Synthesis and Pharmacological Activity of Angiotensin Converting Enzyme Inhibitors: *N*-(Mercaptoacyl)-4-substituted-(*S*)-prolines

Elizabeth M. Smith,*† Gerald F. Swiss,† Bernard R. Neustadt,† Elijah H. Gold,† Jane A. Sommer,‡ Arthur D. Brown,‡ Peter J. S. Chiu,‡ Rosa Moran,‡ Edmund J. Sybertz,‡ and Thomas Baum§

Departments of Medicinal Chemistry and Pharmacology, Schering-Plough Corporation, Bloomfield, New Jersey 07003.
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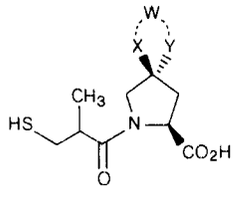
The synthesis of a series of *N*-(mercaptoacyl)-4-substituted-(*S*)-prolines (**2** and **3**) is described. These compounds were evaluated in vitro for inhibition of angiotensin-converting enzyme (ACE), and selected compounds were evaluated in vivo for ACE inhibition. The most potent compounds in vitro are **108**, **109**, **111**, **114**, and **116**, having relative potencies of 1.0, 1.0, 1.3, 1.1, and 2.6 as compared to the potency of captopril. The most potent compounds in vivo intravenously are **108**, **111**, **114**, **116**, **117**, and **97**.

The design and development of captopril[1] (**1**) led to an exciting new treatment for hypertension; captopril exerts its effect by inhibition of angiotensin-converting enzyme (ACE).[1-3] Angiotensin converting enzyme inhibitors have become important therapeutic agents for the treatment of hypertension and congestive heart failure.[4] A review of the current status of the design and development of angiotensin converting enzyme inhibitors discusses recent captopril analogues.[2] We now describe our initial studies of the proline ring in (mercaptoacyl)prolines, in which we identified some of the spatial requirements at the S_2' subsite[2] of ACE (Figure 1) by introduction of mono- and disubstitution (**2** and **3**) at the 4-position. A series of compounds was synthesized and evaluated for angiotensin converting enzyme inhibitory activity in vitro and in vivo and for antihypertensive activity.[5]



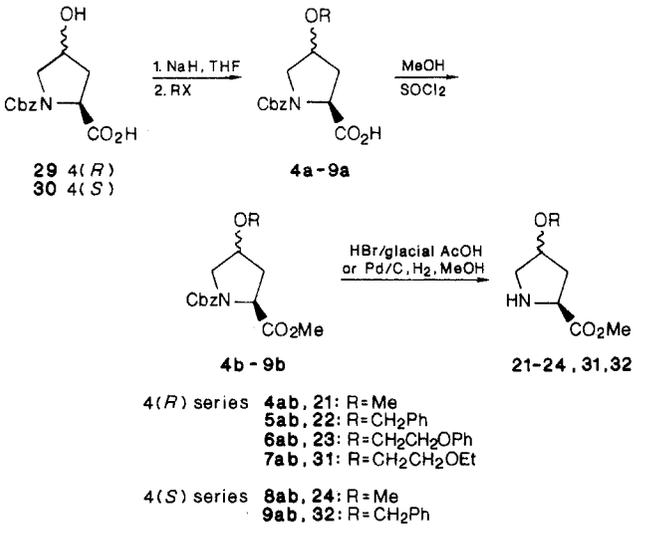
2a: X = H; W = no bond
b: Y = H; W = no bond
W = no bond
3a: W = bond
b: W = no bond

Chemistry. A series of *N*-(mercaptoacyl)-(*S*)-proline derivatives was produced, wherein the proline 4-position is trans (**2a**) or cis monosubstituted (**2b**) or disubstituted (**3**). The synthesis begins with the preparation of a series of *N*-[(phenylmethoxy)carbonyl]-4-substituted-(*S*)-proline esters, which were deblocked to give the respective 4-substituted (*S*)-proline esters. The properties of these compounds are given in Tables I and II, and the synthetic methods are described below.

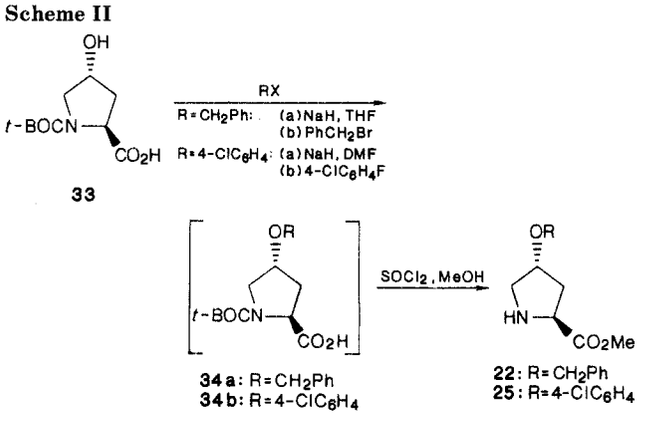
Method 1. Reaction of the *N*-[(phenylmethoxy)-carbonyl]-4(*R*)- or -4(*S*)-hydroxy-(*S*)-proline (**29** and **30**)[6-9] with sodium hydride and then alkyl halide furnished the 4-alkoxy derivatives (**4a–9a**), which were esterified and deblocked, as shown in Scheme I. For certain alkoxy or arylalkoxy groups, an alternative route (see the Experimental Section) using *N*-[(1,1-dimethylethoxy)-carbonyl]-4(*R*)-hydroxy-(*S*)-proline (**33**) was used (Scheme II).

Method 2. A new approach to the synthesis of 4(*R*)-(4-chlorophenoxy)-(*S*)-proline methyl ester (**25**) was developed, as shown in Scheme II. *N*-[(1,1-Dimethylethoxy)carbonyl]-4(*R*)-hydroxy-(*S*)-proline (**33**) was treated with sodium hydride in DMF followed by heating with 4-chlorofluorobenzene to give **34b**, which was deblocked and esterified to **25**.

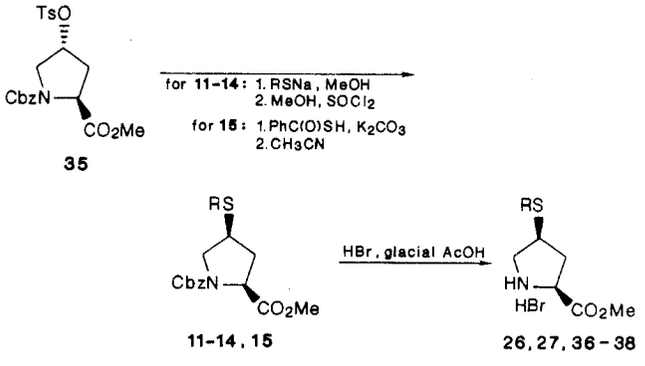
Scheme I



Scheme II



Scheme III



11, 26: R = Me; **12, 36**: R = c-C_6H_{11}; **13, 37**: R = CH_2Ph; **14, 38**: R = Ph; **15, 27**: R = C(O)Ph

† Department of Medicinal Chemistry.
‡ Department of Pharmacology.
§ Deceased February 1985.

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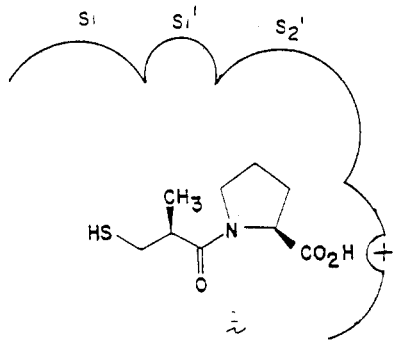
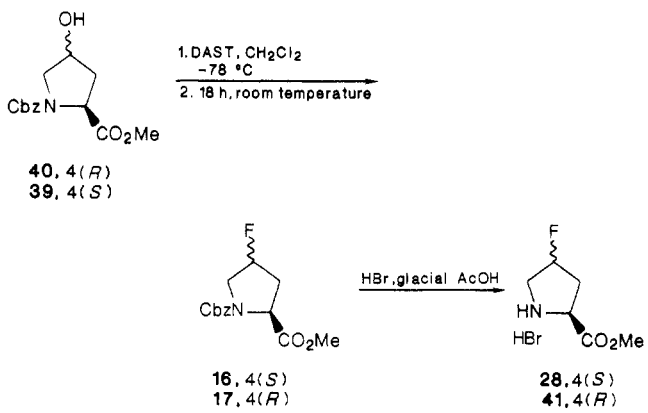


Figure 1. ACE inhibitor with designation of binding sites in ACE.

Scheme IV

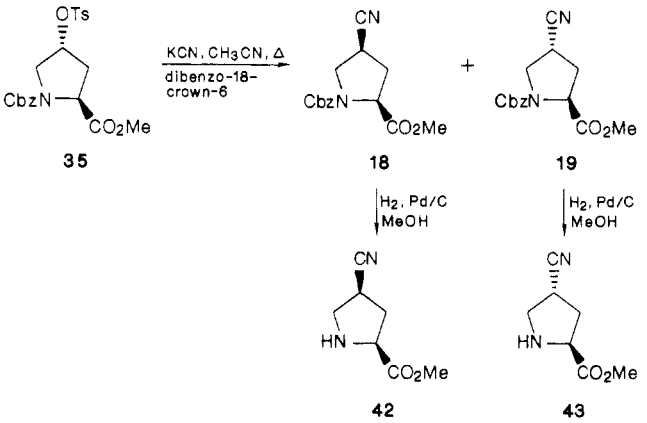


Method 3. 4(*S*)-(Alkylthio)- and 4(*S*)-(arylthio)-(*S*)-proline methyl esters were prepared by reaction of *N*-[(phenylmethoxy)carbonyl]-4(*R*)-(tosyloxy)-(*S*)-proline methyl ester[6] (**35**) with sodium alkyl mercaptide or sodium thiophenolate followed by reesterification and removal of the Cbz group, as shown in Scheme III. The 4(*S*)-benzoylthio derivative was made by reaction of tosylate **35** with thiobenzoic acid and potassium carbonate[10] and subsequent deblocking to **27**.

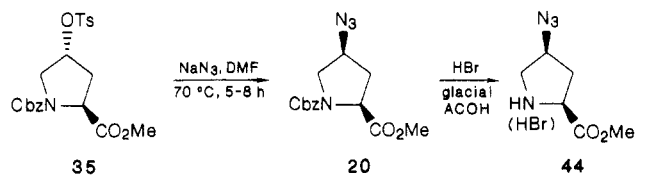
Method 4. Treatment of *N*-[(phenylmethoxy)-carbonyl]-4(*R*)- and -4(*S*)-hydroxy-(*S*)-proline methyl ester (**40** and **39**) with (diethylamido)sulfur trifluoride (DAST)[11a-c] followed by deblocking gave the desired 4(*S*)- and 4(*R*)-fluoro derivatives (**28** and **41**) as shown in Scheme IV. Preparation by fluoride displacement on the tosylates has been reported.[11d]

Method 5. A mixture of 4(*S*)- and 4(*R*)-cyano-(*S*)-proline methyl esters was produced on treatment of the tosylate **35** with potassium cyanide and 18-crown-6 in acetonitrile[12] followed by deblocking, as shown in Scheme V.

Scheme V



Scheme VI



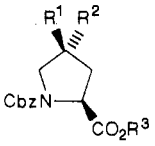
Method 6. Treatment of the tosylate **35** with sodium azide in DMF,[13] followed by HBr in glacial acetic acid, gave (*S*)-azide **44** as shown in Scheme VI.

4,4-Disubstituted (*S*)-proline esters were synthesized from *N*-[(phenylmethoxy)carbonyl]-4-oxo-(*S*)-proline methyl ester (**46**), prepared by oxidation and esterification of *N*-[(phenylmethoxy)carbonyl]-4(*R*)-hydroxy-(*S*)-proline.[6] Ketone **45** was converted to 4,4-disubstituted (*S*)-proline esters as shown in Scheme VII and the following methods. Physical properties are given in Tables III and IV. .

Method A. Ketone **46** was heated with the appropriate diol in toluene (*p*-TSA)[13,14] followed by hydrogenolysis

(1) (a) Ondetti, M. A.; Rubin, B.; Cushman, D. W. *Science (Washington, D.C.)* **1977**, *196*, 441. (b) Cushman, D. W.; Cheung, H. S.; Sabo, E. F.; Ondetti, M. A. *Biochemistry* **1977**, *16*, 5484.
(2) Wyvratt, M. J.; Patchett, A. A. *Med. Res. Rev.* **1985**, *5*, 483 and references cited.
(3) ACE (peptidyl dipeptide hydrolase) EC 3.4.15.1.
(4) (a) Heel, R. C.; Brogden, R. N.; Speight, T. M.; Avery, G. S. *Drugs* **1980**, *20*, 409–452. (b) Maruyama, A.; Ogihara, T.; Naka, T.; Mikami, H.; Hata, T.; Nakamura, M.; Iwanaga, K.; Kumahara, Y. *Clin. Pharmacol. Ther.* **1980**, *28*, 316–323. (c) Biollaz, J.; Brunner, H. R.; Gavras, I.; Waeber, B.; Gavras, H. J. *Cardiovasc. Pharmacol.* **1982**, *4*, 966–972. (d) Chrysant, S. D.; Brown, R. D.; Kem. D. C.; Brown, J. L. *Clin. Pharmacol. Ther.* **1983**, *33*, 741–746. (e) Cody, R. J.; Covit, A. B.; Schaer, G. L.; Laragh, J. H., *J. Am. Coll. Cardiol.* **1983**, *1*, 1154–1159. (f) Biollaz, J.; Burnier, M.; Turini, G. A.; Brunner, D. B.; Porchet, M.; Gomez, H. J.; Jones, K. N.; Ferber, F.; Abrams, W. B.; Gavras, H.; Brunner, H. R. *Clin. Pharmacol. Ther.* **1981**, *29*, 665–670. (g) Rotmensch, H. H.; Vincent, M.; Vlasses, P. H.; Swanson, B. N.; Irvin, J. D.; Hichens, M.; Harris, K. E.; Ferguson, R. K. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1984**, *43*, 1333–1335. (h) Rotmensch, H. H.; Vlasses, P. H.; Swanson, B. N.; Irvin, J. D.; Harris, K. E.; Merrill, D. G.; Ferguson, R. K., *Am. J. Cardiol.* **1984**, *53*, 116–119.
(5) (a) Swiss, G. F.; Smith, E. M.; Neustadt, B. R. *Abstracts*; North American Medicinal Chemistry Symposium, Toronto, Canada, June 1982, Poster III. (b) Swiss, G. F.; Smith, E. M.; Neustadt, B. R.; Gold, E. H.; Chiu, P. J. S.; Brown, A. D.; Sommer, J. *Abstracts*; 184th National Meeting of the American Chemical Society, Kansas City, MO, September 1982, MEDI 77.
(6) Patchett, A. A.; Witkop, B. *J. Am. Chem. Soc.* **1957**, *79*, 185.
(7) Prepared by a different procedure: Adams, E.; Davis, N. C.; Smith, E. L. *J. Biol. Chem.* **1954**, *208*, 573.
(8) Wieland, T.; Schermer, D.; Rohr, G.; Faulstich, H. *Justus Liebigs Ann. Chem.* **1977**, 806.
(9) 4(*R*)-hydroxy is trans, 4(*S*)-hydroxy is cis.
(10) Vebiscar, A. T.; Witkop, B. *J. Org. Chem.* **1970**, *35*, 1924.
(11) (a) Middleton, W. J. *J. Org. Chem.* **1975**, *40*, 574. (b) Rozen, S.; Faust, Y.; Ben-Yakou, H. *Tetrahedron Lett.* **1979**, 1823. (c) Leroy, J.; Hebert, E.; Wakelman, C. *J. Org. Chem.* **1979**, *44*, 3406. (d) Gottlieb, A. A.; Fujita, Y.; Udenfriend, S.; Witkop. B. *Biochemistry* **1965**, *4*, 2507.
(12) Compound **18**: NMR ($CDCl_5$) δ 4.43 (t, C_2-Hα), 3.70 (C_5H, C_5-Hβ, CO_2CH_3), 3.09 (t, C_4-Hα), 2.00–2.77 (br C_3-Hα, C_3-Hβ). Compound **19**: NMR ($CDCl_3$) δ 4.45 (t, br, C_2-Hα), 3.60–3.90 (C_5-Hα, C_5-Hβ, CO_2CH_3), 2.22–2.60 (m, C_3-Hβ).
(13) Andreatta, R. H.; Nair, V.; Robertson, A. V.; Simpson, W. R. J. *Aust. J. Chem.* **1967**, *20*, 1493.

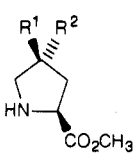
Table I. *N*-[(Phenylmethoxy)carbonyl]-4-substituted-(*S*)-proline Acids and Esters: Preparation and Physicochemical Properties



compd[a-e]	R^1	R^2	R^3	method[f,g]	yield, %	$[\alpha]^{26}_D$[b]	formula[a]	anal.
4a	H	OMe	H	1	87–89[h]	−40.0° (E, *c* 0.1)	$C_{14}H_{17}NO_5 \cdot 0.25H_2O$	CHN
4b	H	OMe	Me	7	87–90[i]	−47.7° (E *c* 0.1)	$C_{15}H_{19}NO_5$	CHN
5b	H	OCH_2Ph	Me	1, 7	56–61[j,l]	−38.2° (E, *c* 0.3)	$C_{21}H_{23}NO_5 \cdot 0.05CHCl_3$	CHN
6a	H	OCH_2CH_2OPh	H	1	27[m]	−32.1° (E, *c* 0.3)	$C_{21}H_{23}NO_6 \cdot 0.25CHCl_3$	CHN
6b	H	OCH_2CH_2OPh	Me	7	76[n]	−43.1° (E, *c* 0.4)	$C_{22}H_{25}NO_6$	CHN
7b	H	OCH_2CH_2OEt	Me	1, 7	36–48[p]	−51.3° (E, *c* 0.4)	$C_{18}H_{25}NO_6$	CHN
8b	OMe	H	Me	1, 7	75[q]	−46.2° (E, *c* 0.1)	$C_{15}H_{19}NO_5 \cdot 0.25CHCl_3$	CHN
9a	OCH_2Ph	H	H	1	*r*	−23.2° (E, *c* 0.2)	$C_{20}H_{21}NO_5 \cdot 0.15CHCl_3$	CHN
9b	OCH_2Ph	H	Me	7	55[s]	−29.7° (E, *c* 0.3)	$C_{21}H_{23}NO_5$	CHN
11[6]	SMe	H	Me	3, 7	39[s,t]	−19.1° (D, *c* 0.4)	$C_{15}H_{19}NO_4S$	CHN[a]
12	SC_6H_{11}-c	H	Me	3, 7	39[s,t]	−15.6° (E, *c* 0.4)	$C_{20}H_{27}NO_4S$	CHN
13	SCH_2Ph	H	Me	3, 7	59[t,v]	−58.5° (D, *c* 0.4)	$C_{21}H_{23}NO_4S$	CHN[w]
14[6]	SPh	H	Me	3, 7	67[t,x]	−21.0° (D, *c* 0.2)	$C_{20}H_{21}NO_4S$	CHN
15	$C_6H_5C(O)S$	H	Me	3	34[s]	−19.6° (E, *c* 0.5)	$C_{21}H_{21}NO_5S$	CHN
16	F	H	Me	4	30[y]	−48.3° (D, *c* 0.4)	$C_{14}H_{16}FNO_4$	CHN
17	H	F	Me	4	33	−63.1° (E, *c* 0.3)	$C_{14}H_{16}FNO_4$	CHN
18	CN	H	Me	5	33[s]	−25.6° (D, *c* 0.4) −27.1° (M, *c* 0.3)	$C_{15}H_{16}N_2O_4$	CHN
19	H	CN	Me	5	15	−21.0° (M, *c* 0.2)	$C_{15}H_{16}N_2O_4$	*z*
20	N_3	H	Me	6	46[aa]	−30.8° (E, *c* 0.4)	$C_{14}H_{16}N_4O_4 \cdot 0.05CHCl_3$	CHN

[a] All compounds had satisfactory C, H, and N elemental analyses (±0.4%, except where indicated) and exhibited IR, ^1H NMR, and mass spectra consistent with the structures. [b] $[\alpha]^{26}_D$ (solvent, concn). Solvent: E, ethanol, M, methanol, D, dioxane. [c] Lobar "C", "B" or RP-8 refers to chromatography on the following: Lobar size C column (440-37) Lichroprep S, 60 (63–125 μm), E. Merck; Lobar size B column (310-25) Lichroprep S, 60 (40–63 μm), E. Merck; and Lobar RP-8 size B column (310-25) Lichroprep RP-8 (40–60 μm), E. Merck, with the indicated eluants. [d] Chromatographic column separations employed Baker silica gel, 60–200 mesh, with the indicated eluants. [e] Analytical and preparative (PLC) thin-layer chromatography were carried out with Analtech silica gel GF plate (50–100 mg of compound per plate) with the indicated eluants. [f] Method 1–6, see text and the Experimental Section. [g] Method 7. Esterification with MeOH/$SOCl_2$. [h] Lobar RP-8 (MeOH–H_2O, 4:1). [i] Lobar "C" ($CHCl_3$–EtOAc, 1:1). [j] From the carboxylic acid. [k] PLC ($CHCl_3$–EtOAc, 3:7). [l] See the Experimental Section for alternate preparation. [m] PLC ($CHCl_3$–MeOH, 23:2). [n] PLC ($CHCl_3$–EtOAc, 3:1). [o] C: calcd, 66.15; found, 67.16. [p] Silica gel (hexane–EtOAc, 5:1). [q] Lobar "C" ($CHCl_3$). [r] PLC ($CHCl_3$–glacial AcOH, 19:1). [s] Silica gel (hexane–EtOAc, 4:1). [t] From tosylate **35**. [u] C: calcd, 58.23; found, 57.50. [v] Silica gel (hexane–EtOAc, 3:1). [w] C: calcd, 65.43; found, 63.94. [x] Silica gel (hexane–EtOAc, 7:1). [y] PLC (hexane–EtOAc, 1:1). [z] MS, *m/z* 288 (M^+). [aa] Lobar "B" ($CHCl_3$).

Table II. 4-Substituted (*S*)-Proline Esters: Preparation and Physicochemical Properties



compd[a-e]	R^1	R^2	method	yield, %	$[\alpha]^{26}_D$[b]	formula	anal.[a]
21[7]	H	OMe	*f*	45–61	−18.0° (E, *c* 0.3)	$C_7H_{13}NO_3 \cdot HBr \cdot H_2O$	CHN[g]
22	H	OCH_2Ph	exp[h]	48[i]	−61.6° (E, *c* 0.3)	$C_{13}H_{17}NO_3$	CHN[j]
23	H	OCH_2CH_2OPh	*k*	93	−20.8° (E, *c* 0.2)	$C_{14}H_{19}NO_4$	CHN[l]
24	OMe	H	*f*	71–89	−3.5° (E, *c* 0.4)	$C_7H_{13}NO_3 \cdot HBr$	CHN[m]
25	H	OC_6H_4Cl-4	exp[h]		−32.2° (E, *c* 0.3)	$C_{12}H_{14}NClO_3$	CHN
26	SMe	H	*f*	72[n]		$C_7H_{13}NO_2S \cdot HBr$	CHN
27	SC(O)Ph	H	*f*	87[o]	−23.5° (E, *c* 0.4)	$C_{13}H_{15}NO_3S \cdot HBr$	CHN[p]
28	F	H	*f*		−46.4° (D, *c* 0.4)	$C_6H_{10}FNO_2 \cdot HBr$	CHN

[a–e] See footnotes in Table I. [f] HBr, glacial AcOH. [g] C: calcd, 32.57; found, 33.06. [h] See the Experimental Section. [i] Mp 124–125 °C. [j] H: calcd, 7.28; found, 6.14. [k] Pd/C, H_2, MeOH. [l] C: calcd, 63.38; found, 60.76. MS, calcd *m/z* 265 (M^+). [m] H: calcd, 5.46; found, 5.96. [n] Mp 128–129 °C. [o] Mp 154–158 °C. [p] C: calcd, 45.10; found, 44.58.

(Pd/C, MeOH) to give **53** or **56**.

Method B. Ketone **46** was heated in methanol (*p*-TSA) followed by removal of the Cbz group as in method A to give **54**.

Method C. Ketone **46** was heated with the appropriate alkanedithiol in glacial acetic acid (*p*-TSA) followed by removal of the Cbz group with HBr in glacial acetic acid to yield **55** or **57**.[15,16]

Method D. Ketone **46** was treated with HCl in ethyl mercaptan at 0 °C followed by removal of the Cbz group as in method C to give **58**.

4-Substituted *N*-(mercaptoacyl)-(*S*)-prolines (**2** and **3**) were generally prepared by reacting the appropriately substituted (*S*)-proline ester (**59** and **60**) with 3-(acetylthio)-2(*RS*)-methylpropanoyl chloride[17] or 3-(acetylthio)-

(14) Cowdhury, A. K. A.; Brown, J. R.; Longmore, R. B. *J. Med. Chem.* **1978**, *21*, 607.

(15) Krapcho, J. U.S. Pat. 4311697, 1982.

(16) Gold, E. H.; Neustadt, B. R.; Smith, E. M. U.S. Pat. 4470972, 1984.

Scheme VII

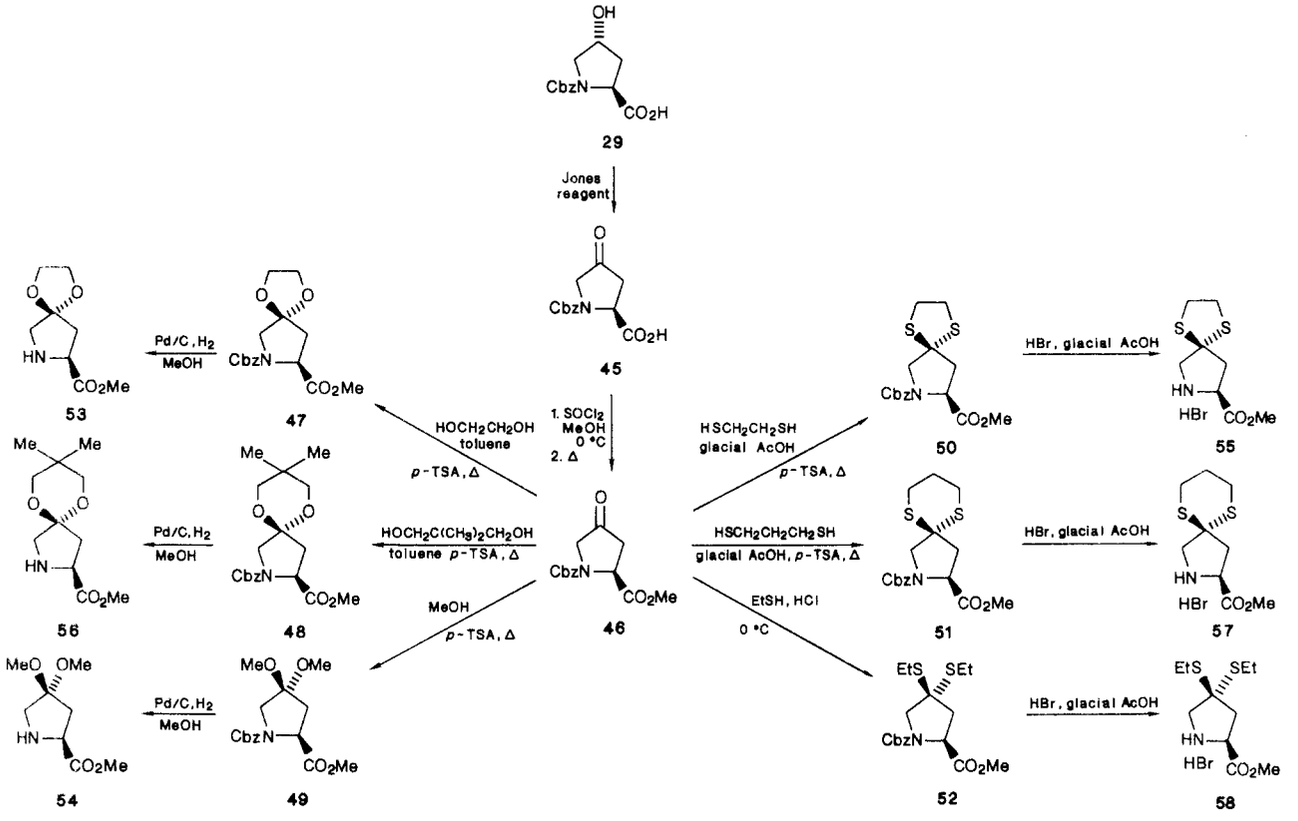
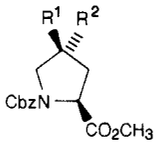


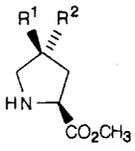
Table III. *N*-[(Phenylmethoxy)carbonyl]-4,4-disubstituted-(*S*)-proline Esters: Preparation and Physicochemical Properties



compd[a-e]	R^1	R^2	method[f]	yield, %	$[\alpha]^{26}_D$[b]	formula	anal.[a]
47[13,14]	OCH_2CH_2O		A	79[g]	−28.2° (D, *c* 0.4)	$C_{16}H_{19}NO_6$	CHN
48	$OCH_2C(CH_3)_2CH_2O$		A	45[g]	−31.8° (E, *c* 0.3)	$C_{19}H_{25}NO_6 \cdot 0.1CH_2Cl_2$	CHN
49[15]	OMe	OMe	B	81	−39.5° (E, *c* 0.6)	$C_{16}H_{21}NO_6$	CHN[h]
50[16]	SCH_2CH_2S		C	48[i]	−12.6° (D, *c* 0.3)	$C_{16}H_{19}NO_4S_2$	CHN
51	$SCH_2CH_2CH_2S$		C	48[j]	−10.2° (D, *c* 0.3)	$C_{17}H_{21}NO_4S_2$	CHN
52	SEt	SEt	D	75	−37.8° (E, *c* 0.6)	$C_{18}H_{25}NO_4S_2$	CHN

[a-e] See footnotes in Table I. [f] See text for method A–D. [g] Silica gel (hexane–EtOAc, 4:1). [h] C: calcd, 59.43; found, 58.33. [i] Silica gel (hexane–EtOAc, 1:1). [j] Silica gel (hexane–EtOAc, 3:1).

Table IV. 4,4-Disubstituted (*S*)-Proline Esters: Preparation and Physicochemical Properties



compd[a-e]	R^1	R^2	method	yield, %	$[\alpha]^{26}_D$[b]	formula	anal.[a]
53[14]	OCH_2CH_2O		*f*	95	−10.9° (E, *c* 0.3)	$C_8H_{13}NO_4$	*g*
54	OMe	OMe	*f*	96	−18.7° (E, *c* 0.2)	$C_8H_{15}NO_4 \cdot 0.05CHCl_3$	CHN[h]
55[16]	SCH_2CH_2S		*i*	71[j]		$C_8H_{13}NO_2S_2 \cdot HBr$	CHN

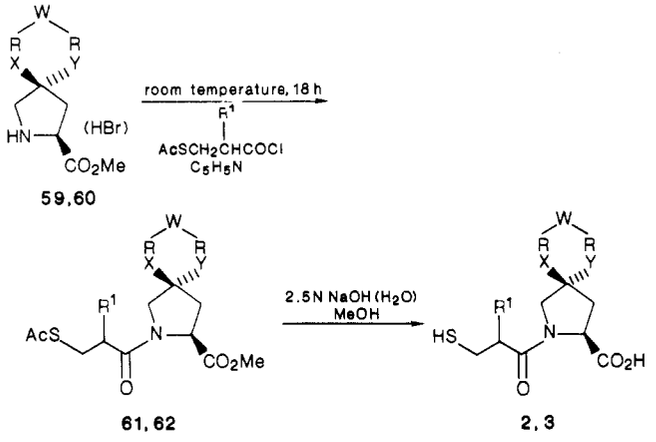
[a-e] See footnotes in Table I. [f] HBr, glacial AcOH. [g] MS, *m/z* 187 (M^+). [h] H: calcd, 7.77; found, 7.29. [i] Pd/C, H_2, MeOH. [j] Mp 156–158 °C.

propanoyl chloride in pyridine to give the *N*-[(acetylthio)acyl]-(*S*)-proline esters (**61** and **62**) (Table V), which were hydrolyzed with sodium hydroxide in aqueous methanol under nitrogen, as shown in Scheme VIII (Table VI). Esters **61** and **62** may be prepared by an alternative procedure involving coupling proline esters **59** and **60** with *S*-acetyl-β-mercaptoisobutyric acid in dimethylformamide in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide and base.

3,4-Dehydro-*N*-(3-mercapto-2(*RS*)-methylpropanoyl)-4-methoxy-(*S*)-proline (**118**) was made by heating ketal **82** in methanol in the presence of HCl and subsequent hydrolysis with sodium hydroxide in aqueous methanol as

(17) Cushman, D. W.; Cheung, H. S. *Biochem. Pharmacol.* **1971**, *20*, 1673.

Scheme VIII



59,61, 2: W = no bond; $R^1 = CH_3$; a, RX = H; b, RY = H
60,62,3: $R^1 = CH_3$, H; a, W = bond; b, W = no bond

shown in Scheme IX. Enol ether **120** was heated in aqueous acetone in the presence of *p*-TSA to yield ketone **121**. Treatment with methoxylamine hydrochloride in pyridine gave the methoxime **122** and the respective disulfide **123**. Hydrolysis of **122** gave acid **119**, as shown in Scheme IX.

Biological Test Methods

Angiotensin Converting Enzyme Inhibition in Vitro. Compounds were initially tested for in vitro ACE-inhibitory activities by the procedure reported by Cushman and Cheung[17] (described in the Experimental Section), and the results are given in Table VI.[18]

Results

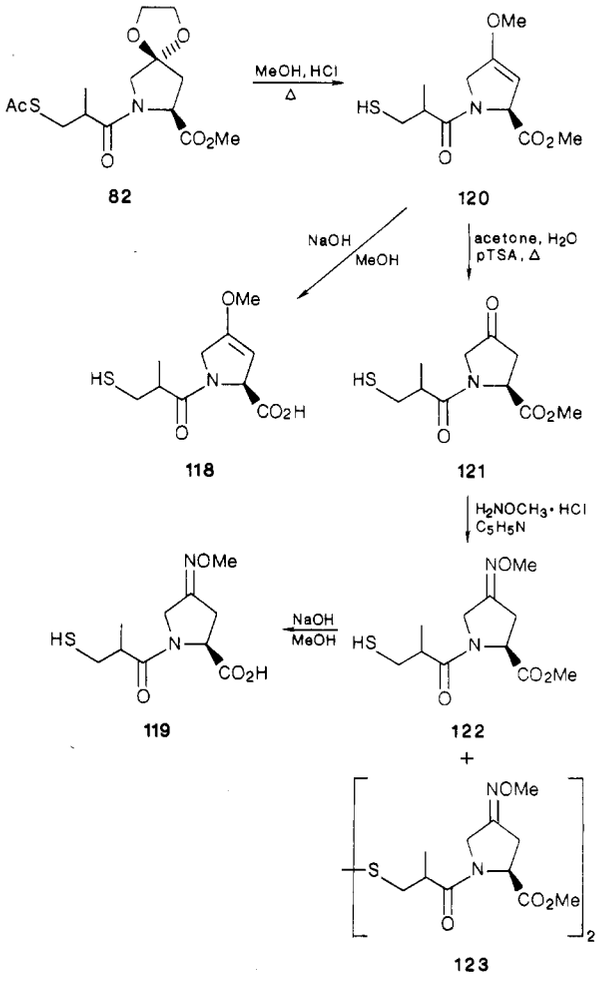
Structure–Activity Relationships for in Vitro ACE Inhibition. Since it has been demonstrated in the case of captopril that the *RS* diastereomer is a much weaker enzyme inhibitor than captopril, the active *SS* diastereomers of the current compounds are likely to possess inhibitory potency almost twice those tested for the mixtures. Table VI suggests the following structure–activity relationships.

4-Monosubstitution. Proline derivatives with a 4-(*S*)-methoxy (**97**), 4(*S*)-benzyloxy (**98**), and 4(*S*)-azido (**107**) substituent were similar in potency to captopril.[18] Introduction of other single substituents at this position led to less active analogues. Interestingly, the 4-*cis*-(*S*)-benzyloxy (**98**), -(*S*)-hydroxy (**96**), and -(*S*)-fluoro (**105**) derivatives exhibited increased enzyme-inhibitory potency relative to the respective trans analogues **92**, **90**, and **104**.

4,4-Disubstitution. Introduction of disubstitution at the 4-position in proline via spiro derivatives **108**, **109**, **111**, and **114** and dimethyl ketal **110** increased enzyme-inhibitory potency relative to captopril.[19] However, the 4,4-bis(ethylthio), 4-(methyloximino), and 4-methoxy-$\Delta^{3,4}$ analogues **115**, **119**, and **118** had greatly diminished inhibitory activity.

Interestingly, only modest differences in potency are evident between the two spiro thioketals **111** and **114**[19] vs their respective analogues **116** and **117**, which lack a methyl group in the acyl side chain. In contrast, removal of the methyl group from the acyl side chain in captopril gives a compound having greatly reduced activity (IC_{50} = 0.20 μM vs captopril IC_{50} = 0.022 μM).[20]

Scheme IX



Angiotensin Converting Enzyme Inhibition in Vivo. The in vivo ACE-inhibitory activity of selected compounds was tested by intravenous administration in the anesthetized rat (Table VI) by use of the method described in the Experimental Section. The cis OMe compound **97** and spiro compounds both with (**108**, **111**, and **114**) and without (**116** and **117**) a methyl group on the side chain showed enhanced potency compared to that of captopril. These compounds did not inhibit angiotensin II.

Bradykinin Potentiation. Selected compounds were tested as bradykinin potentiators (Table VI). A number of compounds showed significant potentiation of bradykinin.

Spontaneously Hypertensive Rat. Selected compounds were tested orally on the spontaneously hypertensive rat. The spiro compound **111** showed significant activity.

Discussion

We conclude that appropriate substitution at the 4-position of proline leads to improved fit at the S_2' subsite of ACE and provides inhibitors with improved potency and biological activity.

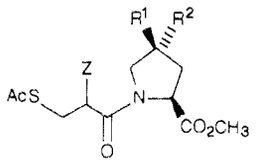
The best fit at the S_2' subsite of ACE was achieved with ketal (**110**), spiro ketal (**108** and **109**) and spiro dithioketal groups (**111**, **113**, **114**, and **116**) at the 4-position. These

(18) The mercapto acids shown in Table VI were obtained as *R,S* and *S,S* mixtures and tested without separation unless otherwise indicated.

(19) These compounds **111** and **114** are a mixture of two diastereomers. The more potent isomeric component is the *S,S* isomer (as with captopril). The *R,S* isomer (as with captopril) is a very weak ACE inhibitor.

(20) Petrillo, E. W.; Ondetti, M. A. *Med. Res. Rev.* **1984**, *2*, 1.

Table V. *N*-[(Acetylthio)acyl]-4-substituted- or -4,4-disubstituted-(*S*)-proline Methyl Esters: Preparation and Physicochemical Properties



compd[a-e]	R^1	R^2	Z	yield,[f] %	$[\alpha]^{26}_D$[b]	formula	anal.[a]
63a	H	OAc	(*RS*)-CH_3	27[g,h]	−35.2° (E, *c* 0.4)	$C_{14}H_{21}NO_6S$	CHN[i]
63b	H	$OC(O)CH(CH_3)CH_2SAc$	(*RS*)-CH_3	11[g,h]	−27.6° (E, *c* 0.3)	$C_{18}H_{27}NO_7S_2$	CHN
64	H	OCH_3	(*RS*)-CH_3	47–51[j]	−51.6° (E, *c* 0.3)	$C_{13}H_{21}NO_5S\cdot0.2CH_3OH$	CHN[k]
65	H	$OCH_2C_6H_5$	(*RS*)-CH_3	22[l,m]	−39.5° (E, *c* 0.4)	$C_{19}H_{25}NO_5S\cdot0.1CHCl_3$	CHN
66	H	$OCH_2CH_2OC_6H_5$	(*RS*)-CH_3	81[j]	−55.0° (E, *c* 0.1)	$C_{20}H_{27}NO_6S$	CHN
67	H	OCH_2CH_2OEt	(*RS*)-CH_3	40[o,p]	−46.5° (E, *c* 0.3)	$C_{16}H_{27}NO_6S\cdot0.2EtOAc\cdot0.2CHCl_3$	CHN
68	H	OC_6H_4Cl-4	(*RS*)-CH_3	68[j,q]	−37.2° (E, *c* 0.6)	$C_{18}H_{22}ClNO_5S$	CHN
69[a]	OAc	H	(*RS*)-CH_3	26[r,h]	−48.5° (E, *c* 0.2)	$C_{14}H_{21}NO_6S$	CHN[s]
69[b]	$OC(O)CH(CH_3)CH_2SAc$	H	(*RS*)-CH_3	22[r,h]	−26.1° (E, *c* 0.2)	$C_{18}H_{27}NO_7S_2$	CHN[t]
70	OCH_3	H	(*RS*)-CH_3	47–64[u]	−50.4° (E, *c* 0.5)	$C_{13}H_{21}NO_5S\cdot0.2CH_3OH$	CHN[v]
71	$OCH_2C_6H_5$	H	(*RS*)-CH_3	40[w,u]	−72.9° (E, *c* 0.1)	$C_{19}H_{25}NO_5S\cdot0.1CHCl_3$	CHN
72	SCH_3	H	(*RS*)-CH_3	57[x,y]	−1.7° (D, *c* 0.5)	$C_{13}H_{21}NO_4S_2$	CHN
73	SC_6H_{11}	H	(*RS*)-CH_3	85[z,j]	−2.2° (E, *c* 0.2)	$C_{18}H_{29}NO_4S_2\cdot0.1CH_2Cl_2\cdot0.2CHCl_3$	CHN[aa]
74	$SCH_2C_6H_5$	H	(*RS*)-CH_3	67[j,bb]	−24.7° (D, *c* 0.2)	$C_{19}H_{25}NO_4S_2\cdot0.75H_2O$	CHN
75	SC_6H_5	H	(*RS*)-CH_3	31[y]	−12.9° (D, *c* 0.2)	$C_{18}H_{23}NO_4S_2$	CHN
76	$SC(O)C_6H_5$	H	(*RS*)-CH_3	90[j]	−26.7° (E, *c* 0.4)	$C_{19}H_{23}NO_5S_2\cdot0.5CHCl_3$	CHN
77	H	F	(*RS*)-CH_3	45[cc]	−62.3° (E, *c* 0.3)	$C_{12}H_{18}FNO_4S\cdot0.25H_2O$	CHN
78	F	H	(*RS*)-CH_3	47[j,dd]	−50.6° (D, *c* 0.2)	$C_{12}H_{18}FNO_4S$	*ee*
79	H	CN	(*RS*)-CH_3	34[ff]		$C_{13}H_{18}N_2O_4S$	*gg*
80	CN	H	(*RS*)-CH_3	50[hh,jj]	−22.3° (D, *c* 0.2)	$C_{13}H_{18}N_2O_4S$	CHN[jj]
81	N_3	H	(*RS*)-CH_3	12–23[kk,ll]	−30.4° (E, *c* 0.5)	$C_{12}H_{18}N_4O_4S\cdot0.3C_4H_8O_2$	CHN[mm]
82	OCH_2CH_2O		(*RS*)-CH_3	69[nn]	−41.2° (E, *c* 0.3)	$C_{14}H_{21}NO_6S$	CHN
83	$OCH_2C(CH_3)_2CH_2O$		(*RS*)-CH_3	95[oo]	−29.1° (E, *c* 0.3)	$C_{17}H_{27}NO_6S\cdot0.2CH_2Cl_2$	CHN
84	OCH_3	OCH_3	(*RS*)-CH_3	74	−40.5° (E, *c* 0.4)	$C_{14}H_{23}NO_6S\cdot0.2CH_2Cl_2$	CHN
85a	SCH_2CH_2S		(*RS*)-CH_3	67[pp,nn]	−25.7° (E, *c* 0.4)	$C_{14}H_{21}NO_4S_3$	CHN
85b	SCH_2CH_2S		(*S*)-CH_3	49	−95.1° (M, *c* 0.3)	$C_{14}H_{21}NO_4S_3$	CHN
86	$SCH_2CH_2CH_2S$		(*RS*)-CH_3	78[qq]	−19.1° (E, *c* 0.2)	$C_{15}H_{23}NO_4S_3$	CHN
87	SEt	SEt	(*RS*)-CH_3	14[rr]	−28.8° (E, *c* 0.1)	$C_{16}H_{27}NO_4S_3$	CHN[ss]
88	SCH_2CH_2S		H	71[pp,tt]	−35.0° (E, *c* 0.3)	$C_{13}H_{19}NO_4S_3$	*uu*
89	$SCH_2CH_2CH_2S$		H	39[qq]	−26.4° (E, *c* 0.3)	$C_{14}H_{21}NO_4S_3$	*ww*

[a-e] See footnotes in Table I. [f] See the Experimental Section. [g] From *N*-[(phenylmethoxy)carbonyl]-4-*trans*-hydroxy-(*S*)-proline methyl ester. [h] Lobar "C" ($CHCl_3$–EtOAc, 3:1). [i] C: calcd, 50.74; found, 50.26. [j] Lobar "C" ($CHCl_3$–EtOAc, 1:1). [k] C: calcd, 51.18; found, 50.74. [l] From *N*-[(phenylmethoxy)carbonyl]-4-*trans*-(benzyloxy)-(*S*)-proline methyl ester. [m] Lobar "B" ($CHCl_3$–EtOAc, 3:1). [n] Lobar "C" ($CHCl_3$–EtOAc, 4:1). [o] From *N*-[(phenylmethoxy)carbonyl]-4-*trans*-(2-ethoxyethoxy)-(*S*)-proline methyl ester. [p] Lobar "B" ($CHCl_3$–EtOAc, 4:1). [q] Mp 97–99 °C. [r] From *N*-[(phenylmethyl)carbonyl]-4-*cis*-hydroxy-(*S*)-proline methyl ester. [s] C: calcd, 50.74; found, 50.23. [t] C: calcd, 49.87; found, 48.16. [u] PLC ($CHCl_3$–EtOAc, 3:2). [v] C: calcd, 51.18; found, 50.65. [w] From *N*-[(phenylmethoxy)carbonyl]-4-*cis*-(benzyloxy)-(*S*)-proline methyl ester. [x] From *N*-[(phenylmethoxy)carbonyl]-4-*cis*-(methylthio)-(*S*)-proline methyl ester. [y] Lobar "C" ($CHCl_3$–EtOAc, 2:1). [z] From *N*-[(phenylmethoxy)carbonyl]-4-*cis*-(cyclohexylthio)-(*S*)-proline methyl ester. [aa] N: calcd, 3.33; found, 2.79. [bb] From *N*-[(phenylmethoxy)carbonyl]-4-*cis*-(benzylthio)-(*S*)-proline methyl ester. [cc] TLC hexane–EtOAc, 1:1, and Lobar "C" ($CHCl_3$). [dd] From *N*-[(phenylmethoxy)carbonyl]-4-*cis*-fluoro-(*S*)-proline methyl ester. [ee] MS, *m/z* 291 (M^+). [ff] TLC hexane–EtOAc, 1:1. [gg] MS, *m/z* 298 (M^+). [hh] From *N*-[(phenylmethoxy)carbonyl]-4-*cis*-cyano-(*S*)-proline methyl ester. [ii] Silica gel column (hexane–EtOAc, 1:1). [jj] N: calcd, 9.39; found, 8.60. [kk] From *N*-[(phenylmethoxy)carbonyl]-4-*cis*-azido-(*S*)-proline methyl ester. [ll] Lobar "B" ($CHCl_3$–EtOAc, 4:1). [mm] N: calcd, 16.44; found, 14.11. [nn] Silica gel (hexane–EtOAc, 2:1). [oo] Silica gel (hexane–EtOAc, 3:2). [pp] From 7-[(phenylmethoxy)carbonyl]-1,4-dithia-7-azaspiro-[4.4]nonane-8(*S*)-carboxylic acid methyl ester. [qq] From 4-[(phenylmethoxy)carbonyl]-6,10-dithia-2-azaspiro[4.5]decane-3(*S*)-carboxylic acid methyl ester. [rr] From *N*-[(phenylmethoxy)carbonyl]-4,4-bis(ethylthio)-(*S*)-proline methyl ester. [ss] C: calcd, 48.82; found, 49.29. [tt] Silica gel column (hexane–EtOAc, 4:1). [uu] MS, *m/z* 349 (M^+). [vv] Silica gel (hexane–EtOAc, 3:1). [ww] MS, *m/z* 363 (M^+).

ketals lie perpendicular to the plane of the proline ring and show that there is considerable space at the S_2' subsite in at least these two directions, thus providing a degree of mapping of this enzymatic site. The fact that the methyl group on the mercaptoacyl side chain is less critical to activity of the spiro ketals **116** and **117** than in the captopril series indicates that the conformational influence of the methyl group is not required for optimization of binding with the spiro ketal compounds.

Experimental Section

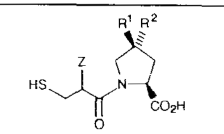
Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. The NMR spectra were recorded on a Varian FT80 instrument at 80 MHz and a T60A instrument at 60 MHz; the IR spectra were recorded on a Perkin-Elmer 180 grating IR instrument; the EI mass spectra were determined with a Finnigan MAT CH-5 instrument and the rotations with a Rudolf Autopol at ambient temperature. Microanalyses were performed by the Physical–Analytical Chemistry Department of Schering-Plough Corpn.

Chemistry. Starting materials were purchased or prepared by literature methods: *N*-[(phenylmethoxy)carbonyl]-4(*R*)-hydroxy-(*S*)-proline (**29**) (Chemical Dynamics or Sigma), *N*-[(phenylmethoxy)carbonyl]-4(*R*)-hydroxy-(*S*)-proline methyl ester (**40**)[14] (Sigma), *N*-[(phenylmethoxy)carbonyl]-4(*R*)-[[(4-methylphenyl)sulfonyl]oxy]-(*S*)-proline methyl ester (**35**),[6] *N*-[(phenylmethoxy)carbonyl]-4(*S*)-hydroxy-(*S*)-proline (**30**),[6,8,9] *N*-[(phenylmethoxy)carbonyl]-4(*S*)-hydroxy-(*S*)-proline methyl ester (**39**),[6] *N*-[(phenylmethoxy)carbonyl]-4-keto-(*S*)-proline (**45**),[6] *N*-[(1,1-dimethylethoxy)carbonyl]-4(*R*)-hydroxy-(*S*)-proline (**33**) DCHA salt (Vega), D-(−)-(*S*)-acetyl-β-*mercaptoisobutyric acid* (Chemical Dynamics), and 3-(acetylthio)-2-methylpropanoic acid chloride.[21]

N-[(Phenylmethoxy)carbonyl]-4(*R*)-methoxy-(*S*)-proline (4a). *N*-[(phenylmethoxy)carbonyl]-4(*R*)-hydroxy-(*S*)-proline (**29**)

(21) Ondetti, M. A.; Cushman, D. A. U.S. Pat. 4 046 889, 1977.

Table VI. *N*-(Mercaptoacyl)-4-substituted- or -4,4-disubstituted-(*S*)-prolines: Preparation, Physiochemical Properties, and in Vitro ACE-Inhibitory Activity



compd[a–e]	R¹	R²	Z[g]	yield,[g] %	$[\alpha]^{26}_{D}$[b]	formula	anal.[a]	ACE inhibition potency: ID_{50}, μg/kg in vitro[h]	in vivo[i]	bradykinin potentiation ΔBP, mm, at 0.1 μg/kg[j]	SHR ΔBP (dec) (dose, mpk po)
90	H	OH	(*RS*)-CH_3	83[k]	−61.3° (E, *c* 0.5)	$C_9H_{15}NO_4S\cdot0.33H_2O$	CHN	0.16	253	48	
91	H	OCH_3	(*RS*)-CH_3	38–43	−25.5° (E, *c* 0.5)	$C_{10}H_{17}NO_4S\cdot0.2CHCl_3$	CHN[l]	0.50	1206		
92	H	$OCH_2C_6H_5$	(*RS*)-CH_3	62[m]	−31.8° (E, *c* 0.3)	$C_{16}H_{21}NO_4S\cdot0.45CHCl_3\cdot0.1AcOH$	CHN	0.35	508	47	
93	H	$OCH_2CH_2OC_6H_5$	(*RS*)-CH_3	55[n]	−49.2° (E, *c* 0.1)	$C_{17}H_{23}NO_5S$	N	0.11	*o*	9	
94	H	OCH_2CH_2OEt	(*RS*)-CH_3	65	−52.5° (E, *c* 0.4)	$C_{13}H_{23}NO_5S\cdot0.005CHCl_3$	CHN[p]	0.19	372	45	
95	H	OC_6H_4Cl-4	(*RS*)-CH_3	72[q]	−27.2° (E, *c* 0.2)	$C_{15}H_{18}NO_4ClS$	CHN	0.27	*o*	23	
96	OH	H	(*RS*)-CH_3	89[r]	−45.6° (E, *c* 0.2)	$C_9H_{15}NO_4S\cdot0.75H_2O$	CHN[s]	0.30	367	40	
97	OCH_3	H	(*RS*)-CH_3	93	−50.0° (M, *c* 0.4)	$C_{10}H_{17}NO_4S\cdot0.1CHCl_3$	CHN	0.53	73	60	
98	$OCH_2C_6H_5$	H	(*RS*)-CH_3	57[t,u]	−18.9° (E, *c* 0.1)	$C_{16}H_{21}NO_4S\cdot0.2CHCl_3$	CHN	0.56	*o*	40	
99	SH	H	(*RS*)-CH_3	23[m,v]	−46.8° (E, *c* 0.3)	$C_9H_{15}NO_3S_2\cdot0.15CHCl_3$	CHN[w]	0.10	NT[x]	NT	
100	SCH_3	H	(*RS*)-CH_3	76	−5.5° (D, *c* 0.8)	$C_{10}H_{17}NO_3S_2\cdot0.15CHCl_3$	CHN[y]	0.23	398		
101	SC_6H_{11}-c	H	(*RS*)-CH_3	87	−7.0° (E, *c* 0.3)	$C_{15}H_{25}NO_3S_2\cdot0.5AcOH\cdot H_2O$	CHN	0.15	*o*	6	
102	$SCH_2C_6H_5$	H	(*RS*)-CH_3	57	−36.7° (D, *c* 0.3)	$C_{16}H_{21}NO_3S_2\cdot0.5AcOH\cdot0.75H_2O$	CHN	0.30	*z*	5	
103	SC_6H_5	H	(*RS*)-CH_3	77	−28.4° (D, *c* 0.2)	$C_{15}H_{19}NO_3S_2\cdot0.4CH_2Cl_2$	CHN	0.39	544	22	
104	H	F	(*RS*)-CH_3	82	−76.1° (E, *c* 0.1)	$C_9H_{14}FNO_3S\cdot0.25H_2O$	CHN	0.15	*o*	53	
105	F	H	(*RS*)-CH_3	54	−46.2° (D, *c* 0.3)	$C_9H_{14}FNO_3S\cdot0.1AcOH\cdot0.2EtOAc\cdot0.75H_2O$	CHN	0.29	581		
106	CN	H	(*RS*)-CH_3	60	−42.0° (D, *c* 0.2)	$C_{10}H_{14}N_2O_3S\cdot0.1AcOH\cdot0.25H_2O$	CHN[aa]	0.12	*o*	47	
107	N_3	H	(*RS*)-CH_3	60	−20.6° (E, *c* 0.1)	$C_9H_{14}N_4O_3S\cdot0.1AcOH\cdot0.1CHCl_3$	CHN[bb]	0.54	NT[x]	NT	
108	OCH_2CH_2O		(*RS*)-CH_3	62	−39.4° (D, *c* 0.16)	$C_{11}H_{17}NO_5S\cdot0.5H_2O$	CHN	1.0	65	43	
109	$OCH_2C(CH_3)_2CH_2O$		(*RS*)-CH_3	65	−28.1° (E, *c* 0.2)	$C_{14}H_{23}NO_5S$	CHN	1.0	359	46	25 (10)
110	OCH_3	OCH_3	(*RS*)-CH_3	85	−32.2° (E, *c* 0.6)	$C_{11}H_{19}NO_5S\cdot0.1AcOH\cdot0.1EtOAc$	CHN[cc]	0.9	NT[x]	NT	
111	SCH_2CH_2S		(*RS*)-CH_3	53	−24.8° (E, *c* 0.3)	$C_{11}H_{17}NO_3S_3\cdot0.2AcOH\cdot0.1EtOAc$	CHN	1.26	26	34	22 (10), 35 (1)
112	SCH_2CH_2S		(*R*)-CH_3	*dd*	−10.0° (E, *c* 0.5)	$C_{11}H_{17}NO_3S_3\cdot0.3CHCl_3$	CHN	0.4	NT[x]		
113	SCH_2CH_2S		(*S*)-CH_3	*dd*	−31.8° (E, *c* 0.3)	$C_{11}H_{17}NO_3S_3$	CHN	4.0	NT[x]	43	17 (1)
114	$SCH_2CH_2CH_2S$		(*RS*)-CH_3	70[q]	−16.9° (E *c* 0.3)	$C_{12}H_{19}NO_3S_3\cdot0.5H_2O$	CHN[ee]	1.07	48		
115	SEt	SEt	(*RS*)-CH_3	90[q]	−30.1° (E, *c* 0.3)	$C_{13}H_{23}NO_3S_3$	CHN[ff]	0.1	NT[x]		
116	SCH_2CH_2S		H	31[gg,hh]	−24.2° (E, *c* 0.5)	$C_{10}H_{15}NO_3S_3\cdot0.33AcOH\cdot0.5H_2O$	CHN	2.6	78	38	
117	$SCH_2CH_2CH_2S$		H	37[v]	−22.2° (E, *c* 0.2)	$C_{11}H_{17}NO_3S_3\cdot0.1CH_2Cl_2\cdot0.2CHCl_3$	CHN[ii]	1.0	10	19	
118	$\Delta^{3,4}$-4-OCH_3		(*RS*)-CH_3	62	−36.3° (D, *c* 0.2)	$C_{10}H_{15}NO_4S\cdot0.2EtOAc\cdot0.1AcOH$	CHN	0.2	*o*	52	
119	=$NOCH_3$		(*RS*)-CH_3	74	+4.0° (E, *c* 0.4)	$C_{10}H_{16}N_2O_4S$	CHN	1.0	NT[x]		
1	H	H	(*S*)-CH_3					1.0	71 ± 25		

[a–e] See footnotes *a–e* in Table I. [f] All compounds were a 1:1 mixture of diastereomers unless otherwise noted. [g] See General Method and the Experimental Section. [h] In vitro ACE inhibitory activity potency relative to captopril equal to 1.00 (IC_{50} = 3.5–25 nm). Mercapto acids were obtained as *RS* and *SS* mixtures and tested without separation unless otherwise indicated. The relative potency of individual compounds is determined with the IC_{50} of captopril measured concomitantly in the same daily assay. This range of IC_{50} for captopril was obtained over a period of almost 2 years, starting with greater values and getting smaller with time. The variation is most likely due to progressive changes in the batches of crude enzyme employed. In cases where a compound was compared relative to captopril with varying IC_{50}, the relative potency showed little variation. [i] Because of the low number of animals on which these ID_{50} values are based, it is difficult to ascribe a precise value of reliability. However, under the conditions of the experiment described above, captopril and enalapril, two standard ACE inhibitors of two different structural classes, possess ID_{50} values associated with low variability, e.g., ID_{50} for captopril is 71 ± 25 (mean ± SEM). [j] A fall of 10–15 mmHg is considered effective. Bradykinin potentiation is seen at doses lower than those that inhibit angiotensin I (absolute increase in bradykinin response). [k] Hydrolysis of 4-acetoxy compound **62a**. [l] C: calcd, 45.17; found, 45.64. [m] Preparative TLC using $CHCl_3$–glacial AcOH, 19:1, followed by 9:1. [n] Lobar RP-8 size B (MeOH–H_2O, 7:3). [o] Inactive at 100 μg/kg. [p] H: calcd, 7.46; found, 7.99. [q] Lobar RP-8 size B column (MeOH–H_2O, 7:3). [r] Hydrolysis of 4-acetoxy compound **68**. [s] H: calcd, 6.73; found, 6.28. N: calcd, 5.67; found, 4.81. [t] PLC ($CHCl_3$–glacial AcOH, 23:2). [u] From 4-benzoylthio **75**. [v] Silica gel column ($CHCl_3$–AcOH, 9:1). [w] H: calcd, 5.71; found, 5.09. [x] NT = not tested. [y] C: calcd, 43.34; found, 42.60. [z] Inactive at 1000 μg/kg. [aa] N: calcd, 11.08; found, 10.16. [bb] N: calcd, 20.28; found, 15.00. [cc] C: calcd, 47.69; found, 46.47. [dd] Silica gel column ($CHCl_3$–AcOH, 98:2). [ee] C: calcd, 43.61; found, 43.11. [ff] C: calcd, 46.26; found, 47.39. [gg] Silica gel column ($CHCl_3$–AcOH, 97:3). [hh] Mp 129–132 °C. [ii] C: calcd, 40.29; found, 39.70.

(9.33 g, 0.035 mol) in anhydrous THF (150 mL) was treated with NaH (50% oil, 3.50 g, 0.073 mol). After 45 min at room temperature, the reaction mixture was treated with MeI (10.36 g, 0.073 mol), and the resulting mixture was heated under reflux for 3 h and then kept at room temperature for 18 h. The reaction mixture was concentrated under reduced pressure and partitioned between H_2O and CH_2Cl_2. The aqueous solution was acidified with dilute HCl and extracted with CH_2Cl_2. The dried ($MgSO_4$) CH_2Cl_2 solution was concentrated in vacuo to give a yellow oil (10.03 g). The yellow oil (0.68 g) was placed on a Lobar RP-8 size B column and eluted with MeOH–H_2O, 4:1, to give **4a** as a colorless oil (0.56 g) (Table I).

The [(phenylmethoxy)carbonyl]-4(*R*)-alkoxy-(*S*)-prolines **5a**, **6a**, and **7a** (Table I) were prepared by using the procedure described above.

***N*-[(Phenylmethoxy)carbonyl]-4(*S*)-alkoxy-(*S*)-prolines (8a and 9a).** Via the procedure for the preparation of *N*-[(phenylmethoxy)carbonyl]-4(*R*)-methoxy-(*S*)-proline (**4a**), *N*-[(phenylmethoxy)carbonyl]-4(*S*)-hydroxy-(*S*)-proline (**30**) was substituted for *N*-[(phenylmethoxy)carbonyl]-4(*R*)-hydroxy-(*S*)-proline (**29**) to give the *N*-[(phenylmethoxy)carbonyl]-4-(*S*)-alkoxy-(*S*)-prolines **8a** and **9a** (Table I).

***N*-[(Phenylmethoxy)carbonyl]-4(*R*)-methoxy-(*S*)-proline Methyl Ester (4b).** Thionyl chloride (2.70 mL, 4.17 g, 0.035 mol) was slowly added to MeOH (40 mL). *N*-[(Phenylmethoxy)-carbonyl]-4(*R*)-methoxy-(*S*)-proline (**4a**) (9.36 g, 0.034 mol) in MeOH (30 mL) was added to the above solution, and the resulting mixture was heated under reflux for 2 h. The reaction mixture was cooled and concentrated under reduced pressure to give a yellow oil (8.58 g). This oil (0.52 g) was placed on a Lobar size C LiChroprep Si 60 column and eluted with $CHCl_3$–EtOAc, 1:1. Fractions containing the title compound were concentrated under reduced pressure to give **4b** as a colorless oil (0.26 g) (Table I). The *N*-[(phenylmethoxy)carbonyl]-4(*R*)-substituted-(*S*)-proline methyl esters **5b**, **6b**, and **7b** (Table I) and *N*-[(phenylmethoxy)carbonyl]-4(*S*)-substituted-(*S*)-proline methyl esters **8b** and **9b** (Table I) were prepared by using the procedure described above.

***N*-[(1,1-Dimethylethoxy)carbonyl]-4(*R*)-(phenylmethoxy)-(*S*)-proline (34a).**[22] *N*-[(1,1-Dimethylethoxy)-carbonyl]-4(*R*)-hydroxy-(*S*)-proline (**33**) (1.13 g, 0.0049 mol) in THF (50 mL) was treated with NaH (50% oil, 0.50 g, 0.0104 mol), and the resulting mixture was stirred at room temperature for 1.5 h. Benzyl bromide (1.70 g, 0.010 mol) was added, and the resulting mixture was heated under reflux for 5 h. The reaction mixture was quenched with ice–water and extracted with hexane. The aqueous solution was acidified with $KHSO_4$ and extracted with EtOAc. The dried ($MgSO_4$) EtOAc solution was concentrated in vacuo to give **34a** as a colorless oil (1.21 g), which was used in the next step.

4(*R*)-(Phenylmethoxy)-(*S*)-proline Methyl Ester (22). *N*-[(1,1-Dimethylethoxy)carbonyl]-4(*R*)-(phenylmethoxy)-(*S*)-proline (**34a**) (1.08 g crude) in MeOH (15 mL) was added to MeOH (15 mL) previously treated with $SOCl_2$ (1.5 mL) at 0–5 °C, and the resulting mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated in vacuo, diluted with CH_2Cl_2, and extracted with 1 N NaOH. The dried ($MgSO_4$) CH_2Cl_2 solution was concentrated in vacuo to give **22** as a yellow oil (0.55 g, 48%) (Table II).

***N*-[(1,1-Dimethylethoxy)carbonyl]-4(*R*)-(4-chlorophenoxy)-(*S*)-proline (34b).** *N*-[(1,1-Dimethylethoxy)carbonyl]-4-(*R*)-hydroxy-(*S*)-proline (**33**) (1.11 g, 0.0048 mol) in anhydrous DMF (10 mL) was added slowly to NaH (50% oil, 0.50 g, 0.01 mol) in DMF (25 mL), and the resulting mixture was heated at 60 °C for 30 min. *p*-Chlorofluorobenzene (1.49 g, 0.008 mol) was added, and the resulting mixture was heated at 95 °C for 18 h. The reaction mixture was cooled, poured into ice–H_2O, and extracted with hexane. The aqueous solution was acidified with $KHSO_4$ and extracted with EtOAc. The dried ($MgSO_4$) EtOAc solution was concentrated at room temperature to give **34b**, an amber oil (0.65 g), which was used in the next step.

4(*R*)-(4-Chlorophenoxy)-(*S*)-proline Methyl Ester (25). To *N*-[(1,1-dimethylethoxy)carbonyl]-4(*R*)-4-(chlorophenoxy)-(*S*)-proline (**34b**) (0.65 g) in MeOH (10 mL) was added a solution of $SOCl_2$ (0.3 mL) in methanol (10 mL) at 0–5 °C, and the resulting mixture was warmed to room temperature and stirred for 18 h. The reaction mixture was concentrated under reduced pressure, and the residue was partitioned between CH_2Cl_2 and saturated aqueous Na_2CO_3. The dried ($MgSO_4$) CH_2Cl_2 solution was concentrated under reduced pressure to give a brown residue (0.27 g), which was placed on preparative thin-layer silica gel plates (2 × 1000 μm) and eluted with EtOAc–MeOH, 19:1, to give **25** as a colorless oil (0.04 g) (Table II).

***N*-[(Phenylmethoxy)carbonyl]-4(*S*)-[(phenylmethyl)-thio]-(*S*)-proline Methyl Ester (13).** Pieces of sodium metal (3.00 g, 0.13 mol) were dissolved in MeOH (25 mL). Benzyl mercaptan (15.9 g, 0.128 mol) was then added, and the resulting mixture was stirred at room temperature for 1 h. *N*-[(Phenylmethoxy)carbonyl]-4(*R*)-[[(4-methylphenyl)sulfonyl]oxy]-(*S*)-proline methyl ester (**35**) (5.5 g, 0.013 mol) was added, and the reaction mixture was heated under reflux for 18 h. The resulting mixture was poured into H_2O (200 mL), and the aqueous solution was extracted with EtOAc. The aqueous solution was acidified with 20% HCl and extracted with EtOAc. The dried ($MgSO_4$) EtOAc solution was concentrated in vacuo to give a residue (3.00 g). This residue was dissolved in MeOH (200 mL) that had previously been treated with $SOCl_2$ (10 mL), and the resulting solution was heated under reflux for 4 h. The reaction mixture was concentrated under reduced pressure, and the residue was placed on a column of silica gel (1 L) and eluted with hexane–EtOAc, 3:1, to give **13** as a colorless oil (2.90 g) (Table I).

The *N*-[(phenylmethoxy)carbonyl]-4(*S*)-alkyl- or -(arylthio)-(*S*)-prolines **11**, **12**, and **14** (Table I) were prepared by using the procedure described above.

***N*-[(Phenylmethoxy)carbonyl]-4(*S*)-(benzoylthio)-(*S*)-proline Methyl Ester (15).** Thiobenzoic acid (1.85 g, 0.0134 mol) was added to K_2CO_3 (5.18 g, 0.0375 mol) in MeCN (100 mL). *N*-[(Phenylmethoxy)carbonyl]-4(*R*)-[[(4-methylphenyl)-sulfonyl]oxy]-(*S*)-proline methyl ester (**35**) (1.76 g, 0.0042 mol) was added, and the resulting mixture was heated under reflux for 44 h. The reaction mixture was cooled and poured into ice–water (400 mL). The aqueous solution was extracted with EtOAc. The organic solution was washed with brine, dried ($MgSO_4$), and concentrated in vacuo to give a residue. Chromatography on silica gel (300 g) (hexane–EtOAc, 4:1) gave **15** as a colorless oil (0.74 g) (Table I).

***N*-[(Phenylmethoxy)carbonyl]-4(*S*)-fluoro-(*S*)-proline Methyl Ester (16).** *N*-[(Phenylmethoxy)carbonyl]-4(*R*)-hydroxy-(*S*)-proline methyl ester (**40**) (1.00 g, 0.036 mol) was dissolved in CH_2Cl_2 (20 mL), cooled to −78 °C, and then treated with DAST (0.58 g, 0.036 mol). The resulting mixture was warmed to room temperature and stirred for 18 h. The reaction mixture was poured into ice–H_2O containing $NaHCO_3$, extracted with CH_2Cl_2, dried ($MgSO_4$), and concentrated under reduced pressure to give an oil. Chromatography on six 1000-μm silica gel plates (hexane–EtOAc 1:1) gave **16** as a colorless oil (0.28 g) (Table I).

***N*-[(Phenylmethoxy)carbonyl]-4(*R*)-fluoro-(*S*)-proline Methyl Ester (17).** *N*-[(Phenylmethoxy)carbonyl]-4(*S*)-hydroxy-(*S*)-proline, methyl ester (**39**) (4.20 g, 0.015 mol) dissolved in CH_2Cl_2 (40 mL) was cooled to 0 °C. DAST (2.64 g, 0.016 mol) was added, and the resulting mixture was stirred at room temperature for 18 h and then treated as described above. The reaction mixture was placed on a column of silica gel (200 g) and eluted with hexane–EtOAc, 4:1, to give **17** as a colorless oil (1.29 g) (Table I).

***N*-[(Phenylmethoxy)carbonyl]-4(*R*)-cyano-(*S*)-proline Methyl Ester (19) and *N*-[(Phenylmethoxy)carbonyl]-4-(*S*)-cyano-(*S*)-proline Methyl Ester (18).** *N*-[(Phenylmethoxy)carbonyl]-4(*R*)-[[(4-methylphenyl)sulfonyl]oxy]-(*S*)-proline methyl ester (**35**) (30.3 g, 0.07 mol) dissolved in MeCN (500 mL) was treated with KCN (33.82 g, 0.52 mol) and dibenzo-18-crown-6 (31.12 g), and the resulting mixture heated under reflux for 44 h. The reaction mixture was poured into ice–water, extracted with EtOAc, dried ($MgSO_4$), and concentrated under reduced pressure to give a residue, which was placed on a column of silica gel (2 kg) and eluted with hexane–EtOAc, 4:1, to give **19** as a

(22) (a) Sakakibara, S.; Inouye, K.; Shudo, K.; Kishida, Y.; Kobayashi, Y.; Prockop, D. J. *Biochem. Biophys. Acta* **1973**, *303*, 198. (b) Weber, R. W.; Nitschmann, H. *Helv. Chim. Acta* **1978**, *61*, 701.

colorless oil (2.20 g), R_f 0.55, and **18** as a colorless oil (3.32 g), R_f 0.45 (Table I).

4(*S*)-Azido-*N*-[(phenylmethoxy)carbonyl]-(*S*)-proline Methyl Ester (20).[13] *N*-[(Phenylmethoxy)carbonyl]-4(*R*)-[[(4-methylphenyl)sulfonyl]oxy]-(*S*)-proline methyl ester (**35**) (0.44 g, 0.001 mol) and NaN_3 (0.085 g, 0.013 mol) in DMF (5 mL) and H_2O (0.5 mL) were heated at 70 °C for 4.5 h. The reaction mixture was quenched with saturated brine and extracted with ether. The ether extract was washed with saturated brine, dried ($MgSO_4$), and concentrated under reduced pressure to give a colorless oil (0.26 g). This oil was chromatographed on a Lobar size B column ($CHCl_3$) to give **20** as a colorless oil (0.14 g) (Table I).

***N*-[(Phenylmethoxy)carbonyl]-4-oxo-(*S*)-proline Methyl Ester (46).**[23] *N*-[(Phenylmethoxy)carbonyl]-4-oxo(*S*)-proline (**45**) (31.69 g, 0.12 mol) in MeOH (400 mL) at 0 °C was treated with $SOCl_2$ (17 mL), and the resulting solution was heated under reflux for 2 h. The reaction mixture was concentrated in vacuo and chromatographed on silica gel (1.5 kg) (hexane–EtOAc, 4:1) to give **46** as a colorless oil (20.90 g), $[\alpha]^{26}_D$ +5.3° (D, *c* 0.3). Anal. ($C_{14}H_{15}NO_5$) H, N; C: calcd, 60.64; found, 59.40.

7-[(Phenylmethoxy)carbonyl]-1,4-dioxa-7-azaspiro[4.4]nonane-8(*S*)-carboxylic Acid Methyl Ester (47). *N*-[(Phenylmethoxy)carbonyl]-4-oxo-(*S*)-proline methyl ester (**46**) (5.54 g, 0.020 mol) was dissolved in toluene (200 mL) and ethylene glycol (22.6 g, 20 mL, 0.36 mol). *p*-Toluenesulfonic acid (0.50 g, 0.0026 mol) was added, and the resulting mixture was heated under reflux for 18 h, cooled, poured into ice–water, extracted with EtOAc, dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel (1.5 L) (hexane–EtOAc, 4:1) gave **47** as a colorless oil (5.10 g) (Table III).

2-[(Phenylmethoxy)carbonyl]-8,8-dimethyl-6,10-dioxa-2-azaspiro[4.5]decane-3(*S*)-carboxylic acid methyl ester (**48**) (Table III) was prepared by using the procedure described above.

***N*-[(Phenylmethoxy)carbonyl]-4,4-dimethoxy-(*S*)-proline Methyl Ester (49).** *N*-[(Phenylmethoxy)carbonyl]-4-oxo-(*S*)-proline methyl ester (**46**) (6.86 g, 0.025 mol) was dissolved in MeOH (200 mL). *p*-Toluenesulfonic acid (0.110 g, 0.0006 mol) was added. The resulting mixture was heated under reflux in the presence of a Dean–Stark trap. MeOH (160 mL) was removed, the reaction mixture was poured into ice–water, extracted with EtOAc, dried ($MgSO_4$), and concentrated in vacuo to give **49** as a colorless oil (6.56 g) (Table III).

7-[(Phenylmethoxy)carbonyl]-1,4-dithia-7-azaspiro[4.4]decane-8(*S*)-carboxylic Acid Methyl Ester (50). A solution of **46** (7.06 g, 0.025 mol) in glacial HOAc (75 mL) was treated with *p*-toluenesulfonic acid (0.70 g, 0.0037 mol) and 1,2-ethanedithiol (2.81 g, 2.50 mL, 0.0298 mol), and the resulting mixture was heated under reflux for 20 h. The reaction mixture was added dropwise to a saturated $NaHCO_3$ solution, extracted with EtOAc, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane–EtOAc, 1:1) to give **50** as a pale yellow oil (4.49 g) (Table III).

2-[(Phenylmethoxy)carbonyl]-6,10-dithia-2-azaspiro[4.5]decane-3(*S*)-carboxylic acid methyl ester (**51**) (Table III) was prepared by using the procedure described above.

***N*-[(Phenylmethoxy)carbonyl]-4,4-bis(ethylthio)-(*S*)-proline Methyl Ester (52).** A solution of (**46**) (6.12 g, 0.022 mol) in ethanethiol (50 mL) at 0 °C was treated with HCl gas and then stirred at 0 °C for 4 h. The reaction mixture was poured into a slightly basic (Na_2CO_3) aqueous solution, extracted with EtOAc, dried ($MgSO_4$), and concentrated to give **52** as a pale yellow oil (6.31 g) (Table III).

4(*S*)-Methoxy-(*S*)-proline Methyl Ester Hydrobromide (24). *N*-[(Phenylmethoxy)carbonyl]-4(*S*)-methoxy-(*S*)-proline methyl ester (**8b**) (0.42 g, 0.0014 mol) was treated with 18% HBr in glacial HOAc (3 mL), and the resulting mixture was stirred at room temperature for 2 h. The reaction was poured into cold ether (300 mL), and the white precipitate was filtered to give **24** (0.31 g), $[\alpha]^{26}_D$ −3.5° (E, *c* 0.4). Anal. ($C_7H_{13}NO_3 \cdot HBr \cdot H_2O$) H, N; C: calcd, 32.57; found, 33.06.

The 4(*R*)- or 4(*S*)-substituted (*S*)-proline methyl ester hydrobromide salts **21**, **22** and **26**, **28**, **31** (Table II)[23] and 4,4-bis(ethylthio)-(*S*)-proline methyl ester (**58**) were prepared by using the procedure described above (Table IV).[24] The 4(*R*)- or 4-(*S*)-substituted (*S*)-proline methyl ester hydrobromide salts **31**, **41**, and **32**, **36**, **37**, **38**, **44**; 1,4-dithia-7-azaspiro[4.4]nonane-8-(*S*)-carboxylic acid methyl ester hydrobromide (**55**); and 6,10-dithia-2-azaspiro[4.5]decane-3(*S*)-carboxylic acid methyl ester hydrobromide (**57**) were prepared by using this method and were used in the next step.

4,4-Dimethoxy-(*S*)-proline Methyl Ester (54). *N*-[(Phenylmethoxy)carbonyl]-4,4-dimethoxy-(*S*)-proline methyl ester (**49**) (5.02 g, 0.016 mol) was dissolved in MeOH (75 mL). Pd/C (10%, 0.54 g) was added, and the resulting mixture was hydrogenated at atmospheric pressure.[25] Upon completion of the hydrogenation, the catalyst was removed by filtration and washed with MeOH. The combined MeOH solutions were concentrated in vacuo to give **54** as a colorless oil (2.84 g), $[\alpha]^{26}_D$ −18.7° (E, *c* 0.2). Anal. ($C_8H_{15}NO_4 \cdot 0.05CHCl_3$[26]) C N; H: Calcd, 7.77; found, 7.29.

The 4(*S*)-substituted (*S*)-proline methyl ester **23** (Table II) and 1,4-dioxa-7-azaspiro[4.4]nonane-8(*S*)-carboxylic acid (**53**) (Table IV) were prepared by using the procedure described above. The 4(*R*)- or 4(*S*)-substituted (*S*)-proline methyl esters **41**, **42**, and **43** and 6,10-dioxa-2-azaspiro[4.5]decane-3(*S*)-carboxylic acid (**56**) were prepared by using this procedure and were used in the next step.

7-[3-(Acetylthio)-2(*RS*)-methylpropanoyl]-1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic Acid Methyl Ester (85a). 1,4-Dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic acid methyl ester hydrobromide salt (**55**) (2.66 g, 0.01 mol) was dissolved in pyridine (100 mL), and 3-(acetylthio)-2-methylpropanoyl chloride (3.00 g, 0.018 mol) was added dropwise. The resulting reaction mixture was stirred at room temperature for 18 h, poured into ice–H_2O, and extracted with EtOAc. The EtOAc solution was extracted with aqueous $CuNO_3$ and then H_2O, dried ($MgSO_4$), and concentrated under reduced pressure to give an oil. Chromatography on silica gel (170 g) (hexane–EtOAc, 2:1) gave the **85a** as a yellow oil (2.20 g) $[\alpha]^{26}_D$ −25.7° (E, *c* 0.4). Anal. ($C_{14}H_{21}NO_4S_3$) C, H, N.

The *N*-[3-(acetylthio)-2-methylpropanoyl]-4,4-disubstituted-(*S*)-proline methyl esters **82**, **83**, **86**, and **87** (Table V) and *N*-[3-(acetylthio)-2-methylpropanoyl]-4(*R* or *S*)-substituted-(*S*)-proline methyl esters **63a,b**, **64–68**, **77**, and **79** and **69a,b**, **70–76**, **78**, **80**, and **81** (Table V) were prepared by using the procedure described above.[27]

7-[3-(Acetylthio)propanoyl]-1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic Acid Methyl Ester (88). 1,4-Dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic acid methyl ester hydrobromide salt (**55**) (1.42 g, 0.0053 mol) was dissolved in pyridine (25 mL) and then treated with 3-(acetylthio)propanoyl chloride (2.00 g, 0.012 mol). The resulting mixture was stirred at room temperature for 18 h, poured into ice–H_2O, and extracted with EtOAc. The organic solution was extracted with saturated aqueous $CuNO_3$ and then H_2O. The dried ($MgSO_4$) organic layer was concentrated under reduced pressure to give an oil (2.71 g). Chromatography on silica gel (100 g) (hexane–EtOAc, 4:1) gave **88** as a pale yellow oil (1.31 g) $[\alpha]^{26}_D$ −35.0° (E, *c* 0.3). Anal. ($C_{13}H_{19}NO_4S$) C, H, N.

4-[3-(Acetylthio)propanoyl]-6,10-dithia-2-azaspiro[4.5]decane-3(*S*)-carboxylic acid methyl ester (**89**) was prepared by using the above procedure (Table V).

7-(3-Mercapto-2(*RS*)-methylpropanoyl)-1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic Acid (111). Nitrogen was bubbled through a solution of 7-[3-(acetylthio)-2-methylpropanoyl]-1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic acid

(23) Procedure used[6] for preparation of **46**. This compound was prepared by oxidation of compound **40**.[14]

(24) If an oily product was obtained, the oil was dissolved in dichloromethane. The dried ($MgSO_4$) dichloromethane solution was concentrated under reduced pressure to give the 4(*R* or *S*)-substituted (*S*)-proline methyl ester hydrobromide, which was used in the next step.

(25) A Parr apparatus at 40–50 psi was used for some compounds.

(26) C, H, N were determined after examining the preliminary NMR spectrum and the compound was dissolved in $CHCl_3$, transferred, and concentrated.

(27) 4(*R* or *S*)-substituted (*S*)-proline methyl ester of 4,4-disubstituted (*S*)-proline methyl ester can be used.

methyl ester (**85**) (2.20 g, 0.0063 mol) in methanol (50 mL), and the resulting solution was cooled to 0–5 °C and treated with 1.0 N NaOH (19.0 mL, 3.0 equiv). The reaction mixture was stirred at room temperature for 4 h and concentrated under N_2. The residue was diluted with H_2O and extracted with EtOAc. The aqueous solution was acidified with 1.0 N HCl, extracted with EtOAc, dried ($MgSO_4$), and concentrated under reduced pressure to give 111 as a colorless oil (0.98 g) (Table VI).

The *N*-(3-mercapto-2-methylpropanoyl)-4,4-disubstituted-(*S*)-prolines **108–110**, **114**, and **115** (Table VI); *N*-(3-mercaptopropanoyl)-4,4-disubstituted-(*S*)-prolines **116** and **117** (Table VI); and *N*-(3-mercapto-2-methylpropanoyl)-4(*R* or *S*)-substituted-(*S*)-prolines **90–94**, and **104** and **96–103**, **105**, **106** (Table VI) were prepared by using the procedure described above.

7-[3-(Acetylthio)-2(*S*)-methylpropanoyl]-1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic Acid Methyl Ester (85b). 1,4-Dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic acid methyl ester hydrochloride (5.10 g, 0.0199 mol), D-(−)-*S*-acetyl-β-mercaptoisobutyric acid (3.449 g, 0.0212 mol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (3.83 g, 0.0199 mol), 1-hydroxybenzotriazole hydrate (2.70 g, 0.0199 mol), and triethylamine (4.06 g, 5.6 mL, 0.0402 mol) in DMF (50 mL) were stirred at room temperature for 72 h. The reaction mixture was concentrated in vacuo, and the residue was partitioned between EtOAc and H_2O. The dried ($MgSO_4$) EtOAc was concentrated in vacuo to give a yellow oil (8.72 g). Chromatography on silica gel (2 L) ($CHCl_3$–EtOAc, 18:2) gave **85b** as a colorless oil (3.59 g) $[\alpha]^{26}_D$ −95.1° (M, *c* 0.35). Anal. ($C_{14}H_{21}NO_4S_3$) C, H, N.

7-(3-Mercapto-2(*S*)-methylpropanoyl)-1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic Acid (113). 7-[3-(Acetylthio)-2(*S*)-methylpropanoyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-(*S*)-carboxylic acid methyl ester (3.37 g, 0.0107 mol) was treated as described in the general procedure to give a colorless viscous oil (2.78 g). The oil was placed on a silica gel column (1.5 L) and eluted with $CHCl_3$–MeOH–NH_4OH, 160:35:5, to give **113** as a colorless oil (1.40 g) $[\alpha]^{26}_D$ −46.0° (M, *c* 0.28). Anal. ($C_{11}H_{17}NO_3S_3 \cdot {}^3/_4NH_3$) C, H, N.

7-(3-Mercapto-2(*S*)-methylpropanoyl)-1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic Acid (113) and 7-(3-Mercapto-2(*R*)-methylpropanoyl)-1,4-dithia-7-azaspiro-[4.4]nonane-8(*S*)-carboxylic Acid (112). The product mixture, 7-(3-mercapto-2(*RS*)-methylpropanoyl)-1,4-dithia-7-azaspiro-[4.4]nonane-8(*S*)-carboxylic acid (**111**) (0.60 g), was chromatographed on silica gel (150 g) ($CHCl_3$–glacial HOAc, 49:1) to give **112** as colorless oil (0.16 g) and its 2(*S*) isomer **113**[28] as a colorless oil (0.08 g) (Table VI).

***N*-(3-Mercapto-2(*RS*)-methylpropanoyl)-$\Delta^{3,4}$-4-methoxy-(*S*)-proline Methyl Ester (120).** Hydrochloric acid (20%, 10 mL) was added to a solution of 7-[3-(acetylthio)-2-methylpropanoyl]-1,4-dioxa-7-azaspiro[4.4]nonane-8(*S*)-carboxylic acid methyl ester (**82**) (1.45 g, 0.0044 mol) in MeOH (50 mL). The mixture was heated under reflux for 6 h, kept at room temperature for 66 h, poured into ice–H_2O (200 mL), and extracted with EtOAc. The dried ($MgSO_4$) EtOAc solution was concentrated in vacuo and chromatographed on silica gel (hexane–EtOAc, 1:1) to give **120** as an oil (0.52 g) $[\alpha]^{26}_D$ −38.5° (D, *c* 0.5), which was used in the next reaction.

***N*-(3-Mercapto-2-methylpropanoyl)-$\Delta^{3,4}$-4-methoxy-(*S*)-proline (118).** Under a N_2 atmosphere, N-(3-mercapto-2-methylpropanoyl)-$\Delta^{3,4}$-4-methoxy-(*S*)-proline methyl ester (**120**) (0.52 g, 0.002 mol) was dissolved in MeOH (20 mL) and cooled to 0–5 °C, and 1.0 N NaOH (3.2 mL) was added. The resulting mixture was stirred for 2 h at room temperature and then concentrated under N_2. The residue was dissolved in 1.0 N NaOH (ice added), and the basic solution was washed with EtOAc. The basic solution was acidified with 20% HCl (ice added) and extracted with EtOAc. The dried ($MgSO_4$) EtOAc solution was concentrated under reduced pressure to give **118** as a colorless oil (0.31 g) (Table VI).

***N*-(3-Mercapto-2-methylpropanoyl)-4-oxo-(*S*)-proline Methyl Ester (121).** *N*-(3-Mercapto-2-methylpropanoyl)-$\Delta^{3,4}$-4-methoxy-(*S*)-proline methyl ester (**120**) (0.60 g, 0.0023 mol) was dissolved in acetone (100 mL), and *p*-toluenesulfonic acid (0.15 g, 0.0008 mol) in H_2O (10 mL) was added. The resulting solution was stirred under reflux for 20 h, poured into ice–H_2O (200 mL), and extracted with EtOAc. The dried ($MgSO_4$) organic solution was concentrated under reduced pressure. Chromatography on silica gel (100 g) (EtOAc–hexane 1:1) gave **121** as an oil (0.18 g), $[\alpha]^{26}_D$ −27.0° (E, *c* 0.4). Anal. ($C_{10}H_{15}NO_2S \cdot 0.05CHCl_3$) C, H, N.[26]

***N*-(3-Mercapto-2-methylpropanoyl)-4-(methoxyimino)-(*S*)-proline Methyl Ester (122).** *N*-(3-Mercapto-2-methylpropanoyl)-4-oxo-(*S*)-proline methyl ester (**121**) (0.50 g, 0.002 mol) was dissolved in pyridine (30 mL), and methoxylamine hydrochloride (0.57 g, 0.0083 mol) was added. The mixture was stirred at room temperature for 20 h, poured into cold 5% HCl (200 mL), and extracted with EtOAc. The organic solution was extracted with 5% HCl, dried ($MgSO_4$), and concentrated under reduced pressure to give a residue (0.33 g). Chromatography on a Lobar size C column ($CHCl_3$–EtOAc, 1:1) gave **122** as a colorless oil (0.16 g) $[\alpha]^{26}_D$ −25.5° (E, *c* 0.4). Anal. ($C_{11}H_{18}N_2O_4S \cdot 0.1CHCl_3$) C, H, N.

***N*-(3-Mercapto-2-methylpropanoyl)-4-(methoxyimino)-(*S*)-proline (119).** Under a N_2 atmosphere, *N*-(3-mercapto-2-methylpropanoyl)-4-(methoxyimino)-(*S*)-proline methyl ester (**122**) (0.26 g, 0.000 94 mol) was dissolved in MeOH (50 mL) and cooled to 0–5 °C, and 2.5 N NaOH (10 mL) was added. The solution was stirred at room temperature for 2 h, poured into ice–H_2O, and washed with EtOAc. The aqueous solution was acidified with 20% HCl, extracted with EtOAc, dried ($MgSO_4$), and concentrated under reduced pressure to give **119** as a colorless oil (0.18 g) (Table VI).

Biology. In Vitro ACE-Inhibitory Activity. The in vitro inhibitory activity was determined by the method of Cushman and Cheung.[17] The crude ACE was prepared by blending rabbit lung acetone powder (Sigma) (10 g) in 50 mM potassium phosphate buffer (100 mL), pH 8.3, and centrifuging for 40 min at 40000*g*; the clear supernatant was kept in the refrigerator. Incubations for the spectrophotometric assay of hippuryl-L-histidyl-L-leucine (HHL; from Sigma) hydrolysis by ACE were carried out at 37 °C in disposable 13 × 100 mm tubes. Each 0.25-mL assay mixture contained the following components at the indicated final concentrations: potassium phosphate buffer, pH 8.3 (100 mM); NaCl (300 mM); HHL (5 mM). The enzyme, in a volume of 0.1 mL, was added last to initiate the reaction, and the tubes were incubated for 30 min. The enzymatic reactions were stopped by adding 0.25 mL of 0.1 N HCl; HCl was added before the enzyme for zero time control. The hippuric acid formed by action of ACE on HHL was extracted with EtOAc (1.5 mL) by vortex mixing for 15 s. After a brief centrifugation, a 1.0-mL aliquot of each EtOAc layer was transferred to a clean tube and evaporated by heating at 120 °C for 30 min in a Temp-Blok module heater. The hippuric acid was redissolved in 1.0 mL of H_2O, and the amount formed was determined from its absorbance at 228 μm. Inhibitory activity was determined as the IC_{50}, the approximate molar concentration of a compound required to cause a 50% inhibition of the control ACE activity. The activity of each compound is compared in relation to captopril, of which the relative potency is designated as 1.0 (IC_{50} = 0.0035–0.025 μM) (lit.[1b] IC_{50} = 0.023 μM). Relative ACE-inhibitory potency is equal to IC_{50} of captopril/IC_{50} of compound X.

In Vivo ACE-Inhibitory Activity. Sprague–Dawley rats were anesthetized with inactin (100 mg/kg) or dial urethane. The carotid artery and jugular vein were cannulated. Blood pressure was measured from arterial cannula. Drugs were injected intravenously. The animals (two to five) were challenged with angiotensin II (0.3 μg/kg), angiotensin I (0.3 μg/kg), and bradykinin (3 μg/kg) during a control period. The sequence of challenges was repeated 5 min after iv administration of the test drug. Each animal received at least two doses (increasing by a factor of 10) of test drug. Angiotensin I responses were expressed as a percent of the control response, and an ID_{50} value was determined by linear regression analysis.

Spontaneously Hypertensive Rat. Drugs are suspended or dissolved in 0.4% methylcellulose vehicle (standard biological vehicle) when possible. Other vehicles include water, physiological saline, or 5% ethanol. Drugs may be solubilized by the addition of 0.1 N HCl or 0.1 N NaOH and diluted with water, saline, or 0.4% methylcellulose. Drugs are usually administered by stomach

(28) TLC and NMR results similar to those of the compound obtained from hydrolysis of **85b**.

tube in volumes of 2 mL/kg. When indicated, intraperitoneal, subcutaneous, or intravenous routes are used. Hypertensive rats are anesthetized with ether. A polyethylene catheter (PE 10 fused to PE 50, 7.5–9.5 cm long depending on body weight) is inserted into the abdominal aorta via the caudal artery. The skin incision is closed with sutures. Animals are then placed into plastic restrainers where they quickly recover consciousness. A 5% dextrose in water solution is infused into the arterial line (0.2 mL/h) via a T-adapter to assure patency of the cannula. The catheter is connected to a P23Gb pressure transducer. Analog blood pressure signals are recorded on an oscillograph. A cardiovascular monitoring system (Buxco Electronics Inc.) and a digital computer may be used to provide averages over 30 min. Mean values are used for comparative purposes. Heart rate is derived from the Buxco system or from the pulse-pressure trace by a tachometer. Animals are removed from the restrainer after approximately 90 min, dosed, returned to the holders, and usually observed for 4 h. Animals are fasted prior to the test. Blood pressure and heart rate values are usually noted at half-hour intervals.

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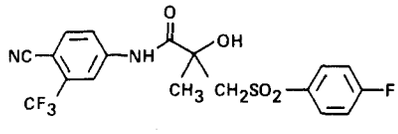
Resolution of the Nonsteroidal Antiandrogen 4′-Cyano-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methyl-3′-(trifluoromethyl)-propionanilide and the Determination of the Absolute Configuration of the Active Enantiomer

Howard Tucker* and Glynne J. Chesterson

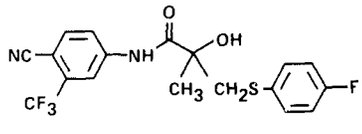
Imperial Chemical Industries PLC, Pharmaceuticals Division, Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TG Great Britain. *Received September 29, 1987*

The nonsteroidal antiandrogen 4′-cyano-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methyl-3′-(trifluoromethyl)-propionanilide (1) (ICI 176334) has been resolved by chromatographic separation of the diastereomeric (*R*)-camphanyl esters of the precursor thioether **2** followed by hydrolysis and oxidation of the isolated enantiomers. In addition, an asymmetric synthesis of (*S*)-3-bromo-2-hydroxy-2-methylpropanoic acid (**11**) and subsequent conversion into the (*S*)-sulfone **6a** has established that the more potent enantiomer of **1** has the *R* absolute configuration.

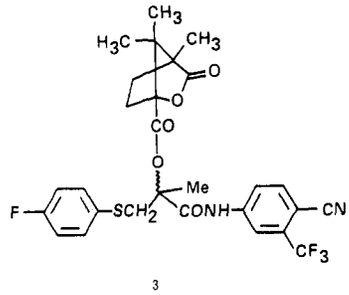
We have reported the discovery of a novel, peripherally selective, nonsteroidal antiandrogen, 4′-cyano-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methyl-3′-(trifluoromethyl)propionanilide (1) (ICI 176334), which is currently being developed for the treatment of androgen-responsive benign and malignant diseases.[1,2] We report here the preparation of the enantiomers of 1 together with their biological activities and the assignment of the absolute stereochemistry of the more active enantiomer.



1

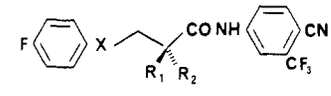


2



3

Our route to the enantiomers of 1 focused on the resolution of the thioether **2**,[2] the enantiomers of which could then be oxidized to the required sulfones by known means.[2] Reaction of **2** with (*R*)-(−)-camphanoyl chloride in pyridine furnished the diastereomeric esters **3**, which were separated by careful flash chromatography on silica gel and were judged to be pure on the basis of TLC and 400-MHz NMR analysis. The individual pure diastereomeric esters were each hydrolyzed, without racemization, with methanolic sodium hydroxide to give the enantiomeric alcohols **4** and **5**. The optical purity of these enantiomeric alcohols was



	X	R_1	R_2	Configuration
4	S	CH_3	OH	S
5	S	OH	CH_3	R
6	SO_2	CH_3	OH	S
7	SO_2	OH	CH_3	R

determined by a HPLC method with use of a Spherisorb 5μ-NH_2 column doped with (*R*)-(−)-*N*-benzoylphenylglycine.[3] This method was able to detect 1% of the (+)-enantiomer in the (−)-enantiomer, but because of unfavorable peak overlap, the limit of detection of the (−)-enantiomer in the (+)-enantiomer was only 5%. The observed rotations of the enantiomeric thioethers **4** and **5** could be consistent with the presence of 5% of the (−)-enantiomer **5** in **4**. Both enantiomeric thioesters **4** and **5** were oxidized to the corresponding sulfones **6** and **7** with use of *m*-chloroperoxybenzoic acid in methylene chloride solution.

Although this method of resolution proved satisfactory for preparing the enantiomers **4** and **5**, we were seeking

(1) Furr, B. J. A.; Valcaccia, B.; Curry, B.; Woodburn, J. R.; Chesterson, G.; Tucker, H. *J. Endocrinol.* **1987**, *113*, R7-9.
(2) Tucker, H.; Chesterson, G. J.; Crook, J. W., submitted for publication in *J. Med. Chem.*
(3) We thank R. Gaskell, Physical Chemistry Section, who carried out this analysis.

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