Symmetry-Based Inhibitors of HIV Protease. Structure-Activity Studies of Acylated 2,4-Diamino-1,5-diphenyl-3-hydroxypentane and 2,5-Diamino-1,6-diphenylhexane-3,4-diol

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The structure-activity relationships in two series of novel, symmetry-based inhibitors of HIV protease, the enzyme responsible for maturation of the human immunodeficiency virus, are described. Beginning with lead compounds **3-6**, the effect of adding polar, heterocyclic end groups to one or both ends of the symmetric or pseudosymmetric inhibitors was probed. Aqueous solubility was enhanced >1000-fold while maintaining potent inhibition of purified HIV-1 protease and anti-HIV activity in vitro. Pharmacokinetic studies in rats indicated a substantial difference in the absorption properties of mono-ol-based and diol-based inhibitors. The oral bioavailability of inhibitor **19** in rats was 19%; however, the C_{max} obtained failed to exceed the anti-HIV EC_{50} in vitro. Substantial plasma levels of potent inhibitors of the diol class were not obtained after oral administration in rats; however, the optimal combination of aqueous solubility and in vitro antiviral activity of several inhibitors support their potential use in intravenous therapy.

The worldwide spread of the human immunodeficiency virus (HIV) has prompted an intense search for agents which have the potential to control or slow the progression of acquired immunodeficiency syndrome (AIDS). Approaches to the chemotherapeutic intervention of HIV disease are hampered by a limited understanding of the pathological mechanisms of HIV, by the lack of reliable, inexpensive animal disease models, and by the heterogeneous nature of HIV in vivo. Nevertheless, the molecular biology of HIV is well-understood, and a significant number of virus-specific targets have been identified.[1] Among these, the virally encoded proteinase of the *pol* gene, HIV protease, provides an attractive target for intervention through the use of chemical inhibitors. The essential role of HIV protease in the specific proteolytic processing of the *gag* and *gag-pol* gene products, resulting in the production of mature, infectious virus particles, has been well-documented.[2] Blocking the action of HIV protease either through site-directed mutagenesis or through the use of inhibitors results in the complete inhibition of HIV in vitro. HIV protease thus represents an attractive target for antiretroviral chemotherapy, and a variety of inhibitors have already been described.[3]

Retroviral proteinases represent a subclass of the more general family of aspartic proteinases, which, although heterologous in primary sequence, retain a significant degree of homology in tertiary structure.[4] Those mammalian and fungal aspartic proteinases which have been characterized structurally are composed of two nonidentical globular domains, each containing a highly conserved amino acid triad (Asp-Thr-Gly) responsible for catalytic activity. In contrast, the retroviral proteinases in general, and HIV protease in particular, have been shown to exist in their active forms as C_2-symmetric homodimers, with each monomer contributing a catalytic triad to a single active site. The high degree of symmetry of the HIV protease structure offers a unique opportunity for the design of C_2-symmetric inhibitors which match the characteristics of the enzyme structure. Inhibitors based on symmetry might provide advantages over more traditional, substrate-based inhibitors in terms of potency, specificity, and reduced peptide character. Recently, we described two such series of symmetric and pseudosymmetric inhibitors.[5,6] These were designed as shown in Figure 1 via a three-step conceptual process: (1) definition of a C_2-axis on the tetrahedral intermediate for cleavage of a peptide substrate; (2) arbitrary deletion of the C-terminus of the substrate; and (3) two-fold rotation of the remaining N-terminus. Placement of the axis of symmetry through the tetrahedral substrate carbon led to the pseudosymmetric core structure **1**, while dissection of the scissile bond with the symmetry axis provided instead the diol **2**, as three stereochemically distinct isomers (two symmetric and one pseudosymmetric). Attachment of peptide residues to **1** and **2** resulted in nanomolar or subnanomolar inhibitors of HIV protease.[6] Initial strucutre-activity studies dedicated to improving aqueous solubility led to the identification of A-77003 (vide infra) as a candidate for intravenous clinical therapy.[7] Here, we provide a full account of the structure-activity relationships of these series of inhibitors, with emphasis not only on inhibitory potency, but on the effect of structural changes upon the antiviral activity, solubility, and pharmacokinetic properties of the inhibitors.[8]

Results and Discussion

The syntheses of diamino alcohol **1** and the three diastereomers of diamino diol **2** from phenylalanine have been described elsewhere.[9] Acylation of **1** with protected amino acids rapidly led to the identification of the bis-(Cbz-valinyl) derivative A-74704 (**3**) as a lead compound with an IC_{50} against HIV-protease in a fluorogenic inhibition assay of 3 nM.[5,6,10] Attachment of the same end piece to each of the diastereomers of **2a-c** led in each case to subnanomolar inhibitors (**4-6**).[6] Being cognizant of the poor pharmacokinetic properties generally encountered with peptide-like agents, we endeavored to examine the pharmacokinetic behavior of a wide variety of structural variants of lead compounds **3-6**, in hopes of iden-

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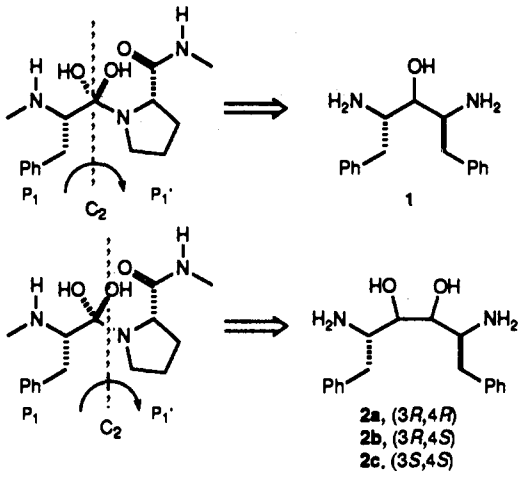
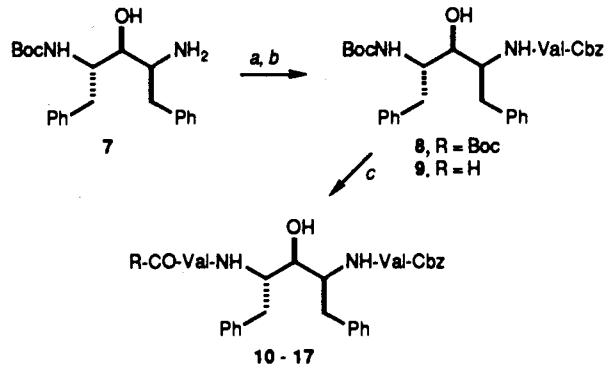


Figure 1. Design of C_2 symmetric HIV protease inhibitors.

Scheme I[a]



[a] (a) Cbz-Val-OH, EDC, HOBT; (b) HCl, dioxane; (c) RCO-Val-OH (method A).

tifying structural motifs which promoted good oral bioavailability. In order to facilitate pharmacokinetic evaluation, we therefore identified as a major goal of our structure–activity studies in these series the improvement of the marginal aqueous solubility of the lead compounds. Accordingly, we anticipated that the terminal phenyl groups of **3–6** might be replaced with polar, heterocyclic functionality without dramatic loss of activity since, in the X-ray crystal structure of the HIV-1 protease/A-74704 complex, those groups lie close to the ends of the active site cleft.[5] In contrast, the symmetry-related P_1 phenyl and P_2 isopropyl side chains, which are imbedded in lipophilic pockets, might be expected to be less tolerant of polar structural changes. Initial structure–activity studies were conducted in the "mono-ol" series exemplified by **3** due to the availability of greater amounts of synthetic diamine **1**. We chose to vary each of the two terminal phenyl groups independently in order to more accurately guage the effect of a particular structural modification upon aqueous solubility, protease inhibition, and, most importantly, in vitro anti-HIV activity. The synthesis of unsymmetrically substituted analogues **10–17** began with monoprotected diamine **7**, available as the precursor to **1**,[9] and is shown in Scheme I. Coupling of **7** to Cbz-Val-OH and subsequent deblocking of **8** led to the monoacylated diamine **9**. Heterocyclic Cbz replacements were attached to L-valine methyl ester via carbamate or urea linkages as described in the Experimental Section. Following ester hydrolysis, coupling to **9** provided analogues **10–17**. Physical properties of each inhibitor are provided in Table I.

The biological activities and estimated solubilities in pH 7.4 (phosphate) buffer of **3** and **10–17** are shown in Table II. IC_{50} values reflect the activities of the inhibitors against purified HIV protease in a fluorogenic assay.[11] EC_{50} values represent the ability of the compounds to inhibit 50% of the cytopathic effect of HIV-1_{3B} in MT4 lymphocytes in vitro.[12] CC_{50} values reflect the concentration at which 50% of the cultured cells remain viable in the absence of HIV infection. As shown in Table II, replacement of a single terminal phenyl group with a pyridyl residue (compounds **10–12**) resulted in a >20-fold improvement in aqueous solubility. Moreover, we were pleased to find that compounds **10** and **11** maintained all of the inhibitory potency of lead compound **3**, whereas the activity of **12** fell off by a factor of 2. Replacement of the terminal phenyl with nonaromatic heterocycles (compounds **13–17**) was less successful in maintaining activity; however, as expected, inclusion of a more basic nitrogen further improved aqueous solubility. Lead compound **3** was ineffective against HIV in MT4 cells, although it had previously shown submicromolar inhibition in H9 cells.[5] This inconsistency is presumably due to the very low solubility of **3**, since inhibitors **10** and **11** showed anti-HIV activity in the low micromolar range. With the exception of compound **15**, inhibitors containing aliphatic heterocycles did not show significant activity against HIV in vitro.

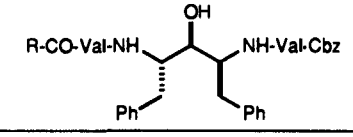
Being unable to achieve aqueous solubilities in excess of 1 μg/mL with a single heterocyclic replacement without sacrificing activity, we turned our attention to inhibitors containing heterocyclic functionality at both termini. In most cases, changes were evaluated in both mono-ol and all three diastereomeric diol series to independently assess the effects of structure upon activity and solubility. The syntheses of these analogues is detailed in Scheme II and is analogous to the preparation of **10–17**. Various end groups were attached either by direct acylation of **1** or **2** with substituted valine residues or, in such cases where racemization might be expected with a substituted valine, by acylation of bis-valinyl derivatives **18a–d**. Physical properties of the resulting inhibitors **19–78** are provided in Table I. Biological activities and estimated solubilities are shown in Table III. As expected, replacement of the phenyl groups in **3** with 2- or 3-pyridinyl (compounds **19** and **23**) had little effect on the inhibition of HIV protease. Moreover, we were gratified that, even with two polar end groups, the anti-HIV activity of **19** and **23** was nearly equivalent to the monopyridinyl inhibitors **10** and **11** and that aqueous solubility was improved to levels well in excess of 1 μg/mL. With diol inhibitors **20–22**, accurate IC_{50} determination at levels less than 1 nM was impossible due to active site titration. However, K_i values for **20–22** were nearly indistinguishable from those of the corresponding diastereomers **10–12**. The estimation of K_i values for selected diol inhibitors and a detailed comparison of the binding affinities of the three diastereomers will be reported separately.[13] The anti-HIV activity of **20–22** paralleled that of **10–12** and reflected the general phenomenon that, between the three diol diastereomers, the highest potency (K_i and anti-HIV) resides in the unsymmetric *R,S*-isomer. The implications of this observation for further inhibitor design are discussed elsewhere.[10,13] As observed with the mono-4-pyridinyl inhibitor **12**, the IC_{50} values of **27–29** were significantly higher than the 2- or 3-pyridinyl analogues against the purified protease, although respectable antiviral activity for each series was still observed. Examination of the estimated solubilities of **19–29** revealed several interesting trends. Whereas the

Table I. Physical Data for HIV Protease Inhibitors

no.	preparation method	mp, °C	R_f (solv)[a]	formula[b]	no.	preparation method	mp, °C	R_f (solv)[a]	formula[b]
3		198–200	0.35 (D)	$C_{43}H_{52}N_4O_7 \cdot 0.5H_2O$	47	C	174–176	0.20 (C)	$C_{44}H_{58}N_8O_6 \cdot H_2O$
4		231–232	0.35 (A)	$C_{44}H_{54}N_4O_8 \cdot 0.25H_2O$	48	C	174–175	0.17 (C)	$C_{44}H_{58}N_8O_6 \cdot H_2O$
5		230–234	0.30 (A)	$C_{44}H_{54}N_4O_8 \cdot 0.5H_2O$	49	C	73–76	0.06 (B)	$C_{46}H_{62}N_8O_6 \cdot H_2O$
6		239–242	0.42 (A)	$C_{44}H_{54}N_4O_8 \cdot H_2O$	50	C	153–155	0.23 (C)	$C_{46}H_{62}N_8O_6 \cdot 0.5H_2O$
10	A	194–195	0.28 (A)	$C_{42}H_{51}N_5O_7 \cdot 0.5H_2O$	51	C	109–111	0.21 (C)	$C_{46}H_{62}N_8O_6 \cdot H_2O$
11	A	177–178	0.21 (A)	$C_{42}H_{51}N_5O_7 \cdot 0.5H_2O$	52	C	67–68	0.61 (D)	$C_{41}H_{56}N_8O_7S_2 \cdot 0.25CHCl_3$[h]
12	A	170–172	0.19 (A)	$C_{42}H_{51}N_5O_7 \cdot 0.5H_2O$	53	C	85–89	0.50 (C)	$C_{42}H_{58}N_8O_6S_2 \cdot H_2O$
13	A	162–163	0.20 (C)	$C_{43}H_{59}N_5O_7 \cdot 0.5H_2O$	54	C	91–93	0.65 (C)	$C_{42}H_{58}N_8O_6S_2 \cdot H_2O$
14	A	160–161	0.37 (C)	$C_{43}H_{59}N_5O_7 \cdot 0.5H_2O$	55	C	75–77	0.54 (C)	$C_{42}H_{58}N_8O_6S_2 \cdot 0.75H_2O$
15	A	159–161	0.34 (B)	$C_{42}H_{57}N_5O_8 \cdot 0.75H_2O$	56	B	187–188	0.44 (C)	$C_{42}H_{58}N_6O_6S_2 \cdot H_2O$
16	A	143–146	0.13 (B)	$C_{42}H_{57}N_5O_7$	57	B	226–227	0.40 (C)	$C_{42}H_{58}N_8O_6S_2$
17	A	175–176	0.15 (B)	$C_{41}H_{56}N_6O_6 \cdot H_2O$	58	B	215–216	0.42 (C)	$C_{42}H_{58}N_6O_6S_2 \cdot 0.5H_2O$
19	B	155–156	0.53 (D)	$C_{41}H_{50}N_6O_7 \cdot 0.5H_2O$	59	B	196–197	0.20 (C)	$C_{42}H_{58}N_8O_6S_2 \cdot 0.5H_2O$
20	C	196–200	0.32 (C)	$C_{42}H_{52}N_6O_8$	60	B	130–131	0.27 (C)	$C_{42}H_{56}N_6O_6S_2 \cdot H_2O$
21	C	238–240	0.23 (A)	$C_{42}H_{52}N_6O_8 \cdot 0.25H_2O$	61	B	127–128	0.31 (C)	$C_{42}H_{58}N_6O_6S_2 \cdot H_2O$
22	C	220–223	0.32 (A)	$C_{42}H_{52}N_8O_8 \cdot 0.5H_2O$	62	D	271–273	0.31 (D)	$C_{43}H_{50}N_6O_5 \cdot 0.75H_2O$[i]
23	B	177–178	0.53 (D)	$C_{41}H_{50}N_8O_7 \cdot 0.5H_2O$	63	D	285–289	0.57 (B)	$C_{44}H_{52}N_6O_6 \cdot 0.75H_2O$
24	C	207–208	0.25 (C)	$C_{42}H_{52}N_6O_8 \cdot 1.25H_2O$	64	D	>260	0.29 (C)	$C_{44}H_{52}N_6O_6 \cdot 1.25H_2O$
25	C	202–207	0.31 (C)	$C_{42}H_{52}N_6O_8 \cdot H_2O$	65	D	265 dec	0.67 (D)	$C_{45}H_{50}N_6O_5 \cdot 2H_2O$[j]
26	C	212–216	0.28 (C)	$C_{42}H_{52}N_6O_8 \cdot H_2O$	66	D	>285	0.57 (C)	$C_{46}H_{52}N_6O_6 \cdot 0.25H_2O$
27	B	158–159	0.44 (D)	$C_{41}H_{50}N_6O_7 \cdot 0.5H_2O$	67	D	82–84	0.68 (D)	$C_{47}H_{50}N_6O_5 \cdot 1.75H_2O$[k]
28	C	221–224	0.11 (C)	$C_{42}H_{52}N_6O_8 \cdot 0.5H_2O$	68	B	115–118	nd	$C_{45}H_{58}N_6O_5 \cdot H_2O$[l]
29	C	190–193	0.25 (C)	$C_{42}H_{52}N_6O_8 \cdot 1.25H_2O$	69	C	92–94	0.33 (C)	$C_{40}H_{54}N_8O_6S_2 \cdot H_2O$
31	B	227–230	0.21 (C)	$C_{42}H_{64}N_6O_{10} \cdot 1.25H_2O$	70	C	165–167	0.31 (C)	$C_{40}H_{54}N_8O_6S_2 \cdot 0.5H_2O$
32	D	243–245	0.17 (C)	$C_{42}H_{64}N_6O_8 \cdot H_2O$	71	C	94–95	0.35 (C)	$C_{40}H_{54}N_8O_6S_2 \cdot 0.5H_2O$
33	D	172–177	0.31 (C)	$C_{30}H_{42}N_4O_6 \cdot 0.5H_2O$	72	C	146–148	0.27 (C)	$C_{40}H_{54}N_8O_6S_2 \cdot 0.5H_2O$
34	D	nd	0.34 (C)	$C_{38}H_{56}N_6O_8 \cdot 0.5H_2O$[c]	73	C	198–200	0.27 (C)	$C_{40}H_{54}N_8O_6S_2$
35	B	172–173	0.45 (C)	$C_{50}H_{66}N_8O_6 \cdot 0.5H_2O$	74	C	176–178	0.29 (C)	$C_{40}H_{54}N_8O_6S_2 \cdot 0.5H_2O$
36	B	178–179	0.11 (A)	$C_{52}H_{70}N_8O_6 \cdot 1.0H_2O$	75	B	nd	0.20 (C)	$C_{48}H_{60}N_{10}O_6 \cdot 2.5H_2O$[m]
37	B	171–173	0.15 (A)	$C_{52}H_{70}N_8O_6 \cdot 1.0H_2O$	76	B	145–146	0.22 (C)	$C_{48}H_{60}N_{10}O_6 \cdot 1.75H_2O$[n]
38	E	198–199	0.46 (E)	$C_{41}H_{52}N_8O_8 \cdot 3H_2O$[d]	77	B	107–109	0.15 (C)	$C_{48}H_{60}N_{10}O_8 \cdot 2H_2O \cdot CHCl_3$
39	D	216–218	0.62 (D)	$C_{43}H_{54}N_6O_5$[e]	78	B	130–134	0.15 (C)	$C_{40}H_{58}N_{10}O_8S_2 \cdot 2.25H_2O$
40	D	234–235	0.20 (D)	$C_{43}H_{54}N_6O_5 \cdot H_2O$	79	F	92–94	0.25 (C)	$C_{44}H_{58}N_8O_6 \cdot 1.5H_2O$
41	D	260–263	0.10 (C)	$C_{44}H_{58}N_6O_6 \cdot 0.75H_2O$[f]	80	F	84–88	0.37 (C)	$C_{42}H_{58}N_8O_6S \cdot 0.5H_2O$
42	B	70–72	0.46 (D)	$C_{43}H_{56}N_8O_5 \cdot 0.2CHCl_3$[g]	81	F	96–97	0.34 (C)	$C_{42}H_{56}N_8O_6S \cdot 0.5H_2O$
43	C	108–111	0.28 (A)	$C_{44}H_{58}N_8O_8 \cdot 1.25H_2O$	82	F	97–99	0.21 (C)	$C_{42}H_{57}N_9O_6S \cdot H_2O$
44	C	159–162	0.36 (A)	$C_{44}H_{58}N_6O_8$	83	F	108–111	0.18 (C)	$C_{40}H_{55}N_9O_6S_2 \cdot 1.5H_2O$
45	C	110–112	0.38 (A)	$C_{44}H_{58}N_8O_6 \cdot H_2O$	84	F	118–120	0.24 (C)	$C_{40}H_{55}N_9O_6S_2 \cdot 0.5CHCl_3$
46	C	95–97	0.31 (C)	$C_{44}H_{58}N_8O_6 \cdot 1.5H_2O$					

[a] Solvent mixtures: A, 5% methanol in chloroform; B, 7.5% methanol in chloroform; C, 10% methanol in chloroform; D, 30% ethyl acetate in chloroform; E, 4.5% methanol/4% isopropylamine in chloroform. [b] Elemental analysis within ±0.4% except where noted. [c] C, H; N: calcd, 11.45; found, 10.91. [d] C, N; H: calcd, 7.39; found 6.74. [e] HRMS: calcd for $C_{43}H_{54}N_6O_5$·Na: 757.4053; found: 757.4073. [f] C, N; H: calcd, 7.68; found, 6.94. [g] C, N; H: calcd, 7.16; found, 6.24. [h] C, N; H: calcd, 6.51; found 6.05. [i] C, N; H: calcd, 6.97; found 6.54. [j] C, H; N: calcd, 10.62; found 11.29. [k] C, H; N: calcd, 10.37; found 11.15. [l] C; H: calcd 7.74; found 7.19; N: calcd, 10.76; found 10.09. [m] C, H; N: calcd, 15.26; found, 14.68. [n] C, H; N: calcd, 15.48; found, 14.45.

Table II. Unsymmetrically Substituted "Mono-ol" Inhibitors of HIV Protease



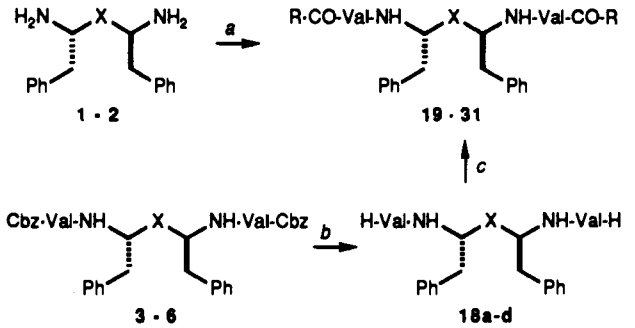
no.	R	IC_{50} (nM)[a]	EC_{50} (μM)[b]	CC_{50} (μM)[c]	solubility (μg/mL)[d]
3	$PhCH_2O$	3	NE[e]	>100	>0.03
10	2-pyridinyl-CH_2O	3	1.5–2.2	>100	0.6
11	3-pyridinyl-CH_2O	4	1.5–2.8	>100	0.9
12	4-pyridinyl-CH_2O	7	1.8–10	>100	0.8
13	(1-Me)piperidin-3-yl-CH_2O	13	NE	17	31
14	(1-Me)piperidin-2-yl-CH_2O	63	nd	nd	19
15	4-morpholinyl-CH_2CH_2O	9	5.4–6.9	>100	6.8
16	1-pyrrolidinyl-CH_2CH_2O	14	NE	21	19
17	(4-Me)piperazin-1-yl	28	NE	55	141

[a] Concentration of test compound at which 50% inhibition of the activity of purified, recombinant HIV-1 protease upon a fluorogenic substrate (ref 11) was observed. [b] Concentration of test compound which inhibited 50% of the cytopathic effect of HIV-1_{3B} in MT-4 lymphocytes in vitro. Values represent the result of a single triplicate assay, using a multiplicity of infection (MOI) of either 0.001 (lower value) or 0.032 (higher value) tissue culture infective dose per cell. [c] Concentration of test compound at which 50% cytotoxicity in MT-4 lymphocytes was observed in vitro, in the absence of HIV infection. [d] Estimated solubility in pH 7.4 (phosphate) buffer. [e] NE: no effect, either up to 100 μM or the cytotoxic concentration.

aqueous solubility of the mono-ol inhibitors declined upon substitution of 2-, 3- and 4-pyridinyl end groups, the corresponding *R,R*-diols showed an enhancement. In each case, the solubility of the *S,S*-diol was substantially lower than that of the *R,R*-diol, reflecting an apparent general phenomenon (vide infra). With the exception of the *S,S*-diols, solubilities in excess of 1 μg/mL were generally achieved without substantial decline in activity.

Use of nonaromatic heterocyclic end groups to further enhance solubility is represented by compounds **30–37**.

Scheme II[a]



[a] (a) RCO-Val-OH (method B or C); (b) H_2, Pd/C; (c) RCO_2H (method D) or $Cl_3COCO_2CCl_3$, $R'NH_2$ (method E).

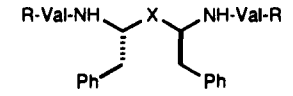
Interestingly, the (4-morpholinyl)ethoxy group, which caused only a 3-fold drop in potency in compound **15**, was clearly deleterious when attached at both ends of mono-ol inhibitor **30**. The corresponding diol **31** showed good solubility, but was less active than **20** by >10-fold. A comparison of **31–34** indicates a preference for a single atom spacer between the heterocyclic and carbonyl groups, in contrast to the trends we have observed with aromatic heterocycles (data not shown). Attachment of lipophilic functionality onto the heterocycle (compounds **35–37**) led to improved potency; however, the favorable combination of solubility and activity observed with the 2- and 3-pyridinyl analogues was not achieved with inhibitors containing nonaromatic heterocycles. We thus focused our attention on the effect of different linking functionality between the heteroaromatic end groups and the symmetry-related P_2-valine residues. Replacement of the carbamate linkages with secondary urea (compound **38**) or amide (compounds **39–41**) functionality resulted in a slight drop in activity, both in protease inhibition and anti-HIV efficacy. The exception was compound **39** which showed excellent activity in vitro in spite of diminished affinity for HIV protease.

A substantial improvement was observed upon modification of the carbamate linkages to *N*-methylurea (compounds **42–48**). Although the potency of mono-ol inhibitor **42** and *R,R*-diols **43** and **46** declined significantly, the antiviral activity of 2-pyridinyl *R,S*- and *S,S*-diols **44** and **45**, respectively, remained high. Most significantly, aqueous solubility was further enhanced by 1 order of magnitude over the corresponding carbamates, with the *S,S*-diol **45** again being the least soluble. The optimal combination of favorable solubility (197 μg/mL) and antiviral activity (0.20 μM) was observed with *R,S*-isomer **44** (A-77003) and became a significant contributing factor in the selection of A-77003 for preclinical development as an intravenous agent.[7] The selective decline in activity of the *R,R*-diastereomer over the *R,S*- and *S,S*-isomers upon changing the linkages from carbamate to *N*-methylurea also suggests the possibility of disparate modes of binding of the three diastereomeric diols to the HIV protease active site. These implications have been discussed in detail elsewhere.[13] Efforts to further enhance solubility while maintaining activity were unsuccessful. The chain-extended compounds **49–51** showed improved solubility, especially for *S,S*-isomer **51**; however, activity decreased by ≥10-fold. Similar potency declines were observed upon changing the *N*-methylurea functionality to *N*-methylsulfamide (**52–55**) or sulfonamide (**56–61**). Compounds **56** and **57**, although maintaining subnanomolar potency in the protease inhibition assay, showed only moderate anti-HIV activity in vitro, presumably reflecting the effect of the polar sulfonamide linkages on the cellular penetration of the inhibitors (vide infra).

The use of a quinoline-2-carbonyl group at the P_3 position has proven very effective in promoting activity of hydroxyethylamine-based HIV protease inhibitors.[14] It was therefore of interest to evaluate the effect of conformational restriction of the end groups upon the potency of present symmetry-based inhibitors. Incorporation of (3-pyridinyl)acryloyl groups enhanced activity in the mono-ol series (compound **62**) but was deleterious to the *R,R*-diol **64**. The corresponding (2-pyridinyl)-acryloyl diol **63** maintained activity, however, perhaps reflecting the difference in length between the mono-ol and diol inhibitors. Incorporation of both quinoline-2-carbonyl and indole-2-carbonyl groups resulted in improved potency in the mono-ol series (compounds **67** and **65**), and the corresponding diol inhibitor **66** exhibited excellent anti-HIV activity (EC_{50} = 50–70 nM). However, a considerable amount of aqueous solubility was sacrificed with these analogues due to the reduced basicity of the heteroaromatic nitrogen atoms, and further structure–activity studies were not pursued. Notably, the conformationally restricted tertiary urea **68** improved substantially on the potency of acyclic urea **42** against HIV protease, although antiviral activity was not observed for **68** due to cellular toxicity at the expected antiviral concentration.

Having determined that carbamate and *N*-methylurea linkages provided the optimal combination of antiviral activity and aqueous solubility, we next directed our attention to other heteroaromatic end groups. Activity in each of the diol diastereomers was maintained by incorporation of 4-thiazolyl (compounds **69–71**) or 2-thiazolyl (compounds **72–74**) groups at the termini. As with previous *N*-methylureas, the potency of the *R,S*- and *S,S*-isomers exceeded that of the *R,R*-diols. Reasonable aqueous solubility was also achieved with these analogues, and the 2-thiazolyl *S,S*-diol **74** was notably more soluble than expected. The more polar benzimidazolyl inhibitors **75–77** exhibited excellent inhibition of HIV protease; however, the anti-HIV activity declined considerably while cellular toxicity increased. A similarly large ratio between the level of inhibitor necessary for inhibition of the enzymatic activity of purified HIV protease and that necessary for antiviral effects in cultured lymphocytes was observed with the polar 2-amino-4-thiazolyl-based inhibitor **78**. This lack of correlation between the two assays has been reported by others and apparently results from decreased cellular penetration by inhibitors containing groups of substantial polarity.[15] To examine this trend in more detail and to gain insight on the contribution of different heterocyclic groups to solubility, we prepared the unsymmetrically substituted inhibitors **79–84**. In order to avoid the issue of regioisomers inherent in the unsymmetric *R,S*-diols and the fall in activity observed with *N*-methylurea derivatives of the *R,R*-diols, we concentrated on the *S,S*-isomer. The "mixed" compounds were conveniently prepared by condensation of the *S,S*-diol **2c** with a limited amount of the *p*-nitrophenyl ester of the desired heterocyclic substituted valine, followed by chromatographic separation of the intermediate monoamine and further acylation with a second end piece. Biological activities and solubilities for **79–84** are presented in Table IV. Compound **79**, containing 2-pyridinyl and 3-pyridinyl groups, retained nearly all of the anti-HIV activity of bis-(2-pyridinyl) inhibitor **45**. Unexpectedly, however, the

Table III. Symmetrically Substituted Inhibitors of HIV Protease[a]



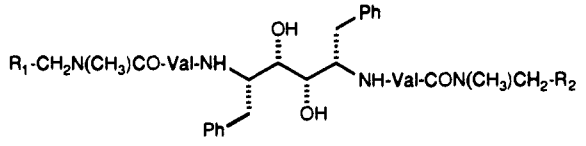
no.	R	X	IC_{50} (nM)	EC_{50} (μM)	CC_{50} (μM)	solubility (μg/mL)
4	$PhCH_2OCO$	(*R*,*R*)-CH(OH)CH(OH)-	<1	0.08–0.11	>100	<0.03
5	$PhCH_2OCO$	(*R*,*S*)-CH(OH)CH(OH)-	<1	0.02–0.09	>100	nd
6	$PhCH_2OCO$	(*S*,*S*)-CH(OH)CH(OH)-	<1	0.05–0.07	>100	nd
19	2-pyridinyl-CH_2OCO	-CH(OH)-	4.8	2.9–4.5	57	51
20	2-pyridinyl-CH_2OCO	(*R*,*R*)-CH(OH)CH(OH)-	<1	0.27–0.45	>100	5.4
21	2-pyridinyl-CH_2OCO	(*R*,*S*)-CH(OH)CH(OH)-	<1	0.05–0.06	>100	2.4
22	2-pyridinyl-CH_2OCO	(*S*,*S*)-CH(OH)CH(OH)-	<1	0.21–0.23	>100	0.21
23	3-pyridinyl-CH_2OCO	-CH(OH)-	6.8	5.0–6.3	62	18
24	3-pyridinyl-CH_2OCO	(*R*,*R*)-CH(OH)CH(OH)-	<1	0.43–0.44	>100	31
25	3-pyridinyl-CH_2OCO	(*R*,*S*)-CH(OH)CH(OH)-	<1	nd	nd	0.50
26	3-pyridinyl-CH_2OCO	(*S*,*S*)-CH(OH)CH(OH)-	<1	nd	nd	0.41
27	4-pyridinyl-CH_2OCO	-CH(OH)-	26	7.5–10	>100	7.8
28	4-pyridinyl-CH_2OCO	(*R*,*R*)-CH(OH)CH(OH)-	1.7	0.53–0.61	>100	55
29	4-pyridinyl-CH_2OCO	(*S*,*S*)-CH(OH)CH(OH)-	2.3	0.47–0.60	>100	0.62
30	4-morpholinyl-CH_2CH_2OCO	-CH(OH)-	180	nd	nd	273
31	4-morpholinyl-CH_2CH_2OCO	(*R*,*R*)-CH(OH)CH(OH)-	20	6.4–14	>100	132
32	4-morpholinyl-CH_2CH_2CO	(*R*,*R*)-CH(OH)CH(OH)-	23	43–53	>100	>1047
33	4-morpholinyl-CH_2CO	(*R*,*R*)-CH(OH)CH(OH)-	7.5	nd	nd	nd
34	4-morpholinyl-CO	(*S*,*S*)-CH(OH)CH(OH)-	43	28–88	>100	nd
35	(4-Ph)piperazin-1-yl-CO	(*R*,*R*)-CH(OH)CH(OH)-	1.4	1.6–1.7	18	1.0
36	(4-$PhCH_2$)piperazin-1-yl-CO	(*R*,*R*)-CH(OH)CH(OH)-	7.8	0.80–4.3	17	9.0
37	(4-$PhCH_2$)piperazin-1-yl-CO	(*S*,*S*)-CH(OH)CH(OH)-	9.0	2.9–9.4	>100	1.7
38	2-pyridinyl-CH_2NHCO	-CH(OH)-	10	6.0–6.1	>100	53
39	2-pyridinyl-CH_2CH_2CO	-CH(OH)-	12	1.2–2.2	>100	nd
40	3-pyridinyl-CH_2CH_2CO	-CH(OH)-	22	10	55	nd
41	3-pyridinyl-CH_2CH_2CO	(*R*,*R*)-CH(OH)CH(OH)-	1.5	7.7–9.5	>100	nd
42	2-pyridinyl-$CH_2N(CH_3)CO$	-CH(OH)-	43	17–25	>100	167
43	2-pyridinyl-$CH_2N(CH_3)CO$	(*R*,*R*)-CH(OH)CH(OH)-	1.66	1.1–1.5	>100	225
44	2-pyridinyl-$CH_2N(CH_3)CO$	(*R*,*S*)-CH(OH)CH(OH)-	<1	0.20–0.27	>100	197
45	2-pyridinyl-$CH_2N(CH_3)CO$	(*S*,*S*)-CH(OH)CH(OH)-	<1	0.17	>100	3.6
46	3-pyridinyl-$CH_2N(CH_3)CO$	(*R*,*R*)-CH(OH)CH(OH)-	1.6	3.8–5.7	>100	338
47	3-pyridinyl-$CH_2N(CH_3)CO$	(*R*,*S*)-CH(OH)CH(OH)-	<1	0.97–1.7	>100	153
48	3-pyridinyl-$CH_2N(CH_3)CO$	(*S*,*S*)-CH(OH)CH(OH)-	<1	0.86–1.4	>100	91
49	2-pyridinyl-$CH_2CH_2N(CH_3)CO$	(*R*,*R*)-CH(OH)CH(OH)-	22	10–13	>100	355
50	2-pyridinyl-$CH_2CH_2N(CH_3)CO$	(*R*,*S*)-CH(OH)CH(OH)-	5.2	2.3–4.2	61	66
51	2-pyridinyl-$CH_2CH_2N(CH_3)CO$	(*S*,*S*)-CH(OH)CH(OH)-	31	7.3–1.2	95	86
52	2-pyridinyl-$CH_2N(CH_3)SO_2$	-CH(OH)-	1300	NE	32	11
53	2-pyridinyl-$CH_2N(CH_3)SO_2$	(*R*,*R*)-CH(OH)CH(OH)-	240	NE	52	23
54	2-pyridinyl-$CH_2N(CH_3)SO_2$	(*R*,*S*)-CH(OH)CH(OH)-	71	NE	56	30
55	2-pyridinyl-$CH_2N(CH_3)SO_2$	(*S*,*S*)-CH(OH)CH(OH)-	38	43	65	40
56	2-pyridinyl-$CH_2CH_2SO_2$	(*R*,*R*)-CH(OH)CH(OH)-	<1	1.7–2.0	>100	26
57	2-pyridinyl-$CH_2CH_2SO_2$	(*R*,*S*)-CH(OH)CH(OH)-	<1	1.5	>100	nd
58	2-pyridinyl-$CH_2CH_2SO_2$	(*S*,*S*)-CH(OH)CH(OH)-	1.6	1.7–1.8	>100	0.047
59	4-pyridinyl-$CH_2CH_2SO_2$	(*R*,*R*)-CH(OH)CH(OH)-	5.7	12–17	>100	5.6
60	4-pyridinyl-$CH_2CH_2SO_2$	(*R*,*S*)-CH(OH)CH(OH)-	24	14–16	>100	32
61	4-pyridinyl-$CH_2CH_2SO_2$	(*S*,*S*)-CH(OH)CH(OH)-	13	17–19	>100	55
62	trans-3-pyridinyl-CH=CHCO	-CH(OH)-	2.2	2.2–2.8	>100	4.9
63	trans-2-pyridinyl-CH=CHCO	(*R*,*R*)-CH(OH)CH(OH)-	<1	0.91–1.1	>100	6.8
64	trans-3-pyridinyl-CH=CHCO	(*R*,*R*)-CH(OH)CH(OH)-	5.3	5.5–6.2	>100	23
65	2-indolyl-CO	-CH(OH)-	1.2	1.6–8.9	58	nd
66	2-indolyl-CO	(*R*,*R*)-CH(OH)CH(OH)-	<1	0.05–0.07	49	nd
67	2-quinolinyl-CO	-CH(OH)-	1.6	1.6–2.1	>100	0.23
68	1,2,3,4-tetrahydroisoquinolin-2-yl-CO	-CH(OH)-	7.1	NE	6.4	nd
69	4-thiazolyl-$CH_2N(CH_3)CO$	(*R*,*R*)-CH(OH)CH(OH)-	<1	0.59–0.63	>100	286
70	4-thiazolyl-$CH_2N(CH_3)CO$	(*R*,*S*)-CH(OH)CH(OH)-	<1	0.18–0.19	>100	159
71	4-thiazolyl-$CH_2N(CH_3)CO$	(*S*,*S*)-CH(OH)CH(OH)-	<1	0.40–0.50	>100	6.5
72	2-thiazolyl-$CH_2N(CH_3)CO$	(*R*,*R*)-CH(OH)CH(OH)-	1.0	1.5–1.7	>100	137
73	2-thiazolyl-$CH_2N(CH_3)CO$	(*R*,*S*)-CH(OH)CH(OH)-	<1	0.24–0.39	>100	101
74	2-thiazolyl-$CH_2N(CH_3)CO$	(*S*,*S*)-CH(OH)CH(OH)-	<1	0.21–0.39	>100	57
75	2-benzimidazolyl-$CH_2N(CH_3)CO$	(*R*,*R*)-CH(OH)CH(OH)-	<1	4.9–5.0	55	12
76	2-benzimidazolyl-$CH_2N(CH_3)CO$	(*R*,*S*)-CH(OH)CH(OH)-	<1	1.5–1.7	27	6.6
77	2-benzimidazolyl-$CH_2N(CH_3)CO$	(*S*,*S*)-CH(OH(CH(OH)-	<1	1.7–1.9	31	18
78	2-aminothiazol-4-yl-$CH_2N(CH_3)CO$	(*R*,*S*)-CH(OH)CH(OH)-	<1	1.8–2.3	>100	184

[a] See Table I for definitions.

aqueous solubility of **79** exceeded that of even the bis(3-pyridinyl) analogue **48** by more than 4-fold. In contrast, although excellent antiviral activity was observed with mixed 2-pyridinyl/4-thiazolyl and 2-pyridinyl/2-thiazolyl analogues **80** and **81**, respectively, solubility enhancement above the corresponding bis(thiazolyl) inhibitors was not observed. Indeed, the estimated solubility of **80** was lower than either of the "parent" inhibitors **45** or **71**. Excellent solubility was obtained with each of the mixed 2-amino-4-thiazolyl analogues **82–84**. In analogy to **78**, however, attenuation of the antiviral activity of **82–84** was observed due to increased polarity, since each inhibited HIV protease activity to an extent equal to that of **80** and **81**.

The improved aqueous solubility of the present het-

Table IV. Unsymmetrically Substituted HIV Protease Inhibitors[a]



no.	R_1	R_2	IC_{50} (nM)	EC_{50} (μM)	CC_{50} (μM)	solubility (μg/mL)
79	2-pyridinyl	3-pyridinyl	<1	0.27–0.48	>100	420
80	2-pyridinyl	4-thiazolyl	<1	0.17–0.20	>100	1.0
81	2-pyridinyl	2-thiazolyl	<1	0.17–0.22	>100	10.9
82	2-pyridinyl	2-aminothiazol-4-yl	<1	0.56	>100	386
83	2-thiazolyl	2-aminothiazol-4-yl	<1	0.83–1.6	>100	196
84	4-thiazolyl	2-aminothiazol-4-yl	<1	1.1–1.5	>100	331

[a] See Table I for definitions.

Table V. Pharmacokinetic Parameters for HIV Protease Inhibitors Following a 5 mg/kg Intravenous or 10 mg/kg Oral Dose in Rats[a]

no.	$t_{1/2}$ (h)	C_{max} (μM)	bioavailability (%)
17	1.1	0.25	13
19	1.9	1.6	19
23	0.31	0.22	2.1
27	0.57	0.20	0.9
42	0.45	1.5	11
52	1.4	2.1	21
48	1.3	0.52	17
55	1.5	1.16	11

[a] Test compounds were administered as 5 mg/mL solutions in 20% ethanol/5% glucose in water containing 2 molar equiv of HCl, with the exception of compound 17, which contained 1 molar equiv of HCl, and compound 48, which contained 2 molar equivalents of methanesulfonic acid. $t_{1/2}$ values represent the terminal half-life after intravenous administration at 5 mg/kg (n = 4). C_{max} values represent the maximum concentration observed after oral administration at 10 mg/kg (n = 4). Percent bioavailability is defined as $AUC_{oral}/2 \times AUC_{iv}$ over the period of 0–6 h.

erocyclic analogues enabled the pharmacokinetic evaluation of many of the compounds in rats. Previously we reported the substantial difference in pharmacokinetic properties between the representatives of the mono-ol and diol classes of inhibitors.[7] Table V lists the pharmacokinetic parameters for six mono-ol inhibitors which have undergone evaluation. Terminal elimination half-lives ($t_{1/2}$) were calculated following a 5 mg/kg intravenous dose; Maximum plasma concentration (C_{max}) and absolute bioavailability were determined following a 10 mg/kg oral dose. In all, four out of the six mono-ol inhibitors showed modest, but significant oral bioavailability (>10%). A preference for 2-pyridinyl substitution over 3- or 4-pyridinyl substitution for oral absorption was observed with this series, as indicated by the significant difference between **19** (19%), **23** (2.1%), and **27** (0.9%). The results in Table V show little effect of the nature of the linking substituent between the 2-pyridinyl groups and the symmetry-related P_2 valine residues, since carbamate **19**, *N*-methylurea **42** and *N*-methylsulfamide **52** each showed C_{max} values in excess of 1.5 μM with substantial absolute bioavailability. Interestingly, although the bioavailability of **19**, **23**, and **27** paralleled their respective solubility values, the solubility of sulfamide **52** was in the same range as **23** and **27**, indicating that factors other than solubility are responsible in part for determining systemic availability. Inhibitor **17**, with high aqueous solubility despite a single polar functionality, also showed reasonable oral bioavailability (13%). In this case, however, absorption was recorded over a much longer period of time, and levels in excess of 0.25 μM were not obtained. In contrast to the mono-ol inhibitors, out of a total of 24 inhibitors of the diol class, including exact analogues of **19**, **42**, and **52**, only two provided significant plasma levels after oral administration in rats (>5% bioavailability) (Table V). Notably, both **48** (17%) and **55** (11%) are *S,S*-diols with appreciable solubility, which led transiently to speculation that absorption of many of the *S,S*-diols might be solubility limited. Compounds **74** and **82** provided, however, examples of equally soluble *S,S*-isomers which were very poorly absorbed.

Conclusion

An important goal in the structure–activity studies on the lead compounds **3–6** was the identification of analogues which retained or improved activity against purified HIV protease and efficacy against HIV induced cytopathic effect in vitro while improving aqueous solubility to levels suitable for pharmacokinetic evaluation in vivo. The results presented here show that solubility can be enhanced considerably without dramatic sacrifice in activity by the introduction of moderately polar, heterocyclic groups at one or both ends of the inhibitors. If the lipophilicity of the inhibitors is excessively attenuated by substitution of more polar groups, however, drastic reductions in anti-HIV activity are observed, apparently due to insufficient cellular penetration. Of the compounds presented here, **44** (A-77003), **70**, **73**, and **79** provided the best balance of activity and solubility. Unfortunately, none of these compounds were orally bioavailable in the rat. Substantial levels (>1 μM) of four other inhibitors (**19**, **42**, **52**, and **55**) were obtained following oral dosing; however, in no case did these levels exceed the concentrations required for effective anti-HIV activity in vitro. In the absence of reliable and inexpensive animal models for AIDS, one must assume that in vivo levels of inhibitor equal to or in excess of in vitro EC_{50} values would be advantageous. As a result, the potential for in vivo efficacy by oral administration is questionable. The combination of good aqueous solubility and antiviral activity of several of the present compounds, however, leaves open the possibility for clinical efficacy via intravenous therapy. Clinical investigations of intravenous A-77003 for HIV infection are currently underway.

Experimental Section

Melting points are uncorrected. ^{1}H NMR spectra were measured on a GE QE-300 (300 MHz) instrument using tetramethylsilane as an internal standard. ^{1}H NMR spectra, mass spectra, and elemental analyses were performed by the Structural Chemistry Department, Abbott Laboratories. Flash column chromatography was performed on silica gel 60, 0.04–0.063 mm (E. Merck). Thin-layer chromatography was performed on precoated silica gel F-254 plates (0.25 mm; E. Merck) and was visualized with phosphomolybdic acid.

4-[*N*-[*N*-[(Benzyloxy)carbonyl]valinyl]amino]-2-[*N*-[(*tert*-butyloxy)carbonyl]amino]-1,5-diphenyl-3-hydroxypen-

tane (8). A solution of 2.24 g (8.92 mmol) of Cbz-Val-OH, 3.00 g (8.11 mmol) of 7,[9] and 1.31 g (9.73 mmol) of 1-hydroxybenzotriazole (HOBT) in 30 mL of dimethylformamide (DMF) was treated sequentially with 0.89 mL (8.1 mmol) of 4-methylmorpholine and 1.87 g (9.73 mmol) of *N*-ethyl-*N'*-[(dimethylamino)propyl]carbodiimide hydrochloride (EDC). After being stirred at ambient temperature overnight, the solution was diluted with ethyl acetate, washed sequentially with 10% citric acid, water, and aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated in vacuo. Flash chromatography using ethyl acetate in chloroform gave 4.20 g (86%) of 8 (R_f 0.30) as a white solid: mp 187–187.5 °C; MS *m/z* 604 $(M + H)^+$.

2-Amino-4-[*N*-[*N*-[(benzyloxy)carbonyl]valinyl]amino]-1,5-diphenyl-3-hydroxypentane (9). Compound 8 (2.90 g, 4.81 mmol) was treated with 20 mL of 4 M HCl in dioxane. After being stirred at ambient temperature for 45 min, the solution was concentrated in vacuo, taken up into $CHCl_3$, washed with 1 N NaOH and H_2O, dried over Na_2SO_4, and concentrated to 2.43 g (100%) of 9 (R_f 0.3; 2.5% methanol/2% isopropylamine/chloroform): mp 158–160 °C; MS *m/z* 504 $(M + H)^+$.

***N*-Carbonylvaline Methyl Ester.** A suspension of L-valine methyl ester hydrochloride (10 g) in toluene (400 mL) was heated to 100 °C and phosgene gas was bubbled into the reaction mixture. After approximately 6 h, the mixture became homogeneous. The bubbling of phosgene was continued for an additional 10 min, then the solution was cooled with the bubbling of N_2 gas. The solvent was evaporated and the residue chased two times with toluene and concentrated to provide crude *N*-carbonylvaline methyl ester.

Representative Procedure for Carbamate Formation. *N*-[[(4-Pyridinyl)methoxy]carbonyl]valine Methyl Ester. A solution of 0.73 g (4.65 mmol) of *N*-carbonylvaline methyl ester and 0.51 g (4.65 mmol) of pyridine-4-methanol in 30 mL of toluene was heated at reflux under N_2 atmosphere for 4 h. The solvent was removed in vacuo, and the residue was purified by silica gel chromatography using 2% methanol in chloroform to give 1.01 g (82%) of the product ester: ^1H NMR ($CDCl_3$) δ 0.91 (d, J = 7 Hz, 3 H), 0.99 (d, J = 7 Hz, 3 H), 2.19 (m, 1 H), 3.76 (s, 3 H), 4.31 (dd, J = 9, 5 Hz, 1 H), 5.12 (s, 2 H), 5.37 (br d, 1 H), 7.25 (d, J = 6 Hz, 2 H), 8.60 (d, J = 6 Hz, 2 H).

***N*-[(2-Pyridinylmethoxy)carbonyl]valine methyl ester:** ^1H NMR ($CDCl_3$) δ 0.91 (d, J = 7 Hz, 3 H), 0.98 (d, J = 7 Hz, 3 H), 2.19 (m, 1 H), 3.75 (s, 3 H), 4.32 (dd, J = 9, 5 Hz, 1 H), 5.24 (s, 2 H), 5.39 (br d, 1 H), 7.23 (ddd, J = 8, 4, 1 Hz, 1 H), 7.37 (d, J = 8 Hz, 1 H), 7.70 (td, J = 8, 2 Hz, 1 H), 8.60 (br d, 1 H).

***N*-[(3-Pyridinylmethoxy)carbonyl]valine methyl ester:** ^1H NMR ($CDCl_3$) δ 0.90 (d, J = 7 Hz, 3 H), 0.98 (d, J = 7 Hz, 3 H), 2.16 (m, 1 H), 3.65 (s, 3 H), 4.30 (dd, J = 9, 5 Hz, 1 H), 5.14 (s, 2 H), 5.30 (br d, 1 H), 7.30 (dd, J = 8, 5 Hz, 1 H), 7.70 (br d, J = 8 Hz, 1 H), 8.58 (dd, J = 4, 1 Hz, 1 H), 8.63 (br s, 1 H).

***N*-[[(1-Methylpiperidin-3-yl)methoxy]carbonyl]valine methyl ester:** ^1H NMR ($CDCl_3$) δ 0.90 (d, J = 7 Hz, 3 H), 0.96 (d, J = 7 Hz, 3 H), 1.55–1.75 (m, 5 H), 1.8–2.0 (m, 2 H), 2.15 (m, 1 H), 2.26 (s, 3 H), 2.7–2.9 (m, 2 H), 3.74 (s, 3 H), 3.89 (m, 1 H), 4.00 (m, 1 H), 4.28 (dd, J = 10, 5 Hz, 1 H), 5.06 (br d, J = 10 Hz, 1 H).

***N*-[[(1-Methylpiperidin-2-yl)methoxy]carbonyl]valine methyl ester:** ^1H NMR ($CDCl_3$) δ 0.90 (d, J = 7 Hz, 3 H), 0.96 (d, J = 7 Hz, 3 H), 1.2–1.35 (m, 1 H), 1.45–1.8 (m, 5 H), 2.0–2.2 (m, 3 H), 2.32 (s, 3 H), 2.88 (br d, 1 H), 3.73 (s, 3 H), 4.03 (m, 2 H), 4.27 (dd, J = 9, 5 Hz, 1 H), 5.33 (br d, J = 9 Hz, 1 H).

***N*-[[[2-(4-Morpholinyl)ethyl]oxy]carbonyl]valine methyl ester:** ^1H NMR ($CDCl_3$) δ 0.89 (d, J = 7 Hz, 3 H), 0.96 (d, J = 7 Hz, 3 H), 2.16 (m, 1 H), 2.50 (br t, 4 H), 2.62 (t, J = 6 Hz, 2 H), 3.72 (t, J = 6 Hz, 4 H). 3.75 (s, 3 H), 4.20 (br t, 2 H), 4.37 (dd, J = 9, 5 Hz, 1 H), 5.25 (br d, 1 H).

***N*-[[[2-(1-Pyrrolidinyl)ethyl]oxy]carbonyl]valine methyl ester:** ^1H NMR ($CDCl_3$) δ 0.90 (d, J = 7 Hz, 3 H), 0.96 (d, J = 7 Hz, 3 H), 1.80 (m, 4 H), 2.15 (m, 1 H), 2.57 (m, 4 H), 2.63 (t, J = 6 Hz, 2 H), 3.74 (s, 3 H), 4.20 (br t, 2 H), 4.28 (dd, J = 9, 5 Hz, 1 H), 5.30 (br d, 1 H).

Representative Procedure for Urea Formation. *N*-[(1-Methylpiperazin-4-yl)carbonyl]valine Methyl Ester. A solution of 0.86 g (5.47 mmol) of *N*-carbonylvaline methyl ester in 10 mL of dichloromethane was treated with 0.73 mL (6.6 mmol) of 1-methylpiperazine. The resulting solution was stirred at ambient temperature for 2.5 h, after which it was concentrated in vacuo. The residue was purified by silica gel chromatography using 5% methanol in chloroform to provide 1.40 g (100%) of the product ester: ^1H NMR ($CDCl_3$) δ 0.91 (d, J = 7 Hz, 3 H), 0.95 (d, J = 7 Hz, 3 H), 2.13 (m, 1 H), 2.31 (s, 3 H), 2.41 (t, J = 5 Hz, 4 H), 3.43 (m, 4 H), 3.74 (s, 3 H), 4.46 (dd, J = 9, 5 Hz, 1 H), 4.93 (br d, 1 H).

***N*-[(4-Morpholin-1-yl)carbonyl]valine methyl ester:** ^1H NMR ($CDCl_3$) δ 0.91 (d, J = 7 Hz, 3 H), 0.96 (d, J = 7 Hz, 3 H), 2.14 (m, 1 H), 3.39 (m, 4 H), 3.70 (m, 4 H), 3.75 (s, 3 H), 4.45 (dd, J = 8, 5 Hz, 1 H), 4.95 (br d, J = 8 Hz, 1 H).

***N*-[(4-Phenylpiperazin-1-yl)carbonyl]valine methyl ester:** ^1H NMR ($CDCl_3$) δ 0.93 (d, J = 7 Hz, 3 H), 0.97 (d, J = 7 Hz, 3 H), 2.15 (m, 1 H), 3.20 (dd, J = 6, 5 Hz, 4 H), 3.58 (m, 4 H), 3.74 (s, 3 H), 4.48 (dd, J = 8, 5 Hz, 1 H), 5.00 (br d, J = 8 Hz, 1 H), 6.90 (t, J = 7 Hz, 1 H), 6.93 (d, J = 7 Hz, 1 H), 7.29 (m, 2 H).

***N*-[[*N*-Methyl-*N*-(2-pyridinylmethyl)amino]carbonyl]-valine Methyl Ester.** A solution of 21.2 g (97 mmol) of di-*tert*-butyl dicarbonate in 200 mL of CH_2Cl_2 was cooled to 0 °C and treated in portions with 10 mL (97 mmol) of 2-(aminomethyl)-pyridine. After being allowed to warm to ambient temperature and stirred overnight, the resulting solution was diluted with CH_2Cl_2, washed with three portions of water, dried over Na_2SO_4, and concentrated in vacuo to provide 19.8 g (98%) of 2-[[*N*-(*tert*-butyloxycarbonyl)amino]methyl]pyridine (R_f 0.28, 5% methanol in chloroform). The crude carbamate was taken up in anhydrous tetrahydrofuran (THF), cooled under N_2 atmosphere to 0 °C, and treated with 4.95 g (124 mmol) of sodium hydride (60% dispersion in oil). The solution was stirred for 15 min, treated dropwise with 7.1 mL (114 mmol) of methyl iodide, stirred at ambient temperature for 2 h, and quenched cautiously with water. The resulting mixture was partitioned between ether and water, dried over Na_2SO_4, and concentrated. Chromatography on silica gel provided 14.9 g (70%) of 2-[[*N*-(*tert*-butyloxycarbonyl)-*N*-methylamino]methyl]pyridine. A portion (3.05 g, 13.7 mmol) was treated with 30 mL of 4 N HCl in dioxane and heated at 40 °C for 0.5 h. After removal of the solvent, the residue was partitioned between $CHCl_3$ and 1 N NaOH, dried over Na_2SO_4, and concentrated to give 2-[(*N*-methylamino)methyl]pyridine. The crude amine was allowed to react with *N*-carbonylvaline methyl ester as above to provide 1.94 g (96%) of the product ester: ^1H NMR ($CDCl_3$) δ 0.93 (d, J = 7 Hz, 3 H), 0.97 (d, J = 7 Hz, 3 H), 2.16 (m, 1 H), 3.03 (s, 3 H), 3.72 (s, 3 H), 4.43 (dd, J = 8, 5 Hz, 1 H), 4.55 (s, 2 H), 6.15 (br, 1 H), 7.22 (dd, J = 8, 6 Hz, 1 H), 7.28 (d, J = 6 Hz, 1 H), 7.69 (br t, 1 H), 8.55 (d, J = 5 Hz, 1 H).

***N*-[[*N*-Methyl-*N*-(3-pyridinylmethyl)amino]carbonyl]-valine Methyl Ester.** 3-[[(*N*-Methylamino)methyl]pyridine was prepared as above and condensed with *N*-carbonylvaline methyl ester: ^1H NMR ($CDCl_3$) δ 0.90 (d, J = 7 Hz, 3 H), 0.96 (d, J = 7 Hz, 3 H), 2.16 (m, 1 H), 2.93 (s, 3 H), 3.74 (s, 3 H), 4.49 (dd, J = 9, 5 Hz, 1 H), 4.54 (s, 2 H), 4.95 (br d, J = 9 Hz, 1 H), 7.28 (td, J = 6, 1 Hz, 1 H), 7.61 (ddd, J = 7, 3, 2 Hz, 1 H), 8.53 (m, 2 H); MS *m/z* 280 $(M + H)^+$.

***N*-[[*N*-Methyl-*N*-[2-(2-pyridinyl)ethyl]amino]carbonyl]-valine methyl ester:** ^1H NMR ($CDCl_3$) δ 0.95 (br t, 6 H), 2.12 (m, 1 H), 2.87 (s, 3 H), 3.06 (m, 2 H), 3.62 (dt, J = 14, 7 Hz, 1 H), 3.70 (s, 3 H), 3.81 (dt, J = 14, 7 Hz, 1 H), 4.38 (dd, J = 9, 6 Hz, 1 H), 5.74 (br, 1 H), 7.16 (m, 2 H), 7.60 (br t, 1 H), 8.54 (br d, 1 H).

***N*-[(1,2,3,4-tetrahydroisoquinolin-2-yl)carbonyl]valine methyl ester:** ^1H NMR ($CDCl_3$) δ 0.94 (d, J = 7 Hz, 3 H), 0.97 (d, J = 7 Hz, 3 H), 2.17 (m, 1 H), 2.90 (m, 2 H), 3.60 (ddd, J = 13, 7, 6 Hz, 1 H), 3.71 (ddd, J = 13, 7, 6 Hz, 1 H), 3.75 (s, 3 H), 4.51 (dd, J = 9, 5 Hz, 1 H), 4.59 (s, 2 H), 4.99 (br d, J = 9 Hz, 1 H), 7.18 (m, 4 H).

***N*-[[*N*-Methyl-*N*-(4-thiazolylmethyl)amino]carbonyl]-valine Methyl Ester.** Aqueous methylamine (100 mL, 40% by weight) was treated with 1.1 g (6.5 mmol) of 4-(chloromethyl)-thiazole hydrochloride.[16] The resulting solution was stirred at ambient temperature for 15 min, concentrated, taken up in 5% methanol in chloroform, dried over Na_2SO_4, and concentrated to provide 0.81 g (97%) of 4-[(*N*-methylamino)methyl]thiazole. A solution of the above amine, 1.0 g (6.3 mmol) of *N*-carbonylvaline methyl ester, and 0.76 mL (7 mmol) of 4-methylmorpholine in 20 mL of dichloromethane was stirred at ambient temperature

for 16 h. The resulting solution was concentrated, and the residue was purified by silica gel chromatography using methanol/chloroform to provide 1.5 g (83%) of the product ester: ^{1}H NMR ($CDCl_3$) δ 0.92 (d, J = 7 Hz, 3 H), 0.97 (d, J = 7 Hz, 3 H), 2.16 (m, 1 H), 3.03 (s, 3 H), 3.73 (s, 3 H), 4.43 (dd, J = 8, 5 Hz, 1 H), 4.61 (s, 2 H), 5.66 (br, 1 H), 7.22 (d, J = 2 Hz, 1 H), 8.79 (d, J = 2 Hz, 1 H).

***N*-[[*N*-Methyl-*N*-(2-thiazolylmethyl)amino]carbonyl]-valine Methyl Ester.** A mixture of 2.0 g (17.7 mmol) of thiazole-2-carboxaldehyde,[17] 4.78 g (71 mmol) of methylamine hydrochloride, 4.36 g (53 mmol) of NaOAc, and 1.67 g (27 mmol) of $NaCNBH_3$ in 50 mL of 2-propanol was stirred at ambient temperture for 3 days. The resulting mixture was concentrated in vacuo, and the residue was taken up in ethyl acetate and extracted with saturated aqueous $NaHCO_3$. The aqueous layer was concentrated in vacuo to a small volume, saturated with NaCl, and extracted with 10% methanol in chloroform until no product remained in the aqueous layer by TLC. The combined organic layers were dried over Na_2SO_4 and concentrated. Silica gel chromatography using methanol in chloroform provided 0.4 g (18%) of 2-[(*N*-methylamino)methyl]thiazole, which was condensed with *N*-carbonylvaline methyl ester as described above to provide the product ester: ^{1}H NMR ($CDCl_3$) δ 0.92 (d, J = 7 Hz, 3 H), 0.97 (d, J = 7 Hz, 3 H), 2.16 (m, 1 H), 3.05 (s, 3 H), 3.73 (s, 3 H), 4.47 (dd, J = 8, 5 Hz, 1 H), 4.78 (AA', J = 16 Hz, 2 H), 5.38 (br d, J = 8 Hz, 1 H), 7.31 (d, J = 4 Hz, 1 H), 7.73 (d, J = 4 Hz, 1 H).

***N*-[[*N*-Methyl-*N*-(2-benzimidazolylmethyl)amino]carbonyl]valine methyl ester:** ^{1}H NMR ($CDCl_3$) δ 0.93 (d, J = 7 Hz, 3 H), 0.98 (d, J = 7 Hz, 3 H), 2.18 (m, 1 H), 3.06 (s, 3 H), 3.77 (s, 3 H), 4.44 (dd, J = 8, 5 Hz, 1 H), 4.65 (AA', J = 15 Hz, 2 H), 5.03 (br d, J = 8 Hz, 1 H), 7.25 (dd, J = 6, 3 Hz, 2 H), 7.57 (dd, J = 6, 3 Hz, 1 H).

***N*-[[*N*-Methyl-*N*-[[2-[*N*-[(*tert*-butyloxy)carbonyl]amino]-4-thiazolyl]methyl]amino]carbonyl]valine Methyl Ester.** *N*-[[*N*-methyl-*N*-[(2-amino-4-thiazolyl)methyl]amino]carbonyl]-valine methyl ester was prepared from 2-amino-4-(chloromethyl)-thiazole hydrochloride[18] using the procedure described above. The resulting ester (1.0 g, 3.3 mmol) in 40 mL of CH_2Cl_2 was treated with 0.87 g (4 mmol) of di-*tert*-butyl dicarbonate and 50 mg of 4-(dimethylamino)pyridine. After being stirred for 3 days, the solution was washed with 10% citric acid, dried over Na_2SO_4, concentrated, and purified by silica gel chromatography to provide 0.65 g (49%) of the product ester: H NMR ($CDCl_3$) δ 0.93 (d, J = 7 Hz, 3 H), 0.97 (d, J = 7 Hz, 3 H), 1.55 (s, 9 H), 2.14 (m, 1 H), 2.97 (s, 3 H), 3.73 (s, 3 H), 4.22 (d, J = 16 Hz, 1 H), 4.46 (dd, J = 8, 5 Hz, 1 H), 4.50 (d, J = 16 Hz, 1 H), 6.27 (br, 1 H), 6.70 (s, 1 H), 8.27 (br, 1 H).

Representative Procedure for Sulfamide Preparation. *N*-[[*N*-Methyl-*N*-(2-pyridinylmethyl)amino]sulfonyl]-valine Methyl Ester. A solution of 15.3 g (90 mmol) of L-valine methyl ester hydrochloride and 22 mL (270 mmol) of sulfuryl chloride in 45 mL of acetonitrile was heated at reflux for 16 h. The resulting solution was concentrated in vacuo to provide crude *N*-(chlorosulfonyl)valine methyl ester, a portion (3.2 g, 14 mmol) of which was combined with 2.75 g (14 mmol) of [(*N*-methylamino)methyl]pyridine dihydrochloride in 100 mL of CH_2Cl_2, cooled to 0 °C, and treated dropwise with 6 mL (50 mmol) of 4-methylmorpholine. After 2 h, the mixture was partitioned between CH_2Cl_2 and aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated in vacuo. Chromatography on silica gel using 30% ethyl acetate in $CHCl_3$ provided 1.7 g (40%) of nearly pure product ester: ^{1}H NMR ($CDCl_3$) δ 0.95 (d, J = 7 Hz, 3 H), 1.03 (d, J = 7 Hz, 3 H), 2.15 (m, 1 H), 2.80 (s, 3 H), 3.76 (s, 3 H), 3.95 (dd, J = 9, 5 Hz, 1 H), 4.50 (d, J = 16 Hz, 1 H), 4.59 (d, J = 16 Hz, 1 H), 6.40 (br d, J = 10 Hz, 1 H), 7.35 (br d, J = 7 Hz, 1 H), 7.71 (m, 1 H), 8.54 (m, 1 H).

Representative Procedure for Sulfonamide Preparation. *N*-[[2-(2-pyridinyl)ethyl]sulfonyl]valine Benzyl Ester. A stirred suspension of 10 g (52 mmol) of 2-pyridineethanesulfonic acid in 40 mL of phosphorus oxychloride was treated under Ar atmosphere over a period of 3 min with 12.32 g (58 mmol) of phosphorus pentachloride. The resulting mixture was heated at 60 °C for 2 h, cooled to 0 °C, and triturated with CCl_4. The mixture was filtered, and the residue was washed with CCl_4, CH_3CN, and anhydrous ether, and air-dried to give 11.3 g (89%) of 2-pyridineethanesulfonyl chloride hydrochloride. A solution of 3.0 g (7.9 mmol) of L-valine benzyl ester *p*-toluenesulfonate in 105 mL of dichloromethane was cooled to 0 °C and treated sequentially with 1.99 g (8.2 mmol) of the sulfonyl chloride and 3.3 mL (23 mmol) of triethylamine. After being stirred for 25 min, a small additional portion of sulfonyl chloride was added to complete the reaction. After 15 additional min, the solution was concentrated in vacuo, and the residue was taken up in dichloromethane. The organic solution was washed with H_2O, and the aqueous layer was back-extracted with 3 portions of dichloromethane. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Chromatography on silica gel using 2% methanol in dichloromethane provided the product ester: ^{1}H NMR ($CDCl_3$) δ 0.86 (d, J = 7 Hz, 3 H), 1.01 (d, J = 7 Hz, 3 H), 2.19 (m, 1 H), 3.30 (m, 2 H), 3.46 (m, 2 H), 4.01 (dd, J = 10, 5 Hz, 1 H), 5.09 (br d, J = 10 Hz, 1 H), 5.19 (s, 2 H), 7.17 (m, 2 H), 7.34 (m, 5 H), 7.62 (td, J = 7, 2 Hz, 1 H), 8.51 (m, 1 H).

***N*-[[2-(4-Pyridinyl)ethyl]sulfonyl]valine methyl ester:** ^{1}H NMR ($CDCl_3$) δ 0.85 (d, J = 7 Hz, 3 H), 1.03 (d, J = 7 Hz, 3 H), 2.23 (m, 1 H), 3.0–3.2 (m, 4 H), 4.01 (dd, J = 10, 5 Hz, 1 H), 4.95 (br d, J = 10 Hz, 1 H), 5.20 (s, 2 H), 7.07 (m, 2 H), 7.33 (s, 5 H), 8.53 (br d, J = 6 Hz, 1 H).

Representative Procedures for Methyl Ester Hydrolysis. *N*-[(2-Pyridinylmethoxy)carbonyl]valine. A solution of 434 mg (1.60 mmol) of *N*-[(2-pyridinylmethoxy)carbonyl]valine methyl ester in 6 mL of dioxane was treated with 6.4 mL of 0.5 M aqueous LiOH. After being stirred for 2 h, the solution was treated with 3.2 mL of 1 M HCl, concentrated to a small aqueous volume, and extracted with two portions of ethyl acetate. The combined organic layers were dried over Na_2SO_4 and concentrated to provide *N*-[(2-pyridinylmethoxy)carbonyl]valine as a white solid.

***N*-[[*N*-Methyl-*N*-(4-thiazolylmethyl)amino]carbonyl]-valine.** A solution of 1.5 g (5.3 mmol) of *N*-[[*N*-Methyl-*N*-[(4-thiazolylmethyl)amino]carbonyl]valine methyl ester in 21 mL of dioxane was treated with 21 mL of 0.5 M aqueous LiOH and stirred for 2 h. The resulting solution was neutralized with 1 M HCl, concentrated to near dryness, taken up in chloroform, dried over Na_2SO_4, and concentrated to provide the product acid as a white solid.

Representative Procedure of Benzyl Ester Hydrogenolysis. *N*-[[2-(2-Pyridinyl)ethyl]sulfonyl]valine. A mixture of 7.9 mmol of *N*-[[2-(2-pyridinyl)ethyl]sulfonyl]valine benzyl ester in 0.3 g of 10% palladium on carbon in 100 mL of methanol was stirred vigorously under H_2 atmosphere for 2.5 h. The resulting mixture was filtered and concentrated in vacuo to provide 2.23 g (98%) of *N*-[[2-(2-pyridinyl)ethyl]sulfonyl]valine.

3-(4-Morpholinyl)propanoic Acid. A solution of 4.9 mL (56 mmol) of morpholine in 50 mL of dichloromethane was treated dropwise with 5.0 mL (56 mmol) of methyl acrylate. The resulting solution was allowed to stand at ambient temperature for 3 days, after which it was concentrated to an oil. Chromatography on silica gel using methanol/isopropylamine/chloroform provided 9.54 g (99%) of methyl 3-(4-morpholinyl)propanoate: ^{1}H NMR ($CDCl_3$) δ 2.45 (m, 4 H), 2.51 (m, 2 H), 2.69 (m, 2 H), 3.69 (s, 3 H), 3.70 (m, 4 H). A portion of the above ester (8.35 g, 48.3 mmol) in 60 mL of dioxane was treated with 40 mL of water and 19.3 mL (58 mmol) of 3 N aqueous NaOH. After being stirred for 4 h, the solution was neutralized with 1 N HCl and concentrated in vacuo to provide crude 3-(4-morpholinyl)-propanoic acid which was used without further purification.

2-(1-Morpholinyl)acetic Acid. A solution of 1.5 mL (17 mmol) of morpholine in 40 mL of CH_2Cl_2 was treated with 1 mL (6.3 mmol) of benzyl 2-bromoacetate. The resulting solution was stirred at ambient temperature for 16 h, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography using $CHCl_3$/ethyl acetate to provide 1.35 g (91%) of benzyl 2-(1-morpholinyl)acetate: ^{1}H NMR ($CDCl_3$) δ 2.59 (m, 4 H), 3.27 (s, 2 H), 3.77 (m, 4 H), 5.17 (s, 2 H), 7.3–7.4 (m, 5 H). The above ester (0.84 g) was taken up in 25 mL of methanol, treated with 0.2 g of 10% palladium on carbon, and stirred under one H_2 atmosphere for 40 h. The resulting solution was filtered through Celite and concentrated to provide 0.51 g (98%) of 2-(1-morpholinyl)acetic acid as a white solid.

***trans*-3-(2-Pyridinyl)acrylic Acid.** A solution of 0.43 g (10.7 mmol) of sodium hydride (60% oil dispersion) in anhydrous THF was cooled under N_2 atmosphere to 0 °C and treated dropwise

with 2.1 mL (10.5 mmol) of triethyl phosphonoacetate. After being sitrred for 10 min, the solution was treated with 1.0 mL of pyridine-2-carboxaldehyde, heated at reflux for 2 h, cooled, partitioned between ether and aqueous ammonium chloride, washed sequentially with water and saturated brine, dried over $MgSO_4$, and concentrated. Silica gel chromatography of the residue using ethyl acetate/hexane provided 1.54 g (83%) of *trans*-ethyl 3-(2-pyridinyl)acrylate: ^1H NMR ($CDCl_3$) δ 1.34 (t, J = 7 Hz, 3 H), 4.28 (q, J = 7 Hz, 2 H), 6.92 (d, J = 15 Hz, 1 H), 7.27 (ddd, J = 8, 5, 2 Hz, 1 H), 7.43 (dt, J = 8, 1 Hz, 1 H), 7.69 (d, J = 15 Hz, 1 H), 7.71 (td, J = 8, 2 Hz, 1 H), 8.66 (dm, 1 H). A solution of 1.36 g (8.2 mmol) of the above ester in 33 mL of dioxane was treated with 33 mL of 0.5 M LiOH. The resulting solution was stirred for 2 h, neutralized with 1 N HCl, concentrated to a volume of 20 mL, and extracted with five 10-mL portions of chloroform. The combined organic layers were dried over Na_2SO_4 and concentrated to give 1.13 g (94%) of *trans*-3-(2-pyridinyl)acrylic acid as a white solid. In the same manner, *trans*-3-(3-pyridinyl)acrylic acid was prepared from pyridine-3-carboxaldehyde.

3-(3-Pyridinyl)propanoic Acid. A mixture of 3 g (20 mmol) of 3-(3-pyridinyl)acrylic acid and 0.3 g of 10% palladium on carbon in 150 mL of ethyl acetate was shaken under 4 atmospheres of hydrogen for 24 h. After filtration, the resulting solution was concentrated to provide 3-(3-pyridinyl)propanoic acid. In the same manner, 3-(2-pyridinyl)propanoic acid was prepared.

Representative Procedure for Preparing Cbz-Valine-Containing Inhibitors. (2*S*,3*R*,4*R*,5*S*)-2,5-Bis[*N*-[*N*-[(benzyloxy)carbonyl]valinyl]amino]-3,4-dihydroxy-1,6-diphenylhexane (4). A solution of 2.5 g (4.8 mmol) of (2*S*,3*R*,4*R*,5*S*)-2,5-diamino-3,4-dihydroxy-1,6-diphenylhexane (**2a**)[9] and 6.0 g (16 mmol) of Cbz-L-valine *p*-nitrophenyl ester in 40 mL of tetrahydrofuran was stirred at ambient temperature for 24 h. The resulting thick mixture was treated with 40 mL of THF and 20 mL of 3 N NaOH, stirred for 3 h, and concentrated in vacuo to a volume of 20 mL. The precipitate was filtered and washed sequentially with aqueous NaOH (until white), H_2O, and diethyl ether. The partially dry solid was then taken up in 10% methanol in chloroform, dried over Na_2SO_4, and concentrated to provide 2.66 g (75%) of 4. Prepared by this method were compounds 3–6.

Representative Procedure for Preparation of 18a–d. (2*S*,3*R*,4*R*,5*S*)-2,5-Bis(*N*-valinylamino)-3,4-dihydroxy-1,6-diphenylhexane (18b). A mixture of 2.21 g of 4 and 0.55 g of 10% palladium on carbon in 150 mL of methanol was shaken under 4 atmospheres of hydrogen for 4 h. The resulting mixture was filtered through Celite and concentrated in vacuo to provide **18b** (R_f 0.07, 10% methanol in chloroform) as a white solid: mp 205–207 °C; Mass spectrum $(M + H)^+$ = 499.

Representative Procedures for Heterocyclic Inhibitor Formation. Method A. (3*S*)-2-[*N*-[*N*-[(Benzyloxy)carbonyl]valinyl]amino]-4-[*N*-[*N*-[(4-pyridinylmethoxy)carbonyl]valinyl]amino]-1,5-diphenyl-3-hydroxypentane (12). A solution of 0.191 mmol of *N*-[(4-pyridinylmethoxy)carbonyl]-valine, 94 mg (0.97 mmol) of **9**, and 31 mg (0.23 mmol) of HOBT in 2 mL of DMF was treated under N_2 atmosphere with 44 mg (0.23 mmol) of EDC and stirred overnight at ambient temperature. The resulting solution was taken up in ethyl acetate, washed sequentially with aqueous $NaHCO_3$, H_2O, and saturated brine, dried over Na_2SO_4, and concentrated in vacuo. Chromatography on silica gel using 3% methanol in chloroform provided 119 mg (87%) of **12**.

Method B. (2*S*,3*R*,4*R*,5*S*)-2,5-Bis[*N*-[*N*-[[[2-(4-morpholinyl)ethyl]oxy]carbonyl]valinyl]amino]-3,4-dihydroxy-1,6-diphenylhexane (31). A solution of 0.55 mmol of *N*-[[[2-(4-morpholinyl)ethyl]oxy]carbonyl]valine, 60 mg (0.20 mmol) of **2a**, and 80 mg (0.59 mmol) of HOBT in 1.5 mL of DMF was treated under N_2 atmosphere with 150 mg (0.78 mmol) of EDC and stirred overnight at ambient temperature. The resulting solution was taken up in ethyl acetate, washed sequentially with aqueous $NaHCO_3$, H_2O, and saturated brine, dried over Na_2SO_4, and concentrated in vacuo. Chromatography on silica gel using 5% methanol in chloroform provided 77 mg (47%) of **31**.

Method C. (2*S*,3*R*,4*R*,5*S*)-2,5-Bis[*N*-[*N*-[(2-pyridinylmethoxy)carbonyl]valinyl]amino]-3,4-dihydroxy-1,6-diphenylhexane (20). A solution of 219 mg (0.87 mmol) of *N*-[(2-pyridinylmethoxy)carbonyl]valine and 133 mg (0.96 mmol) of *p*-nitrophenol in 4 mL of THF and 2 mL of DMF was treated with 183 mg (0.96 mmol) of EDC and stirred at ambient temperature. After 4 h, the solvent was removed in vacuo, and the residue was partially purified by silica gel chromatography using 20% ethyl acetate in chloroform to give 0.34 g of *N*-[(2-pyridinylmethoxy)carbonyl]valine *p*-nitrophenyl ester. A solution of 1.04 g (2.8 mmol) of the above ester and 326 mg (1.1 mmol) of **2a** in 15 mL of THF was stirred at ambient temperature for 16 h. The resulting solution was diluted with H_2O, basified to pH 12 with aqueous NaOH, stirred for 1 h, diluted with ethyl acetate, and washed with aqueous K_2CO_3 until the washes were colorless. The organic layer was dried over Na_2SO_4 and concentrated. Chromatography on silica gel using 2% methanol in chloroform followed by 3.5% methanol in chloroform provided 655 mg (78%) of **20**.

Method D. (2*S*,3*R*,4*R*,5*S*)-2,5-Bis[*N*-[*N*-[3-(4-morpholinyl)propanoyl]valinyl]amino]-3,4-dihydroxy-1,6-diphenylhexane (32). A solution of 0.64 mmol of 3-(4-morpholinyl)-propanoic acid, 106 mg (0.214 mmol) of diamine **18b**, 87 mg (0.64 mmol) of HOBT, and 94 μL (0.86 mmol) of 4-methylmorpholine in 2 mL of DMF was treated with 164 mg (0.86 mmol) of EDC and stirred for 18 h at ambient temperature. The resulting solution was diluted with ethyl acetate, washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated. Silica gel chromatography using 5% methanol in chloroform provided 101 mg (61%) of **32**.

Method E. 2,4-Bis[*N*-[*N*-[[*N*-(2-pyridinylmethyl)amino]-carbonyl]valinyl]amino]-1,5-diphenyl-3-hydroxypentane (38). A solution of 34 mg of triphosgene in 2 mL of THF was cooled under N_2 atmosphere to −78 °C and treated over a period of 2 min with a precooled (−78 °C) solution of 80 mg of 18a and 40 μL of 4-methylmorpholine in 1 mL of THF. After 30 min, the solution was treated with a solution of 37 mg of 2-(aminomethyl)-pyridine and 40 μL of 4-methylmorpholine in 1 mL of THF. The resulting solution was allowed to warm to −10 °C over a 2-h period, after which it was concentrated in vacuo. Silica gel chromatography of the residue using a gradient of 3% methanol in chloroform/4.5% methanol/4.5% isopropylamine in chloroform provided 80 mg (64%) of **38**.

(2*S*,3*R*,4*S*,5*S*)-2,5-Bis[*N*-[*N*-[[*N*-methyl-*N*-[(2-amino-4-thiazolyl)methyl]amino]carbonyl]valinyl]amino]-3,4-dihydroxy-1,6-diphenylhexane (78). A solution of 0.17 g (0.16 mmol) of (2*S*,3*R*,4*S*,5*S*)-2,5-bis[*N*-[*N*-[[*N*-methyl-*N*-[[2-[[(*tert*-butyloxy)carbonyl]amino]-4-thiazolyl]methyl]amino]carbonyl]-valinyl]amino]-3,4-dihydroxy-1,6-diphenylhexane (prepared according to method B above) was taken up in 1 mL of CH_2Cl_2, treated with 1 mL of trifluoroacetic acid, stirred for 2 h, concentrated in vacuo, and partitioned between aqueous $NaHCO_3$ and 10% methanol in chloroform. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Silica gel chromatography of the residue using methanol/chloroform mixtures provided 62 mg (43%) of **78**.

Representative Procedure for Monoacylation of Diamine 2c. (2*S*,3*S*,4*S*,5*S*)-5-Amino-2-[*N*-[*N*-[[*N*-methyl-*N*-(2-pyridinylmethyl)amino]carbonyl]valinyl]amino]-3,4-dihydroxy-1,6-diphenylhexane. Diamine **2c** (0.39 g, 1.3 mmol) was treated with 10 mL of DMF and heated to 60 °C to effect complete dissolution. The resulting solution was treated dropwise with a solution of 1.43 mmol of *N*-[[*N*-methyl-*N*-(2-pyridinylmethyl)-amino]carbonyl]valine *p*-nitrophenyl ester (formed according to method C above) in 5 mL of DMF. After being stirred for 1 h, the solution was diluted with ethyl acetate, washed with 1 N NaOH until colorless, dried over Na_2SO_4, and concentrated. Silica gel chromatography using methanol/chloroform mixtures provided 282 mg (40%) of the product amine (R_f 0.13, 10% methanol in chloroform).

(2*S*,3*S*,4*S*,5*S*)-5-Amino-2-[*N*-[*N*-[[*N*-methyl-*N*-(4-thiazolylmethyl)amino]carbonyl]valinyl]amino]-3,4-dihydroxy-1,6-diphenylhexane: 32% yield, R_f 0.21 (10% methanol in chloroform).

(2*S*,3*S*,4*S*,5*S*)-5-Amino-2-[*N*-[*N*-[[*N*-methyl-*N*-(2-thiazolylmethyl)amino]carbonyl]valinyl]amino]-3,4-dihydroxy-1,6-diphenylhexane: 54% yield, R_f 0.20 (10% methanol in chloroform).

Representative Procedures for Preparation of Unsymmetrically Substituted Inhibitors. Method F. (2*S*,3*S*,4*S*,5*S*)-2-[*N*-[*N*-[[*N*-Methyl-*N*-(2-pyridinylmethyl)amino]carbonyl]-

valinyl]amino]-5-[*N*-[*N*-[[*N*-methyl-*N*-(3-pyridinylmethyl)amino]carbonyl]valinyl]amino]-3,4-dihydroxy-1,6-diphenylhexane (79). A solution of 60 mg (0.11 mmol) of (2*S*,3*S*,4*S*,5*S*)-5-amino-2-[*N*-[*N*-[[*N*-methyl-*N*-(2-pyridinylmethyl)amino]carbonyl]valinyl]amino]-3,4-dihydroxy-1,6-diphenylhexane in 1 mL of THF was treated with 0.13 mmol of *N*-[(3-pyridinylmethoxy)carbonyl]valine *p*-nitrophenyl ester (formed according to method C above) and stirred at ambient temperature for 18 h. The resulting solution was treated with 1 N NaOH, stirred for 1 h, extracted with chloroform, dried over Na_2SO_4, and concentrated. Silica gel chromatography using methanol/chloroform mixtures provided 64 mg (73%) of **79**.

Method G. (2*S*,3*S*,4*S*,5*S*)-2-[*N*-[*N*-[[*N*-Methyl-*N*-(2-pyridinylmethyl)amino]carbonyl]valinyl]amino]-5-[*N*-[*N*-[[*N*-methyl-*N*-[(2-amino-4-thiazolyl)methyl]amino]carbonyl]valinyl]amino]-3,4-dihydroxy-1,6-diphenylhexane (82). A solution of 100 mg (0.18 mmol) of (2*S*,3*S*,4*S*,5*S*)-5-amino-2-[*N*-[*N*-[[*N*-methyl-*N*-(2-pyridinylmethyl)amino]-3,4-dihydroxy-1,6-diphenylhexane, 78 mg (0.20 mmol) of *N*-[[*N*-methyl-*N*-[[2-[[(*tert*-butyloxy)carbonyl]amino]-4-thiazolyl]-methyl]amino]carbonyl]valine, 27 mg (0.20 mmol) of HOBT, and 22 μL (0.20 mmol) of 4-methylmorpholine in 2 mL of DMF was treated with 42 mg (0.22 mmol) of EDC and stirred at ambient temperature for 18 h. The resulting solution was diluted with ethyl acetate, washed with aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated. The crude residue was taken up in 1 mL of CH_2Cl_2, treated with 1 mL of trifluoroacetic acid, and stirred for 3 h. After removal of the solvent, the residue was purified by silica gel chromatography using methanol/chloroform to provide 53 mg (35%) of **82**.

Biological Evaluation. MT4 cells were obtained through the AIDS Research and Reference Reagent Program, AIDS Program, National Institute of Allergy and Infectious Diseases. Inhibition of purified recombinant HIV-1 protease using the fluorogenic substrate DABCYL-Ser-Gln-Asp-Tyr-Pro-Ile-Val-Gln-EDANS[11] and inhibition of the cytopathic effect HIV-1_{3B} in MT4 cells were determined as described previously.[7] Estimated solubilities in pH 7.4 phosphate buffer were determined as previously described.[7] Pharmacokinetic studies in rats were performed in groups of eight, with four rats receiving a 5 mg/kg intravenous dose and four receiving a 10 mg/kg oral dose. Details have been provided elsewhere.[7]

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Supplementary Material Available: ^1H NMR spectra for compounds **3–6, 17, 19–23, 27, 42–45, 48, 52, 55, 70, 73,** and **79** (21 pages). Ordering information is given on any current masthead page.

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