Articles

A Series of Potent HIV-1 Protease Inhibitors Containing a Hydroxyethyl Secondary Amine Transition State Isostere: Synthesis, Enzyme Inhibition, and Antiviral Activity

Thomas J. Tucker,*,† William C. Lumma, Jr.,† Linda S. Payne,† Jenny M. Wai,‖ S. Jane de Solms,† Elizabeth A. Giuliani,† Paul L. Darke,‡ Jill C. Heimbach,‡ Joan A. Zugay,‡ William A. Schleif,§ Julio C. Quintero,§ Emilio A. Emini,§ Joel R. Huff,† and Paul S. Anderson†

Merck Research Laboratories, West Point, Pennsylvania 19486. *Received February 19, 1992*

A series of HIV-1 protease inhibitors containing a novel hydroxyethyl secondary amine transition state isostere has been synthesized. The compounds exhibit a strong preference for the (*R*) stereochemistry at the transition state hydroxyl group. Molecular modeling studies with the prototype compound 11 have provided important insights into the structural requiremens for good inhibitor–active site binding interaction. N-Terminal extension of 11 into the P_2–P_3 region led to the discovery of 19, the most potent enzyme inhibitor in the series (IC_{50} = 5.4 nM). 19 was shown to have potent antiviral activity in cultured MT-4 human T-lymphoid cells. Comparison of analogs of 19 with analogs of 1 (Ro31-8959) demonstrates that considerably different structure–activity relationships exist between these two subclasses of hydroxyethylamine HIV-protease inhibitors.

Introduction

The rapid and relentless spread of the AIDS epidemic and its causative agent, the human immunodeficiency virus type 1 (HIV-1), has created a critical need for effective anti-HIV-1 therapies. The HIV-1-encoded protease responsible for the processing of the *gag* and *pol* gene products has been shown to play a key role in the viral replication process. HIV-1 protease (HIV-PR) is a member of the aspartic acid protease family and exists as a symmetrical dimer.[1] Site-directed mutagenesis designed to inactivate the protease has resulted in the production of noninfective virions.[2] For these reasons, HIV-PR represents an excellent target for potential therapeutic intervention.

Very rapidly, many active inhibitors of HIV-PR have been discovered by applying knowledge obtained in the study of renin[3] and other aspartyl proteases. Potent inhibitors containing the hydroxyethylene isostere of the scissile substrate peptide bond have been described.[4] A variety of other transition state isosteres have also been developed, including α-difluoroketone,[5] phosphinate,[6] reduced amide,[7] and hydroxyethylamine.[8] We were particularly interested in the use of a hydroxyethylamine (HEA) transition state isostere in the design of novel HIV-PR inhibitors. This transition state isostere appeared to be an excellent mimic of the natural Tyr-Pro substrate cleavage site. This cleavage site, specific for retroviral proteases,[9] represents a logical foundation for the design of inhibitors highly selective for HIV-PR.

Several potent HEA transition state containing HIV-PR inhibitors have recently been described (Figure 1, 1 and 2).[8,10] In each of these compounds, the transition state amino group is contained in a five- or six-membered ring in an effort to most accurately mimic the natural cleavage site. No potent inhibitors of HIV-PR have been described in which a noncyclic, secondary amine is used in the transition state isostere.[11] Our approach was based on the use of this novel transition state isostere in a series of

† Department of Medicinal Chemistry.
‡ Department of Biological Chemistry.
§ Department of Virus and Cell Biology.
‖ Department of Molecular Systems.

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(3) Greenlee, W. Renin Inhibitors. *Med. Res. Rev.* 1990, *10*, 173–236.

(4) (a) Richards, A. D.; Roberts, R. F.; Dunn, B. M.; Graves, M. C.; Kay, J. Effective Blocking of HIV-1 Proteinase Activity by Characteristic Inhibitors of Aspartic Proteinases. *FEBS Lett.* 1989, *247*, 113–117. (b) McQuade, T. J.; Tomasselli, A. G.; Liu, L.; Karacostas, V.; Moss, B. A Synthetic HIV-1 Protease Inhibitor with Antiviral Activity Arrests HIV-Like Particle Maturation. *Science* 1990, *247*, 454–456. (c) Ashorn, P.; McQuade, T. J.; Theisrivangs, S.; Tomasselli, A. G.; Tarpley, W. G.; Moss, B. An Inhibitor of the Protease Blocks Maturation of Human and Simian Immunodeficiency Viruses and Spread of Infection. *Proc. Natl. Acad. Sci. U.S.A.* 1990, *87*, 7472–7476. (d) Sigal, I. S.; Huff, J. R.; Darke, P. L.; Vacca, J. P.; Young, S. D.; deSolms, S. J.; Thompson, W. J.; Lyle, T. A.; Graham, S. L.; Ghosh, A. K. European Patent Appl. 0337714, 1989. (e) Vacca, J. P.; Guare, J. P.; deSolms, S. J.; Sanders, W. M.; Giuliani, E. A.; Young, S. D.; Darke, P. L.; Zugay, J.; Sigal, I. S.; Schleif, W. A.; Quintero, J. C.; Emini, E. A.; Anderson, P. S.; Huff, J. R. L-687,908, A Potent Hydroxyethylene-Containing HIV Protease Inhibitor. *J. Med. Chem.* 1991, *34*, 1225–1228. (f) Lyle, T. A.; Wiscount, C. M.; Guare, J. P.; Thompson, W. J.; Anderson, P. S.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Quintero, J. A.; Dixon, R. A. F.; Sigal, I. S.; Huff, J. R. Benzocycloalkyl Amines as Novel C-Termini for HIV Protease Inhibitors. *J. Med. Chem.* 1991, *34*, 1228–1230.

(5) Dreyer, G. B.; Metcalf, B. W.; Tomaszek, T. A., Jr.; Carr, T. V. W.; Chandler, A. C.; III; Hyland, L.; Fakhoury, S. A.; Mogaard, V. W.; Moore, M. L.; Strickler, J. E.; DeBouck, C.; Meek, T. D. Inhibition of HIV-1 Protease in Vitro: Rational Design of Substrate Analog Inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* 1989, *86*, 9752–9756.

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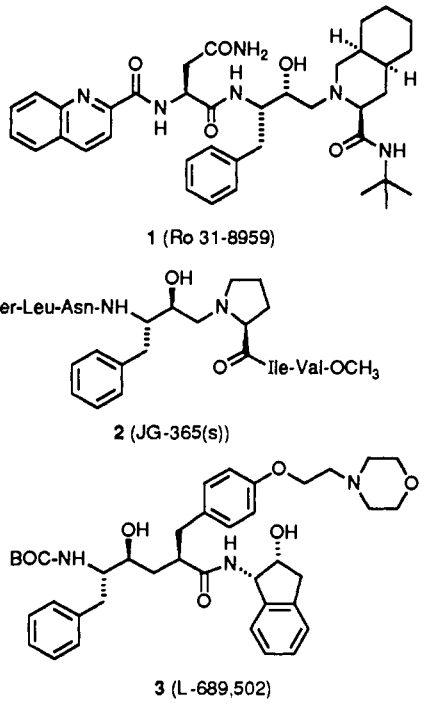


Figure 1.

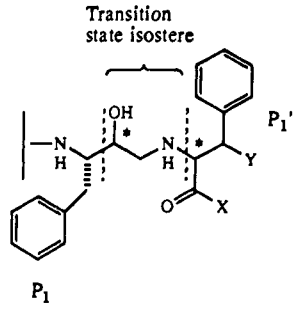
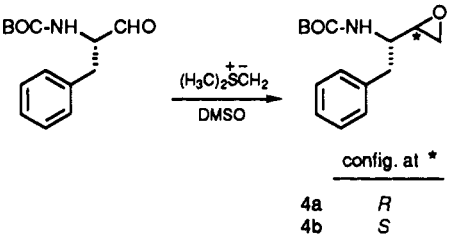


Figure 2.

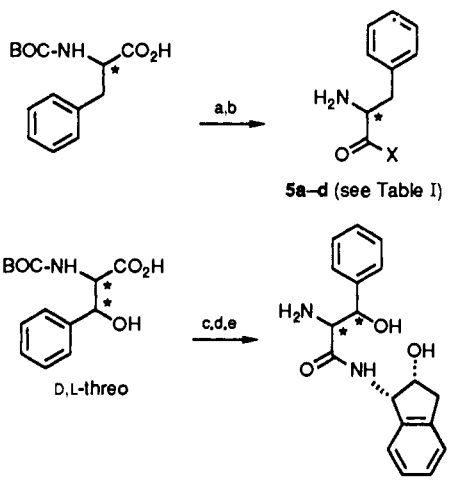
compounds represented by the general structure shown in Figure 2. Structures of this type, consisting of two phenylalanine subunits, offer several potential advantages. The two phenyl rings in the P_1 and P_1' phenylalanine units adjacent to the transition state isostere are available for interaction with the S_1 and S_1' binding pockets of the HIV-PR active site. These structures are also readily accessible synthetically via epoxide opening, and have a backbone amino function to provide desirable solubility characteristics.

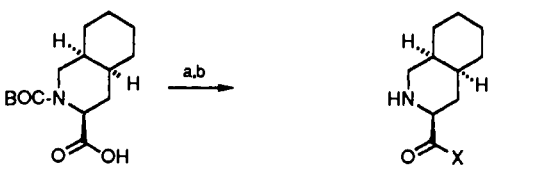
The stereochemical requirements of the HEA class of HIV-PR inhibitors have been the focus of some discussion. 1 and its close analogs demonstrate a strict preference for the (*R*) stereochemistry at the transition state hydroxyl group.[10] Compound 2 shows an approximately 80-fold

Scheme I



Scheme II[a]





	X	% yield
6a	NH(*tert*-butyl)	83
6b	NH[2(*R*)-hydroxy-1(*S*)-indanyl]	67

[a] (a) EDC, HOBT, $N(Et)_3$, X–H, DMF; (b) TFA, $CHCl_3$; (c) EDC, HOBT, $N(Et)_3$, 1(*S*)-amino-2(*R*)-hydroxyindan, DMF; (d) 2 N HCl/MeOH; (e) separate diastereomers.

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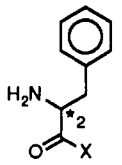
(11) A series of inhibitors of this type has been generically claimed in a patent application for the use of peptide renin inhibitors as anti-retroviral agents: Benz, G.; Bender, W.; Henning, R.; Paesseng, A. European Patent Appl. 0393445, 1990.

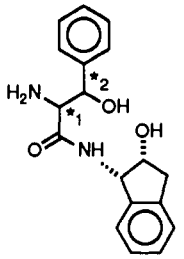
Table I. Structure and Physical Data for Phenylalanine Amides **5a–d**



no.	X	config at *2	yield, %[a]	formula	anal.	mp, °C
5a	NH[2(*R*)-hydroxy-1(*S*)-indanyl]	*R*	65	$C_{18}H_{20}N_2O_2$	C, H, N	183–184
5b	*b*	*S*	89	$C_{18}H_{20}N_2O_2$	C, H, N	184–185
5c	NH[1(*S*)-phenyl-2-hydroxyethan-1-yl]	*S*	62	$C_{17}H_{20}N_2O_2$	C, H, N	112–113
5d	HN(*tert*-butyl)	*S*	72	$C_{13}H_{20}N_2O_2 \cdot 0.4OH_2O$	C, H, N	oil

[a] For the two-step synthesis from BOC-amino acid. [b] Same as above.

Table II. Structure and Physical Data for Phenylserine Amides **5e,f**



no.	configuration at *1 and *2	yield, %[a]	formula	anal.	mp, °C
5e	D,L-threo/diastereomer 1	43	$C_{18}H_{20}N_2O_3 \cdot 0.50H_2O$	C, H, N	171–172
5f	D,L-threo/diastereomer 2	22	$C_{18}H_{20}N_2O_3 \cdot 0.25H_2O$	C, H, N	151–153

[a] Both diastereomers obtained from same reaction mixture.

preference for the (*S*) stereochemistry at the analogous hydroxyl group.[12a] However, various truncations of the C- and N-termini of **2** produce compounds with a reversed stereochemical preference, or compounds in which the (*R*) and (*S*) diastereomers are equipotent.[12a] To probe the stereochemical requirements of our novel inhibitors, we chose to prepare all of the potential diastereomeric permutations at the transition state hydroxyl group, as well as at the P_1' amide functionality. Our initial compounds were synthesized with the goals of minimal peptide character and minimal size as the primary objectives. On the basis of results obtained by other members of our group during the development of several series of potent hydroxyethylene HIV-PR inhibitors, we chose to employ the *tert*-butoxycarbonyl (BOC) group at the N-terminus[4e] and the 1(*S*)-amino-2(*R*)-hydroxyindan functionality at the C-terminus[4f] of our initial compounds. On elucidation of the stereochemical requirements of these molecules, a program of further synthesis was initiated with the goal of obtaining more potent and therapeutically useful HIV-PR inhibitors.

Chemistry

Epoxides **4a,b** were prepared from BOC-L-phenylalanal using the procedure of Evans et al. (Scheme I).[13] The diastereomeric epoxide mixture obtained from the reaction was separated by preparatory HPLC, and each epoxide diastereomer recrystallized to provide pure **4a** and **4b**.

The phenylalanine amides **5a–d** were prepared by standard amino acid coupling of the appropriate amine with the desired BOC-phenylalanine enantiomer, followed by BOC removal with trifluoroacetic acid (Scheme II). The phenylserine amide diastereomers **5e** and **5f** were prepared in an analogous manner by coupling of BOC-D,L-*threo*-phenylserine with 1(*S*)-amino-2(*R*)-hydroxyindan[14] followed by HCl/methanol BOC removal to give a mixture of threo diastereomers (Scheme II). The diastereomers were separated by crystallization of the crude mixture from acetonitrile to give pure more polar diastereomer **5e**. Chromatography of the mother liquor from **5e**, followed by crystallization of the crude product provided the other pure diastereomer **5f**. The absolute stereochemistry of **5e** and **5f** was not determined. Structures and physical data for **5a–f** are contained in Tables I and II. (4a*S*,8a*S*)-decahydroisoquinoline-3(*S*)-carboxylic acid amides **6a**[8a] and **6b** were prepared in an analogous manner from (4a*S*,8a*S*)-*N*-BOC-decahydroisoquinoline-3(*S*)-carboxylic acid[15,16] and the appropriate amine (Scheme II).

(12) (a) Rich, D. H.; Sun, C.-Q.; Prasad, J. V. N. V.; Pathiasseril, A.; Toth, M. V.; Marshall, G. R.; Clare, M.; Mueller, R. A.; Houseman, K. Effect of Hydroxyl Group Configuration in Hydroxyethylamine Dipeptide Isosteres on HIV-Protease Inhibition. Evidence for Multiple Binding Modes. *J. Med. Chem.* **1991**, *34*, 1222–1225. (b) Krohn, A.; Redshaw, S.; Ritchie, J. C.; Graves, B. J.; Hatada, M. H. Novel Binding Mode of Highly Potent HIV-Proteinase Inhibitors Including the (*R*)-Hydroxyethylamine Isostere. *J. Med. Chem.* **1991**, *34*, 3340–3342.

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(14) Hassner, A.; Carber, M. E.; Heathcock, C. Addition of Iodine Isocyanate to Olefins. Scope and Synthetic Utility. *J. Org. Chem.* **1967**, *32*, 540–549.

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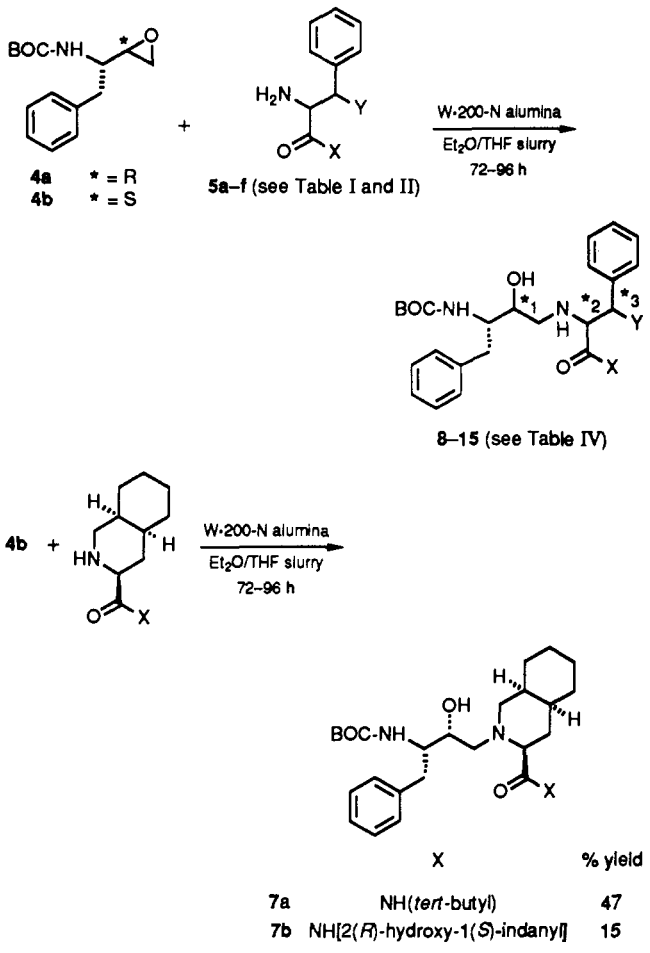
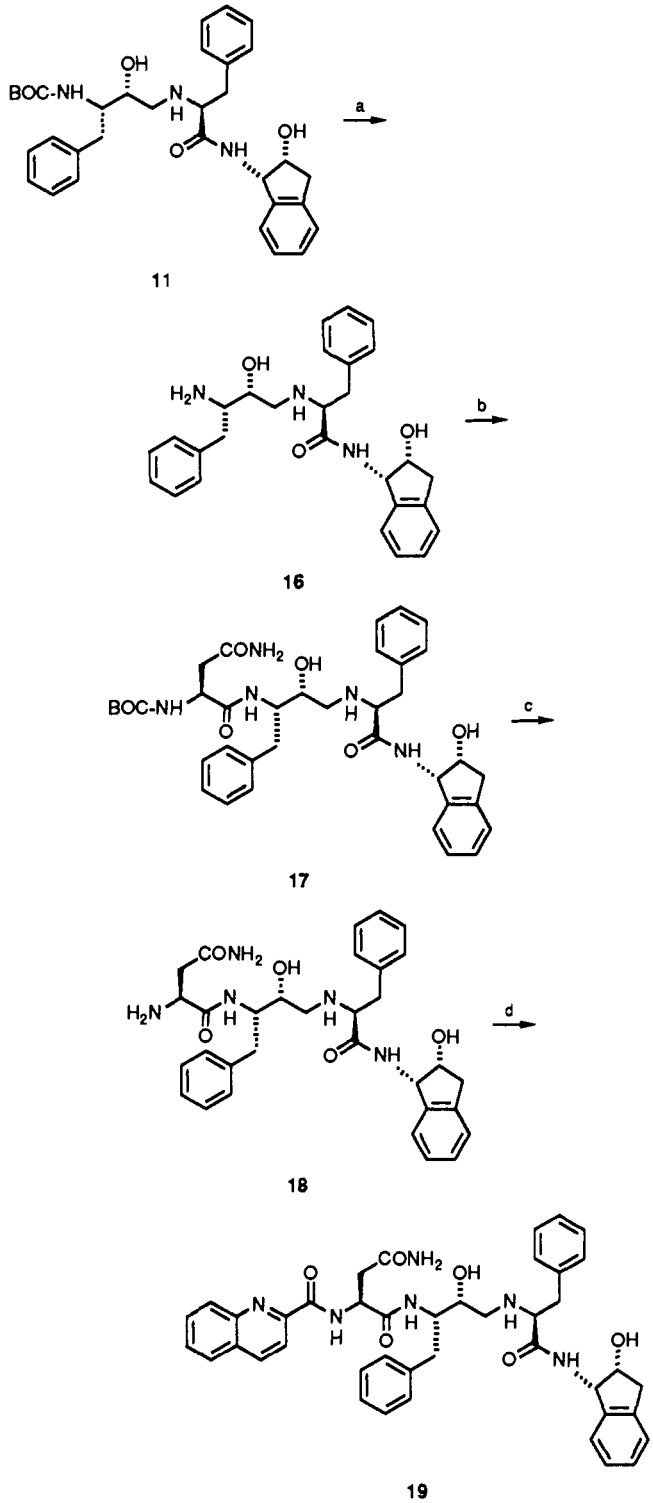
Scheme III



	X	% yield
7a	NH(*tert*-butyl)	47
7b	NH[2(*R*)-hydroxy-1(*S*)-indanyl]	15

Inhibitors 8–15 were synthesized by alumina-catalyzed opening of epoxides **4a** and **4b** with the amino acids amides **5a–f** using a modification of the procedure of Posner et al. (Scheme III).[17] **7a** and **7b**, the N-terminally truncated versions of 1, were prepared via an analogous epoxide-opening methodology. In all epoxide openings, low to moderate yields were obtained, and reaction times of 72–96 h were necessary to obtain maximum yields. Neither heating the reactions nor increasing the amount of amine used produced any increase in reaction rate or yield. Attempts to use other epoxide-opening techniques (metal salt catalysis,[18] Lewis Acid catalysis,[19] heating in protic solvents[20]) failed to provide any of the desired products except in the case of **7a**. **7a** could be prepared in 32% yield by heating **4a** and **6a** at 80 °C in absolute ethanol for 24 h.

Scheme IV[a]



[a] (a) 2 N HCl/MeOH, 60 °C; (b) EDC, HOOBT, DIPEA, Boc-Asn, DMF; (c) 1:1 TFA/$CHCl_3$; (d) EDC, HOBT, $N(Et)_3$, quinoline-2-carboxylic acid, DMF.

Table III. Inhibitory Potency of 1–3, 7a, and 7b

compd	IC_{50}, nM	compd	IC_{50}, nM
1 (Ro31-8959)	0.34	**7a**	>3000
2 (JG-365(s))	0.24[a] (K_i)	**7b**	>3000
3 (L-689,502)	0.45		

[a] Reference 12b.

(16) (4a*S*,8a*S*)-Decahydroisoquinoline-2(*S*)-carboxylic acid was *tert*-butoxycarbonylated using the procedure of R. Houssin, J.-L. Bernier, and J. P. Henichart. A Convenient and General Method for the Preparation of t-Boc Amino Alkane Nitriles and Their Conversion to Mono-t-Boc Alkane Diamines. *Synthesis* **1988**, 259–261.

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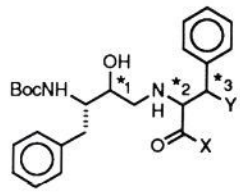
(18) Chini, M.; Crotti, P.; Macchia, F. Metal Salts as New Catalysts for Mild and Efficient Aminolysis of Oxiranes. *Tetrahedron Lett.* **1990**, *31*, 4661–4664.

(19) Overman, L. E.; Flippin, L. A. Facile Aminolysis of Epoxides with Diethylaluminum Amides. *Tetrahedron Lett.* **1981**, *22*, 195–198.

(20) Moller, F. *Houben Weyl. Methoden der Organische Chemie*; Muller, E., Ed.; Thieme-Verlag: Stuttgart, 1957; Vol. 11/1, pp 311–326.

Table IV. Structure, Physical Data, and Enzyme Inhibition Potency of 8–15



no.	X	Y	config at *1	*2	*3	yield, %	formula	anal.	mp, °C	IC_{50}, nM
8	HN[2(*R*)-hydroxy-1(*S*)-indanyl]	H	*S*	*S*	–	40	$C_{35}H_{41}N_3O_5$	C, H, N	168–169	4700
9	*a*	H	*S*	*R*	–	20	$C_{33}H_{41}N_3O_5$	C, H, N	170.5–172.0	23400
10	*a*	H	*R*	*R*	–	40	$C_{33}H_{41}N_3O_5 \cdot 0.25H_2O$	C, H, N	170.0–171.5	7300
11	*a*	H	*R*	*S*	–	35	$C_{33}H_{41}N_3O_5 \cdot 0.25H_2O$	C, H, N	157–158	252 ± 23
12	*a*	OH	*R* D,L-threo/diaster 1			15	$C_{33}H_{41}N_3O_6$	*b*	218–219	8500
13	*a*	OH	*R* D,L-threo/diaster 2			18	$C_{33}H_{41}N_3O_6$	*b*	148 dec	8600
14	HN[1(*S*)-phenyl-2-hydroxyethan-1-yl]	H	*R*	*S*	–	25	$C_{32}H_{41}N_3O_5$	C, H, N	178.0–179.5	38000
15	HN(*tert*-butyl)	H	*R*	*S*	–	59	$C_{28}H_{41}N_3O_4 \cdot H_2O$	C, H, N	120.0–121.5	>100000

[a] Same as above. [b] Elemental analysis not available for these compounds; confirmation of purity by NMR, HPLC, and MS.

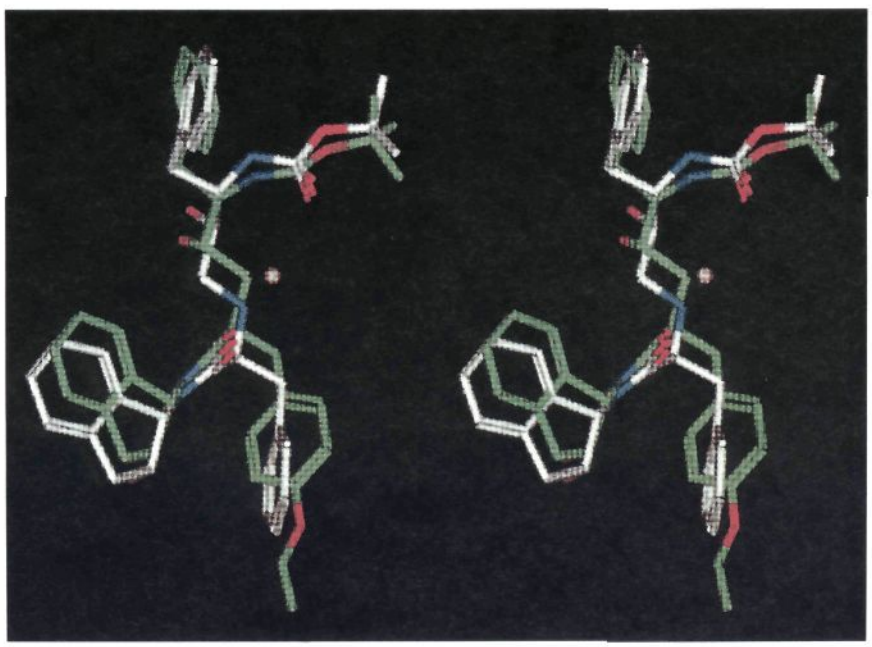


Figure 3. Stereoview of the energy-minimized model of **11** (backbone white, oxygen atoms red, nitrogen atoms light blue) and the X-ray structure of **3** (backbone green, oxygen atoms red, nitrogen atoms light blue) as bound in the HIV-1 protease active site. Hydrogens have been omitted for clarity. The pink sphere indicates the position of the oxygen atom of the active site water molecule.

11 was used as a starting point for the preparation of compounds **16–19** (Scheme IV). Treatment of **11** with 2 N HCl/MeOH gave the amino N-terminal compound **16**. Coupling of **16** with BOC-asparagine using conditions designed to prevent racemization gave **17**. Removal of the BOC group of **17** with trifluoroacetic acid produced **18**, which was coupled with quinoline-2-carboxylic acid to provide the P_2–P_3-extended inhibitor **19** in 56% yield.

Results and Discussion

Compounds **7a–19** were evaluated for their ability to inhibit HIV-PR in vitro. IC_{50} values were determined via a peptide hydrolysis assay that employs the octapeptide substrate H_2N-Val-Ser-Gln-Asn-(β-naphthyl-Ala)-Pro-Ile-Val-OH (430 μM, K_m = 160 μM), enzyme at 0.030 nM, and quantitation via HPLC.[21] Enzyme inhibition data for all compounds is summarized in Tables III, IV, and V. **7–10** demonstrate a clear preference for the (*R*) configuration at the transition state alcohol carbon and the (*S*) configuration at the P1′ α-C atom.

11 represents an interesting lead compound for the development of more potent inhibitors of this subclass. While not nearly as potent as the known HEA-containing HIV-PR inhibitors **1** and **2**, **11** lacks the P_2/P_3 substituents present in **1** and **2** which impart a great deal of the observed potency to these molecules. Comparison of **11** with **7a** and **7b**, the N-terminally truncated versions of **1**, in-

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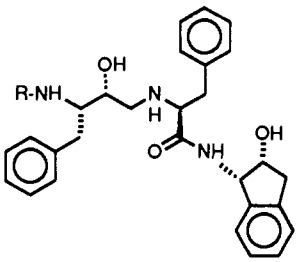
Table V. Effects of N-Terminal Modifications on 11. Structure, Physical Data, and Enzyme Inhibition Data



no.	R	yield, %	formula	anal.	mp, °C	IC_{50}, nM
11	Boc	35	$C_{33}H_{41}N_3O_5 \cdot 0.25H_2O$	C, H, N	157–158	252 ± 23
16	H	72	$C_{28}H_{33}N_3O_3 \cdot 0.25H_2O$	C, H, N	106–108	63 000
17	Boc-Asn	84	$C_{37}H_{47}N_5O_7 \cdot 1.50H_2O$	C, H, N[a]	225–227	295
18	H_2N-Asn	97	$C_{32}H_{39}N_5O_5 \cdot 0.80H_2O$	C, H, N[b]	171–173	1100
19	Qua[c]-Asn	56	$C_{42}H_{44}N_6O_6 \cdot 0.50H_2O$	C, H, N	215 dec	5.4 ± 0.8

[a] H: calcd 7.19; obsd 6.78. [b] N: calcd 11.89; obsd 11.31. [c] Qua = quinoline-2-carbonyl.

dicates that 11 is at least 13× more potent than both **7a** and **7b**. Molecular modeling studies with 11 indicate an interesting binding profile possessing characteristics of several different classes of HIV-PR inhibitors. The X-ray crystal structure of the enzyme–inhibitor complex of the potent hydroxyethyl HIV-PR inhibitor **3** (Figure 1; L-689,502)[22] with HIV-PR provided the model for the docking of 11 into the enzyme active site. Molecular geometry for 11 was created using the modeling program AMF,[23] and was energy-minimized in the static HIV-PR active site using the modified MM2 program OPTIMOL[24] (see Experimental Section). Comparison of **3** and 11 (Figure 3) shows remarkably good overlap between the P_1 and P_1' phenyl groups and the C- and N-terminal regions of these two structurally diverse inhibitors. The crucial interactions of the P_1 Boc carbonyl and the P_1' amide carbonyl with the tight-bound flap water molecule are clearly maintained. However, the longer amine-containing backbone of 11 produces a pronounced deviation in the transition state region as compared with **3**. The geometry of the backbone of 11 allows the (*R*) configuration at the transition state hydroxyl group to be accommodated by the active site in a favorable manner. The model of the backbone of 11 generated here is consistent with that proposed by Rich et al.[12] for HEA-containing inhibitors **1** and **2**. 11 appears to bind to the active site of HIV-PR in a unique "hybrid" manner with characteristics of hydroxyethylene inhibitors like **3**, and also HEA inhibitors like **1** and **2**.

Using 11 as a starting point, our synthetic efforts were expanded in an effort to increase the potency of this series of inhibitors. Incorporation of a hydroxyl group at the P_1' benzylic carbon was a possibility to provide an additional hydrogen bonding interaction with the active site. A preliminary molecular modeling study had indicated that a hydroxyl group of either the (*R*) or (*S*) configuration at this position has the potential to provide an additional hydrogen bond with the active site, with the (*R*) configuration preferred energetically by approximately 2 kcal. The (*R*)-hydroxy compound appeared to be able to form a hydrogen bond of approximately 3.1 Å with the tight-bound flap water molecule. Unfortunately, a study of the torsional angles in the P_1' region of the molecule also indicated that the new hydrogen bond had the potential to disturb the already existing interaction of the P_1' amide carbonyl with the active site water molecule. To address this question, the (*R*) stereochemistry was incorporated at the P_1' benzylic hydroxyl group by using D,L-*threo*-phenylserine as a starting material. The diastereomeric inhibitors **12** and **13** were synthesized, with one of them containing the correct L-threo relationship between the P_1' amide linker and the benzylic hydroxyl group. The other diastereomer would be presumed to be of very low potency, since it was already known that the (*S*) configuration at the P_1' amide functionality was necessary for potent enzyme activity. Results with **12** and **13** showed both compounds to have greatly decreased potency versus 11. This loss of potency may be due to the destabilizing influence of the benzylic hydroxyl group on already existing interactions between the inhibitor and the active site, unfavorable steric interaction with the enzyme, or stabilization of a conformation not well recognized or bound by the enzyme.

Attempted replacement of the 1(*S*)-amino-2(*R*)-hydroxyindan C-terminus of 11 with the more flexible (*S*)-2-phenylglycinol moiety (Table IV, **14**) resulted in a drastic loss of potency. Incorporation of the *tert*-butylamino C-terminus of **1** onto 11 (Table IV, **15**) produced a complete loss of activity. The 2-hydroxy group of the aminoindanol moiety is known to act as a P_2' carbonyl surrogate in hydroxyethylene inhibitors like **3**[25] (Figure 1), and the similarities seen in the molecular modeling comparison of **3** and 11 support a similar binding scheme for 11. Removal of conformational constraint, or of the hy-

(22) Thompson, W. J.; Fitzgerald, P. M. D.; Holloway, M. K.; Emini, E. A.; Drake, P. L.; McKeever, B. M.; Schleif, W. A.; Quintero, J. C.; Zugay, J. A.; Tucker, T. J.; Schwering, J. E.; Homnick, C. F.; Nunberg, J.; Springer, J. P.; Huff, J. R. Synthesis and Antiviral Activity of a Series of HIV-1 Protease Inhibitors with Functionality Tethered to the P_1 or P_1' Phenyl Substituents: X-ray Crystal Structure Assisted Design. *J. Med. Chem.* **1992**, *35*, 1685–1701.

(23) AMF is an extension of work previously described: (a) Gund, P.; Andose, J. D.; Rohodes, J. B.; Smith, G. M. Three-Dimensional Molecular Modeling and Drug Design. *Science* **1980**, *208*, 1425–1431. (b) Smith, G. M.; Hangauer, D. G.; Andose, J. D.; Bush, B. L.; Fluder, E. M.; Gund, P.; McIntyre, E. F. Intermolecular Modeling Methods in Drug Design/Modeling the Mechanism of Peptide Cleavage by Thermolysin. *Drug Inf. J.* **1984**, *18*, 167–178. The major authors of the AMF program are J. A. Andose, R. A. Blevins, E. F. Fluder, and J. Shpungin.

(24) Halgren, T. A.; Merck Sharp and Dohme Research Laboratories; unpublished work on the development of the force field program OPTIMOL. OPTIMOL differs from MM2 mainly in the use of partial charges on atoms, instead of bond dipoles, and in the absence of unshared pairs of electrons on certain nitrogen and oxygen atoms.

(25) Personal communication from Dr. W. J. Thompson.

droxyl group itself, apparently eliminates this crucial interaction between 11 and the active site. Incorporation of the 1(*S*)-amino-2(*R*)-hydroxyindan at the C-terminus of a cyclic-HEA inhibitor as in **7b** did not provide increased potency versus **7a**. This finding indicates distinct differences in the way that the P_1'–P_2' region of 11 interacts with the enzyme active site versus the same region in inhibitors like **1**, **7a**, and **7b**. The binding mode originally proposed by Rich[12a] et al. and confirmed by the X-ray crystal structure of the complex of 1 with HIV-PR[12b] appears to explain these differences. Geometric constraints imposed by the decahydroisoquinoline ring system of 1 cause the P_2' amide group to be accommodated in the S_2' receptor subsite, thereby making any hydrogen bonding groups in this region of the molecule unavailable to act as peptide bond surrogates. In contrast, the P_1'–P_2' region of 11 appears to bind to the active site in a manner similar to hydroxyethylene inhibitors such as **3**. This binding mode places the hydroxyl group of the aminoindanol ring system along the peptide backbone, thus enabling the hydroxyl group to act as a carbonyl surrogate.

N-terminal extension of 11 appeared to hold the most promise for obtaining inhibitors of increased potency. By analogy with **1** and **2**, the P_2–P_3 region appeared to be especially critical for good inhibitory potency. Molecular modeling studies had indicated that extension of the N-terminus of 11 into the P_2–P_3 region might possibly provide additional potency to the compound, while maintaining a preference for the (*R*) stereochemistry at the transition state alcohol. **16–19** were prepared as a stepwise extension of the N-terminus of 11 into the P_2–P_3 region. Removal of the Boc group of 11 (Table V, **16**) completely obliterated activity, owing to the critical role of the Boc carbonyl in interacting with the tight-bound flap water molecule. Reincorporation of a carbonyl via addition of a P_2-Boc-Asn substituent (Table V, **17**) restored the potency. Removal of the Boc group of **17** provided **18**, which showed a surprising 4× loss of inhibitory potency. This free amino group is likely to be charged in the active site, and it is possible that a charged group at the P_2 site is not well tolerated by the enzyme. The fully P_2/P_3-substituted compound **19** proved to be the most potent compound in the series, providing a potency increase of 55× over both 11 and **17**. The quinoline-2-carbonyl P_3 substituent clearly plays a key role in the observed potency enhancement. The importance of aromatic substituents at P_3 has been reported by other authors,[12] and results with **19** appear to support this observation. The data supports the presence of a large, lipophilic binding pocket in the HIV-PR active site that is available for an appropriate P_3 substituent.

The above described potency enhancement observed with **19**, while dramatic, is not nearly equivalent to the magnitude of the enhancement seen by extending the N-terminus of **7a** to give **1**. **1** exhibited a remarkable 8000× potency increase, and literature reports[10] indicate similar potency increases over other N-terminally truncated analogs. Curiously, in their N-terminally truncated form noncyclic-HEA inhibitors like **11** are much more potent than cyclic-HEA inhibitors like **7a** and **7b**. However, when compounds from both subclasses are N-terminally extended with identical P_2/P_3 substituents the cyclic-HEA inhibitors become much more potent than their noncyclic counterparts. A potential explanation of this phenomenon centers on the relative fit of the respective transition state isostere/C-terminal regions in the HIV-1 PR active site. It is apparent from our comparative studies, as well as from the published X-ray data on **1**,[12] that the combination of the P_1-Phe/P_1'-Phe hydroxyethyl secondary amine transition state isostere with the chiral aminoindanol P_2' moiety is better tolerated and more tightly bound by the active site than the same region of **1**. If the transition state isostere–P_1'–P_2' region of **1** is less tightly bound than the equivalent region of **19**, the added overall flexibility would provide more freedom of movement to the P_2–P_3 region of **1**. This added flexibility could enhance the ability of the P_2–P_3 region of **1** to interact with the enzyme active site. A tight binding interaction of the transition state isostere–P_1'–P_2' region of **19** with the enzyme active site may also induce changes in the overall conformation of the enzyme, thereby making the interaction of the P_2–P_3 groups with the active site much less favorable than in **1**. In general, these two closely related subclasses of HIV-1-PR inhibitors appear to bind to the enzyme active site in distinctly different ways, relying on different regions to provide the strongest interactions with the enzyme.

In order to fully evaluate the therapeutic usefulness of **19** as an anti-HIV agent, the compound was evaluated for its ability to inhibit HIV-1 spread in cultured MT-4 human T-lymphoid cells (see Experimental Section). The effectiveness of compounds in this assay is described by the term CIC_{95}, (95% cell culture inhibitory concentration) which is defined as the concentration of test compound which inhibits the production of the viral core protein p24 by 95% relative to untreated controls. In this assay, **19** exhibited a CIC_{95} of 200 nM ($N = 3$), clearly indicating that the compound is effective as a potent anti-HIV agent. In the same assay, **1** had a CIC_{95} of 12.5 nM ($N = 3$). The difference of 16× in the potency of these two compounds as antiviral agents in cell culture is essentially identical to the approximately 14× difference in their enzyme potencies. Therefore, the two compounds appear to have similar ability to penetrate cell membranes and exert their anti-HIV activities.

In conclusion, we have developed a series of novel HIV-PR inhibitors containing the hydroxyethyl "secondary" amine transition state isostere. The compounds exhibit a strict preference for the (*R*) stereochemistry at the transition state hydroxyl group. Comparison of analogs of **1** with analogs of **19** indicate distinct SAR and binding differences between these two subclasses of HIV-PR inhibitors. The most potent enzyme inhibitor in the series is **19**, which exhibited potent antiviral activity in cell culture. This novel subclass of HIV-PR inhibitors further expands our knowledge of the structural requirements for both potent HIV-PR inhibitory activity and potent antiviral activity.

Experimental Section

Mass spectra were taken on a VG Micromass MM7035 spectrometer. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. ^1H NMR spectra were obtained with a Varian XL 300 spectrometer. Chemical shifts are expressed in ppm relative to tetramethylsilane. Flash chromatography was performed on E. Merck silica gel 60 (230–400 mesh) unless otherwise noted. Thin-layer chromatography (TLC) was performed on E. Merck 60F-254 (0.25 mm) precoated silica gel plates. Visualization of TLC plates was accomplished with UV light and/or phosphomolybdic acid stain. Preparatory TLC was performed on E. Merck No. 5717 (2.00 mm) precoated silica plates. Solvents were obtained from Aldrich or Fisher and were used without further purification. When anhydrous solvents were necessary, Aldrich Sure-Seal solvents were used without further drying. W-200-N alumina was obtained from ICN and was used without further drying. 1(*S*)-amino-2(*R*)-hydroxyindan[4f,14] and (4a*S*,8a*S*)-*N*-Boc-decahydroisoquinoline-3(*S*)-carboxylic acid[15,16] were prepared according to literature methods. D,L-*threo*-phenylserine was purchased from Aldrich, and was *tert*-butoxycarbonylated using standard methods.[26] **1** was prepared as a

standard using the literature method.[10]

Epoxides 4a, 4b. The crude **4a/4b** mixture was prepared according to the method of Evans,[13] et al. After flash chromatography of the crude mixture with 3:1 hexane/ethyl acetate to remove sulfur-containing impurities, the 1:1 diastereomeric epoxide mixture was separated by preparatory HPLC on a Waters Prep 400 using a Waters silica gel prep pac and 2% 2-propanol/heptane run isocratically. The separated diastereomers were each recrystallized from boiling hexane to give pure **4a** and **4b** in an overall yield of 36% (18% **4a**, 18% **4b**). **4a**: mp 88–89 °C; NMR ($CDCl_3$) 1.40 (s, 9 H), [2.59 (m), 2.71 (dd), 2.81–3.00 (br m), 3.03 (m); (Total of 5H)], 4.13 (br s, 1 H), 4.46 (br s, 1 H), 7.20–7.40 (m, 5 H). **4b**: mp 123–124 °C; NMR ($CHCl_3$) 1.40 (s, 9 H), [2.78 (br s), 2.81 (t), 2.84–2.93 (m), 2.97 (dd); (Total of 5 H)], 3.70 (br s, 1 H), 4.45 (br s, 1 H), 7.20–739 (m, 5 H).

General Method for the Preparation of Amino Acid Amides 5a–d, (L)-Phenylalanine [2(*R*)-Hydroxyl-1(*S*)-indanyl]amide (5b). To a solution of 2.00 g (13.41 mmol) of 1(*S*)-amino-2(*R*)-hydroxyindan[4f,14] in 50 mL of *N,N*-dimethylformamide was added portionwise 4.09 g of (15.42 mmol) Boc-L-phenylalanine, 2.95 g (15.42 mmol) of 1-ethyl-3-[(3-dimethylamino)propyl]carbodiimide·HCl, and 2.08 g (15.42 mmol) of 1-hydroxybenzotriazole hydrate. After the reaction mixture became homogeneous, 1.56 g (2.15 mL; 15.42 mmol) of triethylamine was added in one portion. The resulting solution was stirred at room temperature for 18 h. The reaction was concentrated in vacuo to remove most of the DMF and the thick residue treated with 100 mL of ice-cold 10% aqueous citric acid. The resulting solution was extracted with 2 × 50 mL of ethyl acetate, and the combined extracts were washed with 3 × 25 mL of water, 2 × 25 mL of saturated aqueous sodium bicarbonate, and 25 mL of brine. Drying (anhydrous Na_2SO_4) and concentration in vacuo gave 5.50 g of white solid. The solid was dissolved in 125 mL of chloroform and the solution treated with 25 mL of trifluoroacetic acid. The resulting solution was stirred at room temperature for three h and was concentrated in vacuo. The residue was redissolved in 80 mL of chloroform, and was carefully washed with 2 × 30 mL 15% ammonium hydroxide, 30 mL water, 30 mL brine, and was dried (anhydrous Na_2SO_4). Concentration in vacuo gave 3.88 g (89%) of product as a white solid: mp 184–185 °C; NMR ($CDCl_3$) 1.79 (br s, 1 H), 2.73–2.85 (m, 2 H), 3.10–3.18 (m, 2 H), 3.36 (br s, 2 H), 3.59 (q, 1 H), 4.42 (br s, 1 H), 5.06 (br s, 1 H), 5.20 (q, 1 H), 7.13 (d, 1 H, J = 4 Hz), 7.11–7.28 (m, 4 H), 7.30 (d, 4 H), 8.12 (d, 1 H, NH). Anal. ($C_{18}H_{20}N_2O_2$) C, H, N.

D,L-*threo*-Phenylserine (2(*R*)-Hydroxy-1(*S*)-indanyl)-amide. Separation of Diastereomers 5e and 5f. An amount of 1.41 g (5.00 mmol) of Boc-D,L-*threo*-phenylserine[26] and 0.82 g (5.50 mmol) of 1(*S*)-amino-2(*R*)-hydroxyindan were coupled according to the previous procedure. An amount of 2.10 g (100%) of white solid product was recovered. The solid was treated with 25 mL of 2 N HCl/MeOH at 60 °C for 2 h. The solution was cooled, and made slightly basic with 1.2 N NaOH. The basic solution was diluted with water, and a white solid precipitate formed. Filtration gave approximately 2 g of a white solid powder, and the filtrate was reserved. The solid was recrystallized from 70 mL of hot acetonitrile to give a first crop of 0.67 g (43%) of white needles corresponding to the pure more polar diastereomer **5e**: mp 171–172 °C dec; NMR ($CDCl_3$) 2.80–3.55 (broad bumps, NH_2, OH), 2.95 (d, 1 H, J = 14 Hz), 3.12 (dd, 1 H), 3.60 (d, 1 H, J = 2 Hz), 4.63 (m, 1 H), 5.31 (m, 1 H), 5.33 (m, 1 H), 7.08 (d, 1 H, J = 6 Hz), 7.14–7.49 (m, 9 H), 7.95 (d, 1 H, J = 7 Hz, NH amide). Anal. ($C_{18}H_{20}N_2O_2 \cdot 0.50H_2O$) C, H, N.

The filtrate reserved from above was extracted with two volumes of EtOAc, and the combined extracts were washed with brine, dried (anhydrous Na_2SO_4), and concentrated in vacuo to give 0.47 g of a gum. The gum was chromatographed over activity III neutral alumina with 5% MeOH/$CHCl_3$ to give a white solid. The solid was recrystallized from CH_3CN to give 0.53 g (23%) of the second diastereomer **5f** as a colorless prism: mp = 151–153 °C; NMR ($CDCl_3$) 2.85–3.94 (very broad s, NH_2 and OH), 2.95 (d, 1 H, J = 14 Hz), 3.12 (dd, 1 H), 3.60 (d, 1 H, J = 4 Hz), 4.54 (t, 1 H), 5.30 (d, 1 H, J = 4 Hz), 5.37 (q, 1 H), 7.18–7.35 (m, 5 H), 7.37 (m, 3 H), 7.46 (d, 2 H), 8.20 (d, 1 H, J = 7 Hz). Anal. ($C_{18}H_{20}N_2O_3 \cdot 0.25H_2O$) C, H, N.

(4a*S*,8a*S*)-Decahydroisoquinoline-3(*S*)-carboxylic Acid Amides (6a, 6b). **6a**[8a] was prepared in 83% yield from (4a*S*,8a*S*)-*N*-Boc-decahydroisoquinolin-3(*S*)-carboxylic acid and *tert*-butylamine according to the general procedure used to prepare **5a–d**. The material was used in the next reaction without further purification or characterization.

6b was prepared in 67% yield from the Boc-amino acid and 1(*S*)-amino-2(*R*)-hydroxyindan via an analogous procedure. The crude material was used as obtained from the reaction mixture without further purification or characterization.

Alumina-Catalyzed Epoxide Opening. General Method for the Preparation of 8–15. *N*-[1-Phenyl-2(*S*)-[(*tert*-butoxycarbonyl)amino]-3(*R*)-hydroxybutan-4-yl]-L-phenylalanine (2(*R*)-Hydroxy-1(*S*)-indanyl)amide (11). A solution of 163.00 mg (0.55 mmol) of **5b** in 1 mL of anhydrous THF and 2 mL anhydrous Et_2O was slurried with 2.80 g (~4% slurry of amine) of W-200-N alumina in an argon atmosphere. After approximately 30 min of vigorous stirring, the slurry was treated with a solution of 70.00 mg (0.27 mmol) of **4b** in 2 mL of anhydrous Et_2O. The resulting suspension was stirred vigorously at room temperature in an argon atmosphere for 72 h (until TLC indicated progress had stopped). The reaction was diluted with 25 mL MeOH and 1 mL H_2O, and was stirred vigorously for 2 h. The suspension was filtered through a Celite pad, and the pad washed thoroughly with MeOH. The filtrate was concentrated in vacuo to give a clear oil. The crude oil was purified via preparative TLC (5% MeOH/CH_2Cl_2). Isolation of the product band gave a clear glass, which was crystallized from Et_2O to give 52.00 mg (35%) of product as a white crystalline solid: mp 157–158 °C; ^1H NMR (DMSO-d_6) 1.29 (s, 9 H), 2.69 (br 8, 2 H), 2.69–2.79 (m, 2 H, 2.78–2.83 (m, 4 H), 3.14 (dd, 1 H), 3.27 (dd, 1 H), 3.45 (br m, 1 H), 3.57 (br m, 1 H), 3.74 (br m, 1 H), 4.54 (d, 2 H), 5.41 (q, 1 H), 7.10–7.38 (complex, 17 H), 7.69–7.78 (br d, 1 H). Anal. ($C_{33}H_{41}N_3O_5 \cdot 0.25H_2O$) C, H, N.

Alumina-Catalyzed Epoxide Opening. Preparation of 7a, 7b. Compound **7a** was prepared in 47% yield from **4b** and **6a** using the procedure detailed above for the preparation of 8–15. Alternatively, **7a** was prepared in 32% yield by heating 490.00 mg (1.86 mmol) of **4b** and 370 mg (1.55 mmol) of **6a** in absolute EtOH at 80 °C for 24 h: mp 93–95 °C; 1 H NMR ($CDCl_3$) 1.37 (s, 18 H), 1.38–1.49 (m, 4 H), 1.49–1.57 (m, 2 H), 1.72–1.89 (m, 2 H), 1.98 (q, 1 H) 2.29 (m, 2 H), 2.59–2.71 (m, 2 H), 2.86–3.10 (m, 3 H), 3.59 (br s, 1 H), 3.81 (br s, 1 H), 3.89 (br s, 1 H), 4.86 (br s, 1 H), 5.90 (s, 1 H), 7.18–7.35 (complex, 5 H). Anal. ($C_{29}H_{47}N_3O_4$) C, H, N.

7b was prepared in 15% yield from **4b** and **6b** via an analogous procedure. The product was isolated as a white foam/solid: NMR ($CDCl_3$) 1.30 (s, 9 H), 1.22–1.45 (m, 4 H), 1.48–1.89 (complex, 9 H) 2.04 (q, 1 H), 2.24–2.38 (m, 2 H), 2.71–2.84 (br m, 2 H), 2.90 (m, 2 H), 2.94 (d, 1 H, J = 4 Hz), 3.09–3.24 (dd, 1 H, J = 4 Hz, 15 Hz and br s, 1 H), 3.75–3.89 (br s, 1 H), 3.90–4.01 (br s, 1 H), 4.62 (t, 1 H), 4.84 (d, 1 H, J = 6 Hz), 5.49 (q, 1 H), 7.14–7.39 (complex, 10 H). Anal. ($C_{29}H_{47}N_3O_4$) C, H, N.

***N*-[1-Phenyl-2(*S*)-amino-3(*R*)-hydroxybutan-4-yl]-L-phenylalanine (2(*R*)-Hydroxy-1(*S*)-indanyl)amide (16).** A solution of 510.00 mg (0.91 mmol) of 11 in 30 mL of 2 N HCl/MeOH was stirred at 60 °C for 2 h. The reaction was cooled to room temperature and concentrated in vacuo. The oily solid residue was partitioned between saturated aqueous $NaHCO_3$ and $CHCl_3$. The aqueous layer was extracted twice with $CHCl_3$, and the combined $CHCl_3$ extracts were washed with brine and dried (anhydrous Na_2SO_4). Concentration in vacuo gave a pale tan foam, which was triturated in Et_2O until a solid formed. Filtration gave 270 mg of off-white crystalline solid product, mp 106–108 °C. Concentration in vacuo and retrituration with a minimum of cold Et_2O gave an additional 30 mg of pure product: yield 72%; ^1H NMR ($CDCl_3$) 2.23–2.48 (m, 2 H), 2.85–3.90 (very broad s, 3 H, NH_2 + NH), 2.59–2.65 (m, 1 H), 2.66 (d, 2 H, J = 6 Hz), 2.80 (d, H, J = 4 Hz), 2.85 (d, 2 H, J = 6 Hz), 2.88–2.92 (m, 1 H), 3.10 (dd, 1 H, J = 4 Hz, 15 Hz), 3.80 (dd, 1 H, J = 4 Hz, 15 Hz), 3.42 (q, 1 H), 3.59 (br s, 1 H), 4.54 (t, 1 H), 5.40 (q, 1 H), 7.04–7.35

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(complex, 14 H), 7.95 (d, 1 H, J = 7.5 Hz). Anal. ($C_{28}H_{33}N_3O_3 \cdot 0.25H_2O$) C, H, N.

N-[1-Phenyl-2(S)-[N-[N-*tert*-butoxycarbonyl)-L-asparaginyl]amino]-3(R)-hydroxybutan-4-yl]-L-phenylalanine (2(R)-Hydroxy-1(S)-indanyl)amide (17). To a suspension of 326.00 mg (1.40 mmol) of Boc-Asn in 15 mL of 1:1 EtOAc/CH_2Cl_2 was added 84.00 mg (0.52 mmol) of 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (HOOBT) and 179.00 mg (0.94 mmol) of 1-ethyl-3-[(3-dimethylamino)propyl]carbodimide·HCl. A solution of 215.00 mg (0.47 mmol) of **16** in 5 mL of EtOAc/CH_2Cl_2 was added to the reaction, and the resulting mixture was treated with 131.00 mg (1.40 mmol) of diisopropylethylamine in 45 μL portions to adjust the pH to 6.5. The resulting solution was stirred at room temperature for approx. 18 h. The reaction was poured into 125 mL of EtOAc, and was washed twice with 75-mL portions of H_2O. The EtOAc layer was washed twice with 50-mL portions of aqueous $NaHCO_3$, and a precipitate came out of the organic layer. The organic layer was filtered, and the solid residue washed with H_2O to remove inorganics. Drying in vacuo gave 266 mg (84%) of the desired product as a white solid: mp 225–227 °C dec; ^{1}H NMR (DMSO) 1.40 (s, 9 H), 2.00 (br s, 1 H), 2.26 (br s, 1 H), 2.75 (d, 2 H, J = 6 Hz), 2.51 (m, 1 H), 2.60 (m, 2 H), 2.80 (dd, 1 H, J = 2 Hz, 14 Hz), 2.85–2.98 (m, 3 H), 3.01–3.22 (m, 3 H), 3.71 (br s, 1 H), 4.28 (q, 1 H), 4.41 (t, 1 H), 4.94 (br s, 1 H), 5.09 (br s, 1 H), 5.17 (q, 1 H), 6.76 (d, 1 H, J = 6 Hz), 6.88 (br s, 1 H), 6.92 (d, 1 H, J = 6 Hz), 7.11–7.36 (complex, 14 H), 7.63 (d, 1 H, J = 7.5 Hz), 8.05 (d, 1 H, J = 7.5 Hz). Anal. ($C_{37}H_{47}N_5O_7 \cdot 1.50H_2O$) C, H, N; H: calcd 7.19; obsd 6.75.

N-[1-Phenyl-2(S)-(N-L-asparaginylamino)-3(R)-hydroxybutan-4-yl]-L-phenylalanine (2(R)-Hydroxy-1-(S)-indanyl)amide (18). To a solution of 250.00 mg (0.37 mmol) of **17** in 10 mL of CH_2Cl_2 cooled to 0 °C was added 10 mL of trifluoroacetic acid. After 1 hr at 0 °C, the reaction was concentrated in vacuo, and placed on a vacuum pump to remove remaining CF_3CO_2H. The residue was dissolved in 100 mL EtOAc and washed with three 20-mL portions of 10% K_2CO_3 solution. The EtOAc layer was washed with brine, dried (anhydrous Na_2SO_4), and concentrated in vacuo to give 205 mg (97%) of product as a white crystalline solid. The crude solid was used as obtained from the reaction without further purification. Recrystallization of a small sample from EtOAc/MeOH gave pure **17** as a white crystalline solid: mp 171–173 °C; ^{1}H NMR (DMSO-d_6) 1.79 (br s, 1 H), 1.93 (dd, 1 H, J = 7.5 Hz, 16 Hz), 2.03 (br s, 1 H), 2.26 (dd, 1 H, J = 4, 12.5 Hz), 2.50 (m, 1 H), 2.61 (m, 1 H), 2.75–2.96 (m, 4 H), 3.06 (d, 1 H, J = 4 Hz), 3.10 (d, 1 H, J = 4 Hz), 3.39 (br m, 2 H), 3.82 (br s, 1 H), 4.42 (t, 1 H), 4.98 (d, 1 H, J = 6 Hz), 5.19 (q, 2 H), 6.86 (s, 1 H), 6.97 (d, 1 H, J = 6 Hz), 7.12–7.40 (complex, 14 H), 7.74 s (d, 1 H, J = 7.5 Hz), 8.04 (d, 1 H, J = 7.5 Hz). Anal. ($C_{32}H_{39}N_5O_5 \cdot 0.8H_2O$) C, H, N; N: calcd 11.89; obsd 11.31.

N-[1-Phenyl-2(S)-[[N-(2-quinolylcarbonyl)-L-asparaginyl]amino]-3(R)-hydroxybutan-4-yl]-L-phenylalanine (2(R)-Hydroxy-1(S)-indanyl)amide (19). To a solution of 166.00 mg (0.29 mmol) of **18** and 60.12 mg (0.35 mmol) of quinoline-2-carboxylic acid in 10 mL of dry DMF was added 53.14 mg (0.35 mL) of 1-hydroxybenzotriazole hydrate, 66.52 mg (0.35 mmol) of 1-ethyl-3-[(3-dimethylamino)propyl]carbodimide·HCl, and 35.11 mg (0.35 mmol) of triethylamine. The resulting solution was stirred at room temperature in an argon atmosphere for 6 h. The reaction was poured into 200 mL of EtOAc and the solution washed with saturated aqueous $NaHCO_3$ (3 × 50 mL), H_2O (2 × 25 mL), and brine (2 × 25 mL). Drying (anhydrous Na_2SO_4) and concentration in vacuo gave 200 mg of a light yellow solid. The solid was purified via flash chromatography over silica gel with 5–8% 2-propanol/$CHCl_3$ to give 119 mg (56%) pure desired product as a white crystalline solid: mp 215–223 °C dec; ^{1}H NMR ($CDCl_3$, 3 drops of CD_3OD) 2.58–2.74 (m, 6 H), 2.88–3.01 (m, 4 H), 3.19 (dd, 1 H, J = 6 Hz, 16 Hz), 3.28 (dd, 1 H, J = 4 Hz, 12.5 Hz), 3.48 (m, 1 H), 3.59 (m, 1 H), 4.02 (m, 1 H), 4.69 (t, 1 H), 4.86 (t, 1 H), 5.39 (d, 1 H, J = 5 Hz), 6.78 (t, 1 H), 6.91 (t, 2 H), 7.07 (d, 2 H, J = 6 Hz), 7.13 (m, 1 H), 7.20–7.39 (complex, 10 H), 7.69 (t, 1 H), 7.82 (t, 1 H), 7.94 (d, 1 H, J = 6 Hz), 8.16 (d, 1 H, J = 7.5 Hz), 8.22 (d, 1 H, J = 8 Hz), 8.36 (d, 1 H, J = 8 Hz). Anal. ($C_{42}H_{44}N_6O_6 \cdot 0.5H_2O$) C, H, N.

Molecular Modeling. All modeled structures were built using the Merck molecular modeling program AMF (Advanced Modeling Facility)[23] and minimized using the Merck molecular force field OPTIMOL,[24] which is a variant of the MM2[27] program. Due to the low-pH optimum of the HIV-1 protease,[28] all titratable residues were charged in the calculations with the exception of one of the pair of catalytic aspartic acids, ASP_{25}.[29] During energy minimizations, the enzyme active site was held fixed at X-ray geometry. Graphics visualization was performed using Quanta.[30]

Inhibition of Virus Spread in Cell Culture. A. Preparation of HIV-Infected MT-4 Cell Suspension. MT-4 human T-lymphoid cells were infected at day 0 at a concentration of 250 000 per milliliter with a 1:2000 dilution of HIV-1 strain IIIb stock (final 125 pg p24/mL; sufficient to yield ≤1% infected cells on day 1 and 25–100% on day 4). Cells were infected and grown in the following medium: RPMI 1640 (Whittaker BioProducts), 10% inactivated fetal bovine serum, 4 mM glutamine (Gibco Labs), and 1:100 penicillin-streptomycin (Gibco Labs). The mixture was incubated overnight at 37 °C in 5% CO_2 atmosphere.

B. **Treatment with Inhibitors.** Serial 2-fold dilutions of compound were prepared in cell culture medium. At day 1, aliquots of 125 μL of compound were added to equal volumes of HIV-infected MT-4 cells (50 000 per well) in a 96-well microtiter cell culture plate. Incubation was continue for 3 days at 37 °C in 5% CO_2 atmospheres.

C. **Measurement of Virus Spread.** Using a multichannel pipettor, the settled cells were resuspended and a 125 μL aliquot harvested into a separate microtiter plate. After the settling of the cells, the plates were frozen for subsequent assay of the supernatant for HIV p24 antigen. The concentration of HIV p24 antigen was measured by a p24 ELISA assay (Coulter Immunology, Hialeah, FL).

The cell culture inhibitory concentration (CIC_{95}) for each compound 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.

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Supplementary Material Available: A table of elemental, mass spectra, and HPLC analytical data for compounds **5a–f** and **7a–19** (2 pages). Ordering information is given on any current masthead page.

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(29) This is based on the pH rate profile for fungal aspartyl proteases (Hofmann, T.; Hodges, R. S.; James, M. N. G. Effect of pH on the Activities of Penicillopepsin and Rhizopus Pepsin and a Proposal for the Productive Substrate Binding Mode in Penicillopepsin. *Biochemistry* **1984**, *23*, 635–643) which suggests that the two catalytic aspartic acids share one proton in the active pH range.

(30) Commercially available from Polygen Corp., 200 Fifth Ave., Waltham, MA 02254.