Inhibitors of HIV-1 Proteinase Containing 2-Heterosubstituted 4-Amino-3-hydroxy-5-phenylpentanoic Acid: Synthesis, Enzyme Inhibition, and Antiviral Activity†

Dieter Scholz,* Andreas Billich, Brigitte Charpiot, Peter Ettmayer, Philipp Lehr, Brigitte Rosenwirth, Erwin Schreiner, and Hubert Gstach

Department of Antiretroviral Therapy, SANDOZ Forschungsinstitut Ges.m.b.H., Brunnerstrasse 59, A-1235 Vienna, Austria

Received April 22, 1994®

A convenient procedure for the synthesis of 2-heterosubstituted statine derivatives as novel building blocks in HIV-protease inhibitors has been developed. The synthesis starts with protected L-phenylalaninols, which were converted to γ-amino α,β-unsaturated esters in a one-pot procedure. A highly diastereoselective epoxidation of the N-protected (*E*)-enoates, followed by regioselective ring opening of the corresponding 2,3-epoxy esters with a variety of heteronucleophiles, resulted in 2-heterosubstituted statine derivatives. The overall stereochemical outcome of the transformations meets the required configuration of HIV-protease inhibitors. The short, synthetically flexible, and highly diastereoselective synthesis of 2-heterosubstituted statines has enabled a broad derivation, covering the S3, S2, and S1′–S3′ sites of the enzyme. In a series of 46 derivatives, several potent inhibitors were obtained with K_i values as low as 3.4 nM and antiviral activity in the lower nanomolar-range. The structural parameters of the compounds which determine the potency of inhibition and selectivity for the viral enzyme are discussed.

Introduction

Inhibition of the proteinase (PR) encoded by human immunodeficiency virus (HIV) is regarded as one of the most promising strategies for design of antiviral agents with potential usefulness for chemotherapy of the acquired immunodeficiency syndrome (AIDS; see refs 1, 2 for reviews). Inhibitors of HIV PR block virus replication *in vitro* when studied in infected lymphocytic or monocytic cell lines, as well as in peripheral blood lymphocytes.[3,4] The mode of action of the inhibitors in infected cells is apparently due to impairment of maturation. This was concluded from the observation that uncleaved gag precursor proteins and immature particles accumulate in the presence of the inhibitors.[5] Recently, first evidence of clinical effectiveness of an HIV PR inhibitor in HIV-infected patients was reported.[6]

The majority of HIV PR inhibitors, including the most potent ones, are based on transition-state analogues as replacements for the dipeptide at the cleavage bond of the substrate. Among these inhibitors, compounds containing statine and its congeners (see Figure 1, **I** and **II**) as a dipeptide mimetic have inhibition constants (K_i) in the range of 10^{-5}–10^{-7} M and show antiviral activity in the micromolar range.[2,7] However, inhibitors containing the hydroxyethylene isostere (see Figure 1, **III**), in most cases, were more potent ($K_i = 10^{-8}$–10^{-9} M, $IC_{50} = 10^{-6}$–10^{-8} M).[2] We wondered whether the lower potency of statine-containing compounds against HIV PR is due to the lack of a hydrophobic side chain at C-2, which favorably interacts with the S1′ subsite of the enzyme.[8] According to structure analyses of enzyme–inhibitor complexes these side chains are obviously occupying the S1′ subsite.[9,10] In fact, the more potent hydroxyethylene isostere based inhibitors contain an alkyl or arylalkyl side chain. Thus, derivatives containing the PheψGly isostere are 100–5000 times weaker

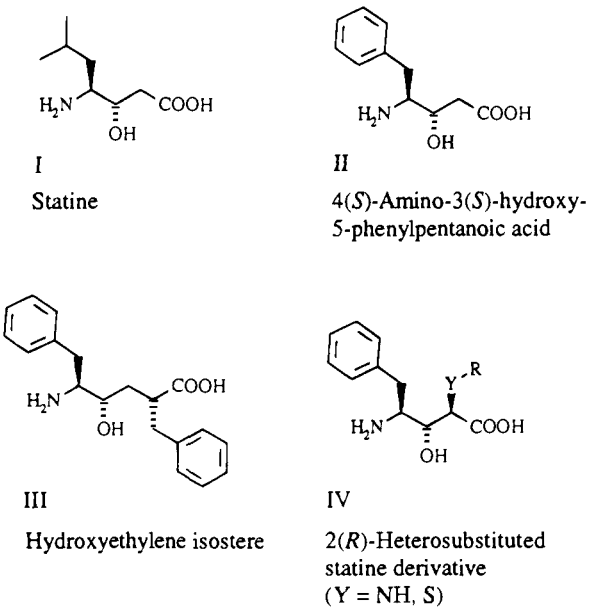


Figure 1. Building blocks of transition state mimics in potent inhibitors of HIV-proteinase.

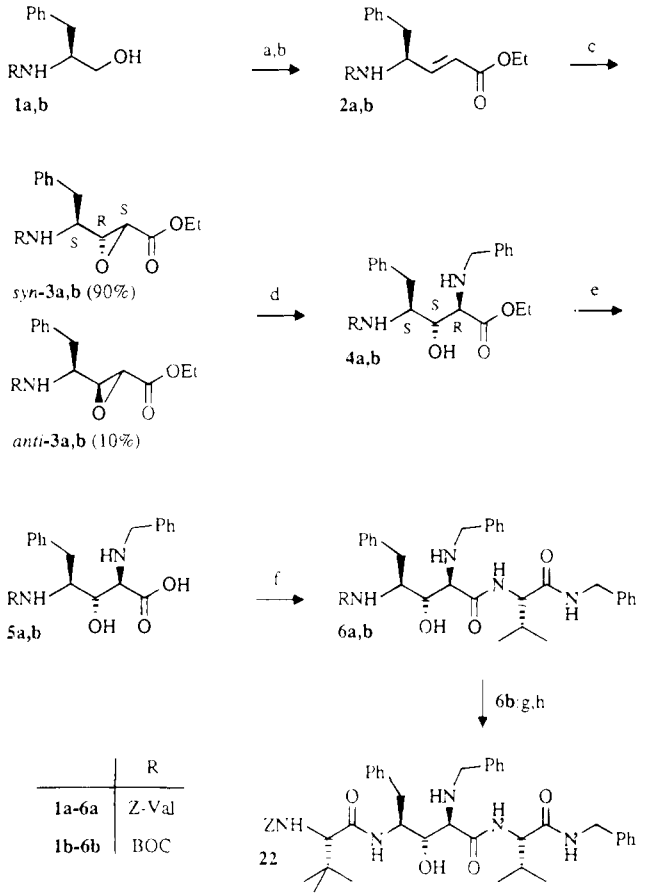
* Author to whom correspondence should be addressed.

† Abbreviations: The abbreviations for the natural amino acids (three letter code) are in accord with the recommendations of the IUPAC–IUB Joint Commission on Biochemical Nomenclature (*Eur. J. Biochem.* **1984**, *138*, 9–37). The symbols represent the L-isomer except where indicated otherwise. In addition: Abu (2(*S*)-aminobutyric acid), AR (analytical research), BOC (*tert*-butyloxycarbonyl; (1,1-dimethylethoxy)carbonyl), CDI (*N,N′*-carbonyldiimidazole), DMF (dimethylformamide), DMSO (dimethyl sulfoxide), EDC·HCl (*N*-ethyl-*N′*-(3-(dimethylamino)propyl)carbodiimide hydrochloride), EDTA (ethylenediaminetetraacetic acid), HOBt (1-hydroxybenzotriazole), HODhbt (3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine), HPTLC (high performance thin-layer chromatography), MES (morpholinoethanesulfonic acid), Nle (norleucine), OSu (*N*-hydroxysuccinimide), Ph (phenyl), Phg ((*S*)-phenylglycine), pNph (4-nitrophenylalanine), Py (pyridyl), RP-HPLC (reversed-phase high-performance liquid chromatography), rt (room temperature), *tert*-leucine ((2*S*)-amino-3,3-dimethylbutanoic acid), TLC (thin-layer chromatography), Tle (*tert*-leucine; 2(*S*)-amino-3,3-dimethylbutanoic acid), Z (benzyloxycarbonyl).

® Abstract published in *Advance ACS Abstracts*, August 15, 1994.

0022-2623/94/1837-3079$04.50/0

Scheme 1. Synthesis of HIV-1 Proteinase Inhibitors Containing 2-Heterosubstituted Statine Analogues[a]



[a] Conditions: (a) $(COCl)_2$, DMSO, CH_2Cl_2, Et_3N, −55 °C → rt; (b) $Ph_3P{=}CHCO_2Et$, $PhCH_3$, 80 °C; (c) MCPBA, CH_2Cl_2, rt; (d) benzylamine, EtOH, reflux; (e) 1.2 equiv of LiOH, THF, H_2O, rt; (f) (*S*)-Val-benzylamide, EDC·HCl, HOBt, DMF, rt; (g) CF_3CO_2H, CH_2Cl_2; (h) (*S*)-Z-Tle-OSu, dioxane, rt.

enzyme inhibitors than those with a PheψPhe isostere.[9] In renin inhibitors, C(2)-alkyl substitution of statines did not increase the potency of these compounds against human renin but rather lowered their activity.[11] However, this may not apply to inhibitors of the viral enzyme.

In this paper, we report the development of a convenient procedure for the synthesis of 2-heterosubstituted statine analogues as a novel dipeptide substitute in HIV PR inhibitors (see Figure 1, **IV**). The substituent at C-2 contains a heteroatom which could lead to favorable interactions within the catalytic center of the enzyme. The structural parameters determining inhibitory potency of this new type of inhibitors against HIV-1 proteinase and HIV replication are described.

Chemistry

Stereochemical control and minimization of the synthetic steps while maintaining maximum synthetic flexibility for derivation were emphasized in the planning of synthesis. The synthesis of 2-heterosubstituted statine derivatives as a novel dipeptide replacement followed the routes described in Scheme 1.

Protected (*S*)-phenylalaninol 1 was subjected to Swern oxidation,[12] and the intermediate aldehyde was transformed by Wittig reaction to the corresponding γ-amino α,β-unsaturated ester **2**. The configuration of the olefinic double bond of **2** is trans, as indicated by a coupling constant of J = 16 Hz between the olefinic protons. Analogous reactions have been reported in the literature, starting from (*S*)-(*N*,*N*-dibenzylamino)phenylalaninal[13] and from (*S*)-*N*-BOC-tyrosine.[14]

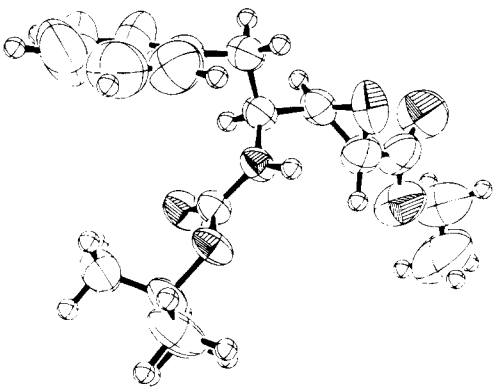


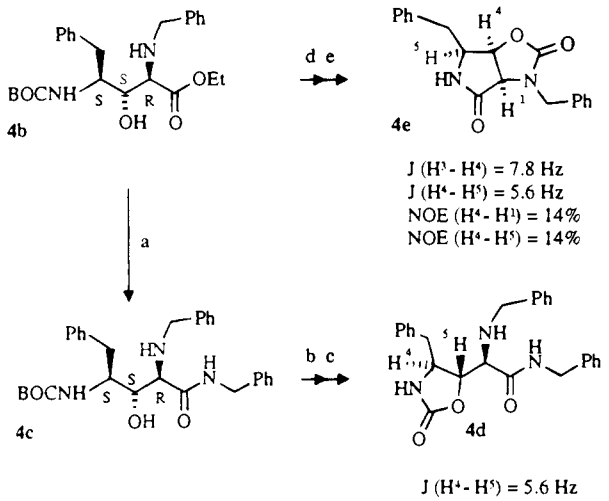
Figure 2. ORTEP representation of epoxide *syn*-**3b**.

Upon treatment of the electron-deficient γ-amino olefin **2** with *m*-chloroperbenzoic acid (MCPBA),[15] a smooth oxidation to the glycidic ester **3** took place. Examination of the crude reaction mixtures (^{1}H-, ^{13}C-NMR; TLC, RP-HPLC) revealed the highly diastereoselective formation of **3** besides unreacted **2**. No undesired byproducts were detected. The diastereomeric mixtures of the corresponding epoxides **3** were isolated by chromatography. The ratio of diastereomers was determined by ^{1}H-NMR spectroscopy (DMSO-d_6) to be 9:1 in both cases (**3a** and **3b**). The predominant diastereomer in the mixture of the *syn*/*anti*-epoxides **3b** could be isolated in diastereomerically pure form (≥99%) and was subjected to crystal structure analysis for determination of the absolute configuration at the oxirane ring relative to the known (*S*)-configuration at the α-carbon of the (*S*)-phenylalanine moiety of the starting material **1**. The stereocenters were established to be 2(*S*), 3(*R*), and 4(*S*) (Figure 2), representing the *syn* product *syn*-**3b**.[16] It was not possible to separate the diastereomers in the case of **3a**, but deprotected *syn*-**3b** could be transformed to *syn*-**3a** by coupling with the Z-valine fragment. The thus obtained *syn*-**3a** was shown to be identical with the main component in the 9:1 mixture of diastereomeric epoxides **3a** by ^{1}H-NMR spectroscopy.[17-19]

The next crucial reaction step in the synthesis of the desired 2-heterosubstituted statine building block was the nucleophilic ring opening of the oxirane **3**. There have been several communications in the literature concerning the ring-opening reactions of 2,3-epoxy esters, amides, and acids.[20,21] For example, 2,3-epoxy acids react with aqueous ammonia to afford only (or predominantly) the C-2 product,[22-24] while 2,3-epoxy esters and amides react under comparable reaction conditions to afford the C-3 product[25-29] (to our knowledge only one example of C-2 ring opening of a 2,3-epoxy amide has been reported[30]). In addition, in the reaction of an epoxy ester with an amine, ester aminolysis can compete with oxirane ring opening.

Upon treatment of **3** with benzylamine in tetrahydrofuran the epoxide ring is attacked at C-2 to give the desired 2-benzylamino-substituted statine derivative **4**. The diastereomerically pure **4**, obtained in high yield after chromatographic workup, was shown to be the

Scheme 2. Determination of Regio- and Stereochemistry of Ring Opening of *syn*-**3b**[a]



[a] Conditions: (a) benzylamine, EtOH, reflux, 3d; (b) 3 N HCl/ether, rt, 1 h; (c) Et_3N, CDI, CH_2Cl_2, 5 °C → rt, 12 h; (d) (i) 3 N HCl/ether, rt 3 h; (ii) *N*-methylmorpholine, EtOH, reflux, 12 h; (e) CH_2Cl_2, pyridine, phosgene, 30 min.

result of ring opening of *syn*-**3**. The regiochemistry of **4** has been confirmed by ^{1}H-NMR and ^{1}H/^{13}C-correlated spectroscopy. The methine proton of the hydroxy-substituted carbon atom is coupled with the C_α-proton of the phenylalanine fragment (for confirmation of stereochemistry, see Scheme 2).

In the course of the reaction of **3b** with benzylamine the formation of a minor byproduct (<5%) was detectable, which became more significant upon prolonged reaction time. The compound was isolated and characterized to be benzylamide **4c** (Scheme 2). Progress of formation of **4c** was accompanied by a decrease of **4b**, indicating that the latter one is the major source of aminolysis rather than the 2,3-epoxy ester **3b**.

An interesting result was obtained by running the ring opening of **3b** with less reactive or sterically hindered benzylamines in dry tetrahydrofuran. Under these conditions **3b** was unaffected by the nucleophiles used. Only in the presence of small amounts of water did a significant product formation (**4**) occur. Under protic reaction conditions (e.g., ethanol) product formation (**4**) is accelerated without influencing the observed regio- and stereoselectivity.

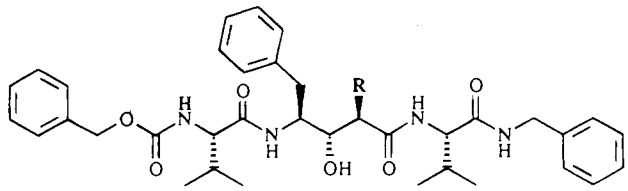
C-2 directed ring opening of **3** could also be achieved with a variety of other nucleophiles (Table 1), e.g., benzyl mercaptans, thiophenols (compare **7** in Table 1), arylamines, secondary amines and amino acid esters (data not shown). In summary the nucleophilic ring opening of 2,3-epoxy esters **3** proceeds regiocontrolled and with inversion at the C-2 carbon. The resulting 2-heterosubstituted statine esters **4** show (*S*)-configuration at the C(3) carbon and (*R*)-configuration at the C(2) carbon.

The highly stereo- and regioselective four-step synthesis of the 2-heterosubstituted statine building block (**4**) allowed a straightforward synthesis of inhibitors **6**–**52** (Tables 1–6). Starting the reaction sequence with **1a** (Scheme 1), the corresponding ester **4a** was saponified (**5a**) and coupled with an appropriate C-protected amino acid (representing P2′–P3′)[8] to give the final inhibitors (Table 1, **6**–**18**; Table 3, **30**–**34**; and Table 5, **41**–**46**).

It is more advantageous to start the synthesis with **1b** since the corresponding BOC-protected intermediate **4b** can be used for derivation in both directions. **4b** was hydrolyzed to give **5b**, which was first flanked by the P2/P3 substituents. After removal of the BOC protection group, synthesis of the inhibitors was completed by coupling with suitable N-protected amino acids (Table 2, **19**–**29**; Table **4**, **35**–**40**; Table 6, **47**–**52**).

We have used **4b** and **4c** for an additional confirmation of the stereo- and regioselectivity of the epoxidation and ring-opening step (Scheme 2). Deprotected **4c** was treated with carbonyldiimidazole to give the corresponding oxazolidinone derivative **4d**. The ^{1}H-NMR spectrum of **4d** displays a vicinal coupling constant of 5.6 Hz between the methine ring protons, consistent with trans configuration of the oxazolidinone.[31] According to the

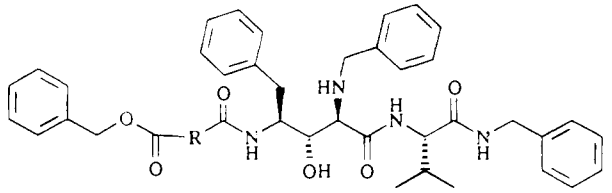
Table 1. Inhibitors Modified in P1′: Inhibitory Activity against HIV-1 Protease and Antiviral Activity against HIV-1, IIIB, in MT4 Cells



no.	R	K_i (nM)	IC_{50} (nM)	mp (°C)	formula[b]
6	$HNCH_2C_6H_5$	6.1	580	81–84	$C_{43}H_{53}N_5O_6 \cdot 0.8H_2O$
7	$SCH_2C_6H_5$	10.0	330	oil	$C_{43}H_{52}N_4O_6S \cdot 0.1H_2O$
8	HNC_6H_5	6.9	1340	120–130	n.d.
9	$HN_1C_2H_4C_6H_5$	34.0	—[a]	127–130	$C_{44}H_{55}N_5O_6$
10	HN-*n*-C_4H_9	23.0	—[a]	149–155	$C_{40}H_{55}N_5O_6 \cdot 0.3H_2O$
11	HNC_6H_{11}	33.5	940	85–95	$C_{42}H_{57}N_5O_6$
12	1-$(HNCH_2)C_{10}H_7$	26.0	—[a]	162–170	$C_{47}H_{55}N_5O_6 \cdot 0.2H_2O$
13	*p*-(HN)biphenyl	59.0	—[a]	168–175	$C_{48}H_{55}N_5O_6$
14	3-(HN-C_2H_4)indolyl	61.0	—[a]	oil	$C_{46}H_{56}N_6O_6 \cdot 0.4H_2O$
15	2(HNC_2H_4)pyridyl	200	—[a]	59–66	$C_{43}H_{54}N_6O_6 \cdot 0.2H_2O$
16	$HNCH_2$-*p*-$(OCH_3)C_6H_4$	7.4	320	72–80	$C_{44}H_{55}N_5O_7 \cdot 1.2H_2O$
17	$HNCH_2$-*p*-$(Cl)C_6H_4$	17.0	400	78–82	$C_{43}H_{52}ClN_5O_6 \cdot 0.7H_2O$[c]
18	$HNCH_2$-*p*-$(Br)C_6H_4$	23.0	350	84–87	$C_{43}H_{52}BrN_5O_6$

[a] >3000 nM. [b] Satisfactory elemental analyses within ±0.4% of the calculated value for C, H, N were obtained unless otherwise noted. [c] C, H; N: calcd, 8.94; found, 8.46.

Table 2. Inhibitors Modified in P2: Inhibitory Activity against HIV-1 Protease and Antiviral Activity against HIV1, IIIB in MT4 Cells



no.	R	K_i (nM)	IC_{50} (nM)	mp (°C)	formula[f]
6	L-Val[a]	6.1	580	81–84	$C_{43}H_{53}N_5O_6 \cdot 0.8H_2O$
19	L-Ala[a]	34	–[b]	98–103	$C_{41}H_{49}N_5O_6 \cdot 0.4H_2O$
20	L-Leu[a]	65	–[b]	157–161	$C_{44}H_{55}N_5O_6 \cdot 0.7H_2O$
21	L-Ile[a]	8.3	–[b]	175–180	$C_{44}H_{55}N_5O_6 \cdot 0.9H_2O$
22	L-Tle[a]	9.2	320	78–82	$C_{44}H_{55}N_5O_6$
23	L-Abu[a]	17.1	2000	153–157	$C_{42}H_{51}N_5O_6$
24	L-Trp[a]	200	–[b]	79–84	$C_{49}H_{54}N_6O_6 \cdot 0.6H_2O$
25	L-Phg[a]	51	–[b]	202–204	$C_{46}H_{51}N_5O_6$[g]
26	L-Asn[c]	10.4	1010	185–193	$C_{42}H_{50}N_6O_7 \cdot 0.4H_2O$
27	L-Ser[d]	153	2500	76–81	$C_{41}H_{49}N_5O_7 \cdot 0.6H_2O$
28	L-Glu[a]	45	2000	178–189	$C_{43}H_{52}N_6O_7 \cdot 1.6H_2O$
29	L-His[e]	29	–[b]	resin	$C_{44}H_{51}N_7O_6$[h]

[a] Coupling Z-R-OSu. [b] >3000 nM. [c] Coupling Z-AsnOH + HODhbt + EDC. [d] Coupling O-(*tert*-butyl)-Ser-OSu and deprotection with CF_3 COOH. [e] Coupling of Z-HisOH by azid method. [f] Satisfactory elemental analyses within ±0.4% of the calculated value for C, H, N were obtained unless otherwise noted. [g] N; C: calcd, 70.47; found, 71.77; H: calcd, 7.39; found, 6.73. [h] H; C: calcd, 68.29; found, 60.78; N: calcd, 12.67; found, 10.46.

known (*S*)-configuration at C-4, C-5 has to be (*R*)-configurated. Deprotection of **4b** and subsequent ring closure between the primary amine and the ethyl ester afforded an intermediate γ-lactam, which was transformed to the bicyclic **4e** upon treatment with phosgene. The measured coupling constants and NOEs of **4e** are in agreement with the expected *cis*-anellation of the two rings.

Results and Discussion

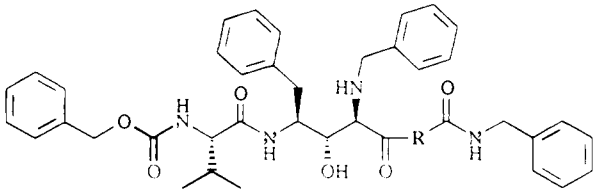
For all compounds reported, we measured the inhibition constant (K_i) for HIV-1 proteinase and determined antiviral activity (IC_{50}) in an assay observing the cytopathic effect induced by HIV-1, strain IIIB, in *de novo* infected MT4 cells (see the Experimental Section for details).

As expected for an inhibitor representing a transition state analogue, the prototype compound **6** exhibited pure competitive inhibition of HIV PR (see ref 32 for the analysis of a derivative of **6**). The K_i for **6** is 6.1 nM and is thus lower than values reported for statine containing inhibitors.[7] Likewise, it shows increased antiviral activity (IC_{50} = 580 nM, mean of 11 experiments) when compared to statine derivatives.

Encouraged by this initial result, we synthesized a large series of derivatives starting with modifications at the P1′ side chain (see Table 1). Replacement of nitrogen in the P1′ benzylamino group by sulfur (**7**) only slightly increased the K_i to 10 nM. This indicates that the P1′ nitrogen in **6** is not crucial for binding to the enzyme. Also changing the P1′ moiety to phenylamino (**8**), thus introducing a less basic nitrogen, influenced K_i only slightly.

We then explored the steric requirements for the P1′ substituent. Elongation of the side chain by one methylene group (**9**) reduced inhibitory potency about 5-fold. Likewise, a small alkyl substituent (butyl in **10**), an aliphatic ring (**11**), α-naphthyl (**12**) and biphenyl groups (**13**), and N-heterocyclic residues (**14**, **15**) were less potent inhibitors of HIV PR than the parent compound **6**. Para substituents at the P1′ benzylamino group (**16**–**18**) also increased the K_i value to some extent.

Table 3. Inhibitors Modified in P2′: Inhibitory Activity against HIV-1 Protease and Antiviral Activity against HIV-1, IIIB, in MT4 Cells



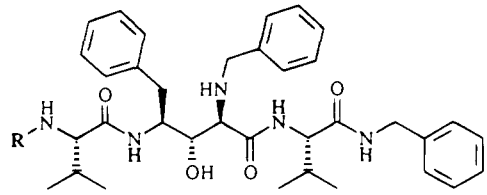
no.	R	K_i (nM)	IC_{50} (nM)	mp (°C)	formula[d]
6	L-Val	6.1	580	81–84	$C_{43}H_{53}N_5O_7 \cdot 0.8H_2O$
30	L-Leu	44	–[a]	190–201	$C_{44}H_{55}N_5O_6$[e]
31	L-Ile	–[a]	–[a]	145–166	$C_{44}H_{55}N_5O_6$[f]
32	L-Tle[b]	20	–[a]	83–87	$C_{44}H_{55}N_5O_6 \cdot 0.4H_2O$
33	D-Tle[c]	–[a]	–[a]	82–86	$C_{44}H_{55}N_5O_6$
34	L-Asn	42	–[a]	208–214	$C_{42}H_{50}N_6O_7 \cdot 0.5H_2O$

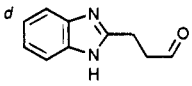
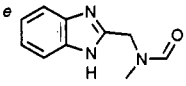
[a] >3000 nM. [b] Mixture of diasteromers 5:1 (L/D-Tle). [c] Mixture of diastereomers 4:1 (D/L-Tle). [d] Satisfactory elemental analyses within ±0.4% of the calculated value for C, H, N were obtained unless otherwise noted. [e] C: calcd, 70.47; found, 65.77; H: calcd, 7.39; found, 6.91; N: calcd, 9.34; found, 8.59. [f] C, N; H: calcd, 7.39; found, 6.86.

No significant correlation between K_i and antiviral activity was observed for these derivatives as well as for other compounds (see below). As an example, compound **10** and **18** exhibit identical K_i values, but **10** was found to be inactive while **18** showed almost the best antiviral activity (IC_{50} = 350 nM) in the series. This illustrates that the antiviral effect is determined not only by the inhibitory potency against the proteinase, but also by other poorly understood factors such as cellular uptake, stability, or intracellular distribution. It was, however, consistently observed that compounds with K_i > 50 nM did not exhibit antiviral activity in our assay system.

Next, we replaced valine in P2 of compound **6** by other amino acids (Table 2). The isopropyl side chain in P2 (**6**), obviously, is optimal for interaction of the inhibitor with the enzyme, since its replacement by smaller (**19**) or larger alkyl groups (**20**–**23**) reduces inhibitory potency. The 8-fold difference in K_i between leucine (**20**) and isoleucine (**21**) reflects the steric restrictions at the S2 subsite of the enzyme. Also aromatic (**24**, **25**), polar (**26**, **27**), and charged (**28**, **29**) side chains are not well tolerated. Only with *tert*-leucine (**22**) an increase in antiviral activity was achieved, which was observed also for other derivatives (see below). Comparing the results obtained by replacing valine in P2 (Table 2) to those obtained for modifications in P2′ (Table 3), it is evident that these two positions are not equivalent. Introduction of isoleucine in P2 yields an 8 times more active enzyme inhibitor than the derivative containing leucine in P2; the compound containing isoleucine in P2′, however, is at least 70-fold less potent than the one containing leucine (compare **30** to **31**). It also appears that the P2′ site is more sensitive to deviation from the isopropyl residue of valine (compare **26** to **34**, **22** to **32**). A D-amino acid is not tolerated at this position (compare **32** to **33**).

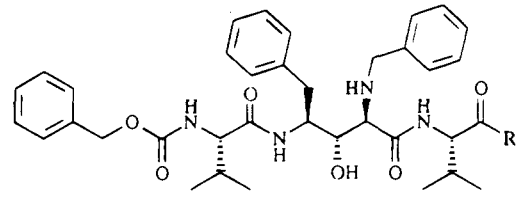
Table 4. Inhibitors Modified in P3: Inhibitory Activity against HIV-1 Protease and Antiviral Activity against HIV-1, IIIB, in MT4 Cells



no.	R	K_i (nM)	IC_{50} (nm)	mp (°C)	formula[f]
6	$C_6H_5CH_2OCO$	6.1	580	81–84	$C_{43}H_{53}N_5O_6 \cdot 0.8H_2O$
35	BOC[a]	215	–[b]	126–129	$C_{40}H_{55}N_5O_6$
36	$H_2N(CH_2)_5CO$	33	–[b]	169–173	n.d.
37	$2PyCH_2OCO$[c]	8.6	350	oil	$C_{42}H_{52}N_6O_6 \cdot 0.7H_2O$
38	2-quinolinoyl[d]	10.2	1610	83–86	$C_{45}H_{52}N_6O_5 \cdot 0.7H_2O$
39		9.9	180	113–118	$C_{45}H_{55}N_7O_5 \cdot 1.6H_2O$
40		10.2	270	175–196	$C_{43}H_{56}N_8O_5$[g]

[a] Synthesized according to Scheme 1, h: instead of Z-Tle-OSu, BOC-Val-OSu was used. [b] >3000 nM. [c] Synthesized according to Scheme 1, h: instead of Z-TleOSu *N*-[(2-pyridylmethyloxy)carbonyl]valine-OSu (ref 43) was used. [d] 2-Quinolinecarboxylic acid or 3-(benzimidazol-2-yl)propionic acid were coupled to valine methyl ester with HODhbt and EDC. Subsequent saponification led to the corresponding acids which were coupled with HODhbt and EDC. [e] Synthesized according to Scheme 1, h: The starting material (*S*/*R*)-[(*N*-(benzimidazol-2-yl)-*N*-methylamino)carbonyl]valine was synthesized analogously to ref 44). [f] Satisfactory elemental analyses within ±0.4% of the calculated value for C, H, N were obtained unless otherwise noted. [g] H; C: calcd, 67.52; found, 66.24; N: calcd, 14.65; found, 13.63.

Table 5. Inhibitors Modified in P3′: Inhibitory Activity against HIV-1 Protease and Antiviral Activity against HIV-1, IIIB, in MT4 Cells



no.	R	K_i (nM)	IC_{50} (nM)	mp (°C)	formula[b]
6	$HNCH_2C_6H_5$	6.1	580	81–84	$C_{43}H_{53}N_5O_7 \cdot 0.8H_2O$
41	OC_2H_5	1200	–[a]	90–96	$C_{38}H_{50}N_4O_7$
42	$NHC(CH_3)_3$	72	–[a]	154–168	$C_{40}H_{55}N_5O_6$
43	2-($NHCH_2$)pyridyl	12.0	1140	129–136	$C_{42}H_{52}N_6O_6$
44	2-($NHCH_2$)benzimidazolyl	13.0	140	201–204	$C_{44}H_{53}N_7O_6 \cdot 0.3H_2O$
45	2-(OCH_2)benzimidazolyl	29	–[a]	86–90	$C_{44}H_{52}N_6O_7 \cdot 0.8H_2O$
46	3-(NHC_2H_4)indolyl	10.6	–[a]	106–114	$C_{46}H_{56}N_6O_6 \cdot 0.8H_2O$

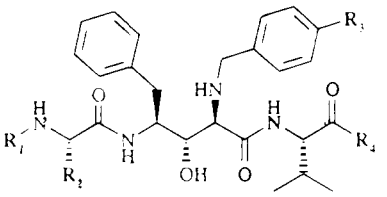
[a] >3000 nM. [b] Satisfactory elemental analyses within ±0.4% of the calculated value for C, H, N were obtained.

A series of compounds with substitutions for the N-terminal benzyloxycarbonyl group in position P3 of **6** were synthesized (Table **4**). Introduction of a *tert*-butoxycarbonyl group (**35**) or of an aliphatic acyl residue (**36**) did not yield good inhibitors. This finding is in accordance with the reported preference of the S3 subsite of HIV PR for aromatic residues.[8] Introducing a pyridyl moiety (**37**), however, was not deleterious to inhibitory potency but slightly enhanced antiviral activity. The 2-quinolinoyl moiety (**38**) which had been incorporated in potent compounds by others[4] led to considerable loss of antiviral activity. Two P3 residues, however, which both contain a benzimidazole moiety (coupled via an acyl chain in **39**, or an urea linkage in **40**) significantly enhanced antiviral activity, while slightly reducing potency against the enzyme. With other compounds, too, an even more pronounced beneficial effect of the benzimidazole group on antiviral activity was observed (see below).

Finally, the P3′ position of **6** was modified (Table 5). Replacement of the aminobenzyl group by ethoxy (**41**) or *tert*-butylamine (**42**) resulted in weak inhibitors. The pyridyl group (**43**), while enhancing antiviral activity when present in P3, was not of advantage in P3′. Again a major increase in antiviral potency but no pronounced effect on enzyme inhibition was observed with a benzimidazole moiety (**44**). This compound, where only one side chain of **6** was modified, was the most potent out of this series of derivatives (IC_{50} = 140 nM). A similar benefical effect of the benzimidazole moiety has been reported by DeSolms et al.[33] Methylbenzimidazole coupled via an ester linkage (**45**) was devoid of antiviral activity. Unexpectedly, an indole moiety (**46**) did not block HIV replication, although the compound has a K_i value lower than that of **44**.

Derivative **44**, which is about **4** times more potent than the lead compound **6**, was further modified (Table 6), based on the results of the single variations reported

Table 6. Inhibitors Modified in P3, P2, P1′, and P3′: Inhibitory Activity against HIV-1 Protease and Antiviral Activity against HIV-1, *IIIB*, in MT4 Cells



no.	R_1	R_2	R_3	R_4	K_i (nM)	IC_{50} (nM)	mp (°c)	formula[a]
44	Z	$CH(CH_3)_2$	H	2-($NHCH_2$)benzimidazolyl	13.0	140	201–204	$C_{44}H_{53}N_7O_6 \cdot 0.3H_2O$
47	Z	$CH(CH_3)_2$	OCH_3	2-($NHCH_2$)benzimidazolyl	3.4	25	205–209	$C_{45}H_{55}N_7O_7 \cdot 0.7H_2O$
48	Z	$CH(CH_3)_2$	Cl	2-($NHCH_2$)benzimidazolyl	6.1	120	206–210	$C_{44}H_{52}ClN_7O_6$
49	Z	$C(CH_3)_3$	Cl	2-($NHCH_2$)benzimidazolyl	7.5	34	114–171	$C_{45}H_{54}ClN_7O_6$[b]
50	Z	$C(CH_3)_3$	OCH_3	2-($NHCH_2$)benzimidazolyl	4.6	14	231–234	$C_{46}H_{57}N_7O_7$
51	*p*-$(OH)C_6H_4C_2H_4CO$	$C(CH_3)_3$	OCH_3	2-($NHCH_2$)benzimidazolyl	13.0	96	198–205	$C_{47}H_{59}N_7O_7 \cdot 0.7H_2O$
52	(*S*)-phenyllactoyl	$C(CH_3)_3$	OCH_3	2-($NHCH_2$)benzimidazolyl	6.0	38	153–157	$C_{47}H_{59}N_7O_7 \cdot 0.8H_2O$

[a] Satisfactory elemental analyses within ±0.4% of the calculated value for C, H, N were obtained unless otherwise noted. [b] C, H, N: calcd, 11.89; found, 12.38.

Table 7. Activity of Aminobenzylstatine-Containing Inhibitors against HIV-1, HIV-2, and Mammalian Aspartic Proteinases and against HIV-1, IIIB, and HIV-2, EHO, Replication in MT4 Cells

	HIV-1		HIV-2		IC_{50} (nM)		
no.	K_i (nM)	IC_{50} (nM)[a]	K_i (nM)	IC_{50} (nM)[a]	porcine pepsin	human renin	human cathepsin D
6	6.1	315	180	>3000	>10,000	>10,000	35
16	7.4	132	39	810	>10,000	>10,000	110
22	9.2	93	54	1400	>10,000	>10,000	60
47	3.4	9.7	120	460	>10,000	>10,000	380
49	7.5	11.3	17	240	>10,000	>10,000	1800
50	4.6	5.4	52	385	>10,000	>10,000	1000
51	13.0	21	63	380	>10,000	>10,000	>10,000
52	6.0	7.9	110	470	>10,000	>10,000	2000

[a] IC_{50} measured by analysis of p24 or p26 antigen concentration for HIV-1 and HIV-2, respectively.

above. Introduction of a methoxy group at the P1′ benzylamino side chain (**47**) lowered the IC_{50} about 6-fold. This was unexpected, since the methoxy group enhanced antiviral activity only slightly in **16** when compared to **6** (Table 1). We had observed that *tert*-leucine was more effective than valine in position P2 (compare **22** to **6**). The effect of this substitution was even more pronounced in the case of compounds **48** and **49**, the latter showing a 4-fold lower IC_{50} value of 34 nM. Finally exchange of the chloro group in **49** to methoxy yielded the most potent inhibitor (**50**), both of the enzyme (K_i = 4.6 nM) and of virus replication (IC_{50} = 14 nM), in this series of derivatives. Additional modifications in P3 (**51**, **52**), intended to change the physicochemical properties of **50**, did not further enhance antiviral potency.

Although we were primarily interested in designing inhibitors of HIV-1 proteinase, we also tested the most active compounds against the HIV-2 enzyme and against HIV-2, strain EHO, replication in MT4 cells. For reasons of comparability, inhibition of HIV-1, strain IIIB, replication was measured under the same experimental conditions. The data in Table 7 show that, in general, the compounds are less potent inhibitors of the HIV-2 enzyme, and, furthermore, that different structure–activity relationships govern the interaction with this enzyme. The most potent HIV-2 PR inhibitor **49** also proved to be the best inhibitor of HIV-2 replication in cell culture (IC_{50} = 240 nM).

To evaluate the selectivity of the HIV-1 protease inhibitors, we also studied their inhibitory potential against mammalian aspartic proteinases. As summarized in Table 7, the compounds do not inhibit human renin or porcine pepsin; the prototype compound **6** is, however, a potent inhibitor of cathepsin D. Inhibition of this enzyme is reduced when proceeding to compounds which contain *tert*-leucine in P2 (compare **6** with **22**), a para substituent in P1′ (**16**), and benzimidazole in P3′ (compare **16** with **47**). Combination of these residues (**49**, **50**) further reduced cathepsin D inhibitory activity, which is also observed for compounds with a modified P3 moiety (**51**, **52**). Thus, the most potent inhibitor of HIV-1 replication reported here (**50**) shows only IC_{50} = 1 μM in cathepsin D inhibition.

Biochemical and pharmacological characterization, including oral bioavailability of these compounds, will be published elsewhere.[34]

Summary

We have developed a flexible synthesis of HIV-1 proteinase inhibitors which contain 2-heterosubstituted 4-amino-3-hydroxy-5-phenylpentanoic acid as a novel central building block. The cornerstone of the synthetic strategy is a two step sequence: The diastereoselective epoxidation of α,β-unsaturated carboxylic esters, obtained by Wittig olefination of appropriately protected α-amino aldehydes, followed by regio- and stereospecific ring opening of the corresponding epoxides with heteronucleophiles leading exclusively to 2-heterosubstituted statine analogues. These compounds are more effective inhibitors of HIV-1 proteinase than related statine-containing compounds. Benzimidazole, placed in either the P3 or P3′ position, considerably enhances antiviral activity of the compounds. Combination of this substitution with other favorable modifications in positions P1′, P2, and P3 led to highly active compounds, the most potent one (**50**) exhibiting a K_i of 4.6 nM and an IC_{50} value of 14 nM. The anti-HIV potency and adequate selectivity especially of derivatives **49**–**51** warrant further pharmacological evaluation of this class of compounds.

Experimental Section

Chemistry. ^{1}H-NMR spectra were recorded with a Bruker WC-250 or AMX-500 spectrometer; chemical shifts are reported in ppm (δ) relative to internal Me_4Si. All *J* values are

given in hertz (Hz). Elemental analyses were performed by Analytical Department, Sandoz Basle, Switzerland, and Mikroanalytisches Laboratorium, Institut für Physikalische Chemie Universität Wien, and are within ±0.4% of the theoretical value. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Analytical thin-layer chromatography was performed on silica gel 60 F_{254} glass plates (HPTLC, Merck). Preparative column chromatography was performed on silica gel (40–63 μm) under pressure (~0.2 mPa). Solvents were AR grade and were used without further purification. All reagents were obtained from commercial suppliers and were used without further purification. Evaporations were carried out *in vacuo* with a rotary evaporator. Melting points were determined with a thermovar apparatus (Reichert-Jung) and are not corrected. The required valine amides (Table 5) were synthesized starting either from BOCValOSu and the free amine, or using BOCVal, free amine and the usual peptide coupling conditions EDC·HCl, HOBt in DMF.

Representative methods for all the compounds synthesized according to Scheme 1 are described. In the case of the mercaptobenzyl derivative **7**, a different procedure for the epoxide opening was developed: The potassium salt of benzyl mercaptan was used, and the solution was stirred at 4 °C under an atmosphere of nitrogen.

***N*-[*N*-[(Benzyloxy)carbonyl]valyl]phenylalaninol (1a).** A 3.2-g (21.2-mmol) sample of (*S*)-phenylalaninol was added to a solution of 7.44 g (20.0 mmol) of *N*-[(benzyloxy)carbonyl]-valine 4-nitrophenyl ester in 50 mL of dimethylformamide. After addition of 2 g (19.8 mmol) of triethylamine, the reaction mixture was stirred at room temperature for 3 d. After evaporation of the solvent the residue was dissolved in dichloromethane, carefully washed several times with 0.1 N NaOH and once with water, and dried. The solution was filtered, the solvent evaporated, and the residue chromatographed on silica gel (eluent: dichloromethane/methanol = 95/5): yield 8.1 g of **1a** (85%); mp 154–156 °C; ^{1}H-NMR ($CDCl_3$) δ 0.82 (2d, 6H), 2.10 (oct, 1H), 2.82 (bd, 2H), 3.45–3.75 (m, 2H), 4.90 (dd, 1H), 4.17–4.25 (m, 1H), 5.17 (s, 2H), 5.30 (d, 1H), 6.30 (d, 1H), 7.10–7.40 (m, 5H). Anal. ($C_{22}H_{28}N_2O_4$) C, H, N.

4(*S*)-[[*N*-[(Benzyloxy)carbonyl]valyl]amino]-5-phenyl-2(*E*)-pentenoic Acid Methyl Ester (2a). A 3.12-mL (36.3-mmol) sample of oxalyl chloride in 40 mL of dry dichloromethane was cooled to −55 °C, and 2.81 mL (32.7 mmol) of dimethyl sulfoxide was added dropwise. Then, 6.98 g (18.1 mmol) of 1a, dissolved in 40 mL of dichloromethane and 3.13 mL (43.9 mmol) of dimethyl sulfoxide were added at −55 °C. The reaction mixture was stirred at −55 °C for 1 h, reacted with triethylamine, and stirred until room temperature was reached. After dilution with 200 mL of dichloromethane, the mixture was washed with 1 N HCl and dried over magnesium sulfate, and the solvent was evaporated. The residue was dissolved in toluene, 6.32 g (18.2 mmol) of [(ethoxycarbonyl)-methylene]triphenylphosphorane was added, and the reaction mixture was heated to 80 °C for 1 h. After evaporation of the solvent, the residue was chromatographed on silica gel (eluent: toluene/ethyl acetate = 4/1): yield 6.7 g of **2a** (82%); mp 161–165 °C; ^{1}H-NMR ($CDCl_3$) δ 0.85 (2d, 6H), 1.24 (t, 3H), 2.10 (sext, 1H), 2.72–3.04 (m, 2H), 3.92 (dd, 1H), 4.18 (q, 2H), 5.00 (q, 1H), 5.10 (s, 2H), 5.18 (d, 1H), 5.86 (d, 1H), 6.17 (bd, 1H, J = 16 Hz), 6.90 (dd, 1H, J = 16 Hz), 7.10–7.22 (m, 10H); $[\alpha]^{20}_D$ −17.4° ($CDCl_3$, c = 1). Anal. ($C_{26}H_{32}N_2O_5$) C, H, N.

4(*S*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-5-phenyl-2-(*E*)-pentenoic Acid Ethyl Ester (2b). Preparation was as described for **2a**, starting from (*S*)-*N*-[(1,1-dimethylethoxy)-carbonyl]phenylalaninol **1b**:[35] yield 4.6 g of **2b** (80%); mp 70–72 °C; ^{1}H-NMR ($CDCl_3$) δ 1.26 (t, 3H), 1.40 (s, 9H), 2.80–2.98 (m, 2H), 4.10–4.25 (m, 3H), 4.56 (d, 1H), 5.86 (dd, 1H), 7.10–7.38 (m, 5H). Anal. ($C_{18}H_{25}NO_4$) C, H, N.

(2*S*/*R*,3*R*/*S*,4*S*)-4-[[*N*-[(Benzyloxy)carbonyl]valyl]amino]-2,3-epoxy-5-phenylpentanoic Acid Ethyl Ester (3a). To a solution of 3 g (6.6 mmol) of **2a** in 30 mL of dichloromethane was added 3.42 g (10.9 mmol) of *m*-chloroperbenzoic acid. The reaction mixture was stirred for 5 d. After evaporation of the solvent, the residue was chromatographed on silica gel (eluent: toluene/ethyl acetate = 4/1): yield 2 g of **3a** (65%); mixture of diastereomers (*syn*-**3a**:*anti*-**3a** = 89:11), introduced in the synthesis of **4a** without further separation; mp 164–167 °C; $[\alpha]^{25}_D$ −10.6° (c = 1, $CHCl_3$); ^{1}H-NMR (DMSO-d_6, 500 MHz, 330 K) δ 0.78, 0.81 (2d, 6H, $CH(CH_3)_2$ of *syn*-**3a**), 0.90, 0.91 (2d, 6H, $CH(CH_3)_2$ of *anti*-**3a**), 1.21 (t, 3H), 1.88 (m, 1H), 2.81 and 2.90 (AB part of ABX, 2H), 3.19 (d, 1H, C(2)*H* of *syn*-**3a**), 3.27 (dd, 1H, C(3)*H* of *syn*-**3a**), 3.30 (m, 2H, C(2)*H* and C(3)*H* of *anti*-**3a**); 3.80 (dd, 1H), 4.14 (m, 3H), 5.04 and 5.08 (AB, 2H), 6.79 (broad, 1H), 7.16–7.37 (m, 10H), 7.70 (d, 1H). Anal. ($C_{26}H_{32}N_2O_6$) C, H, N.

(2*S*,3*R*,4*S*)-4-[[*N*-[(Benzyloxy)carbonyl]valyl]amino]-2,3-epoxy-5-phenylpentanoic Acid Ethyl Ester (*syn*-3a). Trifluoroacetic acid (3 mL) was added to a solution of 1 g (3 mmol) of *syn*-**3b** in 50 mL of dichloromethane. The mixture was stirred for 2 h at room temperature, washed with saturated $NaHCO_3$ solution and brine, and dried over $MgSO_4$. The solvent was evaporated *in vacuo* leaving 0.6 g (86%) of (2*S*,3*R*,4*S*)-4-amino-2,3-epoxy-5-phenylpentanoic acid ethyl ester as an oil: $[\alpha]^{20}_D$ + 32.1° (c = 2.5, methanol); ^{1}H-NMR ($CDCl_3$) δ 1.28 (t, 1 H), 2.43 (bs, 2 H), 2.75–3.05 (m, 3 H), 3.21 (dd, 1 H), 3.26 (m, 1 H), 4.16 and 4.22 (AB part of ABX, 2 H), 7.12–7.38 (m, 5 H). Anal. ($C_{13}H_{17}NO_3 \cdot 0.5H_2O$) C, H, N.

3,4-Dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (0.21 g, 1.28 mmol) and 0.22 g (1.25 mmol) of *N*-ethyl-*N'*-(3-(dimethylamino)propyl)carbodiimide hydrochloride were added to a solution of 0.32 g (1.27 mmol) of *N*-[(benzyloxy)carbonyl]valine and 0.30 g (1.28 mmol) of (2*S*,3*R*,4*S*)-4-amino-2,3-epoxy-5-phenylpentanoic acid ethyl ester in 30 mL of dimethylformamide. The mixture was stirred for 3 d at room temperature. The solvent was evaporated *in vacuo*, and the residue was dissolved in ethyl acetate, washed with 1 N HCl, saturated $NaHCO_3$ solution, and brine, and dried over $MgSO_4$. The mixture was concentrated *in vacuo*; the product crystallized after addition of diethyl ether: yield 0.4 g of *syn*-**3a** (67%); mp 164–169 °C; $[\alpha]^{20}_D$ −6.49° (c = 1, methanol); ^{1}H-NMR (DMSO-d_6, 500 MHz, 330 K) δ 0.74, 0.78 (2d, 6H), 1.18 (t, 3H), 1.85 (m, 1H), 2.78 and 2.89 (AB part of ABX, 2H), 3.16 (d, 1H, C(2)*H*), 3.26 (dd, 1H, C(3)*H*), 3.77 (dd, 1H), 4.11 (m, 3H), 5.03 and 5.07 (AB, 2H), 6.94 (broad, 1H), 7.15–7.37 (m, 10H), 7.77 (d, 1H). Anal. ($C_{26}H_{32}N_2O_6 \cdot 0.2H_2O$) C, H, N.

(2*S*/*R*,3*R*/*S*,4*S*)-4-[[(1,1-Dimethylethoxy)carbonyl]amino]-2,3-epoxy-5-phenylpentanoic Acid Ethyl Ester (3b). Preparation was as described for 3a: yield 1.37 g of **3b** (62%); mixture of diastereomers (*syn*-**3b**:*anti*-**3b** = 9:1), introduced in the synthesis of **4b** without further separation; mp 55–61 °C; $[\alpha]^{25}_D$ +17.5° (c = 1, CH_2Cl_2); ^{1}H-NMR (DMSO-d_6, 500 MHz, 350 K) δ 1.22 (t, 3H), 1.32 (s, 9H, $C(CH_3)_3$ of *anti*-**3b**), 1.34 (s, 9H, $C(CH_3)_3$ of *syn*-**3b**), 2.76–2.88 (m, 2H), 3.16 (dd, 1H, C(3)*H* of *anti*-**3b**), 3.22 (d, 1H, C(2)*H* of *syn*-**3b**), 3.24 (dd, 1H, C(3)*H* of *syn*-**3b**), 3.51 (d, 1H, C(2)*H* of *anti*-**3b**), 3.68–3.74 (m, 1H), 4.12–4.20 (m, 2H), 6.70 (broad, 1H), 7.18–7.29 (m, 5H). Anal. ($C_{18}H_{25}NO_5$) C, H, N.

(2*S*,3*R*,4*S*)-4-[[(1,1-Dimethylethoxy)carbonyl]amino]-2,3-epoxy-5-phenylpentanoic Acid Ethyl Ester (*syn*-3b). **3b** (1.37 g) was dissolved in 10 mL of *n*-hexane/ethyl acetate = 85/15. The solution was transferred to a chromatography column containing 60 g of silica gel. After 6 d the column was eluted with *n*-hexane/ethyl acetate = 85/15. Product containing fractions were collected, and the solvent was evaporated. The crystalline residue was redissolved in seven parts of *n*-hexane by heating. After 12 h at room temperature the crystals were filtered off and dried *in vacuo*: yield 1.09 g of *syn*-**3b** (80%); mp 65–69 °C, $[\alpha]^{25}_D$ +25.5° (c = 1, CH_2Cl_2); ^{1}H-NMR (DMSO-d_6, 500 MHz, 350 K) δ 1.22 (t, 3H), 1.34 (s, 9H), 2.76–2.85 (AB part of ABX, 2H), 3.22 (d, 1H, C(2)*H*), 3.24 (dd, 1H, C(3)*H*), 3.68–3.74 (m, 1H), 4.12–4.17 (m, 2H), 6.70 (broad, 1H), 7.18–7.29 (m, 5H). Anal. ($C_{18}H_{25}NO_5$) C, H, N.

(2*R*,3*S*,4*S*)-2-(Benzylamino)-4-[[*N*-[(benzyloxy)carbonyl]valyl]amino]-3-hydroxy-5-phenylpentanoic Acid Ethyl Ester (4a). **3a** (1.60 g, 3.40 mmol) was dissolved in 6 mL of ethanol; 740 μL (6.8 mmol) of benzylamine was added, and the solution was kept at 60 °C for 2 d. The solvent was evaporated, and the residue was chromatographed on silica gel (eluent: toluene/ethyl acetate = 2/1): yield 1.56 g of **4a** (80%); mp 52–55 °C; ^{1}H-NMR ($CDCl_3$) δ 0.74, 0.86 (2 d, 6H), 1.26 (t, 3H), 2.06 (sext, 1H), 2.88 (m, 2H), 3.27 (d, 1H), 3.56,

3.74 (AB, 2H), 3.70 (d, 1H), 3.90 (dd, 1H), 4.18 (q, 2H), 4.37 (q, 1H), 5.11 (s, 2H), 5.11 (bs, 1H), 6.30 (d, 1H), 7.01–7.40 (m, 15H). Anal. ($C_{33}H_7N_3O_6$) C, H, N.

(2*R*,3*S*,4*S*)-2-(Benzylamino)-4-[[(1,1-dimethylethoxy)carbonyl]amino]-3-hydroxy-5-phenylpentanoic Acid Ethyl Ester (4b). Preparation was as described for **4a**: yield 1.24 g of **4b** (83%); syrup; ^{1}H-NMR ($CDCl_3$) δ 1.28 (t, 3H), 1.38 (s, 9H), 1.72 (broad, 1H), 2.80–3.03 (m, 2H), 3.33 (d, 1H), 3.60 and 3.80 (AB, 2H), 3.72 (d, 1H), 4.04 (q, 1H), 4.18 (q, 2H), 4.80 (d, 1H), 7.15–7.40 (m, 10H). Anal. ($C_{25}H_{34}N_2O_5$) C, H, N.

(2*R*,3*S*,4*S*)-2-(Benzylamino)-4-[[*N*-[(benzyloxy)carbonyl]valyl]amino]-3-hydroxy-5-phenylpentanoic Acid (5a). To a solution of 1.3 g (2.28 mmol) of **4a** in 12 mL of tetrahydrofuran was added 2.51 mL (2.52 mmol) of 1 N NaOH, and the reaction mixture was stirred for 10 h at room temperature. Neutralization with dilute HCl led to a precipitate, which was filtered off and dried: yield 1.12 g of **5a** (89%); mp 192–196 °C; ^{1}H-NMR (DMSO-d_6) δ 0.68 (d, 3H), 0.76 (d, 3H), 1.87 (sext, 1H), 2.70, 2.80 (2d, 2H), 4.34 (q, 1H), 5.07 (s, 2H), 7.08–7.60 (m, 15H), 7.70 (d, 1H). Anal. ($C_{31}H_{37}N_3O_6$) C, H, N.

(2*R*,3*S*,4*S*)-2-(Benzylamino)-4-[[(1,1-dimethylethoxy)carbonyl]amino]-3-hydroxy-5-phenylpentanoic Acid (5b). Preparation was as described for **5a**: yield 0.87 g of **5b** (92%); mp 210–214 °C. ^{1}H-NMR (DMSO-d_6) δ 1.38 (s, 9H), 2.74 (m, 2H), 3.02 (d, 1H), 3.50 (d, 1H), 3.64 and 3.83 (AB, 2H), 4.06 (m, 1H), 6.40 (d, 1H), 7.08–7.40 (m, 10H). Anal. ($C_{23}H_{30}N_2O_5$) C, H, N.

(2*R*,3*S*,4*S*)-*N*-[2-(Benzylamino)-4-[[*N*-[(benzyloxy)carbonyl]valyl]amino]-3-hydroxy-5-phenylpentanoyl]valine Benzylamide (6a). **5a** (920 mg, 1.68 mmol) was dissolved in 20 mL of dimethylformamide; 348 mg (1.68 mmol) of (*S*)-valine benzylamide, 228 mg (1.68 mmol) of 1-hydroxybenzotriazole, and 416 mg (2.00 mmol) of *N*,*N*′-dicyclohexylcarbodiimide were added at room temperature (similar results were obtained when *N*,*N*′-dicyclohexylcarbodiimide was replaced by (1-benzotriazolyloxy)tris(dimethylamino)phosphonium hexafluoride and *N*-methylmorpholine or *N*-ethyl-*N*′-(3-(dimethylamino)propyl)carbodiimide hydrochloride and 1-hydroxybenzotriazole). The reaction mixture was stirred for 24 h at room temperature. The solution was filtered, and the solvent was evaporated. Pure **6a** was obtained as an amorphous solid by chromatography on silica gel (eluent: gradient toluene/ethyl acetate = 1/1 to 1/3): yield 620 mg of **6a** (50%); mp 81–84 °C; ^{1}H-NMR ($CDCl_3$) δ 0.73 (d, 3H), 0.84 (d, 3H), 0.90 (d, 3H), 0.95 (d, 3H), 2.04 (sext, 1H), 2.24 (sext, 1H), 2.95 (d, 2H), 3.26 (d, 1H), 3.56, 3.61 (2d, 2H), 3.84 (t, 1H), 3.90 (d, 1H), 4.17 (m, 1H), 4.20 (m, 1H), 4.35, 4.46 (2dd, 2H), 5.09 (m, 3H), 6.47 (d, 1H), 6.64 (t, 1H), 7.15–7.38 (m, 20H), 8.90 (d, 1H). Anal. ($C_{43}H_{53}N_5O_6$) C, H, N.

(2*R*,3*S*,4*S*)-*N*-[2-(Benzylamino)-4-[[(1,1-dimethylethoxy)carbonyl]amino]-3-hydroxy-5-phenylpentanoyl]valine Benzylamide (6b). Preparation was as described for **6a**: yield 683 mg of **6b** (63%); mp 154–163 °C; ^{1}H-NMR ($CDCl_3$) δ 0.82 and 0.93 (2d, 6H), 1.39 (s, 9H), 2.10–2.28 (m, 1H), 2.80–3.00 (m, 2H), 3.24 (d, 1H), 3.56 and 3.60 (AB, 2H), 3.80 (d, 1H), 3.95 (q, 1H), 4.12–4.20 (m, 1H), 4.40 (d, 2H), 4.71 (s, 1H), 5.08 (d, 1H), 6.60 (t, 1H), 7.12–7.38 (m, 15H), 8.15 (d, 1H). Anal. ($C_{35}H_{46}N_4O_5$) C, H, N.

(2*R*,2′*S*,3*S*,4*S*)-*N*-[2-(Benzylamino)-4-[[2′-[[(benzyloxy)carbonyl]amino]-3′,3′-dimethylbutyryl]amino]-3-hydroxy-5-phenylpentanoyl]valine Benzylamide (22). **6b** (1.83 g, 3 mmol) was dissolved in 30 mL of dichloromethane. Trifluoroacetic acid (3 mL) was added, and the solution was stirred at room temperature for 2 h. The solvent was evaporated, toluene was added, and the mixture was evaporated twice to remove traces of trifluoroacetic acid. The residue was dissolved in dichloromethane, washed with 0.1 N NaOH, dried over $MgSO_4$, and concentrated *in vacuo* to yield 1.37 g of (2*R*,3*S*,4*S*)-*N*-(4-amino-2-(benzylamino)-3-hydroxy-5-phenylpentanoyl)valine benzylamide (90%), mp 138–152 °C, which was used in the next step without further purification: ^{1}H-NMR ($CDCl_3$) δ 0.92 and 0.98 (2d, 6H), 1.65 (broad, 1H), 2.20–2.40 (m, 1H), 2.43–2.62 (m, 1H), 2.92–3.16 (m, 2H), 3.30–3.40 (m, 2H), 3.70 and 3.83 (AB, 2H), 4.23 and 4.51 (AB part of ABX, 2H), 4.41 (dd, 1H), 7.05–7.39 (m, 15H), 8.00 (d, 1H). Anal. ($C_{30}H_{38}N_4O_3$) C, H, N.

2(*S*)-[[(Benzyloxy)carbonyl]amino]-3,3-dimethylbutanoic acid *N*-hydroxysuccinimido ester (0.17 g, 0.46 mmol) was added to a solution of 0.1 g (0.2 mmol) of (2*R*,3*S*,4*S*)-*N*-(4-amino-2-(benzylamino)-3-hydroxy-5-phenylpentanoyl)valine benzylamide in dioxane and was stirred overnight. The solvent was removed *in vacuo*, and the residue was chromatographed on silica gel (eluent: cyclohexane/ethyl acetate = 2/1): yield 0.104 g of **22** (70%); mp 78–82 °C; ^{1}H-NMR ($CDCl_3$) δ 0.86 (s, 9H), 1.86 and 1.94 (2d, 6H), 2.00 (b, 1H), 2.25 (oct, 1H), 2.97 (d, 2H), 3.29 (d, 1H), 3.52–3.63 (m, 2H), 3.73 (d, 1H), 3.90 (d, 1H), 4.12 (q, 1H), 4.29 (dd, 1H), 4.33 and 4.51 (AB part of ABX, 2H), 5.01 and 5.10 (AB, 2H), 5.08 (b, 1H), 5.31 (d, 1H), 6.48 (d, 1H), 6.90–7.03 (m, 1H), 7.08–7.39 (m, 20H), 8.13 (d, 1H). Anal. ($C_{44}H_{55}N_5O_6$) C, H, N.

2(*S*)-[[(Benzyloxy)carbonyl]amino]-3,3-dimethylbutanoic Acid *N*-Hydroxysuccinimido Ester. *N*-Hydroxysuccinimide (2.6 g, 22.6 mmol) and 4.6 g (22.2 mmol) of *N*,*N*′-dicyclohexylcarbodiimide were added to a solution of 6 g (22.6 mmol) 2(*S*)-[[(benzyloxy)carbonyl]amino]-3,3-dimethylbutanoic acid in 70 mL of dioxane. The mixture was stirred for 12 h at room temperature. The solvent was evaporated, and the residue was suspended in ethyl acetate. The urea was removed by filtration, and the solvent was evaporated: yield 6.15 g (75%), which was used without further purification; ^{1}H-NMR ($CDCl_3$) δ 1.10 (s, 9H), 2.84 (s, 4H), 4.52 (d, J = 10 Hz, 1H), 5.03–5.12 (m, 2H), 5.34 (d, J = 10 Hz, 1H), 7.26–7.40 (m, 5H).

(2*R*,3*S*,4*S*)-2-(Benzylamino)-4-[[(1,1-dimethylethoxy)carbonyl]amino]-3-hydroxy-5-phenylpentanoic Acid Benzylamide (4c). A mixture of 5.0 g (14.9 mmol) of **3b** and 3.2 g (29.8 mmol) of benzylamine in 150 mL of tetrahydrofuran was stirred for 3 d at 60 °C. The solvent was evaporated, and the residue was chromatographed on silica gel (eluent: cyclohexane/ethyl acetate = 3/1): yield 3.9 g of **4b** (59%) and 1.7 g of **4c** (24%); mp 50–53 °C; ^{1}H-NMR ($CDCl_3$) δ 1.42 (s, 9H), 1.95 (bs, 1H), 2.95 (d, J = 8 Hz, 2H), 3.16 (d, J = 9.5 Hz, 1H), 3.48 and 3.54 (AB, J_{AB} = 12.5 Hz, 2H), 3.71 (d, J = 9.5 Hz, 1H), 4.08 (q, J = 8 Hz, 1H), 4.34 and 4.44 (AB part of ABX, J_{AB} = 14.5 Hz, 2H), 5.10–5.22 (m, 2H), 7.08–7.38 (m, 15H), 7.96 (t, J = 5.8 Hz, 1H); FAB-MS: MH^+ = 504. Anal. ($C_{30}H_{37}N_3O_4 \cdot 1.3H_2O$) C, H, N.

(2*R*,4*S*,5*S*)-2-(Benzylamino)-2-(4-benzyl-2-oxo-1,3-oxazolidine-5-yl)ethanoic Acid Benzylamide (4d). To 2.25 g (4.5 mmol) of **4c** in 2 mL of dichloromethane was added 20 mL of 3 N hydrochloric acid in ether. The mixture was stirred for 3 h at room temperature. The precipitate was filtered off and dried *in vacuo* to yield 1.4 g of (2*R*,3*S*,4*S*)-4-amino-2-(benzylamino)-3-hydroxy-5-phenylpentanoic acid benzylamide dihydrochloride (65%), mp 131–138 °C, an aliquot of which was used in the next step without further purification: ^{1}H-NMR (DMSO-d_6) δ 2.96 (d, J = 7 Hz, 2H), 3.80–3.95 (m, 1H), 3.96–4.08 (m, 2H), 4.10–4.21 (m, 1H), 4.22–4.50 (m, 3H), 6.78 (bd, 1H), 7.18–7.60 (m, 15H), 8.18 (bs, 3H), 9.05 (bt, 1H), 9.58 (bs, 1H), 9.90 (bs, 1H).

A solution of 81 mg (0.5 mmol) of *N*,*N*′-carbonyldiimidazole in 1 mL of CH_2Cl_2 was added to a mixture of 200 mg (0.42 mmol) of (2*R*,3*S*,4*S*)-4-amino-2-(benzylamino)-3-hydroxy-5-phenylpentanoic acid benzylamide dihydrochloride and 170 mg (1.68 mmol) of triethylamine in 5 mL dichloromethane at 5 °C. The mixture was stirred for 3 h at room temperature, concentrated *in vacuo*, and chromatographed on silica gel (eluent: cyclohexane/ethyl acetate = 1/1): yield 94 mg of **4d** (52%); mp 49–51 °C; ^{1}H-NMR ($CDCl_3$) δ 2.02 (bs, 1H), 2.69 and 2.88 (AB part of ABX, J_{AB} = 13.5 Hz, 2H), 3.29 (d, J = 5.7 Hz, 1H), 3.70 and 3.78 (AB, J_{AB} = 13 Hz, 2H), 4.06 (ddd, J = 13.5 Hz, J = 8.9 Hz, J = 5.6 Hz, 1H), 4.38 and 4.45 (AB part of ABX, J_{AB} = 14.9 Hz, 2H), 4.48 (dd, J = 5.60 Hz, J = 5.6 Hz, 1H), 5.20 (bs, 1H), 7.04–7.42 (m, 16H). Anal. ($C_{26}H_{27}N_3O_3$) C, H, N.

(3a*R*,6a*R*,3*S*,6*S*)-3,6-Dibenzyl-2,4-dioxo-3,3a,6,6a-tetrahydropyrrolo[3,4-*d*]oxozole (4e). **4b** (3 g, 6.8 mmol) was dissolved in 20 mL of ether, 100 mL of a 3 N solution of HCl in ether was added, and the mixture was stirred for 3 h at room temperature. The precipitate was filtered off, washed

with ether, and dried *in vacuo* to yield 2.67 g of (2*R*,3*S*,4*S*)-4-amino-2-(benzylamino)-3-hydroxy-5-phenylpentanoic acid ethyl ester dihydrochloride (95%). The substance was used for the next step without further purification.

A mixture of 415 mg (1 mmol) of (2*R*,3*S*,4*S*)-4-amino-2-(benzylamino)-3-hydroxy-5-phenylpentanoic acid ethyl ester dihydrochloride and 202 mg (2 mmol) of *N*-methylmorpholine in 10 mL of ethanol was stirred for 12 h at 80 °C. The solvent was evaporated *in vacuo*, the residue was taken up in 50 mL of ethyl acetate, washed with 1 N HCl and brine, and dried. After evaporation of the solvent, the residue was chromatographed on silica gel (eluent: cyclohexane/ethyl acetate = 1/2): yield 151 mg of (3*R*,4*S*,5*S*)-5-benzyl-3-(benzylamino)-4-hydroxypyrrolidin-2-one (50%); mp 112–116 °C; ^{1}H-NMR ($CDCl_3$) δ 2.89 and 3.09 (AB part of ABX, J_{AB} = 13.7 Hz, 2H), 3.40 (d, J = 4.6 Hz, 1H), 3.70 (ddd, J = 3.4 Hz, J = 6.2 Hz, J = 8.7 Hz, 1H), 3.80 and 3.88 (AB, J_{AB} = 13.5 Hz, 2H), 4.00 (ddd, J = 4.6 Hz, J = 1.3 Hz, J = 3.4 Hz, 1H), 5.68 (bs, 1H), 7.15–7.40 (m, 10H).

A solution of 50 mg (0.169 mmol) (3*R*,4*S*,5*S*)-5-benzyl-3-(benzylamino)-4-hydroxypyrrolidin-2-one in 10 mL of dichloromethane was treated with 3 mL of pyridine and 100 μL of a 1.9 M solution of phosgene in toluene at −10 °C. After stirring for 30 min, 0.5 M aqueous HCl was added, and the organic layer was washed with saturated aqueous $NaHCO_3$ and dried over magnesium sulfate. The solvent was removed *in vacuo*, and the residue was chromatographed on silica gel (eluent: ethyl acetate/*n*-hexane = 1/1): yield 28 mg of **4e** (48%); mp 167–168 °C; ^{1}H-NMR ($CDCl_3$) δ 2.87 and 3.14 (AB part of ABX, J_{AB} = 13.9 Hz), 3.96 (d, J = 7.8 Hz, 1H), 4.05 (ddd, J = 5.6 Hz, J = 5.7 Hz, J = 9.1 Hz, 1H), 4.91 (dd, J = 5.6 Hz, J = 7.9 Hz, 1H), 4.50 and 4.92 (AB, J_{AB} = 14.7 Hz, 2H), 6.29 (bs, 1H), 7.15–7.40 (m, 10H). Anal. ($C_{19}H_{18}N_2O_3 \cdot 0.1H_2O$) C, H, N.

Biology. HIV Proteinase Inhibition Assay. HIV-1 proteinase was expressed from the plasmid pTZprt$^+$ [36] in *Escherichia coli* strain JM 105 and was purified to homogeneity as published.[37] A peptide cleavage assay was performed using the substrate H-Lys-Ala-Arg-Val-Leu-pNph-Glu-Ala-Nle-NH_2 described by Richards et al.[38] Briefly, HIV-proteinase was incubated at 37 °C in 0.1 M MES, 0.37 M NaCl, 4 mM EDTA, pH 6.25, with 280 μM of substrate in the presence or absence of inhibitors. From the decrease of absorbance at 298 nm, initial rates were calculated. Inhibitors were dissolved in DMSO; the DMSO contents of dilutions in assay buffer did not exceed 5%.

IC_{50} values for test compounds were obtained by fitting the initial velocity data (V) from the inhibition of substrate hydrolysis to the equation $V = V_0(IC_{50})/(I + IC_{50})$ where I denotes the inhibitor concentration and V_0 the velocity of the uninhibited reaction. From the IC_{50} values kinetic constants K_i were calculated:[39] $IC_{50} = E_t/2 + K_i(1 + S/K_m)$, where E_t is the total enzyme concentration, S is the substrate concentration, and K_m is the Michaelis constant for the substrate. K_i values reported here are the mean of two determinations, which yielded the same result within limits of ±20%.

Recombinant HIV-2 proteinase, obtained from P. Strop, Prague, was assayed as described for the HIV-1 enzyme, but in 0.1 M NaAc, pH 4.7, 0.37 M NaCl, 4 mM EDTA.

Assay of Other Proteinases. Cathepsin D was purified from human spleen according to Ikeda et al.[40] Activity was measured at pH 3.5 in citrate buffer with 0.1% bovine hemoglobin as substrate.[41] Recombinant human renin (obtained from J. Evenou, Sandoz Basle) was assayed using the method described by Holzman et al.[42] Porcine pepsin was assayed in 0.1 M glycine–HCl, pH 2.0, with 0.5% hemoglobin as substrate and [^{14}C]methylhemoglobin as tracer.

Inhibition of HIV-1 Induced Cytopathic Effect in MT4 Cells. The assay procedure described by Pauwels et al.[43] was used with minor modifications. The HTLV I transformed cell line MT4 was used as the target cell. Inhibition of HIV-1, strain IIIB, induced cytopathic effect was determined by measuring the viability of both HIV- and mock-infected cells. Viability was assessed spectrophotometrically via *in situ* reaction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Virus-infected and uninfected cultures without compound were included as controls as were uninfected cells treated with compound. The cell concentration was chosen so that the number of cells per milliliter increased by a factor of 10 during the 5 d of incubation in mock-infected cultures. Virus inoculum was adjusted such to cause cell death in 90% of the target cells after 5 d of incubation. The virus was adsorbed to a cell suspension containing 1×10^6 cells/mL at 37 °C for 1 h. Then, the infected cells were added to microtiter plates containing the test compounds to give 1×10^5 cells/mL. Thus, compounds were added post adsorption. The IC_{50} values reported here are the mean of at least five determinations.

Inhibition of HIV-1, Strain IIIB, Replication in MT4 Cells. MT4 cells were infected with HIV-1, strain IIIB, by suspending the cells at a concentration of 1×10^6 cells/mL in virus solution. Adsorption was allowed for 2 h at 37 °C. Virus inoculum was adjusted such to give a linear increase of p24 antigen concentration in the supernatants of infected cells up to day 4 post infection. After adsorption, the cells were spun down, the inoculum was removed by washing, and the infected cells were added to 6-well plates containing the test compounds at the appropriate concentrations to give 1×10^5 cells/mL in a volume of 5 mL. At days 3 and 4 postinfection, aliquots were removed, the cells were spun down, and the supernatants were analyzed for p24 antigen concentration by means of a commercial ELISA kit (Coulter).

Inhibition of HIV-2, Strain EHO, Replication in MT4 Cells. MT4 cells were infected with HIV-2, strain EHO, in the same manner as described for HIV-1. At days 3 and 4 postinfection, aliquots of the cell cultures were removed; the cells were spun down and the supernatants were analyzed for p26 antigen concentration by means of a commercial ELISA kit (Coulter).

Acknowledgment. We thank Dr. R. Datema, Head of Department of Antiretroviral Therapy - SANDOZ Forschungsinstitut, Vienna, for continuous support of this work. We thank R. Csonga, M. Hübner, M. Kern, E. Mlynar, A. Pruckner, H. Schmidt, L. Strommer, K. Wagner, S. Weber-Roth, and G. Winkler for excellent technical assistence and F. Schürch of SANDOZ Pharma AG Basel-Muttenz for performing separation of epoxide diastereomers. E. Bankl is acknowledged for her assistance in the preparation of the manuscript.

References

(1) Dunn, B. M.; Kay, J. Targets for Antiviral Chemotherapy: HIV-Proteinase. *Antiviral Chem. Chemother.* **1990**, *1*, 3–8. Tomasselli, A. G.; Howe, W. J.; Sawyer, T. K.; Wlodawer, A.; Heinrikson, R. L. The Complexities of AIDS: An Assessment of the HIV Protease as a Therapeutic Target. *Chimica Oggi* **1991**, 6–27. Huff, J. R. HIV Protease: A Novel Chemotherapeutic Target for AIDS. *J. Med. Chem.* **1991**, *34*, 2305–2314. Meek, T. D. Inhibitors of HIV-1 Protease. *J. Enzyme Inhib.* **1992**, *6*, 65–98. Robins, T.; Plattner, J. HIV Protease Inhibitors: Their Anti-HIV Activity and Potential Role in Treatment. *J. Aquired Immune Defic. Syndr.* **1993**, *6*, 162–170.

(2) Debouck, Ch. The HIV-1 Protease as a Therapeutic Target for AIDS. *AIDS Res. Hum. Retroviruses* **1992**, *8*, 153–164.

(3) Meek, T. D.; Lambert, D. M.; Dreyer, G. B.; Carr, T. J.; Tomaszek, T. A.; Moore, M. L.; Strickler, J. E.; Debouck, C.; Hyland, L. J.; Matthews, T. J.; Metcalf, B. W.; Petteway, S. R. Inhibition of HIV-1 Protease in Infected T-Lymphocytes by Synthetic Peptide Analogues. *Nature* **1990**, *343*, 90–92. McQuade, T. J.; Tomasselli, A. G.; Liu, L.; Karacostas, V.; Moss, B.; Sawyer, T. K.; Heinrikson, R. L.; Tarpley, W. G. A Synthetic HIV-1 Protease Inhibitor with Antiviral Activity Arrests HIV-like Particle Maturation. *Science* **1990**, *247*, 454–457. Craig, J. C.; Grief, C.; Mills, J. S.; Hockley, D.; Duncan, I. B.; Roberts, N. A. Effects of a Specific Inhibitor of HIV Proteinase (Ro 31-8959) on Virus Maturation in a Chronically Infected Promonocytic Cell Line (U1). *Antiviral Chem. Chemother.* **1991**, *2*, 181–186. Kempf, D. J.; Marsh, K. C.; Paul, D. A.; Knigge, M. F.; Norbeck, D. W.; Kohlbrenner, W. E.; Codacovi, L.; Vasanavonda, S.; Bryant, P.; Wang, X. C.; Wideburg, N. E.; Clemment, J. C.; Plattner, J. J.; Erickson, J. Antiviral and Pharmacokinetic Properties of C_2 Symmetric Inhibitors of the Human Immunodeficiency Virus Type 1 Protease. *Antimicrobial Agents Chemother.* **1991**, *35*, 2209–2214.

(4) Roberts, N. A.; Martin, J. A.; Kinchington, D.; Broadhurst, A. V.; Craig, J. C.; Duncan, I. B.; Galpin, S. A.; Handa, B. K.; Kay, J.; Kröhn, A.; Lambert, R. W.; Merrett, J. H.; Mills, J. S.; Parkes, K. E. B.; Redshaw, S.; Ritchie, A. J.; Taylor, D. L.; Thomas, G. J.; Machin, P. J. Rational Design of Peptide-Based HIV Proteinase Inhibitors. *Science* **1990**, *248*, 358–361.

(5) Overton, H. A.; McMillan, D. J.; Gridley, S. J.; Brenner, J.; Redshaw, S.; Mills, J. S. Effects of Two Novel Inhibitors of the Human Immuno-deficiency Virus Protease on the Maturation of the HIV *gag* and *gag-pol* Polyproteins. *Virology* **1990**, *179*, 508–511. Ashorn, P.; McQuade, T. J.; Thaisrivongs, S.; Tomasselli, A. G.; Tarpley, W. G.; Moss, B. An Inhibitor of the Protease Blocks Maturation of Human and Simian Immuno-deficiency Viruses and Spread of Infection. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 7472–7476. Lambert, D. M.; Pettewa, S. R.; McDanal, C. E.; Hart, T. K.; Leary, J. J.; Dreyer, G. B.; Meek, T. D.; Bugelski, P. J.; Bolognesi, D. P.; Metcalf, B. W.; Matthews, T. J. Human Immunodeficiency Virus Type 1 Protease Inhibitors Irreversibly Block Infectivity of Purified Virions from Chronically Infected Cells. *Antimicrob. Agents Chemother.* **1992**, *36*, 982–988. Kaplan, A. H.; Zack, J. A.; Knigge, M.; Paul, D. A.; Kempf, D. J.; Norbeck, D. W.; Swanstrom, R. Partial Inhibition of the Human Immunodeficiency Virus Type 1 Protease Results in Aberrant Virus Assembly and the Formation of Noninfectious Particles. *J. Virol.* **1993**, *67*, 4050–4055.

(6) Delfraissy, J. F.; Sereni, D.; Brun-Vezinet, F.; Dussaix, E.; Krivine, A.; Dormont, J.; Bragman, K. A. Phase I-II Dose Ranging Study of the Safety and Activity of Ro 31-8959 (HIV Proteinase Inhibitor) in Previously Zidovudine (ZDV) Treated HIV-Infected Individuals. Kitchen, V.; Skinner, C.; Sedwick, A.; Bragman, K.; Pinching, A. J.; Weber, J. A Phase I-II Dose Ranging Study of the Safety and Activity of Ro 31-8959 (HIV-Proteinase Inhibitor) in Asymptomatic or Mildly Symptomatic HIV-Infection. Abstracts at the 9th Int. Conf. on AIDS, Berlin, 1993.

(7) Hui, K. Y.; Manetta, J. V.; Gygi, T.; Bowdon, B. J.; Keith, K. A.; Shannon, W. M.; Lai, M. T. A Rational Approach in the Search for Potent Inhibitors Against HIV Proteinase. *FASEB J.* **1991**, *5*, 2606–2610. Hui, K. Y.; Hermann, R. B.; Mannetta, J. V.; Gygi, T.; Angleton, E. L. Model Peptides to Study the Effects of P2 and P3 Substitutions in Statine-Containing HIV Proteinase Inhibitors. *FEBS Lett.* **1993**, *327*, 355–360. Fehrentz, J. A.; Chromier, B.; Bignon, E.; Venaud, S.; Chermann, J. C.; Nisato, D. Statine Based Tripeptides as Potent Inhibitors of HIV-1 Replication. *Biochem. Biophys. Res. Commun.* **1992**, *188*, 873–878.

(8) Billich, A.; Winkler, G. Analysis of Subsite Preferences of HIV-1 Proteinase Using MA/CA Junction Peptides Substituted at the P3-P1′ Positions. *Arch. Biochem. Biophys.* **1991**, *290*, 186–190. Note: The nomenclature used for describing the individual amino acid residues (P2, P1, P1′, P2′ etc.) of a substrate and the corresponding subsites (S2, S1, S1′, S2′ etc.) of a protease is that of Schechter and Berger: Schechter, I.; Berger, A. On the Size of the Active Site in Proteases. I. Papain. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157–162.

(9) Dreyer, G. B.; Lambert, D. M.; Meek, T. D.; Carr, T. J.; Tomaszek, T. A.; Ferandez, A. V.; Bartus, H.; Caciavillani, E.; Hassell, A. M.; Minnich, M.; Petteway, S. R.; Metcalf, B. W. Hydroxyethylene Isostere Inhibitors of Human Immunodeficiency Virus-1 Protease: Structure-Activity Analysis Using Enzyme Kinetics, X-Ray Crystallography, and Infected T-cell Assays. *Biochemistry* **1992**, *31*, 6646–6659.

(10) Jaskolski, M.; Tomasselli, A. G.; Sawyer, T. K.; Staples, D. G.; Heinrikson, R. L.; Schneider, J.; Kent, S. B. H.; Wlodawer, A. Structure at 2.5 Å Resolution of Chemically Synthesized Human Immunodeficiency Virus Type 1 Protease Complexed with Hydroxyethylene-Based Inhibitors. *Biochemistry* **1991**, *30*, 1600–1609. Murthy, K. H. M.; Winborne, E. L.; Minnich, M. D.; Culp, J. S.; Debouck, C. The Crystal Structures at 2.2 Å Resolution of Hydroxyethylene-Based Inhibitors Bound to Human Immunodeficiency Virus Type 1 Protease Show that the Inhibitors are Present in Two Distinct Orientations. *J. Biol. Chem.* **1992**, *267*, 22770–22778.

(11) Veber, D. F.; Bock, M. G.; Brady, S. F.; Ulm, E. H.; Cochran, D. W.; Smith, G. M.; LaMont, B. I.; Dipardo, R. M.; Poe, M.; Freidinger, R. M.; Evans, B. E.; Boger, J. Renin Inhibitors Containing 2-Substituted Statine. *Biochem. Soc. Transactions* **1984**, *12*, 956–959.

(12) Mancuso, A. J.; Swern, D. Activated Dimethyl Sulfoxide: Useful Reagents for Synthesis. *Synthesis* **1981**, 165–185.

(13) Reetz, M. T.; Röhrig, D. Stereoselective Synthese von γ-Aminocarbonsäureestern. (Stereoselective synthesis of γ-amino acid esters.) *Angew. Chem.* **1989**, *101*, 1732–1734; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 1706.

(14) Hagihara, M.; Schreiber, S. L. Reassignment of Stereochemistry and Total Synthesis of the Thrombin Inhibitor Cyclotheonamide B. *J. Am. Chem. Soc.* **1992**, 6570–6571.

(15) Note: Pure MCPBA (m-chloroperbenzoic acid) is shock sensitive and potentially explosive in the condensed phase: UN test method, Test 2(b)(iii)-ST/AG/AC 10/11.

(16) Weber, H. P.; Scholz, D.; Schmidt, H.; Gstach, H. 4(*S*)-(t-Butyloxycarbonylamino)-2(*S*),3(*R*)-epoxy-5-phenylpentanoic Acid Ethyl Ester. *Acta Crystallogr. C* In press.

(17) Reetz, M. T. Neue Wege zur Nutzung von Aminosäuren als chirale Bausteine in der organischen Synthese. (New methods to use amino acids as chiral synthons in organic synthesis.) *Angew. Chem.* **1991**, *103*, 1559–1573; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1531–1546.

(18) Reetz, M. T.; Lauterbach, E. H. Stereoselective Epoxidation of Chiral Electron-poor γ-Aminoolefins. *Tetrahedron Lett.* **1991**, *32*, 4477–4480. Note: A comparison of our compounds 3 with the epoxidation product of γ-(*N*,*N*-dibenzylamino)-protected analogs of **2** shows identical diastereoselectivity in favor of the *syn* product.

(19) Reetz, M. T.; Lauterbach E. H. Stereoselective [2,3]-Sigmatropic Rearrangement of Chiral Amine Oxides Derived from Amino Acids. *Tetrahedron Lett.* **1991**, *32*, 4481–4482.

(20) Chong, J. M.; Sharpless, K. B. Nucleophilic Openings of 2,3-Epoxy Acids and Amides Mediated by Ti(O-i-Pr)$_4$. Reliable C-3 Selectivity. *J. Org. Chem.* **1985**, *50*, 1560–1563.

(21) Behrens, C. H.; Sharpless, K. B. Selective Transformations of 2,3-Epoxy Alcohols and Related Derivatives. Strategies for Nucleophilic Attack at Carbon-3 or Carbon-2. *J. Org. Chem.* **1985**, 5696–5704.

(22) Scolastico, C.; Conca, E.; Prati, L. Diastereo- and Enantioselective Synthesis of Fluorinated Threonines. *Synthesis* **1985**, 850–855.

(23) Liwschitz, Y.; Robinsohn, Y.; Perera, D. Synthesis of α-Amino-β-hydroxy-acids. Part I. DL-Allothreonine, DL-erytho-β-Hydroxyleucine, and DL-erythro and threo-β-Hydroxy-β-methylaspartic Acid. *J. Chem. Soc.* **1962**, 1116–1119.

(24) Cardani, S.; Bernardi, A.; Colombo, L.; Gennari, C.; Scolastico, C.; Venturini, I. Asymmetric Synthesis of Functionalized α-Amino-β-hydroxy Acids via Chiral Norephedrine-derived Oxazolidines. *Tetrahedron* **1988**, *44*, 5563–5572.

(25) Kamandi, E.; Frahm, A. W.; Zymalkowski, F. Die Synthese von β-Phenyl-isoserinen durch Ammonolyse von β-Phenyl-glycidestern, I. (β-Phenylisoserines by ammonolysis of β-phenylglycidates.) *Arch. Pharm. (Weinheim, Ger.)* **1974**, *307*, 871–878.

(26) Tack, J. W.; Lehmann, J.; Zymalkowski, F. Unterscheidung N-substituierter 3-Phenylserin- und 3-Phenylisoserin-Derivate. (Aminolysis of derivatives of trans-3-phenylglycidic acid, V: Distinction between N-substituted derivatives of 3-phenylserine and 3-phenylisoserine.) *Arch. Pharm. (Weinheim, Ger.)* **1979**, *312*, 138–147.

(27) Elker, A.; Lehmann, J.; Zymalkowski, F. Aminolyse von trans-3-Phenylglycidsäure-Derivaten mit aromatischen Aminen, 4. Mitt. (Aminolysis of derivatives of trans-3-phenylglycidic acid with aromatic amines, IV. *Arch. Pharm. (Weinheim, Ger.)* **1979**, *312*, 26–34.

(28) Kamandi, E.; Frahm, A. W.; Zymalkowski, F. Die Synthese von β-Phenylisoserinen durch Ammonolyse von β-Phenyl-glycidestern, II. (Synthesis of β-phenylisoserines by ammonolysis of β-phenylglycidates. II.) *Arch. Pharm. (Weinheim, Ger.)* **1975**, *308*, 135–141.

(29) Kato, K.; Saino, T.; Nishizawa, R.; Takita, T.; Umezawa, H. Regio- and Stereosepcific Synthesis of threo-3-Amino-2-hydroxy-Acids, Novel Amino-acids contained in Aminopeptidase Inhibitors of Microbial Origin. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1618–1621.

(30) Walborsky, H. M.; Baum, M. E. Chemical Effects of the Trifluoromethyl Group. V. Reactions of Ethyl β-Trifluoromethylglycidate; Syntheses of 2-Amino-3-hydroxy-4,4,4-trifluorobutyric Acid. *J. Am. Chem. Soc.* **1958**, *80*, 187–192.

(31) Futagawa, S.; Inui, T.; Shiba, T. Nuclear Magnetic Resonance Study of Stereoisomeric-2-Oxazolidone and 2-Phenyl-2-oxazoline Derivatives of α-Amino-β-hydroxy Acids. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 3308–3310.

(32) Billich, A.; Aziz, A.; Lehr, P.; Charpiot, B.; Gstach, H.; Scholz, D. Kinetic and Binding Studies on [^{125}I]SDZ-283471, a Radiolabeled Inhibitor of HIV-1 Proteinase. *J. Enzyme Inhib.* **1993**, *7*, 213–224.

(33) DeSolms, S. J.; Giulinai, E.; Guare, J. P.; Vacca, J. P.; Sanders, W. M.; Graham, S. L.; Wiggins, J. M.; Daeke, P. L.; Sigal, I. S.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Quintero, J. G.; Anderson, P. S.; Huff, J. R. Design and Synthesis of HIV Protease Inhibitors. Variations of the Carboxy Terminus of the HIV Protease Inhibitor L-682,679. *J. Med. Chem.* **1991**, *34*, 2852–2857.

(34) Billich, A.; Charpiot, B.; Fricker, G.; Gstach, H.; Lehr, P.; Peichl, P.; Scholz, D.; Rosenwirth, B. HIV Proteinase Inhibitors Containing 2-Aminobenzylstatine as a Novel Scissile Bond Replacement: Biochemical and Pharmacological Characterization. *Antiviral Res.* In press.

(35) Fournié-Zaluski, M.-C.; Coric, P.; Turcaud, S.; Bruetschy, L.; Lucas, E.; Noble, F.; Roques, B. P. Potent and Systemically Active Aminopeptidase M Inhibitors Designed from Active-Site Investigation. *J. Med. Chem.* **1992**, *35*, 1259–1266.

(36) Seelmeier, S.; Schmidt, H.; Turk, V.; von der Helm, K. Human Immunodeficiency Virus has an Aspartic-type Protease that can be Inhibited by Pepstatin-A. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6612−6616.
(37) Billich, A.; Hammerschmid, F.; Winkler, G. Purification, Assay and Kinetic Features of HIV-1 Proteinase. *Biol. Chem. Hoppe-Seyler* **1990**, *371*, 265−272.
(38) Richards, A.; Phylip, L. H.; Farmerie, W. G.; Scarborough, P. E.; Alvarez, A.; Dunn, B. M.; Hirel, P. H.; Konvalinka, J.; Strop, P.; Pavlickova, L.; Kostka, V.; Kay, J. Sensitive, Soluble Chromogenic Substrates for HIV-1 Proteinase. *J. Biol. Chem.* **1990**, *265*, 7733−7736.
(39) Cha, S.; Agarwal, R. P.; Parks, R. E. Tight-binding Inhibitors. *Biochem. Pharm.* **1975**, *24*, 2187−2197.
(40) Ikeda, K.; Suzuki, H.; Okano, T.; Nakagawa, S. Human Spleen Cathepsin D: Its Characterization and Localization in Human Spleen. *Int. J. Biochem.* **1989**, *21*, 317−326.
(41) Böhlen, P.; Stein, S.; Dairman, W.; Udenfriend, S. Fluorometric Assay of Proteins in the Nanogram Range. *Arch. Biochem. Biophys.* **1973**, *155*, 213−220.
(42) Holzman, T. F.; Chung, C. C.; Edalji, R.; Egan, D. A.; Gubbins, E. J.; Rueter, A.; Howard, G.; Yang, L. K.; Pederson, T. M.; Krafft, G. A.; Wang, G. T. Recombinant Prorenin from CHO Cells: Expression and Purification. *J. Protein Chem.* **1990**, 663−672.
(43) Pauwels, R.; Balzarini, J.; Baba, M.; Snoek, R.; Schols, D.; Herdwijn, P.; Desmyter, J.; De Clercq, E. Rapid and Automated Tetrazolium-based Colorimetric Assay for the Detection of Anti-HIV Compounds. *J. Virol. Methods* **1988**, *20*, 309−321.
(44) Kempf, D. J.; Norbeck, D. W.; Erickson, J. W.; Codacovi, L. M.; Sham, H. L.; Plattner, J. J. European Patent Application EP 0 402 646 A1, 1990.