Synthesis of Novel Angiotensin Converting Enzyme Inhibitor Quinapril and Related Compounds. A Divergence of Structure–Activity Relationships for Non-Sulfhydryl and Sulfhydryl Types

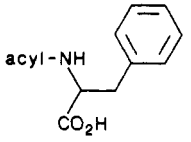
Sylvester Klutchko,*† C. John Blankley,*† Robert W. Fleming,† Jack M. Hinkley,† Ann E. Werner,† Ivan Nordin,† Ann Holmes,† Milton L. Hoefle,† David M. Cohen,‡ Arnold D. Essenburg,‡ and Harvey R. Kaplan‡

Departments of Chemistry and Pharmacology, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan 48106. *Received February 5, 1986*

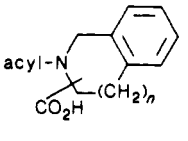
The synthesis and angiotensin converting enzyme (ACE) inhibiting activities of quinapril (CI-906, **22**), its active diacid (CI-928, **33**), and its dimethoxy analogue (CI-925, **25**) are reported. These tetrahydro-3-isoquinolinecarboxylic acid derivatives possess equivalent in vitro potency and in vivo efficacy to enalapril. Sulfhydryl analogues with the same structural variation are also highly potent. In contrast, tetrahydro-1-isoquinolinecarboxylic acid and homologous isoindoline-1-carboxylic acid analogues show a striking divergence in potency between the two types, sulfhydryl analogues being essentially inactive, while non-sulfhydryl analogues are equipotent with the proline prototype. This is the first evidence suggesting that alternate binding modes may exist for the two major structural classes of small molecule ACE inhibitors.

The introduction of agents that block the action of angiotensin converting enzyme (ACE) and hence the production of the potent hypertensive hormone angiotensin II has provided a major advance in the therapy of essential hypertension. Enalapril (1) and captopril (2) represent the prototypes of the non-sulfhydryl and sulfhydryl classes, respectively.[1,2] Many analogues of these compounds have (THIQ) carboxylic been reported and several are under clinical development. Certain aspects of the structure–activity relationships (SAR) of this class of compounds have been reviewed.[3,4]

Our early work in this field explored the concept of introducing conformational restrictions into active prototypes,[5] a strategy which has now been applied successfully in several cases.[6–11] Based on the reported good in vitro ACE inhibitory activity of acyl phenylalanines[2] (structure I), we sought to apply this strategy to the C-terminal position of ACE inhibitor prototypes by synthesizing compounds of type II. This led us to explore tetra-



I

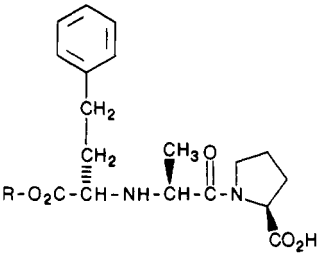


II, n = 0, 1

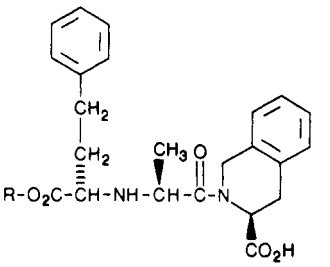
hydroisoquinoline (THIQ) carboxylic acids as proline replacements both in the non-sulfhydryl series and in the sulfhydryl series. The success of this modification and the general utility of bicyclic amino acids as replacements for proline are attested to not only by our own compounds quinapril (CI-906, **22**)[12–17] and its dimethoxy analogue **25** (CI-925), described in this paper, and 1-[2-[[1-(ethoxycarboxyl)-3-phenylpropyl]amino]-1-oxopropyl]octahydro-1*H*-indole-2-carboxylic acid (CI-907, indolapril, SCH 31846) (described separately),[18,19] but also by several other clinical candidates that incorporate this feature.[20–23]

In this paper we wish to report the synthesis and ACE inhibitory activity of quinapril (**22**), its active diacid **33** (CI-928), **25** and certain related compounds in the THIQ-(1 and 3)-carboxylic acid series, and the isoindoline-1-carboxylic acid series (**13–50**), and particularly to note a hitherto unreported divergence in SAR of non-sulfhydryl and sulfhydryl ACE inhibitors.

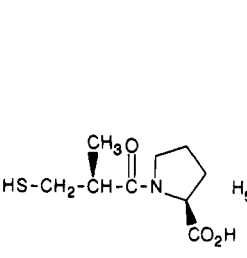
Chemistry. The syntheses of non-sulfhydryl compounds were carried out according to Scheme I. In general, the appropriate amino acid esters **5–12** (Scheme II, Table I) were coupled to the N-substituted alanine side



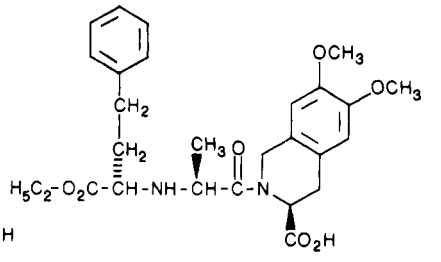
enalapril (**1**), R = C_2H_5
enalaprilat (**1a**), R = H



quinapril (**22**), R = C_2H_5
(**33**), R = H



captopril (**2**)



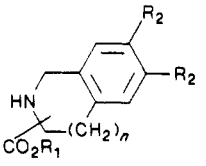
25

(1) Patchett, A. A.; Harris, E.; Tristram, E. W.; Wyvratt, M. J.; Wu, M. T.; Taub, D.; Peterson, E. R.; Ikeler, T. J.; ten Broeke, J.; Payne, L. G.; Ondeyka, D. L.; Thorsett, E. D.; Greenlee, W. J.; Lohr, N. S.; Hoffsommer, R. D.; Joshua, H.; Ruyle, W. V.; Rothrock, J. W.; Aster, S. D.; Maycock, A. L.; Robinson, F. M.; Hirschmann, R.; Sweet, C. S.; Ulm, E. H.; Gross, D. M.; Vassil, T. C.; Stone, C. A. *Nature (London)* **1980**, *288*, 280.
(2) Cushman, D. W.; Cheung, H. S.; Sabo, E. F.; Ondetti, M. A. *Biochemistry* **1977**, *16*, 5484–5491.
(3) Petrillo, E. W.; Ondetti, M. A. *Med. Res. Rev.* **1982**, *2*(1), 1–41.
(4) Wyvratt, M. J.; Patchett, A. A. *Med. Res. Rev.* **1985**, *5*(4), 483–531.
(5) Klutchko, S.; Hoefle, M. L.; Smith, R. D.; Essenburg, A. D.; Parker, R. B.; Nemeth, V. L.; Ryan, M. J.; Dugan, D. H.; Kaplan, H. R. *J. Med. Chem.* **1981**, *24*, 104.
(6) Condon, M. E.; Petrillo, E. W.; Ryono, D. E.; Reid, A.; Neubeck, R.; Puar, M.; Heikes, J. E.; Sabo, E. F.; Losee, K. A.; Cushman, D. W.; Ondetti, M. A. *J. Med. Chem.* **1982**, *25*, 250–258.
(7) Thorsett, E. D.; Harris, E. E.; Aster, S.; Peterson, E. R.; Taub, D.; Patchett, A. A. *Biochem. Biophys. Res. Commun.* **1983**, *111*, 166.
(8) Parsons, W. H.; Davison, J. L.; Taub, D.; Aster, S. D.; Thorsett, E. D.; Patchett, A. A. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 108–113.
(9) Watthey, J. W. H.; Galvin, T.; Desai, M. *J. Med. Chem.* **1984**, *27*, 816–818.

† Department of Chemistry.
‡ Department of Pharmacology.

0022-2623/86/1829-1953$01.50/0

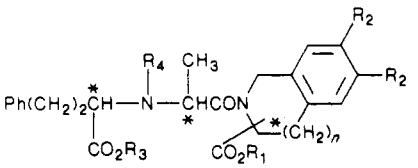
Table I. Esters of Tetrahydro-(1 and 3)-isoquinolinecarboxylic Acids and Isoindoline-1-carboxylic Acids[a]

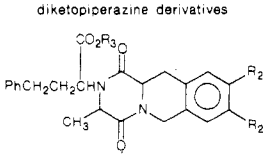


no.	R_1	R_2	n	configuration	CO_2R_1 position	% yield	procedure[b]	$[\alpha]^{23}_D$ deg	mp, °C	recrystn solvent	formula	anal.
5	t-C_4H_9	H	1	*S*	3	47	A	−88.7[c]	190–192	EtOH–Et_2O	$C_{14}H_{19}NO_2$·HCl[d]	C, H, N
6	t-C_4H_9	H	1	*R*	3	52	A	+87.4[c]	295–300 dec	EtOH–Et_2O	$C_{14}H_{19}NO_2$·HCl	C, H, N
7	CH_2Ph	H	1	*S*	3	65	*b*	−83.3[e,f]	190.5–191	EtOH	$C_{17}H_{17}NO_2$·HCl	C, H, N
8	t-C_4H_9	OCH_3	1	*S*	3	50	A	−89.2[g]	72–78	*h*	$C_{16}H_{23}NO_4$	C, H, N
9	CH_2Ph	OCH_3	1	*S*	3	83	*b*	−81.3[i]	255–260	MeOH–Et_2O	$C_{19}H_{21}NO_4$·HCl	C, H, N
10	C_2H_5	H	1	racemic	1	52	*b*		132–134	EtOAc–Et_2O	$C_{12}H_{15}NO_2$·HCl	C, H, N
11	t-C_4H_9	H	0	racemic	1	75	*j*		148–154 dec	EtOH–Et_2O	$C_{13}H_{17}NO_2$·HCl·$0.2H_2O$	C, H, N, Cl, H_2O
12	C_2H_5	H	0	racemic	1	52	*j*		174–176	EtOH–Et_2O	$C_{11}H_{19}NO_2$·HCl	C, H, N, Cl

[a] All compounds exhibited IR and ^1H NMR spectra consistent with assigned structures. [b] See Experimental Section. [c] (c 2, MeOH). [d] Reference 23 described oily free base. [e] (c 1, 1:1 MeOH/1 N HCl). [f] Reference 33 describes *p*-toluenesulfonate salt, $[\alpha]^{10}_D$ −61.2° (c 1, MeOH) and oily base, $[\alpha]^{12}_D$ −88.3°. [g] (c 1, MeOH). [h] Not recrystallized. [i] (c 1, MeOH). [j] Reference 36.

Table II. Intermediate Non-Sulfhydryl Substituted Alanyl-1,2,3,4-tetrahydro-(1 and 3)-isoquinolinecarboxylic Acids and Isoindoline-1-carboxylic Acids and Derivatives[a]



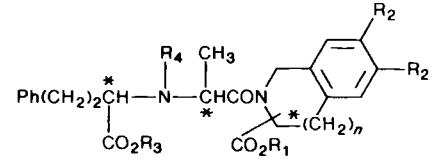
no.	R_1	R_2	R_3	R_4	n	configuration[b]	CO_2R_1 position	% yield	procedure[c]	$[\alpha]^{23}_D$, deg	mp, °C	recrystn solvent	formula	anal.
13	t-C_4H_9	H	C_2H_5	H	1	*S,S,S*	3	78	B	−12.6[d]	71–72	hexane	$C_{29}H_{38}N_2O_5$	C, H, N
15	CH_2Ph	H	C_2H_5	H	1	*S,S,S*	3	61	B	−11.7[e]	152–153	EtOAc	$C_{32}H_{36}N_2O_5$·$C_4H_4O_4$[f]	H, N, C[g]
16	CH_2Ph	H	C_2H_5	H	1	*R,S,S*	3	61	B	−36.0[h]	96–98	EtOAc	$C_{32}H_{36}N_2O_5$·$C_4H_4O_4$[f]	C, H, N
18	t-C_4H_9	OCH_3	C_2H_5	H	1	*S,S,S*	3		B	+22.7[d]	143–144.5	EtOAc	$C_{31}H_{42}N_2O_7$·$C_4H_4O_4$[f]	C, H, N
20	C_2H_5	H	C_2H_5	H	1	*S,S,S*	1	15	B	−15.7[h]	145–146	EtOAc	$C_{27}H_{34}N_2O_5$·$C_4H_4O_4$[f]	C, H, N
21	t-C_4H_9	H	C_2H_5	H	0	*i*	1	74	B	−6.3[j]	*k*	*l*	$C_{28}H_{36}N_2O_5$	*m*
31	t-C_4H_9	H	C_2H_5	CH_3	1	*S,S,S*	3	98	*c*		*k*	*l*	$C_{30}H_{40}N_2O_5$	C, H, N
32	t-C_4H_9	H	H	H	1	*S,S,S*	3	67	G	+3.4[n]	123–126	CH_2Cl_2–hexane	$C_{27}H_{34}N_2O_5$·H_2O	H, N, H_2O, C[o]
	diketopiperazine derivatives													
														
37	R_2 = H, R_3 = C_2H_5					*S,S,S*		80	H	−112.5[p]	121–123	Et_2O	$C_{25}H_{28}N_2O_4$	C, H, N
38	R_2 = R_3 = H					*S,S,S*		50	*c*	−126[q]	117–120	EtOAc	$C_{23}H_{24}N_2O_4$·$0.75H_2O$	C, H, N, H_2O[r]
39	R_2 = OCH_3, R_3 = C_2H_5					*S,S,S*		99	H	−110[q]	*k*	EtOAc	$C_{27}H_{32}N_2O_6$	C, H, N

[a] All compounds exhibited IR and ^1H NMR spectra consistent with assigned structures. [b] Asterisks in structure denote chiral centers. Configuration sequence is from left to right as written. [c] See Experimental Section. [d] (c 2, MeOH). [e] (c 1, MeOH). [f] Maleate salt. [g] Calcd, 67.06; found, 66.58. [h] (c 1.1, MeOH). [i] HPLC; 94% of a 60:40 mixture of *S,S,S* and *S,S,R*. [j] (c 1.2, EtOH). [k] Amorphous solid. [l] Not recrystallized. [m] Not analyzed. [n] (c 0.9, EtOH). [o] Calcd, 66.92; found, 67.90. [p] (c 1, EtOH). [q] (c 1.1, EtOH). [r] Calcd, 3.34; found, 4.11.

chains having the *S,S* or *R,S* configuration (**3** or **4**)[24] using dicyclohexylcarbodiimide as a condensing agent, resulting in the intermediate amino diesters **13**, **15**, **16**, and **18**–**21** (Tables II and Table III) in good yields. The hindered

(10) Weller, H. N.; Gordon, E. M.; Rom, M. B.; Plusced, *J. Biochem. Biophys. Res. Commun.* **1984**, *125*, 82–89.
(11) Hassall, C. H.; Krohn, A.; Moody, C. J.; Thoman, W. A. *J. Chem. Soc., Perkin Trans 1* **1984**, 155–184.
(12) Hoefle, M. L.; Klutchko, S. U.S. Pat. 4 344 949, *1982*.
(13) Kaplan, H. R.; Burmeister, W.; Essenburg, A.; Major, T.; Mertz, T.; Randolph, A. *Pharmacologist* **1982**, *24*(3), 176.
(14) Kaplan, H. R.; Cohen, D. M.; Essenberg, A. D.; Major, T. C.; Mertz, T. E.; Ryan, M. J. *Fed. Proc.* **1984**, *43*(5), 1326–1329.
(15) Ryan, M. J.; Boucher, D. M.; Cohen, D. M.; Olszewski, B. J.; Singer, R. M.; Smith, R. D.; Kaplan, H. R. *Fed. Proc.* **1984**, *43*(5), 1330–1332.
(16) Gavras, I.; Vlahakos, D.; Melby, J. C.; Gavras, H. *J. Clin. Pharm.* **1984**, *24*, 343–350.

Table III. Angiotensin Converting Enzyme Inhibitory Activity and Physical Data on Non-Sulfhydryl Substituted Alanyl-1,2,3,4-tetrahydro-(1 and 3)-isoquinolinecarboxylic Acids and Isoindoline-1-carboxylic Acids and Derivatives[a]



no.	R_1	R_2	R_3	R_4	n	configuration[b]	CO_2R_1 position	% yield	procedure[c]	$[\alpha]^{23}_D$, deg	mp, °C	recrystn solvent	formula	anal.	IC_{50}, μM
14	C_2H_5	H	C_2H_5	H	1	*S,S,S*	3	36	F	+1.50[d]	118–120	EtOAc	$C_{27}H_{34}N_2O_5 \cdot C_4H_4O_4$[e]	C, H, N	0.44
17	C_2H_5	OCH_3	C_2H_5	H	1	*S,S,S*	3	42	F	+19.7[f]	149–151	EtOAc	$C_{29}H_{38}N_2O_7 \cdot C_4H_4O_4$[e]	C, H, N	0.52
19	CH_2Ph	OCH_3	C_2H_5	H	1	*S,S,S*	3	65	B	+3.4[g]	139–141	EtOAc	$C_{34}H_{40}N_2O_7 \cdot C_4H_4O_4$[e]	C, H, N	0.30
22	H	H	C_2H_5	H	1	*S,S,S*	3	88,56	C, D	+14.5[h]	120–130	EtOAc–toluene	$C_{25}H_{30}N_2O_5 \cdot HCl$	C, H, N	0.0083
23	H	H	C_2H_5	H	1	*R,S,S*	3	31	D	−25.5[d]	90–100	*i*	$C_{25}H_{30}N_2O_5 \cdot HCl \cdot 0.5H_2O$	C, H, N, H_2O	0.20
24	H	H	C_2H_5	H	1	*S,S,R*	3		C	+1.50[j]	172–173.5	EtOH–Et_2O	$C_{25}H_{30}N_2O_5 \cdot HCl$	C, H, N	6.10
25	H	OCH_3	C_2H_5	H	1	*S,S,S*	3	88	C, D	+34.2[g]	141–161	EtOH–Et_2O	$C_{27}H_{34}N_2O_7 \cdot HCl$	C, H, N	0.056
26	H	OCH_3	C_2H_5	H	1	*R,S,S*	3		D	−6.1[g]	*k*	*i*	$C_{27}H_{34}N_2O_7 \cdot HCl \cdot 0.25H_2O$	C, H, N	1.1
27	H	OCH_3	C_2H_5	H	1	*S,S,R*	3		C	−26.2[d]	*k*	*i*	$C_{27}H_{34}N_2O_7 \cdot HCl \cdot 0.6H_2O$	C, H, N	90
28	H	H	C_2H_5	H	0	*S,S,S*[l]	1		E	−30.8[d]	*k*	*i*	$C_{24}H_{28}N_2O_5 \cdot HCl \cdot 0.8H_2O$	C, H, N, H_2O, Cl[m]	0.044
29	H	H	C_2H_5	H	0	*S,S,R*[n]	1		E	+28.9[d]	210–214	*i*	$C_{24}H_{28}N_2O_5 \cdot HCl \cdot 0.5H_2O$	C, H, N, H_2O, Cl[o]	>1
30	H	H	C_2H_5	CH_3	1	*S,S,S*	3	96	C	+4.4[d]		*i*	$C_{26}H_{32}N_2O_5 \cdot H_2O$	C, H, N	>10
33	H	H	H	H	1	*S,S,S*	3	79	G	+20.9[p]	166–168	MeOH–Et_2O	$C_{23}H_{26}N_2O_5 \cdot H_2O$	C, H, N, H_2O	0.0028
34	H	OCH_3	H	H	1	*S,S,S*	3	80	G	+37.8[q]	145–170	THF–Et_2O	$C_{25}H_{30}N_2O_7 \cdot HCl \cdot H_2O$	C, H, N, H_2O	0.003
35	H	H	H	H	1	*S,S,S*	1	44	G		60–163	*i*	$C_{23}H_{26}N_2O_5 \cdot H_2O$	C, H, N	0.0058
36	H	H	H	H	0	*S,S,S*	1		G	−113.4[r]	185–192	*i*	$C_{22}H_{24}N_2O_5 \cdot H_2O$	C, H, N, H_2O	0.0031
1	enalapril (MK 421)														0.14
1a	enalaprilat (MK 422)														0.0024

[a] All compounds exhibited IR and ^1H NMR spectra consistent with assigned structures. [b] Asterisks in structure denote chiral centers. Configuration sequence is from left to right as written. [c] See Experimental Section. [d] (*c* 1, EtOH). [e] Maleate salt. [f] (*c* 1.2, MeOH). [g] (*c* 1.1, EtOH). [h] (*c* 1.2, EtOH). [i] Not recrystallized. [j] (*c* 2, MeOH). [k] Amorphous solid. [l] HPLC; 4:1 mixture of *S,S,S* and *S,S,R*. [m] Calcd, 7.46; found, 6.95. [n] HPLC; 100%. [o] Calcd, 7.54, found, 7.98. [p] (*c* 1, MeOH). [q] (*c* 1.1, MeOH). [r] (*c* 1, 0.1*N* NaOH).

Scheme I

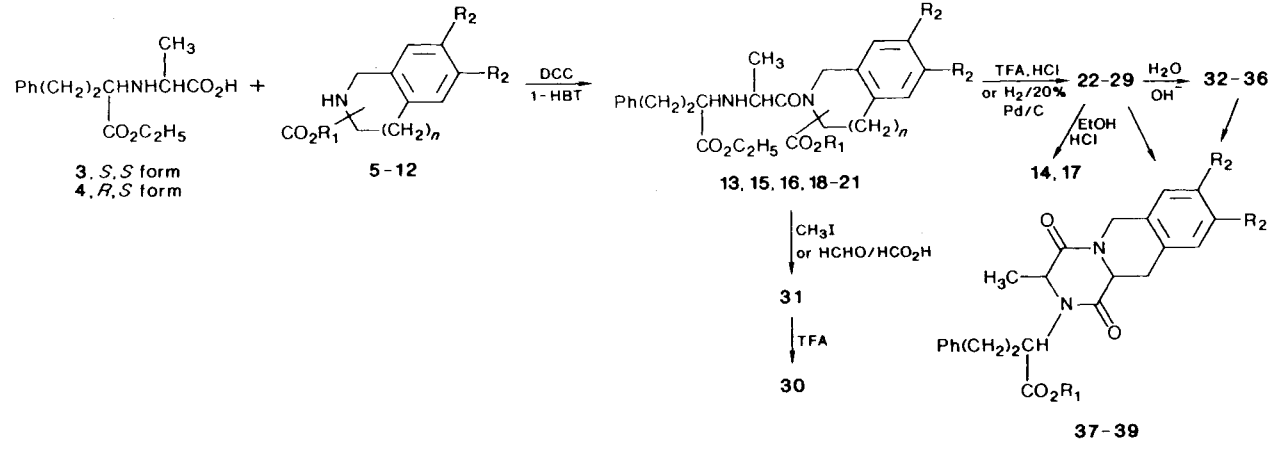
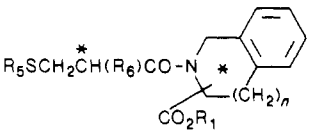


Table IV. Angiotensin Converting Enzyme Inhibitory Activity and Physical Data on Substituted 2-(3-Mercaptopropanoyl)-1,2,3,4-tetrahydro-(1 and 3)-isoquinolinecarboxylic Acids and Isoindoline-1-carboxylic Acids and Derivatives[a]



no.	R_1	R_5	R_6	n	configuration[b]	CO_2R_1 position	% yield	procedure[c]	$[\alpha]^{23}_D$, deg	mp, °C	recrystn solvent	formula	anal.	IC_{50}, μM
40	H	CH_3CO	CH_3	1	(*R,S*), *S*	3	26	I	−46.4[d]	171.5–173.5	2-PrOH	$C_{16}H_{19}NO_4S\cdot C_{12}H_{23}N$[e]	C, H, N	0.13[f]
41	H	H	CH_3	1	*S,S*	3	46	J	−22.9[d,g]	137–139	EtOAc–hexane	$C_{14}H_{17}NO_3S$	C, H, N	0.018[h]
42	*t*-C_4H_9	CH_3CO	H	0	racemic	1	92	K		190–193	MeOH–H_2O	$C_{18}H_{23}NO_4S$	C, H, N, S	*i*
43	H	CH_3CO	H	0	racemic	1	44	L		169–175	EtOH	$C_{14}H_{15}NO_4S$	C, H, N, S	22.0
44	H	H	H	0	racemic	1	14	J		231–263[j]	EtOH–ether	$C_{12}H_{13}NO_3S\cdot C_{12}H_{23}N$[e]$\cdot 0.5H_2O$	C, H, N, S, H_2O	58.0
45	H	CH_3CO	CH_3	0	(*R,S*), (*R,S*)	1	18	L		209–216	CH_3CN	$C_{15}H_{17}NO_4S\cdot C_{12}H_{23}N$[e]	C, H, N	24.0
46	H	H	CH_3	0	(*R,S*), (*R,S*)	1	38	J		*k*	*l*	$C_{13}H_{15}NO_3S\cdot 0.8H_2O$	C, H, N, S, H_2O[m]	14.0
47	H	CH_3CO	CH_3	1	(*R,S*), (*R,S*)	1	54	I		160.5–161.5	2-PrOH	$C_{16}H_{19}NO_4S$	C, H, N	>100
48	H	H	CH_3	1	(*R,S*), (*R,S*)	1	34	J		180[j]	EtOAc	$C_{14}H_{17}NO_3S\cdot C_{12}H_{23}N$[e]	C, H, N	58.0[n]
49		CH_3CO	CH_3	1	racemic	des carboxyl		K		*k*	*l*	$C_{15}H_{19}NO_2S$	H, N, C[o]	49.0
50		H	CH_3	1	racemic	des carboxyl		J		*k*	*l*	$C_{13}H_{17}NOS$	H, N, C[p]	48.0
2	captopril													0.013

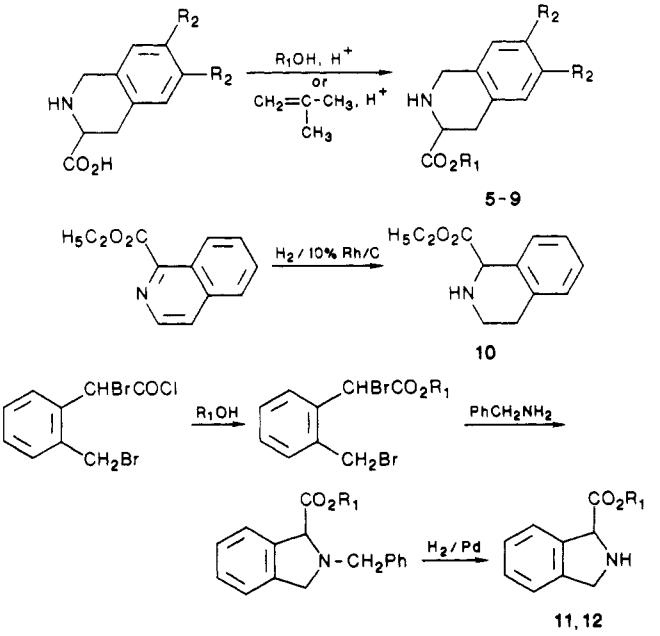
[a] All compounds exhibited IR and ^1H NMR spectra consistent with assigned structures. [b] Asterisks denote chiral centers. [c] See Experimental Section. [d] (*c* 2, MeOH). [e] Dicyclohexylamine salt. [f] Free acid tested. [g] Reference 23 rotation on *S,S* isomer, −23.5° (c 1, MeOH). [h] reference 23 IC_{50} on *S,S* isomer, 0.0086 μM. [i] Not tested. [j] Decomposes. [k] Noncrystalline. [l] Not recrystallized. [m] S: calcd, 11.46; found, 10.34. H_2O: calcd 5.15; found, 7.68. [n] Reference 20 IC_{50} = 1.8 μM for des methyl derivative. [o] Calcd, 64.95; found, 64.45. [p] Calcd, 66.34; found, 65.82.

secondary amine of the side chain did not have to be blocked prior to coupling.[24] In cases of coupling where the amino acid esters (**10** and **11**) were racemic, the resulting mixtures of diastereoisomers were separated either by recrystallization of the maleate salt of the resulting amino diesters giving the pure *S,S,S* isomer in the case of **20** or by recrystallization of the target amino ester acids as in the case of **28** and **29**.

The target amino ester acid products **22–29** (Table III) were isolated as stable hydrochloride salts either by cleavage of intermediate *tert*-butyl ethyl diesters **13**, **18**, and **21** with trifluoroacetic acid or hydrogen chloride in acetic acid or methylene chloride or via hydrogenolysis of benzyl ethyl diesters **15**, **16**, and **19** with 20% palladium on carbon catalyst. Diastereoisomers **28** and **29** were separated by repeated recrystallization of the hydrochloride salts. It was found that cyclization to diketopiperazines such as **37** and **39** could occur if, in the debenzylation to **22**, **23**, and **25**, at least an equivalent amount of a strong acid, such as hydrogen chloride, were not present. For instance, cyclic **37** was isolated even when the benzyl ethyl diester **15** was cleaved in the presence of acetic acid. Subsequently, diketopiperazines **37** and **39** were prepared by a direct cyclization of the free amino ester acids **22** and **25** in toluene at elevated temperatures. Two of the amino ester acids (**22** and **25**) were used to prepare amino diethyl diesters **14** and **17** by direct esterification with ethanolic hydrogen chloride.

The amino diacid products **33**, **34**, and **36** (Table III) were obtained by mild base hydrolysis of the amino ester acids **22**, **25**, and **28** using an equivalent amount of sodium hydroxide in the cold to prevent racemization. The amino diacid **35** was obtained by hydrolysis of the amino diethyl

Scheme II



(17) Cohen, D. M.; Evans, D. B.; Ryan, M. J.; Kaplan, H. R. *New Drugs Annual: Cardiovascular Drugs*; Raven: New York, 1985; Vol 3, pp 71–83.
(18) Blankley, C. J.; Kaltenbronn, J. S.; DeJohn, D. E.; Werner, A.; Bennett, L. R.; Bobowski, G.; Hoefle, M. L.; Essenberg, A.; Pearlman, W. M.; Kaplan, H. R. *Abstracts of Papers*, 185th ACS National Meeting, Seattle, WA, ABS. MEDI 66; American Chemical Society: Washington, DC, 1983.
(19) Ryan, M. J.; Boucher, D. M.; Cohen, D. M.; Essenberg, A. D.; Major, T. C.; Mertz, T. E.; Olszewski, B. J.; Randolph, A. E.; Singer, R. M.; Kaplan, H. R. *J. Pharmacol. Exp. Ther.* **1984**, *228*, 312–318.
(20) Stanton, J. L.; Gruenfeld, N.; Babiarz, J. E.; Ackerman, M. H.; Friedman, R. C. *J. Med. Chem.* **1983**, *26*, 1267–1277.
(21) Kim, D. H.; Guinosso, C. J.; Buzby, G. C.; Herbst, D. R.; McCaully, R. J.; Wicks, T. C.; Wendt, R. L. *J. Med. Chem.* **1983**, *26*, 394–403.
(22) Becker, R. H. A.; Scholkens, B. A.; Unger, Th.; Linz, W. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1983**, 324.
(23) Hayashi, K.; Ozaki, Y.; Nunami, K.; Uchida, T.; Kato, J.; Kinashi, K. Yoneda, N. *Chem. Pharm. Bull.* **1983**, *31*, 570–576.
(24) Kaltenbronn, J. S.; DeJohn, D.; Krolls, U. *Org. Prep. Proced. Int.* **1983**, *15*(1–2), 35–40.

Table V. Summary of the Effects of Certain ACE Inhibitors on Blood Pressure in the Concious Renal (1 Clip/2 Kidney) Hypertensive Rat

compd	dose mg/kg, po	vehicle	no. tested	mean aortic blood pressure	
				baseline, mmHg[a]	max change,[b] mmHg
14	1	*c*	5	208 ± 2	−69 (at 10 h)
22 (quinapril)	0.3	*c*	4	157 ± 2	−50 (at 10 h)
	1	*c*	4	196 ± 10	−54 (at 4 h)
	3	*c*	4	188 ± 6	−62 (at 5 h)
	10	*c*	4	181 ± 10	−87 (at 5 h)
23	30	*c*	5	172 ± 8	−11 (at 10 h)
24	30	*c*	4	187 ± 8	−20 (at 9 h)
25	0.3	*d*	3	201 ± 14	−41 (at 5 h)
	1	*d*	4	182 ± 11	−43 (at 6 h)
	3	*c*	3	179 ± 13	−39 (at 6 h)
	10	*c*	3	205 ± 22	−113 (at 6 h)
26	30	*c*	5	177 ± 6	−15 (at 8 h)
27	30	*c*	5	178 ± 6	−16 (at 8 h)
28	10	*c*	4	200 ± 11	−80 (at 10 h)
33	1	*c*	5	199 ± 8	−44 (at 7 h)
	3	*c*	5	199 ± 8	−45 (at 2 h)
	10	*c*	5	199 ± 8	−76 (at 5 h)
	30	*d*	3	192 ± 13	−89 (at 9 h)
34	1	*c*	5	199 ± 8	−20 (at 8 h)
	3	*c*	5	199 ± 8	−52 (at 8 h)
	10	*c*	5	199 ± 8	−52 (at 6 h)
	30	*d*	3	181 ± 11	−107 (at 8 h)
36	3	*c*	4	195 ± 9	−74 (at 8 h)
37	30	*c*	4	186 ± 8	−28 (at 6 h)
41	1	*c*	6	185 ± 12	−45 (at 1 h)
44	30	*c*	4	174 ± 9	−15 (at 10 h)
46	30	*c*	4	165 ± 9	0
1 (enalapril)	0.3	*c*	4	201 ± 9	−79 (at 5 h)
	1	*c*	4	175 ± 2	−43 (at 8 h)
	3	*c*	4	200 ± 5	−112 (at 5 h)
	10	*c*	4	199 ± 5	−112 (at 4 h)
1a (enalaprilat)	1	*c*	6	188 ± 10	−51 (at 9 h)
	3	*c*	6	188 ± 10	−54 (at 2 h)
	10	*c*	5	188 ± 10	−57 (at 6 h)
	30	*d*	4	176 ± 8	−70 (at 6 h)
2 (captopril)	0.3	*c*	4	189 ± 6	−25 (at 2 h)
	3.0	*c*	4	192 ± 7	−93 (at 6 h)
	30	*c*	4	186 ± 4	−101 (at 6 h)

[a] All values are the mean ± 1 SEM. [b] Average result for 3–5 animals. Vehicle controls average 183 ± 5 mmHg with maximum decreases about 16–20 mmHg, or 10%. Hence, blood pressure decreases in excess of 20 mmHg are taken as significant for the purposes of this comparison. [c] Acacia. [d] EtOH + acacia.

ester **20**. In one case, the side-chain ethyl ester compound **13** was base hydrolyzed leaving the THIQ *tert*-butyl ester **32** intact. Unlike the corresponding free amino ester acids, the crystalline free amino diacids **33–36** were less prone to diketopiperazine formation. However, cyclization of **33** to **38** could be achieved in fair yield on heating in DMF at 100 °C for 30 min.

The N-methylated amino ester acid **30** was prepared either by methyl iodide alkylation of the amino diester **13** or via an Eschweiler–Clarke reaction on **13** using formaldehyde and formic acid and subsequent cleavage of the resulting *tert*-butyl ester **31** with trifluoroacetic acid.

The sulfhydryl class of compounds **40–50** (Table IV) was synthesized as indicated in Scheme III either by direct acylation of the silated THIQ carboxylic acids to give **40** and **47** or by acylation of the amino ester **11** in the presence of pyridine to give compounds like **42**. Subsequent cleavage of the *tert*-butyl group with hydrogen chloride gave **43** and **45**. The *S*-acetyl group was cleaved with ammonia to give **44** and **46**. The des carboxyl THIQ analogues **49** and **50** were prepared similarly from 1,2,3,4-tetrahydroisoquinoline.

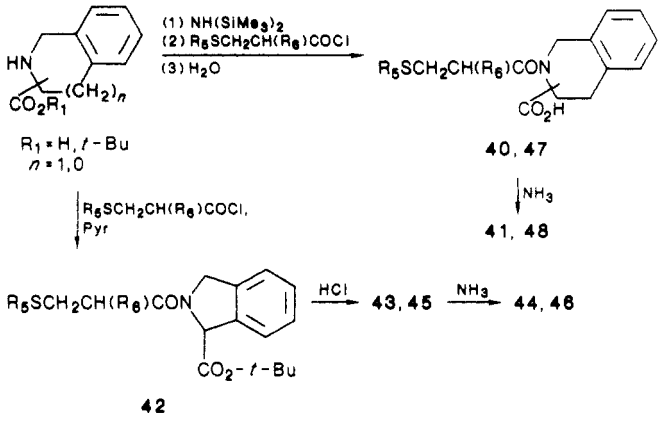
Stereochemistry. The syntheses of target compounds **13–15**, **17–22**, **25**, **28**, and **30–36** were designed to give structures with the *S,S,S* configuration that have been determined in the case of enalapril types[1] to exhibit maximum ACE inhibitory activity. In this paper, the order of the *R* and *S* designations for compounds with multichiral centers is linear (from left to right) for the structures as drawn. Three compounds with the *R,S,S* (**16**, **23**, and **26**) and three with the *S,S,R* configuration (**24**, **27**, and **29**) were prepared from available diastereomeric or enantiomeric fragments for comparison. Reactions were monitored by TLC, analytical HPLC, and optical rotation to ensure that other diastereoisomers did not arise due to possible racemization under the various reaction conditions used. In cases where isomer separation was necessary (**28**, **29**, **35**, and **36**) the configuration of isomers was inferred from biological results and the well-established precedents.

Biological Results and Discussion

The compounds were assayed for ACE inhibitory activity vs. substrate [^{3}H]-Hip-Gly-Gly as described in our earlier papers.[5] The THIQ-3-carboxylic acid series gave compounds **22** and **33** with high in vitro and in vivo ACE inhibitory activity, comparable in all respects to enalapril and more potent in most models than captopril. Similarly, the 6,7-dimethoxy-THIQ analogues gave highly effective ACE inhibitors **25** and **34**. In vitro IC_{50}'s are listed in Table III, while selected in vivo results in renal hypertensive (2 kidney, 1 clip) rats are shown in Table V. As with enalapril, the monoethyl esters (**22** and **25**) show a better oral activity profile than the active diacids (**33** and **34**). Diethyl ester **14** showed similar efficacy to monoester **22**.

Additional pharmacology of compounds **22** and **25** has been reported[13–15,17,25,29] and further studies will be reported

Scheme III



separately. Not surprisingly, the diketopiperazines **37** and **39** obtained from the target compounds showed little if any ACE inhibitory activity. Similarly, the diastereoisomers other than *S,S,S* in the quinapril series (**23** and **24**) and in the dimethoxy series (**26** and **27**) showed the expected decrease in in vitro potency, with the configuration change in the phenylbutyric acid portion of the molecule being less detrimental than inversion at the C-terminal amino acid. Only the analogues with the optimal *S* configuration at all centers showed in vivo efficacy at reasonable doses. Likewise, N-methylation of **22** to **30** destroyed activity.

In agreement with what has been reported from several laboratories, we also found early on that the mercaptopropionyl analogues of THIQ-3-carboxylic acid, e.g., **41**, displayed excellent ACE inhibitory activity, being equivalent in vitro and in vivo to captopril. This activity is not, however, shared by the THIQ-1-carboxylic acid analogue **48**, whose in vitro activity is minimal and not distinguishable from the des carboxy analogue **50**. This observation has also been reported by others[20] and implies a severe steric interference to proper binding at the terminal carboxylic acid binding site. Indeed, the lower homologue isoindoline-1-carboxylic acid (**46**) and its des methyl analogue **44** show a similar lack of activity. Again, by way of contrast, the isomeric indoline-2-carboxylic acid analogue of captopril[20] is one of the most potent sulfhydryl ACE inhibitors known.

When we prepared the isoindoline-1-carboxylic and THIQ-1-carboxylic acid analogues (**28** and **35**), containing the enalapril side chains, we found, unexpectedly, that the compounds thus produced were highly potent in vitro, similar to quinapril and the indoline-2-carboxylic acid analogues currently under investigation by another group.[26] Furthermore, this activity of **28** has been confirmed in vivo in renal hypertensive rats (Table V). These observations suggest either that (a) alternate binding modes exist for the C-terminal residue of sulfhydryl and non-sulfhydryl ACE inhibitor prototypes, (b) the aromatic amino acids have a special rather restricted spatial requirement not shared by other C-terminal amino acids, or (c) the non-sulfhydryl inhibitors have additional binding modes not available to the sulfhydryl inhibitors. Both the perhydroisoindole analogue of **28**[18,27] as well as its perhydroindole isomer[19] are highly potent in vitro and in vivo inhibitors indicating that they do not share the detrimental features of **46**. The alternate binding models a or c thus seem more plausible than b. All currently postulated molecular interaction models have been based on a common conformation of the C-terminal amino acid.[4,11,28] Further study of the effects observed here could allow additional refinement of the receptor mapping for the various classes of ACE inhibitors.

Quinapril (**22**) has proven to have a high margin of safety in animal toxicology studies[29] to date and has shown good efficacy in early clinical trials[16] in essential hypertension. Although the variation in chemical structure from enalapril has provided an analogue with equivalent enzyme inhibition activity, differences can be expected to emerge in the pharmacokinetics and biodistribution of the two compounds due to the increased lipophilicity of **22** relative to **1** and hence, potentially, in the efficacy profile. Preliminary indications of some differences have been reported already[30] and others are currently under investigation. The diacid, **33**, provides the active form of quinapril for investigation as an intravenous formulation.

Conclusion

Quinapril (**22**) and its diacid (**33**) have been prepared and shown to possess potent ACE inhibitory activity and useful in vivo efficacy. In addition, new facets of the SAR of ACE inhibitors have been uncovered by the study of tetrahydroisoquinoline and isoindoline carboxylic acid analogues of active prototypes. In light of these observations, extensions to examine the conjunction of the isomeric tetrahydroisoquinoline carboxylic acids with other active ACE inhibitor side chains seem well warranted.

Experimental Section

Melting points were determined with a Thomas-Hoover capillary melting point apparatus, which was calibrated against known standards. IR spectra were run on a Digilab FTS 14 or Nicolet MX-1 spectrometer. NMR spectra were run on a Varian EM 390 or Bruker WH90 spectrometer using Me_4Si as an internal standard. Optical rotations were run on a Perkin-Elmer 141 polarimeter. Analytical HPLC analyses were run using a C_{18} reverse-phase column, 25 cm × 4.6 mm i.d. with 10-μm particle size and a UV detector at 210 nm. The mobile phase in the case of the amino acid esters was 80:20 MeOH/0.005 M phosphate buffer, pH 7, at a 2 mL/min flow rate. The mobile phase used for the amino acids was 40:60 MeCN/0.05 M $(n\text{-Bu})_4NOH$, pH adjusted to 7.2 with H_3PO_4, at a 2 mL/min flow rate. Satisfactory elemental analyses (±0.4% of calculated values) were obtained for all compounds except where noted otherwise.

Method A. (*S*)-1,2,3,4-Tetrahydro-3-isoquinolinecarboxylic Acid, 1,1-Dimethylethyl Ester, Hydrochloride (5). A quantity of 447 g of isobutylene was passed into a solution of 63.5 g (0.36 mol) of (*S*)-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid[31,32] in 650 mL of dry dioxane and 65 mL of concentrated H_2SO_4 at 0 °C under N_2. The reaction vessel was sealed and shaken for 17 h at room temperature. The vessel was vented and the mixture poured into 2.5 L of cold 2 N NaOH. The product was extracted into Et_2O and the solution washed with water, dried (Na_2SO_4), and concentrated to about 500 mL. This was treated with 180 mL (0.36 mol) of 2 N 2-propanolic hydrogen chloride to precipitate the product. Recrystallization from EtOH/Et_2O gave pure product: wt, 45.5 g; yield, 47%; mp 190–192 °C dec; $[\alpha]^{23}_D$ −88.7° (*c* 2, MeOH). Anal. ($C_{14}H_{19}NO_2$·HCl) C, H, N.

(25) Cohen, D. M.; Essenberg, A. D.; Olszewski, B. J.; Singer, R. M.; Ryan, M. J.; Evans, D. B.; Kaplan, H. R. *Pharmacologist* **1984**, *26*(3), 266.
(26) Schaller, M. D.; Brunner, D. B.; Nussberger, J.; Turini, G. A.; Sen, S. B.; Chen, D.; Waeber, B.; Brunner, H. R. *Eur. J. Clin. Pharmacol.* **1984**, *26*, 419–424.
(27) Blankley, C. J. U.S. Pat. 4503043, 1985.
(28) Andrews, P. R.; Carson, J. M.; Caselli, A.; Spark, M. J.; Woods, R. *J. Med. Chem.* **1985**, *28*, 393–399.
(29) Anderson, J. A.; Fitzgerald, J. E.; Jayasekara, U.; Watkins, J. R.; Aust, A. E.; de la Iglesia, F. A. *Fed. Proc.* **1984**, *43*, 733.
(30) Ryan, M. J.; Singer, R. M.; Bjork, F.; Mertz, T. E.; Randolph, A. E.; Evans, D. B.; Kaplan, H. R. *Pharmacologist* **1983**, *25*(3), 147, (Abstr. 249).
(31) Julian, P. L.; Karpel, W. J.; Magnani, A.; Meyer, E. W. *J. Am. Chem. Soc.* **1948**, *70*, 182.
(32) Hein, G. E.; Niemann, C. *J. Am. Chem. Soc.* **1962**, *84*, 4487.

Compounds **6** and **8** were similarly obtained.

(*S*)-1,2,3,4-Tetrahydro-3-isoquinolinecarboxylic Acid, Phenylmethyl Ester, Hydrochloride (7). Benzyl alcohol, 750 mL, was treated with 150 g of polyphosphoric acid and warmed and stirred at 90 °C to obtain a homogeneous mixture. A quantity of 165.2 g (0.93 mol) of (*S*)-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid was added, and the mixture was stirred for 4 h at 95–105 °C and then allowed to stand at room temperature for 18 h. A solution of 18.5 g of gaseous HCl in 2.5 L of anhydrous Et_2O was added. On cooling overnight the crude benzyl ester hydrochloride separated. Recrystallization from EtOH gave pure material: wt, 184 g; yield, 65%; mp 190.5–191 °C; $[\alpha]^{23}_D$ −83.3° (*c* 1, 1:1 MeOH/1 N HCl). Anal. ($C_{17}H_{17}NO_2$·HCl) C, H, N.

The oily free base of **7**,[33] for use in subsequent reactions, was prepared by treatment of **7**·HCl salt with saturated $NaHCO_3$, extraction with EtOAc, drying (Na_2SO_4), and concentration of the organic solution.

(*S*)-1,2,3,4-Tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic Acid, Phenylmethyl Ester, Hydrochloride (9). A mixture of 34.0 g (0.12 mol) of 1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic acid, hydrochloride (*S* form: mp 281–282 °C dec; $[\alpha]^{23}_D$ −98° (*c* 2.5, 1 N HCl))[34] and 600 mL of benzyl alcohol was saturated with HCl gas. The temperature rose to 45 °C. The mixture was stirred at room temperature for 3 days. A relatively small amount of solid was filtered, and the filtrate was treated with 2 L of Et_2O to precipitate crude product: wt, 37.5 g; yield, 83%. Purification was effected by treatment with excess saturated $NaHCO_3$, extraction of the base into EtOAc, and precipitation of the hydrochloride salt with HCl gas. Recrystallization from MeOH/Et_2O gave pure product: mp 255–260 °C; $[\alpha]^{23}_D$ −81.3° (*c* 1, MeOH); TLC (silica gel/20% MeOH–$CHCl_3$) one spot, R_f = 0.8; IR (KBr pellet) 1742 (ester C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 3.12 (m, 2 H, H-4), 3.68 (s, 6 H, OCH_3), 4.12 (s, 2 H, H-1), 4.48 (m, 1 H, NC*H*CO), 5.21 (s, 2 H, $PhCH_2O$), 6.75 (s, 2 H, H-5 and H-8), 7.24 (s, 5 H, Ar), 10.10 (br s, 2 H, NH·HCl). Anal. ($C_{19}H_{21}NO_4$·HCl) C, H, N.

1,2,3,4-Tetrahydro-1-isoquinolinecarboxylic Acid, Ethyl Ester, Hydrochloride (10). A solution of 10.76 g (0.053 mol) of isoquinoline-1-carboxylic acid, ethyl ester[35] in 100 mL of EtOH and 4 mL of HOAc was hydrogenated at low pressure using 10% Rh/C catalyst. The catalyst was filtered; 5.0 mL of concentrated HCl was added, and the solution was concentrated at reduced pressure. The residue was recrystallized from EtOAc/Et_2O: wt, 6.70 g; yield, 52%; mp 132–134 °C; IR (KBr pellet) 1745 (ester C=O) cm^{-1}; NMR ($CDCl_3$) δ 1.3 (t, 3 H, OCH_2CH_3), 3.1 (m, 2 H, H-4), 3.8 (m, 2 H, H-3), 4.2 (q, 2 H, OCH_2CH_3), 5.35 (s, 1 H, H-1), 7.2 (m, 3 H, H-5,6,7), 7.45 (m, 1 H, H-8). Anal. ($C_{12}H_{15}NO_2$·HCl) C, H, N.

Method B. [3*S*-[2[*R(*R**)]],3*R**]-2-[2-[[1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic Acid, Phenylmethyl Ester, Maleate (19).** A stirred solution of 5.00 g (0.016 mol) of **3** hydrochloride[24] in 200 mL of CH_2Cl_2 at room temperature was treated successively with 1.60 g (0.016 mol) of NEt_3, 2.14 g (0.016 mol) of 1-hydroxybenzotriazole, 5.16 g (0.016 mol) of **9** free base, and 3.26 g (0.016 mol) of dicyclohexylcarbodiimide in 10 mL of CH_2Cl_2. Dicyclohexylurea gradually separated. The mixture was allowed to stand at room temperature overnight. Hexane (300 mL) was added, and the solids were filtered. The filtrate was washed with 250 mL of saturated $NaHCO_3$, dried over Na_2SO_4, and concentrated to remove solvent. The viscous residue was triturated with 50 mL of Et_2O and filtered to remove insolubles. The filtrate was concentrated to give 9.20 g (99%) of crude **19** base. Preparation of maleate salt was as follows: A solution of 9.00 g (0.015 mol) of the above crude base in 50 mL of EtOAc was treated with a warm (40 °C) solution of 1.86 g (0.016 mol) of maleic acid in 50 mL of EtOAc. White crysals separated: wt, 7.20 g; yield, 65%; mp 139–141 °C; TLC of base (generated with aqueous $NaHCO_3$ treatment of the salt and EtOAc extraction) showed one spot, R_f = 0.7 (silica gel/EtOAc). Recrystallization from EtOAc gave pure material of the same melting point: $[\alpha]^{23}_D$ +3.4° (*c* 1.05, EtOH); IR (KBr pellet) 1742 (ester C=O), 1653 (amide C=O) cm^{-1}; NMR ($CDCl_3$) δ 1.18 (t, 3 H, OCH_2CH_3), 1.40 (m, 3 H, CH_3), 2.20 (m, 2 H, $PhCH_2CH_2$), 2.68 (m, 2 H, $PhCH_2$), 3.08 (m, 2 H, H-4), 3.72 (m, 6 H, OCH_3), 4.10 (q, 2 H, OCH_2CH_3), 4.47 (m, 2 H, H-1), 4.65 (m, 1 H, NHC*H*CO), 4.91 (s, 2 H, $PhCH_2O$), 5.40 (m, 1 H, H-3), 6.12 (s, 2 H, vinyl), 6.42 (s, 2 H, H-5 and H-8), 7.10 (m, 10 H, Ar), 10.48 (br s, 3 H, NH and COOH). Anal. ($C_{34}H_{40}N_2O_7$·$C_4H_4O_4$) C, H, N.

Compounds **13**, **15**, **16**, **18**, **20**, and **21** were similarly prepared except that the solvent for preparation of **13** was THF and the solvent for **20** was DMF. Diastereoisomers were separated, in preparation of **20**, by recrystallization of the maleate salt.

Method C. [3*S*-[2[*R(*R**)]],3*R**]-2-[2-[[1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic Acid, Hydrochloride (22).** A quantity of 100 g of trifluoroacetic acid (TFA) was added to 11.6 g (0.023 mol) of **13** base. The mixture was stirred to solution for 1 h at room temperature. The TFA was removed on the rotary vacuum evaporator, and the residual oil was dissolved in 400 mL of dry Et_2O. A solution of 1.0 g (excess) of dry HCl in 20 mL of dry Et_2O was added to precipitate the HCl salt. Recrystallization from EtOAc/toluene gave pure crystalline product: wt, 9.60 g; yield, 88% mp 120–130 °C; TLC (silica gel/20% MeOH–$CHCl_3$) one spot, R_f = 0.5–0.6; $[\alpha]^{23}_D$ +14.5° (*c* 2, EtOH); HPLC, 99.1%; IR (Nujol) 1759 (ester C=O), 1703 (carboxy C=O), 1649 (amide C=O) cm^{-1}; IR (KBr pellet) 1740 (ester and carboxy C=O), 1652 (amide C=O) cm^{-1}; NMR (Me_2SO-d_6 at 75 °C) δ 1.25 (t, 3 H, OCH_2CH_3), 1.52 (d, 2 H, CH_3), 2.22 (t, 2 H, $PhCH_2CH_2$), 2.70 (m, 2 H, $PhCH_2$), 3.20 (m, 2 H, H-4), 3.85 (m, 1 H, NC*H*CO), 4.20 (m, 1 H, NC*H*CO), 4.20 (q, 2 H, OCH_2CH_3), 4.40–4.50 (m, 2 H, H-1), 5.20 (m, 1 H, H-3), 7.20 (m, 9 H, Ar). Anal. ($C_{25}H_{30}N_2O_5$·HCl) C, H, N.

Compounds **24**, **25**, **27**, and **30** were similarly obtained.

Method D. [3*S*-[2[*R(*R**)]],3*R**]-2-[2-[[1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-3-isoquinolinecarboxylic Acid, Hydrochloride (25).** A quantity of 0.046 mol of **19** hydrochloride (see below) dissolved in 100 mL of THF was catalytically debenzylated with hydrogen and 0.5 g of 20% Pd/C at low pressure. The catalyst was filtered, and the product was precipitated as an amorphous solid by addition to a 10-fold quantity of Et_2O: wt, 21.7 g; yield, 88%; mp 120–140 °C; TLC (silica gel/20% MeOH–$CHCl_3$) one spot, R_f = 0.6. Recrystallization from EtOH/Et_2O gave pure **14**: mp 141–161 °C; $[\alpha]^{23}_D$ +34.2° (*c* 1.1, EtOH); IR (KBr pellet) 1745 (ester and carboxy C=O), 1655 (amide C=O) cm^{-1}. Anal. ($C_{27}H_{34}N_2O_7$·HCl) C, H, N.

Compounds **22**, **23**, and **26** were also prepared by this method.

The noncrystalline diester **19** hydrochloride starting material used above was prepared by treatment of 32.4 g (0.046 mol) of **19** maleate salt with excess saturated $NaHCO_3$, extraction of the free base into 50% Et_2O/EtOAc, treatment of this solution with excess HCl, and concentration at reduced pressure.

[3*S*-[2[*R(*R**)]],3*R**]-2-[2-[[1-(Ethoxycarbonyl)-3-phenylpropyl]methylamino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic Acid, 1,1-Dimethylethyl Ester (31). Via Eschweiler–Clarke Methylation of 13.** A solution of 0.99 g (0.002 mol) of **13** base, 0.41 g (0.005 mol) of 37% HCHO solution, 3.0 mL of EtOH, and 5.0 mL of 98–100% HCOOH was heated at 80 °C for 2 h. The reaction mixture was concentrated at reduced pressure, treated with 20 mL of saturated $NaHCO_3$, and extracted with 30 mL of EtOAc. The extract was dried (Na_2SO_4), charcoaled, and concentrated to give a viscous oil: wt, 0.75 g; yield, 74%; HPLC, 86%; IR, TLC, and NMR were similar to product isolated via methyl iodide alkylation method (below).

Via Methyl Iodide Alkylation of 13. A mixture of 0.15 g (0.3 mmol) of **13**, 3 mL of DMF, 1.0 g of methyl iodide, and 1.0 g of powdered $NaHCO_3$ was heated at reflux (pot temp = 75 °C) with stirring for 10 min. Ice water (15 mL) was added, and the separated oil was extracted into 30 mL of Et_2O. The organic phase was concentrated to give a viscous oil: wt, 0.15 g; yield, 98%; TLC (silica gel/EtOAc) one spot, R_f = 0.85; IR (liquid film) 1735 (ester C=O), 1655 (amide C=O) cm^{-1}; NMR (C_5D_5N at 75 °C) δ 1.15–1.40 (m, 9 H, *t*-C_4H_9), 1.50 (d, 3 H, CH_3), 2.25 (m, 2 H, $PhCH_2CH_2$), 2.60 (s, 3 H, NCH_3), 2.80 (m, 2 H, $PhCH_2CH$), 3.30

(33) Hayashi, K.; Ozaki, Y.; Nunami, K.; Yoneda, N. *Chem. Pharm. Bull.* **1983**, *31*, 312.
(34) Sugimoto, N. *J. Pharm. Soc. Jpn.* **1944**, *64*, 27.
(35) Padbury, J. J.; Lindwell, H. G. *J. Am. Chem. Soc.* **1945**, *67*, 1268–1270.

(t, 2 H, H-4), 3.74 (t, 1 H, NC*H*CO), 4.20 (m, 1 H, NCHCO), 4.21 (q, 3 H, OCH_2CH$_3$), 4.86 (d, 1 H, H-1), 5.30 (d, 1 H, H′-1), 6.61 (t, 1 H, H-3), 7.20 (m, 9 H, Ar). Anal. ($C_{30}H_{40}N_2O_5$) C, H, N.

Method E. 2-[2-[[1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-2,3-dihydro-1*H*-isoindole-1-carboxylic Acid, Hydrochloride (28). A quantity of 3.00 g (0.006 mol) of **21** was dissolved in 30 mL of CH_2Cl_2 and the solution cooled at 0 °C in an ice-salt bath. Hydrogen chloride was bubbled in for 15 min. The solution was refrigerated overnight and evaporated at reduced pressure. The residue was taken up in CH_2Cl_2 and filtered to remove solids. The filtrate was treated with charcoal, filtered, and concentrated at reduced pressure to give amorphous product: wt, 1.0 g; yield, 36%; $[\alpha]^{23}_D$ −30.8° (*c* 1.08, EtOH). Anal. ($C_{24}H_{28}N_2O_5$·HCl·0.8H_2O) C, H, N, H_2O; Cl: calcd, 7.46; found, 6.95.

Compound **29** was prepared similarly.

Method F. [3*S*-[2[*R(*R**)]],3*R**]-2-[2-[[1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic Acid, Ethyl Ester, Maleate (14).** A solution of 2.00 g (0.0042 mol) of **22** hydrochloride in 25 mL of absolute EtOH was treated with HCl gas until the temperature reached 60 °C. After cooling and standing at room temperature for 3 days the solvent was removed at reduced pressure and the residue was dissolved in 50 mL of water. The aqueous solution was extracted with Et_2O to remove impurities and then treated with 70 mL of saturated $NaHCO_3$. The diester base was extracted into 50 mL of Et_2O and the solution dried (Na_2SO_4).

A warm solution of 0.48 g (0.004 mol) of maleic acid in 10 mL of EtOAc was added to the above Et_2O solution of base to give crystals of **14** (maleate salt): wt, 0.85 g; yield, 36%; mp 118–120 °C. Recrystallization from EtOAc gave pure product of the same melting point: $[\alpha]^{23}_D$ +1.5° (*c* 1.02, EtOH). Anal. ($C_{27}H_{34}N_2O_5$·$C_4H_4O_4$) C, H, N.

Compound **17** was prepared similarly.

Method G. [3*S*-[2[*R(*R**)]],3*R**]-2-[2-[(1-Carboxy-3-phenylpropyl)amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic Acid, Hydrate (33).** A solution of 0.95 g (0.002 mol) of **22** hydrochloride in 5 mL of water and 5 mL of MeOH was added to a cold (10 °C) solution of 6.4 mL (0.0064 mol) of 1 N NaOH and 5 mL of MeOH. After standing overnight at room temperature this solution was added to 7 mL (0.007 mol) of cold 1 N HCl. The separated crystalline solid was filtered and washed efficiently with cold water: wt, 0.68 g; yield, 79%. Recrystallization from MeOH/Et_2O gave pure product as a hydrate: mp 166–168 °C; $[\alpha]^{23}_D$ +20.9° (*c* 1, MeOH); IR (KBr pellet) 3440 (H_2O of crystallization), 1714 (carboxy C=O), 1650 (amide and carboxy C=O) cm^{-1}; HPLC, 98.4%. Anal. ($C_{23}H_{26}N_2O_5$·H_2O) C, H, N, H_2O.

Compounds **34** and **36** were prepared similarly. Compounds **32** and **35** were also prepared similarly using equivalent amounts of 1 N NaOH, but the starting materials were the diesters **13** and **20**, respectively.

Method H. [3*S*-[2(*R),3α,11aβ]]-1,3,4,6,11,11a-Hexahydro-3-methyl-1,4-dioxo-α-(2-phenylethyl)-2*H*-pyrazino-[1,2-*b*]isoquinoline-2-acetic Acid, Ethyl Ester (37).** A mixture of 0.71 g (0.0015 mol) of **22** hydrochloride and 10 mL of 10% NaOAc solution was extracted with 25 mL of THF. The organic phase was dried (Na_2SO_4), combined with 50 mL of toluene, and heated on the steam bath to remove the THF. After 0.5 h at 95 °C the solution was concentrated at reduced pressure to remove solvent and Et_2O (5 mL) was added to the tacky residue. The product crystallized: wt, 0.55 g; yield, 87%; TLC (silica gel/EtOAc) one spot, R_f = 0.7. Recrystallization from Et_2O gave pure **37**: mp 121–123 °C; $[\alpha 1^{23}_D$ −112.5° (*c* 1.04, EtOH); IR (KBr pellet) 1734 (ester C=O), 1661 (lactam C=O) cm^{-1}; NMR (Me_2SO-d_6) δ 1.19 (t, 3 H, OCH$_2$CH_3), 1.44 (d, 3 H, CCH$_3$), 2.40 (m, 2 H, PhCH$_2$CH_2), 2.70 (m, 2 H, PhCH_2CH$_2$), 3.05 (m, 1 H, H-11), 4.09 (m, 6 H, H′-11, H′-6, H-11a, H-3, OCH_2CH$_3$), 5.22 (d, 1 H, H-6), 7.22 (m, 9 H, Ar). Anal. ($C_{25}H_{28}N_2O_4$) C, H, N.

Compound **39** was prepared similarly.

[3*S*-[2(*R),3α,11aβ]]-1,3,4,6,11,11a-Hexahydro-3-methyl-1,4-dioxo-α-(2-phenylethyl)-2*H*-pyrazino[1,2-*b*]isoquinoline-2-acetic Acid (38).** A solution of 4.0 g (0.0093 mol) of **33** hydrate in 25 mL of DMF was heated at 100 °C for 30 min. The solvent was removed at reduced pressure, and the residue was triturated with water. The gum was warmed with 10 mL of EtOAc to give crystals: wt, 2.6 g; mp 117–120 °C. Recrystallization from 60 mL of EtOAc gave 1.9 g of pure **38**: yield, 50%; mp 117–120 °C; $[\alpha]^{23}_D$ −126° (*c* 1.06, EtOH); TLC one spot, R_f = 0.6 (silica gel/20:80 MeOH/$CHCl_3$); HPLC, 98.5%. Anal. ($C_{23}H_{24}N_2O_4$·0.75H_2O) C, H, N; H_2O: calcd, 3.34; found, 4.11.

Method I. (3*S*)-2-[3-(Acetylthio)-2-methyl-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic Acid (40). A mixture of 35.4 g (0.2 mol) of 1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid (*S* form), 32.3 g (0.2 mol) of hexamethyldisilazane, 150 mL of MeCN, and 0.5 mL of chlorotrimethylsilane as a catalyst was maintained at reflux until ammonia liberation ceased. The resulting solution was cooled to room temperature, and a solution of 36.0 g (0.2 mol) of 3-(acetylthio)-2-methylpropanoyl chloride in 60 mL of MeCN was added over 45 min. A volume of ca. 150 mL of MeCN and volatiles was distilled off at atmospheric pressure. The solution was cooled to room temperature and water (10 mL) was added. After 30 min the filtered solution was concentrated at reduced pressure to remove remaining solvent, and the viscous residue was taken up into 400 mL of warm EtOAc and filtered. An equivalent amount of dicyclohexylamine was added, and the mixture allowed to crystallize in the cold room overnight to give 46.9 g of crude salt: mp 160–167 °C. Recrystallization from MeCN, then 2-propanol, gave 25.9 g of **40** dicyclohexylamine salt; mp 171.5–173.5 °C; yield, 26%; $[\alpha]^{23}_D$ −46.4° (*c* 2, MeOH). Anal. ($C_{16}H_{19}NO_4S$·$C_{12}H_{23}N$) C, H, N.

Compound **47** was prepared by a similar method.

The above dicyclohexylamine salt was converted to the free acid by shaking 23.9 g with a mixture of 200 mL of 5% potassium bisulfate solution and 150 mL of EtOAc. The aqueous layer was separated and extracted with two portions of 100 mL of EtOAc. The EtOAc fractions were combined and dried over anhydrous $MgSO_4$; the solvent was removed on the rotary evaporator, and the oily residue was dissolved in 50 mL of dry, warm Et_2O. Crystallization was induced with the addition of ca. 25 mL of hexane, and the solution was cooled to 0 °C in an ice bath to yield 10.0 g of **40** free acid: mp 131–134.5 °C; $[\alpha]^{23}_D$ −68.7° (*c* 2, MeOH). Two recrystallizations from EtOAc gave product: mp 134.5–137 °C; $[\alpha]^{23}_D$ −70.8° (*c* 2, MeOH).

Method J. [3*S*-[2(*R),3*R**]]-1,2,3,4-Tetrahydro-2-(3-mercapto-2-methyl-1-oxopropyl)-3-isoquinolinecarboxylic Acid (41).** Acetylthio free acid **40** (5.0 g, 0.06 mol) was dissolved in 50 mL of 5 N ammonia in absolute MeOH under a blanket of nitrogen. The solution was allowed to stand at room temperature for 3 h, and the solvent was removed on the rotary evaporator. The resulting solid residue was triturated with 50 mL of EtOAc and filtered: yield, 4.3 g of ammonium salt; mp 163–169 °C dec; $[\alpha]^{23}_D$ −35.1° (*c* 2, MeOH). This salt was converted to the free acid by partitioning between 5% potassium bisulfate and $CHCl_3$. The $CHCl_3$ extracts were dried over magnesium sulfate, filtered, and concentrated to give crude product. Recrystallization from EtOAc/hexane gave 2.06 g of product: yield, 46%; mp 137–139 °C; $[\alpha]^{23}_D$ −22.9° (*c* 1.99, MeOH). Anal. ($C_{14}H_{17}NO_3S$) C, H, N.

Compounds **44**, **46**, **48**, and **50** were prepared similarly.

Method K. 2-[3-(Acetylthio)-1-oxopropyl]-2,3-dihydro-1*H*-isoindole-1-carboxylic Acid, 1,1-Dimethylethyl Ester (42). A mixture of 4.90 g (0.019 mol) of **11** hydrochloride,[36] 50 mL of THF and 3.0 g of pyridine was cooled to 0 °C, and 3.50 g (0.021 mol) of 3-(acetylthio)propanoyl chloride was added slowly enough to keep the temperature at 0 °C. After 1 h at 0 °C the mixture was allowed to warm to room temperature and was stirred for 4 h. Water (50 mL) was added, and the resulting solution was extracted with Et_2O. The extract was dried ($MgSO_4$) and evaporated: wt, 8.6 g. Recrystallization from MeOH/water gave 6.1 g: yield, 92%; mp 190–193 °C. Anal. ($C_{18}H_{23}NO_4S$) C, H, N, S.

Compound **49** was prepared similarly.

Method L. 2-[3-(Acetylthio)-1-oxopropyl]-2,3-dihydro-1*H*-isoindole-1-carboxylic Acid (43). A solution of 5.80 g (0.017 mol) of **42** in 100 mL of CH_2Cl_2 was cooled to 0 °C and treated with a stream of HCl gas for 20 min. After 16 h in the cold, a

(36) Cignarella, G.; Cerri, R.; Grella, G.; Sanna, P. *Gazz. Chim. Ital.* **1976**, *106*, 65–75.

solid had separated: wt, 4.60 g. Recrystallization from EtOH gave pure product: wt, 2.20 g; yield, 44%; mp 169–175 °C. Anal. ($C_{14}H_{15}NO_4S$) C, H, N, S.

Compound **45** was similarly obtained.

Biological Methods. The in vitro ACE inhibitory activity was determined by a radioassay procedure reported previously.[5] Activity is reported as the IC_{50}, which is the approximate molar concentration of test compound causing a 50% inhibition of the control converting enzyme activity.

The test solutions were prepared by dissolving 2–5 mg of test compound in 1 mL of Me_2SO and diluting to the desired concentration with a pH 8 buffer of 0.05 M Hepes (Calbiochem), 0.1 M NaCl, and 0.6 M Na_2SO_4 in H_2O.

Blood Pressure and Heart Rate Test in the Conscious Rat. Hypertension of renal origin was produced in rats by placing a silver clip (0.2-mm gap) around the left renal artery near the aorta and leaving the contralateral kidney intact. Four-week-old Sprague–Dawley male albino rats (Charles River, Wilmington, MA) were clipped soon after arrival, and the hypertension was allowed to develop for 4–8 weeks. The rats were then cannulated for blood pressure monitoring as described previously.[37] Only rats with mean aortic blood pressures of >160 mmHg were used. At the time of cannulation the rats weighed 280–320 g. The rats were given free access to a standard lab show (5012 Purina, Richmond, IN) and tap water and were maintained on a 12-h dark/12-h light cycle.

One-minute running average values of heart rate and aortic blood pressure (mean, systolic, and diastolic) for each rat were recorded every 30th minute by means of a computer-assisted data capture scheme.

Acknowledgment. We wish to thank the following chemists for their contributions of syntheses, development of procedures, and helpful suggestions: Dr. J. Kaltenbronn, Dr. O. Goel, Mr. U. Krolls, Mr. D. de John, Mr. D. Schweiss, Mr. N. Colbry, Dr. R. Meyer, Mr. T. Puls, Mr. L. Bennett, Mr. W. Pearlman, and Mr. D. Johnson. We also thank Dr. F. MacKellar and his staff for analytical data. In particular we thank Mr. E. Schoeb for infrared spectra, Mr. B. Scott and Ms. S. Uhlendorf for NMR spectra, Dr. H. Schneider, Mr. R. Leeds and Mr. M. Young for HPLC data, and Mr. G. Sekerak for elemental analyses and optical rotations. The contributions of the following pharmacologists are also appreciated: Dr. M. Ryan, Mr. T. C. Major, Mr. R. Singer, Ms. B. Olszewski, Ms. F. Bjork, and Dr. R. Smith.

Registry No. **3**, 82717-96-2; **4**, 84324-12-9; **5**, 82586-60-5; **5** (acid), 74163-81-8; **6**, 103733-29-5; **6** (acid), 103733-65-9; **7**, 77497-96-2; **7**·HCl, 103733-30-8; **8**, 103733-31-9; **8** (acid), 103733-66-0; **8**·HCl (acid), 82586-62-7; **9**, 103733-32-0; (±)-**10**, 103733-33-1; (±)-**11**, 96325-07-4; (±)-**12**, 103733-34-2; **13**, 82586-56-9; **14**, 103733-35-3; **14**·$C_4H_4O_4$, 103733-36-4; **15**, 82586-54-7; **16**, 103775-05-9; **17**, 103733-37-5; **17**·$C_4H_4O_4$, 103733-38-6; **18**, 103733-40-0; **19**, 82637-57-8; **19**·$C_4H_4O_4$, 82637-58-9; **19**·HCl, 82586-51-4; **20**, 103733-42-2; (*S,S,S*)-**21**, 103733-43-3; (*S,S,R*)-**21**, 103775-15-1; **22**, 85441-61-8; **22**·HCl, 82586-55-8; **23**, 103775-09-3; **23**·HCl, 89300-89-0; **24**, 103833-16-5; **24**·HCl, 103775-06-0; **25**, 103775-10-6; **25**·HCl, 82586-52-5; **26**, 103775-11-7; **26**·HCl, 103833-14-3; **27**, 103775-12-8; **27**·HCl, 103833-15-4; **28**, 103775-13-9; **28**·HCl, 103733-44-4; **29**, 103833-17-6; **29**·HCl, 103775-07-1; **30**, 103733-45-5; **31**, 103733-46-6; **32**, 103775-08-2; **33**, 85441-60-7; **34**, 103775-14-0; **34**·HCl, 82586-57-0; **35**, 103733-47-7; **36**, 103733-48-8; **37**, 103733-49-9; **38**, 103733-50-2; **39**, 103733-51-3; (*S,R*)-**40**, 100486-65-5; (*S,S*)-**40**, 100486-33-7; (*S,S*)-**40**·$C_{12}H_{23}N$, 103733-52-4; (*S,R*)-**40**·$C_{12}H_{23}N$, 103733-67-1; **41**, 77832-18-9; (±)-**42**, 103733-53-5; (±)-**43**, 103733-54-6; (±)-**44**, 103733-55-7; (±)-**44**·$C_{12}H_{23}N$, 103733-56-8; (±)-(*R*,R**)-**45**, 103733-57-9; (±)-(*R*,R**)-**45**·$C_{12}H_{23}N$, 103733-58-0; (±)-(*R*,R**)-**46**, 103733-59-1; (±)-(*R*,R**)-**47**, 103733-60-4; (±)-(*R*,R**)-**48**, 103733-61-5; (±)-(*R*,R**)-**48**·$C_{12}H_{23}N$, 103733-62-6; (±)-**49**, 103733-63-7; (±)-**50**, 103733-64-8; (±)-$H_3CCOSCH_2CH(CH_3)COCl$, 70354-87-9; isoquinoline-1-carboxylic acid ethyl ester, 50458-78-1; 1,2,3,4-tetrahydroisoquinoline, 91-21-4.

(37) Smith, R. D.; Wood, T. J.; Tessman, D. K.; Olszewski, B. J.; Currier, G.; Kaplan, H. R.; *NIH* **1980**, *41*, 1473.

Tuftsin Analogues: Synthesis, Structure–Function Relationships, and Implications for Specificity of Tuftsin's Bioactivity

Shlomo Dagan,[†‡] Philip Gottlieb,[†] Esther Tzehoval,[‡] Michael Feldman,[‡] Mati Fridkin,*[†] Koichi Yasumura,[§] Kenji Okamoto,[§] and Haruaki Yajima*[∥]

Departments of Organic Chemistry and Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel, and Kyoto College of Pharmacy, Yamashina-Ku, Kyoto, 607 Japan, and Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-Ku, Kyoto, 606 Japan. *Received June 14, 1985*

Thirteen analogues of the natural macrophage activator peptide tuftsin, ten of which are novel, were synthesized with the aim of exploring the relation between their biological potency and their capacity to attach specifically to cellular tuftsin's receptors. The analogues representing modifications and chain extensions at various parts of the parent tuftsin molecule can be classified as (a) N-terminal analogues, (b) C-terminal analogues, (c) "within-chain" derivatives, or (d) dimers of tuftsin and retrotuftsin. The various synthetic routes employed to prepare the analogues are described. A direct correlation was found between the ability of analogues to inhibit [^{3}H-Arg^4]tuftsin specific binding to mice peritoneal macrophages and their capacity to enhance phagocytosis or to inhibit tuftsin-mediated phagocytosis by the cells and to potentiate the cell's immune response.

Tuftsin[1] is an immunoglobulin G associated tetrapeptide of the sequence L-Thr-L-Lys-L-Pro-L-Arg, located in the Fc domain of the protein's heavy chain (residues 289–292).[2] It is released from a unique Ig fraction, leukokinin, through an enzymatic processing.[3] Tuftsin possesses a wide range of activities that it exerts on the phagocytic cells. In fact, the peptide is capable of potentiating most functions of blood granulocytes and tissue macrophages including phagocytosis,[1,4-6] motility,[4,7,8] immunogenic response,[9]

[†] Department of Organic Chemistry, The Weizmann Institute of Science.

[‡] Department of Cell Biology, The Weizmann Institute of Science.

[§] Kyoto College of Pharmacy.

[∥] Kyoto University.

(1) Najjar, V. A.; Nishioka, K. *Nature (London)* **1970**, *228*, 672.
(2) Edelman, G. M.; Cunningham, B. A.; Gall, W. E.; Gottlieb, P. D.; Rutishauser, U.; Waxdal, M. *Proc. Natl. Acad. Sci. U.S.A.* **1969**, *63*, 78.
(3) Najjar, V. A. In *Macrophages and Lymphocytes*; Escobar, M. R., Friedman, H., Eds.; Plenum: New York, 1980; Part A, p 131.

0022-2623/86/1829-1961$01.50/0