1,2-HOPO-6-CO), 110874-37-8; 11 (X = H), 77372-56-6; 12 (X = 1,2-HOPO-6-CO), 110874-38-9; 12 (X = $MeSO_3H$), 138-14-7; 12 (X = H), 70-51-9; 13, 94781-92-7; 13, SRU, 94781-91-6; 14, 101126-68-5; 16, 58569-87-2; 17, 110874-39-0; 18, 110874-40-3; DMAA, 127-19-5; 3,4,3-LICAM(C), 91776-07-7; 3,4,3-LICAM(S), 73487-20-4; $CaNa_3$-DTPA, 12111-24-9; ^{238}Pu, 13981-16-3; $NHMe_2$, 124-40-3; $PhCH_2NH_2$, 100-46-9; propylenediamine, 109-76-2.

Supplementary Material Available: Detailed methods and results of preliminary toxicity screening of HOPO ligands (2 pages). Ordering information is given on any current masthead page.

Design, Structure–Activity, and Molecular Modeling Studies of Potent Renin Inhibitory Peptides Having N-Terminal N^{in}-For-Trp (Ftr): Angiotensinogen Congeners Modified by P_1-P_1' Phe-Phe, Sta, Leuψ[CH(OH)CH_2]Val or Leuψ[CH_2NH]Val Substitutions[⊥1]

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A structure–conformation–activity investigation of several angiotensinogen (ANG) based inhibitors of human renin modified by either Phe-Phe, Sta, Leuψ[CH_2NH]Val, or Leuψ[CH(OH)CH_2]Val at the P_1-P_1' cleavage site and P_5 Trp(N^{in}-For) (Ftr) was performed. Specifically, Ac-Ftr-Pro-Phe-His-Phe-Phe-Val-Ftr-NH_2 (1) provided a potent ($K_I = 2.7 \times 10^{-8}$ M) P_1-P_1' Phe-Phe substituted renin inhibitor to initiate these studies. Substitution of the P_1-P_1' Phe-Phe in compound 1 by Sta effected a 1000-fold increase in biological potency for the resultant octapeptide Ac-Ftr-Pro-Phe-His-Sta-Val-Ftr-NH_2 (10; $K_I = 6.7 \times 10^{-11}$ M). Kinetic analysis of compound 10 showed it to be a competitive inhibitor of human renin catalyzed proteolysis of human ANG. Chemical modifications of the compounds 1 and 10 were evaluated on the basis of comparative human plasma renin inhibitory activities (IC_{50} values) in vitro. Carboxy-terminal truncation studies on compound 10 showed that the P_2' Val and P_3' Ftr residues could both be eliminated without significant loss (ca. 10-fold) in renin inhibitory activity as exemplified by the pentapeptide Ac-Ftr-Pro-Phe-His-Sta-NH_2 (12; $IC_{50} = 3.8 \times 10^{-9}$ M). In addition, the corresponding P_1-P_1' Leuψ[CH(OH)CH_2]Val and Leuψ[CH_2NH]Val derivatives of compound 12 were potent renin inhibitors: Ac-Ftr-Pro-Phe-His-Leuψ[CH(OH)CH_2]Val-NH_2 (13; $IC_{50} = 3.1 \times 10^{-10}$ M) and Ac-Ftr-Pro-Phe-His-Leuψ[CH_2NH]Val-NH_2 (14; $IC_{50} = 2.1 \times 10^{-8}$ M). The structure–conformation–activity properties of the N-terminal Ftr substitution of these human renin inhibitors was examined by (1) comparative analysis of several analogues of 1 and Ac-Ftr-Pro-Phe-His-Sta-Ile-NH_2 (17; $IC_{50} = 1.0 \times 10^{-10}$ M) having P_5 site modifications by Trp, His, D-Ftr, and D-His, (2) deletion of the N-terminal Ftr residue from compounds 12 and 17, to provide Ac-Pro-Phe-His-Sta-Ile-NH_2 (16; $IC_{50} = 3.1 \times 10^{-8}$ M) and Ac-Pro-Phe-His-Sta-NH_2 (15; $IC_{50} = 5.6 \times 10^{-6}$ M), and (3) computer modeling and dynamics studies of compounds 1 and 17 bound to CKH-RENIN, a simulated human renin model, which were focused on identifying potential intermolecular interactions of their common P_5-P_2 sequence, Ac-Ftr-Pro-Phe-His, at the enzyme active site. Finally, the human renin specificity of selected congeners of compound 10 were determined by comparison to porcine kidney renin in vitro.

Renin is an aspartic acid protease (EC 3.4.99.19) that catalyzes the first and rate-limiting step of the enzyme cascade that exists for the biosynthesis of angiotensin II[2-4] (Figure 1). Renin selectively cleaves its polypeptide substrate, angiotensinogen (ANG), to effect the formation of the decapeptide intermediate, angiotensin I. Angiotensin I is then cleaved by angiotensin converting enzyme (ACE) to yield the biologically active octapeptide product, angiotensin II. Angiotensin II possesses multiple bioactivities (e.g., vasoconstriction, aldosterone, and catecholamine secretion) at various target tissues, and the renin–ACE cascade has been implicated to have a physiological role for blood pressure and electrolyte homeostasis. In addition, this pathway apparently is involved in pathophysiological states related to various forms of hypertension.[5,6] Therefore, inhibition of renin may be therapeutically important in the development of novel antihypertensive agents.[7-11]

[⊥] Dedicated to The Upjohn Company in honor of its 100th anniversary, 1886–1986.

[†] Biotechnology-Regulatory Peptide Research.

[‡] Cardiovascular Diseases Research.

[§] Computational Chemistry.

[#] Physical and Analytical Chemistry Units.

[∥] Cardiac Unit.

(1) Symbols and abbreviations are in accord with the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature (*Eur. J. Biochem.* **1984**, *158*, 9). All optically active amino acids are of the L variety unless otherwise specified. Additional abbreviations used are: Ftr, Trp(N^{in}-For); Sta, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid; Leuψ[CH(OH)CH_2]Val, (2*S*,4*S*,5*S*)-4-hydroxy-2-isopropyl-7-methyloctanoic acid; and Leuψ[CH_2NH]Val, (*S*)-4-methylpentyl-L-valine. In accord with nomenclature described by Schechter and Berger (*Biochem. Biophys. Res. Commun.* **1967**, *27*, 157), P_n-P_n' refer to the side-chain positions of the peptide substrate, whereas S_n-S_n' refer to the subsite on the enzyme that binds the corresponding side chain of the substrate. Other abbreviations are referenced as used in text.

(2) Peach, M. J. *Physiol. Rev.* **1977**, *57*, 313.
(3) Ondetti, M. A.; Cushman, D. W. *Annu. Rev. Biochem.* **1982**, *51*, 283.
(4) Skeggs, L. T.; Dorer, F. E.; Levine, M.; Lentz, K. E.; Kahn, J. R. In *The Renin Angiotensin System*; Johnson, J. A.; Anderson, R. R., Eds.; Plenum: New York, 1980; p 1.
(5) Materson, B. J.; Freis, E. D., Anderson, S.; Taguchi, J. T. *Arch. Intern. Med.* **1984**, *144*, 1947.
(6) Davies, R. O.; Irvin, J. D.; Kramsch, D. K.; Walker, J. F.; Moncloa, F. *Am. J. Med.* **1984**, *77*, 23.

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Table I. Comparative Human Plasma Renin IC_{50} Values of Ftr Modified RIP Analogues

	compound	IC_{50}, M
ANG_{6-13}	~His-Pro-Phe-Leu-Val-Ile-His~	
RIP	H-Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys-OH	8.0×10^{-6}
1	Ac-Ftr-Pro-Phe-His-Phe-Phe-Val-Ftr-NH_2	5.0×10^{-7}
2	Ac-Ftr-Pro-Phe-His-Phe-Phe-Val-NH_2	2.1×10^{-6}
3	Ac-Ftr-Pro-Phe-His-Phe-Phe-NH_2	$>1.0 \times 10^{-4}$
4	Ac-Ftr-Gly-Phe-His-Phe-Phe-Val-Ftr-NH_2	$>1.0 \times 10^{-4}$
5	Ac-D-Ftr-Pro-Phe-His-Phe-Phe-Val-Ftr-NH_2	$>1.0 \times 10^{-4}$
6	Ac-Trp-Pro-Phe-His-Phe-Phe-Val-Ftr-NH_2	5.0×10^{-6}
7	Ac-His-Pro-Phe-His-Phe-Phe-Val-Ftr-NH_2	8.3×10^{-6}
8	Ac-D-His-Pro-Phe-Phe-Val-Ftr-NH_2	$\sim 1.0 \times 10^{-4}$
9	Ac-His-Phe-Ftr-Phe-Phe-Val-Ftr-NH_2	$>1.0 \times 10^{-4}$

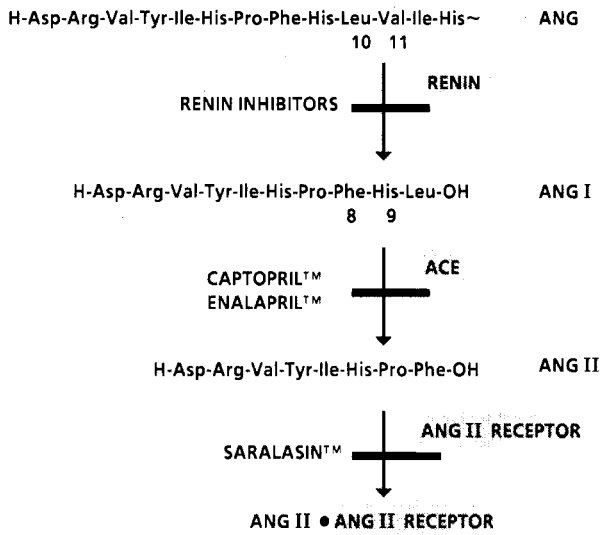

Figure 1. The renin-angiotensin converting enzyme (ACE) cascade and selected inhibitors thereof. ANG, ANG I, and ANG II refer to angiotensinogen, angiotensin I, and angiotensin II, respectively. Captopril and Enalopril represent known ACE inhibitors. Saralasin represents a known competitive receptor antagonist for ANG II.

In retrospect, the development of ANG-based (Figure 2) inhibitors of human renin was significantly advanced by the systematic structure–activity studies of Burton and co-workers[12-16] during the period of 1973–1980. From their efforts the decapeptide H-Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys-OH (RIP; see Figure 3) was synthesized and at the time of its discovery[14] was found to be the most potent ($K_I = 2.0 \times 10^{-6}$ M) renin inhibitory peptide known. The design of RIP was primarily based upon substitution of the dipeptide Phe-Phe for the P_1-P_1' Leu-Leu residues of the corresponding equine ANG_{6-13} template and subsequent addition of N-terminal Pro and C-terminal Lys residues to provide improved solubility in H_2O and in vivo biological activity.[14-16] In particular, the P_1-P_1' Phe-Phe modification most likely elicits important hydrophobic and/or π–π bonding interactions between this competitive inhibitor and renin. Recently, alternative approaches to the P_1-P_1' Phe-Phe modification in the design of more highly potent RIP analogues have been hallmarked by reports[17-27] of various P_1-P_1' pseudosubstrate "transition state" isosteres[28] (e.g., Leuψ[CH_2NH]Val, Leuψ[CH(OH)CH_2]Val, and Sta) and their incorporation within ANG-based peptide templates. Exemplifying such work was the identification of pepstatin (Iva-Val-Val-Sta-Ala-Sta-OH; see Figure 3) as an inhibitor of several aspartyl proteases[29,30] including human renin[20] ($K_I = 2.2 \times 10^{-5}$ M)

(7) Haber, E. J. *Hypertension* **1984**, *2*, 223.
(8) Geiger, R. *Arzneim.-Forsch./Drug Res.* **1984**, *35*, 1386.
(9) Ganten, D.; Unger, T.; Lang, R. E. *Arzneim.-Forsch./Drug Res.* **1984**, *34*, 1391.
(10) Sweet, C. S.; Blain, E. *Cardiovascular Pharmacology*, 2nd ed.; Antonaccio, M., Ed.; Raven: New York, 1984; p 119.
(11) Boger, J. *Annu. Rep. Med. Chem.* **1985**, *20*, 257.
(12) Poulsen, K.; Burton, J.; Haber, E. *Biochemistry* **1973**, *12*, 3877.
(13) Burton, J.; Poulsen, K.; Haber, E. *Biochemistry* **1975**, *14*, 3892.
(14) Cody, R.; Burton, J.; Evin, G.; Poulsen, K.; Herd, J. A.; Haber, E. *Biochem. Biophys. Res. Commun.* **1980**, *97*, 230.
(15) Burton, J.; Cody, R. J., Jr.; Herd, J. A.; Haber, E. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5476.
(16) Burton, J.; Quinn, T.; Poulsen, K. In *Peptides: Synthesis–Structure–Function*, Proceedings of the Seventh American Peptide Symposium; Rich, D. H., Gross, E., Eds.; Pierce Chemical Co.: Rockford, IL, 1981; p 447.
(17) Szelke, M.; Leckie, B.; Hallett, A.; Jones, D. M.; Sueiras, J.; Atrash, B.; Lever, A. F. *Nature (London)* **1982**, *299*, 555.
(18) Szelke, M.; Jones, D. M.; Atrash, B.; Hallett, A.; Leckie, B. In *Peptides: Structure and Function*, Proceedings of the Eighth American Peptide Symposium, Tucson, AZ; Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Co.: Rockford, IL, 1983; p 579.
(19) (a) European Patent Application, 85,305,560.6, 1986. (b) Thaisrivongs, S.; Pals, D. T.; Harris, D. W.; Kati, W. M.; Turner, S. R. *J. Med. Chem.* **1986**, *29*, 2088.
(20) Boger, J.; Lohr, N. S.; Ulm, E. H.; Poe, M.; Blaine, E. H.; Fanelli, G. M.; Lin, T.-Y.; Payne, L. S.; Schorn, T. W.; LaMont, B. I.; Vassil, T. C.; Stabalito, I. I.; Veber, D. F. *Nature (London)* **1983**, *303*, 81.
(21) Boger, J.; Payne, L. S.; Perlow, D. S.; Lohr, N. S.; Poe, M.; Blaine, E. H.; Ulm, E. H.; Schorn, T. W.; LaMont, B. I.; Lin, T.-Y.; Kawai, M.; Rich, D. H.; Veber, D. F. *J. Med. Chem.* **1985**, *28*, 1779.
(22) Sawyer, T. K.; Pals, D. T.; Smith, C. W.; Saneii, H. H.; Epps, D. E.; Duchamp, D. J.; Hester, J. B.; Ten Brink, R. E.; Staples, D. J.; deVaux, A. E.; Affhotter, J. A.; Skala, G. F.; Kati, W. M.; Lawson, J. A.; Schuette, M. R.; Kamdar, B. V.; Emmert, D. E. In *Peptides: Structure and Function*, Proceedings of the Ninth American Peptide Symposium, Toronto, Ontario; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chemical Co.: Rockford, IL, 1985; p 729.
(23) Ryono, D. E.; Free, C. A.; Neubeck, R.; Samaniego, S. G.; Godfrey, J. D.; Petrillo, E. W., Jr. ref 22; p 739.
(24) Dann, J. G.; Stammers, D. K.; Harris, C. J.; Arrowsmith, R. J.; Davies, D. E.; Hardy, G. W.; Morton, J. A. *Biochem. Biophys. Res. Commun.* **1986**, *134*, 71.
(25) Hanson, G. J.; Baran, J. S.; Lindberg, T.; Walsh, G. M.; Papaioannou, S. E.; Babler, M.; Bittner, S. E.; Yang, P. C.; Dal Corobbo, M. *Biochem. Biophys. Res. Commun.* **1985**, *132*, 155.
(26) Guegan, R.; Diaz, J.; Cazaubon, C.; Beaumont, M.; Carlet, C.; Clement, J.; Demarne, J.; Mellet, M.; Richaud, J. P.; Segondy, D.; Vedel, M.; Gagnol, J.-P.; Roncucci, R.; Castro, B.; Corvol, P.; Evin, G.; Roques, B. P. *J. Med. Chem.* **1986**, *29*, 1152.
(27) Kokubu, T.; Hiwada, K.; Nagae, A.; Murakami, E.; Morisawa, Y.; Yabe, Y.; Koike, H.; Iijima, Y. *Hypertension* **1986**, *8* (Suppl. II), II-1.
(28) The term "transition state" has been used to implicate the P_1-P_1' scissile amide bond isosteres of CH_2NH, $CH(OH)CH_2$, and $CH(OH)CH_2NH$ as possible tetrahedral mimics of the substrate P_1-P_1' $C(OH)_2NH$ transition state which is believed to be produced during the renin–ANG reaction. Also refer to: Rich, D. H. *J. Med. Chem.* **1985**, *28*, 263.

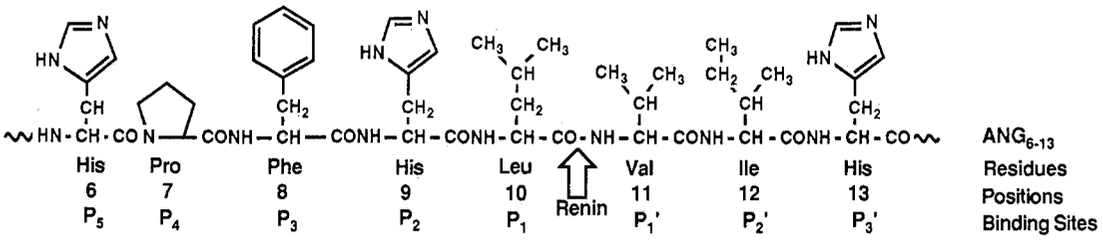

Figure 2. Peptide sequence of human ANG_{6-13}. The position and binding site of each aminoacyl residue are identified (i.e., 6 to 13 and P_5 to P_3', respectively).

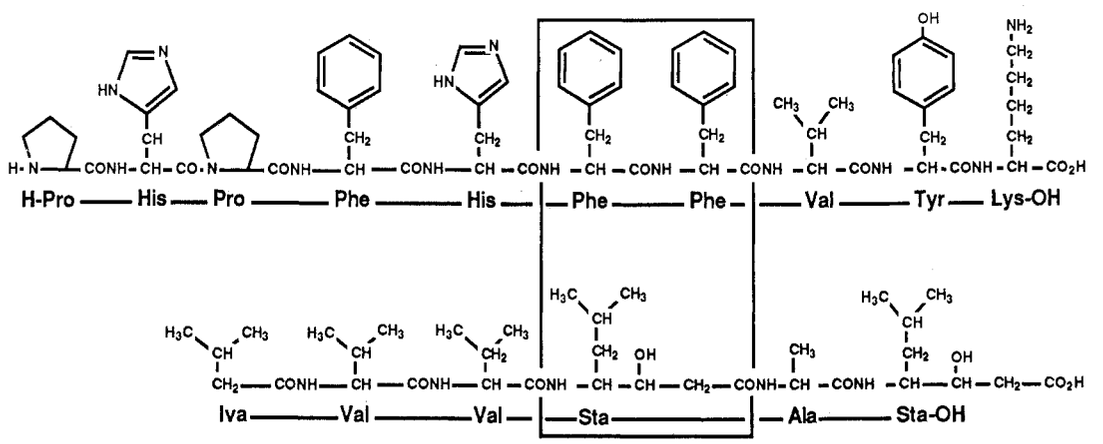

Figure 3. Comparative peptide sequences of RIP (top) and pepstatin (bottom). The P_1-P_1' modifications are designated by the box.

and synthetic tailoring of it to provide potent human renin inhibitors (e.g., Iva-His-Pro-Phe-His-Sta-Leu-Phe-NH_2, SCRIP, which effected[20] a $K_I = 1.9 \times 10^{-8}$ M). Interfacing with these structure–activity studies also have been current research efforts[31-33] directed toward the examination of renin–inhibitor interactions by computer-assisted molecular modeling based upon hypothetical 3-D structures (since the X-ray structure is unknown) of human renin as generated from its primary sequence.[34] In this paper we describe structure–activity studies related to the development of highly potent, N-terminally modified peptide inhibitors of human renin in vitro and molecular modeling with a computer-simulated human renin active site.

Results and Discussion

Design and Structure–Activity Relationships of P_1-P_1' Phe-Phe Substituted RIP Analogues. RIP is predominantly composed of aromatic and/or hydrophobic aminoacyl residues as exemplified by the presence of three Phe, two His, and a Tyr within its decapeptide sequence. The fact that no Trp residues existed in RIP provided us with impetus to explore, as previously reported,[22] the structure–activity relationships of RIP analogues modified by this very hydrophobic, heteroaromatic amino acid.[35] In addition, the N^{in}-formyl-Trp (Ftr) congeners were tested to examine the potential biological significance of this Trp derivative in peptides per se. These studies resulted in the identification of an unexpectedly potent RIP congener, Ac-Ftr-Pro-Phe-His-Phe-Phe-Val-Ftr-NH_2 (**1**; see Table I). Carboxy-terminal truncation was performed on **1** to investigate the significance of the P_3' and P_2' positions (cf. compounds **2** and **3**). The P_3' Ftr residue was not essential for renin-inhibitory activity. However, a stringent structural requirement for the P_2' Val residue apparently did exist as implicated by the significantly diminished (ca. 1000-fold relative to **1**) renin-inhibitory potency of analogue **3**, Ac-Ftr-Pro-Phe-His-Phe-Phe-NH_2. Substitution of the P_4 Pro residue in compound **1** by Gly resulted in markedly reduced (ca. 1000-fold) potency, which implicated a requirement for conformational constraint between the P_5 and P_3 sites (cf. compound **4**). The stereostructural requirements of the P_5 Ftr residue were further examined by substitutions with D-Ftr, Trp, His, and D-His (cf. compounds **6–9**). All of these congeners of **1** showed decreased (ca. 10–100-fold) biological activities. Furthermore, substitution of the P_2 His by Ftr within the template of derivative **5** nearly abolished its renin-inhibitory activity (cf. compound **9**). Thus, the Ftr modification in this series of RIP analogues was regioselective (P_5 site preferred) and stereoselective (L isomer preferred) as related to the enhanced renin-inhibitory potency of **1** (versus RIP).

The kinetic properties of **1** on purified human kidney renin have been previously reported.[22] Compound **1** was shown to be a potent, competitive inhibitor of human renin with a $K_I = 2.7 \times 10^{-8}$ M (versus RIP, $K_I = 2.3 \times 10^{-6}$ M) and apparently is superior to any previously cited[14,36] P_1-P_1' Phe-Phe, Leu-Phe, or Leu-Leu substituted ANG derivatives, including H-Leu-Leu-Val-Phe-OMe, H-His-Leu-Leu-Val-Phe-OMe, H-Pro-His-Pro-Phe-His-Leu-Leu-Val-Tyr-OH, H-Pro-His-Pro-Phe-His-Leu-Phe-Val-Tyr-OH, H-Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-OH, and RIP. The higher relative potency (100-fold) of compound

(29) Tang, J. In *Acid Proteases–Structure, Function and Biology*; Plenum: New York, 1977.
(30) Eid, M.; Evin, G.; Castro, B.; Menard, J.; Corvol, P. *Biochem. J.* **1981**, *197*, 465.
(31) Carlson, W.; Karplus, M.; Haber, E. *Hypertension* **1985**, *7*, 13.
(32) Sibanda, B. L.; Blundell, T.; Hobart, P. M.; Fogliano, M.; Bindra, J. S.; Dominy, B. W.; Chirgwin, J. M. *FEBS Lett.* **1984**, *174*, 102.
(33) Akahane, K.; Umeyama, H.; Nakagawa, S.; Moriguchi, I.; Hirose, S.; Iizuka, K.; Murakami, K. *Hypertension* **1985**, *7*, 3.
(34) Imai, T.; Miyazaki, H.; Hirose, S.; Hori, H.; Hayashi, T.; Kageyama, R.; Ohkuba, H.; Nakanishi, S.; Murakami, K. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 7405.
(35) Fauchere, J. L.; Pliska, V. *Eur. J. Med. Chem.* **1983**, *18*, 369.
(36) Johnson, R. L. *J. Med. Chem.* **1980**, *23*, 666.

Table II. Comparative Human Plasma Renin IC_{50} Values of P_1–P_1' Sta, Leuψ[CH(OH)CH_2]Val, and Leuψ[CH_2NH]Val Modified Analogues of **1**

	compound	IC_{50}, M
ANG_{6-13}	~His~Pro-Phe-His-Leu-Val-Ile-His~	
RIP	H-Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys-OH	8.0×10^{-6}
1	Ac-Ftr-Pro-Phe-His-Phe-Phe-Val-Ftr-NH_2	5.0×10^{-7}
10	Ac-Ftr-Pro-Phe-His-Sta-Val-Ftr-NH_2	3.8×10^{-10}
11	Ac-Ftr-Pro-Phe-His-Sta-Val-NH_2	5.3×10^{-10}
12	Ac-Ftr-Pro-Phe-His-Sta-NH_2	3.8×10^{-9}
13	Ac-Ftr-Pro-Phe-His-Leuψ[CH(OH)CH_2]Val-NH_2	3.1×10^{-10}
14	Ac-Ftr-Pro-Phe-His-Leuψ[CH_2NH]Val-NH_2	2.1×10^{-8}
15	Ac-Pro-Phe-His-Sta-NH_2	5.8×10^{-6}
16	Ac-Pro-Phe-His-Sta-Ile-NH_2	3.1×10^{-8}
17	Ac-Ftr-Pro-Phe-His-Sta-Ile-NH_2	1.0×10^{-10}
18	Ac-Trp-Pro-Phe-His-Sta-Ile-NH_2	1.6×10^{-9}
19	Ac-His-Pro-Phe-His-Sta-Ile-NH_2	6.8×10^{-9}
20	Ac-D-Ftr-Pro-Phe-His-Sta-Ile-NH_2	1.4×10^{-8}

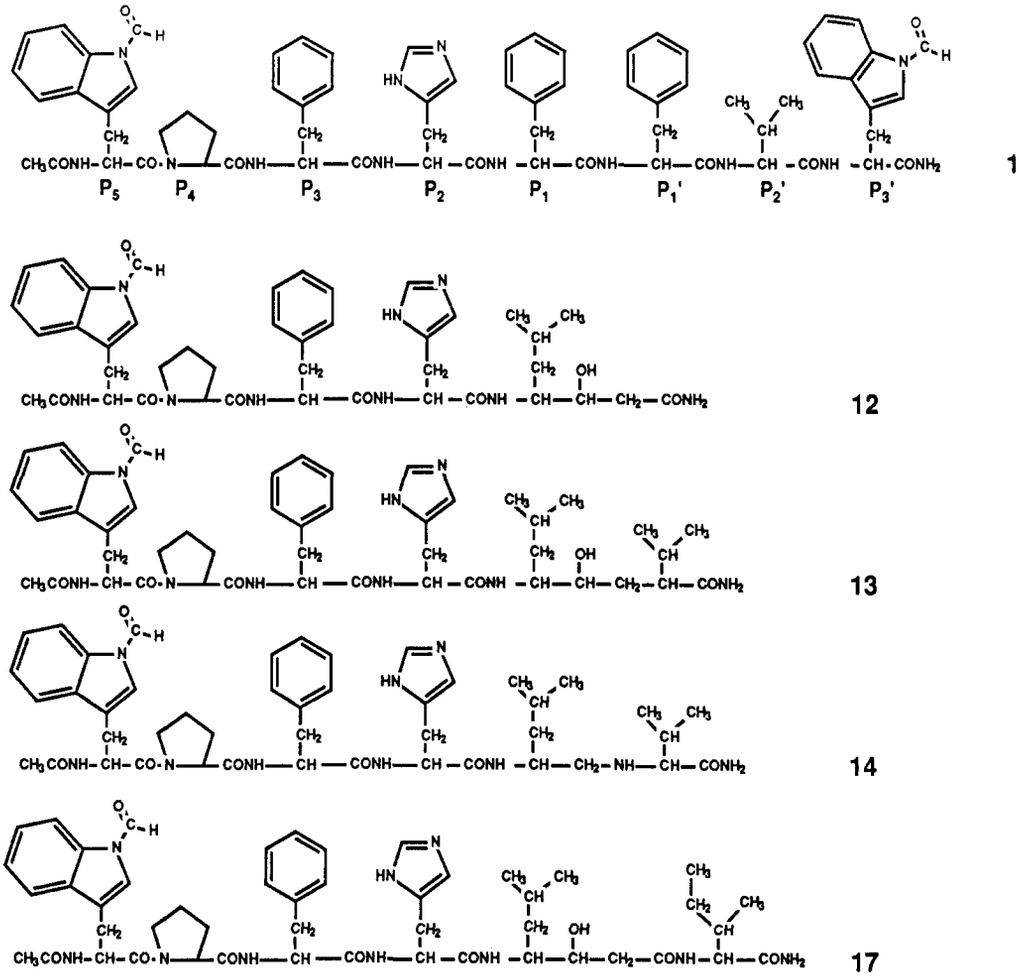

Figure 4. Chemical structures of compounds **1** (U-69010E), **12**–**14**, and **17** (U-70504E). Ligand binding site nomenclature is adapted from that previously designated for ANG_{6-13}.

1 versus RIP in the human kidney renin assay as compared to the above human plasma renin IC_{50} data (Table I) was not surprising as similar relationships for renin inhibitors have been reported[37] previously. Although compound **1** was very hydrophobic, relatively insoluble in H_2O, and labile to metabolism by proteolytic enzymes (data not shown), it did provide a useful lead template for the design of more potent and efficacious congeners in subsequent in vitro (vide infra) and in vivo[38] studies.

Design and Structure–Activity Relationships of P_1-P_1' Sta, Leuψ[CH(OH)CH_2]Val or Leuψ[CH_2NH]-Val Substituted ANG Derivatives. Based on the above data for compound **1**, we first systematically examined several P_1-P_1' Sta substituted congeners (Table II) for their comparative structure–activity relationships. Relative to Ac-Ftr-Pro-Phe-His-Sta-Val-Ftr-NH_2 (**10**; $IC_{50} = 3.8 \times 10^{-10}$ M), carboxy-terminal truncation of the P_2' and P_3'

(37) Evans, B. E.; Rittle, K. E.; Bock, M. G.; Bennett, C. D.; Di-Pardo, R. M.; Boger, J.; Poe, M.; Ulm, E. H.; LaMont, B. I.; Blaine, E. H.; Fanelli, G. M.; Stabilito, I. I.; Veber, D. F. *J. Med. Chem.* **1985**, *28*, 1755.

(38) Preliminary accounts of in vitro and in vivo data on congeners of compound **17** have been recently presented at the ACS Central Regional Meeting (June 4, 1986) at Bowling Green, OH, and the Upjohn Brook Lodge Conference on Peptide Therapeutics (September 26, 1986) at Kalamazoo, MI.

Table III. Comparative Human Plasma and Porcine Renin IC_{50} Values of Selected Inhibitors

	compound	IC_{50}, M	
		human plasma	porcine kidney
RIP	H-Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys-OH	8.0×10^{-6}	16% at 10^{-5}
pepstatin	Iva-Val-Val-Sta-Ala-Sta-OH	2.2×10^{-5}*	1.3×10^{-5}*
15	Ac-Pro-Phe-His-Sta-NH_2	5.8×10^{-6}	3% at 10^{-5}
12	Ac-Ftr-Pro-Phe-His-Sta-NH_2	3.8×10^{-9}	2.4×10^{-6}
17	Ac-Ftr-Pro-Phe-His-Sta-Ile-NH_2	1.0×10^{-10}	1.2×10^{-7}
19	Ac-His-Pro-Phe-His-Sta-Ile-NH_2	6.8×10^{-9}	1.5×10^{-6}
10	Ac-Ftr-Pro-Phe-His-Sta-Val-Ftr-NH_2	3.8×10^{-10}	4.9×10^{-9}
SCRIP[a]	Iva-His-Pro-Phe-His-Sta-Leu-Phe-NH_2	1.6×10^{-8}	3.1×10^{-8}[a]
	H-Pro-Phe-His-Sta-Leu-Phe-NH_2	NR[b]	2.0×10^{-7}[a]
	H-Phe-His-Sta-Leu-Phe-NH_2	NR	1.3×10^{-6}[a]
	H-His-Sta-Leu-Phe-NH_2	NR	3.7×10^{-4}[a]

[a] Pepstatin, SCRIP, and their congeners have been previously described[20] by Boger and co-workers. [b] Not reported.

residues effected a modest (2- and 10-fold, respectively) decrease in renin-inhibitory potency (cf. compounds **11** and **12**). This was in contrast to the data obtained for the structurally homologous P_1-P_1' Phe-Phe substituted congeners **1–3** (refer to Table I). Based on the template of the pentapeptide **12**, Ac-Ftr-Pro-Phe-His-Sta-NH_2 (IC_{50} = 3.8×10^{-9} M), the corresponding P_1-P_1' Leuψ[CH(OH)CH_2]Val and Leuψ[CH_2NH]Val derivatives (cf. **13** and **14**, Figure 4) were tested. The Ac-Ftr-Pro-Phe-His-Leuψ[CH(OH)CH_2]Val-NH_2 was more potent (IC_{50} = 3.1×10^{-10} M) than the pentapeptide **12**, but it was found to be chemically unstable due to acid-catalyzed lactonization at the C-terminal P_1-P_1' Leuψ[CH(OH)CH_2]Val-NH_2 functionality (see the Experimental Section for further details). The Ac-Ftr-Pro-Phe-His-Leuψ[CH_2NH]Val-NH_2 (IC_{50} = 2.1×10^{-8} M) was only slightly less potent than the P_1-P_1' Sta substituted congener **12**. N-Terminal truncation of the P_5 Ftr moiety of pentapeptide **12** resulted in approximately a 1000-fold decrease in renin-inhibitory potency (cf. Ac-Pro-Phe-His-Sta-NH_2, **15**; IC_{50} = 5.8×10^{-6} M). A similar difference in biological activities was observed for the highly potent hexapeptide Ac-Ftr-Pro-Phe-His-Sta-Ile-NH_2 (**17**; IC_{50} = 1.0×10^{-10} M), relative to its des-Ftr derivative **16**. The P_2' Ile residue of compound **17** provided enhanced (ca. 5–40-fold) potency versus congeners having Val or lacking a C-terminal amino acid residue at this site (cf. compounds **11** and **12**). Similar to the P_1-P_1' Phe-Phe substituted RIP series, P_5 Ftr substitution in compound **17** by Trp, His, or D-Ftr resulted in diminished (ca. 10–70-fold) renin-inhibitory activities (cf. compounds **18–20**). This comparison of the structural and stereochemical requirements of the P_5 Ftr substitution in both the templates of compounds **1** and **17** suggested that N-terminal chemical modifications may be introduced independently of the P_1-P_1' substitution to provide for improved biological properties (e.g., renin-inhibitory potency). Based on these structure–activity data, molecular modeling of compounds **1** and **17** was performed (vide infra) to further investigate the structure–conformation relationships of these two inhibitor leads at the active site of a computer-simulated model of human renin.[31]

The in vitro selectivity of several of the above P_1-P_1' Sta substituted peptides to inhibit human renin versus porcine renin was then explored (Table III). Compound **10** was potent (IC_{50} = 4.9×10^{-9} M) against porcine renin, although it was about 10-fold more potent against human renin. Similar trends in the relative order of human or porcine inhibitory potencies were found for two subsets of P_5 modified derivatives of **10**: (1) Ac-Ftr-Pro-Phe-His-Sta-Ile-NH_2 was more potent (ca. 10–60-fold) than Ac-His-Pro-Phe-His-Sta-Ile-NH_2 and (2) Ac-Ftr-Pro-Phe-His-Sta-NH_2 was markedly more potent (ca. 1000-fold) than Ac-Pro-Phe-His-Sta-NH_2. However, striking differences (ca. 100–1000-fold) in the absolute IC_{50} values were noted for each of the above four inhibitors as measured against the two enzymes. For example, compound **17** was highly potent (IC_{50} = 1.0×10^{-10} M) against human plasma renin but was only moderately potent (IC_{50} = 1.2×10^{-7} M) against porcine kidney renin. Similarly, the human renin selectivity of compound **17** was also shown by Ac-Ftr-Pro-Phe-His-Sta-NH_2 (**12**), which was a weak inhibitor (IC_{50} = 2.4×10^{-6} M) of porcine renin versus its high potency (IC_{50} = 3.8×10^{-9} M) against human renin. The C-terminal P_2' and P_3' residues, therefore, were apparently important for the inhibition of porcine renin but not human renin. The lack of selectivity for **10** was similar to data previously described[20] for SCRIP (Table III), which elicited an $IC_{50} \approx 10^{-8}$ M against both human plasma and porcine kidney renin. In addition to the above studies, compound **10** was investigated to determine its kinetic properties (Figure 5) on purified human kidney renin with the natural substrate ANG. Compound **10** was shown to be a potent, competitive inhibitor of human kidney renin and effected a K_I = 6.7×10^{-11} M (versus compound **1**, K_I = 2.7×10^{-8} M).[22] Comparative kinetic analysis of **10** and selected congeners on human recombinant renin[39] is currently in progress to be integrated with computer-assisted molecular modeling studies (vide infra) to identify and quanitiate potential inhibitor–enzyme intermolecular interactions.

Molecular Modeling Analysis of Human Renin–Inhibitor Interactions. The 3-D structure of human renin is yet unknown by crystallographic methods. However, X-ray diffraction studies[40–43] of several aspartic acid proteases (i.e., from *Rhizopus chinensis*, *Penicillium janthanellum*, and *Endothia parasitica*), which exhibit varying degrees (ca. 30–40%) of sequence homology to human renin, have been determined at a resolution of 1.8–3.0 Å. Molecular models[44,45] have been constructed from the electron-density maps for the above proteins and pepstatin-based inhibitory ligands bound at their respective active sites. Based on these data, several 3-D models

(39) Poorman, R. A.; Palermo, D. P.; Post, L. E.; Murakami, K.; Kinner, J. H.; Smith, C. W.; Reardon, I.; Heinrikson, R. L. *Proteins: Struct., Funct., and Genet.* **1986**, *1*, 139.
(40) Subramanian, E.; Swan, I. D. A.; Liu, M.; Davies, D.; Jenkins, J. A.; Tickle, I. J.; Blundell, T. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 556.
(41) James, M. N. G.; Sielecki, A. R. *J. Mol. Biol.* **1983**, *163*, 299.
(42) Pearl, L.; Blundell, T. *FEBS Lett.* **1984**, *174*, 96.
(43) Davies, D. 1.8-Å structure of rhizopuspepsin (personal communication).
(44) Bott, R.; Subramanian, E.; Davies, D. R. *Biochemistry* **1982**, *21*, 6956.
(45) James, M. N. G.; Sielecki, A.; Salituro, F.; Rich, D. H.; Hofman, T. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 6137.

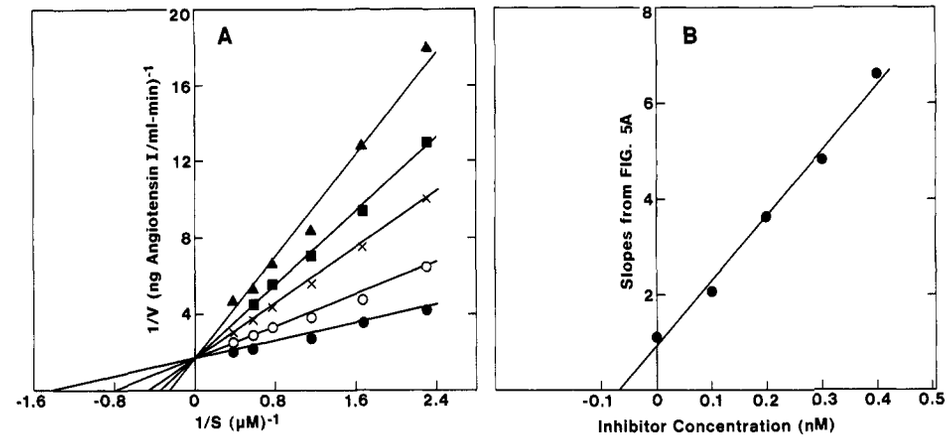

Figure 5. Human renin inhibition kinetic studies of compound **10** at pH 6.0 and 37 °C. (a) Double-reciprocal plot showing competitive inhibition of the reaction by 0 nM (●), 0.10 nM (○), 0.20 nM (X), 0.30 nM (■), and 0.40 nM (▲) of compound **10**. Enzyme and inhibitor were preincubated for one hour at 37 °C and reactions were initiated by the addition of angiotensinogen. (b) Replot of the slopes from panel A against inhibitor concentration to determine the K_I = 67 pM of compound **10** from the abscissa intercept.

of human renin have been constructed[31-33] to provide insight for the design of potent, renin-specific inhibitors. In this study we explored the structure–conformation properties of compounds **1** and **17** by integration of data that included: (1) crystallography studies of structurally homologous aspartic acid protease–inhibitor complexes, (2) structure–activity relationships of peptides tested in this survey, and (3) molecular modeling and dynamics of compounds **1** and **17** to a computer-simulated human renin active site (hereinafter referred to as CKH-RENIN) developed by Carlson, Karplus, and Haber[31] and conformational analysis and potential energy refinement of these two renin inhibitors by the computational programs CHARMM[46] and CONFO.[47] The overall objectives of the molecular modeling of compounds **1** and **17** were to provide insight into their enzyme-bound conformation as well as develop a detailed understanding of the architecture of the binding pockets (i.e., S_5 to S_3') of the CKH-RENIN active site. The tertiary structure of the CKH-RENIN model (Figure 6) illustrates the predicted bilobal 3-D design of the enzyme in which a relatively hydrophobic active site cleft exists between the N-terminal and C-terminal domains. In addition, two Asp residues (i.e., Asp-37 and Asp-215) are juxtaposed at the active site and are proposed to be functionally important for the catalysis of ANG cleavage (i.e., hydrolysis of the P_1-P_1' Leu-Val dipeptidyl moiety). Compounds **1** and **17** were each docked into the active site of the CKH-RENIN model (see the Experimental Section for details) to achieve energetically favorable intermolecular interactions within the ligand–enzyme complex. This modeling included maximization of hydrogen bonding between the inhibitor backbone and CKH-RENIN as well as hydrophobic interactions between the ligand side-chain moieties and the enzyme. In addition, unfavorable steric interactions were minimized. The macromolecular energy refinement program CHARMM was then used to provide the title CKH-RENIN–inhibitor complexes, which were explored by molecular dynamics (Figures 6 and 7). The polyamide backbones of both compounds **1** and **17** are proposed to exist in a twistlike secondary structure and the amino acid side-chain moieties at the P_5, P_3, and P_1 sites (i.e., Ftr, Phe, and Phe/Sta) were observed to constitute an aromatic, hydrophobic surface for each inhibitor that was buried in the CKH-RENIN active site (Figure 7). In particular, the P_5 Ftr residue of both compounds **1** and **17** interacts with a hydrophobic pocket derived from five aromatic residues (i.e., Tyr-14, Tyr-230, Tyr-254, Phe-123, and Tyr-20). The spatial orientation of the P_5 Ftr, P_3 Phe, and P_1 Phe/Sta residues in both CKH-RENIN·**1** and CKH-RENIN·**17** models could provide significant shielding (>50%) of the enzyme subsites (i.e., S_5, S_3, and S_1) from accessible solvent. In the CKH-RENIN·**17** model the backbone torsion angles (e.g., Φ and Ψ) of the ligand were determined (Figure 7). In addition, the following potential H-bond interactions between the enzyme active site and the inhibitor were identified: (1) P_5 Ftr C^α—N—H···O=C—C^α Glu-287, (2) P_3 Phe C^α—C=O···H—N—C^α Ser-229, (3) P_2 His C^{im}—N···H—N—C^α Tyr-230, (4) P_1-P_1' Sta C_3—O—H···O=C-C^β Asp-225, (5) P_1-P_1' Sta C_2—C=O···H—O—C^β Ser-83, and (6) P_2' Ile CONH—H···O=C—C^α Tyr-82. The intermolecular H-bond distances and angles for these specified interactions ranged from 2.9 to 3.5 Å (where H-bond distance is defined between donor and acceptor atoms) and from 3° to 35° (where 0° defines colinearity of the H-bond donor, H-bond acceptor, and H atom), respectively. Noteworthy was the P_1-P_1' Sta C-3 hydroxyl of **17** that was found to be 2.9 Å from the Asp-37 side-chain carbonyl of the CKH-RENIN active site, thus providing possible intermolecular H-bonding directly between the inhibitor and the catalytic center of the enzyme. Overall, the above 3-D molecular models for the CKH-RENIN–inhibitor complexes may explain, in part, the enhanced binding affinities of compounds **1** and **17** versus structurally related compounds (refer to Tables I and II) and account for the observed regioselective and stereoselective intermolecular hydrophobic and/or π–π bonding interactions between the P_5 Ftr side chain and its complementary S_5 binding subsite. In our inhibitor models the P_5 Ftr and P_2 His side chain are not spatially proximate to each other, and the secondary structure of the P_5-P_2 sequence more closely approximates an extended conformation versus the postulated β-turn or reverse-turn conformation previously reported[48,49] for cyclic analogues (i.e., P_5 to P_2 disulfide bridged) of P_1-P_1' Leu-Leu or Sta modified, ANG-based inhibitors of renin. In contrast, the conformational features of the P_1-P_3' sequence of compound **1** were similar to crystallographic structures of H-Leu-Leu-Val-Tyr-OMe and a derivative as previously reported.[50] Finally, a recent

(46) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; Stades, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187.
(47) Duchamp, D. J. In *Computer-Assisted Drug Design*, ACS Symp. Series No. 112; Olson, E. C., Christoffersen, R. E., Eds.; American Chemical Society: Washington, DC, 1979; p 79.
(48) Liepina, I.; Nikiforovich, G. V.; Paiva, A. C. M. *Biochem. Biophys. Res. Commun.* **1984**, *122*, 700.
(49) Boger, J. In ref 22, 1983; p 569.

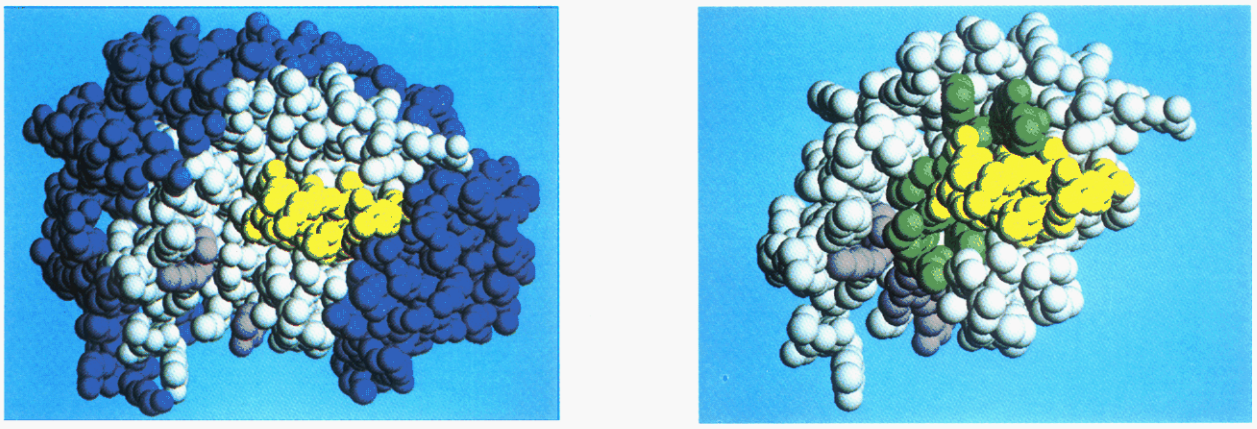

Figure 6. Computer graphics representation of the molecular dynamics averaged 3D structures of the CKH-RENIN·1 model. The active site of the enzyme is shown (CPK space-filling display, left) in which the N-terminal flap region (residues 75–85) is highlighted in yellow and the active site is shown in white. In addition, five aromatic residues (i.e., Tyr-14, Tyr-230, Tyr-254, Phe-123, and Tyr-20) which contribute to the S_5 hydrophobic pocket are colored in purple. The CKH-RENIN·1 active site structure is shown (CPK spacefilling display, right) in which the P_5 Ftr is colored in purple and all other amino acid residues of compound **1** are colored in green. Specific intermolecular interactions of the inhibitor–enzyme complex are further described in the text. The computer graphics shown in this report were generated by using a IRIS 2400/TURBO workstation and an UPJOHN/VIRIS display program written by D. C. Rohrer.

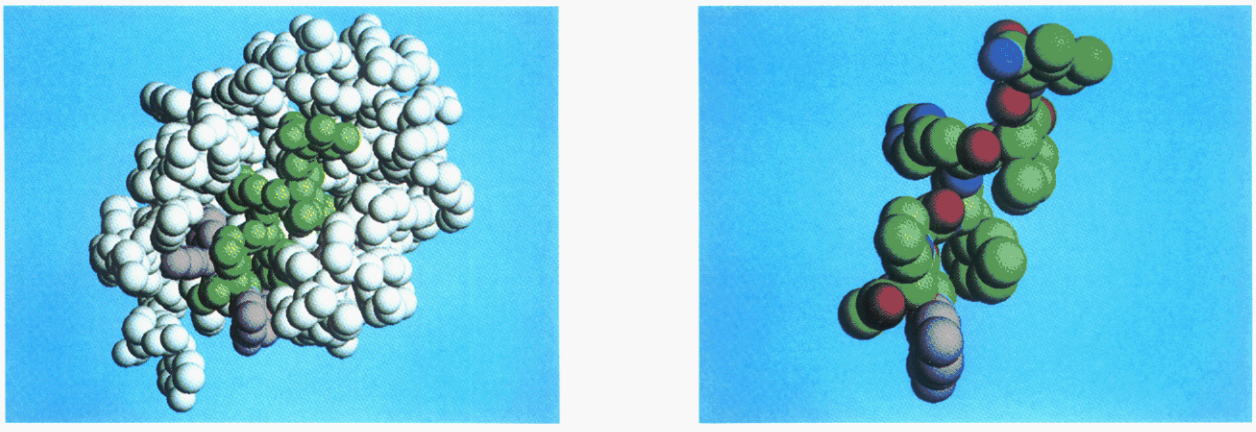

Figure 7. Computer graphics representation of the molecular dynamics averaged 3D structures of the CKH-RENIN-17 model. The CKH-RENIN active site is shown (CPK space-filling display, left) in which the N-terminal flap region (i.e., residues 75-85) of the N-terminal lobe of CKH-RENIN has been eliminated to provide improved visualization of the intermolecular contacts. Compound **17**, itself, is shown (CPK spacefilling display, right) after omission of the enzyme active-site residues. The P_5 Ftr side chain is highlighted in purple, carbon atoms in green, oxygen atoms in red, and nitrogen atoms in blue. The P_5-P_2 backbone torsion angles of **17** are P_5 Ftr, Φ, Ψ (−115°, 105°); P_4 Pro, Φ, Ψ (−70°, 75°); P_3 Phe, Φ, Ψ (−79°, 89°); P_2 His, Φ, Ψ (−136°, 107°).

Table IV. A Synopsis of Reported Renin Inhibitory Peptides

	compound	IC_{50} or K_I, M
RIP	H–Pro–His–Pro–Phe–His–Phe–Phe–Val–Tyr–Lys–OH	2.3×10^{-6}
H-142[a]	H–Pro–His–Pro–Phe–His–Leuψ[CH_2NH]Val–Ile–His–Lys–OH	1.0×10^{-8}
SCRIP[b]	Iva–His–Pro–Phe–His–Sta–Leu–Phe–NH_2	1.6×10^{-8}
CGP2928[c]	Z–Arg–Arg–Pro–Phe–His–Sta–Ile–His–Lys(N^{ϵ}–Boc)–OMe	1.0×10^{-9}
U-71038[d]	Boc–Pro–Phe–N[Me]His–Leuψ[CH(OH)CH_2]Val–Ile–Amp	1.1×10^{-10}
U-70504E	Ac–Ftr–Pro–Phe–His–Sta–Ile–NH_2	1.0×10^{-10}

[a] Reference 51. [b] Reference 52. [c] Reference 53. [d] Reference 54.

crystallographic investigation[43] of rhizopuspepsin in which a P_1-P_1' Pheψ(CH_2NH)Phe substituted renin inhibitor, H-D-His-Pro-Phe-His-Pheψ(CH_2NH)Phe-Val-Tyr-OH, was bound to the enzyme active site has been successfully completed. This provides the opportunity to examine an ANG-based ligand at two different, but structurally homologous, aspartyl proteases in terms of comparative structure–conformation–activity analysis.

Concluding Remarks. In summary, we have explored the structure–activity relationships of ANG-based inhibitory peptides having P_5 His substitution by Ftr, P_1-P_1' Leu-Val replacements by Phe-Phe, Sta, Leuψ[CH(OH)-CH_2]Val, or Leuψ[CH_2NH]Val, and C-terminal modification. The markedly improved potency (ca. 10–1000-fold) of P_5 Ftr versus His or Ac (cf. analogues **12** and **16**–**19**) provided the feasibility of complete C-terminal truncation (i.e., omission of the P_2' and P_3' residues) and examination of the intrinsic properties of P_1-P_1' modifications. For example, relative to Ac-Ftr-Pro-Phe-His-Phe-Phe-NH_2 (RIP derivative **3**; $IC_{50} > 10^{-4}$ M), a potency increase of more than 100 000-fold was obtained by substitution of the P_1-P_1' Phe-Phe by Sta, Leuψ[CH(OH)CH_2]Val, or Leuψ-

(50) Precigoux, G.; Geoffre, S.; Hospital, M. In ref 22, 1983, p 599.

Table V. Repetitive Coupling Procedure for Solid-Phase Peptide Synthesis

step	reagents–solvent[a]	repetitions	time
1	CH_2Cl_2 (15 mL)	3	1.5 min
2a	45% TFA–3% anisole–CH_2Cl_2 (15 mL)	1	2.0 min
2b	45% TFA–3% anisole–CH_2Cl_2 (15 mL)	1[b]	25.0 min
3	CH_2Cl_2 (15 mL)	3	1.5 min
4	10% DIEA–CH_2Cl_2 (15 mL)	2	5.0 min
5	CH_2Cl_2 (15 mL)	3	1.5 min
6	DMF (15 mL)	3	1.5 min
7a	N^{α}–Boc–amino acid derivative (1.5 mmol)–CH_2Cl_2 (5 mL)		
7b	DCC(1.2 mmol)–CH_2Cl_2(5 mL)[c]	1	3 h[d]
7c	HOBT(1.5 mmol)–DMF(5 mL)[c]		
8	DMF (15 mL)	3	1.5 min
9	CH_2Cl_2 (15 mL)	3	1.5 min
10	EtOH (15 mL)	3	1.5

[a] Based on 1 g of initial Boc-amino acid resin and a 0.5-mmol scale of synthesis. [b] For Boc deprotection of His- or Trp-substituted resin intermediates, two 25-min steps were performed. [c] Boc-His(N^{im}–Tos)·Dcha was coupled using BOP. [d] In the case of incomplete acylation (based on ninhydrin assay) a second coupling (steps 3–7) was performed or the peptide–resin intermediate was acetylated with Ac_2O(1.5 mmol) in CH_2Cl_2.

[CH_2NH]Val (cf. compounds **12–14**, respectively). Recently, the in vitro and in vivo biological activities of structurally related, ANG-based renin-inhibitory peptides having such P_1-P_1' modifications have been reported[51-54] (for examples, see Table IV). The correlation of in vitro and in vivo structure–activity relationships remains a challenge in this area of research. However, noteworthy achievements have been made in terms of the design of highly potent and metabolically stable renin inhibitors.[19] Exemplifying this research was the development of the P_2 N[Me]His incorporation in the highly potent renin inhibitor Boc-Pro-Phe-N[Me]His-Leuψ[CH(OH)CH_2]Val-Ile-Amp (U-71038, Table V) as previously described by Thaisrivongs and co-workers,[19b] which provided stability to the peptide against endopeptidase-catalyzed (i.e., chymotryptic) cleavage of the P_3-P_2 Phe-His dipeptidyl moiety. Similarly, we have found[38] (unpublished data) that the P_2 N[Me]His derivative of **17** (i.e., Ac-Ftr-Pro-Phe-N-[Me]His-Sta-Ile-NH_2; $IC_{50} = 2.2 \times 10^{-10}$ M) possesses significant in vivo activity in the sodium-deplete monkey. The comparative renin (human, monkey, and porcine) inhibitory activities in vitro and in vivo of this compound will be described in a future paper.

The paucity of structure–activity studies that have examined Ftr in peptides per se has provided us impetus to more thoroughly investigate this highly hydrophobic, hetereoaromatic aminoacyl derivative in selected renin-inhibitor templates by P_5 side-chain modifications and analysis of these derivatives by biological testing, spectroscopy, and molecular modeling. Preliminary results of a part of this research (e.g., analogues of **17** having P_5 Nal, Tyr, Phe, and Ada substitutions) have been disclosed.[38] Similar approaches to correlate structure–activity relationships of peptides to specific molecular properties (i.e., hydrophobicity, electronic, and/or steric) at selected aminoacyl positions by systematic side-chain modifications have been previously reported.[16,55-59] To further experimentally verify our molecular modeling of specific inhibitors to the CKH-RENIN active site described in this paper, we are currently pursuing human renin (kidney or recombinant[39]) crystal structure complexes in which an inhibitor (e.g., **1** or **17**) is bound at the enzyme active site. Recent fluorescence quenching studies by Epps and co-workers (personal communication) on recombinant human renin, and anti-human renin antibody experiments by Carlson and co-workers[60] have provided data that have been in accord with the predicted 3-D structural properties of the CKH-RENIN model. Other documented molecular modeling studies on ANG-based inhibitors of human renin that may be compared to our studies include (1) molecular modeling of the hexapeptide Boc-Phe-His-Leuψ-[CH_2NH]Val-Ile-His-OMe at a computer-simulated human renin active site as described[61] by Plattner and co-workers, (2) cystallographic evaluation of the decapeptide H-Pro-His-Pro-Phe-His-Leuψ[CH_2NH]Val-Ile-His-Lys-OH at the active site of endothiapepsin as described[62] by Hallett and co-workers, and (3) molecular modeling of the tetrapeptide Boc-Phe-His-Sta-Leu-(4-amido-1-benzylpiperidine) to a physical (i.e., ball-and-stick) model of human renin as described[63] by Akahame and co-workers. In summary, the predicted enzyme–ligand intermolecular interactions (e.g., hydrophobic and H-bond) and conformational properties of the peptide backbones of the above renin inhibitors are in general agreement with our molecular modeling studies on compounds **1** and **17** at the CKH-RENIN active site. Such molecular mapping of renin binding subsites to ANG_{6-13}-based analogues may provide insight to the design of future renin inhibitory peptides and/or peptide mimetics. This general strategy of integrating protein crystallography, comparative model building, and computer graphics to provide insight into rational drug design for various therapeutic objectives has been recently reviewed[64] by Hol. Other computational methodologies that we are currently pursuing to explore the structural and molecular properties of selected P_1-P_1' modified, ANG-based inhibitors of human renin and extend our drug design efforts are similar to those previously documented, including (1) analysis of the conformational and dynamics behavior of the cyclic peptide hormone vasopressin by Hagler and co-workers,[65] (2) application of distance ge-

(51) Leckie, B. J.; Grant, J.; Hallett, A.; Hughes, M.; Jones, D. M.; Szelke, M.; Tree, M. *Scott. Med. J.* **1984**, *29*, 125.
(52) Blaine, E. H.; Schorn, T. W.; Boger, J. *Hypertension* **1984**, *6*, I-111.
(53) Wood, J. M.; Gulati, N.; Forgiarini, P.; Fuhrer, W.; Hofbauer, K. G. *Hypertension* **1985**, *7*, 797.
(54) Pals, D. T.; Thaisrivongs, S.; Lawson, J. A.; Kati, W. M.; Turner, S. R.; DeGraaf, G. L.; Harris, D. W.; Johnson, G. A. *Hypertension* **1986**, *8*, 1105.
(55) Nestor, J. J., Jr.; Tahilramani, R.; Ho, T. L.; McRae, G. I.; Vickery, B. H. *J. Med. Chem.* **1984**, *27*, 1170.
(56) Fauchere, J.-L. *J. Med. Chem.* **1982**, *25*, 1428.
(57) Nadasdi, L.; Medzihradszky, K. *Biochem. Biophys. Res. Commun.* **1981**, *99*, 451.
(58) Pliska, V. *Experientia* **1978**, *34*, 1190.
(59) Jorgensen, E. C.; Weinkam, R. J. In *Peptides 1971*, Proceedings of the 11th European Peptide Symposium; Nesvadba, H., Ed.; North Holland: Amsterdam, 1973; p 311.
(60) Evin, G.; Carlson, W. D.; Hanschumacher, M.; Novotony, J.; Matseuda, G. R.; Haber, E.; Bouhnik, J.; Galen, F. X.; Menard, J.; Corvol, P. *Hypertension* **1986**, *8*, II-72.
(61) Plattner, J. J.; Greer, J.; Fung, A. K. L.; Stein, H.; Kleinert, H. D.; Sham, H. L.; Smital, J. R.; Perun, T. J. *Biochem. Biophys. Res. Commun.* **1986**, *139*, 982.
(62) Hallett, A.; Jones, D. M.; Atrash, B.; Szelke, M.; Leckie, B. J.; Beattie, S.; Dunn, B. M.; Valler, M. J.; Rolph, C. E.; Kay, J.; Foundling, S. I.; Wood, S. P.; Pearl, L. H.; Watson, F. E.; Blundell, T. L. In *Aspartic Proteinases and Their Inhibitors*; Kostka, V., Ed.; deGruyter: New York, 1985; p 467.
(63) Akahane, K.; Umejama, H.; Nakagawa, S.; Moriguchi, I.; Hirose, S.; Iizuka, K.; Murakami, K. *Hypertension* **1985**, *7*, 3.
(64) Hol, W. G. J. *Angew. Chem.* **1986**, *25*, 767.
(65) Hagler, A. T.; Osguthorpe, D. J.; Dauber-Osguthorpe, P.; Hempel, J. D. *Science (Washington, D.C.)* **1985**, *25*, 1309.

Table VI. AAA and FAB-MS Data

compd	amino acid analysis									FAB-MS $(M^* + H)^+$ at m/z
	Pro	His	Phe	Val	Tyr	Lys	Trp	Gly	Ile	
RIP	2.08	2.00	2.94	0.99	0.97	1.02	[c]	[c]	[c]	1318
1	0.99	1.04	3.14	0.95	[c]	[c]	2.05	[c]	[c]	1262
2	0.99	1.00	3.04	0.98	[c]	[c]	1.05	[c]	[c]	1048
3	1.03	1.02	3.01	[c]	[c]	[c]	0.94	[c]	[c]	949
4	[c]	0.96	2.94	1.05	[c]	[c]	1.98	1.00	[c]	1222
5	0.93	1.04	3.00	0.99	[c]	[c]	2.05	[c]	[c]	1262
6	0.99	1.03	2.97	0.88[a]	[c]	[c]	1.95	[c]	[c]	1234
7	1.00	2.00	2.92	0.98	[c]	[c]	1.03	[c]	[c]	1185
8	1.03	2.03	2.97	0.93	[c]	[c]	0.66[b]	[c]	[c]	1185
9	0.98	1.09	3.05	0.83[a]	[c]	[c]	1.97	[c]	[c]	1262
10	0.97	1.00	1.01	1.04	[c]	[c]	1.77	[c]	[c]	1125
11	0.97	1.00	1.00	1.01	[c]	[c]	1.02	[c]	[c]	911
12	0.99	0.95	1.01	[c]	[c]	[c]	1.04	[c]	[c]	812
13	1.02	1.00	1.00	[c]	[c]	[c]	0.90	[c]	[c]	868
14	1.05	0.98	1.03	[c]	[c]	[c]	1.05	[c]	[c]	853
15	0.99	0.91	1.00	[c]	[c]	[c]	[c]	[c]	[c]	598
16	0.99	0.96	1.00	[c]	[c]	[c]	[c]	[c]	1.01	711
17	1.00	1.00	1.02	[c]	[c]	[c]	0.96	[c]	1.00	925
18	0.99	1.01	1.01	[c]	[c]	[c]	0.96	[c]	0.99	897
19	0.95	1.96	1.03	[c]	[c]	[c]	[c]	[c]	1.04	848
20	0.98	1.03	0.97	[c]	[c]	[c]	0.92	[c]	1.01	925

[a] Low values for Val were frequently observed unless prolonged hydrolysis (i.e., 48–72 h) was performed (data not shown). [b] Low recoveries of Trp were periodically observed and are presumedly due to oxidative destruction of the labile indole group. [c] Amino acid not present in title compound.

ometry techniques to the nicotinic pharmacophore by Sheridan and co-workers,[66] and (3) studies on the docking of flexible ligands to macromolecular receptors by molecular shape by Des Jarlais and co-workers.[67]

Experimental Section

Materials. *p*-Methylbenzhydrylamine (*p*-MBHA) and benzhydrylamine (BHA) resins (each ca. 0.5 mequiv of amine g^{-1}) were purchased from U.S. Biochemical Corp. and Peptides International. *p*-Chloromethyl-poly(styrene-co-divinylbenzene) (Merrifield) resin (ca. 0.7 mequiv of chloride g^{-1}) was purchased from Lab Systems Incorporated. N^{α}-*tert*-butyloxycarbonyl (Boc) protected amino acid derivatives were purchased from Pennisula Laboratories and Peptides International. N^{α}-Boc-Sta-OH, N^{α}-Boc-Leuψ[CH(OTBDMS)CH_2]Val-OH, and N^{α}-Boc-Leuψ-[CH_2NH]Val-OH were prepared by reported[19,68,69] procedures. The reactive side chains of the amino acids were protected as follows: His, N^{im}-Tos or N^{im}-Bom; Lys, N^{ϵ}-2-Cl-Z; Tyr, O-2,6-Cl_2-Bzl; and Trp, N^{in}-For. Before use, all amino acid derivatives were tested for authenticity by melting point determination and ninhydrin assay.[70] ACS grade (or higher) methylene chloride (CH_2Cl_2), *N,N*-dimethylformamide (DMF), and absolute ethanol (EtOH) were purchased from Burdick and Jackson. Trifluoroacetic acid (TFA) was purchased from Columbia Organic Chemical. Diisopropylethylamine (DIEA), anisole, acetic anhydride (Ac_2O), and *N*-acetylimidazole were purchased from Aldrich. All reagents and solvents were used without further purification, except the DMF, which was stored over 4-Å molecular sieves, and TFA, which was redistilled prior to use.

Peptide Synthesis. The general methodology used for the preparation of compounds **1–20** is described here. Selected peptides are also described (vide infra) in further detail. Compounds **1–20** were each prepared on either automated (Beckman 990B or Peptides International PS-2000) or manually operated instruments by a solid-phase[71] synthetic procedure outlined in Table V. The coupling reactions were effected by the use of *N,N'*-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT), except for the case of Boc-His(N^{im}-Tos)·dicyclohexylamine (Dcha) salt, which was coupled with benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP). In repeated syntheses of selected compounds described here, it is noted that we have found the use of N^{α}-Boc-His(N^{im}-Bom)-OH to be preferential to its N^{im}-Tos derivative in terms of providing higher purity of crude products. The reactions were monitored by the ninhydrin assay.[70] Subsequent to the coupling reaction, removal of the Boc protecting group was effected by the use of 45% TFA–3% anisole (v/v) in CH_2Cl_2 and the neutralization of the intermediate peptide-resin trifluoroacetate salts was effected by 10% DIEA in CH_2Cl_2. Compound **6** was prepared by incorporating Boc-Trp-OH at the N-terminus. N-Acetylation was achieved by Ac_2O for compounds **1–9** and by *N*-acetylimidizole for compounds **10–20**. This procedure eliminated possible O- or N-acetylations of the P_1-P_1' Sta, Leuψ[CH_2NH]Val, and Leuψ-[CH(OH)CH_2]Val functionalities. The scale of synthesis was derived from the substitution (mmol g^{-1}) value of the starting Boc-amino acid resin either by quantitative amino acid analysis[72] on a Dionex Model D-500 amino acid analyzer following hydrolysis (50% 6 M hydrochloride acid–0.1% phenol in propionic acid in a vacuum sealed tube at 110 °C for 24 h) of a 3–5-mg sample or by weight-gain determination (versus the unacylated resin precursor). Following solid-phase synthesis, the title peptides were cleaved (60 min at 0–4 °C) from their resins by 16% anisole–84% hydrogen fluoride (ca. 15 mL g^{-1} peptide-resin intermediate), which simultaneously removed all side-chain protecting groups except for the N^{in}-For of Ftr-substituted compounds. After evaporation in vacuo of the hydrogen fluoride–anisole, the peptide-resin products were washed with ethyl ether and the crude peptides extracted with aqueous acetic acid (HOAc) and lyophilized to yield amorphous powders. Compound **18** was prepared by alkaline hydrolysis[73] of a portion of a crude (i.e., post HF cleavage) sample

(66) Sheridan, R. P.; Nilakantan, R.; Dixon, J. S.; Venkataraghavan, R. *J. Med. Chem.* **1986**, *29*, 899.
(67) Des Jarlais, R. L.; Sheridan, R. P.; Dixon, J. S.; Kuntz, I. D.; Venkataraghavan, R. *J. Med. Chem.* **1986**, *29*, 2149.
(68) Rich, D. H.; Sun, E. T.; Boparai, A. S. *J. Org. Chem.* **1978**, *43*, 3624.
(69) Parry, M. J.; Russell, A. B.; Szelke, M. In *Chemistry and Biology of Peptides*; Meienhofer, J., Ed.; Ann Arbor Science Publishers: Ann Arbor, MI; p 541.
(70) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.
(71) For a recent review of solid-phase peptide synthesis as well as other related chemistry methods, refer to: Stewart, J. M., Young, J. D. In *Solid Phase Peptide Synthesis*, Pierce Chemical Co.: Rockford, IL, 1984.
(72) Spackman, D. H.; Stein, W. H.; Moore, S. *Anal. Chem.* **1958**, *30*, 1190.
(73) Lemaire, S.; Yamashiro, D.; Li, C. H. *J. Med. Chem.* **1976**, *19*, 373.

of the precurser **17**.

Peptide Purification and Physiochemical Characterization. The crude peptides (ca. 100-mg sample load) were subjected to semipreparative reverse-phase HPLC on a Vydac C18 (15–20-μm particle size) column (2.2 cm i.d. × 29.0 cm length) using a binary solvent system consisting of solvent A (10% acetonitrile–0.2% TFA in H_2O) and solvent B (70% acetonitrile–0.2% TFA in H_2O). An exception to this solvent system was made for the purification of compound **13** in which the TFA was not added to solvents A and B in order to minimize lactonization of the C-terminal Leuψ[CH(OH)CH_2]Val-NH_2, which otherwise was essentially complete within 24 h in aqueous acid solutions. A flow rate of 2.0–2.5 mL min^{-1} (ca. 100 psi back pressure) was used and a typical chromatographic mobile-phase program was as follows: 17% solvent B isocratic (15 min), 17–67% solvent B linear gradient (210 min), and 67% solvent B isocratic (75 min). Elution of the peptide was monitored at 280 (or 254) nm, and 4.0–5.0-mL post column fractions were collected. The title peptides were obtained by pooling of the desired fractions, removal of the acetonitrile by rotary evaporation in vacuo, and lyophilization. AAA of compounds **1–20** (Table VI) were performed as described above after hydrolysis (0.1% phenol in 6 M hydrochloric acid in a vacuum sealed tube at 100 °C for 24 h). No corrections were made for the destruction of amino acids during hydrolysis. Fast atom bombardment mass spectrometry (FAB-MS) of compounds **1–20** (Table VI) were performed with a Varian-MAT-CH5-DF or a VG-ZAB-2F mass spectrophotometer. Thin-layer chromatography (TLC) of compounds **1–20** (Table VII) was performed with silica GF (Analtech) plates (ca. 50–100-μg samples; running lengths were 12–18 cm) and the following solvent system: A, 1-butanol–HOAc–pyridine–H_2O (15:3:10:8, v/v/v/v); and B, EtOAc–pyridine–HOAc–H_2O (5:2:1:1, v/v/v/v). Detection for the TLC was made by iodine vapor or ninhydrin spray. Analytical HPLC on a Beckman-344 gradient liquid chromatography system was used to determine the k' values and percent purities of compounds **1–20** (Table VII) on a Synchropak C18 (6.5-μm particle size) column (4.1 mm i.d. × 250 mm length); other chromatographic protocol included a flow rate of 1.5 mL min^{-1} (ca. 2000 psi back pressure), a standard binary solvent mobile phase program (17% solvent B isocratic for 2 min; 17–100% solvent B linear gradient over 20 min), and detection at 220 and 280 (or 254) nm. Optical rotations of selected compounds were performed with a Perkin-Elmer-141 polarimeter.

Molecular Modeling Methods. The 3-D coordinates of the structural models for the aspartic acid proteases (i.e., rhizopuspepsin, penicillopepsin, and endothiapepsin) were obtained from the Brookhaven Protein Databank and individual investigators (D. Davies and T. Blundell). The structures were displayed with an Evans and Sutherland PS340 high-performance graphics system and FRODO[74] or MOSAIC[75] software. The 3-D structure of pepstatin at the active site of rhizopuspepsin was obtained from electron-density maps calculated by the difference Fourier technique.[44] This 3-D structure for pepstatin provided an initial template for these investigations of compounds **1** and **17** at the active site of the previously described[31] CKH-RENIN model. The P_3-P_3' residues of pepstatin (i.e., Val-Val-Sta-Ala-Sta sequence) were structurally mutated to provide the corresponding Phe-His-Sta-Ile sequence of **17**, and the X_1 and X_2 angles of pepstatin were conserved accordingly. Further structural mutation was required to provide the C-terminal tetrapeptide sequence, Phe-Phe-Val-Ftr, of **1**, and this required computer graphics based modeling at the CKH-RENIN active site. A similar modeling strategy was required to provide the N-terminal Ftr-Pro sequence of both compounds **17** and **1**. This modeling was performed in order to maximize H bonds from the polyamide backbone of each inhibitor to the CKH-RENIN active site as well as hydrophobic interactions from the amino acid side chains of the ligand to the enzyme. In addition, unfavorable steric interactions were minimized. Similar to methods previously reported[31] for the human renin model, the CKH-RENIN·**17** and CKH-RENIN·**1** complexes were refined by using the program CHARMM.[46] Constraints were placed on the spatial disposition of ligand atoms from their initial coordinates and reduced every 25 cycles for the first 100 cycles. An additional 100 cycles were completed without constraints on either the enzyme or ligand.

Torsion angles at the N-terminus of the polyamide backbone of compound **1** were varied in order to explore the P_5 Ftr side chain, in particular, with respect to CKH-RENIN binding subsites, which might provide significant hydrophobic interactions. Specifically, a conformation of **1** was obtained in which the P_5 Ftr side chain attained close contact with several aromatic amino acid residues (e.g., Tyr-230 at 6.0 Å and Tyr-254 at 4.5 Å) derived from the C-terminal lobe of the CKH-RENIN model. To explore the conformation space available to compound **1** in this putative binding geometry, molecular dynamics simulation of the CKH-RENIN·**1** complex was performed in which constraints were placed on residues outside of an approximate 10-Å core of the CKH-RENIN about the docked compound **1**. The energy-refined structure of this enzyme–inhibitor complex was first thermally perturbed to and equilibrated at about 300 K for 20 ps, and then another 20 ps of simulation was conducted to provide the experimental analysis. The average 3-D structure of compound **1** during the second 20-ps simulation showed that although the intermolecular distances between the P_5 Ftr side chain of **1** and specific CKH-RENIN active site residues increased slightly (e.g., from 6.0 to 7.0 Å for Tyr-230, and from 4.5 to 8.0 Å for Tyr-254), close contacts were conserved between those side-chain functionalities that contributed to the P_5–S_5 interaction. This putative P_5–S_5 interaction provided solvent shielding for both the Ftr side chain and aromatic side chains of residues Tyr-14, Tyr-230, Phe-123, Tyr-254, and Tyr-20 of the CKH-RENIN active site. Furthermore, this spatial disposition allowed the P_5 Ftr to exist in an orientation similar to previously described[76] aromatic clusters of other proteins determined by X-ray diffraction studies. A potential energy minimization of each inhibitor alone was also performed with the program CONFO[47] to provide comparative molcular refinement of the 3-D structural data obtained from CHARMM analysis of the CKH-RENIN–peptide complexes.

Human Renin Inhibition Assay. All of the compounds were assayed for their plasma renin inhibitory activity (i.e., IC_{50} determinations) with reconstituted, lyophilized human plasma with 0.1% ethylenediaminetetraacetic acid (EDTA), which was commercially obtained (New England Nuclear). The angiotensin I generation step utilized 250 μL of plasma, 2.5 μL of phenylmethanesulfonyl fluoride, 25 μL of maleate buffer (pH 6.0), and 10 μL of an appropriate concentration of inhibitor in a 1.0% Tween 80 in water vehicle. Incubation was for 90 min at 37 °C. Radioimmunoassay for angiotensin I was carried out with a commercial kit (Clinical Assays). Plasma renin activity values for inhibitor tubes were compared to those for control tubes to estimate the percent inhibition. The IC_{50} values were obtained by plotting three to four inhibitor concentrations on semilog graph paper and estimating the concentration producing 50% inhibition. The K_I values of compound **10** and RIP were also determined by measuring the generation of angiotensin I from partially purified human angiotensinogen by purified human renin (a gift from Dr. V. Dzau and Dr. E. Haber). The human angiotensinogen was prepared from human plasma by a three-step procedure that included ammonium sulfate fractionation and two successive DEAE-Sephacel chromatographies. The final substrate content was 0.15 nmol angiotensinogen mg^{-1} of preparation. The human renin had been isolated from human kidney by a modified protocol of Slater and Strout.[77] The specific activity of this preparation was 800 Goldblatt units mg^{-1} of protein. Each incubation tube contained 49 pmol of renin in a total incubation volume of 480 μL. Compound **10**, for example, was dissolved in 1 mM HCl, diluted to the appropriate concentration with incubation buffer (0.15 M Na_2HPO_4, 3 mM EDTA, 0.16 M NaCl at pH 6.0), and preincubated with the enzyme for 60 min at 37 °C. Following the preincubation period, the reactions were started by the ad-

(74) Jones, T. A. *J. Appl. Crystallogr.* **1978**, *11*, 268.
(75) MOSAIC is the Upjohn-enhanced version of the molecular modeling and energy refinement program known as MACROMODEL, which was developed by C. Still and colleagues at Columbia University.
(76) Burley, S. K.; Petsko, G. A. *Science (Washington, D.C.)* **1985**, *229*, 23.
(77) Slater, E.; Strout, H. V. *J. Biol. Chem.* **1981**, *256*, 8164.

Table VII. TLC and HPLC Data for RIP Analogues

	TLC		HPLC	
compd	R_f, A	R_f, B	k'	% purity
RIP	0.51	*a*	7.7	>98
1	0.77	0.82	14.7	>95
2	0.77	0.76	12.1	>99
3	0.75	0.71	11.6	>95
4	0.76	0.80	13.0	>94
5	0.77	0.88	15.4	>99
6	0.76	0.82	13.8	>96
7	0.71	0.38	10.7	>96
8	0.65	0.39	11.6	>94
9	0.76	0.73	13.4	>95
10	0.76	0.70	11.2	>95
11	0.71	0.60	8.5	>95
12	0.69	0.48	8.2	>95
13	0.69	0.60	9.8	>96
14	0.68	0.57	8.5	>97
15	0.64	0.41	4.2	>95
16	0.71	0.51	6.6	>97
17	0.74	0.65	9.9	>99
18	0.70	0.57	9.4	>98
19	0.64	0.57	5.2	>99
20	0.70	0.66	11.0	>99

[a] No mobility from origin observed.

dition of 20 μL of substrate to the enzyme–inhibitor mixtures at 37 °C. Aliquots were removed at 0, 15, and 30 min and assayed for angiotensin I by utilizing the radioimmunoassay procedure noted above. The K_I values for compound **10** and RIP were then determined by using classical methods.[78,79]

Porcine Renin Inhibition Assay. Several compounds were also assayed for their ability to inhibit porcine renin. One unit of partially purified porcine renin (Sigma; 8.4 unit mg^{-1} protein) was reconstituted and diluted 8100-fold with Tris-acetate buffer. The substrate for porcine renin was the tetradecapeptide H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser-OH (TDP; from Peninsula). TDP was prepared as a 0.16 mg mL^{-1} solution in Na_2HPO_4 buffer at pH 6.0. The renin inhibitors were dissolved in 100 μL of dimethyl sulfoxide and diluted with Na_2HPO_4 buffer to the desired concentration. Each incubation mixture of 340 μL contained 200 μL of Na_2HPO_4 buffer, 10 μL of phenylmethanesulfonyl fluoride, 20 μL of porcine renin, 50 μL of TDP, and 60 μL of the inhibitor solution. Incubation was for 30 min at 37 °C. Following incubation, each mixture was analyzed in duplicate via radioimmunoassay for angiotensin I as described above. The IC_{50} values were also determined as described above.

Ac-Ftr-Pro-Phe-His-Phe-Phe-Val-Ftr-NH_2 (1). The title compound was prepared with 0.6 g of N^{α}-Boc-Val-Ftr-p-MBHA resin (0.31-mmol total scale) as the starting material by the solid-phase synthetic procedure described in Table V. Stepwise coupling of the following N^{α}-Boc amino acids (or derivatives) was performed (in order of addition): N^{α}-Boc-Phe-OH, N^{α}-Boc-Phe-OH, N^{α}-Boc-His(N^{im}-Tos)-Dcha, N^{α}-Boc-Phe-OH, N^{α}-Boc-Pro-OH, and N^{α}-Boc-Trp(N^{in}-For)-OH. The N-terminal N^{α}-Boc protecting group was then removed (steps 1–5, Table V) and the peptide-*p*-MBHA resin intermediate was acetylated with 6.0 mmol of Ac_2O in DMF for 3 h. The resultant Ac-Ftr-Pro-Phe-His-Phe-Phe-Val-Ftr-*p*-MBHA resin was dried in vacuo (1.44 g). Following treatment with HF–anisole (as described above), the crude peptide was extracted from the resin by aqueous HOAc and lyophilized to give 530.0 mg. A portion of the crude peptide (72.0 mg) was dissolved in 3 mL of DMF and chromatographed on a semipreparative reverse-phase column using a mobile phase program consisting of 17% solvent B isocratic for 24 min and 17–67% solvent B linear gradient for 216 min (other HPLC parameters are as specified above). The post column eluent fractions corresponding to the desired peak were then pooled and lyophilized to give 49.9 mg of white powder (overall yield ca. 94%). The AAA and FAB-MS data for compound **1** are given in Table VI. The TLC and HPLC data for the title peptide are given in Table VII. In addition, compound **1** gave a $[\alpha]_D$ −37° (*c* 0.51, EtOH).

Ac-Ftr-Pro-Phe-His-Phe-Phe-Val-NH_2 (2). The title compound was prepared with 2.4 g of N^{α}-Boc-Val-BHA resin (0.89-mmol total scale) as the starting material by the solid-phase synthetic procedure described in Table V. Stepwise coupling of the following N^{α}-Boc amino acids (or derivatives) was performed (in order of addition): N^{α}-Boc-Phe-OH, $N\alpha$-Boc-Phe-OH, N^{α}-Boc-His(N^{im}-Tos)-Dcha, N^{α}-Boc-Phe-OH, N^{α}-Boc-Pro-OH, and N^{α}-Boc-Trp(N^{in}-For)-OH. The N-terminal N^{α}-Boc protecting group was then removed (steps 1–5, Table V) and the peptide-BHA resin intermediate was acetylated with 2.7 mmol of Ac_2O in DMF for 3 h. The resultant Ac-Ftr-Pro-Phe-His-Phe-Phe-Val-BHA resin was dried in vacuo (3.18 g). Following treatment with HF–anisole (as described above), the crude peptide was extracted from the resin by aqueous HOAc and lyophilized to give 419.0 mg. A portion of the crude peptide (117.0 mg) was dissolved in 1.0 mL of DMF and chromatographed on a semipreparative reverse-phase column using a mobile phase program consisting of 17% solvent B isocratic for 15 min and 17–50% solvent B linear gradient for 210 min (other HPLC parameters are as specified above). The post column eluent fractions corresponding to the desired peak were then pooled and lyophilized to give 81.0 mg of white powder (overall yield ca. 28%). The AAA and FAB-MS data for compound **2** are given in Table VI. The TLC and HPLC data for the title peptide are given in Table VII. In addition, compound **2** gave a $[\alpha]_D$ −52° (*c* 0.30, EtOH).

Ac-Ftr-Pro-Phe-His-Phe-Phe-NH_2 (3). The title compound was prepared with 0.56 g of N^{α}-Boc-Phe-BHA resin (0.39-mmol total scale) as the starting material by the solid-phase synthetic procedure described in Table V. Stepwise coupling of the following N^{α}-Boc amino acids (or derivatives) was performed (in order of addition): N^{α}-Boc-Phe-OH, N^{α}-Boc-His(N^{im}-Tos)-Dcha, N^{α}-Boc-Phe-OH, N^{α}-Boc-Pro-OH, and N^{α}-Boc-Trp(N^{in}-For)-OH. The N-terminal N^{α}-Boc protecting group was then removed (steps 1–5, Table V) and the peptide-BHA resin intermediate was acetylated with 1.9 mmol of Ac_2O in DMF for 3 h. The resultant Ac-Ftr-Pro-Phe-His-Phe-Phe-BHA resin was dried in vacuo (1.67 g). Following treatment with HF–anisole (as described above), the crude peptide was extracted from the resin by aqueous HOAc and lyophilized to give 478.0 mg. A portion of the crude peptide (145.0 mg) was dissolved in 1.0 mL of DMF and chromatographed on a semipreparative reverse-phase column using a mobile phase program consisting of 30% solvent B isocratic for 15 min and 30–75% solvent B linear gradient for 210 min (other HPLC parameters are as specified above). The post column eluent fractions corresponding to the desired peak were then pooled and lyophilized to give 115.0 mg of white powder (overall yield ca. 79%). The AAA and FAB-MS data for compound **9** are given in Table VI. The TLC and HPLC data for the title peptide are given in Table VII. In addition, compound **9** gave a $[\alpha]_D$ −41° (*c* 0.50, EtOH).

***N^{α}*-Boc-Sta-OH.** The title compound was prepared by using procedures previously reported,[68] which include conversion of N^{α}-Boc-Leu-OH to N^{α}-Boc-leucinal, aldol condensation with lithium ethyl acetate, and resolution of the resultant diastereomers (i.e., 3*S*,4*S* and 3*R*,4*S*) by silica chromatography. The N^{α}-Boc-Sta-OEt was isolated ($[\alpha]_D$ −38.3°; *c* 1.0 MeOH) and saponified to the desired product, N^{α}-Boc-(3*S*,4*S*)-Sta-OH, which was characterized as follows: $[\alpha]_D$ −38° (*c* 0.81, MeOH); mp 119–120 °C; MS $(M^{\bullet} + H)^+ = 275$; and ^1H NMR (300 MHz, Me_2SO-d_6) δ 6.3 (1 H, d, Boc NH), 3.79 (1 H, m, CHOH), 3.5 (1 H, m, NHCHCOH), 3.33 (1 H, br s, OH), 2.35–2.05 (2 H, ABX, J = 15 Hz, $COHCH_2COOH$), 1.5 (1 H, m, $CH_2CH(CH_3)_2$), 1.37 (9 H, s, Boc), 1.2 (2 H, m, $CHCH_2CH$), 0.85 (6 H, CH_3CH). The N^{α}-Boc-Sta-OH was used directly in solid-phase peptide synthesis using DCC and HOBT as coupling reagents without protection of the C-3 hydroxyl group. It was our experience, however, that O-acetylation could occur with Ac_2O in the N-capping procedure (see Table V). Therefore, *N*-acetylimidazole was used exclusively in the synthesis of peptides **10**–**12** and **15**–**20**.

***N^{α}*-Boc-Leuψ[CH(OTBDMS)CH_2]Val-OH.** The title compound was prepared by using procedures previously reported[19] (and to be documented by J. B. Hester and co-workers in a future detailed manuscript), which included stereocontrolled synthesis of (*S*)-1-[(2*S*,4*E*)-2-isopropyl-7-methyl-1-oxo-4-octenyl]-2-(hy-

(78) Lineweaver, H.; Burk, D. *J. Am. Chem. Soc.* **1934**, *56*, 658.
(79) Dixon, M.; Webb, E. *Enzymes*; Academic: New York, 1958.

droxymethyl)pyrrolidine and subsequent transformation of it to (via a 4*S*,5*S* dihydroxylated derivative) the desired product, N^{α}-Boc-Leuψ[CH(OTBDMS)CH_2]Val-OH, which was characterized as follows: $[\alpha]_D$ −42.0° (*c* 1.0, EtOH); mp 103–104 °C; MS $(M^{\bullet} + H)^+ = 446$; ^1H NMR (300 MHz, Me_2SO-d_6) δ 6.62 (1 H, d, Boc NH), 3.54–3.51 (2 H, m, NHCHCH and CHOSi), 2.2 (1 H, m, $CHCO_2H$), 1.8–0.79 (aliphatic protons). The compound was used directly in synthesis of peptide **13** in a manner described above for N^{α}-Boc-Sta-OH incorporation by solid-phase-chemistry methods.

N^{α}-Boc-Leuψ[CH_2NH]Val-OH. The title compound was prepared by procedures adapted from the literature,[69] which included reaction of N^{α}-Boc-Leu-H (as described above for the synthesis of N^{α}-Boc-Sta-OH) with H-Val-OBzl, reduction of the imine adduct with $NaCNBH_3$ to provide the ester N^{α}-Boc-Leuψ[CH_2NH]Val-OBzl ($[\alpha]_D$ −36.8°, *c* 1.5, MeOH). Hydrogenation of the ester with 10% Pd/C and H_2 in a Paar apparatus (40 psi) provided the desired compound, N^{α}-Boc-Leuψ-[CH_2NH]Val-OH, which was characterized as follows: $[\alpha]$ −34.0° (*c* 0.77, DMF); mp 192–193 °C; MS $(M^{\bullet} + H)^+ = 317$; ^1H NMR (300 MHz, Me_2SO-d_6) δ 6.66 (1 H, d, Boc NH), 3.56 (1 H, q, α-CH Val), 2.85 (1 H, $NHCHCH_2$), 2.6, 2.4 (2 H, 2 dd, $CHCH_2NH$), 1.88 (1 H, m, β-CH Val), 1.55 (1 H, m, $CH_2CH(CH_3)_2$), 1.37 (9 H, s, Boc), 1.2 (2 H, m, $CHCH_2CH$), 0.90–0.83 (12 H, CH_3).

Ac-Ftr-Pro-Phe-His-Sta-NH_2 (12). The title compound was prepared with 0.9 g of N^{α}-Boc-Sta-BHA resin (0.46-mmol total scale) as the starting material by the solid-phase synthetic procedure described in Table V. Stepwise coupling of the following N^{α}-Boc amino acids (or derivatives) was performed (in order of addition): N^{α}-Boc-His(N^{im}-Tos)·Dcha, N^{α}-Boc-Phe-OH, N^{α}-Boc-Pro-OH, and N^{α}-Boc-Trp(N^{in}-For)-OH. The N-terminal N^{α}-Boc protecting group was then removed (steps 1–5, Table V) and the peptide-BHA resin intermediate was acetylated with 3.1 mmol of *N*-acetylimidazole in DMF for 3 h. The resultant Ac-Ftr-Pro-Phe-His-Sta-BHA resin was dried in vacuo (1.52 g). Following treatment with HF–anisole (as described above), the crude peptide was extracted from the resin by aqueous HOAc and lyophilized to give 228.0 mg. A portion of the crude peptide (104.0 mg) was dissolved in 1.5 mL of DMF and chromatographed on a semipreparative reverse-phase column using a mobile phase program consisting of 17% solvent B isocratic for 15 min and 17–60% solvent B linear gradient for 25 min (other HPLC parameters are as specified above). The post column eluent fractions corresponding to the desired peak were then pooled and lyophilized to give 27.0 mg of white powder (overall yield ca. 11%). The AAA and FAB-MS data for compound **12** are given in Table VI. The TLC and HPLC data for the title peptide are given in Table VII. In addition, compound **12** gave a $[\alpha]_D$ −57° (*c* 0.38, EtOH).

Ac-Ftr-Pro-Phe-His-Leuψ[CH(OH)CH_2]Val-NH_2 (13). The title compound was prepared with 3.36 g of N^{α}-Boc-Leuψ[CH-(OH)CH_2]Val-pMBHA resin (0.81-mmol total scale) as starting material by the solid-phase synthetic procedure described in Table V. Stepwise coupling of the following N^{α}-Boc amino acids (or derivatives) was performed (in order of addition): N^{α}-Boc-His-(N^{im}-Tos)·Dcha, N^{α}-Boc-Phe-OH, N^{α}-Boc-Pro-OH, and N^{α}-Boc-Trp(N^{in}-For)-OH. The N-terminal N^{α}-Boc protecting group was then removed (steps 1–5, Table V) and the peptide-*p*-MBHA resin intermediate was acetylated with 3.6 mmol of *N*-acetylimidazole in DMF for 3 h. The resultant Ac-Ftr-Pro-Phe-His-Leuψ[CH(OH)CH_2]Val-*p*-MBHA resin was dried in vacuo (4.05 g). Following treatment with HF–anisole (as described above), the crude peptide was extracted from the resin by aqueous HOAc and lyophilized to give 235.0 mg. A portion of the crude peptide (101.0 mg) was dissolved in 1.2 mL of DMF and chromatographed on a semipreparative reverse-phase column using a modified mobile phase program (i.e., TFA was absent in both solvents A and B) consisting of 17% solvent B isocratic for 15 min and 17–50% solvent B linear gradient for 210 min (other HPLC parameters are as specified above). The post column eluent fractions corresponding to the desired peak were then pooled and lyophilized to give 30.0 mg of white powder (overall yield ca. 9.0%). The AAA and FAB-MS data for compound **13** are given in Table VI. The TLC and HPLC data for the title peptide are given in Table VII. As previously described above (see Results and Discussion), we found this peptide to be chemically unstable due to acid-catalyzed lactonization of the Leuψ[CH(OH)CH_2]Val-NH_2 C-terminal funcationality. A significant proportion (ca. 30%) of the post HF crude precursor was determined (by FAB-MS) to exist as the lactone derivative. This contributed to the low overall percent yield, but it was also determined (by AAA) that the HF cleavage reaction was only about 25% complete to hydrolyze the title compound from the *p*-MBHA resin. Attempts to improve this step by increased reaction times and elevated reaction temperatures were generally unsuccessful. It should be noted that although the C-terminal Leuψ[CH(OH)CH_2]Val-NH_2 functionality of compound **13** was labile to lactonization, it has been previously shown[19] that C-alkylamidation (e.g., by selected alkylamines or amino acids) of such P_1-P_1' Leuψ[CH(OH)CH_2]Val-substituted peptides are relatively stable to moderate acid-base conditions.

Ac-Ftr-Pro-Phe-His-Leuψ[CH_2NH]Val-NH_2 (14). The title compound was prepared with 2.0 g of *p*-MBHA resin (2.6-mmol total scale) as the starting material by using the solid-phase synthetic procedure described in Table V. Stepwise coupling of the following N^{α}-Boc amino acids (or derivates) was performed (in order of addition): N^{α}-Boc-Leuψ[CH_2NH]Val-OH, N^{α}-Boc-His(N^{im}-Tos)·Dcha, N^{α}-Boc-Phe-OH, N^{α}-Boc-Pro-OH, and N^{α}-Boc-Trp(N^{in}-For)-OH. The N-terminal N^{α}-Boc protecting group was then removed (steps 1–5, Table V) and the peptide-*p*-MBHA resin intermediate was acetylated with 11.0 mmol of *N*-acetylimidazole in DMF for 1 h. The resultant Ac-Ftr-Pro-Phe-His-Leuψ[CH_2NH]Val-*p*-MBHA resin was dried in vacuo (3.77 g). Following treatment with HF–anisole (as described above), the crude peptide was extracted from the resin by TFA, evaporated, and lyophilized to give 2.3 g. A portion of the crude peptide (0.5 g) was subjected to Sephadex G-15 chromatography (0.2 M HOAc solvent) which yielded 473 mg product. A portion (79.0 mg) of the post Sephadex G-15 product was dissolved in 1 mL of the HPLC solvent A and chromatographed on a semipreparative reverse-phase column using a mobile phase program consisting of 10% solvent B isocratic for 40 min and 10–50% solvent B linear gradient for 810 min. The post column eluent corresponding to the desired product were then pooled and lyophilized to give 60.0 mg of white powder (overall yield ca. 59%). The AAA and FAB-MS data for compound **14** are given in Table VI. The TLC and HPLC data for the title compound are given in Table VII. In addition, compound **14** gave a $[\alpha]_D$ −32° (*c* 0.28, EtOH).

Ac-Ftr-Pro-Phe-His-Sta-Ile-NH_2 (17). The title compound was prepared with 1.0 g of N^{α}-Boc-Ile-*p*-MBHA resin (0.15-mmol total scale) as the starting material by the solid-phase synthetic procedure described in Table V. Stepwise coupling of the following N^{α}-Boc amino acids (or derivatives) was performed (in order of addition): N^{α}-Boc-Sta-OH, N^{α}-Boc-His(N^{im}-Tos)·Dcha, N^{α}-Boc-Phe-OH, N^{α}-Boc-Pro-OH, and N^{α}-Boc-Trp(N^{in}-For)-OH. The N-terminal N^{α}-Boc protecting group was then removed (steps 1–5, Table V) and the peptide-*p*-MBHA resin intermediate was acetylated with 0.45 mmol of Ac_2O in DMF for 3 h. The resultant Ac-Ftr-Pro-Phe-His-Sta-Ile-*p*-MBHA resin was dried in vacuo (2.08 g). Following treatment with HF–anisole (as described above), the crude peptide was extracted from the resin by aqueous HOAc and lyophilized to give 143.0 mg. A portion of the crude peptide (79.0 mg) was dissolved in 1 mL of DMF and chromatographed on a semipreparative reverse-phase column using a mobile phase program consisting of 17% solvent B isocratic for 15 min and 17–40% solvent B linear gradient for 210 min (other HPLC parameters are as specified above). The post column eluent fractions corresponding to the desired peak were then pooled and lyophilized to give 31.4 mg of white powder (overall yield ca. 39%). The AAA and FAB-MS data for compound **17** are given in Table VI. The TLC and HPLC data for the title peptide are given in Table VII. In addition, compound **17** gave a $[\alpha]_D$ −47° (*c* 0.28, EtOH).

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16, 111113-35-0; 17, 103336-30-7; 18, 111113-36-1; 19, 111113-37-2; 20, 111187-51-0; BOC-Phe-OH, 13734-34-4; BOC-His(Tos)-OH·DCHA, 65057-34-3; BOC-Pro-OH, 15761-39-4; BOC-Trp(CHO)-OH, 47355-10-2; BOC-Sta-OEt, 67010-43-9; BOC-Sta-OH, 58521-49-6; BOC-Leuψ[CH(OTBDMS)CH_2]Val-OH, 103335-80-4; BOC-Leu-H, 58521-45-2; H-Val-OBzl, 21760-98-5; BOC-Leuψ[CH_2NH]Val-OBzl, 82252-38-8; BOC-Leuψ[CH_2NH]Val-OH, 82252-39-9; renin, 9015-94-5.

Synthesis and Biological Properties of α-Mono- and α-Difluoromethyl Derivatives of Tryptophan and 5-Hydroxytryptophan

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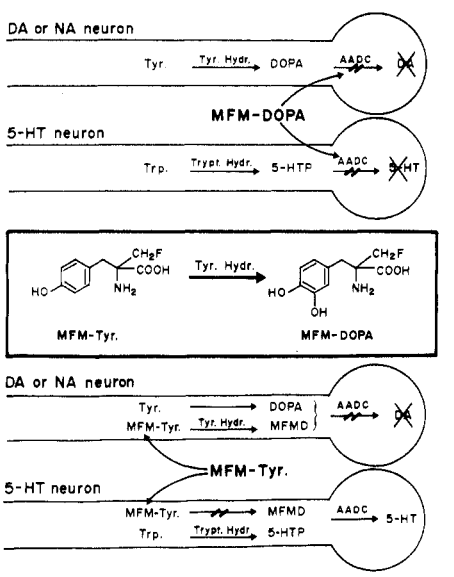
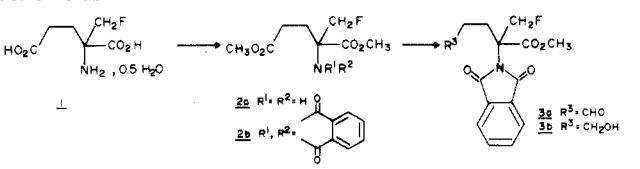
The syntheses of α-mono- and α-difluoromethyl derivatives of tryptophan and 5-hydroxytryptophan are described. In an attempt to selectively regulate serotonin synthesis, α-(mono- and difluoromethyl)tryptophan were tested in vivo as precursors (or prodrugs) of their 5-hydroxy analogues. Although α-(mono- and difluoromethyl)-5-hydroxytryptophans are potent irreversible inhibitors of aromatic amino acid decarboxylase (equipotent to α-difluoromethyl-Dopa), only α-(monofluoromethyl)tryptophan affects the level of serotonin in vivo (small decrease), α-(difluoromethyl)tryptophan being a very poor substrate of the activating (or helper) enzyme, tryptophan hydroxylase.

Over the past years, we and others have reported the synthesis of a number of halomethyl analogues of various amino acids and described their inhibitory properties of the corresponding α-amino acid decarboxylases both in vitro and in vivo.[1-4] Thus, both α-(difluoromethyl)- and α-(monofluoromethyl)-Dopa are time-dependent irreversible inhibitors of aromatic amino acid decarboxylase (AADC)[5,6] although their respective potencies and therefore potential applications are different. Difluoromethyl-Dopa inhibits AADC essentially in peripheral organs but never to the extent where the decarboxylation of endogenous Dopa becomes rate-limiting in catecholamine synthesis.[7] This compound could therefore in conjunction with Dopa replace Carbidopa in the treatment of parkinsonism. α-(Monofluoromethyl)-Dopa on the contrary inhibits AADC in peripheral organs as well as in brain and causes a time- and dose-dependent inhibition of biogenic amine synthesis.[8] In rat brain, for instance, α-(monofluoromethyl)-Dopa depletes in parallel dopamine and norepinephrine as well as serotonin, because the AADC's in the three types of neurons are inhibited to a degree where decarboxylation becomes rate-limiting. We recently demonstrated that α-(fluoromethyl)-Dopa can be generated from the *p*-tyrosine analogue by the action of tyrosine hydroxylase both in vitro and in vivo.[9,10] α-(Fluoromethyl)-*p*-tyrosine functions as a bioprecursor of the potent AADC inhibitor. This compound depletes selectively norepinephrine and, at higher doses, dopamine but has no effect on serotonin even after prolonged administration. The molecular basis for the increased selectivity of α-(monofluoromethyl)-*p*-tyrosine is illustrated in Scheme I.[11a] On the basis of the analogy of that result, we report here the synthesis and biological evaluation of α-(halomethyl)tryptophan and 5-hydroxytryptophan as an attempt to selectively regulate serotonin synthesis. The availability of a means to deplete specifically this important biogenic amine in combination with the use of selective antagonists of the various 5-HT receptors subtypes[11b] should advance our understanding of the role of serotonin.

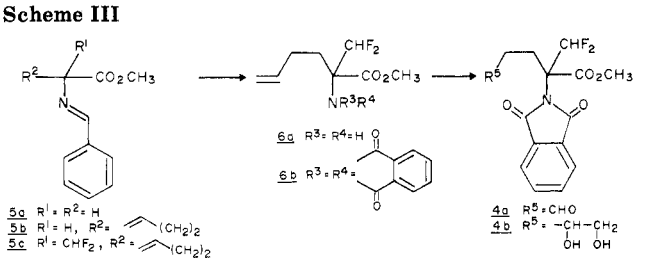
Scheme I. Postulated Mechanism for the Inhibition of AADC in Catecholaminergic and Serotoninergic Neurons by MFMD and MFMT



Scheme II



Scheme III



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