication has suggested several strategies for potential chemotherapeutic intervention. Among those, blockade of the virally encoded protease has become a major target in the quest for an effective antiviral agent.¹

In an earlier communication we described the potent HIV-1 protease inhibitor L-685,434 (1) containing the novel 1(S)-amino-2(R)-hydroxyindan P_{2'} ligand.² The inhibitor was found to block the spread of HIV-1 in T-lymphoid cells at a CIC (100% minimal inhibitory concentration) at 400 nM, but suffered from aqueous insolubility. The ratio of *in vitro* enzyme activity of 1 ($IC_{50} = 0.35$ nM) to antiviral activity in cell culture (CIC) taken as an index of cell penetration ($I/C = 0.0009$) indicates that improvement in antiviral potency might also be achieved through modification of the physical properties of the compound. Thus, molecular sites on 1 were sought for attachment of polar, hydrophilic substituents.

In the absence of an X-ray crystal structure of the enzyme-inhibitor complex, computer-assisted molecular modeling was used to visualize the inhibitor (L-685,434) bound in the native enzyme active site.³ The modeled structure was constructed starting from the X-ray structures of renin inhibitors of the hydroxyethylene isostere class bound in the active site of the fungal aspartyl proteases Endothiapepsin and Rhizopus pepsin.⁴ With the aid of computer graphics the C- and N-termini of the hydroxyethylene isostere were docked to fit the P₂ and P_{2'} sites by visual inspection. The structure was energy minimized in the active site using molecular mechanics. As depicted in Figure 1, the modeled complex revealed that the P₁ and P_{1'} phenyl rings were near the exterior surface of the active site, pointing toward solvent. In the absence of a P₃ or P_{3'} ligand there was sufficient space for tethering a chain terminated with a polar substituent into the aqueous environment. Structure-activity data had further

revealed that hydroxylation of one of the P₁ and P_{1'} phenyls (22 and 23, Table I) generating a Tyr-Phe or Phe-Tyr hydroxyethylene isostere gave a substantial increase in antiviral activity due to apparent increased cell penetration (see Results and Discussion section). Thus, alkylation of the phenols in 22 and 23 with hydrophilic substituents became an attractive design strategy.

Chemistry

The synthesis of the tyrosine- and phenylalanine-based hydroxyethylene isosteres starts with the Peterson olefination⁵ of the *N*-BOC-L-phenylalanal (2) or the *N*-BOC-O-benzyl-L-tyrosinal (3) (Scheme I). The BF₃-catalyzed elimination⁶ of the β-trimethylsilyl alcohols required 4–6 days at room temperature although the starting material disappeared instantaneously. After treatment of the crude amine product with di-*tert*-butyl dicarbonate, the *N*-BOC protected allylic amines 4 and 5 were isolated in good yields. The olefins were converted with monoperoxy-magnesium phthalate⁷ in methanol or MCPBA in methylene chloride into the predominantly threo-epoxides (4:1) 6 and 7.⁸ Alkylation of the sodium salt of diethyl malonate with the epoxides 6 and 7 gave the ester-lactones 8 and 9, which were isolated as single diastereomers by chromatography.⁹ Further alkylation with benzyl bromide or 4-(benzyloxy)benzyl chloride gave the alkylated ester-lactones 10–12. After a saponification-decarboxylation sequence the pure *trans*-lactones 13–15 were isolated by chromatography and converted into the *tert*-butyldimethylsilyl ether (16–18) of the hydroxy acid form (Scheme I).⁹

The racemic *cis*-1-amino-2-hydroxyindan (28) was prepared as first described by Lutz and Wayland¹⁰ in three steps from the racemic *trans*-isomer 25 via 2-phenyloxazoline formation (Scheme II). Resolution of 28 was accomplished through chromatographic separation of the L-phenylalanine amide diastereomers.¹¹ The faster eluting isomer 27 proved to contain the desired (−)-enantiomer of 28 which could be easily liberated from the amide by heating an ethanolic solution of 27 with excess sodium ethoxide. Presumably the neighboring hydroxyl group facilitates cleavage through intramolecular acyl transfer. Coupling (−)-28 with 16–18 in the usual manner gave the inhibitors 19–24 as crystalline solids.

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The phenols 22–24 could be efficiently and selectively alkylated using excess cesium carbonate¹² and 4-(2-chloroethyl)morpholine in dioxane heated to 80 °C (Scheme III). The crystalline morpholinylated inhibitors 29–31 were formed in 82–95% yield. The outcome of the alkylation reaction was highly dependent on the stoichiometry, concentration, temperature, reactivity of alkyl halide, and purity of reactants. In particular, trace contaminants from the commercial tetra-*n*-butylammonium fluoride reagent used to deprotect the silyl ether would

inhibit the reaction, while temperatures in excess of 90 °C would produce significant byproducts resulting from intramolecular rearrangement and polyalkylation.

The relationship of basicity and polarity with antiviral potency could also be explored in this series by simply varying the chloroalkylamine employed in the alkylation reaction of the phenol 22. Thus a series of amine-substituted tetrapeptide mimetics (32–38) structurally analogous to L-689,502 (29) were prepared and evaluated for anti-HIV activity (Table I). Oxidation of the thioether in thiamorpholinyl derivative 38 with sodium metaperiodate of potassium permanganate gave the sulfoxide 39 and sulfone 40. The *N*-oxide 41 could be obtained in essentially quantitative yield by treatment of an ethanolic solution of L-689,502 (29) with 30% aqueous hydrogen peroxide.

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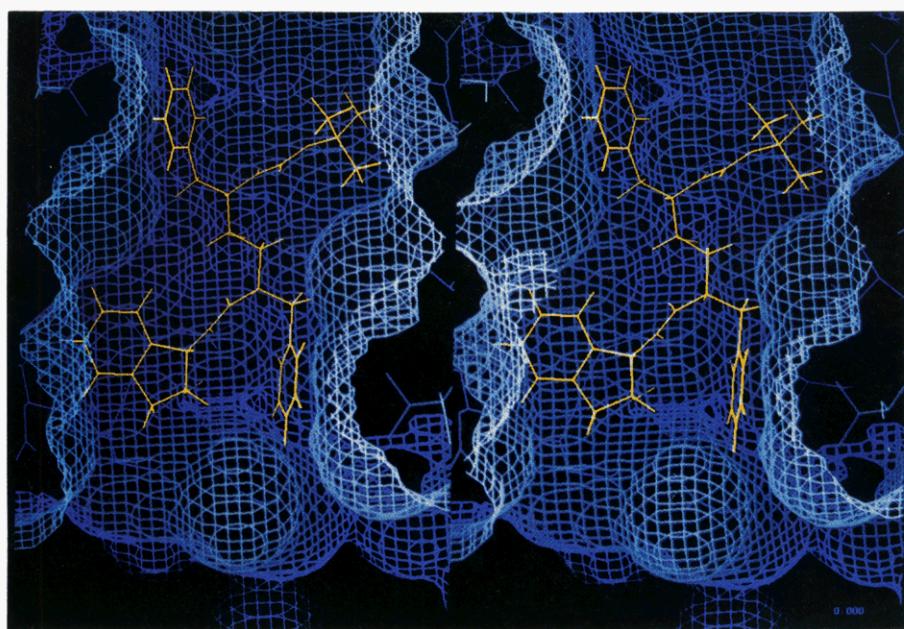
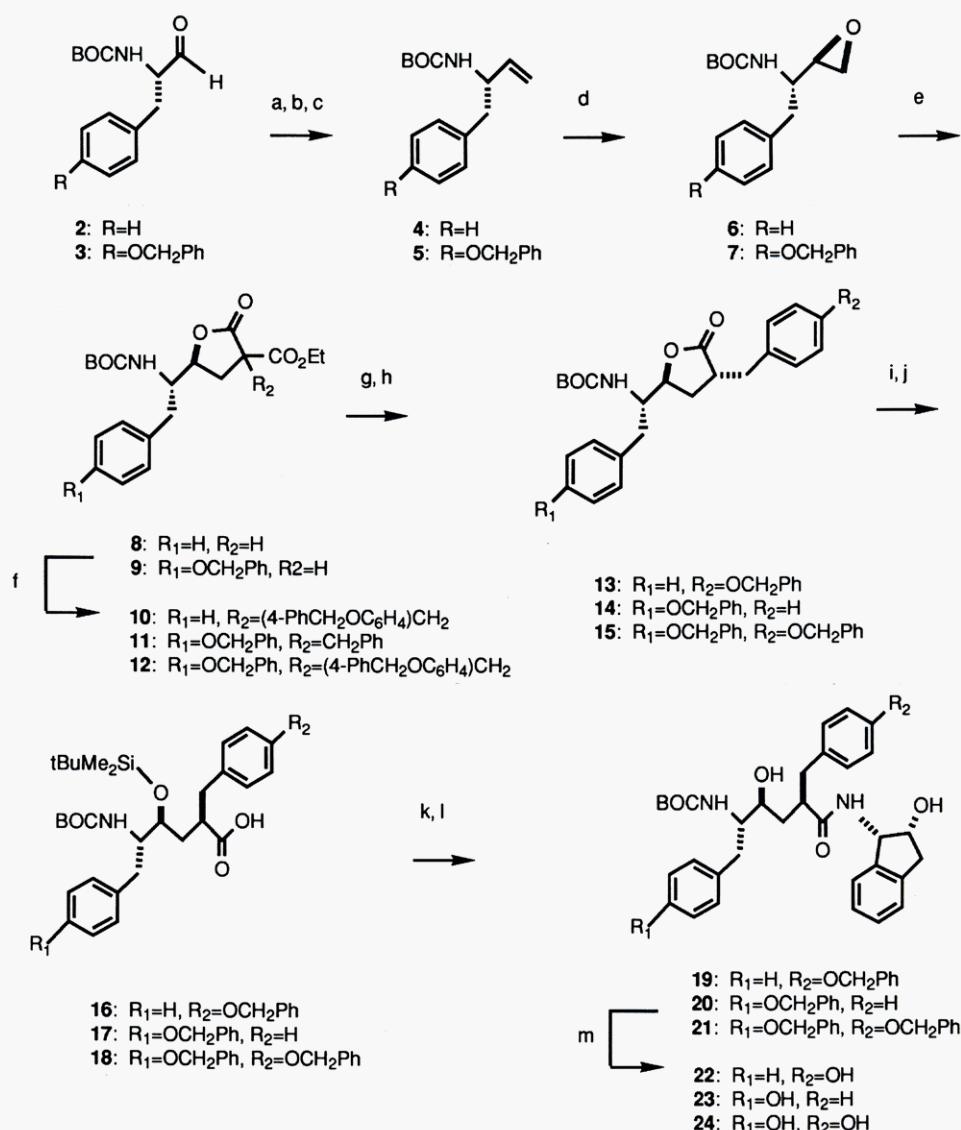
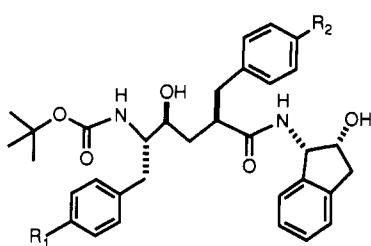


Figure 1. L-685,434 modeled in native HIVP active-site steric surface map.

Scheme I^a



^a (a) $\text{Me}_3\text{SiCH}_2\text{MgCl}$. (b) $\text{BF}_3\text{-OEt}_2$, CH_2Cl_2 . (c) Di-*tert*-butyl dicarbonate. (d) Magnesium monoperphthalate hexahydrate MeOH or MCPBA, CH_2Cl_2 . (e) $\text{CH}_2(\text{CO}_2\text{Et})_2$, Na/EtOH. (f) PhCH_2Br or $(4\text{-PhCH}_2\text{OC}_6\text{H}_4)\text{CH}_2\text{Cl}$, Na/EtOH. (g) LiOH. (h) Toluene, reflux. (i) LiOH. (j) $t\text{BuMe}_2\text{SiCl}$, imidazole, DMF; MeOH. (k) 1(*S*)-Amino-2(*R*)-hydroxyindan, HOBt, EDC, DMF. (l) $(n\text{Bu})_4\text{NF}$, THF. (m) H_2 , 10% Pd/C, THF-MeOH.

Table I. Anti-HIV Activity

compd	R ₁	R ₂	IC ₅₀ , nM	CIC ^{a,b} , nM	I/C
1	H	H	0.35	400	0.0009
19	H	OCH ₂ Ph	0.18	(50)	0.0036
22	H	OH	0.16	25 (50)	0.0032
23	OH	H	0.35	50 (25)	0.007
24	OH	OH	0.15	780	0.0002
29	H	OCH ₂ CH ₂ N(CH ₂) ₃ O	0.45	12 (6-50)	0.038
30	OCH ₂ CH ₂ N(CH ₂) ₃ O	H	0.55	50	0.011
31	OCH ₂ CH ₂ N(CH ₂) ₃ O	OCH ₂ CH ₂ N(CH ₂) ₃ O	1.9	100	0.019
32	H	OCH ₂ CH ₂ CH ₂ N(CH ₂) ₃ O	2.0	200	0.01
33	H	OCH ₂ CH ₂ NMe ₂	2.1	400	0.0025
34	H	OCH ₂ CH ₂ N(CH ₂) ₂	1.6	400	0.004
35	H	OCH ₂ CH ₂ N(CH ₂) ₂	1.8	-	-
36	H	OCH ₂ CH ₂ N(CH ₂) ₂ NMe	0.2	(100)	0.002
37	H	OCH ₂ CH ₂ N-(CH ₂ CH ₂ OMe) ₂	0.9	50	0.018
38	H	OCH ₂ CH ₂ N(CH ₂) ₂ S	0.47	50	0.0094
39	H	OCH ₂ CH ₂ N(CH ₂) ₂ SO	0.07	200	0.00035
40	H	OCH ₂ CH ₂ N(CH ₂) ₂ SO ₂	0.08	(100)	0.0008
41	H	OCH ₂ CH ₂ -N(OCH ₂ CH ₂)-O	0.2	(100)	0.002
43	H	OCH ₂ CO ₂ H	0.04	(>400)	<0.0001
44	H	OCH ₂ CH ₂ OH	0.09	(50)	0.0018
45	H	OCH ₂ -C(=O)-N(CH ₂) ₃ O	0.2	(50)	0.004

^a CIC values in parentheses were determined by p24-ELISA; all other entries were by fixed cell immunofluorescence in H9 cells using the IIb isolate (see Experimental Section). ^b With the exception of 29 (L-689,502) where *n* = 30, and compounds 23, 31, and 37 where *n* = 2, all other entries are *n* = 1. While some variability was observed in the antiviral spread activity for L-689,502 due to factors inherent in the cell-based assay, no other inhibitor in this series was found to exhibit a lower CIC in head to head comparison. The only inhibitors in this series which displayed an anti-HIV activity equal to L-689,502 in the spread assay were 44 and 45.

Under the same reaction conditions, the phenol 22 could also be efficiently converted into the ester 42 by employing ethyl bromoacetate as alkylating agent. Saponification with lithium hydroxide provided the phenoxyacetic acid 43 and reduction with lithium borohydride gave the 2-hydroxyethyl ether 44 (Scheme IV).

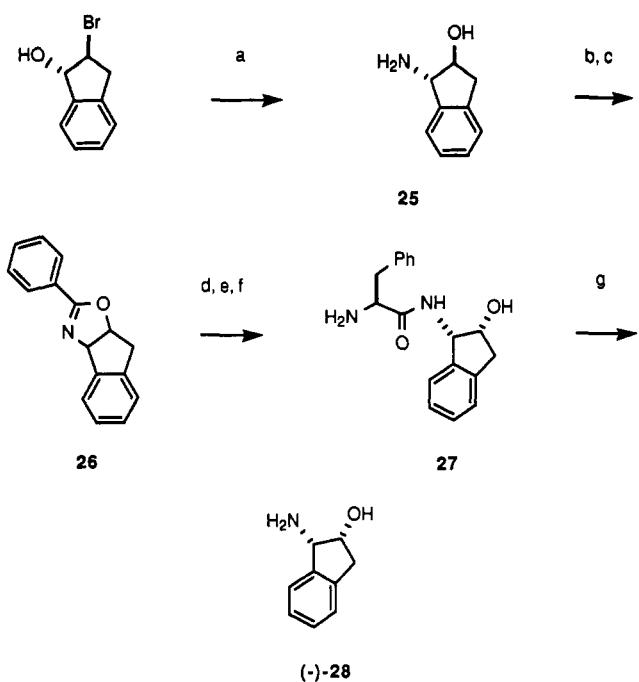
Similarly, alkylation with 4-(2-bromoacetyl)morpholine gave phenoxyacetamide derivative 45.

Molecular Modeling Study

A model of L-685,434 (1) bound in the HIV-1 protease active site was derived from the X-ray structures of the renin inhibitors H-142 and L-363,564 bound in the Endothiapepsin active site⁴ and a reduced peptide renin in-

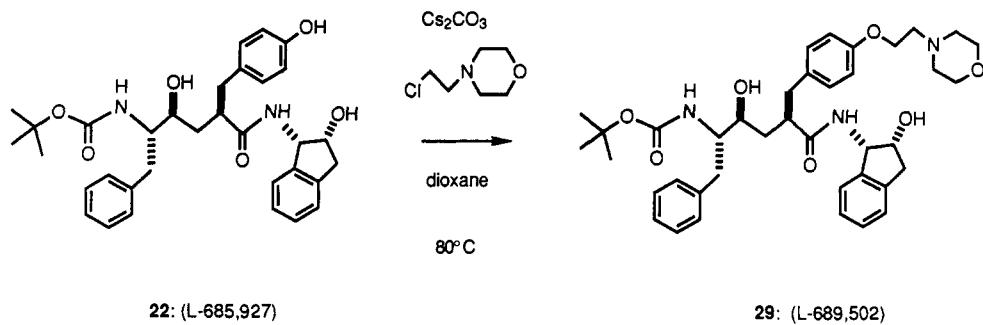
hibitor bound in the Rhizopuspepsin active site.¹³ This model of 1 was then energy minimized in the static native enzyme active site.^{3a} Two low-energy conformations resulted from the minimization; these differed only in the position of the P_{1'} phenyl group due to the open nature of the native enzyme-active site, in which the flaps are involved in crystal packing contacts with another molecule of enzyme. The conformation shown in Figure 1 was

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Scheme II^a

^a (a) Concentrated NH_4OH . (b) PhCOCl , NaOH . (c) SOCl_2 . (d) $\text{6N H}_2\text{SO}_4$, reflux. (e) BOC-Phe-OH , HOBT , DMF . (f) TFA . (g) Na/EtOH , reflux.

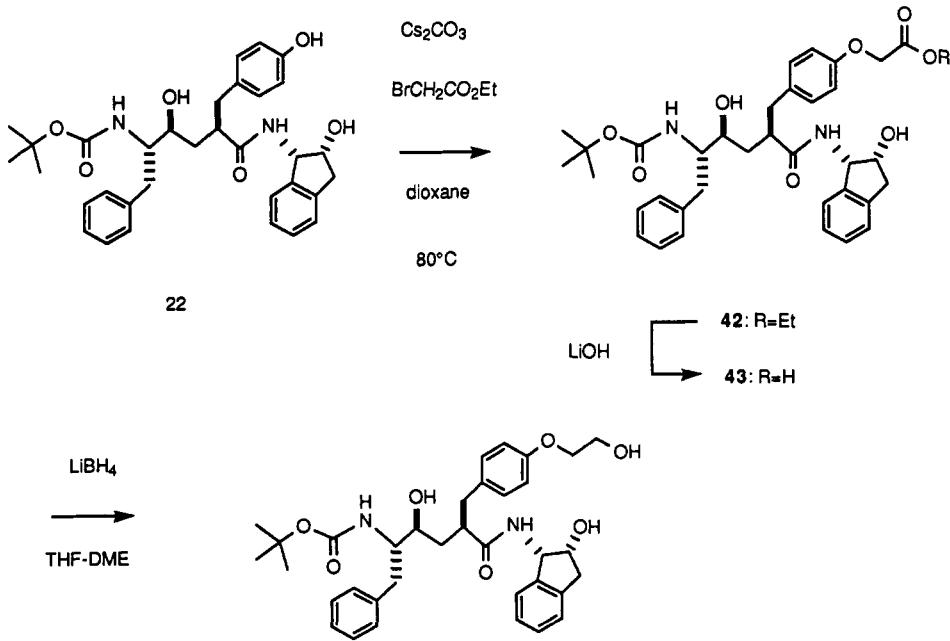
Scheme III. Synthesis of L-689,502



22: (L-685,927)

29: (L-689,502)

Scheme IV



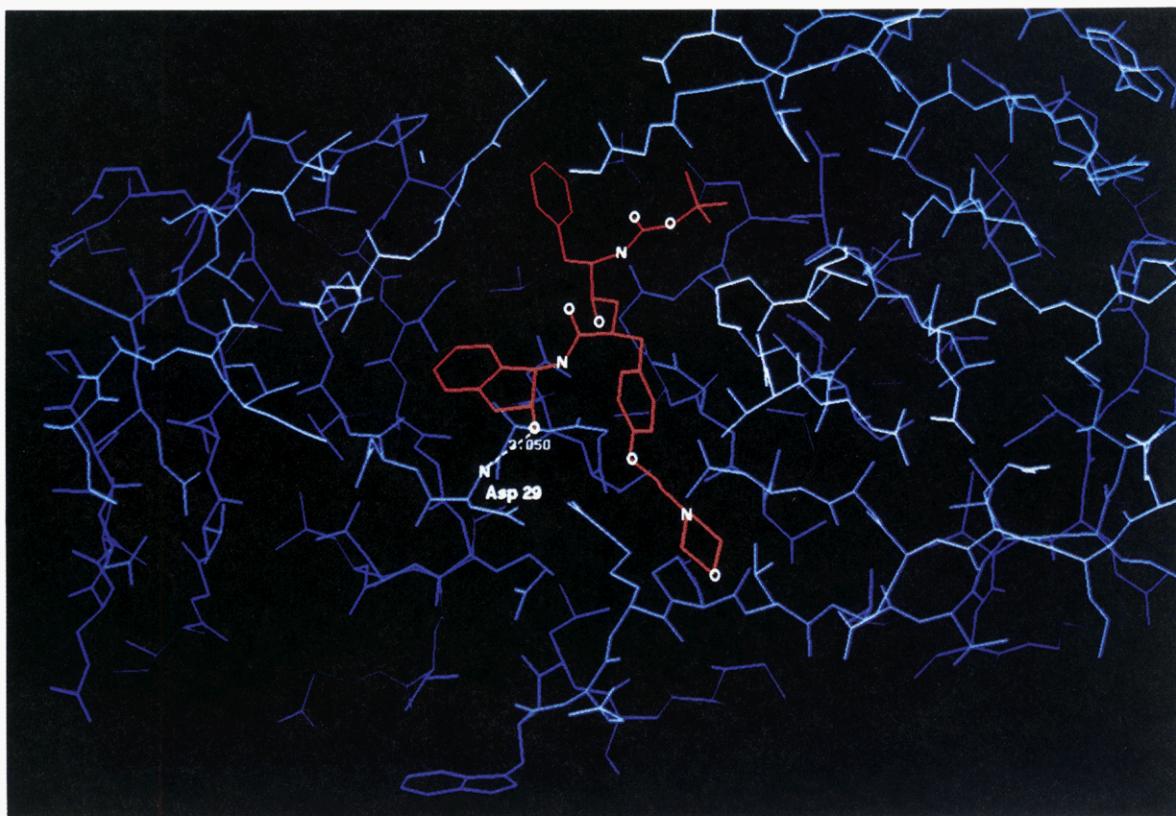


Figure 2. L-689,502 modeled in native enzyme active site.

At the beginning of the structural analysis, it appeared that L-689,502 bound to the nearly-symmetric enzyme-active site in a single orientation, with the N-terminus of the inhibitor interacting with monomer 2 of the enzyme and the C-terminus of the inhibitor interacting with monomer 1. After extensive refinement of the structure, it became apparent that a second orientation of the inhibitor (with the inhibitor N- and C-termini interacting with monomers 1 and 2, respectively) was present. The two orientations of the inhibitor (which will be referred to as orientations 1 and 2) were originally given equal occupancy, but as refinement progressed the occupancies were adjusted in an attempt to equalize the mean thermal factor for the two orientations. The occupancies are 0.55 and 0.45 in the final model. There was no interpretable electron density for the morpholino group of

the P₁' substituent and was therefore not possible to model the position of this group.

Results and Discussion

Consistent with the modeling observations, all inhibitors examined (19, 22–24, and 29–45) were highly potent *in vitro* with IC₅₀'s in the nanomolar range. Consequently, the para position on P₁ and P₁' can be viewed as essentially "neutral" to the thermodynamics of enzyme binding. As can be seen in Figure 4, the modeled structure of 29 is in good agreement with the subsequently determined X-ray structure. The hydrogen-bonding interaction predicted between the hydroxyl of the P₂' indanol and the NH of Asp₂₉ is observed in the X-ray structure as shown in Figure 3 and Table VI. The only major difference between the two structures is in the orientation of the para substituent on the P₁' phenyl. This difference seems to be mostly due to a change in the position of the side chain of Arg₈ between the native and inhibited enzyme structures. This can be seen as a shift in the position of the "channel" which is occupied by this substituent in the active site of the native (Figure 5) and the inhibited (Figure 6) enzyme structures. Although only three atoms may be compared between the modeled and X-ray structures of 29 due to disorder in the X-ray structure at this position (vide supra), it is clear that the terminal methylene seen in the X-ray structure would have an unfavorable steric interaction with the native enzyme-active site. However, it is accommodated within the inhibited enzyme-active site.

The two orientations of the inhibitor observed in the X-ray structure have very similar backbone conformations (Table VI); greater torsional angle differences occur in the conformations of the P₁ and P₁' substituents. In both orientations, the inhibitory hydroxyl group is within hydrogen-bonding distance of all of the side-chain oxygens of the catalytic Asp-25 and Asp-225 residues (Table V). Two of the interactions between the inhibitor and the

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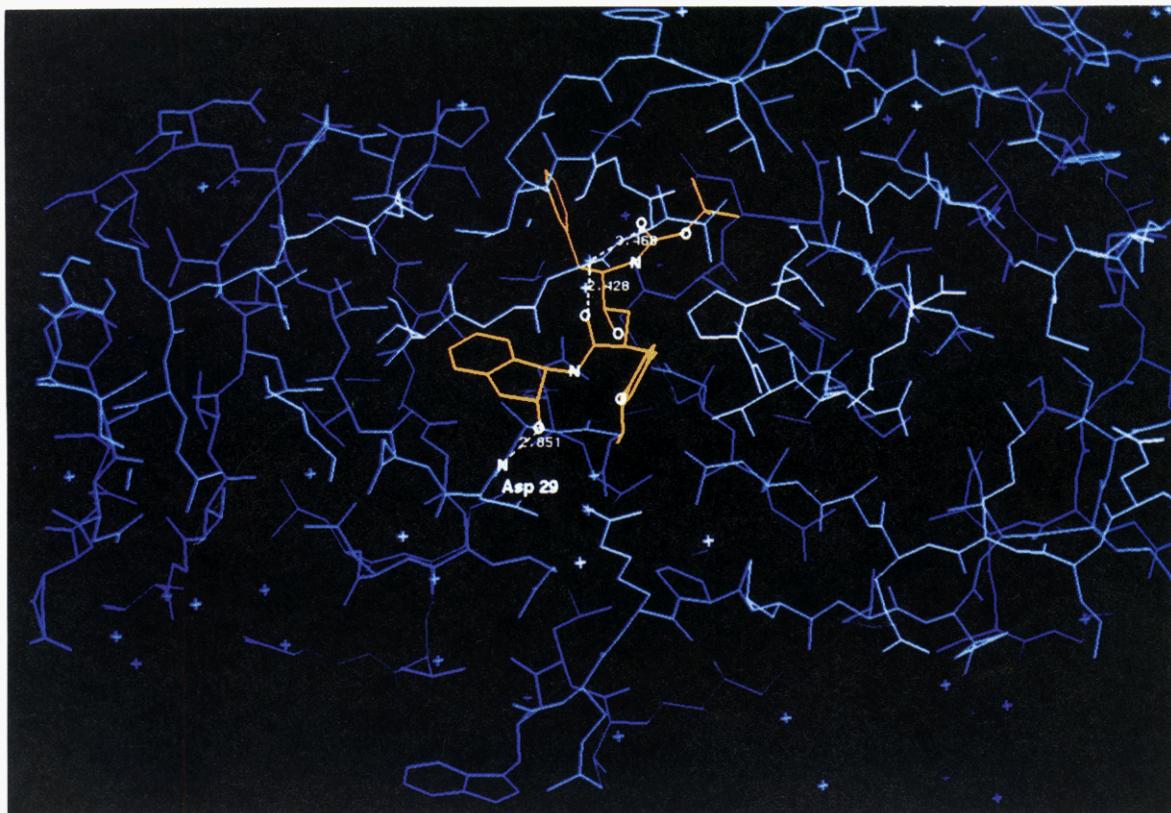


Figure 3. X-ray crystal structure of L-689,502-HIVP complex.

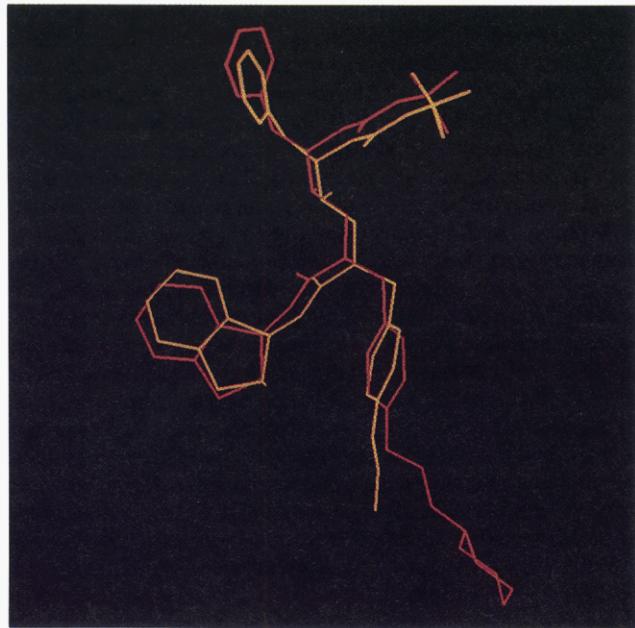


Figure 4. Comparison of L-689,502 active conformation as determined by modeling (red) and X-ray diffraction (yellow).

protein are mediated by solvent molecules. The tightly bound water that bridges between O₇ and O₃₈ of the inhibitor and the amide nitrogens of Ile-50 and Ile-250 is analogous to a similar water molecule that has been observed in the previously determined structures of inhibited complexes of HIV-1 protease.¹⁴⁻¹⁷ The second solvent-mediated interaction, bridging O₄₂ of the inhibitor to the carbonyl oxygen of Gly-227 (or Gly-27) and to the side chain oxygens of Asp-229 (or Asp-29) has no analogue in the previously examined structures.

The increase in antiviral potency for **29** (L-689,502; CIC = 12 nM), relative to the decreased enzyme activity (IC₅₀ = 0.45 nM) in comparison to the other inhibitors in Table I, may be due to the presence of the weakly basic morpholine group ($pK_a \sim 6.6$).¹⁸ The highest value for the ratio of IC₅₀ to CIC ($I/C = 0.0375$) observed in this series for L-689,502 is consistent with increased penetration into the HIV infected lymphocytes. In general, the presence of a weakly basic amine group (compounds **29-38**; $I/C > 0.004$) or *p*-oxygen substituent at P₁ or P_{1'} (compounds **19, 22, 23, 44**, and **45**) enhanced the cell penetration relative to the parent compound **1** ($I/C > 0.003$). Increasing the basicity of the amine¹⁹ (entries **33-36**) generally decreased the potency of the compound both as an enzyme inhibitor and antiviral agent relative to L-689,502.

While the double (P₁-P_{1'}) hydroxylation in **24** was well tolerated by the HIV-1 protease enzyme (IC₅₀ = 0.15 nM), the antiviral activity was markedly decreased (CIC = 780 nM) possibly as a result of increased acidity. In contrast, the bismorpholinyl compound **31** lost more than 10-fold in enzyme inhibitory potency (IC₅₀ = 1.9 nM) and gained in cell penetration (CIC = 100 nM, $I/C = 0.02$), although to a lesser extent than one would expect if the effects were additive.

Not unexpectedly, the neutral hydroxyl or *N*-oxide functionalites in **41, 44**, and **45** induced minimal effects

- (18) The pK_a of **29** was determined by extrapolation of the pH vs solubility curve by Dr. Drazen Ostovic, Department of Pharmaceutical Research.
- (19) Compounds **33-36** and **38** are expected to be more basic than **29** by comparison to the pK_a 's of trimethylamine (9.8), *N*-methylpiperidine (10.2), *N*-methylpyrrolidine (10.2), *N,N'*-dimethylpiperazine (8.54), and *N*-methylmorpholine (7.13), taken from Dean, J. A. *Handbook of Organic Chemistry*; McGraw-Hill: New York, 1987; pp 8-2.

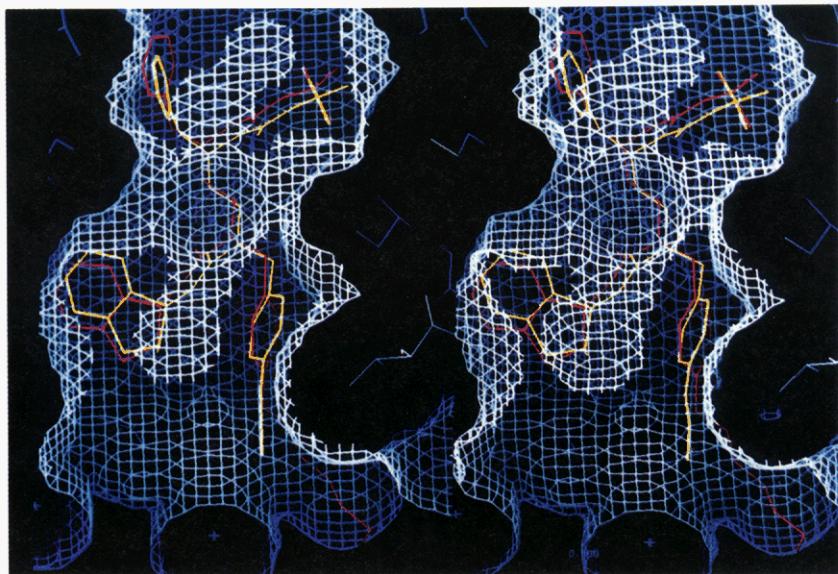


Figure 5. Comparison of L-689,502 active conformation determined by modeling (red) and X-ray diffraction (yellow) docked in native enzyme active site.

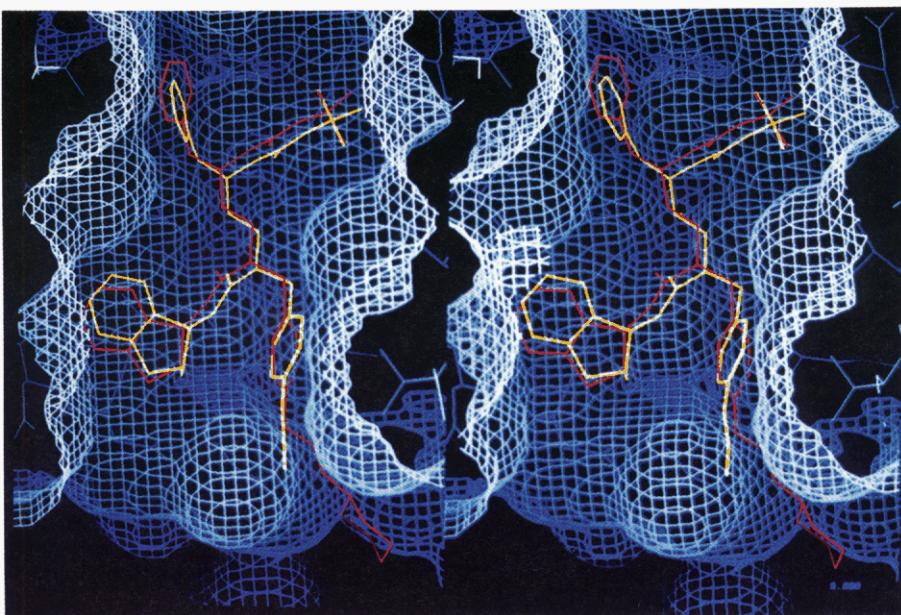


Figure 6. Active site of L-689,502 (red)-HIVP complex as determined by X-ray diffraction compared with active conformation of inhibitor from modeling (yellow).

on cell penetration ($I/C = 0.0072, 0.004$, and respectively vs 0.003 for the phenol 22). The 2-hydroxyethyl ether group in 44 enhanced inhibitory activity against the retroviral protease which parallels the anti-HIV activity in cell culture. Finally, introduction of highly polar groups (entries 39, 40, and 43) generally increased in vitro activity against HIV-1 protease concomitant with decreased cell penetration ($I/C < 0.003$) resulting in overall decreased antiviral activity.

Thus, attachment of 2-substituted ethoxy to the P_1' (or P_1) phenyl ring generally leads to overall enhancement of antiviral activity against HIV-1 in T-lymphoid cells through two possible mechanisms. When the 2-substituent is an electronegative group enhanced protease inhibition can give rise to increased antiviral activity. In the case of weakly basic amine groups, an increase in cell penetration may be responsible for increased blockade of viral spread.

The most potent compound in the series, L-689,502 was further evaluated for anti-HIV activity against five diverse

strains of the HIV-1 and the SIV (simian immunodeficiency virus) in cell culture (Table II). At concentrations of 12–50 nM, L-689,502 completely prevented the spread of the virus in human H9 T-lymphoid cells. By contrast, in identical assays using the IIIb variant of HIV-1, the nucleoside analogues 3'-azidothymidine (AZT) and di-deoxyadenosine (DDI) both exhibited CIC values of 25 mM. L-689,502 was also a potent inhibitor of HIV-1 infection spread in human MT-4 lymphoid cells, primary

- (20) Henderson, L. E.; Benveniste, R. E.; Sowder, R.; Copeland, T. D.; Schultz, A. M.; Oroszlan, S. Molecular Characterization of *gag* Proteins from Simian Immunodeficiency Virus (SIV_{mne}). *J. Virol.* 1988, 62, 2587–95.
- (21) Heimbach, J. C.; Diehl, R. E.; Davis, L. J.; Deana, A. A.; Rooney, C. S.; Guare, J. P.; Giuliani, E. A.; Britcher, S. F.; Thompson, W. J.; Huff, J. R.; Kohl, N. E.; Darke, P. L. Analysis of Inhibitors of the HIV-1 Protease as HIV-2 Protease Inhibitors. Submitted to *Biochem. Biophys. Res. Commun.*

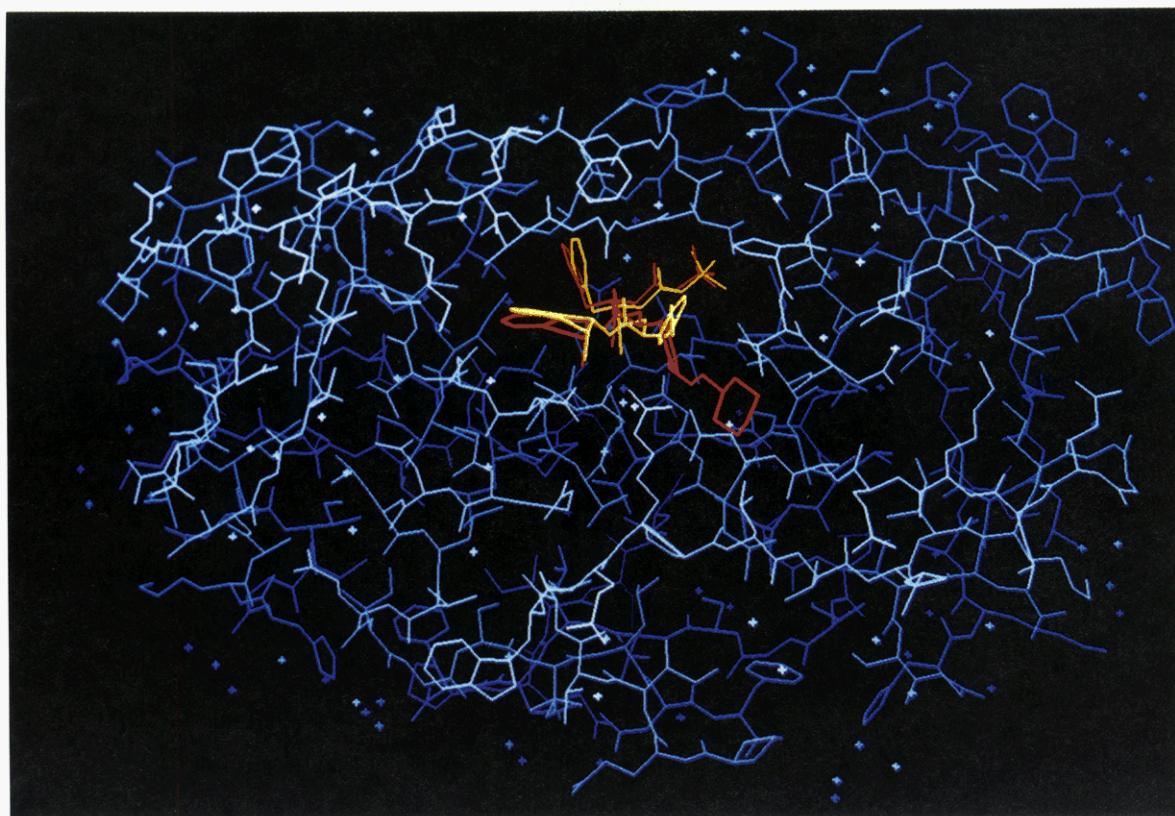


Figure 7. X-ray crystal structure of L-689,502 (red)–HIVP complex compared with active conformation of inhibitor from modeling (yellow).

Table II. Minimum Cell Culture Inhibitory Concentration (CIC) for Preventing Spread of HIV-1 and SIV Infection in Human Cells^a

compound	cell type	virus	CIC, nM		
			fixed-cell immunofluorescence	p24-ELISA	
L-689,502	MT-4 T-lymphoid	HIV-1 (IIIb)	25	6–50	
L-689,502	peripheral blood lymphocytes	HIV-1 (IIIb)	0.78	12–25	
L-689,502	monocytes/macrophages	HIV-1 (SF162)	25–50		
L-689,502	MT-4 T-lymphoid	HIV-1 (RF)	25–50		
L-689,502	MT-4 T-lymphoid	HIV-1 (MN)	25–50		
L-689,502	MT-4 T-lymphoid	HIV-1 (WMJ-2)	6–12		
L-689,502	MT-4 T-lymphoid	HIV-1 (RUTZ)	12		
L-689,502	H9 T-lymphoid	HIV-1 (IIIb)	6–12	12–25	
AZT	MT-4 T-lymphoid	HIV-1 (IIIb)		25	
DDI	H9 T-lymphoid	HIV-1 (IIIb)	25000		
L-689,502	H9 T-lymphoid	SIV (mac251)	800		
L-689,502	H9 T-lymphoid	SIV (mne)	200		

^a Assay was performed as described in Experimental Section.

Table III. Data Measurement

shell		mean: $I/\sigma(I)$	number of observations	number of reflections ^a	percent of possible data	R_{sym}^b
min	max					
∞	3.53	55.9	16 465	3 171	97.3	3.53
3.53	2.81	11.9	10 694	2 835	91.5	7.82
2.81	2.45	3.8	5 270	2 321	75.4	12.19
2.45	2.23	2.4	2 068	1 496	49.1	11.51
2.23	2.07	1.9	893	796	26.2	11.10
2.07	1.95	1.7	205	198	7.2	12.01
totals		20.8	35 595	10 817	59.2	5.69

^a Only those reflections for which $I > s(I)$ were included in the data set. ^b $R_{\text{sym}} = \sum_n \sum_i |F_i - \langle F \rangle| / \sum_n \sum_i |F_i|$, where F_i is the i -th observation of the n -th reflection and $\langle F \rangle$ is the mean of all observations of the n -th reflection.

peripheral blood lymphocytes, and primary peripheral monocytes/macrophages (Table II). In addition, L-689,502 inhibited cell culture infection by the SIV, albeit with a reduced potency. Due to the greater homology of the SIV and HIV-2 proteases,²⁰ a similar reduction in antiviral

activity for L-689,502 would be expected for the latter virus. The lower K_i observed for L-689,502 against the HIV-2 protease confirms this prediction (K_i [HIV-1] = 0.2 nM; K_i [HIV-2] = 5.9 nM).²¹ These results are consistent with the observed sequence differences for the HIV-1 and

Table IV. Summary of Current Restrained Least-Squares Refinement Model

rms deviation	value in		number of constraints		
	σ	current model	total	$>2\sigma$	$>3\sigma$
from ideal distances, Å					
bond distances	0.020	0.018	1647	43	3
angle distances	0.030	0.042	2237	232	73
plant 1-4 distances	0.040	0.045	544	34	9
from ideal planarity, Å	0.020	0.015	266	1	0
from ideal chirality, Å ³	0.150	0.183	262	24	2
from permitted contact distances, Å					
single torsion contacts	0.500	0.229	564	0	0
multiple torsion contacts	0.500	0.264	569	1	0
possible H bonds	0.500	0.273	123	0	0
from ideal torsion angles, deg					
planar groups (0 or 180)	3.0	2.6	203	2	1
staggered groups (± 60 or 180)	15.0	19.6	295	35	9
orthonormal groups (± 90)	20.0	14.9	12	0	0
resolution range, Å	number of reflections			<i>R</i>	
8.00–5.70	503			0.250	
5.70–4.67	616			0.148	
4.67–4.05	720			0.131	
4.05–3.62	780			0.143	
3.62–3.31	866			0.151	
3.31–3.06	921			0.163	
3.06–2.87	922			0.174	
2.87–2.70	936			0.195	
2.70–2.56	938			0.201	
2.56–2.45	799			0.212	
2.45–2.34	688			0.200	
2.34–2.25	626			0.220	
8.00–2.25	9315			0.173	

Table V. Potential Hydrogen Bonds between the HIV-1 Protease Dimer and L-689,502

inhibitor	inhibitor orientation 1				inhibitor orientation 2			
	solvent	distance	protein	distance	solvent	distance	protein	distance
Direct Contacts with Residues in Body of Protein								
502-300 NB			GLY-27 O	3.4			GLY-227 O	3.1
502-300 O18			ASP-25 OD1	2.6			ASP-225 OD1	2.7
502-300 O18			ASP-25 OD2	2.5			ASP-225 OD2	3.3
502-300 O18			ASP-225 OD2	3.0			ASP-25 OD2	2.8
502-300 O18			ASP-225 OD1	2.4			ASP-25 OD1	2.4
502-300 N39			GLY-227 O	2.5			GLY-27 O	3.3
502-300 O42			GLY-227 O	3.0			GLY-27 O	3.4
502-300 O42			ASP-229 N	2.9			ASP-29 N	2.9
502-300 O42			ASP-229 OD2	3.5				
502-300 O42			ASP-229 OD1	3.5			ASP-29 OD1	3.1
Solvent-Mediated Contacts with Residues in Body of Protein								
502-300 O42	WAT-333 O	2.9	GLY-227 O	3.0	WAT-326 O	3.2	GLY-27 O	2.5
502-300 O42	WAT-333 O	2.9	ASP-229 OD2	2.7	WAT-326 O	3.2	ASP-29 OD2	3.0
502-300 O42					WAT-326 O	3.2	ASP-29 OD1	3.5
Solvent-Mediated Contacts with Residues in Flap								
502-300 O7	WAT-309 O	3.5	ILE-50 N	3.5	WAT-309 O	2.4	ILE-250 N	3.3
502-300 O38	WAT-309 O	2.4	ILE-250 N	3.3	WAT-309 O	3.1	ILE-50 N	3.5

HIV-2 proteases which, in the active site, are primarily in the P₁ (and P_{1'}) binding regions.²² It is evident from the X-ray crystal structure that L-689,502 has no interaction with the P₃ or P_{3'} binding regions in the HIV-1 protease. Unlike the longer hexapeptide mimetic inhibitors which utilize the more conserved P₃ binding for inhibitory activity and show smaller differences in activity for the HIV-1 and

HIV-2 proteases,^{1c,23} L-689,502 relies more on interactions with the P₁ and P_{1'} hydrophobic pockets for affinity. Prevention of the viral protease-mediated maturation cleavage of the p55 gag precursor protein into the p24 core and p17 matrix proteins results in the production of noninfectious viral particles.²⁴ The ability of L-689,502

(22) (a) Tomasselli, A. G.; Hui, J. O.; Sawyer, T. K.; Staples, D. J.; Bannow, C.; Reardon, I. M.; Howe, J.; DeCamp, D. L.; Craik, C. S.; Heinrikson, R. L. Specificity and Inhibition of Proteases from Human Immunodeficiency Viruses 1 and 2. *J. Biol. Chem.* 1990, 265, 14675–83. (b) Poorman, R. A.; Tomasselli, A. G.; Heinrikson, R. L.; Kezdy, F. J. A Cumulative Model for Proteases from Human Immunodeficiency Virus Types 1 and 2, Inferred from Statistical Analysis of an Extended Substrate Data Base. *J. Biol. Chem.* 1991, 266, 14554–61.

(23) Tozser, J.; Blaha, I.; Copeland, T. D.; Wondrak, E. M.; Oroszlan Comparison of the HIV-1 and HIV-2 Proteinases Using Oligopeptide Substrates Representing Cleavage Sites in gag and gag-pol Polyproteins. *FEBS Lett.* 1991, 281, 77–80.

(24) Kohn, N. E.; Emini, E. A.; Schleif, W. A.; Davis, L. J.; Heimbach, J. C.; Dixon, R. A. F.; Scolnick, E. M.; Sigal, I. S. Active Human Immunodeficiency Virus Protease is Required for Viral Infectivity. *Proc. Natl. Acad. Sci. U.S.A.* 1988, 85, 4686–90.

(25) Personal communication from Dr. Leeyuan Huang.

Table VI. Torsion Angles in L-689,502

Torsion Angles in Inhibitor Backbone			
atoms defining angle	angle type	angle	
		orientation 1	orientation 2
502-300 C1-O5-C6-N8		177.28	-179.60
502-300 O5-C6-N8-C9	OMG	-179.73	-178.05
502-300 C6-N8-C9-C17	PHI	-111.24	-90.08
502-300 N8-C9-C17-C19	PSI	59.27	68.08
502-300 C9-C17-C19-C20	OMG	166.75	158.54
502-300 C17-C19-C20-C37	PHI	-77.82	-70.23
502-300 C19-C20-C37-N39	PSI	149.22	168.92
502-300 C20-C37-N39-C40	OMG	179.05	175.73
502-300 C37-N39-C40-C41		-150.42	-169.85
502-300 N39-C40-C41-O42		-39.79	-34.53

Torsion Angles in Inhibitor Side Chains			
atoms defining angle	subsite	angle	
		orientation 1	orientation 2
502-300 N8-C9-C10-C11	P1	-83.97	-47.21
502-300 C9-C10-C11-C12	P1	136.02	106.06
502-300 C19-C20-C21-C22	P1	130.62	160.26
502-300 C20-C21-C22-C23	P1'	-113.46	-104.93
502-300 C24-C25-O28-C29	P1'	-147.79	100.03
502-300 C25-O28-C29-C30	P1'	175.17	-134.91

to block processing of p55 supports the conclusion that the antiviral effect is indeed the result of its inhibition of the viral protease enzyme.

The compound L-689,502 was further assayed against several other proteases to examine specificity. Particularly noteworthy was that the compound did not show any inhibition of human renin, another aspartyl protease at concentrations up to 6 mM. In addition, there was no inhibition of pepsin at 250 μ M, papain at 1 mM, or elastase at 7 mM. The in vivo biology of L-689,502 will be reported elsewhere.

Experimental Section

Chemistry. Anhydrous solvents were "anhydrous grade" from Aldrich Chemical Co. "Dry" solvents were distilled from sodium benzophenone ketyl under nitrogen atmosphere. All other solvents were HPLC grade. The abbreviations DME, DMF, and THF refer to 1,2-dimethoxyethane, *N,N*-dimethylformamide, and tetrahydrofuran. The term "concentrated" refers to the removal of volatile solvents under reduced pressure at or below 40 °C, usually with the aid of a rotary evaporator. All products were dried to constant weight. All melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton magnetic resonance spectra were obtained on either a Varian XL-300 (300 MHz) or a Varian VXR-400 (400 MHz) spectrometer using TMS as internal standard. Specific rotations were determined on a Perkin-Elmer 241 polarimeter. Column chromatography was performed with E. Merck 240-400 mesh silica gel under low nitrogen pressure of 5-10 psi. Thin-layer chromatography (TLC) was carried out with E. Merck silica gel 60 F-254 plates. Ultraviolet spectra were obtained on a Beckman Acta MVI spectrophotometer by Matt Zrada. Analyses were performed by John P. Moreau and were within $\pm 0.4\%$ of the theoretical values. Reaction temperatures were monitored internally with an electronic digital thermometer.

3(S)-[(tert-Butyloxycarbonyl)amino]-4-phenyl-1-butene (4). To a stirred suspension of Mg turnings (9.8 g, 0.4 mol) in 200 mL of anhydrous ether was added 50 mL (0.4 mol) of (chloromethyl)trimethylsilane. After initiation by gentle warming, the reaction was maintained at gentle reflux by cooling with an ice bath. When the exotherm was completed, the mixture was aged for 1 h and then cooled to -78 °C. A solution of 19.3 g (77.4 mmol) of *N*-(tert-butyloxycarbonyl)-L-phenylalaninal²⁶ (2) in 120

mL of dry THF was added dropwise maintaining the temperature below -55 °C. After warming to room temperature for 0.5 h, the mixture was poured into 500 g of ice and 500 mL of 10% citric acid. The aqueous layer was extracted with 3 \times 300 mL of ether, and the combined ether extracts were dried ($MgSO_4$) and concentrated. The crude product (26.6 g) was dissolved in 400 mL of CH_2Cl_2 and cooled under nitrogen in an ice bath. To it was added 43 mL (5 equiv) of BF_3 etherate, keeping the temperature below 10 °C. The solution was allowed to warm to room temperature and stir for 5 days, cooled in an ice bath, and quenched by dropwise addition of 400 mL of 10% NaOH. The aqueous phase was extracted with 2 \times 250 mL of CH_2Cl_2 . Combined organic extracts were dried ($MgSO_4$) and concentrated. The oily crude product (3(S)-amino-4-phenyl-1-butene; 14.2 g) and 31 g (2 equiv) of di-*tert*-butyl dicarbonate were dissolved in 200 mL of CH_2Cl_2 and allowed to react at room temperature for 18 h. The solution was washed with 3 \times 100 mL of 10% citric acid, 100 mL of water, and 3 \times 250 mL of saturated $NaHCO_3$, dried ($MgSO_4$), and concentrated. Chromatography on silica gel with 1:4 ethyl/hexanes gave 16.3 g (85%) of the product 4 as a white solid: mp 67.5-68.5 °C. Anal. ($C_{15}H_{21}NO_2$) C, H, N.

***N*-(tert-Butyloxycarbonyl)-O-benzyl-L-tyrosinal (3).** The general procedure of Goel et al.²⁷ was modified as follows. To a stirred solution of 50 g of *N*-BOC-O-benzyl-L-tyrosine in 800 mL of dry CH_2Cl_2 and 200 mL of dry THF cooled to -20 °C was added 20 mL (1.2 equiv) of *N*-methylpiperidine. The solution was allowed to warm to -12 °C, and 21 mL (1.2 equiv) of isobutyl chloroformate was added, maintaining the temperature between -12 and -8 °C. After addition was complete, an ice cold solution of 15.8 g (1.2 equiv) of *N,O*-dimethylhydroxylamine hydrochloride and 20 mL (1.2 equiv) of *N*-methylpiperidine in 120 mL of CH_2Cl_2 was added in one portion. The solution was allowed to warm to room temperature and stir for 15 h, washed with 2 \times 200 mL of 10% citric acid, dried ($MgSO_4$), and concentrated. The crude product (60.2 g) was purified by chromatography through a short pad of silica gel (400 g), with 3 L of ethyl acetate as eluent. The *N*-BOC-O-benzyl-L-tyrosine *N'*-methoxy-*N'*-methylamide (51.8 g, 92.8%) was obtained as a white fluffy solid: mp 107-8 °C; $[\alpha]_D^{25} = +5.6^\circ$ (c = 1.7, MeOH). Anal. ($C_{23}H_{30}N_2O_5$) C, H, N.

To a suspension of 2.22 g (0.058 mol) of $LiAlH_4$ in 200 mL of anhydrous ether cooled to -40 °C was added a solution of 20.5 g (0.05 mol) of the *N*-BOC-O-benzyl-L-tyrosine *N'*-methoxy-*N'*-methylamide in 250 mL of dry THF, maintaining the temperature between -36 °C and -38 °C. After addition was complete, the reaction was warmed to 7 °C and then recooled to -35 °C and quenched by the addition of 40 mL of 2.75 M $KHSO_4$. The mixture was warmed with stirring to 25 °C for 1 h, filtered through diatomaceous earth, and washed with 3 \times 100 mL of Et_2O . The filtrate was washed with 3 \times 100 mL of 10% citric acid, 100 mL of water, 2 \times 200 mL of dilute $NaHCO_3$, and 100 mL of brine, dried ($MgSO_4$), and concentrated. The product 3 (17.7 g, 0.05 mol) was a white crystalline solid which was stored in the freezer: mp 98-99 °C; $[\alpha]_D^{25} = +27.4^\circ$ (c = 1.6, MeOH).

3(S)-[(tert-Butyloxycarbonyl)amino]-4-[4-(benzyloxy)-phenyl]-1-butene (5). By following the olefination procedure described for 4 above, from 13.4 g (0.038 mol) of *N*-(tert-butyloxycarbonyl)-O-benzyl-L-tyrosinal there was obtained 9.9 g (74%) of 5 as a white solid: mp 87-8 °C; $[\alpha]_D^{25} = +6.2^\circ$ (c = 1, MeOH). Anal. ($C_{22}H_{27}NO_3$) C, H, N.

1(R,S)-[1'(S)-[(tert-Butyloxycarbonyl)amino]-2-phenylethyl]oxirane (6). A solution of 97 g (4 equiv) of monoperoxyphthalic acid, magnesium salt hexahydrate (80% by titration), and 19.8 g (80 mmol) of 3(S)-[(tert-butyloxycarbonyl)amino]-4-phenyl-1-butene (4) in 400 mL of absolute MeOH was stirred for 2 days at room temperature. The resulting mixture was filtered and concentrated. The oily residue was partitioned between 800 mL of ether and 200 mL of water. The ethereal extract was washed with 200 mL of water, and 2 \times 200 mL of saturated $NaHCO_3$, dried ($MgSO_4$), and concentrated. Chromatography on silica gel gave 13.5 g (64%) of product 6 as an oil which slowly crystallized.

1(R,S)-[1'(S)-[(tert-Butyloxycarbonyl)amino]-2-[4-(benzyloxy)phenyl]ethyl]oxirane (7). By following the procedure

(26) Fehrentz, J.-A.; Castro, B. An Efficient Synthesis of Optically Active α -(t-Butoxycarbonyl)amino Aldehydes from α -Amino Acids. *Synthesis* 1983, 676-78.

(27) Goel, P.; Krolls, U.; Stier, M.; Kesten, S. *N*-tert-Butyloxycarbonyl-L-leucinal. *Org. Synth.* 1988, 67, 69-75.

as described for 6, there was obtained 13.2 g (8:1 threo/erythro by HPLC) of an oily product 7. The crude product was used in the next step without further purification: ^1H NMR (CDCl_3) δ 1.39 (s, 3 H), 2.59 (m, 1 H), 2.70 (dd, 1 H, J = 4.5, 4.5 Hz), 2.8–3.02 (br m, 1 H), 3.02 (m, 1 H), 4.08 (br m, 1 H), 4.55 (br d, 1 H), 5.02 (s, 2 H), 6.92 (d, 2 H, J = 8 Hz), 7.18 (d, 2 H, J = 8 Hz), 7.3–7.45 (m, 5 H).

3-Carbethoxy-5(S)-[1'(S)-[(*tert*-butyloxycarbonyl)amino]-2-phenylethyl]dihydrofuran-2(3*H*)-one (8). To a stirred solution of 3.08 g (3.7 equiv) of sodium in 200 mL of absolute EtOH was added 24 mL (4.4 equiv) of diethyl malonate followed by 9.5 g (0.036 mol) of epoxide 6 in 100 mL of absolute EtOH. After stirring overnight, the reaction was acidified to pH 4 with 10% citric acid and extracted with 2×500 mL of ether. The combined extracts were washed with 500 mL of water, 500 mL of NaHCO_3 , and 500 mL of brine, dried (MgSO_4), and concentrated. Chromatography on silica gel using 1:1 ether/hexanes gave 12.07 g of 8 as a resin which was homogeneous by TLC and HPLC. Further elution gave 1.5 g of the 5(*R*)-diastereomer.

3-Carbethoxy-5(S)-[1'(S)-[(*tert*-butyloxycarbonyl)amino]-2-[4-(benzyloxy)phenyl]ethyl]dihydrofuran-2(3*H*)-one (9). By following the procedure described for 8 above, from 13.2 g (0.037 mol) of 7 there was obtained 9.5 g (55%) of 9 as a white solid: mp 117–9 °C; $[\alpha]_D^{25} = -11.27^\circ$ ($c = 1.81$, MeOH). Anal. ($\text{C}_{27}\text{H}_{33}\text{NO}_7$) C, H, N.

The lactone 9 was determined to be least 99.99% enantiomerically pure by HPLC on a Pirkle phenylglycine semiprep column. At $\lambda = 215$ nm and flow rate = 2.0 mL/min using linear gradients of hexane/2-propanol from 95:5 to 80:20 over 20 min then to 5:95 over 40 min the retention time of (−)-9 was 31.2 min. Under these conditions, racemic 9 derived from racemized *N*-(*tert*-butyloxycarbonyl)-*O*-benzyl-L-tyrosinal (3) gave nearly equal intensity peaks at retention times of 30.48 and 31.16 min.

3-Carbethoxy-3-[[4-(benzyloxy)phenyl]methyl]-5(S)-[1'(S)-[(*tert*-butyloxycarbonyl)amino]-2-phenylethyl]dihydrofuran-2(3*H*)-one (10). To a stirred solution of 11.27 g (0.03 mol) of 8 in 250 mL of absolute EtOH was added 0.033 mol of 2 M Na/EtOH followed by 8.63 g (0.037 mol) of 4-(benzyloxy)benzyl chloride (Aldrich 97%). The solution was heated to 50 °C for 3.75 h, cooled, and concentrated. The residue was taken up in 1 L of ethyl acetate, washed with 500 mL of 5% citric acid, dried (MgSO_4), and concentrated. Chromatography on silica gel using 1:1 ether/hexanes gave 13.29 g (77%) of 10 as a clear glass essentially homogeneous by TLC.

3-Carbethoxy-3-(phenylmethyl)-5(S)-[1'(S)-[(*tert*-butyloxycarbonyl)amino]-2-[4-(benzyloxy)phenyl]ethyl]dihydrofuran-2(3*H*)-one (11). To a stirred solution of 2.4 g (0.005 mol) of 9 in 25 mL of absolute EtOH was added 0.0052 mol of 1 M Na/EtOH followed by 0.0067 mol of benzyl bromide. The solution was heated to 50 °C for 1 h, cooled, and partitioned between 100 mL of ice cold 10% citric acid and 2×100 mL of ether. The combined ethereal extracts were washed with 50 mL of saturated NaHCO_3 , dried (MgSO_4), and concentrated. The crude product (1.9 g, 0.0038 mol of a foamy solid) contained a small amount of decarbethoxylated product (14) and was used without further purification for the preparation of 14.

3-Carbethoxy-3-[[4-(benzyloxy)phenyl]methyl]-5(S)-[1'(S)-[(*tert*-butyloxycarbonyl)amino]-2-[4-(benzyloxy)phenyl]ethyl]dihydrofuran-2(3*H*)-one (12). To a stirred solution of 3.0 g (0.0062 mol) of 9 in 60 mL of absolute EtOH was added 0.007 mol of 2 M Na/EtOH followed by 1.84 g (0.008 mol) of 4-benzyloxybenzyl chloride (Aldrich 97%). The solution was heated to 50 °C for 2.55 h, cooled, and concentrated. The residue was taken up in 500 mL of ether, washed with 150 mL of 10% citric acid and 50 mL of saturated NaHCO_3 , dried (MgSO_4), and concentrated. The crude product (4.3 g, 0.0062 mol of a foam) contained a small amount of decarbethoxylated product (15) and was used without further purification for the preparation of 15.

3(R)-[[4-(Benzyl)phenyl]methyl]-5(S)-[1'(S)-[(*tert*-butyloxycarbonyl)amino]-2-phenylethyl]dihydrofuran-2(3*H*)-one (13). To a stirred solution of 19.8 g (0.034 mol) of 10 in 245 mL of DME was added 0.122 mol of 1 M LiOH. After 3.5 h, the mixture was concentrated, acidified to pH 3 with 10% citric acid, and extracted into 3×400 mL of ethyl acetate. The combined extracts were dried (MgSO_4) and concentrated. The residue was dissolved in 250 mL of toluene, heated to reflux for 17 h, and

concentrated. Chromatography on silica gel using 15% ethyl acetate/hexanes gave 7.28 g (43%) of 13 as a glass which was 97% pure by HPLC: ^1H NMR (CDCl_3) δ 1.45 (s, 9 H), 1.95 (m, 1 H), 2.2 (m, 1 H), 2.7–3.05 (m, 5 H), 3.8 (br m, 1 H), 4.2 (br m, 1 H), 4.5 (br m, 1 H), 5.0 (s, 2 H), 6.85 (d, 2 H, J = 8 Hz), 7.05 (d, 2 H, J = 8 Hz), 7.15–7.45 (m, 10 H). Further elution gave 10.2 g of mixed fractions containing mostly 3(*S*)-isomer.

Epimerization of the 3(*S*)-diastereomer to a mixture of 3(*R*)- and 3(*S*)-isomers (~1:2) was effected by heating the mixed fractions (10.2 g, 0.02 mol) in 250 mL of DME with 0.5 g (0.15 equiv) of anhydrous cesium carbonate for 2.75 h at 80 °C. The mixture was concentrated, taken up in 250 mL of ethyl acetate, washed with 100 mL of 10% citric acid and 100 mL of brine, dried (MgSO_4), and concentrated. Chromatography as above gave an additional 2.7 g (15.8%) of 13 and 6.08 g (35.6%) of the 3(*S*)-diastereomer. A second epimerization cycle gave another 1.5 g (8.8%) of 13 and 3.6 g (21%) of the 3(*S*)-diastereomer. (Total yield of 13 = 67.6%).

3(R)-[(Phenylmethyl)-5(S)-[1'(S)-[(*tert*-butyloxycarbonyl)amino]-2-[4-(benzyloxy)phenyl]ethyl]dihydrofuran-2(3*H*)-one (14). To a stirred solution of 2.5 g (0.0043 mol) of crude 11 in 30 mL of DME was added 0.017 mol of 0.5 M LiOH. After 12 h, the mixture was concentrated, acidified to pH 3 with 10% citric acid, and extracted into 3×100 mL of ethyl acetate. The combined extracts were dried (MgSO_4) and concentrated. The residue was dissolved in 250 mL of toluene, heated to reflux for 18 h, and concentrated. Chromatography on silica gel using 15% ethyl acetate/hexanes gave 0.7 g (32%) of 14 as a glass: ^1H NMR (CDCl_3) δ 1.35 (s, 9 H), 1.95 (m, 1 H), 2.2 (m, 1 H), 2.7–2.8 (m, 3 H), 2.95 (m, 1 H), 3.1 (dd, 1 H, J = 5, 12 Hz), 3.85 (br m, 1 H), 4.2 (br m, 1 H), 4.7 (br m, 1 H), 5.0 (s, 2 H), 6.85 (d, 2 H, J = 8 Hz), 7.08 (d, 2 H, J = 8 Hz), 7.1–7.45 (m, 10 H). Further elution gave 1.46 g (67%) of mixed fractions containing mostly 3(*S*)-isomer.

Epimerization of the 3(*S*)-diastereomer to a mixture of 3(*R*)- and 3(*S*)-isomers (~1:2) was effected by heating the mixed fractions (1.46 g, 0.003 mol) in 25 mL of 1,2-dimethoxyethane with 0.05 g (0.1 equiv) of anhydrous cesium carbonate for 2.75 h at 80 °C. The mixture was concentrated, taken up in 250 mL of ethyl acetate, washed with 100 mL of 10% citric acid and 100 mL of brine, dried (MgSO_4), and concentrated. Chromatography as above gave an additional 0.49 g (22.7%) of 14 and 0.92 g (42.6%) of the 3(*S*)-diastereomer.

3(R)-[[4-(Benzyl)phenyl]methyl]-5(S)-[1'(S)-[(*tert*-butyloxycarbonyl)amino]-2-[4-(benzyloxy)phenyl]ethyl]dihydrofuran-2(3*H*)-one (15). To a stirred solution of 4.5 g (0.0066 mol) of crude 12 in 60 mL of DME was added 0.066 mol of 1 M LiOH. After 12 h, the mixture was concentrated, acidified to pH 3 with 10% citric acid, and extracted into 3×100 mL of ethyl acetate. The combined extracts were dried (MgSO_4) and concentrated. The residue was dissolved in 100 mL of toluene, heated to reflux for 18 h, and concentrated. Chromatography on silica gel using 15% ethyl acetate/hexanes gave 1.6 g (41%) of 15 as a glass: ^1H NMR (CDCl_3) δ 1.35 (s, 9 H), 1.95 (m, 1 H), 2.2 (m, 1 H), 2.65–3.05 (m, 5 H), 3.85 (br m, 1 H), 4.2 (br m, 1 H), 4.7 (br m, 1 H), 5.0 (s, 2 H), 5.01 (s, 2 H), 7.05 (d, 2 H, J = 8 Hz), 7.08 (d, 2 H, J = 8 Hz), 7.25 (d, 2 H, J = 8 Hz), 7.28 (d, 2 H, J = 8 Hz), 7.45–7.6 (m, 10 H). Further elution gave 1.9 g (49%) of mixed fractions containing mostly 3(*S*)-isomer.

5(S)-[(*tert*-Butyloxycarbonyl)amino]-4(S)-[(*tert*-butyl-dimethylsilyl)oxy]-6-phenyl-2(R)-[[4-(benzyloxy)phenyl]methyl]hexanoic Acid (16). To a stirred solution of 11.25 g (0.0225 mol) of 13 in 350 mL of DME was added 142 mL of 1 N LiOH and 200 mL of water. After 3 h, the solution was concentrated to dryness, and the solids were suspended in 1 L of 5% citric acid. The white solid hydroxy acid was collected by filtration, washed with 4×100 mL of water, and dried at 60 °C in a vacuum oven overnight. To a stirred solution of the hydroxy acid (12 g, 0.0225 mol) in 150 mL of anhydrous DMF was added 57 g of imidazole (37 equiv) and 62 g (18 equiv) *tert*-butyldimethylchlorosilane. After 4.5 days, 170 mL of absolute MeOH was added. After stirring for 30 min, the mixture was diluted with 230 mL of 10% citric acid and extracted with 3×400 mL of ethyl acetate. Combined extracts were washed with 400 mL of water and 400 mL of brine and dried (MgSO_4). Concentration and drying under vacuum gave 13 g (91%) of 16 as a foam: ^1H

NMR (CDCl_3) δ 0.03 + 0.06 + 0.09 + 0.10 (4xs, 2:2:1:1, 6 H total), 0.9 (s, 9 H), 1.18 + 1.35 (2xs, 9 H total), 1.58 (br m, 1 H), 1.8–2.0 (br m, 1 H), 2.4–3.0 (m, 4 H), 3.6–3.8 (m, 3 H), 3.95 (br m, 1 H), 4.73 (br d, J = 8 Hz, 1 H), 5.0 (s, 2 H), 6.85 + 6.9 (1:2, 2xd, J = 8 Hz, 2 H), 7.0 + 7.1 (1:2, 2xd, J = 8 Hz, 2 H), 7.15–7.5 (m, 10 H).

5(S)-[(tert-Butyloxycarbonyl)amino]-4(S)-[(tert-butyl-dimethylsilyl)oxy]-6-[4-(benzyloxy)phenyl]-2(R)-(phenylmethyl)hexanoic Acid (17). By following the procedure described for the preparation of 16, from 1.2 g (0.0024 mol) of lactone 14 there was obtained 1.52 g (quant) of 17 as a white foam: ^1H NMR (CDCl_3) δ 0.03 + 0.05 + 0.08 + 0.10 (4xs, 2:2:1:1, 6 H total), 0.9 (s, 9 H), 1.18 + 1.35 (2xs, 9 H total), 1.58 (br m, 1 H), 1.8–2.0 (br m, 1 H), 2.45–2.8 (m, 3 H), 3.0–3.1 (m, 1 H), 3.6–3.8 (m, 3 H), 3.95 (br m, 1 H), 4.73 (br d, J = 8 Hz, 1 H), 5.0 (s, 2 H), 6.85–7.45 (m, 14 H).

5(S)-[(tert-Butyloxycarbonyl)amino]-4(S)-[(tert-butyl-dimethylsilyl)oxy]-6-[4-(benzyloxy)phenyl]-2(R)-[[4-(benzyloxy)phenyl]methyl]hexanoic Acid (18). By following the procedure described for the preparation of 16 from 0.65 g (0.0011 mol) of lactone 15 there was obtained 0.80 g (quant) of 18 as a white foam which was homogeneous by TLC (5% MeOH in CHCl_3).

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butylcarbonyl)amino]-4(S)-hydroxy-6-phenyl-2(R)-[[4-(benzyloxy)phenyl]methyl]hexanamide (19). To a stirred solution of 13 g (20.5 mmol) of 16 and 3.3 g (22 mmol) of 28 in 250 mL of anhydrous DMF was added 2.8 g (21 mmol) of 1-hydroxybenzotriazole hydrate, 6.2 g (32 mmol) of dimethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride and 22 mmol of *N*-methylmorpholine. After 17 h, the mixture was concentrated and the residue dissolved in 300 mL of ethyl acetate and 150 mL of 10% citric acid. The organic layer was washed with 100 mL of water and 150 mL of saturated NaHCO_3 , dried (MgSO_4), and concentrated. Chromatography of the residue in silica gel using 1:3 ethyl acetate/hexanes gave 10.32 g (66%) of the silyl ether as a glass. The silyl ether was dissolved in 0.130 mol of 1 M tetra-*n*-butylammonium fluoride in THF and aged for 20 h. After concentrating to one half of the volume, the mixture was diluted with 250 mL of 2% citric acid. The white solid product was collected by filtration, washed with 2 × 200 mL of water, and dried at 40 °C in a vacuum oven (5 mm) overnight. Recrystallization from ether/ethyl acetate gave 8.5 g (66% from 16) of 19 as a white solid: mp 190–2 °C. Anal. ($\text{C}_{40}\text{H}_{46}\text{N}_2\text{O}_6$) C, 73.82; H, 7.12; N, 4.30. Found: C, H, N.

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butylcarbonyl)amino]-4(S)-hydroxy-6-[4-(benzyloxy)phenyl]-2(R)-(phenylmethyl)hexanamide (20). By following the procedure described for the preparation of 19, from 0.350 g (0.55 mmol) of silyl acid 17 there was obtained 0.343 g (96%) of 20 as a white solid which was homogeneous by TLC (5% MeOH in CHCl_3).

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butylcarbonyl)amino]-4(S)-hydroxy-6-[4-(benzyloxy)phenyl]-2(R)-[[4-(benzyloxy)phenyl]methyl]hexanamide (21). By following the procedure described for the preparation of 19, from 0.248 g (0.346 mmol) of silyl acid 18 there was obtained 0.256 g (96%) of 21 as a white solid which was homogeneous by TLC (5% MeOH in CHCl_3).

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butylcarbonyl)amino]-4(S)-hydroxy-6-phenyl-2(R)-[(4-hydroxyphenyl)methyl]hexanamide (22). A solution of 12.2 g (0.99 mol) of 19 in 500 mL of MeOH and 500 mL of THF was stirred with 2 g of 10% Pd/C under a hydrogen-filled balloon for 4 days. The mixture was filtered through a pad of diatomaceous earth, washed with 100 mL of MeOH and 100 mL of THF, and concentrated to dryness. Recrystallization of the crude product by dissolving in 1.4 L of hot ethanol, adding 700 mL of water, and cooling gave 10.5 g (98%) of 22 as white solid: mp 218–9 °C. Anal. ($\text{C}_{33}\text{H}_{40}\text{N}_2\text{O}_6$) C, H, N.

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butylcarbonyl)amino]-4(S)-hydroxy-6-(4-hydroxyphenyl)-2(R)-(phenylmethyl)hexanamide (23). By following the procedure described for the preparation of 22, from 0.3 g (0.46 mmol) of 20 there was obtained 0.28 g (quant) of 23 as a white solid: mp 210–11 °C. Anal. ($\text{C}_{33}\text{H}_{40}\text{N}_2\text{O}_6$) C, H, N.

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butylcarbonyl)amino]-4(S)-hydroxy-6-(4-hydroxyphenyl)-2(R)-[(4-hydroxyphenyl)methyl]hexanamide (24). By following the procedure described for the preparation of 22, from 0.256 g (0.33 mmol) of 21 there was obtained 0.196 g (quant) of 24 as a white solid: mp 203–4 °C. Anal. ($\text{C}_{33}\text{H}_{40}\text{N}_2\text{O}_7$) C, H, N.

(±)-trans-1-Amino-2-hydroxyindan (25).²⁸ To 1 L of 12 N NH_4OH cooled to 0 °C was added 50 g (0.235 mol) of 2-bromo-1-indanol. After stirring for 30 min, the mixture was allowed to warm and stir for 24 h. The mixture was concentrated under reduced pressure (water aspirator) to remove excess ammonia and then allowed to stand open at room temperature overnight. The mixture was made basic (pH > 10) by addition of 20% KOH, cooled in an ice bath, and filtered. After the residue was dried in a vacuum oven at 60 °C overnight, there was obtained 24 g (69%) of 25 as a tan solid: mp 128–9 °C. ^1H NMR (CDCl_3) δ 2.0–2.75 (br s, 3 H), 2.72 (dd, J = 7.5, 15 Hz, 1 H), 3.2 (dd, J = 7.5, 15 Hz, 1 H), 4.05–4.2 (m, 2 H), 7.15–7.3 (4 H).

2-Phenyl-3a,8a-dihydro-8*H*-inden[1,2-*d*]oxazole (26). To a vigorously stirred suspension of 34 g (0.23 mol) of 25 in 500 mL of H_2O was added 7 mL of 20% NaOH followed by a solution of 40 mL (0.345 mol) of benzoyl chloride in 300 mL of toluene. The exothermic reaction was diluted with 300 mL of toluene and allowed to stir for 24 h. The product was collected by filtration, washed with 200 mL of H_2O and 100 mL of toluene, and dried in a vacuum oven at 40 °C overnight. The crude hydroxyamide (49.3 g) was suspended in 750 mL of CH_2Cl_2 and cooled in an ice bath. To it was added 72 mL of SOCl_2 . The mixture was allowed to warm and stir at room temperature for 16 h then concentrated to dryness. The tan solid was suspended in 600 mL of saturated NaHCO_3 and extracted into 2 × 800 mL of CHCl_3 . The combined extracts were dried over MgSO_4 , treated with 2 g of activated carbon (Darco 60), and filtered through diatomaceous earth. Concentration of the filtrate to dryness gave 38 g (70%) of 26 as a tan solid: mp 88–90 °C. ^1H NMR (CDCl_3) δ 3.35 (d, J = 15 Hz, 1 H), 3.5 (dd, J = 7, 15 Hz, 1 H), (t, J = 7 Hz, 1 H), 5.75 (d, J = 7 Hz), 7.2–7.5 (m, 6 H), 7.6 (m, 1 H), 7.95 (m, 2 H).

(±)-cis-1-Amino-2-hydroxyindan (28). A stirred mixture of 38 g (0.16 mol) of 26, 150 mL of concentrated H_2SO_4 , and 750 mL of H_2O was heated to reflux for 16 h and then cooled in an ice bath and filtered. The filtrate was adjusted to pH > 10 with 10 N NaOH, concentrated to 500 mL, and extracted with 4 × 500 mL of CHCl_3 . Combined extracts were dried (MgSO_4) and concentrated. After drying overnight in a vacuum oven at 40 °C there was obtained 21.3 g (88%) of 28 as a white solid: mp 132–3 °C. ^1H NMR (CDCl_3) δ 2.0–2.5 (br s, 3 H), 2.95 (dd, J = 3, 15 Hz, 1 H), 3.1 (dd, J = 7, 15 Hz, 1 H), 4.28 (d, J = 7 Hz, 1 H), 4.4 (m, 1 H), 7.2–7.35 (m, 4 H).

N-(L-Phenylalanyl)-1(S)-amino-2(R)-hydroxyindan (27). To a stirred mixture of 37.9 g (0.254 mol) of racemic 28, 37.8 g (0.28 mol) of 1-hydroxybenzotriazole hydrate, and 53.5 g (0.28 mol) of dimethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride in 800 mL of anhydrous DMF was added 39 mL (0.28 mol) of triethylamine. The mixture was allowed to stir for 16 h and then poured into 1 L of 10% citric acid and extracted into 2 × 500 mL of ethyl acetate. The combined extracts were washed with 700 mL of H_2O , 2 × 500 mL of 5% Na_2CO_3 , and dried (MgSO_4). Concentration and drying gave 98.2 g (97%) of a tan solid (BOC-27). To a stirred solution of the solid (BOC-27) in 800 mL of CHCl_3 was added 200 mL of trifluoroacetic acid. After 2.5 h, the solution was concentrated to dryness, the residue taken up in 800 mL of CHCl_3 and washed with 2 × 500 mL of 15% NH_4OH and 500 mL of brine, and dried (MgSO_4). Concentration and drying gave 63.3 g (84% from 28) of a tan solid (L-Phe-(±)-28). Low-pressure chromatography on silica gel, eluting with 8% MeOH in CHCl_3 , gave 30.3 g (40%) of the faster moving diastereomer 27.

Further elution with 15% MeOH in CHCl_3 gave 30 g (40%) of the 1(R),2(S)-diastereomer of 27.

(-)-1(S)-Amino-2(R)-hydroxyindan (28). A mixture of 30

(28) Pope, W. J.; Reid, J. Dihydroxydihydronamine and Its Resolution into Optically Active Compounds. *J. Chem. Soc., Chem. Commun.* 1911, 2071–80.

g (0.1 mol) of 27, 1 L of ethanol, and 265 mL of 20% NaOH was heated to reflux for 16 h. The mixture was concentrated to remove EtOH, diluted with 100 mL of H₂O and 100 mL of saturated NaCl, and extracted 3 × 600 mL of CHCl₃. Combined extracts were dried (MgSO₄) and concentrated. There was obtained 14 g (93%) of 28 as a white solid: mp 114–5 °C, [α]_D²⁵ = −61.2° (c = 1.0, MeOH), ¹H NMR (CDCl₃) δ 2.0–2.5 (br s, 3 H), 2.95 (dd, J = 3, 15 Hz, 1 H), 3.1 (dd, J = 7, 15 Hz, 1 H), 4.28 (d, J = 7 Hz, 1 H), 4.4 (m, 1 H), 7.2–7.35 (m, 4 H).

Recrystallization from ether/hexanes gave 28 as colorless rods: mp 117–177.5 °C; [α]_D²⁵ = −62° (c = 1.0, MeOH). Anal. (C₂₀H₂₁NO) C, H, N.

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butyloxy-carbonyl)amino]-4(S)-hydroxy-6-phenyl-2(R)-[[4-[2-(4-morpholinyl)ethoxy]phenyl]methyl]hexanamide (29). A vigorously stirred mixture of 7.0 g (12 mmol) of the phenol 22, 10 g (30 mmol) of anhydrous cesium carbonate (Aldrich, 99.9%), 33 g (240 mmol) of *N*-(2-chloroethyl)morpholine, free base (prepared by dissolving 50 g of the hydrochloride in 50 mL of water and 50 mL of saturated NaHCO₃, extracting with 500 mL of hexanes, drying the extract over MgSO₄, and concentrating), in 1 L of anhydrous dioxane was heated to 80 ± 2 °C for 36 h under a nitrogen atmosphere. At this time the reaction was complete by TLC. The mixture was allowed to cool to room temperature, filtered through a medium porosity fritted funnel, rinsed in with 50 mL of chloroform, and concentrated to dryness. The solids were triturated with 500 mL of 1:10 ethyl acetate/ether, filtered, and recrystallized from 600 mL of ethyl acetate by heating to dissolution and allowing to cool overnight. After the solids were dried under vacuum (0.1 mm) at 100 °C for 1 h, there was obtained 8.0 g (98.9%) of 29 as colorless needles, 99% pure by HPLC: mp 197–8 °C; UV (MeOH) λ_{max} = 271 (ε = 2167 M^{−1} cm^{−1}); [α]_D²⁵ = +15.9° (c = 1.0, MeOH); ¹H NMR (CDCl₃) δ 1.4 (s, 9 H), 1.85 (br m, 2 H), 2.32 (s, 6 H), 2.6–3.1 (m, 10 H), 3.6–3.72 (br m, 1 H), 3.8 (br m, 1 H), 3.9 (br m, 1 H), 4.02 (t, J = 7 Hz, 2 H), 4.22 (t, J = 6 Hz, 1 H), 4.9 (br d, 10 Hz, 1 H), 5.25 (dd, J = 6, 10 Hz, 1 H), 5.95 (br d, J = 10 Hz, 1 H), 6.85 (d, J = 8 Hz, 2 H), 7.05 (br d, J = 8 Hz, 2 H), 7.15–7.35 (m, 9 H). Anal. (C₃₉H₅₁N₃O₇) C, H, N.

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butyloxy-carbonyl)amino]-4(S)-hydroxy-6-[4-[2-(4-morpholinyl)ethoxy]phenyl]-2(R)-(phenylmethyl)hexanamide (30). A vigorously stirred mixture of 100 mg (0.17 mmol) of 23, 0.2 g (0.62 mmol) of anhydrous cesium carbonate, 0.2 g (1.45 mmol) of *N*-(2-chloroethyl)morpholine, free base, and 10 mL of anhydrous dioxane was heated to 80 ± 2 °C for 3 h under a nitrogen atmosphere. The disappearance of starting material was monitored by TLC on silica gel, eluting with 3:15:200 NH₄OH/MeOH/CHCl₃. The resulting mixture was cooled, filtered, and concentrated to dryness. Preparative TLC on two 20 × 20-cm plates (2 mm) using 1:3:6 MeOH/EtOAc/CHCl₃ for development gave 105 mg (91%) of a white solid after trituration with ether and drying: mp 160–1 °C; ¹H NMR (CDCl₃) δ 1.4 (s, 9 H), 1.85 (br m, 2 H), 2.6 (br m, 4 H), 2.6–3.1 (m, 9 H), 3.6–3.9 (m, 4 H), 3.75 (t, J = 6 Hz, 4 H), 4.1 (t, J = 6 Hz, 2 H), 4.2 (br m, 1 H), 4.9 (d, J = 8 Hz, 1 H), 5.25 (q, J = 6, 8 Hz, 1 H), 5.92 (d, J = 8 Hz, 1 H), 6.92 (d, J = 8 Hz, 2 H), 7.08 (br m, 1 H), 7.15–7.35 (m, 10 H). Anal. (C₃₉H₅₁N₃O₇) C, H, N.

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butyloxy-carbonyl)amino]-4(S)-hydroxy-6-[4-[2-(4-morpholinyl)ethoxy]phenyl]-2(R)-[[4-[2-(4-morpholinyl)ethoxy]phenyl]methyl]hexanamide (31). A vigorously stirred mixture of 250 mg (0.42 mmol) of 24, 0.7 g (2.14 mmol) of anhydrous cesium carbonate, 1.5 g (11 mmol) of *N*-(2-chloroethyl)morpholine, free base, and 50 mL of anhydrous dioxane was heated to 80 ± 2 °C for 4 h under a nitrogen atmosphere. The disappearance of starting material was monitored by TLC on silica gel, with 3:15:200 NH₄OH/MeOH/CHCl₃ as eluent. The resulting mixture was cooled, filtered, and concentrated to dryness. Trituration with ether and drying gave 340 mg (99%) of 31 as hemihydrate: mp 164–6 °C; ¹H NMR (CDCl₃) δ 1.4 (s, 9 H), 1.85 (br m, 2 H), 2.6 (br m, 4 H), 2.6–3.1 (m, 9 H), 3.6–3.9 (m, 4 H), 3.75 (t, J = 6 Hz, 4 H), 4.1 (t, J = 6 Hz, 2 H), 4.25 (br m, 1 H), 4.9 (d, J = 8 Hz, 1 H), 5.25 (q, J = 6, 8 Hz, 1 H), 5.92 (d, 8 Hz, 1 H), 6.92 (t, J = 8 Hz, 4 H), 7.0–7.3 (m, 8 H). Anal. (C₄₅H₆₂N₄O₉·0.5H₂O) C, H, N.

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butyloxy-carbonyl)amino]-4(S)-hydroxy-6-[4-[3-(4-morpholinyl)-

propyl]oxy]phenyl]-2(R)-(phenylmethyl)hexanamide (32). A vigorously stirred mixture of 85 mg (0.13 mmol) of 22, 212 mg (1.3 mmol) of *N*-(3-chloropropyl)morpholine,²⁹ and 108 mg (0.33 mmol) of anhydrous cesium carbonate in 10 mL of anhydrous dioxane was heated to 88 ± 2 °C for 2 h under a nitrogen atmosphere. The disappearance of starting material was monitored by TLC on silica gel, with 3:15:200 NH₄OH/MeOH/CHCl₃ as eluent. The resulting mixture was cooled, filtered, and concentrated to dryness. Preparative TLC on four 20 × 20-cm plates (2 mm) using 5% MeOH/CHCl₃ for development gave 57 mg (59%) of a white solid after trituration with ether and drying.

Maleate salt of 32: mp 101–3 °C. Anal. (C₃₉H₅₁N₃O₇·C₄H₄O₄·0.5CHCl₃) C, H, N.

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butyloxy-carbonyl)amino]-4(S)-hydroxy-6-phenyl-2(R)-[[4-[2-(dimethylamino)ethoxy]phenyl]methyl]hexanamide (33). A vigorously stirred mixture of 200 mg (0.36 mmol) of 22 and 400 mg (1.22 mmol) of anhydrous cesium carbonate in 28 mL of anhydrous dioxane was heated to 40 °C for 3 h under a nitrogen atmosphere. To this suspension was added 2.8 g (26 mmol) of 2-(dimethylamino)ethyl chloride and the internal temperature raised to 80 ± 2 °C for 24 h. The disappearance of starting material was monitored by TLC on silica gel, with 3:15:200 NH₄OH/MeOH/CHCl₃ as eluent. The resulting mixture was cooled, filtered, and concentrated to dryness. Preparative TLC on four 20 × 20-cm plates (2 mm) using 1:4:45 NH₄OH/MeOH/CHCl₃ for development gave 128 mg (56%) of a white solid after trituration with ether and drying: mp 195–7 °C; ¹H NMR (CDCl₃) δ 1.4 (s, 9 H), 1.85 (br m, 2 H), 2.32 (s, 6 H), 2.6–3.1 (m, 10 H), 3.6–3.72 (br m, 1 H), 3.8 (br m, 1 H), 3.9 (br m, 1 H), 4.02 (t, J = 7 Hz, 2 H), 4.22 (t, J = 6 Hz, 1 H), 4.9 (br d, 10 Hz, 1 H), 5.25 (dd, J = 6, 10 Hz, 1 H), 5.95 (br d, J = 10 Hz, 1 H), 6.85 (d, J = 8 Hz, 2 H), 7.05 (br d, J = 8 Hz, 2 H), 7.15–7.35 (m, 9 H). Anal. (C₃₇H₄₉N₃O₆) C, H, N.

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butyloxy-carbonyl)amino]-4(S)-hydroxy-6-phenyl-2(R)-[[4-[2-(1-piperidinyl)ethoxy]phenyl]methyl]hexanamide (34). A vigorously stirred mixture of 56 mg (0.10 mmol) of 22, 148 mg (1.0 mmol) of *N*-(2-chloroethyl)piperidine, and 82 mg (0.25 mmol) of anhydrous cesium carbonate in 8 mL of anhydrous dioxane was heated to 85 ± 2 °C for 2 h under a nitrogen atmosphere. The disappearance of starting material was monitored by TLC on silica gel, eluting with 3:15:200 NH₄OH/MeOH/CHCl₃. The resulting mixture was cooled, filtered, and concentrated to dryness. Preparative TLC on four 20 × 20-cm plates (2 mm) using 2:8:90 NH₄OH/MeOH/CHCl₃ for development gave 56 mg (72%) of a white solid after trituration with ether and drying: mp 190.5–191.5 °C. Anal. (C₄₀H₅₃N₃O₆·0.5H₂O) C, H, N.

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butyloxy-carbonyl)amino]-4(S)-hydroxy-6-phenyl-2(R)-[[4-[2-(1-pyrrolidinyl)ethoxy]phenyl]methyl]hexanamide (35). A vigorously stirred mixture of 56 mg (0.10 mmol) of 22, 134 mg (1.0 mmol) of *N*-(2-chloroethyl)pyrrolidine, and 82 mg (0.25 mmol) of anhydrous cesium carbonate in 8 mL of anhydrous dioxane was heated to 85 ± 2 °C for 1 h under a nitrogen atmosphere. The disappearance of starting material was monitored by TLC on silica gel, with 3:15:200 NH₄OH/MeOH/CHCl₃ as eluent. The resulting mixture was cooled, filtered, and concentrated to dryness. Preparative TLC on four 20 × 20-cm plates (2 mm) using 2:8:90 NH₄OH/MeOH/CHCl₃ for development gave 55 mg (72%) of a white solid after trituration with ether and drying.

Maleate salt of 35: mp 102–4 °C. Anal. (C₃₉H₅₁N₃O₆·C₄H₄O₄·2H₂O) C, H, N.

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butyloxy-carbonyl)amino]-4(S)-hydroxy-6-phenyl-2(R)-[[4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl]methyl]hexanamide (36). A vigorously stirred mixture of 65 mg (0.12 mmol) of 22, 195 mg (1.2 mmol) of *N*-(2-chloroethyl)-4-methylpiperazine,³⁰ and 98 mg (0.30 mmol) of anhydrous cesium carbonate in 8 mL of

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anhydrous dioxane was heated to 85 ± 2 °C for 2 h under a nitrogen atmosphere. The resulting mixture was cooled, filtered, and concentrated to dryness. Preparative TLC on four 20 × 20-cm plates (2 mm) using 2:8:90 NH₄OH/MeOH/CHCl₃ for development gave 39 mg (48%) of a white solid after trituration with ether and drying: mp 181–182.5 °C. Anal. (C₄₀H₅₄N₄O₆·0.7H₂O) C, H, N.

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butyloxy-carbonyl)amino]-4(S)-hydroxy-6-phenyl-2(R)-[[4-[2-[N,N-bis(2-methoxyethyl)amino]ethoxy]phenyl]methyl]hexanamide (37). A vigorously stirred mixture of 60 mg (0.11 mmol) of 22, 200 mg (1.1 mmol) of 2-[N,N-bis(2-methoxyethyl)amino]ethyl chloride,³¹ and 80 mg (0.25 mmol) of anhydrous cesium carbonate in 10 mL of anhydrous dioxane was heated to 80 ± 2 °C for 2 h under a nitrogen atmosphere. The disappearance of starting material was monitored by TLC on silica gel, with 3:15:200 NH₄OH/MeOH/CHCl₃ as eluant. The resulting mixture was cooled, filtered, and concentrated to dryness. Trituration with 25 mL of 1:9 ethyl acetate/ether gave 80 mg of a white solid: mp 176–7 °C; ¹H NMR (CDCl₃) δ 1.4 (s, 9 H), 1.85 (br m, 2 H), 2.65–3.1 (m, 14 H), 3.35 (s, 6 H), 3.5 (br t, J = 8 Hz, 4 H), 3.6–3.72 (br m, 1 H), 3.8–3.9 (br m, 2 H), 4.2 (br t, J = 7 Hz, 2 H), 4.25 (br m, 1 H), 4.9 (br d, 10 Hz, 1 H), 5.25 (dd, J = 6, 10 Hz, 1 H), 5.9 (br d, J = 10 Hz, 1 H), 6.8 (d, J = 8 Hz, 2 H), 7.05 (br d, J = 8 Hz, 2 H), 7.15–7.35 (m, 9 H). Anal. (C₄₁H₅₇N₃O₈·0.5H₂O) C, H, N.

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butyloxy-carbonyl)amino]-4(S)-hydroxy-6-phenyl-2(R)-[[4-[2-(thiamorpholin-4-yl)ethoxy]phenyl]methyl]hexanamide (38). A vigorously stirred mixture of 350 mg (0.63 mmol) of 22, 1.0 g (6.3 mmol) of (2-chloroethyl)thiamorpholine,³² and 513 mg (1.58 mmol) of anhydrous cesium carbonate in 90 mL of anhydrous dioxane was heated to 80 ± 2 °C for 1 h under a nitrogen atmosphere. The disappearance of starting material was monitored by TLC on silica gel, with 1:10 MeOH/CHCl₃ as eluant. The resulting mixture was cooled, filtered, and concentrated to dryness. The residue was triturated with 10% ether in ethyl acetate and the product collected by filtration. Preparative TLC on four 20 × 20-cm plates (2 mm) using 1:10 MeOH/CHCl₃ for development gave 330 mg (75%) of a white solid after trituration with ether and drying: mp 195.5–7 °C; ¹H NMR (CDCl₃) δ 1.4 (s, 9 H), 1.8–1.9 (br m, 2 H), 2.65–2.75 (m, 4 H), 2.76–2.9 (br m, 8 H), 2.95 (m, 2 H), 3.0 (m, 1 H), 3.7 (t, J = 7 Hz, 2 H), 3.8 (m, 1 H), 3.85 (br m, 2 H), 4.1 (m, 2 H), 4.3 (br s, 1 H), 4.9 (d, J = 7 Hz, 1 H), 5.3 (m, 1 H), 5.9 (d, J = 7 Hz, 1 H), 6.85 (d, J = 8 Hz, 2 H), 7.1 (br d, J = 8 Hz, 2 H), 7.15–7.35 (m, 9 H). Anal. (C₃₉H₅₁N₃O₈S·0.25H₂O) C, H, N.

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butyloxy-carbonyl)amino]-4(S)-hydroxy-6-phenyl-2(R)-[[4-[2-(1-oxothiamorpholin-4-yl)ethoxy]phenyl]methyl]hexanamide (39). A mixture of 100 mg (0.15 mmol) of 38, 35 mg (0.17 mmol) of NaIO₄, and 2.5 mL of H₂O in 10 mL of MeOH was stirred for 8 h at room temperature and then concentrated to dryness. The residue was dissolved in 50 mL of chloroform and washed with 50 mL water, dried (MgSO₄), and concentrated. Preparative TLC on four 20 × 20-cm plates (2 mm) using 1:10 MeOH/CHCl₃ for development gave 85 mg (80%) of a white solid after trituration with ether and drying: mp 164–5.5 °C; ¹H NMR (CDCl₃) δ 1.4 (s, 9 H), 1.85 (br m, 2 H), 2.65–3.0 (m, 15 H), 3.0 (m, 1 H), 3.15 (br s, 1 H), 3.7 (t, J = 7 Hz, 1 H), 3.8 (m, 1 H), 4.18 (br m, 2 H), 4.3 (br m, 1 H), 4.94 (d, J = 7 Hz, 1 H), 5.3 (m, 1 H), 6.25 (d, J = 8 Hz, 1 H), 6.85 (d, J = 8 Hz, 2 H), 7.1 (br d, J = 8 Hz, 2 H), 7.15–7.35 (m, 9 H). Anal. (C₃₉H₅₁N₃O₈S·1.25H₂O) C, H, N.

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butyloxy-carbonyl)amino]-4(S)-hydroxy-6-phenyl-2(R)-[[4-[2-(1,1-dioxythiamorpholin-4-yl)ethoxy]phenyl]methyl]hexanamide (40). To a stirred solution of 90 mg (0.13 mmol) of the sulfoxide 39 in 35 mL of MeOH was added 25 mg (0.16 mmol) of KMnO₄. After 15 min at room temperature, the reaction was quenched with 10 mL of saturated aqueous sodium bisulfite. After 10 min,

when the color had discharged, the solution was extracted with 2 × 50 mL of CHCl₃. The combined organic layers were washed with saturated brine, dried (MgSO₄), and concentrated. Preparative TLC on four 20 × 20-cm plates (2 mm) using 1:20 MeOH/CHCl₃ for development gave 50 mg of a white solid. Recrystallization from 7 mL of hot ethyl acetate gave 33 mg (35%) of 40 after drying: mp 157–8.5 °C; ¹H NMR (CDCl₃) δ 1.4 (s, 9 H), 1.85 (br m, 2 H), 2.65–3.0 (m, 16 H), 3.15 (br s, 2 H), 3.7 (t, J = 7 Hz, 1 H), 3.8 (m, 2 H), 4.05 (br m, 2 H), 4.3 (br m, 1 H), 4.9 (d, J = 7 Hz, 1 H), 5.3 (m, 1 H), 5.95 (d, J = 7 Hz, 1 H), 6.85 (d, J = 7 Hz, 2 H), 7.1 (br d, J = 8 Hz, 3 H), 7.15–7.35 (m, 8 H). Anal. (C₃₉H₅₁N₃O₇S·0.80H₂O) C, H, N.

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butyloxy-carbonyl)amino]-4(S)-hydroxy-6-phenyl-2(R)-[[4-[2-(4-oxo-4-morpholinyl)ethoxy]phenyl]methyl]hexanamide (41). A mixture of 60 mg (0.09 mmol) of 29 and 1 mL of 30% H₂O₂ in 5 mL of MeOH was heated to reflux overnight. The mixture was concentrated to dryness, triturated with 25 mL of ethyl acetate, and filtered. Drying at 50 °C for 1 h under vacuum gave 62 mg (91%) of a white solid: mp 142–4 °C; ¹H NMR (CDCl₃) δ 1.4 (s, 9 H), 1.75–2.0 (br m, 2 H), 2.6–3.05 (br m, 7 H), 3.35 (s, 6 H), 3.15–3.4 (br m, 3 H), 3.5 (br m, 2 H), 3.6–3.85 (br m, 5 H), 4.1–4.55 (br m, 8 H), 5.2 (br m, 2 H), 6.75 (d, J = 8 Hz, 2 H), 6.90 (br d, J = 8 Hz, 1 H), 7.05–7.35 (m, 10 H). Anal. (C₃₉H₅₁N₃O₈·2H₂O) C, H, N.

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butyloxy-carbonyl)amino]-4(S)-hydroxy-6-phenyl-2(R)-[[4-[ethoxy-carbonyl]methyl]phenyl]methyl]hexanamide (42). A vigorously stirred mixture of 1.0 g (1.72 mmol) of 22 and 2.0 g (6.13 mmol) of anhydrous cesium carbonate in 140 mL of anhydrous dioxane was aged for 12 h at room temperature under a nitrogen atmosphere. To this suspension was added 9.0 mL (81 mmol) of ethyl bromoacetate and the internal temperature raised to 80 ± 2 °C for 24 h. The disappearance of starting material was monitored by TLC on silica gel, with 8% MeOH in CHCl₃ as eluant. The resulting mixture was cooled, diluted with 100 mL of CHCl₃, filtered, and concentrated to dryness. After drying under vacuum there was obtained 1.1 g (96%) of 42 as a white solid: mp 197–9 °C; ¹H NMR (CDCl₃) δ 1.3 (t, J = 8 Hz, 3 H), 1.4 (s, 9 H), 1.85 (br m, 2 H), 2.65–3.15 (m, 7 H), 3.6–3.9 (m, 3 H), 4.05–4.2 (br m, 2 H), 4.25 (q, J = 8 Hz, 2 H), 4.6 (s, 2 H), 4.95 (d, 8 Hz, 1 H), 5.2 (q, J = 6.8 Hz, 1 H), 6.05 (d, 8 Hz, 1 H), 6.8 (d, J = 8 Hz, 2 H), 7.08 (d, J = 8 Hz, 3 H), 7.1–7.35 (m, 8 H).

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butyloxy-carbonyl)amino]-4(S)-hydroxy-6-phenyl-2(R)-[[4-(carboxymethyl)phenyl]methyl]hexanamide (43). A solution of 140 mg (0.22 mmol) of ester 42 and 50 mg (2.2 mmol) of LiOH in 10 mL of MeOH and 1 mL of H₂O was stirred at room temperature for 2 h. After adjusting the pH to 4 with 85% phosphoric acid, the mixture was concentrated to dryness and the residue triturated with warm THF for 15 min. The suspension was filtered and the filtrate concentrated to dryness. After drying there was obtained 50 mg (34%) of a white solid which was homogeneous by HPLC at $\lambda = 254$ or 210 nM: mp >250 °C. Anal. (C₃₅H₄₄N₂O₇·0.5LiH₂PO₄) C, H, N.

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butyloxy-carbonyl)amino]-4(S)-hydroxy-6-phenyl-2(R)-[[4-(2-hydroxyethoxy)phenyl]methyl]hexanamide (44). To a stirred solution of 1.1 g (1.7 mmol) of the ester 42 in 200 mL of anhydrous 1,2-dimethoxyethane was added 5 mL of 2 M lithium borohydride in THF. After stirring at room temperature for 2 h, the mixture was cooled in an ice bath, quenched by dropwise addition of 50 mL of 10% citric acid, and extracted with 200 mL of CHCl₃. The organic layer was washed with 100 mL of saturated NaHCO₃, dried (MgSO₄), and concentrated. After drying under vacuum there was obtained 1.05 g (quant) of a white solid: ¹H NMR (CDCl₃) δ 1.4 (s, 9 H), 1.85 (br m, 2 H), 2.6–3.2 (m, 8 H), 3.5–4.0 (m, 7 H), 4.05 (t, J = 7 Hz, 2 H), 4.25 (br m, 1 H), 4.95 (br d, 8 Hz, 1 H), 5.25 (q, J = 6, 8 Hz, 1 H), 6.0 (br d, 8 Hz, 1 H), 6.8 (d, J = 8 Hz, 2 H), 7.05 (br d, J = 8 Hz, 3 H), 7.1–7.35 (m, 8 H). Anal. (C₃₄H₄₄N₂O₇·0.25H₂O) C, H, N.

4-(2-Bromoacetyl)morpholine. To an ice-cooled solution of 3 g (34 mmol) of morpholine in 25 mL of anhydrous ether was added 1 mL (11 mmol) of 2-bromoacetyl bromide dropwise. After 10 min, the mixture was filtered and the solids were washed with 25 mL of ether. The filtrate was concentrated and purified by

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chromatography on silica gel, with 8% MeOH in CHCl_3 as eluant. The product, 1.6 g (60%) was obtained as a colorless oil: ^1H NMR (CDCl_3) δ 3.5–3.8 (m, 8 H), 4.12 (s, 2 H).

N-(2(R)-Hydroxy-1(S)-indanyl)-5(S)-[(tert-butyloxycarbonyl)amino]-4(S)-hydroxy-6-phenyl-2(R)-[[4-[2-oxo-2-(4-morpholinyl)ethoxy]phenyl]methyl]hexanamide (45). A vigorously stirred mixture of 100 mg (0.18 mmol) of 22, 375 mg (1.8 mmol) of *N*-(2-chloroethyl)-4-methylpiperazine, and 147 g (0.45 mmol) of anhydrous cesium carbonate in 20 mL of anhydrous dioxane was heated to $90 \pm 5^\circ\text{C}$ for 6 h under a nitrogen atmosphere. The resulting mixture was cooled, filtered, and concentrated to dryness. Preparative TLC on four 20×20 -cm plates (2 mm) using 8% MeOH/ CHCl_3 for development gave 68 mg (55%) of a white solid after trituration with ether and drying: mp 184–5 °C. Anal. ($\text{C}_{39}\text{H}_{49}\text{N}_3\text{O}_8\cdot0.5\text{H}_2\text{O}$) C, H, N.

Biology. Inhibition of HIV-1 Protease. The IC_{50} and K_i values for the compounds were determined using purified HIV-1 protease.³³ Inhibition of the cleavage of the peptide H-Val-Ser-Gln-Asn-(L- β -naphthalalanine)-Pro-Ile-Val-OH was assessed at 30 °C, pH = 5.5 with [Enz] = 30 pM for 1 h, using HPLC with UV detection for quantification of the products. For the IC_{50} data a substrate concentration of 0.4 mg/mL was used, and data was fit to a four parameter sigmoidal equation. K_i data were determined from double reciprocal plots of rate data as a function of substrate and inhibitor concentrations. It has been reported that the dissociation of subunits from the active, dimeric form of the enzyme to inactive monomers has a K_d of 50 nM at pH 7.0³⁴ and a K_d of 3.6 nM at pH 5.0,³⁵ using kinetic methods. Steady-state kinetic treatment of an obligatory active dimer predicts that both specific activity and K_i should be a function of enzyme concentration and K_d . In our assay system, we find constant specific activity for the HIV-1 protease from 0.03 nM to 50 nM enzyme, and no dependence of IC_{50} upon enzyme concentration except when it exceeds the K_i . We conclude that the K_d for monomerization in our assay system is less than 0.03 nM and does not have an important influence upon the IC_{50} values reported here. The solvent conditions used in previous studies, 1 M NaCl³⁵ or pH 7.0,³⁴ may be responsible for the higher K_d values found by those workers.

Inhibition of HIV-1 Infection in Cell Culture. The compounds were initially 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 or SIV 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 5% CO_2 atmosphere. H9 and MT-4 human T-lymphoid cells are described by Popovic et al.³⁶ and Miyoshi et al.,³⁷ respectively. Primary peripheral blood

lymphocytes and primary monocytes/macrophages were obtained from fresh human plasmapheresis 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 phytohemagglutinin (5 mg/mL, Sigma) prior to virus infection and were maintained in medium containing IL-2 (1000 mg/mL, DuPont). The HIV-1 variants are described by reference as follows: IIIb,³⁸ RF,³⁹ MN,⁴⁰ and WMJ-2.⁴¹ RUTZ is an HIV-1 isolate from Zaire (R. C. Gallo, unpublished). The SF162 isolate,⁴² a monocyte/macrophage-tropic variant of HIV-1, was kindly provided by Jay Levy. SIV_{mac}^{251⁴³ and SIV_{mne}⁴⁴ were obtained from the AIDS Research and Reference Reagent Program of the National Institutes of Health.}

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 that 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 streptavidin-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)⁴⁵ and energy minimized using the Merck molecular force field, OPTIMOL,⁴⁶ which is a variant of the MM2 program.⁴⁷ Due to the low pH optimum of the HIV-1 protease,^{1d,48} all titratable

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residues were charged in the calculations with the exception of Tyr₅₉ and one of the pair of catalytic aspartic acids, Asp₂₅.⁴⁹ 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.⁵⁰ All molecular surfaces were generated using a neutral 0.95-Å radius probe with contouring performed at an interaction energy of 0 kcal/mol.

Comparison of the modeled and X-ray structures of 29 was accomplished by aligning the native and inhibited forms of the enzyme using the atoms of the conserved Asp-Thr-Gly motif, *not* by aligning the conformations of 29.

Crystallization and Data Collection. HIV-1 protease from the NY5 isolate⁵¹ (Pro 1 to Phe 99) was expressed as described previously.⁵² The enzyme was purified using an affinity column followed by a hydrophobic interaction column.³⁸ HIV-1 protease at 6 mg/mL was reacted with a 2-fold molar excess of L-689,502 dissolved in dimethyl sulfoxide. The reaction was carried out at 21 °C in a buffer containing 10 mM sodium 2-morpholinoethanesulfonate/2-morpholinoethanesulfonic acid, pH 5.0, 1 mM Na₂EDTA, 1 mM dithiothreitol, 3 mM sodium azide, and a final dimethyl sulfoxide concentration of less than 5% (v/v). Crystals with the external morphology of orthorhombic plates were grown by precipitation with sodium chloride as described previously.¹⁴ A crystal measuring 0.30 × 0.15 × 0.05 mm was mounted for data collection in a sealed glass capillary along with a paper wick wetted with mother liquor. The diffraction pattern displayed the symmetry of space group $P2_12_12$, with unit cell constants of $a = 59.11$

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