stirred for 3 h at room temperature and then made basic by the addition of 25% aqueous sodium hydroxide solution. After stirring 30 min, the mixture was diluted with water and extracted with diethyl ether. The aqueous solution was then acidified with 2 M hydrochloric acid and extracted with ethyl acetate. The organic extracts were then washed with brine and dried. Filtration and concentration provided the crude dihydroxy acid, which was lactonized with azeotropic removal of water by refluxing in toluene (20 mL) for 1 h. This solution was cooled to room temperature and concentrated in vacuo. The residue was flash chromatographed, eluting with 75% ethyl acetate in hexane, to give pure **22** (0.05 g) as a white solid: mp 182–184 °C; ^{1}H NMR ($CDCl_3$) δ 8.20 (d, 1 H), 7.80 (m, 1 H), 7.64 (d, 1 H), 7.12–7.36 (m, 5 H), 5.16 (m, 1 H), 4.48 (m, 1 H), 4.10 (m, 1 H), 3.1 (hept, 1 H), 2.4–2.8 (m, 4 H), 1.6–1.9 (m, 4 H), 1.3 (m, 3 H) ppm. Anal. ($C_{24}H_{26}FN_3O_3$) C, H, N.

Biological Assays. The cholesterol biosynthesis inhibition assay (COR) was performed as previously described.[14] The acute inhibition of cholesterol synthesis (AICS) in rats was also performed as previously described (see Table I).[8]

Hypocholesterolemic Activity in Cholestyramine-Primed Dogs. Male and female dogs (7 to 12 kg) were randomly allocated to treatment groups based on their plasma cholesterol concentrations using the ALLOCATE program (Roy Hammond, Elsevier Science Publishers, Amsterdam). Single meals were provided from 6:30 a.m. to 10:30 a.m. The resin was mixed into the daily meal, and the test compound was given as a single dose (gelatin capsule) at 2:30 p.m. The dogs were treated with resin for a minimum of 4 weeks prior to each experiment. Lovastatin was purchased from local suppliers as Mevacor (Merck Sharpe and Dohme). Tablets (20 mg active drug) were pulverized by mechanical grinding and weighed into capsules.

Blood samples were taken weekly from the jugular vein of unanesthetized animals for total cholesterol determinations using the Abbot VP Analyzer. Statistical differences before and after treatment for each group were determined using paired, two-tailed *t*-tests. Comparisons among different doses of compounds at the same time point were determined using ANOVA followed by Fisher's least significant difference test (using individual percent changes for each group).

Acknowledgment. We thank Dr. F. A. MacKellar and staff for analytical and spectral determinations and Dr. S. Brennan, Mr. T. Hurley, Mr. D. Sherwood, and Ms. A. Bernabei for HPLC analysis. We are also indebted to Mr. G. Kanter for the large-scale preparation of **16**.

Renin Inhibitors Containing C-Termini Derived from Mercaptoheterocycles

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A series of transition-state analogues having heterocyclylthio C-termini has been synthesized and evaluated for inhibition of human renin. Addition of mercaptoheterocycles to a chiral Boc-amino epoxide intermediate led, after several steps, to the target [(2*R*,3*S*)-3-(BocPheHis-amino)-4-cyclohexyl-2-hydroxy-1-butyl]thio derivatives. Oxidation of the thioether to sulfone was also investigated. Several of the compounds, especially those derived from N^1-substituted-5-mercaptotetrazoles or N^4-substituted-3-mercapto-5-(trifluoromethyl)-1,2,4-triazoles, were moderately potent inhibitors of human plasma renin, having IC_{50} values of 30–40 nM. When selected compounds were administered intravenously to sodium-deficient rhesus monkeys at 0.3–1.2 mg/kg, they reduced plasma renin activity by 75–98%. However, this inhibition and the accompanying drop in blood pressure were of short duration.

In recent years the renin–angiotensin system (RAS) has been a major focus for the therapy of cardiovascular disease. The RAS is a hormonal and enzymatic complex which can play a key role in the regulation of blood pressure and electrolyte/fluid balance.[1] In this cascade mechanism, the biologically inactive glycoprotein angiotensinogen, which circulates in the bloodstream, is cleaved by renin specifically at the Leu^{10}–Val^{11} linkage to give the decapeptide angiotensin I (AI). Further transformation of AI by angiotensin-converting enzyme (ACE) provides the active octapeptide, angiotensin II (AII), which is a potent vasoconstrictor and a major mediator of essential hypertension.[2-4]

ACE inhibitors are now widely used for the treatment of hypertension and congestive heart failure.[5] However, AI is not the only peptide substrate for ACE. Bradykinin is also degraded by this enzyme, and some side effects of ACE inhibitors have been attributed to the resulting elevated levels of bradykinin.[6] By virtue of its absolute specificity and its position as the rate-limiting enzyme in the synthesis of AII, renin is an attractive target for inhibition.[2,3] Reports of potent renin inhibitors have now appeared from several laboratories, and this class of compounds has been demonstrated to exert marked antihypertensive effects experimentally and clinically.[7] Unfortunately, renin inhibitors in general have suffered from poor oral bioavailability and a limited duration of action, the latter apparently resulting from rapid biliary excretion

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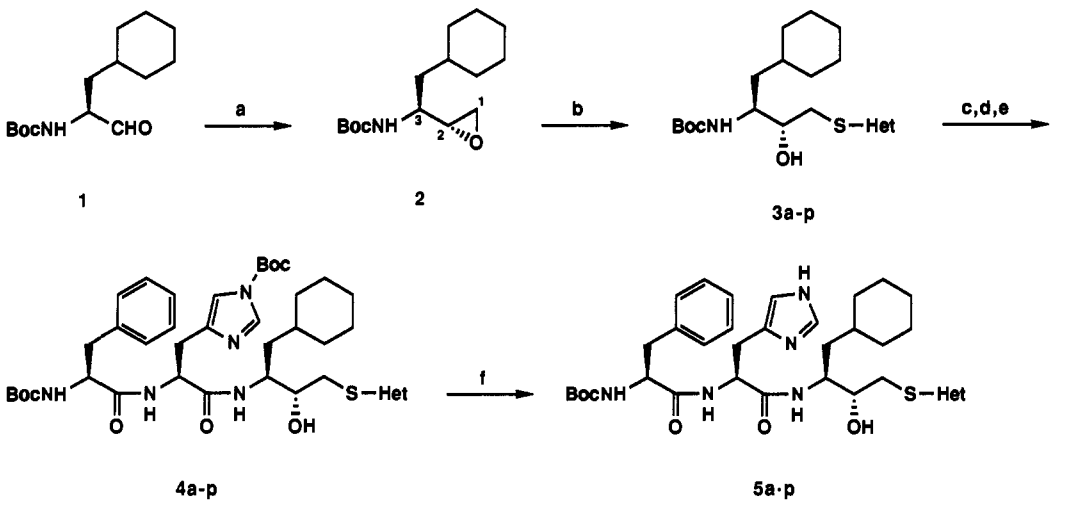
Scheme I[a]



[a] (a) $Me_2S(O)CH_2$ (from $Me_3SO^+I^-$, NaH), DMSO; (b) HS-Het with (1) 2-methoxyethanol or MeCN, Δ, (2) *N*-methylmorpholine + solvent, or (3) catalytic $SnCl_4$, CCl_4, 20 °C; (c) HCl/MeOH or TFA; (d) Et_3N or *N*-methylmorpholine, CH_2Cl_2; (e) Boc-Phe(N^{im}-Boc-His)-OH, DCC/HOBT or BOP, CH_2Cl_2; (f) NH_3/MeOH or K_2CO_3/MeOH.

and/or metabolic breakdown.[7c]

An effective strategy in the design of renin inhibitors has been the insertion of an appropriately configured "hydroxyethylene isostere" at the P_1–$P_{1'}$ locus to mimic the tetrahedral transition state involved in the proteolysis of angiotensinogen.[8,9] Potent renin inhibitors have likewise been achieved by incorporation of statine [(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid][10] or its cyclohexyl analogue ACHPA[11] in place of the scissile Leu–Val bond. Reports from the Abbott group[12,13] described potent, transition-state renin inhibitors having alkylthio or alkylsulfonyl groups at the peptide-equivalent carboxy terminus (for example, **5x**,[13] Table III). Replacement of this C-terminal alkyl or cycloalkyl group by a heterocyclic moiety offered the possibility of increasing aqueous solubility and otherwise modulating the pharmacokinetic properties of the renin inhibitors. Consequently, we have prepared a series of analogues **5a–w** in which the C-terminus is derived from a substituted or unsubstituted mercaptoheterocycle.

Chemistry

The preparation of inhibitors **5a–p** is shown in Scheme I. A key step in the synthesis is the conversion of Boc-cyclohexyl-L-alaninal (1)[11] to the chiral epoxide **2**.[14] An analogous transformation of the corresponding Boc-L-phenylalaninal using dimethylsulfonium methylide[15] was reported[16] to proceed with retention of the chirality originating from the amino aldehyde but essentially without selectivity at the new, adjacent asymmetric center. We verified that treatment of **1** with dimethylsulfonium methylide gave poor diastereoselectivity in the synthesis of **2**. However, we found that substitution of dimethyloxosulfonium methylide, generated in situ in DMSO,[15,17] not only gave a much cleaner reaction but also showed much higher stereoselectivity. Applying the NMR correlations of Evans,[16] the ratio of the 2*R*,3*S* ("threo") epoxide **2** to the 2*S*,3*S* ("erythro") diastereomer formed in the reaction was about 9:1. Upon column chromatography, **2** was readily obtained free of the "erythro" diastereomer. A certain amount of the enantiomeric 2*S*,3*R* epoxide was present at this stage, apparently resulting from partial racemization of **1** either prior to or during the dimethyloxosulfonium methylide reaction. Fortunately, this contaminant could be efficiently removed as the racemate by low-temperature crystallization from petroleum ether,

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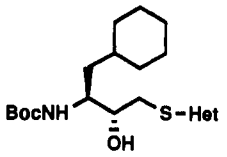
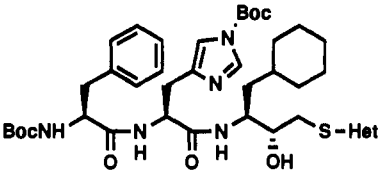
Table I. Physical Properties of Intermediates **3a–p**



no.[a]	HS-Het source[b]	method[c]	% yield	mp, °C	$[\alpha]_D$,[20] deg[c]	formula[d]	FAB-MS *m/e* $(M + H)^+$
3a		C	26	oil	−7.11	$C_{20}H_{32}N_2O_3S$	381
3b		B[e]	38	glass	−19.5	$C_{20}H_{33}N_3O_3S$[f]	396
3c	ref 19	B[e]	60	69–72	−8.72	$C_{23}H_{34}N_4O_3S_2 \cdot 0.2H_2O$	479
3d		A[e]	25	107.5–108.5	−6.85	$C_{19}H_{33}N_3O_3S$	384
3e	ref 20	B[e]	65	>65 (gradual)	−44.8	$C_{22}H_{33}N_3O_3S$[f]	−[g]
3f		B[e]	66	48–50	+4.39	$C_{18}H_{32}N_4O_3S$	385.2266[h]
3g	ref 21	C	65	137–138	+2.99	$C_{19}H_{31}F_3N_4O_3S$	453
3h	7	B[i]	45	150–151	+0.20	$C_{19}H_{31}F_3N_4O_3S \cdot H_2O$	453
3i	ref 22	C	29	gum	+2.53	$C_{21}H_{33}F_3N_4O_3S$[f]	479
3j	12	A[i]	69	glass	+1.26	$C_{25}H_{36}F_3N_5O_3S$	544
3k		B[i]	80	113–115	+2.75	$C_{17}H_{31}N_5O_3S$	386
3l	10a	B[i]	80	>50 (gradual)	−20.1	$C_{24}H_{37}N_5O_4S \cdot 0.5H_2O$[j]	492.2644[k]
3m	10b	A[i]	55	glass	−2.99	$C_{23}H_{36}N_6O_3S$	477
3n	ref 23	B[i]	60	glass	−15.3	$C_{20}H_{36}N_8O_3S$[f]	443
3o	10c	B[i]	51	gum	−1.69	$C_{22}H_{40}N_6O_4S$	485.2906[l]
3p	refs 24 and 25	B[m]	80	glass	−7.49	$C_{20}H_{35}N_5O_5S \cdot 0.2C_4H_8O_2$[n]	458

[a] See Table III for identification of "Het" groups corresponding to **a–p**. [b] Commercially available except as indicated. [c] See Experimental Section for description of general methods. [d] Analyses for C, H, and N within ±0.4% of theory except where characterized by high-resolution mass spectrum or as otherwise indicated. [e] MeCN used as solvent. [f] Characterized spectroscopically. [g] Molecular ion not observed. [h] Calcd for $C_{18}H_{33}N_4O_3S$ $(M + H)^+$: 385.2273. [i] 2-Methoxyethanol used as solvent. [j] Characterized by elemental analysis as well as high-resolution FAB-MS. [k] Calcd for $C_{24}H_{38}N_5O_4S$ $(M + H)^+$: 492.2644. [l] Calcd for $C_{22}H_{41}N_6O_4S$ $(M + H)^+$: 485.2910. [m] EtOH used as solvent. [n] Solvated with EtOAc. [o] (*c* 2, $CHCl_3$).

Table II. Physical Properties of Intermediates **4a–q**



no.[a]	deblocking method[b]	coupling method[b]	overall % yield	mp, °C	formula[c]	FAB-MS *m/e* $(M + H)^+$
4a	D	F	43	>83 (gradual)	$C_{40}H_{56}N_6O_7S \cdot 0.33H_2O$[d]	765
4b	E	G	13	gum	$C_{40}H_{57}N_7O_7S \cdot 0.9H_2O$	780
4c	D	G	5	gum	$C_{43}H_{58}N_8O_7S_2$[e]	863
4d	D	F	38	>100 (gradual)	$C_{39}H_{57}N_7O_7S \cdot 0.5H_2O$	768
4e	E	G	33	97–101	$C_{42}H_{57}N_7O_7S$[e]	804
4f	E	G	28	136–138	$C_{38}H_{56}N_8O_7S \cdot 0.67H_2O$	769
4g	D	F	32	glass	$C_{39}H_{55}F_3N_8O_7S \cdot 0.2C_6H_{14}$[f]	837
4h	E	G	20	97–98	$C_{39}H_{55}F_3N_8O_7S$[e]	837
4i	E	G	28	87–90	$C_{41}H_{57}F_3N_8O_7S \cdot 0.75H_2O$	863
4j	D	G	33	>90 (gradual)	$C_{45}H_{60}F_3N_9O_7S \cdot 0.5H_2O$	928
4k	D	G	20	>96 (gradual)	$C_{37}H_{55}N_9O_7S$	770
4l	E	F	12	>95 (gradual)	$C_{44}H_{61}N_9O_8S \cdot 0.5H_2O$	876
4m	D	G	62	>80 (gradual)	$C_{43}H_{60}N_{10}O_7S \cdot 0.75H_2O$	861
4n	D	G	58	foam	$C_{40}H_{62}N_{10}O_7S \cdot 0.75H_2O$	827
4o	D	G	19	glass	$C_{42}H_{64}N_{10}O_8S \cdot 0.67H_2O$	869
4p	D	G	41	99–100	$C_{40}H_{59}N_9O_9S \cdot 0.75H_2O$	842
4q	J[g]		70	>105 (gradual)	$C_{44}H_{63}IN_{10}O_7S \cdot 0.5H_2O$	875[h]

[a] See Table III for identification of "Het" groups corresponding to **a–p**. [b] See Experimental Section for description of general methods. [c] Analyses for C, H, and N within ±0.4% of theory except where indicated. [d] N: calcd, 10.90; found, 10.27. [e] Characterized spectroscopically. [f] Solvated with hexane. [g] See Experimental Section and Scheme II. [h] M^+ for pyridinium cation.

affording **2** in high optical and diastereomeric purity. A similar preparation of the Cbz-protected analogue of **2** has been reported.[18] However, a 5:1 ratio of diastereomers was obtained, and these evidently were not separated. An alternative, two-step conversion of **1** to **2** via the olefin has

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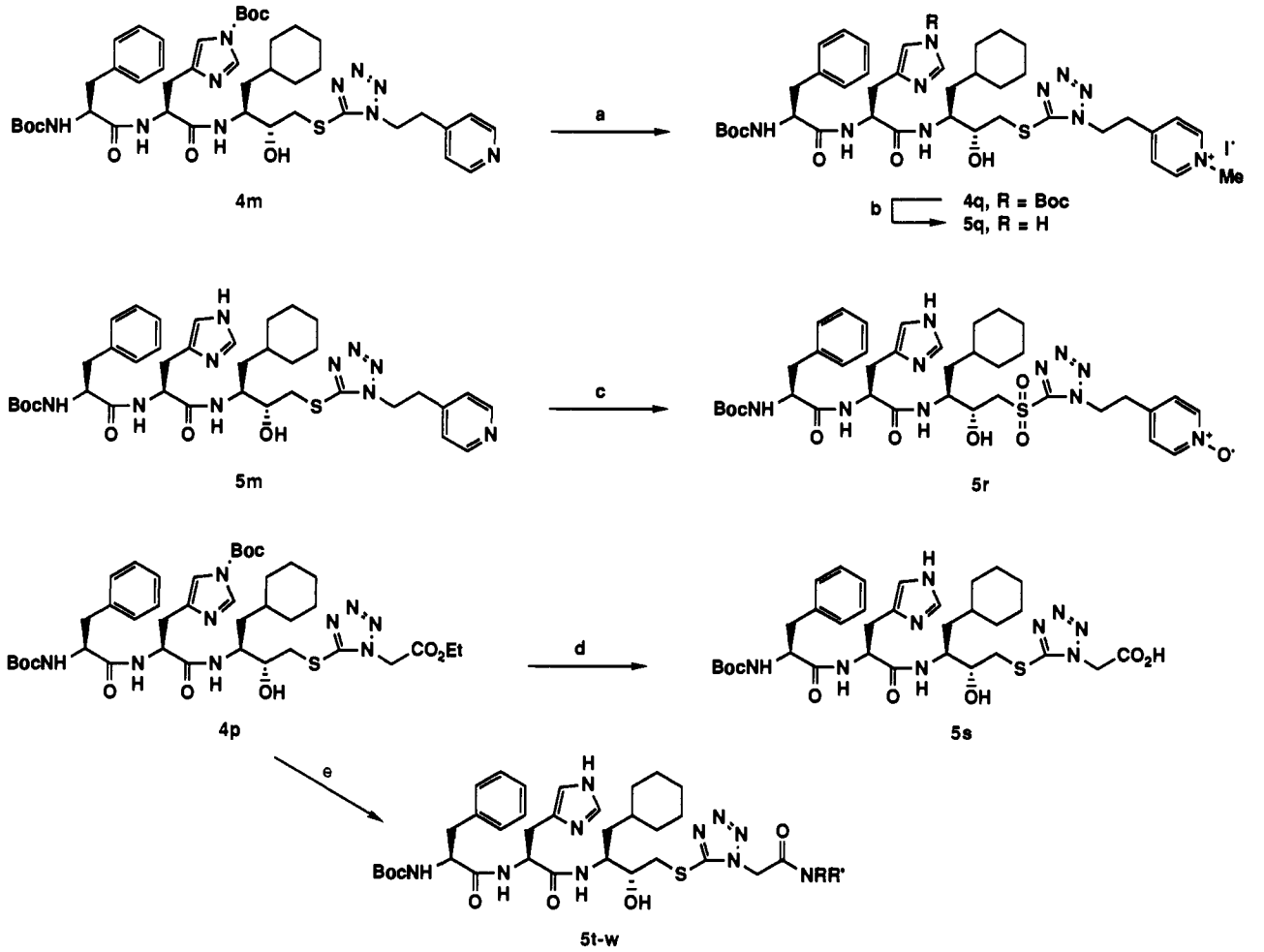
Scheme II[a]

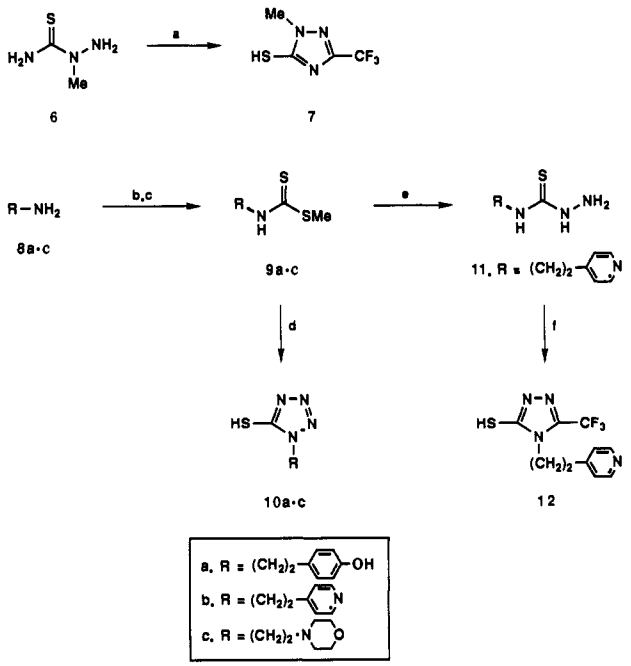


[a] (a) CH_3I; (b) NH_3/MeOH; (c) MCPBA (5 equiv), AcOH; (d) K_2CO_3/MeOH; (e) HNRR'.

been reported,[14] with **2** being favored over the "erythro" diastereomer by a 15:1 ratio. Again, no separation of the isomers was described.

The preferred conditions for addition of the mercaptoheterocycle to epoxide **2** varied from case to case. In some instances, the two reactants could simply be heated together to give **3** (Table I), although for less basic heterocycles or those lacking a basic side chain, it was advantageous to add a base such as *N*-methylmorpholine. In a few cases, better results were obtained by catalyzing the addition with a Lewis acid, $SnCl_4$. Standard conditions were employed for deprotection of **3**, and coupling with the protected dipeptide BocPhe(N^{im}-BocHis)-OH[26] was accomplished by use of BOP reagent[27] or DCC/HOBT[28] to give **4a–p** (Table II). Selective removal of the imidazole Boc group was accomplished either with methanolic ammonia or methanolic K_2CO_3 to yield **5a–p** (Table III).

Scheme III[a]



[a] (a) TFA, toluene, Δ; (b) CS_2, Et_3N; (c) MeI; (d) NaN_3, EtOH-H_2O, Δ; (e) $H_2NNH_2 \cdot H_2O$, $MeO(CH_2)_2OH$, 80 °C; (f) TFA, Δ.

Further transformations were accomplished after coupling with the dipeptide (Scheme II). Treatment of **4m**

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with methyl iodide followed by deprotection gave the quaternized derivative **5q**. Both the sulfone and pyridine *N*-oxide moieties were obtained upon oxidation of **5m** with *m*-chloroperbenzoic acid. Prolonged treatment of **4p** with K_2CO_3 in methanol saponified the ester as well as deblocking the histidine imidazole to give **5s**. Direct reaction of **4p** with amines provided the amide derivatives **5t–w** (Table III).

Routes to new mercaptoheterocycles are shown in Scheme III. The 1-methyl-5-mercaptotriazole **7** was obtained by heating the thiosemicarbazide derivative **6** with trifluoroacetic acid. Amines **8** were converted to dithiocarbamates **9** and then to mercaptotetrazoles **10a–c** by standard conditions. The 4-substituted-3-thiosemicarbazide **11** was also available by reaction of **9** with hydrazine hydrate, and further conversion to the mercaptotriazole **12** was achieved by heating with trifluoroacetic acid.

Biological Results and Discussion

In Vitro Renin Inhibition. Evaluation of **5a–w**, which differ only in the *S*-heterocyclyl moiety, against human plasma renin at pH 7.4 revealed a 100-fold spread of inhibitory potency (Table III). The 6-membered pyridyl and pyrimidyl derivatives **5a** and **5b** displayed modest potency of about 200 nM. It should be noted that the Abbott *S*-cyclohexyl derivative **5x**, our lead compound, was found to have a 289 nM IC_{50} under these conditions, a large discrepancy from the previously reported[13] value of 4 nM, which had been determined against purified human renal renin at pH 6.0.

The major focus was on 5-membered heterocycles. A distinct trend toward greater potency associated with increasing electron deficiency of the heterocycle was apparent, as seen in comparing the identically substituted imidazole (**5d**), 1,2,4-triazole (**5f**), and tetrazole (**5k**) analogues. Further evidence of this trend is seen by the 2-fold increase in potency upon substitution of the triazole **5f** with an electron-withdrawing trifluoromethyl group in **5g**. Electron-poor heterocyclic derivatives such as **5g** (IC_{50} 39 nM) and **5k** (IC_{50} 32 nM) were at least 7-fold more potent than the hydrophobic cyclohexyl lead **5x** in this assay, suggesting a significant electronic interaction between the heterocyclic ring and the enzyme.

Some conclusions can be drawn with regard to steric tolerances for the heterocyclic ring substituents. The N^1-methyl group of the tetrazole **5k** or the equivalent N^4-methyl group of the 1,2,4-triazole **5g** could be replaced with relatively large substituents, as in **5j**, **5l**, **5m**, and **5o** with little effect on inhibitory activity. At other ring positions, steric constraints may be more significant. Compound **5h**, derived from 5-mercapto-1-methyl-3-(trifluoromethyl)-1*H*-1,2,4-triazole (**7**), was 10-fold less potent than **5g** even though it has the same substituents differently disposed about the ring. The weak 3.3 μM IC_{50} of the thiadiazole derivative **5c** may be at least partly the result of unfavorable steric interactions with the bulky anilino substituent positioned more-or-less opposite the alkylthio group. Steric effects may also be responsible for the poor activity of the fused imidazole **5e** compared to **5d**.

Not all tetrazole N^1-substituents were equally well tolerated, and electronic factors appear to play a role in this. A 2-substituted-ethyl group at this position was effective, provided that the 2-substituent was relatively hydrophobic, as in **5l** and **5m**. A basic tertiary amine at this position (**5n**) was unfavorable, although the less basic amine derived from morpholine (**5o**) was satisfactory. Quaternization of the pyridyl group of **5m** led to a 20-fold loss in binding (**5q**). Somewhat surprisingly, **5r**, the corresponding pyridine *N*-oxide containing sulfone in place of thioether adjacent to the heterocycle, was a relatively potent inhhibitor (IC_{50} 37 nM). Substitution of a polar group directly on the N^1-methyl of the tetrazole (**5s–w**) was quite unfavorable, leading in each case to at least a 10-fold drop in potency relative to **5k**.

In Vivo Pharmacology. In order to evaluate effects on in vivo plasma renin activity (PRA), blood pressure, and heart rate, selected compounds were administered intravenously to conscious, sodium-deficient rhesus monkeys (Table IV). At doses (0.3–1.2 mg/kg) proportional to in vitro inhibitory potency, several compounds produced a peak drop in mean arterial pressure ≥10 mmHg. This effect was most pronounced for the (pyridylethyl)tetrazole **5m**, which produced a drop of 22 mmHg. However, the half-life of hypotensive activity was very short, <10 min in each case (borderline for **5o**). Concomitant with the peak hypotensive effect, the analogues inhibited PRA by 75–98%. Effects on heart rate were small except for **5f** and **5r**, which gave average peak increases of 17 and 23 beats/min, respectively. Two compounds (**5m** and **5o**) were tested orally in this monkey model and found to be inactive at 50 mg/kg.

Conclusions

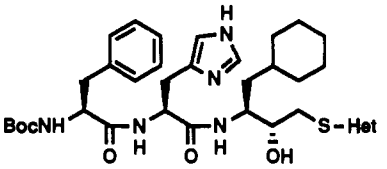
The (hydroxyethyl)thio and (hydroxyethyl)sulfonyl peptide isosteres developed by the Abbott group[12,13] have now been extended to analogues containing C-termini derived from mercaptoheterocycles. Effective renin inhibitors have been obtained from electron-deficient 5-membered heterocycles, especially tetrazole or 3-(trifluoromethyl)-1,2,4-triazole substituted at N^1 or N^4, respectively, with a group having hydrophobic character, at least in proximity to the ring. Several of these compounds had IC_{50} values in the 30–40 nM range. Conceivably a hydrophobic moiety thus positioned on the heterocycle may interact with the renin $S_{1'}$ site[7b] responsible for binding the Val[11] side chain of angiotensinogen. Several of the most potent members of this series strongly inhibited plasma renin activity and produced significant but short-lived reduction in blood pressure upon intravenous administration to sodium-deficient monkeys. Subsequent to the synthesis of these analogues, a patent application from the Yamanouchi group[29] appeared, disclosing some similar heterocyclylthio renin inhibitors including **5k**, which was reported to have a 4 nM IC_{50} against human plasma renin.

Experimental Section

Melting points (uncorrected) were determined in open capillary tubes with a Thomas-Hoover apparatus. ^1H NMR spectra were recorded on Varian XL-400, XL-300, or XL-200 spectrometers, using tetramethylsilane as internal standard. (Note: In the descriptions of the NMR spectra, the designation "br" used alone indicates a broad peak of undetermined multiplicity.) Positive ion fast atom bombardment (FAB) or electron impact (EI) mass spectra (MS) were obtained on Varian MAT 731, Finnigan MAT 90, JEOL HX110, and Varian MAT 212 instruments. Optical rotations at the sodium D line were measured on a Perkin-Elmer 241 polarimeter using water-jacketed cells at 20 °C. Column chromatography was carried out on E. Merck silica gel 60 (70–230 mesh) or grade 62 (60–200 mesh). Compounds showed satisfactory purity by TLC on Analtech silica gel GF plates (visualized by UV light at 254 nm and/or I_2) in the indicated solvent systems. Elemental combustion analyses, where indicated only by the elements, were within ±0.4% of theoretical values.

(29) Nakano, K.; Fujikura, T.; Hara, R.; Ichihara, M.; Fukunaga, Y.; Shibasaki, M. European Patent Appl. 281,316, 1988.

Table III. Physical and Renin-Inhibitory Properties of **5a–x**



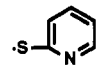
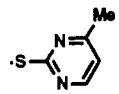
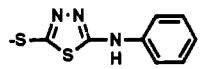
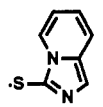
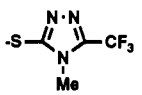
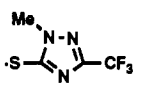
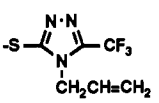
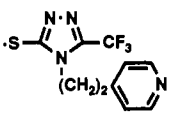
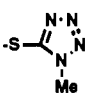
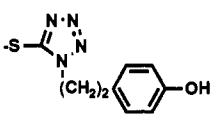
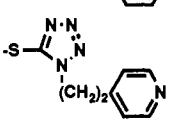
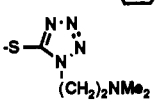
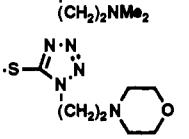
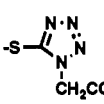
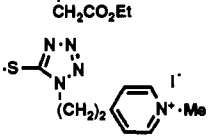
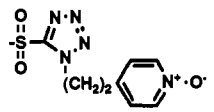
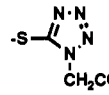
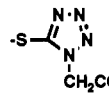
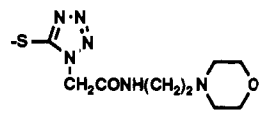
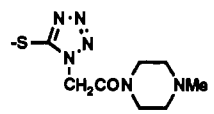
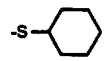
no.	-S(O)$_n$-Het	method[a]	% yield	mp, °C	formula[b]	FAB-MS m/e $(M + H)^+$	human plasma renin IC_{50}, nM
5a		H	61	>166 (gradual)	$C_{35}H_{48}N_6O_5S$	665.3488[c]	198
5b		H	48	172–174	$C_{35}H_{49}N_7O_5S \cdot 1.18H_2O$[d]	680.3566[e]	240
5c		H	31	138–140 dec	$C_{38}H_{60}N_8O_5S_2$	763.3431[f]	3310
5d		H	75	199–20[illegible] dec	$C_{34}H_{49}N_7O_5S \cdot 0.5H_2O$	668	124
5e		H	66	165–166 dec	$C_{37}H_{49}N_7O_5S \cdot H_2O$	704	708
5f		H	80	144–146 dec	$C_{33}H_{48}N_8O_5S$	669.3544[g]	81
5g		H	64	175–177	$C_{34}H_{47}F_3N_8O_5S$	737	39
5h		H	60	182–184	$C_{34}H_{47}F_3N_8O_5S \cdot H_2O$	737	392
5i		H	80	169–171 dec	$C_{36}H_{49}F_3N_8O_5S \cdot 0.9H_2O$	763	206
5j		H	60	135–137 dec	$C_{40}H_{52}F_3N_9O_5S \cdot H_2O$	828	41
5k		H	79	174–176	$C_{32}H_{47}N_9O_5S$	670	32
5l		H	100	197–199 dec	$C_{39}H_{53}N_9O_8S$	776	33
5m		H	91	149–150 dec	$C_{38}H_{52}N_{10}O_5S \cdot 0.5H_2O$	761	31
5n		H	45	gum	$C_{35}H_{54}N_{10}O_5S \cdot 0.67H_2O$	727	145
5o		H	64	156–157	$C_{37}H_{56}N_{10}O_6S$	769	39
5p		I	23	177–178	$C_{35}H_{51}N_9O_7S$	742	207
5q		J, H	85	>120 dec (gradual)	$C_{39}H_{55}IN_{10}O_5S$	775.4077[h]	678

Table III (Continued)

no.	-S(O)$_n$-Het	method[a]	% yield	mp, °C	formula[b]	FAB-MS *m/e* (M + H)$^+$	human plasma renin IC$_{50}$, nM
5r		K	63	157–158 dec	$C_{38}H_{52}N_{10}O_8S\cdot H_2O$	809	37
5s		L	28	>180 (gradual)	$C_{33}H_{47}N_9O_7S$	714.3334[i]	396
5t		M	40	200–201	$C_{33}H_{48}N_{10}O_6S\cdot 0.67H_2O$	713	448
5u		N	40	180–183	$C_{37}H_{57}N_{11}O_6S\cdot 0.75H_2O$[d]	784.4318[j]	1290
5v		N	73	173–176 dec	$C_{39}H_{59}N_{11}O_7S\cdot 0.67H_2O$	826	314
5w		N	32	152–154	$C_{38}H_{57}N_{11}O_6S\cdot 0.8H_2O$	796	359
5x[k]							289[l]

[a] See Experimental Section for description of general methods. [b] Analyses for C, H, and N within ±0.4% of theory except where indicated. [c] Calcd for $C_{35}H_{49}N_8O_5S$ (M + H)$^+$: 665.3485. [d] Characterized by elemental analysis as well as high-resolution FAB-MS. [e] Calcd for $C_{35}H_{60}N_7O_5S$ (M + H)$^+$: 680.3594. [f] Calcd for $C_{38}H_{51}N_8O_5S_2$ (M + H)$^+$: 763.3426. [g] Calcd for $C_{33}H_{49}N_8O_5S$ (M + H)$^+$: 669.3546. [h] Calcd for $C_{39}H_{55}N_{10}O_5S$ (M$^+$ for pyridinium cation): 775.4080. [i] Calcd for $C_{33}H_{48}N_9O_7S$ (M + H)$^+$: 714.3399. [j] Calcd for $C_{37}H_{58}N_{11}O_6S$ (M + H)$^+$: 784.4292. [k] Reference 13. This compound was prepared by Dr. P. Schuda in these laboratories. [l] IC$_{50}$ value of 4 nM against purified human renal renin at pH 6.0 reported for this compound in ref 13.

Table IV. Effects of Intravenously Administered Renin Inhibitors on Conscious, Sodium-Deficient Rhesus Monkeys (N = 2)

no.	dose, mg/kg	peak ΔMAP,[a] mmHg	MAP $T_{1/2}$,[b] min	peak ΔHR,[c] beats/min	plasma renin act., % inhibn[d]
5d	1.19	−8.5	<10	−3	75
5f	0.88	−9	<10	+17	90
5g	0.39	−11	<10	−5	85
5k	0.35	−9	<10	−5	89
5j	0.44	−10	<10	−1	93
5m	0.34	−22	<10	+1	95
5o	0.42	−10.5	<10, 13	−8	90
5r	0.40	−12	<10	+23	98

[a] Peak change in mean arterial pressure (average). [b] Half-life of hypotensive effect. [c] Peak change in heart rate (average). [d] Determined at peak ΔMAP.

Dry tetrahydrofuran (THF) was obtained by distillation from sodium/benzophenone ketyl under N_2. Dry dimethyl sulfoxide (DMSO) was withdrawn directly from Pierce silylation grade Hypo-vials, or HPLC grade DMSO was dried over 4-Å molecular sieves. Reagent grade CH_2Cl_2, MeOH, and EtOH were dried over 3-Å molecular sieves.

(2*R*,3*S*)-3-[(*tert*-Butoxycarbonyl)amino]-4-cyclohexyl-1,2-epoxybutane (2). In an oven-dried three-necked flask, 1.58 g (39.5 mmol) of sodium hydride (60% in oil) was washed with petroleum ether (removed by decantation). The flask was immediately flushed with N_2, and 50 mL of dry DMSO was introduced through a septum. The mixture was stirred at room temperature under N_2 as 8.32 g (38.6 mmol) of trimethyloxosulfonium iodide was added over a period of 5 min via a solid addition funnel. When H_2 evolution had ceased, the resulting clear solution was treated over 8 min with a solution of 8.20 g (32.1 mmol) of *N*-[(*tert*-butoxycarbonyl)amino]-3-cyclohexyl-L-alaninal[11] in 50 mL of dry THF from a dropping funnel. After 1 h, by which time precipitation had occurred, the mixture was concentrated in vacuo to remove the THF. The residual DMSO solution was partitioned between ether and H_2O, and the aqueous phase was further extracted with ether. The combined ether extracts were washed with H_2O, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residual oil was chromatographed on a column of silica gel (elution with 4:1 hexane–EtOAc). Fractions containing exclusively the "threo" epoxide (eluted ahead of the minor "erythro" isomer and detected on TLC by I_2 visualization) were combined and concentrated to yield 4.07 g of waxy semisolid, $[\alpha]^{20}_D$ −5.9° (*c* 2, $CHCl_3$). In a typical procedure to remove the enantiomeric 2*S*,3*R* isomer as a crystalline racemate, a 13.7-g batch of epoxide of comparable optical purity was dissolved in 120 mL of petroleum ether. The flask was placed under N_2 and immersed in an acetone bath, which was cooled by gradual addition of dry ice. Crystallization began at −30 °C and was continued at −40 °C for 1 h. The mixture was rapidly filtered under N_2 to give 6.0 g of slightly gummy, white crystals having low rotation, $[\alpha]^{20}_D$ −2.2° (*c* 2, $CHCl_3$). Concentration of the mother liquor in vacuo provided 7.37 g of **2** as a colorless, viscous residual oil: $[\alpha]^{20}_D$ −12.8° (*c* 2, $CHCl_3$); TLC in 4:1 hexane–ether, FAB-MS *m/e* 270 (M + H)$^+$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.8–1.9 (complex m, 22 H) including 1.45 (s, 9 H), 2.60 (br m, 1 H), 2.73 (t, J = 4.5 Hz, 1 H), 2.98 (br m, 1 H), 4.02 (br m, 1 H), 4.30 (br d, 1 H). These NMR data are in general agreement with those reported by Luly et al.[14] for the preparation of **2** by an alternative route. For the minor "erythro" isomer: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.8–1.9 (complex m, 22 H) including 1.44 (s, 9 H), 2.75 (m, 2 H), 2.84 (br m, 1 H), 3.55

(br, 1 H), 4.37 (br m, 1 H). In several subsequent runs, initial $[\alpha]^{20}_D$ values as high as −10 to −11° were obtained, and crystallization at −40 to −60 °C gave **2** with specific rotations in the range of −12.4 to −13.1°. Rotations of this magnitude appeared consistent with high optical purity for the 2*R*,3*S* epoxide, and petroleum ether solutions of this purified material did not deposit additional crystals of racemate, even when seeded and cooled to −78 °C.

Method A. 5-[[(2*R*,3*S*)-3-[(*tert*-Butoxycarbonyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]-1-[2-(4-pyridyl)ethyl]-1*H*-tetrazole (3m). A mixture of 3.11 g (15 mmol) of **10b**, 4.44 g (16.5 mmol) of **2**, and 45 mL of 2-methoxyethanol was stirred under N_2 in an oil bath at 85 °C. Within 30 min all of the solid had dissolved. After 5 h the solution was cooled and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and chromatographed on silica gel (gradient elution with 1–4% MeOH in CH_2Cl_2) to yield 3.97 g (55%) of **3m** as a very pale green glass: $[\alpha]^{20}_D$ −2.99° (*c* 2, $CHCl_3$); TLC in 95:5 $CHCl_3$–MeOH; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.77 (br m, 1 H), 0.90 (br m, 1 H), 1.0–1.7 (complex m, 19 H) including 1.37 (s, 9 H), 1.74 (m, 1 H), 3.10 (dd, *J* = 8.7 Hz, 12.8 Hz, 1 H), 3.17 (t, *J* = 6.9 Hz, 2 H), 3.27 (m, 1 H), 3.57 (br m, 1 H), 3.65 (br t, 1 H), 4.59 (t, *J* = 6.9 Hz, 2 H), 5.18 (d, *J* = 6.2 Hz, 1 H), 6.44 (d, *J* = 9.4 Hz, 1 H), 7.17 (d, *J* = 5.5 Hz, 2 H), 8.44 (d, *J* = 5.5 Hz, 2 H); FAB-MS *m/e* 477 $(M + H)^+$. Anal. ($C_{23}H_{38}N_8O_3S$) C, H, N.

Method B. Ethyl 2-[5-[[(2*R*,3*S*)-3-[(*tert*-Butoxycarbonyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]-1*H*-tetrazol-1-yl]acetate (3p). A solution of 5.92 g (22 mmol) of **2**, 2.2 mL (2.02 g, 20 mmol) of *N*-methylmorpholine, and 3.76 g (20 mmol) of ethyl 2-(5-mercapto-1*H*-tetrazol-1-yl)acetate[24,25] in 60 mL of absolute EtOH was stirred under N_2 at 85 °C for 2 h. The residue obtained upon concentration of the solution was chromatographed on silica gel (elution with 1:1 hexane–EtOAc) to give 7.6 g (83%) of **3p** as a clear glass: $[\alpha]^{20}_D$ −7.49° (*c* 2, $CHCl_3$); TLC in 2:1 hexane–EtOAc; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.81 (m, 1 H), 0.93 (m, 1 H), 1.05–1.7 (complex m, 22 H) including 1.29 (t, *J* = 7.1 Hz, 3 H) and 1.42 (s, 9 H), 1.78 (br d, *J* = 12 Hz, 1 H), 3.36 (ABX m, 2 H), 3.78 (br m, 1 H), 3.89 (br m, 1 H), 4.26 (q, *J* = 7.1 Hz, 2 H), 4.70 (d, *J* = 9.6 Hz, 1 H), 5.04 (s, 2 H); FAB-MS *m/e* 458 $(M + H)^+$. Anal. ($C_{20}H_{35}N_5O_5S \cdot 0.2C_4H_8O_2$) C, H, N.

Method C. 3-[[(2*R*,3*S*)-3-[(*tert*-Butoxycarbonyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]-4-methyl-5-(trifluoromethyl)-4*H*-1,2,4-triazole (3g). In dried glassware, a mixture of 183 mg (1 mmol) of 3-mercapto-4-methyl-5-(trifluoromethyl)-4*H*-1,2,4-triazole,[21] 296 mg (1.1 mmol) of **2**, and 3 mL of CCl_4 was stirred under N_2 as 30 μL of 1 M $SnCl_4$ in CCl_4 was added through a septum. Stirring was continued at room temperature for 1 h, by which time TLC (2:1 hexane–EtOAc) indicated complete reaction. The mixture was partitioned between 50 mL of ether and 50 mL of NH_4OH. The ether phase was dried over $MgSO_4$, filtered, and concentrated in vacuo. Trituration of the residue followed by filtration yielded 295 mg (65%) of white solid: mp 137–138 °C; $[\alpha]^{20}_D$ +2.99° (*c* 2, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 0.8–1.9 (complex m, 22 H) including 1.49 (s, 9 H), 3.43 (ABX m, 2 H), 3.73 (s, 3 H), 3.86 (br m, 1 H), 3.98 (m, 1 H), 4.81 (br d, *J* = 9 Hz, 1 H); FAB-MS *m/e* 453 $(M + H)^+$. Anal. ($C_{19}H_{31}F_3N_4O_3S$) C, H, N.

Methods D (Deprotection) and G (Coupling). 5-[[(2*R*,3*S*)-3-[(Boc-L-phenylalanyl-N^{im}-Boc-L-histidyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]-1-[2-(4-pyridyl)ethyl]-1*H*-tetrazole (4m). To 30 mL of MeOH, freshly saturated with HCl gas at room temperature, was added a solution of 3.82 g of **3m** in 10–12 mL of MeOH. The solution was stirred at room temperature under a drying tube for 1 h until gas evolution had ceased. The solution was evaporated in a stream of N_2 with mild warming, and the residue was reevaporated twice from MeOH. Final drying in vacuo (oil pump, KOH trap) yielded a stiff foam, which was treated with 30 mL of CH_2Cl_2 followed by 3.34 mL (2.42 g, 24 mmol) of triethylamine. The mixture was agitated in a stoppered flask for a few minutes, then filtered, and concentrated in vacuo at ≤30 °C. The residual gum was treated successively with 3.78 g (8 mmol) of Boc-L-phenylalanyl-N^{im}-Boc-L-histidine,[26] 3.54 g (8 mmol) of (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent), 50 mL of dry CH_2Cl_2, and 1.11 mL (808 mg, 8 mmol) of triethylamine. The resulting solution was stirred under N_2 at room temperature for 3.5 h and concentrated in vacuo at room temperature. The residue was dissolved in EtOAc (30–40 mL) and filtered to remove some insoluble material. The filtrate was diluted to 250 mL with EtOAc and washed with 3 × 50 mL of saturated aqueous $NaHCO_3$ followed by 50 mL of saturated NaCl. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. Chromatography of the residue on silica gel (gradient elution with 2–4% MeOH in CH_2Cl_2) provided 4.31 g (62%) of **4m** as an off-white, stiff foam: mp >80 °C (gradual); TLC in 95:5 $CHCl_3$–MeOH; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.6–1.8 (complex m, 31 H) including 1.32 (s, 9 H) and 1.54 (s, 9 H), 2.86 (m, 2 H), 3.1–3.4 (complex m, 6 H) including 3.26 (t, *J* = 7.5 Hz, 2 H), 3.72 (m, 1 H), 4.10 (br m, 1 H), 4.20 (m, 1 H), 4.50 (t, *J* = 7.5 Hz, 2 H), 4.97 (d, *J* = 4 Hz, 1 H), 6.66 (br d, *J* = 9 Hz, 1 H), 7.1–7.4 (m, 8 H), 7.92 (br s, 1 H), 8.53 (br d, 2 H); FAB-MS *m/e* 861 $(M + H)^+$. Anal. ($C_{43}H_{60}N_{10}O_7S \cdot 0.75H_2O$) C, H, N.

Methods E (Deprotection) and F (Coupling). 5-[[(2*R*,3*S*)-3-[(Boc-L-phenylalanyl-N^{im}-Boc-L-histidyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]-1-(4-hydroxyphenethyl)-1*H*-tetrazole (4l). A solution of 250 mg (0.5 mmol) of **3l** in 3 mL of anhydrous trifluoroacetic acid was stirred at room temperature under protection from moisture for 30 min and then evaporated in a stream of N_2. The residue was reconcentrated three times from t-BuOH and dried in vacuo (oil pump, KOH trap, room temperature). It was then dissolved in some dry CH_2Cl_2, treated with 139 μL (101 mg, 1.0 mmol) of triethylamine, agitated briefly, and concentrated in vacuo at room temperature. This deprotected product was used below in the next step.

A mixture of 236 mg (0.5 mmol) of Boc-L-phenylalanyl-N^{im}-Boc-L-histidine,[26] 84 mg (0.55 mmol) of 1-hydroxybenzotriazole hydrate, and 4 mL of dry CH_2Cl_2 was stirred under N_2 at room temperature for a few minutes and then cooled in an ice–MeOH bath. To this was rapidly added 113 mg (0.55 mmol) of *N,N'*-dicyclohexylcarbodiimide, and stirring in the cooling bath was continued for 30 min. At this time a solution of the deprotected amine prepared above in 2 mL of CH_2Cl_2 was added. The mixture was allowed to warm gradually to room temperature. After 18 h the precipitated *N,N'*-dicyclohexylurea was removed by filtration, and the filtrate was concentrated in vacuo. The residue was dissolved in 50 mL of EtOAc and washed successively with 3 × 20 mL of 0.2 N HCl, 2 × 20 mL of half-saturated Na_2CO_3, and 2 × 20 mL of saturated NaCl. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was chromatographed on silica gel (elution with 98:2 and then 97:3 CH_2Cl_2–MeOH) to give 55 mg (12%) of a white, glassy powder: mp >95 °C; TLC in 95:5 $CHCl_3$–MeOH; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.6–1.8 (complex m, 31 H) including 1.34 (s, 9 H) and 1.58 (s, 9 H), 2.8–3.3 (complex m, 8 H) including 3.04 (t, *J* = 7 Hz, 2 H), 3.62 (m, 1 H), 3.91 (m, 1 H), 4.25 (m, 1 H), 4.34 (t, *J* = 7 Hz, 2 H), 4.66 (br m, 1 H), 5.06 (d, *J* = 5 Hz, 1 H), 6.69 (AB q, *J* = 8 Hz, 4 H), 7.0–7.4 (complex m, 7 H), 8.07 (br, 1 H), 8.40 (br, 1 H); FAB-MS *m/e* 876 $(M + H)^+$. Anal. ($C_{44}H_{61}N_9O_8S \cdot 0.5H_2O$) C, H, N.

Method H. 5-[[(2*R*,3*S*)-3-[(Boc-L-phenylalanyl-L-histidyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]-1-[2-(4-pyridyl)ethyl]-1*H*-tetrazole (5m). A solution of 1.72 g (2 mmol) of **4m** in 100 mL of MeOH was stirred at ambient temperature as a stream of NH_3 gas was passed through it for about 10 min (accompanied by a mild exotherm). Stirring was continued under a drying tube for an additional 2 h. The filtered solution was evaporated, and the residue was thoroughly triturated with hot ether. After cooling, the solid was collected on a filter, washed with ether, and dried to give 1.40 g (91%) of cream-colored solid: mp 149–150 °C; TLC in 9:1 $CHCl_3$–MeOH; ^1H NMR (DMSO-d_6, 300 MHz) δ 0.6–0.9 (complex m, 2 H), 1.0–1.6 (complex m, 19 H) including 1.24 (s, 9 H), 1.66 (br d, 1 H), 2.6–3.1 (complex m, 6 H), 3.13 (t, *J* = 7 Hz, 2 H), 3.53 (br m, 1 H), 3.94 (br m, 1 H), 4.44 (m, 1 H), 4.54 (t, *J* = 7 Hz, 2 H), 5.59 (br, 1 H), 6.80 (s, 1 H), 7.02 (d, *J* = 9 Hz, 1 H), 7.13, (d, *J* = 5.5 Hz, 2 H), 7.2 (m, 5 H), 7.43 (br d, *J* = 9 Hz, 1 H) overlapping 7.48 (s, 1 H), 8.12 (d, *J* = 8 Hz, 1 H), 8.39 (d, *J* = 5.5 Hz, 2 H); FAB-MS *m/e* 761 $(M + H)^+$. Anal. ($C_{38}H_{52}N_{10}O_5S \cdot 0.5H_2O$) C, H, N.

Method I. Ethyl 2-[5-[[(2*R*,3*S*)-3-[(Boc-L-phenylalanyl-L-histidyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]-1*H*-

tetrazol-1-yl]acetate (5p). A solution of 100 mg (0.117 mmol) of **4p** in 3.5 mL of EtOH (warmed to dissolve) was treated with 100 mg of anhydrous K_2CO_3. The mixture was stirred at room temperature for 2 h and then filtered. The residue from evaporation of the filtrate was triturated with CH_2Cl_2 to give a solid, which was isolated by filtration. Chromatography on silica gel (gradient elution with 2–5% EtOH in CH_2Cl_2) afforded 20 mg (23%) of white solid: TLC in 9:1 $CHCl_3$–MeOH; ^1H NMR (DMSO-d_6, 200 MHz) δ 0.6–1.8 (complex m, 25 H) including 1.24 (t, J = 7 Hz, 3 H) and 1.31 (s, 9 H), 2.97 (m, 2 H), 3.16 (m, 2 H), 2.65 (br m, 1 H), 3.9–4.3 (complex m, 4 H) including 4.24 (q, J = 7 Hz, 2 H), 4.53 (m, 1 H), 5.47 (s, 2 H), 6.90 (s, 1 H), 7.09 (d, J = 9 Hz, 1 H, exchangeable with D_2O), 7.28 (m, 5 H), 7.52 (d, J = 9 Hz, 1 H, exchangeable with D_2O), 7.61 (s, 1 H), 8.19 (d, J = 8 Hz, 1 H, exchangeable with D_2O); FAB-MS m/e 742 $(M + H)^+$. The analytical sample (mp 177–178 °C) was obtained by dissolution in hot CH_2Cl_2–acetonitrile, filtration through Celite, and evaporation to dryness. Anal. ($C_{35}H_{51}N_9O_7S$) C, H, N.

Method J. 4-[2-[5-[[(2*R*,3*S*)-3-[(Boc-L-phenylalanyl-N^{im}-Boc-L-histidyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]tetrazol-1-yl]ethyl]-1-methylpyridinium Iodide (4q). To 172 mg (0.2 mmol) of **4m** was added 0.5 mL of methyl iodide. The mixture was stirred at room temperature in a stoppered flask, and a clear solution was soon achieved. After 1.75 h the excess methyl iodide was removed in a gentle stream of N_2, and the residue was dried further in vacuo. The material was next dissolved in 10 mL of CH_2Cl_2 and washed with 10 mL of H_2O. The organic phase was evaporated to dryness, and the glassy residue was triturated with ether. The resulting solid was collected on a filter, washed with ether, and dried to yield 141 mg (70%) of light golden-tan powder: mp >105 °C (gradual); TLC in 80:20:2 $CHCl_3$–MeOH–H_2O; ^1H NMR ($CDCl_3$, 200 MHz) δ 0.6–1.8 (complex m, 31 H) including 1.39 (s, 9 H) and 1.60 (s, 9 H), 2.98 (m, 2 H), 3.1–3.5 (complex m, 4 H), 3.69 (br m, 2 H), 4.06 (m, 1 H), 4.27 (m, 1 H), 4.56 (s, 3 H), 4.78 (br t, 2 H), 5.09 (m, 1 H), 6.77 (d, J = 9 Hz, 1 H), 7.2–7.4 (m, 8 H), 7.97 (m, 2 H), 8.74 (br d, 1 H), 8.99 (m, 2 H); FAB-MS m/e 875 (M^+ for the pyridinium cation). Anal. ($C_{44}H_{63}IN_{10}O_7S\cdot0.5H_2O$) C, H, N.

Method K. 5-[[(2*R*,3*S*)-3-[(Boc-L-phenylalanyl-L-histidyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]-sulfonyl]-1-[2-(1-oxo-4-pyridyl)ethyl]-1*H*-tetrazole (5r). A mixture of 193 mg (0.25 mmol) of **5m**, 255 mg (1.25 mmol) of 85% *m*-chloroperbenzoic acid, and 1.25 mL of glacial AcOH was stirred at room temperature in a stoppered flask. All of the solid dissolved within a few minutes, followed eventually by precipitation. After 18 h the mixture was concentrated in vacuo, and the residue was chromatographed on silica gel (gradient elution with 5–10% MeOH in CH_2Cl_2). The residue from evaporation of the pooled product fractions was dissolved in CH_2Cl_2–acetone, filtered, and reconcentrated. Trituration of this residue with ether afforded 130 mg (63%) of white solid: mp 157–158 °C; TLC in 9:1 $CHCl_3$–MeOH; ^1H NMR (DMSO-d_6, 200 MHz) δ 0.6–1.8 (complex m, 22 H) including 1.29 (s, 9 H), 2.6–3.1 (complex m, 4 H), 3.28 (t, J = 7 Hz, 2 H), 3.7–4.2 (complex m, 5 H), 4.45 (m, 1 H), 4.95 (t, J = 7 Hz, 2 H), 5.83 (br, 1 H), 6.88 (s, 1 H), 7.07 (d, J = 8 Hz, 1 H), 7.26 (m, 7 H), 7.50 (s, 1 H), 7.54 (m, 1 H), 8.14 (d, J = 7.5 Hz, 2 H); FAB-MS m/e 809 $(M + H)^+$. Anal. ($C_{39}H_{52}N_{10}O_8S\cdot H_2O$) C, H, N.

Method L. 2-[5-[[(2*R*,3*S*)-3-[(Boc-L-phenylalanyl-L-histidyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]-1*H*-tetrazol-1-yl]acetic Acid (5s). A solution of 100 mg (0.117 mmol) of **4p** in 4 mL of warm EtOH was treated with 100 mg of anhydrous K_2CO_3, and the resulting suspension was stirred vigorously at room temperature for 18 h. The mixture was concentrated to dryness, and the residue was suspended in H_2O, which was adjusted to pH 2 with dilute HCl. After thorough mixing, the solid was collected on a filter, dried, and chromatographed on silica gel (elution with 90:10:1 and then 80:20:2 CH_2Cl_2–MeOH–H_2O). The product residue was taken up in 4 mL of warm MeOH and filtered to remove insoluble contaminants. Concentration of the filtrate in vacuo yielded 23 mg (28%) of cream-colored solid: mp >180 °C (gradual); TLC in 80:20:2 CH_2Cl_2–MeOH–H_2O; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.73 (m, 1 H), 0.87 (m, 1 H), 1.0–1.6 (complex m, 19 H) including 1.28 (s, 9 H), 1.71 (br d, J = 11.4 Hz, 1 H), 2.69 (dd, J = 10.8 Hz, 13.7 Hz, 1 H), 2.89 (ABX m, 2 H), 2.95–3.1 (complex m, 3 H), 3.64 (br m, 1 H), 3.94 (br m, 1 H), 4.14 (m, 1 H), 4.54 (q, J = 6.8 Hz, 1 H), 4.87 (s, 2 H), 6.97 (s, 1 H), 7.00 (d, J = 8.4 Hz, 1 H), 7.17 (m, 1 H), 7.24 (m, 4 H), 7.62 (d, J = 8.8 Hz, 1 H), 8.04 (s, 1 H), 8.25 (d, J = 7.8 Hz, 1 H); high-resolution FAB-MS m/e 714.3334 [calcd for $C_{33}H_{48}N_9O_7S$ $(M + H)^+$: 714.3399].

Method M. 2-[5-[[(2*R*,3*S*)-3-[(Boc-L-phenylalanyl-L-histidyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]-1*H*-tetrazol-1-yl]acetamide (5t). A stream of NH_3 gas was bubbled through a solution of 88 mg (0.103 mmol) of **4p** in 6 mL of EtOH at room temperature for a few minutes. The resulting saturated solution was stirred in a stoppered flask for 5 h, by which time a gelatinous precipitate had separated. The mixture was concentrated in vacuo, and the residue was stirred overnight with ether. The mixture was filtered, and the solid on the filter was taken up in MeOH. This solution was filtered through Celite and evaporated to give 30 mg (40%) of white solid: mp 200–201 °C; TLC in 9:1 $CHCl_3$–MeOH; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.72 (m, 1 H), 0.84 (m, 1 H), 1.0–1.6 (m, 19 H) including 1.28 (s, 9 H), 1.70 (br d, J = 11.9 Hz, 1 H), 2.70 (dd, J = 11 Hz, 13.5 Hz, 1 H), 2.8–3.1 (complex m, 5 H), 3.60 (br m, 1 H), 3.99 (br m, 1 H), 4.13 (m, 1 H), 4.48 (m, 1 H), 5.03 (s, 2 H), 5.64 (br, 1 H), 6.83 (s, 1 H), 7.06 (d, J = 8.0 Hz, 1 H), 7.17 (m, 1 H), 7.24 (m, 4 H), 7.4–7.6 (m, 3 H), 7.87 (s, 1 H), 8.15 (d, J = 7.7 Hz, 1 H); FAB-MS m/e 713 $(M + H)^+$. Anal. ($C_{33}H_{48}N_{10}O_8S\cdot0.67H_2O$) C, H, N.

Method N. 4-[2-[2-[5-[[(2*R*,3*S*)-3-[(Boc-L-phenylalanyl-L-histidyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]-1*H*-tetrazol-1-yl]acetamido]ethyl]morpholine (5v). A suspension of 107 mg (0.13 mmol) of **4p** in 10 drops of 4-(2-aminoethyl)-morpholine was stirred under N_2 at room temperature for 2 h and then diluted with ether. The precipitated solid was collected on a filter, then washed with saturated aqueous NH_4Cl followed by H_2O, and dried in vacuo over P_2O_5 to yield 77 mg (71%) of white solid: mp 173–176 °C: TLC in 90:10:1 $CHCl_3$–MeOH–concentrated NH_4OH; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.73 (br m, 1 H), 0.86 (br m, 1 H), 1.0–1.6 (complex m, 19 H) including 1.28 (s, 9 H), 1.70 (br d, J = 12 Hz, 1 H), 2.37 (m, 6 H), 2.70 (dd, J = 10.9 Hz, 13.6 Hz, 1 H), 2.8–3.1 (complex m, 5 H), 3.22 (q, J = 6.3 Hz, 2 H), 3.56 (t, J = 4.6 Hz, 4 H), 3.99 (br m, 1 H), 4.13 (m, 1 H), 4.49 (q, J = 6.9 Hz, 1 H), 5.07 (s, 2 H), 5.62 (br, 1 H), 6.87 (s, 1 H), 7.05 (d, J = 8.1 Hz, 1 H), 7.17 (m, 1 H), 7.24 (m, 4 H), 7.48 (d, J = 9.2 Hz, 1 H), 7.63 (s, 1 H), 8.17 (d, J = 7.7 Hz, 1 H), 8.43 (t, J = 5.6 Hz, 1 H); FAB-MS m/e 713 $(M + H)^+$. Anal. ($C_{39}H_{59}N_{11}O_7S\cdot0.67H_2O$) C, H, N.

5-Mercapto-1-methyl-3-(trifluoromethyl)-1*H*-1,2,4-triazole (7). To a suspension of 2.1 g (20 mmol) of 2-methyl-3-thiosemicarbazide in 3 mL of toluene was slowly added 2.0 mL (2.96 g, 26 mmol) of trifluoroacetic acid. The mixture was stirred at reflux under a Dean–Stark trap for a few hours until no additional H_2O was being collected. The solid which precipitated upon cooling was collected on a filter, washed with a small volume of petroleum ether, and recrystallized from nitromethane (about 3 mL) to yield 1.23 g (34%) of solid: mp 117–119 °C; TLC in 95:5 CH_2Cl_2–$MeOH_2$; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.78 (s, 3 H). Anal. ($C_4H_4F_3N_3S$) C, H, N.

Methyl *N*-(4-Hydroxyphenethyl)dithiocarbamate (9a). Using the method described below for **9b**, this material was prepared in 59% yield from tyramine as off-white crystals: mp 79.5–81 °C; TLC in 2:1 hexane–EtOAc; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.62, 2.69 (major and minor s, 3 H total), 2.91 (t, J = 7 Hz, 2 H), 3.67, 3.96 (minor and major dt, J = 7 Hz, 7 Hz, 2 H total, both collapsing to t upon D_2O exchange), 6.80 (d, J = 8 Hz, 2 H), 6.90, 7.58 (major and minor br m, 1 H total, exchangeable with D_2O), 7.08 (d, J = 8 Hz, 2 H); EI-MS m/e 227 (M^+). Anal. ($C_{10}H_{13}NOS_2$) C, H, N.

Methyl *N*-[2-(4-Pyridyl)ethyl]dithiocarbamate (9b). A solution of 7.18 mL (7.33 g, 60 mmol) of 4-(2-aminoethyl)pyridine and 9.01 mL (6.54 g, 64.8 mmol) of triethylamine in 60 mL of ether and 30 mL of MeOH was stirred under N_2 with cooling in an ice bath as a solution of 4.04 mL (5.14 g, 67.5 mmol) of carbon disulfide in 30 mL of ether was added over 15 min. After being stirred overnight at room temperature, the solvent was evaporated in a stream of N_2. The residual oil was dissolved in 120 mL of MeOH and stirred under N_2 at −8 to −5 °C as a solution of 3.74 mL (8.52 g, 60 mmol) of methyl iodide in 30 mL of acetone was added dropwise. After a few minutes, the solution was added to 900 mL of ice-H_2O, and the product was extracted with ether.

The organic phase was dried over $MgSO_4$, filtered, and concentrated. The residual solid was washed with hexane followed by H_2O and then dried to yield 9.92 g (78%) of pale orange crystals: mp 88–90 °C; TLC in 1:1 hexane–EtOAc; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.65 (s, 3 H), 3.02 (t, J = 7 Hz, 2 H), 4.04 (dt, J = 7 Hz, 7 Hz, 2 H), 7.13 (d, J = 6 Hz, 2 H), 7.63 (br m, 1 H), 8.47 (d, J = 6 Hz, 2 H).

Methyl *N*-[2-(4-Morpholino)ethyl]dithiocarbamate (9c). A solution of 7.87 mL (7.81 g, 60 mmol) of 4-(2-aminoethyl)-morpholine in 60 mL of ether was stirred under N_2 at 0 °C as a solution of 4.04 mL (5.14 g, 67.5 mmol) of carbon disulfide in 30 mL of ether was added dropwise. The resulting precipitate was collected on a filter and washed thoroughly with ether. The dried solid (9.3 g, 45 mmol) was dissolved in 15 mL of H_2O, stirred in an ice bath, and treated dropwise with 22.5 mL (45 mmol) of 2 N KOH solution. Next a solution of 3.08 mL (7.03 g, 49.5 mmol) of methyl iodide in 20 mL of acetone was added dropwise. The mixture was stirred for an additional 30 min at 0 °C and then partially evaporated. The remaining aqueous solution was extracted with EtOAc (3 × 200 mL), and the combined EtOAc extracts were washed with saturated NaCl solution, then dried, and filtered. Concentration of the filtrate yielded 6.12 g (46% overall) of a viscous oil: TLC in 1:1 hexane–EtOAc; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.4–2.8 (complex m, 9 H) including 2.64 (s, 3 H), 3.5–3.9 (complex m, 6 H), 7.65 (br m, 1 H).

1-(4-Hydroxyphenethyl)-5-mercapto-1*H*-tetrazole (10a). This material was obtained in 31% yield by the method described below for **10b** except that the aqueous solution was acidified to pH 2 with concentrated HCl to precipitate the product, which was recrystallized from $CHCl_3$ containing a small amount of nitromethane to give white crystals: mp 120.5–121.5 °C: TLC in 80:20:2 $CHCl_3$–MeOH–H_2O; ^1H NMR (DMSO-d_6, 200 MHz) δ 3.04 (t, J = 7.5 Hz, 2 H), 4.39 (t, J = 7.5 Hz, 2 H), 6.66 (d, J = 8 Hz, 2 H), 6.98 (d, J = 8 Hz, 2 H), 9.25 (br s, 1 H, exchangeable with D_2O); EI-MS m/e 222 (M^+). Anal. ($C_9H_{10}N_4OS$) C, H, N.

5-Mercapto-1-[2-(4-pyridyl)ethyl]-1*H*-tetrazole (10b). A mixture of 6.37 g (30 mmol) of **9b**, 2.15 g (33 mmol) of NaN_3, 12 mL of EtOH, and 24 mL of H_2O was stirred at reflux under N_2 for 6 h. The solution was diluted with additional H_2O and neutralized with glacial AcOH, resulting in heavy precipitation. The solid was collected on a filter, washed with a little H_2O, and dried to give 3.99 g (64%) of white crystals: mp 118.5–120 °C (preliminary softening); TLC in 80:20:2 $CHCl_3$–MeOH–H_2O; ^1H NMR (DMSO-d_6, 300 MHz) δ 3.22 (t, J = 7 Hz, 2 H), 4.52 (t, J = 7 Hz, 2 H), 7.30 (d, J = 5 Hz, 2 H), 8.48 (d, J = 5 Hz, 2 H); high-resolution FAB-MS m/e 208.0673 [calcd for $C_8H_{10}N_5S$ ($M + H$)$^+$: 208.0656].

5-Mercapto-1-[2-(4-morpholino)ethyl]-1*H*-tetrazole (10c). A mixture of 6.07 g (27.6 mmol) of **9c**, 1.79 g (27.6 mmol) of NaN_3, 12 mL of EtOH, and 24 mL of H_2O was stirred at reflux under N_2 for 3.5 h. The solution was acidified to pH 2 and applied to a column containing 55 g of AG 50W-X8 cation-exchange resin (H^+ form, 20–50 mesh). The column was eluted with H_2O until the pH of the eluate reached 4.5 and then eluted further with 5% NH_4OH. The product fractions were combined, washed with EtOAc, and concentrated in vacuo. Trituration of the residue with MeOH gave a solid, which was isolated on a filter and dried to give 1.44 g (24%) of pale pink powder: mp 219–223 °C: TLC in 9:1 $CHCl_3$–MeOH; ^1H NMR (DMSO-d_6, 400 MHz) δ 2.93 (br m, 4 H), 3.18 (br m, 2 H), 3.66 (br m, 4 H), 4.46 (t, J = 6.2 Hz, 2 H).

4-[2-(4-Pyridyl)ethyl]-3-thiosemicarbazide (11). A mixture of 3.39 g (16 mmol) of **9b**, 3.10 mL (3.20 g, 64 mmol) of 100% hydrazine hydrate, and 16 mL of 2-methoxyethanol was stirred under N_2 at 80 °C for 4 h. After evaporation of the solution, the residue was triturated thoroughly with EtOAc. The resulting solid was collected on a filter and washed with EtOAc followed by ether to give 2.44 g (84%) of tan crystals: mp 141–144 °C dec; TLC in 9:1 $CHCl_3$–MeOH; ^1H NMR (DMSO-d_6, 300 MHz) δ 2.81 (t, J = 7 Hz, 2 H), 3.56 (dt, J = 7 Hz, 7 Hz, 2 H, collapsing to t upon D_2O exchange), 4.40 (br, 2 H, exchangeable with D_2O), 7.20 (d, J = 5.5 Hz, 2 H), 7.88 (br, 1 H, exchangeable with D_2O), 8.41 (d, J = 5.5 Hz, 2 H), 8.63 (br, 1 H, exchangeable with D_2O). Anal. ($C_8H_{13}N_4S$) C, H, N.

3-Mercapto-4-[2-(4-pyridyl)ethyl]-5-(trifluoromethyl)-4*H*-1,2,4-triazole (12). A mixture of 788 mg (4 mmol) of 11 and 1.0 mL of anhydrous trifluoroacetic acid in a flask fitted with condenser and N_2 inlet was stirred at 75 °C for 1 h, during which time all of the solid dissolved. The bath temperature was gradually raised to 125 °C. After a total of 6.75 h, the viscous oil was cooled and neutralized by gradual addition of saturated aqueous $NaHCO_3$ solution. The addition was accompanied by foaming and precipitation. The mixture was stirred for several minutes until CO_2 evolution had ceased and then filtered. The solid on the filter was washed thoroughly with H_2O and dried to give 858 mg (78%) of slightly off-white crystals: mp 171.5 °C; TLC in 9:1 $CHCl_3$–MeOH; ^1H NMR (DMSO-d_6, 200 MHz) δ 3.09 (t, J = 7.5 Hz, 2 H), 4.27 (t, J = 7.5 Hz, 2 H), 7.26 (d, J = 5.5 Hz, 2 H), 8.51 (d, J = 5.5 Hz, 2 H); FAB-MS m/e 275 ($M + H$)$^+$. Anal. ($C_{10}H_9F_3N_4S$) C, H, N.

In Vitro Plasma Renin Assay. The inhibition of human plasma renin was assayed essentially under the conditions previously described.[11] Briefly, lyophilized human plasma was reconstituted with distilled H_2O on the day of the assay. The radioimmunoassay[30] for angiotensin I used a commercial kit at pH 7.4 (phosphate buffer), 37 °C, with phenylmethanesulfonyl fluoride added to inhibit angiotensinases. Inhibitors were initially dissolved at 1 mM in DMF before serial dilution to 3–5 concentrations bracketing the IC_{50}. Duplicate determinations at each dilution were averaged, and the concentration required to inhibit the plasma renin reaction by 50% (IC_{50}) was calculated using a linear regression method. The relative variability of the IC_{50} measurement in this assay is ±40% (two standard deviations) as determined from 122 runs of a standard inhibitor.

In Vivo Evaluation of Renin Inhibitors. Following the previously described protocol,[31] male and female rhesus monkeys were surgically implanted with chronic arterial vascular access ports for direct monitoring of mean arterial pressure (MAP) and periodic collection of blood samples for determination of plasma renin activity (PRA). Indwelling venous catheters were inserted for intravenous administration of compounds. The monkeys were maintained on a low-sodium diet and treated with furosemide the evening before the experiment. The animals were fasted for 18 h before and during the experiment. Compounds were administered intravenously in 0.05% acetic acid/5% dextrose/H_2O 20 min after a control administration of the vehicle. For oral administration, compounds were suspended or dissolved in 0.1 M citric acid/H_2O and delivered by nasogastric catheter. Percent inhibition of PRA and changes in MAP and heart rate were calculated and plotted against time.

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