Substrate-Based Cyclic Peptidomimetics of Phe-Ile-Val That Inhibit HIV-1 Protease Using a Novel Enzyme-Binding Mode

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Abstract: Results are presented for inhibitors of HIV-1 protease that demonstrate a new strategy for developing peptidomimetics, involving the replacement of flexible segments of peptide substrates with conformationally constrained hydrolytically-stable macrocyclic structural mimics. A 15-membered macrocycle that imitates the tripeptide Phe-Ile-Val was designed and incorporated into the C-terminus of Ac-Leu-Val-Phe-$CHOHCH_2$-{Phe-Ile-Val}-NH_2, an inhibitor of HIV-1 protease derived from a substrate sequence. Advantages of the macrocycle over the acyclic peptide include constraining its components into their bioactive conformation and protecting the amide bonds from enzymatic degradation, the cycle being stable to acid, gastric proteases, and plasma. Molecular modeling and X-ray structural studies reveal that the cyclic inhibitors have a unique enzyme-binding mode, the sterically unencumbered hydroxyethylamine isostere binds via *both* its hydroxyl and *protonated* nitrogen to the anionic Asp25 catalytic residues. The novel macrocycle superimposes well on the linear peptidic inhibitor for which it was designed as a structural mimic. Structural mimicry led to functional mimicry as shown by comparable inhibition of the protease by cyclic and acyclic molecules. Further modification of the acyclic N-terminus (Leu-Val-Phe) gave stable, water-soluble, potent inhibitors of HIV-1 protease. This approach may have general application to the development of mimetics of other bioactive peptides, including inhibitors of other enzymes.

Lytic replication of the Human Immunodeficiency Virus Type 1 (HIV-1) can be inhibited by interfering with viral enzymes required for replication, such as HIV-1 protease (HIVPR),[1] which processes polypeptides containing structural and functional proteins that make up the virus. Either mutagenesis[2a] of the catalytic Asp25 residue of this aspartic protease or binding of inhibitors[2b] to the active site of the protease prevents assembly of viral proteins and results in immature, noninfective virions. Many peptidic and non-peptidic inhibitors of HIVPR are now known,[1] but most suffer as drug candidates from low bioavailability which is sometimes due to hydrolytic degradation. Here we describe a rapid approach to the development of hydrolytically-stable enzyme inhibitors, based upon designing novel macrocycles that structurally mimic portions of the receptor-bound conformation of a peptide substrate. A conformationally constrained cycle that mimics the C-terminal tripeptide of a substrate is used as a template here to produce a range of potent inhibitors of HIVPR. These inhibitors interact with HIVPR using a previously unreported binding mode, involving a positively charged hydroxyethylamine transition state isostere contacting negatively charged catalytic Asp residues of the enzyme.

The peptides Ac-Ser-Leu-Asn-Phe-Pro-Ile-Val-NH_2 and Ac-Leu-Val-Phe-Phe-Ile-Val-NH_2 are HIVPR substrates which are easily converted to potent and competitive inhibitors by replacing the scissile amide bond (−CONH−) with non-cleavable transition state isosteres (e.g. $-CHOHCH_2-N-$). When the sequence Ac-Ser-Leu-Asn-Phe-{$CHOHCH_2$}-Pro-Ile-Val-NH_2 (**1**, JG365), a potent *hepta*peptidic inhibitor of HIVPR with a known binding mode,[3] was truncated to the *hexa*peptidic inhibitor Ac-Leu-Asn-Phe-{$CHOHCH_2$}-Pro-Ile-Val-NH_2 (**2**), there was a loss in activity which is recovered in the modified *hexa*peptide Ac-Leu-Val-Phe-{$CHOHCH_2$}-Phe-Ile-Val-NH_2 (**3**) (Table 1). For synthetic expediency we chose to mimic the C-terminus of the shorter inhibitor **3**.

Figure 1a compares the modeled receptor-bound structures of cyclic inhibitor **6** with the acyclic inhibitor **3**, assuming that the hydroxyethylamine nitrogen is not protonated (uncharged). On the basis of the close proximity of the Phe and Val side chains in the putative enzyme-bound conformation of **3** (Figure 1a, red), we decided to link them together in a 15-membered macrocycle. The resulting cycle in **6** (Figure 1a, white) strongly suggests good structural mimicry with Phe-Ile-Val. The tyrosine, isoleucine, and trimethylene units in **6** superimpose well on the Phe-Ile-Val residues of **3**, both side- and main-chain atoms matching corresponding atoms of the protease-bound acyclic peptide.

However, at physiological pH, the hydroxyethylamine nitrogen is likely to be protonated (p*K*a ∼10 for a secondary amine). When **6** is modeled as the protonated form (Figure 1b, green), the enzyme-binding mode matches that observed in the X-ray crystal structure of **6** bound to HIVPR (Figure 1b, blue, rmsd = 0.4 Å) more closely than does the unprotonated form (white, rmsd = 2.8 Å). Further, when the crystal structure (blue) was deliberately energy minimized with its amine nitrogen uncharged, the modeled structure reverted back to the conformation

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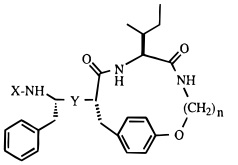
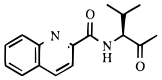
Table 1. Inhibition of HIV-1 Protease by Cyclic Peptidomimetics



	Compound	X	Y	n	K_i, nM[a]	$Log P_{o/w}$[f]
1	Ac-SLNFPIV-NH_2[b]	-	-	-	R 13 S 1	
2	Ac-LNFPIV-NH_2[c]	-	-	-	R 18 S 3	
3	Ac-LVFFIV-NH_2[d]	-	-	-	R 1.5 S 1.5	
4		Ac-Leu-Val-	-CH(OH)CH_2NH	3	R 0.6 S 0.6	2.7
5		Ac-Leu-Val-	-CH(OH)CH_2NH	4	R 1.5	3.4
6			-CH(OH)CH_2NH	3	R 4	3.6
7			-CH_2NH	3	R 44	4.3
8			-CH(OH)CH_2NH	3	R 4	2.4
9			-CH(OH)CH_2NH	3	R+S 15	4.3
10			-CH(OH)CH_2NH	3	R 0.3	4.3
11	DM323[e]		-	-	- 1	

[a] pH 6.5, I = 0.1 M, 37 °C, 50 μM substrate [Abz-NF*-6]; continuous fluorometric assay[7] using synthetic enzyme.[6] Amino acid content of inhibitors was quantified, after decomposition (6 N HCl, 24 h, 110 °C), by HPLC with Nle as the internal standard. Stereochemistry refers to the chiral alcohol. [b] JG-365, acetyl-Ser-Leu-Asn-Phe-{CH-OHCH$_2$}-Pro-Ile-Val-NH_2.[3] [c] Acetyl-Leu-Asn-Phe-{CHOHCH$_2$}-Pro-Ile-Val-NH_2. [d] Acetyl-Leu-Val-Phe-{CHOHCH$_2$}-Phe-Ile-Val-NH_2. [e] See ref 8. [f] Predicted partition coefficients in octanol−water (ref 14).

shown in white (Figure 1). These results strongly suggest that the nitrogen of the hydroxyethylamine isostere in inhibitor **6** is protonated when bound to the enzyme and that this charged nitrogen plays a key role in positioning the inhibitor in the active site of HIVPR.

A novel consequence of creating the cycle one atom removed from the hydroxyethylamine nitrogen, rather than incorporating the nitrogen in the cycle, is that the nitrogen becomes sterically unencumbered and can interact with the catalytic Asp residues in the enzyme. The X-ray crystal structure of **6** and modeling studies on **4**−**10** indicate that the nitrogen, protonated and cationic at pH 5−7, is indeed within hydrogen (or ionic)-bonding distance from both catalytic Asp carboxylates (Figure 2). At the same time the hydroxyl substituent is still close enough to form a hydrogen bond to one Asp. To our knowledge, this binding mode is unique among inhibitors of HIVPR although it has been proposed theoretically for an amino-diol isostere.[4] The effect of this shift in the transition state isostere is that P1 and P1′ residues of **6** (Figure 1c, blue, X-ray structure) occupy very different positions from those of Phe and Pro in JG365 (Figure 1c, red, X-ray structure[3]). However, side chain residues more remote from the transition state isostere (P2, P2′, P3′) occupy positions similar to those of JG365.

Compounds **4**−**10** in Table 1 were synthetically accessible by straightforward methods outlined in Scheme 1 for compounds **6** and **10** and Scheme 2 for compound **5**. Compound **7** was made by reductive amination of an equimolar mixture of the

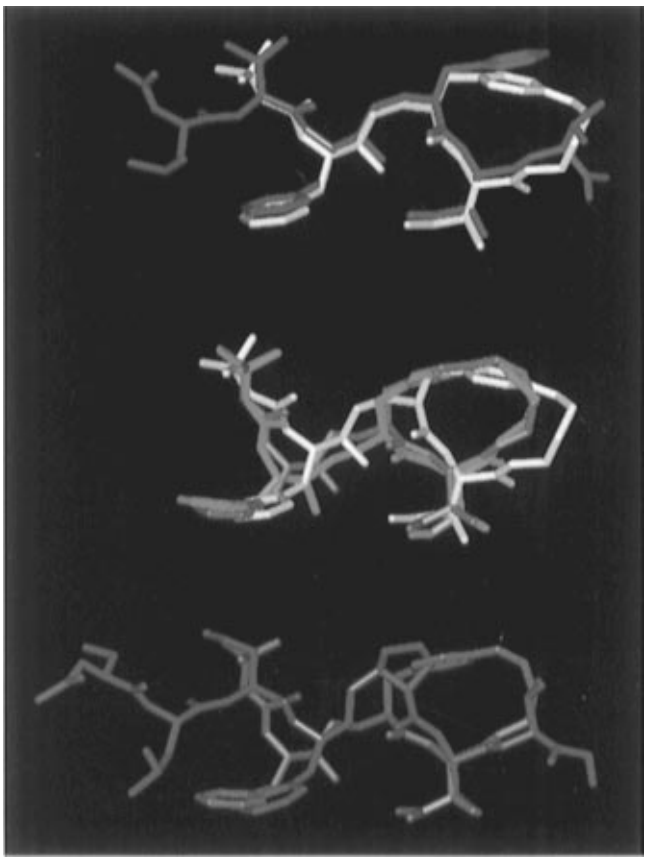


Figure 1. Computer-modeled and X-ray structures for *R*-diasteromeric inhibitors bound to HIV-1 protease: (a, top) inhibitors **3** (red) and **6** (white) modeled as unprotonated amines. (b, middle) modeled structures for **6** with unprotonated amine (white) and protonated amine (green) and compared with its X-ray structure (blue); (c, bottom) superposition of X-ray crystal structures of **6** (blue) and JG-365 (red).[3]

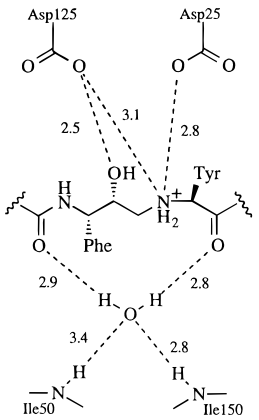


Figure 2. Hydrogen bonding distances (Å) between transition state isostere of **6** and active site residues of HIVPR from the X-ray crystal structure of the HIVPR−**6** complex.

cycle **12**[15] and Boc-Phe-CHO.[5a] Inhibitors **6** and **10** were made by solution phase coupling of the known epoxide from Boc-Phe[5b] with the C-terminal cycle **12**, followed by removal of the Boc group and capping with one of the N-terminal groups depicted in Table 1. The use of the (2*S*,3*S*)-Boc-Phe-epoxide ensured a diastereoselective synthesis of the chiral alcohol. Compounds **4**, **5**, **8**, and **9** were made as diastereomeric mixtures by coupling the cycle to the N-terminal bromomethyl ketone

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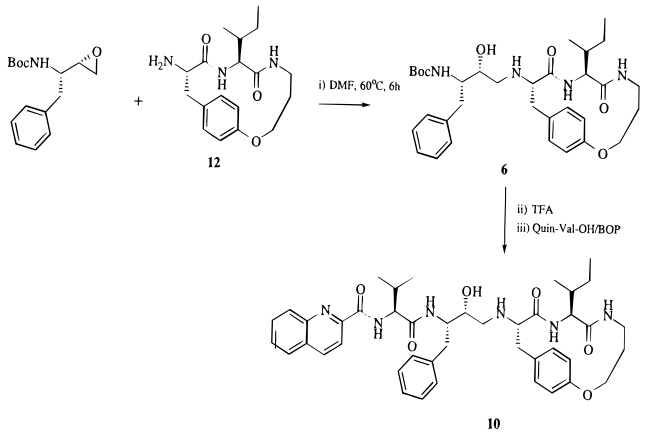
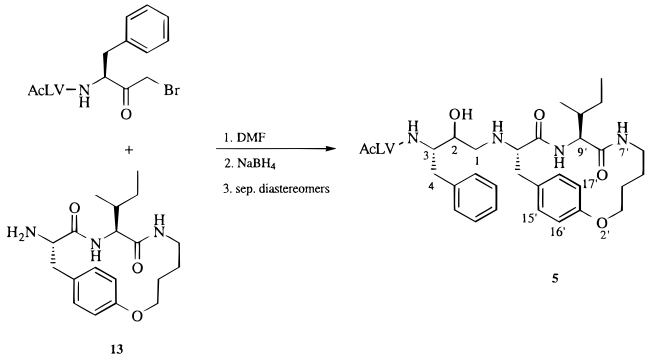
Scheme 1



Scheme 2



derivatives, followed by reduction with $NaBH_4$. The *R*-diastereomer was the major product for **4**, **5**, and **8** and was easily purified by reverse phase HPLC. Compound **9** was obtained as an equal mixture of diastereomeric alcohols that did not separate well under a range of HPLC conditions.

Table 1 reports inhibitor potencies of some molecules containing the macrocyclic mimetic of Phe-Ile-Val against synthetic[6] HIVPR *in vitro*. Under the assay conditions,[7] reference inhibitors JG365[3] and DM323[8] potently inhibit cleavage of a synthetic fluorogenic substrate.[7] The larger 16-membered ring in **5** was no more effective than the 15-membered ring of **4**, and neither ring on its own was a significant inhibitor ($IC_{50} \sim 1-10$ μM). Compounds **6**−**10** combine different N-terminal ends with the C-terminal cycle. Truncating the N-terminal tripeptide LVF to Boc-Phe reduced the potency by 1 order of magnitude (**6** vs **4**), and removing the important hydroxyl substituent of the transition state isostere further reduced potency by a second order of magnitude (**7**), despite the presence of a likely protonated amine in the active site. Compounds **8** and **10** couple the C-terminal cycle to N-terminal groups of known inhibitors.[9,10] The tetrahydrofuran terminus in **8** did not improve potency over the Boc group in **6**, presumably because the cycle in both cases shifts the furan oxygen slightly out of hydrogen-bonding contact distance. On the other hand the longer quinoline-Val-Phe of **10**, which can make more interactions with HIVPR, increased potency significantly. Compound **9** has a benzamide substituent that appears to be comparable to the other N-termini since even the diastereomeric mixture had $K_i \sim 15$ mM.

Macrocycles have previously been used[11] as renin inhibitors, and there are HIV-1 protease inhibitors containing cycles at the N-terminus,[12] but only one receptor-bound structure of these molecules is known.[12a] In this work, the receptor-bound X-ray crystal structure[3] of an acyclic peptidic inhibitor (**1**) has been used to design inhibitors containing a C-terminal macrocycle as a structural mimic that locks the otherwise flexible peptide into a protein-binding conformation. The predicted structural mimicry has been confirmed for the cycle in **6** by X-ray crystallography and can confidently be inferred for other molecules here. The modeling studies indicate that, despite the presence of the flexible trimethylene linker, the 15-membered ring is highly constrained. This is supported by the NMR inequivalence of the four aromatic protons in the cycle, implying that the aromatic ring is not able to rotate freely due to steric congestion.

Like the cyclic renin inhibitors, we find that the macrocycle in compounds **4**−**10** is quite stable to hydrolysis and proteolysis. For example, the cycle survives intact in 3 M HCl solutions at 40 °C for at least 4 days. Similarly, when this 15-membered macrocycle was incubated (37 °C, 1 h) with human cathepsin D or pepsin A (mg/mL) and analyzed by rp-HPLC and electrospray mass spectrometry, there was no evidence of degradation. When the cycle in **4**−**10** was incubated with human plasma (37 °C, 1 h), HPLC−MS analysis indicated no significant cleavage of the macrocycle. The stability of the cycle is also suggested by preliminary antiviral studies, where exposure of **10** for 4 days to cultured cells infected with HIV-1 resulted in appreciable antiviral activity.[13] In each of these experiments, the acyclic peptidic inhibitors (**1**-**3**) of HIVPR are completely degraded under the same conditions and show no antiviral activity at 10 μM concentrations.

In summary, we have described a general strategy for developing a proteolytically stable structural and functional mimic of a tripeptide component of an enzyme inhibitor. The ease of synthesis and control over chiral centers, ready variability of side chains, hydrolytic and proteolytic stability, and the high solubility (in water and octanol) make this a potentially useful strategy for mimicking other bioactive peptides, including peptidic substrates of other enzymes.

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(13) Compound **10** has similar antiviral activity (EC_{50} ~100 nM; PHA-stimulated cord blood mononuclear cells infected with HIV-1 (strain TC354); RT activity assessed 4 days after inoculation) to DM323[8] (EC_{50} 110 nM) under the same conditions.

(14) PALLAS pKalc and PrologP available from Compudrug Chemistry Ltd, Hungary.

Experimental Section

Abbreviations: PIV = Pro-Ile-Val; DIPEA = diisopropylethylamine; DMF = dimethylformamide; BOP = (benzotriazol-1-yloxy)-tris(dimethylamino)phosphonium hexafluorophosphate; HBTU = [(benzotriazolyl)oxy]-*N*′,*N*′,*N*′,*N*′-tetramethyluronium hexafluorophosphate; TFA = trifluoroacetic acid; PHA = phytohaemagglutinin.

General Methods. ^{1}H NMR spectra were recorded on a Bruker ARX 500 MHz NMR spectrometer using CD_3OH as an internal standard. Proton assignments were made using 2D COSY and TOCSY experimental data. Preparative scale HPLC separations were performed on Waters Delta-Pak Prep-Pak C18 40 × 100 mm cartridges (100 Å); analytical reverse phase HPLC was performed on Waters Delta-Pak Radial-Pak C18 8 × 100 mm cartridges (100 Å); gradient mixtures of water/0.1% TFA and water (10%)/acetonitrile (90%)/0.1%TFA were used.

Mass spectra were obtained on a triple-quadrupole mass spectrometer (PE SCIEX API III) equipped with an Ionspray (pneumatically assisted electrospray) atmospheric pressure ionization source (ISMS). Solutions of compounds in 9:1 acetonitrile/0.1% aqueous trifluoroacetic acid were injected by syringe infusion pump at micromolar to picomolar concentrations and flow rates of 2−5 mL/min into the spectrometer. Molecular ions, {[M + *n*H]*n*+/*n*, were generated by the ion evaporation process and focused into the analyzer of the mass spectrometer through a 100 mm sampling orifice. Full scan data were acquired by scanning quadrupole-1 from *m*/*z* 100−900 with a scan step of 0.1 Da and a dwell time of 2 ms. Accurate mass determinations were performed on a KRATOS MS25 mass spectrometer using electron impact ionization.

Amino acid analyses were performed by decomposition of inhibitors with 6 M HCl (24 h, 110 °C) and quantified by rp-HPLC with Nle as an internal standard.

Syntheses. (2*R*,3*S*,11′*S*,8′*S*)-3-[[(Acetyl-(*S*)-leucinyl)-(*S*)-valinyl]-amino]-4-phenyl-1-[[7′,10′-dioxo-8′-(1-methylpropyl)-2′-oxa-6′,9′-diazabicyclo[11.2.2]heptadeca-13′,15′,16′-trien-11-yl]amino]butan-2-ol (4). This compound was synthesized using our previously described procedure.[12a]

(2*R*,3*S*,12′*S*,9′*S*)-3-[[(Acetyl-(*S*)-leucinyl)-(*S*)-valinyl]amino]-4-phenyl-1-[[8′,11′-dioxo-9′-(1-methylpropyl)-2′-oxa-7′,10′-diazabicyclo-[12.2.2]octadeca-14′,16′,17′-trien-12-yl]amino]butan-2-ol (5). To a stirred solution of 12-Amino-8,11-dioxo-9-(1-methylpropyl)-2-oxa-7,-10-diazabicyclo[12.2.2]octadeca-14,16,17-triene (**13**)[15] (23 mg, 67 μmol) in THF (5 mL) was added DIPEA (4 equiv) and 3(*S*)-[[(acetyl-(*S*)-leucinyl)-(S)-valinyl]amino]-1-bromo-4-phenylbutan-2-one[12a] (33 mg, 67 μmol). The reaction mixture was stirred for 60 min at room temperature. The mixture was diluted with ethyl acetate (50 mL), washed with 1 M HCl, and dried and the solvent removed in vacuo. The resultant ketoethylamine was dissolved in methanol (10 mL) and reduced with sodium borohydride (100 μmol) by stirring the solution at −5° for 30 min. The reaction was quenched with acetic acid and evaporated to dryness, and the crude residue was purified by reverse phase HPLC [gradient (water/0.1% TFA) to 0:100 (water/0.1% TFA): (water (10%)/acetonitrile (90%)/TFA (0.1%)) over 35 min (flow rate 1.5 mL/min). Only the *R*-diastereoisomer was isolated (6 mg, 11.7%); retention time = 21.15 min. ^{1}H NMR (500 MHz, 290 K, CD_3OD): δ 8.33 (d, *J* = 5.0 Hz, 1H, Leu-NH), 8.01 (m, 1H, NH), 7.76 (d, *J* = 5.0 Hz, 1H, Val-NH), 7.65−7.73 (m, 2H, Ile-NH, Phe-NH), 6.80−7.30 (m, 9H, ArH), 4.32 (m, 1H, H3′), 4.25 (m, 1H, Leu-αCH), 4.13−4.19 (m, 3H, Tyr-αCH, Phe-αCH, H3′), 3.80−3.87 (m, 3H, Val-αCH, Ile-αCH, H2), 3.46 (m, 1H, H6′), 3.30 (m, 2H, Phe-βCH, Tyr-βCH), 3.00−3.43 (m, 2H, H1, H1), 2.86 (m, 1H, Tyr-βCH), 2.73 (m, 1H, Phe-βCH), 2.63 (m, 1H, H6′), 2.06 (s, 3H, acetyl), 1.97 (m, 1H, H4′),1.85 (m, 1H, Val-βCH), 1.34−1.75 (m, 8H, H4′, H5′, H5′, Leu-γCH, Leu-βCH_2, Ile-βCH, Ile-γCH), 1.04 (m, 1H, Ile-γCH), 0.95 (d, *J* = 6.0 Hz, 3H, Leu-δ CH_3), 0.88 (d, *J* = 6.0 Hz, 3H, Leu-δ CH_3), 0.87 (m, 3H, Ile-δ CH_3), 0.81 (d, *J* = 6.0 Hz, 3H, Ile-γCH_3), 0.75 (d, *J* = 5.0 Hz, 3H, Val-γCH_3), 0.61 (d, *J* = 5.0 Hz, 3H, Val-γCH_3). ISMS: *m*/*z* 765 (M + H). HRMS: calcd for $C_{42}H_{64}N_6O_7$ 764.4837, found 764.4840.

(2*R*,3*S*,11′*S*,8′*S*]-3-(*tert*-Butoxycarbonylamino)-4-phenyl-1-[[7′,-10′-dioxo-8′-(1-methylpropyl)-2′-oxa-6′,9′-diazabicyclo[11.2.2]heptadeca-13′,15′,16′-trien-11′-yl]amino]butan-2-ol (6). 11-Amino-7,10-dioxo-8-(1-methylpropyl)-2-oxa-6,9-diazabicyclo[11.2.2]heptadeca-13,15,16-triene (**12**)[15] (23 mg, 69 μmol) was dissolved in DMF (1 mL), and BocPhe epoxide[5b] (12 mg, 46 μmol) added. The resultant mixture was heated to 70° for 12 h. The crude residue was purified by reverse phase HPLC [gradient (water/0.1% TFA) to 50:50 (water/0.1% TFA): (water (10%)/acetonitrile (90%)/TFA (0.1%)) over 60 min] to give the title compound (5 mg, 18.2%), retention time = 56.20 min, as a white powder after lyophilization. ^{1}H NMR (500 MHz, 290 K, CD_3OD): δ 7.81 (m, 1H, NH), 6.91−7.41 (m, 10H, ArH, Ile-NH), 6.44 (d, *J* = 9.0 Hz, 1H, Phe-NH), 6.06 (m, 1H, Tyr-NH), 4.44 (m, 1H, H3′), 4.27 (m,-1H, H3′), 3.83 (m, 1H, H2), 3.69 (m, 2H, Tyr-αCH, Phe-αCH), 3.51 (m, 1H, Ile-αCH), 3.40 (m, 1H, H5′), 3.10−3.17 (m, 2H, Phe-βCH, Tyr-βCH), 3.03 (m, 1H, H1), 2.86−2.92 (m, 2H, H1, H5′), 2.53−2.61 (m, 2H, Phe-βCH, Tyr-βCH), 2.22 (m, 1H, H4′), 1.84 (m, 1H, H4′), 1.56 (m, 1H, Ile-βCH), 1.38 (m, 1H, Ile-γCH), 1.25 (s, 9H, $(CH_3)_3$), 0.96 (m, 1H, Ile-γCH), 0.83 (t, *J* = 5.0 Hz, 3H, Ile-δCH_3), 0.73 (d, *J* = 5.0 Hz, 3H, Ile-γCH_3). ISMS: *m*/*z* 597 (M + H). HRMS: calcd for $C_{33}H_{48}N_4O_6$ 596.3574, found 596.3579.

(2*S*,11′*S*,8′*S*)-2-(*tert*-Butoxycarbonylamino)-3-phenyl-1-[[7′,10′-dioxo-8′-(1-methylpropyl)-2′-oxa-6′,9′-diazabicyclo[11.2.2]heptadeca-13′,15′,16′-trien-11′-yl]amino]propane (7). The macrocycle **12**[15] (23 mg, 69 μmol) was added to a solution of BocPhe aldehyde[5a] (17 mg, 69 μmol), $MgSO_4$ (100 mg), and sodium cyanoborohydride (47 mg, 76 μmol) in THF/1% acetic acid solution (5 mL). The mixture was stirred overnight at room temperature quenched with 1 M HCl (1 mL), and evaporated in vacuo, and the crude residue was purified by reverse phase HPLC [gradient (water/0.1% TFA) to 0:100 (water/0.1% TFA): (water (10%)/acetonitrile (90%)/TFA 0.1%) over 35 min (flow rate 1.5 mL/min), retention time = 21.24 min. ^{1}H NMR (500 MHz, 290 K, CD_3OD): δ 7.90 (m, 1H, NH), 6.82−7.37 (m, 11H, ArH, Phe-NH, Ile-NH), 6.3 (m, 1H, Tyr-NH), 4.39 (m, 1H, H3′), 4.25 (m,1H, H3′), 4.08 (m, 2H, Tyr-αCH, Phe-αCH), 3.57 (m, 1H, H5′), 3.46 (m, 1H, Ile-αCH), 3.34 (m, 1H, Tyr-βCH), 2.68−3.07 (m, 6H, Tyr-βCH, Phe-βCH_2, H1, H1, H5′), 2.27 (m, 1H, H4′), 1.76 (m, 1H, H4′), 1.50 (m, 1H, Ile-βCH), 1.38−1.42 (m, 10H, Ile-γCH, $(CH_3)_3$), 0.92 (m, 1H, Ile-γCH), 0.86 (t, *J* = 6.0 Hz, 3H, Ile-δCH_3), 0.73 (d, *J* = 5.0 Hz, 3H, Ile-γCH_3). ISMS: *m*/*z* 567 (M + H). HRMS: calcd for $C_{32}H_{46}N_4O_5$ 566.3468, found 566.3447.

(2*R*,3*S*,11′*S*,8′*S*)-3-[[[(3(*S*)-Tetrahydrofuranyl)oxy]carbonyl]amino]-4-phenyl-1-[[7′,10′-dioxo-8′-(1-methylpropyl)-2′-oxa-6′,9′-diazabicyclo-[11.2.2]heptadeca-13′,15′,16′-trien-11′-yl]amino]butan-2-ol (8). The macrocycle **12** (23 mg, 69 μmol) was reacted with 3(*S*)-[[[(3′(*S*)-tetrahydrofuranyl)oxy]carbonyl]amino]-1-bromo-4-phenylbutan-2-one[17] (24 mg, 69 μmol) as described for the synthesis of **5**. Subsequent purification by reverse phase HPLC [gradient (water/0.1% TFA) to 0:100 (water/0.1% TFA):(water (10%)/acetonitrile (90%)/TFA (0.1%)) over 35 min (flow rate 1.5 mL/min) gave the title compound (6 mg, 14.2%) as a white powder, retention time = 18.03 min. The reaction gave only a very small amount of the 2(*S*) diastereomer which could not be isolated. ^{1}H NMR (500 MHz, 290 K, CD_3OD): δ 7.85 (m, 1H, NH), 7.37 (d, *J* = 5.5 Hz, 1H, Ile-NH), 6.83−7.32 (m, 9H, ArH), 6.44 (d, *J* = 9.0 Hz, 1H, Phe-NH), 5.05 (m, 1H, furan-H), 4.40 (m, 1H, H3′), 4.26 (m, 1H, H3′), 3.64−3.89 (m, 6H, H2, Tyr-αCH, Phe-αCH, 3 furan-H), 3.58 (m, 1H, H5′), 3.47−3.53 (m, 2H, Ile-αCH, furan-H), 3.22 (m, 1H, Phe-βCH), 3.12 (m, 1H, H1), 3.00 (m, 1H, H1), 2.91 (m, 2H, Tyr-βCH, Phe-βCH), 2.78−2.84 (m, 2H, Tyr-βCH, H5′), 2.26 (m, 1H, H4′), 2.10 (m, 1H, furan-H), 1.93 (m, 1H, furan-H), 1.76 (m, 1H, H4′), 1.57 (m, 1H, Ile-βCH), 1.42 (m, 1H, Ile-γCH), 0.99 (m, 1H,

(15) A synthesis of cycle **12** has been reported.[12a] In this work, **12** was made in three steps by coupling 3-bromopropylamine to Boc-Ile (94%) with BOP reagent, deprotecting (TFA), coupling with Boc-Tyr (96%), and cyclization with base (50%). 12-Amino-8,11-dioxo-9-(1-methylpropyl)-2-oxa-7,10-diazabicyclo[12.2.2]octadeca-14,16,17-triene (**13**) was made similarly by coupling 4-aminobutanol to Boc-Ile, converting the hydroxyl to bromide with CBr_4/PPh_3,[16] deprotecting (TFA), coupling to Boc-Tyr, and cyclizing (∼50% yield overall). Final deprotection of both cycles was achieved by stirring in a solution of 25% TFA in DCM at room temperature for 15 min. The TFA was evaporated in vacuo and the residue dissolved in saturated $NaHCO_3$ solution and extracted with ethyl acetate.

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(17) 3(*S*)-[[[(3′(*S*)-Tetrahydrofuranyl)oxy]carbonyl]amino]-1-bromo-4-phenylbutan-2-one was synthesized in three steps by reacting 3(*S*)-hydroxytetrahydrofuran and phenylalanine methyl ester·HCl according to the procedure of Ghosh et al.[19] (78%), de-esterification of the resultant ester, preparation of the diazoketone[12a] (95%), and reaction with HBr (91%).[12a]

Ile-γCH), 0.86 (t, J = 5.5 Hz, 3H, Ile-δCH_3), 0.78 (d, J = 5.0 Hz, 3H, Ile-γCH_3). ISMS: m/z 611 (M + H).

(2*R*,3*S*,11′*S*,8′*S*)-3-[[(3-Methylphenyl)carbonyl]amino]-4-phenyl-1-[[7′,10′-dioxo-8′-(1-methylpropyl)-2′-oxa-6′,9′-diazabicyclo[11.2.2]-heptadeca-13′,15′,16′-trien-11′-yl]amino]butan-2-ol (9). The macrocycle **12** (23 mg, 69 μmol) was reacted with 3(*S*)-[[(3′-methylphenyl)carbonyl]amino]-1-bromo-4-phenylbutan-2-one[18] (24 mg, 69 μmol) as described for the synthesis of **5**. Purification by reverse phase HPLC [gradient (water/0.1% TFA) to 0:100 (water/0.1% TFA):(water (10%)/acetonitrile (90%)/TFA (0.1%)) over 35 min (flow rate 1.5 mL/min) gave a diastereomeric mixture of **9** (7 mg, 16.5%), retention time = 20.48 min. The NMR spectra indicated the presence of a 1:1 mixture of diastereomers by a doubling of all resonances at 290 K. The spectra was consistent with the structure. Several attempts to separate diastereomers by reverse phase HPLC were unsuccessful, and the diastereomeric mixture was tested for HIV-1 PR inhibition. ISMS: m/z 615 (M + H). HRMS: calcd for $C_{36}H_{46}N_4O_5$ 614.3468, found 614.3467.

(2*R*,3*S*,11′*S*,8′*S*)-3-[(2-Quinolinylcarbonyl)amino]2-propylmethyl]-carbonyl]amino-4-phenyl-1-[11′-[7′,10′-dioxo-8′-(1-methylpropyl)-2′-oxa-6′,9′-diazabicyclo[11.2.2]heptadeca-13′,15′,16′-triene]amino]-butan-2-ol (10). Compound **6** (3 mg, 5 μmol) was deprotected with 25% TFA in dichloromethane (1 mL) over 15 min and evaporated in vacuo. The residue was dissolved in DMF (1 mL), and to it was added quinaldoyl-(S)-valine[20] (1.6 mg, 6 μmol), BOP (2.6 mg, 6 μmol), and DIPEA (3 equiv). Then the mixture was stirred for 1 h at room temperature. The solvent was evaporated under reduced pressure and the residue purified by reverse phase HPLC [gradient (water/0.1% TFA) to 0:100 (water/0.1% TFA):(water (10%)/acetonitrile (90%)/TFA (0.1%)) over 35 min to give **10** (3 mg, 79%), retention time = 22.42 min, as a white powder. ^{1}H NMR (500 MHz, 290 K, CD_3OD): δ 8.77 (d, J = 7.48 Hz, 1H, Val-NH), 8.44 (d, J = 8.45 Hz, 1H, ArH), 8.25 (d, J = 8.89 Hz, 1H, Phe-NH), 8.21 (d, J = 8.45 Hz, 1H, ArH), 8.16 (d, J = 8.45 Hz, 1H, ArH), 7.99 (d, J =8.45 Hz, 1H, ArH), 7.83−7.91 (m, 2H, ArH, NH), 7.71 (m, 1H, ArH), 7.31 (d, J = 7.33Hz, 1H, Ile-NH), 6.74−7.24 (m, 11H, ArH), 4.36 (m, 1H, H3′), 4.24 (m,1H, H3′), 4.11−4.17 (m, 3H, Val-αCH, Tyr-αCH, Phe-αCH), 3.78 (m, 1H, H2), 3.56 (m, 1H, H5′), 3.47 (m, 1H, Ile-αCH), 3.29−3.33 (m, 2H, Phe-βCH, Tyr-βCH), 3.10 (m, 1H, H1), 3.03 (m, 1H, H1), 2.79 (m, 1H, H5′), 2.53−2.62 (m, 2H, Phe-βCH, Tyr-βCH), 2.25 (m, 1H, H4′), 2.01 (m, 1H, Val-βCH), 1.73 (m, 1H, H4′), 1.58 (m, 1H, Ile-βCH), 1.43 (m, 1H, Ile-γCH), 0.97 (m, 1H, Ile-γCH), 0.85 (d, J = 6.62 Hz, 3H, Val-γCH_3), 0.82 (t, J = 7.33 Hz, 3H, Ile-δCH_3), 0.77 (d, J = 6.74 Hz, 3H, Ile-γCH_3), 0.71 (d, J = 6.62 Hz, 3H, Val-γCH_3). ISMS: m/z 751 (M + H). HRMS: calcd for $C_{43}H_{54}N_6O_6$ 750.4105, found 750.4097.

X-ray Crystallography. Synthetic[6] HIVPR (mutant Q7K, L33I) was mixed with a DMSO solution of **6** (ratio 1:10). Crystals of the HIVPR−**6** complex were grown at 20 °C by vapor diffusion from 45% $(NH_4)_2SO_4$ in 0.1 M acetate buffer (pH 5.4) and were isomorphous with those of the HIVPR−JG365 complex.[6] Crystallographic data were measured and data refined as described[12a] with X-PLOR[21] using an inhibitor−HIVPR complex[12a] (pdb accession code 1CPI) as the starting model. The program O[22] was used for modeling and rebuilding. The final refined structure, including 118 solvent molecules, has an *R*-factor of 0.179 with rms deviations from ideality of 0.010 Å and 1.66°, respectively, for bond distances and bond angles. This structure identified the *R*-configured alcohol of **6** and showed the unusual binding mode depicted in Figures 1 (blue) and 2. A detailed analysis of this crystal structure will be published separately. Atomic coordinates for the structure of the HIVPR−**6** complex have been deposited with the Brookhaven Protein Data Bank (accession code 1MTR).

Computer Modeling. Energy minimization was carried out using Discover (version 2.9.6 [23a]); the CVFF forcefield[23b] parameters were automatically assigned. Of the two catalytic aspartic acids (Asp25 and Asp125),[3] Asp25 was selectively ionized[23c] along with the hydroxyethylamine NH of the inhibitor when the charged model was examined. For the uncharged modeling all acids and bases were neutral. Protein atoms were fixed according to the JG365-bound conformation[3] of HIVPR, a dielectric of 1.0 was employed, and the inhibitor atoms and crystallographic waters were free to move. Calculations were performed using algorithms steepest descents (down to a gradient of ≤10 kcal mol^{-1} Å^{-1}) followed by conjugate gradients (<1 kcal mol^{-1} Å^{-1}) and then Newton Raphson (<0.001 kcal mol^{-1} Å^{-1}). Charged and uncharged models were compared with the crystal structure using an rms comparison over all heavy atoms using Insight II (version 2.3.5[23a]).

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(18) 3(*S*)-[[(3′-Methylphenyl)carbonyl]amino]-1-bromo-4-phenylbutan-2-one was synthesized in three steps by coupling *m*-toluic acid and phenylalanine methyl ester·HCl with BOP (100%), de-esterification of the resultant ester with base, preparation of the diazoketone[12a] (60%), and reaction with HBr (91%).[12a]

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