system-10 computer using mainly the X-ray 72 program system.[34]

The molecular conformations and the atom labeling schemes are shown in Figure 2.

Molecular Mechanics Calculations. The structural modelling was performed by use of the interactive computer graphics program MIMIC (methods for interactive modelling in chemistry).[20] Calculations were performed on a VAX 11/780 computer using Allingers MMP2 force field[35] to which had been added parameters for the phenol[36] and amino groups.[37] Computational times ranged from 1 to 30 min/minimization.

Pharmacology. Materials and Methods. Male Sprague–Dawley rats weighing 200–300 g (ALAB, Stockholm, Sweden) were used. Reserpine and haloperidol were dissolved in a few drops of glacial acetic acid and made up to volume with 5.5% glucose solution. The other test compounds were dissolved in saline immediately before use, occasionally with a few drops of glacial acetic acid and/or moderate heating to obtain complete dissolution. Injection volumes were 5 mL/kg, and injection solutions had approximately neutral pH.

Biochemistry. Brain levels of DOPA and 5-HTP were analyzed by HPLC with electrochemical detection.[4j] For biochemical results and experimental details, see Tables VI and VII and footnotes *a* in Tables VI and VII.

Locomotor Activity. The motor activity was measured by means of photocell recordings (M/P 40 Fc electronic motility meter, Motron Products, Stockholm) as previously described.[21] For experimental details, see footnotes *b* in Tables VI and VII. Each box was equipped with a semitransparent mirror that allowed gross behavior observations of the animals during the experiments. The motor activity results are shown in Tables VI and VII.

Acknowledgment. We thank Gun Andersson, Ingrid Bergh, Ann-Marie Dahlberg, Lucia Gaete, Boel Göransson, and Kirsten Sonniksen for skillful assistance in the pharmacological testing, Anna Krotowska and Maria Lindbäck for skillful synthetic work, Christer Sahlberg for the capillary GC data, Bengt Anderson and Curt Pettersson for the HPLC data, Sven-Olof Larsson for 200-MHz ^{1}H NMR spectra, and Tommy Liljefors and Robert E. Carter for providing access to the MIMIC program. The financial support from Astra Läkemedel AB, The Swedish Board for Technical Development, The Swedish Medical Research Council (Grant No. 155 and 7486), The Swedish Natural Science Research Council, Centrala Försöksdjursnämnden, Ingabritt and Arne Lundbergs Forskningsstiftelse, The Swedish Academy of Pharmaceutical Sciences, IF's Stiftelse, Gyllenstiernska Krapperupsstiftelsen, Torsten and Ragnar Söderbergs Stiftelse, and the Medical Faculty, University of Göteborg, is gratefully acknowledged.

Supplementary Material Available: Lists of X-ray data of (+)-6·HCl and (+)-10·HCl, including thermal parameters for all atoms, positional parameters for the hydrogen atoms, and bond lengths and bond angles for the non-hydrogen atoms (5 pages). Ordering information is given on any current masthead page.

(34) Stewart, J. M.; Kruger, G. J.; Ammon, H. L.; Dickinson, C.; Hall, S. R. "The X-ray System", version of June 1972; Technical Report TR-192; Computer Science Center, University of Maryland, College Park, 1972.
(35) Allinger, N. L.; Yuh, Y. *Quantum Chem. Program Exchange* **1980**, *12*, 395.
(36) Dodziuk, H.; von Voitenberg, H.; Allinger, N. L. *Tetrahedron* **1982**, *38*, 2811.
(37) Profeta, S., Jr.; Allinger, N. L. *J. Am. Chem. Soc.* **1985**, *107*, 1907.
(38) Carlsson, A.; Kehr, W.; Lindqvist, M. *J. Neural Transm.* **1977**, *40*, 99.

α,α-Difluoro-β-aminodeoxystatine-Containing Renin Inhibitory Peptides

Suvit Thaisrivongs,* Heinrich J. Schostarez, Donald T. Pals, and Steve R. Turner

Cardiovascular Diseases Research, The Upjohn Company, Kalamazoo, Michigan 49001. *Received March 30, 1987*

The preparations of sodium 4(*S*)-[(*tert*-butyloxycarbonyl)amino]-2,2-difluoro-3(*S*)- and -3(*R*)-[(4-methoxyphenyl)amino]-6-methylheptanoates (**7a** and **7b**) from sodium 4(*S*)-[(*tert*-butyloxycarbonyl)amino]-2,2-difluoro-3(*R*)- and -3(*S*)-hydroxy-6-methylheptanoates (**1a** and **1b**) are described. The key step involves the stereospecific intramolecular displacement via a Mitsunobu reaction for the conversion of a β-hydroxy hydroxamate to a β-lactam ring. Compounds **7a** and **7b** are useful as synthetic intermediates for the preparation of enzyme inhibitors that contain 3(*S*),4(*S*)- and 3(*R*),4(*S*)-diamino-2,2-difluoro-6-methylheptanoic acid inserts. Angiotensinogen analogues VII and VIII that contain these novel amino analogues of difluorostatine were shown to be inhibitors of the enzyme renin. The α,α-difluoro-β-aminodeoxystatine-containing compounds were shown to be weaker inhibitors than the corresponding difluorostatine-containing congeners.

Pepstatin, Iva-Val-Val-Sta-Ala-Sta, is a naturally occurring pentapeptide that is a general aspartyl protease inhibitor.[1] It has been proposed that the central statine residue (Sta), 4(*S*)-amino-3(*S*)-hydroxy-6-methylheptanoic acid (A), acts as a structural analogue of the tetrahedral species formed during enzymatic hydrolysis of a peptidic bond.[2] Utilization of the concept of transition-state analogue[3] has generated numerous pepstatin-derived inhibitors of aspartyl proteinases.[4]

We have continuing interest in the design of enzyme inhibitors of the aspartyl protease renin. It is a highly specific proteolytic enzyme produced mainly in the juxtaglomerular apparatus of the kidney[5] and cleaves the circulating α-globulin angiotensinogen to form the decapeptide angiotensin I.[6] The N-terminal sequence of human angiotensinogen is shown in Figure 1. The cleaved angiotensin I is further converted to the octapeptide angiotensin II by the converting enzyme by removal of the C-terminal histidylleucine. Angiotensin II is a very potent vasoconstrictor and also stimulates the release of aldosterone from the adrenal gland to induce salt and water retention. The renin–angiotensin system has thus been implicated in several forms of hypertension.[7] Interest in

(1) Umezawa, H.; Aoyagi, T.; Morishima, H.; Matsuzaki, M.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1970**, *23*, 259. Workman, R. J.; Burkitt, D. S. *Arch. Biochem. Biophys.* **1977**, *194*, 157.
(2) Marciniszyn, J.; Hartsuck, J. A.; Tang, J. *J. Biol. Chem.* **1976**, *251*, 7088.
(3) Wolfenden, R. *Transition States of Biochemical Processes*; Gandour, R. D.; Showen, R. L., Eds., Plenum: New York, 1978; p 555.
(4) Rich, D. H. *J. Med. Chem.* **1985**, *28*, 263.
(5) Peach, M. J. *Physiol. Rev.* **1977**, *57*, 313.
(6) Ondetti, M. A.; Cushman, D. W. *Annu. Rev. Biochem.* **1982**, *51*, 283.

0022-2623/87/1830-1837$01.50/0

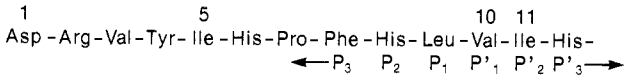


Figure 1. Human angiotensinogen.

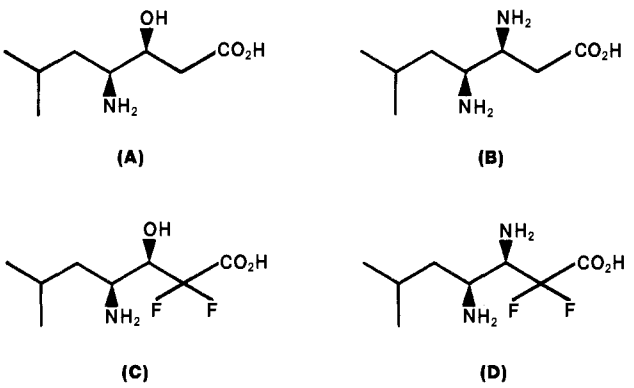


Figure 2. Statine and related analogues.

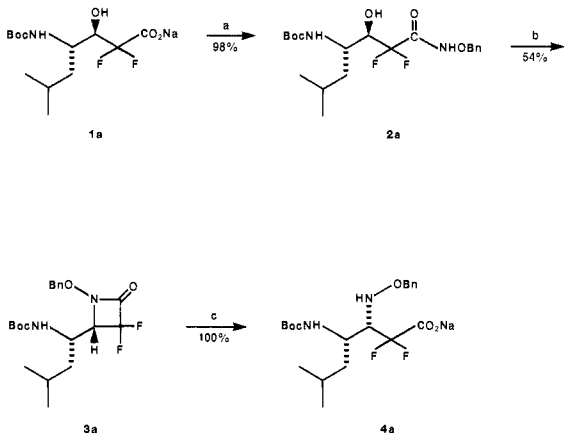
the blockade of renin which is involved in the first and rate-limiting step of the cascade has led to rapid development of inhibitors based on the substrate angiotensinogen sequence.[8] Successful renin inhibitors could provide agents for control of cases of renin-associated hypertension.

Highly potent inhibitors of renin have been reported in which statine was incorporated at the cleavage site of the angiotensinogen sequence.[9] Effort has continued to focus on modifications of the statine residue[10-14] in order to better understand the steric and electronic requirement in these enzyme inhibitors. One particular analogue of interest is 3(*S*),4(*S*)-diamino-6-methylheptanoic acid (B, Figure 2).[11,12,14] Peptides that contain this 3-aminodeoxystatine and its C-3 epimer were prepared to study the importance of hydrogen bonding and electrostatic interaction when the requisite 3(*S*)-hydroxyl group[15] of statine was replaced by a basic amino group. The 3-aminodeoxystatine (B), when incorporated into potential inhibitory peptides, might be expected to protonate on the 3-amino group at physiological pH. The possible favorable electrostatic interaction between the resulting ammonium group and the catalytically essential aspartate residues at the renin active site might be anticipated. Several series of peptides that contain 3-aminodeoxystatine and its C-3 epimer have been reported.[11,12] The 3-aminodeoxystatine-containing peptides, in favorable cases, are half an order of magnitude more effective than the corresponding statine-containing congeners. However, in many instances, the 3-aminodeoxystatine-containing peptides afforded worse inhibitory potency than the related analogues with statine. The relative effectiveness of 3-aminodeoxystatine and its C-3 epimer is dependent upon the peptide templates. This is in contrast to the earlier finding[15] that the 3(*S*)-hydroxy group of statine (A) is much more effective than the 3*R* epimer.

We have continuing interest in the use of the transition-state-analogue concept as an approach in the design of enzyme inhibitors. We have communicated[13] our work on difluorostatone-containing peptides as inhibitors of renin. The readily hydrated α,α-difluorostatone was suggested to mimic the tetrahedral intermediate of peptidic bond hydrolysis. The fluoro ketone proved much more effective than the nonfluorinated ketone congener. During that work we have also prepared α,α-difluorostatine (C) and its C-3 epimer. When incorporated into angiotensinogen analogues, the difluorostatine (C) proved less effective than the parent statine (A). We have suggested that the reduced electron density of the 3-hydroxyl group due to the highly electron withdrawing fluorine atoms diminishes the effectiveness of the hydroxyl function as a hydrogen-bond acceptor.

To complete this series of statine analogues, we have prepared the difluoro-3-aminodeoxystatine (D) in order to mediate the basicity of the 3-aminodeoxystatine group. The synthetic intermediates will be useful in the preparation of various aspartyl protease inhibitors. In the present instance, a congeneric series of peptides that contain this set of statine analogues and their C-3 epimers will be compared in the same template peptide as inhibitors of renin.

Scheme I. Preparation of Compound 4a[a]



[a] (a) NH_2OBn·HCl, DCC, HOBT, CH_2Cl_2; (b) DEAD, Ph_3P, THF; (c) NaOH, H_2O, THF.

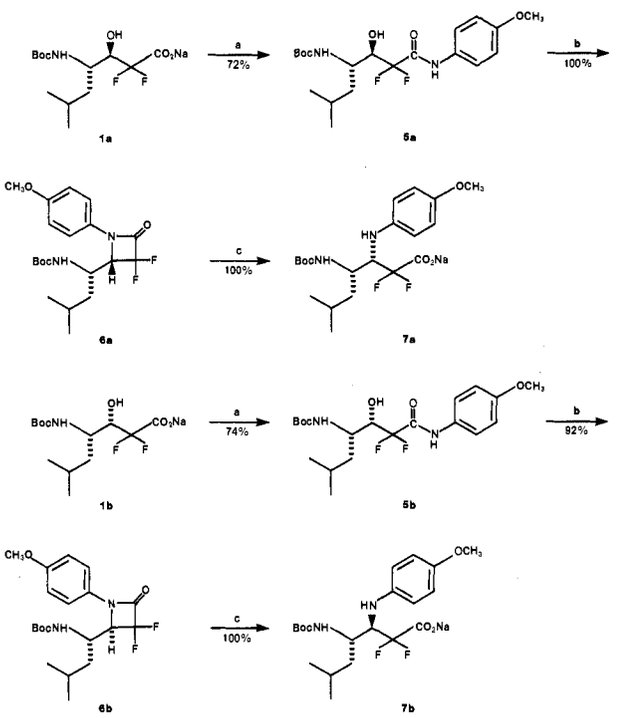
Chemistry[16]

Starting with the previously,[13] reported hydroxyl compounds **1a** and **1b**, a suitable nitrogen nucleophile is

(7) Davis, J. O. *Circ. Res.* **1977**, *40*, 439. Swales, J. D. *Pharmacol. Ther.* **1979**, *7*, 172.
(8) Boger, J. *Ann. Rep. Med. Chem.* **1985**, *20*, 257.
(9) 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.; Stabilito, I. I.; Veber, D. F.; Rich, D. H.; Bopari, A. S. *Nature (London)* **1983**, *303*, 81.
(10) Bartlett, P. A.; Kezer, W. B. *J. Am. Chem. Soc.* **1984**, *106*, 4282. Bock, M. G.; D'Pardo, R. M.; Evans, B. E.; Rittle, K. E.; Boger, J.; Freidinger, R. M.; Veber, D. F. *J. Chem. Soc., Chem. Commun.* **1985**, 109. 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.; Veber, D. F. *J. Med. Chem.* **1985**, *28*, 1779.
(11) Jones, M.; Sueiras-Diaz, J.; Szelke, M.; Leckie, B.; Beattie, S. *Peptides, Structure and Function, Proceedings of the Ninth American Peptide Symposium*; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chemical Co.: Rockford, IL, 1985; p 759.
(12) Arrowsmith, R. J.; Carter, K.; Dann, J. G.; Davies, D. E.; Harris, C. J.; Morton, J. A.; Lister, P.; Robinson, J. A.; Williams, D. J. *J. Chem. Soc., Chem. Commun.* **1986**, 755.
(13) Thaisrivongs, S.; Pals, D. T.; Kati, W. M.; Turner, S. R.; Thomasco, L. M. *J. Med. Chem.* **1985**, *28*, 1553. Thaisrivongs, S.; Pals, D. T.; Kati, W. M.; Turner, S. R.; Thomasco, L. M.; Watt, W. *J. Med. Chem.* **1986**, *29*, 2080.
(14) Schostarez, H. J., manuscript submitted.
(15) Rich, D. H.; Sun, E.; Singh, J. *Biochem. Biophys. Res. Commun.* **1977**, *74*, 762. Rich, D. H.; Sun, E. T. O.; Ulm, E. *J. Med. Chem.* **1980**, *23*, 27.

(16) DCC = *N,N'*-dicyclohexylcarbodiimide, HOBT = 1-hydroxybenzotriazole, DEAD = diethyl azodicarboxylate, BOPCl = bis(2-oxo-3-oxazolidinyl)phosphinic chloride, TFA = trifluoroacetic acid, DEPC = diethylphosphoryl cyanide; Pro = proline, Phe = phenylalanine, His = histidine, Ile = isoleucine, Amp = 2-aminomethylpyridine.

Scheme II. Preparation of Compounds **7a** and **7b**[a]

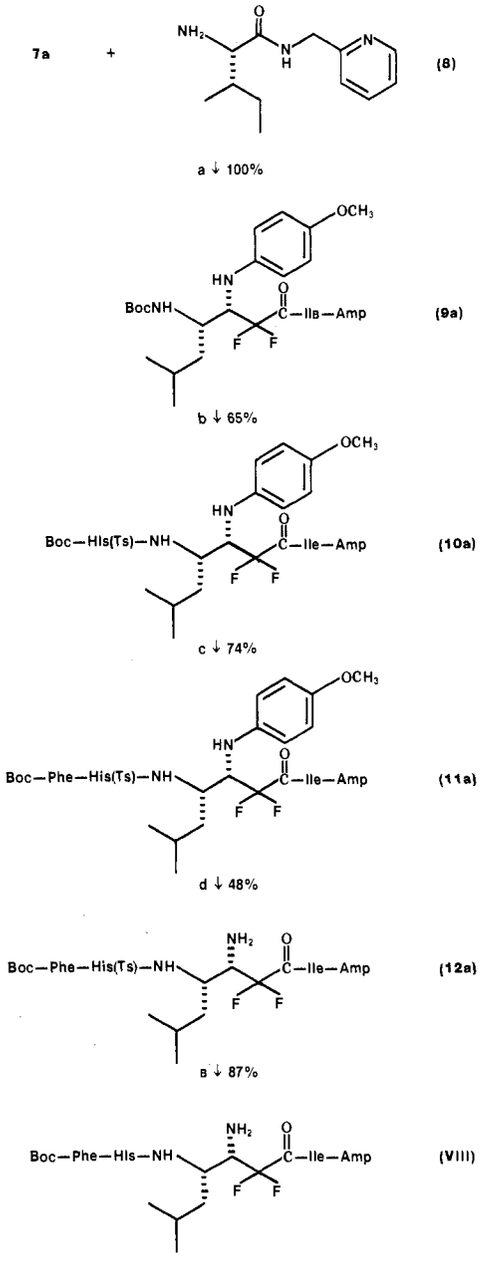


[a] (a) $NH_2C_6H_4OCH_3$, BOPCl, *i*-Pr_2NEt, CH_2Cl_2; (b) DEAD, Ph_3P, THF; (c) NaOH, H_2O, THF.

needed to displace the β-hydroxyl group in order to prepare the corresponding amino analogues. We anticipated difficulty in the intermolecular reaction and chose to employ the intramolecular inversion strategy similar to that described for the synthesis of 3-aminodeoxystatine.[14] This method was based on the stereospecific intramolecular Mitsunobu reaction[17] developed by Miller[18] and modified by Floyd[19] in their syntheses of monolactams. Thus condensation of compound **1a** with *O*-benzylhydroxylamine as shown in Scheme I gave the hydroxamate **2a**. Intramolecular cyclization with triphenylphosphine and diethyl azodicarboxylate afforded the *N*-benzyloxy β-lactam **3a**. However, conventional reductive removal of the benzyloxy group was not successful due to the lability of the resulting α,α-difluoro β-lactam ring with its highly electrophilic carbonyl functionality. The β-lactam **3a** could be hydrolyzed and the product was isolated as the corresponding salt **4a**. This proved useful as synthetic intermediate for incoporation into potential inhibitory peptides. The reductive removal of the benzyloxy group from the resulting peptides did not afford satisfactorily clean reaction however, and this observation necessitated the development of an alternate procedure.

We turned our attention to the 4-methoxyphenyl amide as the vinylogous hydroxamate. The 4-methoxyphenyl group was anticipated to be removed by oxidative procedure. Condensation of compound **1a** with 4-methoxyaniline, as shown in Scheme II, afforded the amide **5a**. Intramolecular Mitsunobu inversion reaction gave the β-lactam **6a** in excellent yield. Alkaline hydrolysis then yielded the salt **7a**, which again proved useful for incoporation into peptides. The C-3 epimeric amide **5b** was obtained from the epimer **1b** and stereospecifically converted in the same manner to the β-lactam **6b**. Alkaline hydrolysis afforded the salt **7b**, which is the C-3 epimer of compound **7a**.

Scheme III. Synthesis of a Representative Peptide VIII[a]



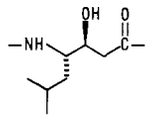
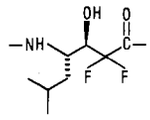
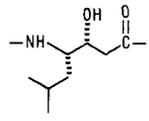
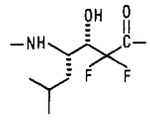
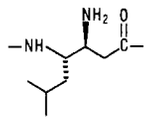
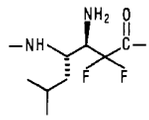
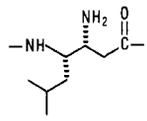
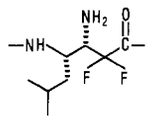
[a] (a) H-Ile-Amp, DCC, HOBT, CH_2Cl_2; (b) TFA, CH_2Cl_2; Boc-His(Ts)-OH, DEPC, *i*-Pr_2NEt, CH_2Cl_2; (c) TFA, CH_2Cl_2; Boc-Phe-OH, DEPC, *i*-Pr_2NEt, CH_2Cl_2; (d) $(NH_4)_2Ce(NO_3)_6$, CH_3CN, H_2O; (e) HOBT, CH_3OH.

The synthetic sequence for a representative peptide VIII is shown in Scheme III. The salt **7a** was coupled to L-isoleucyl-2-pyridylmethylamine (**8**)[13] with dicyclohexylcarbodiimide and 1-hydroxybenzotriazole to give compound **9a**. Sequential addition of suitably protected histidine and then phenylalanine residues with diethylphosphoryl cyanide[20] as the coupling reagent led to compound **11a**. Oxidative removal of the methoxyphenyl group with ceric ammonium nitrate[21] afforded the desired free amine **12a**. Tosyl group removal with 1-hydroxy-

(17) Mitsunobu, O. *Synthesis* **1981**, 1.
(18) Mattingly, P. G.; Kerwin, J. F.; Miller, M. J. *J. Am. Chem. Soc.* **1979**, *101*, 3983.
(19) Floyd, D. M.; Fritz, A. W.; Plusec, J.; Weaver, E. R.; Cimarusti, C. M. *J. Org. Chem.* **1982**, *47*, 5160.
(20) Yamada, S.; Kasai, Y.; Shiori, T. *Tetrahedron Lett.* **1973**, 1595.
(21) Yardely, J. P.; Fletcher, H., III *Synthesis* **1976**, 244. Fukuyama, T.; Frank, R. K.; Jewell, C. F., Jr. *J. Am. Chem. Soc.* **1980**, *102*, 2122. Kronenthal, D. R.; Han, C. Y.; Taylor, M. K. *J. Org. Chem.* **1982**, *47*, 2765.

Table I. Inhibition of Human Plasma Renin

Boc—Phe—His—X—Ile—Amp

X	no.	IC_{50}, nM	X	no.	IC_{50}, nM
	I	1.7		V	12
	II	330		VI	730
	III	15		VII	340
	IV	480		VIII	2800

benzotriazole[22] gave the corresponding peptide VIII. Peptide VII was also prepared in the same manner starting with compound **7b**.

The reference peptide I has been reported previously[13] and the peptide II could be prepared in a similar manner starting with Boc-epistatine. Peptides III and IV were prepared from 3(*S*)- and 3(*R*)-[(benzyloxycarbonyl)-amino]-4(*S*)-[(*tert*-butyloxycarbonyl)amino]-6-methylheptanoic acids.[14] Peptides V and VI were reported previously.[13]

Biology

These peptides were evaluated as inhibitors of human plasma renin, and their IC_{50} values were determined as described previously.[13] The results are shown in Table I as a set of eight compounds in the same peptide template. The amino-containing analogues proved less effective than the corresponding hydroxy-containing congeners, and the fluorinated analogues are less potent than the corresponding nonfluorinated congeners. In this series of peptides, the 3*S* epimers are more potent renin inhibitors than the corresponding 3*R* epimers in the nonfluorinated compounds and, correspondingly, the 3*R* epimers in the fluorinated series are more effective than the 3*S* epimers.

Discussion

It is to be noted that the absolute stereochemistry of the 3(*R*)-amino group in difluoro-3-aminodeoxystatine (D) is the same as that of the 3(*S*)-hydroxyl group in statine (A). The higher effectiveness of the 3*R* epimers in peptide VII over the congeneric peptide VIII is thus in accord with earlier finding[15] that the requisite hydroxy group in statine be in the 3*S* configuration for high potency as an enzyme inhibitor.

As mentioned earlier, the transition-state analogues statine (A) and 3-aminodeoxystatine (B) proved comparably effective when incorporated into inhibitory peptides. The potentially significant difference between a hydroxyl group and an ammonium group in the enzyme–inhibitor interaction did not translate into any appreciable difference in the resulting inhibitory potencies of the resulting peptides. The anticipated increase in binding affinity through the introduction of additional ionic interactions between the ammonium group and the carboxy–carboxylate catalytic pair at the active site was not observed. It has been suggested[12] that the ammonium group replaces a strong hydroxy interaction in statine and this seemingly more favorable ionic interaction is balanced by a large energy requirement for desolvation of the ammonium group as the inhibitor binds to the active site. In the present work, the 3-aminodeoxystatine-containing peptide III was shown to be 1 order of magnitude less active than the statine-containing congener I.

The difluorostatine-containing peptide such as peptide V has been shown to be less potent than the corresponding statine analogue I.[13] The fluorine atoms reduce the electron density on oxygen relative to the hydroxyl group of statine and thus reduce the effectiveness of the difluorostatine as a hydrogen-bond acceptor. The amino analogue of difluorostatine as in compound VII is less potent than the difluorostatine-containing compound V. This might suggest a weaker ionic interaction between the ammonium group of compound VII and the carboxy–carboxylate catalytic pair when compared to the nonfluorinated compound. The reduced basicity of the amino group due to the adjacent electron-withdrawing fluorine atoms is suggested to further diminish the effectiveness of the presumed ammonium ion and carboxylate ion pair.

Since the 3(*R*)-hydroxyl group of statine contributes little to binding, it has been suggested[12] that the increased binding of 3(*R*)-aminodeoxystatine compared with (3*R*)-statine is a consequence of the introduction of an additional ionic interaction due to the ammonium group. However, in the present work, peptides II and IV were shown to bind to renin with comparable affinity. In the corresponding difluoro analogues, the amino compound VIII shows weaker binding affinity than the hydroxy congener VI. Again, it suggests a weakly basic amino function in fluorinated compounds and this is not sufficient to generate noticeable additional ionic interaction with the carboxy–carboxylate catalytic pair in the active site of renin under the test condition.

Summary

We have described in this work, formally, the stereospecific conversions of 4(*S*)-amino-2,2-difluoro-3(*R*)- and -3(*S*)-hydroxy-6-methylheptanoic acids to 3(*S*),4(*S*)- and 3(*R*),4(*S*)-diamino-2,2-difluoro-6-methylheptanoic acids. In their appropriately protected forms, these compounds are novel amino acids useful for peptide preparation. At present, new angiotensinogen analogues that contain the amino analogues of difluorostatine have been prepared as renin inhibitors. A series of congeneric peptides that contain amino and fluorinated analogues were prepared to vary the electrostatic effect of the resulting peptides and their inhibitory activities were then compared as they are bound at the active site. Peptides I–VIII present a congeneric set of renin inhibitors. For each of the four pairs of C-3 epimers, the β-epimer (as drawn in Table I) is more active than the α-epimer. For each of the four pairs of heteroatom analogues at C-3, the hydroxy-containing compound is more active than the amino-containing congener. The four nonfluorinated compounds are more active than the corresponding fluorinated congeners. It is conceivable that different series of peptides that contain difluoro-3-aminodeoxystatine could be generated that might prove to be effective inhibitors of other aspartyl proteases.

Experimental Section

Chemistry. High-resolution mass spectra, infrared spectra, and optical rotations were obtained by the Physical and Analytical Chemistry Department of the Upjohn Co. ^1H NMR spectra were determined on a Varian CFT-20 instrument at 80 MHz and

(22) Fujii, T.; Sakakibara, S. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 3146.

chemical shifts were reported as δ units relative to tetramethylsilane.

Thin-layer chromatography was conducted with Analtech 0.25-mm glass plates precoated with silica gel GF. For column chromatography, E. Merck silica gel 60, 230–400 mesh, was used. All solvents for chromatography were Burdick and Jackson reagent grade distilled in glass.

Tetrahydrofuran was distilled under argon from sodium metal in the presence of benzophenone. Dichloromethane was distilled from phosphorus pentoxide. Diisopropylethylamine was distilled from calcium hydride. Diethylphosphoryl cyanide was freshly distillled before use.

Peptides II, VII, and VIII were analyzed on a Perkin-Elmer Series 4 liquid chromatograph with a Kratos Spectroflow 773 detector (254 nm) and a Perkin-Elmer LCI-100 integrator using a Brownlee RP-18, 10 μm, 25 cm × 4.6 mm analytical column at a flow rate of 1.5 mL/min. The mobile phase was an isocratic mixture of 90% methanol and 10% aqueous phosphate pH 3 buffer. Peptides III and IV were analyzed on a Waters 680 instrument with a Kratos 757 detector (260 nm) using a Synchropak RP-P C-18, 6.5 μm, 25 cm × 4.1 mm analytical column at a flow rate of 2 mL/min. The mobile phase used a binary solvent with a 15-min linear gradient from solvent A (0.5% Et_3N and 20% of 1:1 CH_3CN/CH_3OH in water) to solvent B (0.5% Et_3N and 80% of 1:1 CH_3CN/CH_3OH in water).

***O*-Benzyl 4(*S*)-[(*tert*-Butyloxycarbonyl)amino]-2,2-difluoro-3(*R*)-hydroxy-6-methylheptanohydroxamate (2a).** To a stirred solution of 2.64 g (7.9 mmol) of the sodium salt **1a**, 1.52 g (9.5 mmol) of *O*-benzylhydroxylamine hydrochloride, and 1.28 g (9.5 mmol) of 1-hydroxybenzotriazole in 75 mL of dichloromethane was added 0.33 mL (1.9 mmol) of diisopropylethylamine, followed by 1.96 g (9.5 mmol) of dicyclohexylcarbodiimide.[23] After being stirred at room temperature for 1 day, the reaction mixture was partitioned between saturated aqueous $NaHCO_3$ and dichloromethane. The aqueous phase was further extracted with five portions of dichloromethane. The combined organic phases were dried ($MgSO_4$) and then concentrated. The residue was triturated with ether and then filtered. The filtrate was then concentrated and the resulting residue chromatographed on silica gel with 30% ethyl acetate in hexane to give 3.24 g (7.78 mmol, 98%) of the hydroxamate **2a**: ^1H NMR ($CDCl_3$) δ 0.89 (d, 6 H, J = 6 Hz), 1.41 (s, 9 H), 1.1–2.0 (m, 3 H), 3.7–4.3 (m, 3 H), 4.92 (s, 2 H), 5.0 (d, 1 H), 7.0 (m, 1 H), 7.4 (br s, 5 H); IR (mull) 3300, 1684 cm^{-1}; $[\alpha]_D$ −5° ($CHCl_3$, c 0.694); MS $[M + H]^+$ at m/z 417.2211 (calcd 417.2201).

1-(Benzyloxy)-4(*S*)-[1(*S*)-[(*tert*-butoxycarbonyl)amino]-3-methylbutyl]-3,3-difluoro-2-azetidinone (3a). To a stirred solution of 1.57 g (3.77 mmol) of the hydroxamate **2a** and 1.09 g (4.1 mmol) of triphenylphosphine in 20 mL of dry tetrahydrofuran under argon at room temperature was added a solution of 0.65 mL (4.1 mmol) of diethyl azodicarboxylate in 8 mL of dry tetrahydrofuran. The resulting mixture was then heated in an oil bath at 50 °C for 45 min. The cooled reaction mixture was concentrated and the residue chromatographed on Florisil with 5% ethyl acetate in hexane to give 817 mg (2.05 mmol, 54%) of the β-lactam **3a**: ^1H NMR ($CDCl_3$) α 0.94 (d, 6 H, J = 6 Hz), 1.1–1.9 (m, 3 H), 1.44 (s, 9 H), 3.6–4.5 (m, 3 H), 5.06 (s, 2 H), 7.44 (br s, 5 H); IR (mull) 3351, 1811, 1710 cm^{-1}; $[\alpha]_D$ −56° ($CHCl_3$, c 0.641); MS $[M + H]^+$ at m/z 399.

Sodium 3(*S*)-[(Benzyloxy)amino]-4(*S*)-[(*tert*-butyloxycarbonyl)amino]-2,2-difluoro-6-methylheptanoate (4a). To a stirred solution of 149 mg (0.37 mmol) of the β-lactam **3a** in 0.8 mL of tetrahydrofuran was added 0.41 mL (0.41 mmol) of a 1 M aqueous NaOH solution. After 20 h, tetrahydrofuran was evaporated off and the remaining aqueous solution lyhophilized to give a fluffy white solid.

***N*-(4-Methoxyphenyl)-4(*S*)-[(*tert*-butyloxycarbonyl)amino]-2,2-difluoro-3(*R*)-hydroxy-6-methylheptanamide (5a).** To a stirred solution of 667 mg (2.0 mmol) of the sodium salt **1a** and 271 mg (2.2 mmol) of *p*-anisidine in 10 mL of dichloromethane was added 0.38 mL (2.2 mmol) of diisopropylethylamine, followed by 560 mg (2.2 mmol) of bis(2-oxo-3-oxazolidinyl)phosphinic chloride.[24] After being stirred at room temperature for 15 h, the reaction mixture was partitioned between dichloromethane and dilute aqueous HCl. The aqueous layer was extracted with two more portions of dichloromethane. The combined organic phases were dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 15% ethyl acetate in dichloromethane to give 600 mg (1.44 mmol, 72%) of the amide **5a**: ^1H NMR ($CDCl_3$) δ 0.91 (d, 6 H, J = 6 Hz), 1.1–1.8 (m, 3 H), 1.41 (s, 9 H), 3.80 (s, 3 H), 3.8–4.4 (m, 4 H), 4.91 (br d, 1 H, J = 10 Hz), 6.88 (d, 2 H, J = 9 Hz), 7.55 (d, 2 H, J = 9 Hz); IR (mull) 3378, 1682 cm^{-1}; $[\alpha]_D$ −32° (EtOH, c 0.539); MS $[M + H]^+$ at m/z 416.2118 (calcd 416.2123).

***N*-(4-Methoxyphenyl)-4(*S*)-[(*tert*-butyloxycarbonyl)amino]-2,2-difluoro-3(*S*)-hydroxy-6-methylheptanamide (5b).** By the same procedure for the preparation of the amide **5a**, 333 mg (1 mmol) of the sodium salt **1b**, 135 mg (1.1 mmol) of *p*-anisidine, 0.19 mL (1.1 mmol) of diisopropylethylamine, and 280 mg (1.1 mmol) of bis(2-oxo-3-oxazolidinyl)phosphinic chloride in 5 mL of dichloromethane afforded 307 mg (0.74 mmol, 74%) of the amide **5b** after chromatography: ^1H NMR (CD_3COCD_3) δ 0.91 (d, 6 H, J = 6 Hz), 1.1–1.8 (m, 3 H), 1.32 (s, 9 H), 3.76 (s, 3 H), 3.7–4.5 (m, 4 H), 5.75 (br d, 1 H, J = 9 Hz), 6.90 (d, 2 H, J = 9 Hz), 7.67 (d, 2 H, J = 9 Hz); IR (mull) 3343, 3305, 1687, 1679 cm^{-1}; $[\alpha]_D$ −53° (EtOH, c 0.527); MS $[M + H]^+$ at m/z 416.2106 (calcd 416.2123).

4(*S*)-[1(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-methylbutyl]-3,3-difluoro-1-(methoxyphenyl)-2-azetidinone (6a). To a stirred solution of 600 mg (1.44 mmol) of the amide **5a** and 453 mg (1.73 mmol) of triphenylphosphine in 6 mL of dry tetrahydrofuran under argon at room temperature was added a solution of 0.25 mL (1.6 mmol) of diethyl azodicarboxylate in 2 mL of dry tetrahydrofuran over 10 min. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and the residue chromatographed on silica gel with 20% ethyl acetate in hexane to give 576 mg (1.44 mmol, 100%) of the β-lactam **6a**: ^1H NMR ($CDCl_3$) δ 0.99 (d, 6 H, J = 6 Hz), 1.2–1.9 (m, 3 H), 1.28 (s, 9 H), 3.80 (s, 3 H), 4.2–4.7 (m, 3 H), 6.91 (d, 2 H, J = 9 Hz), 7.39 (d, 2 H, J = 9 Hz); IR ($CHCl_3$) 3450, 1780, 1715 cm^{-1}; $[\alpha]_D$ 51° ($CHCl_3$, c 0.718); MS $[M + H]^+$ at m/z 398.2020 (calcd 398.2017).

4(*R*)-[1(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-methylbutyl]-3,3-difluoro-1-(methoxyphenyl)-2-azetidinone (6b). By the same procedure for the preparation of the β-lactam **6a**, 236 mg (0.567 mmol) of the amide **5b**, 178 mg (0.68 mmol) of triphenylphosphine, and 0.1 mL (0.62 mmol) of diethyl azodicarboxylate in 5 mL of dry tetrahydrofuran afforded 209 mg (0.52 mmol, 92%) of the β-lactam **6b** after chromatoraphy on silica gel: ^1H NMR ($CDCl_3$) δ 0.76 (d, 3 H, J = 6 Hz), 0.87 (d, 3 H, J = 6 Hz), 1.1–1.9 (m, 3 H), 1.42 (s, 9 H), 3.80 (s, 3 H), 4.1–4.7 (m, 3 H), 6.90 (d, 2 H, J = 9 Hz), 7.41 (d, 2 H, J = 9 Hz); IR (mull) 3360, 1773, 1675 cm^{-1}; $[\alpha]_D$ −24° ($CHCl_3$, c 0.565); MS $[M + H]^+$ at m/z 398.2000 (calcd 398.2017).

Sodium 4(*S*)-[(*tert*-Butyloxycarbonyl)amino]-2,2-difluoro-3(*S*)-[(4-methoxyphenyl)amino]-6-methylheptanoate (7a). By the same procedure for the preparation of the salt **4a**, 538 mg (1.35 mmol) of the β-lactam **6a** in 2.8 mL of tetrahydrofuran was treated with 1.4 mL (1.4 mmol) of a 1 M aqueous NaOH solution to afford the salt **7a** after lyophilization.

Sodium 4(*S*)-[(*tert*-Butyloxycarbonyl)amino]-2,2-difluoro-3(*R*)-[(4-methoxyphenyl)amino]-6-methylheptanoate (7b). By the same procedure for the preparation of the salt **4a**, 101 mg (0.253 mmol) of the β-lactam **6b** in 0.53 mL of tetrahydrofuran was treated with 0.27 mL (0.27 mmol) of a 1 M aqueous NaOH solution to afford the salt **7b** after lyophilization.

[4(*S*)-[(*tert*-Butyloxycarbonyl)amino]-2,2-difluoro-3-(*S*)-[(4-methoxyphenyl)amino]-6-methylheptanoyl]-L-isoleucyl-2-pyridylmethylamine (9a). To a stirred solution 219 mg (0.50 mmol) of the sodium salt **7a**, 133 mg (0.60 mmol) of L-isoleucyl-2-pyridylmethylamine and 88 mg (0.65 mmol) of 1-hydroxybenzotriazole in 2.5 mL of dichloromethane was added 124 mg (0.60 mmol) of dicyclohexylcarbodiimide. After 18 h, the reaction mixture was partitioned between dichloromethane and saturated aqueous $NaHCO_3$. The aqueous phase was further

(23) Konig, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788.

(24) Diago-Meseguer, J.; Palomo-Coll, A. L.; Fernandez-Lizarbe, J. R.; Zurgara-Bilbao, A. *Synthesis* **1980**, 547.

Table II

peptides[a]	HPLC[b] k'	amino acid anal.			MS (FAB)	
		Phe	His	Ile	calcd	found
II	7.30	1.05	1.00	1.00	763.4506	763.4522
III	5.97	1.02	0.96	1.00	762.4666	762.4687
IV	6.47	1.00	0.84	1.02	762.4666	762.4649
VII	8.19	1.02	1.02	1.00	798.4478	798.4481
VIII	9.18	1.03	1.00	0.96	798.4478	798.4481

[a] ^{1}H NMR in $CDCl_3$ found consistent with structures. [b] See the Experimental Section for condition; k' is the partition ratio.

extracted with three portions of dichloromethane. The combined organic phases were dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 2:1 ethyl acetate/dichloromethane to give 320 mg (100%) of compound **9a**: ^{1}H NMR ($CDCl_3$) δ 0.91 (m, 12 H), 1.44 (s, 9 H), 3.63 (s, 3 H), 8.47 (br d, 1 H, J = 5 Hz); MS (FAB) $[M + H]^+$ at m/z 620.3603 (calcd 620.3623).

***N*-[*N*-[4(*S*)-[[*N*$^\alpha$-(*tert*-Butyloxycarbonyl)-*N*^{im}-tosyl-L-histidyl]amino]-2,2-difluoro-3(*S*)-[(4-methoxyphenyl)-amino]-6-methylheptanoyl]-L-isoleucyl]-2-pyridylmethylamine (10a).** A solution of 32.2 mg (0.052 mmol) of compound **9a** in 0.5 mL of dichloromethane and 0.5 mL of trifluoroacetic acid was allowed to stir at room temperature for 1 h. The reaction mixture was slowly added to aqueous $NaHCO_3$. The aqueous phase was extracted with three portions of dichloromethane. The combined organic phases were dried ($MgSO_4$) and then concentrated.

To a stirred solution of the above residue, 28.5 mg (0.07 mmol) of N^α-(*tert*-butyloxycarbonyl)-N^{im}-tosyl-L-histidine, and 12 μL (0.07 mmol) of diisopropylethylamine in 0.5 mL of dichloromethane was added 11 μL (0.07 mmol) of diethylphosphoryl cyanide. After being stirred at room temperature for 20 h, the resulting mixture was applied to a silica gel column and then eluted with 3% methanol in dichloromethane to afford 30.6 mg (0.034 mmol, 65%) of compound **10a**: ^{1}H NMR ($CDCl_3$) δ 0.81 (m, 12 H), 1.44 (s, 9 H), 2.40 (s, 3 H), 3.63 (s, 3 H), 8.47 (br d, 1 H, J = 5 Hz); MS (FAB) $[M + H]^+$ at m/z 911.4337 (calcd 911.4301).

***N*-[*N*-[4(*S*)-[[*N*$^\alpha$-[(*tert*-Butyloxycarbonyl)-L-phenylalanyl]-*N*^{im}-tosyl-L-histidyl]amino]-2,2-difluoro-3(*S*)-[(4-methoxyphenyl)amino]-6-methylheptanoyl]-L-isoleucyl]-2-pyridylmethylamine (11a).** By the same procedure for the preparation of compound **10a**, 30.6 mg (0.034 mmol) of compound **10a** was deprotected with dichloromethane/trifluoroacetic acid. The resulting amine, 12 mg (0.045 mmol) of *N*-(*tert*-butyloxycarbonyl)-L-phenylalanine, 8 μL (0.046 mmol) of diisopropylethylamine, and 7 μL (0.046 mmol) of diethylphosphoryl cyanide in 0.3 mL of dichloromethane afforded 26.7 mg (0.025 mmol, 74%) of compound **11a** after chromatography on silica gel with 3% methanol in dichloromethane: ^{1}H NMR ($CDCl_3$) δ 0.81 (m, 12 H), 1.41 (s, 9 H), 2.40 (s, 3 H), 3.56 (s, 3 H), 8.47 (br d, 1 H, J = 5 Hz); MS (FAB) $[M + H]^+$ at m/z 1058.4976 (calcd 1058.4984).

***N*-[*N*-[4(*S*)-[[*N*$^\alpha$-[(*tert*-Butyloxycarbonyl)-L-phenylalanyl]-*N*^{im}-tosyl-L-histidyl]amino]-3(*S*)-amino-2,2-difluoro-6-methylheptanoyl]-L-isoleucyl]-2-pyridylmethylamine (12a).** To a stirred solution of 26.7 mg (0.025 mmol) of compound **11a** in 0.25 mL of acetonitrile at 0 °C was added a solution of 111 mg (0.2 mmol) of ceric ammonium nitrate in a small amount of water. The resulting mixture was allowed to warm to room temperature and continue to stir for 1 h. The resulting mixture was treated with 2 M aqueous triammonium citrate. It was then adjusted to pH 7 with dilute aqueous NH_4OH. The aqueous phase was extracted with five portions of dichloromethane. The combined organic phases were dried ($MgSO_4$) and then concentrated. The resulting residue was chromatoraphed on silica gel with 3% methanol in dichloromethane to give 11.4 mg (0.012 mmol, 48%) of compound **12a**: ^{1}H NMR ($CDCl_3$) δ 0.86 (m, 12 H), 1.41 (s, 9 H), 2.40 (s, 3 H), 8.47 (br d, 1 H, J = 5 Hz); MS (FAB) $[M + H]^+$ at m/z 952.4593 (calcd 952.4566).

***N*-[*N*-[4(*S*)-[[*N*$^\alpha$-[(*tert*-Butyloxycarbonyl)-L-phenylalanyl]-L-histidyl]amino]-3(*S*)-amino-2,2-difluoro-6-methylheptanoyl]-L-isoleucyl]-2-pyridylmethylamine (VIII).** A solution of 11.2 mg (0.012 mmol) of compound **12a** and 6.4 mg (0.047 mmol) of 1-hydroxybenzotriazole in 0.2 mL of methanol was allowed to stir at room temperature for 15 h. The reaction mixture was then concentrated and the residue chromatographed on silica gel with 5% methanol (saturated with gaseous ammonia) in dichloromethane to give 8.2 mg (0.010 mmol, 87%) of the peptide VIII: ^{1}H NMR ($CDCl_3$) δ 0.88 (m, 12 H), 1.36 (s, 9 H), 8.47 (br d, 1 H, J = 5 Hz).

Peptide I has been reported previously[13] and peptide II could be prepared in a similar manner starting with Boc-epistatine. Peptides III and IV were prepared from 3(*S*)- and 3(*R*)-[(benzyloxycarbonyl)amino]-4(*S*)-[(*tert*-butyloxycarbonyl)amino]-6-methylheptanoic acids.[14] Peptides V and VI were reported previously.[13] Peptide VII was prepared in an analogous manner to the preparation of peptide VIII as described above. Physical characteristics for the five new peptides are listed in Table II.

Biology. Inhibition of Human Plasma Renin. These peptides were assayed for plasma renin inhibitory activity as follows: Lyophilized human plasma with 0.1% EDTA was obtained commercially (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% 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 control tubes to estimate percent inhibition. The inhibition results were expressed as IC_{50} values that were obtained by plotting three to four inhibitor concentrations on semilog graph paper and estimating the concentration producing 50% inhibition.

Acknowledgment. We are grateful to Margaret R. Schuette for technical assistance and Ilene M. Reardon for amino acid analyses.

Registry No. **1a**, 103322-63-0; **1b**, 109553-33-5; **2a**, 109553-23-3; **3a**, 109553-24-4; **4a**, 109553-25-5; **5a**, 109553-26-6; **5b**, 109553-34-6; **6a**, 109553-27-7; **6b**, 109584-53-4; **7a**, 109553-28-8; **7b**, 109553-35-7; **8**, 97920-16-6; **9a**, 109553-29-9; **10a**, 109553-30-2; **11a**, 109553-31-3; **12a**, 109553-32-4; I, 97920-11-1; II, 109667-19-8; III, 109553-36-8; IV, 109667-20-1; V, 97920-12-2; VI, 97995-52-3; VII, 109553-37-9; VIII, 109667-21-2; H_2NOCH_2Ph·HCl, 2687-43-6; BOC-His(Tos)-OH, 35899-43-5; BOC-Phe-OH, 13734-34-4; *p*-anisidine, 104-94-9; renin, 9015-94-5.