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Synthesis and Biological Activity of Potent HIV-1 Protease Inhibitors Based on Phe-Pro Dihydroxyethylene Isosteres

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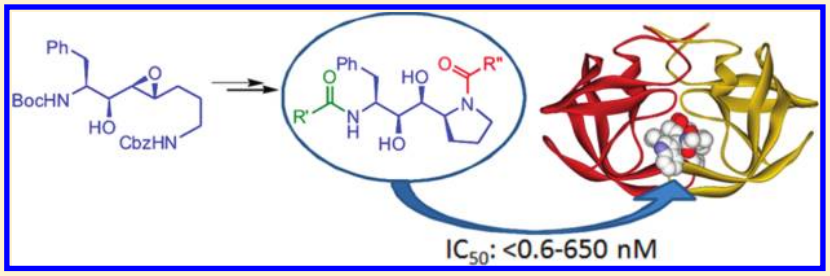
Supporting Information



ABSTRACT: Peptidomimetic inhibitors of HIV-1 PR are still a key resource in the fight against AIDS. Here we describe the synthesis and biological activity of HIV-1 PR inhibitors based on four novel dihydroxyethylene isosteres of the Phe-Pro and Pro-Pro dipeptides. The isosteres, containing four stereogenic centers, were synthesized in high yield and excellent stereoselectivity via the cyclization of epoxy amines derived from α-amino acids. The inhibitors were assembled by coupling the isosteres with suitable flanking groups and were screened against recombinant HIV PR showing activities in the subnanomolar to micromolar range. Two Phe-Pro-based inhibitors active at the nanomolar level were further investigated: both inhibitors combine the ability to suppress HIV-1 replication in infected MT-2 cells with low cytotoxicity against the same cells, thereby displaying a high therapeutic index. These results demonstrate the potential of the new Phe-Pro dihydroxyethylene isostere as a core unit of powerful HIV-1 PR inhibitors.

INTRODUCTION

Since the appearance of saquinavir in clinical practice in 1995, inhibitors of the protease of human type-1 immune deficiency virus (HIV-1 PR) have represented the key weapon in the battle against AIDS pandemics.[1] HIV-PR is responsible for the cleavage of the viral polyprotein precursor to give structural proteins and three viral enzymes. Inhibition of the protease blocks viral replication and maturation, resulting in lowering of the viral load and a general improvement in the clinical state of AIDS patients. The introduction of first generation inhibitors ritonavir, nelfinavir, and indinavir has changed the nature of the AIDS epidemics from a terminal illness to a manageable one.[2] More recently, new protocols based on potent reverse transcriptase (RT) inhibitors have become available;[3] nevertheless, the development of potent protease inhibitors, such as darunavir[4] currently used in combination with reverse transcriptase inhibitors, confirms that protease inactivation remains an essential therapeutic tool. However, the long-term efficacy of PR-targeted therapies is limited by the onset of drug resistance due to mutant viral strains; thus, the need still exists for the development of new, efficient inhibitors.

HIV-1 PR is a dimeric aspartyl protease consisting of two identical monomers of 99 residues.[5] The presence of a two-fold symmetry axis in the homodimer inspired the development of C_2-symmetric inhibitors, and interest has focused on peptidomimetics based on the Phe-Phe isostere **1**[6] (Figure 1)

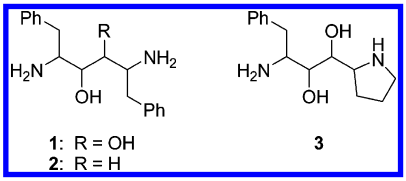


Figure 1. Phe-Phe and Phe-Pro isosteres.

for which several synthetic approaches have been developed.[7,8] Potent inhibitors have been obtained from both (*S*,*S*,*S*,*S*) and (*S*,*R*,*R*,*S*) stereoisomers of **1**.[6−9]

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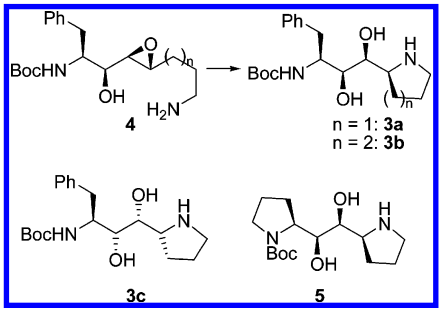
Despite the C_2 symmetry of the protease, it has been observed that nonsymmetrical inhibitors often perform better than symmetrical ones. The superior activity of the nonsymmetric inhibitor may reflect the local lack of symmetry at the catalytic aspartate residues, which are believed to be in different protonation states in the active enzyme.[10] The diol isostere might then be forced to establish nonsymmetrical interactions with the two aspartate residues, thus making symmetry at the core level unimportant. Accordingly, in the solid state, symmetrical inhibitors are often found to bind HIV-Pr in a nonsymmetric fashion.[11]

There are several ways in which the symmetry of the core unit **1** can be perturbed (Figure 1). Removal of a hydroxy group leads to the hydroxyethylene isostere **2**. Ritonavir and lopinavir are significant examples of inhibitors containing this Phe-Phe isostere.[12] Another possibility is to replace one of the benzyl groups of **1** with the side chain of a different amino acid. HIV-PR can recognize Phe-Pro and Tyr-Pro dipeptides as the specific cleavage site, and this specificity can been exploited in the design of selective inhibitors.[13] Replacement of a benzyl group of **1** with the side chain of proline thus leads to the Phe-Pro isostere **3**.

The objective of this study was to investigate the synthesis of nonsymmetric HIV-1 PR inhibitors based on the novel diamino diol Phe-Pro isostere **3** and to compare their biological activities with those of the corresponding inhibitors containing the C_2-symmetric Phe-Phe isostere **1** described earlier.[9] To this end, we have synthesized three nonsymmetric Phe-Pro isosteres (**3a**–**c**) based on the general structure **3** (Scheme 1). The

Scheme 1. Synthesis of Phe-Pro Isosteres by Epoxy Amine Cyclization



synthesis of the isosteres relies on the cyclization of phenylalanine-derived epoxy amines (**4**) for the generation of a five- or six-membered proline mimic, as shown in the scheme for **3a**,**b**.[14] The same approach has also been applied to the synthesis of the monoprotected Pro-Pro isostere **5**. A series of powerful HIV-PR inhibitors with IC_{50} in the nanomolar and subnanomolar range has been obtained by combining isosteres **3** and **5** with selected peptide or nonpeptide flanking residues. We will show that inhibitors containing the nonsymmetric isostere **3a** are superior to the corresponding inhibitors based on the symmetric isosteres **1** and **5**.

RESULTS AND DISCUSSION

Synthesis of the Isosteres. In our synthesis of Xaa-Pro diamino diol isosteres,[14] the carbon skeleton of the isosteres was assembled by the Horner–Emmons reaction between an amino acid-derived phosphonate and a linear aldehyde containing an ω-carboxylate group which was later converted into amine by a Curtius rearrangement. This approach has been modified to reduce the number of steps and avoid temporary protections along the pathway. Thus, phenylalanine-derived phosphonate **6**[8] was used as the nucleophilic partner in a Horner–Emmons olefination with the orthogonally protected 2-hydroxypyrrolidine **7a** (Scheme 2), a synthetic equivalent of 4-aminobutanal. **7a**, in turn, was obtained by diisobutylaluminium hydride (DIBALH) reduction of the corresponding *N*-Cbz-lactam.[15]

The Horner–Emmons reaction requires carefully controlled conditions to avoid aza-Michael cyclization of the product **8a**, giving pyrrolidine **9** as a 1:1 mixture of diastereoisomers (Scheme 2). This side reaction occurs in ethanol with potassium carbonate as a base, and in THF with potassium *tert*-butoxide or 1,8-diazabicycloundec-7-ene (DBU)/lithium chloride. On the contrary, if the reaction is carried out in acetonitrile and potassium carbonate[16] and is stopped at 75% conversion, the required enone **8a** can be readily isolated and purified. The 15.6 Hz coupling constant between the vinyl protons confirms the *E* stereochemistry of the enone. Chelation-controlled reduction of **8a** with lithium tri-*tert*-butoxyaluminium hydride (LTBAH) in ethanol[17] gives the allylic alcohol **10a** in 48% yield from **6**. The synthesis proceeds with peracid epoxidation of **10a**, giving the *syn*-2,3-epoxy alcohol **11a** as a single diastereoisomer. Finally, removal of the Cbz protecting group by catalytic hydrogenation gave the corresponding free amine which spontaneously cyclized onto the epoxide by a 5-exo process, as predicted,[14] giving the mono-Boc-protected isostere **3a** as a single diastereoisomer, in 31% overall yield from the starting phosphonate **6** (Scheme 2). A comparable yield has been obtained when the synthesis was performed on a 100 g scale.

The six-membered proline isostere **3b** was similarly obtained, starting from *N*-Cbz-2-hydroxypiperidine (**7b**) (Scheme 2).[18] Also in this case cyclization was completely regioselective, taking place exclusively in the 6-exo mode.

The *S*,*R*,*R*,*R* diastereoisomer **3c** was synthesized by a modification of this approach (Scheme 3). The *S*,*S* alcohol **10c** was obtained by reduction of the enone **8a** with L-Selectride in methanol. It has been demonstrated that, in polar solvents, L-Selectride reduction of amino ketones gives preferentially the Felkin–Anh product.[19] Although the stereoselectivity was only 3:1 in favor of the desired product, the required alcohol **10c** was easily separated from the *S*,*R* isomer by flash chromatography. Epoxidation of **10c** and cyclization of the epoxy amine **11c**, under the usual conditions for *N*-Cbz deprotection, gave the *S*,*R*,*R*,*R* isostere **3c**.

The symmetrical, monoprotected, Pro-Pro isostere **5** was synthesized by the approach already described for the Phe-Pro isosteres **3a**,**b** (Scheme 4). The proline-derived phosphonate **12** was obtained by the reaction of Boc-protected proline methyl ester with lithiated methyl dimethylphosphonate. Horner–Emmons reaction of phosphonate **12** was carried out in acetonitrile, to prevent the unwanted in situ aza-Michael cyclization of the enone **13**, which did not exceed 8% under these conditions (Scheme 4). Reduction of the carbonyl of **13**, however, proved much more difficult than expected, most likely due to the hindrance of the Boc-protected five-membered ring. Addition of sodium borohydride led to the reduction of the C–C double bond; addition of cerium chloride restored the desired chemoselectivity[20] but without any stereoselectivity. After unsuccessful attempts with lithium borohydride and LTBAH, the alcohol **14** was eventually obtained, as a single stereoisomer, by reduction with DIBALH in toluene. The *S*,*R* stereochemistry was confirmed by NMR analysis of the corresponding oxazolidinone **16**. The value of 7.3 Hz for the coupling constant between the vicinal ring protons and 6% NOE measured for the same protons are both consistent with a syn arrangement of the protons

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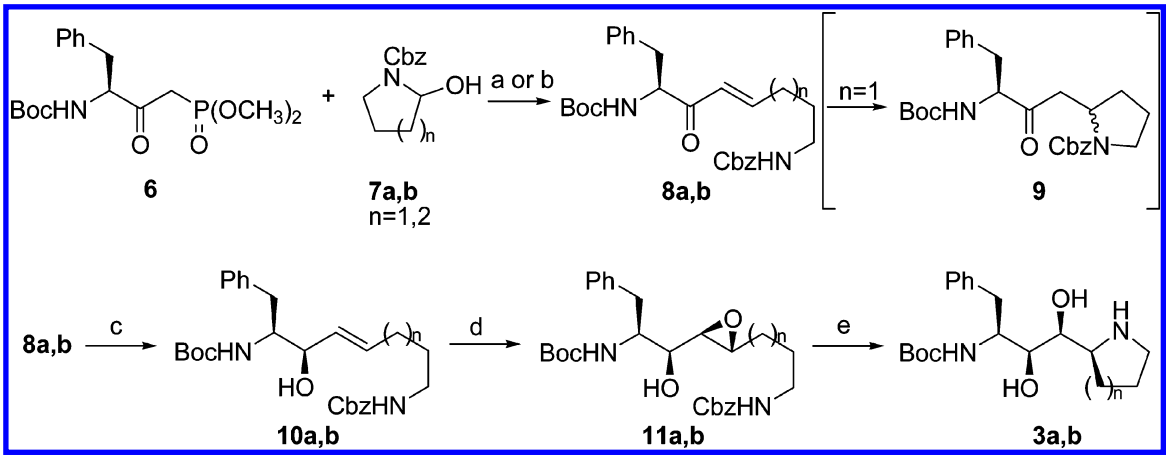
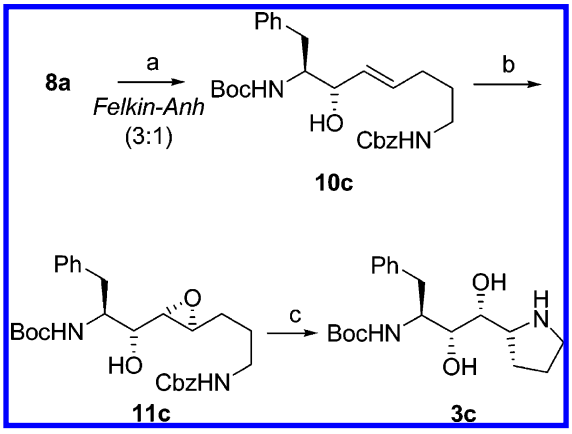
Scheme 2. Synthesis of Isosteres 3a,b[a]



[a]Reagents and conditions: (a) K_2CO_3, CH_3CN, 25 °C, 50% (for **8a**); (b) DBU, LiCl, CH_3CN, 25 °C, 65% (for **8b**); (c) LTBAH, EtOH, −78 °C, 94−96%; (d) *m*CPBA, CH_2Cl_2, 51−68%; (e) H_2, 10% Pd−C, MeOH, 25 °C, 95−96%.

Scheme 3. Synthesis of Isostere 3c[a]



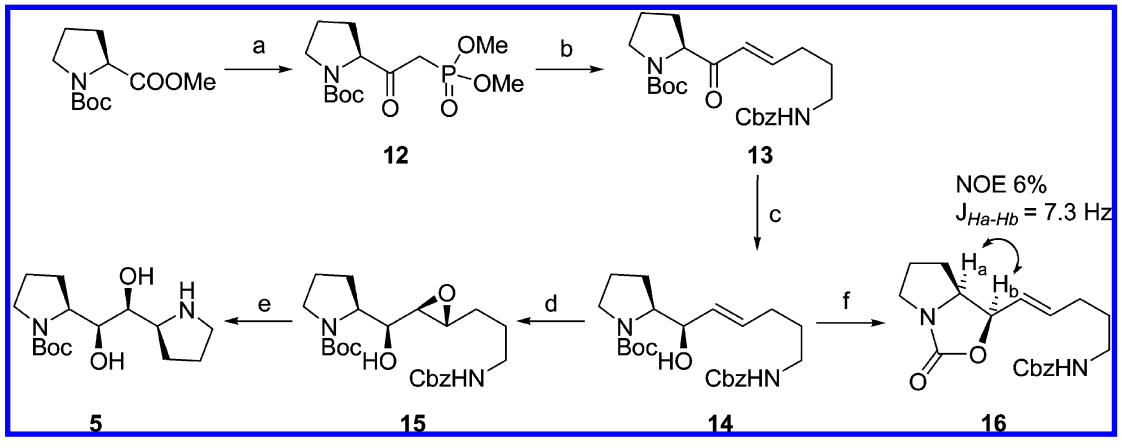
[a]Reagents and conditions: (a) L-Selectride, MeOH, −78 °C, 61%; (b) *m*CPBA, CH_2Cl_2, 0 °C, 72%; (c) H_2, Pd−C 5%, MeOH, 25 °C, 92%.

and therefore with the *S,R* configuration of **14**.[21] Epoxidation and deprotection/cyclization of the epoxy amine **15** finally gave the *S,S,S,S* Pro-Pro isostere **5**.

Synthesis of the Inhibitors. Both symmetrically and unsymmetrically substituted peptidomimetic inhibitors can be obtained from isosteres **3a−c** and **5** by appropriate management of the unprotected and protected amino groups. To test the activity of inhibitors based on these new cores, a small set of 10 inhibitors was synthesized by coupling the isosteres with selected peptide or nonpeptide carboxylic acids (Chart 1). The peptide residues of inhibitor **17** (Chart 1) correspond to a sequence recognized by HIV-PR already exploited for the development of potent inhibitors.[22] Similarly, the AcTrp-Val dipeptide present in inhibitors **18** and **19** was shown to be particularly efficient when combined with a Phe-Phe dihydroxyethylene isostere, as in inhibitor **27**.[9] In inhibitors **19−26** the dihydroxyethylene core is combined with derivatives of phenoxyacetic acid, a ligand specifically targeted at the enzyme's S2 pocket.[23]

Unsymmetrically substituted inhibitors **17**, **19**, **22** were obtained by acylation of the unprotected secondary amino group of the isostere **3**, followed by deprotection and acylation of the primary amine (Scheme 5: method A). The symmetrically substituted inhibitors **18a**, **20**, **21**, **23a−26** were obtained by Boc deprotection of the corresponding isostere followed by the simultaneous acylation of the primary and secondary amino groups (Scheme 5: method B).

Scheme 4. Synthesis of Pro-Pro Isostere 5[a]



[a]Reagents and conditions: (a) $LiCH_2P(O)(OCH_3)_2$, THF, −78 °C, 70%; (b) *N*-Cbz-2-hydroxypyrrolidine (**7a**), K_2CO_3, CH_3CN, 25 °C, 76%; (c) DIBALH, toluene, −78 °C, 51%; (d) *m*CPBA, CH_2Cl_2, 0 °C, 22%; (e) H_2, 5% Pd−C, CH_3OH, 25 °C, 95%; (f) NaH, THF, 25 °C

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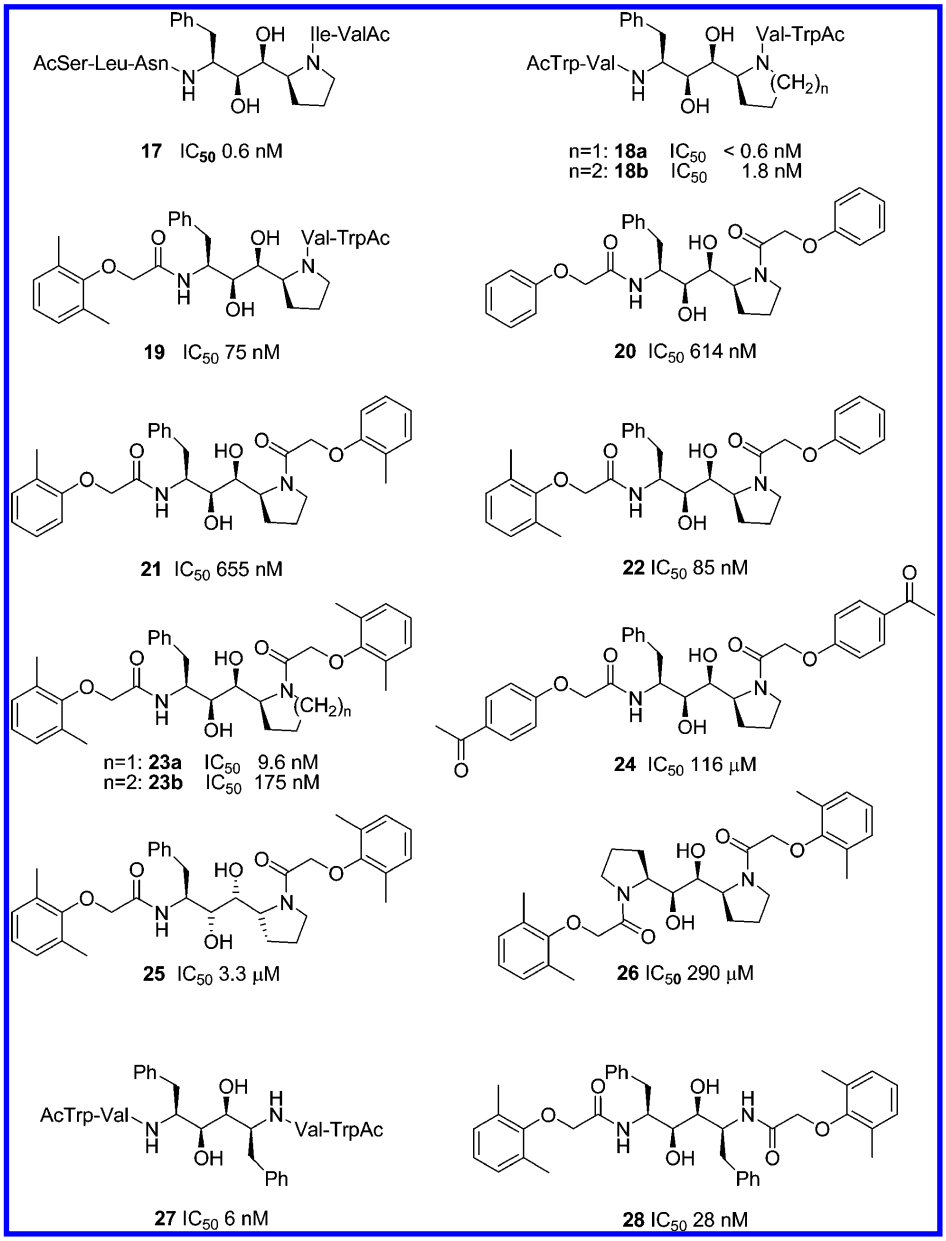
Chart 1. HIV-Pr Inhibitors Based on Phe-Pro and Pro-Pro Dihydroxyethylene Isosteres[a]



[a]Phe-Phe-based inhibitors **27** and **28** are included for comparison.

Acylations were carried out under standard peptide coupling conditions. Assembly of the six-membered inhibitors **18b** and **23b** was more difficult, as the piperidine nitrogen of the core unit **3b** (Scheme 5, $n = 2$) is far less reactive than that of its pyrrolidine analogues **3a** and **3c** ($n = 1$). Thus, inhibitor **18b** was obtained from deprotected **3b** with the mixed anhydride method, using the AcTrpValCOOH peptide and isobutyl chloroformate (method C); **23b** was obtained by acylation of deprotected **3b** with 2,6-dimethylphenoxyacetyl chloride (method D).[23d]

HIV-PR Inhibition and Structure–Activity Relationships. IC_{50} values (chart 1) were determined at pH 5.5 using a commercial recombinant wild-type HIV-1 PR and the fluorogenic substrate 2-aminobenzoyl-Thr-Ile-Nle-Phe(p-NO_2)-Gln-Arg-NH_2 (Abz-NF*-6).

Flanking Residues. Powerful, nanomolar HIV-1 PR inhibitors are obtained when the *S,S,S,S* isosteres **3a,b** are combined with peptide chains that can occupy the protease's S2, S2′ and S3, S3′ subsites (**17**, **18**). Inhibitor **17** is more potent than its analogue JG-365 based on a hydroxyethylamine Phe-Pro isostere,[22] and the efficacy of the Phe-Pro core **3a** is confirmed by inhibitor **18a**, the most powerful compound within this set. Its IC_{50} lies below the limit of our test[24] but is at least 1 order of magnitude lower than that of the corresponding inhibitor **27**, based on a Phe-Phe dihydroxyethylene isostere.[9] Inhibitors **20**–**26**, containing substituted phenoxyacetic acid residues, can only engage the enzyme's S1, S2, S1′, S2′ subsites;[23a–c] nevertheless, they display activities in the nanomolar to micromolar range. The effect of substitution on the phenoxyacetyl residue can be assessed on inhibitors **20**–**24**, based on the same Phe-Pro core. Comparison of inhibitors **20** and **21** indicates that the introduction of a single ortho methyl group has no effect on the inhibitor's activity. On the contrary, replacement of POA with 2,6-dimethylphenoxyacetic acid (DMPOA), as in **22**, results in a 10-fold increase of activity, and a similar improvement is also

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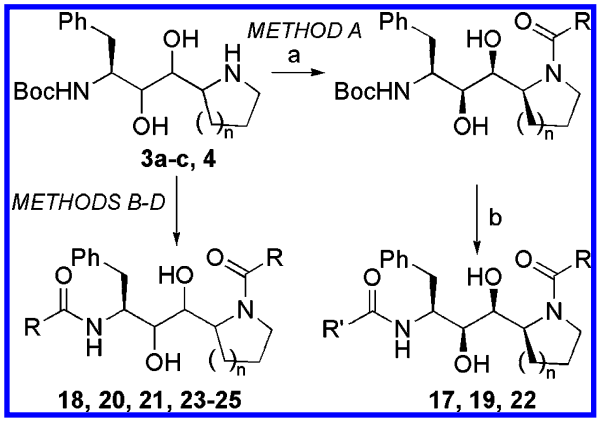
Scheme 5. Synthesis of Inhibitors 17−25[a]



[a]Reagents and conditions: method A: (a) RCOOH, THF, EDC, HOBT, 0−25 °C; (b) i: TFA, DCM, 25 °C; ii: R′COOH, THF, EDC, HOBT, 0−25 °C; method B: as in step b of method A; method C: i: TFA, DCM, 25 °C; ii: RCOOH, isobutyl chloroformate, NMM, EtOAc, 25 °C; method D: i: TFA, DCM, 25 °C; ii: RCOCl, EtOAc, 25 °C.

observed when the second POA group is replaced by DMPOA, as in **23**. A superimposition of the minimum energy conformations of inhibitors **20**, **21**, and **23**, obtained by molecular mechanics (Figure 2), indicates that the phenoxy rings of **20**

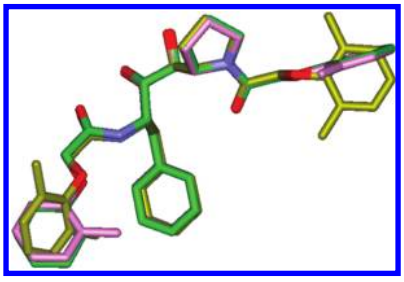


Figure 2. Superimposition of the minimum energy conformations of inhibitors **20** (green), **21** (pink), and **23** (yellow) obtained by molecular mechanics with the MMFF force field.

and **21** adopt a similar orientation, while in **23** the rings are locked by the two methyl groups in an orthogonal conformation close to the bioactive conformation adopted by this ligand in complexes with HIV-PR.[23a] To adopt a similar conformation, the 2-methylphenoxyacetyl ligand of inhibitor **21** is expected to pay a significant entropic penalty that compensates for the enthalpic advantage resulting from hydrophobic interactions between the methyl group of the ligand and residues in the S2/S2′ pockets. The high efficiency of DMPOA as S2 ligand is also confirmed by nanomolar inhibitor **19** in which this residue is present on the primary amino group, while the acetylated Val-Trp dipeptide is linked to the secondary amino group. On the contrary, introduction of an acetyl group in the 4-position of the POA ring, as in **24**, confirms that electron-withdrawing substituents in this position depress the activity, as already observed in a series of inhibitors based on a Phe-Phe hydroxyethylene core.[23b]

Ring Size. Inhibitor **19a** was cocrystallized with HIV-PR, and the X-ray crystal structure of the complex was obtained at 1.3 Å resolution.[25] This structure (Figure 3) shows that the inhibitor's benzyl side chain (middle left) rests nicely in the enzyme's S1 subsite, while the S1′ subsite is only partially filled

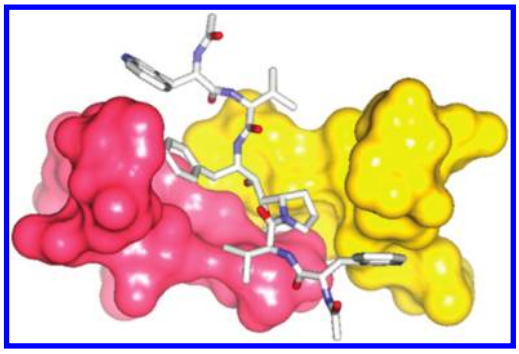


Figure 3. Detail of the X-ray crystal structure of the complex of HIV-1 PR with inhibitor **19a**,[25] showing the interactions of the inhibitor with the S1 (red) and S1′ (yellow) pockets.

by the pyrrolidine ring (middle right). Larger groups in this position should allow further hydrophobic interactions to be established with the subsite and improve the inhibitor's binding properties. However, simple expansion of the pyrrolidine ring to piperidine, as in **19b**, does not improve the inhibitor's activity: on the contrary, **19b** is less active than **19a**. Probably the small increase in the size of the heterocycle is overcome by the rigidity of the six-membered ring, preventing optimal fit of the latter with the S1′ subsite. Introduction of hydrophobic substituents on selected positions of the more flexible pyrrolidine ring may provide a better approach to more efficient inhibitors.

Stereochemistry. A comparison between **23a** and its (*S*,*R*,*R*,*R*) isomer **25** reveals a dramatic drop of activity by 3 orders of magnitude. Changes in the configuration of the hydroxymethyne carbons in hydroxy- and dihydroxyethylene inhibitors of HIV-PR do not generally have such a large effect on the inhibitors' activity.[22,26] The lower activity of **25** is most likely related to the inversion at the pyrrolidine ring carbon, suggesting that it is essential that proline's original configuration is preserved in the isostere.

Symmetry. Inhibitors **23a**, **26**, and **28** (Chart 1) have identical DMPOA flanking groups and configuration but different substituents in the dipeptide isostere core. Although **26** (based on a Pro-Pro isostere) and **28** (based on a Phe-Phe isostere) possess the same C_2 symmetry as the target enzyme, nonsymmetric inhibitor **23a** performs better.

It was mentioned earlier that the preference for nonsymmetric inhibitors may reflect the intrinsic lack of symmetry of the enzyme−inhibitor complex.[10,11] However, a dynamic effect may also operate. The enzyme's binding site is closed by two symmetric loops (flaps) that encompass the ligand upon binding. The flaps can adopt open,[27] semiopen,[28] and closed conformations, the latter being generally adopted in the complexes with small peptides and inhibitors.[5a] Opening of the flaps is necessary for binding the large viral polyproteins, but it is still debated whether the binding of small molecules occurs via the open form and whether it is a single or a multistep process.[29] Recently, the binding of a pentapeptide substrate to HIV-Pr has been studied by molecular dynamics,[30] and it has been found that, in the main binding pathway, the flaps do not open. The ligand enters the tight catalytic channel with a preferred orientation, and at least two nonsymmetric intermediates are formed during the slow rearrangement toward the Michaelis complex. Nonsymmetric species may thus have a kinetic advantage over symmetric ones, in the steps preceding the formation of the final complex.

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Antiviral Activity and Cell Toxicity. Following the remarkable activity of several of the tested inhibitors toward isolated HIV-PR, we further investigated the antiviral activity of the most promising inhibitors **18a** and **23a** on HIV-1_{IIIB}-infected MT-2 cells.

The anti-HIV effect of the two inhibitors has been evaluated by a rapid and sensitive in vitro microtiter infection assay based on cytolysis quantitation by vital dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) uptake as an end point for infection.[31] The effect of inhibitors on endogenous reverse transcriptase activity of HIV-1_{IIIB}-infected MT-2 supernatants was considered as an additional marker for their ability to block HIV-1 replication. MT-2 cells were infected and incubated with **18a** and **23a** for 72−96 h, and then reverse transcriptase activity was measured in the supernatants. To protect the infected cells from cytolysis directly induced from the virus, a multiplicity of infection (MOI) of 0,1 was applied for the test.[31] Results are in Table 1.

Table 1. Antiviral Activity of Inhibitors 18a and 23a

inhibitor	concentration (nM)	infection assay MTT-A540 (% cell survival)[a]	RT activity in supernatants[b] (%)
18a	10	>90	24
	5	>90	50
23a	0.1	78	24
	0.001	80	25
	0.0001	87	26
	0.00001	90	29
	0.000001	>90	28
	0.0000001	>90	26

[a]Percent cell survival of virus-infected cells at different inhibitor concentrations (see Experimental Section). [b]From duplicate experiments. RT activity (%) is the ratio between RT activity in supernatants of inhibitor-treated HIV-1-infected cultures and that in untreated HIV-1-infected MT-2 cells (100%). Abacavir (see ref 32) was used as control (>90% cell survival and >90% inhibition of RT activity).

Data in Table 1 indicate that both **18a** and **23a** protect virus-infected cells for over 90% at nanomolar (**18a**) and subpicomolar (**23a**) concentrations and significantly depress RT activity. Although the virus-induced cytolytic effect is not completely suppressed, it is much lower than that observed when MOI was ≥1 (data not shown), this being a clear indication that the inhibitors had modified de novo-synthesized HIV virions rendering them noninfectious.[31] The extent of protection does not correlate with RT activity (Table 1), and a control experiment with recombinant RT confirms that this enzyme is not a target for the inhibitors. On the contrary, native viral protease obtained by lysis of virions from tissue culture fluids of infected H9/HTLV IIIB cells is efficiently inhibited by **18a** and **23a** with IC_{50} values of 5 ± 1 nM and 1.5 ± 0.9 pM, respectively.[33] The inversion of the inhibitors' activities in this assay with respect to the assay with recombinant HIV-PR (Chart 1) is likely due to the different conditions for the two tests (enzyme concentration, substrate, pH, buffer and ionic strength, presence of albumin; see Experimental Section).

The cytotoxicity of **18a** and **23a** was evaluated on MT-2 cell lines. MNC was evaluated as the maximal concentration which altered neither the morphology of MT-2 cell monolayers nor the cell survival rate, while CC_{50} was evaluated by % cell survival. In vitro cytotoxicities are summarized in Table 2.

Table 2. Cytotoxicity (CC_{50}) and Maximum Nontoxic Concentrations (MNC) of 18a and 23a in MT-2 Cells for 72 and 96 h Cultivation[a]

	MNC (μM)		CC_{50} (μM)	
inhibitor	72 h	96 h	72 h	96 h
18a	0.01	0.0003	0.5	2500
23a	0.0001	0.0001	300	1000

[a]Mean values of six to eight parallels per experiment with standard deviation ≤10%; each experiment was run in triplicate (see Supporting Information).

The two inhibitors express a different degree of cytotoxicity in the tested cells, and this phenomenon is dose-dependent. After a prolonged exposure (96 h), the cytotoxicity of **18a** measured by MNC increases up to 33 times and about 5000 times by CC_{50}, while no significant differences in MNC are observed with **23a** at different incubation times.

CONCLUSIONS

We recently introduced epoxy amine cyclization as a versatile approach for the synthesis of diamino diol Xaa-Pro dipeptide isosteres.[14] This approach has now been optimized by using 2-hydroxypyrrolidine and 2-hydroxypiperidine as synthetic equivalents of the corresponding ω-amino aldehydes in the initial Horner−Emmons olefination. Epoxy amines can thus be obtained in a more direct way and with higher yields. The improved methodology has been applied to the synthesis of three Phe-Pro isosteres (**3a**−**c**) and a C_2-symmetric Pro-Pro isostere (**5**) containing a pyrrolidine or piperidine ring as a proline mimic. All isosteres were obtained in good yields and excellent stereoselectivity.

A set of 12 peptidomimetic HIV-PR inhibitors was synthesized by coupling the isosteres with peptide residues, chosen for their known affinity for the target enzyme, and nonpeptide residues derived from phenoxyacetic acid; several inhibitors display high activity against HIV-1 PR, with IC_{50} in the low nanomolar range. Comparison of inhibitors with identical DMPOA flanking residues grafted onto *S*,*S*,*S*,*S* diamino diol Phe-Pro, Pro-Pro, and Phe-Phe isosteres reveals that the nonsymmetric Phe-Pro-based inhibitor **23a** performs better than the C_2 symmetric partners **26** and **28**, despite the latter two inhibitors having the same symmetry of the target enzyme. The efficiency of nonsymmetric inhibitors based on the Phe-Pro isostere **3a** is confirmed by the higher activity of inhibitor **18a** (Chart 1) with respect to the symmetric Phe-Phe inhibitor **27**.[9] We propose that nonsymmetric binding of the inhibitors with HIV-PR, regardless of the intrinsic symmetry of the inhibitor, is at the origin of this behavior, either through a thermodynamic effect or through a kinetic effect due to the presence of nonsymmetric intermediates along the path to the enzyme−inhibitor complex.

Inhibitors **18a** and **23a**, based on a five-membered proline mimic, were further studied for their ability to inhibit HIV-1 replication. Both inhibitors display high antiviral activity, in the nanomolar to picomolar range, combined with low cytotoxicity, resulting in a high therapeutic index in the 10^3 and 10^4 range, respectively.

In conclusion, we have demonstrated that isostere **3a**, available from phenylalanine in only five steps and excellent yield, is a precursor of efficient HIV-PR inhibitors, when coupled with suitable peptide or nonpeptide residues. The potential of this new peptidomimetic core is well illustrated by inhibitor **23a** that combines low molecular weight with subpicomolar

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antiproliferative activity and low cytotoxicity, thus appearing particularly promising for further development. The synthesis and evaluation of second generation, rationally designed inhibitors based on Phe-Pro isostere **3a** that have high predicted inhibitory potencies, promising ADME profiles, and the potential to avoid drug resistance due to favorable interactions with the PR backbone are currently underway.[34]

EXPERIMENTAL SECTION

Melting points are not corrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 500 MHz spectrometer or a Jeol EX 400 MHz in $CDCl_3$ (unless otherwise stated), with tetramethylsilane as internal standard. Electrospray mass spectra were obtained with a Bruker Daltonics Esquire 4000 spectrometer. Optical activities were measured with a Perkin-Elmer 261 polarimeter, in methanol unless otherwise stated. IR spectra were recorded on a Thermo-Nicolet AVATAR 320 FT-IR instrument. Enzyme kinetics were followed with a fluorogenic substrate on a Perkin-Elmer LS50B spectrofluorimeter. Flash chromatography was performed on silica gel 60 (Merck, 230−400 mesh). THF was distilled, dried over KOH, and then redistilled from sodium−benzophenone ketyl. Dichloromethane was dried over $CaCl_2$ and distilled. Dry acetonitrile and dimethylformamide were purchased from Sigma-Aldrich. Methanol and ethanol were dried over CaH_2 and distilled. All inhibitors were >95% pure, as determined by HPLC analysis (C4, water−acetonitrile).

Dimethyl (*S*)-3-*tert*-Butoxycarbonylamino-2-oxo-4-phenylbutylphosphonate (6). A 2.5 M solution of *n*-butyllithium in hexanes (86 mL, 214 mmol) was added to a solution of methyl dimethylphosphonate (23.0 mL, 214 mmol) in dry THF (200 mL) at −78 °C, under argon atmosphere. The mixture was stirred for 15 min, and then *N*-Boc-L-phenylalanine methyl ester (10 g, 35.8 mmol) in THF (70 mL) was added. The resulting solution was stirred at −78 °C for 2 h and then at −30 °C for 1 h and finally neutralized with 20% aqueous citric acid. The aqueous phase was extracted with ethyl acetate (3 × 75 mL), and the organic phases were extracted with sat. $NaHCO_3$ and brine and then dried over anhydrous sodium sulfate. The solvent was removed, and the crude product was purified over silica to give the phosphonate **6** (12.4 g, 89%) as a white solid, mp 65−67 °C (isopropyl ether). $[\alpha]_D^{25}$ −52 (*c* 0.4). IR (KBr): 3280, 1723, 1713, 1225 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.39 (s, 9H), 2.93 (dd, 1H, *J* = 8.3, 14.2 Hz), 3.18 (dd, 1H, *J* = 6.1, 14.2 Hz), 3.10 (dd, 1H, *J* = 14.2, 22.0 Hz), 3.26 (dd, 1H, *J* = 14.2, 22.0 Hz), 3.75 (d, 3H, *J* = 11.5 Hz), 3.77 (d, 3H, *J* = 11.5 Hz), 4.55 (m, 1H), 5.43 (d, 1H, *J* = 7.8 Hz), 7.24 (m, 5H) ppm. ^{13}C NMR ($CDCl_3$) δ: 28.3, 37.0, 38.3 (d, *J* = 130 Hz), 53.2, 61.3, 80.1, 127.0, 127.8, 128.7, 136. 6, 155.3, 201.0 (d, *J* = 5.5 Hz) ppm. ES-MS *m*/*z* 372 [MH]$^+$.

(*E*,*S*)-10-Benzyloxycarbonylamino-2-*tert*-butoxycarbonylamino-3-oxo-1-phenyloct-4-ene (8a). Phosphonate **6** (3.87 g, 1 mmol) was added to a suspension of dried K_2CO_3 (4.14 g, 3 mmol) in anhydrous acetonitrile (10 mL), and the suspension was stirred at 25 °C for 15 min. *N*-Cbz-2-hydroxypyrrolidine **7a**[15] (2.3 g, 1 mmol) in 10 mL of dry acetonitrile was added dropwise, and the mixture was stirred under argon for 48 h, neutralized with 10% (w/v) citric acid, and extracted with ethyl acetate. The organic phase was extracted with water and brine and dried over anhydrous sodium sulfate. The solvent was evaporated, and the residue was purified over silica giving the enone **8a** (2.3 g, 50%), mp 75−77 °C (isopropyl ether). $[\alpha]_D^{25}$ −0.59 (*c* 0.73). ^{1}H NMR ($CDCl_3$) δ: 1.35 (s, 9H), 1.59 (t, 2H, *J* = 6.9 Hz), 2.18 (q, 2H, *J* = 6.9 Hz), 2.96 (dd, 1H, *J* = 5.9, 13.7 Hz), 3.05 (dd, 1H, *J* = 6.8, 13.7 Hz), 3.15 (q, 2H, *J* = 6.2 Hz); 4.77 (m, 1H), 4.99 (broad, 1H), 5.12 (s, 2H), 5.31 (d, 1H, *J* = 7.7 Hz), 6.09 (d, 1H, *J* = 15.7 Hz), 6.85 (m, 1H), 7.10−7.34 (m, 10H) ppm. ^{13}C NMR ($CDCl_3$) δ: 28.4 (two carbons), 29.8, 38.5, 40.5, 58.5, 66.7, 79.8, 126.9, 127.9, 128.2, 128.4, 128.6, 128.8, 129.5, 136.3, 136.6, 148.4, 155.2, 156.5, 197.5 ppm. ES-MS *m*/*z* 465.1 [MH]$^+$.

(*E*,*S*)-10-Benzyloxycarbonylamino-2-*tert*-butoxycarbonylamino-3-oxo-1-phenylnon-4-ene (8b). LiCl (1.44 g, 3.4 mmol), dried at 220 °C for 4 h, was added to a stirred solution of phosphonate **6** (1.26 g, 3.4 mmol) in 65 mL of dry acetonitrile. *N*-Cbz-2-hydroxypiperidine **7b**[18] (800 mg, 3.4 mmol) and DBU (440 mg, 2.8 mmol) were then added, and the mixture was stirred at 25 °C for 16 h, neutralized with 10% HCl, and extracted with ethyl acetate. Workup as described for **8a**, gave the enone (1.02 g, 65%), mp 72−73 °C (isopropyl ether). $[\alpha]_D^{25}$ −0.62 (*c* 0.73). IR (nujol): 3362, 1743, 1691, 1620 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.40 (m, 13H), 2.16 (m, 2H), 3.05 (m, 4H), 4.77 (m, 2H), 5.08 (s, 2H), 5.25 (d, 1H, *J* = 69 Hz), 6.11 (d, 1H, *J* = 15.7 Hz), 6.85 (m, 1H), 7.29 (m, 10H) ppm. ^{13}C NMR ($CDCl_3$) δ: 25.1, 28.4, 29.6, 32.2, 38.6, 40.7, 58.4, 66.7, 79.8, 126.9, 127.2, 127.9, 128.3, 128.4, 128.5, 128.8, 129.4, 129.6, 136.3, 136.6, 149.0, 155.2, 156.5, 197.5 ppm. ES-MS *m*/*z* 481.2 [MH]$^+$.

(*E*,*2S*,*3R*)-8-Benzyloxycarbonylamino-2-*tert*-butoxycarbonylamino-3-hydroxy-1-phenyloct-4-ene (10a). Enone **8a** (5.0 g, 10.4 mmol), in 40 mL of dry ethanol, was added, under inert atmosphere, to a stirred suspension of LTBAH (6.62 g, 26 mmol) in dry ethanol (30 mL), while keeping the temperature between −78 and −60 °C. When all the enone was reduced, the mixture was quenched with 16 mL of 10% aqueous citric acid. The mixture was diluted with water (50 mL) and extracted with ethyl acetate. The organic phases were extracted with brine and dried over anhydrous sodium sulfate. The solvent was removed, and the residue was purified over silica, giving 4.7 g (96%) of a white solid, mp 113−114 °C (isopropyl ether). $[\alpha]_D^{25}$ −20.23 (*c* 0.44). IR (KBr): 3451, 3341, 1713 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.35 (s, 9H), 1.60 (m, 2H), 2.12 (m, 2H), 2.79 (m, 2H), 3.12 (broad, 1H), 3.22 (m, 2H), 3.79 (m, 1H), 4.12 (m, 1H), 4.78 (broad, 1H), 4.93 (broad, 1H), 5.10 (s, 2H), 5.51 (dd, 1H, *J* = 15.4, 6.6 Hz); 5.67 (m, 1H), 7.34 (m, 10H) ppm . ^{13}C NMR ($CDCl_3$) δ: 28.4, 29.1, 29.2, 36.5, 40.1, 56.8, 66.8, 74.7, 79.7, 126.4, 128.2, 128.3 128.5, 128.6, 129.3, 129.4, 129,5, 130.0, 132.4, 136.7, 138.2, 156.5, 156.6 ppm. ES-MS *m*/*z* 469 [MH]$^+$.

(*E*,*2S*,*3R*)-9-Benzyloxycarbonylamino-2-*tert*-butoxycarbonylamino-3-hydroxy-1-phenylnon-4-ene (10b). Enone **8b** (1.62 g, 3.48 mmol) and LTBAH (2.65 g, 10.2 mmol), as described for the synthesis of **10a**, gave the alcohol **10b** (1.52 g, 94%), mp 76−78 °C (isopropyl ether). $[\alpha]_D^{25}$ −24.3 (*c* 0.41). IR: 3348, 1688, 1620 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.26−1.51 (m, 13H), 2.09 (m, 2H), 2.80 (m, 2H), 3.16 (m, 2H), 3.94 (broad, 1H), 4.13 (m, 1H), 4.78 (broad, 1H), 4.86 (broad, 1H), 5.07 (s, 2H), 5.53 (dd, 1H, *J* = 15.16, 6.2), 5.67 (m, 1H), 7.19−7.33 (m, 10H) ppm. ^{13}C NMR ($CDCl_3$) δ: 28.2, 29.2, 31.7, 36.1, 40.7, 56.6, 66.6, 76.3, 79.5, 126.4, 128.0, 128.2, 128.5, 128.6, 129.0, 129.3, 129.5, 133.2, 136.6, 138.1, 156.4, 156.4 ppm. ES-MS *m*/*z* 483.2 [MH]$^+$.

(*E*,*2S*,*3S*)-8-Benzyloxycarbonylamino-2-*tert*-butoxycarbonylamino-3-hydroxy-1-phenyloct-4-ene (10c). L-Selectride (6.4 mL of a 1.0 M solution in THF, 6.4 mmol) was added at −78 °C and under an inert atmosphere to a solution of **8a** (1.0 g, 2.14 mmol) in 30 mL of dry methanol. After 3 h, the solution was acidified to pH 5 with 1 N HCl, and the solvent was evaporated. The residue was partitioned between ethyl acetate and sat. $NaHCO_3$. The aqueous phase was extracted with ethyl acetate, and the combined organic phases were extracted with brine and dried over anhydrous sodium sulfate. The solvent was removed, and the residue was purified over silica, giving the alcohol **10c** (610 mg, 61%), mp 87−89 °C (isopropyl ether). $[\alpha]_D^{25}$ −36.8 (*c* 0.5). IR (Nujol): 3452, 3341, 1713 cm^{-1}. ^{1}H NMR (CD_3CN, 50 °C) δ: 1.32 (s, 9H), 1.56 (quint, 2H, *J* = 6.9 Hz), 2.05 (q, 2H, *J* = 6.9 Hz), 2.67 (dd, 1H, *J* = 9.2, 13.7 Hz), 2.89 (dd, 1H, *J* = 5.8, 13.7 Hz), 3.12 (q, 2H, *J* = 6.6 Hz), 3.70 (m, 1H), 4.01 (s, 1H), 5.06 (m, 3H), 5.39 (s, 1H), 5.49 (dd, 1H, *J* = 6.2 Hz, 15.4 Hz), 5.63 (m, 1H), 7.34 (m, 10H) ppm. ^{13}C NMR (CD_3CN, 50 °C) δ: 28.0, 29.0, 29.2, 37.4, 40.2, 56.8, 66.0, 72.7, 78.5, 126.1, 127.8, 127.9, 128.3, 128.6, 131.1, 131.5, 137.5, 139.4, 156.0, 156.5 ppm. ES-MS *m*/*z* 469 [MH]$^+$.

(*2S*,*3S*,*4R*,*5R*)-8-Benzyloxycarbonylamino-2-*tert*-butoxycarbonylamino-4,5-epoxy-3-hydroxy-1-phenyloctane (11a). *m*CPBA (314 mg, 1.1 mmol) in DCM (5 mL) was added at 0 °C to a solution of allylic alcohol **10a** (426 mg, 0.91 mmol) in DCM (10 mL). The mixture was stirred at room temperature for 16 h, diluted with 15 mL DCM, extracted with 10% aqueous sodium metabisulfite (2 × 25 mL), sat. $NaHCO_3$ (2 × 25 mL), and brine, and dried over anhydrous sodium sulfate. The solvent was evaporated, and the residue was purified over silica to give the epoxide **11a** (300 mg, 68%), mp 99−101 °C

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(isopropyl ether). $[\alpha]_D^{25}$ +8.33 (*c* 0.3). ^{1}H NMR ($CDCl_3$) δ: 1.35 (m, 11H), 1.61 (m, 2H), 2.63 (d, 1H, *J* = 6.2 Hz), 2.86 (m, 1H), 2.96 (m, 3H), 3.23 (m, 2H), 3.55 (m, 1H), 3.99 (m, 1H), 4.71 (m, 1H), 4.88 (broad, 1H), 5.08 (s, 2H), 7.32 (m, 10H) ppm. ^{13}C NMR ($CDCl_3$) δ: 26.5, 28.3, 28.7, 36.6, 40.6, 55.7, 58.4, 59.0, 66.8, 71.7, 79.7, 126.6, 128.2 (two signals), 128.6 (two signals), 129.5 (two signals), 136.6, 137.6, 156.5 (two signals) ppm. ES-MS *m*/*z* 485 $[MH]^+$, 429 $[MH - C_4H_8]^+$.

(2*S*,3*S*,4*R*,5*R*)-9-Benzyloxycarbonylamino-2-*tert*-butoxycarbonylamino-4,5-epoxy-3-hydroxy-1-phenylnonane (11b). The product (2.10 g, 51%) was obtained from **15b** (4.00 g, 8.3 mmol) and mCPBA (2.90 g, 9.95 mmol), as described for **11a**. Mp 103−105 °C (isopropyl ether). $[\alpha]_D^{25}$ −8.7 (*c* 0.32). IR (nujol): 3341, 1686, 1620 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.34 (m, 13H), 1.49 (m, 2H), 1.97 (d, 1H, *J* = 4.04 Hz), 2.84 (m, 3H), 2.96 (m, 1H), 3.16 (m, 2H), 3.53 (m, 1H), 3.98 (m, 1H), 4.81 (m, 1H), 4.94 (m, 1H), 5.06 (s, 2H), 7.20−7.33 (m, 10H) ppm. ^{13}C NMR ($CDCl_3$) δ: 23.1, 28.4, 29.7, 31.0, 36.3, 40.9, 55.0, 56.1, 59.1, 66.7, 72.0, 79.6, 126.5, 128.5, 128.6 (multiple overlapping signals), 129.5, 136.7, 137.8, 155.8, 156.5 ppm. ES-MS *m*/*z* 499.2 $[MH]^+$.

(2*S*,3*R*,4*S*,5*S*)-8-Benzyloxycarbonylamino-2-*tert*-butoxycarbonylamino-4,5-epoxy-3-hydroxy-1-phenyloctane (11c). Epoxidation of **10c** (770 mg, 1.64 mmol) with *m*CPBA (567 mg, 1.98 mmol), as described for **11a**, gave the product **11c** (571 mg, 72%) as a colorless oil. $[\alpha]_D^{25}$ −14.4 (*c* 0.25). ^{1}H NMR (CD_3CN, 70 °C) δ: 1.33 (s, 9H), 1.59 (m, 4H), 2.72−2.91 (m, 4H), 3.14 (m, 2H), 3.25 (broad, 1H), 3.32 - 3.44 (2s, 1H, rotamers), 3.88 (m, 1H), 5.06 (s, 2H), 5.15 (broad, 1H), 5.53 (m, 1H), 7.37 (m, 10H) ppm. ^{13}C NMR (CD_3CN, 70 °C) δ: 26.1, 27.7, 28.6, 37.7, 40.6, 55.4 and 55.7 (rotamers), 58.6, 59.9, 65.9, 71.1 and 72.6 (rotamers), 78.7, 127.7, 127.9, 128.3 (2 overlapping signals), 128.4, 129.3, 137.7, 139.1, 155.8 and 156.5 (rotamers) ppm. ES-MS *m*/*z* 485 $[MH]^+$.

(1*S*,2*S*,3*S*)-3-*tert*-Butoxycarbonylamino-4-phenyl-1-((*S*)-pyrrolidin-2-yl)butane-1,2-diol (3a). The Cbz-protected epoxy alcohol **11a** (100 mg, 0.206 mmol) in methanol (5 mL) was stirred for 16 h over 5% Pd−C under a hydrogen atmosphere. The mixture was filtered over Celite, and the solvent was evaporated to give the crude product (69 mg, 95%), mp 167 °C (toluene−hexane). $[\alpha]_D^{25}$ −38.8 (*c* 0.35). ^{1}H NMR ($CDCl_3$) δ: 1.33 (s, 9H), 1.57 (m, 1H), 1.76 (m, 2H), 1.91 (m, 1H), 2.86 (m, 1H), 2.92 (m, 1H), 2.98 (m, 1H), 3.13 (d, 1H, *J* = 13.9 Hz), 3.53−3.66 (m, 3H), 3.91 (m, 1H), 4.62 (broad, 3H), 7.34 (m, 5H) ppm. ^{13}C NMR ($CDCl_3$) δ: 25.2, 27.3, 28.3, 36.5, 45.9, 52.8, 61.9, 69.6, 73.1, 80.2, 126.4, 128.4, 129.5, 137.9, 157.1 ppm. ES-MS *m*/*z* 351 $[MH]^+$.

(1*S*,2*S*,3*S*)-3-*tert*-Butoxycarbonylamino-4-phenyl-1-((*S*)-piperidin-2-yl)butane-1,2-diol (3b). The product (350 mg, 96%) was obtained from epoxy alcohol **11b** (500 mg, 1 mmol) with the procedure described for the synthesis of **6a**. Mp 120−123 °C (toluene−hexane). $[\alpha]_D^{25}$ −39.3 (*c* 0.4). IR (nujol): 3385, 3190 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.33 (s, 9H), 1.43−1.81 (m, 6H), 2.48 (td, 2H, *J* = 9.16, 2.56 Hz), 2.85 (m, 1H), 3.05 (m, 1H), 3.14 (m, 1H), 3.40 (m, 1H), 3.46 (m, 1H), 3.95 (m, 1H), 4.40 (m, 1H), 7.26 (m, 5H) ppm. ^{13}C NMR ($CDCl_3$) δ: 24.6, 25.9, 28.1, 28.3, 36.4, 46.5, 51.7, 60.3, 70.6, 74.1, 80.5, 126.5, 128.5, 129.6, 137.7, 157.5 ppm. ES-MS *m*/*z* 365.3 $[MH]^+$.

(1*R*,2*R*,3*S*)-3-*tert*-Butoxycarbonylamino-4-phenyl-1-((*R*)-pyrrolidin-2-yl)butane-1,2-diol (3c). The oily product (65.4 mg, 92%) was obtained from epoxy alcohol **11c** (98 mg, 0.20 mmol) with the procedure described for the synthesis of **3a**. $[\alpha]_D^{25}$ −17.1 (*c* 0.3). ^{1}H NMR ($CDCl_3$) δ: 1.23−1.37 (m, 11H), 1.64 (m, 2H), 2.85−2.89 (m, 4H), 3.29 (m, 1H), 3.47 (m, 1H), 3.58 (m, 1H), 3.82 (m, 1H), 3.95 (broad, 2H), 5.22 (d, 1H, *J* = 8.4 Hz); 7.12−7.25 (m, 5H) ppm. ^{13}C NMR ($CDCl_3$) δ: 24.8, 25.7, 28.4, 39.1, 46.1, 53.3, 60.3, 71.1, 72.4, 79.6, 126.4, 128.4, 128.4, 129.5, 138.4, 156.1 ppm. ES-MS *m*/*z* 351 $[MH]^+$.

Dimethyl 2-((*S*)-1-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl)-2-oxoethylphosphonate (12). The phosphonate (6.85 g, 70%, yellow oil) was obtained from *N*-Boc-L-proline methyl ester (7.0 g, 30.5 mmol), methyl dimethylphosphonate (9.8 mL, 91.7 mmol), and *n*-butyllithium (36.6 mL, 91.7 mmol) as described previously for **6**. $[\alpha]_D^{25}$ −54.2 (*c* 0.5). IR (film): 1693, 1255 cm^{-1}. ^{1}H NMR (DMSO-d_6, 90 °C) δ: 1.39 (s, 9H), 1.78 (m, 2H), 1.98 (m, 1H), 2.13 (m, 1H), 3.26 (dd, 2H, *J* = 3.5, 21.2 Hz), 3.36 (m, 2H), 3.69 (d, 6H, *J* = 10.6 Hz), 4.36 (dd, 1H, *J* = 4.8, 8.8 Hz) ppm. ^{13}C NMR (DMSO-d_6 90 °C) δ: 23.8, 28.6 (two carbons), 36.6 (d, *J* = 132.7 Hz), 47.1, 53.0, 66.1 (d, *J* = 3.8 Hz), 79.6, 154.7, 201.6 (d, J = 6.9 Hz) ppm. ES-MS *m*/*z* 322 $[MH]^+$.

(*E*,*S*)-6-Benzyloxycarbonylamino-1-(*N*-*tert*-butoxycarbonylpyrrolidin-2-yl)hex-2-en-1-one (13). The enone (1.8 g, 76%, oil) was obtained from phosphonate **12** (1.82 g, 5.69 mmol), *N*-Cbz-2-hydroxypyrrolidine **7a**[15] (334 mg, 1.51 mmol), and K_2CO_3 (2.36 g, 17 mmol), as described for the synthesis of **8a**. $[\alpha]_D^{25}$ −29.6 (*c* 0.58). ^{1}H NMR ($CDCl_3$, pair of rotamers) δ: 1.31−1.40 (s, 9H), 1.61−1.80 (m, 5H), 2.21 (m, 1H), 2.22 (q, 2H, *J* = 7.3 Hz), 3.17 (m, 2H), 3.48 (m, 2H), 4.31−4.51 (m, 1H), 5.04 (m, 3H), 6.20 (d, 1H, *J* = 15.7 Hz), 6.90 (m, 1H) ppm. ^{13}C NMR ($CDCl_3$, pair of rotamers) δ: 23.8−24.3, 28.3−28.5, 29.3, 29.8−30.4, 40.5, 46.8−46.9, 63.4−64.3, 66.7, 79.7−80.0, 126.0, 127.0, 128.1−128.6, 136.6, 147.3, 154.0−154.1, 156.5, 198.5, 198.8 ppm. ES-MS *m*/*z* 417.2 $[MH]^+$.

(*R*,*E*)-6-Benzyloxycarbonylamino-1-((*S*)-*N*-*tert*-butoxycarbonylpyrrolidin-2-yl)hex-2-en-1-ol (14). DIBALH in toluene (5.2 mmol) was added at −78 °C to a stirred solution of enone **13** (2.16 g, 5.18 mmol) in 50 mL of toluene. The reaction was monitored by TLC and neutralized with acetic acid when all the reagent was consumed. The solvent was evaporated, and the residue was partitioned between ethyl acetate and sat. $NaHCO_3$. The aqueous phase was extracted with ethyl acetate, and the combined organic phases were extracted with brine and dried over anhydrous sodium sulfate. The solvent was evaporated, and the residue was purified over silica to give a colorless oil (1.1 g, 51%). $[\alpha]_D^{25}$ −43.8 (*c* 0.5). ^{1}H NMR ($CDCl_3$, pair of rotamers) δ: 1.43 (s, 9H), 1.57−1.85 (m, 5H), 1.99−2.04 (m, 3H), 3.13 (m, 2H), 3.16 (broad, 1H), 3.41 (m, 1H), 3.76−3.87 (m, 1H), 5.04 (m, 3H), 5.39 (dd, 1H, *J* = 5.8, 15.7 Hz), 5.62 (m, 1H) ppm. ^{13}C NMR ($CDCl_3$, pair of rotamers) δ: 24.0, 25.4, 28.3−28.5, 29.4, 29.4−29.5, 40.6−41.1, 47.5−48.2, 62.7, 66.6, 75.6, 80.4−80.5, 127.9−131.3, 132.4, 156.5, 157.9 ppm. ESI-MS *m*/*z* 419.2 $[MH]^+$.

(1*S*,2*R*,3*R*)-6-Benzyloxycarbonylamino-1-((*S*)-*N*-*tert*-butoxycarbonylpyrrolidin-2-yl)-2,3-epoxyhexanol (15). The colorless oil (223 mg, 53%) was obtained from **14** (407 mg, 0.97 mmol) and *m*CPBA (336 mg, 1.17 mmol), as described for **11a**. $[\alpha]_D^{25}$ 9.7 (*c* 0.3). ^{1}H NMR ($CDCl_3$) δ: 1.42 (s, 9H), 1.61−1.98 (m, 5H), 2.77 (m, 1H), 2.89 (m, 1H), 3.20 (m, 2H), 3.42 (broad, 1H), 3.62 (m, 1H), 3.91 (m, 1H), 5.08 (m, 3H), 7.18−7.32 (m, 10H) ppm. ^{13}C NMR ($CDCl_3$) δ: 24.3, 25.7, 28.5, 28.9, 29.8, 40.6, 47.6, 55.2, 58.8, 61.0, 66.6, 72.4, 80.0, 128.0−128.6, 136.7, 156.2, 156.5 ppm. ESI-MS *m*/*z* 434.2 $[MH]^+$.

(*S*)-1-*tert*-Butoxycarbonyl-2-((1*S*,2*S*)-1,2-dihydroxy-2-((*S*)-pyrrolidin-2-yl)ethyl)pyrrolidine (5). Deprotection of *N*-Cbz epoxy amine **15** (73 mg, 0.17 mmol), as described for **3a**, gave 47.9 mg (95%) of a colorless oil. $[\alpha]_D^{25}$ −18.8 (*c* 0.35). ^{1}H NMR ($CDCl_3$) δ: 1.42 (s, 9H), 1.86−2.19 (m, 8H), 3.35 (m, 4H), 3.64 (m, 1H), 3.78 (m, 1H), 3.87 (m, 1H), 3.91 (m, 1H) ppm. ^{13}C NMR ($CDCl_3$) δ: 23.3, 24.2, 27.1, 27.4, 28.4, 45.2, 47.2, 58.6, 62.0, 68.5, 71.1, 80.6, 157.0 ppm. ES-MS *m*/*z* 301 $[MH]^+$.

General Procedure for the Preparation of Inhibitors 17, 19, and 22 (method A). The acid (peptide or phenoxyacetic acid derivative) (1.1 equiv) was dissolved in the minimum amount of anhydrous THF; HOBT (1.1 equiv), diisopropylethylamine (1.1 equiv), and isostere (1 equiv) were added. The solution was cooled to 0 °C, and EDC (1.2 equiv) was added. The mixture was stirred for 1 h at 0 °C and for a further 16 h at 25 °C. The solvent was removed, and the residue was dissolved in ethyl acetate, extracted with 10% aqueous citric acid, sat. $NaHCO_3$, and brine and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo, and the crude product was purified on silica with a 9: 1 mixture of DCM−methanol. The monoacylated product was Boc-deprotected immediately prior to use by treatment with 40% TFA in DCM for 1 h at 25 °C, followed by coevaporation of TFA with ethyl ether. Acylation of the primary amine was carried out as previously, and the product was purified by flash chromatography and/or preparative HPLC. Details on the synthesis and characterization of the inhibitors are in the Supporting Information.

General Procedure for the Preparation of Inhibitors 18, 20, 21, and 23−25 (method B). The isostere (**3a** or **3c**) was Boc-deprotected and the crude diamine was bis-acylated with 2 equiv of the acid and purified, as described in method A. Details on the synthesis and characterization of the inhibitors are in the Supporting Information.

Inhibitor 18b (method C). Isobutyl chloroformate (84 μL, 0.65 mmol) was added, at 0 °C, to a solution of dipeptide N-Ac-TrpVal-COOH (224 mg, 0.65 mmol) and NMM (183 μL, 1.3 mmol) in 5 mL of ethyl acetate; the solution was allowed to reach 25 °C, and Boc-deprotected diol **3b** (118.5 mg, 0.32 mmol) in 5 mL of ethyl acetate was added dropwise. The solution was kept at 25 °C for 2 h, and aqueous workup gave the solid product, 146 mg (49%). ES-MS m/z 832 $[MH]^+$.

Inhibitor 23b (method D). DMPOA (65.3 mg, 0.36 mmol) was dissolved in thionyl chloride, and the solution was heated at 50 °C for 5 h. This solution was then added to a solution of Boc-deprotected diol **3b** (55.2 mg, 0.15 mmol) in 1:1 ethyl acetate and aqueous sodium hydrogen carbonate (0.6 mmol). After 2 h, the organic layer was washed with 5% aqueous sodium hydrogen carbonate and brine. The solution was dried over anhydrous sodium sulfate, the solvent was removed, and the crude product was crystallized from acetone−hexane. White solid; 41 mg (46%). ES-MS m/z 590 $[MH]^+$.

Inhibition of Recombinant HIV-Pr. IC_{50} values were determined at pH 5.5 using recombinant wild-type HIV-1 PR from Bachem and the fluorogenic substrate Abz-Thr-Ile-Nle-Phe(p-NO_2)-Gln-Arg-NH_2 (Abz-NF*-6; Bachem AG, Bubendorf, CH). Atazanavir (IC_{50} = 20 pM) was used in this assay as reference inhibitor for titration of the active enzyme. Full details are given in the Supporting Information.

Cellular Assays. The MT-2 cell line was obtained from Dr. Sylvie Liu, Imperial College, Faculty of Medicine, London, UK. Supernatants of H9/HTLV III B line obtained by Dr. R. Gallo (NIH, Bethesda, MD) were used as a source of HIV-1. The cells were chronically infected and produced a high titer HIV-1. The supernatants were collected and centrifuged to remove the cells, and virus stocks were prepared with known p24 antigen content (460 pg/mL, Murex HIV Antigen mAB test), RT activity (565.3 pg RT/mL, HS-Lenti RT Activity Kit, Cavidi, Sweden), and infectivity (2×10^6 infectious virions/mL, microtiter infection assay).[31] MT-2 and H9/HTLV III B cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum (Invitrogen). CC_{50} and MNC were detected by the MTT-uptake assay.[35] Antiviral activity was studied only on MT-2 cells by a microtiter infection assay, exploring the protection of cells from the cytopathic effect of HIV measured by the MTT test. Native HIV-1 protease[33] was obtained from a suspension of concentrated viral stock (50×) from chronically infected H9/HTLV IIIB cell supernatants. Endogenous RT activity of supernatants of HIV-1 infected/uninfected MT-2 cells treated/untreated with inhibitors was tested by HS-Lenti Kit-RT assay (Cavidi, Sweden). The RT activity after treatment with the inhibitors was expressed as % relative to the viral control (100%). Details are given in the Supporting Information.

Modeling. Lowest energy conformations for inhibitors **21−23** were obtained from a Monte Carlo conformational search with the MMFF force field[36] as implemented in Spartan '10 (Wave Function, Inc.)

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis and characterization of the inhibitors. Description of the recombinant HIV-PR inhibition assay. Average IC_{50} values and standard errors for inhibitors **17−26**. Detailed description of the antiviral activity and cytotoxicity assays. Description of the native protease inhibition assay. This material is available free of charge via the Internet at http://pubs.acs.org

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

DMPOA, 2,6-dimethylphenoxyacetic acid; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBT, hydroxybenzotriazole; LTBAH, lithium tri-*tert*-butoxyaluminium hydride; MNC, maximum nontoxic concentration; MOI, multiplicity of infection; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NMM, *N*-methylmorpholine; PR, protease; RT, reverse transcriptase

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