Table IV. Effect of a Single Oral Dose of **1b** on the Appearance of Orally Administered [^{14}C]Cholesterol in the Plasma of Rats

time post-dosing, h	treatment	plasma [^{14}C]cholesterol (dpm/mL)
2.5	control	1507
	1b	818
6.0	control	6609
	1b	3884

Table V. Effect of Orally Administered **1b** on ACAT Activity in Rabbit Aorta and Rat Liver (dpm Cholesteryl [^{14}C]Oleate Formation/100 Wet Wt)

tissue		ACAT activity	% reduction
rabbit aorta	control	530	-
	1b (50 mg/kg)	305	42
rat liver	control	1420	-
	1b (30 mg/kg)	535	62

rabbits that received **1b** (50 mg kg^{-1} day^{-1} for 6 days) following a 10-day cholesterol feeding regiment designed to increase arterial ACAT activity; ACAT activity was reduced 42% by **1b** treatment. Additionally, hepatic ACAT was evaluated in vitro in liver minces from rats that received **1b** in the diet (30 mg kg^{-1} day^{-1}) for 8 days; ACAT activity was reduced 62% by **1b** treatment (Table V). This evidence of the systemic action of **1b** on the ACAT enzyme in arterial tissue, and its hypocholesterolemic action, are important to its continued development as an antiatherosclerotic agent.

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(13) New Zealand rabbits (2.2-2.3 kg) were fed a cholesterol-rich diet[15] for 10 days to induce increases in arterial ACAT activity[16] and then switched to Purina chow ± **1b** (calculated to provide 50 mg kg^{-1} day^{-1}). After 6 days, the aortas were excised, incubated in vitro with [1-^{14}C]oleate for 3 h, and ACAT activity was evaluated by the formation of cholesteryl [^{14}C]-oleate.[15] Liver minces[17] from male Sprague-Dawley rats (435-460 g) that received a Purina chow diet ± **1b** (calculated to provide **30** mg kg^{-1} day^{-1}) for 8 days were incubated in vitro with [1-^{14}C]oleate for 90 min and ACAT activity was evaluated by the formation of cholesteryl [^{14}C]oleate.[18] ACAT data are presented as dpm cholesteryl [^{14}C]oleate formed/100 mg wet weight of aorta or liver; all values are means of 2 animals per group.

(14) The effects of orally administered **1b** on rabbit arterial ACAT and rat hepatic ACAT are unlikely to be attributable to reduced substrate (cholesterol) availability resulting from inhibition of reabsorption of biliary cholesterol for the following reasons: (a) virtually all arterial cholesterol in the rabbit is derived from plasma (St. Clair, R. W. *Atheroscler. Rev.* **1976**, *1*, 61) and plasma cholesterol levels were ca. 10-fold above normal levels in the **1b** group; (b) inhibitors of gut cholesterol absorption in rats on normal chow diets have essentially no effect on plasma cholesterol or hepatic cholesterol levels (Heider, J. G. In *Pharmacological Control of Hyperlipidaemia*; J. R. Prous: Barcelona, Spain, 1986; p 423.)

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Structure-Based, C_2 Symmetric Inhibitors of HIV Protease

The genome of the human immunodeficiency virus (HIV-1) encodes a proteinase (HIV protease) which proteolytically processes the *gag* and *gag-pol* polyproteins.[1] Blockade of these processing steps results in the production of progeny virions which are immature and noninfectious.[2] Chemical inhibition of this critical viral enzyme therefore represents a powerful strategy for the development of an effective therapy for AIDS. Already, potent and selective inhibitors of HIV protease have been shown to prohibit the spread of HIV infection in vitro.[3] However, all of these inhibitors utilize known transition-state analogues[4] as replacements for the P_1-P_1' substrate cleavage sites. We report here the synthesis and antiviral properties of two novel classes of inhibitors specifically designed to capitalize on the unique symmetric structure of HIV protease.

The initial suggestion[5] and subsequent crystallographic demonstration[6] that HIV protease functions as a C_2 symmetric homodimer prompted us to utilize the concept of symmetry for the design of novel inhibitor structures. Inherently less peptide-like than inhibitors based on classical transition-state analogues, symmetric inhibitors might be expected to exhibit greater stability in vivo. Moreover, symmetric inhibitors should confer high specificity for retroviral proteinases over the related mammalian aspartic proteinases, whose substrate binding sites are less symmetric. The design of a C_2 symmetric inhibitor from the tetrahedral intermediate for cleavage of an asymmetric substrate, (e.g. -Phe-Pro-) hinges on three

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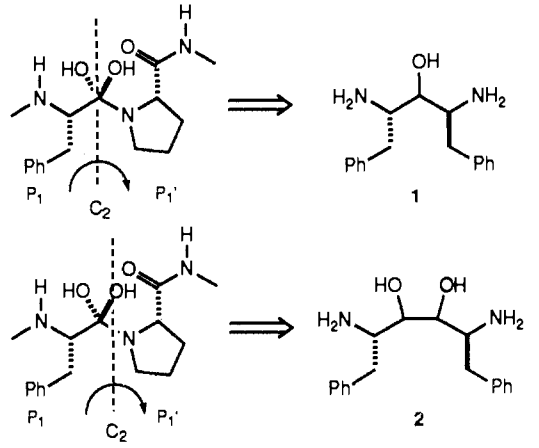
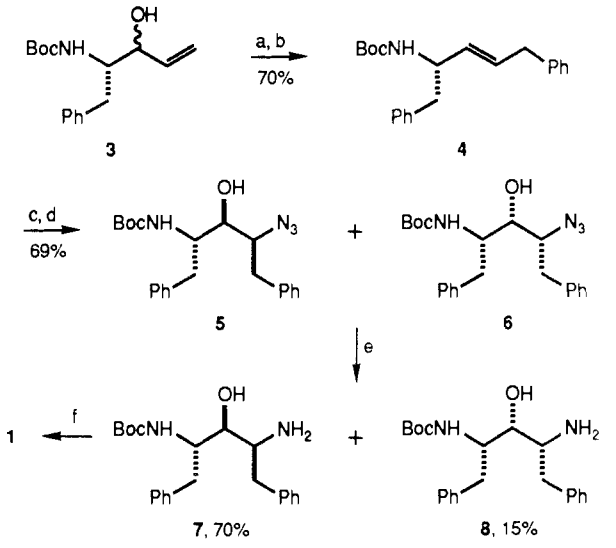


Figure 1. Design of C_2 symmetric HIV protease inhibitors.

Scheme I[a]

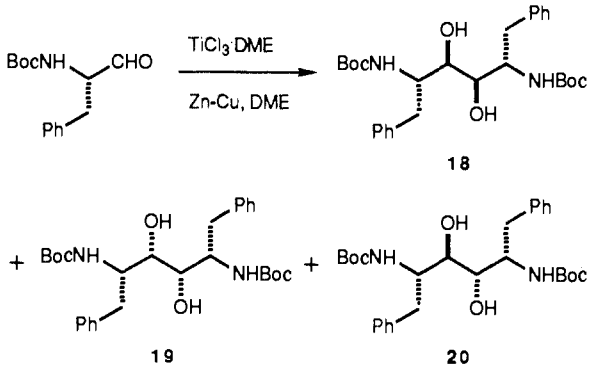


[a] (a) MsCl, $(i\text{-Pr})_2$NEt; (b) PhMgBr, cat. CuCN; (c) MCPBA; (d) LiN_3, NH_4Cl, DMF/H_2O; (e) 10% Pd/C, ammonium formate; (f) HCl, dioxane; NaOH.

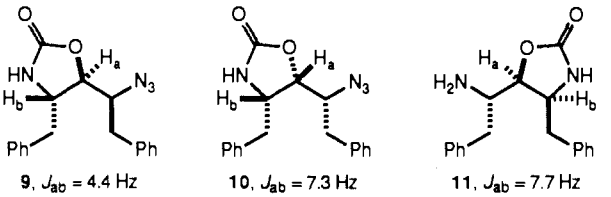
operations (Figure 1). First, a hypothetical axis of symmetry in the substrate is defined based on the C_2 axis *of the enzyme*. Our placement of the symmetry axis on or near the carbonyl carbon undergoing cleavage is based upon a modeling experiment[7] and upon the fact that the catalytically active aspartate residues reside close to the C_2 axis of the enzyme. Second, one "half" of the substrate is arbitrarily deleted. Deletion of the P′ region is guided by the greater importance of the P region previously observed for the binding of renin inhibitors.[8] Third, a C_2 operation is performed on the remainder of the substrate to generate a symmetric inhibitor. Application of these operations with the axis oriented either through the carbonyl carbon or through the middle of the scissile bond provides two distinct, chemically stable, pseudosymmetric or symmetric core units (**1** and **2**, respectively).

The stereoselective synthesis of diamino alcohol **1** is shown in Scheme I. Allylic alcohol **3**, obtained from Boc-phenylalanine methyl ester by DIBAL reduction and addition of vinylmagnesium bromide, undergoes clean

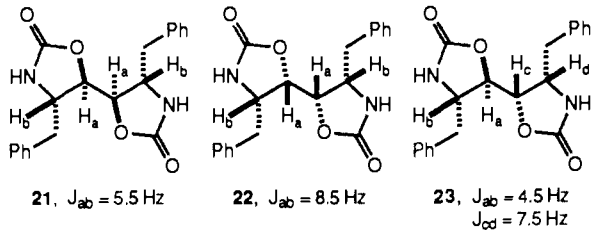
Scheme II



mesylation and S_N2' displacement with phenylmagnesium bromide/catalytic cuprous cyanide. Stereoselective epoxidation of the resulting *trans*-olefin **4** gives a ca. 4:1 (β:α) mixture of epoxides which are regioselectively opened with lithium azide. Reduction of the resulting mixture **5**/**6** provides the diastereomeric monoprotected diamines **7** and **8**, which are readily separated by silica gel chromatography. The stereochemistry of **5**–**7** (and thus **8**) is established through conversion to the corresponding oxazolidinones **9**–**11**, respectively. The NMR spectra of both **10** and **11** display coupling constants consistent with cis substitution while the spectrum of **9** is clearly consistent with the trans stereochemistry shown.[9] Deprotection of the major amino



alcohol **7** leads to the desired core unit **1**, which can be symmetrically acylated on both nitrogen atoms to provide inhibitors **12**–**17**.[10] Alternately, unsymmetric inhibitors can be prepared by monoacylation of **7**. The synthesis of the three distinct stereoisomers of **2** is shown in Scheme II. McMurry pinacol coupling[11] of Boc-phenylalaninal provides a ca. 2:1:1 mixture of **18**–**20**, respectively. The stereochemistry of each isomer is confirmed through independent conversion to bis-oxazolidinones **21**–**23** and



through a single crystal X-ray determination of **19**.[12] Deprotection of **18**–**20** provides **2a**–**c**, respectively, which can be acylated to provide inhibitors **24**–**26**.[10]

The inhibition of recombinant HIV-1 protease[13] by acylated diamino alcohols **12**–**17**, expressed as IC_{50} values,

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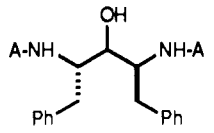
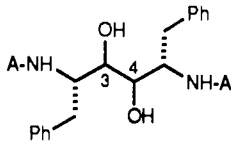
Table I. Inhibition of HIV-1 Protease by Acylated Diamino Alcohols



no.	A	IC_{50}, nM
12	Ac	>10000[a]
13	Ac-Val	12
14	Ac-Val-Val	10
15	Cbz-Val	3.0
16	Cbz-Ile	4.9
17	Cbz-Leu	>100[b]

[a] 12% inhibition at 10 μM. [b] 15% inhibition at 100 nM.

Table II. Inhibition of HIV-1 Protease by Acylated Diaminodiols



no.	A	config	IC_{50}, nM
18	Boc	3*R*,4*R*	40
19	Boc	3*S*,4*S*	280
20	Boc	3*R*,4*S*	12
24	Cbz-Val	3*R*,4*R*	0.22
25	Cbz-Val	3*S*,4*S*	0.38
26	Cbz-Val	3*R*,4*S*	0.22

is shown in Table I. Compound **12**, which can interact only with the S_1 and S_1' subsites, is only weakly active; however, the addition of P_2/P_2' residues gives the nanomolar inhibitor **13**. Additional amino acids in the P_3/P_3' sites (compound **14**) do not further increase activity, but replacement of acetyl with Cbz to give **15** results in another 4-fold boost in potency. Substitution of isoleucine for valine at the P_2/P_2' sites is tolerated (compound **16**), but less conservative changes such as leucine (compound **17**) are not. The corresponding ketone of **15** is >1000-fold less active, emphasizing the importance of the tetrahedral geometry (which abolishes the perfect 2-fold symmetry) at the center carbon of this series of inhibitors. Compound **15** shows dose-dependent inhibition of HIV-1 in H9 cells (IC_{50} = 0.4 μM). Details of the activity of **15** in vitro and the crystal structure of this inhibitor complexed to HIV-1 protease have recently been described.[7]

Inhibitors derived from core unit **2** (Table II) are generally 10-fold more potent than those from **1**. Boc-protected intermediates **18–20**, which contain no amino acids, are respectable inhibitors in their own right. Replacement of Boc with Cbz-valine leads to the highly potent inhibitors **24–26** with IC_{50} values of <0.4 nM. Surprisingly, the activity of **24–26** is not highly dependent on the stereochemistry of the two hydroxyl groups, in dramatic contrast to inhibitors based on traditional transition-state analogues.[3] Moreover, it is noteworthy that the asymmetric inhibitors **20** and **26** show equivalent potency to the diastereomeric C_2 symmetric inhibitors **18**, **19** and **24**, **25**, respectively. Although this implies that the inhibitors possess considerable flexibility at the central carbons, definition of the exact nature of the interactions of **24–26** with HIV protease must await crystallographic analysis.

The ability of inhibitors **24–26** to block the spread of acute HIV infection in two immortalized human T-lymphocytic cell lines is shown in Table III. Dose-dependent

Table III. Inhibition of HIV-1 in Vitro by Symmetric Protease Inhibitors

	H9 cells[a]		MT4 cells[b]	
no.	IC_{50}, μM	TC_{50}, μM	IC_{50}, μM	TC_{50}, μM
24	0.02	10	0.08	>100
25	0.06	>100	0.15	>100
26	0.02	60	0.016	>100

[a] H9 cells (2×10^4) were preinfected with 100 $TCID_{50}$ of HIV-1_{3B} for 2 h, washed, and cultured at 37 °C in medium containing inhibitor. Virus replication was monitored at days 7 and 10 by p24 antigen production. Cell viability in uninfected cultures was monitored by trypan blue dye exclusion. IC_{50}(AZT) = 0.001 μM. [b] MT4 cells (1×10^4) were infected with 10 $TCID_{50}$ of HIV-1_{3B} for 2 h, washed, and cultured at 37 °C in medium containing inhibitor. Cytopathic effect and toxicity were monitored at day 5 by MTT uptake. IC_{50}(AZT) = 0.007 μM.

inhibition of HIV-1 in H9 cells, as measured by p24 ELISA assay,[14] is observed at concentrations of 20–60 nM. The cytopathic effect of HIV in MT4 cells[15] is also blocked by **24–26**, with IC_{50} values ranging from 20 to 150 nM. Toxicity in these cell lines, expressed as TC_{50} values, ranges from 10 to >100 μM, resulting in therapeutic indeces of 500–>5000. Preliminary results of radioimmunoprecipitation studies show that the antiviral action of **24** is accompanied by a buildup of unprocessed $Pr55^{gag}$ in the newly assembled virions, suggesting that the antiviral action is indeed due to inhibition of HIV protease.[16]

These results demonstrate that consideration of the unique C_2 symmetric nature of HIV protease for the design of inhibitors leads to compounds which not only show potent protease inhibition but also effectively prohibit the spread of HIV-1 in vitro. Compounds **15** and **24–26** do not inhibit human renin at 10 μM,[17] indicating that the symmetric nature of the inhibitors confers high specificity over other aspartic proteinases. X-ray crystallographic studies have confirmed that **15** makes highly symmetric interactions with HIV-1 protease.[7] The novelty and high potency of these series of compounds warrents their consideration as agents for the therapeutic intervention of AIDS.

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