# Articles

## Aminodiol HIV Protease Inhibitors. 1. Design, Synthesis, and Preliminary SAR

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A series of HIV protease inhibitors containing a novel $C_2$ symmetrical "aminodiol" core structure were prepared from amino acid starting materials. The ability of the aminodiols to inhibit HIV replication in cell culture is comparable to their ability to inhibit the isolated enzyme, a result compatible with good cell membrane penetration by this class of compounds. Optimization of the structure–activity in this series led to aminodiol **9a** ($K_i$ = 100 nM; $ED_{50}$ (HIV-1) = 80 nM) containing $P_1/P_{1'}$ benzyl and $P_2/P_{2'}$ Boc substituents. Compound **9a** is a selective inhibitor of HIV protease versus other aspartyl proteases such as human renin, human cathepsin D, and porcine pepsin. In addition, **9a** is equipotent against HIV-1 and HIV-2 in cell culture and demonstrates similar activity in infected T-lymphocytes and PBMCs. After iv and oral administration in rats, **9a** displayed significant oral bioavailability (ca. 40%) and a promising plasma elimination half-life (4 h).

The acquired immunodeficiency syndrome (AIDS) pandemic has prompted an intensive effort to identify chemotherapeutics directed at its causative agent, the human immunodeficiency virus (HIV).[1] Among the possible targets for intervention, the virally encoded protease of the *pol* gene has attracted considerable attention due to its critical role in the production of mature infectious virus.[2] The identification of HIV protease as an aspartyl protease provided the impetus for the design of transition-state analog peptide mimetics based on previously applied pharmacophores.[3] Further demonstration that HIV protease is a $C_2$ symmetrical homodimer led to the rational design of potent and selective symmetrical inhibitors which complement the symmetry of the enzyme active site.[4] Herein, we disclose a series of HIV protease inhibitors which contain a novel $C_2$ symmetrical core.

Kempf et al.[4a] were first to report the synthesis of structure-based symmetrical inhibitors of HIV protease. The design of their inhibitors was based on placement of a $C_2$ axis of symmetry in the vicinity of a prototypical scissile peptide bond (e.g., -Phe-AA-; Figure 1). Deletion of the P′ region followed by a $C_2$ operation on the remaining P regon provided the symmetrical, chemically stable compounds monoalcohol **1** (axis *A*) and diol **2** (axis *B*). Direct application of the symmetry operation along axis *C*, however, would result in a chemically unstable entity, **3**. One possible remedy to this instability entails insertion of a methylene group between the nitrogen and the carbinol on each side of **3**, leading to the stable "aminodiol" **4**. A similar strategy led to the discovery of the hydroxyethylamine (HEA) pharmacophore for inhibition of angiotensin converting enzyme.[5] Potent HEA inhibitors of renin[6] as well as HIV protease[7] have since been reported. These considerations have prompted us to prepare inhibitors that incorporate the $C_2$ symmetrical aminodiol core unit.

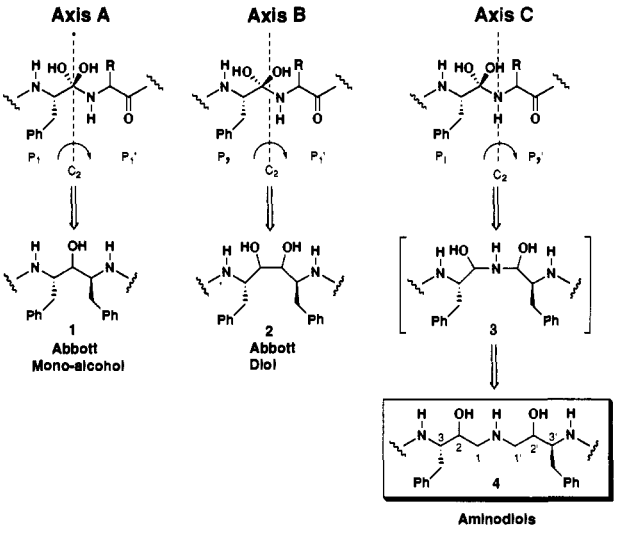


**Figure 1.** Design of $C_2$ symmetric HIV protease inhibitors.

Analogs containing **4** are readily accessible as shown in Scheme 1. Treatment of epoxides[8] **6a** or **6b** with $NH_3$/MeOH provides amino alcohols **7a** or **7b**, respectively. Reaction of **7a** and **7b** with **6a** or **6b**, respectively, gives the symmetrical aminodiols **9** and **10**. Symmetrical aminodiols **12** with terminal groups other than Boc or Cbz can be prepared by reaction of **6a** with the benzyl-protected amino alcohol **8** (prepared from epoxide **6a** and excess benzylamine) to give the *N*-benzylaminodiol **11**. Protected aminodiol **11** can also be prepared by reaction of 1 equiv of epoxide **6a** with 0.5 equiv of benzylamine. Removal of the Boc groups, formation of the desired carbamate (using

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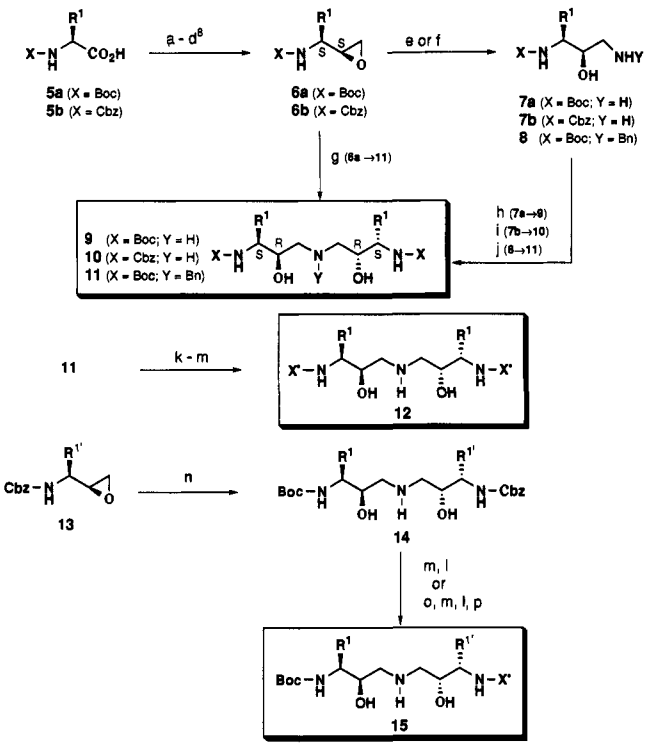
**Scheme 1**[a]




[a] (a) *i*-$BuO_2CCl$, 4-methylmorpholine, THF, then $CH_2N_2$, $Et_2O$, 0 °C; (b) HCl, dioxane, 0 °C; (c) $NaBH_4$ (3:1 diastereoselectivity); (d) KOH, EtOH, room temperature; (e) **6** to **7**, $NH_3$, MeOH; (f) **6a** to **8**, excess $BnNH_2$, DMF, 100 °C; (g) 0.5 equiv of $BnNH_2$, DMF, 100 °C; (h) **6a**, DMF, 100 °C (or MeOH, 50 °C); (i) **6b**, DMF, 100 °C; (j) **6a**, DMF, 100 °C (or MeOH, 50 °C); (k) HCl, dioxane, 0 °C; (l) +X′; see discussion; (m) $H_2$, $Pd(OH)_2/C$; (n) **7a**, DMF, 100 °C (or MeOH, 50 °C); (o) 2-(trimethylsilyl)ethyl chloroformate, *i*-$Pr_2NEt$, DMF, 0 °C, or 9-fluorenylmethyl chloroformate, *i*-$Pr_2NEt$, DMF, 0 °C; (p) *n*-$Bu_4NF$, THF, 50 °C, or piperidine, $CH_2Cl_2$, room temperature.

a chloroformate or (*p*-nitrophenyl)carbonate), urea (using an isocyanate), or amide (using a carboxylic acid and EDC/HOBT), and subsequent debenzylation gives **12**.

Synthesis of unsymmetrical aminodiols **15** proceeds from amino alcohol **7a** and epoxide **13**. Selective removal of the Cbz group and attachment of the X′ group[9] as described above provide **15**. Alternatively, the central amine can be protected prior to replacement of the Cbz group followed by deprotection.

Analogs designed to investigate simple modifications at $P_1/P_{1'}$ and $P_2/P_{2'}$ are shown in Table 1.[10] Activity against HIV protease is determined by inhibition of the cleavage of the peptidic substrate V-S-Q-N-(β-naphthylalanine)-P-I-V.[11] As observed with other classes of HIV protease inhibitors,[3] $P_1/P_{1'}$ aromatic groups (e.g., **9a**) are favored over saturated, branched aliphatic groups (**9b,c**). Replacement of one of the benzyl groups of **9a** with a phenethyl group (**15a**) results in a 3–4-fold loss in activity against the enzyme. However, 4-substitution of the aromatic ring by a polar group (**15b**) is tolerated, suggesting that the hydrophobic $S_1$ subsite can accommodate a hydroxyl group or that the *para* position of the $P_1$ aromatic ring may be accessible to solvent (as seen with other classes of HIV protease inhibitors[12]). Comparison of Boc (**9a**) and Cbz (**10a**) groups at $P_2/P_{2'}$ shows that a branched aliphatic group is preferred over an aromatic group. The optimal fit of the Boc groups was further demonstrated by evaluation of the bis-isopropyl carbamate **12a** and the bis-neopentyl carbamate **12b** which are 6-fold and 40-fold less potent than **9a**, respectively. Several modifications of the linker between the $P_1$ and $P_2$ groups were examined, including the thiocarbamate (**15c**), urea (**15d**), amide (**15e**), and *N*-methyl carbamate (**16**)[13] analogs, and in each case, the *O*-alkyl, N–H carbamate is favored.

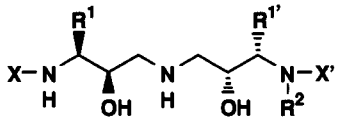
Extensive studies of HIV protease inhibitors containing the HEA isostere have shown that the stereochemical requirement at the hydroxyl center is dependent upon the peptide framework.[7b,d] In view of the unique structure of the aminodiols, we prepared the two alternative hydroxyl group diastereomers of **9a** (Figure 2) to determine the optimum configuration for this series of inhibitors. These compounds were synthesized from epoxide **6c**, the minor isomer formed in the conversion of *N*-Boc-L-phenylalanine to **6a** ($R^1$ = Bn). Inversion of one of the two hydroxyl groups to give the *S,R,S,S* isomer **9d** leads to a 40-fold decrease in activity against the enzyme, while the doubly-inverted *S,S,S,S* isomer **9e** displays no inhibition at 10 μM. Furthermore, the *S* configuration of the benzyl groups of **9a** is favored as demonstrated by the relatively poor activity of isomers **9f–h** (prepared from *N*-Boc-D-phenylalanine *via* the mixture of epoxides **6d**).

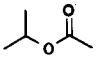
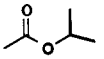
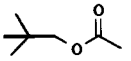
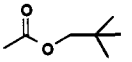
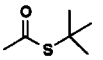
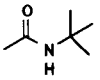
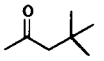
The novel structure of the aminodiol isostere prevents our inferring a binding mode from that determined for the diols **2** or from nonsymmetrical HEA-containing inhibitors such as Ro 31-8959[7c] or JG-365[7a]. In fact, the spacing between the $P_1$ and $P_{1'}$ groups is greater in **4** than in any other HIV protease inhibitor disclosed to date.[15] A clearer picture of the binding begins to emerge from the results of modifications of the aminodiol core. The importance of the central secondary amine was tested by the synthesis of the tertiary amine **18** and the ether **22** (Scheme 2). Preparation of **18** proceeded *via* the amino alcohol **17** by alkylation with the chloromethyl ketone derived from *N*-Boc-L-phenylalanine followed by reduction. The symmetrical ether analog **22** was prepared by reaction of the alkoxide derived from **20** with the corresponding triflate **21** and subsequent deprotection. Both **18** and **22** show little inhibition (<15%) of HIV protease at 10 μM, suggesting that the N–H of **9a** may be involved in a critical hydrogen-bond donor interaction with one of the catalytic aspartic acids.

Unlike most other reported inhibitors of HIV protease, compounds related to **9a** do not have two carbonyl groups optimally positioned to interact with the structural water molecule that binds inhibitors to the "flaps" of the enzyme dimer.[17,21] Assuming that one of the carbamate carbonyl groups binds to the structural water and that the central amine and one of the hydroxyl groups interact with the catalytic aspartic acids in analogy to other HEA inhibitors, we investigated the significance of the "second" hydroxyl group by the preparation of the methyl ether **24** and the deshydroxy analog **27** (Scheme 2). Synthesis of **24** proceeded *via* the amino ether **23** which in turn was prepared from **6a** ($R^1$ = Bn) by azide opening of the epoxide[22] and alkylation of the resulting alcohol. Amino alcohol **27** was prepared by reductive amination of the aldehyde **26** which was derived from the known[23] olefin **25**. Amino alcohol **27** is 20-fold less potent than **9a** against the protease, while the methyl ether **24** displays only 30% inhibition at 10 μM. In combination with the result for **9d** described above, these findings suggest that the second hydroxyl group plays a critical role in binding to the enzyme.[24]

Antiviral activity for the analogs in Table 1 was determined in CEM cells (T-lymphocytes) using the HIV-

**Table 1.** Structure–Activity at $P_1/P_{1'}$ and $P_2/P_{2'}$ Positions of Aminodiols



| compd | $R^1$ | $R^{1'}$ | X | X′ | $R^2$ | $IC_{50}$ (nM)[a] | $ED_{50}$ (nM)[b,c] |
|---|---|---|---|---|---|---|---|
| **9a** | Bn | Bn | Boc | Boc | H | 125[d] | 80[e] |
| **9b** |  |  | Boc | Boc | H | 800 | 660 |
| **9c** |  |  | Boc | Boc | H | 4500 | 5300 |
| **15a** | Bn |  | Boc | Boc | H | 500 | 690 |
| **15b** | Bn |  | Boc | Boc | H | 100 | 60[f] |
| **10a** | Bn | Bn | Cbz | Cbz | H | 3400 | >5000[g] |
| **12a** | Bn | Bn |  |  | H | 900 | 1000 |
| **12b** | Bn | Bn |  |  | H | 6000 | ND[h] |
| **15c** | Bn | Bn | Boc |  | H | 700 | 800 |
| **15d** | Bn | Bn | Boc |  | H | 400 | 160 |
| **15e** | Bn | Bn | Boc |  | H | 4000 | 1200 |
| **16** | Bn | Bn | Boc | Boc | Me | 8500 | >3000[g] |

[a] Concentration needed to inhibit cleavage of V-S-Q-N-(β-naphthylalanine)-P-I-V by 50%[11] (see ref 26 for comparison to standards). [b] Concentration needed to inhibit virus replication by 50% as determined by an XTT endpoint;[25] average of two determinations ($n = 2$) unless otherwise noted (see ref 26 for comparison to standards). [c] Unless indicated, not cytotoxic at antiviral concentrations. [d] $K_i$ = 100 nM. [e] $n$ = 45; $ED_{50}$ values ranged from 30 to 180 nM. [f] $n = 6$. [g] No inhibition at noncytotoxic concentrations. [h] Not determined.

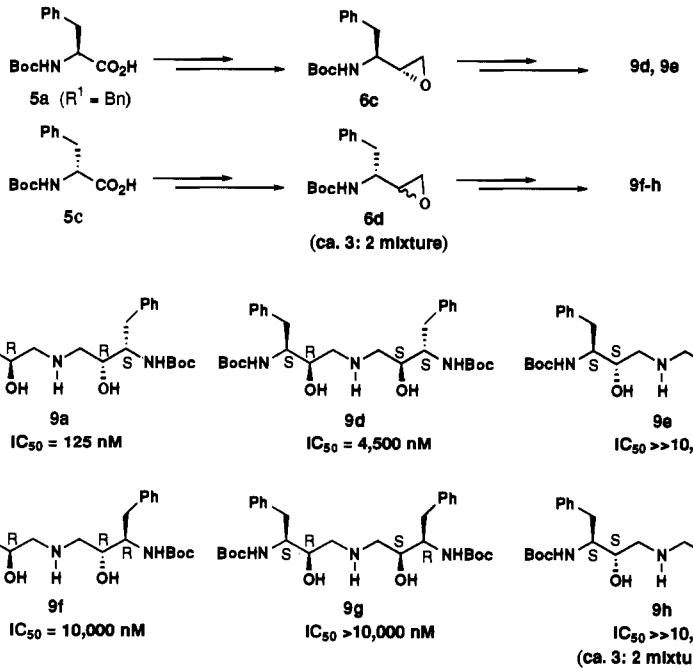
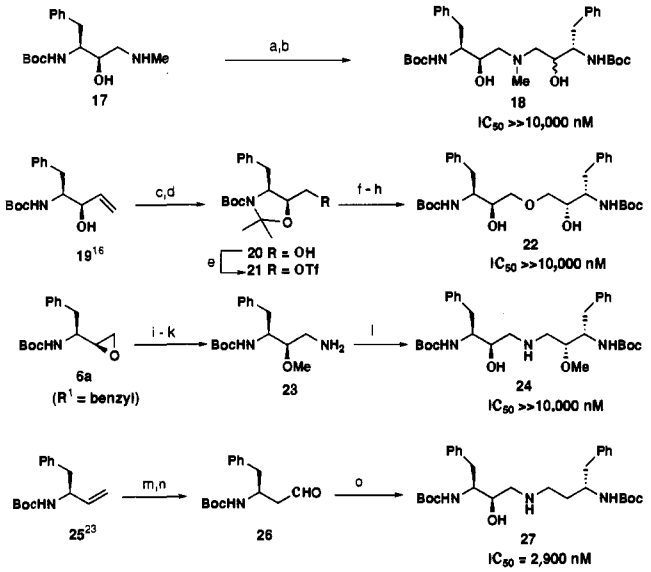



**Figure 2.** Inhibition of HIV protease by stereoisomers of **9a**.

$1_{RF}$ laboratory strain.[25] The ability of the aminodiols to inhibit HIV replication in cell culture is comparable to their ability to inhibit isolated HIV protease. Although, in general, $ED_{50}$ values are dependent on the assay conditions (e.g., duration of the assay, host cell, viral strain, and multiplicity of infection, etc.), this observation is

**Scheme 2.** Core Unit Modifications of **9a**[a]




[a] (a) Boc-phenylalanine chloromethyl ketone, $NaHCO_3$, NaI, DMF, room temperature; (b) $NaBH_4$, EtOH, $H_2O$, 0 °C; (c) $Me_2C(OMe)_2$, *p*-TsOH, PhH, reflux; (d) $O_3$, MeOH, −60 °C, then $NaBH_4$; (e) $Tf_2O$, pyr, $CH_2Cl_2$, 0 °C; (f) **20**, **21**, $NaN(TMS)_2$, THF, −20 °C; (g) 10% HCl, HOAc, THF, 50 °C; (h) $(Boc)_2O$, $Et_3N$, acetone, $H_2O$, room temperature; (i) $NaN_3$, $NH_4Cl$, MeOH, reflux,[22] (j) NaH, THF, then MeI, room temperature; (k) $H_2$, 10% Pd/C, MeOH, room temperature; (l) **6a** ($R^1$ = benzyl), DMF, 135 °C; (m) 9-BBN, THF, room temperature, then 3 N NaOH, 30% $H_2O_2$; (n) $(COCl)_2$, DMSO, $CH_2Cl_2$, −60 °C, then $Et_3N$; (o) $NaBH_3CN$, **7a** ($R^1$ = benzyl), HOAc, MeOH, room temperature.

unusual[26] and may be a reflection of good cell membrane penetration by the aminodiols.[28] Compound **9a** was chosen as a prototype for further biochemical and virological evaluation. Selectivity of >250-fold is observed for **9a** against HIV protease versus that of other aspartyl proteases such as human renin, human cathepsin D, and porcine pepsin (Table 2). In addition, **9a** has comparable activity against HIV-1 and HIV-2 in CEM cells as well as against $HIV\text{-}1_{IIIB}$ in peripheral blood mononuclear cells (PBMCs).

The attractive *in vitro* properties of **9a** prompted the evaluation of its pharmacokinetics in rats (Table 3). Upon iv administration, the elimination half-life of **9a** averaged 4 h, and after oral administration, the bioavailability averaged 40%. The peak plasma concentration of **9a** occurred at >6 h for all orally dosed animals, and a maximal plasma concentration ($C_{max}$) of 3 times the *in vitro* $ED_{50}$ was achieved at normalized oral doses of 10 mg/kg.

In summary, the aminodiols are a novel class of HIV protease inhibitors containing a $C_2$ symmetrical core which can easily be prepared from readily available starting materials. In general, the ability of the aminodiols to inhibit HIV replication in cell culture is comparable to their ability to inhibit the isolated enzyme, a result that may indicate good cell membrane permeability by this class of compounds. Optimization of the structure–activity in this series led to aminodiol **9a** containing $P_1/P_{1'}$ benzyl and $P_2/P_{2'}$ Boc substituents. Compound **9a** is a selective inhibitor of HIV protease versus other aspartyl proteases, is equipotent against HIV-1 and HIV-2 in cell culture, and demonstrates similar activity in infected T-lymphocytes and PBMCs. After iv and oral administration in rats, **9a** demonstrated significant oral bioavailability and a promising elimination half-life. Modifications directed at improving the potency of **9a** against the enzyme while maintaining its favorable *in vitro* and *in vivo* properties will be the subject of future reports.

## Experimental Section

**Biological Assays. HIV Protease Assay.** HIV-1 protease activity was determined by a peptide substrate cleavage assay. Protease products were analyzed on reverse-phase HPLC by a variation of the method of Heimbach et al.[11] The inhibitor (5 mM stock solution in DMSO) was diluted to assay concentrations with reaction buffer consisting of 50 mM sodium acetate and 1 mg/mL BSA, pH 5.5. A 20-μL aliquot of inhibitor solution was mixed with a 20-μL aliquot of peptide substrate $H_2N$-Val-Ser-Gln-Asn-(β-naphthylalanine)-Pro-Ile-Val-OH (final substrate concentration of 0.45 mM). The assay was initiated with purified enzyme diluted with reaction buffer to a final concentration of 1–10 nM. After 30 min at 37 °C, reactions were quenched with 150 μL of 5% aqueous phosphoric acid. Controls substituted the reaction buffer for test compounds. Inhibitor concentrations of 0.1, 1, and 10 μM were initially evaluated to aid in choosing the concentrations (five) of inhibitor used for determination of the $IC_{50}$ (concentration of compound which inhibits 50% cleavage of substrate).

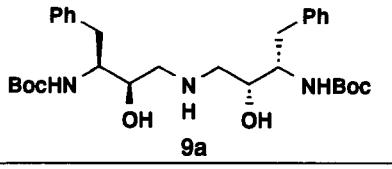
The $K_i$ determination of **9a** was done by measuring the rate of substrate to product conversion with varying amounts of inhibitor. A final substrate concentration of 0.284 mM was used with a final enzyme concentration of 1–10 nM. Six inhibitor concentrations were assayed in a 4-fold range around the $IC_{50}$ value. Product was measured at three time intervals. The rate of product formed with no inhibitor ($V_o$) was divided by the rates of conversion with inhibitor ($V_i$). These values were graphed $V_o/V_i$ versus inhibitor concentration, and $[I]/K_i = V_o/V_i - 1$ was used to determine the $K_i$.

HIV-1 protease was purified by the method of Louis et al.[29] *Escherichia coli* containing the expressed protease–maltose-binding protein fusion was resuspended in 50 mL of lysis buffer (50 mM Tris/HCl, pH 6.5, 30 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 0.5% Nonidet P-40, and 1 mM toluenesulfonyl fluoride) and sonicated. The suspension was clarified, diluted 4-fold with column loading buffer (10 mM sodium phosphate, pH 6.5, 30 mM NaCl, 0.25% Tween-20, and 1 mM dithiothreitol) and loaded onto an amylose resin column (New England Biolabs). The column was first rinsed with column loading buffer and then wash buffer (10 mM sodium phosphate, pH 6.5, 0.5 M NaCl, 1 mM dithiothreitol, and 1 mM EDTA). Fusion protein was eluted with wash buffer containing 10 mM maltose. All fractions containing over 100 μg/mL protein were pooled and diluted 1:1 with 10 M urea in phosphate buffer (100 mM sodium phosphate, pH 6.5, 60 mM NaCl, 20 mM dithiothreitol, and 2 mM EDTA). The protein was dialyzed against 1 L of decreasing amounts of urea (4, 2, 1, 0.5, and 0.25 M) in phosphate buffer for 45–60 min. Dialysis was then done against a phosphate buffer containing 25 mM sodium phosphate, pH 6.5, 1 mM dithiothreitol, and 1 mM EDTA for 1 h and then against 2 L of 50 mM MES, pH 6.5, 1 mM EDTA, 1 mM dithiothreitol, and 5% glycerol (buffer A) for 3–4-h. Enzyme activity was verified by the peptide substrate cleavage assay. Protease was separated from maltose-binding protein on a Mono-S (Pharmacia) column using a gradient program of 0–1 M NaCl in buffer A. Protease eluted at 0.5 NaCl and was 99% pure.

**Human Renin Assay.** The assay was performed as described in ref 30.

**Human Cathepsin D and Porcine Pepsin Assays.**[31] Inhibition of cathepsin D (human liver; Calbiochem, #219401) and pepsin (porcine stomach mucosa; Sigma, P6887) was measured by a spectrophotometric assay. The assay consisted of 1.6 mL of reaction buffer (0.1 M formate, pH 3), a 10-μL aliquot of peptide substrate H-Pro-Thr-Glu-Phe-*p*-nitro-Phe-Arg-Leu-OH (Protogen, #03-30-0025; final concentration 0.32 mM), and a 10-μL aliquot of **9a** diluted with reaction buffer for assay test concentrations. Substrate and inhibitor were added to buffer already in a cuvette in the cell holder of a spectrophotometer that was maintained at 37 °C. Control reactions substituted the reaction buffer in place of **9a**. Assays were started by the addition of enzyme, 0.36 units of cathepsin D and 0.03 units for pepsin. Absorbance was measured at 310 nm for 20 min.

Table 2. Enzyme Inhibition and Antiviral Profile of 9a




9a

| IC50 (nM) | | | | ED50 (nM) | | |
|---|---|---|---|---|---|---|
| HIV-Pr[a] | H-renin[b] | H-cathepsin D[c] | P-pepsin[d] | HIV-$1_{RF}$[e] | HIV-$2_{CBL-20}$[f] | HIV-$1_{IIIB}$[g] |
| 125 | >100 000 | ≫31 000 | ≫31 000 | 80 | 170 | 25 |

[a] $K_i$ = 100 nM. [b] 15% inhibition of human renin[30] at 100 000 nM. [c] No inhibition of human cathepsin D[31] at the highest concentration tested (31 000 nM). [d] No inhibition of porcine pepsin[31] at the highest concentration tested (31 000 nM). [e] $n$ = 45; $ED_{50}$ values ranged from 30 to 180 nM. [f] $n$ = 7 (see the Experimental Section). [g] Determined in PBMCs (see the Experimental Section); $n$ = 2.

Table 3. Pharmacokinetic Data for 9a in Rats

| parameter[a] | units | intravenous | oral |
|---|---|---|---|
| half-life ($t_{1/2}$) | hr | 4.2 ± 0.2[b] | - - |
| $C_{max}$ | nM | - - | 240 ± 67[c] |
| oral bioavailability | % | - - | 40 ± 8[d] |

[a] Mean ± SEM ($n$ = 5); pharmacokinetic data from three rats given [3H]-9a (0.7 mg/kg iv and po) and two rats given nonradiolabeled 9a (5 mg/kg iv and 12 mg/kg po) were combined (see experimental for dosing and assay details). [b] On the basis of 6- to 24-h data. [c] Values for $C_{max}$ are normalized to a 10 mg/kg dose. [d] On the basis of the individual dose-normalized $AUC_{0\infty}$ values after each oral dose relative to the *average* $AUC_{0\infty}$ value for the iv route.

Table 4. Physical Data for HIV Protease Inhibitors

| compd | mp (°C) | [α]D | formula[a] | anal. |
|---|---|---|---|---|
| 9a | 178–80 | -7.1° (c 0.1, MeOH) | $C_{30}H_{45}N_3O_6 \cdot 0.8H_2O$ | C,H,N |
| 9b | 94–95 | -33.7° (c 0.18, MeOH) | $C_{30}H_{57}N_3O_6$ | HRMS[b] |
| 9c | oil | +8.0° (c 0.1, MeOH) | $C_{24}H_{49}N_3O_6$ | HRMS[b] |
| 9d | 151–53 | -22.9° (c 0.23, MeOH) | $C_{30}H_{45}N_3O_6 \cdot 0.67H_2O$ | C,H,N |
| 9e | 140–41 | -42.4° (c 0.29, MeOH) | $C_{30}H_{45}N_3O_6 \cdot 1.45H_2O$ | C,H,N |
| 9f | 158–60 | +14.9° (c 0.35, MeOH) | $C_{30}H_{45}N_3O_6 \cdot 0.67H_2O$ | C,H,N |
| 9g | 200–01 | +6.0° (c 0.1, DMSO) | $C_{30}H_{45}N_3O_6 \cdot 0.32H_2O$ | C,H,N |
| 9h | 156–59 | ND[c] | $C_{30}H_{45}N_3O_6 \cdot 0.16H_2O$ | C,H,N |
| 10a | 167–71 | -27.7° (c 0.13, HOAc) | $C_{36}H_{41}N_3O_6 \cdot 1.43H_2O$ | C,H,N |
| 12a | 195–200 | -13.2° (c 0.09, MeOH) | $C_{28}H_{41}N_3O_6 \cdot 0.63H_2O$ | C,H,N |
| 12b | 152–54 | -12.5° (c 0.1, MeOH) | $C_{32}H_{49}N_3O_6$ | HRMS[b] |
| 15a | 145–48 | -6.0° (c 0.3, MeOH) | $C_{31}H_{47}N_3O_6 \cdot 0.44H_2O$ | C,H,N |
| 15b | 151–53 | -1.5° (c 0.2, $CHCl_3$) | $C_{30}H_{45}N_3O_7 \cdot 0.70H_2O$ | C,H,N |
| 15c | 140–41 | -20.0° (c 0.05, MeOH) | $C_{30}H_{45}N_3O_5S$ | HRMS[b] |
| 15d | 110–12 | -3.5° (c 0.2, MeOH) | $C_{30}H_{46}N_4O_5 \cdot 1.39H_2O$ | C,H,N |
| 15e | 153–56 | -3.3° (c 0.1, MeOH) | $C_{31}H_{47}N_3O_5 \cdot 0.54H_2O$ | C,H,N |
| 16 | foam | -19.6° (c 0.6, MeOH) | $C_{31}H_{47}N_3O_6 \cdot 0.33H_2O$ | C,H,N |
| 18 | 62–70 | ND[c] | $C_{31}H_{47}N_3O_6 \cdot 0.18H_2O$ | C,H,N |
| 22 | 158–60 | +1.9° (c 0.16, MeOH) | $C_{30}H_{44}N_2O_7$ | C,H,N |
| 24 | foam | -3.9° (c 0.67, MeOH) | $C_{31}H_{47}N_3O_6 \cdot 0.78H_2O$ | C,H,N |
| 27 | 95–100 | +3.0° (c 0.33, MeOH) | $C_{30}H_{45}N_3O_5 \cdot 1.40H_2O$ | C,H,N |

[a] Water content was not experimentally determined. [b] High-resolution mass spectrum determined due to compound availability. [c] Not determined.

**Cell Culture Activity against HIV-$1_{RF}$ and HIV-$2_{CBL-20}$ in CEM Cells.**[25] Suspensions of CEM-SS cells (5000/well) in a growth medium (RPMI without phenol red, 10% fetal calf serum, 10 mM HEPES buffer, 2 mM L-glutamine, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin) were infected with the virus (HIV-$1_{RF}$ or HIV-$2_{CBL-20}$) at a multiplicity of infection of 0.04 in a 96-well plate. Inhibitors were dissolved in DMSO and then diluted to the appropriate concentration with growth medium (maximum 0.125% final DMSO concentration). Following incubation for 6 days at 37 °C, 5% $CO_2$ and XTT/PMS solution were added to each well. Plates were reincubated for 4 h at 37 °C to allow the PMS-coupled reduction of the pale yellow tetraazolium reagent XTT to its soluble orange formazan product. Cell viability was quantified by the visable-light absorbance at 450 nm and a reference wavelength at 650 nm. Data were expressed as a percentage of formazan produced in drug test wells compared to formazan produced in wells of untreated control cells. The $ED_{50}$ value was calculated as the concentration of drug that increases the percentage of formazan production in virally infected cells to 50% of that produced by control cells. At least one standard was used in each assay; standards included AZT, DDI, and the HIV protease inhibitors listed in ref 26.

**Cell Culture Activity against HIV-$1_{IIIB}$ in PBMCs.**[28] PHA-stimulated PBMCs were infected with viruses at a m.o.i. of 0.001–0.02. Infected PBMCs were incubated in the presence of varying concentrations of **9a**. Three days after infection, half of the volume of each well was removed and replaced with fresh medium containing compound. Levels of p24 were determined in culture supernatants 7 days after infection by p24 ELISA. The $ED_{50}$ values were calculated as the dose of **9a** that resulted in a 50% reduction in p24 levels as compared to those in control wells.

**Rat Study.** The *in vivo* dispostion of **9a** was evalulated in male Sprague–Dawley rats (200–350 g; Harlan Industries, Indianapolis, IN). Rats were instrumented with an indwelling jugular vein cannula on the day before dosing.[32] The rats were fasted overnight, and water was provided *ad libitum* throughout the experiment. Intravenous doses of **9a** were administered as an ethanol solution, and about 0.3 mL was infused over a 5-min period. Oral doses were administered by gavage and were dosed as either suspensions in 0.2% carboxymethyl cellulose or solutions in ethanol. Blood samples (0.5 mL) were collected into heparinized syringes and immediately centrifuged to obtain plasma.

Intravenous and oral doses of [3H]-**9a**[33] (specific activity 0.6 Ci/mmol; 1.3 μmol/kg) were administered ($n$ = 3 rats for each route), and plasma samples were analyzed for intact **9a** by a specific thin-layer radiochromatographic (TLRC) assay, described in detail below. In addition, iv and oral doses of nonradiolabeled **9a** (9 μmol/kg (iv) and 22 μmol/kg (oral)) were administered ($n$ = 2 rats for each route), and plasma samples were analyzed by a selective liquid chromatography/mass spectormetry/mass spectrometry (LC/MS/MS) assay, described below.

Plasma proteins were precipitated by mixing with 2 volumes of ethanol, and the supernatant was transferred and evaporated to dryness. For the samples involving LC/MS/MS analysis, an internal standard was used. For plasma samples containing [3H]-**9a**, the reconstituted samples were spotted onto TLC plates (Silica Gel GF; Analtech, Newark, DE) and developed with $CH_2Cl_2$:$CH_3OH$:$NH_4OH$ (87:12:1). Nonradiolabeled **9a** was also spotted and used as a reference standard ($R_f \approx 0.6$). The radioactivity corresponding to **9a** was removed from the plate and quantitated by liquid scintillation counting. For analysis of plasma samples containing nonradiolabeled **9a**, the reconstituted samples were applied to a reverse-phase HPLC column (Partisil, octadecasilyl; Keystone Scientific, Bellefonte, PA) and eluted with 50% $CH_3CN$ in water (containing 0.05% TFA). The eluate was split 20:1 and analyzed by multiple-reaction monitoring with a SCIEX API*III* mass spectrometer (PE Sciex, Thornhill, Ontario, Canada) equipped with an ionspray interface. On each day of analysis, standard curves were prepared with either peak area ratios (LC/MS/MS) or DPM (TLRC). The lowest limits of quantitation were 10 nM (LC/MS/MS) and 1 nM (TLRC).

Pharmacokinetic parameters were calculated with standard model-independent methods.[34] Areas under the curve (AUC) were calculated using either Lagrange integration (iv) or the trapezoidal rule (oral) up to 24 h after dosing and were extrapolated to infinity with the iv half-life.

**Chemistry. General Experimental.** Nuclear magnetic resonance ($^1H$ and $^{13}C$ NMR) spectra were obtained on a GE QE300, JEOL GX-270, or GSX-400 spectrometer with tetram-

ethylsilane as an internal reference, unless otherwise specified. Chemical shifts are expressed in δ units (parts per million). Chemical ionization mass spectra were obtained on a Finnigan TSQ-4600 mass spectrometer. High- and low-resolution FAB mass spectra were obtained on either a JEOL HX-110 or SX-102 mass spectrometer. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter spectrometer. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Flash chromatography was performed on silica gel (Merck silica gel 60, 230–400 mesh). TLCs were run on Merck silica gel 60 F254 plates.

**General Method for the Preparation of Epoxides 6.**[8] To a solution of *N*-Boc-L-phenylalanine (14.34 g, 54.05 mmol) in dry THF (80 mL) cooled at −20 to −25 °C was added isobutyl chloroformate (7.01 mL, 54.05 mmol) over 5 min followed by 4-methylmorpholine (5.94 mL, 54.05 mmol), and the mixture was stirred for 20 min. The white precipitate was removed by filtration under argon atmosphere and washed with ca. 70 mL of dry THF. The combined THF solution of mixed anhydride was cooled to −5 °C and poured into diazomethane in $Et_2O$ (prepared from 1-methyl-3-nitro-1-nitrosoguanidine (23.85 g, 162.2 mmol), 40% KOH (75 mL), and $Et_2O$ (255 mL)) cooled to 0 °C. The resulting solution was kept at 0 °C for 2 h and then at room temperature overnight. $N_2$ was then bubbled through the light yellow solution for 30 min, and $Et_2O$ (400 mL) was added. The solution was washed with $H_2O$, saturated $NaHCO_3$, and brine and dried ($MgSO_4$). Concentration *in vacuo* afforded a yellow residue, which was triturated with hexane to give, after drying over $P_2O_5$ overnight under high vacuum, 14.72 g (94%) of the α-diazo ketone as a pale yellow solid. This material was used immediately in the next step without further purification.

To a solution of the crude α-diazo ketone prepared above (14.72 g, 50.87 mmol) in dry $Et_2O$ (500 mL) at 0 °C was added, dropwise, a solution of 4 N HCl in dioxane (12.72 mL, 50.87 mmol) while maintaining the temperature below 5 °C. The reaction mixture was then stirred at 0 °C for 1 h. TLC (hexane–EtOAc 4:1) showed that trace amounts of the starting α-diazo ketone remained. Additional 4 N HCl in dioxane (636 μL, 0.05 equiv, 2.54 mmol) was added, and the mixture was stirred at 0 °C for 1 additional hour. Concentration *in vacuo* gave a residue which was dissolved in hot $Et_2O$ (60 mL). Hexane (200 mL) was slowly added and the mixture allowed to stand for 2 h at 5 °C. The solid was filtered and dried over $P_2O_5$ under high vacuum to afford 9.58 g (first crop) of the α-chloro ketone. The filtrate was concentrated to dryness, and the residue was again recrystallized from $Et_2O$–hexane to give an additional 4.41 g (second crop) of the above α-chloro ketone. Total yield: 13.99 g (92%).

$NaBH_4$ (1.59 g; 42 mmol) was added to a solution of the α-chloro ketone prepared above (5 g, 16.8 mmol) in 84 mL of THF and 9 mL of $H_2O$ at 0 °C. After stirring at 0 °C for 45 min, the reaction mixture was concentrated to dryness. The residue was stirred at 0 °C with EtOAc (150 mL) and $H_2O$ (25 mL) while saturated $KHSO_3$ solution was carefully added until the pH was ~1.5. This mixture was then diluted with 350 mL of EtOAc, and the layers were separated. The organic layer was washed with $H_2O$ and brine, dried over $MgSO_4$, and concentrated to a white solid. A portion of this solid (4.89 g) was recrystallized from 70 mL of hot EtOAc to afford 2.47 g (50%) of the (*S*)-chlorohydrin as a white solid containing <5% of its diastereomer.

KOH (0.71 M) in EtOH (14.7 mL, 10.4 mmol) was added to a suspension of the chlorohydrin (2.6 g, 8.67 mmol) in 87 mL of EtOH at room temperature. The reaction mixture was stirred for 1.5 h at room temperature, at which time the EtOH was removed *in vacuo* and the residue was partitioned between EtOAc and $H_2O$. The organic layer was washed with saturated $NH_4Cl$ solution, $H_2O$, and brine and dried over $MgSO_4$ and the solvent removed *in vacuo*. The resulting solid white residue was recrystallized by dissolving in 10 mL of refluxing EtOAc and adding 190 mL of hexane. The resulting crystalline suspension was cooled to −40 °C and allowed to stand overnight. Filtration, rinsing with hexane, and drying under high vacuum for 2 h afforded 1.92 g (84%) of **6a** ($R^1$ = Bn) as a colorless crystalline solid. This material was 99.1% diastereomerically pure by HPLC. $^1H$ NMR ($CDCl_3$): δ 1.31 (s, 9H), 2.68–2.74 (m, 2H), 2.76–2.93 (m, 3H), 3.63 (m, 1H), 4.37 (m, 1H), 7.14–7.26 (m, 5H).

**6a ($R^1$ = $CH_2$cyclohexyl).** $^1H$ NMR ($CDCl_3$): δ 0.85 (m, 1H), 0.95 (m, 1H), 1.10–1.39 (m, 6H), 1.41 (s, 9H), 1.58–1.85 (m, 5H), 2.75 (m, 2H), 2.82 (m, 1H), 3.55 (br s, 1H), 4.38 (br s, 1H). 93% diastereomerically pure by $^1H$ NMR.

**6a ($R^1$ = (*2R*)-methylpropyl).** $^1H$ NMR ($CDCl_3$): δ 0.94 (t, *J* = 7 Hz, 3H), 1.01 (d, *J* = 7 Hz, 3H), 1.15 (m, 1H), 1.45 (s, 9H), 1.55–1.72 (m, 2H), 2.70 (m, 2H), 2.87 (m, 1H), 3.28 (br s, 1H), 4.47 (br s, 1H). 98% diastereomerically pure by HPLC.

**6b ($R^1$ =Bn).** $^1H$ NMR ($CDCl_3$): δ 2.79 (m, 2H), 2.93 (m, 2H), 3.00 (dd, *J* = 4.5, 13.5 Hz, 1H), 3.76 (m, 1H), 4.68 (m, 1H), 5.40 (s, 2H), 7.19–7.37 (m, 10H). ≥99.4% diastereomerically pure by HPLC.

**6b ($R^1$ = phenethyl).** $^1H$ NMR ($CDCl_3$): δ 1.78 (m, 1H), 1.98 (m, 1H), 2.75 (m, 4H), 2.95 (br s, 1H), 3.56 (m, 1H), 4.70 (br s, 1H), 5.10 (s, 2H), 7.05–7.40 (m, 10H). 98.5% diastereomerically pure by HPLC.

**6b ($R^1$ = $CH_2$-(*p*-*O*-benzyl)phenyl).** $^1H$ NMR ($CDCl_3$): δ 2.65–2.90 (m, 5H), 3.71 (m, 1H), 4.68 (br s, 1H), 5.03 (s, 2H), 5.04 (s, 2H), 6.91 (d, *J* = 8.5 Hz, 2H), 7.11 (d, *J* = 8.5 Hz, 2H), 7.25–7.44 (m, 10H). ≥97% diastereomerically pure by HPLC.

**6c**: prepared by an analogous procedure to that described for **6a** ($R^1$ = Bn) except that the minor chlorohydrin diastereomer was isolated and treated with base. $^1H$ NMR ($CDCl_3$): δ 1.39 (s, 9H), 2.58 (m, 1H), 2.69 (m, 1H), 2.80–3.02 (m, 3H), 4.13 (m, 1H), 4.51 (m, 1H, NH), 7.21–7.33 (m, 5H). >99% diastereomerically pure by HPLC.

**6d**: prepared as a 3:2 mixture of isomers by an analogous procedure to that described for **6a** ($R^1$ = Bn) except that *N*-Boc-D-phenylalanine was used and the chlorohydrin diastereomers were not separated before conversion to the epoxides.

**General Methods for the Preparation of Aminodiols 9–12 and 14. Preparation of [(*S*,*R*)-3-[[(*R*,*S*)-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-hydroxy-4-phenylbutyl]amino]-2-hydroxy-1-(phenylmethyl)propyl]carbamic Acid, 1,1-Dimethylethyl Ester (9a).** Method A (0.5 equiv of benzylamine). A DMF (2.5 mL) solution of **6a** ($R^1$ = Bn) (1.97 g, 7.48 mmol) and benzylamine (0.41 mL, 3.74 mmol) was heated under argon for 7 h at 105 °C, and then stirred overnight at room temperature. After heating for an additional 1.5 h at 105 °C, the volatiles were evaporated *in vacuo*. The resulting residue was coevaporated twice with MeOH/$CH_2Cl_2$ and purified by flash column chromatography ($SiO_2$; graident from 0.5% to 10% MeOH:$CH_2Cl_2$) to give **11** ($R^1$ = Bn) (1.39 g, 56%) as a colorless solid. $^1H$ NMR ($CD_3OD$): δ 1.27 (1.14 rotamer) (s, 18H), 2.30–2.61 (m, 6H), 2.98–3.04 (m, 2H), 3.45–3.82 (m, 6H), 7.05–7.37 (m, 15H).

To an EtOH (5 mL) solution of **11** ($R^1$ = Bn) (90 mg, 0.142 mmol) and cyclohexene (2.5 mL) at room temperature was added $Pd(OH)_2$ (20% on carbon; 41 mg). The reaction mixture was refluxed at 90 °C for 1 h and filtered hot through Celite, and the volatiles were removed *in vacuo* to leave a colorless solid (74 mg). The residue was purified by flash chromatography ($SiO_2$; gradient from 0.5% to 9% MeOH:$CH_2Cl_2$) to give **9a** (53 mg, 69%) as a colorless solid. $^1H$ NMR ($CD_3OD$, 45 °C): δ 1.30 (s, 18H), 2.56–2.68 (m, 2H), 2.72 (dd, *J* = 8, 12 Hz, 2H), 2.79 (dd, *J* = 3, 12 Hz, 2H), 3.08 (dd, *J* = 3, 14 Hz, 2H), 3.61–3.66 (m, 2H), 3.67–3.75 (m, 2H), 7.13–7.29 (m, 10H). $^{13}C$ NMR ($CD_3OD$): δ 28.7, 37.9, 52.9, 56.8, 72.9, 80.0, 127.1, 129.2, 130.5, 140.2, 158.1. Anal. ($C_{30}H_{45}N_3O_6$) C, H, N.

Method B (*via* **8**). To a solution of epoxide **6a** ($R^1$ = Bn) (5.0 g, 19.0 mmol) and benzylamine (10.5 mL, 96.0 mmol) in 22 mL of $CH_3CN$ was added $LiClO_4$ (2.0 g, 19.0 mmol) in one portion, and the resulting mixture was stirred at room temperature for 22 h. $H_2O$ (250 mL) was added and the resulting white solid filtered, washed with $H_2O$ and hexane, and dried under vacuum to afford 6.74 g (96%) of **8** ($R^1$ = Bn). $^1H$ NMR ($CD_3OD$): δ 1.26 (1.14 rotamer) (s, 9H), 2.40–2.58 (m, 2 H), 2.76 (m, 1 H), 3.10 (m, 1 H), 3.55–3.90 (m, 4 H), 7.10–7.45 (m, 10 H).

A mixture of **8** ($R^1$ = Bn) (200 mg, 0.54 mmol) and **6a** ($R^1$ = Bn) (149 mg, 0.56 mmol) in 0.55 mL of DMF was heated to 105 °C for 20 h. After cooling to room temperature, the reaction mixture was partitioned between EtOAc and $H_2O$. The organic layer was washed with $H_2O$ and brine. After drying ($MgSO_4$), the organic layer was concentrated to a residue which was purified by flash column chromatography ($SiO_2$; gradient from 1% to 3% MeOH:$CH_2Cl_2$) to afford 254 mg (74%) of **11** ($R^1$ = Bn).

Method C. **Preparation of (*R*-(*R**,*S**))-[Iminobis[2-hydroxy-1-(phenylmethyl)-3,1-propanldiyl]]bis[carbamic acid], 1,1-Dimethylethyl Phenylmethyl Ester (14 ($R^1$,$R^{1'}$ = Bn)).** Epoxide **6a** ($R^1$ = Bn) (15.0 g, 56.96 mmol) dissolved in 350 mL of EtOH was added, with stirring, over 1 h to 350 mL of concentrated $NH_4OH$ at 0 °C. $NH_3$ gas was bubbled through the reaction mixture during the addition and for 1 h after. The reaction was then warmed to room temperature and stirred overnight. The resulting slurry was diluted with 800 mL of EtOAc and the organic layer washed repeatedly with brine. The organic extracts were dried ($MgSO_4$) and concentrated to give a white solid which was triturated with 10% *i*-PrOH/EtOAc to give 4.37 g of **7a** ($R^1$ = Bn). The mother liquors were evaporated and triturated again as above to give an additional 5.73 g of **7a** ($R^1$ = Bn). Total yield: 10.1 g (63%). Mp 172–73 °C. $^1$H NMR ($CD_3OD$): δ 1.29 (s, 9H), 2.55 (dd, *J* = 10.5, 13.5 Hz, 1H), 2.63 (dd, *J* = 7, 13.5 Hz, 1H), 2.76 (dd, *J* = 3, 13.5 Hz, 1H), 3.11 (dd, *J* = 3, 13.5 Hz, 1H), 3.40 (m, 1H), 3.65 (m, 1H), 7.10–7.30 (m, 5H).

To a solution of lithium perchlorate (16.4 g, 0.155 mol) in 500 mL of $CH_3CN$ was added **7a** ($R^1$ = Bn) (43.5 g, 0.155 mol) as a solid followed by **6b** ($R^1$ = Bn) (41.2 g, 0.147 mol) in 150 mL $CH_3CN$. The resulting mixture was stirred at 30 °C for 2 h and at 40 °C for 30 h at which point it was cooled to room temperature and then added to 1.5 L of $H_2O$. The resulting precipitate was filtered and triturated with *i*-PrOH followed by 1:1 EtOAc:*i*-PrOH (2×) to give 42.1 g (49%) of 14 ($R^1$, $R^{1'}$ = Bn) as a white solid. Mp 169–73 °C. $^1$H NMR ($CD_3OD$): δ 1.29 (s, 9H), 2.59 (m, 2H), 2.70 (m, 4H), 3.10 (m, 2H), 3.64 (m, 3H), 3.73 (m, 1H), 4.92 (m, 2H), 7.10–7.32 (m, 15H). $^{13}$C NMR ($CD_3OD$): δ 28.8, 37.8, 38.0, 53.0, 56.9, 57.6, 67.2, 73.0, 73.1, 80.0, 127.1, 127.2, 128.5, 128.8, 129.2, 129.3, 129.5, 130.4, 130.5, 138.5, 140.2, 140.3, 158.1, 158.6 (1 aliphatic carbon unresolved).

**Preparation of (*R,S*)-[Iminobis[1-(cyclohexylmethyl)-2-hydroxy-3,1-propanediyl]]bis[carbamic acid], Bis(1,1-dimethylethyl ester) (9b):** from **6a** ($R^1$ = $CH_2$cyclohexyl) (method A). $^1$H NMR ($CDCl_3$): δ 0.81 (m, 2H), 0.97 (m, 2H), 1.05–1.60 (m, 12H), 1.44 (s, 18H), 1.55–1.76 (m, 8H), 1.83 (m, 2H), 2.60–2.80 (m, 4H), 3.00 (br s, 4H), 3.58 (m, 2H), 4.66 (d, *J* = 9.0 Hz, 2H). $^{13}$C NMR ($CDCl_3$): δ 26.1, 26.5, 28.4, 32.3, 34.1, 38.1, 51.0, 51.6, 72.7, 79.5, 156.4. Accurate mass measurement $(M + H)^+$ calcd for $C_{30}H_{58}N_3O_6$: 556.4325. Found: 556.4302 ($\Delta_{ppm}$ = 4.1).

**Preparation of (*R,S*)-[Iminobis[2-hydroxy-1-(1-methylpropyl)-3,1-propanediyl]]bis[carbamic acid], 1,1-Dimethylethyl Ester (9c):** from **6a** ($R^1$ = (2*R*)-methylpropyl) (method B). $^1$H NMR ($CD_3OD$): δ 0.95 (m, 12H), 1.02 (m, 2H), 1.44 (s, 18H), 1.55 (m, 2H), 1.80 (m, 2H), 2.71 (dd, *J* = 8.0, 12.0 Hz, 4H), 3.41 (m, 2H), 3.74 (m, 2H). $^{13}$C NMR ($CD_3OD$): δ 12.1, 16.7, 24.1, 28.8, 36.1, 52.8, 59.6, 69.6, 80.1, 158.9. Accurate mass measurement $(M + H)^+$ calcd for $C_{24}H_{50}N_3O_6$: 476.3696. Found: 476.3699 ($\Delta_{ppm}$ = 0.6).

**Preparation of [(*S,S*)-3-[[(*R,S*)-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-hydroxy-4-phenylbutyl]amino]-2-hydroxy-1-(phenylmethyl)propyl]carbamic Acid, 1,1-Dimethylethyl Ester (9d):** from **8** ($R^1$ = Bn) and **6c** (method B). $^1$H NMR ($CD_3OD$): δ 1.23 (1.17 rotamer) (s, 9H), 1.34 (1.24 rotamer) (s, 9H), 2.41–2.92 (m, 6H), 3.10–3.12 (m, 2H), 3.55–3.68 (m, 2H), 3.70–3.80 (m, 2H), 7.13–7.29 (m, 10H). $^{13}$C NMR ($CD_3OD$): δ 28.7 (28.4 rotamer), 37.8, 53.4, 56.0, 56.9, 71.4, 73.3, 80.0, 80.2, 127.1, 127.2, 129.2, 129.3, 130.3, 130.4, 140.0, 140.2, 158.1, 158.2 (3 aliphatic carbons unresolved). Anal. ($C_{30}H_{45}N_3O_6$) C, H, N.

**Preparation of [(*S,S*)-3-[[(*S,S*)-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-hydroxy-4-phenylbutyl]amino]-2-hydroxy-1-(phenylmethyl)propyl]carbamic Acid, 1,1-Dimethylethyl Ester (9e):** from **6c** (method A). $^1$H NMR ($CD_3OD$): δ 1.34 (1.22 rotamer) (s, 18H), 2.61–2.79 (m, 6H), 2.83–2.89 (m, 2H), 3.68–3.80 (m, 4H), 7.13–7.29 (m, 10H). $^{13}$C NMR ($CD_3OD$): δ 28.7 (28.4 rotamer), 38.8, 53.0, 56.0, 70.9, 80.2, 127.2, 129.3, 130.4, 140.0, 158.2. Anal. ($C_{30}H_{45}N_3O_6$) C, H, N.

**Preparation of [(*R,R*)-3-[[(*R,S*)-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-hydroxy-4-phenylbutyl]amino]-2-hydroxy-1-(phenylmethyl)propyl]carbamic Acid, 1,1-Dimethylethyl Ester (9f) and [(*R,S*)-3-[[(*R,S*)-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-hydroxy-4-phenylbutyl]amino]-2-hydroxy-1-(phenylmethyl)propyl]carbamic Acid, 1,1-Dimethylethyl Ester (9g):** from **7a** ($R^1$ = Bn) and **6d** (method C). The isomers were separated by flash chromatography ($SiO_2$; MeOH:$CH_2Cl_2$:$NH_4OH$ gradient from 2:97.8:0.2 to 5:94.5:0.5). **9f.** $^1$H NMR ($CD_3OD$): δ 1.28 (1.16 rotamer) (s, 9H), 1.34 (1.23 rotamer) (s, 9H), 2.41–2.82 (m, 6H), 2.89 (dd, *J* = 6.0, 13.7 Hz, 1 H), 3.02–3.12 (m, 1H), 3.55–3.68 (m, 2H), 3.68–3.78 (m, 2H), 7.10–7.29 (m, 10H). $^{13}$C NMR ($CD_3OD$): δ 27.4 (27.1 rotamer), 36.5, 37.6, 51.6, 51.8, 54.6, 55.5, 69.6, 71.6, 78.7, 78.8, 125.7, 125.9, 127.8, 129.0, 129.1, 138.7, 138.8, 156.7, 156.8 (1 aliphatic and 1 aromatic carbon unresolved). Anal. ($C_{30}H_{45}N_3O_6$) C, H, N. **9g.** $^1$H NMR ($CD_3OD$): δ 1.29 (1.18 rotamer) (s, 18H), 2.40–2.65 (m, 4H), 2.80–2.90 (m, 2H), 3.05–3.15 (m, 2H), 3.58–3.72 (m, 4H), 7.10–7.27 (m, 10H). $^{13}$C NMR (DMSO-$d^6$): δ 28.1 (27.8 rotamer), 36.0, 52.9, 55.1, 72.2, 77.2, 125.5, 127.8, 129.0, 139.7, 155.2. Anal. ($C_{30}H_{45}N_3O_6$) C, H, N.

**Preparation of (*1S*-(*1R**,*2R*(*3S**)))-[3-[[3-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-hydroxy-4-phenylbutyl]amino]-2-hydroxy-1-(phenylmethyl)propyl]carbamic Acid, 1,1-Dimethylethyl Ester (9h).** Epoxide **6c** was reacted with $NH_3$ as described for the preparation of **7a** ($R^1$ = Bn). The resulting amino alcohol was then reacted with **6d** as described in method C to give, after purification by flash chromatography ($SiO_2$; MeOH:$CH_2Cl_2$:$NH_4OH$ gradient from 2:97.8:0.2 to 4:95.6:0.4), **9h** as a ca. 3:2 mixture of isomers. Anal. ($C_{30}H_{45}N_3O_6$) C, H, N.

**Preparation of (*1S*-(*1R**,*2S**(*2S**,*3R**)))-[3-[[3-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-hydroxy-4-phenylbutyl]amino]-2-hydroxy-1-(2-phenylethyl)propyl]carbamic Acid, 1,1-Dimethylethyl Ester (15a).** (Trimethylsilyl)ethyl chloroformate (0.11 mL, 0.61 mmol; contains 10% toluene by weight) was added in one portion to a solution of 14 ($R^1$ = Bn, $R^{1'}$ = phenethyl) (prepared from **6a** ($R^1$ = Bn) and **6b** ($R^1$ = phenethyl) by method C) (290 mg, 0.5 mmol) and diisopropylethylamine (0.14 mL, 0.81 mmol) in 4.9 mL of DMF at 0 °C. After the mixture was stirred for 1 h at 0 °C, 10 mL of saturated $NaHCO_3$ solution was added and the resulting mixture was extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ solution, $H_2O$, aqueous 5% $KHSO_4$ solution, $H_2O$, and brine. After drying over $MgSO_4$, the organic layer was concentrated to yield 400 mg of a crude oil which was purified by flash chromatography ($SiO_2$; EtOAc:hexane 6:4) to afford 341 mg (95%) of a colorless oil. $^1$H NMR ($CD_3OD$): δ 0.00 (s, 9H), 0.98 (m, 2H), 1.26 (s, 9H), 1.70 (m, 1H), 1.95 (m, 1H), 2.53 (m, 2H), 2.69 (m, 1H), 3.03 (m, 1H), 3.20 (m, 1H), 3.53 (m, 1H), 3.57–3.85 (m, 6H), 4.10 (m, 2H), 5.08 (s, 2H), 6.37 (d, *J* = 7 Hz, 1N-H), 7.00–7.40 (m, 15H).

A solution of the TEOC carbamate (340 mg, 0.46 mmol) in 5 mL of MeOH was hydrogenated for 2 h over 20% $Pd(OH)_2$/C (102 mg) at room temperature using a hydrogen balloon. The catalyst was removed by filtration, and the filtrate was concentrated to dryness, affording 273 mg (98%) of a white foam.

To a solution of the above amine (273 mg, 0.45 mmol) in 1,4-dioxane (2 mL) and $H_2O$ (1 mL) was added triethylamine (95 μL, 0.68 mmol) followed by 2-[[(*tert*-butoxycarbonyl)oxy]imino]-2-phenylacetonitrile (Boc-ON) (134 mg, 0.54 mmol). The reaction mixture was stirred at room temperature overnight, after which time it was diluted with EtOAc and extracted with $H_2O$. The aqueous layer was extracted with EtOAc, and the combined extracts were washed with saturated $NaHCO_3$ and brine. After drying over $Na_2SO_4$, the organic layer was concentrated to yield 414 mg of crude oil which was purified by flash chromatography ($SiO_2$; $CH_2Cl_2$ than a gradient from 1% to 5% MeOH/$CH_2Cl_2$) to afford 276 mg (87%) of a solid. $^1$H NMR ($CD_3OD$): δ 0.00 (s, 9H), 0.98 (m, 2H), 1.26 (s, 9H), 1.41 (s, 9H), 1.65 (m, 1H), 1.92 (m, 1H), 2.53 (m, 2H), 2.67 (m, 1H), 3.01 (m, 1H), 3.17 (m, 1H), 3.43 (m, 1H), 3.57–3.75 (m, 6H), 4.10 (m, 2H), 7.05–7.25 (m, 10H).

*n*-$Bu_4NF$ (349 mg, 1.33 mmol) was added, in one portion, to a solution of the TEOC-protected amine (275 mg, 0.39 mmol) in 4 mL of THF at room temperature. The reaction mixture was warmed to 50 °C and stirred for 4 h. After cooling to room temperature and removal of the THF *in vacuo*, the residue was purified by flash chromatography ($SiO_2$; gradient from 97.8:2:0.2 to 92.3:7:0.7 $CH_2Cl_2$:MeOH:$NH_4OH$) to afford 120 mg (55%) of **15a** as a white solid. $^1$H NMR ($CD_3OD$): δ 1.29 (s, 9H), 1.47 (s, 9H), 1.65 (m, 1H), 2.01 (m, 1H), 2.55 (m, 2H), 2.72 (m, 5H), 3.10 (m, 1H), 3.44 (m, 1H), 3.51–3.71 (ms, 3H), 7.22 (m, 10H). $^{13}$C NMR ($CD_3OD$): δ 29.0, 29.2, 33.7, 34.1, 38.2, 53.2, 55.1, 57.2, 73.1, 73.5, 80.3, 80.5, 127.1, 127.4, 129.5, 129.7, 129.8, 130.8, 140.5, 143.6, 158.4, 158.8 (1 aliphatic carbon unresolved). Anal. ($C_{31}H_{47}N_3O_6$) C, H, N.

**Preparation of (*1S*-(*1R**,*2S**(*2S*,*3R**)))-[3-[[3-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-hydroxy-4-(4-hydroxyphenyl)butyl]amino]-2-hydroxy-1-(phenylmethyl)propyl]carbamic Acid, 1,1-Dimethylethyl Ester (15b)**: from 14 ($R^1$ = Bn, $R^{1'}$ = $CH_2$-(*p*-*O*-benzyl)phenyl) (prepared from **6a** ($R^1$ = Bn) and **6b** ($R^1$ = $CH_2$-(*p*-*O*-benzyl)phenyl) by method C) by a procedure analogous to that for **15a** above. $^1H$ NMR ($CD_3OD$): $\delta$ 1.20 (s, 9H), 1.23 (s, 9H), 2.35–2.75 (m, 6H), 2.87 (m, 1H), 2.98 (m, 1H), 3.44–3.66 (m, 4H), 6.58 (d, $J$ = 8.55, 2H), 6.93 (d, $J$ = 8.55, 2H), 7.02–7.23 (m, 5H). $^{13}C$ NMR ($CD_3OD$): $\delta$ 28.4, 28.7, 37.0, 37.9, 53.1, 56.9, 57.0, 73.1, 73.2, 79.5, 79.9, 116.0, 127.1, 129.2, 130.5, 130.7, 130.9, 131.4, 140.3, 156.7, 158.1 (1 aliphatic carbon unresolved). Anal. ($C_{30}H_{45}N_3O_7$) C, H, N.

**Preparation of (*R*-(*R**,*S**))-[Iminobis[2-hydroxy-1-(phenylmethyl)-3,1-propanediyl]]bis[carbamic acid], Bis(phenylmethyl ester) (10a)**: from **6b** ($R^1$ = Bn) (method B). $^1H$ NMR (DMSO-$d^6$): $\delta$ 2.55 (m, 4H), 2.69 (m, 2H), 3.01 (m, 2H), 3.54 (m, 2H), 3.62 (m, 2H), 4.88 (d, $J$ = 13 Hz, 2H), 4.95 (d, $J$ = 13 Hz, 2H), 4.85–5.10 (br m, 2H), 7.16–7.33 (m, 22H). $^{13}C$ NMR (DMSO-$d^6$): $\delta$ 35.9, 51.9, 55.8, 64.6, 70.9, 125.7, 127.0, 127.4, 127.9, 128.1, 129.0, 137.3, 139.3, 155.7. Anal. ($C_{36}H_{41}N_3O_6$) C, H, N.

**Preparation of (*R*-(*R**,*S**))-[Iminobis[2-hydroxy-1-(phenylmethyl)-3,1-propanediyl]]bis[carbamic acid], Bis(1-methylethyl ester) (12a).** A solution of 11 ($R^1$ = Bn) (211 mg, 0.38 mmol) and 3.5 mL (14.1 mmol) of 4 N HCl was stirred at 0 °C for 1 h. The reaction mixture was warmed to room temperature and the volatiles were removed *in vacuo*. The residue was coevaporated with dry $Et_2O$ to give 165 mg of a white solid.

To a solution of the resulting dihydrochloride salt (165 mg, 0.35 mmol) and 0.27 mL (1.56 mmol) of dry diisopropylethylamine in 0.35 mL of dry DMF at 0 °C was added 0.78 mL (0.78 mmol) of isopropyl chloroformate. The reaction mixture was stirred at room temperature for 72 h, the reaction quenched with saturated $NaHCO_3$, and the mixture extracted with $Et_2O$. The organic layer was washed with brine and dried over $Na_2SO_4$. The salts were filtered, and the filtrate was evaporated to a residue. Purification on silica gel (25–50% EtOAc–hexane) afforded 100 mg (47% over two steps) of the bis-isopropyl carbamate. $^1H$ NMR ($CDCl_3$): $\delta$ 1.11 (d, $J$ = 6.4 Hz, 6H), 1.17 (d, $J$ = 4.7 Hz, 6H), 2.52–2.61 (m, 3H), 2.67–2.84 (m, 4H), 3.36–3.52 (m, 3H), 3.58–3.82 (m, 6H), 4.64–4.73 (br d, 2H), 4.76 (dt, $J$ = 6.4, 4.7 Hz, 2H), 7.13–7.35 (m, 15H).

To a solution of the bis-isopropyl carbamate (87 mg, 0.14 mmol) in 3.2 mL of EtOH were added 25 mg of $Pd(OH)_2/C$ and 1.6 mL (15 mmol) of cyclohexene. The reaction mixture was heated to reflux for 2 h, filtered through a Celite plug, and rinsed with EtOH. Evaporation gave a residue which was purified by trituration with $Et_2O$ to afford 60 mg (82%) of **12a** as a white solid. $^1H$ NMR (DMSO-$d_6$, 80 °C): $\delta$ 0.8–1.2 (m, 12H), 2.4–2.6 (m, 6H), 2.9–3.1 (m, 6H), 4.0 (m, 3H), 6.4 (m, 2H), 7.1–7.3 (m, 10H). $^{13}C$ NMR (DMSO-$d_6$, 80 °C): $\delta$ 21.4, 35.9, 52.1, 55.3, 65.9, 71.3, 125.1, 127.3, 128.6, 139.3, 155.0. Anal. ($C_{28}H_{41}N_3O_6$) C, H, N.

**Preparation of (*1S*-(*1R**,*2S**))-[Iminobis[2-hydroxy-1-(phenylmethyl)-3,1-propanediyl]]bis[carbamic acid], Bis(2,2-dimethylpropyl ester) (12b)**: from 11 ($R^1$ = Bn) by a procedure analogous to that for **12a**. $^1H$ NMR ($CD_3OD$, 45 °C): $\delta$ 0.84 (s, 18H), 2.81–2.61 (m, 6H), 3.13 (dd, $J$ = 4, 14 Hz, 2H), 3.55–3.77 (m, 8H), 7.22 (m, 10H). $^{13}C$ NMR ($CD_3OD$, 45 °C): $\delta$ 26.7, 32.4, 37.7, 53.0, 57.3, 73.1, 75.0, 127.1, 129.2, 130.4, 140.2, 159.0. Accurate mass measurement (M + H)$^+$ calcd for $C_{32}H_{50}N_3O_6$: 572.3700. Found: 572.3673 ($\Delta_{ppm}$ = 4.7).

**Preparation of (*1S*-(*1R**,*2S**(*2S**,*3R**)))-[3-[[3-[[[(1,1-Dimethylethyl)thio]carbonyl]amino]-2-hydroxy-4-phenylbutyl]amino]-2-hydroxy-1-(phenylmethyl)propyl]carbamic Acid, 1,1-Dimethylethyl Ester (15c)**. To a 0 °C solution of *tert*-butyl mercaptan (2 mL, 17.74 mmol) in 10 mL of dry $CH_2Cl_2$ was added 5 mL of dry pyridine followed by dropwise addition fo a solution of *p*-nitrophenyl chloroformate (4.29 g, 21.28 mmol) in 6 mL of $CH_2Cl_2$. The reaction mixture was stirred at 5 °C overnight and then diluted with EtOAc and washed with saturated $NaHCO_3$ and brine and finally dried ($MgSO_4$) and concentrated. The crude product was purified by flash chromatography on silica gel eluting with a gradient of hexane to 20% EtOAc–hexane to provide 0.5 g (11%) of the carbonate as a white solid.

A suspension of 0.3 g (0.52 mmol) of 14 ($R^1$, $R^{1'}$ = Bn) and 0.125 g of $Pd(OH)_2/C$ in 50 mL of EtOH was stirred under a hydrogen atmosphere for 3.5 h. Filtration and evaporation gave 0.21 g (100%) of the diamine as a white solid.

To a solution of the above diamine (0.2 g, 0.45 mmol) in 1.5 mL of DMF was added ($i$-Pr)$_2$NEt (0.157 mL, 0.9 mmol) followed by a solution of the above carbonate (0.138 g, 0.54 mmol), and the solution was stirred at room temperature for 40 h. The reaction mixture was diluted with EtOAc, washed with 1 N NaOH, dried ($MgSO_4$), and concentrated. The crude residue was purified by flash chromatography on silica gel (eluting with a gradient of $CH_2Cl_2$ to 89:10:1 $CH_2Cl_2$:MeOH:$NH_4OH$) followed by preparative TLC using silica gel (20 × 20 × 1 mm$^3$; eluting with 90:9:1 $CH_2Cl_2$:MeOH:$NH_4OH$) and trituration with ether to afford 25 mg (10%) of **15c** as a white solid. $^1H$ NMR ($CD_3OD$, 45 °C): $\delta$ 1.35 (s, 9H), 1.36 (s, 9H), 2.75–2.55 (m, 6H), 3.12 (m, 2H), 3.70–3.62 (m, 3H), 3.95 (m, 1H), 7.3–7.1 (m, 10H). Accurate mass measurement (M + H)$^+$ calcd for $C_{30}H_{46}N_3O_5S$: 560.3158. Found: 560.3165 ($\Delta_{ppm}$ = 1.3).

**Preparation of (*1S*-(*1R**,*2S**(*2S**,*3R**)))-[3-[[3-[[[(1,1-Dimethylethyl)amino]carbonyl]amino]-2-hydroxy-4-phenylbutyl]amino]-2-hydroxy-1-(phenylmethyl)propyl]carbamic Acid, 1,1-Dimethylethyl Ester (15d)**: from 14 ($R^1$, $R^{1'}$ = Bn) analogous to preparation of **15a** except that the urea was formed as follows. To a solution of the amine (75 mg, 0.128 mmol) in 1 mL of $CH_2Cl_2$ was added *tert*-butyl isocyanate (16 $\mu$L, 0.14 mmol). The reaction mixture was stirred at room temperature for 1 h, the volatiles were removed *in vacuo*, and the residue was purified by flash chromatography ($SiO_2$; 2% MeOH/$CHCl_3$) to afford 84 mg (95%) of the urea. $^1H$ NMR ($CD_3OD$): $\delta$ 1.20 (s, 9H), 1.30 (s, 9H), 2.60 (m, 2H), 2.80–3.00 (m, 4H), 3.10 (m, 2H), 3.70 (m, 3H), 3.80 (m, 1H), 7.10–7.30 (m, 10H). $^{13}C$ NMR ($CD_3OD$): $\delta$ 28.7, 29.7, 37.8, 38.1, 50.8, 52.7, 55.1, 56.8, 72.0, 72.4, 80.2, 127.2, 127.3, 129.2, 129.3, 130.4, 130.6, 139.9, 140.0, 158.2, 160.2 (1 aliphatic carbon unresolved). Anal. ($C_{30}H_{46}N_4O_5$) C, H, N.

**Preparation of (*1S*-(*1R**,*2S**(*2S**,*3R**)))-[3-[[3-[(3,3-Dimethyl-1-oxybutyl)amino]-2-hydroxy-4-phenylbutyl]amino]-2-hydroxy-1-(phenylmethyl)propyl]carbamic Acid, 1,1-Dimethylethyl Ester (15e)**: from 14 ($R^1$, $R^{1'}$ = Bn) analogous to preparation of **15a** except that the amide was formed as follows. To a suspension of *tert*-butylacetic acid (23.2 mL, 0.18 mmol) and HOBT (32 mg, 0.237 mmol) in 1 mL of $CH_2Cl_2$ cooled at 0 °C was added EDC (34.9 mg, 0.18 mmol). The reaction mixture was stirred at 0 °C for 45 min before addition of the amine (93 mg, 0.158 mmol). The reaction mixture was stirred at 0 °C for 1 h and overnight at room temperature and then diluted with 20 mL of $CH_2Cl_2$, washed with saturated $NaHCO_3$, 5% aqueous citric acid, and brine, dried ($MgSO_4$), and concentrated *in vacuo* to give 112 mg of an oily residue, which was deprotected without purification. $^1H$ NMR ($CD_3OD$): $\delta$ 0.80 (s, 9H), 1.30 (s, 9H), 1.95 (m, 2H), 2.45–2.90 (m, 6H), 3.15 (m, 2H), 3.65 (m, 3H), 4.12 (m, 1H), 7.10–7.30 (m, 10H). $^{13}C$ NMR ($CD_3OD$): $\delta$ 27.4, 28.9, 30.2, 36.0, 36.5, 49.3, 51.8, 53.9, 55.5, 71.7, 71.8, 78.6, 125.7, 125.8, 127.8, 127.9, 129.0, 129.1, 138.7, 138.9, 156.7, 173.0 (1 aliphatic carbon unresolved). Anal. ($C_{31}H_{47}N_3O_5$) C, H, N.

**Preparation of [(*S*,*R*)-3-[[(*R*,*S*)-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-hydroxy-4-phenylbutyl]amino]-2-hydroxy-1-(phenylmethyl)propyl]methylcarbamic Acid, 1,1-Dimethylethyl Ester (16).** A solution of 2.4 g (4.15 mmol) of 14 ($R^1$, $R^{1'}$ = Bn), 1.24 g (4.8 mmol) of 9-fluorenylmethyl chloroformate and 695 mg (5.4 mmol) of diisopropylethylamine in 25 mL of DMF was stirred at 0 °C for 1 h. The reaction mixture was evaporated to dryness and the residue diluted with 100 mL of EtOAc. The solution was washed with 1 N HCl, brine, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated to an oily residue. The crude material was purified by flash chromatography on a 500-mL column of silica gel. Elution with a stepwise gradient of 25%, 50%, and 75% EtOAc–hexane afforded 3.0 g (3.74 mmol, 90%) of a white solid foam.

A solution of 1.69 g (2.1 mmol) of the FMOC carbamate in 2.0 mL of 1,4-cyclohexadiene and 60 mL of EtOH containing 225 mg of 10% Pd/C catalyst was stirred under a hydrogen atmosphere for 3.5 h. The catalyst was filtered through Celite, 0.5 mL of HOAc was added to the filtrate, and the solution was evaporated to dryness to yield 1.8 g (assumed 100% yield) of a white solid.

Formic acetic anhydride was prepared by the addition of 260 μL of $HCO_2H$ to 745 μL of $Ac_2O$ at 0 °C. The solution was then heated at 50 °C for 2 h. A solution of the anhydride in 5 mL of THF was added to a slurry of 1.8 g (assumed 2.1 mmol) of the above amine in 20 mL of THF at 0 °C. After 30 min at 0 °C and 30 min at room temperature, the reaction mixture was evaporated to dryness to afford the crude product as a white foam. This material was purified by flash chromatography ($SiO_2$; elution with 50% EtOAc–hexane followed by 100% EtOAc) to give 1.16 g (80% yield over two steps) of a solid white foam.

To a solution of 785 mg (1.13 mmol) of the above formamide in 10 mL of THF at 0 °C was added dropwise 2.85 mL of 2 M $BH_3$·$Me_2S$[14] in THF. After heating at 50 °C for 1 h, the solution was cooled to 0 °C and excess borane hydrolyzed by the dropwise addition of 10 mL of MeOH; 0.5 mL of HOAc was then added and the solution heated at 50 °C for 6 h to destroy the amine–boron complex. The solution was evaporated to dryness and the oil residue purified by flash chromatography ($SiO_2$; elution with a gradient of 100% EtOAc, 10% MeOH/EtOAc, and 20% MeOH/EtOAc). After the appropriate fractions were combined, 0.5 mL of HOAc was added and the solvent removed to yield 625 mg (0.84 mmol, 75% yield) of a solid white foam containing approximately 3 equiv of HOAc by $^1H$ NMR.

To a solution of 160 mg (0.166 mmol) of the above acetate salt and 137 mg (0.63 mmol) of di-*tert*-butyl dicarbonate in 1 mL of DMF at 0 °C was added 146 μL of triethylamine. After 30 min, an additional 65 mg of di-*tert*-butyl dicarbonate and 75 μL of triethylamine were added, and stirring was continued for an additional 30 min. The reaction mixture was diluted with EtOAc, washed with $H_2O$ and brine, and dried ($MgSO_4$), and the solvent was removed to yield an oily residue which was purified by flash chromatography ($SiO_2$; elution with 25% EtOAc/hexane followed by 50% EtOAc/hexane) to provide 129 mg (89%) of a solid white foam.

A solution of 230 mg (0.288 mmol) of the above FMOC carbamate and 0.25 mL of piperidine in 5 mL of $CH_2Cl_2$ was stirred at room temperature for 1.5 h. The reaction mixture was purified by flash chromatography ($SiO_2$; elution with 95:5:0.5 $CH_2Cl_2$:MeOH:$NH_4OH$) to afford 155 mg (72% yield) of **16** as a solid white foam. $^1H$ NMR (DMSO-$d_6$, 110 °C): δ 1.24 (s, 9H), 1.26 (s, 9H), 2.59 (s, 3H), 2.60 (m, 4H), 2.75 (m, 2H), 2.90–3.20 (m, 3H), 3.51 (m, 1H), 3.55–3.75 (m, 2H), 4.00 (m, 1H), 4.40 (m, 2H), 6.05 (m, 1H), 7.10–7.25 (m, 10H). Anal. ($C_{31}H_{47}N_3O_6$) C, H, N.

**Preparation of [(*S*)-3-[[(*R,S*)-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-hydroxy-4-phenylbutyl]methylamino]-2-hydroxy-1-(phenylmethyl)propyl]carbamic Acid, 1,1-Dimethylethyl Ester (18).** Epoxide **6a** ($R^1$ = Bn) (0.38 g, 1.44 mmol) in 10 mL of absolute EtOH was stirred with 10 mL of methylamine in EtOH (33% solution) for 18 h. Evaporation gave 0.42 g (100%) of the amino alcohol as an oily residue. The amino alcohol (0.36 g, 1.22 mmol) was reacted in 3 mL of DMF at 0 °C with 0.181 g (1.22 mmol) of NaI, excess solid $NaHCO_3$, and the Boc-Phe chloromethyl ketone intermediate described in the preparation of **6a** ($R^1$ = Bn) above (0.365 g, 1.22 mmol). After 24 h at room temperature, the mixture was partitioned between EtOAc and saturated $NaHCO_3$ and the combined organic extracts were washed with $H_2O$ and dried ($Na_2SO_4$). Evaporation and purification by flash chromatography ($SiO_2$; $CHCl_3$ and then 1% MeOH/$CHCl_3$) gave 0.425 g (68%) of **17** as a white foam.

To a solution of **17** (0.225 g, 0.4 mmol) in 3 mL of 95% EtOH and 0.3 mL of $H_2O$ at 0 °C was added solid $NaBH_4$ (0.015 g, 0.4 mmol). After 2 h, the reaction mixture was concentrated *in vacuo* and purified by flash chromatography ($SiO_2$; 1% MeOH/$CHCl_3$) to give 0.085 g (38%) of **18** (ca. 1:1 mixture of isomers) as a white amorphous solid. Anal. ($C_{31}H_{47}N_3O_6$) C, H, N.

**Preparation of (*S*-(*R*,R**))-[Oxybis[2-hydroxy-1-(phenylmethyl)-3,1-propanediyl]]bis[carbamic acid], Bis(1,1-dimethylethyl ester) (22).** A solution of the allylic alcohol **19**[16] (2.2 g, 7.93 mmol; >95% diastereomerically pure by HPLC and $^1H$ NMR), dry benzene (35 mL), 2,2-dimethoxypropane (5.65 mL, 45.2 mmol), and *p*-toluenesulfonic acid·$H_2O$ (17 mg) was refluxed for 7 h with azeotropic removal of $H_2O$. The reaction mixture was cooled to room temperature and poured into saturated $NaHCO_3$ solution (85 mL). The aqueous layer was extracted with $Et_2O$, and the combined organic extracts were dried ($MgSO_4$) and evaporated. The solvents were removed *in vacuo* to give a crude oil which was purified by flash chromatography ($SiO_2$; EtOAc:hexane gradient from 5:95 to 20:80) to afford the ketal (2.2 g, 88%) as an oil.

The above ketal (500 mg, 1.58 mmol) in MeOH (9 mL) was stirred at −60 °C, and ozone was bubbled into the mixture for 15 min until a faint blue color persisted. After purging the mixture with $N_2$, a solution of sodium borohydride (125 mg, 3.3 mmol) in cold MeOH (2 mL) was added. The reaction mixture was stirred at room temperature for 15 min and cooled to 0 °C and the reaction quenched with $H_2O$ (2 mL). The pH was adjusted to pH 5 with 5% aqueous citric acid, the volatiles were removed *in vacuo*, and the slurry was extracted with EtOAc. The combined organic extracts were washed with 5% $NaHCO_3$ and brine and then concentrated to a white gummy solid which was purified by flash chromatography ($SiO_2$; EtOAc:hexane gradient from 10:90 to 30:70) to give the alcohol **20** (448 mg, 86%). $^1H$ NMR (benzene-$d_6$, 70 °C): δ 1.37 (s, 9H), 1.57 (br s, 3H), 1.70 (br s, 3H), 2.75–2.80 (m, 1H), 2.95 (br s, 1H), 3.35–3.42 (m, 1H), 3.57–3.65 (m, 1H), 3.96–4.03 (m, 1H), 4.22 (br s, 1H), 7.0–7.3 (m, 5H).

To the above alcohol (250 mg, 0.78 mmol) in $CH_2Cl_2$ (5 mL) at 0 °C was added pyridine (94 μL, 1.17 mmol) followed by triflic anhydride (158 μL, 0.93 mmol). After 2 h, the reaction mixture was treated with 0.1 N HCl (10 mL) and the organic layer was washed with cold 0.1 N HCl and brine and then dried ($MgSO_4$). Removal of solvent *in vacuo* gave the triflate **21** (269 mg, 76%) as an oil. $^1H$ NMR (benzene-$d_6$, 70 °C): δ 1.35 (s, 9H), 1.45 (br s, 3H), 1.62 (br s, 3H), 2.40–2.50 (m, 1H), 2.90 (br s, 1H), 3.85–3.95 (m, 2H), 4.07 (br s, 1H), 4.30–4.40 (m, 1H), 7.0–7.2 (m, 10H).

To a solution of **20** (190 mg, 0.59 mmol) in dry THF (1.2 mL) at −78 °C was added sodium bis(trimethylsilyl)amide (653 μL, 0.65 mmol). After the mixture was stirred for 30 min at −78 °C, a solution of **21** (269 mg, 0.59 mmol) in THF (1.5 mL) was added slowly, keeping the temperature below −70 °C. The reaction mixture was stirred at −78 °C for 90 min and then warmed slowly to room temperature and stirred for 4 h. The reaction mixture was cooled to −20 °C, the reaction quenched with HOAc (510 μL), and the mixture warmed to room temperature and diluted with EtOAc (9 mL) and $H_2O$ (9 mL). The pH was adjusted to pH 7 with saturated $NaHCO_3$ solution, and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with $H_2O$ and brine and dried over $MgSO_4$. Removal of the solvent *in vacuo* afforded a dark brown oil which was purified by flash chromatography ($SiO_2$; elution with EtOAc:hexane using a gradient from 5:95 to 100:0) to afford the ether (110 mg, 30%). Deprotection was carried out by heating a solution of the above ether (76 mg, 0.12 mmol), 10% HCl (800 μL, 2.2 mmol), and HOAc (400 μL, 6.7 mmol) in THF (1.2 mL) at 50 °C for 1 h. The organic solvent was removed *in vacuo* to give a crude oil (41 mg, 0.12 mmol) which was dissolved in acetone (1.9 mL) and $H_2O$ (1.9 mL) to which was added triethylamine (75 μL, 0.54 mmol) and di-*tert*-butyl dicarbonate (58 mg, 0.26 mmol) at room temperature. The reaction mixture was stirred for 5 h and concentrated *in vacuo* and the crude product purified by flash chromatography ($SiO_2$; MeOH:$CHCl_3$ gradient from 1:99 to 5:95) to give **22** (50 mg, 77%) as a white solid. $^1H$ NMR ($CDCl_3$): δ 1.36 (s, 18H), 2.85–3.0 (m, 2H), 3.0–3.1 (m, 2H), 3.4–3.7 (br m, 6H), 3.91 (d, $J$ = 7.7 Hz, 2H), 4.05–4.2 (br m, 2H), 4.59 (d, $J$ = 9.8 Hz, 2NH), 7.2–7.4 (m, 5H). $^{13}C$ NMR ($CDCl_3$): δ 28.3, 36.8, 52.6, 71.7, 79.6, 126.4, 128.4, 129.7, 137.8, 155.9 (1 aliphatic carbon unresolved). Anal. ($C_{30}H_{44}N_2O_7$) C, H, N.

**Preparation of (*1S*-(*1R*,2S*(2S*,3R**))-[3-[[3-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-hydroxy-4-phenylbutyl]-amino]-2-methoxy-1-(phenylmethyl)propyl]carbamic Acid, 1,1-Dimethylethyl Ester (24).** A solution of 300 mg (1.14 mmol) of **6a** ($R^1$ = Bn), 186 mg (2.85 mmol) of sodium azide,[22] and 111 mg (2.05 mmol) of $NH_4Cl$ in 6 mL of MeOH was stirred at reflux overnight. The white solid residue obtained on removal of solvent was dissolved in EtOAc and washed with $H_2O$ and brine. Drying ($MgSO_4$) and concentration gave 300 mg (86%) of the azido alcohol as a white solid.

To a suspension of 48 mg (1.2 mmol) of 60% NaH (hexane washed) in 1 mL of dry THF at room temperature was added a solution of 295 mg (0.96 mmol) of the above azido alcohol in 4 mL of THF. The reaction mixture was stirred for 2 h at which time 89 μL (1.44 mmol) of methyl iodide was added. After 1 h,

the reaction mixture was diluted with EtOAc and washed with $H_2O$, 10% $Na_2S_2O_3$, and brine. After drying ($MgSO_4$), removal of solvent gave a white solid residue which was purified by flash chromatography ($SiO_2$; elution with 20% EtOAc/hexane) to give 206 mg (67%) of the methyl ether as a white solid.

A suspension of 150 mg (0.47 mmol) of the above azido methyl ether in 3 mL of MeOH containing 45 mg of 10% Pd/C was hydrogenated at room temperature and 1 atm for 1.5 h. The reaction mixture was diluted with additional MeOH, 0.5 mL of $NH_4OH$ was added, and stirring was continued for 15 min. The catalyst was removed by filtration through Celite (MeOH wash) and the filtrate concentrated. The residue was taken up in $CH_2Cl_2$ and dried ($MgSO_4$) and the solvent evaporated to afford 122 mg (88%) of the amino methyl ether **23** as a white solid. $^1H$ NMR (DMSO-$d_6$, 150 °C): $\delta$ 1.28 (s, 9H), 1.29 (s, 9H), 2.6–2.8 (m, 8H), 2.86 (dd, $J$ = 5, 14 Hz, 1H), 2.97 (dd, $J$ = 5, 14 Hz, 1H), 3.30 (m, 1H), 3.36 (s, 3H), 3.59 (m, 1), 3.64 (m, 1H), 3.87 (m, 1H), 5.84 (br s, 1H), 5.99 (br s, 1H), 7.13–7.22 (m, 10H).

Compounds **23** and **6a** ($R^1$ = Bn) were converted to **24** by method C above. $^1H$ NMR (270 MHz, DMSO-$d_6$, 110 °C): $\delta$ 1.24 (s, 9H), 1.26 (s, 9H), 2.59 (s, 3H), 2.60 (m, 4H), 2.75 (m, 2H), 2.9–3.2 (m, 3H), 3.51 (m, 1H), 3.55–3.75 (m, 2H), 4.00 (m, 1H), 4.4 (m, 2H), 6.05 (m, 1H), 7.10–7.25 (m, 10H). Anal. ($C_{31}H_{47}N_3O_6$) C, H, N.

**Preparation of (*1S*-(*1R**(*2S**,*3R**)))-[3-[[3-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-hydroxy-4-phenylbutyl]amino]-1-(phenylmethyl)propyl]carbamic Acid, 1,1-Dimethylethyl Ester (27).** To a solution of olefin **25**[23] (300 mg, 1.21 mmol) in 1.5 mL of THF was added a total of 4.84 mL (2.42 mmol) of 0.5 M 9-BBN in THF at room temperature. After 1 h, the reaction mixture was cooled to 0 °C and the reaction quenched with 0.82 mL of 3 N aqueous NaOH and 0.82 mL of 30% $H_2O_2$. After 30 min at 0 °C, the excess peroxide was reduced with $Na_2SO_3$ (220 mg) and diluted with 10 mL of EtOAc. Brine was added, and the aqueous layer was extracted with EtOAc. The combined organic extracts were dried ($Na_2SO_4$) and concentrated to give, after flash chromatography ($SiO_2$; gradient from 2:1 hexane:EtOAc to 1:1 hexane:EtOAc), 240 mg (75%) of the alcohol as a white solid.

To a solution of oxalyl chloride (76 $\mu$L, 0.87 mmol) in 0.73 mL of dry $CH_2Cl_2$ at –60 °C was added 82 $\mu$L (1.15 mmol) of DMSO in 0.36 mL of $CH_2Cl_2$; 154 mg (0.58 mmol) of the above alcohol in 4.35 mL of $CH_2Cl_2$ was then added. After 50 min at –60 °C, 357 $\mu$L (2.56 mmol) of triethylamine in 0.71 mL of $CH_2Cl_2$ was added. The reaction mixture was warmed to room temperature and stirred for 30 min, and then the reaction was quenched with 1 mL of 10% citric acid and the mixture immediately poured into 20 mL of 1:1 hexane:$Et_2O$ and 9 mL of 10% citric acid. The aqueous layer was extracted with $Et_2O$, and the combined organic extracts were washed with saturated $NaHCO_3$, $H_2O$, and brine and dried ($Na_2SO_4$). Concentration gave 124 mg (81%) of the crude aldehyde **26** as a yellow solid. $^1H$ NMR ($CDCl_3$): $\delta$ 1.40 (s, 9H), 2.49–2.66 (m, 2H), 2.87–2.99 (m, 2H), 4.29 (m, 1H), 4.78 (m, 1H), 7.16–7.34 (m, 5H), 9.71 (t, $J$ = 1.2 Hz, 1H).

A solution of **26** (60 mg, 0.22 mmol) in 0.5 mL of HOAc/MeOH (pH = 6.0) was added to 64 mg (0.22 mmol) of **7a** ($R^1$ = Bn). A few pulverized 4-Å sieves were added followed by 14 mg (0.22 mmol) of $NaBH_3CN$, and the reaction mixture was stirred at room temperature overnight; 1 mL of saturated $NaHCO_3$ and 5 mL of EtOAc were added, and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with brine, dried ($Na_2SO_4$), concentrated, and purified by flash chromatography ($SiO_2$; 95:5 $CH_2Cl_2$:MeOH + 0.1% $NH_4OH$) to afford 55 mg (28%) of **27** as a white solid. $^1H$ NMR ($CD_3OD$, 50 °C): $\delta$ 1.30 (s, 9H), 1.37 (s, 9H), 1.71 (m, 1H), 1.89 (m, 1H), 2.61 (m, 1H), 2.78 (d, $J$ = 6.8 Hz, 2H), 2.73–3.09 (m, 4H), 3.13 (d, $J$ = 3.4 Hz, 1H), 3.68–3.72 (m, 2H), 3.81 (m, 1H), 7.15–7.29 (m, 10H). $^{13}C$ NMR (DMSO-$d_6$, 70 °C): $\delta$ 28.1, 28.2, 31.9, 35.9, 45.5, 49.8, 50.8, 55.1, 69.8, 77.7, 77.8, 125.7, 125.9, 127.9, 128.0, 129.1, 138.8, 139.5, 155.3, 155.6 (2 aliphatic and 2 aromatic carbons unresolved). Anal. ($C_{30}H_{45}N_3O_5$) C, H, N.

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**Supplementary Material Available:** Molecular modeling coordinates for a preferred binding mode of **9a** to HIV protease (25 pages). Ordering information is given on any current masthead page.

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(17) Although it can not be ruled out, modeling of **9a** into HIV protease shows that a bound complex in which both carbamate carbonyls interact with $H_2O_{(301)}$ is energetically less favorable than one where one of the carbamate carbonyls and one of the hydroxyl oxygens from hydrogen bonds to $H_2O_{(301)}$. Modeling was carried out as follows: The X-ray crystal structure of the HIV protease–JG-365 complex (7HVP) was used as the starting enzyme structure. Bound conformations of **9a** were generated using distance geometry (DGEOM[18]). Crystal structures of other HIV protease complexes as well as the SAR of aminodiol analogs were used to guide the distance geometry calculations. All conformations were subjected to molecular dynamics and geometry optimization using the Amber[19] force field in Macromodel.[20] The inhibitor as well as the side chains in the active site was allowed to freely move during this procedure while the backbone atoms were held fixed. Potential binding conformations were evaluated by the quality of the hydrogen bonds, especially those to the structural water, and how well the hydrophobic pockets were filled. Although we cannot unambiguously distinguish between binding modes of **9a** with the benzyl groups in $S_1/S_{1'}$ and the *tert*-butyl groups in $S_2/S_{2'}$ or the reverse,[7e] future ligand modifications may help to differentiate between the two models. Further details of the modeling will be published elsewhere. We are also continuing our efforts to obtain an X-ray crystal structure of an enzyme–inhibitor complex containing an aminodiol.

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| | $IC_{50}$ (nM) | $ED_{50}$ (nM) |
|---|---|---|
| A-77003 (Abbott) | 2.3 (<1) | 50 (200) |
| Ro 31-8959 (Roche) | 0.85 (<0.4) | 4 (2) |
| Sc-52151 (Monsanto-Searle) | 4.0 (6.0) | 20 (21) |
| L-735,524 (Merck) | 1.1 (0.4) | 10 (50 [$ClC_{95}$]) |

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