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Design of Orally Bioavailable, Symmetry-Based Inhibitors of HIV Protease

Dale J. Kempf,* Kennan C. Marsh, Lynnmarie Codacovi Fino, Pamela Bryant, Adrienne Craig-Kennard, Hing L. Sham, Chen Zhao, Sudthida Vasavanonda, William E. Kohlbrenner, Norman E. Wideburg, Ayda Saldivar, Brian E. Green, Thomas Herrin and Daniel W. Norbeck

Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL 60064, U.S.A.

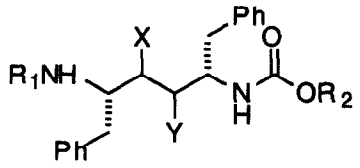
Abstract—A series of novel inhibitors of HIV-1 protease with excellent oral bioavailability is described. Differential acylation of the two amino groups of symmetry-based diamine core groups **2–5** led to unsymmetrically substituted inhibitors **17–43**, many of which inhibited HIV protease at subnanomolar concentrations. Anti-HIV activity *in vitro* was observed at 0.1–1 μM. A systematic evaluation of the pharmacokinetic behavior of these inhibitors in rats identified the influence of aqueous solubility, molecular size and hydrogen-bonding functionality. Compound **30** (A-80987) was selected for further evaluation based on a favorable C_{max}/ED_{50} ratio (> 20) and half-life (> 2 h).

Introduction

The aspartic proteinase encoded by the *pol* gene of the human immunodeficiency virus type 1 (HIV-1) protease has been the subject of intense scrutiny because of its essential role in HIV replication. In the life cycle of HIV, the structural proteins of the viral core and the essential viral enzymes are translated from the *gag* and *pol* genes as single (*gag* and *gag-pol*) polyprotein gene products. For newly formed HIV particles to mature into an infectious form, a series of post-translational proteolytic cleavages, mediated by HIV protease, are required.[1] Inhibitors of HIV protease, which block *gag* and *gag-pol* polyprotein processing, have been shown to be effective in preventing the spread of HIV *in vitro*.[2,3] The inhibition of HIV protease thus represents an attractive therapeutic strategy for the clinical intervention of acquired immunodeficiency syndrome (AIDS), and several compounds have entered human trials.[4–9] In general, however, clinical development of HIV protease inhibitors has been hampered by the poor physicochemical properties and low oral bioavailability of many inhibitors due to their peptidomimetic character.[9–11] The discovery of compounds with improved pharmacokinetic properties would overcome a major obstacle to the development of these agents. Furthermore, a more general understanding of the structural properties which affect the oral bioavailability of HIV protease inhibitors may broadly impact the use of peptidomimetic agents in diverse therapeutic areas.

Structurally, HIV protease has been shown to exist as a C_2-symmetric homodimer with a single active site. The wide availability of three-dimensional structural information on HIV-protease/inhibitor complexes from X-ray crystallographic studies has made HIV protease an attractive target for structure-based drug design.[9,10,12] We recently described several series of novel inhibitors derived from the C_2-symmetric or pseudo-C_2-symmetric diamines **1–4**, designed to match the unique symmetry characteristics of the HIV protease active site.[5,13–15] Symmetry-based inhibitors, being intrinsically less peptide-like, might be expected to display pharmacokinetic properties superior to analogous inhibitors derived from substrate sequences. In our early work, we were tantalized by the observation that diaminoalcohol inhibitors typified by A-75912 (**6**), while significantly less potent than analogous diaminodiols **7–9**, demonstrated consistently superior oral bioavailability in animal studies.[5,15] In order to examine the structural basis for this difference, we studied inhibitors derived from the diaminoalcohol **5**, which contains the molecular framework of the more potent diols but lacks the second hydroxyl group.[16,17] Although the initial inhibitors in this series (**10, 11**) were poorly bioavailable in rats, inhibitory potency was improved over **7–9**, an observation recently explained on the basis of X-ray crystal structures of **7–10** bound to HIV protease.[18] The increased potency of inhibitors derived from **5** prompted us to examine unsymmetrically substituted derivatives of **2–5** with reduced molecular weight in hopes of identifying compounds with improved pharmacokinetic properties. Structural leads for size reduction included Boc protected diamines **12–15**[14,16] and (3-pyridinyl)carbamate **16**, the latter of which showed both potency and oral bioavailability (*ca* 20 % in rats) equivalent to that of **6**. Accordingly, we combined the end groups of symmetric inhibitors **7–11** and **12–16** to design asymmetric 'hybrid' analogues **17–43** (Table 1) which contained the longer substituted P_2 aminoacyl residue attached to one amino group of **2–5** and the shorter carbamate groups present in **12–16** at the other terminus. Here we report that **17–43** represent a novel series of HIV protease inhibitors with potent anti-HIV activity *in vitro* and substantial oral bioavailability in animal models. Furthermore, the relationship of structural features of **17–43** to pharmacokinetic behavior provides insight which may have broader application to the development of peptidomimetics as therapeutic agents.

Table 1. Structure and activity of unsymmetrically substituted inhibitors of HIV protease



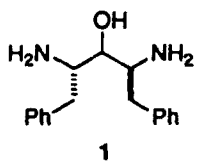
No.	R_1[a]	X	Y	R_2[a]	IC_{50} (nM)	K_i (nM)	EC_{50} (μM)	$CCIC_{50}$ (μM)
10					< 1	0.09	0.24-0.25	>100
11					< 1	0.02	0.01-0.14	44
12[b]	Boc	*R*-OH	*R*-OH	*t*-Bu	40		nd	nd
13[b]	Boc	*S*-OH	*S*-OH	*t*-Bu	280		nd	nd
14[b]	Boc	*R*-OH	*S*-OH	*t*-Bu	12		6.5-12	48
15[c]	Boc	*S*-OH	H	*t*-Bu	5		5.7-5.8	18
16	3-Pyr-CH_2OCO-	*S*-OH	H	3-Pyr-CH_2	3.0		1.7-2.8	>100
17	2-Pyr-CH_2OCO-Val-	*R*-OH	*R*-OH	3-Pyr-CH_2	2.2		1.8-2.1	>100
18	2-Pyr-$CH_2N(CH_3)CO$-Val-	*S*-OH	*S*-OH	3-Pyr-CH_2	5.2		3.2-5.2	>100
19	2-Pyr-CH_2OCO-Val-	*S*-OH	*S*-OH	3-Pyr-CH_2	4.7		2.3	>100
20	2-Pyr-$CH_2N(CH_3)CO$-Val-	*R*-OH	*S*-OH	*t*-Bu	< 1	0.75	0.42-0.51	60
21	2-Pyr-$CH_2N(CH_3)CO$-Val-	*S*-OH	*R*-OH	*t*-Bu	1.5	3.9	1.7-1.9	54
22	2-Pyr-CH_2OCO-Val-	*S*-OH	*R*-OH	*t*-Bu	2.4		2.3-2.5	>100
23	2-Pyr-$CH_2N(CH_3)CO$-Val-	*R*-OH	*S*-OH	3-Pyr-CH_2	1.3		0.26-0.73	>100
24	2-Pyr-$CH_2N(CH_3)CO$-Val-	*S*-OH	*R*-OH	3-Pyr-CH_2	< 1		0.73-0.81	>100
25	2-Pyr-CH_2OCO-Val-	*R*-OH	*S*-OH	3-Pyr-CH_2	< 1		0.14-0.23	>100
26	2-Pyr-CH_2OCO-Val-	*S*-OH	*R*-OH	3-Pyr-CH_2	< 1		0.71-0.85	>100
27	2-Pyr-$CH_2N(CH_3)CO$-Val-	*S*-OH	H	*t*-Bu	< 1		0.62-0.63	60
28	2-Pyr-$CH_2N(CH_3)CO$-Val-	*S*-OH	H	3-Pyr-CH_2	< 1	0.75	0.74-0.82	>100
29	2-Pyr-$CH_2N(CH_3)CO$-Val-	H	*S*-OH	3-Pyr-CH_2	< 1	0.33	0.55-0.61	>100
30	2-Pyr-CH_2OCO-Val-	*S*-OH	H	3-Pyr-CH_2	< 1	0.25	0.13-0.25	>100
31	2-Pyr-CH_2OCO-Val-	H	*S*-OH	3-Pyr-CH_2	< 1		0.23-0.55	64
32	2-Pyr-$CH_2N(CH_3)CO$-Ile-	*S*-OH	H	3-Pyr-CH_2	< 1	0.69	0.58-0.81	60
33	2-Pyr-$CH_2N(CH_3)CO$-Ile-	H	*S*-OH	3-Pyr-CH_2	< 1	0.19	0.38-0.55	>100
34	2-Pyr-CH_2OCO-Ile-	*S*-OH	H	3-Pyr-CH_2	< 1	0.17	0.27-0.57	63
35	2-Pyr-CH_2OCO-Ile-	H	*S*-OH	3-Pyr-CH_2	< 1		0.20-0.23	87
36	2-Pyr-$CH_2N(CH_2CH_3)CO$-Val-	*S*-OH	H	3-Pyr-CH_2	2.0		1.7-3.6	59
37	2-Pyr-$CH_2N(CH_2CH_3)CO$-Val-	H	*S*-OH	3-Pyr-CH_2	< 1		0.53-0.77	>100
38	3-Pyr-CH_2OCO-Val-	*S*-OH	H	3-Pyr-CH_2	< 1		0.14-0.32	59
39	3-Pyr-CH_2OCO-Val-	H	*S*-OH	3-Pyr-CH_2	< 1		0.11-0.13	56
40	(6-Me)-2-Pyr-$CH_2N(CH_3)CO$-Val-	*S*-OH	H	3-Pyr-CH_2	< 1		0.31-0.35	81
41	(6-Me)-2-Pyr-$CH_2N(CH_3)CO$-Val-	H	*S*-OH	3-Pyr-CH_2	< 1		0.22-0.29	59
42	(6-Me)-2-Pyr-CH_2OCO-Val-	*S*-OH	H	3-Pyr-CH_2	< 1		0.082-0.114	>100
43	(6-Me)-2-Pyr-CH_2OCO-Val-	H	*S*-OH	3-Pyr-CH_2	< 1		0.026-0.075	>100

[a]Pyr = pyridinyl, Me = methyl; [b]Reference 14; [c]Reference 16.

Results and Discussion

Chemistry

Syntheses of diaminodiols **2–4** have been reported previously.[19] (3*S*)-Diaminoalcohol **5** was prepared from *N*-protected (3*R*,4*R*)-diol **44** as shown in Scheme I. Previously we reported that treatment of **44** with α-acetoxyisobutyryl bromide[20] in acetonitrile resulted in cyclization to the oxazolidinone **45** via opening of the activated cyclic acetal **46** by the neighboring Cbz carbonyl oxygen.[19] Formation of **45** could be nearly completely suppressed in favor of the competing intermolecular displacement by bromide ion either by addition of lithium bromide to the reaction mixture in acetonitrile or by reducing the solvent polarity. Thus, use of 2:1 CH_2Cl_2:hexane provided the desired bromoacetate **47** almost exclusively. Reductive debromination and complete hydrolysis of the resulting acetate **48** led to diamine **5**.[16]



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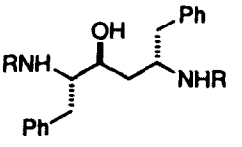
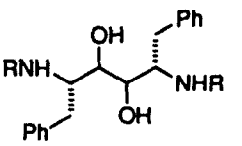
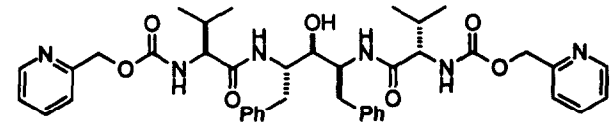


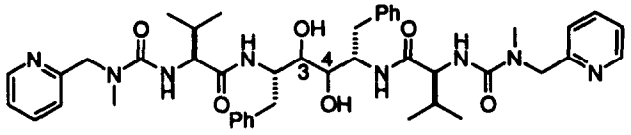
5, R = H
15, R = Boc
16, R = CO_2CH_2(3-pyr)



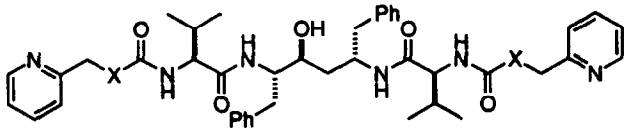
2, 3*R*,4*R*, R = H
3, 3*S*,4*S*, R = H
4, 3*R*,4*S*, R = H
12, 3*R*,4*R*, R = Boc
13, 3*S*,4*S*, R = Boc
14, 3*R*,4*S*, R = Boc



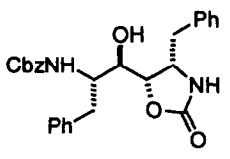
6, A-75912



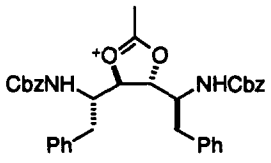
7, A-76889, 3*R*,4*R*
8, A-76928, 3*S*,4*S*
9, A-77003, 3*R*,4*S*



10, A-78791, X = NCH_3
11, A-78624, X = O

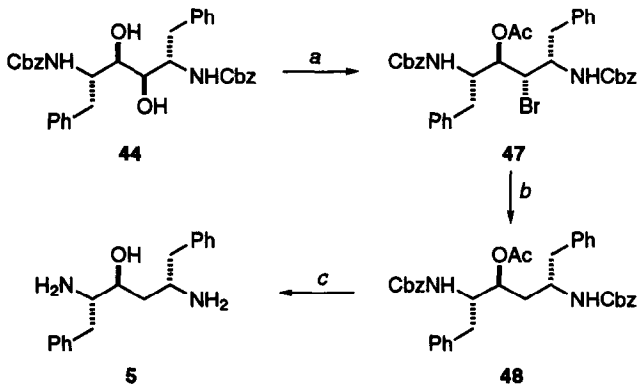


45



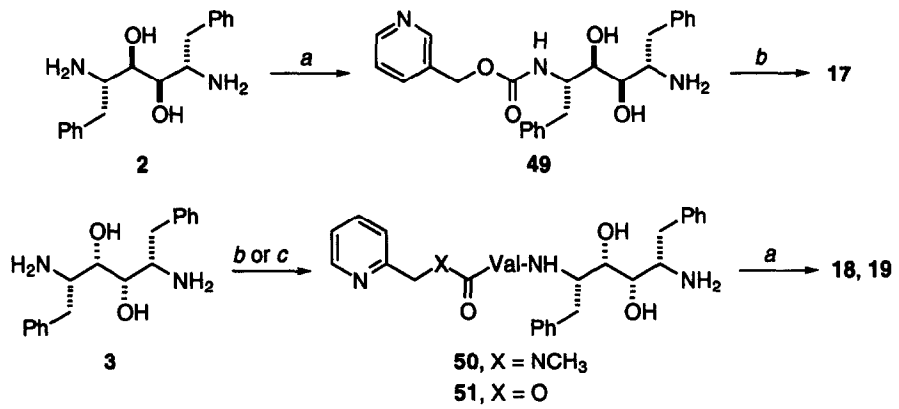
46

Conversion of diamines **2**–**5** to the unsymmetrically substituted protease inhibitors required differentiation of the two amino groups. Statistical monoacylation of **2** and **3** was accomplished by reaction with a limited amount of (3-pyridinyl)methyl *p*-nitrophenyl carbonate or *N*-substituted valine *p*-nitrophenyl esters to generate mixtures of monacylated (**49**–**51**) and diacylated products along with the recovered diamines (Scheme II). Inhibitors **17**–**19** were prepared in straightforward manner by subsequent acylation of the remaining free amine of **49**–**51**. Monofunctionalization of pseudosymmetric diamines **4** and **5** was more complicated due to the regioisomeric nature of the two products resulting from acylation at the two nonidentical amino groups. Regioselection in the reaction of **5** with either (3-pyridinyl)methyl *p*-nitrophenyl carbonate or *N*-[[*N*-methyl-*N*-[(2-pyridinyl)methyl]amino]-carbonyl]valine *p*-nitrophenyl ester[15] was low, leading to a *ca* 1:2 mixture of **52**:**53** and **54**:**55**, respectively, along with diacylated products (Scheme III). The identity of **54** and **55** was established from the 2D NMR spectra, in which protons at C_2 and C_5 were distinguished on the basis of chemical shift, coupling to the amide NH, and coupling to the upfield C_4 methylene protons. Further acylation of **54** and **55** with (3-pyridinyl)methyl p-nitrophenyl carbonate led to **28** and **29**, respectively. Alternately, **28** and **29** were prepared by acylation of **53** and **52**, respectively with *N*-[[*N*-methyl-*N*-[(2-pyridinyl)methyl]-amino]carbonyl]valine *p*-nitrophenyl ester, which served to confirm the structures of **52** and **53**.

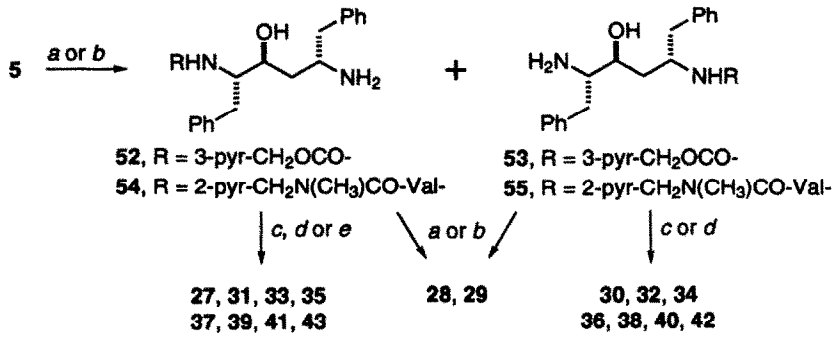


Scheme I. *a*, α-acetoxyisobutyryl bromide, 2:1 CH_2Cl_2:hexane; *b*, $(n\text{-}Bu)_3SnH$, AIBN, THF; *c*, $Ba(OH)_2, H_2O$, dioxane.

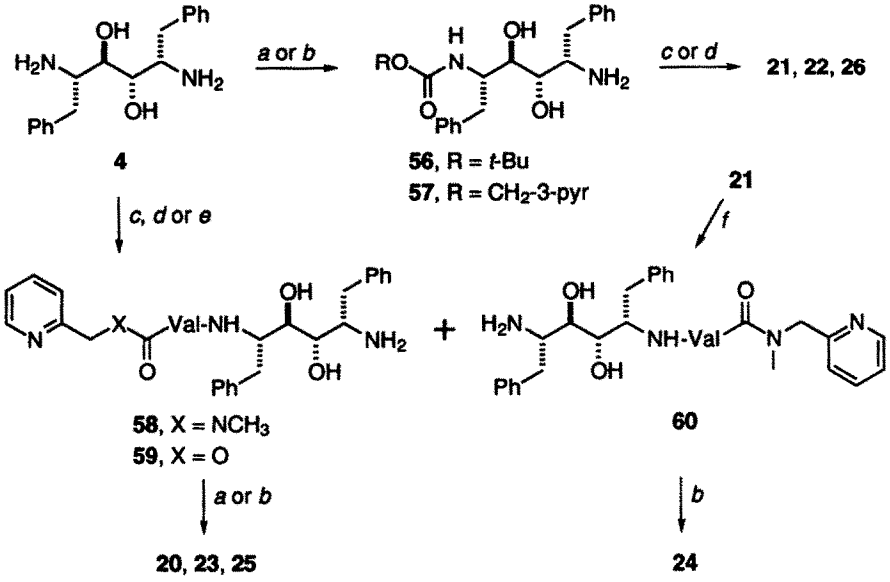
In contrast to **5**, monoacylation of (3*R*,4*S*)-diaminodiol **4** under most conditions (*p*-nitrophenyl esters, *p*-nitrophenyl-carbonates and di-*t*-butyldicarbonate), occurred primarily at the 2-amino group to give **56**–**59** as the only isolated monoacyl products (Scheme IV). Carbodiimide coupling to **5** was less selective and led to a *ca* 2:1 mixture of **58** and **60**. The regioisomeric identity of **56**, which was established by single crystal X-ray analysis, was correlated to minor isomer **60** by conversion to inhibitor **21** and removal of the Boc group, thus establishing that the regioselectivity for the acylation of **4** was the same with both activated esters and carbonates. Thus, by simple selection of the order of acylating agents, the



Scheme II. *a*, [(3-pyridinyl)methyl]-(4-nitrophenyl)carbonate, THF; *b*, *N*-[(2-pyridinyl)methoxycarbonyl]valine *p*-nitrophenyl ester, THF; *c*, *N*-[[*N*-methyl-*N*-[(2-pyridinyl)methyl]amino]carbonyl]valine *p*-nitrophenyl ester, THF.



Scheme III. *a*, [(3-pyridinyl)methyl]-(4-nitrophenyl)carbonate, THF; *b*, *N*-[[*N*-methyl-*N*-[(2-pyridinyl)methyl]amino]carbonyl]valine *p*-nitrophenyl ester; *c*, R-CO-Val *p*-nitrophenyl ester; *d*, R-CO-Val-OH, EDC, HOBT; *e*, di-*t*-butyldicarbonate, CH_2Cl_2.



Scheme IV. *a*, di-*t*-butyldicarbonate, CH_2Cl_2; *b*, [(3-pyridinyl)methyl]-(4-nitrophenyl)carbonate, THF; *c*, *N*-[[*N*-methyl-*N*-[(2-pyridinyl)methyl]amino]carbonyl]valine *p*-nitrophenyl ester; *d*, *N*-[(2-pyridinyl)methoxycarbonyl]valine *p*-nitrophenyl ester; *e*, *N*-[[*N*-methyl-*N*-[(2-pyridinyl)methyl]amino]carbonyl]valine, EDC, HOBT, CH_2Cl_2; *f*, HCl, dioxane, ethanol.

unsymmetrically substituted *R,S*-diols **20–26** were prepared in a regioselective fashion. Physical data and yields for the last step in the preparation of **17–43** are given in Table 2.

HIV Protease inhibition

The inhibitory potencies of compounds **17–43** against purified, recombinant HIV protease were evaluated using a fluorogenic assay as described previously[5] and are presented in Table 1. IC_{50} values < 1 nM did not accurately represent inhibitory potency due to active site titration. The K_i values of selected inhibitors were thus evaluated using methods for tight-binding inhibitors.[21] In general, the activities of **17–43** approximated the midpoint between analogous longer (**7–11**) and shorter (**12–16**) inhibitors. For example, elimination of one valine residue from **11** to give **30** resulted in a loss of 1.4 kcal/mol of binding. An additional 1.5 kcal/mol decline in potency was observed upon elimination of a second valine residue (compound **16**). This relationship implies that the overall symmetry of binding observed in the X-ray crystal structures of **7–10** bound to HIV protease[18] is generally conserved in the unsymmetrically substituted inhibitors.

In detail, however, the trends in potency for **17–43** resembled more closely that of the di-Boc derivatives **12–15**[14,16] than that of the longer, symmetrically substituted inhibitors. Thus, within **17–43**, potency between classes of inhibitors generally increased in the order *S,S*-diol < *R,R*-diol < *R,S*-diol < *S*-monool, whereas in longer inhibitors derived from **2–5**, the potency of the *S,S*-diols equaled or exceeded that of the *R,R*-diols.[15,16] This difference may arise from the substantial conformational divergence observed between the *S,S*- and *R,R*-diols upon binding.[18] The resulting subtle differences between the orientation of the C–N bonds of the core diamines might be expected to affect the contribution of the adjacent P_2 carbamate moieties present in both **2–3** and **17–19** to overall binding affinity. Alternatively, the positional asymmetry in the binding orientation of the *R,R*-diol core (observed for **7**) with respect to the enzyme diad[18] may allow for more favorable peripheral interactions in unsymmetrically substituted inhibitors than the *S,S*-diol, which (in compound **8**) binds in a symmetric orientation.[18]

Table 2. Physical data for HIV protease inhibitors **17–43**

No.	Prep.[a]	Yield[b]	mp, °C	R_f (solv)[c]	Formula[d]
17	A	58	189-190	0.32	$C_{37}H_{43}N_5O_7 \cdot 0.25H_2O$
18	B	85	84-85	0.42	$C_{38}H_{46}N_6O_6 \cdot 0.5H_2O$[e]
19	B	69	84-85	0.33	$C_{37}H_{43}N_5O_6 \cdot 1.0H_2O$
20	C	72	105-107	0.53	$C_{36}H_{49}N_5O_6 \cdot 0.25H_2O$
21	A	79	91-93	0.55	$C_{36}H_{49}N_5O_6$
22	A		202-204	0.39	$C_{35}H_{46}N_4O_7 \cdot 0.9CHCl_3$
23	B	83	86-88	0.33	$C_{38}H_{46}N_6O_6 \cdot 0.5H_2O$
24	B	76	82-83	0.33	$C_{38}H_{46}N_6O_6 \cdot 0.75H_2O$
25	B	75	166-168	0.32	$C_{37}H_{43}N_5O_7$
26	A	57	184-185	0.32	$C_{37}H_{43}N_5O_7 \cdot 0.5H_2O$
27	A	92	84-85	0.60	$C_{36}H_{49}N_5O_5 \cdot 0.5H_2O$
28	A, B	92	69-71	0.28	$C_{38}H_{46}N_6O_5 \cdot 0.5H_2O$
29	A, B	94	58-61	0.26	$C_{38}H_{46}N_6O_5 \cdot 1.1H_2O$
30	A	95	169-171	0.30	$C_{37}H_{43}N_5O_6 \cdot 0.5H_2O$
31	A	94	172-174	0.30	$C_{37}H_{43}N_5O_6 \cdot 0.5H_2O$
32	A	96	71-73	0.50[f]	$C_{39}H_{48}N_6O_5 \cdot 0.5H_2O$
33	A	96	66-67	0.49[f]	$C_{39}H_{48}N_6O_5 \cdot 0.5H_2O$
34	A	77	176-177	0.56[f]	$C_{38}H_{45}N_5O_6 \cdot 0.75H_2O$
35	A	56	166-168	0.54[f]	$C_{38}H_{45}N_5O_6 \cdot 0.5H_2O$
36	A	93	64-69	0.28	$C_{38}H_{46}N_6O_5$
37	A	88	64-66	0.28	$C_{38}H_{46}N_6O_5$[g]
38	A	81	160-162	0.30	$C_{37}H_{43}N_5O_6$
39	A	87	125-128	0.30	$C_{37}H_{43}N_5O_6$
40	D	91	68-69	0.39[f]	$C_{39}H_{48}N_6O_5$
41	D	88	70-71	0.41[f]	$C_{39}H_{48}N_6O_5 \cdot 0.5H_2O$
42	D	79	175-176	0.44[f]	$C_{38}H_{45}N_5O_6 \cdot 0.25H_2O$
43	D	70	165-166	0.46[f]	$C_{38}H_{45}N_5O_6$

[a]Method of preparation (see Experimental Section); [b]Yield for last step of synthesis; [c]10 % MeOH/$CHCl_3$ unless otherwise noted; [d]Elemental analysis within ± 0.4 % except where noted; [e]N: calcd, 12.15; Found, 11.70; [f]10 % MeOH/CH_2Cl_2; [g]H: calcd, 6.95, Found 7.38.

Crystallographic results showed similar, asymmetric binding modes for **9** and **10**, derived from the pseudosymmetric diamines **4** and **5**, respectively.[18] The effect of asymmetric binding in unsymmetrically substituted derivatives of **4** and **5** was manifested by significant differences in inhibitory potency between pairs of regioisomeric P_3–P_2 *N*-methylurea-linked inhibitors (**20/21**, **23/24**, **28/29**, **32/33**, **36/37**). In contrast, pairs of regioisomeric carbamate-linked inhibitors (**30/31**, **34/35**, **38/39**) showed identical inhibition of HIV protease.[22] The consistent effect of this structural difference which is remote from the position of asymmetry is not well understood, but may result from an interaction between the urea methyl group and the Gly_{48} carbonyl group of the enzyme active site, as observed in the crystal structures of **7–10**.[18] The absence of this interaction in the carbamate-linked inhibitors might allow a more symmetric binding orientation and a corresponding equivalence in the potency of sets of regioisomeric inhibitors.

In view of their superior potency, derivatives of diaminomonool **5** became the focus of structure–activity studies. In general, the structure–activity relationships of this series paralleled that observed previously with longer, symmetric inhibitors[15] and is summarized by the following trends: (1) incorporation of a carbamate linkage resulted in slightly better potency than use of the *N*-alkylurea linkage (*c.f.* **28/30**, **32/34**); (2) an *N*-ethylurea linkage was less tolerated than an *N*-methylurea linkage (*c.f.* **28/36**, **29/37**); (3) inhibitors with isoleucine at the P_2 position showed slightly improved potency over those with valine (*c.f.* **28/32**, **29/33**, **30/34**); (4) use of 2-pyridinyl or 3-pyridinyl groups at the P_3 position gave inhibitors of equivalent potency;[22] (5) methyl substitution on the P_3 pyridinyl group was tolerated without loss of potency.

In vitro *anti-HIV activity*

Inhibitors **17–43** were evaluated for the ability to block the spread of HIV-1_{3B} in immortalized human (MT4) lymphocytes as described previously.[5] *In vitro* activity, expressed as ED_{50}, and cytotoxicity, expressed as $CCIC_{50}$, are shown in Table 1. Antiviral activity for the more potent inhibitors was generally observed at 0.1–0.5 μM. All of the inhibitors lacked cytotoxicity to uninfected MT4 cells at concentrations < 50 μM, leading to *in vitro* therapeutic windows > 100 in many cases. In general, anti-HIV activity was observed at concentrations approximately 1,000-fold higher than those required for inhibition of purified HIV protease. This gap apparently reflects in part the limited ability of the protease inhibitors to penetrate infected cells and/or newly formed virus particles; however, the magnitude of the gap is presumably artificially high due to the highly optimized conditions (high ionic strength, low pH) under which protease inhibition was assayed. Under physiological conditions, higher K_i values might be anticipated, thereby reducing this observed difference between observed activities in the two assays.

The general relationship of structure and anti-HIV activity resembled the trends observed with protease inhibition. Compounds containing carbamate linkages between the P_3

heterocycle and P_2 amino acid were consistently more potent than the corresponding *N*-methylureas. Importantly, methyl substitution on the terminal 2-pyridinyl group resulted in reproducible enhancement of antiviral potency (*c.f.* **28–31**, **40–43**). Compound **43**, containing a 6-methyl-2-pyridinyl end group and carbamate linkage, was the most potent of the series, with an ED_{50} of 0.026–0.075 μM. The results of a systematic investigation of this enhancement of activity will be reported in due course.

Pharmacokinetic analysis

The pharmacokinetic behavior of many of the protease inhibitors in rats was evaluated by quantitation of plasma levels following either a 5 mg/kg intravenous or 10 mg/kg oral dose ($n \geq 3$).[5] Rapid absorption of the inhibitors ($T_{max} < 0.5$ h) was observed. Terminal half-life ($T_{1/2}$) following iv administration and maximum levels (C_{max}) after oral administration are listed in Table 3, along with the estimated aqueous solubility of each inhibitor in pH 7.4 phosphate buffer.[5] Percent bioavailability (F) was calculated from the dose normalized AUC values [$(AUC/Dose)_{oral}/(AUC/Dose)_{iv}$]. A sizeable majority (16 out of 21) of the compounds displayed C_{max} levels in excess of 1 μM, demonstrating that as a class, this series of inhibitors offers significant advantages over the larger, symmetrically substituted inhibitors **7–11**.[5,15] The data in Table 3 suggest several relationships between the structural or physicochemical features of the inhibitors and the observed oral bioavailability. Within the range of solubilities of these compounds (2–200 μg/mL), little overall correlation between aqueous solubility and oral bioavailability was found. However, as shown in Figure 1, inhibitors with an *N*-methylurea linkage between the pyridinyl group and the P_2 aminoacyl residue showed greater average percent bioavailability (18.3 %) and more consistent absorption than those with a carbamate linkage (12.6 %). Of the two sets of inhibitors, the *N*-methylureas were also significantly more soluble. Furthermore, related compounds with solubilities significantly less than 1 μg/mL consistently showed very low bioavailability in rats (data not shown). Taken together, these results suggest a subtle relationship between these two parameters and that a threshold (*ca* 1 μg/mL) of solubility must be exceeded in this series for good oral bioavailability.

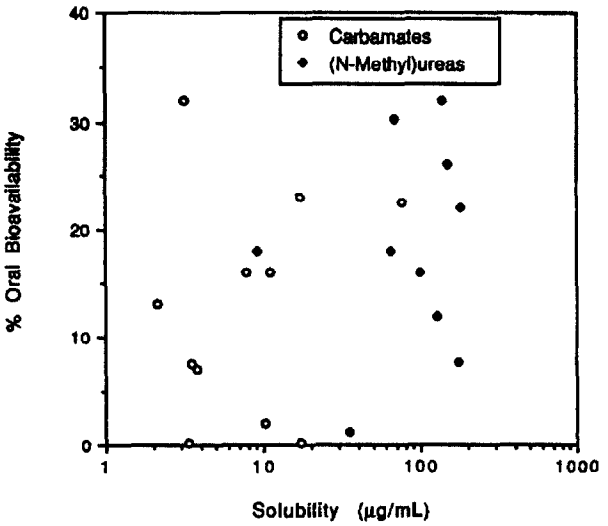


Figure 1. Relationship of aqueous solubility (pH 7.4 phosphate buffer) and oral bioavailability in rats of HIV protease inhibitors **17–43**.

A detailed examination of the data in Table 3 reveals still another correlation between aqueous solubilitity and oral bioavailability. Within most pairs of regioisomeric inhibitors derived from diaminomonool **5**, a significant (2- to 3-fold) difference in calculated oral bioavailability was observed (compounds **28/29**, **30/31**, **32/33**, **38/39**,**40/41**, **42/43**). As shown in Figure 2, in each

Table 3. Aqueous solubility and pharmacokinetic parameters for HIV protease inhibitors

No.	Sol. (μg/mL)	$T_{1/2}$ (h)	C_{max} (μM)	F (%)	C_{max}/ED_{50}
17	8.7				
18	196				
19	76	1.54	1.69	23	0.7
20	35	0.36	0.11	1.2	0.2
21	80				
22	3.3		trace	<1	
23	127	1.04	1.30	12	2.6
24	174	0.76	1.04	7.7	1.4
25	10	0.38	0.56	2.1	3.0
26	17	1.00	0.06	0.2	0.1
28	148	0.19	3.58	26	3.1
29	178	1.22	2.18	22	3.8
30	3.2	2.3	4.11	32	21.6
31	2.1	1.54	1.42	13	3.6
32	9.3	1.03	1.29	18	1.9
33	68	0.40	3.52	30	7.6
34	7.8	2.50	2.99	16	7.1
36	28				
37	66	0.34	2.14	18	3.3
38	3.8	0.40	0.85	7.1	3.7
39	17	1.59	1.00	23	8.4
40	101	1.0	2.42	16	7.3
41	137	0.68	3.40	32	13.3
42	3.5	0.74	1.16	7.5	11.8
43	11	1.95	1.35	16	26.8

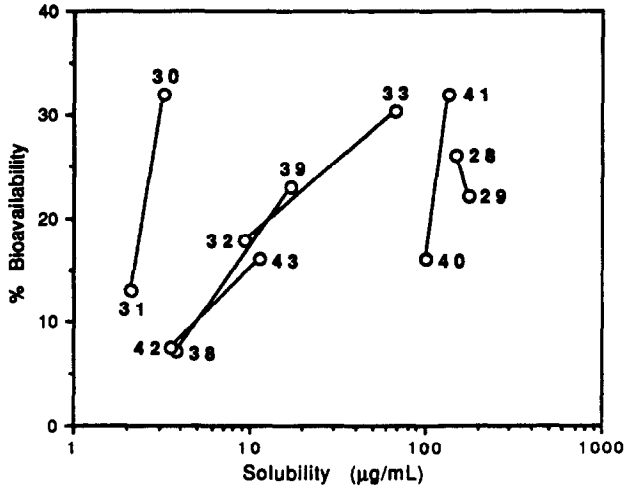


Figure 2. Relationship of aqueous solubility and bioavailability of regioisomeric derivatives of **5**.

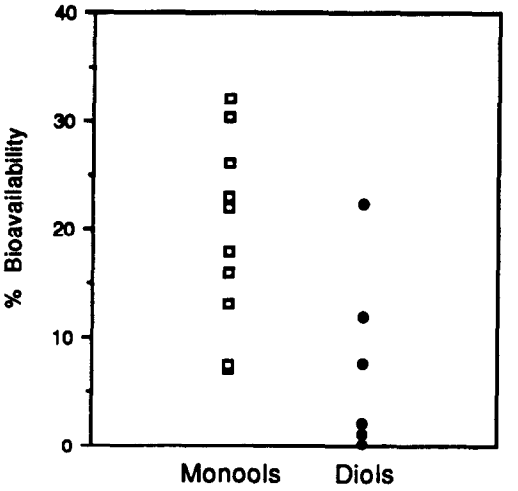


Figure 3. Effect of the number of core hydroxyl groups on oral bioavailability of symmetry-based HIV protease inhibitors.

case but one, the regioisomer with the highest solubility also showed the higher bioavailability. The only exception is the set **28/29**, in which both compounds are quite soluble, and the bioavailability of each is nearly equivalent. This correlation suggests that between compounds of very similar structure and physical properties (see Table 2), kinetic solubility can indeed be a determining factor in the observed pharmacokinetic profiles.

Within the series of inhibitors **17–43**, one structural feature showed clear correlation with oral bioavailability. A comparison of the sets of analogous inhibitors **23–26** and **28–31**, derived from diamines **4** and **5**, respectively, indicates that the presence of a second hydroxyl group in the core diamine is clearly deleterious to good oral bioavailability. For example, inhibitors **25/30** and **26/31** showed a > 7-fold difference in C_{max} and > 15-fold difference in absolute bioavailability. A comparison of the percent bioavailability of all the inhibitors in this study (Figure 3) indicates that average bioavailability of the inhibitors based on monool **5** (20 %) was 3-fold higher than the average of those derived from diols **2–4** (6.5 %). Whether this marked change is due to differences in absorption or biliary excretion is unknown. It is likely, however, that the presence of the additional hydrogen-bonding functionality in **18–21** contributes to lower overall intestinal absorption. In previous studies, removal of the hydrogen-bond donating capability of amide bonds resulted in improved absorption properties of linear peptides.[23,24] The present results suggest that abrogating the need for desolvation of the second hydroxyl group of **17–26** may be responsible for a similarly beneficial effect. Alternatively, intramolecular hydrogen-bonding of diols **17–26** might influence the overall molecular shape in a manner disadvantageous to absorption. It is noteworthy that the observed trend between the present derivatives parallels that found previously with analogues of monool **6** and diols **7–9**,[5,15] and that the original design rationale for the preparation of inhibitors containing diaminomonool **5**, i.e. that removal of the second hydroxyl group in **7–9** might result in inhibitors with improved oral bioavailability, has proven valid.

In general, inhibitors containing a carbamate linkage showed longer $T_{1/2}$ values than did those containing urea linkages. This feature, combined with the overall potency advantage of the carbamates, made up for the slightly better bioavailability observed with the *N*-methylureas. One measure of the overall potential utility of HIV protease inhibitors utilizes the comparison of plasma levels achieved *in vivo* to the concentration required for anti-HIV activity *in vitro*. Ratios of C_{max} to the median ED_{50} for each inhibitor are provided in Table 3. Using this criterion, the superiority of **30** (A-80987) and **43**, in which plasma concentrations > 20-fold in excess of the antiviral ED_{50} were obtained, to other inhibitors of this series was manifest. While the C_{max}/ED_{50} ratio for **43** was primarily a result of potent anti-HIV activity, that of **30** represented a combination of good activity and excellent pharmacokinetic behavior. A-80987 demonstrated equal potency *in vitro* to the clinical agent A-77003 (**9**)[5] in spite of a substantial (140 g/mol) reduction in molecular weight. The results of detailed evaluation of A-80987 will be described elsewhere.

Conclusion

A major obstacle to the development of inhibitors of HIV protease for clinical use has been the identification of agents with acceptable pharmacokinetic behavior. The present results confirm that potent inhibitors with substantial oral bioavailability can be obtained in spite of significant peptidomimetic character. These results also provide insight into factors which can enhance oral bioavailability, including increasing aqueous solubility, reducing molecular size, and reducing the hydrogen-bonding potential. The individual combination of these and other factors toward pharmacokinetic behavior are normally difficult to decipher. In the present work, however, detailed comparison of inhibitors of very similar structure, which tends to equalize most physicochemical characteristics, allowed the effects of parameters such as aqueous solubility and hydrogen-bonding potential to be identified in a systematic manner. These results, along with the recent

description of orally active, peptidomimetic inhibitors of human renin[25] provide the basis for potentially useful strategies for the design of peptidomimetic drugs in general. Optimization of the activity and pharmacokinetics of this series of inhibitors led to the identification of A-80987 (**30**), which upon oral dosing in rats, provided plasma levels > 20-fold in excess of the anti-HIV ED_{50}. The results of further preclinical and clinical evaluation of A-80987 will be reported in due course.

Experimental Section

Melting points are uncorrected. ^1H NMR spectra were recorded at 300 MHz using a GE QE-300 instrument using tetramethylsilane as an internal standard. ^1H NMR spectra and mass spectra 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; E. Merck) and was visualized with phosphomolybdic acid or ceric ammonium molybdate. Solvents were of reagent grade or higher. Methods of preparation, yields for the last synthetic step, and physical data (mp, R_f and combustion analysis) for compounds **17–43** are provided in Table 2.

p-Nitrophenyl esters of *N*-substituted amino acids were prepared in crude form from the corresponding methyl esters by hydrolysis and activation (*p*-nitrophenol, dicyclohexylcarbodiimide) as described previously.[15]

N-[[N-Methyl-N-[(2-pyridinyl)methyl]amino]carbonyl]isoleucine methyl ester

Prepared by reaction of *N*-carbonylisoleucine methyl ester and 2-[(*N*-methylamino)methyl]pyridine.[15] ^1H NMR ($CDCl_3$) δ 0.92 (t, *J* = 7 Hz, 3H), 0.94 (d, *J* = 7 Hz, 3H), 1.21 (m, 1H), 1.46 (m, 1H), 1.90 (m, 1H), 3.02 (s, 3H), 3.71 (s, 3H), 4.46 (dd, *J* = 8, 5 Hz, 1H), 4.53 (s, 2H), 6.15 (br, 1H), 7.22 (dd, *J* = 7, 5 Hz, 1H), 7.27 (d, *J* = 7 Hz, 1H), 7.69 (td, *J* = 7, 2 Hz, 1H), 8.55 (br d, 1H).

N-[(2-Pyridinyl)methoxycarbonyl]isoleucine methyl ester

Prepared by reaction of *N*-carbonylisoleucine methyl ester and pyridine-2-methanol.[15] ^1H NMR ($CDCl_3$) δ 0.93 (t, *J* = 7 Hz, 3H), 0.94 (d, *J* = 9 Hz, 3H), 1.19 (m, 1H), 1.44 (m, 1H), 1.90 (m, 1H), 3.74 (s, 3H), 4.36 (dd, *J* = 9, 4 Hz, 1H), 5.23 (s, 2H), 5.41 (br d, *J* = 9 Hz, 1H), 7.22 (m, 1H), 7.36 (d, *J* = 8 Hz, 1H), 7.70 (td, *J* = 8, 2 Hz, 1H), 8.59 (br d, *J* = 5 Hz, 1H).

N-[[N-Ethyl-N-[(2-pyridinyl)methyl]amino]carbonyl]valine methyl ester

A mixture of 4.5 g of 2-pyridinecarboxaldehyde, 45 mL of ethylamine and 0.45 g of 10 % Pt/C in 150 mL of CH_3OH was shaken under 4 atmospheres of H_2 for 8 h. The resulting mixture was filtered and concentrated *in vacuo* to provide crude *N*-(2-pyridinyl)-*N*-ethylamine, which upon reaction with *N*-carbonylvaline in the manner previously described gave *N*-[[*N*-ethyl-*N*-[(2-pyridinyl)methyl]amino]-carbonyl]valine methyl ester. ^1H NMR ($CDCl_3$) δ 0.92 (d, *J* = 7 Hz, 3H), 0.96 (d, *J* = 7 Hz, 3H), 1.17 (t, *J* = 7 Hz, 3H), 2.16 (m, 1H), 3.43 (AA', 2H), 3.71 (s, 3H), 4.42 (dd, *J* = 8, 5 Hz, 1H), 4.51 (AA', 2H), 6.29 (br, 1H), 7.22 (ddd, *J* = 7, 5, 1 Hz, 1H), 7.30 (d, *J* = 8 Hz, 1H), 7.69 (td, *J* = 8, 2 Hz, 1H), 8.55 (m, 1H). CIMS m/z 294 $(M + H)^+$.

N- [[N- Methyl-N- [(6-methyl-2-pyridinyl) methyl] amino]-carbonyl]valine methyl ester

Prepared by reaction of *N*-carbonylvaline methyl ester and 2-[(*N*-methylamino)methyl]-6-methylpyridine.[15] ^1H NMR ($CDCl_3$) δ 0.96 (d, *J* = 7 Hz, 3H), 0.98 (d, *J* = 7 Hz, 3H), 2.17 (m, 1H), 2.55 (s, 3H), 3.00 (s, 3H), 3.71 (s, 3H), 4.37 (d, *J* = 16 Hz, 1H), 4.40 (dd, *J* = 9, 5 Hz, 1H), 4.55 (d, *J* = 16 Hz, 1H), 6.53 (br, 1H), 7.05 (d, *J* = 8 Hz, 1H), 7.08 (d, *J* = 8 Hz, 1H), 7.57 (t, *J* = 8 Hz, 1H).

N-[(6-Methyl-2-pyridinyl)methoxycarbonyl]valine methyl ester

Prepared by reaction of *N*-carbonylvaline methyl ester and 6-methylpyridine-2-methanol.[15] ^1H NMR ($CDCl_3$) δ 0.91 (d, *J* = 7 Hz, 3H), 0.97 (d, *J* = 7 Hz, 3H), 2.17 (m, 1H), 2.56 (s, 3H), 3.74 (s, 3H), 4.31 (dd, *J* = 9, 5 Hz, 1H), 5.19 (s, 2H), 5.38 (br d, *J* = 9 Hz, 1H), 7.08 (d, *J* = 8 Hz, 1H), 7.16 (d, *J* = 8 Hz, 1H), 7.58 (t, *J* = 8 Hz, 1H).

(2S,3R,4S,5S)-3-Acetoxy-2,5-bis-[N-(benzyloxycarbonyl)-amino]-3-bromo-1,6-diphenylhexane (47)

A suspension of 25 g (44 mmol) of **44**[19] in 500 mL of 2:1 CH_2Cl_2:hexane was treated with 23 g of α-acetoxyisobutyryl bromide. The resulting mixture was stirred at ambient temperature until the reaction clarified, washed with two 200 mL portions of saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated *in vacuo* to give 30.8 g of crude **47**. A portion was purified by flash chromatography using 9:1 CH_2Cl_2:EtOAc to provide the pure desired compound as a white solid. ^1H NMR ($CDCl_3$) δ 2.21 (s, 3H), 2.62 (dd, *J* = 13, 11 Hz, 1H), 2.75 (d, *J* = 7 Hz, 2H), 2.95 (br d, *J* = 15 Hz, 1H), 4.03 (br t, *J* = 10 Hz, 1H), 4.40 (br d, *J* = 10 Hz, 1H), 4.6–5.0 (m, 6H), 5.12 (br d, *J* = 13 Hz, 1H), 5.33 (br d, *J* = 11 Hz, 1H), 7.0–7.4 (m, 10H). CIMS m/z 690, 692 $(M + NH_4)^+$.

(2S, 3S, 5S) -3-Acetoxy -2,5 -bis -[N- (benzyloxycarbonyl)-amino]-1,6-diphenylhexane (48)

A solution of 30.8 g (44 mmol) of crude **47** in 600 mL of THF was treated with 17.8 mL (66 mmol) of tri-*n*-butyltin hydride and 1.45 g (8.8 mmol) of 2,2'-azobis-[2-methylpropionitrile]. The resulting solution was heated at reflux under N_2 atmosphere for 1.5 h. After being allowed to cool, the solution was concentrated *in vacuo*, and the residue was taken up into acetonitrile and washed with four portions of hexane. The acetonitrile layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to provide 32 g of crude **48**. CIMS m/z 612 $(M + NH_4)^+$.

(2S,3S,5S)-2,5-Diamino-1,6-diphenyl-3-hydroxyhexane (5)

A suspension of 32 g of crude **48** and 55.5 g (176 mmol) of barium hydroxide octahydrate in 400 mL of 1,4-dioxane and 400 mL of water was heated at reflux for 4 h. The resulting mixture was filtered, and the residue was rinsed with dioxane. The combined filtrates were concentrated to a volume of approximately 200 mL and extracted with four 400 mL portions of $CHCl_3$. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography using first 2 % *i*-$PrNH_2$ in $CHCl_3$ and then 2 % *i*-$PrNH_2$/2 % CH_3OH in $CHCl_3$ to provide 10.1 g (81 %) of pure **5** as a white solid. ^1H NMR ($CDCl_3$) δ 1.54 (dt, *J* = 14, 10 Hz, 1H), 1.67 (dt, *J* = 14, 3 Hz, 1H), 2.50 (dd, *J* = 13, 8 Hz, 1H), 2.58 (dd, *J* = 13, 8 Hz, 1H), 2.8 (m, 2H), 2.91 (dd, *J* = 13, 5 Hz, 1H), 3.10 (m, 1H), 3.72 (ddd, *J* = 11, 3, 2 Hz, 1H), 7.1–7.4 (m, 10H). CIMS m/z 285 $(M + NH_4)^+$.

[(3-Pyridinyl)methyl]-(4-nitrophenyl)carbonate (61)

A solution of 20 g (0.1 mol) of (4-nitrophenyl)chloroformate in 150 mL of CH_2Cl_2 was cooled to 0 °C and treated sequentially with 8.0 mL (0.083 mol) of pyridine-3-methanol and 11 mL (0.1 mol) of 4-methylmorpholine. After addition, the solution was allowed to come to ambient temperature, stirred for 0.5 h, diluted with CH_2Cl_2, washed sequentially with aqueous $NaHCO_3$ and water, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was broken up, triturated with 3:1 hexane:EtOAc, and filtered. The resulting solid was dissolved in a minimum amount of boiling EtOAc/hexane, filtered hot to remove an insoluble dark oil, and allowed to cool. The crystalline product (18.65 g, 82 %) was collected by filtration. ^1H NMR ($CDCl_3$) δ 5.33 (s, 2H), 7.36 (ddd, *J* = 8, 5 Hz, 1H), 7.39 (m, 2H), 7.80 (dt, *J* = 8, 2 Hz, 1H), 8.28 (m, 2H), 8.66 (dd, *J* = 5, 1 Hz, 1H), 8.72 (br d, *J* = 2 Hz, 1H).

(2S, 3S, 5S) -2,5- Bis-[N- [(3-pyridinyl) methoxycarbonyl]-amino]-1,6-diphenyl-3-hydroxyhexane (16)

A solution of 90 mg (0.32 mmol) of **5** and 217 mg (0.8 mmol) of **61** in 1.0 mL of DMF was stirred at ambient temperature for 21 h. After removal of the solvent *in vacuo*, the crude product was purified by flash chromatography using $CH_3OH/CHCl_3$ to provide 116 mg (66 %) of **16** (R_f 0.59, 10 % CH_3OH in $CHCl_3$). ^1H NMR (DMSO-d_6) δ 1.49 (m, 2H), 2.55 (m, 1H), 2.60 (m, 1H), 2.70 (m, 2H), 3.55 (m, 1H), 3.88 (m, 2H), 4.96 (s, 2H), 4.98 (s, 2H), 6.95 (br d, 1H), 7.1–7.25 (br envelope, 11H), 7.35 (m, 2H), 7.60 (br d, 2H), 8.50 (m, 4H). CIMS m/z 555 $(M + H)^+$.

(2S, 3R, 4R, 5S) -5- Amino-2- [N- [(3-pyridinyl) methoxy-carbonyl]amino]-3,4-dihydroxy-1,6-diphenylhexane (49)

A solution of 0.4 g (1.33 mmol) of **2**[19] in 25 mL of THF was treated with 0.44 g (1.6 mmol) of **61**, stirred at ambient temperature for 16 h, and concentrated *in vacuo*. Flash chromatography of the residue using CH_3OH in $CHCl_3$ gave 238 mg (41 %) of **49** (R_f 0.10, 10 % CH_3OH in $CHCl_3$) along with 0.28 g of the diacylated byproduct. CIMS m/z 436 $(M + H)^+$.

(2S, 3S, 4S, 5S)-5-Amino-2-[N- [N-[[N-methyl-N-[(2-pyridinyl) methyl] amino] carbonyl] valinyl] amino]-3,4-dihydroxy-1,6-diphenylhexane (50)

Prepared as previously reported.[15] ^1H NMR (DMSO-d_6) δ 0.64 (d, *J* = 7 Hz, 3H), 0.65 (d, *J* = 7 Hz, 3H), 1.85 (m, 1H), 2.39 (dd, *J* = 14, 8 Hz, 1H), 2.67 (dd, *J* = 14, 10 Hz, 1H), 2.95 (dd, *J* = 14, 5 Hz, 1H), 3.08 (m, 1H), 3.14 (dd, *J* = 14, 3 Hz, 1H), 3.63 (br d, 1H), 3.90 (dd, *J* = 9, 7 Hz, 1H), 3.99 (m, 1H), 4.51 (AA', 2H), 4.55 (m, 1H), 6.16 (d, *J* = 9 Hz, 1H), 7.1–7.3 (br envelope, 14H), 7.76 (td, *J* = 7, 2 Hz, 1H), 7.78 (d, *J* = 9 Hz, 1H), 8.49 (m, 1H).

(2S,3S,4S,5S)-5-Amino-2-[N- [N- [(2-pyridinyl) methoxycarbonyl]valinyl]amino]-3,4-dihydroxy-1,6-diphenylhexane (51)

A suspension of 0.5 g (1.67 mmol) of **3**[19] in 15 mL of THF was heated to reflux and treated with a solution of 0.74 g (2.0 mmol) of *N*-[(2-pyridinyl)methoxycarbonyl]-valine *p*-nitrophenyl ester[15] in 5 mL of THF. The resulting mixture was heated at reflux for 2 h, then stirred at ambient temperature for 16 h. After addition of 2 mL of 1 N NaOH, the solution was stirred for 30 min, partitioned between $CHCl_3$ and 1 N NaOH, dried over Na_2SO_4, and concentrated *in vacuo*. Flash chromatography using CH_3OH in $CHCl_3$ provided 340 mg (38 %) of **51** (R_f 0.20, 10 % CH_3OH in $CHCl_3$). ^1H NMR (DMSO-d_6) δ 0.67 (d, *J* = 7 Hz, 3H), 0.72 (d, *J* = 7 Hz, 3H), 1.79 (m, 1H), 2.41 (dd, *J* = 14, 9 Hz, 1H), 2.66 (dd, *J* = 14, 9 Hz, 1H), 2.66 (dd, *J* = 14, 9 Hz, 1H), 2.94 (dd, *J* = 14, 4 Hz, 1H), 3.1 (m, 2H), 3.2 (m, 1H), 3.30 (s, 1H), 3.63 (m, 1H), 3.75 (dd, *J* = 9, 8 Hz, 1H), 3.98 (m, 1H), 4.52 (m, 1H), 5.09 (AA', 2H), 7.1–7.35 (br envelope, 10H), 7.39 (d, *J* = 8 Hz, 1H), 7.30 (td, *J* = 7, 1Hz, 1H), 7.35 (m, 1H), 8.54 (m, 1H).

(2S,3S,5S)-5-Amino-2-[N-[(3-pyridinyl)methoxycarbonyl]-amino]-1,6-diphenyl-3-hydroxyhexane (52) and (2S,3S,5S) -2-amino-5-[N-[(3-pyridinyl)methoxycarbonyl]amino]-1,6-diphenyl-3-hydroxyhexane (53)

A solution of 1.5 g (5.28 mmol) of **5** in 10 mL of THF was treated dropwise over a 5 h period with a solution of 1.6 g (5.8 mmol) of **61** in 10 mL of THF. After addition, the resulting solution was stirred at ambient temperature for 16 h and concentrated *in vacuo*. Flash chromatography using CH_3OH in $CHCl_3$ provided a mixture of **52** and **53**. Further chromatography of the mixture using first 2 % *i*-$PrNH_2$ in CH_2Cl_2 followed by 2 % *i*-$PrNH_2$/2 % CH_3OH in CH_2Cl_2 provided 0.38 g (16 %) of **52** and 0.87 g (36 %) of **53**. **52**: ^1H NMR ($CDCl_3$) δ 1.50 (m, 2H), 2.45 (dd, *J* = 14, 8 Hz, 1H), 2.78 (dd, *J* = 14, 5 Hz, 1H), 2.88 (d, *J* = 8 Hz, 2H), 3.05 (m, 1H), 3.73 (br q, 1H), 3.82 (br d, 1H), 5.10 (s, 2H), 5.41 (d, *J* = 10 Hz, 1H), 7.07 (br d, 2H), 7.15–7.3 (br envelope, 9H), 7.64 (dt, *J* = 8, 1 Hz,

1H), 8.56 (dd, J = 5, 2 Hz, 1H), 8.61 (d, J = 2 Hz, 1H). CIMS m/z 420 $(M + H)^+$. **53**: ^1H NMR ($CDCl_3$) δ 1.60 (dt, J = 14, 8 Hz, 1H), 1.75 (m, 1H), 2.52 (dd, J = 14, 9 Hz, 1H), 2.83 (m, 2H), 2.92 (m, 2H), 3.52 (m, 1H), 4.01 (br q, 1H), 5.08 (s, 2H), 5.33 (br d, 1H), 7.1–7.3 (br envelope, 11H), 7.64 (br d, 1H), 8.53 (dd, J = 5, 2 Hz, 1H), 8.59 (d, J = 2 Hz, 1H). CIMS m/z 420 $(M + H)^+$.

(2S,3S,5S)-5-Amino-2-[N-[N-[[N-methyl-N-[(2-pyridinyl)-methyl] amino] carbonyl] valinyl] amino] -1,6- diphenyl-3-hydroxyhexane (54) and (2S,3S,5S)-2-amino-5-[N-[N-[[N-methyl-N-[(2-pyridinyl) methyl] amino] carbonyl] valinyl]-amino]-1,6-diphenyl-3-hydroxyhexane (55)

A solution of 3.85 g (14 mmol) of **5** in 50 mL of THF was treated with 5.8 g (15 mmol) of *N*-[[*N*-methyl-*N*-[(2-pyridinyl)methyl]amino]carbonyl]valine *p*-nitrophenyl ester[15] and stirred at ambient temperature for 16 h. The resulting solution was concentrated *in vacuo*, and the residue was purified by flash chromatography using $CH_3OH/CHCl_3$. A second column using *i*-$PrNH_2/CH_3OH$ in $CHCl_3$ provided 0.77 g (10 %) of **54** (R_f 0.28) and 1.36 g (18 %) of **55** (R_f 0.20, 2 % *i*-$PrNH_2$/2 % CH_3OH in $CHCl_3$). **54**: ^1H NMR ($CDCl_3$) δ 0.82 (d, J = 7 Hz, 3H), 0.96 (d, J = 7 Hz, 3H), 1.5 (m, 2H), 2.75–2.9 (m, 4H), 2.99 (s, 3H), 3.05 (m, 1H), 3.83 (m, 1H), 4.04 (m, 1H), 4.19 (dd, J = 7, 5 Hz, 1H), 4.40 (d, J = 16 Hz, 1H), 4.61 (d, J = 16 Hz, 1H), 6.42 (br, 1H), 6.80 (br, 1H), 7.1–7.3 (br envelope, 12H), 7.72 (d, J = 8, 2 Hz, 1H), 8.55 (m, 1H). **55**: ^1H NMR (DMSO-d_6, 500 MHz) δ 0.74 (d, J = 7 Hz, 3H, H_γ), 0.75 (d, J = 7 Hz, 3H, H_γ), 1.52 (dt, J = 14, 8 Hz, 1H, H_4), 1.64 (dt, J = 14, 6 Hz, 1H, $H_{4'}$), 1.89 (octet, J = 7 Hz, 1H, H_β), 2.42 (dd, J = 13, 9 Hz, 1H, H_1), 2.64 (m, 2H, $H_{1',6}$), 2.69 (m, 1H, H_2), 2.75 (dd, J = 14, 5 Hz, 1H, $H_{6'}$), 2.89 (s, 3H, NCH_3), 3.44 (br, 1H, H_3), 3.94 (dd, J = 8, 6 Hz, 1H, H_α), 4.06 (m, 1H, H_5), 4.40 (m, 1H, OH), 4.51 (AA', 2H, NCH_2), 6.08 (d, J = 9 Hz, 1H, urea NH), 7.1–7.3 (br envelope, 12H, Ar), 7.68 (d, J = 9 Hz, 1H, amide NH), 7.75 (dt, J = 8, 2 Hz, 1H, pyr), 8.50 (m, 1H, pyr). 2D spectrum of **55** showed the following coupling relationships: H_5 to $H_{4,4'}$, $H_{6,6'}$ and amide NH; H_3 to $H_{4,4'}$, H_2 and OH; H_2 to H_3 and $H_{1,1'}$; H_α to H_β and urea NH; H_β to H_γ.

(2S, 3R, 4S, 5S) -5- Amino -2- [N- (tert-butyloxycarbonyl)-amino]-3,4-dihydroxy-1,6-diphenylhexane (56)

A solution of 0.70 g (2.33 mmol) of **4**[19] and 0.61 g (2.8 mmol) of di-*tert*-butyldicarbonate in 20 mL of CH_2Cl_2 was stirred at ambient temperature for 16 h. After removal of the solvent *in vacuo*, flash chromatography using $CH_3OH/CHCl_3$ provided 0.67 g (72 %) of **56** (R_f 0.32, 10 % CH_3OH in $CHCl_3$): ^1H NMR ($CDCl_3$) δ 1.43 (s, 9H), 2.77 (dd, J = 13, 6 Hz, 1H), 2.85–2.95 (m, 2H), 2.95–3.05 (m, 2H), 3.10 (dd, J = 13, 3 Hz, 1H), 3.51 (dd, J = 8, 1 Hz, 1H), 4.24 (qd, J = 10, 1 Hz, 1H), 4.80 (m, 2H), 7.15–7.3 (br envelope, 10H). CIMS m/z 401 $(M + H)^+$.

(2S,3R,4S,5S)-5-Amino-2-[N-[(3-pyridinyl)methoxy-carbonyl]amino]-3,4-dihydroxy-1,6-diphenylhexane (57)

A solution of 250 mg (0.83 mmol) of **4**[19] and 251 mg (0.916 mmol) of **61** in 20 mL of THF was stirred at ambient temperature for 16 h. The resulting solution was concentrated *in vacuo*, and the residue was purified by flash chromatography using CH_3OH in $CHCl_3$ to provide 142 mg (57 %) of **57** (R_f 0.15, 10 % CH_3OH in $CHCl_3$) along with 110 mg of the diacylated product.

(2S,3R,4S,5S)-5-Amino-3,4-dihydroxy-1,6-diphenyl-2-[N-[N-[[N-methyl -N- [(2-pyridinyl) methyl] amino] carbonyl]-valinyl]amino]hexane (58) and (2S,3R,4S,5S)-2-amino-3,4-dihydroxy-1,6-diphenyl-5-[N-[N-[[N-methyl-N-[(2-pyridinyl)methyl]amino]carbonyl]valinyl]amino]hexane (60)

A mixture of 1.94 g (7.3 mmol) of *N*-[[*N*-methyl-*N*-[(2-pyridinyl)methyl]amino]carbonyl]valine,[15] 2.08 g (6.7 mmol) of **4**,[19] and 1.08 g (8.0 mmol) of 1-hydroxybenzotriazole hydrate (HOBT) in 50 mL of CH_2Cl_2 was treated with 1.54 g (8.0 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and stirred at ambient temperature for 16 h. The resulting solution was diluted with EtOAc, washed sequentially with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated *in vacuo*. Careful flash chromatography using $CH_3OH/CHCl_3$ provided 0.83 g (23 %) of **58** (R_f 0.14) and 0.52 g (14 %) of **60** (R_f 0.05, 10 % CH_3OH in $CHCl_3$). **58**: ^1H NMR (DMSO-d_6) δ 0.74 (d, J = 7 Hz, 3H), 0.75 (d, J = 7 Hz, 3H), 1.97 (octet, J = 7 Hz, 1H), 2.39 (dd, J = 13, 9 Hz, 1H), 2.8–2.95 (m, 3H), 2.90 (s, 3H), 3.4–3.5 (m, 2H), 4.04 (dd, J = 8, 7 Hz, 1H), 4.24 (br q, J = 8 Hz, 1H), 4.52 (AA', 2H), 4.92 (d, J = 5 Hz, 1H), 6.29 (d, J = 9 Hz, 1H), 7.15–7.3 (br envelope, 12H), 7.72 (d, J = 9 Hz, 1H), 7.77 (td, J = 8, 6 Hz, 1H), 8.51 (dm, 1H); CIMS m/z 548 $(M + H)^+$. **60**: ^1H NMR (DMSO-d_6) δ 0.63 (d, J = 7 Hz, 3H), 0.66 (d, J = 7 Hz, 3H), 1.86 (octet, J = 7 Hz, 1H), 2.6 (m, 2H), 2.74 (dd, J = 14, 6 Hz, 1H), 2.87 (s, 3H), 2.91 (dd, J = 16, 3 Hz, 1H), 3.23 (m, 1H), 3.41 (m, 1H), 3.55 (m, 1H), 3.89 (dd, J = 9, 8 Hz, 1H), 4.21 (m, 1H), 4.51 (AA', 2H), 4.88 (br, 1H), 6.13 (d, J = 9 Hz, 1H), 7.1–7.3 (br envelope, 12H), 7.60 (d, J = 9 Hz, 1H), 7.77 (td, J = 8, 6 Hz, 1H), 8.50 (dm, 1H). CIMS m/z 548 $(M + H)^+$.

(2S,3R,4S,5S)-5-Amino-2- [N- [N- [[N-methyl -N- [(2-pyridinyl) methyl] amino] carbonyl] valinyl] amino]-3,4-dihydroxy-1,6-diphenylhexane (58)

A solution of 0.40 g (0.133 mmol) of **4**[19] and 0.57 g (0.147 mmol) of *N*-[[*N*-methyl-*N*-[(2-pyridinyl)methyl]-amino]carbonyl]valine *p*-nitrophenyl ester[15] in 10 mL of THF was stirred at ambient temperature for 16 h. The resulting solution was diluted with 50 mL of $CHCl_3$, washed with several portions of 3 N aqueous NaOH, dried over Na_2SO_4, and concentrated *in vacuo*. Flash chromatography using CH_3OH in $CHCl_3$ provided 0.41 g (56 %) of **58** (R_f 0.15, 10 % CH_3OH in $CHCl_3$) which was identical to **58** produced above.

(2S,3R,4S,5S)-5-Amino-2-[N-[N-[(2-pyridinyl) methoxycarbonyl]valinyl]amino]-3,4-dihydroxy-1,6-diphenylhexane (59)

A solution of 0.5 g (1.66 mmol) of **4**[19] in 50 mL of THF was treated with 0.75 g (2.0 mmol) of *N*-[(2-pyridinyl)methoxycarbonyl]valine *p*-nitrophenyl ester[15] and stirred at ambient temperature for 16 h. After removal of the solvent *in vacuo*, flash chromatography using CH_3OH in $CHCl_3$ provided 210 mg (24 %) of **59** (R_f 0.20, 10 % CH_3OH in $CHCl_3$).

Representative procedure for preparation of inhibitors: (A) acylation with amino acid p*-nitrophenyl esters*

(2S,3S,5S)-5-[N-[N-[[N-Methyl-N-[(2-pyridinyl) methyl]-amino] carbonyl] valinyl] amino]-2-[N-[(3-pyridinyl) methoxycarbonyl]amino]-1,6-diphenyl-3-hydroxyhexane (29). A solution of 0.95 g (2.27 mmol) of **52** in 15 mL of THF was treated with 1.22 g (3.17 mmol) *N*-[[*N*-methyl-*N*-[(2-pyridinyl)methyl]amino]carbonyl]valine *p*-nitrophenyl ester.[15] The resulting solution was stirred at ambient temperature for 24 h, treated with aqueous $NaHCO_3$, extracted with $CHCl_3$, dried over Na_2SO_4, and concentrated *in vacuo*. Flash chromatography using first 2 % then 4 % CH_3OH in $CHCl_3$ provided 1.46 g (94 %) of **29** as a white solid. CIMS m/z 667 $(M + H)^+$.

Representative procedure for preparation of inhibitors: (B) acylation with p*-nitrophenyl carbamates*

(2S, 3R, 4S, 5S) -2- [N- [N- [[N- Methyl-N- [(2-pyridinyl)-methyl]amino]carbonyl]valinyl]amino]-5-[N-[(3-pyridinyl)-methoxycarbonyl]amino]-3,4-dihydroxy-1,6-diphenylhexane (23). A solution of 70 mg (0.13 mmol) of **58** and 42 mg (0.15 mmol) of **61** in 1 mL of THF was stirred at ambient temperature for 16 h. The resulting solution was concentrated *in vacuo*, and the residue was purified by flash chromatography using CH_3OH in $CHCl_3$ to provide 72 mg (83 %) of **23** as a white solid. CIMS m/z 683 $(M + H)^+$.

*Representative procedure for preparation of inhibitors: (C) acylation with di-*tert*-butyldicarbonate*

(2S, 3R, 4S, 5S) -2- [N- [N- [[N- Methyl-N- [(2-pyridinyl)-methyl] amino] carbonyl] valinyl] amino] -5-[N-(tert-butyl-oxycarbonyl) amino] -3,4- dihydroxy -1,6- diphenylhexane (20). A solution of 110 mg (0.20 mmol) of **58** in 1 mL of dichloromethane was treated with 53 mg (0.24 mmol) of di-*tert*-butyldicarbonate. The resulting solution was stirred for 16 h at ambient temperature, concentrated *in vacuo*, and purified by flash chromatography using first 1.5 % then 2 % CH_3OH in $CHCl_3$ to provide 93 mg (72 %) of **20** as a white solid, mp 105-107 °C. CIMS m/z 648 $(M + H)^+$.

Representative procedure for preparation of inhibitors: (D) acylation with activated amino acids

(2S, 3S, 5S) -2- [N- [(6-Methyl-2-pyridinyl) methoxycarbonyl] valinyl] amino]-5-[N-[(3-pyridinyl)methoxycarbonyl]-amino]-1,6-diphenyl-3-hydroxyhexane (40). A mixture of 100 mg of *N*-[[*N*-methyl-*N*-[(6-methyl-2-pyridinyl)methyl]-amino]carbonyl]valine (prepared by hydrolysis of the corresponding methyl ester), 100 mg (0.24 mmol) of **53**, 113 mg of HOBT and 0.084 mL of triethylamine in 3 mL of DMF was treated with 116 mg of EDC and stirred at ambient temperature for 16 h. The resulting solution was diluted with EtOAc, washed sequentially with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated *in vacuo*. Flash chromatography using CH_3OH/CH_2Cl_2 provided 150 mg (91 %) of **40** as a white solid, mp 68-69 °C.

HIV Protease inhibition

Inhibition of purified, recombinant HIV-1 protease was determined using the fluorogenic substrate DABCYL-GABA-Ser-Gln-Asp-Tyr-Pro-Ile-Val-Gln-EDANS[26] as described previously.[5] In cases where IC_{50} values suggested a K_i of less than 1 nM, inhibitor levels were varied at several enzyme concentrations and the data were analyzed by the method of Morrison and Stone for tight-binding inhibitors to obtain valid K_i values.[21] Fluorescence versus time data were acquired using a QuickBasic program developed by the authors to drive the fluorescence well plate reader of a Perkin–Elmer LS50 Luminescence Spectrometer. Individual assays were run in 200 μL volumes in microtiter plate wells under previously described assay conditions[5] with three exceptions: all assays contained 10 % DMSO, enzyme and inhibitor were incubated for 10 min prior to initiation of the reaction with substrate, and the concentration of HIV protease was varied from 0.05–0.3 nM depending on the potency of the inhibitor. Substrate concentrations were increased when necessary to obtain reasonable reaction rates at low enzyme concentrations. However, due to limited substrate solubility and a pronounced inner filter effect at high substrate concentrations,[26] 15 μM was the highest substrate level used. It was assumed that the addition of substrate did not significantly alter the enzyme–inhibitor equilibrium because the levels of substrate used were far below the K_m. In all cases, rates were determined from the linear portions of the progress curves generated for each well. All tight-binding inhibitor data sets were fit by nonlinear regression analysis to published equations.[21]

Anti-HIV activity

MT4 cells were obtained through the AIDS Research and Reference Reagent Program, AIDS Program, National Institute of Allergy and Infectious Diseases. Inhibition of the cytopathic effect of HIV-1_{3B} in MT4 cells was determined using uptake of MTT[27] as described previously.[5] Cytotoxicity was measured simultaneously with activity in uninfected MT4 cells using MTT uptake.

Pharmacokinetic studies

Estimated solubilities of the inhibitors in pH 7.4 phosphate buffer were determined as previously described.[5] Pharmacokinetic behavior of the inhibitors in rats was evaluated as described elsewhere in detail.[5]

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