Potent Renin Inhibitory Peptides Containing Hydrophilic End Groups[1]

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A previously reported renin inhibitor, Boc-Pro-Phe-N(Me)His-Leuψ[CHOHCH$_2$]Val-Ile-Amp (U-71038), was altered by the incorporation of polar, hydrophilic moieties at either end, e.g., tris(hydroxymethyl)aminomethane (THAM) or glucosamine urea groups at the N-terminus, and the THAM amide or aminomethylpyridine *N*-oxide at the C-terminus. These modified analogues, with dramatically improved water solubility, all retained the potent renin inhibitory activity of U-71038 in vitro. The fact that good activity was maintained in these new analogues, which possess hydrophilicity and steric bulk considerably different from the parent compound, suggests that neither end of these molecules is critical for recognition and binding of the inhibitors by renin. These modified analogues were evaluated in a rat model, and several exhibited hypotensive activity after both oral and iv administration which was greater in magnitude and longer in duration than that caused by equimolar doses of U-71038. Furthermore, unlike U-71038, the oral activity of these analogues was not dependent upon administration in a citric acid vehicle.

The enzyme renin, an aspartyl protease, is responsible solely for the cleavage of the protein angiotensinogen, thereby releasing the decapeptide angiotensin I. The latter intermediate is further transformed by angiotensin converting enzyme (ACE) into the octapeptide angiotensin II, which, by a number of mechanisms, is a potent hypertensive agent. A promising, relatively new approach to the treatment of hypertension involves the inhibition of renin,[2-5] which is responsible for the initial step in this enzymatic cascade.

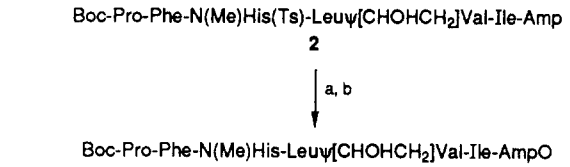
Upjohn's first clinical entry into this field, U-71038[6,7] (Figure 1), is an analogue of the key N-terminal portion of human angiotensinogen in which the Leu-Val amide bond normally cleaved by human renin has been replaced by the isosteric, but more stable, hydroxyethylene moiety.[8] In vitro, U-71038 is a potent inhibitor of human plasma renin, with an IC_{50} of 0.26 nM.[6] In vivo, oral administration of U-71038 at 50 mg/kg to conscious, sodium-depleted monkeys elicits a pronounced hypotension and decrease in plasma renin activity that persists for 5 h.[7] However, despite its promising pharmacological profile, its specificity for renin,[6] and its proteolytic stability,[6] U-71038 does not exhibit satisfactory oral activity in the absence of citric acid and is not sufficiently soluble in water to be formulated in the usual aqueous vehicles.

Rational attempts to improve the oral absorption of U-71038 are complicated by the fact that the mechanisms by which medium-sized peptides are transported intact from the GI tract into the blood are unknown.[9-11] Likewise unknown are the mechanisms by which the oral absorption of peptides appears to be enhanced by coadministration of adjuvants such as citric acid,[7] glucose,[12] or aromatic hydroxy acids.[13] Finally, very little information is available regarding structural features of the peptides themselves which might correlate with enhanced oral absorption. The new analogues described herein were synthesized to determine whether increasing the polarity and water solubility of U-71038 might provide agents which would retain the desirable pharmacological profile of U-71038 and also possess more manageable physical and pharmacokinetic properties.

Chemistry

The oxidation of His(Ts)-protected intermediate **2**[6] (Scheme I) to the corresponding pyridine *N*-oxide was accomplished in good yield with either hydrogen peroxide in methanol or (preferably) with *m*-chloroperbenzoic acid (MCPBA) in chloroform. The oxidation of unprotected His intermediates proceeded analogously, with only slightly decreased yields.

Scheme I



[a] (a) MCPBA; (b) HOBT, MeOH.

Since the pyridine *N*-oxide **3** exhibited only a marginal improvement in water solubility relative to U-71038 (3 mg/mL vs <1 mg/mL, respectively), we next investigated a more dramatic structural modification—namely, re-

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(1) Abbreviations for the amino acids, and the notation ψ[x], indicating that x replaces the amide CONH unit, all follow IUPAC-IUB Commission on Biochemical Nomenclature guidelines: *Eur. J. Biochem.* **1984**, *138*, 9. Additional abbreviations used herein include: Ac, acetyl; Amp, 2-(aminomethyl)-pyridine; AmpO, 2-(aminomethyl)pyridine *N*-oxide; Boc, *tert*-butyloxycarbonyl; DMAP, 4-(dimethylamino)pyridine; DEPC, diethylphosphoryl cyanide; FAB, fast-atom bombardment; HOBT, 1-hydroxybenzotriazole; MCPBA, *m*-chloroperbenzoic acid; MSTFA, *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide; TBS, *tert*-butyldimethylsilyl; TFA, trifluoroacetic acid; TMS, trimethylsilyl; THAM, tris(hydroxymethyl)-aminomethane.

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(12) Bloom, S., Royal Postgraduate Medical School, Hammersmith Hospital, London. Unpublished data.
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Boc-Pro-Phe-N(Me)His-Leuψ[CHOHCH$_2$]Val-Ile-Amp
1 (U-71038)

Figure 1.

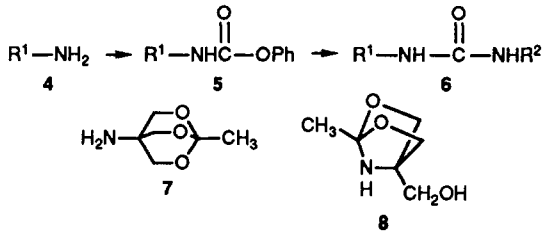
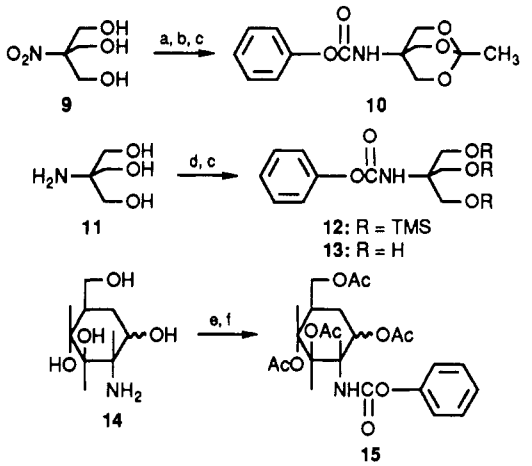


Figure 2.

Scheme II[a]



[a] (a) Triethyl orthoacetate, pTsA; (b) H_2, PtO_2, dioxane; (c) $ClCO_2Ph$, pyridine; (d) MSTFA, THF; (e) $ClCO_2Ph$, aqueous $NaHCO_3$; (f) Ac_2O, pyridine.

placement of the lipophilic N-terminal Boc group by a hydrophilic tris(hydroxymethyl)aminomethane (THAM) urea moiety. The most frequently encountered literature protocol for the conversion of amines to the corresponding THAM ureas involves conversion of primary amines (with phosgene) to the isocyanates and subsequent reaction with THAM itself.[14] Since an analogous procedure was anticipated to be difficult with proline (i.e. via the carbamyl chloride) and, at best, disagreeable with the other amino acids,[15] an alternative preparative scheme was sought.

Adamiak and Stawinski[16] reported a versatile approach to the preparation of unsymmetrical N,N′-disubstituted ureas, involving first treatment of an amine with excess (phenoxycarbonyl)tetrazole to yield a phenyl urethane, followed by addition of a second amine component under more vigorous conditions (Figure 2, **4** → **5** → **6**). However, when the ortho ester protected amine **7**[17] was used as the first amine component, it proved impossible to utilize most of the amine **7** without obtaining unacceptable amounts of the corresponding symmetrical urea. By using the more reactive phenyl chloroformate in place of the tetrazole reagent, it was possible to prepare the crystalline intermediate **10** in good yield (Scheme II). This key intermediate **10** should be generally useful for the conversion of most primary and secondary amines into the corresponding THAM ureas. (For the preparation of bicyclic ortho ester **10**, it was necessary to start with commercially available nitro triol **9**. Despite a literature reference to the contrary,[18] treatment of THAM itself (**11**) with trimethyl orthoacetate clearly afforded *only* the undesired bicyclic product **8**.)

Scheme III[a]

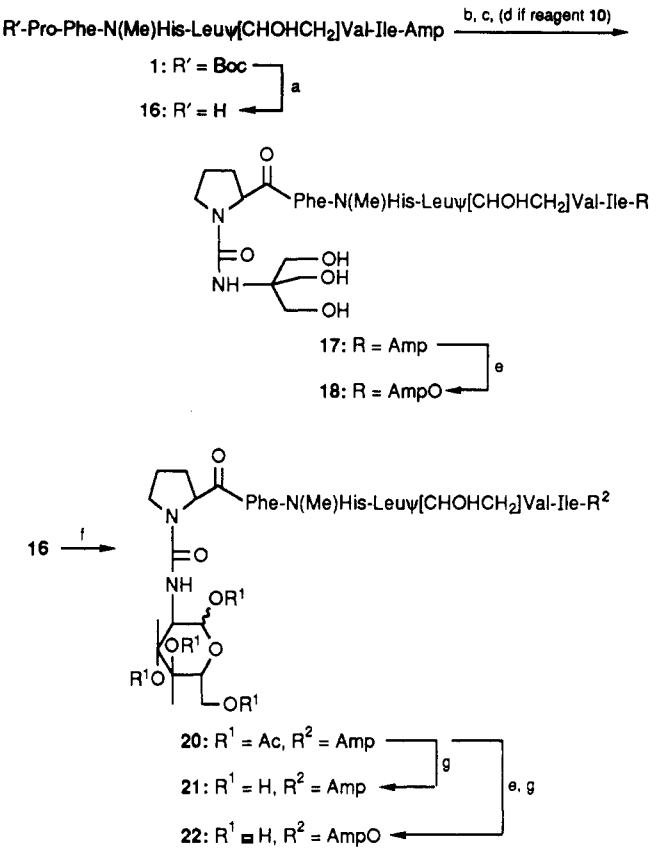


Figure 3.

For larger scale THAM urea preparations, the silylated urethane **12** could also be employed. Silylated reagent **12** was less reactive with amines than ortho ester **10** and was too hydrolytically unstable to allow thorough analytical characterization. However, by using **12**, one could avoid the potentially hazardous conversion of **9** to the ortho ester, a reaction which proceeded very smoothly on a moderate scale, but which required precise temperature control and might have been difficult to control on a large scale. (Interestingly, the triol **13** which resulted from the mild acid hydrolysis of **12**, as well as the corresponding tris-TBS ether, were both unreactive toward amines.)

The use of reagents **10** or **12** in the conversion of N-terminal deprotected U-71038 into the THAM urea **17** is

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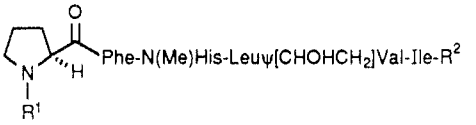
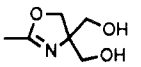
(18) Deffner, J. F. U.S. Patent 3,686,224. 1972; *Chem. Abstr.* **1972**, *77*, 140171w.

Table I. Characterization of Water-Soluble Renin Inhibitors



	R¹	R²	inhibition of plasma renin activity in vitro: IC_{50}, M	hypotensive activity recombinant human renin infused rats: max, mmHg; $t_{1/2}$, min		solubility in H_2O, mg/mL		FAB high-resolution mass spectrum:[b] observed/calculated, $[M + H]^+$
				po	iv	25 °C	37 °C	
3	CO_2tBu	AmpO	3.3×10^{-10}	40; 58	19; 28	3	3	946.5756/946.5766
17	$CONHC(CH_2OH)_3$	Amp	3.3×10^{-10}	38; 85	40; 30	1	1	977.5827/977.5824
18	$CONHC(CH_2OH)_3$	AmpO	5.8×10^{-10}	48; >120	43; 60	80	20	993.5785/993.5773
19		AmpO	1.1×10^{-9}	nt	nt	30	10	975.5692/975.5667
21	$CONH(C_6H_{11}O_5)$	Amp	4.9×10^{-10}	37; >120	36; >120	<2	<2	1035.583/1035.588
22	$CONH(C_6H_{11}O_5)$	AmpO	1.1×10^{-9}	39; >120	35; >120	>100	>100	1051.584/1051.583
30	CO_2tBu	$-NHC(CH_2OH)_3$	2.6×10^{-10}	35; >120	30; 19	1	<1	943.5856/943.5868
1[a]	CO_2tBu	Amp	2.6×10^{-10}	24; 60	25; 27	<1	<1	930.5789/930.5816

[a] Reference 6. [b] All substances were completely homogeneous by TLC in 4 M NH_3–MeOH/$CHCl_3$ solvent systems chosen to give an R_f in the 0.2–0.5 range.

outlined in Scheme III. Essentially the same yield could be obtained by using either 2 equiv of reagent **10** (dioxane; 50 °C, 20 h) or 5 equiv of reagent **12** (dimethylformamide; 64 h, 35 °C or 40 h, 50 °C). Oxidation of **17** with MCPBA as described earlier yielded **18**, which has hydrophilic modifications at both ends of the molecule. The order of the steps in this short sequence was not critical. Good results have also been obtained by performing the MCPBA oxidation at the beginning (on **1**) or prior to the two-step, one-pot hydrolysis[19] of the ortho ester protecting group. If the latter sequence were followed, however, it was necessary to buffer the MCPBA oxidation with solid sodium bicarbonate. Exposure of the ortho ester protected THAM urea intermediates to acidic, aprotic conditions led to the formation of the bis(hydroxymethyl)-substituted oxazoline byproduct **19** (Figure 3), which proved to be separable from **18** only with difficulty. The same byproduct was encountered in several other instances, depending upon the precise conditions employed for the ortho ester hydrolysis. The tentative structural assignment for **19** was based primarily on FAB mass spectroscopy. The mass spectrum of THAM urea **18** exhibited a peak at m/z 245, which could be ascribed to cleavage at the Pro-Phe amide bond of **18**. In the byproduct **19**, the 245 peak was absent, replaced by a peak at 227, indicating that the loss of water which occurred, leading to **19**, took place on the N-terminal side of the Pro-Phe amide bond. This information, coupled with several literature precedents for the facile cycloelimination of ureas or amides with leaving groups β to the nitrogen,[20] rendered the proposed oxazoline structure a reasonable possibility. When the synthesis of **18** was carried out by using silylated urethane **12** (instead of ortho ester **10**), only trace amounts of oxazoline **19** were formed during the final deprotection steps.

THAM urea **18** and the bis(hydroxymethyl)oxazoline **19** were not interconvertible under moderately acidic (0.1 M HCl/MeOH, 50 °C, 24 h) or moderately basic conditions (1:1 MeOH/1 M aqueous KOH, 50 °C, 3 h). Furthermore, both **18** and **19** were completely stable to prolonged standing on silica gel, with or without solvent. Thus, the oxazoline **19** appeared to arise only via ortho ester intermediates and not from THAM urea **18**.

The Scheme III sequence has also been carried out with His(Ts)-protected intermediate **2** as starting material.[6] The protected histidine route offered the advantages of slightly higher overall yield, greater ease of extraction following the proline deprotection step, and a slightly cleaner crude product following MCPBA oxidation. (No extra steps were required with the His(Ts) intermediates, as the tosyl group was removed during the ortho ester hydrolysis procedure following MCPBA oxidation.)

The synthesis of analogues **21** and **22**, in which the glucosamine moiety is attached to the N-terminus of U-71038 via a urea linkage, is also included in Scheme III. The crystalline urethane intermediate **15** (Scheme II) required for the formation of the unsymmetrical urea was prepared in 72% yield (two steps) from commercially available D-glucosamine. In this series, (**16** → **20** → **22**) the MCPBA oxidation of the pyridine ring was best performed prior to deacylation of the glucosamine moiety, since **22** possessed only limited solubility in chloroform. By effecting the subsequent deacylation under nonaqueous ester exchange conditions, it was possible to avoid the potentially very difficult extractive recovery of **22** from aqueous solutions. As anticipated, **21** and **22** both consisted of mixtures at the anomeric carbon, generally unresolvable by chromatographic methods. Following an extended search, conditions were finally developed by which the preparative scale separation of the anomers of **22** could be effected (silica gel, 0.5 M NH_3MeOH/$CHCl_3$, 30:70). The purified anomers were sufficiently stable to obtain NMR spectra but interconverted too readily in aqueous solution to allow meaningful biological evaluation of each one separately. Hence, the data for **21** and **22** in Table I refer to the anomeric mixtures.

The synthesis of the C-1 THAM amide of U-71038 (**30**, Scheme IV) was undertaken in order to determine whether the retention of renin inhibiting activity was dependent upon the point of attachment of the hydrophilic THAM moiety to the U-71038 backbone. The dicyclohexylcarbodiimide-mediated formation of Boc-Ile THAM amide

(19) Corey, E. J.; Raju, N. *Tetrahedron Lett.* **1983**, *24*, 5571.
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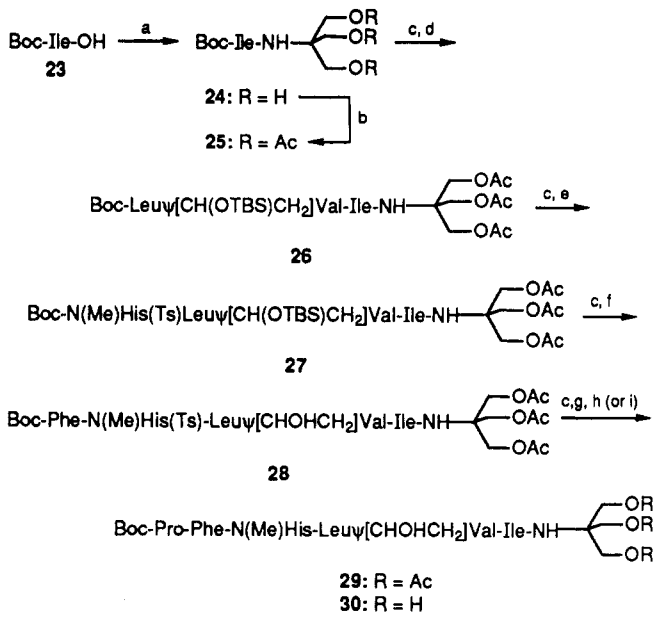
Scheme IV[a]



[a] (a) $H_2NC(CH_2OH)_3$, DCC; (b) Ac_2O, DMAP, pyridine; (c) CF_3CO_2H, CH_2Cl_2; (d) Boc-LeuΨ[CH(OTBS)CH_2]Val-OH, Et_3N, DEPC; (e) Boc-N(Me)His(Ts)-OH, Et_3N, DEPC; (f) Boc-Phe-OH, Et_3N, DEPC; (g) Boc-Pro-OH, Et_3N, DEPC; (h) HOBT, MeOH; (i) aqueous KOH, MeOH.

24 proceeded in only very modest yield (23%). (The major product from this reaction resulted from ester formation between one of the hydroxyl groups of 24 and another molecule of Boc-Ile-OH.) Although the THAM amide 24 could be carried through the remaining steps to 30 unprotected, the simple expedient of acylating the THAM hydroxyls (→ 25) led to improved yields and easier purification at every stage. The remainder of the sequence to build up the peptide backbone followed very closely that reported earlier[6] for the preparation of U-71038 itself. At the end, deprotection of the histidine could be accomplished either selectively (with HOBT) or simultaneously with the acetate hydrolysis to afford 29 or 30, respectively. Although the Scheme IV preparation of 30 required several steps, the yields were good throughout (with the aforementioned exception of the 23 → 24 conversion). Furthermore, the simpler alternative approach, viz. amide formation between the C-terminal acid corresponding to U-71038 and either THAM itself or ortho ester protected intermediate 7, was unsatisfactory.

Solubility

As anticipated, the addition of hydrophilic groups to the ends of the peptidic backbone of U-71038 did indeed produce analogues with increased water solubility (Table I). The increases ranged from rather modest for the analogues with modifications at only one end (3, 17, 30) to dramatic for those with changes at both ends (18, 22). The water solubility of 22 was sufficiently high that normal extraction with organic solvents such as chloroform became difficult. In fact, the Scheme III deprotection protocol was chosen intentionally to avoid the necessity of an aqueous workup at the final stage.

Several of the compounds in Table I (including the parent 1, U-71038) exhibited the curious property that they were more soluble at 25 °C than they were at 37 °C. This behavior is not unusual for peptides containing hydrophobic amino acids.

Biology

The water soluble derivatives described above were evaluated as inhibitors of human renin following an experimental protocol described earlier[6] (Table I). All of the compounds in Table I retained the potent renin inhibitory activity of the parent U-71038. The range of IC_{50} values is fairly narrow and does not appear to correlate in either a positive or negative manner with solubility of the test compound. Given the diversity in terms of hydrophilicity and steric bulk represented by the compounds in Table I (all consistent with good renin inhibitory activity in vitro), it would appear that neither the N-terminal nor the C-terminal portions of U-71038 are critical for its recognition and binding by human renin.

The in vivo renin inhibiting activity of the analogues in Table I was investigated via experiments in a rat model.[21] Recombinant human renin was infused into anesthetized nephrectomized, ganglion-blocked Sprague-Dawley rats. The magnitude of the human renin dependent blood pressure component was approximately 60 mmHg. For the compounds in Table I, the intravenous dose was equimolar to 0.05 mg/kg of compound 1 and the oral dose was equimolar to 5.0 mg/kg of compound 1. These doses were located on the linear portion of U-71038's dose–response curves and allowed small changes in apparent potency to be reflected in relatively large changes in blood pressure as a result of the inhibition of human renin. Potency was expressed as the maximum hypotensive effect (max) in millimeters of mercury caused by an equimolar dose while the duration of effect was expressed as the duration ($t_{1/2}$) in minutes required for a 50% return of the blood pressure from its maximum effect to its starting point.

As summarized in Table I, most of the water soluble analogues, when administered iv or orally at doses equimolar to U-71038, produced hypotensive effects of greater magnitude and duration than U-71038. Particularly noteworthy were compounds 18 and 22, which after oral administration produced blood pressure drops of 39–48 mm which did not return to within 50% of pretreatment levels during the 2-h time course of the experiment. (The fact that 22 was among the least potent of the compounds in this series in vitro shows that IC_{50} values in vitro are not sufficient to predict the relative in vivo hypotensive activities of fairly similar analogues.)

The in vivo data in Table I (iv and oral) were obtained by using solutions of the test drugs in 0.1 M citric acid because the comparison standard U-71038 was insufficiently soluble in water to allow meaningful comparisons in that vehicle. Further studies with compound 18 showed that it also retained excellent hypotensive activity when administered iv in 5% dextrose or orally in sterile water (data not shown). The hypotensive responses were diminished only slightly in the absence of citric acid, and good dose–response correlations could be obtained. An oral dose of 5 mg/kg of 18 in sterile water produced a 37 mm drop in blood pressure which had not begun to return to pretreatment levels during the 2-h experiment.

Summary

We have synthesized a series of hydrophilic derivatives of the renin inhibitor U-71038, some of which improved the water solubility of the parent by a factor of >100. Within a factor of 10, all of these derivatives retained the potent in vitro human renin inhibitory activity of U-71038. Several (18, 22), at doses equimolar to U-71038, produced hypotensive responses in the rat of greater magnitude and longer duration than U-71038. Furthermore, because of

(21) Pals, D. T.; Lawson, J. A.; Couch, S. J. *J. Pharmacol. Methods*, in press, 1990.

their greater water solubility, they did not need to be administered in vehicles containing citric acid. Additional studies are underway to determine the mechanistic basis for the improved pharmacological profile of these water soluble analogues.

Experimental Section

Melting points (uncorrected) were determined in open capillary tubes with a Thomas-Hoover Unimelt apparatus. NMR spectra were recorded on either an EM-390 90 MHz or a Bruker AM300 spectrometer; chemical shifts are reported, for $CDCl_3$ solutions, as δ values (ppm) relative to tetramethylsilane as the internal standard. FAB mass spectra (high resolution, see Table I) were obtained by the Physical and Analytical Chemistry Department of the Upjohn Co., by using a Varian MAT CH5-DF spectrometer. Spectral data for all new compounds were consistent with the structures assigned (not all reported herein). Unless otherwise noted, thin-layer chromatography was carried out on Analtech silica gel GF plates (250 μm layer). Preparative purifications were performed in open columns packed with EM Science Kieselgel 60 (40–63 μm). Solutions of ammonia in methanol (4 M or 7 M), used for TLC and preparative chromatographic purification, were prepared by bubbling anhydrous ammonia gas into reagent grade methanol with cooling and stirring, until titration of aliquots with aqueous 1 M HCl showed that the desired concentration had been reached. In tightly sealed bottles, these NH_3/MeOH solutions remained the same concentration for several weeks at 25 °C. All organic solvents were Burdick and Jackson reagent grade, distilled in glass. These were stored over the appropriate molecular sieves (3A, 4A) after the bottles were opened.

General Procedure A. MCPBA Oxidation of Pyridines to Pyridine *N*-Oxides. A solution of the substrate in chloroform (30 mL/mmol substrate) was treated with a 20% excess of *m*-chloroperbenzoic acid (MCPBA). The resulting solution was stirred at 25 °C until TLC analysis indicated that the reaction was complete (1–2 h). The reaction mixture was then poured into a mixture of brine and aqueous sodium sulfite and extracted thoroughly with chloroform. The combined extracts were dried (anhydrous Na_2SO_4) and concentrated.

General Procedure B. Removal of *tert*-Butyloxycarbonyl Group with Trifluoroacetic Acid. The substrate was dissolved in methylene chloride (3 mL/g), and the stirred solution was treated with an equal volume of trifluoroacetic acid (TFA). After 4 h at 25 °C, the reaction mixture was added dropwise to a stirred mixture of [10 mL of H_2O, 1.25 g of $NaHCO_3$, 5 mL of methylene chloride]/mL of TFA. The resulting mixture was transferred to a separatory funnel and extracted thoroughly with additional methylene chloride. The extracts were dried (Na_2SO_4) and concentrated in vacuo.

General Procedure C. Peptide Bond Formation with Diethylphosphoryl Cyanide (DEPC). To a stirred solution of the acid component and the amide component in dichloromethane was added a slight excess of triethylamine, followed by slow addition of a slight excess of diethylphosphoryl cyanide. After being stirred at room temperature for 2–12 h, the reaction mixture was diluted with dichloromethane and then washed with saturated aqueous $NaHCO_3$. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was then purified by chromatography on silica gel.

General Procedure D. Removal of the *p*-Tolylsulfonyl Group from Histidine. A solution of the peptide substrate and 3–5 equiv of 1-hydroxybenzotriazole in methanol was allowed to stir at room temperature for 12–24 h. The reaction mixture was then concentrated and the residue purified by chromatography on silica gel.

Boc-Pro-Phe-*N*(Me)His-Leuψ[CHOHCH$_2$]Val-Ile-AmpO (3). According to general procedure A, 3.0 g (2.76 mmol) of intermediate **2**[6] and 580 mg (3.37 mmol) of *m*-chloroperbenzoic acid in 90 mL of chloroform afforded the corresponding pyridine *N*-oxide. Removal of the Ts protecting group according to general procedure D (2.2 g, 16.3 mmol of HOBT hydrate; 6 mL of MeOH), followed by chromatography on silica gel with 6% 4 M NH_3–MeOH/94% $CHCl_3$ afforded 2.25 g (86%) of *N*-oxide **3** as an amorphous white solid: ^{1}H NMR ($CDCl_3$) δ 8.3–8.2 (m, 1 H; for C-6 of the pyridine ring; corresponding signal for **1** was at 8.5 ppm); R_f 0.33 (4 M NH_3–MeOH/$CHCl_3$, 12:88).

1-Methyl-4-[(phenoxycarbonyl)amino]-2,6,7-trioxabicyclo[2.2.2]octane (10). In the manner of the general procedure of Yokoyama, et al.,[17] a solution of 52 g (0.321 mol) of triethyl orthoacetate in 160 mL of dioctyl phthalate was treated with 48.32 g (0.320 mol) of 2-(hydroxymethyl)-2-nitro-1,3-propanediol, added with stirring in one portion. The resulting homogeneous mixture was heated at 120 °C for 2.5 h, during which time 27 mL of ethanol was removed by short path distillation. The mixture was then cooled to room temperature, treated with 200 mg of *p*-toluenesulfonic acid in 80 mL of dioctyl phthalate and finally divided into two equal portions, each in a 1000-mL round-bottomed flask. Each half was subjected to sublimation in a Kugelrohr apparatus at 150 °C, 0.2 mm for 3 h (most of the material sublimed during the first 2 h). The combined sublimed material was recrystallized from acetone/hexane, thereby affording 17 g (30%) of the cyclic orthoacetate as white crystals with mp 124–125 °C (lit.[17] mp 124–126 °C; in Table I of this reference, the data for compounds **5** and **6** are incorrectly interchanged with those for **15** and **16**). An additional 5–6 g of product would be available, if desired, via chromatography of the mother liquors from the above crystallization: ^{1}H NMR ($CDCl_3$) δ 4.42 (s, 6 H), 1.50 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 110.23, 67.72, 61.22, 22.08; R_f 0.81 (Et_3N/EtOAc, 1:99).

A solution of 17 g of the nitro orthoacetate from the preceding paragraph in 140 mL of dioxane was hydrogenated over 600 mg of platinum oxide at 50 psi for 2.5 h. The mixture was filtered through Celite in an argon atmosphere, and the catalyst was washed with additional dioxane. After removal of the dioxane on the rotary evaporator, the residue was recrystallized from ethyl acetate. The crystalline amino orthoacetate, after drying in a vacuum desiccator for 18 h (0.05 mm), weighed 10.2 g and exhibited mp 105–109 °C (lit.[17] mp 110–114 °C; note same interchange of data in literature reference as mentioned in preceding paragraph). Chromatography of the mother liquors on silica gel (4 M NH_3–MeOH/$CHCl_3$, 5:95) afforded an additional 1.72 g of pure amino orthoacetate (total yield, 11.92 g, 85%): ^{1}H NMR ($CDCl_3$) δ 3.89 (s, 6 H), 1.46 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 108.26, 72.65, 44.92, 22.87; R_f 0.27 (Et_3N/EtOAc, 1:99).

A 250-mL, 3-necked, round-bottomed flask equipped with an addition funnel and an air stirrer was charged with 100 mL of dry pyridine. With rapid stirring, 6.85 mL (8.55 g; 54.6 mmols, a 4-fold excess) of phenyl chloroformate was added dropwise over 5 min. The resulting thick (but stirrable) suspension was stirred for 10 min, then treated dropwise over 5 min with a solution of 2.0 g (13.79 mmols) of the above amino orthoacetate in 10 mL of pyridine. After an additional 15 min, the reaction mixture was cooled in an ice bath and treated with 25 mL of water (the first 5 mL of which was added slowly—gas evolution). The mixture was stirred for 15 min, then poured into half-saturated aqueous sodium bicarbonate and extracted with 3 × 100 mL of 1:1 ethyl acetate/hexane. The extracts were washed with additional aqueous sodium bicarbonate and brine, dried over anhydrous sodium sulfate, and evaporated. A vacuum pump was employed at the end to remove small amounts of pyridine. The crude product, a white solid, was recrystallized from approximately 25 mL of 30% ethyl acetate/hexane and dried at 25 °C, 0.01 mm, for 3 h, thereby affording pure phenyl urethane **10**: 2.62 g; mp 186–188 °C. Chromatography of the mother liquors on 200 g of silica gel (EtOAc/hexane, 35:65) yielded an additional 665 mg of clean urethane **10** (total 3.285 g, 90%): ^{1}H NMR ($CDCl_3$) δ 7.55–7.04 (m, 5 H), 4.96 (br s, 1 H), 4.22 (s, 6 H), 1.47 (s, 3 H); R_f 0.40 (EtOAc/hexane, 35:65). Anal. ($C_{13}H_{15}NO_5$) C, H, N.

[Tris[[(trimethylsilyl)oxy]methyl]methyl]carbamic Acid, Phenyl Ester (12). A solution of 24.2 g (0.20 mole) of tris(hydroxymethyl)aminomethane in 1250 mL of tetrahydrofuran was treated with 121.4 g (0.61 mol) of *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA), and the resulting mixture was stirred at 25 °C for 22–24 h (sustained but gentle exotherm). The reaction mixture was treated with 16.9 mL (0.21 mol) of pyridine, cooled to 3–7 °C in an ice bath, and finally treated dropwise over 15 min with a solution of 25.0 mL (0.20 mol) of phenyl chloroformate in a small amount of tetrahydrofuran. The mixture was stirred for 30 min at 5 °C and then 5–6 h at room temperature. The solids (pyridine hydrochloride) were removed by filtration, and the filtrate was concentrated in vacuo. The material was maintained on a Kugelrohr apparatus (0.2 mm) until sublimation

of the byproduct acetamide was complete. The remaining semisolid weighed 89.4 g (97.6% of theory) and, due to its hydrolytic instability, was used in subsequent coupling reactions without further purification: ^{1}H NMR ($CDCl_3$) δ 7.3–6.9 (m, 5 H), 3.66 (s, 6 H). Attempts to crystallize urethane **12** at low temperature were unsuccessful. Due to its instability in air, satisfactory C, H, N analyses could not be obtained.

***N*-(Phenoxycarbonyl)-D-glucosamine, Tetra-*O*-acetate (15).** In the manner of a procedure based on that of Chargaff and Bovarnick,[22] 5.25 g (62 mmol) of solid sodium bicarbonate was added over 2 min (CO_2 evolution) to a stirred solution of 5.4 g (25 mmol) of D-glucosamine hydrochloride (Aldrich) in 25 mL of water. To this stirred mixture was added 4.7 mL (37.5 mmol) of phenyl chloroformate (0.4 mL every 5 min). By the eighth addition, the mixture had become very difficult to stir, and control of foaming also became a problem. At this point the reaction mixture was diluted with 75 mL of water and transferred to a 400-mL beaker. The use of an air stirrer then solved both the stirring and foam control problems. The suspension was stirred at 25 °C for 10 min after the last of the phenyl chloroformate had been added. The solids were isolated by filtration through a medium-porosity sintered glass funnel and were washed with 2 × 10 mL of acetone and 10 mL of methanol. The crude phenyl carbamate, after drying for 16 h at 25 °C and 0.1 mm, weighed 6.0 g (80% yield), and was used immediately in the acetylation step without further purification or characterization.

A solution of 5.32 g of the crude phenyl carbamate in 30 mL of pyridine and 10 mL of acetic anhydride was stirred at 25 °C for 3 h. The mixture was then cooled in an ice bath, treated with 5 mL of methanol, and stirred for 1 h at 0 °C and 1 h at 25 °C. The mixture was poured into ice and water containing 52 g of sodium bisulfate and extracted with 1:1 ethyl acetate/hexane. The extracts were washed with water, aqueous sodium bicarbonate, and brine, dried (Na_2SO_4), and evaporated. The crude product **15** (10.4 g) was chromatographed on a 700-g column of silica gel, packed and eluted with 50% ethyl acetate/hexane (1 × 2000 mL; then 230-mL fractions). Fractions 4–12 were combined, and, upon evaporation of the solvent, the product crystallized readily. The product was triturated with 25 mL of ether, then diluted with 15 mL of pentane, and filtered. The solids were washed with 10 mL of 1:1 ether/pentane and dried at 25 °C, 0.05 mm for 3 h, thereby affording 7.48 g (90% yield) of pure phenyl carbamate tetra acetate **15** with mp 146–148 °C: ^{1}H NMR ($CDCl_3$) δ 7.45–7.08 (m, 5 H), 6.35 (d, 1 H), 5.45–5.13 (m, 3 H), 4.40–3.80 (m, 4 H), 2.30–2.10 (m, 12 H). Anal. ($C_{21}H_{25}NO_{11}$) C, H, N.

***N*-[[[2-Hydroxy-1,1-bis(hydroxymethyl)ethyl]amino]carbonyl]-Pro-Phe-N(Me)His-Leuψ[CHOHCH$_2$]Val-Ile-AMP (17).** According to general procedure B, 3.0 g (3.22 mmol) of peptide **1**[6] was converted to the N-deprotected intermediate in quantitative yield: R_f 0.13 (4 M NH_3–MeOH/$CHCl_3$, 8:92).

The N-deprotected product from the preceding paragraph (3.22 mmol) was dissolved in 35 mL of dioxane which had been dried immediately prior to use by percolation through 100 g of Woelm activity I alumina. The stirred solution was treated with 0.5 mL of triethylamine and 1.71 g (6.44 mmol) of urethane reagent **10**, and the resulting homogeneous solution was stirred under an argon atmosphere at 50 °C for 20 h. After removal of the dioxane on a rotary evaporator (30 °C bath temperature), the residue was diluted with 150 mL of chloroform and washed with 70 mL of saturated aqueous sodium bicarbonate. The aqueous layer was then extracted with three 50-mL portions of chloroform. The extracts were finally dried over anhydrous sodium sulfate and concentrated in vacuo. Chromatographic purification of the crude product on 400 g of silica gel (4 M NH_3–MeOH/$CHCl_3$ 4.5:95.5) yielded 2.40 g (74.5% from **1**) of pure ortho ester corresponding to **17**: ^{1}H NMR ($CDCl_3$) δ 4.18 (s, 6 H), 1.49 (s, 3 H), diagnostic for the bicyclic ortho ester moiety; mass spectrum, $[M + H]^+$ observed at m/z 1001.586 (calcd for $C_{52}H_{77}N_{10}O_{10}$, 1001.582); R_f 0.29 (4 M NH_3–MeOH/$CHCl_3$, 10:90).

The ortho ester product from the preceding paragraph was dissolved in 100 mL of methanol containing 0.5 mL of 4 M NH_3/MeOH (pH 9–10). The stirred mixture was treated with 15 mL of 1 M methanolic HCl, and the resulting clear solution was stirred for 2 h at ambient temperature. The mixture was then treated with 25 mL of aqueous 1 M potassium hydroxide and allowed to stand at 25 °C for 30 min. The methanol was removed on the rotary evaporator (bath temperature 30 °C), and the aqueous residue was saturated with sodium chloride and extracted with three 200-mL portions of chloroform. The combined extracts were dried over anhydrous sodium sulfate and concentrated. TLC analysis of the crude product showed only the desired derivative **17**, sufficiently pure for further chemical transformations. For characterization purposes, chromatographic purification on 180 g of silica gel (4 M NH_3–MeOH/$CHCl_3$, 12:88) afforded 2.03 g (87%) of pure triol **17** as a white, amorphous solid (overall yield from **1**, 65%.): ^{1}H NMR (DMSO) δ 3.51 (s, 6 H, CH_2OH); R_f 0.26 (4 M NH_3–MeOH/$CHCl_3$, 15:85).

When this sequence was performed starting from His(Ts) intermediate **2**, the tosyl protecting group was also removed during the above ortho ester hydrolysis step.

***N*-[[[2-Hydroxy-1,1-bis(hydroxymethyl)ethyl]amino]carbonyl]-Pro-Phe-N(Me)His-Leuψ[CHOHCH$_2$]Val-Ile-AmpO (18).** Either following or prior to ortho ester hydrolysis in the preceding experiment, the intermediate **17** (or the corresponding bicyclic orthoester) was oxidized according to general procedure A. (If the oxidation was performed with the ortho ester intact, it was necessary to buffer the MCPCA oxidation with solid sodium bicarbonate to prevent formation of the oxazoline byproduct.) In one run, 2.0 g of intermediate **1**[6] (2.15 mmol) was converted by the procedure in the preceding experiment (without purification of triol **17**), followed by the general procedure A oxidation (550 mg of MCPBA), into AmpO derivative **18**. Chromatographic purification of the crude product on 430 g of silica gel (4 M NH_3–MeOH/$CHCl_3$, 12:88) yielded, after combination of chromatography fractions and concentration, a white foam. The foam was dissolved in 12 mL of hot chloroform and added dropwise to 70 mL of rapidly stirred pentane. The solids were isolated by filtration and dried (65 °C, 0.05 mm, 18 h), thereby affording 1.02 g (48% yield from **1**) of pure *N*-oxide **18**, an easily handled, amorphous, white solid: ^{1}H NMR (CD_3OD) δ 8.40–8.33 (m, 1 H, C-6 pyridine signal), 3.75 (m, 6 H, CH_2OH); melt solvate, no $CHCl_3$, <0.01% pentane; KF water, 0.9% H_2O; R_f 0.21 (7 M NH_3–MeOH/$CHCl_3$, 16:84); HPLC major component 98.81% (10 × 300 mm Waters C_{18} 5 μ column; 0.05 M aqueous ammonium acetate/MeOH/CH_3CN, 50:25:25; 1 mL/min; t_R 9.2 min); $[\alpha]_D$(MeOH) −87°.

Preparation of 18 via Silylated Intermediate 12. A stirred solution of 465 mg (0.56 mmol) of intermediate **16** and 0.56 mL of triethylamine (4 mmol) in 4 mL of anhydrous dimethylformamide was treated dropwise with 1.28 g (2.8 mmol) of the tris-[[(trimethylsilyl)oxy]methyl] urethane **12**, and the resulting mixture was stirred in a nitrogen atmosphere at 35 °C for 64 h. The dimethylformamide was then removed in vacuo, and the residue was dissolved in 20 mL of methanol containing a trace of ammonia (pH 9–10). The solution was stirred and treated with 2 mL of 1 M HCl/methanol (resulting solution pH <1). After 1 h at 25 °C, 5 mL of 1 M aqueous potassium hydroxide was added, and the methanol was removed under reduced pressure. The residue was taken up in brine and chloroform and then extracted with three 100-mL portions of chloroform. The extracts were dried over anhydrous sodium sulfate and evaporated, thereby affording a crude product weighing 902 mg. MCPBA oxidation of this material according to general procedure A, followed by chromatographic purification and precipitation of the product as described above, afforded 210 mg of pure *N*-oxide **18**, identical in all respects with the material prepared in the preceding experiment (38% overall yield from **16**).

Isolation of *N*-[4,5-Dihydro-4,4-bis(hydroxymethyl)-2-oxazolyl]-Pro-Phe-N(Me)His-Leuψ[CHOHCH$_2$]Val-Ile-AmpO (19). In an early preparation of **18**, as described above except that the MCPBA oxidation was performed *without* buffering before hydrolysis of the ortho ester, the crude product (2.84 g) appeared as an elongated spot in the usual TLC system (4 M NH_3–MeOH/$CHCl_3$, 16:84). When the ammonia concentration was increased to 7 M, the product was found to contain two components in approximately equal amounts, the more polar of which corresponded to authentic samples of *N*-oxide **18**. Chromatography of this mixture on 550 g of silica gel (7 M NH_3–MeOH/$CHCl_3$, 15:85) afforded, after $CHCl_3$/pentane pre-

(22) Chargaff, E.; Bovarnick, M. *J. Biol. Chem.* **1937**, *118*, 421.

cipitation of the product as described earlier, 1.62 g of pure oxazoline **19**, a white, amorphous solid: R_f 0.26 (7 M NH_3–MeOH/$CHCl_3$, 16:84); HPLC same conditions as described above for **18**, t_R 13.0 min. Continued elution of the above chromatogram yielded 1.78 of pure **18**, identical in all respects with standard samples.

***N*-[(D-Glucosylamino)carbonyl]-Pro-Phe-N(Me)His-Leuψ[CHOHCH$_2$]Val-Ile-AMP (21).** A stirred solution of 178 mg (0.215 mmol) of intermediate **16**, 300 mg (0.64 mmol) of reagent **15**, and 0.04 mL of triethylamine in 2.5 mL of anhydrous dioxane was heated at 50 °C for 42 h under nitrogen. The cooled reaction mixture was then poured into aqueous $NaHCO_3$, and the product was isolated by extraction with chloroform. Chromatographic purification of the crude product on 20 g of silica gel (4 M NH_3–MeOH/$CHCl_3$, 6:94) yielded 210 mg (81%) of pure tetraacetate **20** as an amorphous solid: R_f 0.54 (4 M NH_3–MeOH/$CHCl_3$, 10:90; starting intermediate **16** exhibited R_f 0.31, and the urethane reagent **15**, R_f 0.85 when authentic samples were spotted on the same plate).

The tetraacetate **20** (210 mg) was dissolved in 5 mL of methanol and treated with 1 mL of 4 M ammonia in methanol, and the resulting yellow solution was stirred under nitrogen for 18 h at 25 °C. The reaction mixture was then evaporated to dryness, and the crude product (160 mg; >95% pure by TLC) was chromatographed on 21 g of silica gel (4 M NH_3–MeOH/$CHCl_3$, 25:75). Precipitation of the chromatographically pure product from $CHCl_3$/pentane and drying of the recovered solids (0.1 mm, 25 °C, 3 h) afforded 95 mg (53%) of pure glucosamine urea derivative **21**, an amorphous white solid: R_f 0.37 (4 M NH_3–MeOH/$CHCl_3$, 30:70).

***N*-[(D-Glucosylamino)carbonyl]-Pro-Phe-N(Me)His-Leuψ[CHOHCH$_2$]Val-Ile-AmpO (22).** A 2.85 g (3.44 mmol) portion of intermediate **16** was converted, as described in the preceding experiment to the glucosylamine urea tetraacetate **20**. Without purification, the crude **20** was oxidized according to general procedure A, and the crude oxidation product was chromatographed on 450 g of silica gel (4 M NH_3–MeOH/$CHCl_3$, 8:92). The chromatographically pure product (2.2 g, 53%) dissolved in 50 mL of methanol was treated with 10 mL of 4 M NH_3/MeOH, and the resulting solution was stirred for 18 h at 25 °C. (The solution darkened considerably during this time.) Removal of the methanol in vacuo afforded a dark residue (2.5 g), which was chromatographed on 200 g of silica gel (4 M NH_3–MeOH/$CHCl_3$, 25:75). The chromatographically pure **22**, a white foam, was dissolved in 6 mL of 10% MeOH/$CHCl_3$ and precipitated by dropwise addition of this solution to 150 mL of rapidly stirred pentane. Filtration and drying (0.05 mm, 25 °C, 6 h) yielded pure *N*-oxide **22** as a free-flowing, white, amorphous solid (1.15 g, 32% yield overall from **16**): R_f 0.14 (10 M NH_3–MeOH/$CHCl_3$, 25:75).

Boc-Ile, THAM Amide (24). A mixture containing 12 g of Boc-Ile·$^1/_2H_2O$, 7.0 g of tris(hydroxymethyl)aminomethane (THAM), 7.1 g of HOBT hydrate, 10.85 g of dicyclohexylcarbodiimide, and 15 g of crushed 3A molecular sieves in 500 mL of methylene chloride was stirred vigorously (air stirrer) over a 64-h weekend at 25 °C. The suspension was then cooled to 0 °C and filtered through a medium-porosity sintered glass funnel, and the filtrate was concentrated in vacuo. Chromatographic purification of the residue on 1.2 kg of silica gel (MeOH/$CHCl_3$, 4:96) afforded 3.08 g (23%) of the desired THAM amide **24** as a white solid. Recrystallization of a small portion of this material from $CHCl_3$ gave pure **24** with mp 126–128 °C: ^{1}H NMR ($CDCl_3$) δ 7.1 (broad s, 1 H), 5.10–5.05 (d, 1 H), 4.10 (broad s, 3 H), 3.90 (m, 1 H), 3.70 (s, 6 H, CH_2OH), 1.46 (s, 9 H); mass spectrum, $[M + H]^+$ observed at m/z 335.2157, (calcd for $C_{15}H_{31}N_2O_6$, 335.2182); R_f 0.31 (MeOH/$CHCl_3$, 8:92). The major product from this reaction (6 g), which eluted faster than **24**, resulted from ester formation between one of the hydroxyl groups of **24** and a second molecule of Boc-Ile-OH ($[M + H]^+$ observed at m/z 548.3629; R_f 0.49, same solvent as for **24**). No effort was expended to optimize the yield of **24**.

Boc-Ile, THAM Amide Triacetate (25). A stirred 5 °C solution of 2.59 g (7.75 mmol) of intermediate **24** in 15 mL of pyridine was treated with 7.5 mL of acetic anhydride and 80 mg of 4-(dimethylamino)pyridine, and the resulting homogeneous mixture was stirred for 1 h at 25 °C under argon. The reaction mixture was then cooled to 0 °C, treated with 7 mL of methanol, and stirred for 30 min at 0 °C and 30 min at 25 °C. The mixture was poured into 1:1 brine/water and extracted with ethyl acetate. The extracts were washed with cold 0.5 M hydrochloric acid, water, sodium bicarbonate, and brine, dried over sodium sulfate, and evaporated. The crude product (3.6 g, 100% yield) was completely clean by TLC and was used in the next step without purification: R_f 0.45 (1:1 EtOAc/hexane.

Boc-Leuψ[CH(OTBS)CH$_2$]Val-Ile, THAM Amide Triacetate (26). The Boc protecting group was removed from the product in the preceding experiment according to general procedure B. Then, by following general procedure C, the deprotected amine (7.75 mmol) was converted to **26** with 3.45 g (7.75 mmol) of Boc-Leuψ[CH(OTBS)CH$_2$]Val-OH[6], 1.13 mL (8.10 mmol) of triethylamine and 1.24 mL (8.14 mmol) of diethylphosphoryl cyanide in 60 mL of methylene chloride. Chromatographic purification of the crude coupled product on 700 g of silica gel (EtOAc/hexane, 45:55) afforded 5.0 g (82%) of pure **26**: ^{1}H NMR ($CDCl_3$) δ 6.30 (m, 1 H), 6.06–5.96 (m, 1 H), 4.55–4.10 (m, 8 H), 3.80–3.45 (m, 2 H), 2.10 (s, 9 H), 1.45 (s, 9 H); mass spectrum, $[M + H]^+$ observed at m/z 788.5117 (calcd for $C_{39}H_{74}N_3O_{11}Si$, 788.5092); R_f 0.25 (acetone/CH_2Cl_2, 10:90); R_f 0.53 (EtOAc/hexane, 1:1).

Boc-N(Me)His(Ts)-Leuψ[CH(OTBS)CH$_2$]Val-Ile, THAM Amide Triacetate (27). The BOC protecting group was removed from **26** according to general procedure B. The deprotected amine (6.35 mmol) was then converted to **27** by following general procedure C, with 4.03 g (6.67 mmol) of Boc-N(Me)His(Ts)OH dicyclohexylamine salt, 0.93 mL (6.67 mmol) of triethylamine, and 1.01 mL (6.67 mmol) of diethylphosphoryl cyanide in 60 mL of methylene chloride. Chromatographic purification of the crude product on 700 g of silica gel (EtOAc/hexane, 65:35) afforded 5.5 g (79%) of pure **27** as a viscous, colorless oil: R_f 0.20 (EtOAc/hexane, 65:35). Continued elution of the above chromatogram yielded 0.95 g (15%) of desilylated **27**: R_f 0.30 (EtOAc). Since desilylation occurred quantitatively during the next Boc removal, the combined products were used in the next experiment.

Boc-Phe-N(Me)His(Ts)-Leuψ[CHOHCH$_2$]Val-Ile, THAM Amide Triacetate (28). The combined product from the preceding reaction (6.0 mmol) was N-deprotected (and simultaneously desilylated) according to general procedure B. Then, by following general procedure C, the deprotected substrate was converted to **28** with 3.18 g (12 mmol) of Boc-Phe-OH, 1.67 mL (12 mmol) of triethylamine, and 1.82 mL (12 mmol) of diethylphosphoryl cyanide in 60 mL of methylene chloride. Chromatographic purification of the crude coupling product on 700 g of silica gel (EtOAc/hexane, 90:10) gave 3.0 g (44% yield) of pure **28**, a viscous, colorless oil: R_f 0.50 (EtOAc). Although the low yield was due, in part, to a fraction collector malfunction, TLC analysis of the crude coupling product also showed appreciable (15–25%) starting peptide remaining.

Boc-Pro-Phe-N(Me)His-Leuψ[CHOHCH$_2$]Val-Ile, THAM Amide Triacetate (29). Deprotection of 3.0 g (2.66 mmol) of **28** was carried out according to general procedure B. Then coupling via general procedure C, with 744 mg (3.46 mmol) of Boc-Pro-OH, 0.48 mL (3.44 mmol) of triethylamine, and 0.52 mL (3.43 mmol) of diethylphosphoryl cyanide in 30 mL of methylene chloride yielded the crude product. Chromatography on 450 g of silica gel (acetone/hexane, 45:55) gave 2.30 g (70%) of pure product (the His(*Ts*) derivative corresponding to **29**): R_f 0.24 (acetone/hexane, 45/55). Removal of the Ts protecting group on histidine from 400 mg (0.327 mmol) of the intermediate was accomplished via general procedure D. Chromatographic purification of the crude product was performed on 80 g of silica gel (0.4 M NH_3–MeOH/$CHCl_3$, 4.5:95.5). The combined fractions, after evaporation were precipitated by dissolving in 1 mL of CH_2Cl_2 and adding the solution dropwise to 25 mL of well-stirred hexane. Filtration and drying (25 °C, 0.1 mm, 64 h) afforded 250 mg (72%) of pure triacetate **29** as a white amorphous solid: ^{1}H NMR ($CDCl_3$) δ 4.50–4.30 (m, 6 H, CH_2OAc), 2.08 (s, 9 H), 1.46 (s, 9 H); mass spectrum, $[M + H]^+$ observed at m/z 1069.623 (calcd for $C_{54}H_{85}N_8O_{14}$, 1069.619); R_f 0.46 (4 M NH_3–MeOH/$CHCl_3$, 8:92).

Boc-Pro-Phe-N(Me)His-Leuψ[CHOHCH$_2$]Val-Ile, THAM Amide (30). Both Ts removal and acetate hydrolysis could be carried out simultaneously as follows. A stirred solution of 1.9

g (1.55 mmoles) of the purified Boc-Pro-OH coupling product above (before Ts removal) in 50 mL of methanol was treated with 10 mL of water and 10 mL of 1 M aqueous potassium hydroxide, and the resulting clear solution was stirred at 25 °C for 1 h under nitrogen. (By TLC, the reaction was complete at this time. A small aliquot allowed to react under the same conditions for 18 h underwent no further change.) Most of the methanol was removed in vacuo, and the aqueous residue was saturated with solid sodium chloride and extracted with methylene chloride (5 × 100 mL) and chloroform (2 × 100 mL). The combined organic layer was dried over magnesium sulfate and concentrated. Chromatographic purification of the crude product on 180 g of silica gel (4 M NH_3–MeOH/$CHCl_3$, 12:88), followed by precipitation from CH_2Cl_2/hexane, afforded 1.27 g (87%) of pure THAM amide **30** as an easily handled, white, amorphous solid: ^1H NMR ($CDCl_3$) δ 3.80–3.60 (m, 6 H, CH_2OH), 1.45 (s, 9 H); R_f 0.28 (4 M NH_3–MeOH/$CHCl_3$, 15:85).

Biology. Inhibition of Human Plasma Renin Activity. The compounds in Table I 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 (Baxter). 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, which were obtained by plotting three to four inhibitor concentrations on semilog graph paper and estimating the concentration producing 50% inhibition.

Estimation of Solubility in Water. The solubility of each compound (Table I) in water was estimated visually. Each compound was added to a series of clear test tubes containing distilled water to make concentrations of compound ranging from 1–100 mg/mL. Each tube was shaken and observations initially made with the tubes at 25 °C and again after 30 min in a 37 °C water bath.

Evaluation in Recombinant Human Renin Infused Rats. Sprague-Dawley rats were anesthetized with methoxyflurane and bilaterally nephrectomized. Approximately 18 h later, each animal was reanesthetized with dial urethane, 100 mg/kg ip; tracheostomized, and bilaterally vagotomized. One carotid artery and both jugular veins were catheterized. In some animals that had fasted for 24 h prior to nephrectomy, an infant feeding tube was passed into the stomach through the mouth for the oral administration of compounds. Mecamylamine at 1.25 mg/kg iv was utilized to elicit ganglionic blockade. an intravenous infusion of recombinant human renin was administered at 2.4 GU/kg per min for 10 min and was followed by a sustained infusion at 0.6 GU/kg per min for the duration of the experiment. The compounds in Table I were dissolved in 0.1 M citric acid and infused intravenously at 0.05 mL/min for 10 min or administered into the stomach as a 5 mL/kg bolus approximately 30–35 min postinitiation of the renin infusion.

Resolution, Absolute Stereochemistry, and Enantioselectivity of 2-Methyl-4-phenyl-1,2,3,4-tetrahydroisoquinolin-4-ol

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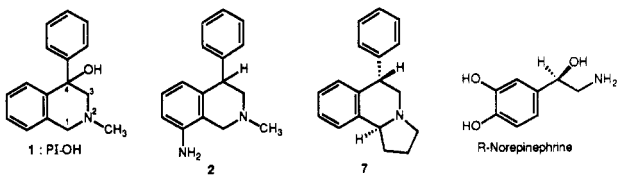
Racemic 2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinolin-4-ol (PI-OH) (1) was found to be an effective potentiator of the contractile response of norepinephrine (NE) on rat anococcygeus muscle. This paper describes the resolution of racemic PI-OH by an HPLC method to give the optically pure enantiomers (+)-1 and (−)-1. The absolute configuration of (+)-1 was *R* as determined by CD analysis and by single-crystal X-ray diffractometric analysis of the methiodide **6** derived from (+)-1. Examination of the effects of the enantiomers to potentiate the contraction of the rat anococcygeus muscle by NE showed a high degree of enantioselectivity. The NE potentiation was found to reside exclusively in (*R*)-(+)-1; the activity ratio being 21 at 3 × 10^{-6} M, whereas (*S*)-(−)-1 did not show any potentiating and inhibiting activity.

In the previous paper,[1] we reported a convenient synthesis of racemic 2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinolin-4-ol (PI-OH) (1), a compound with a chemical structure similar to that of nomifensine (2) (Chart I).[2] Racemic 1 potentiated the concentration-dependent responses of rat anococcygeus muscle to norepinephrine (NE) and electrical nerve stimulation. The potency of PI-OH was greater than that of cocaine, nomifensine (2), or desipramine in both responses.[3] Nomifensine and desipramine potentiated the response to NE at low concentration, which was progressively masked by an inhibitory effect as the concentration of the two drugs is increased.[3] However, PI-OH (1) had no side effects such as postsynaptic inhibition, and therefore it may be an ideal potentiator of the response to NE in adrenergically innervated tissues.[3] The potentiation produced by these drugs and by cocaine in NE-sensitive anococcygeus muscle to NE was consistent with the view that by inhibiting the neural uptake mechanism they cause supersensitivity in adrenergically innervated tissues.[4]

Chart I



PI-OH (1) has an asymmetric center at position 4, that structurally corresponds to β-hydroxyphenethylamines such as norepinephrine. Thus, the optical resolution of

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