Potent HIV-1 Protease Inhibitors: Stereoselective Synthesis of a Dipeptide Mimic

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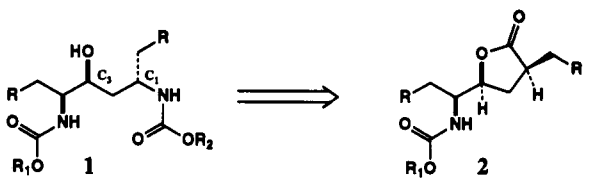
The synthesis of a differentially protected dipeptide mimic **10** in enantiomerically pure form is described. The key step involves the epimerization of the C-2 center of the lactone **4**, hydrolysis and protection of the resulting hydroxy acid, followed by Curtius rearrangement to introduce the urethane functionality. The scope and versatility of this isostere has been demonstrated by its conversion to potent HIV-1 protease inhibitors with nanomolar potencies. Also, established through the synthesis of compound **13** and **14**, the 3*S* hydroxyl configuration of the dipeptide isostere **1** is the preferred configuration for its potency. The present synthesis is efficient and provides an access to other dipeptide mimics with a great deal of structural diversity.

Introduction

Since the recognition of the molecular events critical to the replication of the human immunodeficiency virus (HIV-1),[1] numerous therapeutic strategies have been proposed for the treatment of acquired immunodeficiency syndrome (AIDS). Among them, inhibition of the virally encoded protease has emerged as a major target for the chemotherapy of AIDS.[2] Indeed, many potent and selective inhibitors of HIV-1 protease have now been reported based on a transition-state mimetic concept which incorporates nonhydrolyzable hydroxyethylene and hydroxyethylamine isosteres[3] at the P_1–P_1' substrate cleavage sites. Also, a new class of symmetric inhibitors with a C_2-axis of symmetry were designed and synthesized[4] based on the symmetric disposition of the HIV-1 protease structure.[5] More recently, the Abbott group has reported[6] a series of protease inhibitors which were synthesized based on the deoxygenation of the symmetric diols structure. Their report prompted us to disclose our research efforts in this area. Herein we report a stereoselective synthesis of this dipeptide mimic, its conversion to potent HIV-1 protease inhibitors, and some relevant biological results (IC_{50} values).



Results and Discussion

The key element of our synthetic strategy for dipeptide isostere of general structure **1** involved establishing the stereochemistry of the C-1 center stereoselectively. Since Hoffmann and Curtius type rearrangements of carboxylic acids to introduce nitrogen functionality with complete retention of configuration are well precedented, an alkylated lactone such as **2** would be our desired synthetic intermediate. Thus, the lactone **3** was alkylated with benzyl bromide according to the previously reported procedure[7] to afford the known[8] alkylation product **4** (84% yield) along with a small amount of isomeric desired product **5** (<4%). Initial attempts to obtain epimer **5** by a deprotonation and reprotonation sequence of lactone **4** were unsuccessful.[9] Nevertheless, the epimerization of the C-2 stereochemistry was effectively carried out by the following three-step sequence: (1) selenylation of lactone **4** with lithium diisopropylamide (2.1 equiv) and diphenyl

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(9) Deprotonation with lithium diisopropylamide or lithium hexamethyldisilazide (2.1 equiv) in THF at −78 to −55 °C and subsequent quenching with propionic acid or with saturated ammonium chloride solution yielded low proportion (<10%) of desired epimeric lactone **5**.

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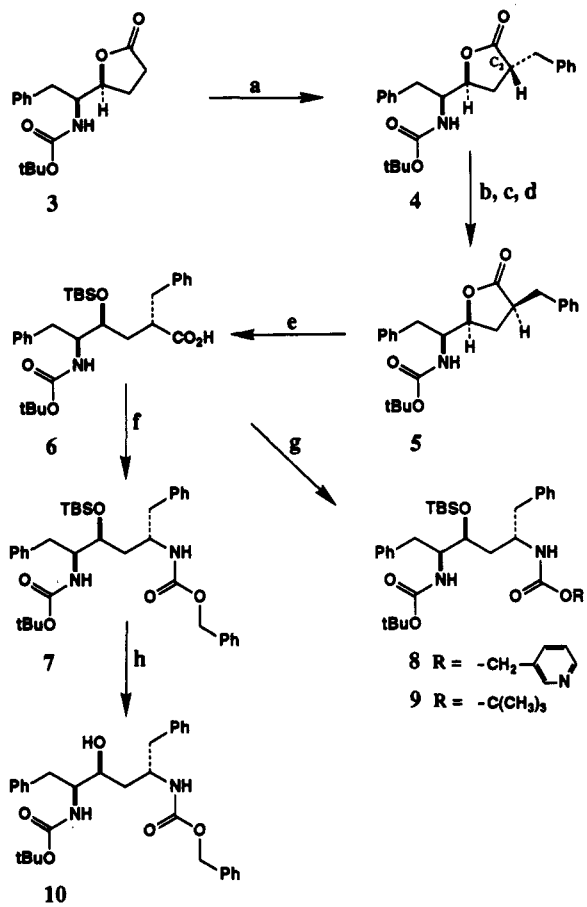
Scheme I[a]



[a] Key: (a) $(TMS)_2NLi$, THF, −78 °C, 30 min; $PhCH_2I$, −78 °C, 30 min; then $MeCH_2CO_2H$, −78 to 23 °C, 15 min; (b) lithium diisopropylamide, THF, −78 to −55 °C, 1 h; PhSeSePh, HMPA, −78 to −40 °C, 3 h; (c) MCPBA, CH_2Cl_2, 0 °C, 15 min; (d) 10% Pd–C, H_2, EtOAc–MeOH; (e) aqueous LiOH, DME, 12 h; TBDMSCl, imidazole, DMF, 12 h; MeOH, 10% citric acid; (f) $(PhO)_2P(O)N_3$, Et_3N, PhMe, 115 °C, 1 h, then $PhCH_2OH$, 115 °C, 12 h; (g) $(PhO)_2P(O)N_3$, Et_3N, PhMe, 115 °C, 1 h, then ROH, 115 °C, 12 h; (h) n-$Bu_4N^+F^-$, THF, 3 h.

diselenide (1.5 equiv) according to the Grieco procedure[10] to provide the corresponding phenyl selenide (85% yield); (2) oxidative dehydroselenenylation with *m*-chloroperbenzoic acid (1.2 equiv) in methylene chloride at 0 °C for 15 min; and (3) catalytic hydrogenation of the resulting olefin[11] with 10% palladium on charcoal in a mixture (3:1) of ethyl acetate and methanol under a hydrogen-filled balloon for 1 h to furnish the epimeric lactone **5** exclusively (71% overall yield from **3**). Lactone **5** was then hydrolyzed with lithium hydroxide (5 equiv) in aqueous dimethoxyethane, and the resulting acid obtained after acidification with 10% citric acid (pH 4.0) was converted to the silyl-protected acid **6** by reaction with *tert*-butyldimethylsilyl chloride (10 equiv) and imidazole (12 equiv) in DMF at 23 °C for 12 h, followed by workup with methanol and citric acid solution.

Conversion of acid **6** to the corresponding urethane derivative was readily accomplished by a modified Curtius-type rearrangement.[12] Accordingly, acid **6** was reacted with diphenyl phosphorazidate (1.2 equiv) and triethylamine (1.2 equiv) in refluxing toluene for 1 h. Benzyl alcohol (2 equiv) was added, and the resulting mixture was continued to reflux for 12 h to furnish the urethane **7** (Cbz derivative) in 65% yield after silica gel chromatography. Here the stereochemistry of the urethane was unambiguously assigned since the Curtius rearrangements are well-known to proceed with complete retention of configuration.[13] The above procedure turned out to be quite general and therefore provided a convenient access to other suitable urethanes in good yield. For example, reaction of acid **6** under the above reaction conditions in the presence of 3-pyridylcarbinol[14] provided the corresponding urethane **8** in 71% yield. Similarly, reaction of *tert*-butyl alcohol afforded the corresponding *tert*-butyloxycarbonyl derivative **9** in 64% isolated yield. The silyl protecting group of urethane **7** was removed by treatment with tetrabutylammonium fluoride in THF for 12 h to provide the differentially functionalized dipeptide mimic **10** in 90% isolated yield.

The protected urethane derivative **10** is a versatile intermediate for the synthesis of suitably substituted HIV-1 protease inhibitors. For example, removal of the *tert*-butyloxycarbonyl group of compound **10** with trifluoroacetic acid in methylene chloride at 0 °C for 30 min afforded the corresponding amine in 85% isolated yield. Reaction of the resulting amine and carbobenzoxy-L-valine using a standard peptide coupling procedure[8a] with *N*-ethyl-*N*′-(dimethylaminopropyl)carbodiimide hydrochloride (1.2 equiv) and 1-hydroxybenzotriazole hydrate (1.2 equiv) in the presence of triethylamine in DMF resulted in compound **11** (white solid, mp 188–190 °C) in 65% yield after chromatography (R_f 0.75, 5% methanol in chloroform). On the other hand, catalytic hydrogenation of **10** with Pearlman's catalyst under a hydrogen-filled balloon in a mixture (3:1) of ethyl acetate and methanol afforded the corresponding amine in quantitative yield. Coupling of this amine with carbobenzoxy-L-valine according to the above-mentioned conditions furnished the compound **12** (white solid, mp 191–194 °C) in 61% yield after chromatography.

Furthermore, the carbobenzoxy moiety of **10** was readily exchanged with the *tert*-butyloxycarbonyl group. Accordingly, hydrogenation[15] of **10** with 10% palladium on charcoal in tetrahydrofuran in the presence of *tert*-butyl dicarbonate and triethylamine for 12 h afforded the bis(*tert*-butyloxycarbonyl) derivative **13** (white solid, mp 139–142 °C) exclusively in 72% yield. In order to ascertain the importance of C-3 hydroxyl configuration for binding in the enzyme active site, the hydroxyl stereochemistry was conveniently inverted by an oxidation/reduction sequence. Thus, pyridinium dichromate oxidation of **13** in glacial acetic acid furnished the corresponding ketone which upon reduction with sodium borohydride in methanol at 0 °C for 1 h provided the alcohol **14** along with a

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(11) Dehydroselenelylation with *m*-chloroperbenzoic acid yielded a 4:1 mixture of exocyclic and endocyclic olefins.

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(14) 3-Pyridylmethanol has been previously utilized as a surrogate for the P_2 amino acid in HIV protease inhibitors; please see ref 6.

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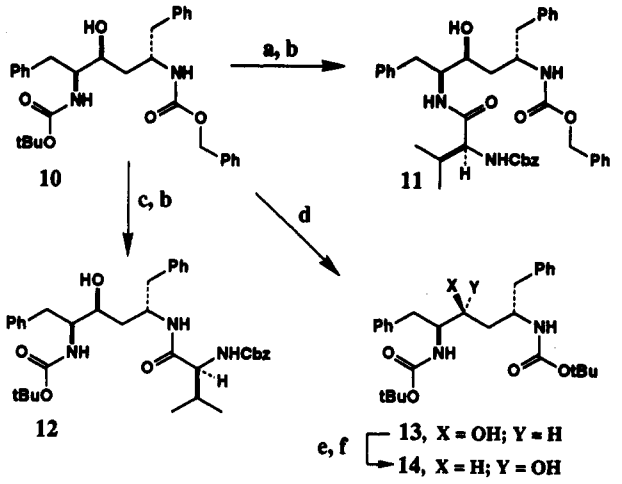
Scheme II[a]



[a] Key: (a) CF_3CO_2H, CH_2Cl_2, 0 °C, 30 min; (b) Cbz-Val-acid, EDC, HOBt, Et_3N, DMF, 23 °C, 12 h; (c) Pearlman's catalyst, H_2, EtOAc–MeOH (3:1); (d) 10% Pd–C, H_2, BOC_2O, Et_3N, THF; (e) PDC, glacial acetic acid; (f) $NaBH_4$, MeOH, 0 °C, 1 h.

small amount of starting alcohol 13 (mixture ratio 9:1)[16] in 88% isolated yield. The isomers were separated by column chromatography over silica gel (25% ethyl acetate in hexanes) to furnish the pure alcohol 14 (mp 162–164 °C).

The inhibitory potencies of compounds 11–14 were measured using purified HIV-1 protease as described previously by Darke et. al.[17] The IC_{50} values are the concentration of compound required to halve the amount of substrate cleaved during the allotted reaction period. Compounds 11 and 12 are potent inhibitors of HIV-1 protease with IC_{50} values of 3.8 and 3.1 nM, respectively. Through the synthesis of compounds 13 and 14, it has been established that the *S* configuration of the C-3 hydroxyl group was necessary for its inhibitory potency (compound 13, IC_{50} value 110 nM). When the stereochemistry of the C-3 hydroxyl group was inverted to the *R* configuration (compound 14), the inhibitory potency dropped significantly (IC_{50} value >3000 nM).

Conclusion

A convenient and stereoselective synthetic route to a differentially protected novel dipeptide mimic 10 has been developed. Compound 10 has been converted to potent HIV-1 protease inhibitors 11 and 12. The 3*S* hydroxyl configuration of the new dipeptide mimic 1 was the preferred configuration for its potency and was demonstrated through the synthesis of compound 13 and 14. Since the C-1 benzyl group was introduced stereoselectively by an alkylation procedure and the starting lactone could be derived from a natural amino acid or from a carbohydrate precursor, the present synthesis provides access to a variety of compounds with a great deal of structural diversity. Synthesis and structure–activity relationship studies of a host of other HIV-1 protease inhibitors are currently in progress.

(16) Similar stereochemical results have been reported elsewhere: (a) Hamada, Y.; Kondo, Y.; Shibata, M.; Shioiri, T. *J. Am. Chem. Soc.* **1989**, *111*, 669. (b) Jouin, P.; Poncet, J.; Dufour, M.-N.; Pantaloni, A.; Castro, B. *J. Org. Chem.* **1989**, *54*, 617. (c) Maibaum, J.; Rich, D. H. *Ibid.* **1988**, *53*, 869.

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Experimental Section

All melting points were recorded on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton magnetic resonance spectra were recorded on a Varian XL-300 spectrometer using tetramethylsilane as internal standard. Significant ^1H NMR data for representative compounds are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons, coupling constant(s) in hertz. FAB mass spectra were recorded on a VG Model 7070 mass spectrometer and relevant data are tabulated as *m/z*. Elemental analysis were performed by the analytical department, Merck Research Laboratories, West Point, PA, and were within ±0.4% of the theoretical values. Anhydrous solvents were obtained as follows: methylene chloride, distillation from P_4O_{10}; tetrahydrofuran, distillation from sodium/benzophenone; dimethylformamide and pyridine, distillation from CaH_2. All other solvents were HPLC grade. Column chromatography was performed with E. Merck 240–400-mesh silica gel under low pressure of 5–10 psi. Thin-layer chromatography (TLC) was carried out with E. Merck silica gel 60 F-254 plates.

Preparation of (3*R*,5*S*,1′*S*)-3-Benzyl-5-[1′-[[(1,1-dimethylethoxy)carbonyl]amino]-2′-phenylethyl]dihydrofuran-2-(3*H*)-one (4). Lithium hexamethyldisilazide (2 mmol) was prepared by the dropwise addition of 1.5 M *n*-BuLi in hexane (1.35 mL) to hexamethyldisilazane (0.46 mL, 2.2 mmol) in THF (2 mL) at 0 °C for 5 min and then warmed to 23 °C for 15 min. The resulting solution was cooled to −78 °C, and lactone 3 (305 mg, 1 mmol) in THF (2 mL) was added dropwise for 5 min. The mixture was stirred at −78 °C for 30 min, and benzyl iodide (218 mg, 1 mmol) in THF (1 mL) was added slowly for 2 min. The resulting reaction mixture was stirred at −78 °C for 30 min and then quenched with propionic acid (0.25 mL) in THF (1 mL), stirred for 5 min, and warmed to 23 °C for 15 min. Aqueous 10% citric acid (10 mL) and ethyl acetate (50 mL) were added, and the layers were separated. The aqueous layer was extracted with additional ethyl acetate (30 mL), and the combined extracts were washed with aqueous $NaHCO_3$ and brine and dried (Na_2SO_4). Evaporation of solvents gave a residue which was flash chromatographed over silica gel (1:3 ethyl acetate/hexane) to provide 4 (332 mg, mp 76–78 °C) and 5 (15 mg, mp 112–115 °C). Compound 4: ^1H NMR (300 MHz, $CDCl_3$) δ 7.1–7.3 (m, 10 H), 4.5 (br d, 1 H, J = 10.1 Hz), 4.2 (m, 1 H), 3.95 (br q, 1 H, J = 9 Hz), 3.12 (dd, 1 H, J = 5, 14 Hz), 2.98 (m, 1 H), 2.85 (m, 2 H), 2.75 (dd, 1 H, J = 8, 13.2 Hz), 2.2 (m, 1 H), 1.95 (m, 1 H), 1.35 (s, 9 H); IR (neat) 1768, 1710, 1490 cm^{-1}; MS (70 eV) *m/z* 396 (M^+ + H). Anal. Calcd for $C_{24}H_{29}NO_4$: C, 72.89; H, 7.39; N, 3.54. Found: C, 72.38; H, 7.42; N, 3.52.

Preparation of (3*S*,5*S*,1′*S*)-3-Benzyl-5-[1′-[[(1,1-dimethylethoxy)carbonyl]amino]-2′-phenylethyl]dihydrofuran-2-(3*H*)-one (5). Lithium diisopropylamide (2.1 equiv) was prepared by the dropwise addition of 1.6 M *n*-BuLi in hexane (1.3 mL, 2.1 mmol) to diisopropylamine (0.322 mL, 2.3 mmol) in THF (2 mL) at 0 °C for 5 min and then warmed to 23 °C for 15 min. The mixture was cooled to −78 °C, and lactone 4 (395 mg, 1.0 mmol) in THF (3 mL) was added dropwise for 5 min. The mixture was stirred at −78 °C and allowed to warm to −55 °C for 1 h. The resulting mixture was recooled to −78 °C, and a mixture of diphenyl diselenide (470 mg, 1.5 mmol) and HMPA (0.260 mL, 1.5 mmol) in THF (2 mL) was added dropwise over a period of 5 min. After stirring for 30 min at −78 °C, the reaction was warmed to −40 °C for 2 h, quenched with saturated aqueous NH_4Cl (4 mL), and allowed to warm to 23 °C. The mixture was then extracted with ethyl acetate (2×), and the organic layers were combined, washed with brine, and dried over Na_2SO_4. Filtration and evaporation of the solvents under reduced pressure gave a residue which was chromatographed over silica gel (25% ethyl acetate–hexane) to provide the selenylation product (485 mg).

The above lactone (485 mg, 0.88 mmol) was dissolved in dry CH_2Cl_2 (10 mL), and saturated aqueous $NaHCO_3$ (10 mL) was added. The mixture was cooled to 0 °C, 60% MCPBA (350 mg, 1.0 mmol) was added, and the resulting mixture was stirred for 15 min at 0 °C. The reaction was diluted with CH_2Cl_2 and water, and the layers were separated. The organic layer was dried over

Na_2SO_4, filtered, and concentrated to give a mixture of isomeric olefins (300 mg, mixture ratio 4:1) which was used without further purification.

The above mixture of olefins (300 mg) was dissolved in ethyl acetate (12 mL) and methanol (4 mL), and 10% Pd–C (75 mg) was added. Hydrogenation under balloon pressure for 1 h followed by filtration through Celite to remove catalyst and evaporation of the solvents under reduced pressure provided compound **5** (280 mg, 71%) as a foamy, white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.1–7.3 (m, 10 H), 4.6 (br d, 1 H, J = 10 Hz), 4.3 (m, 1 H), 3.94 (br q, 1 H, J = 9.1 Hz), 3.25 (dd, 1 H, J = 3, 14 Hz), 2.82–3.0 (m, 3 H), 2.69 (dd, 1 H, J = 9, 13 Hz), 2.1 (m, 1 H), 1.8 (br q, 1 H, J = 12 Hz), 1.40 (s, 9 H); IR (neat) 1765, 1707, 1490 cm^{-1}; MS (70 eV) m/z 396 (M^+ + H). Anal. Calcd for $C_{24}H_{29}NO_4$: C, 72.89; H, 7.39; N, 3.54. Found: C, 72.69; H, 7.69; N, 3.54.

Preparation of (2*S*,4*S*,5*S*)-5-[[(1,1-Dimethylethoxy)carbonyl]amino]-4-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-6-phenyl-2-(phenylmethyl)hexanoic Acid (6). The lactone **5** (2.6 g, 6.6 mmol) was dissolved in DME (45 mL), and an aqueous lithium hydroxide solution (1 N, 45 mL) was added. The resulting mixture was stirred at 23 °C for 12 h. The mixture was then carefully acidified with 10% aqueous citric acid to pH 4 and extracted thoroughly with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with brine and then dried (Na_2SO_4). Evaporation of the solvent gave a residue which was dissolved in dry DMF (25 mL), and then *tert*-butyldimethylsilyl chloride (9.9 g, 66 mmol) and imidazole (5.4 g, 80 mmol) were added. The resulting mixture was stirred at 23 °C for 12 h. After this period, MeOH (20 mL) was added, and the mixture was stirred for an additional 2 h. The solvents were removed under reduced pressure, and the residue was partitioned between ethyl acetate and 10% aqueous citric acid. The layers were separated, and the organic phase was washed with brine and then dried (Na_2SO_4). Evaporation of the solvent gave a residue which was chromatographed over silica gel (1:3 ethyl acetate/hexane) to furnish the acid **6** (2.9 g, 90%) as a foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.1–7.4 (m, 10 H), 4.65 (br d, J = 8.5 Hz, 1 H), 4.0 (m, 1 H), 3.7 (m, 1 H), 3.0–2.6 (m, 4 H), 1.6–1.9 (m, 2 H), 1.35 (s, 9 H), 0.9 (s, 9 H), 0.15 (s, 3 H), 0.1 (s, 3 H); MS (70 eV) m/z 528 (M^+ + H).

Preparation of (2*S*,4*S*,5*S*)-2-[[(Phenylmethoxy)carbonyl]amino]-4-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-5-[[(1,1-dimethylethoxy)carbonyl]amino]-1,6-diphenylhexane (7). To a solution of silyl acid **6** (1.2 g, 2.3 mmol) in toluene (50 mL) were added diphenyl phosphorazidate (0.58 mL, 2.7 mmol) and triethylamine (0.38 mL, 2.7 mmol). This mixture was heated to reflux for 1 h, benzyl alcohol (0.48 mL, 4.6 mmol) was added, and refluxing was continued for 12 h. After cooling and evaporation of the solvents under reduced pressure, the resulting residue was partitioned between ethyl acetate and saturated aqueous $NaHCO_3$. The layers were separated, and the organic phase was washed with brine (1 × 50 mL) and then dried (Na_2SO_4). Evaporation of the solvent gave a residue which was chromatographed over silica gel (1:3 ethyl acetate/hexane) to furnish compound **7** (0.94 g, 65%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.1–7.5 (m, 15 H), 5.0–5.2 (m, 3 H), 4.5–4.7 (m, 2 H), 4.0 (m, 1 H), 3.8 (m, 1 H), 2.6–2.8 (m, 4 H), 1.6–1.8 (m, 2 H), 1.4 (s, 9 H), 0.95 (s, 9 H), 0.5 (s, 6 H); IR (neat) 3310, 2970, 1698 cm^{-1}; MS (70 eV) m/z 633 (M^+ + H).

Preparation of (2*S*,4*S*,5*S*)-2-[[(Phenylmethoxy)carbonyl]amino]-5-[[(1,1-dimethylethoxy)carbonyl]amino]-1,6-diphenyl-4-hydroxyhexane (10). To a solution of **7** (800 mg, 1.3 mmol) in THF (5 mL) was added a solution of tetrabutylammonium fluoride (1 M solution in THF) in THF (6 mL). The resulting solution was stirred at 23 °C for 12 h. After this period, the solvent was removed under reduced pressure, and the residue was diluted with water and extracted with ethyl acetate (2 × 50 mL). The combined extracts were washed sequentially with brine and water and dried (Na_2SO_4). Evaporation of the solvent and chromatography over silica gel (50% ethyl acetate–hexane) afforded compound **10** (596 mg, 90%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.1–7.4 (m, 15 H), 5.05 (d, J = 2.0 Hz, 2 H), 4.7–4.9 (m, 2 H), 3.95 (br q, J = 7.5 Hz, 1 H), 3.6–3.7 (m, 2 H), 2.7–2.9 (m, 4 H), 1.65 (m, 2 H), 1.35 (s, 9 H); IR (neat) 3302, 3025, 2945, 1690 cm^{-1}; MS; 519 (M^+ + H). Anal. Calcd for $C_{31}H_{38}N_2O_5 \cdot 0.5H_2O$: C, 70.56; H, 7.45; N, 5.31. Found: C, 70.51; H, 7.13; N, 5.69.

Preparation of (2*S*,4*S*,5*S*)-2-[[(Phenylmethoxy)carbonyl]amino]-5-[[[(phenylmethoxy)carbonyl]-L-valinyl]amino]-1,6-diphenyl-4-hydroxyhexane (11). To a solution of **10** (80 mg, 0.15 mmol) in dry CH_2Cl_2 (1.5 mL) at 0 °C was added trifluoroacetic acid (1.5 mL). After stirring for 30 min at 0 °C, the solvents were removed under reduced pressure, and the residue was taken up in ethyl acetate (25 mL) and washed with saturated aqueous $NaHCO_3$ (10 mL). The layers were separated, and the organic phase was washed with brine (10 mL) and then dried (Na_2SO_4). Evaporation of the solvent gave the free amine (72 mg) as a white, flakey solid.

To a solution of the above amine (30 mg, 0.07 mmol) in dry DMF (5 mL) were added HOBt (48 mg), EDC (68 mg), and CBZ-L-valine (22 mg, 0.09 mmole) followed by triethylamine until the pH of the solution was 8.5. The resulting mixture was then stirred at 23 °C for 12 h, and the reaction mixture was poured into water (6 mL) and extracted with ethyl acetate (2 × 25 mL). The combined organic extracts were washed with 10% citric acid, saturated aqueous $NaHCO_3$ solution, and brine and dried (Na_2SO_4). Evaporation of the solvent and flash chromatography over silica gel (75% ethyl acetate–hexane) afforded compound **11** (30 mg, 65%) as a white solid: mp 188–190 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.1–7.4 (m, 20 H), 6.3 (d, J = 9.3 Hz, 1 H), 5.2 (d, J = 8.3 Hz, 1 H), 5.08 (s, 2 H), 5.01 (d, J = 5.3 Hz, 2 H), 4.95 (m, 1 H), 4.1 (m, 1 H), 3.95 (m, 2 H), 3.7 (m, 1 H), 2.8 (d, J = 7.7 Hz, 2 H), 2.7 (d, J = 5.9 Hz, 2 H), 2.1 (m, 1 H), 1.6 (m, 2 H), 0.9 (d, J = 6.6 Hz, 3 H), 0.75 (d, J = 6.9 Hz, 3 H); IR (neat) 3296, 2957, 1687 cm^{-1}; MS (70 eV) m/z 652 (M^+ + H). Anal. Calcd for $C_{39}H_{45}N_3O_6 \cdot 0.25H_2O$: C, 71.37; H, 6.99; N, 6.40. Found: C, 71.20; H, 6.81; N, 6.34.

Preparation of (2*S*,4*S*,5*S*)-2-[[[(Phenylmethoxy)carbonyl]-L-valinyl]amino]-5-[[(1,1-dimethylethoxy)carbonyl]amino]-1,6-diphenyl-4-hydroxyhexane (12). To a solution of **10** (45 mg, 0.09 mmol) in ethyl acetate (3 mL) and methanol (1 mL) was added 25 mg of 20% palladium hydroxide on carbon (Pearlman's catalyst-moist). Hydrogenation under balloon pressure for 1 h followed by filtration through Celite to remove the catalyst and evaporation of the solvents under reduced pressure provided the desired amine, which was used directly for coupling.

To a solution of the above amine (37 mg, 0.1 mmol) in dry DMF (5 mL) were added HOBt (20 mg, 0.144 mmol), EDC (28 mg, 0.144 mmol), and CBZ-L-valine (33 mg, 0.13 mmol), followed by triethylamine until the pH of the solution was 8.5. The resulting mixture was then stirred at 23 °C for 12 h, and the reaction mixture was poured into water (6 mL) and extracted with ethyl acetate (2 × 25 mL). The combined organic extracts were washed with 10% citric acid, saturated aqueous $NaHCO_3$ solution, and brine and dried (Na_2SO_4). Evaporation of the solvent and flash chromatography over silica gel (75% ethyl acetate–hexane) afforded compound **12** (38 mg, 61%) as a white solid: mp 191–194 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.1–7.4 (m, 15 H), 6.2 (d, J = 7.7 Hz, 1 H), 5.15 (m, 1 H), 5.1 (s, 2 H), 4.8 (d, J = 9.7 Hz, 1 H), 4.15 (m, 1 H), 3.88 (dd, J = 5.8, 8.5 Hz, 1 H), 3.6 (m, 2 H), 2.7–2.9 (m, 4 H), 2.1 (m, 1 H), 1.6–1.7 (m, 2 H), 1.5 (s, 9 H), 0.9 (d, J = 6.6 Hz, 3 H), 0.75 (d, J = 6.3 Hz, 3 H); IR (neat) 3300, 2970, 1690 cm^{-1}; MS (70 eV) m/z 618 (M^+ + H). Anal. Calcd for $C_{39}H_{45}N_3O_6$: C, 69.99; H, 7.67; N, 6.80. Found: C, 69.81; H, 7.66; N, 6.94.

Preparation of (2*S*,4*S*,5*S*)-2,5-Bis[[(1,1-dimethylethoxy)carbonyl]amino]-1,6-diphenyl-4-hydroxyhexane (13). To a solution of compound **10** (50 mg, 0.1 mmol) in tetrahydrofuran (3 mL) were added 10% Pd–C (25 mg), *tert*-butyl dicarbonate (28 mg, 0.13 mmol), and triethylamine (40 μL). This mixture was stirred under a hydrogen-filled balloon for 12 h. Filtration through Celite to remove catalyst and evaporation of the solvents under reduced pressure provided a residue, which was chromatographed over silica gel (25% ethyl acetate–hexane) to give compound **13** (35 mg, 72%) as a white solid: mp 139–142 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.1–7.3 (m, 10 H), 4.8 (d, J = 8.0 Hz, 1 H), 4.55 (m, 1 H), 3.85 (m, 1 H), 3.6–3.7 (m, 2 H), 2.7–2.9 (m, 4 H), 1.65 (m, 2 H), 1.45 (s, 9 H), 1.35 (s, 9 H); IR (neat) 3360, 2976, 1687 cm^{-1}; MS (70 eV) m/z 485 (M^+ + H). Anal. Calcd for $C_{28}H_{40}N_2O_5$: C, 69.39; H, 8.32; N, 5.78. Found: C, 69.50; H, 8.57; N, 5.80.

Preparation of (2*S*,4*R*,5*S*)-2,5-Bis[[(1,1-dimethylethoxy)-carbonyl]amino]-1,6-diphenyl-4-hydroxyhexane (14). To a solution of **13** (42 mg, 0.09 mmol) in glacial acetic acid was added pyridinium dichromate (100 mg, 0.27 mmol). After stirring for 3 h, ethyl acetate (25 mL) was added, and the mixture was carefully washed with saturated aqueous $NaHCO_3$ solution (2×) and brine and dried (Na_2SO_4). Filtration and evaporation of the solvent afforded the ketone which was used directly for the reduction.

To a solution of $NaBH_4$ (5 mg, 0.13 mmol) in methanol (5 mL) at 0 °C was added a solution of the above ketone (38 mg, 0.08 mmol) in methanol (1 mL). After stirring for 1 h, the solvents were evaporated, and the residue was taken up in ethyl acetate and water. The layers were separated, and the organic layer was washed with brine and then dried (Na_2SO_4). Filtration and evaporation of the solvent gave a mixture (9:1) of alcohols which were separated by chromatography over silica gel (25% ethyl acetate–hexane) to provide compound **14** as the main isomer (31 mg, 88%) as a white solid: mp 162–164 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.1–7.3 (m, 10 H), 4.6 (d, J = 8.0 Hz, 1 H), 4.4 (d, J = 9.1 Hz, 1 H), 4.1 (m, 1 H), 3.8 (m, 1 H), 3.55 (m, 1 H), 2.7–2.9 (m, 4 H), 1.6–1.7 (m, 2 H), 1.4 (s, 9 H), 1.35 (s, 9 H); IR (neat) 3365, 2985, 1690 cm^{-1}; MS (70 eV) m/z 485 (M^+ + H). Anal. Calcd for $C_{28}H_{40}N_2O_5$: C, 69.39; H, 8.32; N, 5.78. Found: C, 69.12; H, 8.34; N, 5.86.

Inhibition of HIV-1 Protease. The IC_{50} values were determined using purified HIV-1 protease.[16] Inhibition of the cleavage of the peptide H-Val-Ser-Gln-Asn-(L-β-naphthylalanine)-Pro-Ile-Val-OH was assessed at 30 °C, pH = 5.5 with [Enz] = 30 pM and BSA along with the inhibitor. The reaction is quenched with H_3PO_4, and the products were analyzed by using HPLC with UV detection (225 nm) for quantification of the products. For IC_{50} determination, a substrate concentration of 0.4 mg/mL was used and the data was fit to a four-parameter sigmoidal equation.

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