43, 96576-38-4; 43·HCl, 96576-39-5; 44, 96615-43-9; 44·HCl, 96576-40-8; 45, 96576-41-9; 45·HCl, 96576-42-0; 46, 96576-43-1; 47, 96576-44-2; 48, 96576-45-3; 49, 96576-46-4; 50, 96576-47-5; 50·HCl, 96576-48-6; 51, 96576-49-7; 51·HCl, 96576-50-0; 52, 96576-51-1; 53, 96576-52-2; 54, 96576-53-3; 55, 96576-54-4; 55·HCl, 96576-55-5; EDA, 107-15-3; $ClCH_2COCH_3$, 78-95-5; $BrCH_2COCH_2CH_3$, 816-40-0; $BrCH_2CO(CH_2)_2CH_3$, 817-71-0; $2\text{-}ClC_6H_4CH_2OH$, 17849-38-6; $Cl(CH_2)_2OH$, 107-07-3; EtSH, 75-08-1; MeCHBrCHBrCOOEt, 609-11-0; $CH_3(CH_2)_2OH$, 71-23-8; $(CH_3)_2CHOH$, 67-63-0; $CH_3(CH_2)_3OH$, 71-36-3; $CH_3(CH_2)_4OH$, 71-41-0; $CH_2{=}CHCH_2OH$, 107-18-6; $HC{\equiv}CCH_2OH$, 107-19-7; $PhCH_2OH$, 100-51-6; $2\text{-}MeOC_6H_4CH_2OH$, 612-16-8; $4\text{-}MeOC_6H_4CH_2OH$, 105-13-5; $4\text{-}ClC_6H_4CH_2OH$, 873-76-7; $Ph(CH_2)_2OH$, 60-12-8; $HO(CH_2)_2OH$, 107-21-1; $HO(CH_2)_4OH$, 110-63-4; $MeO(CH_2)_2OH$, 109-86-4; PhOH, 108-95-2; $3\text{-}MeC_6H_4OH$, 108-39-4; $4\text{-}MeOC_6H_4OH$, 150-76-5; $Cl_3CCH_2OH$, 115-20-8; $CH_3CH(NH_2)CH_2NH_2$, 78-90-0; 2-(hydroxymethyl)-2-methyl-1,4-benzodioxan, 16163-83-0; 3-hydroxy-3-methyl-2*H*-1,5-benzodioxepin, 68281-26-5; 2-methyl-1,4-benzodioxan-2-carboxylic acid, 68281-27-6; 2-methyl-1,4-benzodioxan-2-carbonyl chloride, 77156-57-1; 2-methyl-1,4-benzodioxan-2-carboxamide, 84141-79-7; catechol, 120-80-9; catechol monosodium salt, 34789-97-4; 2-cyano-2-isopropenyl-1,4-benzodioxan, 84141-86-6; 2-acetyl-2-cyano-1,4-benzodioxan, 96576-56-6; 2-cyano-2-(1-hydroxyethyl)-1,4-benzodioxan, 96576-57-7; 2-cyano-2-vinyl-1,4-benzodioxan, 96576-58-8; 3-methyl-1,4-benzodioxan-2-carboxamide, 96576-59-9; 2,3-dibromo-1,4-benzodioxan-2-carbonitrile, 96576-60-2; 2-(ethylthio)-1,4-benzodioxan-2-carbonitrile, 96576-61-3; 2-(chloromethyl)-2-methyloxirane, 598-09-4; 2-butyl-2-(chloromethyl)oxirane, 86488-91-7; 2-(chloromethyl)-2-heptyloxirane, 96576-62-4; 2-(chloromethyl)-2-phenyloxirane, 1005-91-0; 2-butyl-2-(hydroxymethyl)-1,4-benzodioxan, 96576-65-7; 2-heptyl-2-(hydroxymethyl)-1,4-benzodioxan, 96576-66-8; 2-(hydroxymethyl)-2-phenyl-1,4-benzodioxan, 84141-83-3; cyclobutanone, 1191-95-3; cyclopentanone, 120-92-3; cyclohexanone, 108-94-1; ethyl 3-methyl-1,4-benzodioxan-2-carboxylate, 67770-59-6; cyclopentanol, 96-41-3; 2-(chloromethyl)-2-vinyloxirane, 96576-69-1; 2-bromo-6,7-dimethoxy-1,4-benzodioxan-2-carbonitrile, 96576-70-4.

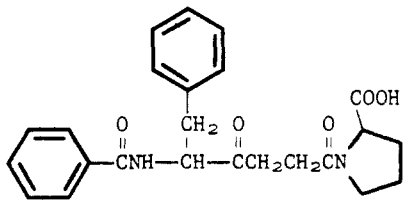
# Synthesis and Biological Activity of Pentapeptide Analogues of the Potent Angiotensin Converting Enzyme Inhibitor 5(*S*)-Benzamido-4-oxo-6-phenylhexanoyl-L-proline

Ronald G. Almquist,* Clive Jennings-White, Wan-Ru Chao, Thomas Steeger,† Kevin Wheeler,† James Rogers,† and Chozo Mitoma†

*Bio-Organic Chemistry Laboratory and Biomedical Research Laboratory, SRI International, Menlo Park, California 94025.*
*Received October 15, 1984*

Two pentapeptide analogues (14 and 15) of the ketomethylene-containing angiotensin converting enzyme (ACE) inhibitor 5(*S*)-benzamido-4-oxo-6-phenylhexanoyl-L-proline (1) were synthesized and evaluated as ACE inhibitors and antihypertensive agents. Compounds 14 and 15 were very potent ACE inhibitors with $I_{50}$ values of 7.0 and 3.0 nM, respectively, compared to an $I_{50}$ value of 70 nM for 1. Neither 14 nor 15 showed significant blood pressure lowering activity in renal hypertensive rats. Investigations conducted on a tritiated analogue of 14 showed that 70% of an oral dose of this compound is absorbed but is rapidly excreted from the blood with a half life of 24 min. Thin-layer chromatography of bile and urine contents in rats given tritiated 14 orally showed that it is excreted in greater than 90% unchanged form. This implies that a ketomethylene linkage can stabilize peptide amide linkages adjacent to it to peptidase degradation.

The ketomethylene tripeptide analogue 5(*S*)-benzamido-4-oxo-6-phenylhexanoyl-L-proline (1) has been shown to be a potent in vitro angiotensin converting enzyme (ACE) inhibitor, $I_{50}$ = 70 nM.[1] Unfortunately this compound has poor activity as a blood pressure lowering agent when given either orally or intravenously to renal hypertensive rats.[2]




1

Recent studies[3] of radiolabeled derivatives of 1 suggest two possible explanations for its poor in vivo activity. First, only 20% of an oral dose of 1 is absorbed into the blood stream over a 24-h period. Second, the half-life of 1 in the blood in rats was only 10 min because it is rapidly excreted into the bile. Compound 1, however, is only slowly metabolized by the rat in which 80% of the excreted radioactivity was in the form of unchanged 1.

One possible method to improve the antihypertensive activity of 1 in the rat would be to develop analogues of it that have greatly increased ACE inhibition activity. Such compounds would be expected to require lower blood levels than 1 to achieve total ACE inhibition in vivo. Therefore, even though these new compounds may also be rapidly excreted in the bile, the blood level required to achieve significant blood pressure reduction with them in the rat may be lower because of their increased potency.

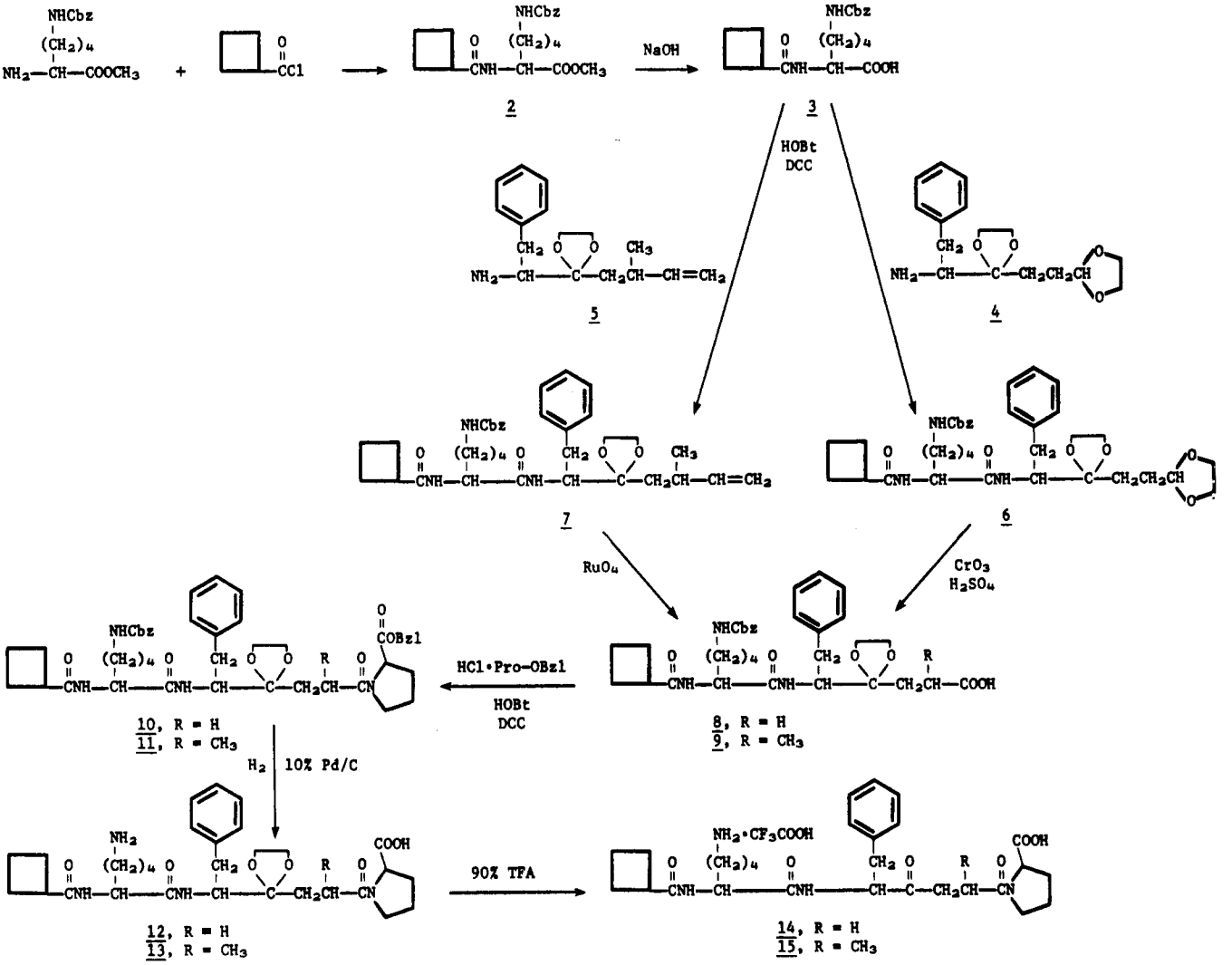
An examination of the ACE inhibition data published by Cushman et al.[4] on various peptide analogues of the original snake venom peptide ACE inhibitors indicates that the pentapeptide pGlu-Lys-Phe-Ala-Pro is almost 30 times more potent than the tripeptide Phe-Ala-Pro. By analogy, extending 1 to a pentapeptide might greatly increase its

† Biomedical Research Laboratory.

(1) Almquist, R. G.; Chao, W.-R.; Ellis, M. W.; Johnson, H. L. *J. Med. Chem.* **1980**, *23*, 1392.
(2) Meyer, R. F.; Nicolaides, E. D.; Tinney, F. J.; Lunney, E. A.; Holmes, A.; Hoefle, M. L.; Smith, R. D.; Essenburg, A. D.; Kaplan, H. R.; Almquist, R. G. *J. Med. Chem.* **1981**, *24*, 964.
(3) Almquist, R. G.; Steeger, T.; Jackson, S.; Mitoma, C. *Life Sci.* **1985**, *37*, 299.
(4) Cushman, D. W.; Pluscec, J.; Williams, N. J.; Weaver, E. R.; Sabo, E. F.; Kocy, O.; Cheung, H. S.; Ondetti; M. A. *Experientia* **1973**, *29*, 1032.

0022-2623/85/1828-1062$01.50/0 

**Scheme I**




ACE inhibition activity. It is also reported by Ondetti et al.[5] that replacing pyroglutamic acid in pGlu-Lys-Phe-Ala-Pro with cyclobutanecarboxylic acid (Cbc) yields a pentapeptide analogue with slightly better ACE inhibition activity. Since Cbc would also be expected to be more stable to peptidase degradation than pGlu, it was used in the pentapeptide analogues that we have prepared. The two analogues chosen for synthesis, **14** and **15** (Scheme I), are analogues of Cbc-Lys-Phe-Gly-Pro and Cbc-Lys-Phe-Ala-Pro, respectively, in which the amide linkage connecting Phe-Gly and Phe-Ala have been replaced with ketomethylene linkages.

**Chemistry.** As outlined in Scheme I, initially $N^{\epsilon}$-Cbz-L-Lysine-$OCH_3$[6] was acylated with cyclobutanecarbonyl chloride to give **2** in 84% yield. The methyl ester **2** was readily saponified to the desired acid **3**. Compound **3** was condensed with use of 1-hydroxybenzotriazole (HOBT) and dicyclohexylcarbodiimide (DCC) as additives with either the amino acetal **4**[1] or the amino olefin **5**[7] to yield compounds **6** and **7**, respectively. Reaction of the acetal in compound **6** with Jones reagent and the olefin in compound **7** with ruthenium tetraoxide yielded the two acids **8** and **9**, respectively. Coupling of **8** and **9** with L-proline benzyl ester followed by removal of the blocking groups as shown in Scheme I yielded the desired final products **14** and **15**.

**Table I.** Inhibition Results with Porcine Plasma Angiotensin Converting Enzyme[a]

| inhibitor | $I_{50}$,[b] nM | inhibitor | $I_{50}$,[b] nM |
|---|---|---|---|
| 1 | 70 | 15 | 3.0 |
| 14 | 7.0 | captopril | 300 |

[a] The fluorometric assay of porcine plasma ACE that was used is described in ref 1. [b] All values are the average of results obtained in two or more experiments.

## Biological Activity and Discussion

The ACE inhibition activities of **14** and **15** are shown in Table I. Both compounds are at least 10 times more potent than the parent compound **1**. The addition of (cyclobutylcarbonyl)-L-lysine to the amino terminus of **1** must provide additional binding points for **14** and **15** to the angiotensin converting enzyme. Addition of a methyl group to **14** to give **15** does not increase the ACE inhibition activity of **15** to the same extent as that seen when a methyl group was attached to **1**.[7]

Both **14** and **15** were tested for their ability to lower blood pressure in renal hypertensive rats. As seen in Table II, neither compound lowered blood pressure to a statis-

(5) Ondetti, M. A.; Pluscec, J.; Weaver, E. R.; Williams, N.; Sabo, E. F.; Kocy, O. "Chemistry and Biology of Peptides"; Meienhofer, J., Ed.; Ann Arbor Science: Ann Arbor, MI, 1972; pp 525–531.
(6) Shiba, T.; Kaneka, T. *Bull. Chem. Soc. Jpn.* **1960**, *33*, 1721.
(7) Almquist, R. G.; Crase, J.; Jennings-White, C.; Meyer, R. F.; Hoefle, M. L.; Smith, R. D.; Essenburg, A. D.; Kaplan, H. R. *J. Med. Chem.* **1982**, *25*, 1292.

**Table II.** Effect of ACE Inhibitors on Blood Pressure in the Conscious Renal (One Clip/Two Kidney) Hypertensive Rat

| compd | dose, mg/kg po | no. tested | mean aortic blood pressure, mmHg | | |
|---|---|---|---|---|---|
| | | | base line | max effect | change |
| 1[a] | 30 | 4 | 190 ± 6 | 178 ± 6 | −12 (at 8 h) |
| **14** | 3[b] | 4 | 195 ± 7 | 173 ± 23 | −22 (at 4 h) |
| **15** | 6.4[b] | 4 | 186 ± 20 | 181 ± 12 | −5 (at 8 h) |
| captopril[a] | 3 | 4 | 192 ± 7 | 99 ± 7 | −93 (at 1 h) |

[a] Data taken from ref 7. [b] Vehicle employed: 5 mL of 0.25% methylcellulose aqueous solution/kg of body weight.

**Table III.** Percent Recovery of Orally Administered **16** in the Bile and Urine of Rats over 24 h

| rat wt, g | oral dose: mg; μCi | recovered radioactivity, % | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | in bile over time, h | | | | | | | | | | in urine, 0–24 h | total recov |
| | | 0–1 | 1–2 | 2–3 | 3–4 | 4–5 | 5–6 | 6–7 | 7–8 | 8–24 | total | | |
| 250 | 1.3; 2.45 | 7.50 | 3.25 | 4.29 | 4.51 | 4.15 | 2.84 | 4.32 | 3.75 | 11.01 | 45.62 | 22.49 | 68.11 |
| 241 | 4.3; 4.09 | 7.38 | 11.72 | 10.80 | 7.05 | 5.30 | 1.42 | 2.50 | 1.85 | 10.55 | 58.57 | 18.81 | 77.38 |

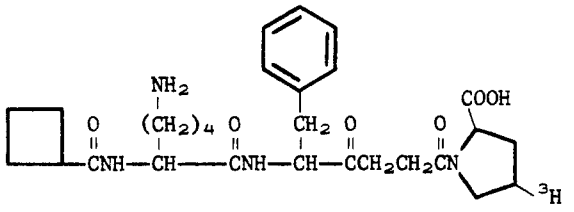
**Table IV.** Half-Life of **16** and $^3$H-Labeled 1[3] in Rat Blood

| time, min | blood level, counts/min per 0.2 mL | | | | | |
|---|---|---|---|---|---|---|
| | **16** | | | | $^3$H-labeled 1 | |
| | rat 1 | rat 2 | rat 3 | rat 4 | rat 1 | rat 2 |
| 5[b] | 1750 | 2180 | 4616 | 4385 | 7568 | 5803 |
| 10 | 1334 | 1813 | 3921 | 4221 | 5217 | 3824 |
| 15 | 1234 | 1574 | 3429 | 3357 | 3817 | 2501 |
| 20 | 1003 | 1466 | 3051 | 2956 | 2576 | 2039 |
| 30 | 818 | *c* | *c* | 2771 | 1777 | 1229 |
| $t_{1/2}$ | 23.6 | 26.0 | 23.3 | 24.6 | 11.8 | 11.4 |
| av $t_{1/2}$ | 24.4 | | | | 11.6 | |

[a] Male Sprague–Dawley rats weighing ca. 200 g were intravenously dosed with 2 mg containing approximately 3 μCi of radioactivity. [b] The variation in the cpm at 5 min, reflects that iv injection into the tail vein was not consistent. [c] These 30-min values for **16** were too high to be included in the linear regression analysis. This indicates that the $^3$H label on **16** was more metabolically unstable than that on 1.

tically significant extent at the doses tested. Because of compound limitations testing at higher doses was not possible, although based on the ACE inhibition activities of **14** and **15** one would have expected to see significant antihypertensive activity at the doses tested.

In order to gain a better understanding of the reasons for the low antihypertensive activity of **14** and **15**, a radiolabeled derivative of **14** was synthesized in which the 4-position in the proline ring was tritiated (compound **16**).




**16**

Unlike 1,[3] **16** is well absorbed after oral administration in rats as shown in Table III in which approximately 50% and 20% of an oral dose appear in the bile and urine, respectively, over a 24-h period. The blood half-life of **16** (Table IV) is only 24 min following intravenous injection. This is longer than the 11-min blood half-life found for 1. Because of its higher molecular weight, **16** would be expected to undergo biliary excretion at least as rapidly as 1,[8] but on the basis of its better oral absorption (3.5 times compound 1), the longer blood half-life of **16** vs. 1 could be due to the much better ability of **16** to be reabsorbed into the blood following its rapid biliary excretion.

Another interesting finding was that the pentapeptide analogue **16** was metabolically stable. Aliquots of bile collected at 1, 2, 3, and 4 h and of total 24 h urine after oral administration were chromatographed on an Analtech silica gel G preabsorbent plate and the plate was developed in 1-butanol/water/acetic acid/ethyl acetate (1:1:1:1, v/v/v/v). The autoradiogram of the plate revealed only a single spot corresponding to **16** for each sample. This indicates that the lysyl–phenylalanine amide bond in **16** is not significantly cleaved after oral administration.

To further investigate the resistance of the Lys–Phe amide bond to peptidase cleavage, **14** was stirred at room temperature with trypsin, the specific Lys-X cleaving peptidase that is present in the gastrointestinal tract. The reaction with trypsin was monitored by TLC. Conditions were used for the reaction with trypsin that completely decomposed a lysine-containing tridecapeptide (synthesized in our lab), Ala-Ser-Ser-Gly-Glu-Arg-Trp-Met-Phe-Lys-Asn-Asp-Gly, in 30 min. After 5 h in the presence of trypsin, only 20–30% of **14** had decomposed to other products. This study shows that **14** is cleaved by trypsin, but only slowly. This would allow **14** to be absorbed after oral administration without being cleaved significantly by trypsin.

The stability of **16** and **14** to oral administration and trypsin cleavage indicates that insertion of a ketomethylene linkage in place of the normal amide linkage connecting Phe$^3$-Gly$^4$ not only prevents the cleavage of Phe from Gly but also greatly increases the stability of the adjacent Lys$^2$–Phe$^3$ bond to peptidase cleavage. Peptidases capable of cleaving such a Lys–Phe bond may have a strong requirement for hydrogen bonding with the NH group of the next amide bond on the C-terminal side, an NH group not present in **14** or **16**.

At the present time the best explanation for the lack of antihypertensive activity after oral administration of **14** is that a blood level of **14** necessary to cause significant blood pressure lowering cannot be achieved due to rapid

(8) Klaassen, C. D.; Watkins, J. B. *Pharmacol. Rev.* **1984**, *36*, 1.

excretion of 14 into the bile and urine. Although the blood half-life of the radiolabeled derivative of 14, compound 16, after intravenous administration is 24 min, the blood levels achieved after oral administration are much lower. The absorption of 16 after oral dosing when based on biliary excretion values over time (Table III) occurs at a fairly constant rate over the first 5 h and continues at a slower rate over the next 19 h. Unlike the intravenous case in which a large bolus of 16 is injected directly into the blood, a much smaller amount of orally administered 16 is absorbed from the GI tract into the blood over a given time period and this small amount can rapidly be excreted by the liver and kidneys.

## Experimental Section

Melting points were determined on a Thomas-Hoover Uni-melt instrument and are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 automatic polarimeter. Mass spectra were taken on a LKB 9000 GC–MS spectrometer. High-resolution mass spectra were taken on a CEC 21-110B spectrometer. $^1$H NMR spectra were taken with a Varian EM390 spectrometer. Thin-layer chromatography was carried out on Uniplates from Analtech coated with 250 μm of silica gel GF. Preparative TLC was carried out on 20 cm × 20 cm plates from Analtech coated with 1500 μm of silica gel GF. Evaporations were performed at 40 °C under house vacuum on a Büchi rotavapor. Elemental analyses were conducted by Eric Meier, Stanford University, Palo Alto, CA, or Galbraith Labs, Knoxville, TN. Analytical high-performance LC was carried out on a Waters ALC-201 HPLC, with a Waters μBondapak C18 column with UV visualization at 260 nm with a Schoeffel GM 770 UV spectrometer. Some of the compounds could not be totally freed of solvent even on heating under reduced pressure. The elemental analyses of these compounds have been recorded with solvent present. The existence of solvents of crystallization was confirmed by $^1$H NMR whenever possible.

**$N^{\epsilon}$-(Benzyloxycarbonyl)-$N^{\alpha}$-(cyclobutylcarbonyl)-L-lysine Methyl Ester (2).** A solution of $N^{\epsilon}$-(benzyloxycarbonyl)-L-lysine methyl ester[6] (2.61 g, 8.87 mmol) and HPLC-grade dichloromethane (100 mL) was stirred in an ice bath and triethylamine (1.86 mL, 13.4 mmol) followed by cyclobutanecarboxylic acid chloride (1.40 mL, 13.0 mmol) was added. Stirring was continued at ice-bath temperature for 1 h and at room temperature for 2 h. The reaction mixture was then washed successively with 0.3 N NaOH (100 mL), water (100 mL), 0.4 N HCl (100 mL), and water (100 mL). The organic layer was dried ($CaSO_4$) and evaporated to a yellow solid 3.49 g. This solid was triturated in hot ether (20 mL) and a white solid was collected by filtration and dried as 2, 2.60 g, mp 87–89 °C. The mother liquor yielded more crystalline product from ether: 0.219 g; mp 85–87 °C (total yield 84.4%); $[\alpha]^{22}_D$ −15° (*c* 0.13, ethanol); $^1$H NMR ($CDCl_3$) δ 1.20–2.50 (m, 12, cyclobutane $CH_2$s and lysine $(CH_2)_3$), 2.80-3.30 (m, 3, cyclobutane CH and $CH_2NHCO$), 3.70 (s, 3, $OCH_3$), 4.60 (m, 1, NHC*H*CO), 5.10 (s, 2, benzyl $CH_2$), 7.32 (s, 5, phenyl). Anal. ($C_{20}H_{28}N_2O_5$) C, H, N.

**$N^{\epsilon}$-(Benzyloxycarbonyl)-$N^{\alpha}$-(cyclobutylcarbonyl)-L-lysine (3).** A solution of 2 (2.00 g, 5.31 mmol) in methanol (20 mL) was stirred and 1 N NaOH (8.00 mL, 8.00 mmol) was added dropwise. After stirring at room temperature for 30 min, the solution was acidified by addition of 2 N HCl. The resulting orange solution was evaporated partially to remove methanol. The remaining aqueous mixture was extracted three times with chloroform (40 mL and 2 × 20 mL). The chloroform extracts were combined, dried ($Na_2SO_4$) and evaporated to an orange gum, 2.21 g. This gum was crystallized from chloroform–ether to off-white crystals of 3, 1.63 g, mp 103–105 °C. Additional product was obtained by crystallization of the mother liquor to white crystals of 3: 0.167 g (total yield 93.2%); mp 102–104 °C; $[\alpha]^{22}_D$ +1.6 (*c* 0.13, ethanol); $^1$H NMR ($CDCl_3$) δ 1.20–2.40 (m, 12, cyclobutane $CH_2$'s and lysine $CH_2$'s), 2.90–3.40 (m, 3, $CH_2NHCO$ and cyclobutane CH), 4.60 (m, 1, NHC*H*CO), 5.10 (s, 1, $CH_2Ph$), 7.30 (s, 5, phenyl), 10.27 (brs, 1, COOH). Anal. ($C_{19}H_{26}N_2O_5$) C, H, N.

**2-[2-(1,3-Dioxolan-2-yl)ethyl]-2-[1(*S*)-[[$N^{\epsilon}$-(benzyloxycarbonyl)-$N^{\alpha}$-(cyclobutylcarbonyl)-L-lysyl]amino]-2-phenylethyl]-1,3-dioxolane (6).** A mixture of 3 (2.81 g, 7.75 mmol), 4[1] (2.29 g, 7.80 mmol), and 1-hydroxybenzotriazole hydrate (1.16 g, 7.80 mmol) was dissolved in HPLC-grade dichloromethane (60 mL) and stirred in an ice bath while dicylcohexylcarbodiimide (1.61 g, 7.80 mmol) in HPLC dichloromethane (20 mL) was added. This mixture was stirred at ice-bath temperature for 1 h and at room temperature for 64 h. The mixture was cooled in an ice bath and filtered. The yellow filtrate was diluted to 100 mL with chloroform and washed successively with ice-cold 2 N HCl (100 mL), 0.3 N NaOH (100 mL), and water (100 mL). The organic layer was dried ($CaSO_4$) and evaporated to a straw-colored solid foam, 4.74 g. This foam was crystallized from chloroform–ether to a white powdery solid (6): 4.28 g (86%); mp 148–149.5 °C; $[\alpha]^{22}_D$ −40 (*c* 0.16, ethanol); $^1$H NMR ($CDCl_3$) δ 3.76–4.10 (m, 8, ethylene ketal and acetal), 5.10 (s, 2, benzyl $CH_2$), 7.10 (s, 5, phenyl), 7.30 (s, 5, benzyl). Anal. ($C_{35}H_{47}N_3O_8$) C, H, N.

**2-(2-Methyl-3-butenyl)-2-[1(*S*)-[[$N^{\epsilon}$-(benzyloxycarbonyl)-$N^{\alpha}$-(cyclobutylcarbonyl)-L-lysyl]amino]-2-phenylethyl]-1,3-dioxolane (7).** With the use of the same method outlined for the preparation of 6, compounds 3 and 5[7] were condensed to yield 7 (52%): mp 141–143 °C (EtOAc); $^1$H NMR ($CDCl_3$) δ 1.03 (d, 3, $J$ = 4.0 Hz, $CH_3$), 3.97 (brs, 4, ethylene ketal), 4.85–5.03 (m, 2, olefin $CH_2$), 5.07 (s, 2, benzyl $CH_2$), 5.83 (dd, 1, olefin CH), 7.13 (s, 5, phenyl), 7.33 (s, 5, benzyl). Anal. ($C_{35}H_{47}N_3O_6$) C, H. N.

**2-(2-Carboxyethyl)-2-[1(*S*)-[[$N^{\epsilon}$-(benzyloxycarbonyl)-$N^{\alpha}$-(cyclobutylcarbonyl)-L-lysyl]amino]-2-phenylethyl]-1,3-dioxolane (8).** A solution of 7 (4.20 g, 6.58 mmol) and acetone (840 mL) was stirred in an ice bath and chromium trioxide (6.58 g, 65.8 mmol) in 35% $H_2SO_4$ (275 mL) was slowly added over a 20-min period. The mixture was stirred an additional 20 min at ice-bath temperature and then was poured into a separatory funnel containing ice (60 g) and chloroform (2.1 L). After shaking of the funnel, the chloroform layer was separated and the aqueous layer was reextracted with chloroform (2.1 L). Each chloroform extract was washed separately with water (600 mL) and then dried ($Na_2SO_4$). The dried chloroform extracts were combined and evaporated to a white foam, 4.14 g. This foam was crystallized from ethyl acetate (100 mL) to white crystalline 8: 3.09 g (77.0%); mp 154–155 °C; $[\alpha]^{22}_D$ −39 (*c*, 0.12, ethanol); $^1$H NMR ($CDCl_3$) δ 4.00 (brs, 4, ethylene ketal), 5.10 (s, 2, benzyl $CH_2$), 7.10 (s, 5, phenyl), 7.30 (s, 5, benzyl). Anal. ($C_{33}H_{43}N_3O_8$) C, H, N.

**2-(2-Carboxypropyl)-2-[1(*S*)-[[$N^{\epsilon}$-(benzyloxycarbonyl)-$N^{\alpha}$-(cyclobutylcarbonyl)-L-lysyl]amino]-2-phenylethyl]-1,3-dioxolane (9).** A solution of 13 mg of hydrated ruthenium dioxide (51%) and sodium periodate (265 mg, 1.24 mmol) in water (2 mL) was rapidly added to a solution of 7 (100 mg, 0.165 mmol) in acetone (5 mL) at room temperature, and the mixture was stirred vigorously for 15 min. 2-Propanol (1 mL) was added and the mixture was stirred for 10 min. The black precipitate was removed by filtering through Celite and the filtrate was poured onto ice (15 g) and was extracted with chloroform (3 × 15 mL). The organic extracts were washed with saturated sodium chloride solution (15 mL), combined, dried ($Na_2SO_4$), and evaporated in vacuo to give 70 mg of crude material. This was purified by preparative TLC, eluting with acetic acid/ethyl acetate (1:99), to give 43 mg of 9 (42%) as a colorless gum: $^1$H NMR ($CDCl_3$) δ 11.05 (1 H, b), 7.30 (5 H, s), 7.11 (5 H, s), 7.00 (3 H, m), 5.05 (2 H, s), 4.45 (2 H, m), 3.97 (4 H, s); mass spectrum, *m/e* 606 (M − OH), 515 (M − $PhCH_2OH$). Anal. ($C_{34}H_{45}N_3O_8$) C, H, N.

**2-[3-oxo-3-[2(*S*)-(benzyloxycarbonyl)pyrrolidin-1-yl]-propyl]-2-[1(*S*)-[[$N^{\epsilon}$-(benzyloxycarbonyl)-$N^{\alpha}$-(cyclobutylcarbonyl)-L-lysyl]amino]-2-phenylethyl]-1,3-dioxolane (10).** To a mixture of 8 (3.00 g, 4.92 mmol), L-proline benzyl ester hydrochloride (2.38 g, 9.84 mmol), HPLC-grade dichloromethane (40 mL), 1-hydroxybenztriazole hydrate (0.807 g, 5.41 mmol), and triethylamine (1.37 mL, 9.84 mmol) stirring in an ice bath was added dicyclohexylcarbodiimide (1.12 g, 5.41 mmol) in HPLC-grade dichloromethane (10 mL). This mixture was stirred at ice-bath temperature for 1 h and at room temperature for 64 h. The yellow mixture was cooled in an ice bath and acetic acid (0.700 mL) was added to convert any remaining dicyclohexylcarbodiimide to dicyclohexylurea. After stirring at 5 °C for 15 min, the mixture was filtered. The filtrate was diluted to 60 mL with chloroform and was washed successively with ice-cold 2 N HCl (50 mL), 0.3 N NaOH (50 mL), and water (2 × 50 mL). Saturated $NaHCO_3$ (30 mL) was added to the last water wash to break the emulsion.

The organic layer was dried ($CaSO_4$) and evaporated to a pale yellow solid, 3.69 g. This solid was crystallized from chloroform–ether to white crystalline **10**: 2.47 g (63.0%); mp 160–161 °C; $[\alpha]^{22}_D$ −52° (*c* 0.14, ethanol); $^1H$ NMR ($CDCl_3$) δ 4.00 (s, 4, ethylene ketal), 5.04 (s, 2, benzyl $CH_2$), 5.12 (s, 2, benzyl $CH_2$), 7.10 (s, 5, phenyl), 7.30 (s, 10, 2 × benzyl). Anal. ($C_{45}H_{56}N_4O_9$) C, H, N.

**2-[2-Methyl-3-oxo-3-[2(*S*)-(benzyloxycarbonyl)-pyrrolidin-1-yl]propyl]-2-[1(*S*)-[[*N*$^{\epsilon}$-(benzyloxycarbonyl)-*N*$^{\alpha}$-(cyclobutylcarbonyl)-L-lysyl]amino]-2-phenylethyl]-1,3-dioxolane (11).** Compound **9** was condensed with L-proline benzyl ester hydrochloride by the method described for the preparation of **10**. The crude product was purified by preparative TLC, eluting with ethyl acetate, to give **11** as a colorless gum (51%): $^1H$ NMR ($CDCl_3$) δ 7.30 (10 H, s), 7.13 (5 H, s), 6.85 (1 H, d, *J* = 9 Hz), 6.02 (1 H, d, *J* = 8 Hz), 5.56 (1 H, m), 5.05 (4 H, m), 4.45 (3 H, m), 3.92 (4 H, m), 3.56 (2 H, m), 1.08 (3 H, d, *J* = 6 Hz); mass spectrum, *m/e* 702 (M − $PhCH_2OH$). Anal. ($C_{46}H_{58}N_4O_9 \cdot 0.5H_2O$) C, H, N.

**2-[3-Oxo-3-(*N*-L-prolyl)propyl]-2-[1(*S*)-[[*N*$^{\alpha}$-(cyclobutylcarbonyl)-L-lysyl]amino]-2-phenylethyl]-1,3-dioxolane (12).** A mixture of **10** (2.70 g, 3.39 mmol), 10% palladium on carbon (2.20 g), and acetic acid (60 mL) was stirred under 1 atm of hydrogen gas at room temperature for 16 h. This mixture was filtered through Celite, and the clear filtrate was evaporated to a clear syrup. This syrup was mixed with water (60 mL) and filtered. The filtrate was lyophilized to a white solid, 2.00 g. This solid was triturated in ether, and white crystals were collected by filtration. These crystals were recrystallized from absolute ethanol to white crystals of **12**: 1.65 g (84.0%); $[\alpha]^{22}_D$ −29° (*c* 0.14, ethanol); mp softens 165 °C, resolidifies 195 °C, melts 231–233 °C with gas evolution; $R_f$ 0.70 (1:1:1:1, water–1-butanol–acetic acid–ethyl acetate); high-resolution mass spectrum calcd for $M^+$ of $(Me_3Si)_2$ derivative ($C_{36}H_{60}N_4O_7Si_2$) 716.3999, found 716.3983. Anal. ($C_{30}H_{44}N_4O_7 \cdot H_2O \cdot CH_3COOH$) C, H, N.

**5(*S*)-[[*N*$^{\alpha}$-(Cyclobutylcarbonyl)-L-lysyl]amino]-4-oxo-6-phenylhexanoyl-L-proline Trifluoroacetic Acid Salt (14).** A mixture of **12** (40 mg, 0.070 mmol) and 90% trifluoroacetic acid in water (0.33 mL) was left to stand at room temperature for 7.5 h. It was then diluted with water (20 mL) and evaporated at 40 °C at 1 mmHg. The resulting clear residue was reevaporated from water (20 mL) at 40 °C at 1 mmHg. The resulting residue was dissolved in water (6 mL) and lyophilized to a white fluffy solid, 48 mg. This solid was slurried in ether and a white solid (hygroscopic) was collected by filtration and dried. This solid was crystallized from methanol–ethyl acetate to white crystalline **14**: 28 mg (63%); mp 152–154 °C; $[\alpha]^{23}_D$ −84.3° (*c* 0.1, water); $R_f$ 0.75 (1:1:1:1, water–butanol–acetic acid–ethyl acetate). Anal. ($C_{28}H_{40}N_4O_6 \cdot CF_3COOH$) C, H, N.

**5(*S*)-[[*N*$^{\alpha}$-(Cyclobutylcarbonyl)-L-lysyl]amino]-2-methyl-4-oxo-6-phenylhexanoyl-L-proline Trifluoroacetic Acid Salt (15).** A mixture of **11** (249 mg, 0.308 mmol), methanol (25 mL), and 10% palladium on carbon (200 mg) was stirred at room temperature under hydrogen for 16 h. The mixture was filtered through Celite, the filter cake being washed with methanol (10 mL), and the filtrate was evaporated in vacuo to give 146 mg of gummy solid **13**. A portion of this (100 mg) was dissolved in 90% aqueous trifluoroacetic acid (1 mL) and the mixture was stirred at room temperature under nitrogen for 7.5 h. Water (5 mL) was added and the mixture was evaporated in vacuo. The residue was dissolved in water (5 mL) and the mixture was lyophilized to give a white powder. This was mixed with dry diethyl ether (10 mL) and the mixture was filtered. The solid was dried in vacuo to give 114 mg of **15** (81%) as a white powder, which could be recrystallized from methanol/ethyl acetate to give white crystals: mp 132–134 °C; $^1H$ NMR ($CDCl_3/CD_3OD$) δ 7.88 (1 H, m), 7.22 (6 H, s), 4.37 (7 H, s), 3.71 (4 H, m), 2.95 (6 H, m), 1.11 (3 H, m). Anal. ($C_{31}H_{43}N_3O_8 \cdot H_2O$) C, H, N.

**Radiolabeled Synthesis of 16.** A solution containing 5 mCi of L-[4-$^3$H(N)]proline in 0.01 N HCl (purchased from New England Nuclear) was dissolved in $H_2O$ with 5.00 mmol of L-proline and evaporated (azeotroping with ethanol) to an off-white solid. The resulting proline sample was converted to its benzyl ester with use of benzyl alcohol and hydrochloride gas in 71.0% yield. With use of the method outlined in Scheme I, the tritiated L-proline benzyl ester was condensed with **8** and deprotected to yield **16** as white crystals in 62% overall yield. Examination of **16** by autoradiography and HPLC showed it to have the same chromatography characteristics as **14** and a greater than 95% purity.

**Blood Pressure Measurements in Renal Hypertensive Rats.** The testing of compounds for antihypertensive activity in renal hypertensive rats was performed by Pharmakon Laboratories in Waverly, PA. The testing method they employed is described below.

Hypertension of renal origin was produced in rats by placing a silver clip around the left renal artery near the aorta and leaving the contralateral kideny intact. Several weeks later, the rats were cannulated for blood pressure monitoring by the method of Weeks and Jones.[9] Rats with mean blood pressure greater than 160 mmHg were used for the studies. Four rats received the test compound orally in a 0.25% methylcellulose aqueous solution at 5 mL/kg. Compounds **14** and **15** dissolved totally in this solvent. Two rats were administered the 0.25% methylcellulose aqueous solution alone at 5 mL/kg orally and served as the controls. Systolic, diastolic, and mean blood pressure and heart rate were monitored prior to dosing and hourly for 8 h and at 24 h after test or control article administration.

**Metabolic Disposition Methods.** Adult male Sprague–Dawley rats were purchased locally from Simonsen Laboratories, Inc., Gilroy, CA.

The animals were given Purina Laboratory Chow and water ad libitum except that the food was withdrawn from them the night before the experiment was to be conducted. After dosing of the animals with radiolabeled compounds, serial blood samples were taken by the orbital bleeding technique from the rats. The blood half-time for decline of the radioactivity level was calculated by subjecting the blood data to linear regression analysis with use of a Hewlett-Packard 9815A desktop computer. Compound **16** wad dissolved in physiological saline for intravenous injections and in dilute $NaHCO_3$ solution for oral administration. The compound dissolved readily in these two solvents.

For biliary cannulation, rats were anesthetized with Nembutal, and the common bile duct was cannulated with Silastic medical-grade tubing after a midline abdominal incision was made. The rats were kept in modified Bollman cages for the collection of bile.

For radioactivity assays, aliquots of urine, bile, and blood which was decolorized with a few drops of 30% $H_2O_2$ were counted directly in a Searle Analytic Mark III liquid scintillation spectrometer. Correction for quenching was made with automatic external standardization and the use of a previously determined quench curve.

**Assay of Trypsin Cleavage of Peptides.** Trypsin (1 mg) type III from Sigman was dissolved in 50 mL of 0.001 N HCl. A solution containing **14** (1 mg) and Tris buffer (pH 8.1, 0.300 mL) was stirred at room temperature and 0.140 mL of the above trypsin solution was added. This mixture was stirred and monitored by TLC on RP18 TLC plates from EM Reagents using double elutions in 35% acetonitrile, 0.1% trifluoroacetic acid in water. The $R_f$ of **14** in this system is 0.35.

**Acknowledgment.** This work was supported by NIH Grant HL19538.

(9) Weeks, J. R.; Jones, J. A. *Proc. Soc. Exptl. Biol. Med.* **1960**, *104*, 646.