

Crystal-Structure-Based Design and Synthesis of Novel C-Terminal Inhibitors of HIV Protease

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The X-ray crystal-structure-based design, synthesis, computational evaluation, and activity of a novel class of HIV protease inhibitors are described. The initial lead compounds **2** and **3** were designed by modeling replacement groups for the C-terminal Val-Val-OCH₃ of a known hydroxyethylene inhibitor into the active site of the reported crystal structure of HIV protease complexed with MVT-101. The lead compound **2** was found to be a modest inhibitor with a $K_i = 1.67 \mu\text{M}$. The X-ray crystal structure of compound **2** complexed with HIV protease was solved and used for subsequent design. The lead compound **3** was found to be a more potent inhibitor with $K_i = 0.2 \mu\text{M}$, and the structure of it complexed with HIV protease was also solved and used for subsequent design. Modification of both the C-terminus and N-terminus of indole **3** resulted in compounds with $K_i = 30 \text{ nM}$. Using the crystal structure of compounds **2** and **3** with HIV protease as a starting point, the thermodynamic cycle perturbation molecular dynamics method was applied to a select group of compounds in order to test the accuracy of this type of computation within a series of closely related compounds.

Introduction

Human immunodeficiency virus (HIV) is a member of the greater family of retroviruses and is the cause of the debilitating and fatal disease acquired immune deficiency syndrome (AIDS).² Given the array of processes required to sustain the viral lifecycle, it is clear that there are numerous intervention points that could be exploited in the development of anti HIV drugs. Among these, the viral encoded protease has emerged as one of the most interesting targets.³ Critical to the proliferation and maturation of the virus, this enzyme is involved in the proteolytic processing of the *gag-pol* polyprotein gene product producing the functional enzymes integrase, reverse transcriptase, and protease.⁴ Blockage of this processing step by inhibiting the activity of the protease results in immature noninfectious viral particles.⁵

A wide range of approaches have been taken in the effort to discover HIV protease inhibitors. These include the screening of natural product and chemical libraries,⁶ design of simple substrate analogues,⁷ and most recently, structure-based design⁸ using the reported X-ray structure of HIV protease in both its apo and complexed forms.⁹ We have recently reported the successful application of an iterative protein crystallographic inhibitor design approach to the discovery of potent inhibitors of the enzyme thymidylate synthase,¹⁰ and we felt that such an approach was well-suited for the HIV protease because the enzyme was relatively small (a homodimer of 99 residues), easily expressed and purified,¹¹ and that crystal structures of ligand complexes were readily obtainable.⁹ In addition, such structural information would allow us to test and validate current computational design methods that require as starting points protein crystal structures.¹² Overall, this type of structure-based design approach seems uniquely suited for

the discovery of potent nonpeptidic inhibitors of proteases which in general have been difficult to find.¹³

In order to exploit this method for the discovery of nonpeptidic inhibitors, we considered three possible strategies: (1) design of novel C-terminal derivatives of a known peptide inhibitor, (2) design of novel N-terminal derivatives of a known peptide inhibitor, and (3) complete de novo design into the empty active site. The first two approaches could theoretically be used in sequence to obtain completely novel compounds. We chose as our initial effort the first of these strategies. Herein we describe the crystal-structure-based design, synthesis, computational analysis, and crystallographic and biochemical evaluation of a class of novel C-terminal containing HIV protease inhibitors.

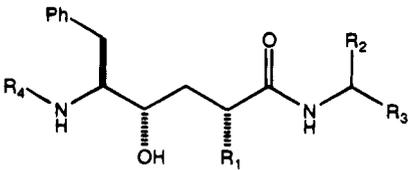
Design

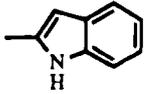
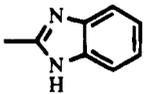
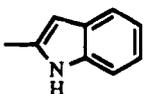
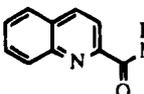
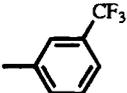
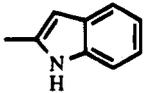
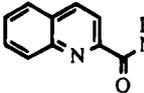
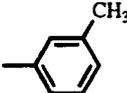
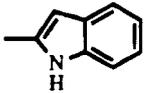
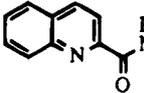
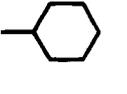
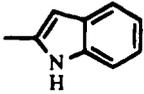
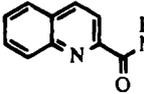
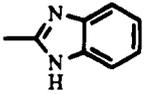
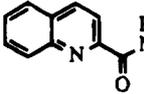
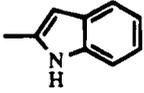
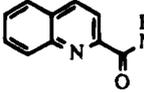
We used as our starting point a model of the unsubstituted hydroxyethylene-based inhibitor **1**^{7a} that was created by simply overlaying an AM1¹⁴ minimized structure of the compound on to the MVT-101 HIV protease complex reported by Wlodawer.^{9b} At the time this work was initiated, 2-benzyl-substituted hydroxyethylene groups were known to yield the most active inhibitors;¹⁵ however, the ease of synthesis of the unsubstituted hydroxyethylene moiety allowed us to test our initial design ideas more quickly, and therefore we used the unsubstituted hydroxyethylene as the inhibitor core in all the compounds except one.

The design exercise consisted of removing the C-terminal Val-Val methyl ester which occupied the P2' and P3' sites in the protein and replacing it with a simple scaffold that filled the space occupied by the two Valine side chains. Initially, the N-terminal portion of the molecule was left as the Ala-Ala free NH₂ to confer increased water solubility which would be critical to the success of the protein crystallization process. Eventually, as potency increased, the amine was replaced with other functional groups.

[⊗] Abstract published in *Advance ACS Abstracts*, June 15, 1994.

Table 1. Structure Activity of HIV Protease Inhibitors



no.	R ₁	R ₂	R ₃	R ₄	K _i , μM ^a	formula ^c	mp, °C
1					0.018±0.002 ^b		
2	H	Ph	Ph	H ₂ N-ala-ala	1.67±0.25	C ₃₁ H ₃₈ N ₄ O ₄ •H ₂ O	174-177
3	H	Ph		H ₂ N-ala-ala	0.20±0.07	C ₃₃ H ₃₉ N ₅ O ₄ •1.1H ₂ O	206-208
4	H	Ph		H ₂ N-ala-ala	5.37±1.45	C ₃₃ H ₃₉ N ₅ O ₄ ^d	oil
5	H	Ph		 -NH-CO-CH(CH ₃)-CO-NH ₂	0.043±0.01	C ₄₁ H ₄₀ N ₆ O ₅ •H ₂ O	211-218
6	H			 -NH-CO-CH(CH ₃)-CO-NH ₂	0.033±0.01	C ₄₂ H ₃₉ F ₃ N ₆ O ₅	205-215
7	H			 -NH-CO-CH(CH ₃)-CO-NH ₂	0.039±0.01	C ₄₂ H ₄₂ N ₆ O ₅	215-219
8	H			 -NH-CO-CH(CH ₃)-CO-NH ₂	0.18±0.047	C ₄₁ H ₄₆ N ₆ O ₅	220-227
9	H	Ph		 -NH-CO-CH(CH ₃)-CO-NH ₂	0.48±0.14	C ₄₀ H ₃₉ N ₇ O ₅	150-152
10	Benzyl	Ph		 -NH-CO-CH(CH ₃)-CO-NH ₂	1.28±0.20	C ₄₈ H ₄₆ N ₆ O ₅	210-215

Pepstatine

3.1

^a HIV protease K_i values were determined by the continuous chromogenic assay method. See the Experimental Section for a detailed description. ^b This K_i is that reported in ref 7a and was measured using a different assay method in which pepstatin had a K_i = 1.4 μM. ^c Unless stated otherwise, combustion analysis were performed for all elements except oxygen and are within 0.4% of the theoretical values. ^d This formula was determined using high-resolution mass spectrometry.

The first compound chosen for consideration was compound 2 (Table 1). It is a simple diphenylhydramine

amide derivative in which the two phenyl groups fill the p2' and p3' side-chain binding pockets. It was modeled

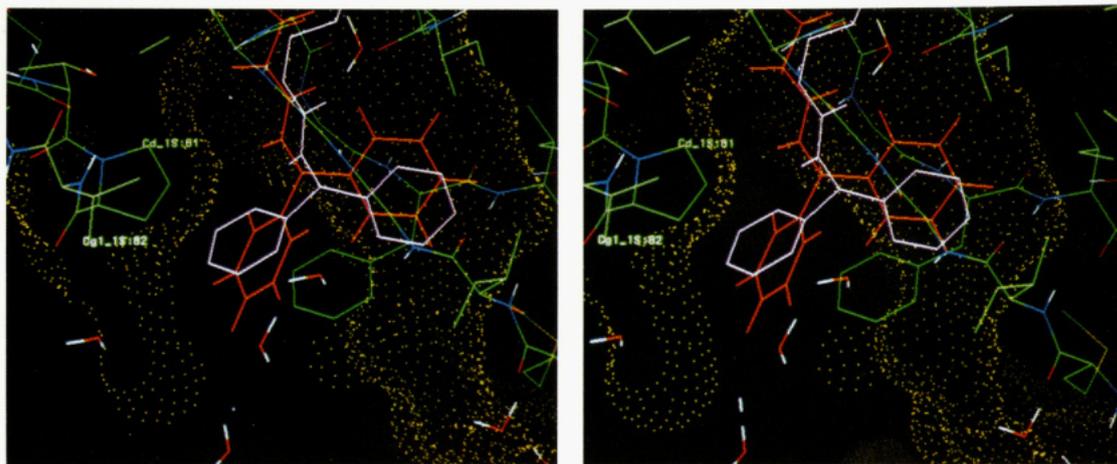


Figure 1. Stereodrawing showing the X-ray structure of compound **2** complexed with HIV protease. The actual fitted structure is shown in magenta and the modeled structure is shown in red. Important residues that make up the p3' side-chain pocket are labeled.

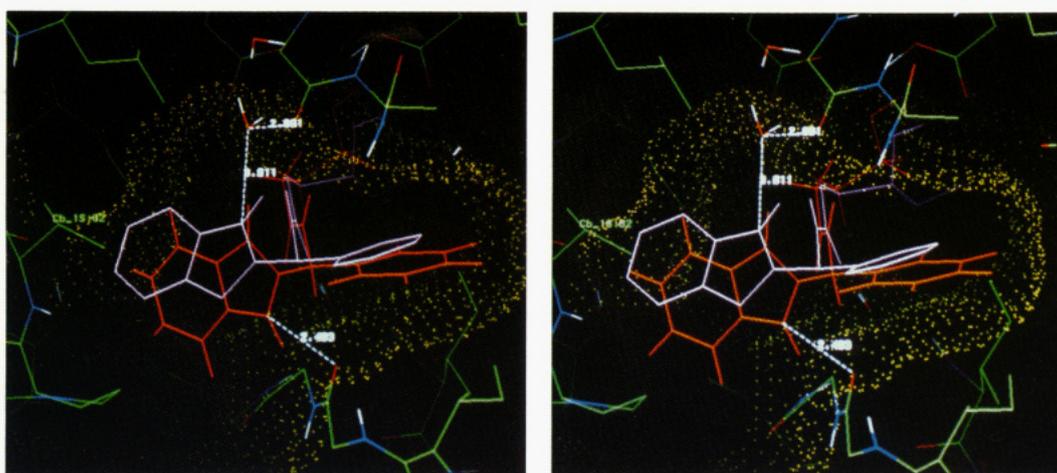
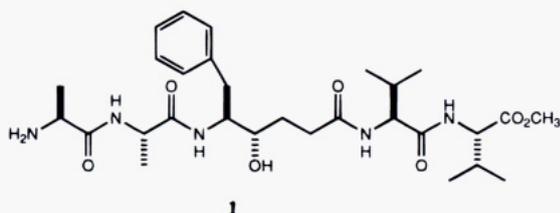


Figure 2. Stereodrawing showing the X-ray structure of compound **3** complexed with HIV protease. The actual fitted structure is shown in magenta and the modeled structure is shown in red. Important hydrogen-bonding interactions are indicated with white lines. The key feature is the water-mediated hydrogen bond from the indole NH to the Gly 27 carbonyl oxygen.

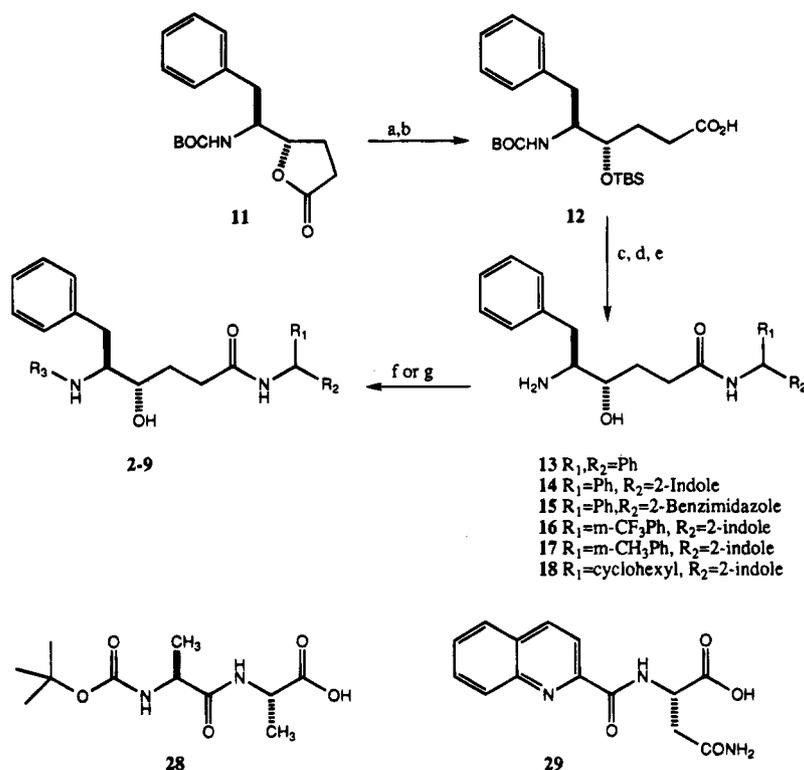
by first minimizing the molecule using AM1 and then overlaying the hydroxyethylene and N-terminal portions on to the model of compound **1**.



The two phenyl groups were placed to optimally fill the p2' and p3' side-chain pockets without causing major movement of the rest of the molecule. Compound **2** was synthesized and was found to be a modest inhibitor of HIV protease with a $K_i = 1.67 \mu\text{M}$. The crystal structure of HIV protease complexed with compound **2** was solved, and the diphenhydramine portion is shown in Figure 1. The magenta shows the actual fit of the molecule to the electron density and the red shows the model. Overall, the compound binds similar to the model. The major difference between the two is a shift of the inhibitor in the direction of the p3' pocket. This shift places the phenyl closer to the hydrophobic wall that is created by side chains Pro 81, Val 82, and Leu

23, and in addition creates a small space in the p2' pocket around the edge of the other phenyl group.

Using the same model of compound **1** described above, the more elaborate inhibitor **3** was designed. This involved replacement of the phenyl group of compound **2** occupying the p3' pocket with an indole ring. The NH of the indole was modeled to make an H-bond to the carbonyl oxygen of Gly 48. The indole ring was intended to occupy a larger portion of the hydrophobic space that makes up the p3' pocket than was seen with **2**. In the model, the indole ring does not lie directly against the hydrophobic wall in the p3' pocket, but it was felt that a minor inward movement of this region would allow for a close fit. Compound **3** was synthesized as a mixture of diastereomers and determined to be 8 times better as an enzyme inhibitor than compound **2** having a binding constant of $0.20 \mu\text{M}$. The crystal structure of HIV protease complexed with compound **3** and overlaid onto the model is shown in Figure 2. Interestingly, the enzyme binds only one of the diastereomers (the one having the *R* configuration) in such a manner that the indole ring (the experimental structure is in magenta) is situated in the p3' binding pocket, but is flipped 180° from the model. Instead of making an H-bond to Gly 48 carbonyl oxygen at the bottom of the

Scheme 1.^a General Synthesis of Protease Inhibitors

^a (a) 2 N LiOH, THF (94%); (b) imidazole, TBSCl, DMF (88%); (c) DCC, amine, CH₂Cl₂; (d) TFA, CH₂Cl₂; (e) TBAF, THF; (f) (i) DCC, acid **28**, DMF; (ii) TFA, CH₂Cl₂; (g) acid **29**, DMF, HOBT, TEA.

active site, it makes a water-mediated H-bond to Gly 27 carbonyl oxygen at the top. Careful analysis revealed that the p3' hydrophobic wall did not move inward as was hoped. As a result, in order for the NH to make the H-bond to Gly 48, the indole ring would have to move off the surface of the p3' pocket. Instead, the indole ring packs very tightly with the hydrophobic wall in the p3' pocket, similar to compound **2**, and the only way the NH can H-bond is by flipping the ring and wedging a water molecule next to the Gly 27 carbonyl oxygen. The factor of 8 in binding is believed to be the result of both the increased surface area of contact and the hydrogen bond. The critical nature of complemented H-bonds is illustrated by compound **4**. Replacement of the 3-carbon of the indole with a nitrogen atom decreased binding by a factor of over 20. This is believed to be due to desolvation of the new nitrogen atom which does not appear to be capable of making an additional H-bond to the protein.

At this point, the N-terminal alanines were replaced with the asparagine-2-quinolinecarbonyl moiety reported from Roche.^{7b} The remainder of the structure-activity work included this change in hopes of finding compounds with *in vitro* antiviral activity in addition to enzyme inhibition.

With the crystal structure of compounds **2** and **3** in hand, we next turned our attention to the phenyl ring in the p2' pocket. There remained some unfilled space in the p2' pocket that could be reached from the meta position. Small substituents looked best in the models, and a methyl and trifluoromethyl group were placed at the meta position leading to compounds **6** and **7**. Both of these changes showed a slight increase in binding (see Table 1). Replacement of the phenyl group with a cyclohexyl group decreased binding by a factor of 6. Finally, substitution of the hydroxyethylene at the

2-position with a benzyl group (to give compound **10**) decreased activity by a factor of 28.

Chemistry

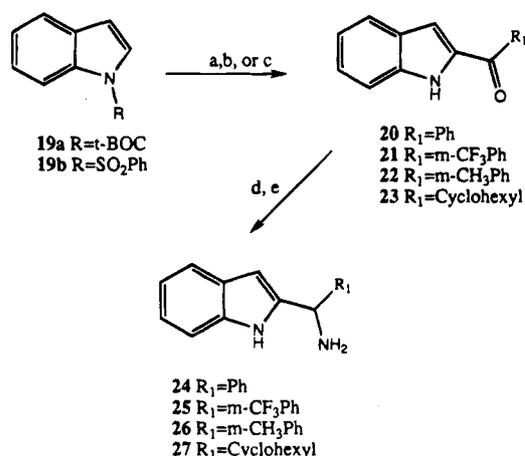
All of the compounds reported were synthesized using the same general approach shown in Scheme 1. The protected lactone **11** was prepared using known methods.¹⁶ The lactone was opened with hydroxide and the alcohol was protected with the *tert*-butyldimethylsilyl group¹⁷ to give the acid **12**. The acid **12** was coupled with the appropriate amine using standard peptide coupling techniques,¹⁸ and the protecting groups were removed to give the N-terminal amines **13**–**18**. To complete the syntheses, the amines **13**–**18** were coupled either to BOC-Ala-Ala acid **28**¹⁹ followed by deprotection or to the asparagine-2-quinoloyl acid **29**²⁰ to give the inhibitors **2**–**9**.

Inhibitor **10** was prepared from the known 2-benzyl-substituted derivative of lactone **11**³¹ in a manner analogous to that described in Scheme 1.

The synthesis of the substituted amines is shown in Scheme 2. This involved the lithiation of the 2-position of the protected indole **19** or benzimidazole (only the indole compounds are shown) and subsequent trapping of the anion with the appropriate acid chloride.²¹ The nitrogen protecting group was removed, and the introduction of the nitrogen was accomplished via the oxime.²² The free amines **24**–**27** were prepared by reduction with either catalytic Pd/C and hydrogen²³ or zinc and ammonium acetate.²⁴

Computational Chemistry

Understanding the interactions that any particular compound makes with the solvent and with the enzyme of interest is of critical importance in the development

Scheme 2.^a General Synthesis of Indole C-Terminal Amines

^a (a) *t*-BuLi, THF, acid chloride; (b) TFA, CH₂Cl₂; (c) NaOH, EtOH; (d) NH₂OH·HCl, EtOH, H₂O, NaOH; (e) 10% Pd/C, H₂, MeOH; or Zn, NH₄OAc.

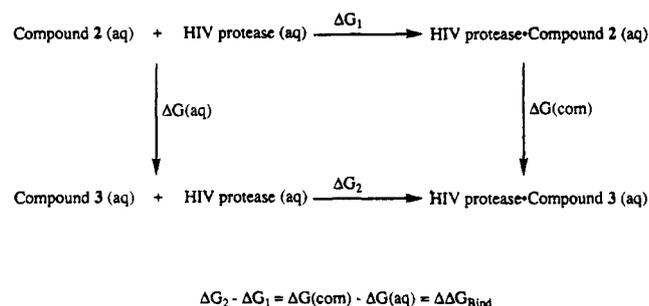


Figure 3. Thermodynamic cycle used to calculate relative binding free energies of HIV protease inhibitors. Cycle shows how the nonphysical transformation of compound 2 into compound 3 in both aqueous and complexed states yields $\Delta\Delta G_{\text{Bind}}$.

of and the prioritization of new design ideas. For the last several years the thermodynamic cycle perturbation approach (TCP), in conjunction with molecular dynamics or Monte Carlo simulations, has been successfully used for calculating the relative binding free energy associated with solvation and binding of two closely related ligands in both aqueous and enzyme-bound states.²⁵ The method consists of performing the nonphysical computational mutation of one ligand into another in both an aqueous unbound state and an enzyme-bound state. Because free energy is a state function, a thermodynamic cycle can be constructed that allows for the calculation of the relative binding free energies of any two mutated ligands. Figure 3 shows how the relative free energy of binding difference can be calculated for compounds 2 and 3.

This TCP method was applied to the C-terminus of a select group of the inhibitors in Table 1. The purpose of this study was to determine the limitations of the method within a series of closely related compounds, and in addition, to aid in the understanding of solvation effects on protein-ligand interactions. The details of the calculations have been described separately,²⁶ and the results are shown in Table 2. The results show that in the replacement of a phenyl group with an indole ring (compound 2 to compound 3) the molecule must overcome a large desolvation penalty. That is, it is 3 kcal/mol more difficult to remove compound 3 from the solvent than compound 2. Given that compound 3 binds

8 times more tightly to the enzyme than compound 2, this penalty must be overcome and the enhanced binding is probably a combination of the increased surface area of contact the inhibitor makes with the protein and the water-mediated H-bond to Gly 27 carbonyl oxygen. In the mutation of compound 3 to compound 4, the additional nitrogen atom also causes an increase in desolvation energy (in this case 2 kcal/mol). However, the inhibitor must never recoup this increase in desolvation energy because compound 4 is 20 times less inhibitory than compound 3. A lack of optimal H-bonding to the protein on the part of compound 4 is the most probable explanation for this lack of good activity. In the case of the substituents on the phenyl ring of compound 3, the calculations in which the hydrogen is mutated into either a methyl or a trifluoromethyl group indicated that no major changes in binding were expected. This was indeed the case.

Overall, this set of calculations did show the TCP method to be relatively accurate in reproducing experimental relative differences in binding in a closely related series of compounds. In addition, by breaking down the relative contributions to the free energy of binding into its component parts, this type of calculation provided both qualitative and quantitative insights into the important role that solvation effects play in protein-ligand binding.

Crystallography

Crystals for compounds 2 and 3 were grown as complexes with HIV protease using the hanging drop method. The crystals grow in the orthorhombic space group *P*2₁2₁2₁ with *a* = 66.38 Å, *b* = 92.44 Å, and *c* = 29.24 Å. The structures were solved as described in the experimental section and the inhibitors were fitted into an *F*_o - *F*_c electron density map and refined.²⁷ Water molecules were added at each step of the refinement using *F*_o - *F*_c and 2*F*_o - *F*_c electron density maps as well as chemical environment as a guide.

Biochemistry

The compounds in Table 1 were evaluated for their inhibition of purified recombinant HIV1 protease. Protease activity was measured by continuous chromogenic assay using a modification of the previously described method.²⁸ The chromogenic peptide His-Lys-Ala-Arg-Val-Leu-(*p*-NO₂-Phe)-Glu-Ala-Nleu-Ser (Bachem) was used as a substrate. Experimental results were analyzed by the nonlinear regression analysis program TIGHTFIT using a mathematical model for tight binding inhibitors.²⁹ The observed inhibition patterns are competitive. Pepstatine was included as a control.

Select compounds from Table 1 were tested for their *in vitro* anti-HIV activity in both CEM and MT2 cells. In all cases, ED₅₀ values were higher than 2 μM.

Discussion and Conclusion

During the course of these studies, an approach to a novel class of nonpeptidic HIV protease inhibitors was taken in which the C-terminus of a known inhibitor was replaced with a simple bifurcated organic moiety. The approach consisted of filling the available space in the p2' and p3' side-chain binding pockets with different types of aromatic rings to give the lead compounds 2 and 3. Once the protein crystal structure of these leads

Table 2. Calculation of Relative Solvation and Binding Free-Energy Differences (kcal/mol) for Selected Inhibitors of HIV Protease

property	phenyl-indole ^a	indole-benzindole	hydrogen-trifluoromethyl	hydrogen-methyl
solvation free energy $\Delta\Delta G$ (sol)	3.1 ± 0.4	2.0 ± 0.5	1.0 ± 0.4	-0.04 ± 0.20
binding free energy $\Delta\Delta G$ (bind)	2.3 ± 0.6	-1.3 ± 0.6	-0.2 ± 0.5	-0.40 ± 0.50
experimental $\Delta\Delta G$	1.25 ± 0.03	-1.94 ± 0.31	0.16 ± 0.24 ^b	0.06 ± 0.26 ^b

^a All calculations were performed on the Ala-Ala-hydroxyethylene containing inhibitors. ^b These experimental values are for the asparagyl quinoloyl containing inhibitors **6**, **7**, **9**.

had been solved, they were analyzed and used to design more elaborate inhibitors. The common feature of the complexed structures of compounds **2** and **3** was the close contact between the flat aromatic groups and the p3' hydrophobic pocket. Analysis of the crystal structure of compound **3** revealed that in order for the indole ring to pack against the p3' wall it had to shift the NH of the indole out of reach of the Gly 48 carbonyl oxygen. The result is a 180° rotation of the indole ring and a water-mediated H-bond to Gly 27 carbonyl oxygen. Addition of hydrophobic substituents to the phenyl group occupying the p2' pocket and replacement of the N-terminal Ala-Ala resulted in compounds with inhibition constants in the 30 nM range (compounds **6** and **7**).

Once this initial set of compounds had been synthesized, the more highly substituted hydroxyethylene containing compound **10** was prepared. On the basis of precedent,¹⁵ this change was expected to confer an additional 2 orders of magnitude in binding and result in a subnanomolar inhibitor. Surprisingly, the 2-benzyl substituent in compound **10** actually decreased binding by over a factor of 28. Unfortunately, due to the poor inhibition constant, we were unable to obtain a cocrystal of this compound with the protease. The explanation for this complete reversal of activity seems to lie in the manner in which the new C-terminal indole substituent interacts with the protein. Retrospective modeling studies indicate that the 2-benzyl group of compound **10** extends from the p1' side-chain pocket to the edge of the p3' pocket which could interfere with the placement of the indole ring against the p3' hydrophobic wall. From these results one can conclude that the substituents in the p1' and p3' positions are not independent and illustrate the pitfalls of treating different substituents on a core structure as independent variables.

Computational analysis of a select group of these inhibitors using the thermodynamic cycle perturbation method proved useful in understanding relative binding affinities.

Experimental Section

Proton magnetic resonance spectra were determined using a General Electric QE-300 spectrometer operating at a field strength of 300 MHz. Chemical shifts are reported in parts per million (δ) and setting the references such that in CDCl₃ and CHCl₃ is at 7.26 ppm and in *d*₆-DMSO the DMSO is at 2.49 ppm. Standard and peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; brs, broad singlet; brd, broad doublet; br, broad signal; m, multiplet. Mass spectra were determined at either the University of California Riverside or the University of California Berkeley Mass Spectrometry Centers. Infrared absorption spectra were taken on either a Perkin-Elmer 457 spectrometer or a MIDAC Corp. FTIR. Elemental microanalysis were performed by Atlantic Microlab Inc. Norcross, GA, or MHW Laboratories Phoenix, AZ, and gave results for the elements stated with ±0.4% of the theoretical values. *N,N*-Dimethylformamide (DMF) was dried over activated (250 °C) 4-Å molecular sieves; *N,N*-dimethylacetamide (DMA) (Aldrich

Gold Label grade) was similarly dried. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under nitrogen. Ether refers to diethyl ether. Pet. ether refers to petroleum ether of bp 36–53 °C. Flash chromatography was performed using silica gel 60 (Merck Art 9385). Thin-layer chromatographs (TLC) were performed on precoated sheets of silica 60 F₂₅₄ (Merck Art 5719). Melting points were determined on a Mel-Temp apparatus and are uncorrected.

Computational Methods. The details of the TCP study have been described in a separate account.²⁶

Biochemical Assays. Protease activity was measured by continuous chromogenic assay using a modification of a previously described procedure.²⁸ The chromogenic peptide His-Lys-Ala-Arg-Val-Leu-(*p*-NO₂-Phe)-Glu-Ala-Nleu-Ser (Bachem) was used as a substrate, and the reactions were run at 37 °C in buffer containing 0.2 M NaCl, 50 mM MES (2-[*N*-morpholino]ethanesulfonic acid pH 5.5), 3 mM EDTA, 5 mM mercaptoethanol, and DMSO (dimethyl sulfoxide) at final concentration not exceeding 5%. The reactions were run either in the absence of inhibitor or in the presence of inhibitor at concentrations ranging, at a minimum, between 0.5K_i and 10K_i except when the solubility of inhibitor was limiting. Reactions were followed by the decrease of absorbance at 304 nm using 1 Perkin-Elmer spectrophotometer. Experimental results were analyzed by the nonlinear regression analysis program TIGHTFIT using a mathematical model for tight binding inhibitors.²⁹

Crystallization and Structure Solution. Crystals of HIV protease inhibitor complexes were grown by hanging drop vapor diffusion. Protease, at 8 mg/mL in 50 mM sodium citrate, pH 5.5, containing 5 mM dithiothreitol, was mixed with inhibitor in a 1:5 molar ratio at a dioxane concentration of 5%. After overnight incubation at room temperature, any insoluble excess of inhibitor was removed by centrifugation. The crystallization well buffer consisted of 40% saturated ammonium sulfate, 50 mM sodium citrate pH 6.0, 10 μ L of 1.0 M mercaptoethanol, and 12% dioxane. Drops were seeded with microcrystals prior to sealing, and crystals grew at room temperature over a period of few weeks. Crystals of complexes of HIV protease with compound **2** and **3** were grown in the orthorhombic space group *P*2₁2₁2₁ with *a* = 66.38 Å, *b* = 92.44 Å, and *c* = 29.24 Å. This crystal form contains one HIV protease/inhibitor complex in the asymmetric unit and was first observed for the complex of HIV protease with a peptidic inhibitor.³⁰ X-ray diffraction data were collected at room temperature on a 9-kW Rigaku AFC-6 rotating anode generator equipped with a graphite monochromator and dual area detectors from San Diego Multiwire System. Coordinates of the protein portion were used to provide starting phases for the structure determination of complexes with **2** and **3**. In both cases, a single SA-refinement cycle with the enzyme only as a starting model was performed using the program XPLOR.²⁷ Inhibitors were fitted into the resulting *F*_o - *F*_c electron density map, and the complex was refined with several additional cycles of SA-refinement followed by individual atom B-factor refinement.

Water molecules were added at each step of the refinement using *F*_o - *F*_c and 2*F*_o - *F*_c electron density maps.

2-Benzoylindole-1-carboxylic Acid *tert*-Butyl Ester. To a stirred solution of 17.57 g (80.9 mmol) of *N*-(*tert*-butoxycarbonyl)indole (**19a**) in 120 mL of dry THF under argon and cooled to -78 °C were added 60.0 mL (102.0 mmol) of 1.7 M *tert*-butyllithium. After being stirred for 1 h at -78 °C, this solution was transferred via cannula to a -78 °C solution of 11.3 mL (97.3 mmol) of benzoyl chloride in 50 mL of dry THF. The reaction mixture was stirred for 15 min and then allowed to warm to room temperature. After quenching with 10 mL

Chart 1. Summary of X-ray Data Collection and Refinement

	compound 2	compound 3
resolution	10.0–2.7	10.0–2.4
reflections (total/unique)	13 604/4203	22 504/7307
R_{sym} (%) ^a ($ F > 0.0$)	4.51	5.16
R factor	14.6	15.2
no. water molecules	39	51
rms bonds (Å)	0.015	0.016
rms angle (deg)	2.62	2.71

^a $R_{\text{sym}} = |I - \langle I \rangle| / I$, where I is the observed intensity, and $\langle I \rangle$ is the average intensity obtained from multiple observations of symmetry related reflections.

of 0.5 N NaOH, the reaction was concentrated in vacuo to 75 mL, diluted with saturated NaCl solution, and extracted with EtOAc. The organic layer was dried (MgSO₄) and concentrated under reduced pressure. The resulting oil was refluxed in hexanes and cooled to give 8.95 g of product. A second crop gave 3.28 g. The mother liquors were concentrated and flash chromatographed on silica eluting with hexanes:EtOAc (20:1) to give an additional 3.86 g. Overall: 16.09 g (61%). Mp: 88–91 °C. IR (KBr): 1740, 1665, 1327, 1238, 1157, 1087 cm⁻¹. NMR (CDCl₃): δ 1.35 (s, 9H), 6.94 (s, 1H), 7.30 (t, 1H, $J = 7.5$ Hz), 7.48 (m, 3H), 7.62 (m, 2H), 7.91 (d, 2H, $J = 7.7$ Hz), 8.21 (d, 1H, $J = 8.4$ Hz). Anal. (C₂₀H₁₉NO₃) C, H, N.

(1H-Indol-2-yl)phenylmethanone (20). To a stirred solution of 8.95 g (27.9 mmol) of the above ketone in 50 mL of CH₂Cl₂ was added 6 mL of trifluoroacetic acid. After 4 h at room temperature, 3 N NaOH was added until the aqueous layer was basic, and the product was extracted with CH₂Cl₂, dried (MgSO₄), and concentrated under reduced pressure. The residue was recrystallized from hexanes to give 4.45 g (72%) of the desired indole as an off-white solid. Mp: 147–149 °C. IR (KBr): 3310, 1610, 1570, 1460, 1340, 1315, 1255 cm⁻¹. NMR (CDCl₃): δ 7.17 (m, 2H), 7.38 (t, 1H, $J = 7.6$ Hz), 7.53 (m, 4H), 7.71 (d, 1H, $J = 7.8$ Hz), 7.99 (d, 2H, $J = 7.0$ Hz), 9.30 (brs, 1H). Anal. (C₁₅H₁₁NO) C, H, N.

(N-(Phenylsulfonyl)-1H-indol-2-yl)-m-tolylmethanone. A solution of 4.00 g (15.55 mmol) of *N*-(phenylsulfonyl)-indole (19b) in dry THF (25 mL) was treated with 1.7 M *t*-BuLi (11.0 mL, 18.66 mmol) dropwise via a gas-tight syringe at –78 °C. The red anion solution was cannulated directly into a solution of *m*-toluoyl chloride (2.5 mL, 18.66 mmol) in THF (10 mL) cooled to –78 °C. After 1 h at –78 °C the reaction was quenched with saturated NH₄Cl (10 mL) and diluted with EtOAc. The organic layer was washed H₂O and brine, dried, and evaporated under reduced pressure. The crude residue was purified by flash chromatography (25% EtOAc/Hex) to afford the desired ketone (4.00 g, 68% yield) as a white solid. NMR (CDCl₃): δ 2.42 (s, 3H), 6.93 (s, 1H), 7.31–7.75 (m, 8H), 7.79 (d, 1H, $J = 7.5$ Hz), 7.81 (s, 1H), 8.10 (m, 3H).

(1H-Indol-2-yl)-m-tolylmethanone (22). The above protected indole (3.80 g, 10.12 mmol) was suspended in EtOH (20 mL) and 2.2 N NaOH (15 mL, 33 mmol) and heated to reflux. After 3 h the reaction mixture was cooled to room temperature and diluted with EtOAc and H₂O. The organic layer was washed with brine, dried, and concentrated under reduced pressure. The solid residue was recrystallized from EtOH to give the desired unprotected indole (1.59 g, 67%) as a light yellow solid. Mp: 132–133 °C. NMR (CDCl₃): δ 2.47 (s, 3H), 7.17 (dd, 2H, $J = 6.6, 8.0$ Hz), 7.36–7.50 (m, 4H), 7.72 (d, 1H, $J = 8.1$ Hz), 7.80 (m, 1H), 9.33 (brs, 1H).

(N-(Phenylsulfonyl)-1H-indol-2-yl)cyclohexylmethanone. This compound was prepared from the protected indole 19b using the procedure described above to give the desired product as a white solid in 77% yield. Mp: 95–97 °C. NMR (CDCl₃): δ 1.2–1.6 (m, 5H), 1.65–2.01 (m, 5H), 3.21 (m, 1H), 7.01 (s, 1H), 7.28 (dd, 1H, $J = 7.5, 8.0$ Hz), 7.40–7.60 (m, 6H), 7.98 (d, 1H, $J = 8.7$ Hz), 8.07 (d, 1H, $J = 8.0$ Hz).

(1H-Indol-2-yl)cyclohexylmethanone (23). This compound was prepared using the procedure described for ketone 22 to give the desired product as a tan solid in 95% yield. Mp: 168–170 °C. NMR (CDCl₃): δ 1.2–2.0 (m, 10H), 3.20 (m, 1H), 7.15 (t, 1H, $J = 7.5$ Hz), 7.23 (s, 1H), 7.34 (dd, 1H, $J = 7.5, 8.0$ Hz), 7.71 (d, 1H, $J = 8.1$ Hz), 9.20 (brs, 1H).

(N-(Phenylsulfonyl)-1H-indol-2-yl)(m-(trifluoromethyl)phenyl)methanone. This compound was prepared from the protected indole 19b using the procedure described above to give the desired product as a yellow solid in 70% yield. Mp: 115–116 °C. NMR (CDCl₃): δ 7.00 (s, 1H), 7.33 (dd, 1H, $J = 7.5, 8.0$ Hz), 7.50 (m, 3H), 7.62 (m, 3H), 7.88 (d, 1H, $J = 7.7$ Hz), 8.04 (m, 2H), 8.15 (m, 2H), 8.27 (s, 1H).

(1H-Indol-2-yl)(m-(trifluoromethyl)phenyl)methanone (21). This compound was prepared using the procedure described for ketone 22 to give the desired product as a yellow solid in 83% yield. Mp: 130–131 °C. NMR (CDCl₃): δ 7.17 (m, 2H), 7.42 (m, 2H), 7.71 (m, 2H), 7.88 (d, 1H, $J = 7.9$ Hz), 8.17 (d, 1H, $J = 7.7$ Hz), 8.25 (s, 1H), 9.39 (brs, 1H).

(1H-Indol-2-yl)phenylmethanone Oxime. To a suspension of 4.02 g (18.2 mmol) of ketone 20 in a mixture of 40 mL of EtOH/8 mL of H₂O was added 1.90 g (27.3 mmol) of NH₂OH·HCl. To this was added in portions 3.63 g (90.8 mmol) of powdered NaOH. When the addition was complete, the reaction mixture was refluxed for 35 min, cooled, and poured into 1 N HCl, and the product was extracted with EtOAc. After drying (MgSO₄) and removal of the solvent under reduced pressure, the residue was flash chromatographed on silica, eluting with CH₂Cl₂ to give 4.10 g (95%) of the oxime as a tan solid. Mp: 169–172 °C. IR (KBr): 3440, 3010 (broad), 1335, 1305, 1260, 1020, 940, 915 cm⁻¹. NMR (CDCl₃): δ 6.57 (s, 1H), 7.10 (t, 1H, $J = 7.1$ Hz), 7.30 (t, 1H, $J = 7.1$ Hz), 7.45 (m, 4H), 7.61 (m, 3H), 9.70 (brs, 1H), 10.24 (s, 1H). Anal. (C₁₅H₁₄N₂O·0.1H₂O) C, H, N.

(1H-Indol-2-yl)-m-tolylmethanone Oxime. This compound was prepared with ketone 22 using the procedure described above to yield the desired material in 86% as a yellow oil. NMR (CDCl₃, major isomer): δ 2.43 (s, 3H), 6.30 (d, 1H, $J = 1.8$ Hz), 7.04–7.4 (m, 7H), 7.56 (d, 1H, $J = 8.0$ Hz), 10.24 (brs, 1H).

(1H-Indol-2-yl)cyclohexylmethanone Oxime. This compound was prepared with ketone 23 using the procedure described above to yield the desired material as a white solid in 53% yield. Mp: 208–210 °C. NMR (CDCl₃, major isomer): δ 1.25–1.58 (m, 5H), 1.85 (m, 3H), 2.09 (m, 2H), 2.88 (m, 1H), 6.89 (s, 1H), 7.13 (dd, 1H, $J = 7.8, 7.8$ Hz), 7.27 (dd, 1H, $J = 8.0, 7.6$ Hz), 7.45 (d, 1H, $J = 7.7$ Hz), 7.68 (d, 1H, $J = 8.0$ Hz), 10.39 (brs, 1H).

(1H-Indol-2-yl)(m-(trifluoromethyl)phenyl)methanone Oxime. This compound was prepared with ketone 21 using the procedure described above to yield the desired material as an oil in 93% yield. NMR (CDCl₃, major isomer): δ 6.49 (s, 1H), 7.13 (m, 2H), 7.30 (m, 2H), 7.52–7.84 (m, 4H), 7.93 (s, 1H), 10.22 (brs, 1H).

[(1H-Indol-2-yl)-m-tolylmethyl]amine (26). To a suspension of activated Zn dust (2.2 g, 33.6 mmol) and NH₄OAc (1.55 g, 20.2 mmol) in concentrated NH₄OH (20 mL) was added a solution of the corresponding oxime (1.68 g, 6.72 mmol) in EtOH (10 mL), and the mixture was heated to reflux. After 1 h the reaction mixture was diluted with EtOAc and filtered to remove excess Zn. The filtrate was washed with H₂O and brine, dried, and concentrated at reduced pressure. The crude residue was purified by flash chromatography (2.5% MeOH/1% Et₃N/96.5% CH₂Cl₂) to afford the desired amine (1.18 g, 86% yield) as a light brown solid. Mp: 83–86 °C. NMR (CDCl₃): δ 2.34 (s, 3H), 5.30 (s, 1H), 6.29 (s, 1H), 7.04–7.30 (m, 7H), 7.53 (d, 1H, $J = 7.6$ Hz), 8.42 (brs, 1H).

[(1H-Indol-2-yl)cyclohexylmethyl]amine (27). This compound was prepared using the procedure described for amine 26 to give the desired material as an oil in 67% yield. NMR (CDCl₃): δ 1.09 (m, 5H), 1.75 (m, 8H), 3.94 (d, 1H, $J = 6.1$ Hz), 6.29 (s, 1H), 7.07 (dd, 1H, $J = 7.2, 7.5$ Hz), 7.09 (dd, 1H, $J = 7.2, 8.4$ Hz), 7.35 (d, 1H, $J = 8.1$ Hz), 7.56 (d, 1H, $J = 7.6$ Hz), 8.59 (brs, 1H).

[(1H-Indol-2-yl)(m-(trifluoromethyl)phenyl)methyl]amine (25). This compound was prepared using the procedure described for amine 26 to give the desired material as a tan solid in 50% yield. Mp: 120–123 °C. NMR (CDCl₃): δ 5.42 (s, 1H), 6.26 (s, 1H), 7.10 (m, 2H), 7.32 (d, 1H, $J = 8.0$ Hz), 7.44 (d, 1H, $J = 7.7$ Hz), 7.55 (m, 4H), 7.73 (s, 1H), 8.47 (s, 1H).

[(1*H*-Indol-2-yl)phenylmethyl]amine (24). A solution of 328 mg (1.39 mmol) of the above oxime, 37 mg of 10% palladium on carbon, and two drops of concentrated HCl in 10 mL of MeOH was placed under 50 psi of H₂ for 18 h. The catalyst was filtered off, and the volatiles were removed under reduced pressure. The residue was dissolved in EtOAc (50 mL) and extracted with 0.5 N HCl (3 × 50 mL). The aqueous layer was basified with 6 N NaOH and extracted with EtOAc. The organic layer was dried (MgSO₄) and the solvent removed under reduced pressure to give 192 mg (62%) of the desired amine as a tan solid. Mp: 98–101 °C. IR (KBr): 3175, 3079, 1586, 1493, 1456, 1427, 1312, 953 cm⁻¹. NMR (CDCl₃): δ 1.85 (brs, 2H), 5.36 (s, 1H), 6.33 (s, 1H), 7.1 (m, 2H), 7.40 (m, 6H), 7.58 (d, 1H, *J* = 7.6 Hz), 8.40 (brs, 1H). Anal. (C₁₅H₁₄N₂) C, H, N.

5-((*tert*-Butyloxycarbonyl)amino)-4-hydroxy-6-phenylhexanoic Acid. To a stirred solution of 1.63 g (5.34 mmol) of lactone 11 in 10 mL of THF was added 4 mL of 2 N LiOH. After 2 h at room temperature, the reaction was poured into EtOAc/H₂O, and the layers were separated. The organic layer was washed with saturated NaHCO₃ solution. The combined aqueous layers were acidified with 4 N HCl and re-extracted with EtOAc. This organic layer was dried (MgSO₄) and concentrated under reduced pressure to give 1.63 g (94%) of the desired acid as a white solid. Mp: 118–119 °C. IR (KBr): 3460, 3395, 2980, 2925, 1690, 1665, 1520, 1245, 1165 cm⁻¹. NMR (CDCl₃): δ 1.34 and 1.39 (s, s, 9H), 1.55 (m, 1H), 1.82 (m, 1H), 2.05 (m, 1H), 2.44–2.61 (m, 2H), 2.89 (d, 2H, *J* = 7.2 Hz), 3.55 (m, 2H), 4.93 (d, 1H, *J* = 8.6 Hz), 6.80 (d, 1H, *J* = 8.6 Hz), 7.25 (m, 5H). Found as a mixture of rotamers. Anal. (C₁₇H₂₅NO₅) C, H, N.

5-((*tert*-Butyloxycarbonyl)amino)-4-((*tert*-butyldimethylsilyloxy)-6-phenylhexanoic Acid (12). To a stirred solution of 1.57 g (4.86 mmol) of the above acid and 1.66 g (24.38 mmol) of imidazole in 20 mL of DMF were added 2.94 g (19.49 mmol) of chloro-*tert*-butyldimethylsilane. After 8 h of heating at 55 °C, the reaction mixture was poured out 0.5 N HCl and extracted twice with EtOAc. The combined organic layers were washed with brine, dried (MgSO₄), and concentrated at reduced pressure. The resulting oil was dissolved in 10 mL of THF/H₂O/HOAc (1:1:0.5), stirred at room temperature for 4.5 h, poured into water, and extracted with EtOAc. The organic layer was dried (MgSO₄), concentrated under reduced pressure, and flash chromatographed on silica, eluting with CH₂Cl₂-EtOAc (5:1) to give 2.37 g (88%) of the desired silylated alcohol as a colorless oil which solidified on standing: Mp: 97–99 °C. IR (KBr): 2940, 2860, 1700, 1650, 1365, 1250, 1165 cm⁻¹. NMR (CDCl₃): δ 0.11 and 0.13 (s, s, 6H), 0.95 (s, 9H), 1.20 and 1.35 (s, s, 9H), 1.79 (m, 2H), 2.36 (m, 2H), 2.80 (m, 2H), 3.73 (t, 1H, *J* = 5.8 Hz), 3.90 (m, 1H), 4.70 and 5.69 (d, d, 1H, *J* = 9.4 Hz), 7.22 (m, 5H). Found as a mixture of rotamers. Anal. (C₂₃H₃₉NO₅Si) C, H, N.

5-((*tert*-Butyloxycarbonyl)amino)-4-((*tert*-butyldimethylsilyloxy)-6-phenylhexanoic Acid Benzhydrylamide. To a stirred solution of 1.88 g (4.30 mmol) of acid 12 and 1.5 mL (8.70 mmol) of aminodiphenylmethane in 15 mL of CH₂Cl₂ was added 1.77 g (8.58 mmol) of DCC. After 3 h at room temperature, the reaction mixture was diluted with EtOAc and washed sequentially with 0.5 N HCl, saturated NaHCO₃, and brine. The organic layer was dried (MgSO₄) and the solvent removed at reduced pressure. The residue was chromatographed on silica, eluting with CH₂Cl₂-EtOAc (25:1) to give 1.47 g (57%) of the desired amide as a white amorphous solid. Mp: 43–50 °C. IR (KBr): 3455, 3304, 2930, 2857, 1713, 1649, 1530, 1497, 1171 cm⁻¹. NMR (CDCl₃): δ 0.09 (s, 6H), 0.91 and 0.93 (s, s, 9H), 1.27 and 1.57 (s, s, 9H), 1.75 (m, 1H), 1.92 (m, 1H), 2.18 (m, 1H), 2.34 (m, 1H), 2.74 (d, 2H, *J* = 7.1 Hz), 3.69 (m, 1H), 3.90 (m, 1H), 4.68 (m, 1H), 6.22 (s, 2H), 7.20 (m, 15H). Found as a mixture of rotamers. Anal. (C₃₈H₅₀N₂O₄Si·0.1H₂O) C, H, N.

5-Amino-4-hydroxy-6-phenylhexanoic Acid Benzhydrylamide (13). A solution of 1.40 g (2.33 mmol) of the above amide and 1 mL of TFA in 10 mL of CH₂Cl₂ was allowed to react for 3 h at room temperature. The volatiles were removed under reduced pressure and the residue diluted with EtOAc and washed with saturated NaHCO₃. The organic layer was

dried (MgSO₄) and concentrated. To a stirred solution of the resulting residue in 10 mL of THF was added 3.2 mL (3.5 mmol) of a 1.1 M solution of tetrabutylammonium fluoride in THF. After 3 h at 45 °C, the THF was removed under reduced pressure. The crude product was diluted with EtOAc, washed with saturated NaHCO₃ solution, and dried (MgSO₄). After removed of the solvent, the residue was chromatographed on silica, eluting with CH₂Cl₂-MeOH (9:1) to give 515 mg (57%) of the desired amino alcohol as a white solid. Mp: 161–163 °C. IR (KBr): 3305, 3030, 2920, 1635, 1530, 1490, 1325 cm⁻¹. NMR (CDCl₃): δ 1.80 (m, 1H), 1.92 (m, 1H), 2.51 (m, 3H), 2.91 (brm, 5H), 3.41 (m, 1H), 6.23 (d, 1H, *J* = 8.0 Hz), 6.66 (d, 1H, *J* = 8.1 Hz), 7.25 (m, 15H). MH⁺ HRMS (C₂₅H₂₉N₂O₂)⁺ calcd 389.2229, found 389.2226.

5-((*L*-((*tert*-Butyloxycarbonyl)amino)alanyl-*L*-alanyl)-amino)-4-hydroxy-6-phenylhexanoic Acid Benzhydrylamide. To a stirred solution of 298 mg (0.77 mmol) of the amino alcohol 13 and 220 mg (0.85 mmol) of *N*-*t*-BOC-*L*-Ala-*L*-alanine in 10 mL of DMF was added 238 mg (1.15 mmol) of DCC. The reaction mixture was stirred for 3 days at room temperature, poured into saturated NaCHO₃ solution, and extracted with EtOAc. The organic layer was washed with 0.5 N HCl and then with saturated NaCl solution and dried (MgSO₄), and the solvent was removed at reduced pressure. The residue was chromatographed on silica, eluting with a gradient of EtOAc in CH₂Cl₂ (50–75%) to give 338 mg (70%) of the coupled product as a white solid. Mp: 133–135 °C. IR (KBr): 3300, 3060, 3030, 2980, 2930, 1635, 1495, 1450, 1245, 1160 cm⁻¹. NMR (CDCl₃): δ 1.26 (m, 9H), 1.46 (s, 6H), 1.75 (m, 3H), 2.36 (m, 2H), 2.88 (m, 2H), 3.59 (m, 1H), 4.00 (m, 2H), 4.27 (m, 1H), 5.08 and 5.25 (d, d, 1H, *J* = 5.6 Hz), 6.21 (d, 1H, *J* = 8.0 Hz), 6.57 (d, 1H, *J* = 6.3 Hz), 6.71 (m, 1H), 6.95 (m, 1H), 7.25 (m, 15H). Anal. (C₃₆H₄₅N₄O₆·0.5H₂O) C, H, N.

5-((*L*-Alanyl-*L*-alanyl)amino)-4-hydroxy-6-phenylhexanoic Acid Benzhydrylamide (2). A stirred solution of 205 mg (0.33 mmol) of the above *t*-BOC-protected compound and 0.3 mL of TFA in 5 mL of CH₂Cl₂ was allowed to react at room temperature for 1 h. The volatiles were removed at reduced pressure. The residue was diluted with EtOAc and washed twice with 0.5 N HCl solution. The combined aqueous washes were made basic with 6 N NaOH and extracted twice with EtOAc. The organic layer was dried (MgSO₄) and the solvent removed under reduced pressure. The residue was dissolved in 2 mL of CH₂Cl₂-MeOH (9:1) and triturated with Et₂O. The precipitate which formed was collected and dried to give 110 mg (64%) of the desired product as a white solid. Mp: 174–177 °C. IR (KBr): 3290, 3060, 3030, 2960, 2930, 1640, 1510, 1450, 1255 cm⁻¹. NMR (CDCl₃): δ 1.14 and 1.22 and 1.25 (d, d, d, 6H, *J* = 7.0 Hz), 1.75 (m, 2H), 2.10 (brs, 2H), 2.38 (m, 2H), 2.89 (d, 2H, *J* = 7.7 Hz), 3.33 (m, 1H), 3.60 (m, 1H), 4.01–4.33 (m, 2H), 6.19 (d, 1H, *J* = 8.0 Hz), 6.73 (m, 2H), 7.26 (m, 15H), 7.62 (d, 1H, *J* = 7.3 Hz). Anal. (C₃₁H₃₉N₄O₄·H₂O) C, H, N. MH⁺ HRMS (C₃₁H₃₉N₄O₄)⁺ calcd 531.2971, found 531.2987.

5-((*tert*-Butyloxycarbonyl)amino)-4-((*tert*-butyldimethylsilyloxy)-6-phenylhexanoic Acid ((1*H*-Indol-2-yl)phenyl)methyl)amide. To a stirred solution of 1.96 g (6.07 mmol) of acid 12 and 1.35 g (6.07 mmol) of amine 24 in 20 mL of CH₂Cl₂ was added 1.75 g (8.48 mmol) of DCC. After 4 h, another 0.20 g (0.62 mmol) of the acid was added, and the reaction was allowed to stir at room temperature for 18 h more. The reaction mixture was diluted with EtOAc, washed with saturated NaHCO₃ and 0.5 N HCl, dried (MgSO₄), and concentrated at reduced pressure. The residue was chromatographed on silica, eluting with CH₂Cl₂-EtOAc (30:1) to give 2.29 g (59%) of the coupled product as an inseparable mixture of diastereomers as a white brittle foam. Neutralization of the acidic aqueous wash and extraction with EtOAc gave a recovery of 0.45 g of starting indolamine. Mp: 76–79 °C with foaming. IR (KBr): 3304, 2930, 2857, 1694, 1655, 1497, 1456, 1169 cm⁻¹. NMR (CDCl₃): δ 0.09 and 0.10 (s, s, 6H), 0.94 and 0.95 (s, s, 9H), 1.27 (m, 11H), 1.85 (m, 2H), 2.21 (m, 1H), 2.74 (m, 1H), 3.80 (m, 2H), 4.73 (m, 1H), 5.99 and 6.05 (s, s, 1H), 6.29 and 6.33 (d, d, 1H, *J* = 7.9 Hz), 6.46 and 6.54 (d, d, 1H, *J* = 7.9 Hz), 7.05–7.50 (m, 14H), 8.93 and 9.08 (s, s, 1H). Anal. (C₃₈H₅₁N₃O₄Si·0.4H₂O) C, H, N.

5-((*tert*-Butyloxycarbonyl)amino)-4-((*tert*-butyldimethylsilyloxy)-6-phenylhexanoic Acid ((1*H*-indol-2-yl)-(3-(trifluoromethyl)phenyl)methyl)amide. This compound was prepared with the acid **12** and the amine **25** using the method described above to give the desired amide in 74% yield as an inseparable mixture of diastereomers as a gummy oil. IR (KBr): 3310, 2955, 2932, 1692, 1500, 1331, 1167, 1128, 837, 400 cm^{-1} ; $^1\text{H NMR}$ (CDCl_3 , 300 MHz): δ 9.46 and 8.91 (s, s, 1H), 7.69–7.48 (m, 4H), 7.37–7.04 (m, 11H), 6.81 and 6.49 (d, d, 1H, $J = 7.95$ and 7.85 Hz), 6.38 and 6.33 (d, d, 1H, $J = 7.92$ and 7.57 Hz), 6.06 and 6.94 (s, s, 1H), 4.79 and 4.67 (d, d, 1H, $J = 9.36$ and 9.76 Hz), 3.87 (m, 1H), 3.63 (m, 1H), 2.74 (m, 2H), 2.46–2.25 (m, 2H), 2.1–1.6 (m, 2H), 1.35 (s, 9H), 0.98 (s, 9H), 0.11 (s, 6H). Anal. ($\text{C}_{39}\text{H}_{50}\text{N}_3\text{O}_4\text{SiF}_3$) C, H, N.

5-((*tert*-Butyloxycarbonyl)amino)-4-((*tert*-butyldimethylsilyloxy)-6-phenylhexanoic Acid ((1*H*-Indol-2-yl)(3-methylphenyl)methyl)amide. This compound was prepared with the acid **12** and the amine **26** using the method described above to give the desired amide in 79% yield as an inseparable mixture of diastereomers as a gummy oil. IR (KBr): 3316, 2932, 1694, 1499, 1252, 1169, 1092, 837, 777, 700 cm^{-1} . $^1\text{H NMR}$ (CDCl_3 , 300 MHz): δ 9.24 and 8.93 (s, s, 1H), 7.54–7.02 (m, 15H), 6.82 and 6.38 (d, d, 1H, $J = 7.89$ and 7.84 Hz), 6.38 and 6.32 (d, d, 1H, $J = 7.91$ and 7.54 Hz), 6.06 and 6.87 (s, s, 1H), 4.70 and 4.62 (d, d, 1H, $J = 9.34$ and 9.82 Hz), 3.75 (m, 1H), 3.6 (m, 1H), 2.75 (m, 2H), 2.1–1.6 (m, 2H), 1.35 (s, 9H), 0.98 (s, 9H), 0.12 (s, 6H). Anal. ($\text{C}_{39}\text{H}_{53}\text{N}_3\text{O}_4\text{Si}$) C, H, N.

5-((*tert*-Butyloxycarbonyl)amino)-4-((*tert*-butyldimethylsilyloxy)-6-phenylhexanoic Acid ((1*H*-Indol-2-yl)-cyclohexylmethyl)amide. This compound was prepared with the acid **12** and the amine **27** using the method described above to give the desired amide in 76% yield as an inseparable mixture of diastereomers as a gummy oil. IR (KBr): 3315, 2932, 2857, 1696, 1655, 1499, 1366, 1254, 1169, 1089, 837, 777, 700 cm^{-1} . $^1\text{H NMR}$ (CDCl_3 , 300 MHz): δ 9.01 (s, 1H), 7.6 (d, 1H, $J = 7.46$ Hz), 7.42–7.06 (m, 10H), 6.32 (s, 1H), 4.72–4.63 (m, 2H), 3.85–3.63 (m, 2H), 2.77–2.72 (m, 2H), 1.98–1.71 (m, 9H), 1.38–1.26 (m, 14H), 0.97 (s, 9H), 0.12 (s, 6H). Anal. ($\text{C}_{38}\text{H}_{57}\text{N}_3\text{O}_4\text{Si}$) C, H, N.

5-((*tert*-Butyloxycarbonyl)amino)-4-((*tert*-butyldimethylsilyloxy)-6-phenylhexanoic Acid ((1*H*-benzimidazol-2-yl)phenylmethyl)amide. This compound was prepared with the acid **12** and [(1*H*-Benzimidazol-2-yl)phenylmethyl]amine using the method described above to give the desired amide in 73% yield as an inseparable mixture of diastereomers as a gummy oil. IR (KBr): 3254, m 2953, 2930, 1664, 1497, 1454, 1365, 1254, 1169, 1094, 837, 777, 742 cm^{-1} . $^1\text{H NMR}$ (DMSO, 300 MHz): δ 12.33 (s, 1H), 8.94 (d, 1H, $J = 8.63$ Hz), 7.61–7.12 (m, 14H), 6.67 (d, 1H, $J = 8.5$ Hz), 6.26 (d, 1H, $J = 8.11$ Hz), 3.67 (brs, 2H), 2.46–2.25 (m, 2H), 1.82–1.67 (brm, 1H), 1.61–1.43 (brm, 1H), 1.35 (s, 9H), 0.97 (s, 9H), 0.11 (s, 6H). HRMS (FAB) $\text{C}_{37}\text{H}_{50}\text{N}_4\text{O}_4\text{Si}$ 642.9259 MW; MH^+ found 643.5732.

5-Amino-4-hydroxy-6-phenylhexanoic Acid ((1*H*-Indol-2-yl)phenylmethyl)amide (14). To a stirred solution of 2.07 g (3.23 mmol) of 5-((*tert*-butyloxycarbonyl)amino)-4-((*tert*-butyldimethylsilyloxy)-6-phenylhexanoic acid (1*H*-indol-2-yl)-phenylmethyl amide in 10 mL of THF was added 4.4 mL (4.84 mmol) of 1.1 M tetrabutylammonium fluoride in THF. After 4 h at 55 °C, the reaction was cooled to 40 °C for 18 h. The solvent was removed at reduced pressure. The residue was diluted with EtOAc, washed with 0.5 N HCl and brine, and dried (MgSO_4). After removal of the solvent under reduced pressure, the residue was dissolved in 10 mL of CH_2Cl_2 at 0 °C, and 0.5 mL of TFA was added. Another 0.5 mL of TFA was added after 1 h. The reaction, when complete, was poured into 2 N NaOH and extracted with EtOAc (3 \times). The combined organic layer was washed with brine and dried (MgSO_4) and the solvent removed under reduced pressure. The residue was chromatographed on silica, eluting with a gradient of MeOH in EtOAc (7–10%) to give 0.50 g (36%) of the amino alcohol as an inseparable mixture of diastereomers as a white solid. Mp: 147–149 °C. IR (KBr): 3316, 3027, 2924, 1651, 1528, 1495, 1456, 1292 cm^{-1} . $^1\text{H NMR}$ (CDCl_3): δ 1.79 (m, 2H), 2.33 (m, 7H), 2.78 (m, 2H), 3.28 (m, 1H), 6.02 and 6.06 (s, s, 1H),

6.36 (d, 1H, $J = 8.1$ Hz), 7.15 (m, 7H), 7.45 (d, 1H, $J = 8.1$ Hz), 9.30 and 9.39 (s, s, 1H). Anal. ($\text{C}_{27}\text{H}_{29}\text{N}_3\text{O}_2$) C, H, N.

5-Amino-4-hydroxy-6-phenylhexanoic Acid (1*H*-Benzimidazol-2-yl)phenylmethyl)amide (15). The compound was prepared using the procedure described for amine **14**. Product was collected in 68% yield as an inseparable mixture of diastereomers as a yellow solid. Mp: 74–76 °C. $^1\text{H NMR}$ (CDCl_3 , 300 MHz): δ 12.43 (brs, 1H), 7.51–7.12 (m, 14H), 6.41 (t, 1H, $J = 7.9$ Hz), 3.39 (m, 1H), 2.67 (m, 2H), 2.51 (m, 4H), 1.98 (m, 3H).

5-Amino-4-hydroxy-6-phenylhexanoic Acid ((1*H*-indol-2-yl)(3-(trifluoromethyl)phenyl)methyl)amide (16). The compound was prepared using the procedure described for amine **14**. Product was collected in 76% yield as an inseparable mixture of diastereomers as a tan solid. Mp: 64–65 °C. $^1\text{H NMR}$ (CDCl_3 , 300 MHz): δ 9.35 (s, 1H), 7.75–7.01 (m, 13H), 6.44 (t, 1H, $J = 7.4$ Hz), 6.01 (d, 1H, $J = 8.1$ Hz), 3.35 (m, 1H), 2.74 (m, 2H), 2.36 (m, 6H), 1.92 (m, 2H).

5-Amino-4-hydroxy-6-phenylhexanoic Acid ((1*H*-Indol-2-yl)(3-methylphenyl)methyl)amide (17). The compound was prepared using the procedure described for amine **14**. The product was isolated in 73% yield as an inseparable mixture of diastereomers as a tan solid. Mp: 71–75 °C. $^1\text{H NMR}$ (CDCl_3 , 300 MHz): δ 9.36 (s, 1H), 7.54–7.02 (m, 13H), 6.47 (t, 1H, $J = 7.5$ Hz), 6.11 (d, 1H, $J = 8.1$ Hz), 3.27 (m, 1H), 2.68 (m, 2H), 2.46 (s, 3H) 2.35 (m, 6H), 1.90 (m, 2H).

5-Amino-4-hydroxy-6-phenylhexanoic Acid ((1*H*-Indol-2-yl)cyclohexylmethyl)amide (18). The compound was prepared using the procedure described for amine **14**. The product was isolated in 72% yield as an inseparable mixture of diastereomers as a tan solid. Mp: 85–86 °C. $^1\text{H NMR}$ (CDCl_3 , 300 MHz): δ 9.41 (s, 1H), 7.59–7.10 (m, 9H), 6.41 (m, 1H), 6.03 (d, 1H, $J = 8.2$ Hz), 3.32 (m, 1H), 2.65 (m, 2H), 2.34 (m, 6H), 1.76 (m, 12H).

5-((*L*-*tert*-Butyloxycarbonyl)-alanyl-*L*-alanyl)amino-4-hydroxy-6-phenylhexanoic Acid ((1*H*-Indol-2-yl)phenylmethyl)amide. To a stirred solution of 361 mg (0.84 mmol) of amino alcohol **14** and 253 mg (0.97 mmol) of *N*-*t*-BOC-*L*-Ala-*L*-alanine in 10 mL of a 5:1 mixture of CH_2Cl_2 :DMF was added 261 mg (1.27 mmol) of DCC. After 24 h at room temperature, another 25 mg (0.10 mmol) of BOC-Ala-Ala and 26 mg (0.13 mmol) of DCC were added. After 4 h more at room temperature, the reaction mixture was diluted with EtOAc and washed sequentially with 0.5 N HCl, saturated NaHCO_3 , and brine. The organic layer was dried (MgSO_4) and the solvent removed at reduced pressure. The residue was chromatographed on silica, eluting with CH_2Cl_2 -MeOH (15:1) to give 277 mg (49%) of the desired product as an inseparable mixture of diastereomers as a white brittle foam. Mp: 114–117 °C with foaming. IR (KBr): 3304, 3061, 2978, 2932, 1655, 1532, 1454, 1252, 1165 cm^{-1} . NMR (DMSO- d_6): δ 0.90 and 1.11 (m, 6H), 1.35 (s, 9H), 1.53 (m, 2H), 2.25 (m, 2H), 2.70 (m, 2H), 3.41 (m, 1H), 3.92 (m, 2H), 4.22 (m, 1H), 4.94 (m, 1H), 6.08 (s, 1H), 6.24 (m, 1H), 6.97 (m, 4H), 7.19–7.42 (m, 11 H), 7.63 (m, 2H), 8.78 (m, 1 H), 10.99 (brs, 1H). Found as a single pair of diastereomers. M^+ HRMS ($\text{C}_{38}\text{H}_{47}\text{N}_5\text{O}_6$) $^+$ calcd 669.3526, found 669.3525. Anal. ($\text{C}_{38}\text{H}_{47}\text{N}_5\text{O}_6 \cdot 0.6\text{DMF}$) C, H, N.

5-(*L*-Alanyl-*L*-alanyl)amino-4-hydroxy-6-phenylhexanoic Acid ((1*H*-Indol-2-yl)phenylmethyl)amide (3). To a stirred solution of 263 mg (0.39 mmol) of the BOC protected compound above dissolved in 3 mL of hot CH_2Cl_2 and then cooled to 0 °C was added 1 mL of TFA. The ice bath was removed, and the reaction mixture was warmed to room temperature. After 30 min, it was poured into 1 N NaOH and extracted twice with EtOAc. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was chromatographed on silica, eluting with CH_2Cl_2 -6% NH_3 in MeOH (15:1) to give 110 mg (50%) of the desired product as an inseparable mixture of diastereomers as a white solid. Mp: 206–208 °C. IR (KBr): 3283, 3061, 2926, 1651, 1528, 1454, 1292, 1229 cm^{-1} . NMR (DMSO- d_6) δ 0.95 and 1.06 and 1.10 and 1.30 (d, d, d, d, 6H, $J = 7.1$ Hz), 1.53 (m, 2H), 2.27 (m, 3H), 2.63 (m, 1H), 2.80 (m, 1H), 3.21 (m, 2H), 3.43 (m, 1H), 3.86–4.26 (m, 2H), 4.98 (m, 1H), 6.17 (s, 1H), 6.23 (m, 1H), 6.92 (t, 1H, $J = 7.4$ Hz), 7.00 (t, 1H, $J = 7.4$ Hz), 7.10–7.43 (m, 12H), 7.66 (brs, 1H), 7.93 (brs, 1H), 8.82 (brs, 1H),

11.02 (brs, 1H). Found as a mixture of rotamers and a pair of diastereomers with individual resonances. MH⁺ HRMS (C₃₃H₄₀N₅O₄)⁺ calcd 570.3080, found 570.3053. Anal. (C₃₃H₃₉N₅O₄·1.1H₂O) C, H, N.

5-(L-Alanyl-L-alanyl-amino)-4-hydroxy-6-phenylhexanoic Acid ((1*H*-Benzimidazol-2-yl)phenylmethyl)amide (4). The compound was prepared from the amine 15 and *N*-*t*-BOC-L-Ala-L-alanine using the procedure described for compound 2 to give (96%) of the desired product as a clear oil as an inseparable mixture of diastereomers. IR (KBr): 3283, 1651, 1528, 1454, 1292, 1066, 700 cm⁻¹; ¹H NMR (DMSO, 300 MHz): δ 12.13 (s, 1H), 8.77 (m, 1H), 7.64 (m, 2H), 7.63–6.95 (m, 14H), 6.27 (m, 1H), 4.95 (m, 1H), 4.27 (m, 1H), 3.92 (m, 2H), 3.43 (m, 1H), 2.71 (m, 2H), 2.25 (m, 2H), 1.52 (m, 2H), 1.30 (d, 3H, *J* = 7.1 Hz), 1.06 (d, 3H, *J* = 7.1 Hz). HRMS: calcd for (C₃₂H₃₉N₅O₄)⁺ 571.3033, found FAB M⁺ 571.3009.

4-Hydroxy-5-((*N*-2-quinoloyl-L-asparagyl)amino)-6-phenylhexanoic Acid ((2-Indolyl)benzyl)amide (5). To a stirred solution of 0.076 g (0.18 mmol) of the alcohol 14 in 1 mL of DMF at 0 °C was added 0.077 g (0.21 mmol) of the acid 29. To this mixture was added 0.024 g (0.18 mmol) of HOBT followed by 0.034 g (0.18 mmol) of EDC·HCl. After 5 min, 0.12 mL (0.89 mmol) of TEA was added. The mixture was allowed to stir at 0 °C for 1 h and then for 16 h at room temperature. The reaction mixture was poured into H₂O and extracted with EtOAc. The combined organic layers were dried (MgSO₄), and the solvent was removed under reduced pressure. The crude residue was chromatographed on silica with MeOH/CH₂Cl₂ (3:97) to give 0.11 g (88%) of the desired product as an inseparable mixture of diastereomers as a white solid. Mp: 211–218 °C. IR (KBr): 3387, 3302, 1659, 1528, 1499, 1292, 750, 700 cm⁻¹. ¹H NMR (DMSO, 300 MHz) δ 10.99 (s, 1H), 9.04 (d, 1H, *J* = 7.4 Hz), 8.81 (d, 1H, *J* = 8.23 Hz), 8.51 (d, 1H, *J* = 7.68 Hz), 8.27–8.06 (m, 3H), 7.87–7.72 (m, 3H), 7.46–6.93 (m, 17H), 6.27 (d, 1H, *J* = 8.26 Hz), 6.09 (s, 1H), 4.92 (brs, 1H), 4.82 (brd, 1H, *J* = 6.94 Hz), 3.99 (brs, 1H), 3.52 (s, 1H), 2.79–2.51 (m, 4H), 2.49–2.26 (m, 2H), 1.64 (brs, 1H). Anal. (C₄₁H₄₀N₆O₅·H₂O) C, H, N.

4-Hydroxy-5-((*N*-2-quinoloyl-L-asparagyl)amino)-6-phenylhexanoic Acid (α-(2-Indolyl)-*m*-(trifluoromethyl)benzyl)amide (6). The compound was prepared using the method described for compound 5. White solid, inseparable mixture of diastereomers, 83% yield. Mp: 205–215 °C. IR (KBr): 3339, 1659, 1526, 1500, 1331, 1167, 1124, 775, 702 cm⁻¹. ¹H NMR (DMSO, 300 MHz) δ 11.09 (s, 1H), 9.20 (dd, 1H, *J* = 8.20 Hz), 8.93 (d, 1H, *J* = 8.3 Hz), 8.54 (d, 1H, *J* = 8.5 Hz), 8.19–8.07 (m, 3H), 7.87 (t, 1H, *J* = 7.33 Hz), 7.79–7.63 (m, 6H), 7.59–7.42 (m, 4H), 7.36–6.91 (m, 9H), 6.36 (d, 1H, *J* = 8.26 Hz), 6.06 (s, 1H), 4.92 (d, 1H, *J* = 3.91 Hz), 4.91 (s, 1H), 3.94 (brs, 1H), 3.44 (brs, 1H), 2.83–2.21 (m, 6H), 1.65–1.61 (m, 2H). Anal. (C₄₂H₃₉F₃N₆O₅) C, H, N.

4-Hydroxy-5-((*N*-2-quinoloyl-L-asparagyl)amino)-6-phenylhexanoic Acid (α-(2-indolyl)-*m*-methylbenzyl)amide (7). The compound was prepared using the method described for compound 5. White solid, inseparable mixture of diastereomers, 86.5% yield. Mp: 215–219 °C. IR (KBr): 3325, 1658, 1524, 1499, 1427, 1292, 748, 702 cm⁻¹; ¹H NMR (DMSO, 300 MHz) δ 10.90 (s, 1H), 9.25 (d, 1H, *J* = 8.1 Hz), 8.76 (d, 1H, *J* = 8.6 Hz), 8.54 (d, 1H, *J* = 8.43 Hz), 8.19–8.11 (m, 3H), 7.90–7.70 (m, 3H), 7.46–6.90 (m, 16H), 6.22 (d, 1H, *J* = 8.5 Hz), 6.19 (s, 1H), 4.91 (dd, 1H, *J* = 5.51 Hz), 4.85–4.78 (m, 1H), 3.94 (brd, 1H, *J* = 7.24 Hz), 3.45 (brs, 1H), 3.32 (s, 3H), 2.83–2.33 (m, 6H), 1.65–1.60 (m, 2H). Anal. (C₄₂H₄₂N₆O₅) C, H, N.

4-Hydroxy-5-((*N*-2-quinoloyl-L-asparagyl)amino)-6-phenylhexanoic Acid (α-(2-indolyl)cyclohexylmethyl)amide (8). The compound was prepared using the method described for compound 5. White solid, inseparable mixture of diastereomers, 86% yield. Mp: 220–227 °C. IR (KBr): 3326, 2929, 1657, 1524, 1499, 1427, 777, 700 cm⁻¹. ¹H NMR (DMSO, 300 MHz) δ 10.83 (s, 1H), 9.02 (d, 1H, *J* = 7.95 Hz), 8.54 (d, 1H, *J* = 7.83 Hz), 8.34–8.1 (m, 4H), 7.92–7.73 (m, 3H), 7.48–6.92 (m, 12H), 6.36 (s, 1H), 4.99–4.72 (m, 3H), 3.87 (brs, 1H), 3.45 (brs, 1H), 2.68–2.51 (m, 4H), 2.35–2.12 (m, 2H), 1.65 (brs, 1H), 1.56–1.46 (m, 5H), 1.33–0.86 (m, 5H). Anal. (C₄₁H₄₆N₆O₅) C, H, N.

4-Hydroxy-5-((*N*-2-quinoloyl-L-asparagyl)amino)-6-phenylhexanoic Acid (α-(2-benzimidazolyl)benzyl)amide (9). The compound was prepared using the method described for compound 5. White solid, inseparable mixture of diastereomers. Mp: 150–152 °C. 83% yield. IR (KBr): 3302, 1659, 1524, 1499, 1427, 1273, 744, 700 cm⁻¹. ¹H NMR (DMSO, 300 MHz) δ 12.46 (s, 1H), 9.04 (d, 1H, *J* = 8.42 Hz), 8.97 (d, 1H, *J* = 7.98 Hz), 8.56 (d, 1H, *J* = 8.02 Hz), 8.19–8.07 (m, 3H), 7.89–7.72 (m, 3H), 7.56–7.00 (m, 17H), 6.31 (d, 1H, *J* = 8.01 Hz), 4.92 (m, 1H), 4.72 (m, 1H), 3.89 (m, 1H), 3.5 (brs, 1H), 2.75–2.43 (m, 4H), 2.33–2.25 (m, 2H), 1.66 (brs, 2H). Anal. (C₄₀H₃₉N₇O₅) C, H, N.

2-Benzyl-4-hydroxy-5-((*N*-2-quinoloyl-L-asparagyl)amino)-6-phenylhexanoic Acid (α-(2-indolyl)benzyl)amide (10). This compound was prepared from the known 2-*R*-benzyl substituted derivative of lactone 11³¹ using a sequence identical to that used to prepare compound 5. White solid, inseparable mixture of diastereomers. Mp: 210–215 °C. 83% yield. IR (KBr): 3376, 1660, 1528, 1496, 1456, 749, 700 cm⁻¹. ¹H NMR (DMSO, 300 MHz) δ 10.96 (s, 1H), 8.97 (d, 1H, *J* = 7.53 Hz), 8.59 (d, 1H, *J* = 8.23 Hz), 8.45 (d, 1H, *J* = 7.52 Hz), 8.19–8.08 (m, 3H), 7.87–7.73 (m, 3H), 7.45–6.90 (m, 22H), 6.20–6.17 (m, 2H), 4.84–4.72 (m, 2H), 3.75 (brs, 1H), 3.52 (brs, 1H), 2.95–2.51 (m, 8H). Anal. (C₄₈H₄₆N₆O₅) C, H, N.

[(1*H*-Benzimidazol-2-yl)phenylmethyl]amine (30). To a stirring suspension of lithium hexamethylsilazane (0.093 mol) was added benzaldehyde (0.062 mol) at 0 °C under Ar. At the end of this dropwise addition a clean solution is observed. This was concentrated and the silyl imine is distilled under reduced pressure. The silyl imine (10.9 g, 0.062 mol) was added in 20 mL of dry THF to 0.031 mol of 2-lithio-*N*-MOM-benzimidazole (prepared by the dropwise addition of 0.034 mol of 1.6 M *n*-BuLi/Hex to *N*-MOM-benzimidazole (0.031 mol) in dry THF (50 mL) at –78 °C under argon) at –78 °C under Ar. This was warmed to –10 °C and stirred for 2 h. The reaction mixture was poured into H₂O, extracted 2× w/EtOAc, and concentrated to a yellow oil which was stirred in 30 mL of MeOH containing 10 mL of concentrated HCl. The resulting mixture was heated to reflux for 16 h. The acidic solution was cooled to room temperature, made basic with 6 N NaOH, and then extracted with EtOAc. The organics were dried (MgSO₄), filtered, and concentrated to give a 4.6 g (67%) of the desired amine as a white solid which was recrystallized from EtOAc/Hex. Mp: 189–191 °C. IR (KBr): 3045, 2879, 2750, 2679, 1427, 1271, 870, 754, 706, 621 cm⁻¹. ¹H NMR (DMSO, 300 MHz): δ 12.26 (brs, 1H), 7.52 (brs, 5H), 7.42–7.01 (m, 4H), 5.26 (s, 1H), 2.35 (brs, 2H). Anal. (C₁₄H₁₃N₃) C, H, N.

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