A Novel Constrained Reduced-Amide Inhibitor of HIV-1 Protease Derived from the Sequential Incorporation of γ-Turn Mimetics into a Model Substrate[1]

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C_7 mimetics, designed to lock three amino acid residues of a peptide chain into a γ-turn conformation, were introduced sequentially between the P_3 to P_2' positions of a model HIV-1 protease substrate I (resulting in compounds **II–IV**) to probe its conformational requirements in binding to HIV-1 protease. Of these, compound **IIIa** with the C_7 mimetic replacing Asn-Tyr-Pro, corresponding to the P_2 through P_1' positions of substrate, was found to be an inhibitor with a K_i of 147 μM. Reduction of the amide bond in the C_7 mimetic of **IIIa** resulted in a novel constrained reduced-amide mimetic **VIa** with a K_i of 430 nM. This corresponds to over a 300-fold improvement in inhibitory activity over the original C_7 mimetic. The inhibitory activity of mimetic **VIa** was in addition found to be 44-fold better than a similar linear reduced-amide containing inhibitor **V**. The synthesis of these mimetics are described.

Introduction

An understanding of the conformational requirements for a peptide substrate binding to the active site of its enzyme is fundamental for the design of high-affinity inhibitors. Unfortunately for most peptides this task is made difficult by the inherent flexibility of the peptide itself. Insights regarding the biologically active conformation therefore have usually been obtained by introducing conformational constraints at selected sites in the backbone of the peptide substrate. A number of such constraints have been applied over the past few years that replace either an amide bond or several residues in a peptide chain.[2] Of special interest are those mimetics which force linear peptide sequences into various defined, turn conformations.[3] Previously our laboratory described the rational design and conformation of a C_7 mimetic which locks three amino acid residues of a peptide sequence into a γ-turn conformation (Figure 1).[4] In a study designed to explore the utility of C_7 mimetics to probe conformational features about the cleavage site of a peptide substrate, HIV-1 protease and its substrate **I** were selected as a model system.

HIV-1 protease, which is a member of the aspartic protease family, specifically processes high molecular weight viral polyproteins into individual structural proteins and enzymes.[5] A likely element for protease recognition and processing is its substrate conformation[6] since amino acid sequence requirements, on the other hand, appear to be less important. Although no consensus amino acid sequence has been deduced, the occurrence of a proline residue at the P_1' position is a common feature in many substrates.[7,8] Because proline is also often found in turns in a number of other proteins, its common occurrence in the HIV-1 protease substrate suggested that a turn may be involved in protease recognition.[9] γ-Turn mimetics were therefore incorporated sequentially in this proposed turn region, between the P_3 to P_2' positions of model HIV-1 protease substrate **I**,[7] to probe its conformational requirements in binding to HIV-1 protease (see Table I).

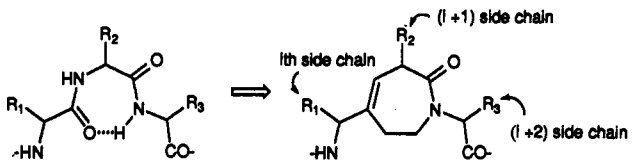

Figure 1. γ-Turn mimetic.

Substitution of a γ-turn mimetic in the P_1 to P_2' positions of substrate **I** afforded peptidomimetic **II**. Since the C_7 itself was designed to mimic the potential γ-turn induced by proline, the proline ring (at the putative $i + 1$ position) was considered unnecessary and therefore eliminated. Additional changes in **II**, previously shown in linear HIV substrates[7,10] to result in at most a 2–3-fold difference in K_m, include a Phe for Tyr and Ala for Asn substitution, the addition of a serine at the N-terminus, and a methyl ester for amide replacement at the C-terminus. Incorporating a γ-turn mimetic in the P_2 to P_1' positions of substrate **I** gave peptidomimetic **III**, which incorporates the mimetic frame-shifted one residue toward the N-terminus. In the design of **III** the proline ring was again eliminated and replaced with a methyl side chain. Lastly, as a means to further probe the conformational requirements of the active site, a γ-turn mimetic was also inserted into the P_3 to P_1 sequence of substrate **I** to give compound **IV**, where the nonessential asparagine side chain at the $i + 1$ position was eliminated for synthetic ease. Herein, we report the results obtained from this incorporation of γ-turn mimetics into model HIV-1 substrate **I**.

Chemistry

Three synthetic approaches were used in the synthesis of the C_7 mimetics in compounds **II–IV**. Each of these C_7 mimetics will illustrate a different synthesis. The choice of synthesis was dependent on the number of side chains in the mimetic and the chemical compatibility of the side chains in the mimetic to the reaction conditions. All methods involved modification of a synthetic scheme described earlier, based on the Birch reduction and selective ozonolysis of a appropriately substituted benzene.[4b]

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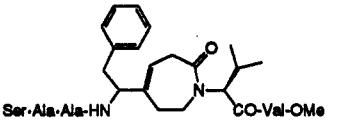
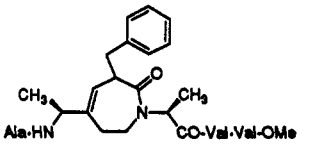
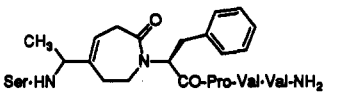
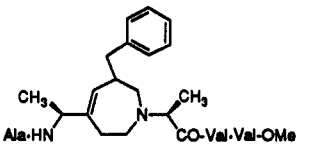
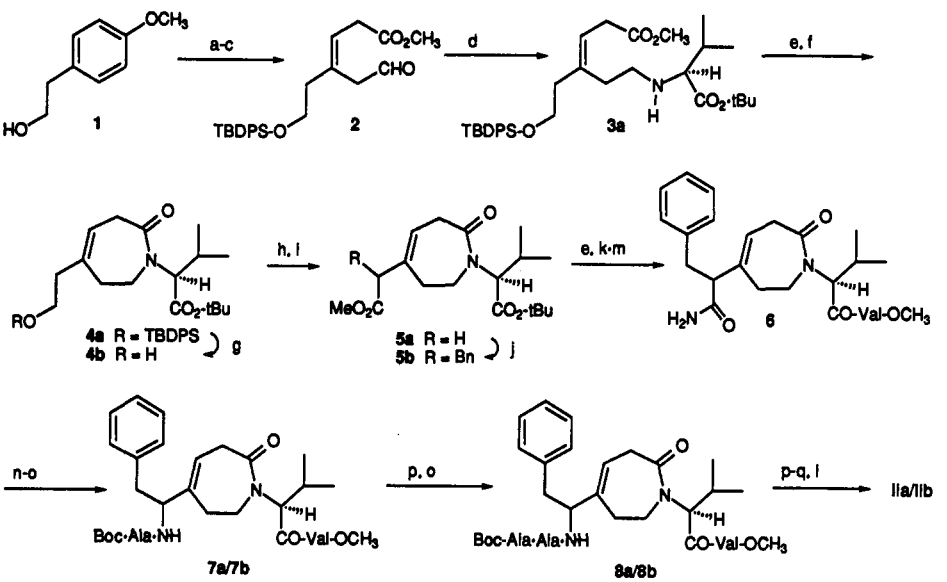
Table I. HIV-1 Protease Inhibition Results

no.[a]	structure	activity (μM)[b]
I	Ala-Asn-Tyr-Pro-Val-Val-NH_2	K_m = 17700[c]
IIa IIb		K_i = 1700 ± 300 >2.0 mM
IIIa IIIb		K_i = 147 ± 4 K_i = 372 ± 17
IVa IVb		>2.0 mM >2.0 mM
V	Ser-Ala-Ala-PheΨ[CH_2N]Pro-Val-Val-NH_2	K_i = 18.8 ± 2[d]
VIa VIb		K_i = 0.43 ± 0.07 K_i = 3.89 ± 0.67

[a] a and b denote single diastereomers of unassigned absolute stereochemistry. [b] K_m and K_i values were determined using the protocol described in refs 7 and 10. [c] Personal communication; see refs 7 and 10. [d] Reference 28 and 10.

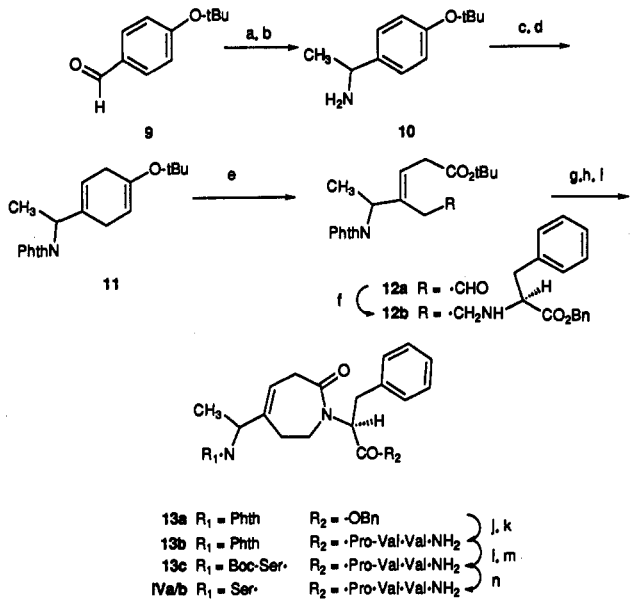
Scheme I[a]



[a] Reaction conditions: (a) Li, NH_3, tBuOH, EtOH; (b) TBDPSiCl, imidazole, DMF; (c) O_3, MeOH; Me_2S; (d) HCl-Val-OtBu, Et_3N $NaBH_3CN$, MeOH; (e) aq NaOH, dioxane; HCl; (f) DPPA, Et_3N, DMF; (g) TBAF, THF; (h) Jones oxidation; (i) CH_2N_2, Et_2O; (j) KN(TMS)$_2$, THF; BnBr; (k) EtOCOCl, Et_3N, THF; NH_4OH; (l) TFA, CH_2Cl_2; (m) Val-OMe, DCC, HOBt, DMF, CH_2Cl_2; (n) PhI(OCOCF$_3$)$_2$, H_2O, CH_3CN; (o) (Boc-Ala)$_2$O, Et_3N, DMF; (p) HCl, dioxane; (q) Boc-Ser, DCC, HOBt, Et_3N, DMF.

The synthesis of the C_7 mimetic in compound **II** is shown in Scheme I. Starting with 4-methoxyphenethyl alcohol (1), Birch reduction and protection of the resulting alcohol as its TBDPS ether, followed by ozonolysis and reductive amination with valine *tert*-butyl ester, afforded amine **3a** as an approximately 80:20 mixture of desired material and over-reduced saturated product **3b**. All attempts to modify the Birch reduction conditions varied the amount of over-reduced product present but could not completely eliminate it. Saponification of the mixture followed by cyclization with diphenyl phosphorazidate (DPPA) gave **4a**, which was treated with tetrabutylammonium fluoride to remove the silyl ether, affording alcohol **4b**. The over-reduced side product was removed at this stage by column chromatography. Treatment of alcohol **4b** with Jones reagent followed by excess ethereal diazomethane gave ester **5a**. Selective alkylation α to the ester with benzyl bromide afforded **5b** as a mixture of diastereomers at the *i*th position of the mimetic. Saponification of **5b** followed by ammonolysis of the intermediate mixed anhydride gave the primary amide, which was converted to **6** upon treatment with TFA followed by a DCC coupling to valine methyl ester. A modified Hofmann rearrangement of **6** using [bis(trifluoroacetoxy)iodo]benzene afforded the corresponding amine,[11] which was then coupled with the symmetrical anhydride of Boc-alanine to yield diastereomers **7a** and **7b**. These diastereomers were conveniently separated at this point. Final deprotection and coupling

Scheme II[a]



[a] Reaction conditions: (a) $LiN(TMS)_2$, THF, 0 °C; (b) MeMgBr reflux; (c) Li, NH_3, THF, tBuOH, EtOH; (d) *N*-(ethoxycarbonyl)phthalimide, Et_3N, THF; (e) O_3, CH_2Cl_2, MeOH; Me_2S; (f) TSA-Phe-OBn, Et_3N, $NaBH_3CN$, MeOH; (g) TFA, CH_2Cl_2; (h) HCl, dioxane; (i) DPPA, Et_3N, $NaHCO_3$, DMF; (j) HF, 0 °C 45 min; (k) TFA-Pro-Val-Val-NH_2, Et_3N, HOBt, Bop, DMF, 0 °C, 2 days; (l) NH_2NH_2-H_2O, EtOH; (m) Boc-Ser, HOBt, DCC, DMF; (n) TFA, CH_2Cl_2.

of the remaining alanine residue to each diastereomer gave compounds **IIa** and **IIb**.[12]

For the syntheses of the C_7 mimetic in **IV**, an alternate strategy was used which allowed for the early introduction of the *i*th side chain (Scheme II). This change simplified the overall synthesis but could not be used for mimetics containing a side chain in the *i*th position of the C_7 that was incompatible to the Birch reaction, such as the benzyl side chain in **II**. Beginning the synthesis of **IV**, *p*-*tert*-butoxybenzaldehyde (**9**) was converted into its TMS imine with lithium hexamethyldisilazide and then treated with methyl Grignard to give the requisite substituted benzylamine **10**.[13] Birch reduction of **10** as before followed by protection of the amine with the phthalimide group afforded diene **11**.[14] Selective ozonolysis and reduction of **11** afforded aldehyde **12a**, which was partially purified[15] and subjected to reductive amination with phenylalanine benzyl ester to yield amine **12b**. Removal of the *tert*-butyl ester with TFA, conversion of the amine to its hydrochloric acid salt, and cyclization with DPPA afforded protected mimetic **13**. Finally, removal of the benzyl ester with HF, coupling to Pro-Val-Val-NH_2 with Bop reagent,[16] and removal of the phthalimide protecting group using hydrazine, followed by coupling of Boc-serine with DCC and TFA deprotection, gave compounds **IVa** and **IVb** as a separable mixture of diastereomers.[12]

Synthesis of the C_7 mimetic in **III** was substantially more involved. Since the mimetic contains three side chains, a synthesis was required that allowed at least two of the three side chains to be introduced stereoselectively, in order to obtain a manageable mixture of two diastereomers in the final product. This synthesis was accomplished using Evans' methodology to synthesize intermediate **10** enantioselectively[17] (Scheme III). Evans' amide **15d** was obtained by conversion of **14** to the *tert*-butyl ether **15a**[18] with isobutylene and catalytic triflic acid,[19] saponification of the methyl ester to give acid **15b**, and conversion to the acid fluoride **15c** using cyanuric fluoride,[20] followed by treatment with the lithium salt of (*S*)-4-benzyl-2-oxazolidinone.[21] Alkylation of **15d** with methyl iodide using lithium hexamethyldisilazide gave **16a** in 82% d.e. which was purified by flash chromatography to greater than 95% d.e. by HPLC analysis. Removal of the chiral auxiliary with lithium hydroperoxide gave acid **16b**, which was converted to its Cbz-protected amine **16c** by a modified Curtius rearrangement using DPPA and benzyl alcohol.[22] The Cbz protecting group was removed by catalytic hydrogenolysis to give amine **16d** as a single enantiomer. Birch reduction followed by selective ozonolysis and reduction was repeated as before to give aldehyde **18a**.

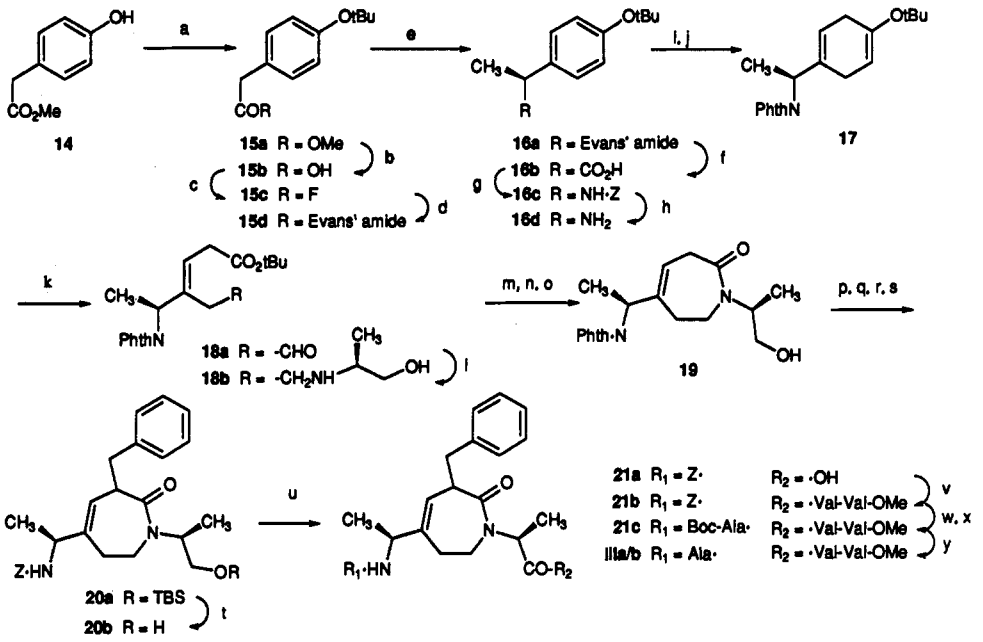
Initially, alanine methyl ester was used in the reductive amination reaction with aldehyde **18a**. It was found, however, during later alkylation of the ring to introduce the $i + 1$ side chain, that complete racemization of the $i + 2$ alanine side chain took place as determined by ^1H NMR. Consequently L-alaninol was used in place of alanine to eliminate this problem and then later oxidized to its acid after introduction of the $i + 1$ side chain. Reductive amination of aldehyde **18a** with L-alaninol therefore afforded amine **18b**, which was deprotected with TFA, converted to its hydrochloric acid salt as before, and cyclized with DPPA to yield compound **19** as a single isomer. After protection of the alcohol in **19** as its *tert*-butyldimethylsilyl ether, the phthalyl protecting group was removed using hydrazine and then replaced with the Cbz protecting group.[23] Alkylation of the Cbz-protected seven-membered ring with benzyl iodide afforded the fully substituted mimetic **20a** as a mixture of diastereomers at the $i + 1$ position. Although these diastereomers were separable by careful column chromatography at this point, the benzyl side chain was found susceptible to epimerization in subsequent reactions and was therefore carried on as a mixture through the rest of the synthesis, and then separated in the final purification. With the benzyl side chain introduced, the silyl protecting group was removed with acid to afford alcohol **20b**. The alcohol was then oxidized with Jones reagent, without epimerization, to yield acid **21**, which was taken on via sequential couplings and deprotections of the remaining amino acid residues to afford compounds **IIIa** and **IIIb** as a separable mixture of diastereomers.[12]

For the synthesis of reduced amine mimetic **VI**, γ-turn intermediate **21** was employed. Using a previously reported method,[24] (Scheme IV) **21** was first esterified with diazomethane to yield methyl ester **22a** and then selectively converted to thioamide **22b** with Lawesson's reagent. S-Alkylation with Meerwein's reagent and reduction of the resulting iminium salt with sodium borohydride gave amine **23a** as a mixture of diastereomers at the benzyl position.[25] Amine **23a** was then carried on to give diastereomeric reduced-amide mimetics **VIa** and **VIb** using methods already described.[12]

Results and Discussion

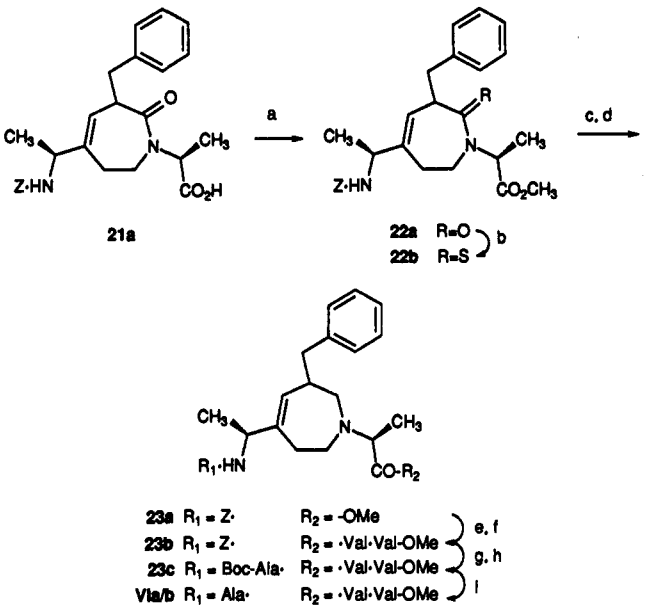
Of the compounds containing γ-turn mimetics (compounds **II**–**IV**, see Table I), compound **IIIa** with the C_7 mimetic replacing the P_2 to P_1' sequence of substrate **I** resulted in the highest inhibitory activity with a K_i of 147 μM. Enzyme assays also confirmed that **IIIa** was a competitive inhibitor with respective to substrate, but was not a substrate itself since no proteolytic amide-bond cleavage was observed by HPLC analysis (data not shown).[26] Compound **IIa** with the C_7 mimetic replacing

Scheme III[a]



[a] Reaction conditions: (a) isobutylene, cat. TfOH, CH_2Cl_2, −28 °C; (b) dioxane, 1 N NaOH; HCl; (c) cyanuric fluoride, pyridine, CH_3CN; (d) (*S*)-4-benzyl-2-oxazolidinone, BuLi, THF, −78 °C; (e) $LiN(TMS)_2$, THF; CH_3I; (f) LiOH, H_2O_2, THF, H_2O; Na_2SO_3; HCl; (g) DPPA, Et_3N, toluene, 80 °C; BnOH; (h) H_2, Pd/C, MeOH; (i) Li, NH_3, THF, tBuOH; EtOH; (j) *N*-(ethoxycarbonyl)phthalimide, Et_3N, THF; (k) O_3, CH_2Cl_2, MeOH; Me_2S; (l) L-alaninol, HOAc, $NaBH_3CN$, MeOH; (m) TFA, CH_2Cl_2; (n) HCl, dioxane; (o) DPPA, Et_3N, $NaHCO_3$, DMF; (p) TBSOTf, 2,6-lutidine, CH_2Cl_2; (q) NH_2NH_2–H_2O, EtOH; (r) ZOSu, Et_3N, THF; (s) $LiN(TMS)_2$, THF; BnI; (t) 3:1:1 HOAc/THF/H_2O; (u) Jones oxidation; (v) HCl-Val-Val-OMe, Bop, Et_3N, HOBt, DMF; (w) HF, anisole, 45 min, 0 °C; (x) Boc-Ala, DCC, Et_3N, DMF; (y) HCl, dioxane.

Scheme IV[a]



[a] Reaction conditions: (a) CH_2N_2, Et_2O, THF; (b) Lawesson's reagent, toluene, 80 °C, 4 h; (c) Et_3OBF_4, CH_2Cl_2, 0 °C to rt; (d) $NaBH_4$, MeOH; (e) 1 N aq NaOH, MeOH; HCl; (f) HCl-Val-Val-OMe, Bop, Et_3N, HOBt, DMF; (g) HF, anisole, 45 min, 0 °C; (h) Boc-Ala, DCC, Et_3N, DMF; (i) TFA, CH_2Cl_2.

the P_1 to P_2' sequence of **I**, by contrast, displayed 1 order of magnitude less inhibition and was shown to be noncompetitive with substrate (data not shown). Compounds **IVa** and **IVb** likewise were found to have negligible activity. Because of its improved binding relative to compound **I** and the competitive nature of its inhibition, it is tempting to suggest that mimetic **IIIa** effectively constrains linear substrate **I** into its biologically active conformation. However, for steric reasons, it is highly improbable that the proline residue in **I** would occupy the $i + 2$ position of a γ-turn. One possible explanation for the improved activity exhibited by **IIIa** is that the C_7 mimetic locks the substrate **I** into a conformation which, while not favored in the ground state of the linear proline-containing substrate, binds to the enzyme more effectively by adopting a conformation similar to the transition-state conformation of bound substrate. Distortions in substrate conformation upon substrate binding to rhizopuspepsin has been postulated from the crystal structure of a reduced-amide inhibitor complexed to rhizopuspepsin.[32a] The significant increase in binding affinity found for compound **IIIa** over substrate **I** could be explained by this hypothesis, since it has been shown that enzymes have a higher binding affinity for substrates at their transition-state conformation as compared to their ground-state conformation.[27] Another possible explanation is that compound **IIIa** is accessing different receptor sites in the enzyme active site and happens to bind more effectively to the enzyme than substrate **I**. In order to test the first hypothesis and to modify **IIIa** into a more effective inhibitor, the carbonyl of the C_7 mimetic in **IIIa** was next replaced with an sp^3 methylene group, affording constrained reduced-amide **VI**. This modification incorporates the tetrahedral transition-state geometry found during amide-bond hydrolysis.[28] Peptidomimetic **VIa** indeed was a significantly better inhibitor of HIV-1 protease with a K_i of 430 nM. This activity was over 300-fold better than the original amide-containing mimetic **IIIa**, providing support for the transition-state hypothesis. **VIa**, in addition, was found to have over 1 order of magnitude (44-fold) better inhibitory activity than a similar linear reduced-amide inhibitor, **V**,[10,29] suggesting that the reduced-amide C_7 mimetic may be adopting the bioactive conformation of linear reduced-amide type inhibitors. In fact a preference for the C_7 conformation has been shown by X-ray diffraction studies of a linear reduced-amide-containing peptide[30] and, more recently, by the determination of a

local minimum in the C_7^{eq} region of conformational space available to a linear reduced-amide-containing dipeptide.[31] Examination of X-ray crystal structures of linear substrate inhibitors bound to HIV-1 protease also indicate that a C_7-like backbone would be compatible with the structure for the P_2 to P_1' sequence.[32]

Conclusion

A strategy to reduce the conformational mobility of oligopeptides involves the rational design and synthesis of rigid, cyclic scaffolds to mimic and replace peptide secondary structural elements. One ultimate goal of an effective mimetic is to retain or preferably increase biological activity when incorporated into its peptide. In this study we have shown that the incorporation of a C_7 mimetic replacing the P_2 to P_1' positions of a model HIV-1 protease substrate **I** with a K_m in the millimolar range resulted in constrained inhibitor **IIIa** with significantly improved binding affinity to HIV-1 protease (K_i = 147 μM). We have also shown that reduction of the C_7 amide in compound **IIIa** resulted in novel constrained reduced-amide inhibitor **VIa** now with a K_i in the high nanomolar range (K_i = 430 nM). This corresponds to over a 300-fold improvement in inhibitory activity over the original amide containing mimetic **IIIa**. Lastly we have shown that cyclic reduced-amide **VIa** is over 1 order of magnitude better than a similar linear reduced-amide inhibitor **V**, suggesting that the reduced-amide C_7 mimetic may be an effective conformational mimetic of linear reduced-amide type inhibitors. Using the conformational mimetics approach to probe the sequence of a model HIV-1 protease substrate we have obtained a novel constrained reduced-amide-type inhibitor, which, through additional modifications, can possibly lead to even more potent inhibitors of HIV-1 protease. Further studies into the conformation and exact binding mode of these novel turn-mimetic-containing peptides are being pursued and will be reported later.

Experimental Section

General. ^{1}H NMR spectra were recorded at 90 MHz on a Varian EM390 spectrometer and at 250 MHz on a Bruker AM250 spectrometer. Chemical shifts are recorded in δ units from the internal standard tetramethylsilane. Mass spectra were taken on a Finnigan Model 3300 mass spectrometer. High-resolution mass spectra were taken on a VG 70 SE mass spectrometer. Ozone was generated from a Ozone Research Equipment Corp. Model 03V10-0 ozonator. Gas chromatography was performed on a Hewlett-Packard 5890 instrument using an HP-1 (0.53 mm × 10 m) methyl silicone gum capillary column. TLC was carried out on Analtech silica gel GF plates and visualized by using 10% bleach/1% starch KI. Both flash and gravity chromatography were carried out on Merck 60 (230–400 mesh) silica gel. Tetrahydrofuran was distilled from sodium ketyl immediately before use. All final compounds were shown to be a single homogeneous peak both by isocratic and gradient HPLC on a Beckman series 342 liquid chromatograph with ultraviolet detection at both 254 and 220 nm.

Methyl [2-[4-(*tert*-Butyldiphenylsilyl)oxy]ethyl]-6-oxo-3-hexenoate (2). Liquid ammonia (520 mL) was condensed in a three-necked flask which was fitted with a cold finger and overhead stirrer and kept at −78 °C with a dry ice/2-propanol bath. A solution of 1 (50 g, 329 mmol) in tetrahydrofuran (140 mL) was added to the reaction flask followed by the addition of small pieces of lithium wire (10.3 g, 1.48 mol) over a period of about 15 min. The reaction mixture was stirred an additional 30 min at −78 °C and then quenched by the slow addition of absolute ethanol (400 mL). After the reaction was completely quenched (white color), it was allowed to warm to room temperature overnight to allow most of the NH_3 to evaporate. The residue was partitioned between water and diethyl ether, and the organic layer was collected, dried over anhydrous $MgSO_4$, filtered, and evaporated at reduced pressure. The residue, which still contained some water, was dissolved in methylene chloride, dried over anhydrous $MgSO_4$, filtered, and evaporated at reduced pressure to give crude diene as an oil (40.3 g) contaminated with about 20–25% of the over-reduced cyclohexene product: ^{1}H NMR δ ($CDCl_3$) δ (major component) 2.12 (1H, br s), 2.28 (2H, t, J = 7.5 Hz), 2.78 (4H, br s), 3.58 (3H, s), 3.73 (2H, t, J = 7.5 Hz), 4.68 (1H, br s), 5.55 (1H, br s).

The crude diene was dissolved in DMF (150 mL) and treated at room temperature with imidazole (44.8 g, 658 mmol) and *tert*-butyldiphenylsilyl chloride (85.6 mL, 329 mmol). The resulting solution was stirred at room temperature for 2 d (though the reaction was essentially complete after a few hours), after which time it was diluted with 10% ethyl acetate in hexane, washed (3×) with water, dried over anhydrous $MgSO_4$, filtered, and evaporated to give crude protected diene still contaminated with ene side product: ^{1}H NMR ($CDCl_3$) δ (major component) 1.07 (9H, s), 2.25 (2H, t, J = 6.0 Hz), 2.70 (4H, s), 3.55 (3H, s), 3.77 (2H, t, J = 6.0 Hz), 4.62 (1H, br s), 5.43 (1H, br s), 7.22–7.93 (10H, m).

The above crude protected diene (~164 mmol) was dissolved in a mixture of methanol and dichloromethane (4:1, 500 mL), the resulting solution was cooled to −78 °C and treated with O_3 until the starting material disappeared by TLC. The reaction mixture was then reduced with methyl sulfide (25 mL) and slowly brought to room temperature, where it was stirred for 18 h. The reaction mixture was evaporated at reduced pressure and the residue purified by flash chromatography (silica gel, 10 × 20 cm column, 15% ethyl acetate/hexane) to yield 26.77 g (38%) of **2** contaminated with its corresponding saturated aldehyde: ^{1}H NMR ($CDCl_3$) δ (major component) 1.05 (9H, s), 2.33 (2H, t, J = 6.0 Hz), 2.97–3.17 (4H, m), 3.67 (3H, s), 3.73 (2H, t, J = 6.0 Hz), 5.77 (1H, t, J = 7.5 Hz), 7.27–7.87 (10H, m), 9.63 (1H, t, J = 2.4 Hz).

***N*-[5-(Methoxycarbonyl)-3-[[2-[(*tert*-butyldiphenylsilyl)oxy]ethyl]-(*Z*)-pent-3-enyl]valine *tert*-Butyl Ester (3).** The aldehyde **2** (36.81 g, 86.7 mmol), contaminated with saturated aldehyde, was dissolved in methanol (300 mL) and treated at −20 °C with a mixture of valine *tert*-butyl ester (9.69 g, 55.9 mmol) and valine *tert*-butyl ester hydrochloride (11.7 g, 55.9 mmol) followed by sodium cyanoborohydride (6.0 g, 95.4 mmol). The resulting reaction mixture was stirred at room temperature for 24 h. At this time, the reaction mixture was evaporated at reduced pressure, the residue dissolved in ethyl acetate, washed with 5% Na_2CO_3 (aqueous), dried over anhydrous $MgSO_4$, filtered, and evaporated at reduced pressure. The residue was purified by flash chromatography (silica gel, 10 × 22 cm column, 10% ethyl acetate–hexane) to give 30.53 g (61%) of **3**, contaminated with saturated material: ^{1}H NMR ($CDCl_3$) δ (major component) 0.93 (d, 6H, J = 7.5 Hz), 1.05 (s, 9H), 1.47 (s, 9H), 1.63–2.87 (m, 9H), 3.07 (d, 2H, J = 7.5 Hz), 3.60 (s, 3H), 3.72 (t, 2H, 7.5 Hz), 5.42 (t, 1H, J = 7.5 Hz), 7.27–7.77 (m, 10 H).

1-[1(*S*)-(*tert*-Butoxycarbonyl)-2-methylpropyl]-5-[2-[(*tert*-butyldiphenylsilyl)oxy]ethyl]-2,3,6,7-tetrahydro-1*H*-azepin-2-one (4a). The amino ester **3** (30.5 g, 52.5 mmol) was dissolved in dioxane (130 mL) and the resulting solution was treated with aqueous 1 N NaOH (63 mL) at room temperature for 4 h. The reaction mixture was then treated with aqueous 3 N HCl (63 mL) and evaporated under high vacuum. The residue was re-evaporated from toluene (3×) and the residue dried under vacuum. The resulting amino acid hydrochloride was dissolved in dry DMF (1800 mL), cooled to 0 °C, and treated sequentially with triethylamine (22 mL, 157.5 mmol) and DPPA (22.6 mL, 105 mmol). The reaction mixture was brought to room temperature and stirred for 48 h. After this time, the reaction mixture was evaporated under high vacuum and the residue purified by flash chromatography (silica gel, 10 × 24 cm column, 15–20% ethyl acetate/hexane) 19.83 g (69%) **4a**, contaminated with saturated product: ^{1}H NMR ($CDCl_3$) δ (major component) 0.83 (d, 3H, J = 6.9 Hz), 0.97 (d, 3H, J = 6.9 Hz), 1.05 (s, 9H), 1.42 (s, 9H), 1.87–2.30 (m, 5H), 3.18 (br d, 2H, J = 6 Hz), 3.60 (t, 2H, J = 7.5 Hz), 3.68 (t, 2H, J = 7.5 Hz), 4.73 (d, 1H, J = 11 Hz), 5.33 (t, 1H, J = 6 Hz), 7.20–7.73 (m, 10H).

1-[1(*S*)-(*tert*-Butoxycarbonyl)-2-methylpropyl]-5-(2-hydroxyethyl)-2,3,6,7-tetrahydro-1*H*-azepin-2-one (4b). The

protected alcohol **4a** (19.83 g, 36.1 mmol) was dissolved in tetrahydrofuran (75 mL) treated with 72 mL of 1 M nBu_4NF (THF) at room temperature for 5 h. The reaction mixture was evaporated and the residue was purified using flash chromatography (silica gel, 10 × 22 cm, 60–100% ethyl acetate/hexane) to give **4b** still partially contaminated with its saturated isomer. Further purification by column chromatography (silica gel, 4 × 50 cm, 75% ethyl acetate/hexane) gave 7.63 g (67.9%) pure **4b**: ^{1}H NMR ($CDCl_3$) δ 0.87 (d, 3H, J = 7.5 Hz), 1.00 (d, 3H, J = 7.5 Hz), 1.45 (s, 9H), 1.93–2.43 (m, 6H), 3.17–3.37 (m, 2H), 3.53–3.83 (m, 4H), 4.73 (d, 1H, J = 10.5 Hz), 5.47 (t, 1H, J = 7.5 Hz).

1-[1(*S*)-(*tert*-Butoxycarbonyl)-2-methyl-1-propyl]-5-[(methoxycarbonyl)methyl]-2,3,6,7-tetrahydro-1*H*-azepin-2-one (5a). The alcohol **4b** (4.91 g, 15.8 mmol) in acetone (60 mL) was treated with 11 mL of Jones reagent at 0 °C for 1 h. The reaction mixture was quenched with 2-propanol, diluted with saturated NaCl solution, and extracted with ethyl acetate. The combined organic extracts were dried over $MgSO_4$ and evaporated at reduced pressure. The residue was then dissolved in CH_2Cl_2 and treated with excess ethereal CH_2N_2 at 0 °C. After the excess CH_2N_2 was decomposed, the reaction mixture was evaporated. Purification of the crude residue by flash chromatography (silica gel, 5 × 20 cm, 25–35% ethyl acetate/hexane) gave 3.50 g (65%) of **5a**: ^{1}H NMR ($CDCl_3$) δ 0.88 (d, 3H, J = 7.5 Hz), 1.00 (d, 3H, J = 7.5 Hz), 1.45 (s, 9H), 1.77–2.74 (m, 3H), 2.93 (s, 2H), 3.27 (br d, 2H, J = 6 Hz), 3.62 (s, 3H), 3.60–3.83 (m, 2H), 4.77 (d, 1H, J = 11 Hz), 5.53 (t, 1H, J = 6 Hz).

1-[1(*S*)-(*tert*-Butoxycarbonyl)-2-methyl-1-propyl]-5-[1(*R,S*)-(methoxycarbonyl)-2-phenyl-1-ethyl]-2,3,6,7-tetrahydro-1*H*-azepin-2-one (5b). A solution of **5a** (1.15 g, 3.39 mmol) in dry THF (10 mL) was cooled to −78 °C and treated with 3.5 mL of 1 M $KN(TMS)_2$ in THF and stirred for 20 min. Benzyl bromide (806 μL, 6.78 mmol) was added and the reaction mixture was stirred at −78 °C for 10 min and then slowly warmed to room temperature. The reaction was quenched with 10% NH_4Cl (aqueous), diluted with H_2O, and extracted with ethyl acetate. The combined organic extracts were dried over $MgSO_4$ and evaporated at reduced pressure. The residue was purified by flash chromatography (silica gel, 3 × 18 cm, 25% ethyl acetate/hexane) to give 528 mg (36%) of **5b** as an inseparable set of diastereoisomers: ^{1}H NMR ($CDCl_3$) δ 0.82/0.83 (2d, 3H, J = 6.9 Hz), 0.98 (d, 3H, J = 6.9 Hz), 1.45 (s, 9H), 1.88–2.45 (m, 3H), 2.57–3.33 (m, 5H), 3.53–3.82 (m, 2H), 3.57 (s, 3H), 4.75/4.77 (2d, 1H, J = 10.5 Hz), 5.45–5.73 (m, 1H), 7.00–7.40 (m, 5H).

***N*-[2-[(1(*R,S*)-Carbamoyl-2-phenyl-1-ethyl)-2-oxo-2,3,6,7-tetrahydro-1*H*-azepin-1-yl]-3-methylbutyryl]valine Methyl Ester (6).** A solution of **5b** (528 mg, 1.23 mmol) in dioxane (4 mL) was treated with 1.4 mL of 1 N NaOH (aqueous) at room temperature for 24 h. The reaction mixture was acidified with 3 N HCl (aqueous) and evaporated at reduced pressure. The residue was dissolved in ethyl acetate, washed with H_2O, dried over $MgSO_4$, and evaporated to give 544 mg of crude product, which was carried on without purification.

A solution of the crude acid in THF (14 mL) was cooled to −20 °C and treated with triethylamine (686 μL, 4.92 mmol) and ethyl chloroformate (470 μL, 4.92 mmol) and stirred for 30 min. At this time, a mixture of concentrated NH_4OH (2 mL) and THF (10 mL) was added and the reaction mixture was stirred at −20 °C for 30 min and left standing at 5 °C for 18 h. The reaction mixture was then poured into ice-cold 3 N HCl (aqueous) and extracted with ethyl acetate. The combined organic extracts were dried over $MgSO_4$ and evaporated at reduced pressure. Partial purification of the residue by flash chromatography (silica gel, 3 × 20 cm, 96:4:0.1 $CHCl_3$/MeOH/HOAc) gave 553 mg of amide as a mixture of diastereoisomers contaminated with ethyl carbamate: ^{1}H NMR ($CDCl_3$) δ 0.83 (d, 3H, J = 6 Hz), 0.97 (d, 3H, J = 6 Hz), 1.45 (s, 9H), 2.10–3.33 (m, 8H), 3.63 (d, 2H, J = 6 Hz), 4.70 (d, 1H, J = 10.5 Hz), 5.53 (t, 1H, J = 6 Hz), 5.97–6.30 (m, 2H), 6.97–7.37 (m, 5H).

The carboxamide from above was dissolved in a mixture of 80% TFA in CH_2Cl_2 (12 mL) and stirred at room temperature for 1 h. The reaction was then evaporated at reduced pressure, evaporated from toluene, and then dried under vacuum. This acid was dissolved in DMF (5 mL) and the pH of the resulting solution adjusted to ~7 with triethylamine. This solution was then treated with valine methyl ester (520 mg, 3.96 mmol), HOBt (250 mg, 1.85 mmol), and DCC (279 mg, 1.35 mmol). After stirring for 44 h, the reaction mixture was filtered and evaporated under vacuum. The residue was purified by flash chromatography (silica gel, 3 × 20 cm, 98:2 $CHCl_3$/MeOH) to give 486 mg (84%) **6** as an inseparable mixture of diastereoisomers: ^{1}H NMR ($CDCl_3$) δ 0.67–1.13 (m, 12H), 1.17–3.40 (m, 8H), 3.53–3.90 (m, 2H), 3.70 (s, 3H), 3.95–4.83 (m, 3H), 5.57 (t, 1H, J = 6 Hz), 5.70–6.00 (m, 2H), 6.80–7.40 (m, 6H).

***N*-[2-[5-[1(*R,S*)-[[*N*-(*tert*-Butoxycarbonyl)alaninyl]amino]-2-phenyl-1-ethyl]-2-oxo-2,3,6,7-tetrahydro-1*H*-azepin-1-yl]-3-methylbutyryl]valine Methyl Ester (7a/7b).** The solution of **6** (486 mg, 1.03 mmol) in CH_3CN/H_2O (4:1, 10 mL) was treated at room temperature with [bis(trifluoroacetoxy)iodo]-benzene (500 mg, 1.16 mmol) for 4 h. The reaction mixture was evaporated at reduced pressure and then evaporated from $CHCl_3$/toluene. A solution of Boc-Ala-OH (1.17 g, 6.18 mmol) in CH_2Cl_2 (50 mL) was treated with DCC (607 mg, 2.94 mmol) at 0 °C for 30 min and then filtered and evaporated to give $(Boc\text{-}Ala)_2O$. The crude amine salt, from above, was dissolved in DMF (10 mL), basified (pH 8) with triethylamine, and treated with a solution of $(Boc\text{-}Ala)_2O$ in DMF at room temperature for 18 h. After evaporation at reduced pressure, the residue was first purified by flash chromatography (silica gel, 3 × 20 cm, 45–60% ethyl acetate/hexane) and then by gravity chromatography (silica gel, 2.5 × 50 cm, 50–60% ethyl acetate/hexane) to yield the two diastereoisomers **7a** (201 mg, 32%) and **7b** (142 mg, 22%). Compound **7a**: ^{1}H NMR ($CDCl_3$) δ 0.73–1.07 (m, 12H), 1.10 (d, 3H, J = 7.5 Hz), 1.43 (s, 9H), 1.87–3.83 (m, 10H), 3.70 (s, 3H), 3.87–4.67 (m, 4H), 4.80–5.23 (m, 1H), 5.58 (t, 1H, J = 7.5 Hz), 6.37 (d, 1H, J = 9 Hz), 6.73 (d, 1H, J = 9 Hz), 7.00–7.30 (m, 5H); MS (FAB) m/z 615 $(M + H)^+$. Compound **7b**: ^{1}H NMR ($CDCl_3$) δ 0.73–1.10 (m, 12H), 1.20 (d, 3H, J = 7.5 Hz), 1.45 (s, 9H), 1.97–3.80 (m, 10H), 3.72 (s, 3H), 3.90–4.67 (m, 4H), 4.70–4.97 (m, 1H), 5.53 (t, 1H, J = 6 Hz), 6.35 (d, 1H, J = 9 Hz), 6.75 (d, 1H, J = 9 Hz), 7.00–7.37 (m, 5H); MS (FAB) m/z 615 $(M + H)^+$.

***N*-[2-[5-[1(*R/S*)-[[*N*-[*N*-(*tert*-Butoxycarbonyl)alaninyl]-alaninyl]amino]-2-phenyl-1-ethyl-2-oxo]-2,3,6,7-tetrahydro-1*H*-azepin-1-yl]-3-methylbutyryl]valine (8a).** The protected peptide **7a** (117 mg, 0.190 mmol) was treated with 4 N HCl in dioxane (5 mL) at room temperature for 1.5 h. The reaction mixture was evaporated at reduced pressure, evaporated from toluene, and dried under vacuum. The residue was dissolved in DMF (4 mL) basified (pH ~8) with triethylamine and treated with a solution of $(Boc\text{-}Ala)_2O$ (2 equiv) in DMF at room temperature for 24 h. After evaporation at reduced pressure, the residue was purified by flash chromatography (silica gel, 3 × 20 cm, 50–100% ethyl acetate/hexane) to yield 61 mg (47%) of **8a**: ^{1}H NMR ($CDCl_3$) δ 0.67–1.60 (m, 18H), 1.43 (s, 9H), 1.80–3.87 (m, 10H), 3.70 (s, 3H), 3.90–4.70 (m, 5H), 4.93–5.27 (m, 1H), 5.43–5.73 (m, 1H), 6.57–6.97 (m, 3H), 7.00–7.37 (m, 5H).

***N*-[2-[5-[1(*R/S*)-[[*N*-[*N*-(*tert*-Butoxycarbonyl)alaninyl]-alaninyl]amino]-2-phenyl-1-ethyl]-2-oxo-2,3,6,7-tetrahydro-1*H*-azepin-1-yl]-3-methylbutyryl]valine Methyl Ester (8b).** The protected peptide **7b** (66 mg, 0.107 mmol) was treated with 4 N HCl in dioxane (5 mL) at room temperature for 1.5 h. The reaction mixture was evaporated at reduced pressure, evaporated from toluene, and dried under vacuum. The residue was dissolved in DMF (4 mL) basified (pH ~8) with triethylamine, and treated with a solution of $(Boc\text{-}Ala)_2O$ (2 equiv) in DMF at room temperature for 96 h. After evaporation at reduced pressure, the residue was purified by flash chromatography (silica gel, 2 × 20 cm, 75–90% ethyl acetate/hexane) to yield 50 mg (68%) of **8b**: ^{1}H NMR ($CDCl_3$) δ 0.73–1.13 (m, 12H), 1.17–1.43 (m, 6H), 1.45 (s, 9H), 1.63–3.80 (m, 10H), 3.70 (s, 3H), 3.83–4.63 (m, 5H), 4.92–5.25 (m, 1H), 5.53 (t, 1H, J = 6 Hz), 6.47–6.93 (m, 3H), 6.98–7.30 (m, 5H).

***N*-[2-[5-[1(*R/S*)-[[(Serinylalaninyl)alaninyl]amino]-2-phenyl-1-ethyl]-2-oxo-2,3,6,7-tetrahydro-1*H*-azepin-1-yl]-3-methylbutyryl]valine Methyl Ester (IIa).** The protected peptide **8a** (61 mg, 0.089 mmol) was treated with 4 N HCl in dioxane (3 mL) at room temperature for 2 h. The reaction mixture was evaporated at reduced pressure, evaporated (2×) from DMF/toluene, and dried under vacuum. The residue was dissolved in DMF (4 mL); neutralized (pH ~7.5) with triethylamine; treated with Boc-Ser-OH (55 mg, 0.267 mmol), HOBt (40 mg, 0.294 mmol), and DCC (55 mg, 0.267 mmol); and stirred at room temperature

for 20 h. After evaporation at reduced pressure, the residue was treated with a mixture of TFA and CH_2Cl_2 (2:1, 6 mL) at room temperature for 4 h. After evaporation the residue was purified first by partition chromatography (G-25, 2.5 × 1000 cm, eluted with 4:1:5 butanol/acetic acid/water, upper phase) and then twice by preparative HPLC (5 μm Apex-ODS, Jones Chromatography; 55:45 water/acetonitrile–0.1% trifluoroacetic acid; 65:35 water/acetonitrile–0.1% trifluoroacetic acid; UV detection at 220 nm) to give 5.2 mg of **IIa**. HRMS(FAB) calcd for $C_{34}H_{52}N_6O_8$ 672.3846, found 672.3858; HPLC k' 2.31 (5 μm Apex-ODS, Jones Chromatography; 65:35 water/acetonitrile–0.1% trifluoroacetic acid, UV detection at 200 nm); HPLC k' 6.73 (5 μm Apex-ODS; gradient: A, water/0.1% trifluoroacetic acid; B, acetonitrile/0.1% trifluoroacetic acid; 90–50% A during 20 min, hold 10 min; UV detection at 220 nm); TLC R_f 0.45, silica gel, 4:1:1 butanol/acetic acid/water; R_f 0.61 silica gel, 1:1:1:1 butanol/acetic acid/water/ethyl acetate.

***N*-[2-[5-[(*R*/*S*)-[[(Serinylalaninyl)alaninyl]amino]-2-phenyl-1-ethyl]-2-oxo-2,3,6,7-tetrahydro-1*H*-azepin-1-yl]-3-methylbutyryl]valine Methyl Ester (IIb).** The protected peptide **8b** (50 mg, 0.073 mmol) was treated with 4 N HCl in dioxane (3 mL) at room temperature for 2 h. The reaction mixture was evaporated at reduced pressure, evaporated (2×) from DMF/toluene and dried under vacuum. The residue was dissolved in DMF (5 mL); neutralized (pH ~7.5) with triethylamine; treated with Boc-Ser-OH (45 mg, 0.219 mmol), HOBt (33 mg, 0.241 mmol), and DCC (45 mg, 0.219 mmol); and stirred at room temperature for 72 h. After evaporation at reduced pressure, the residue was purified by flash chromatography (silica gel, 2 × 17 cm, 5% methanol/chloroform) to give 28 mg (68%) of protected peptide. The protected peptide was treated with a mixture of TFA and CH_2Cl_2 (1:1, 4 mL) at room temperature for 4 h and then evaporated at reduced pressure. The residue was purified by preparative HPLC (5 μm Apex-ODS, Jones Chromatography; 65:35 water/acetonitrile–0.1% trifluoroacetic acid; UV detection at 220 nm) to give 4.5 mg of **IIb**. HRMS (FAB) calcd for $C_{34}H_{52}N_6O_8$ 672.3846, found 672.3831; HPLC k' 1.85 (5 μm Apex-ODS, Jones Chromatography, 65:35 water/acetonitrile–0.1% trifluoroacetic acid, UV detection at 220 nm); HPLC k' 6.42 (5 μm Apex-ODS, gradient: A, water/0.1% trifluoroacetic acid; B, acetonitrile/0.1% trifluoroacetic acid; 90–50% A during 20 min, hold for 10 min; UV detection at 220 nm); TLC R_f 0.43, silica gel, 4:1:1 butanol/acetic acid/water; R_f 0.60, silica gel, 1:1:1:1 butanol/acetic acid/water/ethyl acetate.

1(*R*,*S*)-(*p*-*tert*-Butoxyphenyl)ethylamine (10). To a stirred solution of *p*-*tert*-butoxybenzaldehyde (**9**) (45 g, 250 mmol) in dry THF (100 mL) under Ar at 0 °C was added a solution of lithium hexamethyldisilazine (280 mL, 1 N in THF). The reaction was allowed to warm to room temperature and stirred for 15 min. A solution of methylmagnesium bromide (170 mL, 3 N in ether) was next added and the reaction heated at reflux, under argon, for 2 days. The reaction was slowly poured into cold saturated ammonium chloride (500 mL) with swirling, extracted with ether (2 × 300 mL), washed with brine, dried over sodium sulfate, and evaporated to dryness. Short-path distillation under high vacuum (bp 100 °C, 0.005 mmHg) afforded product **10** as a clear liquid (32.3 g, 71%): GC t_R 6.88 min (He carrier flow rate 20 mL/min, 100 °C initial temperature, hold 3 min, 10 °C/min increase rate, 180 °C final temperature, hold for 1 min; ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 9H), 1.35 (d, 3H, J = 7 Hz), 2.3 (br s, 2H), 4.07 (q, 1H), 6.93 (d, 2H, J = 8 Hz), 7.22 (d, 2H, J = 8 Hz).

1-(*tert*-Butyloxy)-4-[1(*R*,*S*)-(phthalylamino)-1-ethyl]-1,4-cyclohexadiene (11). To a stirred solution of the above amine **10** (32.3 g) and *tert*-butyl alcohol (14.5 g) in dry THF (200 mL) at −78 °C was condensed NH_3 (1000 mL) via a cold finger. Lithium wire (3.3 g) was then added in portions with vigorous stirring. The now blue reaction mixture was stirred for 1 h at −78 °C then slowly quenched with ethanol until the reaction turned white. The reaction was allowed to go to room temperature and warmed to allow the NH_3 to evaporate. The slurry was taken up in ether, washed with water, dried over sodium sulfate, and evaporated to give the diene as a clear liquid (32.6 g, >95% pure): GC t_R 7.26 min (He carrier flow rate 20 mL/min, 100 °C initial temperature, hold for 3 min, 10 °C/min increase rate, 180 °C final temperature, hold for 1 min); ^{1}H NMR ($CDCl_3$) δ 1.15 (d, 3H, J = 6 Hz), 1.3 (s, 9H), 1.4 (s, 2H), 2.7 (m, 4H), 3.45 (q, 1H), 5.1 (s, 1H), 5.6 (s, 1H).

To a stirred solution of the above diene (32.6 g) in THF (300 mL) was added *N*-(ethoxycarbonyl)phthalimide (36.6 g). After 2 h, triethylamine (24 mL) was added and the reaction stirred overnight at room temperature. TLC indicated the reaction was complete. The reaction was evaporated to dryness and re-evaporated several times with toluene to remove any excess triethylamine. The crude phthalated diene was purified by flash chromatography on silica gel eluted with 20% ethyl acetate in *n*-hexane to give **11** as a white solid (43.1 g, 79%): ^{1}H NMR ($CDCl_3$) δ 1.3 (s, 9H), 1.65 (d, 3H, J = 7 Hz), 2.73 (br s, 4H), 4.82 (t, 1H), 4.97 (m, 1H), 5.78 (m, 1H), 7.8 (m, 4H).

***N*-[5-(*tert*-Butoxycarbonyl)-3-[1(*R*,*S*)-(phthalylamino)-1-ethyl]-(*Z*)-pent-3-enyl]phenylalanine Benzyl Ester (12b).** The above diene **11** (43.1 g) was taken up in 3:1 methanol/CH_2Cl_2 (500 mL) and with stirring at −78 °C, O_3 was passed through the reaction until most of the diene was consumed by TLC. The reaction was then flushed with Ar to remove excess O_3 and dimethyl sulfide (30 mL) added. The reaction was allowed to warm to room temperature and stirred for 3 h. After evaporation of the solvent, the aldehyde **12a** (contaminated with conjugated aldehyde) was obtained as an oil by flash chromatography on silica gel eluted with 20% ethyl acetate in *n*-hexane (23.4 g, 50%): ^{1}H NMR ($CDCl_3$) δ 1.5 (s, 9H), 1.7 (d, 3H, J = 7 Hz), 3.05 (d, 2H, J = 7 Hz), 3.3 (s, 2H), 5.05 (q, 1H), 6.2 (t, 1H), 7.85 (m, 4H), 9.65 (t, 1H).

To the above crude aldehyde **12a** (5.0 g) in methanol (150 mL) under Ar was added L-phenylalanine benzyl ester *p*-toluenesulfonic acid salt (12 g), NEt_3 (2 mL), and $NaBH_3CN$ (1.0 g) in portions over 5 min. The reaction was stirred for 16 h, evaporated to dryness, taken up in ethyl acetate, washed with water, dried over Na_2SO_4, and re-evaporated. The crude product was purified by flash chromatography on silica gel eluted with 15% ethyl acetate in *n*-hexane to obtain **12b** as a mixture of diastereomers. (3.55 g, 43%); TLC R_f 0.57 silica, 30% EtOAc, *n*-hexane; R_f 0.32 silica, 80:15:5 *n*-hexane/EtOAc/pyridine.

1-[1(*S*)-(Benzyloxycarbonyl)-2-phenyl-1-ethyl]-5-[1(*R*,*S*)-(phthalylamino)ethyl]-2,3,6,7-tetrahydro-1*H*-azepin-2-one (13a). To the above diester (**12b**, 3.55 g) was added 80% TFA in CH_2Cl_2 (75 mL). After stirring for 1 h at room temperature, the reaction was evaporated to dryness and re-evaporated from toluene several times to remove trace amounts of TFA. To a stirred solution of the above in DMf (300 mL) at 0 °C under Ar was added NEt_3 (0.9 mL), $NaHCO_3$ (2.6 g), and diphenyl phosphorazidate (2.7 mL). The reaction was stirred under Ar at 0 °C for 2 days. After removal of the solvent, the residue was taken up in ethyl acetate, washed with water, dried over $MgSO_4$, evaporated, and purified by flash chromatography on silica gel eluted with 35% EtOAc in *n*-hexane, affording product, a mixture of diastereomers, as an oil (1.39 g, 45%): TLC R_f 0.17 silica, 30% EtOAc, *n*-hexane; ^{1}H NMR ($CDCl_3$) δ 1.45 (d, 3H, J = 7 Hz), 2.0 (m, 2H), 3.1–3.6 (m, 6H), 4.6 (t, 1H), 5.05 (m, 1H), 5.2 (d, 2H, J = 3 Hz), 5.75 (br s, 1H), 7.2 (s, 5H), 7.35 (s, 5H), 7.8 (m, 4H).

Synthesis of *N*-[2-[2-Oxo-5-[1(*R*,*S*)-(serinylamino)-1-ethyl]-2,3,6,7-tetrahydro-1*H*-azepin-1-yl]-1-oxo-3-phenylpropyl]prolylvalinylvaline Amide (IVa/IVb). To the above benzyl ester **13a** (1.39 g) in an HF apparatus was added anisole (2 mL) followed by anhydrous HF (30 mL) at −78 °C. The solution was stirred for 45 min at 0 °C and then evaporated to dryness by a water aspirator. The product was obtained as a white solid by flash chromatography on silica gel eluted with a gradient from chloroform to 98:2:1 $CHCl_3$/MeOH/HOAc (1.18 g, 100%): ^{1}H NMR ($CDCl_3$) δ 1.5 (d, 3H, J = 7 Hz), 2.0 (m, 2H), 3.1–3.6 (m, 6H), 4.6 (dd, 1H), 5.05 (m, 1H), 5.2 (d, 2H, J = 3 Hz), 5.75 (br s, 1H), 7.2 (s, 5H), 7.8 (m, 4H), 10.4 (s, 1H); MS (DCI, CH_4) m/z 433 $(M + H)^+$.

To a stirred solution of the above acid (0.5 g) in DMF (25 mL) was added Et_3N (1 mL), TFA-Pro-Val-Val-NH_2 (0.8 g), HOBt (0.3 g), and lastly Bop reagent (1.0 g). After stirring at room temperature for 16 h the reaction was evaporated to dryness. The residue which remained was loaded onto a flash silica gel column and eluted with (98:2) $CHCl_3$/MeOH to obtain **13b** as a white solid (0.78 g, 92%): TLC R_f 0.35, silica, 10% MeOH/$CHCl_3$.

To a stirred solution of **13b** (0.78 g) in ethanol (30 mL) was added hydrazine monohydrate (0.22 mL). The reaction was stirred at room temperature for 24 h during which the solution became a cloudy white suspension. The reaction was then evaporated to dryness, triturated with chloroform and filtered to remove the insoluble materials. The clear filtrate was evaporated to give the free amine, which was taken up in DMF (25 mL) and treated with Boc-Ser (0.34 g), HOBt (0.3 g), and finally DCC (0.36 g). After stirring at room temperature for 16 h the reaction was evaporated to dryness and loaded onto a flash silica gel column, which was eluted with 7% MeOH in $CHCl_3$ to yield **13c** as a white solid (0.44 g, 52%); TLC R_f 0.32, silica, 9:1 $CHCl_3$/MeOH.

To **13c** above was added 90% trifluoroacetic acid in methylene chloride. The solution was stirred at room temperature for 45 min and then evaporated to dryness. Trituration with diethyl ether and filtration left a mixture of diastereomers **IVa** and **IVb**, which were separated by preparative HPLC chromatography on a (10 × 250 mm) C_{18} column eluted with 25% CH_3CN/0.1% TFA–H_2O/0.1% TFA.

IVa: TLC R_f 0.48, silica, 1:1:1:1 *n*-butanol/ethyl acetate/HOAc/H_2O; R_f 0.48, silica, 4:1:5 *n*-butanol/HOAc/H_2O; HPLC k' 4.11 25% CH_3CN/0.1% TFA–H_2O/0.1% TFA, 4.6 × 250 mm C_{18} column; MS (FAB, DTT/DTE) m/z 684 $(M+H)^+$; HRMS (FAB) calcd for $C_{35}H_{53}N_7O_7$ 683.4006, found 683.4020.

IVb: TLC R_f 0.48, silica, 1:1:1:1 *n*-butanol/ethyl acetate/HOAc/H_2O; R_f 0.48, silica, 4:1:5 *n*-butanol/HOAc/H_2O; HPLC k' 4.87 25% CH_3CN/0.1% TFA–H_2O/0.1% TFA, 4.6 × 250 mm C_{18} column; MS (FAB, DTT/DTE) m/z 684 $(M+H)^+$; HRMS (FAB) calcd for $C_{35}H_{53}N_7O_7$ 683.4006, found 683.4016.

Methyl 4-*tert*-Butoxyphenylacetate (15a). To a solution of methyl 4-hydroxyphenylacetate (100 g) in 400 mL of dichloromethane was condensed an equal volume of isobutylene via a cold finger at −78 °C. To the cloudy solution at −78 °C with vigorous stirring was next carefully added dropwise trifluoromethanesulfonic acid (4.2 mL). The now pale yellow solution was stirred for 15 min at −78 °C and then at −20 °C for 45 min, at which time TLC indicated that the reaction was complete. After the addition of 12 mL of triethylamine, the reaction was evaporated to dryness. Purification by flash chromatography on silica gel eluted with 10% ethyl acetate in *n*-hexane gave product **15a** (128.4 g, 96%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.30 (s, 9H), 3.55 (s, 2H), 3.65 (s, 3H), 6.96 (d, 2H, J = 8 Hz), 7.20 (d, 2H, J = 8 Hz).

4-*tert*-Butoxyphenylacetic Acid (15b). To a stirred solution of **15a** (128.4 g, 578 mmol) in 500 mL of dioxane was added aqueous 1 N NaOH (694 mL). After stirring at room temperature for 3 h, the reaction was acidified with HCl, extracted with ethyl acetate, washed with brine, dried ($MgSO_4$), and evaporated to dryness. Trituration with petroleum ether and filtration gave product **15b** (116.75 g, 97%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 9H), 3.6 (s, 2H), 6.96 (d, 2H, J = 8 Hz), 7.2 (d, 2H, J = 8 Hz), 9.47 (br s, 1H).

4-*tert*-Butoxyphenylacetyl Fluoride (15c). To a stirred solution of *tert*-butoxyphenylacetic acid (**15b**) (50 g) in 500 mL of dry acetonitrile was added pyridine (20 mL) followed by cyanuric fluoride (15 mL). After stirring for 2 h at room temperature the now white suspension was evaporated to dryness, taken up in diethyl ether, triturated, and filtered. The precipitate was washed with diethyl ether, and the filtrates were combined and transferred to a separatory funnel, washed with cold water, dried ($MgSO_4$), and evaporated to give 49.76 g (99%) **15c** as a pale yellow oil which solidified in a freezer: ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 9H), 3.74 (d, 2H, J = 3 Hz), 7.0 (d, 2H, J = 8 Hz), 7.21 (d, 2H, J = 8 Hz).

***N*-[(4-*tert*-Butoxyphenyl)acetyl]-4(*S*)-benzyl-2-oxazolidinone (15d).** To a stirred solution of (*S*)-benzyloxazolidinone (47 g, 265 mmol) in 500 mL of dry THF at −78 °C under Ar was added 2.5 N BuLi in *n*-hexane (100 mL). After stirring for 15 min at −78 °C, a solution of **15c** (49.76 g, 237 mmol) in THF (25 mL) was added via syringe. After stirring for 1 h at −78 °C, the reaction was quenched with saturated aqueous NH_4Cl and extracted with ethyl acetate. Any precipitate which formed was filtered through a pad of Celite. The ethyl acetate phase was washed with brine, dried ($MgSO_4$), and evaporated. Purification by flash chromatography on silica gel eluted with 20% ethyl acetate in *n*-hexane gave product **15d** (76.91 g, 87%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 9H), 2.77 (d, 1H), 3.28 (dd, 1H), 4.20 (d, 2H, J = 6 Hz), 4.3 (s, 2H), 4.72 (m, 1H), 6.95–7.45 (m, 9H).

***N*-[1-Oxo-2(*S*)-(4-*tert*-butoxyphenyl)propyl)]-4(*S*)-benzyl-2-oxazolidinone (16a).** To a stirred solution of **15d** (34 g, 93 mmol) in 250 mL of dry THF at −78 °C under Ar was added 1 N lithium hexamethyldisilazane in THF (100 mL) via syringe. After stirring for 30 min, methyl iodide (11.5 mL) was added in one portion. The reaction was allowed to warm to 0 °C, stirred for an additional 1 h, quenched with saturated aqueous NH_4Cl, extracted with ethyl acetate, washed with brine, dried ($MgSO_4$), and evaporated. Analysis of the crude reaction mixture showed a 82% diastereomeric excess by silica gel HPLC. Purification by flash chromatography on silica gel eluted with 20% ethyl acetate in *n*-hexane gave product **16a** (30.68 g, 87%) as a crystalline solid as well as a slower eluting fraction corresponding to the minor diastereomer. ^{1}H NMR ($CDCl_3$) δ (major diastereomer) 1.32 (s, 9H), 1.53 (d, 3H, J = 7 Hz), 2.80 (dd, 1H), 3.36 (dd, 1H), 4.10 (m, 2H), 4.60 (m, 1H), 5.10 (q, 1H), 6.92 (m, 2H), 7.3 (m, 7H); ^{1}H NMR ($CDCl_3$) δ (minor diastereomer) 1.37 (s, 9H), 1.52 (d, 3H, J = 7 Hz), 2.59 (dd, 1H), 3.06 (dd, 1H), 4.08 (dd, 1H), 4.20 (t, 1H), 4.76 (m, 1H), 5.11 (q, 1H), 6.98 (m, 4H), 7.22 (m, 3H), 7.33 (m, 2H).

2(*S*)-(4-*tert*-Butoxyphenyl)propionic Acid (16b). To a stirred solution of **16a** (63.2 g, 166 mmol) in 1 L of 3:1 THF/H_2O at 0 °C was added dropwise over 30 min a solution of lithium hydroperoxide made by adding 84 mL of 30% H_2O_2 to a solution of 10.4 g lithium hydroxide monohydrate in 250 mL of H_2O. After stirring for an additional 1 h, excess peroxide was destroyed by the slow addition of a solution of Na_2SO_3 (103 g) in 500 mL of H_2O while keeping the reaction cool in an ice bath. After the addition most of the THF was evaporated and the remaining solution washed with methylene chloride (2 × 400 mL) to recover Evans' amide. The aqueous phase was acidified with 3 N HCl and extracted with ethyl acetate (2 × 400 mL), washed with brine, dried ($MgSO_4$), and evaporated to dryness to give **16b** (36.47 g, 99%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 9H), 1.50 (d, 3H, J = 7 Hz), 3.70 (q, 1H), 6.95 (d, 2H, J = 8 Hz), 7.22 (d, 2H, J = 8 Hz), 9.3 (br s, 1H).

***N*-(Carbobenzyloxy)-1(*S*)-(4-*tert*-butoxyphenyl)-1-ethylamine (16c).** To a stirred solution of **16b** (36.0 g, 162 mmol) in dry toluene (300 mL) and triethylamine (24.9 mL) at room temperature under Ar was added diphenyl phosphorazidate (36.6 mL). After stirring for 5 min, a reflux condenser was attached and the solution was heated to 80 °C, in an oil bath behind a safety shield, at which point vigorous gas evolution ensued. After gas evolution subsided (approximately 30 min) benzyl alcohol (34 mL) was added and the reaction stirred for an additional 3 h. The reaction was then evaporated to dryness, taken up in ethyl acetate, washed with saturated aqueous sodium bicarbonate, 1 N aqueous HCl, brine, dried ($MgSO_4$), and evaporated to dryness. Purification by flash chromatography on silica gel eluted with 10% ethyl acetate in *n*-hexane gave **16c** (48.80 g, 92%) as a waxy solid: ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 9H), 1.45 (d, 3H, J = 7 Hz), 4.85 (m, 2H), 5.1 (s, 2H), 6.95 (d, 2H, J = 8 Hz), 7.2 (d, 2H, J = 8 Hz), 7.34 (s, 5H).

1(*S*)-(4-*tert*-Butoxyphenyl)-1-ethylamine (16d). A solution of **16c** (48.80 g, 149 mmol) in methanol (250 mL) was hydrogenated on a Parr apparatus over 4 g of 5% Pd on carbon at 50 psi of H_2 for 4 h, filtered free of catalyst, and evaporated at reduced pressure to yield **16d** (28.8 g, 100%): ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 9H), 1.35 (d, 3H, J = 7 Hz), 2.3 (br s, 2H), 4.07 (q, 1H), 6.93 (d, 2H, J = 8 Hz), 7.22 (d, 2H, J = 8 Hz); $[\alpha]^{25}_D$ = −15.27°.

1-(*tert*-Butyloxy)-4-[1(*S*)-(phthalylamino)-1-ethyl]-1,4-cyclohexadiene (17). Compound **17** (38.42 g, 79%) was prepared from **16d** (28.80 g, 149 mmol) using synthetic procedures analogous to that used in preparing **11**: ^{1}H NMR ($CDCl_3$) δ 1.3 (s, 9H), 1.65 (d, 3H, J = 7 Hz), 2.73 (br s, 4H), 4.82 (t, 1H), 4.97 (m, 1H), 5.78 (m, 1H), 7.8 (m, 4H).

***N*-[5-(*tert*-Butoxycarbonyl)-3-[1(*S*)-(phthalylamino)-1-ethyl]-(*Z*)-pent-3-enyl]-2(*S*)-amino-1-propanol (18b).** Compound **18a** (41.33 g, 70% pure) was prepared from **17** (38.42 g, 118 mmol) by synthetic procedures analogous to that used to prepare **12a**: ^{1}H NMR ($CDCl_3$) δ 1.5 (s, 9H), 1.7 (d, 3H, J = 7

Hz), 3.05 (d, 2H, J = 7 Hz), 3.3 (s, 2H), 5.05 (q, 1H), 6.2 (t, 1H), 7.85 (m, 4H), 9.65 (t, 1H).

To a stirred solution of 2(*S*)-amino-1-propanol (20 mL, 257 mmol) in methanol (200 mL) was added glacial acetic acid (14.8 mL) followed by, after 5 min, the aldehyde **12a** obtained above. After stirring for an additional 30 min, the reaction was cooled to 0 °C in an ice bath and sodium cyanoborohydride (8 g, 127 mmol) was added portionwise over 15 min. After stirring for 16 h the reaction was evaporated at reduced pressure, taken up in ethyl acetate, washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated. The crude product was purified by flash chromatography on silica gel eluted with 5% methanol in chloroform to yield **18b** (9.83 g, 20%): ^{1}H NMR ($CDCl_3$) δ 1.07 (d, 3H, J = 7 Hz), 1.43 (s, 9H), 1.68 (d, 3H, J = 7 Hz), 2.23 (dt, 1H), 2.43 (dt, 1H), 2.71 (dt, 1H), 2.77–3.05 (br m, 4H), 3.11 (dd, 2H), 3.30 (dd, 1H), 3.60 (dd, 1H), 4.92 (q, 1H), 5.84 (t, 1H), 7.72 (m, 2H), 7.82 (m, 2H); MS (DCI/NH_3) m/z 417.4 $(M + H)^+$.

1-[1-Hydroxy-2(*S*)-propyl]-5-[1(*S*)-(phthalylamino)-1-ethyl]-2,3,6,7-tetrahydro-1*H*-azepin-2-one (19). To a stirred solution of **18b** (8.62 g, 21 mmol) in dichloromethane (50 mL) was added 200 mL of trifluoroacetic acid. After stirring for 30 min at room temperature the reaction was evaporated at reduced pressure, re-evaporated two times from HCl saturated dioxane (150 mL), and then precipitated from ether to give a white hygroscopic solid (8.22 g, 100%). This material was taken up in dry dimethylformamide (300 mL) and with stirring at 0 °C under Ar were added sequentially triethylamine (6 mL) and $NaHCO_3$ (9 g), followed by diphenyl phosphorazidate (9.3 mL). After stirring for 48 h in a Dewar flask kept at 0 °C, the reaction was evaporated at reduced pressure, taken up in ethyl acetate, washed with H_2O and brine, dried ($MgSO_4$), and then evaporated to dryness. Purification by flash chromatography on silica gel eluted with 3% methanol in chloroform gave product **19** (6.54 g, 88%): ^{1}H NMR ($CDCl_3$) δ 1.14 (d, 3H, J = 7 Hz), 1.62 (d, 3H, J = 7 Hz), 2.36 (br dd, 2H), 3.1–3.4 (m, 3H), 3.45–3.70 (m, 4H), 4.57 (m, 1H), 4.80 (q, 1H), 5.80 (t, 1H), 7.72 (m, 2H), 7.82 (m, 2H).

1-[1-[(*tert*-Butyldimethylsilyl)oxy]2(*S*)-propyl]-3(*R,S*)-benzyl-5-[1(*S*)-[(carbobenzyloxy)amino]-1-ethyl]-2,3,6,7-tetrahydro-1*H*-azepin-2-one (20a). To a stirred solution of **19** (3.21, 9.4 mmol) in dry dichloromethane (30 mL) at 0 °C under Ar was added 2,6-lutidine (1.5 mL) followed by *tert*-butyldimethylsilyl triflate (2.6 mL). The reaction was stirred at 0 °C for 1 h, quenched with saturated aqueous NH_4Cl, washed with aqueous 1 N HCl and brine, dried ($MgSO_4$), and evaporated under reduced pressure. Purification by flash chromatography on silica gel eluted with 35% ethyl acetate in *n*-hexane gave 3.51 g (82%) of the silyl ether as an oil: ^{1}H NMR ($CDCl_3$) δ 0.02 (s, 6H), 0.85 (s, 9H), 1.1 (d, 3H, J = 7 Hz), 1.6 (d, 3H, J = 7 Hz), 2.3 (br dd, 2H), 3.2 (dd, 1H), 3.3 (dd, 1H), 3.54 (m, 4H), 4.66 (m, 1H), 4.75 (q, 1H), 5.77 (t, 1H), 7.72 (m, 2H), 7.82 (m, 2H).

To the above in ethanol (75 mL) was added hydrazine monohydrate (1.1 mL). After stirring at room temperature for 48 h the now white suspension was evaporated under reduced pressure, triturated with chloroform (100 mL), and filtered free of insoluble material. After washing of the precipitate with fresh chloroform (20 mL), the combined clear filtrates were evaporated, taken up in dry THF (75 mL), and treated with *N*-[(benzyloxycarbonyl)oxy]succinimide (2.3 g, 9.2 mmol). After stirring for 15 min triethylamine (1.3 mL) was added and the reaction stirred for an additional 16 h. The reaction was evaporated under reduced pressure, taken up in ethyl acetate, washed with saturated aqueous Na_2CO_3 and brine, dried ($MgSO_4$), and evaporated to dryness. Purification by flash chromatography on silica gel eluted with 50% ethyl acetate in *n*-hexane gave the Cbz-protected silyl ether (3.15 g, 89%): ^{1}H NMR ($CDCl_3$) δ 0.02 (s, 6H), 0.86 (s, 9H), 1.12 (d, 3H, J = 7 Hz), 1.18 (d, 3H, J = 7 Hz), 2.24 (m, 2H), 3.21 (m, 2H), 3.58 (m, 4H), 4.08 (m, 1H), 4.67 (m, 1H), 4.83 (br d, 1H), 5.08 (dd, 2H), 5.55 (t, 1H), 7.32 (s, 5H).

To 3.05 g (6.6 mmol) of the above in dry THF (100 mL) with stirring at −78 °C under Ar was added via syringe a solution of 1 N lithium hexamethyldisilazide in THF (14 mL). After stirring for 30 min at −78 °C, benzyl iodide (2 mL) was added in one portion. After stirring for an additional 2 h, the reaction was quenched with saturated aqueous NH_4Cl, extracted with ethyl acetate, washed with brine, dried ($MgSO_4$), and evaporated to dryness. Purification by flash chromatography on silica gel eluted with 25% ethyl acetate in *n*-hexane gave **20a** (2.98 g, 82%) as a 2:1 mixture of diastereomers. Pure isomers were separated by a gravity column (3 × 100 cm) on silica gel eluted with 25% ethyl acetate in *n*-hexane: ^{1}H NMR ($CDCl_3$) δ (major diastereomer) 0.03 (d, 6H, J = 3.5 Hz), 0.88 (s, 9H), 1.14 (d, 3H, J = 7 Hz), 1.16 (d, 3H, J = 7 Hz), 2.1–2.45 (m, 2H), 2.74 (dd, 1H), 3.33 (dd, 1H), 3.43 (dt, 1H), 3.61 (t, 1H), 3.6–3.9 (m, 3H), 4.02 (br t, 1H), 4.55–4.9 (m, 2H), 5.02 (dd, 2H), 5.34 (d, 1H, J = 1.7 Hz), 7.1–7.45 (m, 10H); MS (DCI/NH_3) m/z 551.4 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ (minor diastereomer) 0.01 (d, 6H, J = 3.4 Hz), 0.87 (s, 9H), 1.15 (d, 6H, J = 7 Hz), 2.19 (br s, 2H), 2.74 (dd, 1H), 3.45–3.15 (m, 3H), 3.7–3.95 (m, 2H), 4.08 (br t, 1H), 4.59 (br d, 1H), 4.74 (m, 1H), 5.06 (s, 2H), 5.37 (s, 1H), 7.0–7.4 (m, 10H); MS (DCI/NH_3) m/z 551.4 $(M + H)^+$.

1-[1-Carboxy-1(*S*)-ethyl]-3(*R,S*)-benzyl-5-[1(*S*)-[(carbobenzyloxy)amino]-1-ethyl]-2,3,6,7-tetrahydro-1*H*-azepin-2-one (21a). The above **20a** (7.59 g, 13.8 mmol) was treated with 3:1:1 acetic acid/THF/H_2O (250 mL) and stirred at room temperature for 16 h. After evaporation at reduced pressure, the remaining residue was purified by flash chromatography on silica gel eluted with 80% ethyl acetate in *n*-hexane to give **20b** (6.01 g, 100%).

To a stirred solution of the above **20b** (6.01 g, 13.8 mmol) in acetone (60 mL) was added dropwise a solution of 10 mL of Jones reagent in 25 mL of acetone. After stirring for 3 h the reaction was quenched with 5 mL of 2-propanol and stirred for 15 min. The reaction was then evaporated to half its volume under reduced pressure, diluted with H_2O, and extracted with ethyl acetate (2 × 200 mL). The clear ethyl acetate phases were combined, washed with H_2O, dried ($MgSO_4$), and evaporated to dryness to give crude product **21a** (6.44 g, 100%) as a white solid which was used as is in the next reaction. MS (DCI/NH_3) m/z 417.5 $(M + H)^+$.

Synthesis of *N*-[2(*S*)-[2-Oxo-3(*R,S*)-benzyl-5-[1(*S*)-(alanylamino)ethyl]-2,3,6,7-tetrahydro-1*H*-azepin-1-yl]-1-oxopropyl]valinylvaline Methyl Ester (IIIa/IIIb). To a stirred solution of the above acid **21a** (300 mg, 0.67 mmol) in DMF (15 mL) was added sequentially HCl-Val-Val-OMe (266 mg), HOBt (180 mg), NEt_3 (0.56 mL), and Bop reagent (0.59 g). The reaction was stirred for 16 h at room temperature and evaporated to dryness. The product **21b**, as a mixture of diastereomers, was obtained as a solid foam by flash chromatography on silica gel eluted with 65% ethyl acetate in *n*-hexane (330 mg, 75%). Pure diastereomers were obtained from the individual coupling of each pure isomer of acid **21a**. ^{1}H NMR ($CDCl_3$) δ (one diastereomer) 0.67 (m, 12H), 0.93 (d, 3H, J = 7 Hz), 1.14 (d, 3H, J = 7 Hz), 1.92 (m, 2H), 2.05 (br m, 2H), 2.55 (dd, 1H), 3.08 (dd, 2H), 3.37 (br m, 2H), 3.48 (s, 3H), 3.5–3.8 (m, 3H), 4.0 (t, 1H), 4.23 (dd, 1H), 4.8 (dd, 2H), 5.12 (br s, 1H), 5.63 (br d, 1H), 7.02 (m, 8H), 7.11 (s, 5H); ^{1}H NMR ($CDCl_3$) δ (other diastereomer) 0.53 (d, 3H, J = 8 Hz), 0.65 (m, 9H), 0.92 (d, 3H, J = 7 Hz), 1.13 (d, 3H, J = 7 Hz), 1.87 (m, 2H), 2.02 (br s, 2H), 2.47 (dd, 1H), 3.07 (dd, 2H), 3.24 (br d, 2H), 3.44 (s, 3H), 3.55–3.9 (m, 3H), 4.04 (dd, 1H), 4.15 (dd, 1H), 4.8 (m, 3H), 5.12 (br s, 1H), 5.97 (br d, 1H), 6.81 (d, 1H), 6.98 (m, 5H), 7.07 (s, 5H), 7.19 (d, 1H).

The above **21b** (330 mg, 0.5 mmol) was treated with anhydrous HF (25 mL) in an HF apparatus for 1 h at 0 °C and evaporated to dryness. Trituration with diethyl ether, filtration, and drying under vacuo gave the amine hydrofluoride as a white solid (213 mg). To this solid in DMF (10 mL) was added triethylamine (70 μL) followed by Boc-Ala symmetrical anhydride made from treating Boc-alanine (284 mg) in CH_2Cl_2 (25 mL) with DCC (155 mg), stirring for 30 min at room temperature, filtration, and evaporation under reduced pressure. After stirring for 16 h the reaction was evaporated and purified by flash chromatography on silica gel eluted with 2% methanol in chloroform to give product **21c** (276 mg, 79%).

To the above **21c** was added 50 mL of saturated HCl in dioxane. After stirring for 1 h at room temperature the reaction was evaporated to dryness, triturated with ethyl ether, filtered, and dried under vacuo to give products **IIIa** and **IIIb** as a mixture of diastereomers which were separated by preparative HPLC on a (10 × 250 mm) Hamiltonian PRP-1 column eluted with 30% CH_3CN/0.1% TFA–H_2O/0.1% TFA.

IIIa: TLC R_f 0.62, silica, 1:1:1:1 *n*-butanol/ethyl acetate/HOAc/H_2O; R_f 0.63, silica, 4:1:5 *n*-butanol/HOAc/H_2O; R_f 0.80, silica, 15:3:12:10 *n*-butanol/HOAc/H_2O/pyridine; HPLC k' 4.05

30% CH_3CN/0.1% TFA–H_2O/0.1% TFA, 4.6 × 250 mm Hamilton PRP-1 column; MS (FAB, DTT/DTE) m/z 600.2 $(M + H)^+$; HRMS (FAB) calcd for $C_{32}H_{49}N_5O_6$ 599.3683, found 599.3693.

IIIb: TLC R_f 0.62, silica, 1:1:1:1 *n*-butanol/ethyl acetate/HOAc/H_2O; R_f 0.63, silica, 4:1:1 *n*-butanol/HOAc/H_2O; R_f 0.80 silica, 15:3:12:10 *n*-butanol/HOAc/H_2O/pyridine; HPLC ,′ 4.89 30% CH_3CN/0.1% TFA–H_2O/0.1% TFA, 4.6 × 250 mm Hamilton PRP-1 column; MS (FAB, DTT/DTE) m/z 600.3 $(M + H)^+$; HRMS (FAB) calcd for $C_{32}H_{49}N_5O_6$ 599.3683, found 599.3695.

1-[1-(Methoxycarbonyl)-1(*S*)-ethyl]-3(*R,S*)-benzyl-5-[1(*S*)-[(carbobenzyloxy)amino]-1-ethyl]-2,3,6,7-tetrahydro-1*H*-azepin-2-one (22a). To a stirred solution of **21a** (5.14 g, 11.4 mmol) in methanol (50 mL) at 0 °C was added a solution of diazomethane generated from 3.5 g of Diazald (16.3 mmol) in ethyl ether. The reaction was allowed to warm to room temperature over 30 min, quenched of excess diazomethane with acetic acid, and evaporated to dryness. Purification by flash chromatography on silica gel eluted with 40% ethyl acetate in *n*-hexane gave product **22a** (4.19 g, 79%). Analytical samples of pure diastereomers were obtained by HPLC separation on silica gel: ^1H NMR ($CDCl_3$) δ (major diastereomer) 1.20 (d, 3H, J = 7 Hz), 1.4 (d, 3H, J = 7 Hz), 2.15–2.55 (br m, 2H), 2.77 (dd, 1H), 3.32 (dd, 1H), 3.39 (dt, 1H), 3.67 (s, 3H), 3.87 (br m, 2H), 4.04 (br t, 1H), 4.88 (br d, 1H), 5.03 (dd, 2H), 5.1 (q, 1H), 5.38 (br s, 1H), 7.24 (m, 5H), 7.33 (s, 5H); ^1H NMR ($CDCl_3$) δ (minor diastereomer) 1.15 (d, 3H, J = 7 Hz), 1.42 (d, 3H, J = 7 Hz), 2.23 (br m, 2H), 2.73 (dd, 1H), 3.27 (dt, 1H), 3.34 (dd, 1H), 3.68 (s, 3H), 3.8–4.15 (br m, 3H), 4.82 (d, 1H), 5.05 (s, 3H), 5.1 (q, 1H), 5.38 (br s, 1H), 7.22 (m, 5H), 7.3 (s, 5H).

1-[1-(Methoxycarbonyl)-1(*S*)-ethyl]-3(*R,S*)-benzyl-5-[1(*S*)-[(carbobenzyloxy)amino]-1-ethyl]-2,3,6,7-tetrahydro-1*H*-azepin-2-thione (22b). To a stirred solution of the above **22a** (3.6 g, 7.8 mmol) in dry toluene (100 mL) was added Lawesson's reagent (2.2 g). The suspension was stirred under Ar at 80 °C for 4 h (after 15 min the reaction became clear), evaporated, and purified by flash chromatography eluted with 30–40% EtOAc in *n*-hexane. A fraction (1.66 g, 44%) which contained mostly thioamide with some starting material was used in the next reaction. Both pure diastereomers of **22a** yielded an identical mixture of diastereomeric thioamides by ^1H NMR analysis: TLC R_f 0.45, silica, 40% EtOAc/*n*-hexane; ^1H NMR ($CDCl_3$) δ 1.13, 1.17 (2d, 3H), 1.51 (2d, 3H), 2.35 (m, 2H), 3.02 (m, 1H), 3.45 (dd, 1H), 3.53 (m, 1H), 3.71, 3.75 (2s, 3H), 3.8–4.2 (m, 2H), 4.2–4.5 (m, 1H), 4.5–4.9 (m, 1H), 4.9–5.2 (m, 2H), 5.4 (t, 1H), 6.4, 6.52 (2q, 1H), 7.23 (m, 5H), 7.32 (m, 5H); MS (DCI/NH_3) m/z 481.2 $(M + H)^+$.

1-[1-(Methoxycarbonyl)-1(*S*)-ethyl]-3-(*R,S*)-benzyl-5-[1(*S*)-[carbobenzyloxy)amino]-1-ethyl]-2,3,6,7-tetrahydro-1*H*-azepine (23a). To a stirred solution of **22b** (1.66 g, 3.5 mmol) in CH_2Cl_2 (5 mL) at 0 °C under Ar was added dropwise triethyloxonium tetrafluoroborate (4.5 mL, 1 N in CH_2Cl_2). The reaction was stirred for 5 min at 0 °C then for 45 min at room temperature, evaporated to a foam, dissolved in methanol (6 mL), and cooled to 0 °C. $NaBH_4$ (0.37 g) was then added portionwise with stirring. (Exothermic, stench!) The reaction was stirred at 0 °C for 10 min and then at room temperature for 2 h, quenched with aqueous 1 N HCl (10 mL), and evaporated to dryness. The residue was taken up in EtOAc, washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated. Flash chromatography on silica gel eluted with 40% EtOAc in *n*-hexane afforded product **23a** as a mixture of diastereomers (729 mg, 46%): TLC R_f 0.34 (silica, 30% EtOAc/*n*-hexane); ^1H NMR ($CDCl_3$) δ 1.18 (2d, 3H), 1.25 (d, 3H, J = 7 Hz), 2.1–2.5 (m, 4H), 2.5–2.9 (m, 5H), 3.4 (m, 1H), 3.6, 3.64 (2s, 3H), 4.12 (m, 1H), 4.74 (br d, 1H), 5.1 (m, 2H), 5.56 (br d, 1H), 7.21 (m, 5H), 7.36 (s, 5H); MS (DCI/NH_3) m/z 451.2 $(M + H)^+$.

Synthesis of *N*-[2(*S*)-[3(*R,S*)-Benzyl-5-[1(*S*)-(alaninylamino)-1-ethyl]-2,3,6,7-tetrahydro-1*H*-azepin-1-yl]-1-oxopropyl]valinylvaline Methyl Ester (VIa/VIb). To a stirred solution of the above **23a** (729 mg, 1.6 mmol) in MeOH (10 mL) was added aqueous 1 N NaOH (3.5 mL). After stirring for 16 h, aqueous 1 N HCl was added (6 mL) and the reaction evaporated to dryness. After drying under vacuum overnight the residue was taken up in DMF (20 mL) and HCl·H-Val-Val-OMe (0.9 g), HOBt (0.45 g), NEt_3 (1.4 mL), and Bop reagent (1.5 g) were added with stirring. The reaction was stirred for 16 h and evaporated to dryness. Flash chromatography on silica gel eluted with 50% EtOAc in *n*-hexane afforded product **23b** as a mixture of diastereomers (400 mg, 38%): TLC R_f 0.42 silica, 50% EtOAc/*n*-hexane.

The above **23b** (400 mg, 0.6 mmol) was treated with anhydrous HF (20 mL) in an HF apparatus for 1 h at 0 °C and evaporated to dryness. Trituration with diethyl ether, filtration, and drying under vacuo gave the amine dihydrofluoride as a white solid (245 mg). To this solid in DMF (10 mL) was added triethylamine (260 μL) followed by Boc-Ala symmetrical anhydride made from treating Boc-alanine (284 mg) in CH_2Cl_2 (25 mL) with DCC (155 mg), stirring for 30 min at room temperature, filtration, and evaporation under reduced pressure. After stirring for 16 h the reaction was evaporated and purified by flash chromatography on silica gel eluted with 2% methanol in chloroform to give product **23c** (323 mg, 77%): TLC R_f 0.23 silica, 60% EtOAc/*n*-hexane, R_f 0.68 silica, 5% MeOH/$CHCl_3$.

The above **23c** (323 mg, 0.5 mmol) was treated with 80% TFA in CH_2Cl_2 (20 mL) with stirring for 30 min and evaporated to dryness. Trituration and filtration from ether gave products **VIa** and **VIb** as a mixture of diastereomers (390 mg), which were separated by preparative HPLC on a (10 × 250 mm) Hamilton PRP-1 column eluted with 25% CH_3CN/0.1% TFA–H_2O/0.1% TFA.

VIa: TLC R_v 0.47, silica, 1:1:1:1 *n*-butanol/ethyl acetate/HOAc/H_2O; R_f 0.46, silica, 4:1:1 *n*-butanol/HOAc/H_2O; R_f 0.75, silica, 15:3:12:10 *n*-butanol/HOAc/H_2O/pyridine; HPLC k' 7.12 23% CH_3CN/0.1 TFA–H_2O/0.1% TFA, 4.6 × 250 mm Hamilton PRP-1 column; MS (DCI/NH_3) m/z 586.4 $(M + H)^+$; HRMS (FAB) calcd for $C_{32}H_{51}N_5O_5$ 585.3890, found 585.3869.

VIb: TLC R_f 0.47, silica, 1:1:1:1 *n*-butanol/ethyl acetate/HOAc/H_2O; R_f 0.46, silica, 4:1:1 *n*-butanol/HOAc/H_2O; R_f 0.75 silica, 15:3:12:10 *n*-butanol/HOAc/H_2O/pyridine; HPLC k' 5.75 23% CH_3CN/0.1% TFA–H_2O/0.1% TFA, 4.6 × 250 mm Hamilton PRP-1 column; MS (DCI/NH_3) m/z 586.4 $(M + H)^+$; HRMS (FAB) calcd for $C_{32}H_{51}N_5O_5$ 585.3890, found 585.3915.

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