New Modified Heterocyclic Phenylalanine Derivatives. Incorporation into Potent Inhibitors of Human Renin[1,2]

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Modified heterocyclic phenylalanine analogues designed as replacements for the P3-P4 region were synthesized and incorporated into renin inhibitors. These inhibitors were found to have significant activity versus human recombinant renin, as well as in vivo activity. The compounds proved to be very resistant to chymotrypsin degradation, as exemplified by compound 8, which remained greater than 60% intact after a 24-h exposure to chymotrypsin. In contrast, the Boc-Phe analogue was nearly completely degraded after 1 h. Compound 6 proved to be the most potent renin inhibitor with an IC_{50} = 8.9 nM. These stable cyclized phenylalanines should prove to be generally useful as a substitute for Boc-Phe in protease inhibitors.

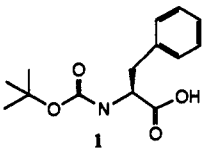
It is now well-established that the renin-angiotensin system is an appropriate target for antihypertensive therapy as evidenced by the clinical success of angiotensin converting enzyme (ACE) inhibitors. Renin inhibitors are attractive since they may provide advantages over ACE inhibitors due to their high specificity for a single substrate. Work has continued on the development of renin inhibitors for a number of years,[3] though no drug has yet successfully overcome the clinical barriers facing this set of compounds. Many of the difficulties encountered in the renin inhibitor field are well-known.[4-10] The molecules studied to this point have been beset by a number of problems including large molecular weight, a significant amount of peptidic character, metabolic instability, and poor water solubility. Generally, all of the above factors combine to result in very poor oral bioavailability of these inhibitors. While several of these issues have been successfully addressed, efforts continue to develop novel renin inhibitors that incorporate all of the features necessary to overcome the important problem of bioavailability.

tert-(Butyloxycarbonyl)-L-phenylalanine (Boc-Phe; 1) is a very effective P3-P4 substructural unit[11] for renin inhibitors, resulting in tight-binding inhibitors.[12] However, renin inhibitors containing Boc-Phe-X at the N-terminus (P2-P4 region) are highly unstable when exposed to chymotrypsin.[13] We have prepared heterocyclic phenylalanine derivatives (e.g. 2) and incorporated them into inhibitors which bind well to renin yet are specifically modified to prevent chymotrypsin degradation (e.g. 5). This has been accomplished by cyclizing about the nitrogen atom of phenylalanine, thus removing the critical proton of the NH of Phe which forms a hydrogen bond with the ^{214}Ser carbonyl of chymotrypsin (Figure 1).[14]

The use of these modified phenylalanines has enabled us to restrict the molecular weight, increase water solubility through the use of heteroatoms, and reduce the overall peptidic nature of the compounds. We have incorporated these stable phenylalanine derivatives into new inhibitors of renin, which have been tested for in vitro renin inhibition, chymotrypsin stability, and in vivo activity. The results of these studies, along with the modeling of one of these compounds in the active site of human renin, are presented herein.

Results

Chemistry. The choice for the P1 binding group was norstatine (or ACHBA), since extended P′ binding sites are not required[15,16] and the overall size of the inhibitor is thus reduced. Synthesis of the norstatine unit followed those of the literature.[17-19] Briefly, Boc-cyclohexylalanine



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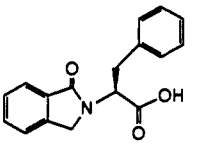

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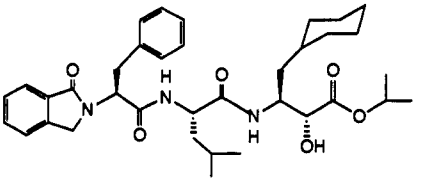

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was reduced to the aldehyde via the methoxymethyl amide, converted to the cyanohydrin, and hydrolyzed to the 2-

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(1) Portions of this work were presented in August 1990 at the 200th National Meeting of the American Chemical Society, see: *Abstract of Papers*; Ocain, T. D.; Deininger, D. D.; McCaully, R. J. A New Series of Renin Inhibitors Containing Heterocyclic P3-P4 Replacements. 200 National Meeting of the American Chemical Society: American Chemical Society: Washington, DC, 1990; MEDI 106.

(2) Abbreviations used follow the IUPAC-IUB commission on Biochemical Nomenclature recommendations. Additional abbreviations are as follows: ACHBA, norstatine, (3-amino-4-cyclohexyl-2-hydroxybutanoic acid); TMS, trimethylsilyl; THF, tetrahydrofuran; DIBAL-H, diisobutylaluminum hydride; Boc, *tert*-butyloxycarbonyl; TFA, trifluoroacetic acid; Sta, 4-amino-3-hydroxy-6-methylheptanoic acid; DMSO, dimethyl sulfoxide.

(3) Ocain, T. D.; Abou-Gharbia M. Renin-Angiotensin System (RAS) Dependent Antihypertensive Agents: I. Renin Inhibitors. *Drugs Future* **1991**, *16*, 37-51.

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(6) Kleinert, H. D. Renin Inhibitors: Discovery and Development. An Overview and Perspective. *Am. J. Hypertens.* **1989**, *2*, 800-808.

(7) Kokubu, T.; Hiwada, K. Human Renin Inhibitors. *Drugs Today* **1987**, *23*, 101-108.

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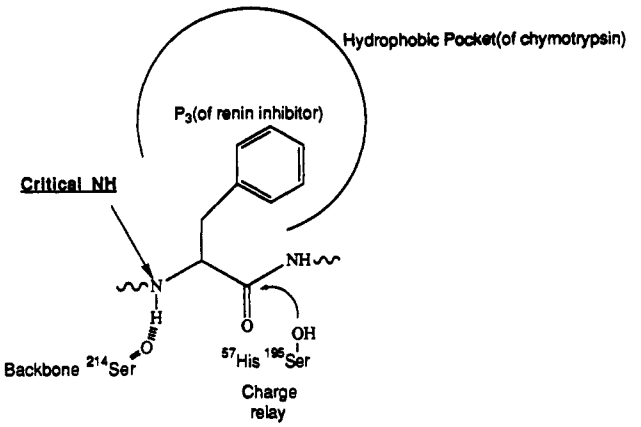
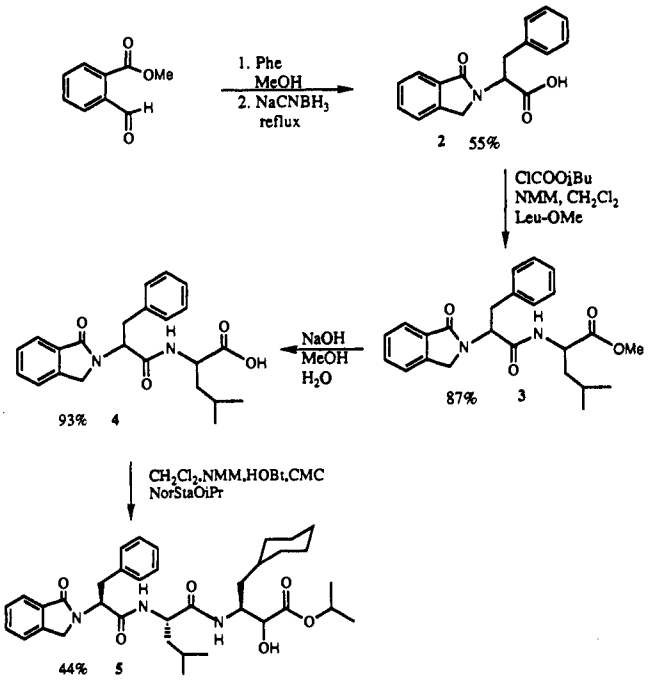


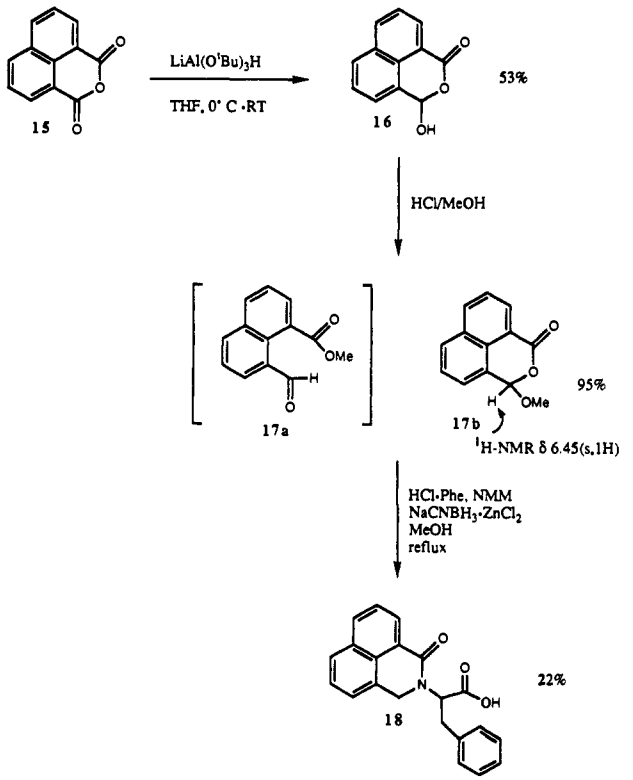
Figure 1. Chymotrypsin binding site.

Scheme I

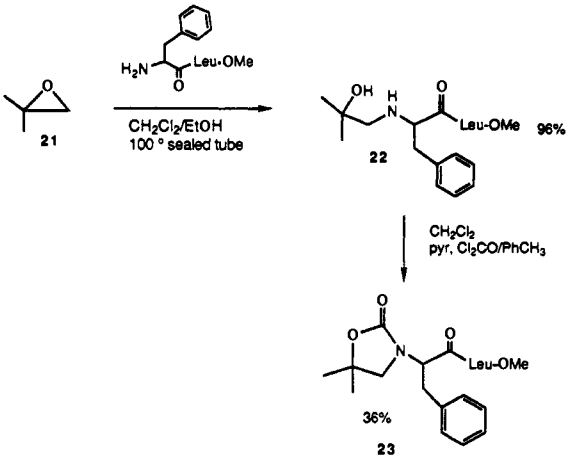


hydroxy-3-amino acid. A Boc group was introduced at the N-terminus and esters were generated either with TMS–diazomethane or 2-propanol–diethyl azodicarboxylate–triphenylphosphine. The stereochemistry of the norstatine

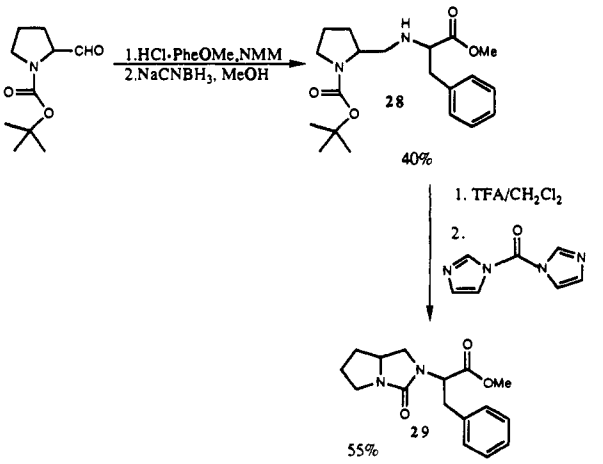
Scheme II



Scheme III



Scheme IV



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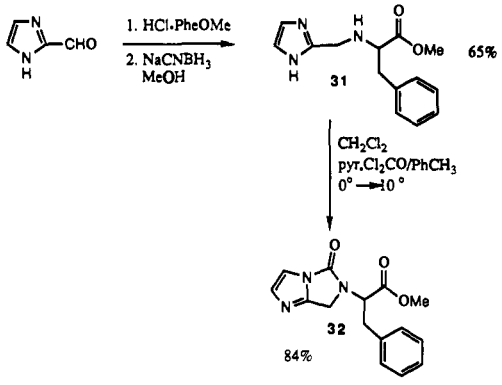
(12) Boger, J.; Lohr, N. S.; Ulm, E. H.; Poe, M.; Blaine, E. H.; Fanelli, G. M.; Lin, T.-U.; Payne, L. S.; Schorn, T. W.; LaMont, B. I.; Vassil, T. C.; Stabilito, I. I.; Veber, D. F.; Rich, D. H.; Bopari, A. S. Novel Renin Inhibitors Containing the Amino Acid Statine. *Nature* **1983**, *303*, 81–84.
(13) Rosenberg, S. H.; Plattner, J. J.; Woods, K. W.; Stein, H. H.; Marcotte, P. A.; Cohen, J.; Perun, T. J. Novel Renin Inhibitors Containing Analogues of Statine Retro-Inverted at the C-Termini: Specificity at the P_2 Histidine Site. *J. Med. Chem.* **1987**, *30*, 1224–1228.
(14) Blow, D. M. Structure and Mechanism of Chymotrypsin. *Acc. Chem. Res.* **1976**, *9*, 145–152.
(15) Iizuka, K.; Kamijo, T.; Kubota, T.; Akahane, K.; Umeyama, H.; Kiso, Y. New Human Renin Inhibitors Containing an Unnatural Amino Acid, Norstatine. *J. Med. Chem.* **1988**, *31*, 701–704.

Scheme V



residue was fixed at C-3 (3*S*) and was generally a mixture at the C-2 hydroxyl group in varying ratios. We found that the more abundant isomer was also the more active one, and we assume that to be the 2*R* isomer on the basis of literature precedence.[19] For example, compound **5** is a single diastereomer isolated in much greater abundance than the other diastereomer from the reaction mixture. It was shown to be more active (98% inhibition at 1 μM) than a 7:1 mixture of the less abundant isomer and **5** (65% inhibition at 1 μM). Norstatine esters enriched in the more active isomer were used in all of the syntheses and resulted in a variety of final diastereomeric ratios (see Table I and the Experimental Section).

The synthesis of one of the most active renin inhibitors incorporating a modified phenylalanine derivative is shown in Scheme I. Methyl 2-formylbenzoate and phenylalanine were reacted in methanol under reductive amination conditions with sodium cyanoborohydride, followed by heating in the same pot to effect the cyclization. The resulting phenylalanine derivative **2** could then be treated as an ordinary N-protected amino acid and thus coupled to give dipeptide **3**. Saponification of the ester gave free acid **4**, which was incorporated with norstatine isopropyl ester in the final inhibitor structure to yield **5**.

A number of modified phenylalanine derivatives were synthesized as shown in Schemes II–V. The benzisoquinolinyl analogue was prepared (Scheme II) starting from anhydride **15**, which was reduced with lithium tri-*tert*-butoxyaluminohydride in THF; DIBAL-H reduction in this case did not yield the desired product. When the resulting hemiacetal **16** was subjected to methanolysis, methylated hemiacetal **17b** was observed by NMR [δ 6.45 (s, 1 H)], not ester aldehyde **17a**. The hemiacetal then underwent a sluggish reductive amination and cyclization in the presence of $ZnCl_2$, to give the modified phenylalanine **18** in 22% yield (in the absence of $ZnCl_2$ no product was observed).

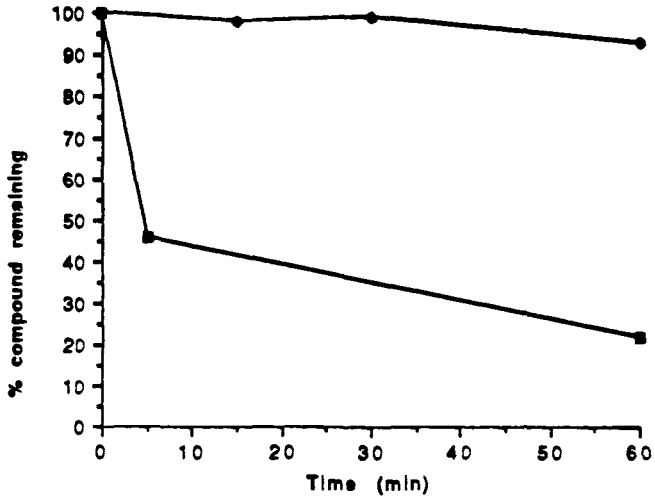


Figure 2. Chymotrypsin stability (**8**, ●; Boc-Phe-Leu-ACHBA-OMe, ■).

Since the Boc derivatives are very good inhibitors, we undertook the synthesis of a cyclic Boc-Phe analogue which is shown in Scheme III. Methylpropylene oxide **21** was heated in a sealed tube with the dipeptide to give amino alcohol **22** in very good yield. The compound was then cyclized with phosgene (in a toluene solution) to give cyclized Boc-phenylalanine **23**. The cyclized proline derivative was also made and is shown in Scheme IV. Boc-prolinal was subjected to reductive amination conditions to yield pseudodipeptide **28**. Removal of the Boc group with TFA and cyclization with 1,1′-carbonyldiimidazole gave the cyclized Pro-Phe analogue **29** in 55% yield. Finally, an imidazole analogue was synthesized (Scheme V) starting from imidazole-2-carboxaldehyde. Reductive amination gave amine **31**, which was subsequently cyclized with phosgene (in toluene) to give imidazolyl-Phe analogue **31** in 84% yield. Apparently epimerization occurred under these conditions since the fully elaborated compound **33** was determined to be mixture of four diastereomers, not two as would arise from the ACHBA group. All of the aforementioned modified phenylalanines were then elaborated to give the renin inhibitors shown in Table I.

In Vitro Results. The results of in vitro inhibition of the modified phenylalanine-containing inhibitors with recombinant human renin are shown in Table I. Isoindolones **5–9** were effective inhibitors in general, and the compound with norvaline substituted in the P2 position (**6**) proved to be the most active inhibitor synthesized with an IC_{50} of 8.9 nM. A comparison with the BocPhe derivative (IC_{50} = 8.0 nM) showed that the isoindolones had nearly the same in vitro potency. The introduction of an additional carbonyl group, as in phthalimido derivative **10**, resulted in a much less active compound. Pyridine analogue **12** possessed increased water solubility but was less active in the in vitro assay than the corresponding carbon analogue. The addition of an aromatic ring, as in **19**, was relatively well-tolerated as demonstrated by an IC_{50} of 80 nM for that compound. The cyclized Boc-Phe mimic **24** did not display outstanding inhibition. Better activity was seen with **30**, a proline mimic in the P4 position (IC_{50} = 141 nM). Finally, imidazole derivative **33** demonstrated good inhibition with an IC_{50} of 100 nM. The statine-containing inhibitor SCRIP in this assay gave an IC_{50} of 24 nM, consistent with the published value of 16 nM.[4]

Chymotrypsin Stability Results. Several of the inhibitors were tested for chymotrypsin stability at 22 °C and assayed by HPLC. Boc-Phe-Leu-ACHBA-OMe was severely degraded in this test while the modified pheny-

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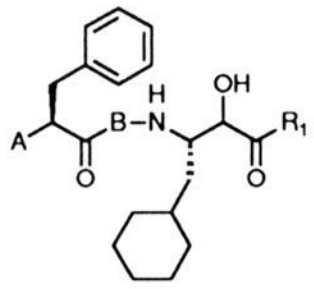
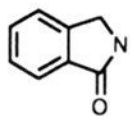
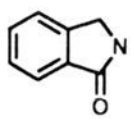
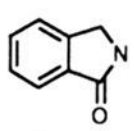
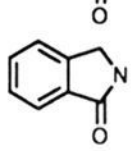
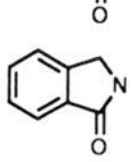
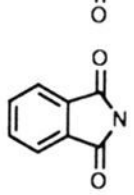
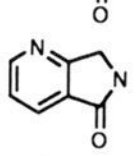
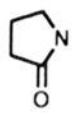
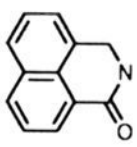
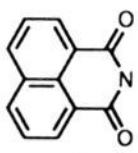
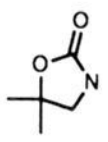
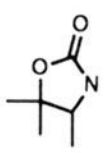
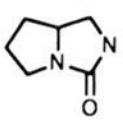
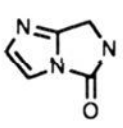
Table I. In vitro Renin Inhibition[a]



no.	A	B	R/S ratio[b]	R_1	IC_{50} (μM)
5		Leu	*h*	O^{i}Pr	0.036
6		Nva	4.8:1	O^{i}Pr	0.0089
7		Nle	15.7:1	O^{i}Pr	0.016
8		Leu	17:1	OMe	0.023
9		His	17:1	OMe	0.186
10		Leu	28:1	OMe	*c*
12		Leu	ND[d]	OMe	0.207
14		Leu	3.8:1	O^{i}Pr	0.157
19		Leu	4.3:1	O^{i}Pr	0.080
20		Leu	*h*	OMe	0.391
24		Leu	9:1	OMe	*e*
27		Leu	2:1	OMe	*f*
30		Leu	3.2:1	O^{i}Pr	0.141
33		Leu	g	O^{i}Pr	0.100
SCRIP	Boc-HN	Leu	24:1	OMe	0.008 0.024

[a] In vitro RIA assay measuring inhibition of human recombinant renin. [b] Ratio at C-2 hydroxyl group. [c] 45% inhibition at 1 μM. [d] ND = not determined. [e] 86.4% inhibition at 1 μM. [f] 76% inhibition at 1 μM. [g] Isolated as a mixture of four diastereomers in a ratio of 5:3:1:1. [h] Single diastereomer.

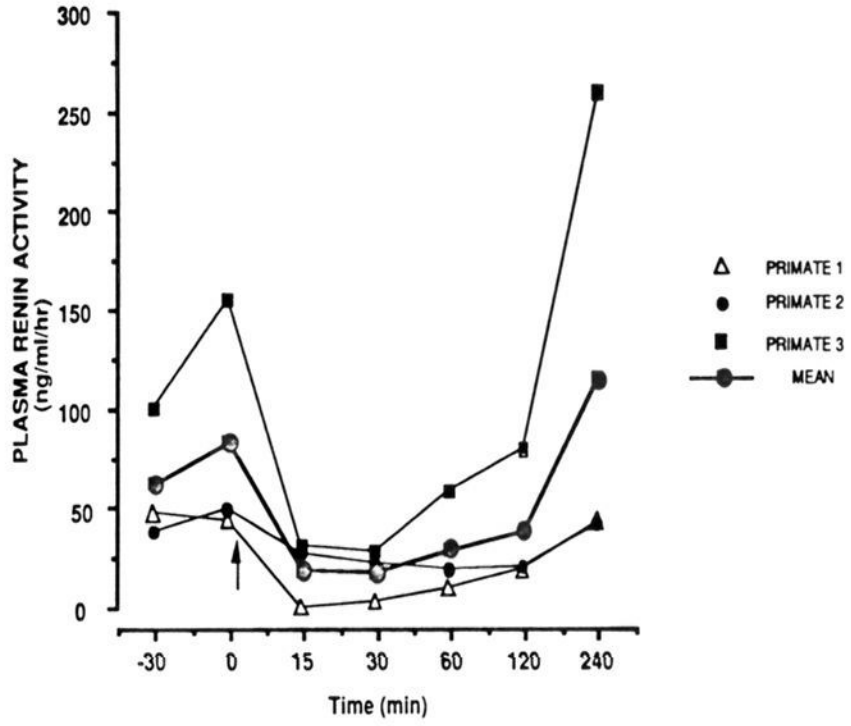


Figure 3. Effects of compound 8 (5 mg/kg iv) on PRA in conscious salt-depleted rhesus monkeys.

lalanines were very stable. This contrast is demonstrated in Figure 2, comparing the Boc derivative versus isoindolone **8**. Clearly **8** is the most stable compound since after 1 h the Boc compound is nearly completely degraded while the isoindolone remains greater than 90% intact; even after 24 h greater than 60% of **8** remained. In addition to compound **8**, greater than 90% of the original compound remained after a 1 h exposure to chymotrypsin for *all* of the following inhibitors: compounds **6**, **12**, **19**, **24**, and **33** (data not shown).

Effects on Plasma Renin Activity (PRA) and Blood Pressure. As shown in Figure 3, intravenous administration of compound **8** at 5 mg/kg to conscious, salt-depleted rhesus monkeys decreased baseline mean PRA by 75% within 15 min of drug administration. By 120 min PRA had returned to approximately 50% of baseline and by 240 min PRA had returned to baseline. Blood pressure was decreased by 10 mmHg at 30 min and rapidly returned to baseline. Oral administration (po) to four rhesus monkeys under similar conditions at 50 mg/kg led to a 75% decrease in PRA within 60 min of drug administration. PRA remained inhibited for the duration of the experiment and was still inhibited by 75% 24 h after drug administration. However, no effects on mean arterial pressure (MAP) nor heart rate (HR) were observed after po administration. Finally, oral administration of compound **6**, a slightly better enzyme inhibitor, at the much lower dose of 10 mg/kg, showed no effect on PRA, MAP, or HR (data not shown).

Molecular Modeling Studies

Recently, the X-ray crystal structure of recombinant human renin has been determined at 2.5-Å resolution.[20] We have carried out docking studies in order to understand how the compounds shown in Table I may interact with the active site of human renin.[21] A great deal of information has been obtained concerning the binding regions within the human renin structure by carrying out GRID

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(21) A more extensive analysis of the modeling with the renin structure will be presented elsewhere.

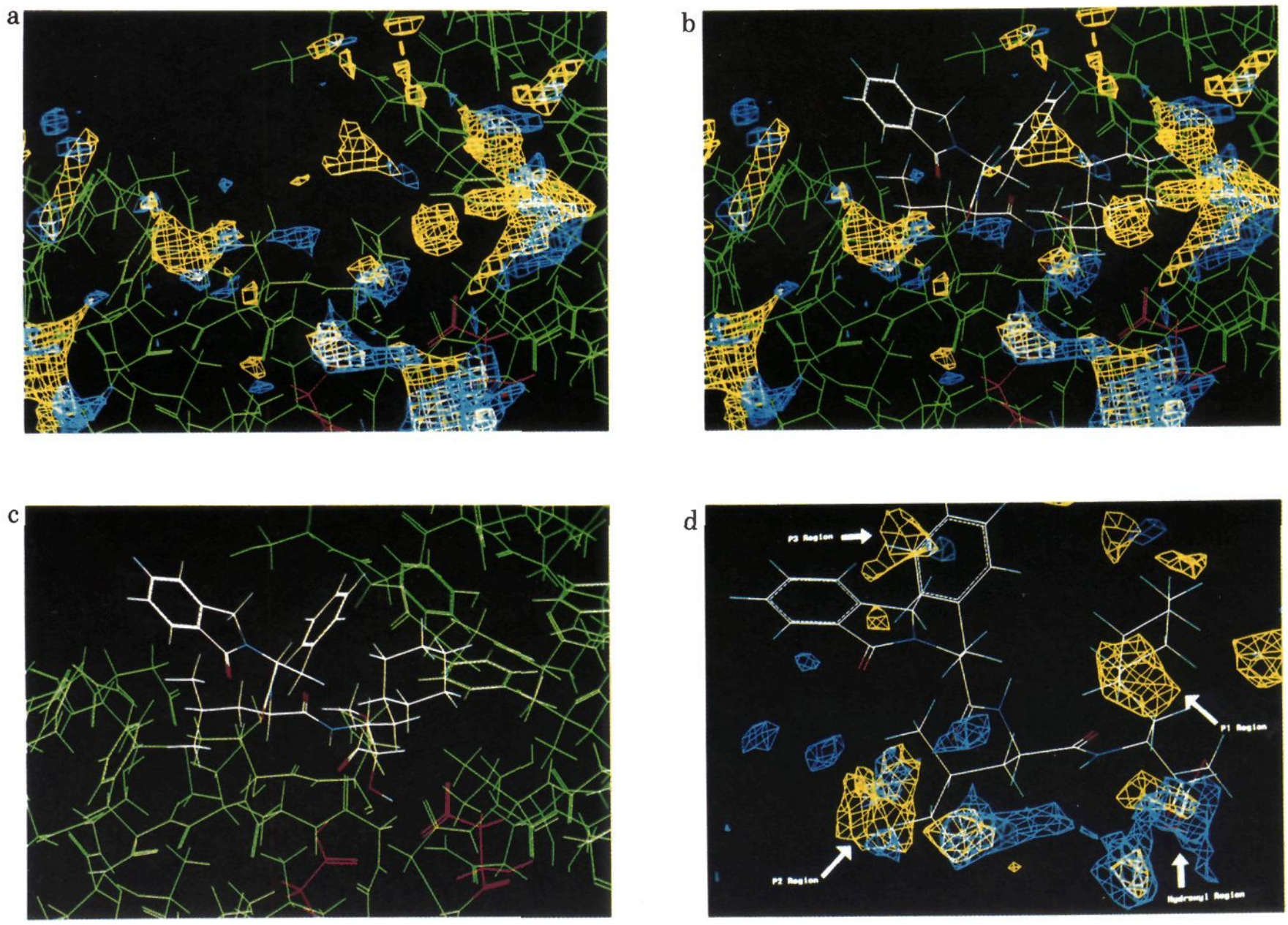

Figure 4. (a) Methyl (yellow) and hydroxyl (blue) grids calculated for the X-ray structure of human recombinant renin. The catalytic residues (Asp^{38} and Asp^{226}) are shown in red; (b) crystal structure of human renin with proposed binding orientation of compound 8 shown with methyl and hydroxyl grid regions; (c) Same as b without the grid regions displayed; (c) compound 8 fit to the methyl and hydroxyl grid regions calculated for the renin X-ray structure.

calculations[22] with a variety of probes. GRID contours were generated using methyl, hydroxyl, carbonyl oxygen, water, and cationic NH_2 probes. The renin crystal structure with the corresponding methyl and hydroxyl grids is shown in Figure 4a. Based on the results of these GRID calculations, we have proposed conformations and orientations of the inhibitors within the active site which maximize the overlap of the key functional groups within the molecule and the corresponding GRID regions within the active site.

To simplify the problem, we have used a crystal structure of a pepstatin fragment–penicillopepsin complex[23] as a template to provide a reasonable starting orientation of our inhibitors within renin. The X-ray coordinates for human renin[24] were overlapped with those of penicillopepsin by fitting both aspartic acid residues in the active site. Pepstatin was then extracted from the complex, inserted into the renin structure and, for example, transformed into compound 8. The newly formed side chains were rotated in order to better fit the nearby GRID regions, and the resulting complex was minimized.[25]

The final orientation of compound 8 in the active site of human renin is shown with (Figure 4b) and without (Figure 4c) the methyl and hydroxyl GRID contours. We found that compound 8 could adopt a low-energy conformation in which the cyclohexyl, isobutyl, and benzyl side chains overlap with methyl grids which may correspond with the S1, S2, and S3 sites, respectively (see Figure 4d). The norstatine hydroxyl group was found to fit nicely within a hydroxyl probe region only when one of the active site aspartic acids is protonated. When both of the aspartic acid residues are left unprotonated, the hydroxy group is displaced from the hydroxyl region by almost 1 Å.

Discussion

The problem of metabolism of renin inhibitors has received considerable attention, particularly the complications of chymotrypsin instability inherent in some of the earlier potent renin inhibitors. The most problematic compounds in that regard were those containing Boc-

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(23) James, M. N. B.; Sielecki, A.; Salituro, F.; Rich, D. H.; Hofmann, T. Conformational Flexibility in the Active Sites of Aspartyl Proteinases Revealed by a Pepstatin Fragment Binding to Penicillopepsin. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 6137–6141.

(24) The crystal structures of human renin and the penicillopepsin–pepstatin complex were obtained from Dr. M. James, University of Alberta.

(25) Molecular Graphics were displayed within the Sybyl Program (Tripos Associates, St. Louis, MO). Minimizations were carried out using the Amber force field as implemented within the Macromodel Program (Columbia University).

Phe-His- at the N-terminus. Modifications in the P2-P4 region of renin inhibitors by a number of workers have resulted in structures that bear little resemblance to phenylalanine, and thus chymotrypsin degradation is not a problem. However, the excellent binding affinity realized upon incorporation of Boc-Phe in the P3-P4 region[12] demonstrates the advantage of utilizing amino acid derivatives that more closely resemble phenylalanine but are modified to impart chymotrypsin resistance. Profound alterations that have been made to impart proteolytic stability to renin inhibitors include cyclization between the P2 and P4 regions,[26] resulting in potent inhibitors of human renin completely stable to chymotrypsin degradation. Utilizing a more general modification, Abbott workers found that replacement of Phe with OMe-Tyr resulted in effective chymotrypsin resistance.[13,27] Also, several groups[27,28] found that by replacing the α NH of Phe with oxygen or carbon, chymotrypsin recognition was again lost while renin recognition was retained. Apparently, a critical hydrogen bond for chymotrypsin binding is disrupted when the proton on the nitrogen is removed (see Figure 1).

We capitalized on the important interaction of the Phe NH with chymotrypsin and gained enzymatic resistance by cyclizing P4 structure onto the nitrogen of Phe to give structures such as **5**. These modified phenylalanine analogues contain the necessary hydrophobic recognition elements essential for renin binding, but do not bind well to chymotrypsin due to the disruption of binding between the critical NH and the ^{214}Ser carbonyl group. Incorporation of several structurally diverse cyclized phenylalanine derivatives in general yields renin inhibitors with a high degree of chymotrypsin stability. Utilizing this strategy, we were able to synthesize a cyclized Boc-Phe isostere **24** which, although not extremely potent when incorporated with Leu-Norsta-OMe, was very stable to chymotrypsin. This general modification also can be utilized to address the other problems common to renin inhibitors, such as water solubility. The pyridine analogue **12** and the imidazo analogue **33** both contain heteroatoms which result in enhanced polarity of the inhibitors (though still not freely water soluble), as demonstrated by HPLC. Unfortunately, the more polar compounds did not have the excellent binding characteristics of compounds such as **6**, which we have shown to have a high log P (4.86).[29]

Since we have removed the peptidic amide bond at P3-P4 and have utilized norstatine in the P1 position, we have reduced the overall peptidic nature of the molecules to two standard amide bonds and a single standard amino acid. It has been shown by others that incorporation of norleucine (Nle) and norvaline (Nva) at the P2 position can improve in vitro and in vivo activity of renin inhibitors.[30] We have confirmed this finding by substituting Nva and Nle at the P2 position, substitutions which yield the tightest binding compounds of the series (**6** and **7**). Unfortunately, the Nva substitution in **6** did not result in a significant increase in the in vivo activity of the compound. As mentioned earlier, the utilization of norstatine in the P1-P1′ position also greatly reduced the overall size of these renin inhibitors. Although more potent inhibition could likely be achieved utilizing tighter-binding C-terminal components (e.g. Sta-Phe-Leu-NH_2), the group of compounds reported here (e.g. **6**) include some of the lowest molecular weight renin inhibitors known to date (MW of **6** = 605).

In conclusion, we have synthesized modified analogues of phenylalanine which impart good activity to renin inhibitors and are very resistant to chymotrypsin degradation. Utilizing these new amino acid derivatives allows incorporation of many of the desired features of an optimal renin inhibitor, namely reduced peptidic character, metabolic stability, and water solubility. The general utility of these modified phenylalanines should be emphasized since, once prepared, they can be treated as protected amino acids and incorporated into many types of inhibitor molecules where chymotrypsin stability is required.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian VXR-400, a Bruker AM-400, or a Varian XL-200. Chemical shifts are reported in ppm from an internal tetramethylsilane standard ($CDCl_3$) or from an internal DMSO peak (DMSO-d_6). Fast atom bombardment mass spectra (FAB-MS) were recorded on a Kratos MS50TC. Elemental analyses were determined inhouse on a Perkin-Elmer elemental analyzer. Reverse-phase HPLC was carried out utilizing a Waters Novapak C_{18} or phenyl column. TLC was performed on 0.25 mm thickness silica gel plates. Flash chromatography was carried out with Merck silica gel (grade 60, 230–400 mesh). All reagents and solvents were HPLC, analytical, or reagent grade.

General Procedure A. Saponification of Esters with Sodium Hydroxide. The ester was dissolved in MeOH (2 mL/mmol) and 1 N NaOH (1.1 equiv) was added. The reaction was monitored by TLC and upon completion H_2O (2–5× by volume) was added to the reaction mixture and then most of the MeOH was removed in vacuo. The aqueous layer was washed with EtOAc, acidified with solid $KHSO_4$ (pH = 1–2), and extracted (2×) with EtOAc. The EtOAc layer was washed with brine, dried (anhydrous $MgSO_4$), and concentrated in vacuo.

General Procedure B. Removal of Boc Group with Trifluoroacetic Acid. The N-Boc amino acid or peptide was dissolved in CH_2Cl_2 (0.2 mM) and the solution was chilled to about 0 °C with an ice bath. Trifluoroacetic acid (10 equiv) was added to the solution; the solution was allowed to warm to room temperature and stirred until TLC showed no remaining starting material. The solution was then concentrated in vacuo. The resulting TFA salt could be used directly or converted to the free base by first adding 1 N HCl, washing with Et_2O, raising the pH with solid $NaHCO_3$, extracting with EtOAc, washing with brine, drying with $MgSO_4$, and concentration in vacuo.

General Procedure C. Coupling Reaction with 1-Cyclohexyl-3-(2-morpholinoethyl)carbodiimide Metho-*p*-toluenesulfonate (CMC). The free acid was dissolved in CH_2Cl_2 (2 mL/mmol) and cooled in an ice bath. The amine component (1 equiv) and HOBt (1.2 equiv) were added to the chilled solution, which was stirred for 10 min. A solution of CMC (1 equiv) in CH_2Cl_2 (2 mL/mmol) was added and the reaction mixture was allowed to gradually warm to room temperature and was stirred overnight. The solution was concentrated, taken up in EtOAc,

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and washed with H_2O, 1 N HCl, saturated $NaHCO_3$, and brine. It was then dried ($MgSO_4$), filtered, and concentrated in vacuo.

General Procedure D. Cyclization with Phosgene. The compound was dissolved in CH_2Cl_2 (0.2 mM) and chilled to about 0 °C with an ice bath. Pyridine (1.5 equiv) was added and then a solution of phosgene in toluene (1.3 equiv, 1.93 M solution) was added. The reaction was stirred about 1 h (held between 0 and 10 °C) and then quenched with saturated $NaHCO_3$. The aqueous layer was extracted with EtOAc (3×); the organic layers were combined and washed with 1 N HCl and brine, dried over $MgSO_4$, filtered, and concentrated.

(*S*)-2-(1-Oxo-1,3-dihydroisoindol-2-yl)-3-phenylpropionic Acid (2). General Procedure E. To a slurry of phenylalanine (23.06 g, 139.6 mmol) in MeOH (400 mL) was added methyl 2-formylbenzoate (27.5 g, 167.5 mmol) and after 5 min $NaCNBH_3$ (10.53 g, 167.5 mmol). After refluxing overnight the reaction mixture was partially concentrated. The reduced-volume reaction mixture was quenched with saturated $NaHCO_3$ (~1 L) and washed with Et_2O (2 × 500 mL). The aqueous layer was adjusted to pH 2.5 with solid $NaHSO_4$ and extracted into EtOAc (4 × 400 mL). The combined organic extracts were washed with brine (500 mL), dried over $MgSO_4$, filtered, concentrated, precipitated out of EtOAc with Et_2O, washed with Et_2O, and dried in vacuo to provide 20.57 g (55%) of the title compound as a white solid. ^1H NMR ($CDCl_3$, 200 MHz) δ 7.84 (d, 1 H, J = 7.04 Hz), 7.16–7.58 (m, 8 H), 5.17 (dd, 1 H, J = 10.94 Hz, 5.08 Hz), 4.19–4.40 (m, 2 H), 3.25–3.60 (m, 2 H); FAB-MS $(M + H)^+$ m/e 282.

(2*S*)-4-Methyl-2-[[(2*S*)-2-(1-oxo-1,3-dihydroisoindol-2-yl)-3-phenylpropionyl]amino]pentanoic Acid Methyl Ester (3). In flame-dried glassware under an N_2 atmosphere, acid **2** (0.33 g, 1.24 mmol) was dissolved in anhydrous EtOAc (10 mL), followed by the addition of *N*-methylmorpholine (0.14 mL, 1.24 mmol) at 0 °C. After further cooling to −10 °C, isobutyl chloroformate (0.16 mL, 1.24 mmol) was added. After 5 min leucine methyl ester hydrochloride (0.23 g, 1.24 mmol) was added and then the white slurry was basified with *N*-methylmorpholine (0.14 mL, 1.24 mmol, pH ≈ 7.5). The reaction mixture was warmed to room temperature and after 45 min quenched with saturated $NaHCO_3$ (~30 mL) and extracted into EtOAc (3 × 50 mL). The combined organic extracts were washed with saturated $NaHCO_3$ (1 × 50 mL), 1 N HCl (2 × 50 mL), and brine (1 × 50 mL), dried over $MgSO_4$, filtered, concentrated, and flash chromatographed (10–20% EtOAc/CH_2Cl_2 gradient) to provide 0.44 g (87%) of the title compound as a white foam: ^1H NMR ($CDCl_3$, 200 MHz) δ 7.83 (d, 1 H, J = 7.03 Hz), 7.39–7.54 (m, 3 H), 7.23 (m, 5 H), 6.68 (d, 1 H, J = 8.29 Hz), 5.15 (dd, 1 H, J = 9.60 Hz, 7.03 Hz), 4.50 (m, 1 H), 4.43 (s, 2 H), 3.67 (s, 3 H), 3.34 (m, 2 H), 1.5 (m, 3 H), 0.75 (d, 6 H, J = 6.86 Hz); FAB-MS $(M + H)^+$ m/e 409.

(2*S*)-4-Methyl-2-[[(2*S*)-2-(1-oxo-1,3-dihydroisoindol-2-yl)-3-phenylpropionyl]amino]pentanoic Acid (4). A solution of ester **3** (0.43 g, 1.06 mmol) was subjected to general procedure A to provide 0.39 g (93.4%) of the acid as a white solid: mp 136–140 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.80 (d, 1 H, J = 6.95 Hz), 7.41–7.58 (m, 3 H), 7.14–7.23 (m, 5 H), 5.32 (q, 1 H, J = 8.60 Hz), 4.56 (m, 1 H), 4.50 (s, 2 H), 3.31 (m, 2 H), 1.56 (m, 3 H), 0.78 (d, 6 H, J = 5.39 Hz); FAB-MS $(M + H)^+$ m/e 395. Anal. ($C_{23}H_{26}N_2O_4 \cdot {}^1/_4H_2O$) C, H, N.

(3*S*)-4-Cyclohexyl-2-hydroxy-3-[[(2*S*)-4-methyl-2-[[(2*S*)-2-(1-oxo-1,3-dihydroisoindol-2-yl)-3-phenylpropionyl]amino]pentanoyl]amino]butyric Acid Isopropyl Ester (5). A solution of Boc-ACHBA-O^{i}Pr[16] was deprotected according to general procedure B to provide 0.11 g (100%) of the TFA salt as a foam: ^1H NMR ($CDCl_3$, 200 MHz) δ 7.7 (bs, 2 H), 5.1 (m, 1 H), 4.2–5.05 (2 H), 3.81 (1 H), 0.8–1.8 (19 H); FAB-MS $(M + H)^+$ m/e 244 (free base).

The amine salt (0.11 g, 0.29 mmol) was coupled to acid **4** (0.123 g, 31 mmol) according to general procedure C and then flash chromatographed first in 25–35% EtOAc/CH_2Cl_2 then 45% EtOAc/hexane to provide two diastereomers, A (**5**, 0.067 g) and B (0.012 g) (combined 43.6%), as foams (A = 100%, B = 1:7 A/B mixture by HPLC).

Diastereomer A (**5**): ^1H NMR ($CDCl_3$, 400 MHz) δ 7.83 (d, 1 H, J = 7.5 Hz), 7.38–7.57 (m, 3 H), 7.17–7.25 (m, 5 H), 6.80 (d, 1 H, J = 7.73 Hz), 6.12 (d, 1 H, J = 9.70 Hz), 4.96–5.19 (m, 2 H), 4.30–4.45 (m, 3 H), 4.21 (m, 1 H), 4.05 (dd, 1 H, J = 4.8 Hz, 1.9 Hz), 3.28–3.43 (m, 2 H), 3.19 (d, 1 H, J = 4.8 Hz), 1.82 (d, 1 H, J = 2.26 Hz), 1.28–1.69 (m, 10 H), 1.26 (d, 3 H, J = 2.3 Hz), 1.24 (d, 3 H, J = 2.3 Hz), 0.83–1.21 (m, 5 H), 0.76 (d, 3 H, J = 6.5 Hz), 0.71 (d, 3 H, J = 6.5 Hz); FAB-MS $(M + H)^+$ m/e 620. Anal. ($C_{36}H_{49}N_3O_6 \cdot {}^1/_2H_2O$) C, H, N.

Diastereomer B (12.5% diastereomer A): ^1H NMR ($CDCl_3$, 400 MHz) δ 7.83 (d, 1 H, J = 7.16 Hz), 7.24–7.56 (m, 3 H), 7.16–7.25 (m, 5 H), 6.98 (d, 1 H, J = 7.03 Hz), 6.22 (d, 1 H, J = 9.36 Hz), 5.07 (m, 2 H), 4.24–4.44 (m, 3 H), 4.16 (d, 1 H, J = 7.16 Hz), 3.30–3.44 (m, 2 H), 1.82 (d, 1 H, J = 2.30 Hz), 0.70–1.70 (m, 21 H) [includes 1.27 (d, 3 H, J = 4.3 Hz), 1.25 (d, 3 H, J = 4.2 Hz), 0.80 (d, 3 H, J = 6.5 Hz), and 0.73 (d, 3 H, J = 6.5 Hz)]; FAB-MS $(M + H)^+$ m/e 620.

(3*S*)-4-Cyclohexyl-2-hydroxy-3-[[(2*S*)-2-[[(2*S*)-2-(1-oxo-1,3-dihydroisoindol-2-yl)-3-phenylpropionyl]amino]pentanoyl]amino]butyric Acid Isopropyl Ester (6). Boc-Nva-ACHBA-O^{i}Pr (0.36 mmol) was deprotected by utilizing general procedure B to yield the TFA salt [FAB-MS $(M + H)^+$ m/e 343 (free base)] which was coupled to acid **2** by utilizing general procedure C to yield, after flash chromatography (1:10:10 v/v/v MeOH/Et_2O/CH_2Cl_2), 0.15 g (71%) of the title compound as a white solid. HPLC indicated a diastereomeric ratio of 4.8:1: ^1H NMR ($CDCl_3$, 200 MHz) δ 7.83 (d, 1 H, J = 7.83 Hz), 7.38–7.57 (m, 2 H), 7.39 (d, 1 H, J = 7.5 Hz), 7.16–7.31 (m, 5 H), 6.89 and 7.09 (2 d, 1 H, J = 7.4 Hz), 6.07 and 6.18 (2 d, 1 H, J = 9.6 Hz), 4.98–5.08 (m, 2 H), 4.34–4.45 (m, 3 H), 4.17 (m, 1 H), 4.06 (bs, 1 H), 3.20–3.43 (m, 3 H), 0.75–1.84 (m, 26 H); FAB-MS $(M + H)^+$ m/e 606. Anal. ($C_{35}H_{47}N_3O_6$) C, H, N.

(3*S*)-4-Cyclohexyl-2-hydroxy-3-[[(2*S*)-2-[[(2*S*)-2-(1-oxo-1,3-dihydroisoindol-2-yl)-3-phenylpropionyl]amino]hexanoyl]amino]butyric Acid Isopropyl Ester (7). Boc-Nle-ACHBA-O^{i}Pr (0.32 mmol) was deprotected by utilizing general procedure B to yield the TFA salt [FAB-MS $(M + H)^+$ m/e 357 (free base)] which was coupled directly to acid **2** by utilizing general procedure C to yield, after flash chromatography (1:20:140 v/v/v MeOH/Et_2O/CH_2Cl_2), 0.084 g (42%) of the title compound as a white solid. HPLC indicates a diastereomeric ratio of 15.7:1: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.83 (dd, 1 H, J = 7.5 Hz, 0.9 Hz), 7.17–7.53 (m, 8 H), 6.83 (d, 1 H, J = 7.68 Hz), 6.04 (d, 1 H, J = 9.34 Hz), 4.98–5.09 (m, 2 H), 4.38–4.45 (m, 3 H), 4.16 (m, 1 H), 4.04 (m, 1 H), 3.08–3.40 (m, 3 H), 0.64–1.86 (m, 28 H), 0.66 (t, 3 H, J = 6.84 Hz); FAB-MS $(M + H)^+$ m/e 620; Anal. ($C_{36}H_{49}N_3O_6 \cdot {}^1/_2H_2O$) C, H, N.

(3*S*)-4-Cyclohexyl-2-hydroxy-3-[[(2*S*)-4-methyl-2-[[(2*S*)-2-(1-oxo-1,3-dihydroisoindol-2-yl)-3-phenylpropionyl]amino]pentanoyl]amino]butyric Acid Methyl Ester (8). Boc-Phe-Leu-ACHBA-OMe was deprotected according to general procedure B to provide 0.097 g (>100%) of the TFA salt: ^1H NMR ($CDCl_3$, 200 MHz) δ 7.68 (bs, 1 H), 7.08–7.38 (m, 6 H), 4.23–4.39 (m, 3 H), 3.68 (s, 3 H), 3.23 (m, 2 H), 0.79–1.68 (m, 22 H).

The crude amine salt (1.52 g, 2.58 mmol) was reacted with methyl 2-formylbenzoate according to procedure E and flash chromatographed (1% MeOH/$CHCl_3$) to provide 0.657 g (58%) of the title compound as a white solid. HPLC indicated a diastereomeric ratio of 17:1: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.80 (d, 1 H, J = 7.85 Hz), 7.16–7.56 (m, 8 H), 6.79 and 6.98 (2 d, 1 H, J = 7.06 Hz), 6.14 and 6.25 (2 d, 1 H, J = 9.25 Hz), 5.05 (m, 1 H), 4.12–4.58 (m, 4 H), 3.72 and 3.74 (2 s, 3 H), 3.24–3.46 (m, 3 H), 0.71–1.82 (m, 22 H); FAB-MS $(M + H)^+$ m/e 592. Anal. ($C_{34}H_{45}N_3O_6$) C, H; N: calcd 7.10; found, 6.69.

(3*S*)-4-Cyclohexyl-2-hydroxy-3-[[(2*S*)-3-(1*H*-imidazol-4-yl)-2-[[(2*S*)-2-(1-oxo-1,3-dihydroisoindol-2-yl)-3-phenylpropionyl]amino]propionyl]amino]butyric Acid Methyl Ester (9). Boc-(Ts)-His-ACHBA-OMe was deprotected according to general procedure B to give the TFA salt (>100%, no Boc group by 200-MHz NMR), which was coupled directly to *N*-(1-oxo-2*H*-isoindol-2-yl)-L-phenylalanine (**2**, 0.11 g, 0.39 mmol) by utilizing general procedure C. Some detosylation occurred in the coupling reaction. Column chromatography (8% MeOH/$CHCl_3$) of the crude material gave 0.040 g (21%) of the title detosylated final product [an additional 0.2 mmol (67%) of tosylated product was also recovered]: ^1H NMR ($CDCl_3$, 400 MHz) δ 6.68–7.92 (m, 11 H), 6.34 (d, 1 H, J = 8.7 Hz), 5.04–5.16 (m, 1 H), 4.24–4.72 (m, 5 H), 3.76 and 3.71 (2 s, 3 H), 3.48–3.68 (m, 2 H), 2.82–3.36 (m, 3 H), 0.75–1.80 (m, 13 H); FAB-MS $(M + H)^+$ m/e 616. Anal. ($C_{34}H_{41}N_5O_6 \cdot 1{}^3/_4H_2O$) C, N; H: calcd, 6.93; found, 6.26.

(3*S*)-4-Cyclohexyl-3-[[(2*S*)-2-[[(2*S*)-2-(1,3-dioxo-1,3-dihydroisoindol-2-yl)-3-phenylpropionyl]amino]-4-methylpentanoyl]amino]-2-hydroxybutyric Acid Methyl Ester (10). The title compound was prepared from Pht-Phe (0.028 g, 95 μmol) and TFA-Leu-ACHBA-OCH_3 (81.7 μmol) by utilizing general procedure C. Column chromatography (2% MeOH/$CHCl_3$) yielded 0.036 g (73%) of pure compound with a diastereomeric ratio of 28:1: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.69–7.82 (m, 4 H, aromatic), 7.11–7.23 (m, 5 H, aromatic), 6.51 and 6.44 (2 d, 1 H, J = 7.7 Hz), 6.27 and 6.15 (2 d, 1 H, J = 9.3 Hz), 5.13 (dd, 2 H, J = 9.3 Hz, 7.2 Hz), 4.12–4.43 (m, 3 H), 3.72 (s, 3 H), 3.26–3.54 (m, 2 H), 0.90–1.82 (m, 22 H); FAB-MS (M + H)$^+$ m/e 606. Anal. ($C_{34}H_{43}N_3O_7 \cdot 1.5H_2O$) C, H, N.

(2*S*)-4-Methyl-2-[[(2*S*)-2-(5-oxo-5,7-dihydropyrrolo[3,4-*b*]pyridin-6-yl)-3-phenylpropionyl]amino]pentanoic Acid (11). TFA-Phe-Leu-OMe (0.188 g, 0.392 mmol) was reacted with ethyl 2-formyl-3-pyridinecarboxylate (0.070 g, 0.392 mmol) according to general procedure E and flash chromatographed (20% EtOAc/CH_2Cl_2) to provide 0.062 g (38%) of the title compound as a foam: ^1H NMR ($CDCl_3$, 200 MHz) δ 8.71 (d, 1 H, J = 5.21 Hz), 8.07 (d, 1 H, J = 7.98 Hz), 7.14–7.37 (m, 6 H), 6.75 (d, 1 H, J = 7.81 Hz), 5.26 (dd, 1 H, J = 8.51 Hz, 7.51 Hz), 4.56 (m, 3 H), 3.68 (s, 3 H), 3.18–3.50 (m, 2 H), 1.51 (m, 3 H), 0.79 (d, 6 H, J = 6.34 Hz).

(3*S*)-4-Cyclohexyl-2-hydroxy-3-[[(2*S*)-4-methyl-2-[[(2*S*)-2-(5-oxo-5,7-dihydropyrrolo[3,4-*b*]pyridin-6-yl)-3-phenylpropionyl]amino]pentanoyl]amino]butyric Acid Methyl Ester (12). A solution of methyl ester 11 (0.062 g, 0.151 mmol) was deprotected according to general procedure A to provide 0.056 g (91%) of acid as a white solid [FAB-MS (M + H)$^+$ m/e 396]. The acid (0.054 g, 0.136 mmol) was coupled to TFA-Leu-ACHBA-OMe according to general procedure C and flash chromatographed (1:10:89 MeOH/EtOAc/CH_2Cl_2) to provide 0.045 g (55%) of the title compound as a foam: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.70 (dd, 1 H, J = 4.92 Hz, 1.30 Hz), 8.08 (dd, 1 H), 7.37 (m, 1 H), 7.15–7.25 (m, 5 H), 6.61 and 6.82 (2 d, 1 H, J = 7.84 Hz), 6.07 and 6.18 (2 d, 1 H, J = 9.42 Hz), 5.11 (m, 1 H), 4.35–4.52 (m, 2 H), 4.15–4.30 (m, 1 H), 4.22 (m, 1 H), 4.12 (dd, 1 H), 3.72 and 3.75 (2 s, 3 H), 3.22–3.55 (m, 3 H), 0.73–1.80 (m, 22 H); FAB-MS (M + H)$^+$ m/e 593. Anal. ($C_{33}H_{44}N_4O_6 \cdot H_2O$) C, H, N.

(*S*)-2-(2-Oxopyrrolidin-1-yl)-3-phenylpropionic Acid Methyl Ester (13). To a slurry of L-phenylalanine methyl ester hydrochloride (4.64 mmol) in CH_3CN (10 mL) was added K_2CO_3 (16.2 mmol) and ethyl 4-bromobutyrate (0.73 mL, 5.1 mmol). The reaction mixture was stirred overnight at room temperature. Another 0.2 mL (1.4 mmol) of bromide was added, and the reaction mixture was stirred for 3 days at 50 °C and then quenched with H_2O. The aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were then extracted with 0.5 N HCl (3 × 50 mL). The pH of the aqueous layer was raised to ~8 with solid $NaHCO_3$, and this layer was then extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, concentrated, and flash chromatographed (5–10% Et_2O/CH_2Cl_2) to yield 0.577 g of amino diester as a pale yellow oil [FAB-MS (M + H)$^+$ m/e 294]. This compound was dissolved in toluene (10 mL), refluxed for 46 h, diluted with Et_2O, washed with 0.5 N HCl and brine, dried over $MgSO_4$, filtered, and concentrated to provide 0.322 g (75%) of the title compound as a pale yellow oil: ^1H NMR ($CDCl_3$, 200 MHz) δ 7.25 (m, 5 H), 5.10 (dd, 1 H, J = 10.05 Hz, 5.38 Hz), 3.72 (s, 3 H), 3.29–3.45 (m, 3 H), 2.99 (dd, 1 H, J = 14.37, 10.46 Hz), 1.85–2.35 (m, 4 H); FAB-MS (M + H)$^+$ m/e 284.

(3*S*)-4-Cyclohexyl-2-hydroxy-3-[[(2*S*)-4-methyl-2-[[(2*S*)-2-(2-oxopyrrolidin-1-yl)-3-phenylpropionyl]amino]pentanoyl]amino]butyric Acid Isopropyl Ester (14). Ester 13 (1.3 mmol) was deprotected by utilizing general procedure A to yield 0.286 g of the acid, which was directly coupled to TFA-Leu-ACHBA-O^{i}Pr (0.36 mmol) by utilizing general procedure C to yield, after flash chromatography (1:20:80 v/v/v MeOH/EtOAc/CH_2Cl_2), 0.16 g (50%) of the title compound as a white solid. HPLC indicated a diastereomeric ratio of 3.8:1: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.20–7.34 (m, 5 H), 6.62 and 6.82 (2 d, 1 H, J = 7.45 Hz), 6.17 and 6.26 (2 d, 1 H, J = 9.67 Hz), 5.03–5.10 (m, 1 H), 4.71–4.84 (m, 1 H), 4.18–4.48 (m, 2 H), 4.07 (bs, 1 H), 3.09–3.44 (m, 3 H), 2.31 (m, 2 H), 1.90 (m, 2 H), 0.84–1.74 (m, 30 H); FAB-MS (M + H)$^+$ m/e 572. Anal. ($C_{32}H_{49}N_3O_6 \cdot {}^1/_2H_2O$) C, H, N.

8-Formyl-1-naphthoic Acid (16). To a slurry of 1,8-naphthalic anhydride (15.1 mmol) in anhydrous THF at 0 °C was added 3.85 g (15.1 mmol) of lithium tri-*tert*-butoxyaluminohydride. The reaction mixture was allowed to warm to room temperature and stirred for 1 h, at which time an additional 0.25 g of the hydride reagent was added. The reaction mixture was stirred an additional 0.5 h, quenched with 2 N HCl, and extracted into CH_2Cl_2. The organic layer was back-extracted with 1 N NaOH and reacidified with 2 N HCl. The aqueous layer was extracted with CH_2Cl_2 (with 5% MeOH), washed with brine, dried ($MgSO_4$), and concentrated to one-third volume, at which time a white solid began to precipitate which was collected by filtration. The filtrate was concentrated to dryness, reslurried in CH_2Cl_2, and filtered. The two crops were combined to provide 1.62 g (53%) of product as a white solid: mp 170–172 °C; ^1H NMR (CD_3OD, 200 MHz) δ 8.38 (d, 1 H, J = 7.6 Hz), 8.26 (dd, 1 H, J = 8.7 Hz, 1.4 Hz), 8.05 (dd, 1 H, J = 7.5 Hz, 1.9 Hz), 7.72 (m, 3 H), 6.86 (s, 1 H), 5.49 (s, 1 H); EI-MS m/e 200.

Methyl 8-Formyl-1-naphthoate (17). To a solution of 16 (2.5 mmol) in MeOH was added 2 mL of HCl/MeOH (pH = 1; exact concentration unknown). The reaction mixture was stirred at room temperature overnight, quenched with saturated $NaHCO_3$, and extracted into CH_2Cl_2. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated to yield 0.51 g (95%) of product as a white solid: mp 106–108 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.43 (dd, 1 H, J = 7.5 Hz, 1.4 Hz), 8.12 (dd, 1 H, J = 8.21 Hz, 1.4 Hz), 7.92 (m, 1 H), 7.62 (m, 3 H), 6.45 (s, 1 H), 3.72 (s, 3 H); EI-MS m/e 214.

(2*S*)-2-(1-Oxo-2,3-dihydro-1*H*-benz[*de*]isoquinolin-2-yl)-3-phenylpropionic Acid (18). The title compound was prepared from L-phenylalanine and compound 17 (2.37 mmol) by utilizing a modification of general procedure E where a complex, $ZnCl_2$–$NaCNBH_3$ (1:10), was used in the reaction mixture. Flash chromatography (1:10:80 v/v/v $CH_3CO_2H/Et_2O/CH_2Cl_2$) provided pure product (0.170 g) in 22% yield: ^1H NMR ($CDCl_3$, 200 MHz) δ 8.32 (d, 1 H, J = 7.5 Hz), 7.96 (d, 1 H, J = 8.2 Hz), 7.76 (d, 1 H, J = 7.2 Hz), 7.54 (t, 1 H, J = 7.5 Hz), 7.45 (t, 1 H, J = 7.5 Hz), 7.21 (m, 6 H), 4.57–5.09 (m, 3 H), 3.53 (m, 2 H); EI-MS m/e 331.

(3*S*)-4-Cyclohexyl-2-hydroxy-3-[[(2*S*)-4-methyl-2-[[(2*S*)-2-(1-oxo-2,3-dihydro-1*H*-benz[*de*]isoquinolin-2-yl)-3-phenylpropionyl]amino]pentanoyl]amino]butyric Acid Isopropyl Ester (19). Compound 18 (0.51 mmol) was coupled to TFA-Leu-ACHBA-O^{i}Pr by utilizing general procedure C to yield, after flash chromatography (35% EtOAc/hexane), 0.218 g (63%) of the title compound as an off-white solid. HPLC indicated a diastereomeric ratio of 4.25:1: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.59 and 8.29 (2 d, 3 H, J = 7.5 Hz), 8.23 and 7.95 (2 d, 1 H, J = 8.1 Hz), 7.75 (d, 1 H, J = 8.1 Hz), 7.57 (t, 1 H, J = 8.51 Hz), 7.47 (t, 1 H, J = 7.93 Hz), 7.13–7.36 (m, 6 H), 6.70 and 6.86 (2 d, 1 H, J = 8.1 Hz), 6.27 and 6.38 (2 d, 1 H, J = 8.5 Hz), 5.50 (m, 1 H), 4.82–5.09 (m, 3 H), 4.15–4.45 (m, 3 H), 4.04 (d, 1 H, J = 1.8 Hz), 3.28–3.50 (m, 2 H), 0.60–1.84 (m, 26 H). FAB-MS (M + H)$^+$ m/e 670. Anal. ($C_{40}H_{51}N_3O_6 \cdot {}^3/_4H_2O$) C, H, N.

(3*S*)-4-Cyclohexyl-3-[[(2*S*)-2-[[(2*S*)-2-(1,3-dioxo-2,3-dihydro-1*H*-benz[*de*]isoquinolin-2-yl)-3-phenylpropionyl]amino]-4-methylpentanoyl]amino]-2-hydroxybutyric Acid Methyl Ester (20). A solution of Boc-Phe-Leu-ACHBA-OMe (0.318 g, 0.553 mmol) was deprotected via general procedure B to provide 0.219 g (83.4%) of the free amine as a white foam [FAB-MS (M + H)$^+$ m/e 476], which was used directly in the next reaction.

To a solution of the above amine (0.08 g, 0.17 mmol) in toluene (5 mL) was added 1,8-naphthalic anhydride (0.03 g, 0.17 mmol). After 2 h at reflux, $MgSO_4$ (0.050 g) was added. After refluxing overnight the reaction was cooled to room temperature, quenched with 1 N HCl (25 mL), and extracted into CH_2Cl_2 (3 × 30 mL). The combined extracts were washed in 1 N HCl (1 × 30 mL), saturated $NaHCO_3$ (2 × 30 mL), and saturated NaCl (1 × 30 mL), dried over $MgSO_4$, filtered, concentrated, and flash chromatographed (20–30% EtOAc/CH_2Cl_2 gradient) to provide 0.03 g (28%) of the title compound as a hard foam: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.60 (d, 2 H, J = 7.26 Hz), 8.24 (d, 1 H, J = 7.83 Hz), 7.77 (t, 2 H, J = 7.85 Hz), 7.20–7.35 (m, 5 H), 6.73 (d, 1 H, J =

9.23 Hz), 6.09 (dd, 1 H, J = 8.60 Hz, 6.99 Hz), 5.94 (d, 1 H, J = 7.38 Hz), 4.45 (m, 1 H), 4.29 (m, 1 H), 4.17 (dd, 1 H, J = 8.27, 2.05 Hz), 3.88 (d, 1 H, J = 8.26 Hz), 3.04–3.95 (m, 5 H) [includes 3.66 (s, 3 H)], 0.80–1.88 (m, 16 H), 0.76 (t, 6 H, J = 6.96 Hz). FAB-MS $(M + H)^+$ m/e 656. Anal. ($C_{38}H_{45}N_3O_7 \cdot 1.5H_2O$) C, H, N.

(2*S*)-2-[[(2*S*)-2-[(2-Hydroxy-2-methylpropyl)amino]-3-phenylpropionyl]amino]-4-methylpentanoic Acid Methyl Ester (22). To a solution of Phe-Leu-OMe (0.69 mmol) in CH_2Cl_2 (1 mL) was added methylpropylene oxide (0.075 mL). The reaction mixture was stirred at room temperature overnight; however, no change was observed. Two additional milliliters of the oxide was added and then the reaction mixture was transferred with EtOH to a closed tube reactor, heated to 100 °C for 5 h, stirred at room temperature for 2 days, and concentrated to yield 0.24 g (95%) of product: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.76 (d, 1 H, J = 8.98 Hz), 4.62 (m, 1 H), 3.69 (s, 3 H), 3.13–3.35 (m, 2 H), 2.36–2.74 (m, 3 H), 1.57 (m, 2 H), 0.80–1.30 (m, 15 H); FAB-MS $(M + H)^+$ m/e 365.

(2*S*)-[[(2*S*)-2-(5,5-Dimethyl-2-oxooxazolidin-3-yl)-3-phenylpropionyl]amino]-4-methylpentanoic Acid Methyl Ester (23). Hydroxyamine **22** (0.63 mmol) was cyclized by utilizing general procedure D to yield, after flash chromatography (5% Et_2O/CH_2Cl_2), 0.089 g (36%) of the title product as a white solid (upon standing compound became syruplike): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.25 (m, 5 H), 6.48 (d, 1 H, J = 8.20 Hz), 4.60 (m, 2 H), 3.70 (s, 3 H), 3.15 (m, 4 H), 1.61 (m, 3 H), 1.34 (s, 3 H), 1.17 (s, 3 H), 0.92 (d, 6 H, J = 5.08 Hz); FAB-MS $(M + H)^+$ m/e 391.

(3*S*)-4-Cyclohexyl-3-[[(2*S*)-2-[[(2*S*)-2-(5,5-dimethyl-2-oxooxazolidin-3-yl)-3-phenylpropionyl]amino]-4-methylpentanoyl]amino]-2-hydroxybutyric Acid Methyl Ester (24). Ester **23** (0.23 mmol) was deprotected by utilizing general procedure A to yield 0.043 g of the acid, which was directly coupled to TFA-ACHBA-OMe (0.12 mmol) by utilizing general procedure C to yield, after flash chromatography (5–10% Et_2O/CH_2Cl_2), 0.029 g (56%) of the title compound. HPLC showed a diastereomeric ratio of approximately 9:1: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.23–7.33 (m, 5 H), 6.55 and 6.70 (2 d, 1 H, J = 7.85 Hz), 6.02 and 6.18 (2 d, 1 H, J = 9.44 Hz), 4.59 (m, 1 H), 4.41 (m, 1 H), 4.27 (m, 1 H), 4.14 (dd, 1 H, J = 4.9 Hz, 1.8 Hz), 3.77 and 3.79 (2 s, 3 H), 3.04–3.28 (m, 4 H), 1.11–1.84 (m, 16 H), 0.79–0.97 (m, 6 H); FAB-MS $(M + H)^+$ m/e 574. Anal. ($C_{31}H_{47}N_3O_7 \cdot {}^1/_2H_2O$) C, H, N.

(2*S*)-2-[[(2*S*)-2-[(2-Hydroxy-1,2-dimethylpropyl)amino]-3-phenylpropionyl]amino]-4-methylpentanoic Acid Methyl Ester (25). To a solution of TFA-Phe-Leu-OMe (0.56 mmol) in MeOH was added *N*-methylmorpholine (0.93 mL, pH = 7), 3-hydroxy-3-methyl-2-butanone (0.84 mmol), and $NaCNBH_3$ (0.72 mmol) under an N_2 atmosphere. The reaction mixture was stirred overnight at room temperature and quenched with 50 mL of saturated $NaHCO_3$. The aqueous layer was extracted three times with EtOAc (25 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, concentrated, and then flash chromatographed (1–3% $MeOH/CH_2Cl_2$) to yield 0.17 g (80%) of the title compound as an oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.32 (d, 1 H, J = 9.46 Hz), 7.22–7.53 (m, 6 H), 4.64 (m, 1 H), 3.71 and 3.72 (2 s, 3 H), 2.98–3.47 (m, 3 H), 2.25–2.77 (m, 2 H), 0.65–1.67 (m, 18 H); FAB-MS $(M + H)^+$ m/e 379.

(2*S*)-4-Methyl-2-[[(2*S*)-3-phenyl-2-(4,5,5-trimethyl-2-oxooxazolidin-3-yl)propionyl]amino]pentanoic Acid Methyl Ester (26). Hydroxyamine **25** (0.34 mmol) was cyclized according to general procedure D and flash chromatographed (3–5% EtOAc/CH_2Cl_2) to provide 0.107 g (79.4%) of the title compound as a light yellow oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.58 (m, 1 H), 7.20 (m, 5 H), 4.47 (m, 1 H), 4.28 (m, 1 H), 3.65 and 3.66 (2 s, 3 H), 3.30–3.81 (m, 3 H), 0.75–1.71 (m, 18 H); FAB-MS $(M + H)^+$ m/e 405.

(3*S*)-4-Cyclohexyl-2-hydroxy-3-[[(2*S*)-4-methyl-2-[[(2*S*)-3-phenyl-2-(4,5,5-trimethyl-2-oxooxazolidin-3-yl)-propionyl]amino]pentanoyl]amino]butyric Acid Methyl Ester (27). Ester **26** (0.26 mmol) was subjected to the conditions of general procedure A to yield 0.0965 g (96.8%) of the acid [FAB-MS $(M + H)^+$ m/e 391] which was used directly in the following reaction. The acid was coupled to TFA-ACHBA-OMe (0.28 mmol) by utilizing general procedure C and then flash chromatographed (10–20% EtOAc/CH_2Cl_2) to provide 0.0699 (42%) of the title compound as an off-white solid. HPLC indicated a diastereomeric ratio of 2:1: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.26–7.38 (m, 5 H), 6.99 and 7.45 (2 d, 1 H, J = 9.0 Hz), 6.19 and 6.27 (2 d, 1 H, J = 12 Hz), 4.47 (m, 1 H), 4.32 (m, 1 H), 4.17 (m, 1 H), 3.94 (m, 1 H), 3.84 (s, 3 H), 3.22–3.68 (m, 4 H), 0.83–1.92 (m, 31 H); FAB-MS $(M + H)^+$ m/e 588. Anal. ($C_{32}H_{49}N_3O_7 \cdot {}^1/_4H_2O$) C, H, N.

(2*S*)-2-[[[(1*S*)-1-(Methoxycarbonyl)-2-phenylethyl]amino]methyl]pyrrolidine-1-carboxylic Acid *tert*-Butyl Ester (28). The title compound was prepared from L-phenylalanine methyl ester hydrochloride (4.13 mmol) and Boc-prolinal[31] (4.13 mmol) utilizing a modification of general procedure **E** (reductive amination without heating) to yield, after flash chromatography (15–25% Et_2O/CH_2Cl_2), 0.588 g (40%) of pure product: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.22 (m, 5 H), 3.19 (bs, 1 H), 3.64 (s, 3 H), 3.50 (bs, 1 H), 3.28 (bs, 2 H), 2.91 (m, 2 H), 2.59 (m, 3 H), 1.76 (m, 4 H), 1.44 (s, 9 H); FAB-MS $(M + H)^+$ m/e 385.

(*S*)-2-(3-Oxohexahydro-3*H*-pyrrolo[1,2-*c*]imidazol-2-yl)-3-phenylpropionic Acid Methyl Ester (29). Ester **28** (1.19 mmol) was deprotected by utilizing general procedure B to give the free amine (0.253 g, 81%), which was used directly in the cyclization reaction. To the crude amine in THF (25 mL) was added 1,1′-carbonyldiimidazole (1.25 mmol). The reaction mixture was stirred overnight at room temperature, quenched with 0.5 N HCl, and extracted with EtOAc (3 × 80 mL). The combined organic layers were washed with 0.5 N HCl, saturated bicarbonate, and brine, dried over $MgSO_4$, filtered, concentrated, and flash chromatographed (first in 100% CH_2Cl_2, then 100% Et_2O) to yield 0.151 g (55%) of the title compound: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.24 (m, 5 H), 4.90 (dd, 1 H, J = 5.47, 5.47 Hz), 3.71 (s, 3 H), 3.29–3.61 (m, 5 H), 2.99 (m, 2 H), 1.69–1.93 (m, 3 H), 1.41 (m, 1 H).

(3*S*)-4-Cyclohexyl-2-hydroxy-3-[[(2*S*)-4-methyl-2-[[(2*S*)-2-(3-oxohexahydro-3*H*-pyrrolo[1,2-*c*]imidazol-2-yl)-3-phenylpropionyl]amino]pentanoyl]amino]butyric Acid Isopropyl Ester (30). Ester **29** (0.52 mmol) was deprotected utilizing general procedure A to yield 0.137 g (95%) of the acid which was directly coupled to TFA-Leu-ACHBA-O^{i}Pr (0.46 mmol) by utilizing general procedure C to yield, after flash chromatography (1:9:90 v/v/v $MeOH/Et_2O/CH_2Cl_2$), 0.157 g (56%) of the title compound as a white solid. HPLC indicated a diastereomeric ratio of 3.2:1: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.19–7.31 (m, 5 H), 6.90 and 6.99 (2 d, 1 H, J = 7.9 Hz), 6.12 and 6.24 (2 d, 1 H, J = 9.75 Hz), 4.93–5.11 (m, 1 H), 4.04–4.61 (m, 5 H), 3.51–3.65 (m, 3 H), 3.34 (d, 1 H), 2.99–3.30 (m, 3 H), 0.71–1.91 (m, 32 H); FAB-MS $(M + H)^+$ m/e 613. Anal. ($C_{34}H_{52}N_4O_6 \cdot 1.5H_2O$) C, H; N: calcd, 8.76; found, 9.17.

(*S*)-2-[(1*H*-Imidazol-2-ylmethyl)amino]-3-phenylpropionic Acid Methyl Ester (31). The title compound was prepared from L-phenylalanine methyl ester hydrochloride (3.30 mmol) and 2-formylimidazole (3.63 mmol) by utilizing a modification of general procedure E (reductive amination without heating) to yield, after flash chromatography (3:50:50 v/v/v MeOH/EtOAc/CH_2Cl_2), 0.55 g (65%) of pure product: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.25 (m, 5 H), 6.79 (s, 2 H), 3.86 (m, 2 H), 3.73 (s, 3 H), 3.26 (dd, 1 H, J = 9.29 Hz, 4.08 Hz), 3.08 (dd, 1 H, J = 13.76 Hz, 4.69 Hz), 2.75 (dd, 1 H, J = 13.76, 9.21 Hz); EI-MS m/e 259.

(2*S*)-(5-Oxo-6,7-dihydro-5*H*-imidazol[1,5-*a*]imidazol-6-yl)-3-phenylpropionic Acid Methyl Ester (32). Imidazo compound **31** (2.12 mmol) was cyclized by utilizing general procedure D to yield, after flash chromatography (30% EtOAc/CH_2Cl_2), 0.51 g (84%) of the title compound as a yellow oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.23 (m, 5 H), 6.99 and 7.14 (2 s, 2 H), 5.15 (dd, 1 H, J = 10.04 Hz, 5.56 Hz), 4.43 (m, 2 H), 3.78 (s, 3 H), 3.50 (dd, 1 H, J = 14.84 Hz, 5.08 Hz), 3.11 (dd, 1 H, J = 14.28, 10.86 Hz); EI-MS m/e 285.

(3*S*)-4-Cyclohexyl-2-hydroxy-3-[[(2*S*)-4-methyl-2-[[(2*S*)-2-(5-oxo-6,7-dihydro-5*H*-imidazol[1,5-*a*]imidazol-6-

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yl)-3-phenylpropionyl]amino]pentanoyl]amino]butyric Acid Isopropyl Ester (33). Ester **32** (0.60 mmol) was deprotected by utilizing general procedure A to yield 0.075 g (46%) of the acid which was directly coupled to TFA-Leu-ACHBA-O^{i}Pr (0.34 mmol) by utilizing general procedure C to yield, after flash chromatography (1–3:20:80 v/v/v MeOH/EtOAc/CH_2Cl_2), 0.101 g (49%) of the title compound as a white solid. HPLC indicated a mixture of four diastereomers in a ratio of 5:3:1:1: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.13–7.33 (m, 7 H), 6.42 and 6.50 (2 d, 1 H, J = 8.09 Hz), 5.99–6.16 (m, 1 H), 4.81–5.13 (m, 2 H), 3.99–4.62 (m, 4 H), 3.08–3.37 (m, 2 H), 0.71–1.87 (m, 28 H); FAB-MS $(M + H)^+$ m/e 610. Anal. ($C_{33}H_{47}N_5O_6 \cdot {}^3/_4H_2O$) C, H, N.

Biological Methods. Chymotrypsin Stability Studies. Stability studies were carried out essentially according to the procedure of Thaisrivongs et al.[32] Analysis of chymotrypsin digestion products was carried out by determining peak area by HPLC, utilizing a C_{18} or phenyl column. Solvent systems used were either an acetonitrile/0.01 M $NH_4H_2PO_4$ or a methanol/0.01 M $NH_4H_2PO_4$ mixture eluting at 1 mL/min. Products were detected by UV at 214 nm.

In Vitro Assay for Renin Inhibition. Compounds were first dissolved in DMSO and then diluted 1:10 with distilled water to give a 10% DMSO working solution. The 10% DMSO working solution was further diluted when added to the assay to give a final concentration of 1% DMSO. The method is adapted from the "Gamma Coat [^{125}I] Plasma Renin Activity RIA Kit" from Baxter Health Care Corp. The assay is a two-part procedure consisting of an initial angiotensin I generation step, followed by a quantification of angiotensin I by radioimmunoassay (RIA). Briefly, the two steps were as follows.

Angiotensin I Generation. Two sets of 12 × 75 mm polypropylene tubes were numbered. One set was designated 4 °C and one set 37 °C. Both sets were placed in a 4 °C ice bath. The 37 °C set of tubes received 50 μL of test compound or DMSO, 310 μL of prechilled distilled water, 50 μL of 20 μM human renin substrate (Bachem Biochemicals, Inc.), 50 μL of 10× buffer (500 mM sodium phosphate, monobasic, 3.0% w/v BSA, and 30 mM EDTA; pH adjusted to 5.7), 10 μL of phenylmethylsulfonyl fluoride (PMSF), and 30 μL of 0.05 GU/mL recombinant human renin (California Biotechnology Inc.) prepared in 3.0% BSA adjusted to generate 30–40 ng of A_1/mL per h prior to assaying. After mixing of the above mixture, 250 μL was transferred to the corresponding 4 °C tube. The 37 °C set of tubes was placed in a 37 °C water bath and incubated for 1 h. The 4 °C tubes were maintained in an ice bath. After the incubation, the 37 °C tubes were returned to the ice bath.

Angiotensin I RIA. The assay procedure included the preparation of a standard curve from which the unknown angiotensin I content in both 37 and 4 °C sets of samples is interpolated. Into appropriate duplicated tubes, 100 μL of standard or assay buffer, 25 μL of sample, 75 μL of assay buffer, and 1000 μL of tracer buffer were pipetted. All tubes were mixed and incubated at room temperature for 3 h and then all tubes were decanted except for the "total counts" tubes. All tubes were counted in a γ counter for 1 min. Data reduction was performed on-line utilizing the γ counter software to determine angiotensin I levels. Subsequent calculations were then transcribed into a Lotus spreadsheet where in vitro renin inhibition was expressed in terms of percent inhibition or IC_{50} (concentrations of compound which resulted in 50% inhibition of enzyme activity).

Pharmacologic Activity in Acute Salt-Depleted Conscious Primates. The ability of test compounds to inhibit plasma renin activity (PRA) was used as an index of renin inhibitory activity. Rhesus monkeys (either sex, weight range 3.0–8.0 kg) were obtained from a colony of animals in which vascular access ports with the catheter tip located in the abdominal aorta had been implanted at least 2 weeks before testing. The experimental protocol for testing renin inhibitors was as follows: animals were treated with furosemide 4 mg/kg sc for 2 days. On day 3, animals were randomized to receive either test drug or vehicle, three or four animals were used in each treatment group. Animals were placed in a primate restraining chair and an iv catheter was placed in the saphenous vein for drug administration. Blood pressure was recorded from a 22-gauge needle placed in the vascular access port and connected to a Gould P50 pressure transducer. Heart rate was derived electronically from the pulse pressure signal using a Beckman cardiotachometer (Model 9857B). Arterial blood pressure and heart rate were displayed continuously on a Beckman R611 polygraph. The animals were allowed to stabilize for 30 min and a baseline blood sample was obtained at time designation −30 min. At time zero, baseline blood pressure and heart rate measurements were obtained and test drug was administered either iv (in saline) or po (in 0.5% (carboxymethyl)cellulose solution). Blood pressure and heart rate measurements were obtained hourly for 5 h and again at 24 h. Blood samples were obtained from the vascular access ports in heparinized tubes and were stored on ice until centrifugation. Plasma was stored at −70 °C until assay for PRA. Compound **8** was administered at 5 mg/kg iv and 50 mg/kg po and compound **6** was administered at 10 mg/kg po.

Assay for Plasma Renin Activity. The method used was that of Gamma Coat [^{125}I] Plasma Renin Activity Radioimmunoassay Kit (Baxter Healthcare Corp., MA), as described above.

(32) Thaisrivongs, S.; Pals, D. T.; Lawson, J. A.; Turner, S. R.; Harris, D. W. α-Methylproline-Containing Renin Inhibitory Peptides: In Vivo Evaluation in an Anesthesized, Ganglion-Blocked, Hog Renin Infused Rat Model. *J. Med. Chem.* **1987**, *30*, 536–541.