Angiotensin-Converting Enzyme Inhibitors. Mercaptan, Carboxyalkyl Dipeptide, and Phosphinic Acid Inhibitors Incorporating 4-Substituted Prolines

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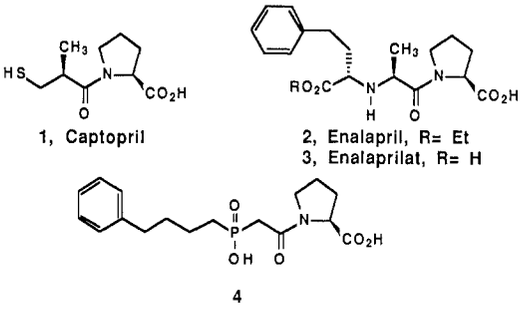
Analogues of captopril, enalaprilat, and the phosphinic acid [[hydroxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline incorporating 4-substituted proline derivatives have been synthesized and evaluated as inhibitors of angiotensin-converting enzyme (ACE) in vitro and in vivo. The 4-substituted prolines, incorporating alkyl, aryl, alkoxy, aryloxy, alkylthio, and arylthio substituents were prepared from derivatives of 4-hydroxy- and 4-ketoproline. In general, analogues of all three classes of inhibitors with hydrophobic substituents on proline were more potent in vitro than the corresponding unsubstituted proline compounds. 4-Substituted analogues of captopril showed greater potency and duration of action than the parent compound as inhibitors of the angiotensin I induced pressor response in normotensive rats. The *S*-benzoyl derivative of *cis*-4-(phenylthio)captopril, zofenopril, was found to be one of the most potent compounds of this class and is now being evaluated clinically as an antihypertensive agent. In the phosphinic acid series, the 4-ethylenethioketal and *trans*-4-cyclohexyl derivatives were found to be the most potent compounds in vitro and in vivo. A prodrug of the latter compound, fosinopril, is also being evaluated in clinical trials.

Captopril (1) was the first nonpeptidic orally active ACE inhibitor to be introduced for the treatment of hypertension and congestive heart failure. Its disclosure in 1977,[1] and the subsequent reports of its efficacy in a substantial proportion of patients with essential hypertension, stimulated extensive research in many laboratories aimed at new ACE inhibitors.[2] This research has been guided by the same hypothetical model of the active site of ACE that was instrumental in the design of captopril. Many compounds have been disclosed that differ from captopril by the substitution of another C-terminal amino acid for proline, and some of these compounds have entered clinical trials. The most noteworthy result among non-mercaptan ACE inhibitors was the discovery of the carboxyalkyl dipeptides, of which enalapril (2) is the premier example.[3] Enalapril, the ethyl ester prodrug of the active inhibitor enalaprilat (3), was found to be more potent and longer acting than captopril in animals, but the profile of therapeutic utility for enalapril is similar to that of captopril. Despite the diversity of structure among these inhibitors, relatively few novel molecular entities among ACE inhibitors have entered clinical trials. Several classes of ACE inhibitors incorporating phosphorus-containing functional groups have been described, and we chose the phosphinic acid 4, [[hydroxy(4-phenylbutyl)phosphinyl]acetyl]-L-proline, as the prototype for the present study.[4]

The work described herein began as an investigation of the effects of substitution at the 4-position of the proline



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ring of captopril. 4-Substituted prolines are readily accessible due to the availability of 4-hydroxyproline,[5] and we anticipated that the binding requirements of ACE would tolerate changes at the position corresponding to P_2'. Most of the emphasis was placed on lipophilic alkyl and aromatic substituents, since these initially appeared to have the greatest effect on biological activity. Subsequently, 4-substituted prolines were incorporated into carboxyalkyl dipeptide and phosphinic acid inhibitors. This paper summarizes the results of these investigations, which have led to a captopril analogue, zofenopril, with improved potency and longer duration of action, and a new class of phosphinic acid inhibitors with high intrinsic potency and long duration of action that paved the way for the development of the phosphinic acid prodrug ester fosinopril.

Synthesis of Inhibitors

4-Substituted Prolines. At the time this work began, protected derivatives of 4-hydroxyproline and 4-ketoproline had been elaborated to various 4-substituted prolines by O-alkylation[6] and nucleophilic displacement[7] and by Wittig reaction and hydrogenation leading to 4-methylproline[8] and 4-alkylprolines of interest in connection with lincomycin.[9] We have extended this chemistry to the preparation of selected alkylthio-, arylthio-, aryloxy-, and ketal-substituted compounds. We also developed syntheses of *cis*- and *trans*-4-phenyl- and cyclohexylprolines.

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(7) Patchett, A. A.; Witkop, B. *J. Am. Chem. Soc.* **1957**, *79*, 185.

(8) Gray, D. O.; Fowden, L. *Nature (London)* **1962**, *193*, 1285.

(9) Magerlein, B. J.; Birkenmeyer, R. D.; Hess, R. R.; Kagan, F. *J. Am. Chem. Soc.* **1967**, *89*, 2459.

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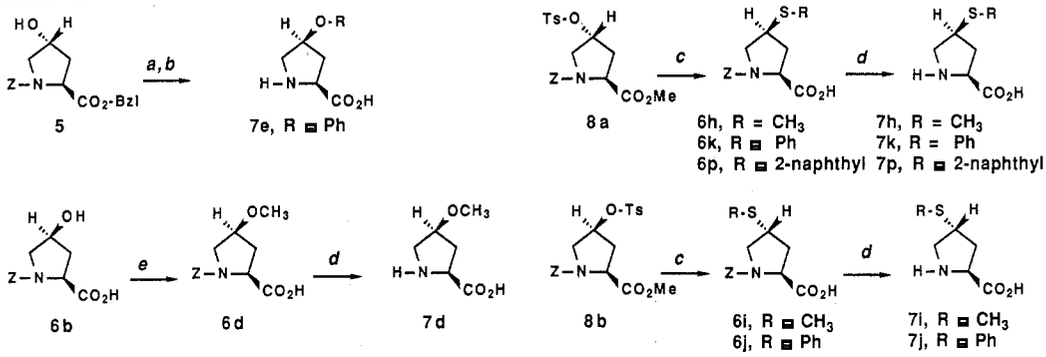
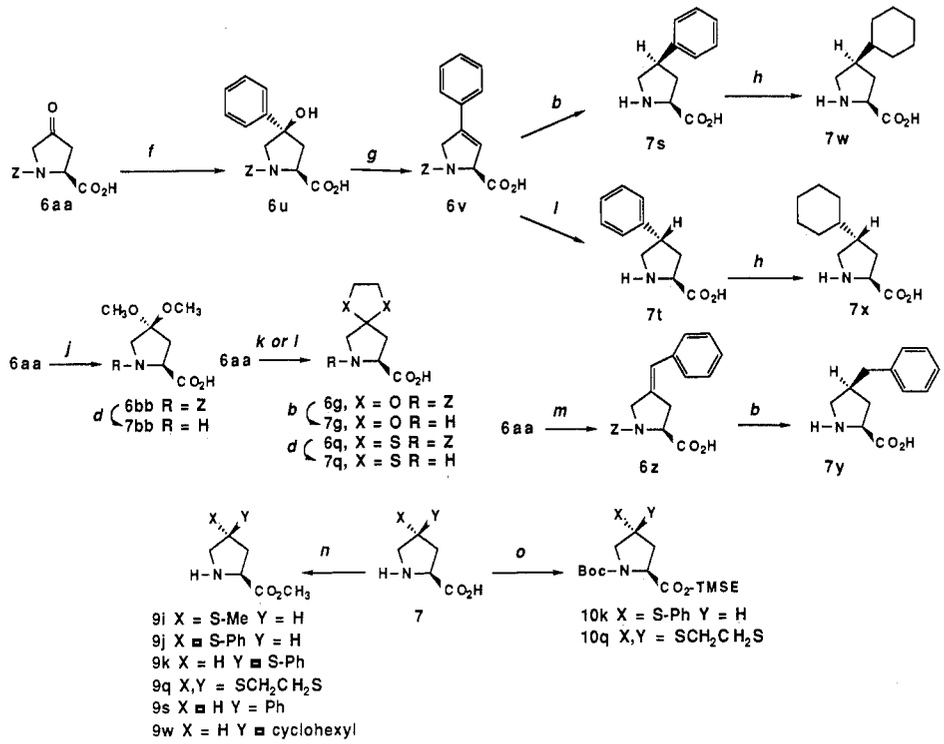
Scheme I. Synthesis of 4-Substituted Prolines[a]



[a] Notes to Schemes I–V: (a) PhOH or 2-naphthyl-OH, Ph_3P, DEAD; (b) H_2, Pd/C; (c) NaSR; NaOH; (d) HCl or HBr, AcOH; (e) Ag_2O, CH_3I; KOH; (f) PhMgBr; (g) CF_3CO_2H; (h) H_2, PtO_2; (i) Li, NH_3; NH_4Cl; (j) $(CH_3O)_3CH$, H_2SO_4; KOH; (k) *p*-TsOH, $HOCH_2CH_2OH$; (l) BF_3-Et_2O, $HSCH_2CH_2SH$; (m) $Ph_3P{=}CHPh$; (n) CH_3OH, $SOCl_2$; (o) BOCON, Et_3N; $(CH_3)_3SiCH_2CH_2OH$, DMAP, DCC; (p) 11, NaOH; (q) NH_3, H_2O; (r) $NaIO_4$; (s) HCl, H_2O; (t) 12, NaOH; (u) CH_3OH, *p*-TsOH; (v) *N*-arylthiosuccinimide, Bu_3P; KOH, H_2O; (w) Et_3N, DPPA; NaOH, H_2O; (x) *p*-TsOH, CH_3CN reflux; **17**, DCC, HOBT, $EtN(i\text{-}Pr)_2$; Bu_4NF; (y) CDI, Et_3N; $(CH_3)_3SiBr$; (z) CDI, Et_3N; NaOH, H_2O.

Scheme II. Additional Syntheses of 4-Substituted Prolines



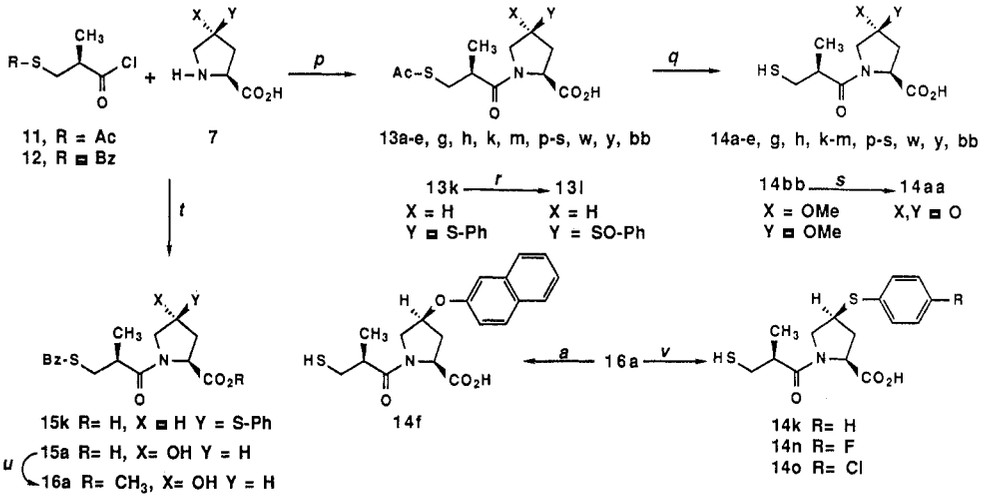
Scheme I illustrates the synthesis of several compounds by nucleophilic substitution on derivatives of *cis*- and *trans*-hydroxyproline. (All of the proline derivatives prepared herein have the natural L-configuration; cis and trans refer to the configuration of the substituent relative to the carboxyl group.) The known methyl ester tosylates **8a** and **8b**[7] were reacted with thiolate anions to give the expected displacement products **6h–k,p**, which were deprotected by using standard methods to obtain the substituted prolines **7h–k,p**. Introduction of phenoxy substitution was accomplished by Mitsunobu reaction[10] of phenol with hydroxy ester 5.[11] The *cis*-methoxy derivative **7d** was prepared by O-alkylation of **6b**.

The 4-ketoproline derivative **6aa**[7] was a useful starting material for a number of additional substituted prolines (Scheme II). The ketone could be ketalized with methanol, ethylene glycol, or ethanedithiol to give the desired ketal amino acids **7bb**, **7g**, and **7q**.

Addition of phenylmagnesium bromide to ketone **6aa** gives a moderate yield of the phenylcarbinol **6u**. The stereochemistry of this carbinol, which is apparently the only diastereomer formed, was established by facile closure to a lactone by treatment with DCC. Dehydration leads to the olefin **6v**, which undergoes catalytic hydrogenation exclusively from the side opposite the carboxylic acid to give *cis*-phenylproline **7s**. Alternatively, lithium–liquid ammonia reduction of **6v** gives a mixture of *trans*-phenylproline **7t** in a 9:1 ratio to the minor cis isomer.[12] The stereochemistry of **7t** was established by X-ray diffraction.[13] The phenylprolines were reduced to the corresponding cyclohexylprolines **7w** and **7x** on platinum catalyst. The synthesis of *cis*-4-benzylproline **7y** was modeled after the reported synthesis of *cis*-4-propylproline.[9] Wittig reaction of **6aa** gives benzylidene derivative **6z**, which was reduced to a single isomer assigned the cis stereochemistry

(10) Mitsunobu, O. *Synthesis* **1981**, 1.
(11) Baer, E.; Stedman, R. J. *Can J. Biochem. Physiol.* **1959**, *37*, 583.
(12) For an alternative synthesis of **7t**, see: Thottathil, J. K.; Moniot, J. L. *Tetrahedron Lett.* **1986**, *27*, 151.
(13) We thank J. Z. Gougoutas and M. Malley of the Squibb Institute for this determination.

Scheme III. Synthesis of Thioacyl 4-Substituted Prolines

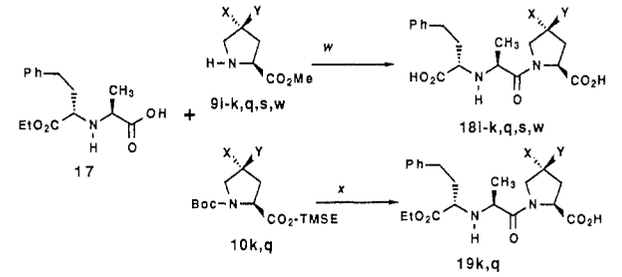


by analogy with the reduction of **6v** and other alkylideneproline derivatives.[9] In most of the chemistry described below, the substituted prolines could be employed without carboxyl protection. However, methyl esters **9** were readily prepared for those instances where carboxyl protection was desired. For the preparation of enalapril analogues, we found it necessary to choose a protecting group for the proline carboxylic acid that would be removable in the presence of the side chain ethyl ester. Because of the presence of sulfur-containing proline substituents in the targets we chose, hydrogenolytically labile protecting groups were not considered suitable. The 2-(trimethylsilyl)ethyl ester function met all of the requirements for this protecting group problem.[14] The fully protected intermediates **10k** and **10q** were prepared by (dimethylamino)pyridine-catalyzed esterification of the Boc-amino acids with DCC and (trimethylsilyl)ethanol. Table I lists physical properties for all fully characterized 4-substituted prolines and intermediates.

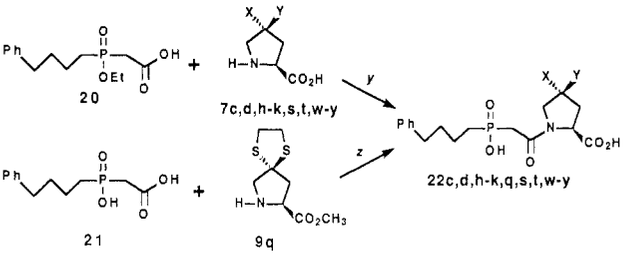
Mercaptoacyl Amino Acids. As shown in Scheme III, captopril analogues were prepared by acylation of the 4-substituted prolines with acid chlorides **11**[1b] or **12**.[15] The *S*-acetyl derivatives **13** were deprotected to give captopril analogues **14**. In certain cases, the *S*-acetyl derivatives or mercaptans were further transformed to afford compounds with other 4-substitution patterns. Thus, the ketone **14aa** was prepared by acid hydrolysis of ketal **14bb**, and sulfoxide **14l** was prepared by oxidation of **13k** followed by removal of the mercaptan protecting group from **13l**. Hydroxy compound **15a** was esterified to give **16a**, which proved to be a useful intermediate for the introduction of aryloxy or arylthio substituents. *N*-(Arylthio)succinimides[16] were reacted with **16a** to give protected derivatives, which were carried forward to **14k**, **14n**, and **14o**. The Mitsunobu reaction mentioned above for the synthesis of **7e** was applied with 2-naphthol for the synthesis of **14f** from **16a**. Several of the mercaptoacyl prolines were found to be noncrystalline and were therefore handled as salts with adamantylamine or arginine. Control experiments established that neither amine counterion significantly affected the outcome of in vitro or in vivo experiments.

Carboxyalkyl Dipeptides. Analogues of enalaprilat were prepared by coupling the enalapril side chain synthon **17** with the appropriate substituted proline methyl ester

Scheme IV. Synthesis of Carboxyalkyl Dipeptides



Scheme V. Synthesis of Phosphinic Acids



(Scheme IV). Compound **17** was obtained in diastereomerically pure form by essentially the same methods which have recently been published.[17] Saponification of the ester functions in the coupled intermediate occurs nonselectively to give the desired diacids. For the preparation of enalapril analogues **19k** and **19q**, the (trimethylsilyl)ethyl esters were generated from Boc derivatives **10k** and **10q** by treatment with *p*-toluenesulfonic acid in refluxing acetonitrile and coupled to **17**. Removal of the (trimethylsilyl)ethyl ester group was accomplished with fluoride ion in DMF.[14]

Phosphinic Acids. The substituted phosphinic acids were prepared by two variations of the method used to prepare **4** (Scheme V).[4] The side chain derivative **20**,[18] in which the phosphinic acid group was protected as an ethyl ester, could be activated with carbonyldiimidazole and coupled to unprotected amino acid **7**. The ethyl ester was removed from the coupling product by using bromotrimethylsilane. Alternatively, the diacid side chain synthon **21**[18] could be coupled to amino acid methyl esters with the same activation scheme, followed by saponifica-

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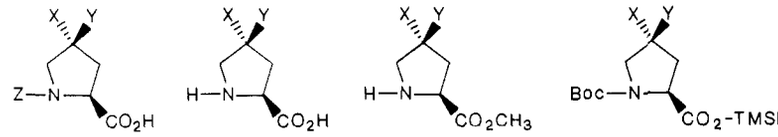
(17) (a) Kaltenbronn, J. S.; DeJohn, D.; Kroll, U. *Org. Prep. Proced. Int.* **1983**, *15*, 35. (b) Urbach, H.; Henning, R. *Tetrahedron Lett.* **1984**, *25*, 1143.
(18) Thottathil, J. K.; Przybyla, C. A.; Moniot, J. L. *Tetrahedron Lett.* **1984**, *25*, 4737.

Table I. 4-Substituted Proline Derivatives



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no.[a]	X	Y	formula[b]	mp, °C	$[\alpha]_D$, deg (*c*, solvent)	anal.[c]
7c[d]	MeO	H				
6d	H	OMe	$C_{14}H_{17}NO_5$·CHA	150–152	−34 (1, EtOH	C, H, N
7d			$C_6H_{11}NO_3$	222–224	−42 (1, MeOH)	C, H, N
7e	H	OPh	$C_{11}H_{13}NO_3$·0.25H_2O	239–241	nd	C, H, N
6g	OCH_2CH_2O		$C_{15}H_{17}NO_6$·CHA	182–184	−21.0 (1, EtOH)	C, H, N
7g			$C_7H_{11}NO_4$·0.25H_2O	245–247	−32.0 (0.5, MeOH/H_2O)	C, H, N
6h	H	SMe	$C_{14}H_{17}NO_4S$·CHA	124–127	−11.0 (1, MeOH)	C, H, N, S
7h			$C_6H_{11}NO_2S$·HCl	117–119	−1.7 (1, MeOH)	C, H, N, S, Cl
6i	MeS	H	$C_{14}H_{17}NO_4S$·CHA	140–143	−14.0 (1, MeOH)	C, H, N, S
7i			$C_6H_{11}NO_2S$·HCl	179–182	−13.0 (1, MeOH)	C, H, N, S, Cl
9i			$C_7H_{13}NO_2S$·HCl	155–156	−11.3 (1.71, MeOH)	*e*
6j	PhS	H	$C_{19}H_{19}NO_4S$·CHA	154–156	−9.0 (1, $CHCl_3$)	C, H, N, S, Cl
7j			$C_{11}H_{13}NO_2S$·HCl	181–184	−18.0 (1, pyr)	C, H, N, S, Cl
9j			$C_{12}H_{15}NO_2S$·HCl	103–104	−1.0 (2, MeOH)	*e*
6k	H	SPh	$C_{19}H_{19}NO_4S$·CHA	152–155	−24.0 (1, EtOH)	C, H, N, S
7k			$C_{11}H_{13}NO_2S$·HBr·0.75H_2O	106–109	−3.0 (1, MeOH)	C, H, N, S, Br
9k			$C_{12}H_{15}NO_2S$·HCl	87–89	+3.7 (2.05, MeOH)	C, H, N, S, Cl
10k			$C_{21}H_{33}NO_4SSi$	oil	nd	nd
6m	H	SO_2Ph	$C_{19}H_{19}NO_6S$·Ad	225–227	−31.0 (1, EtOH)	C, H, N
7m			$C_{11}H_{13}NO_4S$·HBr	200–202	+7.0 (1, MeOH)	C, H, N, S, Br
6p	H	S-2-naphthyl	$C_{23}H_{21}NO_4S$·CHA·0.25H_2O	126–128	−20.0 (1, EtOH)	C, H, N, S
7p			$C_{15}H_{15}NO_2S$·HBr	180–183	−4.0 (1, MeOH)	C, H, N, S, Br
6q	SCH_2CH_2S		$C_{15}H_{17}NO_4S_2$·CHA	207–209	−15.0 (1, $CHCl_3$)	C, H, N, S
7q			$C_7H_{11}NO_2S_2$·HCl	229–231	+7.8 (1, MeOH)	C, H, N, S, Cl
9q			$C_8H_{13}NO_2S_2$·HCl	137–138	+2.7 (1.63, MeOH)	nd
10q			$C_{17}H_{31}NO_4S_2Si$	70–72	−26.6 (2.5, $CHCl_3$)	C, H, N, S
7r[f]	H	Me				
7s	H	Ph	$C_{11}H_{13}NO_2$·HCl·0.25H_2O	165–168	+5.0 (1, MeOH)	C, H, N, Cl
9s			$C_{12}H_{15}NO_2$·HCl	132–133	+5.0 (2.12, MeOH)	nd
7t	Ph	H	$C_{11}H_{13}NO_2$	>300	+2.3	C, H, N, Cl
6u	Ph	OH	$C_{19}H_{19}NO_5$	121–123	−32.0 (1, $CHCl_3$)	C, H, N
6v	Ph	3,4-dehydro	$C_{19}H_{17}NO_4$	152–153	nd	C, H, N
7w	H	C_6H_{11}	$C_{11}H_{19}NO_2$·HCl·0.25H_2O	165–167	−16.0 (1, MeOH)	C, H, N, Cl
9w			$C_{12}H_{21}NO_2$·HCl	146–147	−12.7 (2.09, MeOH)	nd
7x	C_6H_{11}	H	$C_{11}H_{19}NO_2$·HCl	172–174	−20.1 (1, pyr)	C, H, N, Cl
7y	H	CH_2Ph	$C_{12}H_{15}NO_2$·0.25H_2O	200–201	−3.5 (1, 1 N HCl)	C, H, N
6z	$=CH_2Ph$[g]		$C_{20}H_{19}NO_4$·DCHA	150–155	+7.7 (1, $CHCl_3$)	C, H, N
6bb	MeO	OMe	$C_{15}H_{19}NO_6$·CHA	157–159	−33 (1, EtOH)	C, H, N
7bb			$C_7H_{13}NO_4$	197–199	−49 (1, MeOH)	C, H, N

[a] In this and subsequent tables, compounds sharing a common letter suffix have identical substituents X and Y. [b] Amine salts are indicated by Ad, 1-adamantanamine, $C_{10}H_{17}N$; CHA, cyclohexanamine, $C_6H_{13}N$; DCHA, *N*-cyclohexylhexanamine, $C_{12}H_{23}N$. [c] Indicated analyses (±0.4%) and IR and ^1H and ^{13}C NMR spectra were consistent with assigned structures. [d] See ref. 6. [e] No analysis; precise mass determined. [f] See ref 8. [g] Mixture of olefin isomers. nd = not determined.

tion of the methyl ester function. Many of the substituted phosphinic acids were isolated as crystalline solids, but in certain instances we found it more convenient to handle them as dilithium salts.

ACE Inhibition

Mercaptans. The substituted analogues of captopril were initially compared to the parent compound by using two criteria: inhibition of ACE in vitro[19] and inhibition of the angiotensin I (AI) induced pressor response in normotensive rats after oral administration[20] (Table II). Most of the substituted compounds were somewhat more potent than captopril in vitro. The *cis*-methoxy (**14d**), ethylene ketal (**14g**), methyl (**14r**), cyclohexyl (**14w**), and benzyl (**14y**) analogues were the most potent compounds in vitro, while the phenoxy (**14e**) and naphthylthio (**14p**) compounds were among the least potent. As discussed below, the phenylthio analogue **14k** was especially interesting on the basis of in vivo activity, so several analogues were prepared. The oxidized analogues **14l** and **14m** were both less potent than **14k** in vitro, while the two halogenated analogues **14n** and **14o** differ very little from **14k** in vitro.

It is readily apparent that introduction of substituents into the 4-position of the proline moiety of captopril could have marked effects on ACE inhibitory activity in vitro. In selecting members of the present series for potential clinical study, we decided to employ an in vivo assay that would highlight duration of action. The standard protocol employed for the normotensive rat AI pressor response assay involved measurement of the AI pressor response at regular intervals for at least 170 min after oral dosing. Figure 1 presents the percent maximum inhibition observed for each compound as well as the percent inhibition remaining after 170 min.

Since the duration of action of the more active compounds as measured by this assay was so great, we sought another method for comparing compounds that would

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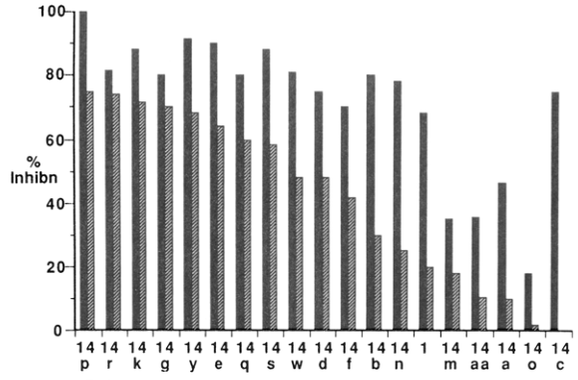


Figure 1. Inhibition of the angiotensin I pressor response in normotensive rats by mercaptan inhibitors. Solid bar, maximal inhibition observed during the 170-min experiment; striped bar, inhibition remaining at 170 min. All compounds were administered to two animals at oral doses equivalent to 1.0 mg/kg of **1** (4.6 μmol/kg) except for **14g** (3.6 μmol/kg), **14e** (3.2), **14q** (2.9), **14f** (2.8).

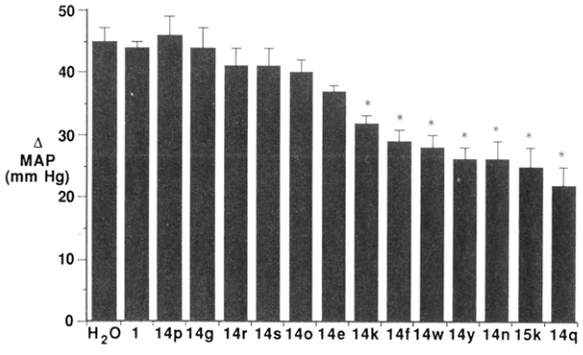


Figure 2. Angiotensin I pressor response 24 h after oral dosing of inhibitors or water. All compounds were administered at a dose equivalent to 10 mg/kg of **1**. (*) $p < 0.05$ versus H_2O.

emphasize duration of action. Therefore, animals were dosed with the inhibitor, and their pressor responses to AI were measured 24 h later and compared to AI pressor responses in a group of animals receiving only water. Compounds were selected for evaluation in this assay if they showed significant levels of inhibition of the AI pressor response 170 min after dosing. These results are shown in the bar graph in Figure 2. Pressor responses in animals treated with 10 mg/kg captopril do not differ from responses in the control animals. The mercaptans **14k** (phenylthio), **14f** (naphthyloxy), **14w** (cyclohexyl), **14y** (benzyl), **14n** [(*p*-fluorophenyl)thio], and **14q** (thioketal) showed levels of inhibition of the pressor response that were significantly different from control levels. Among these compounds, the phenylthio-substituted compound **14k** was judged to be most suitable for further development on the basis of synthetic accesibility and pharmacokinetic considerations. Because this compound was found to be an amorphous solid, its *S*-benzoyl ester **15k**, which forms crystalline potassium and calcium salts, was evaluated. The potassium salt was also evaluated in the 24-h AI pressor response protocol and found to be markedly active.

Carboxyalkyl Dipeptides. The 4-substituted analogues of enalaprilat that we investigated (Table III) did not differ markedly from the parent compound when evaluated in vitro. The two phenylthio analogues **18j** and **18k** and the thioketal **18q** were at most twice as potent as enalaprilat.

Compounds **18k and 18q** show activity very similar to that of enalaprilat after oral administration in the normotensive rat AI pressor response test (Table IV). We

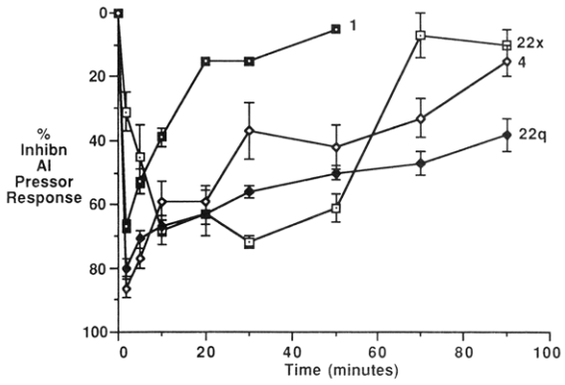


Figure 3. Inhibition of the AI pressor response versus time for phosphinic acid inhibitors and captopril (**1**). Compounds **1**, **22q**, and **22x** were dosed at 0.15 μmol/kg iv and compound **4** at 1.5 μmol/kg.

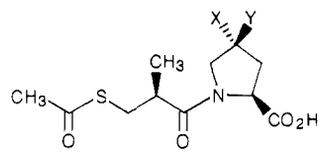
prepared ethyl esters **19k** and **19q** of these compounds for comparison with enalapril, and these compounds are also indistinguishable from enalapril when evaluated in the normotensive rat AI pressor response.

Phosphinic Acids. As reported previously,[4] phosphinic acid **4** has about one-tenth the in vitro ACE inhibitory activity of captopril, but it seemed especially well suited to further development because its in vivo activity after iv administration was about one-third that of captopril. Examination of the in vitro ACE inhibitory activities of the 12 4-substituted proline analogues we prepared reveals a much greater effect on activity than was the case with either mercaptans or carboxyalkyl dipeptides (Table V). All of the substituted analogues prepared were more active than the unsubstituted parent compound. The effect of methoxy substitution (**22c** and **22d**) was slight, while methylthio substitution (**22h** and **22i**) has a greater effect. However, thioketal (**22q**), phenylthio (**22j** and **22k**), cyclohexyl (**22w** and **22x**), benzyl (**22y**), and *cis*-phenyl (**22s**) substitution led to increases in in vitro activity of as much as 45-fold. The stereochemistry of substitution did not have a marked effect on activity except in the case of the *trans*-phenyl compound **22t**, which was about 15 times less active than the cis isomer **22s**. Thus, the effect of replacing proline by a substituted proline in this series led to compounds that are as potent in vitro as carboxyalkyl dipeptides and mercaptans.

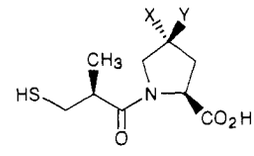
When the substituted phosphinic acids were evaluated by intravenous administration in the normotensive rat AI pressor model, most of the compounds were found to be more potent than the parent phosphinic acid **4**. These data are presented in Table VI. The thioketal **22q** gave levels of inhibition comparable to those found for compound **4** at doses that were 10 times lower. Among the cis-substituted compounds, the phenyl (**22s**) and cyclohexyl (**22w**) compounds were the most potent, in accord with their in vitro activity. The cyclohexyl analogue **22x** was the most potent among the trans-substituted compounds. The *cis*- and *trans*-phenylthio analogues **22k** and **22j** showed lower levels of inhibition than did the unsubstituted parent compound **4**, despite the fact that the in vitro activity of these two compounds was about 10 times greater.

Figure 3 shows the time course of inhibition of the AI pressor response after administration of 0.15 μmol/kg doses of captopril and phosphinic acids **22q** and **22x**, and of a 10-fold higher dose of parent phosphinic acid **4**. These doses were chosen to show the duration of effect of each compound at comparable levels of maximal inhibition. The inhibition of the pressor response by captopril has diminished to insignificant levels after 30 min, while the

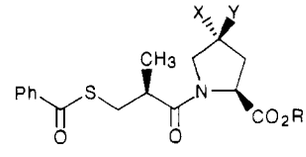
Table II. 4-Substituted Thioacyl Proline Derivatives



13



14



15, R = H
16, R = CH_3

no.	X	Y	formula[a]	mp, °C	$[\alpha]_D$, deg (*c*, solvent)	anal.[b]	ACE I_{50},[c] nM
1	H	H					23
13a	HO	H	$C_{11}H_{17}O_5NS$·DCHA	199–200	−79 (2, MeOH)	C, H, N, S	
14a			$C_9H_{15}O_4NS$	oil	−119 (2, MeOH)	C, H, N, S	26
16a			$C_{17}H_{21}NO_5S$	65–76	−158 (1, MeOH)	C, H, N, S	
13b	H	OH	$C_{13}H_{20}N_2O_6S$	141–143	−145 (1, MeOH)	C, H, N, S	
14b			$C_9H_{15}O_4NS$	oil	−114 (1, MeOH)	C, H, N, S	8.6
13c	MeO	H	$C_{12}H_{19}NO_5S$·DCHA	179–181	−62 (1, EtOH)	C, H, N, S	
14c			$C_{10}H_{17}NO_4S$·0.25H_2O	oil	−80 (1, EtOH)	C, H, N, S	12
13d	H	OMe	$C_{12}H_{19}NO_5S$·DCHA	173–175	−60 (1, EtOH)	C, H, N, S	
14d			$C_{10}H_{17}NO_4S$	oil	−88 (1, EtOH)	C, H, N, S	2.8
13e	H	OPh	$C_{17}H_{21}NO_5S$	136–138	−122 (1, $CHCl_3$)	C, H, N, S	
14e			$C_{15}H_{19}NO_4S$	125–135	−109 (1.2, $CHCl_3$)	C, H, N, S	97
14f	H	O-2-naphthyl	$C_{19}H_{21}NO_4S$·Ad	244–246	−30 (1, MeOH)	C, H, N	13
13g	OCH_2CH_2O		$C_{13}H_{19}NO_6S$·DCHA	187–189	−59 (1, EtOH)	C, H, N, S	
14g			$C_{11}H_{17}NO_5S$	131–133	−67 (1, EtOH)	C, H, N, S	2.9
13h	H	SMe	$C_{12}H_{19}NO_4S_2$·DCHA	171–173	−52 (1, EtOH)	C, H, N, S	
14h			$C_{10}H_{17}NO_4S_2$·Ad	198–201	−20 (1, EtOH)	C, H, N, S	3.0
13k	H	SPh	$C_{17}H_{21}NO_4S_2$	103–105	−92 (1, EtOH)	C, H, N, S	
14k			$C_{15}H_{19}NO_3S_2$·0.5H_2O	oil	−43 (1, EtOH)	C, H, N, S	8.0
14k·Ad			$C_{15}H_{19}NO_3S_2$·Ad	247–249	−57 (0.5, MeOH)	C, H, N, S	
14k·Arg			$C_{15}H_{19}NO_3S_2$·Arg	126–128	−44 (1, MeOH)	C, H, N, S	
15k			$C_{22}H_{23}NO_4S_2$	42–44	−37 (1, MeOH)	C, H, N	
15k·Ca			$C_{22}H_{22}NO_4S_2$·0.5Ca	235–237	nd	C, H, N	
15k·K			$C_{22}H_{22}NO_4S_2$·K·1.5H_2O	195–205	−14 (1, H_2O)	C, H, N, S, K	
13l	H	SOPh[d]	$C_{17}H_{21}NO_5S_2$·DCHA	198–200	−72 (1, EtOH)	C, H, N, S	
14l			$C_{15}H_{19}NO_4S_2$	47–50	−67 (1, EtOH)	C, H, N, S	46
13m	H	SO_2Ph	$C_{17}H_{21}NO_6S_2$·DCHA	236–238	−71 (1, MeOH)	C, H, N, S	
14m			$C_{15}H_{19}NO_5S_2$·0.25H_2O	59–62	−64 (1, EtOH)	C, H, N, S	28
14n	H	SPh-4-F	$C_{15}H_{18}FNO_3S_2$·Arg·H_2O	100 dec	−43 (1, H_2O)	C, H, N, S	17
14o	H	SPh-4-Cl	$C_{15}H_{18}ClNO_3S_2$·Arg	132 dec	−44 (1, H_2O)	C, H, N, S	11
13p	H	S-2-naphthyl	$C_{21}H_{23}NO_4S_2$·DCHA·0.5H_2O	157–159	−46 (1, EtOH)	C, H, N, S	
14p			$C_{19}H_{21}NO_3S_2$·0.75H_2O	48–51	−19 (1, EtOH)	C, H, N, S	96
13q	SCH_2CH_2S		$C_{13}H_{19}NO_4S_3$·DCHA	176–178	−56 (1, EtOH)	C, H, N, S	
14q			$C_{11}H_{17}NO_3S_3$	116–118	−44 (1, EtOH)	C, H, N, S	6.5
13r	H	Me	$C_{12}H_{19}NO_4S$·DCHA	182–184	−69.3 (1.8, $CHCl_3$)	C, H, N, S	
14r			$C_{10}H_{17}NO_3S$·Ad	214–216	−67.6 (1, MeOH)	C, H, N, S	2.0
13s	H	Ph	$C_{17}H_{21}NO_4S$·DCHA	179–181	−74 (1, EtOH)	C, H, N, S	
14s			$C_{15}H_{19}NO_3S$·0.25H_2O	52–55	−55 (1, EtOH)	C, H, N, S	30
14s·Ad			$C_{15}H_{19}NO_3S$·Ad·0.25H_2O	237–239	−37 (1, MeOH)	C, H, N, S	
14s·Arg			$C_{15}H_{19}NO_3S$·Arg·1.34H_2O	140–143	−35 (1, H_2O)	C, H, N, S	
13w	H	C_6H_{11}	$C_{17}H_{27}NO_4S$·DCHA	191–193	−64 (1, EtOH)	C, H, N, S	
14w			$C_{15}H_{25}NO_3S$·0.25H_2O	52–55	−55 (1, EtOH)	C, H, N, S	2.0
14w·Arg			$C_{15}H_{25}NO_3S$·Arg·0.25H_2O	126–129	−45 (1, EtOH)	C, H, N, S	
13y	H	CH_2Ph	$C_{18}H_{23}NO_4S$·DCHA	169–171	−80 (1, $CHCl_3$)	C, H, N, S	2.0
14y			$C_{16}H_{21}NO_3S$·0.25H_2O	43–48	−85 (1, EtOH)	C, H, N, S	
14aa	O=		$C_9H_{13}NO_4S$·Ad·0.25H_2O	188–190	−13 (1, MeOH)	C, H, N, S	16
13bb	MeO	OMe	$C_{13}H_{21}NO_6S$·DCHA	158–160	−69 (1, EtOH)	C, H, N, S	
14bb			$C_{11}H_{19}NO_5S$	108–110	−77 (1, EtOH)	C, H, N, S	7.2

[a] Amine salts as in Table I plus Arg, L-arginine, $C_6H_{14}N_4O_2$. [b] Indicated analyses (±0.4%) and IR and ^1H and ^{13}C NMR spectra were consistent with assigned structures. [c] Not determined for *S*-acyl and ester derivatives. [d] Mixture of sulfoxide diastereomers. nd = not determined.

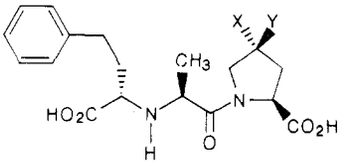
phosphinic acids continue to show significant inhibition after that time. While the substituted compounds are more potent than compound **4**, they do not appear to be significantly longer acting.

Table VI also showed the effect of oral doses of captopril and phosphinic acids **4**, **22q**, **22w**, and **22x** in the normotensive rat AI pressor response assay. The substituted phosphinic acids yielded comparable levels of inhibition at 10-fold lower doses than the unsubstituted parent **4**. The dose/response relationship for the phosphinic acids is much steeper than that of captopril, so that low doses of the mercaptan inhibitor show maximal inhibition comparable to 3- to 10-fold higher doses of the phosphinic acids.

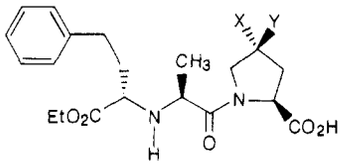
Determination of Kinetics of Enzyme Inhibition for Selected Inhibitors. In order to allow a more meaningful comparison of the effects of proline substitution on the affinity of inhibitors for ACE, we studied the kinetics of inhibition of ACE by selected compounds using the fluorometric assay previously developed.[21] Inhibitory constants (K_i) for the inhibitors were determined at pH 8.3 in the presence of 300 mM sodium chloride, the optimum conditions for cleavage of the hippurylhistidylleucine substrate (Table VII). Inhibitors were preincubated for 20 min to assure attainment of equilibrium. Under these

(21) Cheung, H. S.; Cushman, D. W. *Biochim. Biophys. Acta* **1973**, *293*, 451.

Table III. 4-Substituted Proline Carboxyalkyl Dipeptides



18



19

no.	X	Y	formula[a]	mp, °C	$[\alpha]_D$, deg (c, solvent)	anal.	I_{50}, nM
3	H	H					4.0[b]
18i	MeS	H	$C_{19}H_{26}N_2O_5S\cdot0.5H_2O$	am[c]	−45.3 (1.02, pyr)	C, H, N, S	5.0
18j	PhS	H	$C_{24}H_{28}N_2O_5S\cdot0.5H_2O$	am	+3.0 (1.05, MeOH)	C, H, N, S	2.0
18k	H	SPh	$C_{24}H_{28}N_2O_5S\cdot0.34H_2O$	am	−24.3 (1, pyr)	C, H, N, S	2.0
19k			$C_{26}H_{32}N_2O_5S\cdot0.24H_2O$	am	−32.7 (1.31, pyr)	C, H, N, S	*d*
19k·HCl			$C_{26}H_{32}N_2O_5S\cdot HCl$	140–142	+13.9 (1, MeOH)	C, H, N, S	
18q	SCH_2CH_2S		$C_{20}H_{26}N_2O_5S_2$	am	+2.0 (1.5, MeOH)	C, H, N, S	2.0
19q			$C_{22}H_{30}N_2O_5S_2$	am	−21.3 (1.1, pyr)	C, H, N, S	*d*
18s	H	Ph	$C_{24}H_{28}N_2O_5\cdot0.25H_2O$	am	−26.0 (1.02, pyr)	C, H, N	5.0
18w	H	C_6H_{11}	$C_{24}H_{34}N_2O_5\cdot0.2H_2O$	am	−64.7 (1.08, pyr)	C, H, N	5.0

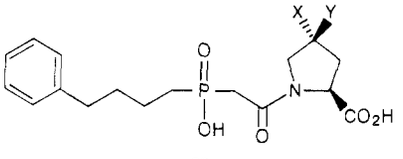
[a] Indicated analyses (±0.4%) and IR and ^1H and ^{13}C NMR spectra were consistent with assigned structures. [b] This work; for other determinations see ref. 3, 22. [c] Amorphous lyophilate. [d] Not determined for ester derivatives.

Table IV. Inhibition of the Angiotensin I Induced Pressor Response in Normotensive Rats after Intravenous and Oral Dosing of Carboxyalkyl Dipeptides

		dose, μmol/kg							
no.	route[a]	0.015	0.05	0.15	0.5	1.5	5.0	15.0	5.0
1	iv		18 ± 3	66 ± 4	87 ± 1	91 ± 2	93 ± 5		
1	po				42 ± 8	61 ± 10	79 ± 7	78 ± 6	93 ± 6
2	po				50 ± 6	84 ± 2	83 ± 2		
3	po						46 ± 11	85 ± 3	
3	iv	11 ± 2	67 ± 3	90 ± 5					
18i	iv	29 ± 7	69 ± 8	77 ± 12					
18j	iv	57 ± 8	94 ± 2						
18k	iv	20 ± 3	43 ± 11	85 ± 3					
18k	po				23 ± 18	31 ± 1	67 ± 0	78 ± 3	
19k	po				29 ± 10	81 ± 4	86 ± 2		
18q	iv	15 ± 6	81 ± 2	91 ± 1					
18q	po					25 ± 7	73 ± 9	93 ± 7	
19q	po			15 ± 6	41 ± 16	87 ± 3			
18w	iv	21 ± 19	48 ± 9	92 ± 4					

[a] Vehicle: 5% $NaHCO_3$.

Table V. 4-Substituted Phosphinic Acids



22

no.	X	Y	formula	mp, °C	$[\alpha]_D$, deg (c, solvent)	anal.	T_{50}, nM
4	H	H					180
22c·Li	MeO	H	$C_{18}H_{24}Li_2NO_6P\cdot H_2O$	am[b]	−29.3 (1, MeOH)	C, H, N, P	100
22d·Li	H	OMe	$C_{18}H_{24}Li_2NO_6P\cdot H_2O$	am	−38.3 (1, MeOH)	C, H, N, P	60
22h	H	SMe	$C_{18}H_{26}NO_5PS\cdot0.5H_2O$	48–51	−5.8 (1, pyr)	C, H, N, P	32
22i	MeS	H	$C_{18}H_{26}NO_5PS\cdot0.5H_2O$	48–51	−30.0 (1, MeOH)	C, H, N, P, S	29
22j	PhS	H	$C_{23}H_{28}NO_5PS$	62–65	−17.0 (1, MeOH)	C, H, N, P, S	17
22k·Li	H	S-Ph	$C_{23}H_{26}Li_2NO_5PS\cdot1.5H_2O$	am	−32.0 (1, MeOH)	C, H, N, P, S	20
22q	SCH_2CH_2S		$C_{20}H_{26}NO_5PS_2\cdot0.25H_2O$	110–120	−5.5 (1, MeOH)	C, H, N, P, S	2.0
22q·Li			$C_{19}H_{24}Li_2NO_5PS_2\cdot2H_2O$	am	−13.2 (1.35, MeOH)	C, H, N, P, S	4.0
22s	H	Ph	$C_{23}H_{28}NO_5P$	118–128	−11.5 (1, MeOH)	C, H, N, P	7.0
22t	Ph	H	$C_{23}H_{28}NO_5P\cdot0.5H_2O$	85–115	−23.1 (1, MeOH)	C, H, N, P	120
22w	H	C_6H_{11}	$C_{23}H_{34}NO_5P$	174–176	−43.1 (1, MeOH)	C, H, N, P	7.0
22x	C_6H_{11}	H	$C_{23}H_{34}NO_5P$	149–153	−24.0 (1, MeOH)	C, H, N, P	11
22x·Li			$C_{23}H_{32}Li_2NO_5P\cdot H_2O$	>250	−25.9 (1, MeOH)	C, H, N, P	4.0
22y·Li	H	CH_2Ph	$C_{24}H_{28}Li_2NO_5P\cdot1.5H_2O$	am	−33.6 (1.1, MeOH)	C, H, N, P	10

[a] Indicated analyses (±0.4%) and IR and ^1H and ^{13}C NMR spectra were consistent with assigned structures. [b] Amorphous lyophilate.

conditions, all of the inhibitors were found to exhibit purely competitive inhibition of ACE. We find that enalaprilat (**3**) is only slightly more active than captopril, whereas measurement of the K_i of these inhibitors at pH 7.5 showed enalaprilat to be somewhat more potent (captopril, K_i = 0.498 nM; enalaprilat, K_i = 0.214 nM).[22] Comparison of the K_i for **14k** with that of captopril shows

(22) Bull, H. G.; Thornberry, N. A.; Cordes, M. H. J.; Patchett, A. A.; Cordes, E. H. *J. Biol. Chem.* **1985**, *260*, 2952.

Table VI. Inhibition of the Angiotensin I Induced Pressor Response in Normotensive Rats after Intravenous and Oral Dosing of Phosphinic Acids

		dose, μmol/kg								
no.	route[a]	0.015	0.05	0.15	0.5	1.5	5.0	15.	50.	150
1	iv		18 ± 3	66 ± 4	87 ± 1	91 ± 2	93 ± 5			
1	po				42 ± 8	61 ± 10	79 ± 7	78 ± 6	93 ± 6	
4	iv				56 ± 4	86 ± 2	92 ± 1			
4	po							13 ± 3	47 ± 4	75 ± 2
22c	iv	23 ± 8	33 ± 12		73 ± 9					
22d	iv		16 ± 3	55 ± 2	90 ± 0					
22i	iv	7 ± 8	44 ± 7	27 ± 7	79 ± 4					
22j	iv		20 ± 10	19 ± 6	48 ± 8	69 ± 3	78 ± 6			
22h	iv			29 ± 10	69 ± 4	89 ± 1				
22k	iv			27 ± 4	34 ± 13	65 ± 5	88 ± 3			
22q	iv	20 ± 2	47 ± 2	80 ± 3	90 ± 8					
22q	po						28 ± 4	74 ± 3	94 ± 2	
22s	iv		16 ± 5	69 ± 8	97 ± 3					
22t	iv		17 ± 4	37 ± 4	76 ± 3					
22x	iv		21 ± 7	68 ± 5	87 ± 1					
22w	po						13 ± 3	72 ± 7		
22w	iv		26 ± 5	73 ± 2	84 ± 8					
22x	po						28 ± 9	71 ± 7		

[a] Vehicle: H_2O for Li salts; 5% $NaHCO_3$ for acids.

Table VII. Inhibitory Constants versus Rabbit Lung ACE for Selected Inhibitors

compd	K_i, nM	compd	K_i, nM
1	1.7 ± 0.5	**4**	15.
14k	0.4	**22x**	1.5
3	1.4		

that the 4-phenylthio substitution increases affinity for the enzyme by about 4-fold. The *trans*-cyclohexyl substituent on **22x** increases the affinity of this phosphinic acid 10-fold over that of the unsubstituted parent, compound **4**.

Discussion

Angiotensin-converting enzyme is known to hydrolyze at least two substrates of physiological importance: angiotensin I and bradykinin.[23] The P_2' position of these substrates is occupied by leucine and arginine residues, respectively. Studies on the hydrolysis of synthetic substrates by ACE[24] and inhibition of ACE by dipeptides[25] and hippuryl di- and tripeptides[26] have shown that the enzyme will tolerate considerable variation at the P_2' position of substrates, with aromatic amino acids being especially favored. Only residues with acidic side chains were not tolerated. The naturally occurring venom peptide ACE inhibitors such as BPP_{5a} provided the first indication that proline was especially well tolerated in the P_2' position of inhibitors.[27] Replacement of the C-terminal proline by other amino acids in mercaptans and carboxyalkyl dipeptides has been by far the most frequently explored aspect of ACE medicinal chemistry. Among captopril analogues, proline has been replaced by thiazolidinecarboxylic acids,[15,28] *N*-alkyl- and *N*-arylglycines,[29] and indoline- and isoquinolinecarboxylic acids.[30] Generally, these substitutions, when compared to the analogous proline-containing inhibitors, increase in vitro potency. Among carboxyalkyl dipeptides, similar substitutions lead to modest increases in in vitro potency, which sometimes have been associated with greater potency or longer duration of action in vivo.[31]

While clinical experience with captopril has shown that it can be an effective antihypertensive when give once or twice a day,[32] analogues of captopril with longer duration of action after oral administration might offer the potential for antihypertensive efficacy at longer dosing intervals. This goal has been sought by others, who have reported derivatives of captopril whose longer duration of activity may be due to slow conversion of a prodrug form to captopril in vivo.[33] Data from the present investigation demonstrate that substitution at the 4-position of proline by lipophilic groups increases the in vitro activity of captopril, and that the effect of this increase in potency is to increase the duration of action of the resulting compound in vivo. Thus, the *cis*- phenylthio-substituted compound **15k** and several analogues inhibit the AI-induced pressor response in rats 24 h after oral administration of a dose at which captopril shows no effect. In the form of its calcium salt, this compound has been evaluated extensively as an antihypertensive under the name zofenopril, and preliminary details of its pharmacology[34] and disposition[35] have appeared elsewhere. Incorporation of the same substituents into analogues of enalapril did not result in a marked change in either in vitro or in vivo activity. During the course of this investigation, we leaned that compound **19q** is identical with spirapril, which is being evaluated in clinical trials by others.[36]

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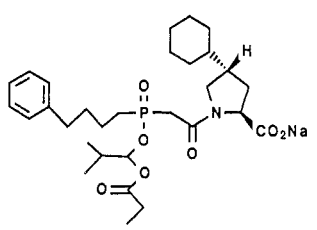
The most profound effect we discovered for 4-substituted prolines was the enhancement in activity in vitro for compounds in the phosphinic acid series such as **22q**, **22y**, **22s**, **22x**, and **22w**. This marked effect of proline replacement in a moderately active series of proline-based ACE inhibitors has also been described for carboxyalkyl ureas,[37] glutaryl prolines,[38] and (trifluoroketo)acyl prolines.[39] The large increases in activity for these substituted phosphinic acids relative to the parent **4** led to improved activity in vivo as well. However, the phenylthio-substituted compounds **22k** and **22j** suffered a decrease in in vivo activity relative to **4** despite increases in potency in vitro. The reason for this anomaly cannot lie solely in the nature of the 4-phenylthio substitution, since the *cis*-4-phenylthio substituent enhanced the activity of captopril in vivo and yielded an analogue of enalapril with a very similar profile of activity. Clearly, however, the presence of the phenylthio substituent and the phosphinic acid side chain has a detrimental effect on ACE inhibitory activity in vivo, which cannot be explained by the in vitro activity of the substituted compounds. Apparently, the properties of a given substituted proline compound that influence in vivo activity are not the same as those that influence affinity for rabbit lung ACE in vitro.

The poor oral activity of the 4-substituted phosphinic acids made these compounds unsuitable for further development as oral antihypertensive agents. However, the potential of these compounds was ultimately realized in a subsequent investigation that culminated in the design of fosinopril (**23**),[40] an (acyloxyl)alkyl prodrug of compound **22x**, which has entered clinical trials as an orally active antihypertensive. The details of this investigation will be reported in a separate paper.



23, Fosinopril

Experimental Section

All reactions were run under an atmosphere of nitrogen or argon. Standard reaction workup procedures consisted of evaporation of the reaction solvent where appropriate, dissolution of the residue in the indicated solvent, and washing with saturated sodium chloride solution or other solutions as indicated: bicarbonate (saturated $NaHCO_3$), bisulfate (5% $KHSO_4$), or citric acid (10%). Workup solvents were dried over magnesium sulfate and evaporated. 1-Adamantanamine (Ad), cyclohexylamine (CHA), and dicyclohexylamine (DCHA) salts were prepared by adding an excess of the amine to the acid in ethyl acetate or other indicated solvent and cooling to induce crystallization. Acids were liberated from these salts by shaking an ethyl acetate solution (or suspension) of the salt with 5% $KHSO_4$ and working up as usual. L-Arginine (Arg) salts were prepared by shaking a solution of the acid in $EtOAc/Et_2O$ with an aqueous solution of slightly less than 1 equiv of L-arginine and then lyophilizing the aqueous solution. Hydrogenations were performed in a Parr shaker at 30–45 psi over 10% Pd/C catalyst unless otherwise specified until gas uptake ceased. Esters were saponified by stirring with 1.1 equiv of 1 N sodium or potassium hydroxide solution, washing with ether, acidifying with concentrated HCl, and working up in the usual manner. Thin-layer chromatography was performed on Whatman MK6F or EM silica gel plates; mercaptans were visualized with nitroprusside reagent and other compounds were visualized with phosphomolybdic acid. Preparative chromatography was performed with Whatman LPS-1 silica gel by using the flash chromatography method. Melting points (uncorrected) were determined on a Thomas-Hoover apparatus. Infrared and proton and carbon NMR spectra were obtained for all final compounds. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter with a 10-cm microcell.

4-Substituted Prolines. Preparation of 6d, *N*-(Benzyloxycarbonyl)-*cis*-4-methoxy-L-proline, from 6b. Silver(I) oxide (40 g, 0.17 mol) and methyl iodide (40 mL, 0.18 mol) were added to a solution of **6b** (13.9 g, 0.052 mol) in 100 mL of acetone. The reaction temperature was maintained below 35 °C with a cooling bath. After being stirred overnight, the suspension was filtered, the filtrate evaporated, and the residue was subjected again to the same procedure. The residue (17.5 g) was saponified overnight and worked up (EtOAc) to yield a residue (15 g), which was converted to a CHA salt (EtOH). Crystallization (CH_3CN) yielded **6d** (8.8 g, 60%).

Preparation of 7d, *cis*-4-Methoxy-L-proline, from 6d. The free acid of **6d**, liberated from its CHA salt (6.8 g, 0.023 mol), was hydrogenated in 67% methanol/water (210 mL) under 3 atm of hydrogen to give **7d** (3.2 g, 93%) after crystallization from methanol/ether.

Preparation of 7e, *cis*-4-Phenoxy-L-proline, from 5. Phenol (3.3 g, 0.035 mol) and triphenylphosphine (9.3 g, 0.035 mol) were added to **5**[11] (8.4 g, 0.024 mol) in THF (75 mL). This solution was cooled to 0 °C and treated dropwise with diethyl azodicarboxylate (6.2 g, 0.035 mol) over a period of 1 h. After standing overnight at room temperature, the mixture was concentrated, and the residue was suspended in 100 mL of ether. The mixture was filtered, the filtrate was concentrated, and the residue was chromatographed (1:1 ether/pentane) to give the intermediate phenoxy derivative (7.5 g, 73%), which was not further characterized. Hydrogenation of 6.6 g of this material gave **7e** (2.8 g, 87%).

Preparation of 6j, *N*-(Benzyloxycarbonyl)-*trans*-4-(phenylthio)-L-proline Cyclohexanamine Salt, from 8b. Tosylate **8b**[7] (17.0 g, 0.039 mol) was added to a solution of thiophenol (8.4 mL, 0.082 mol) and sodium (1.9 g, 0.083 g-atom) in EtOH (100 mL), and the mixture was stirred for 20 h, concentrated, and worked up (dichloromethane). The residue was taken up in ether and filtered to remove unreacted **8b**. The residue was saponified, and the mixture worked up to give a residue, which was converted to the CHA salt of **6j** (10.7 g, 60%).

Preparation of 6h, *N*-(Benzyloxycarbonyl)-*cis*-4-(methylthio)-L-proline Cyclohexanamine Salt, from 8a. Tosylate **8a**[7] and methanethiol were reacted according to the procedure given for **6j** to give **6h** in 77% yield.

Preparation of 6i, *N*-(Benzyloxycarbonyl)-*trans*-4-(methylthio)-L-proline Cyclohexanamine Salt, from 8b. Tosylate **8b** and methanethiol were reacted according to the procedure given for **6j** to give **6i** in 77% yield.

Preparation of 6k, *N*-(Benzyloxycarbonyl)-*cis*-4-(phenylthio)-L-proline Cyclohexanamine Salt, from 8a. Tosylate **8a** and thiophenol were reacted according to the procedure given for **6j** to give **6k** in 65% yield.

Preparation of 6p, *N*-(benzyloxycarbonyl)-*cis*-4-(2-naphthalenylthio)-L-proline cyclohexanamine salt, from 8a was accomplished in 46% yield by substituting 2-naphthalenethiol for thiophenol in the procedure given for **6k**.

Preparation of 6m, *N*-(Benzyloxycarbonyl)-*cis*-4-(phenylsulfonyl)-L-proline 1-Aminoadamantane Salt, from 6k. Hydrogen peroxide (30%, 15.6 g, 0.14 mol) was added dropwise to a solution of **6k** (8.2 g, 0.023 mol) in 95 mL of acetic acid, and the mixture was stirred overnight. The solution was concentrated

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and worked up (CH_2Cl_2) to give 8.0 g of foam, which was converted to an $AdNH_2$ salt (EtOAc) (10.9 g, 88%).

Preparation of 6bb, *N*-(Benzyloxycarbonyl)-4,4-dimethoxy-L-proline Cyclohexanamine Salt, from 6aa. Ketone **6aa**[7] (7.8 g, 0.03 mol) and H_2SO_4 (0.6 mL) were dissolved in 96 mL of trimethyl orthoformate and 60 mL of CH_3OH and stirred overnight. Potassium carbonate (1.5 g) was added, and the mixture was concentrated. The residue was worked up ($CHCl_3$) to give 8.8 g of oil, which was saponified and worked up (CH_2Cl_2) to give an oil, which was converted to **6bb** as its CHA salt (8.5 g, 70%).

Preparation of 6g, 7-Aza-1,4-dioxaspiro[4.4]nonane-7,8-dicarboxylic Acid 7-(Phenylmethyl ester) Cyclohexanamine Salt, from 6aa. Ketone **6aa** (22.3 g, 0.085 mol) and *p*-toluenesulfonic acid (1.3 g) were dissolved in 93 mL of ethylene glycol and 1200 mL of benzene and refluxed for 7 h under a Dean–Stark trap. The mixture was partitioned, and the benzene layer worked up to give 27.5 g of residue, which was saponified and worked up to yield a residue, which was crystallized from ether, mp 101–103 °C, and then converted to its CHA salt (CH_3CN) (14.5 g, 42%).

Preparation of 6q, 7-Aza-1,4-dithiaspiro[4.4]nonane-7,8-dicarboxylic Acid 7-(Phenylmethyl ester) Cyclohexanamine Salt, from 6aa. Boron trifluoride ethyl ether (3 mL, 0.024 mol) was added to a cold (8 °C) solution of **6aa** (3.9 g, 0.014 mol) and ethanedithiol (3 mL, 0.036 mol) in 60 mL of CH_2Cl_2, and the mixture was stirred overnight at room temperature. Ice was added, and the mixture was worked up (CH_2Cl_2) to give 6.0 g of residue, which was saponified to an oil, which was converted to a CHA salt (4.4 g, 69%).

Preparation of 7j, *trans*-4-(Phenylthio)-L-proline, from 6j. Free acid **6j** (8.0 g, 0.021 mol) and concentrated HCl (48 mL) in acetic acid (125 mL) were heated at reflux for 1 h and concentrated, and the residue was taken up in 2-propanol and concentrated again. The residue was triturated with ether to give **7j** (5.4 g, 97%).

Compounds 7h, 7i, 7k, 7m, 7p, and 7q were also prepared by this procedure (concentrated HBr was used for **7k**, **7m**, and **7p**).

Preparation of 9i, *trans*-4-(Methylthio)-L-proline Methyl Ester Hydrochloride, from 7i. A solution of **7i** (1.5 g, 7.59 mmol) and concentrated HCl (0.5 mL) in methanol (40 mL) was stirred at −30 °C under argon for 2 h. The mixture was warmed to room temperature, stirred for 24 h, and worked up (CH_2Cl_2, bicarbonate). The final solution was not concentrated but was treated with HCl/methanol, whereupon a crystalline precipitate was formed, which was recrystallized (acetonitrile/ether) to give **9i** as a colorless solid (0.63 g, 39%).

Compounds **9j**, **9k**, **9q**, **9s**, and **9w** were also prepared by this method.

Preparation of 10k, *N*-[[(1,1-Dimethylethyl)oxy]carbonyl]-*trans*-4-(phenylthio)-L-proline (Trimethylsilyl)ethyl Ester, from 7k. Amino acid **7k** (4.9 g, 0.022 mol), triethylamine (6.57 mL, 0.047 mol), and BOC-ON (5.11 g, 0.021 mol) in 50% aqueous acetone (75 mL) were stirred for 24 h. The solution was diluted with water, washed with ether, acidified (0.5 M citric acid, 80 mL), and worked up (ether) to give the Boc derivative of **7k** as an oil (6.73 g, 97%). This substance, 2-(trimethylsilyl)ethanol (2.85 g, 0.024 mol), 4-(dimethylamino)pyridine (0.27 g, 0.0020 mol), and dicyclohexylcarbodiimide (4.98 g, 0.024 mol) in CH_2Cl_2 (70 mL) were stirred for 3 h, and the mixture was filtered, concentrated, and then worked up (ether; bicarbonate, citric acid washes) to give a residue, which was chromatographed (5% acetone/hexane) to give **10k** as an oil (7.53 g, 81%): TLC R_f 0.37 (20% acetone/hexane); ^{13}C NMR (15 MHz, $CDCl_3$) δ 176.9 (COO), 153.4 (OCON), 133.9 (arom C), 128.9, 131.8, 127.3 (arom CH), 80.0 (C), 63.2 (CH_2), 58.3 (CH), 52.3 (CH_2), 43.5 (CH), 36.9 (CH_2), 28.2 (CH_3), −1.6 (CH_3Si).

Compound **10q** was also prepared by the above procedure.

Preparation of 6z, *N*-(Benzyloxycarbonyl)-4-(phenylmethylidene)-L-proline, from 6aa. Benzyltriphenylphosphonium chloride (61.6 g, 0.16 mol) suspended in 150 mL of DMSO was added to a solution of sodium hydride (50% oil dispersion, 7.6 g, 0.16 mol) in 150 mL of DMSO, and the mixture was heated to 70 °C until homogeneous. The solution was cooled to 25 °C, and a solution of **6aa** (13.2 g, 0.025 mol) in 40 mL of DMSO was added over 20 min. The mixture was heated at 70 °C for 4 h, allowed to stand overnight, and poured into 1000 mL of ice water containing 10 g of $KHCO_3$. The solution was washed with ether, acidified, and then worked up ($CHCl_3$) to give 102 g of residue, which was triturated with ether. The ether was decanted from a solid residue and extracted with 5% $NaHCO_3$. The aqueous solution was acidified and worked up (ether) to yield 8.9 g of foam, which was converted to **6z** as its DCHA salt (CH_3CN) (9.5 g, 38%).

Preparation of 7y, *cis*-4-Benzyl-L-proline, from 6z. The free acid of **6z** (6.1 g, 0.018 mol) was hydrogenated over 5% Pd/C (2 g) in methanol to give **7y** (3.3 g, 89%).

Compounds **7g** and **7bb** were also prepared from **6g** and **6bb**, respectively, by the above procedure.

Preparation of 6u, *N*-(Benzyloxycarbonyl)-*cis*-4-hydroxy-*trans*-4-phenyl-L-proline, from 6aa. Phenylmagnesium bromide was prepared by the addition of bromobenzene (78.6 g, 0.50 mol) to magnesium turnings (12.6 g, 0.52 mol) in THF (300 mL). The cooled Grignard solution was added over 30 min to a mechanically stirred solution of ketone **6aa** (52.6 g, 0.20 mol) in THF (700 mL) at 0 °C. Additional THF (300 mL) was added when the reaction mixture, containing a thick white precipitate, became difficult to stir. The mixture was stirred at room temperature for 18 h, after which it was nearly homogeneous. Ammonium chloride solution (10%, 600 mL) was added slowly at 0 °C, and the mixture was stirred for 1 h, acidified (6 N HCl, to pH 2), and worked up (EtOAc) to give a brown gummy residue, which was dissolved in 2% NaOH. The aqueous solution was washed with ether, filtered through Celite, acidified, and worked up (EtOAc) to give a residue, which was crystallized from ether to give the carbinol **6u** (29.8 g, 44%). Additional product could be obtained by treating the concentrated Et_2O filtrate with excess 1-$AdNH_2$ in EtOH to give the salt, mp 243–245 °C, which was converted back to the free acid in the usual manner: ^1H NMR of **6u** (270 MHz, CD_3OD) δ 2.4–2.9 (m, 2 H), 3.79 (s, 2 H), 4.57 (d, J = 8.2, 1H), 5.14 (d, J = 0.8, 2 H), 7.3, 7.4 (2 br s, 10 H); ^{13}C NMR (15 MHz, $CDCl_3$) δ (43.7, 42.6), 60.0, 67.4 (CH_2), 58.7 (CH), (80.1, 79.5) (CO), 125.2, 127.9, 128.4 (arom CH), 141.2, 136.0 (arom C), (155.5, 155.0), 175.5 (C=O); IR (KBr) 1714, 3450 cm^{-1}.

Confirmation of the Stereochemistry of Carbinol 6u by Conversion to a Lactone. Carbinol **6u** (1.0 g, 2.9 mmol) and dicyclohexylcarbodiimide (0.73 g, 3.5 mmol) were stirred in THF (30 mL) overnight. The reaction mixture was filtered, and the filtrate was concentrated to an oil, which was chromatographed (20% acetone/hexane) to give 0.70 g (74%) of the corresponding lactone as a colorless glass: ^{13}C NMR of lactone (15 MHz, $CDCl_3$) δ 42.8, 54.2, 67.4 (CH_2), 59.1 (CH), 89.8 (CO), 125.4, 127.7, 128.0, 128.2, 128.6, 129.3 (arom CH), 132.7, 135.6 (arom C), 153.8, 169.8 (C=O); MS, m/e 323; IR (neat) 1716, 1800 cm^{-1}.

Preparation of 6v, *N*-(Benzyloxycarbonyl)-3,4-dehydro-4-phenyl-L-proline, from 6u. Carbinol **6u** (7.60 g, 22.3 mmol) was stirred for 18 h in dichloromethane (20 mL) and trifluoroacetic acid (20 mL). The reaction mixture was concentrated, and the residue was crystallized from ether to give olefin **6v** (6.43 g, 91%): ^1H NMR (100 MHz, $CDCl_3$) δ 4.65 (br s, 2 H), 5.16 (s, 2 H), 5.20 (s, 1 H), 6.24 (t, J = 2, 1 H), 7.36 (br s, 10 H).

Preparation of 7s, *cis*-4-Phenyl-L-proline Hydrochloride, from 6v. Hydrogenation of **6v** in the usual manner gave an oil, which was dissolved in 1 N HCl and evaporated to a residue, which was crystallized from acetonitrile/ether to give a 74% yield of **7s**: ^1H NMR (400 MHz, DMSO-d_6) δ 2.24 (dd, J = 8, 11, 1 H), 2.94 (ddd, J = 7.0, 7.0, 14, 1 H), 3.48 (t, J = 12, 1 H), 3.78 (m, 1 H), 4.05 (t, J = 8.0, 12, 1 H), 4.94 (dd, J = 8.0, 9.0, 1 H).

Preparation of 7t, *trans*-4-Phenyl-L-proline, from 6v. Olefin **6v** (9.0 g, 0.025 mol) in THF (150 mL) was added over 5 min to a solution of lithium metal (1.16 g, 0.167 g-atom) in ammonia (315 mL) at −78 °C. The blue solution was stirred for 10 min and quenched by the slow, careful addition of ammonium chloride (25 g). The colorless mixture was allowed to stand for 18 h in a water bath to evaporate all of the ammonia, and then acetic acid (150 mL) was added, and the mixture was stirred for 2 h. The mixture was filtered, and the filtrate was concentrated to an oily residue, which was taken up in water and concentrated again. The solid residue (4.13 g, 78%) was shown to contain a 94:6 mixture of *trans*- and *cis*-4-phenylproline by NMR analysis. Pure (>99% trans) **7t** was obtained after two recrystallizations from water: ^1H NMR (400 MHz, CD_3CO_2D) δ 2.49 (ddd, J = 10, 11, 14, 1 H), 2.73 (ddd, J = 2.6, 7.0, 14, 1 H), 3.57 (m, 1 H), 3.39 (t, J = 11, 1 H), 3.97 (dd, J = 7.0, 11, 1 H), 4.68 (dd, J = 2.6, 9.8).

Table VIII. ^{13}C NMR Chemical Shifts for Substituted Prolines

no.	X	Y	solvent	CO_2H	2	3	4	5	other
7c	MeO	H	D_2O	171.6	58.4	34.2	78.9	50.6	56.3
7e	H	OPh	CD_3CN	174.2	60.9	35.3	76.2	51.8	156.6, 117.2, 130.8, 120.1
7f	H	ONp	CD_3CN	181.1	62.0	38.3	79.1	52.6	155.7, 120.1, 135.1, 127.4, 127.6 124.8, 128.4, 129.7, 130.5, 109.6
7i	MeS	H	CD_3OD	170.8	60.0	35.8	43.3	52.1	14.5
7j	PhS	H	CD_3OD	170.6	60.0	35.8	45.0	52.2	134.1, 133.4, 130.6, 129.3
7k	H	SPh	CD_3OD	169.8	59.0	34.9	44.6	52.5	134.1, 133.2, 132.1, 129.2, 127.7
7m	H	SO_2Ph	DMSO	168.7	58.8	28.7	60.0	44.5	136.8, 134.9, 130.0, 128.3
7q	SCH_2CH_2S		DMSO	169.0	58.8	44.2	65.9	57.6	39.8, 39.5
7r	H	Me	CD_3OD	174.4	62.8	38.5	34.8	53.0	17.0
7s	H	Ph	DMSO	169.6	58.8	35.7	42.4	50.2	138.9, 128.6, 127.2
7t	Ph	H	CD_3OD	178.4	62.6	38.1	43.9	53.7	140.0, 130.9, 129.6, 129.1
7w	H	C_6H_{11}	CD_3CO_2D	no[a]	60.3	33.3	43.7	50.7	41.2, 32.3, 31.9, 26.5
7x	C_6H_{11}	H	D_2O	172.6	60.4	33.6	45.0	50.3	41.2, 32.1, 31.7, 26.7, 26.4
7y	H	CH_2Ph	DMSO	170.4	59.0	34.0	29.4	49.8	37.6, 139.7, 128.8, 128.6

[a] No = not observed.

Determination of the Stereochemistry of 7t by X-ray Diffraction. The following crystallographic parameters were determined for **7t**: a = 8.771 (1), b = 5.725 (1), and c = 9.968 (1) Å; β = 99.94 (1)°; v = 493.0 (2) Å^3; $P2_1$, z = 2; d_{obsd} = 1.31, d_{calcd} = 1.29 for $C_{11}H_{13}NO_2$. Cu Kα data (I = 1.5418 Å), $2\theta_{max}$ = 115°; number of observed reflections with $I \geq 3\sigma(I)$ = 710, R = 0.049, R_w = 0.068. Tables of atomic coordinates, bond distances, and angles and thermal parameters are included as supplementary material.

Preparation of 7w, *cis*-4-Cyclohexyl-L-proline, from 7s. Amino acid **7s** (3.8 g, 0.017 mol) was hydrogenated over platinum oxide (0.6 g) in ethanol (150 mL) to give **7w** (3.5 g, 88%).

Preparation of 7x, *trans*-4-Cyclohexyl-L-proline, from 7t. Amino acid **7t** was converted to its hydrochloride salt with ethanolic HCl and hydrogenated as described for **7w** to give **7x** in 91% yield: ^1H NMR (400 MHz, CD_3CO_2D) δ 1.00 (m, 2 H), 1.10–1.37 (m, 4 H), 1.65 (m, 5 H), 2.05–2.2 (m, 2 H), 2.40 (m, 1 H), 3.19 (t, J = 12, 1 H), 3.68 (dd, J = 8.0, 12), 4.73 (dd, J = 4.0, 10).

^{13}C NMR Spectra of 4-Substituted Prolines. Table VIII shows chemical shift assignments for most of the novel 4-substituted prolines prepared herein.

Mercaptoacyl Amino Acids. Preparation of 13k, (1*S*,4*S*)-1-[3-(Acetylthio)-2-methyl-1-oxopropyl]-4-(phenylthio)-L-proline, from 11 and 7k. A solution of amino acid **7k** (3.0 g, 0.0094 mol) in water (60 mL) at 5 °C was adjusted to pH 8.5 by the addition of 25% sodium carbonate. (*R*)-3-(Acetylthio)-2-methylpropanoyl chloride **11**[1b] (2.0 g, 0.011 mol) was added portionwise while the pH was maintained between 7.5 and 8.5 by the addition of sodium carbonate solution. After being stirred for 1 h, the mixture was extracted with ethyl acetate, and the aqueous solution was acidified (6 N HCl, pH 2) and worked up (EtOAc) to give a residue, which was treated with excess dicyclohexylamine in EtOAc to give **13k** as the DCHA salt after crystallization from acetonitrile. The salt was converted to the free acid to give **13k** (3.5 g, 59%).

Compounds **13a–e**, **13g**, **13h**, **13m**, **13p–s**, **13w**, **13y**, and **13bb** were all prepared by this procedure.

Preparation of 14d, (1*S*,4*S*)-1-(3-Mercapto-2-methyl-1-oxopropyl)-4-methoxy-L-proline, from 13d. A solution of **13d** (2.0 g, 0.0069 mol) in water (8.5 mL) and concentrated ammonia (3.5 mL) was stirred for 2 h and then extracted with ethyl acetate. The aqueous solution was acidified (6 N HCl, pH 2) and worked up to give **14d** (1.7 g, 100%) as an oil.

Compounds **14a–c**, **14e**, **14g**, **14l**, **14m**, **14p**, **14q**, **14s**, **14w**, **14y**, and **14bb** were all prepared by this procedure. Compounds **14h**, **14r**, and **14s** were prepared in a similar fashion and characterized as $AdNH_2$ salts; compounds **14s** and **14w** were additionally characterized as L-arginine salts.

Preparation of 13l, (1*S*,4*S*)-1-[3-(Acetylthio)-2-methyl-1-oxopropyl]-4-(phenylsulfinyl)-L-proline, from 13k. Sodium periodate (1.4 g, 0.0065 mol) in 12 mL of H_2O was added to a cooled solution of **13k** (2.0 g, 0.0054 mol) in 28 mL of methanol, and the mixture was stirred at room temperature overnight. The mixture was filtered, and the filtrate was concentrated and worked up (EtOAc). The residue was converted to a DCHA salt (EtOAc), which was crystallized from *i*-PrOH (1.9 g, 60%). ^{13}C NMR of the free acid showed it to be a 1:1 mixture of diastereomers.

Preparation of 14f, (1*S*,4*S*)-1-(3-Mercapto-2-methyl-1-oxopropyl)-4-(2-naphthalenyloxy)-L-proline 1-Adamantanamine Salt, from 16a. Diethyl azodicarboxylate (0.882 g, 5.1 mmol) in 2 mL of THF was added to a solution of ester **16a** (1.19 g, 3.38 mmol), 2-naphthol (0.733 g, 5.1 mmol), and triphenylphosphine (1.33 g, 5.1 mmol) in 10 mL of THF, and the mixture was stirred for 3 days. The reaction mixture was filtered, the filtrate was concentrated, and the residue was chromatographed (petroleum ether/ether) to yield 1.0 g (63%) of the intermediate ester. The ester was saponified and worked up to yield an intermediate oil product, which was converted to an $AdNH_2$ salt (EtOAc) (0.50 g, 46%).

Preparation of 14k, (1*S*,4*S*)-1-(3-Mercapto-2-methyl-1-oxopropyl)-4-(phenylthio)-L-proline, and the Corresponding 1-Adamantanamine and L-Arginine Salts, from 16a. Ester **16a** (16.0 g, 0.0455 mol) was added to a solution of *N*-(phenylthio)succinimide[16] (10.6 g, 0.0511 mol) and tri-*n*-butylphosphine (10.3 g, 0.0511 mol) in 15 mL of benzene, and the mixture was stirred for 22 h and then worked up (ether) to give 36.2 g oil. The oil was chromatographed (ether, alumina) to yield 16.0 g (67%) of the intermediate ester. The ester (8.5 g, 0.0154 mol) was saponified and worked up to yield 6.15 g of product, which could be converted to an $AdNH_2$ salt (MeOH/ether). The free acid liberated from this salt could be converted to an Arg salt.

Compounds **14n** and **14o** were prepared by the above procedure from *N*-[(4-chlorophenyl)thio]succinimide and N-[(4-fluorophenyl)thio]succinimide,[16] respectively.

Preparation of 15k, (1*S*,4*S*)-1-[3-(Benzoylthio)-2-methyl-1-oxopropyl]-4-(phenylthio)-L-proline, from 12 and 7k. Substitution of (*S*)-3-(benzoylthio)-2-methylpropanoyl chloride[15] (**12**) for **11** in the procedure described for **13k** gave **15k** in 76% yield.

Preparation of the Potassium Salt of 15k. Free acid **15k** (21.5 g, 0.05 mol) in absolute ethanol (100 mL) and 1 N potassium hydroxide in ethanol (50 mL) were combined, chilled, and filtered to give the potassium salt as an initial ethanol solvate, mp 212–213 °C, which after air-drying for 2 days gave the ethanol-free potassium salt of **15k**: ^1H NMR (270 MHz, DMSO-d_6) δ 1.01 (d, 3 H), 1.86 (m, 1 H), 2.56 (t, 1 H), 2.75 (m, 1 H), 2.95 (m, 1 H), 3.1–3.3 (m, 2 H), 3.65 (t, 1 H), 3.97 (d, 2 H), 7.20–7.93 (m, 10 H).

Preparation of the Calcium Salt of 15k. The free acid of **15k** (0.859 g, 0.002 mol) was dissolved in ethanol (50 mL) and combined with a suspension of calcium oxide (0.056 g, 0.001 mol) in water (50 mL), and the mixture was stirred overnight. The mixture was concentrated to remove ethanol, washed with ether, and lyophilized to give the calcium salt of **15k**.

Preparation of 14aa, (*S*)-1-(3-Mercapto-2-methyl-1-oxopropyl)-4-oxo-L-proline 1-Adamantanamine Salt, from 14bb. A solution of ketal **14bb** (0.40 g, 1.4 mmol) in 8 mL of 1 N HCl was allowed to stand overnight, saturated with NaCl, and worked up (CH_2Cl_2). The residue was converted to the $AdNH_2$ salt in CH_3CN (0.36 g, 69%).

Preparation of 16a, (1*S*,4*R*)-1-[3-(Benzoylthio)-2-methyl-1-oxopropyl]-4-hydroxy-L-proline Methyl Ester. A solution of *trans*-4-hydroxy-L-proline (32.7 g, 0.25 mol) in water (250 mL) was adjusted to pH 9.3 by addition of 10% sodium carbonate. The solution was warmed to 30 °C, and to it was added a solution of (*R*)-3-(benzoylthio)-2-methylpropanoyl chloride (**12**)

(63.0 g, 0.26 mol) in toluene (75 mL) over 1 h while the pH was maintained at 9.0 by addition of 10% sodium carbonate. After being stirred for 1.5 h, the two-phase mixture was separated, the aqueous phase was cooled and acidified (concentrated HCl), and the crystalline product was filtered to give the free acid (71.4 g, 85%). A portion of the acid (33.7 g, 0.10 mol) was refluxed in methanol (1 L) with *p*-toluenesulfonic acid (0.5 g) for 18 h. The mixture was worked up (ether), and the residue was triturated with petroleum ether to give crystalline **16a** (27.3 g, 78%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.28 (d, 3 H), 2.07 (m, 1 H), 2.30 (m, 2 H), 2.90 (q, 1 H), 3.22 (ddd, 2 H), 3.73 (s, 3 H), 3.75 (d, 2 H), 4.57 (br, 1 H), 4.63 (t, 1 H), 7.41–7.97 (m, 5 H).

Carboxyalkyl Dipeptides. Preparation of 18k, (1*S*,4*S*)-1-[*N*-(1-Carboxy-3-phenylpropyl)-L-alanyl]-4-(phenylthio)-L-proline, from 17 and 9k. (*S*,*S*)-Ethyl α-[(1-carboxyethyl)amino]benzenebutanoate (**17**)[17] (500 mg, 1.8 mmol) and ester **9k** (540 mg, 2.0 mmol) were stirred in DMF (8 mL) at 0 °C and diphenyl phosphorazidate (0.43 mL, 1.8 mmol) was added dropwise, followed by dropwise addition of triethylamine (0.55 mL, 4.0 mmol) in DMF (1.5 mL) over 10 min. The mixture was stirred at 0 °C for 3 h and at room temperature for 19 h. The mixture was worked up (EtOAc, bicarbonate wash), and the residue was chromatographed (preparative TLC, CH_2Cl_2/35% EtOAc/5% acetone) to give 0.55 g (62%) of the diester of **18k** as an oil, R_f 0.37 (EtOAc), used without further purification. The diester was stirred in EtOH (7 mL) and 1 N NaOH (2.4 mL) for 4 h, and the reaction mixture was concentrated. The residue was applied to an AG50W-X2 acidic ion exchange column (30 mL), and the column washed with several volumes of water. Elution with 3% aqueous pyridine and lyophilization of the product-containing fractions gave 455 mg (90%) of **18k** as an amorphous solid, R_f 0.56 (*n*-BuOH/16% AcOH/16% H_2O): ^{1}H NMR (400 MHz, DMSO) δ 1.16 (d, 3 H), 1.84 (m, 2 H), 2.62 (m, 2 H), 2.72 (m, 1 H), 3.13 (t, 1 H), 3.30 (t, 1 H), 3.74 (M, 1 H), 3.91 (m, 2 H), 4.18 (dd, 2 H), 4.35 (t, 1 H), 7.15–7.45 (m, 10 H).

Compounds **18i**, **18j**, **18q**, **18s**, and **18w** were also prepared by this method.

Preparation of 19k, (1*S*,4*S*)-1-[*N*-[1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-4-(phenylthio)-L-proline, from 17 and 10k. Compound **10k** (18.7 g, 0.044 mol) and *p*-toluenesulfonic acid (10.5 g, 0.055 mol) in acetonitrile were refluxed for 1.75 h. The reaction mixture was washed with saturated $NaHCO_3$ and extracted with 1 N HCl. The aqueous solution was made basic with $NaHCO_3$ and worked up (ether) to give the amino acid (trimethylsilyl)ethyl ester as an oil (8.4 g, 59%). A solution of this ester (7.34 g, 23 mmol), **17** (5.96 g, 19 mmol), dicyclohexylcarbodiimide (3.9 g, 19 mmol), 1-hydroxybenzotriazole hydrate (2.56 g, 19 mmol), and diisopropylethylamine (3.3 mL, 19 mmol) in THF (15 mL) was stirred for 1.5 h at 0 °C and at room temperature for 18 h. The reaction mixture was filtered and concentrated, and the residue was worked up (EtOAc, bicarbonate wash). The residue was flash chromatographed (CH_2Cl_2/5% acetone) to give 7.74 g (70%) of the (trimethylsilyl)ethyl ester of **19k**. A solution of this ester (1.49 g, 2.5 mmol) and tetrabutylammonium fluoride trihydrate (1.60 g, 5 mmol) in DMF (4 mL) was stirred for 15 min and then worked up (EtOAc, water wash). The residue was applied to an AG50W-X2 acidic ion exchange column (50 mL) (with 50% EtOH and 10% H_2SO_4 to aid solubility), and the column was washed with 3 volumes of water. Elution with 3% aqueous pyridine and lyophilization gave 0.99 g (79%) of **19k**, R_f 0.24 (EtOAc/5% pyridine/2% AcOH/3% H_2O).

Compound **19q** was also prepared by this method.

Phosphinic Acids. Preparation of 22t, *trans*-1-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-4-phenyl-L-proline, from 7t and 20. Carbonyldiimidazole (584 mg, 3.6 mmol) was added at 0 °C to a solution of [ethoxy(4-phenylbutyl)phosphinyl]acetic acid (**20**)[18] (853 mg, 3.0 mmol) in acetonitrile (50 mL). After 1 h, triethylamine (303 mg, 3.0 mmol) and *trans*-4-phenyl-L-proline (**7t**) (574 mg, 3.0 mmol) were added, and the mixture stirred at room temperature for 48 h. The reaction mixture was concentrated, and the residue was worked up (EtOAc, bisulfate, water and brine wash) to give 1.3 g of crude product, which was flash chromatographed (CH_2Cl_2/5% MeOH/5% AcOH) to give 1.1 g (80%) of the ethyl ester of **22t**, R_f 0.5 (CH_2Cl_2/5% MeOH/5% AcOH). This intermediate (246 mg, 0.54 mmol) and bromotrimethylsilane (230 mg, 1.5 mmol) were stirred overnight in CH_2Cl_2 (10 mL). The reaction mixture was concentrated, dissolved in 0.3 N NaOH, and washed with ether. The mixture was acidified and worked up (EtOAc) to give a semisolid, which was triturated with ether to give 170 mg of **22t**, R_f 0.25 (CH_2Cl_2/5% MeOH/5% AcOH): ^{1}H NMR (270 MHz, CD_3OD) δ 1.65 (br, 4 H), 1.83 (br, 2 H), 2.35 (d, 1 H), 2.50 (m, 1 H), 2.62 (t, 2 H), 2.97 (dd, 2 H), 3.58 (m, 1 H), 3.69 (t, 1 H), 4.18 (t, 1 H), 4.59 (d, 1 H), 7.00–7.35 (m, 10 H).

Compounds **22d**, **22h–k**, **22s**, **22t**, and **22w–y** were also prepared by this method.

Preparation of 22q, (*S*)-7-[[Hydroxy(4-phenylbutyl)-phosphinyl]acetyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic Acid and Its Lithium Salt, 22q·Li, from 21 and 9q. [Hydroxy(4-phenylbutyl)phosphinyl]acetic acid (**21**)[18] (3.0 g, 12 mmol) and carbonyldiimidazole (1.9 g, 12 mmol) in CH_2Cl_2 (50 mL) were stirred at 0 °C for 1 h. Triethylamine (3.5 g, 35 mmol) and **9q** (3.0 g, 12 mmol) were added, and the mixture was stirred at room temperature for 16 h, concentrated, and worked up (EtOAc, phosphate wash) to give 4.9 g (92%) of the methyl ester of **22q**, R_f 0.58 (CH_2Cl_2/10% MeOH/10% AcOH). A solution of the methyl ester (4.9 g, 11 mmol) in 1 N NaOH was stirred for 30 min. The reaction mixture was washed with ether, acidified, and worked up (EtOAc, using large volumes). The extraction solution was dried and only partially concentrated to allow crystallization of the product **22q** (4.0 g, 84%), R_f 0.33 (CH_2Cl_2/10% MeOH/10% AcOH). The lithium salt **22q**·Li was prepared by loading a solution of **22q** in excess 1 N LiOH onto a column of AG-50W X8 resin (Li^+ form), eluting with distilled water, and lyophilizing the product-containing fractions: ^{1}H NMR (270 MHz, CD_3OD) δ 1.69 (br, 4 H), 1.88 (br, 2 H), 2.64 (ddd, 2 H), 2.66 (t, 2 H), 3.00 (dd, 2 H), 3.40 (s, 4 H), 4.13 (dd, 2 H), 4.50 (t, 1 H); ^{13}C NMR (15 MHz, D_2O; C–P coupling constants (Hz) in parentheses) δ 178.3 (COO^-), 167.9 (CO), 143.8 (arom C), 129.3, 128.3, 126.5 (arom CH), 66.4 (C), 63.2 (CH_2), 61.9 (CH), 45.7 (CH_2), 40.4 (CH_2), 39.8 (CH_2), 38.8 (CH_2, 76), 35.7 (CH_2), 33.3 (CH_2, 17), 31.1 (CH_2, 94), 22.6 (CH_2).

Compound **22c** was also prepared by this method.

Determination of Inhibition Constants. Enzyme incubations[21] were carried out by combining a substrate/buffer solution (Hip-His Leu, sodium salt, 12.5 mM; phosphate buffer (K_2HPO_4/KH_2PO_4), 250 mM; NaCl, 750 mM; pH 8.3; 0.1 mL), inhibitor or H_2O (0.05 mL), and purified ACE (0.10 mL, final concentration 0.026 nM). Inhibitors were preincubated with the enzyme for 20 min before the reaction was initiated by addition of the substrate. The reactions were incubated at 37 °C for 10 or 30 min and stopped by adding 0.280 N NaOH (1.45 mL). The enzymic product was converted to a fluorescent derivative by adding 1% phthalaldehyde in MeOH (0.1 mL), incubating for 10 min, adding 3 N HCl (0.2 mL), and incubating for 30 min. Relative fluorescent intensity (RFI) was measured with a Ferrand System 3 spectrofluorometer at excitation wavelength 364 nm, emission wavelength 486 nm, maximum gain, and 2-mm slit widths. The rate of product formation was determined from a standard curve of RFI versus His-Leu concentration. Inhibition constants were determined from double-reciprocal plots of initial reaction rates versus substrate concentration in the absence of the inhibitor and in its presence at the standard I_{60} concentration.

Inhibition of Angiotensin I Induced Pressor Response, Standard Protocol. Male Sprague–Dawley rats (225–275 g) were equipped with indwelling abdominal aorta and vena caval catheters by using a modification of the method of Weeks and Jones.[41] The animals were allowed to recover for at least 2 weeks before experimentation, during which they were housed individually and maintained on rat chow and tap water ad libitum. On the day of experimentation, aortic blood pressures were monitored directly by pressure transducers and recorded on a Beckman Dynograph. The venous catheter was used for drug injections. During all experiments the rats were conscious and unrestrained in their cages. Pressor responses were obtained for AI (310 ng/kg, iv) and angiotensin II (AII) (100 ng/kg iv) before administration of the compounds. For intravenous testing, compounds were admin-

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

istered in 0.1 mL of water or 5% $NaHCO_3$, and AI and AII pressor responses were evaluated for up to 70 min. For oral testing, compounds were administered in 0.75 mL of water, 5% $NaHCO_3$, or 1% agar suspension and AI and AII pressor responses were evaluated for up to 280 min. Maximum percent inhibition was determined as the mean of the responses for four animals per dose.

Inhibition of Angiotensin I Induced Pressor Response, 24 h Predosing. Male Sprague–Dawley rats were prepared as described for the experiments above. The rats were fasted for 24 h before and 2 h after the oral administration of water (control group) or drug. Twenty-four hours after drug administration, each rat was given AI (310 ng/kg), and the pressor response was recorded as described above. The mean of the responses for each drug-treated group were compared to the responses of groups given only water.

Registry No. **5**, 13500-53-3; **6b** (free acid), 13504-86-4; **6d** (free acid), 75176-20-4; **6d** (methyl ester), 75176-19-1; **6d**·CHA, 75176-21-5; **6g** (free acid), 75776-49-7; **6g** (methyl ester), 16217-17-7; **6g**·CHA, 75776-50-0; **6h**·CHA, 113949-39-6; **6i**·CHA, 113949-41-0; **6j** (free acid), 83552-11-8; **6j**·CHA, 83552-12-9; **6k**·CHA, 75176-29-3; **6m** (free acid), 94898-97-2; **6m**·$AdNH_2$, 94898-98-3; **6p**·CHA, 81806-15-7; **6g** (free acid), 75776-77-1; **6g** (methyl ester), 75776-76-0; **6q**·CHA, 75776-78-2; **6a** (free acid), 78464-03-6; **6u** (lactone), 113949-45-4; **6u**·$AdNH_2$, 78464-14-9; **6v** (free acid), 82087-66-9; (*E*)-**6z** (free acid), 114029-40-2; (*Z*)-**6z** (free acid), 114029-41-3; (*E*)-**6z**·DCHA, 114029-42-4; (*Z*)-**6z**·DCHA, 114029-43-5; **6aa** (free acid), 64187-47-9; **6bb** (free acid), 75776-55-5; **6bb** (methyl ester), 75776-54-4; **6bb**·CHA, 75785-37-4; **7d** (free acid), 75176-22-6; **7e** (free acid), 113949-37-4; **7g** (free acid), 75776-51-1; **7h**·HCl, 113949-42-1; **7i**·HCl, 83552-27-6; **7j**·HCl, 83552-13-0; **7k**·HBr, 105107-84-4; **7k** (*N*-BOC derivative), 83623-88-5; **7m**·HBr, 94898-99-4; **7p**·HBr, 81806-16-8; **7q**·HCl, 83552-41-4; **7s**·HCl, 82087-67-0; *trans*-**7t** (free acid), 96314-26-0; *cis*-**7t** (free acid), 103290-40-0; **7w**·HCl, 82087-68-1; **7x**·HCl, 90657-55-9; **7y** (free acid), 82087-73-8; **7bb** (free acid), 75776-56-6; **8a**, 57653-38-0; **8b**, 77345-53-0; **9i**·HCl, 83552-28-7; **9j**·HCl, 83552-14-1; **9k**·HCl, 83552-04-9; **9q**·HCl, 83552-42-5; **9s**·HCl, 83552-24-3; **10k**, 113949-43-2; **10k** (BOC-deblocked), 113949-53-4; **10q**, 113949-44-3; **11**, 74345-73-6; **12**, 74654-91-4; **13a**·DCHA, 114127-21-8; **13b** (free acid), 114029-44-6; **13c**·DCHA, 75197-32-9; **13d**·DCHA, 75246-74-1; **13e** (free acid), 113949-46-5; **13g**·DCHA, 75785-36-3; **13h**·DCHA, 113975-20-5; **13k** (free acid), 75176-36-2; **13k**·DCHA, 75197-36-3; **13l**·DCHA (diastereomer 1), 114029-50-4; **13l**·DCHA (diastereomer 2), 114029-52-6; **13m**·DCHA, 94899-01-1; **13p**·DCHA, 81814-77-9; **13q**·DCHA, 75776-81-7; **13r**·DCHA, 113949-48-7; **13s**-DCHA, 82165-74-0; **13w**·DCHA, 82092-95-3; **13y**·DCHA, 82092-98-6; **13bb**·DCHA, 75785-38-5; **14a** (free acid), 113564-51-5; **14b** (free acid), 113564-58-2; **14c** (free acid), 75176-14-6; **14d** (free acid), 75197-34-1; **14e** (free acid), 113949-49-8; **14f** (free acid), 81814-74-6; **14f** (methyl ester, *S*-benzoyl derivative), 81814-75-7; **14f**·$AdNH_2$, 81872-08-4; **14g** (free acid), 75776-53-3; **14h** (free acid), 113564-63-9; **14h**·$AdNH_2$, 114029-46-8; **14k** (free acid), 75176-37-3; **14k** (methyl ester, *S*-benzoyl derivative), 113949-52-3; **14k**·$AdNH_2$, 114029-53-7; **14k**·Arg, 81872-09-5; **14l** (free acid, diastereomer 1), 114029-45-7; **14l** (free acid, diastereomer 2), 114029-55-9; **14m** (free acid), 94899-02-2; **14n** (free acid), 81814-88-2; **14n**-Arg, 81872-11-9; **14o** (free acid), 81814-64-4; **14o**·Arg, 81814-65-5; **14p** (free acid), 81814-78-0; **14q** (free acid), 75776-82-8; **14r** (free acid), 113949-50-1; **14r**·$AdNH_2$, 113949-51-2; **14s** (free acid), 82092-93-1; **14s**·$AdNH_2$, 114029-47-9; **14s**·Arg, 114029-48-0; **14w** (free acid), 82092-96-4; **14w**·Arg, 82165-75-1; **14y** (free acid), 82092-99-7; **14aa** (free acid), 77282-50-9; **14aa**·$AdNH_2$, 77282-51-0; **14bb** (free acid), 75802-72-1; **15k** (free acid), 81872-10-8; **15k**·$^1/_2Ca$, 81938-43-4; **15k**·k, 81938-42-3; **16a**, 80586-33-0; **16a** (free acid), 81814-84-8; **17**, 82717-96-2; **18i**, 83552-30-1; **18j**, 83602-04-4; **18k**, 83552-06-1; **18k** (ethyl methyl ester), 83552-05-0; **18q**, 83602-05-5; **18s**, 83552-23-2; **18w**, 83552-26-5; **19k** (free acid), 83551-92-2; **19k** (trimethylsilyl)ethyl ester, 113949-54-5; **19k**·HCl, 114029-54-8; **19q** (free acid), 83647-97-6; **20**, 83623-46-5; **21**, 83623-61-4; **22c** (free acid), 113949-61-4; **22c**·2Li, 113949-60-3; **22d** (free acid), 113949-62-5; **22d**·2Li, 83624-12-8; **22h** (free acid), 83624-60-6; **22i** (free acid), 83745-60-2; **22j** (free acid), 113949-57-8; **22k**·2Li, 113949-58-9; **22q** (free acid), 83623-49-8; **22q** (methyl ester), 83623-48-7; **22q**·2Li, 83623-50-1; **22s** (free acid), 83624-30-0; **22t** (free acid), 113949-56-7; **22t** (*P*-ethyl ester), 113949-55-6; **22w** (free acid), 83624-20-8; **22x** (free acid), 95399-71-6; **22x**·2Li, 113975-21-6; **22y**·2Li, 113949-59-0; ACE, 9015-82-1; PhOH, 108-95-2; PhSH, 108-98-5; MeSH, 74-93-1; $HOCH_2CH_2OH$, 107-21-1; $HSCH_2CH_2SH$, 540-63-6; $Me_3SiCH_2CH_2OH$, 2916-68-9; $PhCH_2P^+Ph_3{\cdot}Cl^-$, 1100-88-5; *N*-(benzyloxycarbonyl)-*cis*-4-phenoxy-L-proline benzyl ester, 113949-36-3; 2-naphthalenethiol, 91-60-1; 2-naphthol, 135-19-3; *N*-(phenylthio)succinimide, 14204-24-1; *N*-(*P*-chlorophenylthio)succinimide, 42839-20-3; *N*-(*P*-fluorophenylthio)succinimide, 779-73-7; *trans*-4-hydroxy-L-proline, 51-35-4.

Supplementary Material Available: Structure factor tables for compound **7t** (2 pages). Ordering information is given on any current masthead page.

Binding of Steroids to the Progestin and Glucocorticoid Receptors Analyzed by Correspondence Analysis

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The relative binding affinities of over 30 steroids have been measured for the cytosol glucocorticoid receptor (GR) of thymus, liver, and hepatoma tissue culture cells and for progestin, androgen, and mineralocorticoid receptors. The data have been analyzed by correspondence analysis to reveal the singularities among the receptors of different hormonal classes, the similarities in GR of different origins, and the different specificities of the ligands. Additional data on new steroids have been injected into the system as well as results on a further parameter, namely the induction of tyrosine aminotransferase (TAT) activity, to illustrate the power and flexibility of the methodology. The analysis has confirmed previous correlations between GR binding and TAT response but also highlighted the antiglucocorticoid activity of progestins. This method should prove to be a substantial aid to the interpretation of increasingly complex data, in particular with regard to the action of existing and newly synthesized steroids on glucocorticoid systems of differential sensitivity.

In the early 1970s we set up a screening system for measuring the relative binding affinities (RBAs) of test steroids to the estrogen (ER), progestin (PR), androgen (AR), glucocorticoid (GR), and mineralocorticoid (MR) receptors. This system yields two types of information: first, whether a steroid can recognize one or more receptors (specificity profile) and second, from data measured at short and long incubation times, whether interaction kinetics are faster or slower than for reference hormones.[1-3]

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