mg/kg, dissolved or suspended in 0.5% [(hydroxypropyl)-methyl]cellulose in distilled water, 10 mL/kg. If a compound was incompatible with this vehicle, an alternative vehicle was used.

Mean arterial pressures (MAP) and heart rates were recorded for 10 s, every 5 min, with an electronic switching computer system. Fifteen or 30 min averages were tabulated and reported as percent changes from the 0.5-h average values just prior to dosing. Compounds were evaluated at each time period by comparing the mean percent changes oberved for MAP with those of a cumulative control group composed of all rats which received the vehicle alone in previous runs. Values which fell outside the range of the mean ± 2SD were considered significantly different from controls. The duration of action was the continuous length of time during which test values were below or above those of the historical controls. If the results in the two test rats were inconsistent, one or two additional rats were used.

D. Diuretic Testing in the Rat. Male rats weighing 175–250 g were used. Water and food were withheld for 18 h prior to and during the experiment. The rats were housed in metabolism cages, two to a cage.

Eight rats each were dosed with the test compound or vehicle alone. Compounds were administered iv, ip, or po at the indicated dose in Tris buffer (pH 8.5, 3.5 mL/kg). Each animal was given a fluid load of 0.9% saline, 20 mL/kg, by gavage after the test drug.

Urine was collected from each cage for 0–5- and 5–24-h post-dosing. Volume (mL), Na^+ (mequiv), and K^+ (mequiv) per kilogram of body weight were recorded and reported as the means ± SD.

Nonpaired "t" tests were used to determine a significant difference in the volume or Na^+ or K^+ excretions between the test and control groups.

E. Diuretic Activity in Dogs. The procedure is a modification of ref 25. Mongrel dogs of either sex were fasted overnight and then anesthetized with pentobarbital sodium (35 mg/kg, iv) and respired with 20 mg/kg of room air at 12 breaths/min. Arterial pressure (femoral artery; Statham Instruments Model P23ID pressure transducer) was recorded.

After a ventral midline incision of the lower abdomen was made, the left and right ureters were cannulated for urine collection. Mannitol (4% solution, 2.2 mL/min) was infused via the left external jugular vein to maintain urine flow. Urine samples were collected every 15 min and analyzed for electrolytes with NOVA 4+4 ion sensitive electrodes.

Tests compounds were infused in 3.5 mL of saline or pH 8.5 Tris buffer through a right femoral venous cannula at 1.5 mL/min. Drug effects were monitored for a minimum for 1 h. Mean values of $N \geq 3$ animals (volume, Na^+, K^+) were compared to mean ± 2SD of pooled historical controls. Values outside the historical range were deemed significant. Duration of action refers to the time of return of treated animals to control values.

Acknowledgment. We are indebted to Raul Calvo, Joseph Bruno, and Brian Kornberg for expert technical assistance; Dr. John Lloyd and Richard Gedamke for mass spectral assistance, and Michael Fitzgerald for elemental analyses. Thanks are also due Drs. Bill Mann, Atul Khāndwala, Ira Weinryb, Ronald D. Smith, Peter Wolf, and John Barrett for helpful discussions and guidance. Finally we thank Tammy Gibble for expert manuscript preparation.

Supplementary Material Available: Synthetic procedures for the intermediates 4 (41 pages). Ordering information is given on any current masthead page.

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Angiotensin Converting Enzyme Inhibitors. 10. Aryl Sulfonamide Substituted *N*-[1-Carboxy-3-phenylpropyl]-L-alanyl-L-proline Derivatives as Novel Antihypertensives†

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Compounds **1a–g** consisting of enalaprilat covalently bonded to aryl sulfonamides, including several known thiazide diuretics, were synthesized and tested for ACE inhibitory and diuretic and overall antihypertensive effects. All compounds were potent ACE inhibitors in vitro, with IC_{50} = 6.5–85 nM. At 10 mg/kg iv or ip in the rat, **1a–g** inhibited the AI pressor response by 76–100%; inhibition declined significantly upon oral dosing. Compounds **1a** and **1f** at 100 mg/kg ip in the sodium-depleted, spontaneously hypertensive rats reduced blood pressure 28–35% and 41–42%, respectively. Compounds **1a** and **1f** elicited natriuresis and kaliuresis without accompanying volume increases in the rat; **1c** at 25 mg/kg iv induced delayed diuresis. Compound **1f** has been chosen for further development.

Angiotensin converting enzyme (ACE) inhibitors have been safely and effectively applied in the treatment of nearly all forms of hypertension, regardless of severity or etiology, and have also become the major therapy in the treatment of refractory congestive heart failure.[1] Numerous reviews of the chemistry and pharmacology[2] and clinical applications[3] of these drugs have appeared.

Recently, synergism between ACE inhibitors and diuretics has been investigated. The effects of diuretics on plasma renin levels have long been of interest.[4] It has been shown that diuretics could act to potentiate an enhanced antihypertensive response to ACE inhibitors by stimulating the renin–angiotensin–aldosterone system.[5] Clinical in-

† See ref 18.

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(2) (a) Wyvratt, M. J.; Patchett, A. A. *Med. Res. Rev.* **1985**, *5*, 483. (b) Brunner, H. R.; Nussberger, J.; Waeber, B. *J. Cardiovasc. Pharm.* **1985**, *7* (Suppl. 1), S2. (c) Andrew, M.; Julius, F.; Julius, S. *J. Cardiovasc. Pharm.* **1985**, *7* (Suppl. 1), S35. (d) Hollenberg, N. K. *J. Cardiovascular Pharm.* **1985**, *7* (Suppl. 1), S40. (e) Kostis, J. B., DeFelice, E. A., Eds. *Angiotensin Converting Enzyme Inhibitors* New York: Alan R. Liss, Inc., 1987. (f) Nelson, E. B.; Pool, J. L.; Taylor, A. A. *Am. J. Med.* **1986**, *81* (Suppl. 4C), 13. (g) Cushman, D. W.; Ondetti, M. A.; Gordon, E. M.; Natarajan, S.; Karenewsky, D. S.; Krapcho, J. *J. Cardiovasc. Pharmacol.* **1987**, *10* (Suppl. 7), 517–530.

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vestigations involving lisinopril[6,7] and enalapril[8,9] indicate that larger percentages of patient populations respond to combined ACE inhibitor and diuretic therapy than to monotherapy with ACE inhibitors alone. Monotherapy with enalapril was effective in 55–65% of patients in long-range trials, while coadministration with a diuretic increased this to 85–95%.[10] Similarly, captopril's[11] capture rate increased from 48% for monotherapy to 85% for combined ACE inhibitor/diuretic treatment.[12]

In view of these clinical observations, a single drug entity possessing both diuretic and ACE inhibitory activity could provide a useful alternative to combination therapy with two drugs.[13] The occurrence of two mutually complementary biological activities into a single molecule has been observed clinically in the dipharmacophore labetalol[14] and sought chemically through covalent combination of separate, biologically symbiotic agents.[13,15] The merits and pitfalls of this "symbiotic approach to drug design",[16] as well as the principles guiding such research, have been discussed elsewhere.[15,16]

In previous papers we reported investigations concerning N-substituted glycine derivatives as ACE inhibitors[17] and efforts toward single molecules expressing ACE inhibition and diuresis.[18] In this paper we discuss the synthesis and biological activity of compounds **1a–g** (Table I), which were derived from chemical combination of enalaprilat[8] with various aryl sulfonamides, including several known thiazide diuretics. These were assessed for degree of ACE inhibitory, diuretic, and overall antihypertensive effect.

Chemistry

Key steps in the preparation of all seven targets were the connection of a proline derivative to *N*-[1(*S*)-(ethoxycarbonyl)-3-phenylpropyl]-L-alanine[19] (**2a**) and the attachment of a thiazide diuretic to proline (Scheme I). Acid **2**, generally in its (trichloroethyl)formamoyl (TROC) protected form[17d] **2b**, was condensed with the proline derivative via either its acid chloride or through the use of 1,1-carbonyldiimidazole (CDI).[21] One adduct, alcohol **3** (Scheme I), served as the starting point for compounds **1b–g**. Chromate oxidation of alcohol **3** smoothly afforded oxoproline derivative **4**, from which targets **1b**, **1e**, and **1f** were prepared. Reductive aminations of ketone **4** with (3-sulfamyl-4-chlorobenzoyl)hydrazine and ketone **5** with 3-[(methylamino)methyl]-6-chloro-7-sulfamyl-3,4-dihydrobenzo-1,2,4-thiadiazine 1,1-dioxide[22] using sodium cyanoborohydride[23] provided the completed frameworks of targets **1b** and **1f** as diastereomeric mixtures at the proline 4-position.[24] Condensation of ketone **4** with 1-amino-3-chloro-4,6-benzenedisulfonamide under acid catalysis afforded the spiro[25] linkage which completed the skeleton of **1e** as a roughly 1:1 mixture of diastereomers.

The frameworks of targets **1c** and **1d** were constructed by condensation of amine **13** with sulfonyl chloride **14**[26] (Scheme II). The requisite amine was obtained by a highly selective reduction[27] of the azide, **12**, derived from alcohol **3**.

Synthesis of **1a** (Scheme III) began with the alkylation of *N*-(benzyloxycarbonyl)-4-hydroxyproline ethyl ester (**17**) with 2-(2-iodoethyl)-1,3-dioxolane to provide ether acetal **19**. Hydrogenolysis of the nitrogen protecting group gave acetal **20**. Condensation of **20** with acid **2a** via CDI[21] provided dipeptide **21**, which upon treatment with 1-

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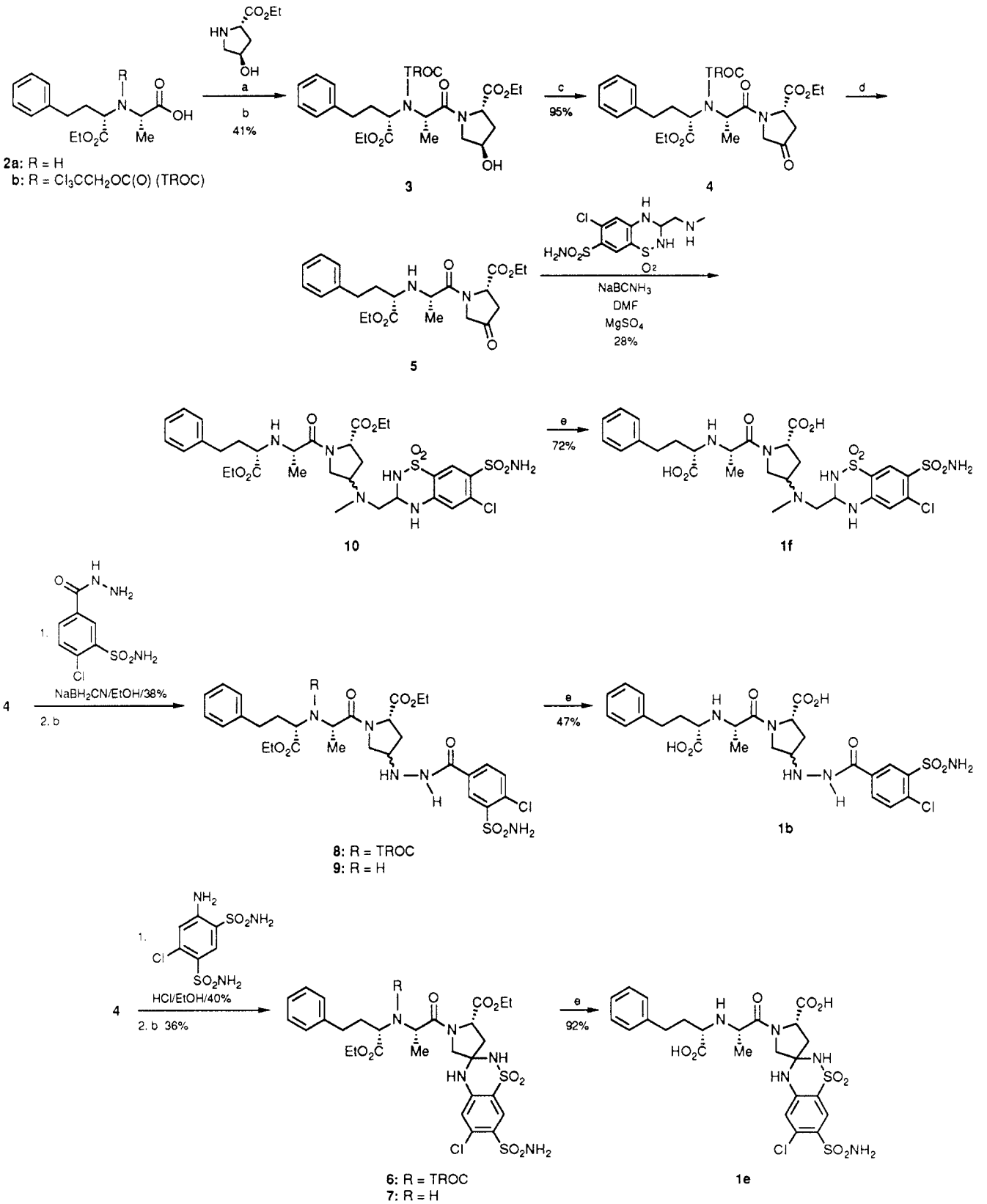
Scheme I[a]



[a] (a) Cf. ref 20; (b) $COCl_2/CH_2Cl_2$, cat. DMF; (c) PCC/CH_2Cl_2; (d) Zn/HOAc; (e) (1) N aqueous NaOH/EtOH; (2) 2 N aqueous HCl.

amino-3-chloro-4,6-benzenedisulfonamide under mild catalysis gave penultimate diester **22**.

For the synthesis of **1g** (Scheme IV), aldehyde **23**[28] was condensed with cysteine ethyl ester to afford the stereochemically mixed thioproline **24**; acylation of **24** with the acid chloride of **2b** provided **25**, which comprised the requisite framework.

Standard treatment of TROC-protected intermediates **4**, **6**, **8**, **15**, and **25** with an excess of zinc in acetic acid (method A) liberated the alanine nitrogen. For the synthesis of compound **1a**, condensation of acid **2a** with de-

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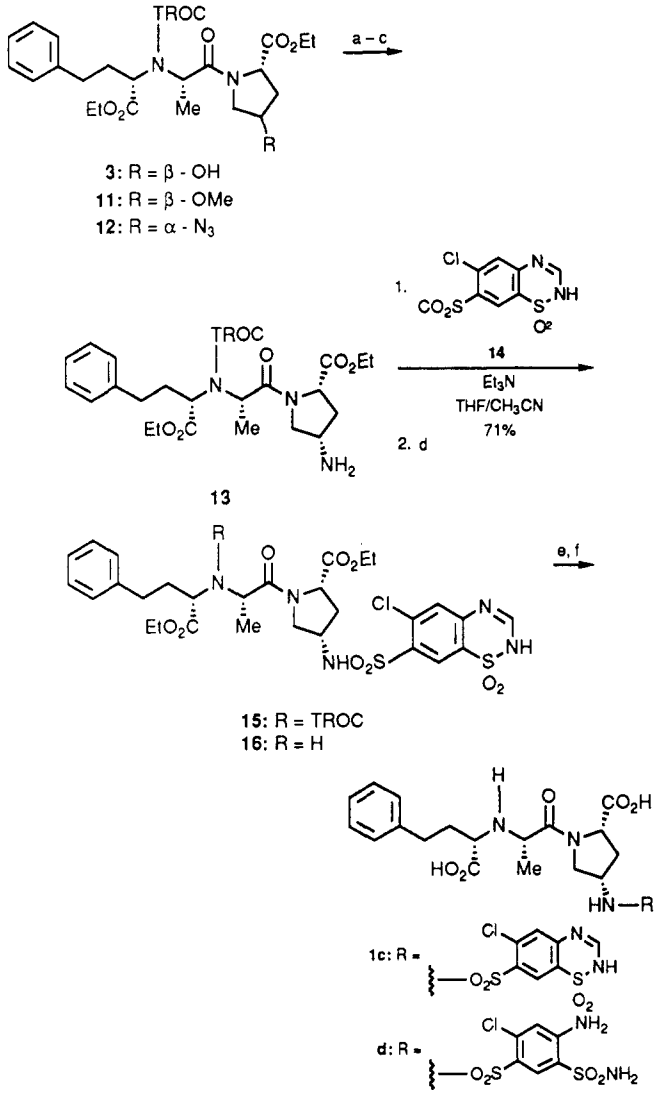
Scheme II[a]



[a] (a) $MsCl/Et_3N/CH_2Cl_2$, 89%; (b) NaN_3 (2 equiv)/22% $EtOH/H_2O$, heat, 94%; (c) $HS(CH_2)_3SH$ (5 equiv)/Et_3N/CH_3OH, quant.; (d) Zn (12.5 equiv)/HOAc, 79%; (e) 52% aqueous 1 N NaOH/EtOH, 98%; (f) 5% aqueous NaOH, 90 °C, 2 h, 86%.

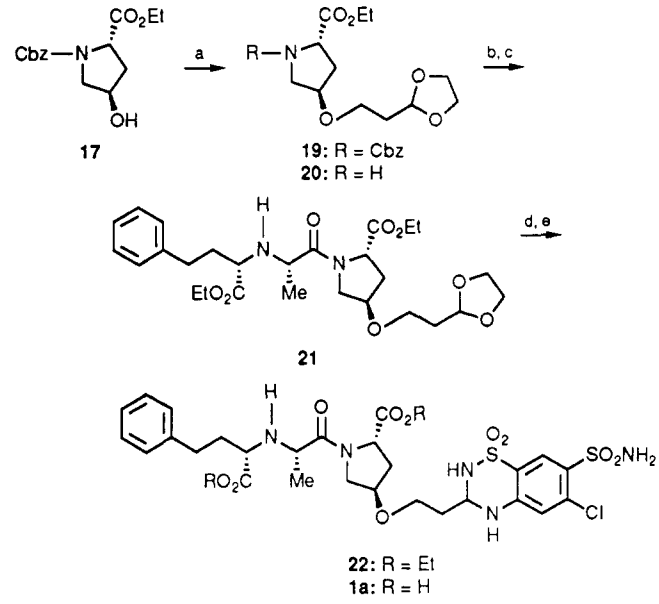
rivatized proline averted complications arising from treatment of advanced intermediates with zinc. In the synthesis of **1f**, TROC was removed prior to reductive amination of ketone **4** for similar reasons. Final saponification of the diesters of **1a–f** followed by acidification of the reaction mixture (methods B and C) provided in each case the desired dicarboxylic acid as the hydrochloride or trifluoroacetate salt. Orthanilamide **1d** was obtained from **1c** after more vigorous treatment with hot aqueous base.[26]

Results and Discussion

Compounds **1a–g** were potent ACE-inhibitors in vitro, with IC_{50} values ranging from 6.5 to 85 nm (Table II). By comparison, enalaprilat and captopril had IC_{50} values of 1.2 and 20 nm, respectively.[9b] When administered to rats iv or ip at 10 mg/kg, all produced a 76–100% inhibition of the pressor response to angiotensin I (AI). Maximum inhibition upon iv dosing typically occurred after 0.3–1 h, and the inhibitory effect endured from 1 to over 4 h.

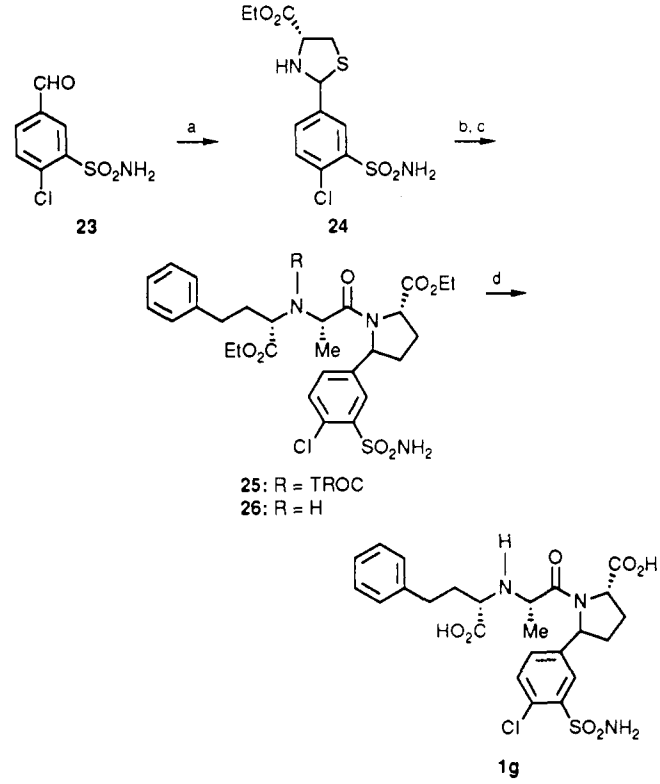
A comparison of iv or ip with po dosing (Table II) in the rat at 10 mg/kg revealed a drop in the inhibition of the AI pressor response from the 76–100% range to the 33–58% range. These effects were attributed to poor oral

Scheme III[a]



[a] (a) 2-(2-Iodoethyl)-1,3-dioxolane (18), Ag_2O, DMF, 25 °C, 18 h, then 45 °C, 5 h; (b) H_2 (40 psi), 10% Pd/C Et_3N, EtOH, quant.; (c) **2a**, CDI/CH_2Cl_2, 71%; (d) 1-amino-3-chloro-4,6-benzenedisulfonamide/CSA/EtOH, 87%; (e) (1) 1:1 aqueous 2 N NaOH/EtOH; (2) TFA, 62%.

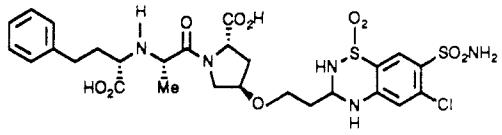
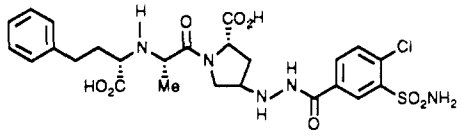
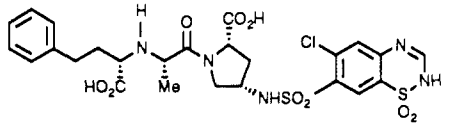
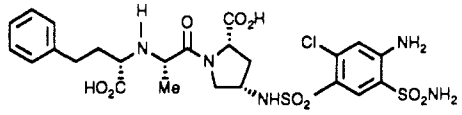
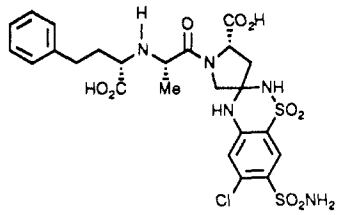
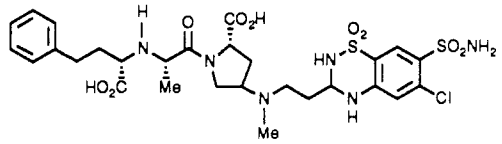
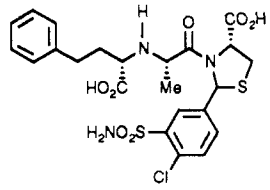
Scheme IV[a]



[a] (a) $H_2NCH(CH_2SH)CO_2Et/HCl/EtOH$, Δ, 94%; (b) **2b**, $(COCl)_2/DMF$, 27%; (c) Zn/HOAc, 87%; (d) (1) $NaOH/H_2O$; (2) aqueous HCl, 65%.

absorption. Time until maximal effect showed no consistent trend in going from iv/ip to po administration; duration of action generally declined upon oral dosing. Two illustrative cases bear mention. Compound **1b**, when administered to rats at 1 mg/kg iv, produced an 85–94% inhibition of the AI pressor response, but oral dosing at 10 times this level produced a modest 52% inhibition. Compound **1a** at 10 mg/kg iv in the rat effected a 100%

Table I

		elemental composition of test compound	mp, °C
1a		$C_{27}H_{34}ClN_5O_{10}S_2 \cdot CF_3CO_2H \cdot 2H_2O$[a]	150 dec[b]
1b		$C_{25}H_{30}ClN_5O_8S \cdot HCl \cdot 2H_2O$[c]	188 dec
1c		$C_{25}H_{28}ClN_5O_9S_2 \cdot CF_3CO_2H$	193–195 dec
1d		$C_{24}H_{30}ClN_5O_9S_2 \cdot CF_3CO_2H \cdot H_2O$[d]	168–180 dec
1e		$C_{24}H_{28}ClN_5O_9S_2 \cdot HCl \cdot 0.75H_2O$[e]	198 dec
1f		$C_{27}H_{35}ClN_6O_9S_2 \cdot 2HCl \cdot 3H_2O$	190–195 dec
1g		$C_{23}H_{26}ClN_3O_7S_2 \cdot 0.75H_2O$	165–167

[a] H: calcd, 4.68; found, 4.18. N: calcd 8.36; found, 7.77. [b] Melting points are uncorrected. [c] N: calcd, 10.48; found, 9.91. [d] C: calcd, 39.92; found, 40.34. [e] N: calcd, 10.30; found, 9.53.

Table II. In Vitro and in Vivo ACE Inhibition

compd	in vitro ACE inhibn: IC_{50},[a] μM	in vivo ACE inhibn: dose, mg/kg, route	max % inhibn	n[b]	time to max inhibn, h	duration,[c] h
captopril	0.018	0.3, po	69	5	0.7	2
	(0.025)[e]	(0.3, po)[e]	(64)[e]	(4)[e]	(0.2)[e]	(1.5)[e]
enalapril	0.8	0.3, po	90	6	0.7–1	>4
	(1.2)[d]	(0.3, po)[d]	(55)[d]	(6)[d]	(1.5)[d]	(>3)[d]
enalaprilat	0.0058					
	(0.0012)[d]	(10, po)[d]	(74)[d]	(6)[d]	(1)[d]	(>6)[d]
1a	0.022	10, iv	100	2	0.3	3.7
		10, po	38, 56	2	2.6, 3	1.6, 2.3
1b	0.026	1.0, iv	85, 94	2	0.3	3, 4
		10, po	52, 21	2	0.3	0, 3.7
1c	0.023	10, iv	78	6	0.3	1.25
1d	0.0065	10, iv	76, 86	2	0.3	3.7
1e	0.085	10, po	54	3	1	2.1
1f	0.018	10, iv	96	3	2.7	>4
		10, po	37	3	3	3.7
1g	0.008	10, ip	96	6	0.3	>4
		10, po	44	6	0.3	1.3

[a] Concentration inhibiting 50% of the activity of rabbit lung ACE at pH 8.3 in 0.05 M KH_2PO_4 buffer containing 0.30 M NaCl with the substrate Hip-His-Leu at a concentration of 2 mM; cf. ref 17d. [b] Number of rats used at each dose. [c] Time during which ACE inhibition was ≥30%. [d] Hog plasma ACE; cf. ref 8a. [e] Hog plasma ACE; cf. ref 11.

inhibition of the pressor response, but upon oral dosing at the same level inhibition dropped to 38–56%.

Compounds **1a–g** were assayed for diuretic, kaliuretic, and natriuretic activity in the fasted rat (Table III). In

Table III. Rat Diuretic Data[a]

compd	n[b]	dose, mg/kg, route	% change in volume at 0–5 h (5–24 h)	% change in K^+ at 0–5 h (5–24 h)	% change in Na^+ at 0–5 h (5–24 h)
chloro-	8	10, iv	+83	+100	60
thiazide	8	30, po	+94	+71	0
1a	8	82, iv	0	+80	+60
1b	8	100, iv		inactive	
1c	8	25, iv	0	+40	0
			(+61)	(+67)	(0)
1d	8	100, iv	-49	0	-57
			(+60)	(0)	(+38)
1e	8	30, ip		inactive	
		30, ip		inactive	
1f	8	30, iv	0	0	0
		100, po	0	+40	+36
1g	8	100, iv		inactive	
		100, po	-43	0	0
			(-42)	(-45)	(-22)

[a] + indicates excretion, - indicates retention; see the Experimental Section. [b] Number of rats used at each dose. [c] Table entries lacking 5–24-h data reflect absence of activity in that time period.

some cases, an unexplained reduction in urinary volume was observed. Compound **1g** was inactive in the rat at 1 mg/kg iv, but at 100 mg/kg po, a 42 and 43% decrease in urinary volume occurred during the 0–5-h and 5–24-h period, respectively. Potassium and sodium ion excretion was reduced by 45 and 22%, respectively, over the 5–24-h interval. Compound **1d** in the rat at 100 mg/kg iv caused a 49% attenuation in urinary volume and a 57% reduction in sodium excretion during the 0–5-h period.

Compounds **1b** and **1e** were devoid of any ion- or water-excretion activity up to doses of 100 and 30 mg/kg iv, respectively. Three of the seven test compounds elicited diuretic and/or natriuretic and kaliuretic effects in the rat. Compound **1c** at 25 mg/kg iv caused a 40% increase in K^+ excretion in the 0–5-h interval, while 61 and 67% elevations in urinary volume and K^+ excretion were observed in the 5–24-h period. Compound **1a** at 82 mg/kg iv produced an 80% elevation in urinary potassium and a 60% increase in urinary sodium during the first 5 h; no increase in urinary volume was noted. Compound **1f** also elicited this response: **1f** was inactive at 10 and 30 mg/kg iv, but a 100 mg/kg po dose elevated K^+ and Na^+ excretion during the 0–5-h period by 36 and 40%, respectively, with no accompanying urinary volume increase.

Target molecules were also tested for antihypertensive efficacy in the sodium-depleted, spontaneously hypertensive rat (SHR) (Table IV). A 28–35% sustained (24 h) drop in mean arterial pressure (MAP) was observed upon administration of **1a** at 100 mg/kg ip. However, oral dosing of **1a** at the same level greatly attenuated its antihypertensive effect. Compound **1f**, at 100 mg/kg ip, produced a 41–42% drop in blood pressure which persisted for over 24 h. Upon oral dosing at the same level, a 20–22% drop in pressure occurred after 0.5–1 h and persisted for 42 h.

Conclusion

The potent in vitro and iv/ip in vivo ACE-inhibitory (ACE-I) activities of **1a–g** imply that connections of aryl sulfonamides at the 4-position of the proline of enalaprilat are not deleterious to ACE inhibition. However, rat ACE-I data for **1a–g** and SHR data for **1a** and **1f** show that ACE inhibition and antihypertensive potency decline markedly upon oral administration. This implies that oral bioavailability suffers when an aryl sulfonamide is attached to enalaprilat.

Certain of compounds **1a–g** increased Na^+ and K^+ excretion in the rat, while **1c** exerted a delayed diuretic effect. Thiazide diuretics generally produce responses within 30–60 min of oral dosing, with the effect normally lasting less than 5 h.[29] Compounds **1a–g** upon iv and po testing in the rat exhibited ACE inhibition within 0.5–3 h, indicating bioavailability under both treatment regimens well within the 0.5–5 h range during which the thiazide diuretics incorporated within them would be expected to act. For this reason, a diuretic and/or natriuretic or kaliuretic effect occurring after 5-h postadministration (e.g. **1c**) was ascribed to active metabolites or toxic effects of the test compound. The delayed diuretic effect of **1c** was thus discounted.

In view of its encouraging primary ACE inhibitory (IC_{50} = 0.018 μM), SHR antihypertensive (100 mg/kg po; MAP, -20%, -22%), and diuretic (100 mg/kg po; K^+, +40%; Na^+, +36%; 0–5 h) data, compound **1f** has been chosen for further development.[30]

Experimental Section

Melting points were determined with a Thomas-Hoover capillary melting point apparatus or a Mel-Temp apparatus and are uncorrected. NMR spectra were measured on a Varian EM-360, Varian EM-390, or JEOL JNM FX-270 Fourier Transform NMR spectrometer using tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ) from the Me_4Si resonance in the indicated solvent. Infrared (IR) data were recorded on a Perkin-Elmer Model 298 spectrometer. Mass spectra were run on a Varian MATT 112. TLC analytical separations were conducted with E. Merck silica gel 60 F-254 plates of 0.25 mm thickness and were visualized with UV or I_2. Normal-phase chromatographies were run by using gravity (Woelm Activity III/30 mm silica gel) or flash[31] (E. M. Science Kieselgel 60, 70–230 mesh) conditions or on a Waters Prep LC/System 500 using Prep Pak-500/Silica cartridges and indicated solvents. Reverse-phase MPLC chromatography was performed with C-18 (Waters, 15 μm) at 30 psi in glass columns. Reverse-phase analytical HPLC was run with twin Waters M-6000 pumps, a Waters Model 660 Solvent Programmer, a Unimetrics GM770 polychromatic UV detector, and C-8 Bondapak or Vydak C-18 protein/peptide columns with UV detection at 215, 220, 254, or 280 nm. The commercially available starting materials were used as obtained. Tetrahydrofuran was dried using sodium and benzophenone; acetonitrile and methylene chloride were distilled from calcium hydride. Protocols for determining ACE inhibition in vitro and in vivo in the rat were reported earlier.[17d]

***N*-[*N'*-[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]-*N'*-[(2,2,2-trichloroethoxy)carbonyl]-L-alanyl]-L-4-hydroxyproline Ethyl Ester (3).** A solution of **2b** (5.94 g, 13.1 mmol), oxalyl chloride (5.70 mL, 65.3 mmol), and DMF (20 μL) in 50 mL of methylene chloride was stirred at room temperature for 3.5 h and diluted with toluene, and the volatiles were removed in vacuo. The residue was diluted in 30 mL of methylene chloride and cooled to 0–5 °C. A mixture of 4-hydroxy-L-proline ethyl ester hydrochloride[20] (1.96 g, 10.1 mmol) and anhydrous triethylamine (6.99 mL, 50.3 mmol) in 40 mL of methylene chloride was added dropwise. After the addition was complete, the mixture was slowly warmed to room temperature and stirred overnight, and the volatiles were removed in vacuo. The residue was diluted with ether, washed with aqueous HCl, H_2O, 1 N NaOH, and brine, and dried ($MgSO_4$). Removal of the volatiles in vacuo provided a residue which was purified by HPLC using 50% ethyl acetate in hexanes as the eluent. Concentration in vacuo of the product-rich fractions provided 2.44 g (41%) of the oily product: MS *m/e* 594 (M); >99% pure by HPLC analysis (C-18, 54% CH_3CN/H_2O, 0.1% TFA, 280, 254, 220 nm); ^1H NMR ($CDCl_3$) 7.15 (m, 5 H), 4.67 (s, 2 H), 1.24 (m, 9 H). Anal. ($C_{25}H_{33}Cl_3N_2O_8$) C, H, N.

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Table IV. Sodium-Deficient, Spontaneously Hypertensive Rat[a]

compd	dose, mg/kg, route (vehicle)	n^b	% change in[a] MAP	onset, h	duration,[a] h
enalapril[c]	(3, po)[c]	(5)[c]	(−10%)[c]	(1)[c]	(4)[c]
1a	100, ip (0.5% HPMC)[d]	2	−35, −28	0.25	14, >24
	100, po (0.5% HPMC)	2	−19, −25	0.25	19, 24
1c	100, ip (0.5% HPMC)	2	−11, −15	<0.25	3
1f	100, ip (0.5% HPMC)	3	−42, −41, −41	0.5–1	>24
	100, po (0.5% HPMC)	2	−20, −22	0.5–1	>42

[a] See the Experimental Section. [b] Numbers of rats used at each dose. [c] Reference 8c. [d] HPMC = [(hydroxypropyl)methyl]cellulose.

***N*-[*N'*-[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]-*N'*-[(2,2,2-trichloroethoxy)carbonyl]-L-alanyl]-L-prolin-4-one Ethyl Ester (4).** A mixture of **3** (2.21 g, 3.71 mmol) and pyridinium chlorochromate (3.20 g, 14.9 mmol) in 30 mL of methylene chloride was stirred at room temperature for 3 days, diluted with ether, and filtered through a plug of SiO_2. Removal of the volatiles in vacuo provided 2.1 g (95%) of the product which was taken forward without further purification: MS *m/e* 592 (M); ^{1}H NMR ($CDCl_3$) 7.13 (m, 5 H), 5.15 (m, 1 H), 4.9–4.55 (m, 4 H), 4.37–4.15 (m, 7 H), 2.9–2.5 (m, 5 H), 1.4–1.15 (m, 9 H); IR (neat) 1765, 1725, 1710 cm^{-1}.

1'-[*N'*-[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]-*N'*-[(2,2,2-trichloroethoxy)carbonyl]-L-alanyl]-5'-(ethoxycarbonyl)-7-(aminosulfonyl)-6-chlorospiro-[2*H*-1,2,4-benzothiadiazine-3(4*H*),3'-pyrrolidine] *S*,*S*-Dioxide (6). A mixture of **4** (1.91 g, 3.22 mmol) and 1-amino-3-chloro-4,6-benzenedisulfonamide (0.988 g, 3.46 mmol) in 30 mL of 2 N HCl/ethanol was heated at 65 °C for 90 min and the volatiles were removed in vacuo. Purification of the residue by HPLC using 40% ethyl acetate in hexanes as the eluent provided 1.10 g (40%) of a 1:1 mixture of diastereomers: ^{1}H NMR (acetone-d_6) 8.15 (s, 1 H), 7.78 (b s, 1 H), 7.47 (b s, 1 H), 7.24 (b s, 5 H), 7.00 (b s, 1 H), 6.67 (b s, 2 H), 4.70 (d, 2 H), 1.25 (m, 9 H).

***N*-[*N'*-[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-(4*RS*)-4-[*N*-[(6-chloro-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazin-3-yl)methyl]-*N*-methylamino]-L-proline Ethyl Ester *S*,*S*-Dioxide (10).** To a solution of 3.7 g (8.2 mmol) of **5** and 3.6 g (9.5 mmol) of 3-[(methylamino)methyl]-6-chloro-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazine 1,1-dioxide hydrochloride in 25 mL of DMF was added 4 g of $MgSO_4$. The mixture was stirred for 30 min at room temperature and then treated with a solution of 0.6 g (0.01 mol) of sodium cyanoborohydride in 5 mL of DMF. After stirring overnight the mixture was partitioned between water and EtOAc. The aqueous phase was washed with EtOAc and then with CH_2Cl_2; the combined organics were washed with H_2O and brine.

The combined organic extracts were stirred with 2.3 g of oxalic acid overnight and the resulting paste was rinsed with clean EtOAc. The precipitate was redissolved in water, neutralized with excess K_2CO_3, and extracted with two portions of EtOAc. The extracts were concentrated in vacuo to give 1.7 g (28%) of product as a mixture of diastereomers: mp 79–85 °C; ^{1}H NMR ($CDCl_3$) 8.2 (s, 1 H), 7.2 (b s, 6 H), 4.2 (dq, 4 H), 2.0, 2.05 (s, isomeric, 3 H), 1.3 (m, 8 H).

***N*-[*N'*-[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-prolin-4-one Ethyl Ester (5).** To a solution of 8.1 g (13.64 mmol) of **4** in 100 mL of EtOH was added 25 mL of HOAc, followed by 10.7 g (0.164 mol) of zinc dust. The mixture was stirred at room temperature overnight, filtered, and concentrated in vacuo. The residue was redissolved in EtOAc, washed with H_2O (2×), saturated $NaHCO_3$, and brine. The organic phase was then diluted with 1 volume of hexane and treated with excess 4 N HCl/dioxane dropwise. Oily hydrochloride **5** was separated, rinsed with hexane, and used directly: ^{1}H NMR ($CDCl_3$) 7.1–7.3 (m, 5 H), 5.06 (dd, 1 H), 3.50 (q, 1 H), 3.25 (m, 2 H), 2.95 (dd, 1 H), 2.08 (d, 3 H); IR (neat) 1770, 1735 cm^{-1}.

***N*-[*N'*-[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]-*N'*-[(2,2,2-trichloroethoxy)carbonyl]-L-alanyl]-4-[N^2-(3-sulfamyl-4-chlorobenzoyl)hydrazino]-L-proline Ethyl Ester (8).** A solution of **4** (3.90 g, 6.57 mmol) and (3-sulfamyl-4-chlorobenozyl)hydrazine (1.63 g, 6.67 mmol) in 40 mL of absolute ethanol was stirred at room temperature for 3 h and cooled to 0–5 °C. Sodium cyanoborohydride (0.74 g, 19.7 mmol) was added portionwise. The mixture was stirred for 45 min at 0–5 °C, diluted with H_2O and extracted with ethyl acetate. The combined organic layers were washed with brine and dried ($MgSO_4$). Removal of the volatiles in vacuo provided a residue which was purified by HPLC using 25% ethyl acetate in hexanes as the eluent. Concentration in vacuo of the product-rich fractions provided a residue which was triturated with 30% hexanes in ether and furnished 2.82 g (52%) of the solid product as a mixture of diastereomers: MS (FAB) *m/e* 827 (M + H); ^{1}H NMR (DMSO-d_6) 8.45 (s, 1 H), 8.03 (d, 1 H), 1.15 (m, 6 H), 0.83 (d, 3 H).

***N*-[*N'*-[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]-*N'*-[(2,2,2-trichloroethoxy)carbonyl]-L-alanyl]-(4*R*)-4-[(methylsulfonyl)oxy]-L-proline Ethyl Ester (11).** To a 0 °C solution of 16.5 g (27.7 mmol) of **3** and 3.0 g (30 mmol, 4.2 mL) of freshly distilled triethylamine in 170 mL of dry methylene chloride under N_2 was added dropwise 3.3 g (28.5 mmol, 2.2 mL) of methanesulfonyl chloride. The mixture was stirred under N_2 at 0 °C for 1 h and then was poured into 200 mL of water. The two-phase mixture was shaken well, and the two re-formed phases were separated. The organic layer was washed successively with two portions of 1 N aqueous HCl, one portion of H_2O, two portions of concentrated aqueous sodium bicarbonate, and one portion of brine. The organic phase was dried ($MgSO_4$) and concentrated in vacuo to provide 16.6 g (24.7 mmol, 89%) of the crude mesylate, which was used without purification: ^{1}H NMR ($CDCl_3$) 7.2 (s, 5 H), 4.75 (m, 2 H), 3.05 (s, 3 H), 1.0–1.35 (m, 9 H).

***N*-[*N'*-[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]-*N'*-[(2,2,2-trichloroethoxy)carbonyl]-L-alanyl]-(4*S*)-4-azido-L-proline Ethyl Ester (12).** A homogeneous solution of 11.3 g (17.0 mmol) of **11** and 2.2 g (34 mmol) of sodium azide (freshly reprecipitated from water/acetone) in 160 mL of 22% water/ethanol was heated at reflux for 24 h. The reaction mixture was concentrated by rotary evaporation over a 35 °C water bath. The residue was taken up with swirling in water. The aqueous solution was extracted once with ethyl acetate, and the phases were separated. The aqueous phase was salted, and extracted again with ethyl acetate. The organic layers were combined, washed with brine, dried ($MgSO_4$), and concentrated in vacuo to afford 10.0 g (16.1 mmol, 94%) of the azide. An analytical sample was prepared by silica gel chromatography (40% EtOAc/hex.): ^{1}H NMR ($CDCl_3$) 7.26 (s, 5 H), 4.70 (s, 2 H), 4.0–4.4 (m, 4 H), 1.1–1.35 (m, 9 H); IR (neat) 2100 cm^{-1}. Anal. ($C_{25}H_{32}Cl_3N_5O_6$) C, H, N.

***N*-[*N'*-[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-(4*S*)-4-amino-L-proline Ethyl Ester (13).** To a solution of 19.0 g (30 mmol) of **12** in 450 mL of freshly distilled methanol was added dropwise and successively 16 g (150.0 mmol, 21.3 mL) of freshly distilled triethylamine and 12.4 g (150.0 mmol, 15.4 mL) of propanedithiol. The resulting solution was stirred for 20 h under a nitrogen purge; effluent gases were passed through 6% sodium hypochlorite solution.

A 175-g portion of Dowex (50X2-100) resin was washed with distilled water until the filtrates were neutral to litmus. The residue was then washed with three portions of methanol and then was suspended in methanol and transferred to a chromatography column.

The methanolic reaction mixture was transferred via cannula under slight pressure onto the Dowex column. The column was eluted with methanol (850 mL) until all the propanedithiol and sulfur-containing contaminants had eluted (TLC detection, acetone, alkaline permanganate detection). The column was then eluted with 10% triethylamine/methanol (500 mL) to remove the desired product. Solvent removal in vacuo afforded 18.2 g (30 mmol, quant.) of the amine (TLC, R_f = 0.53, acetone) which was used without further purification: ^{1}H NMR ($CDCl_3$) 7.3 (s, 5 H), 4.9 (m, 2 H), 4.0–3.5 (m, 8 H), 1.0 (t, 6 H).

N-[N'-[(1S)-1-(Ethoxycarbonyl)-3-phenylpropyl]-N'-[(2,2,2-trichloroethoxy)carbonyl]-L-alanyl]-(4S)-4-[[[6-chloro-1,2,4-benzothiadiazin-7-yl]sulfonyl]amino]-L-proline Ethyl Ester S,S-Dioxide (15). To a 0 °C solution of 5.94 g (10 mmol) of **13** in 120 mL of dry CH_3CN was added dropwise under N_2 1.26 g (12.5 mmol, 1.74 mL) of freshly distilled triethylamine. To the resulting solution at 0 °C was added dropwise over 90 min 3.9 g (12.3 mmol) of **14** in 49% dry tetrahydrofuran/acetonitrile. The mixture was stirred for 20 h as it warmed to room temperature, then it was stripped of solvent. The residue was taken up in 700 mL of ethyl acetate and washed with one 100-mL portion of 5% aqueous HCl, one portion of water, one 50-mL portion of saturated aqueous sodium bicarbonate, and once with brine. The separated aqueous phases were back-extracted with ethyl acetate. The combined organic phases were dried over magnesium sulfate, filtered, and concentrated to afford 7.0 g of crude product. The crude product was purified by HPLC (two 500-g silica gel columns, 3% methylene chloride/ethyl acetate) to provide 6.17 g (7.1 mmol, 71%) of the desired product: ^1H NMR ($CDCl_3$) 8.25 (s, 1 H), 8.1 (s, 1 H), 7.55 (s, 1 H), 7.2 (s, 5 H), 4.78 (m, 2 H), 0.9–1.35 (m, 9 H). Anal. ($C_{32}H_{37}Cl_4N_5O_{11}$) C, H, N.

Method A. N-[N'-[(1S)-1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-(4S)-4-[[[6-chloro-1,2,4-benzothiadiazin-7-yl]sulfonyl]amino]-L-proline Ethyl Ester S,S-Dioxide (16). To a solution of 3.5 g (4.0 mmol) of **15** in 70 mL of glacial HOAc was added 4.0 g (57.7 mmol) of finely powdered Zn. The suspension was stirred for 22 h and then was filtered through Celite; the Celite filter was washed thoroughly with HOAc. The filtrate was concentrated by rotary evaporation over a tepid-water bath. The residue was reconcentrated twice from toluene and then was purified by flash chromatography (EtOAc, then 1:1 EtOAc/acetone) to afford 2.2 g (3.15 mmol, 79%) of **16**: ^1H NMR ($CDCl_3$) 8.5 (s, 1 H), 8.1 (s, 1 H), 7.75 (s, 1 H), 7.25 (s, 5 H), 4.2 (m, 4 H), 1.0–1.35 (m, 9 H). Anal. ($C_{29}H_{36}ClN_5O_9S_2$) C, H; N: calcd, 10.03; found, 9.47.

2-(2-Iodoethyl)-1,3-dioxolane (18). A mixture of 2-(2-bromoethyl)-1,3-dioxolane (51.5 g, 0.285 mol) and sodium iodide (183 g, 1.2 mol) in 425 mL of anhydrous acetone was heated at reflux for 30 min, cooled, filtered, and concentrated in vacuo. The residue was diluted with ether, washed with H_2O, saturated $NaHSO_3$, saturated $NaHCO_3$, and brine, and dried ($MgSO_4$). Removal of the volatiles in vacuo provided 47.4 g (73%) of 2-(2-iodoethyl)-1,3-dioxolane, which was used without further purification: ^1H NMR ($CDCl_3$) 4.88 (t, 1 H, J = 4 Hz), 3.9 (m, 9 H), 3.17 (t, 2 H, J = 8 Hz), 2.33–2.05 (m, 2 H).

(4R)-N-(Carbobenzyloxy)-4-[3-(1,3-dioxolan-2-yl)propoxy]-L-proline Ethyl Ester (19). A mixture of *N*-Cbz-4-hydroxy-L-proline ethyl ester (**17**; 15.5 g, 52.8 mmol), **18** (47.4 g, 211 mmol), and silver oxide (42.3 g, 185 mmol) in 100 mL of anhydrous DMF was stirred under N_2 at 25 °C for 18 h, heated at 45 °C for 5 h, cooled, and filtered over Celite. The filtrate was diluted with ether, washed with H_2O and brine, and dried ($MgSO_4$). Removal of the volatiles in vacuo provided a residue which was purified by HPLC using 33% ethyl acetate in hexanes as the eluent. Concentration in vacuo of the product-rich fractions provided 2.80 g (14%) of the oily product: MS m/e 393; ^1H NMR ($CDCl_3$) 7.22 (d, 5 H), 5.06 (s, 2 H), 4.84 (t, 1 H), 4.44–3.40 (m, 12 H), 2.4–1.75 (m, 4 H), 1.4–1.0 (dt, 3 H).

N-[N'-[(1S)-1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-(4R)-4-[3-(1,3-dioxolan-2-yl)propoxy]-L-proline Ethyl Ester (21). A mixture of **19** (3.50 g), triethylamine (0.31 mL), and 10% palladium on carbon (0.7 g) in 35 mL of ethanol was hydrogenated at 40 psi for 3 h and filtered. Removal of the volatiles in vacuo provided 2.49 g of **20** as an oil.

To a 0–5 °C solution of **2a** (2.50 g, 8.91 mmol) in 25 mL of CH_2Cl_2 was added portionwise CDI (1.45 g, 8.91 mmol). After 30 min a solution of **20** (2.31 g, 8.91 mmol) in 25 mL of CH_2Cl_2 was added and the solution was allowed to warm to 25 °C while the solution stirred overnight. Removal of the volatiles in vacuo provided a residue which was diluted with ether and filtered. Concentration in vacuo of the filtrate left a residue which was purified by HPLC using 50% ethyl acetate in hexanes as the eluent. Concentration in vacuo of the product-rich fractions provided 3.30 g (71%) of the oily product: MS m/e 520; ^1H NMR ($CDCl_3$) 7.17 (m, 5 H), 4.90 (t, 1 H), 4.53 (t, 1 H), 4.13 (q, 2 H), 3.23 (t, 1 H), 2.7–1.77 (m, 8 H), 1.33–1.17 (m, 9 H).

N-[N'-[(1S)-1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-(4R)-4-[3-(6-chloro-7-sulfamyl-3,4-dihydro-2H-1,2,4-benzothiadiazin-3-yl)propoxy]-L-proline Ethyl Ester S,S-Dioxide (22). A mixture of **21** (1.50 g, 2.88 mmol), 1-amino-3-chloro-4,6-benzenedisulfonamide (0.75 g, 2.62 mmol), and camphorsulfonic acid (1.22 g, 5.24 mmol) in 15 mL of ethanol was stirred at 25 °C for 48 h. Removal of the volatiles in vacuo provided a residue which was diluted with ethyl acetate, washed with saturated $NaHCO_3$ and brine, and dried ($MgSO_4$). Concentration of the volatiles in vacuo provided 1.7 g (87%) of **22** as a brownish solid that was used without further purification: 94–97% pure by HPLC analysis (254 nm): ^1H NMR (DMSO-d_6) 8.25 (d, 1 H), 7.98 (s, 1 H), 7.87 (m, 1 H), 7.52 (s, 1 H), 7.30–7.10 (m, 7 H), 4.84 (m, 2 H), 1.89 (m, 3 H), 1.36–1.14 (m, 6 H).

Ethyl 2-(4-Chloro-3-sulfamylphenyl)-4(R)-thiazolidinecarboxylate (24). L-Cysteine ethyl ester hydrochloride (2.78 g, 15 mmol) in water (5.6 mL) was added to 4-chloro-3-sulfamylbenzaldehyde (**23**; 3.29 g, 15 mmol) in warm ethanol (30 mL), heated for 15 min on the steam bath, and then allowed to stand overnight. The mixture was adjusted to pH 4.5 with aqueous sodium bicarbonate solution and then concentrated in vacuo. The residue was partitioned between saturated $NaHCO_3$ solution and ethyl acetate. The organic layer was washed with brine and dried (Na_2SO_4). Concentration provided the product (4.9 g, 94%) as an oil: ^1H NMR ($CDCl_3$) 7.85 (dd, 1 H), 6.95–7.4 (m, 2 H), 3.6–4.1 (m, 3 H), 1.05 (t, 3 H).

Ethyl N-[[(1S)-1-(Ethoxycarbonyl)-3-phenylpropyl]-N-[(2,2,2-trichloroethoxy)carbonyl]-L-alanyl]-2-(4-chloro-3-sulfamylphenyl)-4(R)-thiazolidinecarboxylate (25). Oxalyl chloride (4.04 g, 31.8 mmol) was added to **2b** (5.09 g, 11.2 mmol) in methylene chloride (71 mL), followed by DMF (76 μL). After 4 h the solution was concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (25 mL) and added in portions over 10 min to **24** (3.93 g, 11.2 mmol) and triethylamine (1.25 g, 12.3 mmol) in CH_2Cl_2 (150 mL) at 0 °C. The solution was stirred overnight and then concentrated in vacuo. The residue was dissolved in ethyl acetate, washed with 1 N HCl, saturated $NaHCO_3$ solution, and brine, then dried (Na_2SO_4), and concentrated to an oil. Purification by HPLC (35/15 hexanes/ethyl acetate) gave 2.4 g (27%) of pure product: ^1H NMR ($CDCl_3$) 8.83 (s, 1 H), 8.5 (d, 1 H), 7.5 (d, 1 H), 7.1 (s, 5 H), 4.0–4.45 (m, 6 H), 1.0–1.4 (m, 7 H). Anal. ($C_{30}H_{35}N_3O_9S_2$) C, N; H: calcd, 5.33; found, 4.88.

1'-[N-[(1S)-1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-5'-(ethoxycarbonyl)-7-(aminosulfonyl)-6-chlorospiro-[2H-1,2,4-benzothiadiazine-3(4H),3'-pyrrolidine] S,S-Dioxide (7). Carboxylate **7** was prepared from 1.10 g (1.28 mmol) of **6** according to method A, using 3.6 g of Zn dust in 23 mL of 26% EtOH/HOAc. Trituration of the crude product (20% EtOAc/hex.) provided 0.83 g (90%) of **7** as an amorphous solid: mp 100 °C dec. Anal. ($C_{28}H_{36}ClN_5O_9S_2 \cdot 0.5C_6H_{14}$) C, H, N.

Ethyl N-[[(1S)-1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-2-(4-chloro-3-sulfamylphenyl)-4(R)-thiazolidinecarboxylate (26). Carboxylate **26** was prepared from 1.88 g (2.39 mmol) of **25** according to method A, using 2.11 g (32.2 mmol) of powdered Zn in 12.8 mL of glacial HOAc. Compound **26** (1.28 g, 2.08 mmol) as a gum was obtained. An analytical sample was prepared by silica gel chromatography. Anal. ($C_{27}H_{34}ClN_3O_7S_2$) C, H, N.

N-[N'-[(1S)-1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-4-[N^2-(3-sulfamyl-4-chlorobenzoyl)hydrazino]-L-proline Ethyl Ester (9). Carboxylate **9** was prepared from 2.70 g (3.26 mmol) of **8** and 2.12 g (32.6 mmol) of Zn in 30 mL of 2:1 EtOAc/HOAc according to method A. Crude product was purified by HPLC (EtOAc) to afford 0.80 g (1.24 mmol, 38%) of **9** as an amorphous solid: mp 92–101 °C. Anal. ($C_{28}H_{38}ClN_5O_8S$) C, H; N: calcd, 10.94; found, 10.28.

Method B. N-[N'-[(1S)-1-Carboxy-3-phenylpropyl]-L-alanyl]-(4S)-4-[[[6-chloro-1,2,4-benzothiadiazin-7-yl]-sulfonyl]amino]-L-proline Trifluoroacetate S,S-Dioxide (1c). A solution of 850 mg (1.2 mmol) of diester **16** in 52% 1 N aqueous sodium hydroxide/ethanol was stirred overnight. The reaction mixture was then adjusted to pH 2 by addition of 25% aqueous HCl and concentrated by rotary evaporation. The wet solid was triturated with 5 mL of water and then filtered. The residue was dried in vacuo to provide 800 mg (1.18 mmol, 98%) of the white hydrochloride salt as an amorphous solid (91% pure by HPLC:

33% CH_3CN/0.1% aqueous TFA). A 357-mg sample was purified by reverse-phase MPLC using a gradient elution of 20–30% CH_3CN in 0.1% aqueous TFA to provide 190 mg of TFA salt: mp 193–195 °C dec; >99% by HPLC analysis (C-8, 33% CH_3CN/0.1% TFA/H_2O, 254 nm); ^1H NMR (DMSO-d_6) 8.27 (s, 1 H), 8.15 (s, 1 H), 7.65 (s, 1 H), 7.02–7.40 (m, 5 H), 4.16 (t, 1 H), 1.4 (d, 3 H). Anal. ($C_{25}H_{28}ClN_5O_9 \cdot CF_3CO_2H$) H, N; C: calcd, 42.89; found, 42.31.

***N*-[*N'*-[(1*S*)-1-Carboxy-3-phenylpropyl]-L-alanyl]-(4*R*)-4-[3-(6-chloro-7-sulfamyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazin-3-yl)propoxy]-L-proline Trifluoroacetate *S*,*S*-Dioxide (1a).** Diacid **1a** was obtained from 429 mg (0.577 mmol) of **22** according to method B, using 1:1 2 N NaOH/EtOH, followed by acidification with TFA. Reverse-phase MPLC purification (12–18% CH_3CN/0.3% aqueous TFA) provided 0.270 g (0.358 mmol, 62%) of solid **1a** as its TFA salt: mp 150 °C dec; MS (FAB) *m/e* 688 (M + H); >98% pure by HPLC analysis (C-8, 254 nm); ^1H NMR (DMSO-d_6) 7.98 (s, 1 H), 7.53 (s, 1 H), 6.98 (s, 1 H), 1.40 (d, 3 H). Anal. ($C_{27}H_{34}ClN_5O_{10}S_2 \cdot CF_3CO_2H \cdot H_2O$) C; H: calcd, 4.68; found, 4.18. N: calcd, 8.36; found, 7.77.

***N*-[*N'*-[(1*S*)-1-Carboxy-3-phenylpropyl]-L-alanyl]-(4*RS*)-4-[*N*-[(6-chloro-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazin-3-yl)methyl]-*N*-methylamino]-L-proline *S*,*S*-Dioxide (1f).** Diacid **1f** was prepared from 1.7 g (2.3 mmol) of **10** according to method B, using 25% EtOH/3% aqueous NaOH, followed by acidification with 2 N aqueous HCl; 1.1 g (1.65 mmol, 72%) of crude product as its hydrochloride salt was isolated. A pure sample for biological testing was obtained by reverse-phase MPLC (10–30% CH_3CN/0.1 N aqueous HCl): mp 190–195 °C dec; 98% pure by HPLC analysis (C-8, 254 nm). Anal. ($C_{27}H_{35}ClN_6O_9S_2 \cdot 2HCl \cdot 3H_2O$) C, H, N.

***N*-[[(1*S*)-1-Carboxy-3-phenylpropyl]-L-alanyl]-2-(4-chloro-3-sulfamylphenyl)-(4*R*)-4-thiazolidinecarboxylic Acid (1g).** Treatment of 0.75 g (1.22 mmol) of **26** with 10% 8.9 M aqueous NaOH/CH_3OH according to method B, followed by acidification with 1.0 N aqueous HCl and recrystallization of the crude product (aqueous EtOH) provided 0.44 g (0.535 mmol, 65%) of **1g**: mp 161–163 °C; ^1H NMR (DMSO-d_6) 7.0 (b s, 5 H), 6.5 (s, 1 H), 6.1 (s, 1 H), 4.6 (t, 1 H), 1.2 (d, 3 H), 0.75 (d, 3 H). Anal. ($C_{23}H_{26}ClN_3O_7S$) C, H, N.

Method C. *N*-[*N'*-[(1*S*)-1-Carboxy-3-phenylpropyl]-L-alanyl]-4-[N^2-(3-sulfamyl-4-chlorobenzoyl)hydrazino]-L-proline Hydrochloride (1b). A solution of **9** (1.17 g, 1.83 mmol) and NaOH (18.3 mL of a 1 N solution) was stirred at room temperature overnight, cooled to 0–5 °C, acidified to pH 1–2 with dilute, aqueous HCl, and extracted with 5% ethanol in ethyl acetate. The combined organic layers were washed with brine and dried ($MgSO_4$). Removal of the volatiles in vacuo provided a residue which was purified by trituration with acetonitrile and provided 0.54 g (47%) of the solid product: mp 188 °C dec; >98% pure by HPLC analysis (215, 270 nm); ^1H NMR (DMSO-d_6) 8.65 (s, 1 H), 8.47 (s, 1 H), 8.17 (d, 1 H), 8.05 (m, 1 H), 8.0–7.0 (m, 6 H), 1.40 (d, 3 H). Anal. ($C_{25}H_{30}ClN_5O_8S \cdot HCl \cdot 2H_2O$) C, H; N: calcd, 10.48; found, 9.91.

1'-[*N'*-[(1*S*)-1-Carboxy-3-phenylpropyl]-L-alanyl]-5'-carboxy-7-(aminosulfonyl)-6-chlorospiro[2*H*-1,2,4-benzothiadiazine-3(4*H*)-3'-pyrrolidine] *S*,*S*-Dioxide (1e). Diacid **1e** was prepared from 0.60 g (0.83 mmol) of **7** according to method C, using 38% EtOH/1 N aqueous NaOH. Trituration of the crude product with 50% EtOAc/ether provided 0.51 g (0.77 mmol, 93%) of **1e** as its hydrochloride salt: mp 198 °C dec; ^1H NMR (DMSO-d_6) 8.70 (m, 1 H), 8.00 (s, 1 H), 7.57 (d, 2 H), 7.05 (d, 1 H), 1.43 (m, 3 H). Anal. ($C_{24}H_{28}ClN_5O_9S_2 \cdot HCl \cdot 0.75H_2O$) C, H; N: calcd, 10.30; found, 9.53.

***N*-[*N'*-[(1*S*)-1-Carboxy-3-phenylpropyl]-L-alanyl]-(4*S*)-4-[2-chloro-5-sulfamylsulfanilamido]-L-proline Monotrifluoroacetate (1d).** A solution of 800 mg (1.2 mmol) of **1c** in 11 mL of 5% aqueous sodium hydroxide was stirred at 90 °C for 2 h. The reaction mixture was cooled to 10 °C and brought to pH 2 by the addition of 25% aqueous HCl. The resulting solid was removed by filtration. The residue was washed with water and dried in air. Traces of water were removed by drying in vacuo at room temperature to afford 700 mg (1.03 mmol, 86%) of the desired orthanilamide.

A 250-mg sample of this was purified by reverse-phase MPLC (20–30% CH_3CN/0.1% aqueous TFA) to provide 272.5 mg (0.35 mmol) of **1d** as its TFA salt: mp 168 dec; >98% relative purity by HPLC (C-8, 5–50% CH_3CN/0.5% aqueous TFA): ^1H NMR (DMSO-d_6) 8.16 (s, 1 H), 7.20–7.34 (m, 5 H), 7.03 (s, 1 H), 1.37 (d, 3 H). Anal. ($C_{25}H_{30}ClN_5O_9S_2 \cdot CF_3CO_2H \cdot 2H_2O$) H, N; C: calcd, 39.92; found, 40.34.

Antihypertensive Screen in Sodium-Deficient, Spontaneously Hypertensive Rats. Sixteen week old, male, spontaneously hypertensive rats were used. They were maintained on a sodium-deficient diet and distilled water for 4 weeks prior to experimentation.

One week prior to experimentation, polyethylene catheters were implanted in the rats' abdominal aortae, with the external ends emerging from between the scapulae. At the time of experimentation, the rats were harnessed and their catheters were attached to a recording system that allowed the animals to roam freely in individual cages while their pressures were monitored. Water and food were available throughout the experiment.

After a 24-h acclimation period, two rats were dosed with the test compound. Compounds were administered ip or po at 100 mg/kg, dissolved or suspended in 0.5% [(hydroxypropyl)-methyl]cellulose in distilled water, 10 mL/kg. If a compound was incompatible with this vehicle, an alternative vehicle was used.

Mean arterial pressures (MAP) and heart rates were recorded for 10 s, every 5 min, with an electronic switching computer system. Fifteen or 30 min averages were tabulated and reported as percent changes from the 0.5-h average values just prior to dosing. Compounds were evaluated at each time period by comparing the mean percent changes observed for MAP with those of a cumulative control group composed of all rats which received the vehicle alone in previous runs. Values which fall outside the range of the mean ± 2SD were considered significantly different from controls. The duration of action was the continuous length of time during which test values were below or above those of the historical controls. If the results in the two test rats were inconsistent, one or two additional rats were used.

Diuretic Testing in the Rat. Male rats, weighing 175–250 g, were used. Water and food were withheld for 18 h prior to and during the experiment. The rats were housed in metabolism cages, two to a cage.

Eight rats were dosed with the test compound or vehicle alone. Compounds were administered iv, ip, or po at the indicated dose in Tris buffer (pH 8.5, 3.5 mL/kg). Each animal was given a fluid load of 0.9% saline, 20 mL/kg, by gavage after the test drug.

Urine was collected from each cage for 0.5- and 5–24-h post-dosing. Volume (mL), Na^+ (mequiv) and K^+ (mequiv) per kilogram of body weight were recorded and reported as the means ± SD.

Nonpaired "*t*" tests were used to determine whether there was a significant difference in the volume or Na^+ or K^+ excretions between the test and control groups.

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Registry No. **1a**·TFA, 126257-73-6; **1a** (free base), 126257-72-5; **1b**·HCl (diastereomer 1), 126451-55-6; **1b**·HCl (diastereomer 2), 126451-56-7; **1b** (free base, diastereomer 1), 126373-72-6; **1b** (free base, diastereomer 2), 126373-73-7; **1c**·TFA, 126451-57-8; **1c** (free base), 126373-74-8; **1d**·TFA, 126373-67-9; **1d** (free base), 103580-36-5; **1e**·HCl (diastereomer 1), 126451-58-9; **1e**·HCl (diastereomer 2), 126451-59-0; **1e** (free base, diastereomer 1), 126373-75-9; **1e** (free base, diastereomer 2), 126373-76-0; **1f**·2HCl (diastereomer 1), 126373-68-0; **1f**·2HCl (diastereomer 2), 126373-69-1; **1f** (free base, diastereomer 1), 103580-40-1; **1f** (free base, diastereomer 2), 103665-11-8; **1g** (free base, diastereomer 1), 126373-70-4; **1g** (free base, diastereomer 2), 126373-71-5; **2a**, 82717-96-2; **2b**, 92893-50-0; **3**, 103654-87-1; **4**, 103580-24-1; **5**·HCl, 103580-43-4; **6** (diastereomer 1), 126373-56-6; **6** (diastereomer 2), 126373-57-7; **7** (diastereomer 1), 126373-61-3; **7** (diastereomer 2), 126373-62-4; **8** (diastereomer 1), 126373-58-8; **8** (diastereomer 2), 126373-59-9; **9** (diastereomer 1), 126257-69-0; **9** (diastereomer 2), 126373-63-5; **10** (diastereomer 1), 103580-39-8; **10** (diastereomer 2), 103665-10-7; **11**, 126294-84-6; **12**, 126257-61-2; **13**, 126257-62-3; **14**, 4194-47-2; **15**, 126257-63-4; **16**, 126257-64-5; **17**, 103667-57-8;

18, 83665-55-8; 19, 126257-65-6; 20, 126257-66-7; 21, 126257-67-8; 22, 126257-68-9; 23, 3279-81-0; 24 (diastereomer 1), 126451-71-6; 24 (diastereomer 2), 126373-60-2; 25 (diastereomer 1), 126257-70-3; 25 (diastereomer 2), 126373-66-8; 26 (diastereomer 1), 126373-64-6; 26 (diastereomer 2), 126373-65-7; ACE, 9015-82-1; H-Cys-OEt·HCl, 868-59-7; H-Hyp-OEt·HCl, 33996-30-4; 1-amino-3-chloro-4,6-benzenedisulfonamide, 121-30-2; (3-sulfamyl-4-chlorobenzoyl)-hydrazine, 5378-62-1; 2-[(methylamino)methyl]-6-chloro-7-sulfamyl-3,4-dihydrobenzo-1,2,4-thiadiazine 1,1-dioxide hydrochloride, 126257-71-4; 2-(2-bromoethyl)-1,3-dioxolane, 18742-02-4.

Structural Study of the N-Terminal Segment of Neuropeptide Tyrosine†

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A series of analogues of neuropeptide tyrosine (NPY) was synthesized by solid-phase peptide synthesis using BOP as a coupling reagent for the complete synthesis. A structure–activity study of the N-terminal portion of the molecule was performed with the analogues obtained by the successive replacement of the first 10 amino acids by the residue L-alanine. NPY and its analogues [Ala$^{1-10}$]hNPY were tested for their potency on rat vas deferens and for their affinity to central nervous system receptors on a rat brain membrane preparation. The results suggest that the hypothetical polyproline type II helix structure of the N-terminal segment is involved in both potency and affinity. Indeed, the substitution by L-Ala of proline residues in position 2, 5, or 8 showed important losses of activity and affinity. The more important losses were observed with the replacement of Pro-5 or Pro-8. A critical loss of potency of hNPY was also observed after the substitution of the Tyr-1 residue by L-Ala, thus confirming the important role played by this residue for the full expression of the biological activity of NPY.

Neuropeptide Y (NPY), a 36 amino acid peptide containing a N-terminal tyrosine and a C-terminal tyrosine amide, was isolated from porcine brain by Tatemoto et al.[1,2] in 1982. The elucidation of the primary structure showed that this peptide has a high degree of homology with peptide YY (70%) and pancreatic polypeptides (47–56%).[3] Several studies confirmed that NPY is largely distributed in peripheral anatomical structures, such as the heart,[4,5] the gastrointestinal tract,[6,7] the reproductive organs,[8] and the perivascular nerve fibers.[8] NPY has also been found in various areas of the central nervous system[9,10] (CNS) where it would be involved in the control of blood pressure and appetite.[11,12] In the peripheral nervous system, NPY exhibits a potent vasoconstrictor activity and participates to the tonus control of smooth muscle by playing a co-transmitter role with norepinephrine in noradrenergic neurons.[13]

Syntheses of NPY were reported by various groups using solution techniques,[14] the conventional DCC method,[15] or the symmetrical anhydride procedure.[3,16,17] Molecular analyses[17-21] and structure–activity studies[3,15,22-24] were also carried out with NPY itself and closely related peptides. These investigations allowed the elaboration of a structural model containing a N-terminal polyproline type II helix and a C-terminal α-helix, connected by a type II β-turn.

Table I. Protocol for a Synthetic Cycle Using BOP Reagent[a]

step	reagent	time
1	CH_2Cl_2	2 × 1 min
2	50% TFA/CH_2Cl_2[b]	1 × 5 min
3	50% TFA/CH_2Cl_2	1 × 20 min
4	CH_2Cl_2	3 × 1 min
5	DMF	3 × 1 min
6	3 equiv of Boc-AA-COOH/DMF + 3 equiv of BOP reagent/DMF + 5 equiv of DIEA	1 ×[c]
7	DMF	3 × 1 min
8	CH_2Cl_2	2 × 1 min

[a] Solvent for all washings and couplings were measured to volumes of 10–20 mL/g of resin. [b] 1% DMS was added after the incorporation of the methionine residue. [c] Couplings are usually achieved in less than 30 min.

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† Abbreviations: The abbreviations for the amino acids are in accord with the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature (*Eur. J. Biochem.* **1984**, *138*, 9–37). L-isomers of amino acids were used. In addition: hNPY, human neuropeptide tyrosine; Boc, *tert*-butoxycarbonyl; BHA, benzhydrylamine, Tos, *p*-tolylsulfonyl; OcHx, cyclohexyl ester; ClZ, [(2-chlorobenzyl)oxy]carbonyl; Bzl, benzyl ester; Dcb, 2,6-dichlorobenzyl; DMF, dimethylformamide; BOP, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; TFA, trifluoroacetic acid; DMS, dimethyl sulfide; DIEA, diisopropylethylamine; DCC, dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; SAR, structure–activity relationship.

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