Renin Inhibitors. Design of Angiotensinogen Transition-State Analogues Containing Novel (2*R*,3*R*,4*R*,5*S*)-5-Amino-3,4-dihydroxy-2-isopropyl-7-methyloctanoic Acid

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A highly stereoselective synthesis of 2(*R*)-[5(*R*)-[1(*S*)-[(*tert*-butyloxycarbonyl)amino]-3-methylbutyl]-2,2-dimethyl-4(*R*)-dioxolanyl]-3-methylbutanoic acid (**11**) is described. This is a suitably protected carboxylic acid useful as an intermediate for the preparation of renin inhibitory peptides. Angiotensinogen analogues such as peptides IX and X that contain the dipeptide isostere (2*R*,3*R*,4*R*,5*S*)-5-amino-3,4-dihydroxy-2-isopropyl-7-methyloctanoic acid residue at the scissile site are shown to be potent inhibitors of human plasma renin. The glycol moiety in this novel acid, dihydroxyethylene isostere, is suggested to act as a transition-state analogue and mimics the tetrahedral intermediate formed during the enzyme-catalyzed hydrolysis of the peptidic bond.

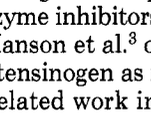
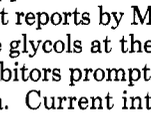
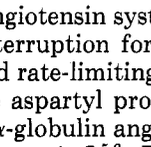
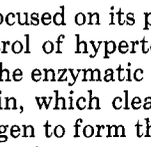
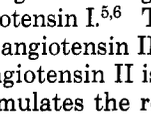
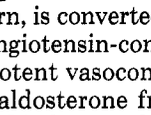
We have a continuing interest in the use of the transition-state-analogue concept as an approach in the design of potent enzyme inhibitors.[1] Recent reports by Matsueda et al.[2] and Hanson et al.[3] on peptide glycols at the scissile site of angiotensinogen as renin inhibitors prompted us to report our related work in this area. Current interest in the renin–angiotensin system has focused on its pharmacological interruption for the control of hypertension.[4] The first and rate-limiting step of the enzymatic cascade involves the aspartyl protease renin, which cleaves the circulating α-globulin angiotensinogen to form the decapeptide angiotensin I.[5,6] This, in turn, is converted to the octapeptide angiotensin II by the angiotensin-converting enzyme. Angiotensin II is a very potent vasoconstrictor and also stimulates the release of aldosterone from the adrenal gland. This mineralocorticoid induces sodium and water retention, and this, in conjunction with vasoconstriction, can lead to an increase in blood pressure. Renin is a highly specific proteolytic enzyme with only one known natural substrate.[6] Consequently, interruption of the renin–angiotensin system by inhibition of renin offers a very specific pharmacological intervention.[7]

Interest in the blockade of renin has led to rapid development of potent inhibitors based on the angiotensinogen sequence. The N-terminus of human angiotensinogen is shown in Figure 1. The most successful approach is based on the concept of the transition-state analogues[8] of the scissile bond -Leu[10]-Val[11]-. Modifications at the cleavage site A to mimic the tetrahedral species B that forms during the enzyme-catalyzed hydrolysis of the peptidic bond have generated analogues of the minimum substrate with high inhibitory potency (Figure 2).[9]

Following the report of Umezawa and Aoyagi[10] and Ito et al.[11] on C-terminal aldehydic peptides as potent protease inhibitors, Kokubu and co-workers[12] and Fehrentz et al.[13] prepared peptide analogues of angiotensin I with C-terminal aldehydes which were shown to inhibit renin. The tripeptide aldehydes C such as peptides I[2,12] and II[2,12] in Table I were postulated to bind renin as the corresponding hydrates D shown in Figure 3. The tetrahedral species D was suggested to act as a transition-state analogue mimicking the tetrahedral intermediate B during the amide bond hydrolysis. The aldehydes were shown to be much more effective enzyme inhibitors than the corresponding carbinols such as peptides III[2,12] and IV.[2,12]

Since aldehydes are anticipated to be metabolically unstable, the homologous glycol has been proposed to mimic the functioning of the geminal diol of the parent aldehyde hydrate.[2,3] The glycol, such as E, when placed at the C-terminus of angiotensin I, such as the tripeptides V[3] and VI,[2] provided renin inhibitors with IC_{50} in the micromolar range. Our effort on a possible transition-state

Table I. Inhibitory Potencies against Human Renin[a]

		IC_{50} (μM)			IC_{50} (μM)
I		0.75	II		0.06
III		22% inhibition at 10^{-4} M	IV		3.6
V		2.6	VI		1

[a] Boc = *tert*-butyloxycarbonyl; Z = benzyloxycarbonyl; 1-Nal = 3-(1′-naphthyl)alanine; see references in text.

(1) 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.
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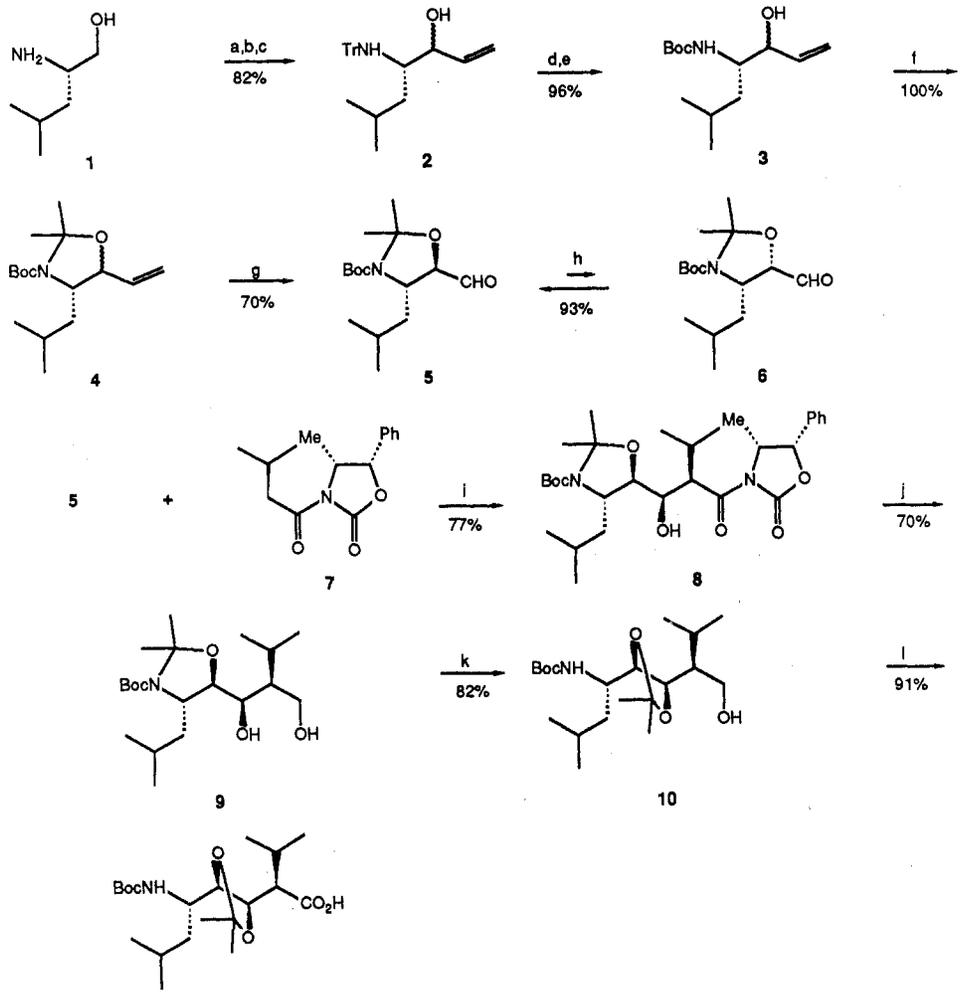
Scheme I. Synthesis of the Dipeptide Isostere 11[a]



[a] Key: (a) TrCl, Et_3N, CH_3Cl_2. (b) $(COCl)_2$, CH_3SOCH_3, CH_2Cl_2; Et_3N. (c) CH_2=CHMgBr, THF. (d) DOWEX 50 W-X8, CH_3OH. (e) $(t\text{-BuOCO})_2O$, THF. (f) CH_2=$C(OCH_3)CH_3$, pyr TsOH, CH_2Cl_2. (g) O_3, CH_2Cl_2/CH_3OH; Zn, AcOH. (h) K_2CO_3, CH_3OH. (i) n-Bu_2BOTf, i-Pr_2NEt, CH_2Cl_2; H_2O_2. (j) n-Bu_2BOTf, i-Pr_2NEt, THF; $LiBH_4$. (k) Camphorsulfonic acid, CH_2Cl_2; $CH_3C(OCH_3)_3$. (l) $RuCl_3 \cdot H_2O$, H_5IO_6, CH_3CN, CCl_4, H_2O.

1 6 10 11
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-

Figure 1. Human angiotensinogen.

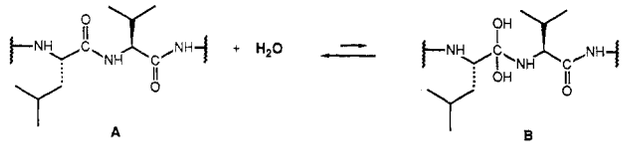


Figure 2. Hydration of a peptidic bond.

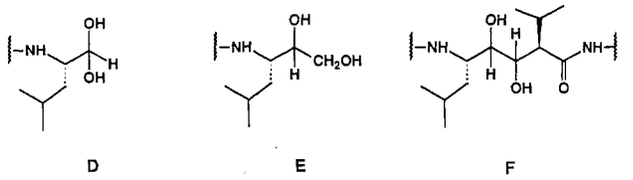


Figure 3. Postulated transition-state analogues.

analogue has focused on the dihydroxyethylene dipeptide isostere F as a mimic of the tetrahedral hydrated species B.

We decided to examine one diastereomer out of several possible diastereomers of the dipeptide isostere F with due consideration of prior knowledge. The absolute stereochemistry at C-2 and C-5 follows that of L-amino acids. The preferred configuration at C-4 should be R according to precedence from earlier work on similar dipeptide isosteres such as statine and the hydroxyethylene isostere.[9] Finally, qualitative molecular modeling with a computer graphic model of the human renin active site[14] suggested the *R* configuration at C-3 in order that the C-3 hydroxyl group might be appropriately positioned for possible favorable interaction with the catalytic pair of aspartic acid residues of the enzyme active site. The acid **11** was chosen as the suitably protected dipeptide isostere synthetic building block for the -Leu[10]-Val[11]- scissile bond replacement. The relative stereochemistry at C-2 and C-3 then suggested a possible synthesis by a stereocontrolled aldol addition[15] between the aldehyde **5** and the oxazolidinone **7** to give the *syn*-aldol adduct with control of the absolute stereochemistry.

Chemistry

The synthetic sequence is shown in Scheme I. L-Leucinol (**1**) was tritylated on the amino function, and the remaining hydroxyl group was then oxidized[16] to the

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(15) Evans, D. A.; Nelson, J. V.; Vogel, E.; Taber, T. R. *J. Am. Chem. Soc.* **1981**, *103*, 3099.

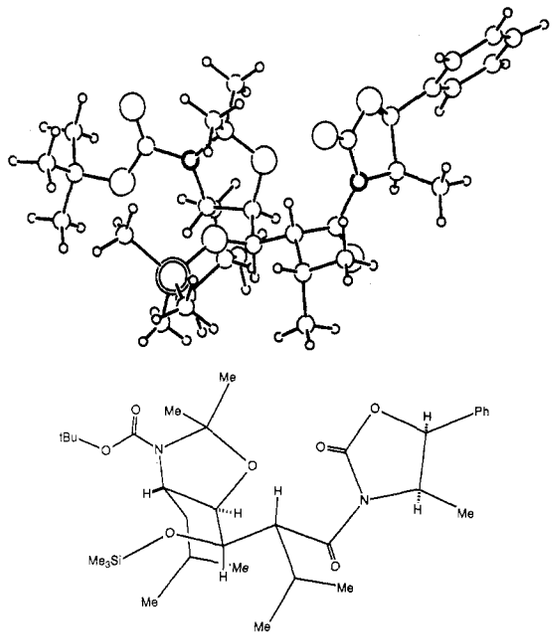


Figure 4. Trimethylsilyl derivative of the aldol adduct 8a.

corresponding aldehyde. Addition of vinylmagnesium bromide afforded a nearly equal mixture of epimeric alcohols **2**. The trityl group was then removed under acidic hydrolysis, and the resulting amines were protected with the *tert*-butyloxycarbonyl group as in compounds **3**. The addition of vinylmagnesium bromide to (*tert*-butyloxycarbonyl)-L-leucinal that would have afforded compounds **3** directly unfortunately resulted in much lower yield and also partial racemization, and this consequence necessitated the use of a temporary trityl protecting group.

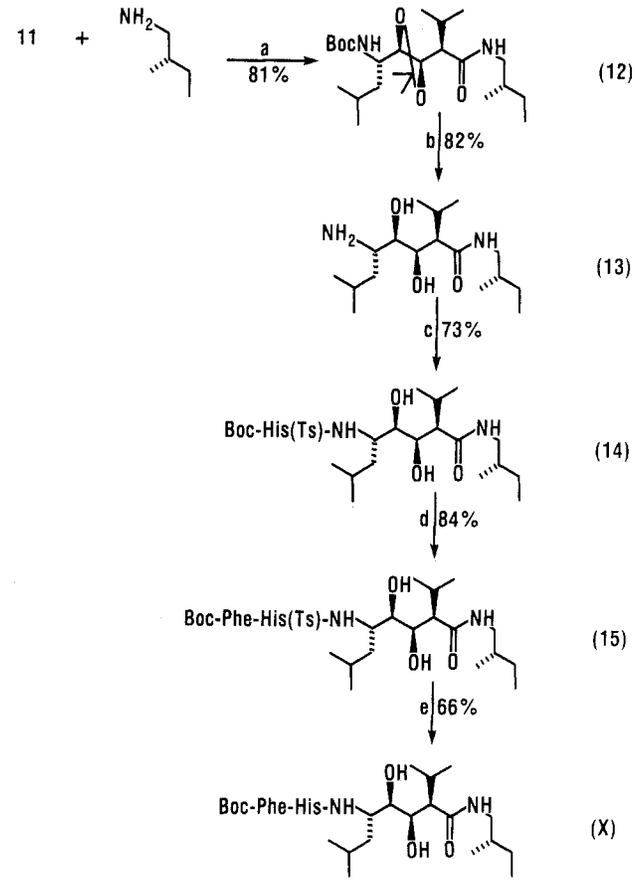
Formation of the isopropylidenes **4** effected protection for both acidic protons in compounds **3**. Treatment of compounds **4** with ozone afforded a mixture of aldehydes **5** and **6**. This mixture, with the strategically placed oxazolidine ring, could be favorably equilibrated under basic conditions to give the desired aldehyde **5** (≥10:1 by ^{1}H NMR). As already mentioned, the requirement for the stereochemistry of the hydroxyl group as in compound **5** assumed earlier precedence in related dipeptide isosteres.[9]

Stereocontrolled aldol addition[15] of the aldehyde **5** to the acyloxazolidinone **7** afforded the adduct **8**, the ^{13}C NMR spectrum of which indicated one diastereomer. The corresponding trimethylsilyl ether **8a** offered a crystalline derivative, which was subjected to a single-crystal X-ray analysis, and the result as shown in Figure 4 confirmed the predicted stereochemistry as that shown in compound **8**. The chiral auxiliary was then reductively removed[17] to give the diol **9**. To differentiate the two hydroxyl groups in compound **9**, the acetonide could be equilibrated to afford the alcohol **10** and the remaining primary alcohol was oxidized[18] to the desired carboxylic acid **11**.

It is to be noted that the overall synthetic sequence is highly stereoselective and convergent. The two side chains, which represent leucine and valine in the present instance, can be varied, and a wide variety of dipeptide isosteres with this general structure should be readily prepared.

Angiotensinogen analogues that contain this dihydroxyethylene isostere were prepared as potential renin

Scheme II. Synthesis of the Peptide X[a]



[a] Key: (a) $(EtO)_2P(O)CN$, Et_3N, CH_2Cl_2. (b) HCl, CH_3OH. (c) Boc-His(Ts)-OH, $(EtO)_2P(O)CN$, Et_3N, CH_2Cl_2. (d) CF_3CO_2H; Boc-Phe-OH, $(EtO)_2P(O)CN$, Et_3N, CH_2Cl_2. (e) 1-Hydroxybenzotriazole, CH_3OH.

inhibitors as shown in Scheme II for a representative peptide X. The carboxylic acid **11** was coupled to 2(*S*)-methylbutylamine to give the amide **12** by using diethylphosphoryl cyanide.[19] The hydrolyzed free amine was coupled to N^{α}-(*tert*-butyloxycarbonyl)-N^{im}-tosyl-L-histidine to give compound **14**. The corresponding hydrolyzed free amine was then coupled to N^{α}-(*tert*-butyloxycarbonyl)-L-phenylalanine to give compound **15**. Removal of the tosyl protecting group gave the renin inhibitory peptide X. Peptide IX was synthesized in a similar manner by starting with L-isoleucyl-2-pyridylmethylamine in place of 2(*S*)-methylbutylamine. Peptide VIII was also prepared by using 4(*S*)-[(*tert*-butyldimethylsilyl)oxy]-5-(*S*)-[(*tert*-butyloxycarbonyl)amino]-2(*S*)-isopropyl-7-methyloctanoic acid[20] in place of the acid **11**. Peptide VII was prepared by the oxidation of the corresponding carbinol VIII as described previously.[1]

Results and Discussion

Peptides VII through X were evaluated as inhibitors of renin with IC_{50} values as shown in Table II. Peptides that contain the dihydroxyethylene isostere such as peptides IX and X are potent renin inhibitors. The increase of 3 orders of magnitude in potency in the dihydroxyethylene-isostere-containing peptide X when compared, for example, to the glycol V[3] suggests substantial importance of the additional binding sites, presumably S_1' and

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(17) Bartroli, J. Ph.D. Thesis, California Institute of Technology, 1985.
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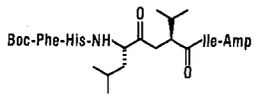
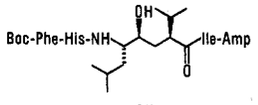
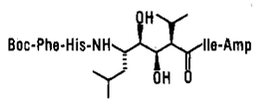
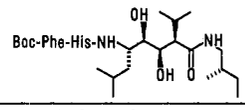
Table II. Inhibitory Potencies against Human Renin[a]

		IC_{50}, nM
VII		3.1
VIII		0.22
IX		0.35
X		3.4

[a] Amp = 2-aminomethylpyridine.

S_2', in compound X, which is still a relatively low molecular weight inhibitor.

C-Terminal aldehydes such as peptides I[2,12] and II[2,12] were proposed to be readily hydrated and resulted in a geminal diol as a transition-state mimic of the peptide bond hydrolysis. The corresponding carbinols such as peptides III[2,12] and IV[2,12] with sp^3 hybridization are, however, much less effective in mimicking the tetrahedral intermediate. The ketoethylene isostere,[21] such as the ketone VII, is also expected to bind to the active site as the corresponding hydrate.[22] A ketone functionality is normally less likely, when compared to an aldehyde, to be predominantly in the hydrated form, and this consequence might reflect the greater binding affinity of the corresponding carbinol VIII with its tetrahedral geometry. The tremendous increase in binding affinity of peptide VIII over that of peptide III,[2,12] for example, again reflects substantial importance of the additional binding sites for enzyme recognition.

The homologous glycols such as peptide V[3] and VI[2] were prepared as functioning mimics of the corresponding aldehydes I[2,12] and II.[2,12] The effectiveness of these glycols can be significantly influenced by other binding sites on the peptides. The peptide aldehyde II[2,12] with a naphthylmethyl group for the P_3 site is an active renin inhibitor. The corresponding glycol VI[2] is substantially less effective, and it is not much different from the carbinol IV[2,12] in its binding affinity to renin. The glycol such as peptide IX can be viewed as a functioning mimic of the hydrated ketone in the ketoethylene-isostere-containing peptide VII, and as can be seen in Table II, the dihydroxyethylene isostere is more effective than the ketoethylene isostere. The glycol IX is comparable to the carbinol VIII in its binding affinity to renin in this template peptide. This result suggests the effectiveness of the hydroxyethylene isostere and that the additional hydroxyl group at C-3 in the dihydroxyethylene isostere as proposed by qualitative molecular modeling did not result in any noticeable influence on the binding affinity to the enzyme.

Summary

A highly stereoselective and convergent synthesis of 2(*R*)-[5(*R*)-[1(*S*)-[(*tert*-butyloxycarbonyl)amino]-3-methylbutyl]-2,2-dimethyl-4(*R*)-dioxolanyl]-3-methylbutanoic acid (**11**) is described. It should prove generally applicable to the preparation of dipeptide isosteres with this representative structure. The glycol moiety in this novel acid is suggested to act as a transition-state analogue mimicking the tetrahedral species formed during the hydrolysis of a peptidic bond. Angiotensinogen analogues such as peptides IX and X that contain the dihydroxyethylene isostere F were proven to be effective inhibitors of the targeted enzyme renin. The transition-state-analogue concept continues to be a valuable approach in the design of efficient enzyme inhibitors. This work supports the value of an understanding of enzymatic mechanism and exploits this knowledge to create effective inhibitors of therapeutically important enzymes.

Experimental Section

Chemistry. Mass spectra, infrared spectra, optical rotations, melting point, and combustion analyses were obtained by the Physical and Analytical Chemistry Department of The Upjohn Company. ^1H NMR spectra were recorded at 80 MHz and ^{13}C NMR at 20 MHz with a Varian Model CFT-20 Fourier transform spectrometer. Chemical shifts were reported as δ units relative to tetramethylsilane as internal standard.

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. Oxalyl chloride, dimethyl sulfoxide, and trimethylamine were distilled from calcium hydride. Diethylphosphoryl cyanide was freshly distilled before use. Dibutylboron triflate was freshly prepared and distilled.

Peptides VII through X 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.

4-Methyl-2(*S*)-[(triphenylmethyl)amino]-1-pentanol. To a stirred solution of 3.0 g (25.6 mmol) of L-leucinol in 26 mL of anhydrous dichloromethane was added 2.84 g (28.1 mmol) of triethylamine followed by 7.13 g (25.6 mmol) of triphenylmethyl chloride. After 70 min at room temperature, the reaction mixture was poured into 200 mL of ethyl acetate and extracted with two 75-mL portions of half-saturated aqueous NaCl. The aqueous phase was extracted with two 100-mL portions of ethyl acetate. The combined organic phases were dried ($MgSO_4$), filtered, and concentrated. Flash chromatography of the residue on silica gel using 15% ethyl acetate in hexane with 0.1% added pyridine afforded 7.17 g (20.0 mmol, 85%) of the desired alcohol: ^1H NMR ($CDCl_3$) δ 0.65 (m, 6 H), 3.15 (m, 1 H), 7.25 (m, 15 H); IR (neat) 3336, 2954, 1490 cm^{-1}; $[\alpha]_D$ +27° (*c* 0.27, $CHCl_3$); FAB-HRMS, *m/z* 360.2327 (calcd for $C_{25}H_{29}NO$, 360.2327). Anal. ($C_{25}H_{29}NO$) C, H, N.

4-Methyl-2(*S*)-[(triphenylmethyl)amino]-1-pentanal. To a stirred solution of 3.13 g (24.7 mmol) of distilled oxalyl chloride in 50 mL of dry dichloromethane, cooled to −78 °C, was added dropwise 3.70 g (47.4 mmol) of dry dimethyl sulfoxide in 1.0 mL of dichloromethane. After 10 min, 6.81 g (19.0 mmol) of 4-methyl-2(*S*)-[(triphenylmethyl)amino]-1-pentanol in 50 mL of dry dichloromethane was added dropwise via cannula. After 15 min, the reaction mixture was treated with 13.7 mL (98.6 mmol) of triethylamine. After 5 min, the reaction mixture was warmed to room temperature and then poured into 500 mL of pentane and 200 mL of water to dissolve the salts. The reaction mixture was then partitioned between saturated aqueous $NaHCO_3$ and pentane. The aqueous phase was extracted with five portions of pentane. The organic phases were combined, dried ($MgSO_4$), filtered, and then concentrated to afford 6.76 g (19.0 mmol, 100%) of the desired aldehyde, which was immediately used in the next reaction.

3(*R*)- and 3(*S*)-Hydroxy-6-methyl-4(*S*)-[(triphenylmethyl)amino]-1-heptene (2). To 38 mL (38 mmol) of a 1.0 M solution of vinylmagnesium bromide in tetrahydrofuran, cooled

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to −30 °C, under argon with stirring, was carefully added dropwise via cannula 6.76 g (19.0 mmol) of the freshly prepared 4-methyl-2(*S*)-[(triphenylmethyl)amino]-1-pentanal in 38 mL of dry tetrahydrofuran. After 5 min, the reaction mixture was poured into a stirred ice-cold solution of saturated aqueous NH_4Cl. After 5 min, the reaction mixture was warmed to room temperature, then diluted with ether, and washed successively with saturated aqueous NH_4Cl, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl. The organic phase was dried ($MgSO_4$), filtered, and then concentrated. The resulting yellow thick oil was passed through a pad of Florisil using 20% ethyl acetate in hexane. Concentration of the filtrate afforded 7.11 g (96%) of compound **2**: ^1H NMR ($CDCl_3$) δ 0.5 (d, 3 H, J = 4 Hz), 0.7 d, 3 H, J = 4 Hz), 1.25 (m, 3 H), 2.5 (m, 1 H), 5.5 (m, 3 H), 7.5 (m, 15 H); IR (neat) 3345, 2955, 1490 cm^{-1}; FAB-HRMS, m/z 386.2503 (calcd for $C_{27}H_{31}NO$, 386.2484).

4(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3(*R*)- and -3(*S*)-hydroxy-6-methyl-1-heptene (3). To a stirred solution of 7.11 g (18.5 mmol) of compound **2** in 185 mL of dry methanol was added 7.10 g of DOWEX 50W-X8 resin. After 14 h at room temperature, 9.0 g (84.9 mmol) of solid sodium carbonate was added. After 40 min, at room temperature, the reaction mixture was filtered through Celite and the filtrate was concentrated. The resulting residue was triturated with dichloromethane, filtered, and then concentrated. To this residue were added 75 mL of dry tetrahydrofuran and 4.22 g (19.6 mmol) of di-*tert*-butyl dicarbonate. After the reaction mixture was stirred for 14 h at room temperature, it was concentrated. Flash chromatography of the resulting residue on silica gel using 20% ethyl acetate in hexane afforded 4.13 g (17.0 mmol, 92%) of compound **3**: ^1H NMR ($CDCl_3$) δ 0.92 (2 d, 6 H, J = 7 Hz), 1.43 (s, 9 H), 5.25 (m, 2 H), 5.78 (m, 1 H); IR (neat) 3361, 1690 cm^{-1}; FAB-HRMS, m/z 244.1898 (calcd for $C_{13}H_{25}NO_3$, 244.1913).

3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-5(*R*)- and -5-(*S*)-ethenyl-4(*S*)-(2-methylpropyl)oxazolidine (4). To a stirred solution of 3.81 g (15.7 mmol) of compound **3** in 31 mL of dry dichloromethane were added 11.29 g (156.6 mmol) of 2-methoxypropene and 197 mg (0.78 mmol) of pyridinium *p*-toluenesulfonate. After 4 h at room temperature, the reaction mixture was neutralized with excess solid sodium bicarbonate. After 1 h, the reaction mixture was filtered and then concentrated. Flash chromatography of the residue on silica gel with hexane and then 3% ethyl acetate in hexane afforded 4.43 g (15.7 mmol, 100%) of compound **4**: ^1H NMR ($CDCl_3$) δ 0.94 (2 d, 6 H, J = 7 Hz), 1.48 (s, 9 H), 1.51 (s, 3 H), 1.54 (s, 3 H), 1.56 (s, 3 H), 1.60 (s, 3 H), 5.25 (m, 2 H), 5.75 (m, 1 H); IR (neat) 1700 cm^{-1}; MS, m/z 268. Anal. ($C_{16}H_{29}NO_3$) C, H, N.

3-(*tert*-Butyloxycarbonyl)-5(*R*)- and -5(*S*)-formyl-2,2-dimethyl-4(*S*)-(2-methylpropyl)oxazolidine (5 and 6). To a stirred solution of 4.43 g (15.7 mmol) of compound **4** in 74 mL of dry dichloromethane and 18.5 mL of dry methanol cooled to −78 °C was added 5 drops of Sudan III (0.1% solution in dichloromethane). The reaction mixture was purged with nitrogen, and then ozone was passed into the stirred solution until red color discharged (ca. 45 min). The solution was then purged with a stream of nitrogen. The reaction mixture was then cannulated into a stirred −45 °C solution containing 92.5 mL of dry methanol, 92.5 mL of water, 3.7 mL of glacial acetic acid, and 3.7 g of zinc dust. After 5 min, the reaction mixture was warmed to room temperature. The aqueous phase was extracted with four 100-mL portions of dichloromethane. The combined organic phases were dried ($MgSO_4$), filtered, and then concentrated. Flash chromatography of the residue on silica gel using 15% ethyl acetate in hexane afforded 3.17 g (11.1 mmol, 70%) of the aldehydes **5** and **6** as a 1.7:1 mixture of epimers: ^1H NMR ($CDCl_3$) δ 0.96 (m, 6 H), 1.46 (s, 9 H), 1.48 (s, 3 H), 1.56 (s, 3 H), 1.61 (s, 3 H), 4.24 (m, 2 H), 9.72 (d, 1 H, J = 1 Hz), 9.82 (s, 1 H); FAB-HRMS, m/z 286.2051 (calcd for $C_{15}H_{27}NO_4$, 286.2018).

Equilibration of the Aldehydes 5 and 6. To a stirred solution of 264.8 mg (0.92. mmol) of the aldehydes **5** and **6** in 3.7 mL of methanol was added 128.4 mg (0.929 mmol) of powdered anhydrous potassium carbonate. After 2 h at room temperature, the reaction mixture was cooled to 0 °C and treated with 105 μL (1.83 mmol) of glacial acetic acid. After 5 min, the reaction mixture was warmed to room temperature and 1 M pH 7 phosphate buffer was added. After 30 min, the reaction mixture was concentrated and then the aqueous phase was extracted with five portions of ether. The combined ethereal phases were dried ($MgSO_4$), filtered, and then concentrated. Flash chromatography of the residue on silica gel using 15% ethyl acetate in hexane afforded 246.2 mg (0.863 mmol, 93%) of the equilibrated aldehydes (**5:6** > 10:1): ^1H NMR ($CDCl_3$) δ 0.96 (m, 6 H), 1.46 (s, 9 H), 1.57 (s, 3 H), 1.61 (s, 3 H), 4.13 (m, 2 H), 9.82 (s, 1 H).

4(*R*)-Methyl-3-(1-oxo-3-methylbutyl)-5(*S*)-phenyl-2-oxazolidinone (7). To a stirred solution of 6.04 g (34.1 mmole of 4(*R*)-methyl-5(*S*)-phenyl-2-oxazolidinone in 76 mL of dry tetrahedrofuran, cooled to −78 °C, was slowly added, via addition funnel, 22.8 mL (35.8 mmol) of 1.57 M *n*-butyllithium in hexane. After the mixture was stirred for 5 min, 4.93 g (40.9 mmol) of isovaleryl chloride was added dropwise. The reaction mixture was warmed to room temperature. After 30 min, the reaction mixture was treated with 15 mL of saturated aqueous $NaHCO_3$ and concentrated. The residue was partitioned between ether and 1 N aqueous NaOH. The aqueous layer was extracted with 75 mL of ether. The combined organic phases were washed with 30 mL of water and 30 mL of saturated aqueous NaCl, dried ($MgSO_4$), filtered, and then concentrated. Evaporative distillation (0.3 torr, 200 °C) of the yellow residue afforded 8.45 g (32.3 mmol, 95%) of a thick oil, which solidified upon standing: mp 52–53 °C; ^1H NMR ($CDCl_3$) δ 0.88 (d, 3 H, J = 7 Hz), 0.97 (d, 3 H, J = 7 Hz), 0.98 (d, 3 H, J = 7 Hz), 2.12 (m, 1 H, J = 4 Hz), 2.75 (dd, 1 H, J = 15, 19 Hz), 2.81 (dd, 1 H, J = 16, 20 Hz), 4.77 (dq, 1 H, J = 7 Hz), 5.59 (d, 1 H, J = 7 Hz), 7.45 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 18.46, 26.33, 29.05, 47.91, 58.63, 82.78, 129.57, 132.60, 137.36, 176.33; IR (mull) 1770 cm^{-1}; $[\alpha]_D$ +50° (c 0.95, $CHCl_3$); MS, m/z 261.1371 (calcd for $C_{15}H_{19}NO_3$, 261.1365). Anal. ($C_{15}H_{19}NO_3$) C, H, N.

3-[3(*R*)-[3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*R*)-oxazolidinyl]-3-hydroxy-2(*R*)-isopropyl-1-oxopropyl]-4(*R*)-methyl-5(*S*)-phenyl-2-oxazolidinone (8). To a stirred solution of 229.5 mg (0.878 mmol) of the acyloxazolidinone **7** in 0.9 mL of dry dichloromethane, cooled to −78 °C, under argon, was added dropwise 0.24 mL (0.96 mmol) of dibutylboron triflate followed by 0.18 mL (1.04 mmol) of diisopropylethylamine. After 30 min, the reaction mixture was recooled to −78 °C and 227.6 mg (0.798 mmol) of the aldehyde **5** in 0.5 mL of dichloromethane was added dropwise. After 30 min, the reaction mixture was warmed to room temperature. After 90 min, the reaction mixture was cooled to 0 °C and treated with 0.63 mL of pH 7 phosphate buffer, 1.26 mL of methanol, and 0.63 mL of 30% aqueous H_2O_2 in 1.26 mL of methanol. After 1 h, the reaction mixture was partitioned between dichloromethane and pH 7 phosphate buffer. The aqueous phase was extracted with four portions of dichloromethane. The combined organic phases were dried ($MgSO_4$), filtered, and then concentrated. Flash chromatography of the residue on silica gel using 15% ethyl acetate in hexane afforded 337.2 mg (0.617 mmol, 77%) of the desired aldol product **8**: ^1H NMR ($CDCl_3$) δ 1.00 (m, 15 H), 1.47 (s, 9 H), 1.56 (s, 3 H), 1.63 (s, 3 H), 4.0 (m, 4 H), 4.8 (m, 1 H), 5.6 (d, 1 H, J = 7 Hz), 7.35 (m, 5 H); ^{13}C NMR ($CDCl_3$ δ 18.59, 23.42, 23.90, 25.38, 28.24, 28.79, 30.82, 31.44, 32.38, 33.01, 47.47, 54.72, 58.78, 60.65, 74.71, 82.38, 83.75, 85.64, 98.19, 129.54, 132.62, 137.25, 155.51, 156.42, 177.46; IR (mull) 3493, 1795, 1702, 1693 cm^{-1}; $[\alpha]_D$ + 13° (c 0.94, $CHCl_3$); FAB-HRMS, m/z 547.3396 (calcd for $C_{30}H_{46}N_2O_7$, 547.3383).

1(*R*)-[3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4(*S*)-isobutyl-5(*R*)-oxazolidinyl]-2(*S*)-isopropyl-1,3-propanediol (9). To a stirred solution of 350.9 mg (0.642 mmol) of the compound **8** in 1.2 mL of dry tetrahydrofuran, cooled to 0 °C, under argon, was added dropwise 0.14 mL (0.802 mmol) of diisopropylethylamine followed by 0.18 mL (0.706 mmol) of dibutylboron triflate. After 30 min, 0.80 mL (1.60 mmol) of a 2.0 M solution of lithium borohydride in tetrahydrofuran was added dropwise. After 90 min, the reaction mixture was warmed to room temperature. After 30 min, the reaction mixutre was recooled to 0 °C and carefully quenched with a solution containing 1.2 mL of methanol, 1.2 mL of pH 7 phosphate buffer, and 0.6 mL of 30% aqueous H_2O_2. After 10 min, the reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was then partitioned between dichloromethane and pH 7 buffer. The aqueous phase was extracted with five portions of dichloromethane. The combined organic phases were dried ($MgSO_4$), filtered, and then

Table III. Characterization of Renin Inhibitory Peptides

peptides[a]	HPLC[b] k'	amino acid anal.			formula	FAB-MS	
		Phe	His	Ile		calcd	found
VII	6.36	1.00	1.04	0.71	$C_{44}H_{65}N_8O_7$	817.4976	817.4962
VIII	6.30	1.00	1.01	1.00	$C_{44}H_{67}N_8O_7$	819.5132	819.5126
IX	6.01	1.04	1.00	1.05	$C_{44}H_{66}N_8O_8$	835.5082	835.5089
X	6.05	1.09	0.94		$C_{37}H_{60}N_6O_7$	701.4601	701.4634

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

concentrated. Flash chromatography of the residue on silica gel using 20% ethyl acetate in hexane afforded 165 mg (0.442 mmol, 70%) of the desired diol **9**: ^{1}H NMR ($CDCl_3$) δ 0.99 (m, 12 H), 1.47 (s, 9 H), 1.51 (s, 3 H), 1.65 (s, 3 H), 2.57 (br s, 1 H), 3.78 (br s, 2 H), 4.05 (m, 2 H); IR ($CHCl_3$) 3500, 2940, 1690 cm^{-1}; $[\alpha]_D$ + 10.6° (*c* 0.62, $CHCl_3$); FAB-HRMS, *m/z* 374.2893 (calcd for $C_{20}H_{39}NO_5$, 374.2096). Anal. ($C_{20}H_{39}NO_5$) C, H, N.

2(*S*)-[5(*R*)-[1(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-methylbutyl]-2,2-dimethyl-4(*R*)-dioxolanyl]-3-methylbutanol (10). A solution of 549.7 mg (1.47 mmol) of the diol **9** in 6.0 mL of a 0.1 M solution of camphorsulfonic acid in dichloromethane was stirred at room temperature for 45 min. The reaction mixture was then diluted with 8.0 mL of reagent grade acetone. After the reaction mixture was stirred at room temperature overnight, it was neutralized with excess solid sodium bicarbonate. After 30 min, the reaction mixture was filtered through celite and then concentrated. Flash chromatography of the residue on silica gel using 5–10% ethyl acetate in hexane afforded 452.8 mg (1.21 mmol, 82%) of the desired alcohol **10**: ^{1}H NMR ($CDCl_3$) δ 1.00 (m, 12 H), 1.37 (s, 3 H), 1.44 (s, 9 H), 3.82 (m, 4 H); IR ($CHCl_3$) 3450, 2950, 1700 cm^{-1}; $[\alpha]_D$ −32.7° (*c* 0.99, $CHCl_3$); FAB-HRMS, *m/z* 374.2886 (calcd for $C_{20}H_{39}NO_5$, 374.2906). Anal. ($C_{20}H_{39}NO_5$) C, H, N.

2(*R*)-[5(*R*)-[1(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-methylbutyl]-2,2-dimethyl-4(*R*)-dioxolanyl]-3-methylbutanoic Acid (11). To a stirred solution of 235 mg (0.63 mmole of the alcohol **10** in 1.2 mL of acetonitrile, 1.2 mL of carbon tetrachloride, and 1.8 mL of water were added 574.4 mg (2.52 mmol) of periodic acid and 3.3 mg (0.012 mmol) of ruthenium trichloride trihydrate. The entire biphasic mixture was stirred vigorously for 2 h at room temperature. The reaction mixture was then diluted with dichloromethane. The aqueous phase was extracted with five portions of dichloromethane. The combined organic phases were dried ($MgSO_4$), filtered, and then concentrated. The resulting residue was chromatographed on 15 g of silica gel using 40% ethyl acetate in hexane to afford 221.3 mg (0.57 mmol, 91%) of the desired acid **11**: ^{1}H NMR ($CDCl)_3$ δ 1.00 (m, 12 H), 1.41 (s, 3 H), 1.44 (s, 9 H), 9.95 (6 s, 1 H); IR ($CHCl_3$) 3450, 2960, 1710 cm^{-1}; FAB-HRMS, *m/z* 388.2680 (calcd for $C_{20}H_{37}NO_6$, 388.2699).

2(*R*)-[5(*R*)-[1(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-methylbutyl]-2,2-dimethyl-4(*R*)-dioxolanyl]-3-methylbutanoic Acid 2(*S*)-Methylbutylamide (12). To a stirred solution of 112.1 mg (0.289 mmol) of the acid **11** and 50.4 mg (0.578 mmol) of 2(*S*)-methylbutylamine in 1.2 mL of dry dichloromethane was added 40 μL (0.35 mmol) of triethylamine, followed by 50 μL (0.35 mmol) of diethylphosphoryl cyanide. After stirring at room temperature for 2 h, the concentrated reaction mixture was then chromatographed on silica gel with 10% ethyl acetate in hexane to give 107.1 mg (0.235 mmol, 81%) of the amide **12**: ^{1}H NMR ($CDCl_3$) δ 0.87–1.12 (m, 18 H), 1.32 (s, 3 H), 1.38 (s, 3 H), 1.43 (s, 9 H); FAB-HRMS, *m/z* 457.3662 (calcd for $C_{25}H_{48}N_2O_5$, 457.3641).

[5(*S*)-[[*N*-(*tert*-Butyloxycarbonyl)-N^{im}-tosyl-L-histidyl]amino]-3(*R*),4(*R*)-dihydroxy-2(*R*)-isopropyl-7-methyloctanoyl]-2(*S*)-methylbutylamine (14). A solution of 105.9 mg (0.23 mmol) of compound **12** in 2 mL of methanol containing 140 μL (2 mmol) of acetyl chloride was stirred at room temperature for 14 h. Excess solid $NaHCO_3$ was slowly added, and after 10 min, the mixture was diluted with 5 mL of dichloromethane. The resulting mixture was filtered through Celite, and the concentrated filtrate was chromatographed on silica gel with 5% methanol (saturated with ammonia) in dichloromethane to give 60 mg (0.19 mmol, 82%) of the amine **13**.

To a stirred solution of this amine, 100 mg (0.24 mmol) of *N*-(*tert*-butyloxycarbonyl)-N^{im}-tosyl-L-histidine, and 40 μL (0.29 mmol) of triethylamine in 2 mL of dichloromethane was added 40 μL (0.26 mmol) of diethylphosphoryl cyanide. After 14 h, the concentrated reaction mixture was chromatographed on silica gel with 75% ethyl acetate in hexane to give 98 mg (0.138 mmol, 73%) of compound **14**: ^{1}H NMR ($CDCl_3$) δ 0.85–1.0 (m, 18 H), 1.42 (s, 9 H), 2.43 (s, 3 H), 7.27–7.89 (m, 6 H).

[5(*S*)-[[[*N*-(*tert*-Butyloxycarbonyl)-L-phenylalanyl]-N^{im}-tosyl-L-histidyl]amino]-3(*R*),4(*R*)-dihydroxy-2(*R*)-isopropyl-7-methyloctanoyl]-2(*S*)-methylbutylamine (15). A solution of 49 mg (0.069 mmol) of compound **14** in 0.5 mL of dichloromethane and 0.5 mL of trifluoroacetic acid was stirred at room temperature for 30 min. The reaction mixture was then slowly added to a stirred mixture of 2 g of $NaHCO_3$ in 10 mL of water. The resulting mixture was extracted with several portions of dichloromethane. The combined organic phases were dried ($MgSO_4$) and then concentrated to give 40 mg of the amine.

To a stirred solution of this amine, 25 mg (0.094 mmol) of *N*-(*tert*-butyloxycarbonyl)-L-phenylalanine, and 15 μL (0.11 mmol) of triethylamine in 0.5 mL of dichloromethane was added 15 μL (0.10 mmol) of diethylphosphoryl cyanide. After 14 h, the reaction mixture was partitioned between dichloromethane and saturated aqueous $NaHCO_3$. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with ethyl acetate to give 50 mg (0.058 mmol, 84%) of compound **15**: ^{1}H NMR ($CDCl_3$) δ 0.78–1.02 (m, 18 H), 1.40 (s, 9 H), 2.43 (s, 3 H), 7.23–7.80 (m, 11 H).

[5(*S*)-[[[*N*-(*tert*-Butyloxycarbonyl)-L-phenylalanyl]-L-histidyl]amino]-3(*R*),4(*R*)-dihyrdoxy-2(*R*)-isopropyl-7-methyloctanoyl]-2(*S*)-methylbutylamine (X). A solution of 50 mg (0.058 mmol) of compound **15** and 32 mg (0.24 mmol) of 1-hydroxybenzotriazole in 0.5 mL of methanol was stirred at room temperature for 14 h. The concentrated reaction mixture was chromatographed on silica gel with 5% methanol (saturated with ammonia in dichloromethane to give 26.8 mg (0.038 mmol, 66%) of the peptide X: ^{1}H NMR ($CDCl_3$) δ 0.75–1.06 (m, 18 H), 1.34 (s, 9 H), 7.2 (m, 7 H).

Peptides VIII and IX were prepared in a manner similar to that of the preparation of peptide X. Peptide VII was prepared by the oxidation of the corresponding alcohol as described below.

[5(*S*)-[[[*N*-(*tert*-Butyloxycarbonyl)-L-phenylalanyl]-N^{im}-tosyl-L-histidyl]amino]-2(*S*)-isopropyl-4-oxo-7-methyloctanoyl]-L-isoleucyl-2-pyridylmethylamine (N^{im}-Tosyl-VII). To a stirred solution of 0.015 mL (0.17 mmol) of distilled oxalyl chloride in 0.25 mL of dry dichloromethane, cooled to −78 °C, was added dropwise 0.025 mL (0.35 mmol) of dry dimethyl sulfoxide. After 10 min, 75 mg (0.08 mmol) of [5(*S*)-[[[*N*-(*tert*-butyloxycarbonyl)-L-phenylalanyl]-N^{im}-tosyl-L-histidyl]amino]-4-(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl-2-pyridylmethylamine (N^{im}-tosyl-VIII) in 0.25 mL of dry dichloromethane and 0.25 mL of dimethyl sulfoxide was added dropwise. The resulting solution was stirred at −30 °C for 45 min and then treated with 0.1 mL (0.57 mmol) of diisopropylethylamine. After the reaction mixture was to 0 °C for 15 min, it was then partitioned between saturated aqueous $NaHCO_3$ and dichloromethane. The organic phases were combined, dried ($MgSO_4$), filtered, and then concentrated. The resulting residue was chromatographed on silica gel with 3% methanol in dichloromethane to give 63 mg (0.064 mmol, 80%) of the ketone (N^{im}-tosyl-VII).

Physical characteristics of peptides VII–X are listed in Table III.

Crystallography. Compound 8a: $C_{33}H_{58}N_2O_7Si$, M_r = 617.88, monoclinic, $P2_1$, a = 10.699 (1) Å, b = 17.018 (1) Å, c = 10.411 (1) Å, β = 103.25 (1) Å, V = 1845.1 (2) Å^3, Z = 2, D_{calcd} = 1.11 gm/cm^3, Cu Kα, λ = 1.5418 Å, μ(Cu Kα) = 0.8 cm^{-1}, $F(000)$ = 670, T = 123 K, R = 0.041 for 2753 unique reflections.

A clear, chunky prism of dimensions 0.20 × 0.40 × 0.30 mm was used for intensity measurement on a Syntex P1 diffractometer controlled by a Harris computer. Cu Kα radiation and a graphite monochromator were used for intensity measurement. The step scan technique was used with a scan speed of 2°/min, a scan width of 3.4°, and a $2\theta_{max}$ of 138°. Ten reflections periodically monitored showed no loss of intensity during the data collection. Of the 2753 unique reflections measured, 2584 had intensities greater than 3σ; standard deviations in the intensities were approximated by the equation

$$\sigma^2(I) = \sigma^2(I)_{\text{countingstatistics}} + (0.020I)^2$$

where the coefficient of I was calculated from the variations in intensities of the monitored reflections. Unit cell parameters were determined accurately by least-squares fit of Cu $K\alpha_1$ 2θ values (λ(Cu kα_1) = 1.5402) for 25 high 2θ reflections.[23] Lorentz and polarization corrections appropriate for a monochromator with 50% perfect character were applied without absorption correction. A partial trial solution, 30 atoms, was obtained by direct methods, using RANTAN81.[24] The remaining atoms were found by successive Fourier syntheses. Hydrogen atom positions were found in difference maps very close to positions generated by using planar or tetrahedral geometry, so generated positions were used. The structure was refined by least squares with coordinates including hydrogens and anisotropic temperature factors for nonhydrogen atoms included in the refinement. The function minimized in the refinement was $\sum w(F_o^2 - F_c^2)^2$, where weights w were $1/\sigma^2(F_o^2)$. Atomic form factors were from Doyle and Turner,[25] except for hydrogen, which was from Stewart et al.[26] In the final refinement cycle, all shifts were $<0.84\sigma$. The final R was 0.041, and the standard deviation of fit was 2.79. A final difference map showed no peaks >0.2 e Å^{-3}. The CRYM system of computer programs was used.[27]

Biology

Inhibition of Human Plasma Renin. Peptides VII–X were assayed for plasma renin inhibitory activity as follows: Lyophiized 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 those for control tubes to estimate percent inhibition. The inhibition results were expressed as IC_{50} values, which were obtained by plotting three to four inhibitor concentrations on semilog graph paper and estimating the concentration producing 50% inhibition.

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Supplementary Material Available: Tables of fractional coordinates, bond lengths, bond angles, torsion angles, close intermolecular contacts, and thermal parameters (6 pages). Ordering information is given on any current masthead page.

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Systematic Synthesis and Biological Evaluation of α- and β-D-Lyxofuranosyl Nucleosides of the Five Naturally Occurring Nucleic Acid Bases

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The α- and β-D-lyxofuranosyl analogues of the naturally occurring nucleosides have been synthesized and their antiviral properties examined. The α anomers were prepared by glycosylation of purine and pyrimidine aglycons with tetra-*O*-acetyl-α-D-lyxofuranose, followed by removal of the blocking groups. The β anomers were obtained by sequential oxidation and reduction of 3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-xylofuranosyl nucleosides. The lyxofuranosyl nucleosides were tested for their activity against a variety of RNA and DNA viruses and for inhibition of cell growth. One compound, 9-α-D-lyxofuranosyladenine, showed activity against herpes simplex virus types 1 and 2 both in vitro and in vivo.

During the last decades there has been considerable interest in the synthesis and biological evaluation of sugar-modified nucleoside analogues.[1] In order to define structure–activity relationships, we initiated a comprehensive program to systematically study anomeric D-pentofuranosyl nucleosides.[2,3] Thus, in an earlier paper we have discussed the α- and β-D-xylofuranosyl nucleosides, and it was found that three of these compounds, 9-β-D-xylofuranosyladenine and -guanine as well as 1-β-D-xylofuranosylcytosine, showed marked biological activity.[4] These findings prompted us to investigate in the current study the α- and β-D-lyxofuranosyl nucleosides of the five naturally occurring nucleic acid bases. A thorough literature survey revealed that little attention has been given to the synthesis and biological evaluation of this class of analogues.[5] For the β (cis-1′,2′) anomers this can be

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