# Regioselective Structural and Functional Mimicry of Peptides. Design of Hydrolytically-Stable Cyclic Peptidomimetic Inhibitors of HIV-1 Protease

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**Abstract:** Hydrolytically-stable cyclic mimetics of the tripeptides Leu-Asn-Phe and Phe-Ile-Val were designed and incorporated into peptidic inhibitors, Ac-{Leu-Asn-Phe}-$CHOHCH_2$-Pro-Ile-Val-$NH_2$ and Ac-Leu-Val-Phe-$CHOHCH_2$-{Phe-Ile-Val}-$NH_2$, of HIV-1 protease. Structural mimicry has been established through molecular modeling and X-ray crystallographic studies of inhibitors bound to HIV-1 protease. Cyclic and acyclic inhibitors had similar conformations that were superimposable and formed similar interactions with the enzyme. Functional mimicry was demonstrated by comparable inhibition of the protease by acyclic and cyclic molecules. Further substitution of the residual acyclic Pro-Ile-Val or Leu-Val-Phe inhibitor components, with Pip-NHtBu or Boc-Phe, respectively, gave hydrolytically stable, water-soluble, lipophilic inhibitors of similar potency. The use of cycles to fix the conformations of amino acid sequences in peptides allows regioselective structural mimicry leading to functional mimicry and also permits localized structure−activity optimization in inhibitors of HIV-1 protease. This approach might be usefully applied to inhibitors of other proteins.

Therapeutic agents that potently and selectively inhibit the replication of human immunodeficiency viruses (HIV) are needed to treat the 20 million people worldwide who are infected with HIV. HIV-1 protease (HIVPR)[1] is an aspartic protease[2] which processes polypeptides (Pr160 and Pr50) transcribed from *gag* and *pol* genes and is essential for replication of HIV (type 1). Inhibitors of this enzyme prevent assembly of viral proteins and maturation of HIV-1 and result in noninfective virions.[3] They are consequently potential candidates for drug development. The leading inhibitor[4] is now in third-phase clinical trials despite low oral bioavailability, a problem shared by many peptidic inhibitors of HIVPR due in part to their hydrolyses by peptidases in the gut, blood, and cells.[1]

A new strategy for rationally developing hydrolytically-stable protease inhibitors involves independent structural (hence functional) mimicry of peptide segments of protease substrates. Such regioselective optimization of amino acid sequences in peptidic inhibitors of HIVPR has not previously been successful[1,5] because of unpredictable cooperative effects on conformations of both inhibitor and enzyme caused by multiple variations in side chains. The goal of this work was instead to *copy* precisely the three-dimensional positions of side- and main-chain atoms in amino acids of a single peptidic substrate/inhibitor using hydrolytically-stable isostructural replacements. This approach avoids conformational rearrangement of either inhibitor or enzyme by mimicking a specific enzyme-binding conformation of the inhibitor. We now describe the rational design and application of hydrolytically-stable macrocycles as structural replacements for the Leu-Asn-Phe and Phe-Ile-Val components of HIVPR substrates {Leu-Asn-Phe}-Pro-Ile-Val and Leu-Val-Phe-{Phe-Ile-Val}. Incorporating these cycles into peptidic inhibitors that contain a transition state isostere[5] replacement for cleavable Phe-Pro and Phe-Phe amide bonds, Ac-Leu-Asn-Phe-{*S*-$CHOH-CH_2$}-Pro-Ile-Val-OMe and Ac-Leu-Val-Phe-{*R*-$CHOH-CH_2$}-Phe-Ile-Val-$NH_2$, resulted in similar inhibitor potencies against HIVPR. Thus, structural mimicry has been demonstrated to lead to functional mimicry.

The 2.4 Å resolution X-ray crystal structure[6] of Ac-Ser-Leu-Asn-Phe-{(*S*)-$CHOH-CH_2$}-Pro-Ile-Val-OMe (JG365) bound to HIVPR was used for computer modeling studies.[7] Alternating amino acid side chains (e.g. Leu and Phe) were observed to be close enough to be connected in a cyclic 15-membered ring. Macrocycles incorporated into renin inhibitors[8] were previously found to protect peptide bonds from peptidases, so it was anticipated that cyclic mimics of HIVPR inhibitors would also be hydrolytically stable. It was expected that inhibitor−enzyme

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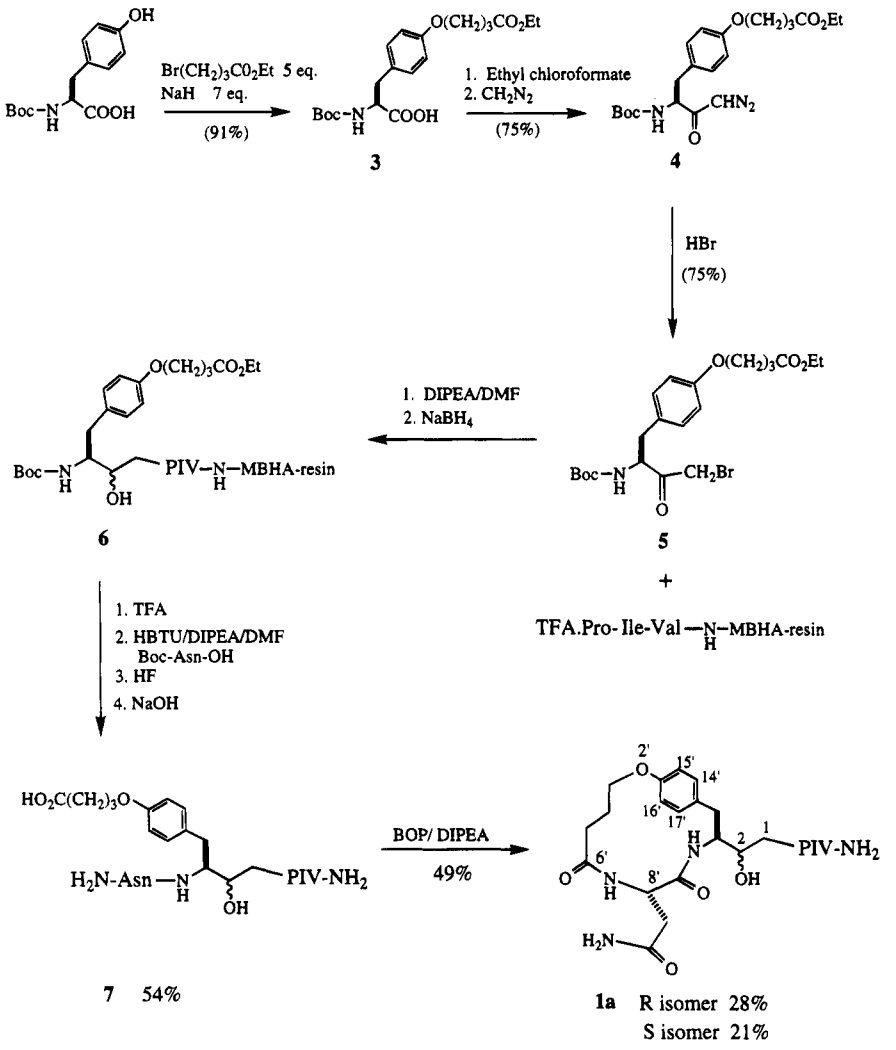
**Scheme 1.** Solid Phase Synthesis of **1a**




affinity would be increased by cyclic peptides that conformationally constrain otherwise flexible amino acids to an enzyme-binding conformation.

Structure **1a**, with *S*-stereochemistry at the chiral alcohol, was found from modeling studies to superimpose well (not shown) upon the HIVPR-bound conformation of JG365, suggesting structural mimicry. While there was a good match for these two structures, the *R*-isomer of the chiral alcohol superimposed poorly. Scheme 1 summarizes a solid phase synthesis[9] of macrocycle **1a**, modified slightly[10] for **1c**. Boc-tyrosine was O-alkylated and then converted in two steps to bromomethyl ketone **5** (51%). The C-terminus of **5** was coupled to the tripeptide PIV (assembled on MBHA resin using HBTU), the ketone reduced with $NaBH_4$, and the N-terminus extended with Asn, before cleavage from resin (HF). De-esterification with NaOH gave a mixture of diastereomeric alcohols (**7**, 54%). Dilute (mM) solutions of **7** were cyclized with BOP to **1a**, and diastereomers were separated by HPLC.

The 2.0 Å crystal structure of **1a** bound to HIVPR was determined to establish how well **1a** mimicked the enzyme-bound conformation[6] of JG365. Electron density for **1a** bound in the active site of the HIVPR homodimer was continuous and well defined; the average temperature factor for inhibitor atoms was 18 Å$^2$. The structure established *S*-stereochemistry for the more active alcohol diastereomer and revealed that the HIVPR-bound inhibitor conformation was very similar to that observed[6] for HIVPR-bound JG365 (Figure 1a). Hydrogen bonds between HIVPR and **1a** were comparable to those in the enzyme complex of JG365, including interactions between the hydroxyl group of the chiral alcohol and the catalytic aspartates (25, 125) of HIVPR, as well as between inhibitor main-chain atoms and enzyme flap residues and the flap water molecule.

Energy-minimized computer models of **2a** and Ac-Leu-Val-Phe-{(*R*)-CHOH-$CH_2$}Phe-Ile-Val-$NH_2$ superimpose well (Figure 1b). In **2a**, the cycle based upon Phe-Ile-Val is thus predicted to be isostructural with the C-terminal tripeptide. The benzylic component in the cycle of **2a** and its acyclic mimic runs along the enzyme wall rather than filling the P1′ pocket. Scheme 2 summarizes a solution synthesis of **2a** (15% yield) through coupling of components **17** and **11**, each prepared in five steps (83%) and three steps (68%), respectively, from Boc-

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(10) **1c** was synthesised in solution by reacting bromomethyl ketone **5** with the *tert*-butyl amide of L-pipecolinic acid, reducing with $NaBH_4$ to give the hydroxyethylamine analogue of **6**, coupling **6** to Asn with HBTU, and elaboration as in Scheme 1. **2b** was synthesized after reduction of BocPhe-$COCH_2Br$ to the bromohydrin, chromatographic separation of diastereomers; one was then treated with alcoholic KOH, giving the *S,S*-epoxide[11] which was subsequently added to the cycle **11**.

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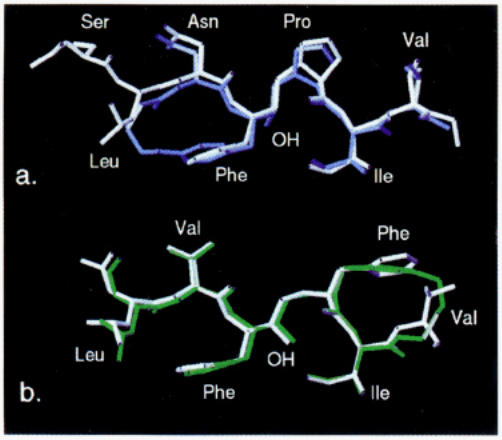


**Figure 1.** (a) Overlayed structures of the *S*-diastereomers of JG365 (white) and **1a** (purple) taken from X-ray crystal structures of their complexes with HIVPR. Side-chain residues of JG365 are labeled. (b) Overlayed structures of the *R*-diastereomers of Ac-Leu-Val-Phe-{CHOH-$CH_2$}-Phe-Ile-Val-$NH_2$ (white) and **2a** (green) taken from energy minimized computer models of the complexes with HIVPR.[7] Side chains of the acyclic inhibitor are labeled.

(Ile/Val)OH. **2b** was made stereoselectively by a similar method.[10] Functional mimicry for **1a** and **2a** was established by measuring inhibition of synthetic[12] HIV-1 protease *in vitro*.[13] Table 1 compares assay data for inhibition of HIVPR under conditions where JG365 and DM323[14] potently inhibit cleavage of a synthetic fluorogenic substrate.[13] The *S*-isomer of **1a**, containing a Leu-Asn-Phe mimetic, has activity similar to that of Ac-Leu-Asn-Phe-{(*S*)-CHOH-$CH_2$}-Pro-Ile-Val-$NH_2$ ($IC_{50}$ 9 nM). Consistent with modeling and structural data, the *S*-isomer of **1a** was a far more potent inhibitor of HIVPR than the *R*-isomer (Table 1) and truncation (**1b**) diminished the activity further. Similarly **2a**, containing a Phe-Ile-Val mimetic, had activity comparable to that of Ac-Leu-Val-Phe-{(*R*)-CHOH-$CH_2$}-Phe-Ile-Val-$NH_2$ ($IC_{50}$ 5 nM), but in this case the diastereomers had similar activity.

All of these compounds are susceptible to hydrolytic cleavage of their acyclic peptide bonds, but the cyclic components of **1a** and **2a** were not cleaved by the hydrolytic enzymes pepsin A, gastricsin, and cathepsin D.[15] While all compounds were water soluble, several do not have sufficient lipophilicity (e.g., calculated[16] log *P*(o/w) < 0, Table 1) to penetrate cell membranes. To reduce peptidase activity and increase lipid solubility, the acyclic Pro-Ile-Val residues in **1a** were replaced with the hydrolytically more stable and more lipophilic *tert*-butyl amide of L-pipecolic acid, giving **1c**, which had inhibitor potency similar to that of **1a**. The *R*-configuration in **1c** was more potent than the *S*-isomer. Modeling studies indicated that the bulky NtBu substituent in the P2′ position affects the preferred stereochemistry; an *S*- to *R*-inversion for isomer preference by HIVPR was previously reported for Ro-318959, where the bulky decahydroisoquinoline substitutes for Pro.[4] On the other hand, replacing the hydrolytically unstable acyclic portion of **2a** with the hydrolytically stable and more lipophilic Boc-Phe led to an equipotent inhibitor **2b**, for which the *R*-diastereomer was the more potent isomer.

Compounds **1** and **2** are examples of HIV-1 protease inhibitors which contain constrained 15-membered rings. Other cyclic inhibitors of aspartic proteases, namely HIV-1 protease[19] and renin,[20] have been synthesized with even larger rings, but while their activities are known, their receptor-bound conformations have not been determined. One of these compounds[19] does however exemplify to some extent this concept of regioselective optimization since it consists of a macrocycle attached to a difluoroketone transition state isostere.

In summary, the use of cycles to fix the conformations of tripeptide components of HIVPR inhibitors allows regioselective structural and functional mimicry. It also permits predictable regioselective structure−activity optimization which may lead to even more potent optimized structures. The ease of synthesis, variability of side chains, hydrolytic stability, ease of incorporating chiral centers, and water and lipid solubility make this an attractive approach for developing HIVPR inhibitors. This method may have wider application for drug development.

## Experimental Section

**General Methods.** All materials were obtained commercially as reagent grade unless otherwise stated. Melting points were determined on a Reichert hot stage apparatus and are uncorrected. $^1$H NMR spectra were recorded on a Bruker ARX 500 MHz spectrometer using $D_2O$ as internal standard for water-soluble samples or TMS for spectra recorded in $CDCl_3$. Proton assignments were determined by 2D COSY and TOCSY experiments. $^{13}$C NMR spectra were measured on a Varian Gemini 300 NMR spectrometer, and chemical shifts are reported in parts per million relative to $CD_3OH$, EtOH, or $CDCl_3$. Preparative scale reverse phase HPLC separations were performed on Waters Delta-Pak PrepPak $C_{18}$ 40 mm × 100 mm cartridges (100 Å) and analytical reverse phase HPLC on Waters Delta-Pak Radial-Pak $C_{18}$ 8 mm × 100 mm cartridges (100 Å), using gradient mixtures of water/0.1%TFA and water (10%)/acetonitrile (90%)/TFA (0.1%).

Mass spectra were obtained on a triple-quadrupole mass spectrometer (PE SCIEX API III) equipped with an ion spray (pneumatically assisted electrospray)[21] atmospheric pressure ionization source (ISMS). Solutions of compounds in 9:1 acetonitrile/0.1% aqueous trifluoroacetic acid were injected by syringe infusion pump at micromole to picomole concentrations and flow rates of 2−5 μL/min into the spectrometer. Molecular ions, $\{[M + nH]^{n+}\}/n$, were generated by the ion evaporation process[22] 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.

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(15) Macrocyclic inhibitors were incubated (37 °C, 1 h, 5 units of enzyme) with human cathepsin D, pepsin A, and gastricsin and the product(s) monitored by RP-HPLC and electrospray mass spectrometry. While the cyclic components survived intact, the acyclic components were hydrolyzed under these conditions (E. Blair, private communication).

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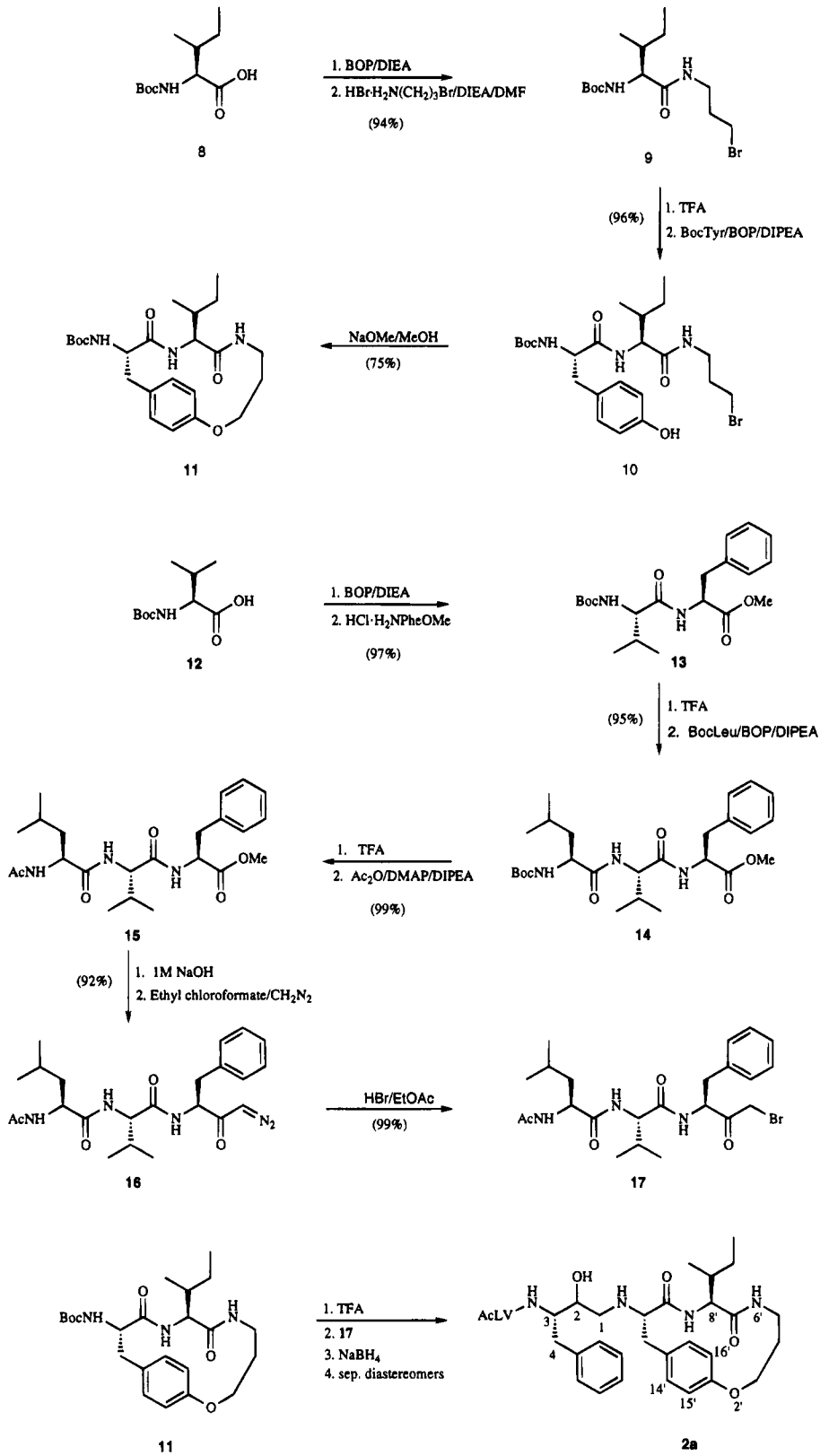
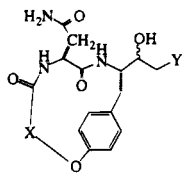
**Scheme 2.** Synthesis of **2a**



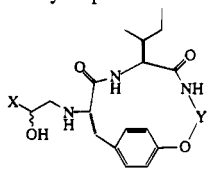

**Abbreviations:** LNF = Leu-Asn-Phe; PIV = Pro-Ile-Val; DIPEA = diisopropylethylamine; MBHA = *p*-methylbenzhydrylamine resin·HCl, 0.79 mequiv/g; DMF = dimethylformamide; BOP = [(benzotriazol-1-yloxy)tris(dimethylamino) phosphonium]hexafluorophosphate; HBTU = *O*-benzotriazole *N*′,*N*′,*N*′,*N*′-tetramethyluronium hexafluorophosphate; TFA = trifluoroacetic acid.

**Ethyl 4-[4′-[[2-[(*tert*-Butoxycarbonyl)amino]-2-carboxy]ethyl]-phenoxy]butanoate (3).** To a suspension of NaH (1.2 g, 50 mmol) in dry THF (80 mL) was slowly added Boc-tyrosine (3.5 g, 12.46 mmol), under an argon atmosphere. The solution was stirred at room temperature for 5 min, after which ethyl 4-bromobutanoate (7.28 g, 37.35 mmol) was added in one portion and the suspension heated at reflux for 16 h. A further 3 equiv of both NaH and ethyl 4-bromobutanoate was added, and heating was continued for 5 h. Solvent was removed under reduced pressure, the residue dissolved in water (100 mL), and the basic solution extracted with diethyl ether (3 × 40 mL) to remove unreacted ethyl 4-bromobutanoate and ethyl cyclopropanecarboxylate side product. The aqueous layer was acidified (pH = 2) with 1 M $KHSO_4$ and extracted with ethyl acetate (4 × 50 mL). The organic phase was dried and evaporated to give the title compound

**Table 1.** Inhibition Of HIV-1 Protease by Peptidomimetics



1a-c



2a-b

| compd | X | Y | | $IC_{50}$, nM[a] | log $P_{o/w}$[b] |
|---|---|---|---|---|---|
| Ac-SLNFPIV-$NH_2$[c] | | | *R* | 22 | −1.8 |
| | | | *S* | 1 | |
| Ac-LNFPIV-$NH_2$ | | | *R* | 60 | −0.1 |
| | | | *S* | 9 | |
| **1a** | $-(CH_2)_3-$ | PIV-$NH_2$ | *R* | 1580 | −0.7 |
| | | | *S* | 39 | |
| **1b** | $-(CH_2)_3-$ | PI-$NH_2$ | *R* | 8500 | −1.0 |
| | | | *S* | 9500 | |
| **1c** | $-(CH_2)_3-$ | Pip-NtBu[d] | *R* | 16 | 0.1 |
| | | | *S* | 5600 | |
| Ac-LVFFIV-$NH_2$[e] | | | *R* | 5 | 2.7 |
| | | | *S* | 5 | |
| **2a** | Ac-LVF− | $-(CH_2)_3-$ | *R* | 2 | 2.7 |
| | | | *S* | 2 | |
| **2b** | Boc-F− | $-(CH_2)_3-$ | *R* | 15 | 3.6 |
| | | | *R* + *S* | 50 | |
| DM-323[f] | | | | 1 | 4.1 |

[a] pH 6.5, $I$ = 0.1 M, 37 °C, 50 μM substrate [Abz-NF*-6]; fluorometric assay (ref 13) using synthetic enzyme (ref 12). Amino acid content of inhibitors was quantified, after decomposition (6 N HCl, 24 h, 110 °C), by HPLC with Nle as internal standard. [b] Octanol−water partition coefficient calculated using program of ref 15. [c] JG-365, acetyl-Ser-Leu-Asn-Phe-{CHOH-$CH_2$}-Pro-Ile-Val-$NH_2$ (ref 6). [d] *tert*-Butyl amide of L-pipecolinic acid. [e] Acetyl-Leu-Val-Phe-{CHOH-$CH_2$}-Phe-Ile-Val-$NH_2$. [f] Cyclic urea inhibitor (ref 14) tested as reference compound.

(4.2 g, 91%) as a colorless oil. Although used directly for subsequent reactions, a small amount was purified by reverse phase HPLC (70:30, water/acetonitrile/0.1% TFA) to give a colorless oil. $^1$H NMR ($CDCl_3$) AA′BB′ system: δ 7.08 (d, $J_{AB} + J_{AB'}$, 2H, 8.61 Hz, ArH ortho to $CH_2$), 6.81 (d, $J_{AB} + J_{AB'}$, 8.61 Hz, 2H, ArH ortho to O), 6.60 (br, s., 1H, COOH), 4.94 (d, 7.6 Hz, 1H, NH), 4.55 (m, 1H, Tyr αCH), 4.14 (q, $J$ = 7.2 Hz, 2H, $OCH_2CH_3$), 3.98 (t, $J$ = 6.1 Hz, 2H, $OCH_2CH_2CH_2$), 3.14 (dd, $J$ = 12.8, 5.1 Hz, 1H, Tyr βCH), 3.02 (dd, $J$ = 12.8, 6.0 Hz, 1H, Tyr βCH), 2.50 (t, $J$ = 7.3 Hz, 2H, $CH_2COOEt$), 2.09 (m, 2H, $CH_2CH_2CH_2$), 1.42 (s, 9H *tert*-butyl), 1.26 (t, $J$ = 7.2 Hz, 3H, $OCH_2CH_3$). $^{13}$C NMR ($CDCl_3$) δ 14.19 ($OCH_2CH_3$), 24.62 ($CH_2$-$CH_2$-$CH_2$), 28.12 (*tert*-butyl $CH_3$), 30.74 ($CH_2COOEt$), 36.88 (Tyr βCH), 54.35 (Tyr αCH), 60.47 ($OCH_2$), 66.66 ($OCH_2CH_3$), 80.18 (*tert*-butyl), 114.44 (ArC, ortho to $CH_2$), 127.78 (Ar−$CH_2$), 130.37 (ArC, ortho to O), 155.36 (ArC−O), 157.93 (C=O, carbamate), 173.35 (C=O, ethyl ester), 176.25 (C=O, COOH). ISMS 413 (M + $NH_4$), 396 (M + H), 340 (M + H − isobutene), 296 (M + H − Boc). HRMS calcd for $C_{20}H_{29}NO_7$ 395.1944, found 395.1943.

**(*S*)-3-[(tert-Butoxycarbonyl)amino]-1-diazo-4-[4′-[(3-carbethoxypropyl)oxy]phenyl]-2-butanone (4).** To a solution of alkylated Boc-tyrosine **3** (4 g, 10.1 mmol) in dry THF (50 mL) was added *N*-methylpiperidine (1.58 mL, 12.1 mmol). The solution was cooled to −10 °C under an atmosphere of dry nitrogen, and ethyl chloroformate (1.2 g, 1.05 mL, 11.1 mmol) was added in one portion. The solution was stirred for 10 min, during which *N*-methylpiperidine hydrochloride precipitated. An ethereal solution of diazomethane (excess) was added dropwise to this suspension over 30 min at −5 °C. The reaction mixture was allowed to warm to room temperature over 1 h, after which a slow stream of nitrogen was bubbled through the solution for 15 min to remove any unreacted diazomethane. The solution was diluted with ether (150 mL) and washed with water (3 × 100 mL), saturated $NaHCO_3$ (2 × 100 mL), and brine (1 × 100 mL). The organic phase was dried with $MgSO_4$ and evaporated *in vacuo* to give the title compound as a light yellow oil (3.2 g, 75%). While sufficiently pure for synthetic purposes, a small amount was purified by radial chromatography (ethyl acetate/light petroleum, 1:3) and subsequently recrystallized from hexane to give colorless needles, mp 73−75 °C. $^1$H NMR ($CDCl_3$) AA′BB′ system: δ 7.08 (d, $J_{AB} + J_{AB'}$ = 8.52 Hz, 2H, ArH ortho to $CH_2$), 6.82 (d, $J_{AB} + J_{AB'}$ = 8.52 Hz, 2H, ArH ortho to O), 5.21 (s, 1H, $CHN_2$), 5.12 (d, $J$ = 8.1 Hz, 1H, NH), 4.36 (m, 1H, Tyr αC-H), 4.15 (q, $J$ = 7.2 Hz, 2H, $OCH_2CH_3$), 3.98 (t, $J$ = 6.1 Hz, 2H, O-$CH_2$), 2.95 (d, $J$ = 6.4 Hz, 2H, Tyr βCH), 2.51 (t, $J$ = 7.3 Hz, 2H, $CH_2COOEt$), 2.10 (m, 2H, $CH_2CH_2CH_2$), 1.41 (s, 9H, *tert*-butyl), 1.26 (t, $J$ = 7.2 Hz, 3H, $OCH_2CH_3$). $^{13}$C NMR ($CDCl_3$) δ 14.22 ($OCH_2CH_3$), 24.63 ($CH_2CH_2CH_2$), 28.28 (*tert*-butyl $CH_3$), 30.79 ($CH_2$-COOEt), 37.73 (Tyr βCH), 54.41 (Tyr αC-H), 58.50 ($CHN_2$), 60.43 ($OCH_2$), 66.69 ($OCH_2CH_3$), 80.07 (*tert*-butyl), 114.58 (ArC, ortho to $CH_2$), 128.21 ($ArCH_2$), 130.34 (ArC, ortho to O), 155.13 (ArC−O), 157.85 (C=O, carbamate), 173.20 (C=O, ethyl ester), 193.13 (diazoketone). ISMS: 437 (M + $NH_4$), 420 (M + H), 336 (M + H − isobutene-$N_2$), 292 (M + H − BOC − $N_2$). Anal. Calcd for $C_{21}H_{29}N_3O_6$: C, 60.13%; H, 6.97%; N, 10.02%. Found: C, 60.16%; H, 6.97%; N, 9.85%.

**1-Bromo-(*S*)-3-[(*tert*-butoxycarbonyl)amino]-4-[4′-[(3′-carbethoxypropyl)oxy]phenyl]-2-butanone (5).** A saturated solution of HBr in ethyl acetate was diluted 1:9 with ethyl acetate and added in 1 mL aliquots to a cold solution (0 °C) of the diazoketone (**4**) (1.75 g, 4.18 mmol) in ethyl acetate (40 mL). The progress of the reaction was followed by thin layer chromatography. On completion the reaction mixture was washed with a 0.1 M $NaHSO_3$ (2 × 50 mL), saturated $NaHCO_3$ (2 × 40 mL), and brine (1 × 40 mL). The organic phase was dried with $MgSO_4$ and evaporated to dryness to give the title compound as a colorless solid (1.5 g, 75%). While sufficiently pure for synthetic purposes, a small amount was purified by radial chromatography (ethyl acetate/light petroleum, 1:3) and subsequently recrystallized from hexane/dichloromethane to give colorless needles, mp 92−94 °C. $^1$H NMR ($CDCl_3$) AA′BB′ system: δ 7.07 (d, $J_{AB} + J_{AB'}$ = 8.60 Hz, 2H, ArH ortho to $CH_2$), 6.83 (d, $J_{AB} + J_{AB'}$ = 8.60 Hz, 2H, ArH ortho to O), 5.03 (d, $J$ = 7.2 Hz, 1H, −NH), 4.67 (m, 1H, Tyr αC-H), 4.15 (q, $J$ = 7.2 Hz, 2H, $OCH_2CH_3$), 3.99 (t, $J$ = 6.1 Hz, 2H, O−$CH_2$), 3.97 (d, $J$ = 13.9 Hz, 1H, −CHBr), 3.83 (d, $J$ = 13.9 Hz, 1H, −CHBr), 2.95 (m, 2H, Tyr βCH), 2.51 (t, $J$ = 7.3 Hz, 2H, $CH_2COOEt$), 2.10 (m, 2H, $CH_2CH_2CH_2$), 1.42 (s, 9H, *tert*-butyl), 1.26 (t, $J$ = 7.2 Hz, 3H, $OCH_2CH_3$). $^{13}$C NMR ($CDCl_3$): δ 14.15 ($OCH_2CH_3$), 24.54 ($CH_2CH_2CH_2$), 28.19 (*tert*-butyl $CH_3$), 30.71 ($-CH_2$-COOEt), 33.37 ($-CH_2Br$), 37.00 (Tyr βCH), 58.61 (Tyr αCH), 58.50 ($CHN_2$), 60.37 ($OCH_2$), 66.66 ($OCH_2CH_3$), 80.34 (*tert*-butyl), 114.77 (ArC, ortho to $CH_2$), 127.58 (Ar−$CH_2$), 130.09 (ArC, ortho to O), 155.15 (ArC−O); 157.99 (C=O, carbamate), 173.12 (C=O, ethyl ester), 200.95 (C(O)$CH_2Br$). ISMS: 491/489 (M + $NH_4$, 1:1), 474/472 (M + H, 1:1), 418/416 (M + H − isobutene,1:1), 374/372 (M + H − BOC, 1:1). Anal. Calcd for $C_{21}H_{30}BrNO_6$: C, 53.40%; H, 6.40%; N, 2.97%. Found: C, 53.37%; H, 6.30%; N, 2.84%.

**(2*S*)- and (2*R*)-(*S*)-3-((*S*)-Asparaginylamino)-4-[4′-[(3′-carbethoxypropyl)oxy]phenyl]-1-((*S*)-*N*-prolyl-(*S*)-isoleucyl-(*S*)-valine amide)-butan-2-ol (7).** Assembly of the uncyclized peptidomimetic (**7**) was accomplished by solid phase peptide synthesis.[9] MBHA resin (2.15 g, 2 mmol, SV = 0.93 mequiv/g) was shaken with DIPEA (0.7 mL, 4.0 mmol) in DMF (12 mL) for 2 min. The resin was filtered, and to it was added a solution of Boc-valine (1.74 g, 8 mmol), HBTU (0.5 M solution in DMF, 16 mL, 8 mmol), and DIPEA (2.75 mL, 16 mmol). The resin was shaken with this solution for 10 min, and the reaction was monitored by the negative ninhydrin test, which indicated that a 99.65% coupling was achieved. The resin was washed with DMF and treated with TFA (2 × 10 mL, 1 min each) to give the deprotected valine on resin, and the procedure was repeated for Boc-isoleucine (99.99%) and Boc-proline (99.96%). The hydroxyethylamine isostere was introduced by shaking the resin with a solution of the ketobromide **5** (2.36 g, 5 mmol) and DIPEA (1.72 mL, 10 mmol) in DMF (16 mL) for 30 min. The resultant ketone was reduced by shaking the resin, at room temperature for 1 h, with sodium borohydride (0.6 g, 16.2 mmol) in THF (16 mL). Boc-Asn was coupled to the peptide using the same procedure descibed above (99.66%). The weight of dried resin was 3.8 g (theoretical weight = 3.85 g). The peptide was cleaved from resin (0.8 g, 0.42 mmol) with HF, lyophilized, treated with a 0.1 M ammonium carbonate solution at room temperature for 15 min, and again lyophilized to give 279 mg of a powder consisting of a diastereomeric mixture of peptides, ISMS 734 (M + H). The diastereomeric mixture was de-esterified without further purification

by dissolving in a mixture of water (3 mL) and 0.57 M NaOH solution (2.16 mL, 12.3 mmol). The suspension was made homogeneous by the gradual addition of THF, and the resulting solution stirred at room temperature for 30 min. The mixture was neutralized with 1 M HCl to pH = 7 and the solvent evaporated *in vacuo*. Purification by HPLC (gradient, water 0.1% TFA to 50:50 water/acetonitrile, 0.1% TFA over 75 min) gave a diastereomeric mixture of **7** (156 mg, 53% overall yield from beginning of the solid phase synthesis). ISMS: 706 (M + H).

**[2(*S*),11′(*S*),8′(*S*)]-2-[6′,9′-Dioxo-8′-ethanamido-2′-oxa-7′,10′-diazabicyclo[11.2.2]heptadeca-13′,15′,16′-trien-11-yl]-1-(*N*-(*S*)-prolyl-(*S*)-isoleucyl-(*S*)-valine amide)ethan-2-ol (1a-*S*).** The diastereomeric mixture **7** (66 mg, 0.093 mmol) was dissolved in DMF (800 mL, $C = 1.16 \times 10^{-4}$ M), BOP reagent (61.6 mg, 0.14 mmol) and DIPEA (0.1 mL, 0.58 mmol) were added, and the solution was stirred at room temperature for 1 h. The solvent was evaporated *in vacuo* and the residue redissolved in distilled water (20 mL). An insoluble precipitate was filtered from the solution, and the diastereomeric mixture 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 75 min) to give a pure sample of both **1a-*R*** (18 mg, 28%,) and **1a-*S*** (13 mg, 21%,) as white powders, after lyophilization. The two diastereomers were pure by analytical HPLC analysis (gradient, water 0.1% TFA to 50:50 (water/0.1%TFA)/(water 10%/acetonitrile 90%/TFA 0.1%) over 50 min), **1a-*S*** rt = 39.9 min; **1a-*R*** rt = 42.2 min. $^1$H NMR of **1a-*S*** ($H_2O/D_2O$, 8:2, 313 K): $\delta$ 8.69 (br. s., 1H, Ile-NH), 8.22 (d, $J$ = 7.23 Hz, 1H, Val-NH), 7.71 (d, $J$ = 9.8 Hz, 1H, 10′-NH), 7.65 (br. s., 1H, Val-1° amide), 7.47 (br. s., 1H, Asn-1° amide), 7.21 (d, $J$ = 8.8 Hz, 1H, Asn-NH), 7.20 (dd, $J$ = 2.1, 8.4 Hz, 1H, H17′), 7.15 (dd, $J$ = 2.2, 8.4 Hz, 1H, H14′), 7.09 (br. s., 1H, Val-1° amide), 7.00 (dd, $J$ = 2.7, 8.4 Hz, 1H, H16′), 6.95 (dd, $J$ = 2.7, 8.4 Hz, 1H, H15′); 6.72 (br. s., 1H, Asn-1° amide), 4.42−4.48 (m, 1H, H-3′), 4.29−4.38 (m, 3H, Asn-αCH, Ile-αCH and H-3′), 4.22−4.29 (m, 1H, H-11′), 4.19 (m, 1H, Val-αCH), 4.10−4.16 (m, 2H, Pro-αCH and H-2), 3.8 (m, 1H, Pro-δCH), 3.15−3.28 (m, 3H, Pro δCH and 2 × H-1), 3.12 (dd, $J_{H12'-H12'}$ = 3.5 Hz, $J_{H12'-H11'}$ = 5.6 Hz, 1H, H-12′), 2.78 (dd, $J_{H12'-H11'}$ = 13.5 Hz, $J_{H12'-H12'}$ = 13.5 Hz, 1H, H-12′), 2.55 (m, 1H, Pro-βCH), 2.42−2.51 (m, 3H, Asn-$\beta CH_2$ and H-5′), 2.29 (ddd, $J_{H5'-H5'}$ = 16.2 Hz, $J_{H5'-H4'}$ = 7.6 Hz, $J_{H5'-H4'}$ = 3.6 Hz, 1H, H-5′), 2.19 (m, 1H, Pro-γCH), 1.98−2.14 (m, 6H, Val-βCH, Pro-βCH, Pro-γCH, Ile-γCH & 2 × H-4′), 1.89−1.98 (m, 1H, Ile-βCH), 1.51−1.61 (m, 1H, Ile-γCH), 1.20−1.30 (m, 1H, Ile-γCH), 1.02 (d, $J$ = 6.8 Hz, 6H, Val-$\gamma CH_3$), 0.98 (d, $J$ = 6.8 Hz, 3H, Ile-$\gamma CH_3$), 0.94 (t, 3H, $J$ = 7.4 Hz, Ile-$\delta CH_3$). $^{13}$C NMR: ($H_2O/D_2O$, 8:2, ref EtOH); 10.59, 15.06, 18.21, 18.69, 23.08, 24.25, 24.98, 30.04, 30.23, 31.50, 35.63, 36.51, 38.86, 50.51, 53.84, 55.49, 58.19, 59.18, 59.74, 68.42, 68.78, 78.50, 114.98, 117.23, 117.83, 129.45, 131.27, 132.44, 156.90, 158.00, 171.93, 173.71, 174.50, 176.13. ISMS: 688 (M + H).

**3-[[*N*-(*tert*-Butoxycarbonyl)-(*S*)-isoleucinyl]amino]-1-bromopropane (9).** To a solution of Boc-isoleucine hemihydrate (2.4 g, 10 mmol) and BOP reagent (4.42 g, 10 mmol) in dry THF (50 mL) was added DIPEA (1.29 g, 10 mmol), and the solution was stirred for 5 min. 3-Bromopropylamine.HBr (2.4 g, 11 mmol) and DIPEA (1.56 g, 12 mmol) were then added to the solution. After 30 min, the solvent was removed under vacuum and the residue redissolved in ethyl acetate (100 mL), thoroughly washed with 1 M hydrochloric acid (4 × 50 mL), saturated sodium bicarbonate (2 × 50 mL), and brine (1 × 50 mL), and dried over sodium sulphate. Purification by column chromatography (silica; 40% ethyl acetate in hexane ($R_f$ = 0.8)) provided the title compound (3.3 g, 94%) as a white solid. $^1$H NMR ($CDCl_3$): $\delta$ 6.50 (br. s, 1H, NH), 5.15 (br. s, 1H, Ile-NH), 3.85 (m, 1H, Ile-αCH), 3.40 (m, 4H, $NCH_2$, $BrCH_2$), 2.05 (m, 2H, $CH_2$), 1.85 (m, 1H, Ile-βCH), 1.40 (m, 10H, Ile-γCH and $(CH_3)_3$), 1.10 (m, 1H, Ile-γCH), 0.90 (d, $J$ = 6.82 Hz, 3H, Ile-$\gamma CH_3$), 0.86 (t, $J$ = 7.73 Hz, 3H, Ile-$\delta CH_3$); ISMS (M + H) 351/353.

**3-[[[*N*-(*tert*-Butoxycarbonyl)-(*S*)-tyrosinyl]-(*S*)-isoleucinyl]amino]-1-bromopropane (10).** Compound **9** (3.51 g, 10 mmol) was dissolved in a solution of 25% TFA in DCM (10 mL) and stirred at room temperature for 30 min. The solution was evaporated to dryness *in vacuo* and the residue evaporated from DCM three more times to remove residual traces of TFA. This residue was coupled to Boc-tyrosine (2.8 g, 10 mmol) using the procedure described for the synthesis of compound **9**. The resultant product was purified by column chromatography (silica; 50% ethyl acetate in hexane ($R_f$ = 0.19)) as a colorless powder (4.9 g, 96%), mp 96−100 °C. $^1$H NMR ($CDCl_3$): $\delta$ 6.7−7.1 (m, 6H, ArH, Ile-NH, NH), 5.15 (d, $J$ = 8.2 Hz, 1H, Tyr-NH), 4.30 (m, 1H, αCH), 4.2 (m, 1H, αCH), 3.35−3.50 (m, 1H, NCH), 3.40 (t, $J$ = 6.0 Hz, 2H, $CH_2Br$), 3.25 (m, 1H, NCH), 3.01 (dd, $J$ = 6.1, 13.6 Hz, 1H, Tyr-βCH), 2.93 (dd, $J$ = 6.1, 13.6 Hz, 1H, Tyr-βCH), 2.05 (m, 2H, $CH_2$), 1.9 (m, 1H, Ile-βCH), 1.4 (m, 10H, Ile-γCH, $(CH_3)_3$), 1.05 (m, 1H, Ile-γCH), 0.89 (d, $J$ = 6.32 Hz, 3H, Ile-$\gamma CH_3$), 0.83 (t, $J$ = 7.37 Hz, 3H, Ile-$\delta CH_3$). ISMS: 514/516 (M + H). HRMS calcd for $C_{23}H_{35}N_3O_5$ (M − HBr) 433.2577, found 433.2573.

**(11*S*,8*S*)-11-[(*tert*-Butoxycarbonyl)amino]-7,10-dioxo-8-(1-methylpropyl)-2-oxa-6,9-diazabicyclo[11.2.2]heptadeca-13,15,16-triene (11).** The dipeptide **10** (1 g, 1.9 mmol) was added to a solution of sodium methoxide (0.1 g, 1.9 mmol) in methanol (40 mL) and stirred at room temperature for 96 h. The solution was evaporated to dryness and diluted with ethyl acetate (50 mL) and washed with 1 M hydrochloric acid (1 × 25 mL), saturated sodium bicarbonate (1 × 25 mL), and brine (1 × 25 mL). The organic phase was dried and the solvent removed. The crude product was purified by column chromatography (silica; 50% ethyl acetate in hexane ($R_f$ = 0.17)) providing the title compound (0.63 g, 75%) as a colorless solid, mp 248−252 °C. $^1$H NMR ($CDCl_3$): $\delta$ 7.85 (m, 1H, NH-6), 7.16 (d, $J$ = 8.75 Hz, 1H, ArH), 6.97 (d, 1H, $J$ = 7.50 Hz, Ile-NH), 6.94 (d, $J$ = 7.50 Hz, 1H, Tyr-NH), 6.83−6.90 (m, 3H, ArH), 4.38 (dt, $J_{H3-H3}$ = 12.5 Hz $J_{H3-H4}$ = 5.00 Hz, 1H, H-3), 4.22 (m, 1H, H-3), 4.14 (m, 1H, H-11), 3.45−3.55 (m, 2H, H-8 & H-5), 3.08 (dd, $J_{H12-H12}$ = 13.1 Hz $J_{H11-H12}$ = 6.25 Hz, 1H, H-12), 2.84 (m, 1H, H-5), 2.59 (m, 1H, H-12), 2.19 (m, 1H, H-4), 1.79 (m, 1H, H-4), 1.57 (m, 1H, Ile-βCH), 1.43−1.49 (m, 10H, Ile-γCH and *tert*-butyl), 0.98 (m, 1H, Ile-γCH), 0.83 (d, $J$ = 7.5 Hz, 3H, Ile-$\gamma CH_3$), 0.76 (d, $J$ = 6.3 Hz, 3H, Ile-$\delta CH_3$). ISMS: 434 (M + H). HRMS calcd for $C_{23}H_{35}N_3O_5$ 433.2577, found 433.2584.

**Methyl (*S*)-2-[[(*tert*-Butoxycarbonyl)-(*S*)-valinyl]amino]-3-phenylpropanoate (13).** The title compound was prepared by coupling Boc-valine (2.17 g, 10 mmol) and phenylalanine methyl ester·HCl (2.3 g, 11 mmol) with BOP reagent according to the procedure described for **9**. Purification of the crude residue by column chromatography (silica; 50% ethyl acetate in hexane) gave **13** (3.66 g, 97%) as a white solid, mp 103−106 °C. $^1$H NMR ($CDCl_3$): $\delta$ 7.1−7.35 (m, 5H, ArH), 6.35 (d, $J$ = 8.33 Hz, 1H, Phe-NH), 5.05 (d, $J$ = 7.7 Hz, 1H, Boc-NH), 4.85 (ddd, $J$ = 6.3, 6.3, 8.33 Hz, 1H, Phe-αCH), 3.9 (dd, $J$ = 7.7, 7.7 Hz, 1H, Val-αCH), 3.7 (s, 3H, $OCH_3$), 3.15 (dd, $J$ = 6.3, 14.1 Hz, 1H, Phe-βCH), 3.08 (dd, $J$ = 6.3, 14.1 Hz, 1H, Phe-βCH), 2.1 (m, 1H, Val-βCH), 1.45 (s, 9H, $(CH_3)_3$), 0.9 (d, $J$ = 5.0 Hz, 3H, Val-$\gamma CH_3$), 0.85 (d, $J$ = 5.0 Hz, 3H, Val-$\gamma CH_3$). ISMS: 379 (M + H). HRMS calcd for $C_{20}H_{30}N_2O_5$ 378.2155, found 378.2151.

**Methyl (*S*)-2-[[(*tert*-Butoxycarbonyl)-(*S*)-leucinyl]-(*S*)-valinyl]-amino]-3-phenylpropanoate (14).** The dipeptide **13** (3.5 g, 9.2 mmol) was deprotected at the N-terminus with a solution of 25% TFA in DCM (10 mL) and the resultant amine coupled to Boc-leucine (2.49 g, 10 mmol) using the same procedure described for **9** above. Column chromatography (silica; 50% ethyl acetate in hexane) provided **14** (4.3 g, 95%) as a white solid, mp 133−134 °C. $^1$H NMR ($CDCl_3$): $\delta$ 7.00−7.30 (m, 5H, ArH), 6.70 (d, $J$ = 9.30 Hz, 1H, Val-NH), 6.55 (d, $J$ = 8.14 Hz, 1H, Phe-NH), 5.00 (d, $J$ = 8.20 Hz, 1H, Bocleu-NH), 4.85 (m, 1H, Phe-αCH), 4.27 (m, 1H, αCH), 4.10 (m, 1H, αCH), 3.50 (s, 3H, $OCH_3$), 3.10 (m, 2H, Phe-$\beta CH_2$), 2.10 (m, 1H, Val-βCH), 1.35−1.70 (m, 12H, $(CH_3)_3$, Leu-$\beta CH_2$, Leu-γCH), 0.70−1.00 (m, 12H, Val-$\gamma CH_3$, Leu-$\delta CH_3$). ISMS: 492 (M + H). HRMS calcd for $C_{26}H_{41}N_3O_6$ 491.2995, found 491.2996.

**Methyl (*S*)-2-[[(Acetyl-(*S*)-leucinyl)-(*S*)-valinyl]amino]-3-phenylpropanoate (15).** The tripeptide (**15**) was prepared by deprotection of (**14**) (4 g, 8.1 mmol) with 25% TFA in DCM. The solvent and excess TFA was evaporated before diluting the residue with THF (100 mL). To the solution were added DIPEA (approximately 10 mL, excess) and DMAP (1 mol %). The resulting solution was then cooled to 0 °C in an ice bath before adding acetic anhydride (5 equiv). The mixture was warmed to room temperature and stirred for a further 30 min. The solvent was removed and the residue redissolved in ethyl acetate (200 mL). The organic phase was washed with 1 M hydrochloric acid (1 × 50 mL), saturated sodium bicarbonate (1 × 50 mL), brine (1 × 50 mL) and dried and the solvent evaporated. The

product was recrystallized from ethyl acetate to provide the title compound (3.45 g, 99%) as a white solid, mp 216−217 °C. $^1$H NMR ($CDCl_3$): $\delta$ 7.10−7.30 (m, 6H, ArH, NH), 7.0 (d, $J$ = 9.0 Hz, 1H, NH), 6.5 (d, $J$ = 9.1 Hz, 1H, NH), 4.9 (m, 1H, Phe-αCH), 4.60 (m, 1H, Leu-αCH), 4.45 (m, 1H, Val-αCH), 3.70 (s, 3H, O-$CH_3$), 3.10 (m, 2H, Phe-$\beta CH_2$), 2.10 (m, 1H, Val-$\beta$CH), 2.00 (s, 3H, $CH_3C$(O)), 1.45−1.70 (m, 3H, Leu-$\gamma$CH and Leu-$\beta CH_2$), 0.90−1.05 (m, 12H, Val-$\gamma CH_3$ and Leu-$\delta CH_3$). $^{13}$C NMR: ($CDCl_3$): 18.18, 18.99, 22.32, 22.92, 22.99, 24.82, 31.14, 35.07, 41.42, 51.74, 52.23, 52.26, 58.41, 127.11, 128.57, 129.17, 135.82, 170.20, 170.80, 171.84, 172.35. ISMS: 434 (M + H). HRMS calcd for $C_{23}H_{35}N_3O_5$ 433.2577, found 433.2576.

**(*S*)-2-[[(Acetyl-(*S*)-leucinyl)-(*S*)-valinyl]amino]-1-diazo-3-phenylbutan-2-one (16).** The tripeptide (**16**) (1 g, 2.3 mmol) was deesterified by dissolving in a mixture of dioxan (50 mL) and 1 M sodium hydroxide (10 mL) and stirring at room temperature for 30 min, before neutralizing with 1 M hydrochloric acid. The solvent was removed under vacuum to yield (*S*)-2-[[acetyl-(*S*)-leucinyl)-(*S*)-valinyl]-amino]-3-phenylpropanoic acid (0.92 g, 95%). This compound (0.87 g, 2 mmol) and *N*-methyl piperidine (0.25 g, 2.5 mmol) were dissolved in a mixture of dry THF (50 mL) and DMF (5 mL) at room temperature under an atmosphere of nitrogen. The resultant solution was cooled to −15 °C and ethyl chloroformate (0.24 g, 2.2 mmol) added. After stirring for 5 min, an ethereal solution of diazomethane (excess) was added and the mixture allowed to warm gradually to room temperature over 2 h. The excess diazomethane was then removed by purging the solution with a stream of $N_2$ for 20 min and evaporated to dryness. The residue was redissolved in ethyl acetate (100 mL), washed with saturated sodium carbonate (2 × 50 mL) and brine (1 × 50 mL), and dried over sodium sulphate. The product was purified on a silica column pretreated with triethylamine to give the diazoketone **16** (0.81 g, 92%) as a light yellow solid, mp 220−230 °C (dec). $^1$H NMR (DMSO-$d_6$): $\delta$ 8.30 (d, $J$ = 6.70 Hz, 1H, Phe-NH), 8.03 (d, $J$ = 7.05 Hz, 1H, Leu- NH), 7.6 (d, $J$ = 8.9 Hz, 1H, Val-NH), 7.23 (m, 5H, ArH), 6.03 (br. s, 1H, $CHN_2$), 4.48 (m, 1H, Phe-αCH), 4.27 (m, 1H, Leu-αCH), 4.05 (dd, $J$ = 7.3, 8.9 Hz, 1H, Val-αCH), 3.02 (dd, $J$ = 4.8, 16.1 Hz, 1H, Phe-$\beta$CH), 2.75 (dd, $J$ = 4.8, 16.1 Hz, 1H, Phe-$\beta$CH), 1.85 (m, 1H, Val-$\beta$CH), 1.83 (s, 3H, acetyl), 1.55 (m, 1H, Leu-$\gamma$CH), 1.36 (m, 1H, Leu-$\beta CH_2$), 0.86 (d, $J$ = 6.73 Hz, 3H, Leu-$\delta CH_3$), 0.81 (d, $J$ = 6.73 Hz, 3H, Leu-$\delta CH_3$), 0.75 (d, $J$ = 4.8 Hz, 3H, Val-$\gamma CH_3$), 0.72 (d, $J$ = 4.8 Hz, 3H, Val-$\gamma CH_3$). ISMS 444 (M + H). HRMS calcd for $C_{23}H_{33}N_3O_4$ (M − $N_2$) 415.2583, found 415.2578.

**(*S*)-2-[[(Acetyl-(*S*)-leucinyl)-(*S*)-valinyl]amino]-1-bromo-3-phenylbutan-2-one (17).** The diazoketone **16** (0.75 g, 1.7 mmol) was dissolved in ethyl acetate (10 mL) and treated with HBr under conditions identical to those used for the preparation of **5.** Purification by column chromatography (silica; 50% ethyl acetate in hexane) afforded the ketobromide **17** (0.82 g, 99%) as a colorless solid, mp 184−185 °C. $^1$H NMR (DMSO-$d_6$): $\delta$ 8.5 (d, $J$ = 6.8 Hz, 1H, Phe-NH), 8.0 (d, $J$ = 6.6 Hz, 1H, Leu-NH), 7.7 (d, $J$ = 7.0 Hz, 1H, Val-NH), 7.2 (m, 5H, ArH), 4.67 (m, 1H, Phe-αCH), 4.38 (d, $J$ = 14.6 Hz, 1H, CHBr), 4.30 (d, $J$ = 14.6 Hz, 1H, CHBr), 4.25 (m, 1H, Leu-αCH), 4.03 (m, 1H, Val-αCH), 3.11 (dd, $J$ = 6.1, 15.9 Hz, 1H, Phe-$\beta$CH), 2.78 (dd, $J$ = 6.1, 15.9 Hz, 1H, Phe-$\beta$CH), 1.8 (s, 3H, acetyl), 1.55 (m, 1H, Leu- $\gamma$CH), 1.35 (m, 2H, Leu-$\beta CH_2$), 0.86 (d, $J$ = 6.5 Hz, 3H, Leu-$\delta CH_3$), 0.81 (d, $J$ = 6.5 Hz, 3H, Leu-$\delta CH_3$), 0.76 (d, $J$ = 6.7 Hz, 3H, Val-$\gamma CH_3$), 0.72 (d, $J$ = 6.7 Hz, 3H, Val-$\gamma CH_3$). ISMS: 496/498 (M + H).

**(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 (2a).** The macrocycle **11** (30 mg, 69 μmol) was deprotected at the N-terminus with 25% TFA in DCM according to the procedure used for the synthesis of **10**. The residue was diluted with DCM (50 mL) and washed with 1 M sodium hydroxide to generate the free amine, and the solvent was evaporated. The residue was redissolved in THF (5 mL), to which DIPEA (0.015 mL, 76 μmol) and **17** (37 mg, 76 μmol) were added, the solution stirred at room temperature for 4 h, the solvent evaporated *in vacuo*, the residue redissolved in ethyl acetate (20 mL), washed with 0.5 M hydrochloric acid (5 mL), and dried, and the solvent removed. The ketone intermediate was not purified but reduced directly to the title compound with $NaBH_4$ (40 mg, 100 mmol) in MeOH (10 mL), stirred at room temperature for 30 m before quenching the reaction with 1 M hydrochloric acid (1 mL). Subsequent purification of the crude residue by reverse phase HPLC [gradient, (water 0.1%TFA to 40:60 (water/ 0.1%TFA)/(water 10%/acetonitrile 90%/TFA 0.1%) over 50 min], rt = 55.8 min, and lyophilization gave **2a** as a colorless powder (7 mg, 15%) and the 2(*S*)-diastereomer as the minor product. $^1$H NMR ($CD_3OH$, 293 K): $\delta$ 8.30 (d, $J$ = 6.05 Hz, 1H, Leu-NH), 7.85 (m, 1H, 6′-NH), 7.73 (d, $J$ = 5.8 Hz, 1H, Val-NH), 7.70 (m, 1H, Phe-NH), 7.34 (d, $J$ = 7.6 Hz, 1H, Ile-NH), 7.23 (m, 4H, Ph), 7.17 (m, 1H, Ph), 7.11 (dd, $J$ = 8.3, 1.9 Hz, 1H, H-14′), 6.96 (dd, $J$ = 8.4, 1.9 Hz, 1H, H-17′), 6.88 (dd, $J$ = 8.3, 2.6 Hz, 1H, H-16′), 6.83 (dd, $J$ = 8.3, 2.6 Hz, 1H, H-15′), 4.38 (m, 1H, H-3′), 4.20−4.26 (m, 2H, Leu-α(CH) and H-3′), 4.13 (m, 2H, H-11′ and H-3), 3.81 (m, 2H, Val-αCH and H-2), 3.54 (m, 1H, H-5′), 3.45 (m, 1H, Ile-αCH), 3.39 (dd, $J_{H11'-H12'}$ = 7.0 Hz, $J_{H12'-H12'}$ = 12.1 Hz, 1H, H-12′), 3.28 (m, 1H, H-4), 3.08 (dd, $J_{H1-H1}$ = 12.7 Hz, $J_{H1-H2}$ = 1 Hz, 1H, H-1), 3.00 (dd, $J_{H1-H1}$ = 12.7 Hz, $J_{H1-H2}$ = 6.0 Hz, 1H, H-1), 2.79 (m, 2H, H-5′ and H-12′), 2.70 (dd, $J_{H4-H4}$ = 14.0 Hz, $J_{H4-H3}$ = 11.0 Hz, 1H, H-4), 2.26 (m, 1H, H-4′), 2.04 (s, 3H, acetyl), 1.80−1.85 (m, 1H, Val-$\beta$CH), 1.72 (m, 1H, H-4′), 1.61−1.64 (m, 1H, Leu-$\gamma$CH), 1.53−1.59 (m, 2H, Leu-$\beta$CH and Ile-$\beta$CH), 1.43−1.49 (m, 2H, Leu-$\beta$CH and Ile-$\gamma$CH), 0.94−1.00 (m, 1H, Ile-$\gamma$CH), 0.93 (d, $J$ = 6.5 Hz, 3H, Leu-$\delta CH_3$), 0.88 (d, $J$ = 6.5 Hz, 3H, Leu-$\delta CH_3$), 0.85 (t, $J$ = 7.4 Hz, 3H, Ile-$\delta CH_3$), 0.76 (d, $J$ = 6.7 Hz, 3H, Ile-$\gamma CH_3$), 0.74 (d, $J$ = 6.7 Hz, 3H,Val-$\gamma CH_3$), 0.59 (d, $J$ = 6.7 Hz, 3H, Val-$\gamma CH_3$). $^{13}$C NMR ($CD_3OH$): 11.69, 14.97, 18.82, 19.29, 21.79, 22.52, 23.32, 25.74, 26.47, 27.46, 30.86, 36.60, 37.25, 37.64, 39.97, 41.12, 50.72, 54.28, 54.75, 59.99, 62.17, 63.82, 68.52, 70.74, 118.57, 118.73, 127.53, 127.90, 129.46, 130.05, 130.43, 132.35, 139.36, 159.98, 167.78, 171.39, 174.19, 174.79, 175.67. ISMS: 751(M + H). HRMS calcd for $C_{41}H_{62}N_6O_7$ 750.4680, found 750.4682.

**Crystallography.** Synthetic[12] HIVPR was mixed with a DMSO solution of **1a** (ratio 1:10). Crystals of the HIVPR−**1a** complex were grown at 20 °C by vapor diffusion from 40% $(NH_4)_2SO_4$ in acetate buffer, pH 5.5, and were isomorphous with those of the HIVPR-JG365 complex[6] ($a$ = 51.3 Å $b$ = 58.8 Å $c$ = 62.3 Å, spacegroup $P_12_12_12_1$). Crystallographic data were measured at 16 °C using a RAXIS IIC imaging plate area detector. A total of 11 539 unique reflections were obtained from 34 832 observations with an *R*-merge 0.072 (94% of data to 2.0 Å). All reflections between 8.0 and 2.0 Å were used in structure refinement with X-PLOR[17] with the HIVPR-JG365 complex (pdb accession code 7HVP) used as the starting model, and O was used for modeling and rebuilding.[18] The final refined structure, including 103 solvent molecules, has an *R*-factor of 0.174 with rms deviations from ideality of 0.011 Å and 1.925° for bond distances and bond angles, respectively. A detailed analysis of this crystal structure will be published separately. Atomic coordinates for the structure of HIVPR−**1a** complex have been deposited with the Brookhaven Protein Data Bank.

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