alyzed for cholesterol levels.[11] triglyceride levels (Bio-Dynamics/bmc triglyceride kit), neutral lipid content,[30] and phospholipid content.[31] In Sprague–Dawley rats after 14 days of dosing the liver and small intestine, a 24-h fecal collection was extracted for the lipid and analyzed as outlined above.

[^{3}H]Cholesterol Distribution in Rats. Sprague–Dawley rats (~300 g) were administered 2-(4-methoxyphenyl)indan-1,3-dione for 14 days orally. On day 13, 10 μCi of [^{3}H]cholesterol was administered orally by intubation needle to male rats, and according to the procedures described previously,[13] some tissue samples were combusted in a Packard tissue oxidizer or plated on filter paper, dried, and digested for 24 h in Hyamine hydroxide (New England Nuclear) at 40 °C and counted (Fisher Scintiverse in a Packard scintillation counter). Results were expressed as disintegration/min (dpm) per total organ.

Plasma Lipoprotein Fractions. Sprague-Dawley male rats (~300 g) were administered test drugs at 20 mg/kg per day, orally. Blood was collected from the abdominal vein, and lipoprotein fractions were obtained by the method of Hatch and Lees[32] and Havel et al.[33] Each of the fractions was analyzed for cholesterol,[13] triglyceride (Bio-Dynamics/bmc triglyceride kit),[12] neutral lipids,[30] and protein levels.[27]

Acknowledgment. This work was supported by a National Heart, Lung, and Blood Institute grant (HL25690).

Registry No. 1, 606-23-5; 2, 876-83-5; 3, 27606-61-7; 4, 14570-43-5; 5, 91909-55-6; 6, 97920-50-8; 7, 83-12-5; 8, 1470-39-9; 9, 6149-23-1; 10, 117-37-3; 11, 15432-97-0; 12, 15432-98-1; 13, 7561-48-0; 14, 1470-42-4; 15, 1470-44-6; 16, 1146-99-2; 17, 1146-98-1; 18, 19055-67-5; 19, 55994-28-0; 20, 6549-60-6; 21, 7561-62-8; 22, 22445-53-0; 23, 1470-38-8; 24, 19147-02-5; 25, 97920-51-9; 26, 27533-97-7; 27, 1786-03-4; 28, 2156-11-8; 29, 2156-14-1; 30, 6549-63-9; dimethyl phthalate, 131-11-3; phthalide, 87-41-2; 3-pentanone, 96-22-0; 4-heptanone, 123-19-3; 5-nonanone, 502-56-7; 6-undecanone, 927-49-1; di-*n*-hexyl ketone, 462-18-0; dibenzyl ketone, 102-04-5; α-naphthaldehyde, 66-77-3; β-naphthaldehyde, 66-99-9; 4-biphenylcarboxaldehyde, 3218-36-8; 9-anthraldehyde, 642-31-9; benzaldehyde, 100-52-7; 2-methoxybenzaldehyde, 135-02-4; 3-methoxybenzaldehyde, 591-31-1; 2-methylbenzaldehyde, 529-20-4; 3-methylbenzaldehyde, 620-23-5; 4-methylbenzaldehyde, 104-87-0; 2-chlorobenzaldehyde, 89-98-5; 3-chlorobenzaldehyde, 587-04-2; 4-chlorobenzaldehyde, 104-88-1; 4-bromobenzaldehyde, 1122-91-4; 2,4-dichlorobenzaldehyde, 874-42-0; 2,6-dichlorobenzaldehyde, 83-38-5; 3,4-dichlorobenzaldehyde, 6287-38-3; 2,4-dimethylbenzaldehyde, 15764-16-6; 2,4-dimethoxybenzaldehyde, 613-45-6; 3,4-dimethoxybenzaldehyde, 120-14-9; 2-carboxbenzaldehyde, 119-67-5; 4-carboxybenzaldehyde, 619-66-9; 4-ethoxybenzaldehyde, 10031-82-0; 4-methoxybenzaldehyde, 123-11-5; acetyl-CoA synthetase, 9012-31-1; HMG CoA reductase, 9028-35-7; acyl cholesterol acyl transferase, 9027-63-8; cholesterol 7α-hydroxylase, 9037-53-0; acetyl-CoA carboxylase, 9023-93-2; *sn*-glycerol-3-phosphate acyl transferase, 9029-96-3; phosphatidate phosphohydrolase, 9025-77-8; lipoprotein lipase, 9004-02-8; citrate lyase, 9012-83-3.

Supplementary Material Available: Analyses of variance followed by Duncan's multiple-range test of serum cholesterol and serum triglyceride levels (2 pages). Ordering information is given on any current masthead page.

Synthesis and Pharmacology of the Potent Angiotensin-Converting Enzyme Inhibitor *N*-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-(*S*)-alanyl-(*S*)-pyroglutamic Acid†

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Structure **3a**, a potent angiotensin-converting enzyme inhibitor, was prepared in five steps from L-(+)-α-amino-4-phenylbutyric acid by construction of the activated side-chain ester **16**, displacement with L-pyroglutamate ester anion, and deblocking. Diastereomer separation was accomplished by chromatography at the diester stage, **17**. Pharmacological assays established that **3a** parallels enalapril in its ability to inhibit converting enzyme and lower blood pressure.

Recent research activity on antihypertensive agents has been concerned with the rational design of angiotensin-converting enzyme inhibitors,[1] the best known examples of which are captopril,[2] **1**, and enalapril,[3] **2**. We were interested in knowing if the L-pyroglutamic acid analogue (**3a**) of enalapril possesses similar biological properties. However, a thorough search of the existing literature did not reveal **3a** to be a known compound. Since we were convinced that **3a** was synthetically accessible, we sought reasons for this omission because we reasoned that it was a novel structure that ought to possess interesting biological properties. While the 2-oxopyrrolidine and pyrrolidine rings are geometrically similar, there are profound differences in acid–base properties, and we sought to determine if this was important to interaction of the proposed non-sulfhydryl inhibitor with converting enzyme. It turned out that the synthetic method[3] used to prepare **2** is not practical for the preparation of **3a** because of the hydrolytic instability of L-alanyl-L-pyroglutamic acid, **4**. Structure **3a** was prepared by an alternative method and found to be a potent angiotensin-converting enzyme inhibitor and hypotensive agent.[4]

Synthesis of Structure 3a

Structure **3a** consists of three units: L-pyroglutamic acid (A), L-alanine (B), and 2-substituted 4-phenylbutyrate (C). Therefore, its assembly has two possible routes: (A + B) + C and A + (B + C), depending on which final connection is made. Enalapril, **2**, was prepared by the (A + B) + C

† Contribution No. 84-P26.

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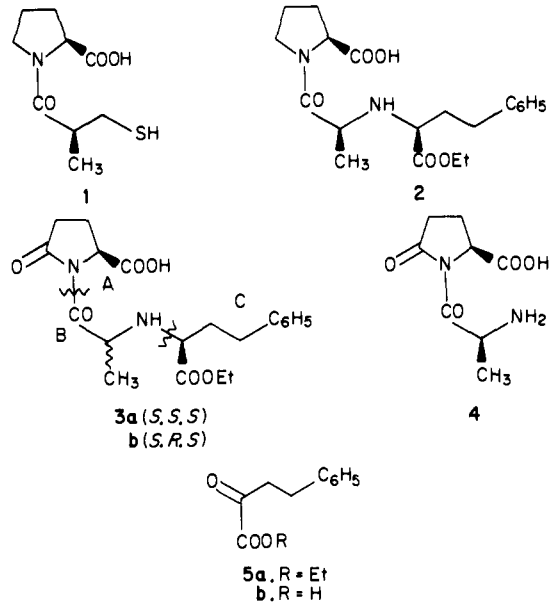
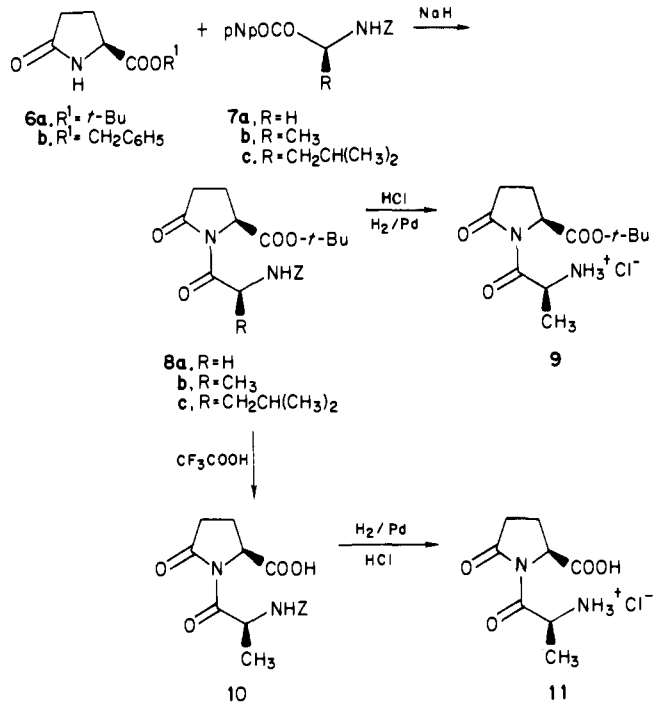


route,[3] and we decided to try this approach first although substitution of "amide-like" L-pyroglutamic acid for "amino-like" L-proline was expected to demand some different reaction conditions. Scheme I shows the preparation of L-alanyl-L-pyroglutamic acid derivatives analogous to L-alanyl-L-proline used as starting material in the Merck synthesis[3] of **2**. Scheme II shows the successful A + (B + C) route used to prepare multigram amounts of **3a**.

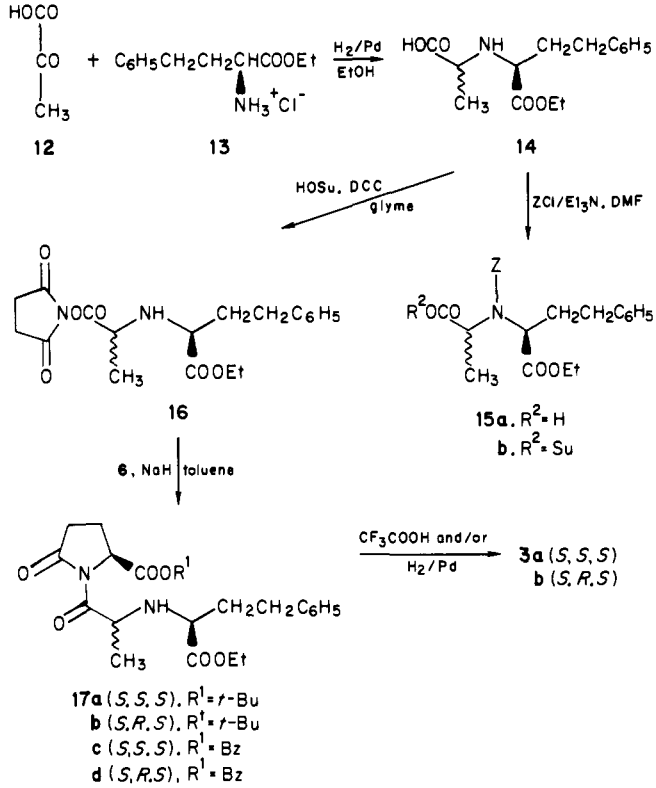
In Scheme I our plan was to reductively couple the C-unit starting material, ethyl 2-keto-4-phenylbutyrate, **5a**,[5] with L-alanyl-L-pyroglutamic acid hydrochloride, **11**. Starting materials for **11** are readily available: *tert*-butyl L-pyroglutamate, **6a**, is made from L-pyroglutamic acid, while various *N*-carbobenzyloxyamino acid 4-nitrophenyl esters, **7a–c**, can be purchased.[6] We envisaged the preparation of many analogues of this system with various R groups derived from different amino acids. The reaction of the anion of **6a** with the respective glycine, **7a**, alanine, **7b**, and leucine, **7c**, derivatives proceeds in good yields in toluene at room temperature to form compounds **8a–c**. The functional groups of **8b** can be separately deprotected to ester, **9**, or acid, **10**. Acid **10** is readily converted to hydrochloride **11**, but attempts to use **11** or material derived from it by base treatment for further structural elaboration gave decomposition products. The lability of the peptide bond of **9** toward alkali can be illustrated by the isolation of **6a** from the attempted reductive coupling of **9** with **5a** or **5b** with use of $NaBH_4$. Attempts to reductively couple **11** with **5a**,**5b** in the presence of $NaBH_3CN$ or H_2/Pd/C were likewise unsuccessful. Thus, the early promise of the (A + B) + C approach for generating a family of analogues of **3a** by selection of an appropriate amino acid for the B unit remained unfulfilled and this route was abandoned.

In the A + (B + C) approach the new chiral center corresponding to the alanine unit is generated from pyruvic acid, **12**, rather than from the 4-phenyl-2-oxobutyrate unit **5a**,**b** used in the synthesis of **2**. Both enantiomers of α-

Scheme I. (A + B) + C



Scheme II. A + (B + C)



amino-4-phenylbutyric acid[5d,8] are available; the desired *S* configuration corresponds to the L-(+) acid.[8d] We remeasured rotations of commercial L-(+)-α-amino-4-

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Table I. Effects of 3a and Enalapril on Angiotensin I Vasopressor Response in Anesthetized Normotensive Rats

dose, mg/kg cum iv	angiotensin I pressor response			
	3a		enalapril	
	mean ± SE, mmHg	% decrease	mean ± SE, mmHg	% decrease
0.010	45 ± 2.4	6	37 ± 4.8	14
0.030	39 ± 3.2	19	27 ± 3.0	37
0.090	33 ± 4.2	31	17 ± 4	60
0.27	18 ± 6.3	63	8 ± 3	81
0.81	4 ± 4.2	92	7 ± 2	84
control AI response, mmHg	+48 ± 3 (N = 12)		+43 ± 4 (N = 9)	
ED_{50},[a] mg/kg	0.13		0.066	

[a] Dose required for a 50% change from the control response.

phenylbutyric acid for direct comparison and confirmation of stereochemistry with the L-(+) acid ($[\alpha]_D$ +48.1° *c* 1.0, 1 N HCl) prepared enzymatically by the method of Tanaka and Izumiya.[8d] We elected to separate the product diastereomers at the diester stage, **17a–d**, because this is readily done by flash chromatography, whereas the diastereomers of **2** are separated by crystallization of the maleic acid salt.[3,7b] The reductive amination of pyruvic acid, **12**, with L-(+)-α-amino-4-phenylbutyric acid ethyl ester hydrochloride, **13**, proceeds in moderate yield (66%) to form the side-chain (B + C) unit **14**. This reaction resembles that of **5a,b** with L-alanyl-L-proline to form **2** in that it is necessary to use a 2–3 molar excess of the ketonic reagent[3] due to competitive reduction to the hydroxy acid derivative.[7] Initially, the secondary amine function of **14** was protected as its Cbz derivative before activation of the free carboxyl group and displacement with L-pyroglutamate ester anion. In several attempts, protected compound **15a** was obtained in low yield and successfully converted to its *N*-oxysuccinimidyl ester **15b**. However, the sluggish reaction of **14** with benzyl chloroformate suggested that this protection step and the subsequent deprotection step were unnecessary. Direct reaction of **14** with *N*-hydroxysuccinimide and DCC gave a quantitative conversion to ester **16**. This activated ester should be used immediately in order to obtain good yields of ester products **17a–d**. Attempts to purify or store **16** for more than a few days, especially in large-scale runs, causes severe reduction in the yields of **17a–d**. Both *tert*-butyl L-pyroglutamate, **6a**, and benzyl L-pyroglutamate, **6b**, were successfully used as the A-unit synthon and permitted chromatographic separation of the diastereomers. However, the subsequent deprotection and isolation of final products **3a** and **3b** is more conveniently done as the hydrochloride salts formed by one-step hydrogenolysis of **17c** and **17d** in EtOH/HCl than by using CF_3CO_2H required to deprotect **17a** and **17b**. In both cases (**17a**:**17b** and **17c**:**17d**), the diastereomeric ratio was 3–4:1 in favor of the *S,S,S* isomer.

Pharmacology

A. Angiotensin-Converting Enzyme (ACE) Inhibition Test in Rats. Intravenous dosing of **3a** to anesthetized normotensive rats produced a dose-related inhibition of the vasopressor response caused by intravenous injection of angiotensin I (AI) (Table I). The ED_{50} for **3a** was calculated to be 0.13 mg/kg cum iv compared to an ED_{50} of 0.066 mg/kg cum (time required for response to drop to 50% of peak value) iv for enalapril. At equipotent doses, the AI inhibition half-lives determined from time of peak effect were about 2 h for **3a** and 2.5 h for enalapril (0.08 mg/kg iv). The AI inhibition half-life is the time required for the response to fall to 50% of the peak value. No effect on the angiotensin II (AII) vasopressor response was produced by either compound. The ED_{50} value for the *S,R,S* isomer **3b** was 4.0 mg/kg for the ACE inhibition test, consistent with its assigned stereochemistry. Compound **3a** caused potentiation of the bradykinin vasodepressor response (Table II). ED_{50} values (dose to increase the vasodepressor response by 50%) of 0.012 and 0.0097 mg/kg cum iv were obtained for **3a** and enalapril, respectively. Both compounds increased the duration of the vasodepressor effect.

Table II. Effects of 3a and Enalapril on Bradykinin Depressor Response in Anesthetized Normotensive Rats

dose, mg/kg cum iv	bradykinin depressor reponse			
	3a		enalapril	
	mean ± SE,[b] mmHg	% change	mean ± SE,[b] mmHg	% change
control	−36 ± 3		−29 ± 3	
0.0033	−44 ± 4	+22	−27 ± 2	−7
0.010	−58 ± 5	+61	−45 ± 4	+55
0.030	−61 ± 6	+69	−61 ± 2	+110
0.090	−61 ± 4	+69	−60 ± 5	+107
ED_{50},[a] mg/kg	0.012		0.0097	

[a] Dose required for a 50% change from the control response. [b] *N* = 8.

Table III. Blood Pressure and Heart Rate Effects in SHR Pretreated with Hydrochlorothiazide

drug	dose, mm/kg po	systolic blood pressure, mmHg		heart rate, bpm	
		mean ± SE	ΔC[a]	mean ± SE	ΔC[a]
vehicle		236 ± 8		371 ± 9	
3a	3.0	211 ± 4	−25	374 ± 15	+3
3a	10	189[b] ± 10	−17	369 ± 16	−2
3a	30	190[b] ± 6	−46	381 ± 15	+10
enalapril	3.0	199[b] ± 7	−37	378 ± 14	+7
enalapril	10	169[b] ± 5	−67	380 ± 22	+9
enalapril	30	163[b] ± 10	−73	386 ± 14	+15
HCTZ	25	231 ± 9	−5	379 ± 12	+8

[a] Difference from vehicle-treated control group. [b] Significantly different from vehicle-treated control group at 0.05 level or beyond.

B. Blood Pressure and Heart Rate Effects in Spontaneous Hypertensive Rats (SHR) Pretreated with Hydrochlorothiazide and 2-Kidney–1-Clip (2K–1C) Renal Hypertensive Rats. Orally administered **3a** produced a dose-related decrease in systolic blood pressure of SHR pretreated with hydrochlorothiazide (Table III). Oral ED_{30} values (dose required to reduce systolic blood pressure by 30 mm Hg) of 3.5 and 1.5 mg/kg were calculated for **3a** and enalapril, respectively, in the same test. No significant change in heart rate was produced by either agent. Compound **3a** significantly lowered the systolic blood pressure of 2K–1C renal hypertensive rats at 10–100 mg/kg without significant change in heart rate (Table IV). The oral ED_{30} values were 5.9, 10, and 7 mg/kg respectively for **3a**, enalapril, and captopril.

Table IV. Effects of **3a** on Blood Pressure and Heart Rate in Renal-Hypertensive Rats

drug	dose mg/kg po	systolic blood pressure, mmHg mean ± SE	ΔC^a	heart rate, bpm mean ± SE	ΔC^a
3a	vehicle	243 ± 8		463 ± 14	
	3.0	220 ± 12	−23	460 ± 13	−3
	10	204[b] ± 10	−39	438 ± 9	−25
	30	204[b] ± 17	−39	481 ± 27	+18
	100	186[b] ± 13	−57	454 ± 28	−9
	ED_{30}, mg/kg	5.9			

[a] Difference from vehicle-treated control group. [b] Significantly different from vehicle-treated control group at the 0.05 level or beyond.

Table V. Hypotensive Effects of **3a** and Enalapril in Rats Fed a Low-Sodium Diet

drug	dose, mg/kg po	systolic blood pressure, mmHg 2 h mean ± SE	ΔC^a	5 h mean ± SE	ΔC^a
vehicle		170 ± 9		174 ± 7	
3a	3.0	154 ± 3	−16	153[b] ± 3	−21
3a	10	148[b] ± 5	−22	143[b] ± 8	−31
3a	30	135[b] ± 4	−35	132[b] ± 9	−42
enalapril	3.0	148[b] ± 8	−22	128[b] ± 7	−46
enalapril	10	138[b] ± 5	−32	123[b] ± 7	−51
enalapril	30	134[b] ± 4	−36	128[b] ± 5	−46

[a] Difference from vehicle-treated control group. [b] Significantly different from vehicle-treated control group at the 0.05 level or beyond.

C. Hypotensive Effect of 3a in Low-Sodium-Treated Rats. A hypotensive effect was produced by orally administered **3a** in rats fed a low-sodium diet (Table V). Significant decreases in systolic blood pressure were found at 10 mg/kg (2-h posttreatment) and 3 mg/kg (5 h). Oral ED_{30}'s of 19 and 9.7 mg/kg were calculated for **3a** and enalapril, respectively, from the 2-h data, tested concurrently. In a separate and more sensitive test in which blood pressure was directly recorded, both **3a** and enalapril produced the maximum hypotensive effect 45 min after oral treatment and the effect persisted throughout the 6-h period of measurements. By 6 h, some decrease in activity was apparent for **3a**, whereas none was observed for enalapril, compared to the control group. This demonstrates rapid oral adsorption of both compounds.

D. Effect of Orally Administered 3a on the Pressor Responses to Angiotensin I and II in Conscious Dogs. Compound **3a** produced a dose-related decrease in the pressor response to angiotensin I; the oral ED_{50} values for AI inhibition are 0.77 mg/kg for **3a** (Table VI) and 0.29 mg/kg for enalapril (data not shown). Maximum AI inhibition occurred between 45 and 60 min after dosing and the duration at 1 and 2 mg/kg was greater than 4 h. The pressor responses to angiotensin II (Table VI) and norepinephrine (data not shown) were not reduced by **3a**.

E. Effect of Intravenously Administered 3a on the Pressor Responses to Angiotensin I and II in Anesthetized Dogs. Compound **3a** produced a dose-related decrease in the AI pressor response and an increase in the AII pressor response. The ED_{50} values for AI inhibition are 0.62 mg/kg iv for **3a** (Table VII) and 0.30 mg/kg iv for enalapril (data not shown).

Conclusions

We have developed a practical synthesis to the new angiotensin-converting enzyme inhibitor **3a** in which we have effectively substituted the L-pyroglutamic acid residue for L-proline in the enalapril structure. The potency of **3a** is approximately one-half that of enalapril, **2**, in the five pharmacological assays we used to evaluate the compound. These assays demonstrate that **3a** lowers blood pressure by ACE inhibition. Compound **3a** is similar to enalapril in its duration of action;[9] both **2** and **3a** have a significantly longer duration of action than captopril, **1**. We used the observation that the most potent converting enzyme inhibitors in the enalapril series have the *S,S,S* configuration[3,7b] to establish the stereochemistry of **3a** and **3b**. This assumption is justified in view of the established criteria for highly complementary relationships between active sites and bioactive agents[10] that apply to enalapril-type converting enzyme inhibitors.[3,7b] Because stereoselectivity is important to this class of compounds, using ED_{50} values for cumulative intravenous dosing in the ACE inhibition test in rats, we assign the *S,S,S* configuration to diastereomer **3a** (0.13 mg/kg) and the *S,R,S* configuration to diastereomer **3b** (4.0 mg/kg). This biological evaluation relates **3a** to the absolute stereochemistry of enalapril, **2**, which has been established by X-ray crystallography.[7b]

Experimental Section

Chemistry. Melting points (uncorrected) were determined in open glass capillaries on a Thomas-Hoover apparatus. Spectra were obtained on the following instruments: ^1H NMR, Varian 390, IBM FT 80, Nicolet NT 360 WB, IBM FT 200; ^{19}F NMR, Varian XL-100; IR, Nicolet 7199; UV, Carey 17; MS, Consolidated CEC-110; $[\alpha]_D$, Perkin-Elmer 241MC. Elemental analyses (C, H, and N) for new substances were within ±0.4% of the theoretical values, except where noted otherwise.

L-(−)-Pyroglutamic Acid *tert*-Butyl Ester (6a). A mixture of L-(−)-pyroglutamic acid (12.91 g, 0.10 mol), CH_2Cl_2 (150 mL), H_2SO_4 (8.0 mL, 0.15 mol), and 2-methylpropene (150 mL, 90 g, 1.6 mol) was stirred in an autoclave under autogenous pressure at 25 °C for 48 h. The mixture from the autoclave was washed with 3 × 100 mL of 5% $NaHCO_3$ and 100 mL of brine, dried over Na_2SO_4, and then evaporated to leave the crude ester. Recrystallization from EtOAc/hexane, yield 12.08 g (65.2 mmol, 65%) of **6a**: mp 106–107 °C; IR ν_{max} ($CHCl_3$) 3440, 1735, 1702 cm^{-1}; $[\alpha]_D$ +6.3° (*c* 0.99, EtOH); NMR ($CDCl_3$/Me_4Si) δ 7.00 (br, NH), 4.08 (m, $NCHCO_2R$), 2.30 (m, CH_2CH_2), 1.46 (s, $C(CH_3)_3$). Anal. ($C_9H_{15}NO_3$) C, H, N.

Benzyl L-Pyroglutamate (6b). A mixture of C_6H_6 (500 ml), L-pyroglutamic acid (Sigma; 129.1 g, 1.0 mol), benzyl alcohol (150 g, 1.4 mol), and TsOH (2 g) was stirred at reflux for 4 h in a 2-L, three-neck, round-bottom flask equipped with a mechanical stirrer, reflux condenser, Dean–Stark trap, and nitrogen cap. The collected water (18 mL, 1.0 mol) was discarded, and the benzene solution washed with 2 × 200 mL of 5% $NaHCO_3$ and dried over Na_2SO_4. The residual sirup from rotary evaporation of the filtrate was distilled (Kugelrohr), bp 175 °C (0.06 mm) (123.4 g, 0.561 mol, 56%). Product **6b** is a heavy sirup with the following: IR ν_{max} ($CHCl_3$) 3435, 1742, 1701 cm^{-1}; UV λ_{max} (EtOH) 252 nm (ε 170), 257 (221), 263 (188), 268 (124); $[\alpha]_D$ −7.4° (*c* 1.03, EtOH); NMR ($CDCl_3$/Me_4Si) δ 7.42 (s, C_6H_5), 7.30 (s, NH), 5.23 (s, OCH_2), 4.33 (m, NCH), 2.37 (m, CH_2CH_2). Anal. ($C_{12}H_{13}NO_3$) C, H, N.

***N*-(*N*-Carbobenzyloxyglycyl)-L-pyroglutamic Acid *tert*-Butyl Ester (8a).** Compound **6a** (9.26 g, 50 mmol) in 75 mL of toluene was added to a suspension of hexane-washed 50% NaH (2.46 g, 55 mol) in 50 mL of toluene at 25 °C. After 30 min of

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Table VI. Effect of Oral 3a on the Pressor Responses to Angiotensin I (AI) and II (AII) in Conscious Dogs

		AI			AII		
oral dose, mg/kg	group size	control response, mmHg	response at 60–65 min following dosing, mmHg	% change from control response	control response, mmHg	response at 60–65 min following dosing, mmHg	% change from control response
0.5	4	61 ± 4	47 ± 6	−23	46 ± 6	48 ± 7	+4
1.0	4	59 ± 7	23 ± 6	−61	41 ± 1	48 ± 4	+21
2.0	4	53 ± 5	8 ± 6	−85	41 ± 2	40 ± 5	−2

Table VII. Effect of Intravenously Administered 3a on the Pressor Responses to Angiotensin I (AI) and II (AII) in Anesthetized Dogs (n = 4)

	AI		AII	
cumulative iv dose, mg/kg	pressor response, mmHg	% change from control	pressor response, mmHg	% change from control
control	68 ± 17		59 ± 13	
0.1	60 ± 17	−12	64 ± 12	+8
0.3	46 ± 14	−32	68 ± 12	+15
0.9	28 ± 10	−59	73 ± 13	+24

stirring at 25 °C, a solution of **7a** (16.61 g, 50 mmol, Sigma) in 400 mL of toluene was added. The mixture was stirred overnight at 25 °C and filtered free of suspended sodium 4-nitrophenolate; the filtrate was washed with 2 × 50 mL of 10% NaOH and 50 mL of brine, dried, and evaporated to leave 12.07 g (32.1 mmol, 64%) of crude **8a**. Recrystallization (2:1 hexane/benzene, 300 mL) gave 8.48 g of colorless solid 8a: mp 134–135 °C; IR ν_{max} ($CHCl_3$) 3440, 1740, 1709 cm^{-1}; UV λ_{max} (EtOH) 251 nm (ϵ 290), 257 (290), 264 (220), 267 (140); $[\alpha]_D$ −61.7° (c 1.01, EtOH); NMR ($CDCl_3/Me_4Si$) δ 7.33 (s, C_6H_5), 5.44 (br, NH), 5.11 (s, OCH_2Ar), 4.52 (m, $NCHCO_2Ar$, $COCH_2N$), 2.72–1.78 (m, CH_2CH_2), 1.44 (s, $C(CH_3)_3$). Anal. ($C_{19}H_{24}N_2O_6$) C, H, N.

***N*-(*N*-Carbobenzyloxy-L-alanyl)-L-pyroglutamic acid *tert*-butyl ester (8b)** was prepared similarly from **6a** and **7b** (50-mmol scale, yield 14.23 g, 36.5 mmol, 73%) as a colorless solid: mp 94–95 °C; IR ν_{max} ($CHCl_3$) 3400, 3025, 2980, 2940, 1748, 1708, 1230 cm^{-1}; UV λ_{max} (EtOH) 257 nm (ϵ 895), 263 (860), 267 (780); $[\alpha]_D$ −84.6° (c 1.00, EtOH); NMR ($CDCl_3/Me_4Si$) δ 7.30 (s, C_6H_5), 5.00 (s, OCH_2), 4.56 (m, $NCHCO_2R$), 2.70–1.89 (m, CH_2CH_2), 1.48 (s, $C(CH_3)_3$), 1.39 (d, J = 5 Hz, CH_3). Anal. ($C_{20}H_{26}N_2O_6$) C, H, N.

***N*-(*N*-Carbobenzyloxy-L-leucyl)-L-pyroglutamic acid *tert*-butyl ester (8c)** was prepared similarly from **6a** and **7c** (13.5-mmol scale, yield 3.87 g, 8.94 mmol, 66%): colorless crystals from hexane; mp 85–86 °C; IR ν_{max} ($CHCl_3$) 3440, 1745, 1702 cm^{-1}; UV λ_{max} (EtOH) 252 nm (ϵ 428), 258 (410), 264 (310), 267 (210); $[\alpha]_D$ −64.9° (c 1.01, EtOH); NMR ($CDCl_3/Me_4Si$) δ 7.30 (s, C_6H_5), 5.59–5.17 (m, 2 NCHCO), 5.06 (s, OCH_2Ar), 4.59 (br, NH), 2.70–1.56 (m, CH_2CH_2, CH_2CH), 1.41 (s, $C(CH_3)_3$), 1.00 (d, J = 6 Hz), 0.89 (d, J = 6 Hz, 2 CH_3).

***N*-L-Alanyl-L-pyroglutamic Acid *tert*-Butyl Ester Hydrochloride (9)**. A mixture of **8b** (10.0 g, 25.6 mmol), EtOH (200 mL), concentrated HCl (5 mL, 55 mmol), and 5% Pd/C (2 g) was reduced with hydrogen (Parr shaker, 40 psig, 25 °C, uptake 25.6 mmol) for 35 min. Compound **9** (8.07 g, 100%) is a colorless water-soluble solid: mp 181–182 °C dec; IR ν_{max} (KBr) 1750, 1740, 1705 cm^{-1}; UV λ_{max} (EtOH) 213 nm (ϵ 9100); $[\alpha]_D$ −94.0° (c 1.007, H_2O); NMR (Me_2SO-d_6) δ 8.80 (br, NH_3^+), 4.72 (br m, NCHCO), 2.67–1.67 (m, CH_2CH_2), 1.48 (s + m, $C(CH_3)_3$, CH_3). Anal. ($C_{12}H_{21}N_2O_4Cl$) C, H, N.

The stability of **9** toward alkali was evaluated by proton NMR spectra in Me_2SO-d_6, with and without added NaOD. The spectra were stable in Me_2SO-d_6 alone, but the addition of NaOD darkened the solution immediately, changed the chemical shifts of the NCHCOR and alanine CH_3 groups, and degraded spectral quality. In experiments with this compound in slightly alkaline solution in which reductive coupling with 2-keto-4-phenylbutyric acid in the presence of $NaBH_3CN$ was attempted, *tert*-butyl L-pyroglutamate was isolated from the reaction mixture.

***N*-(*N*-Carbobenzyloxy-L-alanyl)-L-pyroglutamic Acid and Its Dicyclohexylamine Salt (10)**. A mixture of **8b** (5.69 g, 14.58 mmol), CF_3CO_2H (50 mL), and anisole (50 mL) was stirred at 25 °C for 2 h. The colorless sirup left after evaporation of the solvents lacked the proton NMR signal for the *tert*-butyloxy group. Treatment with dicyclohexylamide (CH_3CN, to pH 9) gave 7.0 g (14.36 mmol, 98%) of crude dicyclohexylamine salt. After recrystallization (CH_3CN), the colorless crystalline salt had the following: mp 201–203 °C dec; UV λ_{max} (EtOH) 257 nm (ϵ 535), 268 (390), 272 (220); $[\alpha]_D$ −46.6° (c 1.03, EtOH); NMR (Me_2SO-d_6) δ 7.33 (s, C_6H_5), 5.22 (t, J = 6 Hz, ring NCHCO), 5.03 (s, OCH_2Ar), 4.44 (m, side-chain NCHCO), 2.92 (m, NCH), 2.00–1.00 (m, CH_2, CH_3). Anal. ($C_{28}H_{41}N_3O_6$) C, H, N.

***N*-L-Alanyl-L-pyroglutamic Acid Hydrochloride (11)**. The dicyclohexylamine salt of **10** (15.85 g, 32.5 mmol) was converted to **10** by shaking with a mixture of H_2O (100 mL), $NaHSO_4$ (12.5 g, 90.5 mmol), and 3 × 100 mL of EtOAc. The combined EtOAc layers were extracted with 5% HCl (50 mL), dried (Na_2SO_4), and evaporated. A solution of **10** in EtOH (200 mL) containing concentrated HCl (6 mL, 66 mmol) was hydrogenated for 1 h (2 g of 10% Pd/C, Parr, 40 psig, 25 °C) to give **11** (5.77 g, 24.4 mmol, 75%): mp 180–182 °C; IR ν_{max} (KBr) 3420, 1740, 1703 cm^{-1}; λ_{max} (H_2O) 217 nm (ϵ 7170); $[\alpha]_D$ −74.5° (c 1.047, H_2O); NMR (D_2O/DSS) δ 5.00 (m, NCHCOR, exchange peak), 2.89–2.08 (m, CH_2CH_2), 1.56 (d, J = 6 Hz, CH_3); MS, m/z 182.0682 (calcd for $C_8H_{10}N_2O_3$ fragment, 182.0691). Anal. Calcd for $C_8H_{13}N_2O_4Cl$: C, 40.60; H, 5.54; N, 11.84. Found: C, 39.34, H, 5.36, N, 11.25, C, 39.51; H, 5.46; N, 11.34.

L-(+)-α-Amino-4-phenylbutyric Acid Ethyl Ester Hydrochloride (13). A mixture of L-(+)-α-amino-4-phenylbutyric acid (Chemical Dynamics Corp. No. 05-4520-00, $[\alpha]_D$ +38.8° (1 N HCl) (25.0 g, 0.139 mol) and EtOH (500 mL) was treated with a vigorous stream of HCl at 25 °C and then stirred at reflux for 2 h. The mixture was cooled slightly and evaporated to give **13** as a colorless solid (32.2–35.3 g, 95–100%). Recrystallization (EtOH) gave pure **13**: mp 157–158 °C IR; ν_{max} (KBr) 3440, 1745 cm^{-1}; UV λ_{max} (EtOH) 247 nm (ϵ 119), 252 (165), 258 (203), 263 (161), 267 (129); $[\alpha]_D$ +40.0° (c 1.04, EtOH). Anal. ($C_{12}H_{18}NO_2Cl$) C, H, N.

***N*-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-(*RS*)-alanine (14)**. A mixture of **13** (10.2 g, 39.1 mmol), **12** (7.78 g, 6.1 mL, 88.4 mmol), EtOH (300 mL), NaOH (2.62 g, 65.3 mmol, predissolved in the ethanol) and 5% Pd/C (1 g) was reduced with hydrogen (Parr, 42 psig, 25 °C, 90-psig uptake). The colorless crystalline solid **14**, resulting from trituration of the crude product with ether is satisfactory for continuation of the synthesis, yield 6.6–7.0 g, (23.6–25.1 mmol, 60–64%). Recrystallized **14** (glyme) has the following: mp 148–151 °C; IR ν_{max} (KBr) 3430, 1745, 1620 cm^{-1}; UV λ_{max} (EtOH) 242 nm (ϵ 150) 247 (162), 253 (192), 258 (222), 261 (199), 264 (175), 268 (153); $[\alpha]_D$ +13.2° (c 1.00, EtOH); NMR ($CDCl_3/Me_4Si$) δ 7.20 (m, C_6H_5), 7.10 (br, NH, OH), 4.20 (q, J = 7 Hz, OCH_2), 3.40 (t, J = 8 Hz) and 3.37 (q J = 8 Hz) (2 NCH), 2.73 (t, J = 8 Hz, $ArCH_2$), 2.10 (m, CH_2), 1.42 (d, J = 8 Hz, alanine CH_3), 1.25 (t, J = 8 Hz, ester CH_3). Anal. ($C_{15}H_{21}NO_4$) C, H, N.

***N*-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-(*RS*)-*N*-carbobenzyloxyalanine (15a)**. A mixture of 14 (0.5 g, 1.6 mmol), DMF (2 mL), and Et_3N (1.01 g, 10 mmol) was treated at 25 °C with benzyl chloroformate (0.79 g, 5 mmol) for 2 h. The mixture was diluted with water (15 mL) and extracted with 3 × 10 mL of ether. The aqueous layer was acidified with hydrochloric acid and reextracted with 3 × 10 mL of ether to leave 0.1679 g (0.41 mmol, 26%) of colorless sirupy product after drying at 50 °C (0.1 mm). The carbobenzyloxy derivative is characterized by TLC (R_f 0.84, silica gel, 10% Me_2CO/90% $CHCl_3$) and NMR [($CDCl_3/Me_4Si$) δ 7.97 (s, CO_2H), 7.30 (s, C_6H_5), 7.22 (m, C_6H_5),

5.14 (s, OCH_2Ar), 4.67–4.11 (m, $NCHCO_2R$ and OCH_2), 2.70–1.89 (m, CH_2CH_2), 1.50 (d, J = 6 Hz, alanine CH_3), 1.24 (t, J = 7 Hz, ethyl CH_3)].

***N*-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-(*RS*)-*N*-carbobenzyloxyalanine *N*-Oxysuccinimidyl Ester (15b).** A mixture of **15a** (0.16 g, 0.39 mmol), glyme (3 mL), *N*-hydroxysuccinimide (46 mg, 0.4 mmol), and DCC (90.6 mg, 0.44 mmol) was left at 0 °C for 24 h. The colorless crystalline precipitate was filtered (DCHU, 69.3 mg, 0.31 mmol) and the filtrate evaporated to leave 0.2156 g of crude **15b**. Compound **15b** has the following: IR (film) 1810, 1780, 1730, 1720, 1700 cm^{-1}; NMR ($CDCl_3/Me_4Si$) δ 7.33 (m, C_6H_5), 7.22 (m, C_6H_5), 5.17 (s, OCH_2Ar), 4.70–3.89 (m, OCH_2, 2 $NCHCO_2R$), 2.81 (m, succinimide CH_2CH_2), 2.59–1.9 (m, CH_2CH_2), 1.61 (d, J = 8 Hz, alanine CH_3), 1.19 (t, J = 7 Hz, ester CH_3).

***N*-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-(*RS*)-alanine *N*-Oxysuccinimidyl Ester (16).** A mixture of **14** (11.19 g, 40.0 mmol), glyme (150 mL), $CHCl_3$ (150 mL), and *N*-hydroxysuccinimide (4.60 g, 40.0 mmol) was stirred at 25 °C and DCC (8.25 g, 40.0 mmol) was added in 1-g portions. The reaction mixture was allowed to stir at 25 °C overnight after the addition was complete. The precipitated DCHU (8.66 g, 38.6 mmol, 97%) was filtered and the filtrate was diluted with 750 mL of chloroform, extracted with 3 × 200 mL of 10% Na_2CO_3, dried (Na_2SO_4), and then evaporated to leave **16** as a pale yellow oil. Ester **16** can be used directly without further purification. **16**: IR ν_{max} (film) 3300, 1820, 1790, 1750 cm^{-1}; NMR ($CDCl_3/Me_4Si$) δ 7.22 (m, C_6H_5), 4.19 (q, J = 7 Hz, OCH_2), 3.78 (2 m, 2 NCH), 2.83 (s, $COCH_2CH_2CO$), 2.19–1.74 (m, CH_2CH_2), 1.56 (d, J = 7 Hz, alanine CH_3), 1.30 (t, J = 7 Hz, ester CH_3; UV λ_{max} (THF) 253 nm (ϵ 448), 258 (380), 262 (362), 268 (288); $[\alpha]_D$ +28.4° (c 1.00, THF); MS, m/z calcd 376.1634, found 376.1597. Anal. ($C_{19}H_{24}N_2O_6$) C, H, N.

[*N*-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-(*RS*)-alanyl]-(*S*)-pyroglutamic Acid *tert*-Butyl Ester (17a,b). A slurry of 60% NaH (0.48 g, 11.9 mmol) in toluene (21.6 mL) was treated with a solution of **6a** (2.29 g, 12.4 mmol) in toluene (21.6 mL) at 25 °C. After 30 min of stirring, the mixture was treated with a solution of **16** (4.32 g, 11.5 mmol) in toluene (21.6 mL) and allowed to stir overnight. The reaction mixture was extracted with 3 × 50 mL of water, dried, and evaporated to leave 5.59 g (100%) of **17a,b**: R_f 0.80 (minor, **17b**), 0.63 (major, **17a**) (silica gel, 3:1 CH_2Cl_2/EtOAc). Flash chromatography (silica gel, 3:1 CH_2Cl_2/EtOAc) gave 3.03 g (6.79 mmol, 59%) of **17a** as a thick sirup with R_f 0.63: IR ν_{max} (neat) 1740 cm^{-1}; UV λ_{max} (EtOH) 252 mm (ϵ 567), 258 (549), 261 (518), 264 (460), 268 (425); $[\alpha]_D$ -54.4° (c 1.00, EtOH); ^1H NMR (360 MHz) δ 7.26 (t, J = 7 Hz) and 7.18 (t, J = 7 Hz), (C_6H_5), 4.57 (t, J = 7 Hz, 2 q, J = 7 Hz, NCH), 4.13 (2 q, J = 7 Hz, OCH_2), 3.22 (t, J = 7 Hz, NCH), 2.8–2.2 (m, 3 CH_2), 2.04 (s, NH), 2.1–1.8 (m, CH_2), 1.46 (s, $C(CH_3)_3$), 1.31 (d, J = 7 Hz, alanine CH_3), 1.24 (t, J = 7 Hz, ethyl CH_3). Anal. ($C_{24}H_{34}N_2O_6$) C, H, N.

[*N*-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-(*RS*)-alanyl]-(*S*)-pyroglutamic Acid (3a,b). A mixture of **17a,b** (1.82 g, 4.08 mmol) and CF_3CO_2H (50 mL) was stirred at 25 °C for 1 h and then evaporated to leave a sirup, which was dried at 25 °C 0.1 mm); yield 2.0 g (4.0 mmol, 98%) of the trifluoroacetate salt of **3a,b**: IR ν_{max} (neat) 1750 cm^{-1}; NMR ($CDCl_3/Me_4Si$) δ 7.57 (br, NH, CO_2H), 7.23 (m, C_6H_5), 5.00 (br, $NCHCO_2R$), 4.20 (q, J = 7 Hz, OCH_2), 3.83 (m, $NCHCO_2R$), 3.0–2.0 (m, CH_2CH_2), 1.47 (m, alanine CH_3), 1.17 (t, J = 7 Hz, ethyl CH_3); ^{19}F NMR ($CDCl_3$-F11) δ -76.51 (s, CF_3CO_2).

[*N*-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-(*RS*)-alanyl]-(*S*)-pyroglutamic Acid Benzyl Ester (17c,d). A slurry of 60% NaH (2.31 g, 57.8 mmol) in toluene (100 mL) was treated dropwise over a period of 10 min with a solution of **6b** (11.50 g, 52.5 mmol) in toluene (50 mL); the mixture was stirred for 30 min at 25 °C and then treated with a solution of **16** (19.75 g, 52.5 mmol) in 50 mL of toluene. The mixture was stirred overnight at 25 °C and then extracted with 3 × 100 mL of water and 3 × 100 mL of brine. The organic layer was dried (Na_2SO_4) and evaporated, first on a rotary evaporator and second by Kugelrohr at 40 °C (0.1 mm) to leave 20.67 g (43.0 mol 82%) of diastereomer mixture **17c,d** as a pale yellow sirup. Flash chromatography (silica gel 60, 85:15 CH_2Cl_2/EtOAc) was used to separate 33.74 g (70.3 mmol) of crude **17c,d** into the *S,S,S* and *S,R,S* diastereomers. Material with R_f 0.92 (4.95 g, 10.30 mol, 15%) was the *S,R,S* diastereomer, **17d**; that with R_f 0.72 (19.49 g, 40.6 mol, 58%) was the *S,S,S* diastereomer, **17c**; total recovery 24.44 g (50.9 mol, 72%).

The major diastereomer **17c** is a colorless sirup with the following: IR ν_{max} (film) 1745 cm^{-1}; UV λ_{max} (c 0.93, EtOH) 247 nm (ϵ 615), 252 (634), 258 (573), 261 (606), 264 (567), 268 (485); $[\alpha]_D$ -53.9° (c 1.00, EtOH); 360-MHz ^1H NMR ($CDCl_3/Me_4Si$) δ 7.329 (m, C_6H_5), 7.250 (t, J = 7.5 Hz) and 7.167 (d, J = 7.5 Hz) (C_6H_5), 5.164 (AB, J = 12 Hz, OCH_2Ar), 4.767 (AB, J = 4 Hz, NCH), 4.594 (q, J = 7 Hz, NCOCH), 4.128 (2 q, J = 7 Hz, ester OCH_2), 3.106 (AB, J = 6 Hz, NCH), 2.622 (m, $COCH_2CH_2$), 2.317 (m), 2.018 (m), and 1.892 (m) (CH_2CH_2), 1.253 (d, J = 7 Hz, alanine CH_3), 1.250 (t, J = 7 Hz, ester CH_3); MS, m/z, calcd 480.2260, found 480.2263. Anal. ($C_{27}H_{32}N_2O_6$) C, H, N.

The minor diastereomer **17d** is a colorless sirup with the following: IR ν_{max} (film), 1730 cm^{-1}; UV λ_{max} (EtOH) 247 nm (ϵ 567), 252 (610), 258 (678), 262 (610), 264 (577), 268 (560); $[\alpha]_D$ -16.8° (c 0.93, EtOH); 360-MHz ^1H NMR ($CDCl_3/Me_4Si$) δ 7.340 (brs, C_6H_5), 7.249 (m) and 7.175 (m) (C_6H_5), 5.140 (AB, J = 12 Hz, OCH_2Ar), 4.157 (2 q, J = 7 Hz, m, ester OCH_2 + NCH), 3.423 (q, J = 7 Hz, CONCH), 3.327 (AB, J = 6 Hz, ring NCH), 2.675 (m) and 2.05–1.80 (m) (2 CH_2CH_2), 1.343 (d, J = 7 Hz, alanine CH_3), 1.226 (t, J = 7 Hz, ethyl CH_3). Compound **17d** was not obtained in an analytically pure state. Anal. Calcd for $C_{27}H_{32}N_2O_6$: C, 67.48; H, 6.71; N, 5.83. Found: C, 71.05; H, 7.49; N, 4.45; C, 71.21; H, 7.39; N, 4.75.

[*N*-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-(*S*)-alanyl]-(*S*)-pyroglutamic Acid Hydrochloride (3a·HCl). A mixture of EtOH (250 mL), **17c** (18.34 g, 38.2 mmol), concentrated HCl (6.0 mL, 66.0 mmol), and 5% Pd/C (1.0 g) was hydrogenated (Parr shaker, 25 °C, 2 h). Compound **3a** isolated by evaporation of the filtrate is hygroscopic. **3a**: mp 90 °C dec; IR ν_{max} (KBr) 3400, 1740 cm^{-1}; UV λ_{max} (EtOH) 252 nm (ϵ 451), 258 (419), 263 (306), 267 (235); $[\alpha]_D$ -31.8° (c 0.96, EtOH); NMR ($CDCl_3/Me_4Si$) δ 7.22 (s, C_6H_5), 5.07 (m) and 4.89 (m) (2 $NCHCO_2R$), 4.28 (q, J = 7 Hz, OCH_2), 3.86 (m, NCH), 2.63 (m, 2 CH_2CH_2), 1.67 (d, J = 6 Hz, alanine CH_3), 1.33 (t, J = 7 Hz, ethyl CH_3). Anal. ($C_{20}H_{26}N_2O_6Cl$) C, H, N.

[*N*-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-(*R*)-alanyl]-(*S*)-pyroglutamic Acid Hydrochloride (3b·HCl). The hydrogenation procedure was repeated with **17d** (4.95 g, 10.30 mmol), yield 2.92 g (6.86 mmol, 57%) of **3b**. **3b**: mp 189–191 °C (from $CHCl_3$); IR ν_{max} (KBr) 3080, 3060, 1745 cm^{-1}; UV λ_{max} (EtOH) 247 nm (ϵ 282), 253 (301), 258 (323), 264 (256), 268 (207); $[\alpha]_D$ +23.9° (c 0.90, EtOH); NMR ($CDCl_3/Me_4Si$) δ 7.30 (m, C_6H_5), 4.22 (q, J = 8 Hz, OCH_2), 4.07 (br m, OCH_2NCHCO_2R), 2.90–2.67 (m, CH_2CH_2), 1.80 (d, J = 7 Hz, alanine CH_3), 1.43 (t, J = 8 Hz, ester CH_3. Compound **3b**·HCl was not obtained in an analytically pure state. Anal. Calcd for $C_{20}H_{26}N_2O_6Cl$: C, 56.27; H, 6.37; N, 6.56. Found: C, 56.92; H, 7.26; N, 4.80; C, 57.26; H, 7.12; N, 4.65.

Pharmacology. Pharmacological methods are based on those described by Rubin et al.[11a] and Sweet et al.[11b] In ACE inhibition tests challenged by AI, the effective dose required to increase vasodepressor response by 50% (ED_{50}) is used as the indicator of effectiveness; in measuring hypotensive effects, the effective dose required to lower systolic blood pressure by 30 mmHg (ED_{30}) is used.

A. ACE Inhibition Test in Rats. Male rats (Charles River CD) weighing 380–500 g were anesthetized with a mixture of pentobarbital NA (15 mg/kg) and barbital (220 mg/kg) via intraperitoneal injection. Each rat was tracheotomized, and cannulas were inserted into the right femoral artery and vein. Blood pressure was recorded from the femoral artery via a Statham pressure transducer (P23GC) and recorded on a Grass polygraph. All dosing was done with the venous cannula. Rats were held on a heating board at 37 °C during the experiment.

To establish the control vasopressor response to angiotensin I (AI) in each rat, AI (Sigma) at 0.3 μg/kg was injected, and the blood pressure at maximum increase was recorded and compared to the predose blood pressure. At 5 min after the AI dose, the control vasopressor response to angiotensin II (AII) (Ciba) given at 0.1 μg/kg was determined as above. The first dose of test drug was administered at 5 min after the AII injection, and beginning at 5 min after drug dosage, blood pressure responses to injections of AI and AII were determined at 5-min intervals as above. The drug–AI–AII sequence was repeated to cover a cumulative dosage range of **3a** and enalapril from 0.01 to 0.81 mg/kg.

Duration of inhibition of the AI vasopressor response was determined for **3a** and enalapril with use of intravenous doses of 0.2 and 0.08 mg/kg, respectively, using basically the same procedure. After obtaining the control response to AI, rats were challenged with AI at 5 min after dosing with test drug and at every 10 min thereafter for a total of 210 min.

To establish the control vasodepressor response to bradykinin (Bk) in each rat in tests separate from the angiotensin study, Bk (Sigma) at 1 μg/kg was injected, and the maximum decrease in blood pressure was determined and compared to the predose pressure. At 10 min after the dosage with Bk, the first dose of test drug was given followed at 5 min by a Bk injection. The vasodepressor response and duration of the response were determined. The drug–Bk sequence was repeated to cover a cumulative dose range of **3a** and enalapril of 0.0033–0.27 mg/kg.

All compounds were administered intravenously in 0.9% saline solution at a volume to body weight ratio of 1 mL/kg. Doses of **3a** (hydrochloride salt) and enalapril (maleate salt) were given on the free base basis. ED_{50} values, that dose producing a 50% change from the control response were calculated by using linear regression analysis.

B. Hypotensive Effect in Hydrochlorothiazide (HCTZ) Treated Spontaneously Hypertensive Rats (SHR) and Renal Hypertensive Rats (2K–1C). Male SHR (Charles River), 13 weeks old, were each treated orally once daily for 3 days with 25 mg/kg HCTZ (Merck) suspended in 0.25% methylcellulose solution. At 1 h after the third dose, rats were administered **3a** or enalapril orally, and systolic blood pressures and heart rates were determined 1.5 h later. Groups of rats given HCTZ or methylcellulose vehicle for 3 days were dosed with water to serve as controls. To prepare renal hypertensive rats, male rats (Charles River CD), 4 weeks old and weighing 90–120 g, were anesthetized by intraperitoneal injection with pentobarbital–barbital solution, the left renal artery was exposed by flank incision, and a silver clip with a 0.22-mm-diameter opening was placed on the artery. The right kidney and artery were left untouched. Rats were held for 6 weeks to allow the hypertension to develop. At this time, groups of overnight-fasted rats were treated orally with **3a** in aqueous solution. Animals treated with water served as the hypertensive control group. Systolic blood pressures and heart rates were determined at 2 h after treatment.

Compound **3a** (hydrochloride salt) and enalapril (maleate) were dosed on the free base basis in water solutions. Oral dosing was done at a volume to body weight ratio of 5 mL/kg.

The systolic arterial blood pressure of each rat was measured by an indirect method with use of an inflatable occluding cuff and a piezoelectric pulse transducer placed on the rat's tail. The cuff pressure and pulse sounds were recorded with a sphygmomanometer preamplifier and Grass polygraph. Heart rate was determined by counting pulse waves for a 6-s period. Rats were warmed for 20 min at 38 °C before blood pressure determinations.

Blood pressure and heart rate results were statistically analyzed with use of one-way analysis of variance and Duncan's multiple range test. ED_{30} values, that dose producing a 30-mmHg decrease in blood pressure, were calculated, using linear regression analysis.

C. Hypotensive Effects of 3a in Low-Sodium-Treated Rats. Male rats (Charles River, CD), 11 weeks of age, were placed on a sodium-deficient diet (Hartcraft Sodium Test Diet, U.S. Biochemical Corp.) for 3 weeks. Groups of these rats, after overnight fasting, were dosed orally with aqueous solutions of **3a** and enalapril, and one group was treated with water and served as control. Compound **3a** (hydrochloride salt) and enalapril (maleate) were dosed on the free base basis in water solutions. Oral dosing was done at a volume to body weight ratio of 5 mL/kg. Blood pressures and heart rates were determined at 2 h after treatment. The systolic arterial blood pressure of each rat was measured by the indirect method described above. Blood pressure and heart rate results were statistically analyzed as above.

Three groups of overnight-fasted low-sodium rats were cannulated via the caudal artery under ether anesthesia. Rats were placed in restraining holders, and blood pressure was measured with a Statham pressure transducer (P23GC) and recorded on a Grass polygraph. After recovery from the anesthesia, rats were dosed with aqueous solutions of **3a** and enalapril, and blood pressure and heart rate were recorded for a 6-h period. A group treated with water served as the control.

D. ACE Inhibition Test in Dogs. Oral Administration. Mongrel dogs of both sexes (6.8–15.3 kg) had polyethylene cannulas implanted in the femoral artery and vein under thiopental sodium anesthesia (120 mg/kg iv). The cannulas were passed subcutaneously, exteriorized through a small incision in the patellar region, and the leg was bandaged. On the following morning the fasted animals were placed in canvas slings. Blood pressure was recorded through the femoral artery cannula attached to a Statham pressure transducer; heart rate was determined from lead II of the electrocardiogram. Both were measured continuously on a Grass polygraph.

Beginning at 30 min prior to dosing, intravenous bolus challenges of angiotensin I (AI, 0.3 μg/kg), angiotensin II (AII, 0.1 μg/kg), and norepinephrine (NE, 1 μg/kg) were administered 5 min apart.

Two sets of challenges preceded the dose of **3a**; the second served as each dog's control responses. Groups of four dogs each were administered **3a** at either 0.5, 1, or 2 mg/kg orally (calculated upon base weight) at a volume to body weight ratio of 2 mL/kg. Compound **3a** was suspended in a 0.25% methylcellulose vehicle with glycerin added as a wetting agent to a concentration of 3%. Challenges of AI were administered every 5 min up to 60 min after dosing; sequences of AI, AII, and NE challenges were administered every 30 min up to 240 min after dosing.

Intravenous Administration. Mongrel dogs of both sexes (7.3–15.5 kg) were anesthetized with a mixture of barbital sodium (220 mg/kg iv) and pentobarbital sodium (15 mg/kg iv), tracheotomized, and bilaterally vagotomized. Arterial blood pressure and heart rate were measured as described for oral administration tests.

Intravenous bolus challenges of angiotensin I (AI, 1 μg/kg) and angiotensin II (AII, 1 μg/kg) were administered 10 min apart. Two sets of challenges preceded the first dose of **3a**; the second set served as each dog's control responses. A group of four dogs was administered **3a** at cumulative doses of 0.1, 0.3, and 0.9 mg/kg (calculated upon base weight) at a volume to body weight ratio of 0.2 mL/kg. Compound **3a** was dissolved in saline and administered through an indwelling femoral vein cannula. A set of AI and AII challenges followed each dose of **3a**; doses of **3a** were thus spaced 30 min apart.

Registry No. **3a**·trifluoroacetate salt, 98049-16-2; **3a**·HCl, 90940-59-3; **3b**·trifluoroacetate salt, 98049-18-4; **3b**·HCl, 90940-60-6; **6a**, 35418-16-7; **6b**, 94885-52-6; **7a**, 1738-86-9; **7b**, 1168-87-2; **7c**, 1738-87-0; **8a**, 98013-97-9; **8b**, 97998-60-2; **8c**, 97998-61-3; **9**, 97998-62-4; **10**, 97998-63-5; **10**·dicyclohexylamine salt, 97998-64-6; **11**, 97998-65-7; **12**, 127-17-3; **13**, 90891-21-7; **14**, 90988-18-4; **15a**, 97998-66-8; **15b**, 97998-67-9; **16**, 97998-68-0; **17a**, 98049-19-5; **17b**, 98049-20-8; **17c**, 90891-25-1; **17d**, 90940-58-2; 2-methylpropene, 115-11-7; benzyl chloroformate, 501-53-1; *N*-hydroxysuccinimide, 6066-82-6; angiotensin-converting enzyme, 9015-82-1.