Inhibitors of Human Renin with C-Termini Derived from Amides and Esters of α-Mercaptoalkanoic Acids

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New transition-state analogues bearing C-termini derived from α-mercaptoalkanoic acids, esters, and amides were prepared and evaluated as inhibitors of human renin. Addition of α-mercaptoalkanoate esters to a chiral Boc-amino epoxide intermediate led ultimately to the target [(2*R*,3*S*)-3-(BocPheHis-amino)-4-cyclohexyl-2-hydroxy-1-butyl]thio derivatives. The corresponding sulfoxide and sulfone analogues were also investigated. Some of these derivatives, including one with a stable BocPhe replacement, were relatively potent inhibitors of human plasma renin, having IC_{50} values below 10 nM. When selected compounds were administered intravenously to sodium-deficient rhesus monkeys (*Macaca mulatta*) at 0.06–1 mg/kg, they reduced plasma renin activity by 87–94%. However, the accompanying drop in blood pressure was of short duration.

The renin–angiotensin system (RAS) can play a key role in blood pressure regulation and consequently is a major focus for the therapy of cardiovascular disease.[1] The circulating glycoprotein angiotensinogen, which is biologically inactive, 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]

While ACE inhibitors are now widely used for the treatment of hypertension and congestive heart failure,[5] occasional side effects have been attributed to elevated levels of bradykinin,[6] which is also a substrate for the enzyme. In contrast, angiotensinogen is the only known natural substrate for renin. This specificity and its position as the rate-limiting enzyme in the synthesis of AII make renin an attractive target for inhibition.[2,3] Numerous reports of renin inhibitors have appeared in the literature, and the antihypertensive activity of this class of compounds has been demonstrated experimentally and clinically.[7] Yet to be overcome for renin inhibitors in general are problems of poor oral bioavailability and limited duration of action, the latter presumably resulting from rapid biliary excretion and/or metabolic breakdown.[7c]

The tetrahedral transition state involved in the proteolysis of angiotensinogen at the scissile Leu–Val bond has been effectively mimicked by inhibitors containing a "hydroxyethylene isostere"[8] or by incorporation of statine [(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid][9] or its cyclohexyl analogue ACHPA[10] at the P_1-$P_{1'}$ locus.[11] Similarly, the Abbott group[12,13] has obtained potent, transition-state renin inhibitors bearing C-terminal alkylthio or alkylsulfonyl groups. We[14] have recently described a related series of inhibitors containing a heterocyclylthio C-terminus. This study showed that rather large substituents could be accommodated on the heterocycle, depending on ring position and hydrophobic character. It was of interest to further explore modifications of the end group in an effort to improve inhibitory potency and oral bioavailability. We have now prepared a series of potential transition-state renin inhibitors in which the C-terminus is derived from an α-mercaptoalkanoic acid or, especially, an amide or ester thereof.

Chemistry

Physical properties of most intermediates are listed in Tables I and II and those of the final products in Table III. In order to prepare these inhibitors, ethyl α-mercaptoalkanoates **2** were added to the previously described chiral epoxide **1**[14,15] in the presence of triethylamine[16] to

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(6) Lindgren, B. R.; Andersson, R. G. G. Angiotensin-Converting Enzyme Inhibitors and Their Influence on Inflammation, Bronchial Reactivity and Cough. *Med. Toxicol. Adverse Drug Exp.* **1989**, *4*, 369–380.
(7) For recent reviews, see: (a) Kleinert, H. D. Renin Inhibitors: Discovery and Development. An Overview and Perspective. *Am. J. Hypertens.* **1989**, *2*, 800–808. (b) Corvol, P.; Chauveau, X. J.; Ménard, J. Human Renin Inhibitor Peptides. *Hypertension* **1990**, *16*, 1–11. (c) Greenlee, W. J. Renin Inhibitors. *Med. Res. Rev.* **1990**, *10*, 173–236. (d) Ocain, T. D.; Abou-Gharbia, M. Renin–Angiotensin System (RAS) Dependent Antihypertensive Agents: I. Renin Inhibitors. *Drugs Future* **1991**, *16*, 37–51.
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(9) Boger, J. Renin Inhibitors. Design of Angiotensinogen Transition-State Analogs Containing Statine. In *Peptides: Structure and Function. Proceedings of the Eighth American Peptide Symposium*; Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Co.: Rockford, IL, 1984; pp 569–578.
(10) Boger, J.; Payne, L. S.; Perlow, D. S.; Lohr, N. S.; Poe, M.; Blaine, E. H.; Ulm, E. H.; Schorn, T. W.; LaMont, B. I.; Lin, T.-Y.; Kawai, M.; Rich, D. H.; Veber, D. F. Renin Inhibitors. Syntheses of Subnanomolar, Competitive, Transition-State Analogue Inhibitors Containing a Novel Analogue of Statine. *J. Med. Chem.* **1985**, *28*, 1779–1790.
(11) Rich, D. H.; Salituro, F. G.; Holladay, M. W.; Schmidt, P. G. Design and Discovery of Aspartyl Protease Inhibitors. Mechanistic and Clinical Implications. In *Conformationally Directed Drug Design: Peptides and Nucleic Acids as Templates of Targets*; Vida, J. A., Gordon, M., Eds.; ACS Symposium Series 251; American Chemical Society: Washington, D.C., 1984; pp 211–237.
(12) Luly, J. R.; Yi, N.; Soderquist, J.; Stein, H.; Cohen, J.; Perun, T. J.; Plattner, J. J. New Inhibitors of Human Renin That Contain Novel Leu-Val Replacements. *J. Med. Chem.* **1987**, *30*, 1609–1616.

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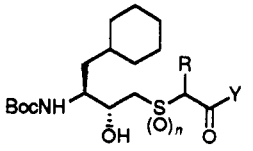
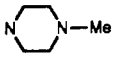
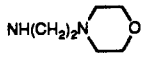
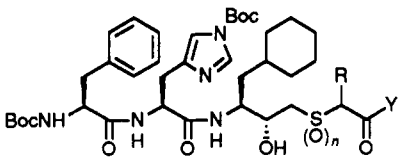
Table I. Physical Properties of Intermediates **3**, **8**, **12**, and **17**

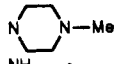
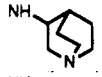
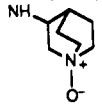


no.	*n*	R (config)	Y	method[a]	% yield	mp, °C	$[\alpha]^{20}_D$, deg[b]	formula[c]	FAB-MS, *m/e* $(M + H)^+$
3a	0	H	OEt	A	84	oil	−43.8	$C_{19}H_{35}NO_5S$	390
3b	0	*i*-Bu (*R*)	OEt	A	81	oil	+3.59	$C_{23}H_{43}NO_5S$[d]	446
3c	0	*i*-Bu (*S*)	OEt	A	85	oil	−63.9	$C_{23}H_{43}NO_5S$[e]	446
8	2	H	OEt	B	89	102–104	−28.0	$C_{19}H_{35}NO_7S$	422
12a	0	H		C[f]	100	foam	−22.5	$C_{22}H_{41}N_3O_4S$	444.2895[g]
12b	0	H		C	75	105–106	−20.7	$C_{24}H_{43}N_3O_4S \cdot 1.75H_2O$	470
17	2	H		D	92	foam	−16.9	$C_{23}H_{43}N_3O_7S$	506

[a] See Experimental Section for description of general methods. [b] (*c* 2, $CHCl_3$). [c] Analyses for C, H, and N within ±0.4% except where characterized by high-resolution mass spectrum or as otherwise indicated. [d] H: calcd, 9.73; found, 8.91. [e] H: calcd, 9.73; found, 8.93. [f] Reaction conducted at 80 °C. [g] Calcd for $C_{23}H_{44}N_3O_7S$ $(M + H)^+$: 444.2896.

Table II. Physical Properties of Intermediates **4**, **9**, **10**, **13**, and **14**



no.	*n*	R (config)	Y	method[a]	% yield	mp, °C	formula[b]	FAB-MS, *m/e* $(M + H)^+$
4a	0	H	OEt	E	47	>60 (gradual)	$C_{39}H_{59}N_5O_9S \cdot 0.5H_2O$	774
4b	0	*i*-Bu (*R*)	OEt	E	56	75–78	$C_{43}H_{67}N_5O_9S \cdot 0.5H_2O$	830
4c	0	*i*-Bu (*S*)	OEt	E	56	82–83	$C_{43}H_{67}N_5O_9S \cdot 0.5H_2O$	830
9a	2	H	OEt	E	46	foam	$C_{39}H_{59}N_5O_{11}S \cdot 0.5H_2O$	806
9b	2	*i*-Bu (*R*)	OEt	B	26	>73 (gradual)	$C_{43}H_{67}N_5O_{11}S \cdot 1.5H_2O$[c]	862
10a	1	H	OEt	F	68	140–142 dec	$C_{39}H_{59}N_5O_{10}S \cdot 0.67H_2O$[d]	790
10b	1	*i*-Bu (*S*)	OEt	F	72	oil	$C_{43}H_{67}N_5O_{10}S \cdot 0.67H_2O$[e]	846
13a	0	H		E[f]	31	>80 (gradual)	$C_{42}H_{65}N_7O_8S \cdot H_2O$	828
13b	0	H		E[f]	38	>120 dec (gradual)	$C_{44}H_{67}N_7O_8S$	854.854[g]
14	2	H		B[h]	40	>140 dec (gradual)	$C_{44}H_{67}N_7O_{11}S$	902.4702[i]

[a] See Experimental Section for description of general methods. [b] Analyses for C, H, and N within ±0.4% except where characterized by high-resolution mass spectrum or as otherwise indicated. [c] N: calcd, 7.88; found, 7.37. [d] N: calcd, 8.73; found, 8.01. [e] N: calcd, 8.16; found, 7.69. [f] MeOH substituted for EtOH. [g] Calcd for $C_{44}H_{68}N_7O_8S$ $(M + H)^+$: 854.4850. [h] Purified by preparative silica gel TLC (developed with 85:15:1.5 $CHCl_3$–MeOH–concentrated NH_4OH). [i] Calcd for $C_{44}H_{68}N_7O_{11}S$ $(M + H)^+$: 902.4697.

give the adducts **3** (Scheme I). The chiral mercapto esters **2b,c** were obtained by acid-catalyzed esterification of the optically active mercapto acids,[17] which were readily available by transformation of the corresponding amino acids of opposite configuration as reported.[18,19] Inter-

(13) Dellaria, J. F.; Maki, R. G.; Bopp, B. A.; Cohen, J.; Kleinert, H. D.; Luly, J. R.; Merits, I.; Plattner, J. J.; Stein, H. H. Optimization and in Vivo Evaluations of a Series of Small, Potent, and Specific Renin Inhibitors Containing a Novel Leu-Val Replacement. *J. Med. Chem.* **1987**, *30*, 2137–2144.

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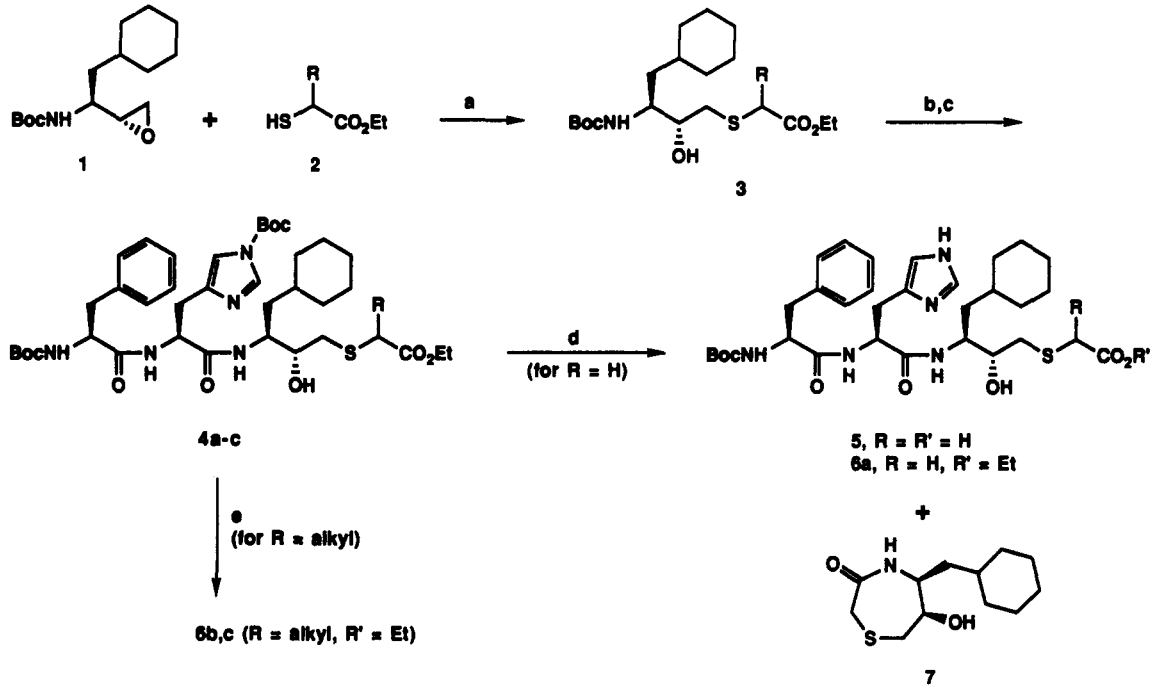
(16) Bernstein, P. R.; Snyder, D. W.; Adams, E. J.; Krell, R. D.; Vacek, E. P.; Willard, A. K. Chemically Stable Homocinnamyl Analogues of the Leukotrienes: Synthesis and Preliminary Biological Evaluation. *J. Med. Chem.* **1986**, *29*, 2477–2483.

(17) Noe, C. R. Chirale Lactole, III. Eine enantioselektive Alkylierung der Mercaptoessigsäure. *Chem. Ber.* **1982**, *115*, 1607–1616.

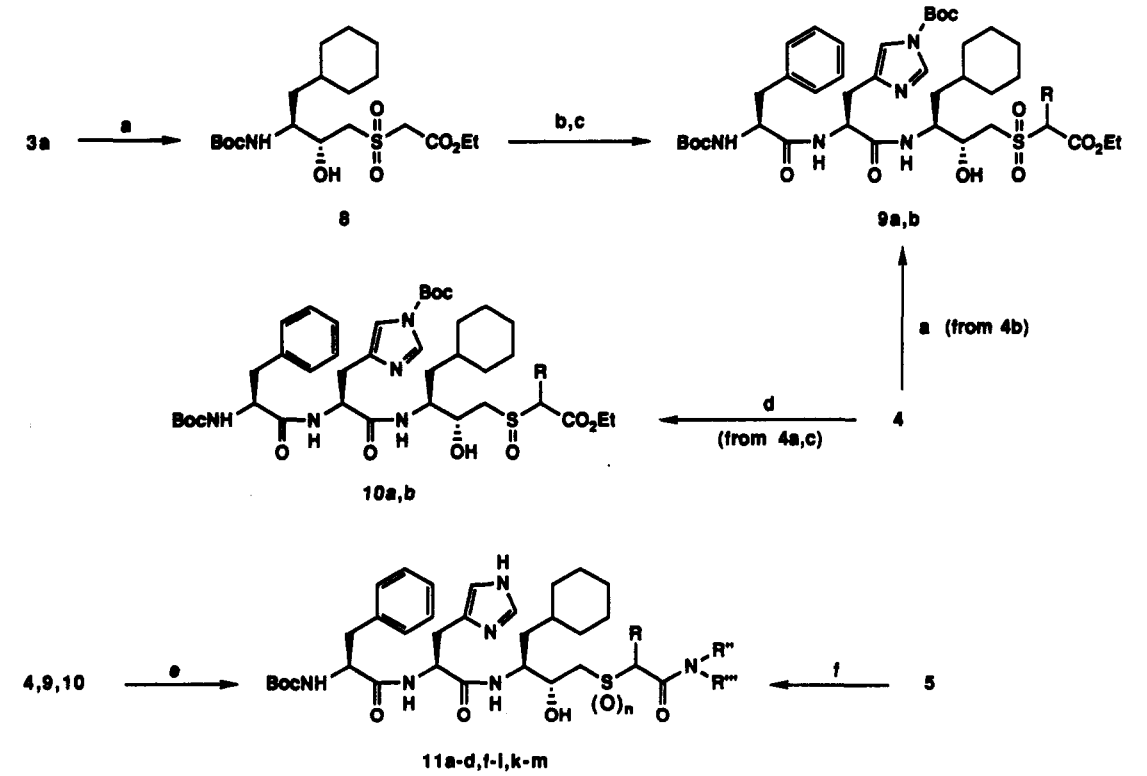
(18) Yankeelov, J. A., Jr.; Fok, K.-F.; Carothers, D. J. Peptide-Gap Inhibitors. 2. Stereoselective Synthesis of Enantiomeric Dipeptide Analogues of Glycylleucine Which Contain Methylene Thioether Groups Substituted for Peptide Linkages. *J. Org. Chem.* **1978**, *43*, 1623–1624.

Scheme I[a]



[a] (a) Et_3N, EtOH, 20 °C; (b) HCl/EtOH or TFA; (c) BocPheHis(N^{im}-Boc)-OH, BOP, Et_3N; (d) K_2CO_3, EtOH; (e) $H_2NNH_2 \cdot H_2O$, EtOH, 20 °C.

Scheme II[a]



[a] (a) MCPBA (2.4-3 equiv); (b) HCl/EtOH or TFA; (c) BocPheHis(N^{im}-Boc)-OH, BOP, Et_3N; (d) MCPBA (1.1 equiv); (e) HNR″R‴; (f) HNR″R‴, BOP, Et_3N.

mediates **3** were deblocked with either ethanolic HCl or anhydrous trifluoroacetic acid and then coupled with the protected dipeptide BocPhe(N^{im}-BocHis)-OH[20] in the presence of BOP reagent[21] to give **4a–c**. In the case of the mercaptoacetate derivative **4a**, treatment with ethanolic K_2CO_3 at room temperature removed the imidazole Boc

(19) Feder, J.; Weingarten, H. I., European Patent Appl. 149,593, 1986.

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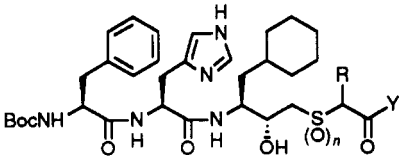
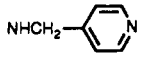
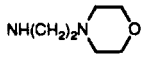
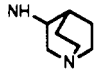
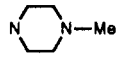
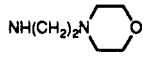
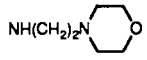
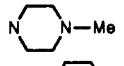
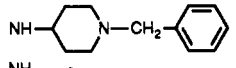
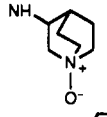
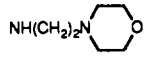
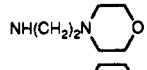
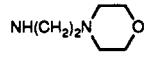
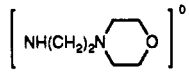
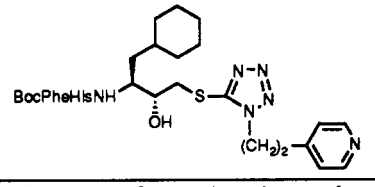
Table III. Physical and Renin-Inhibitory Properties of **5**, **6**, **11**, and **19**



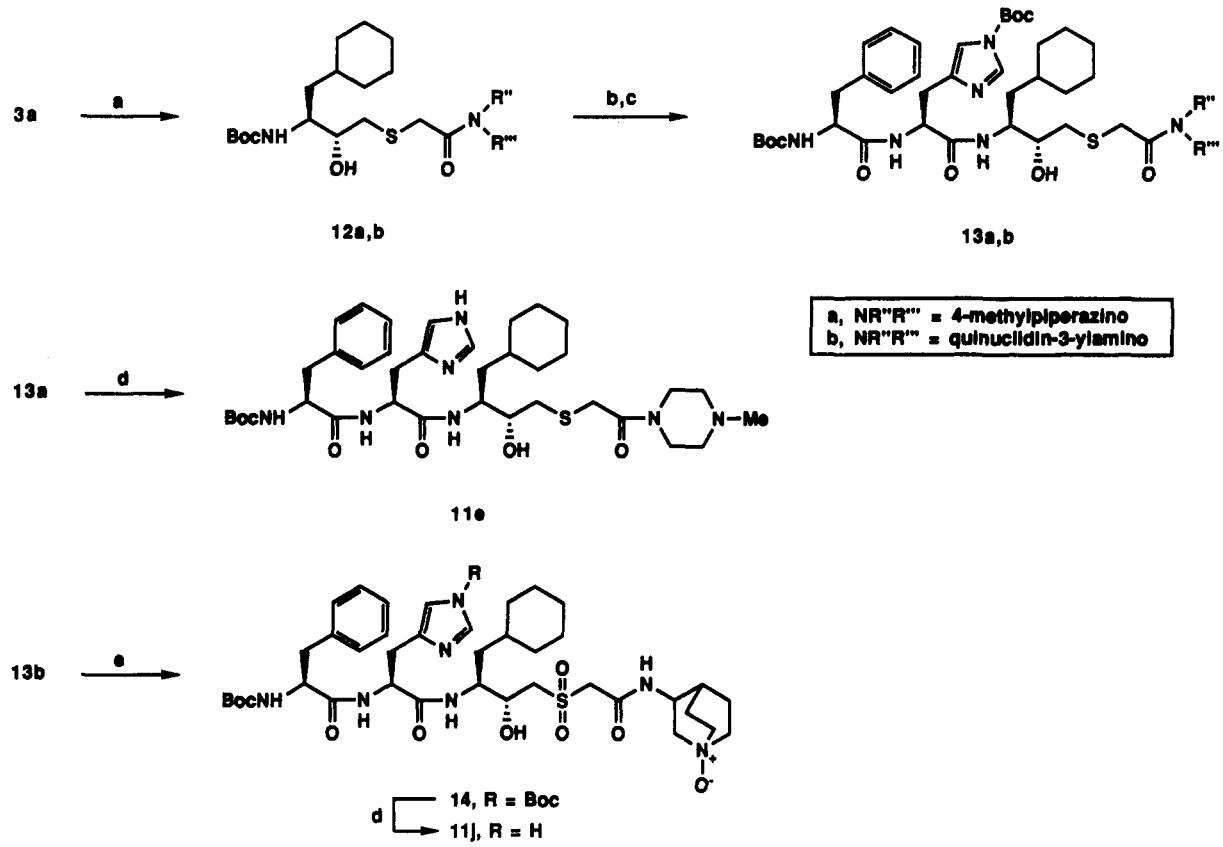
no.	n	R (config)	Y	method[a]	% yield	mp, °C	formula[b]	FAB-MS, m/e (M + H)+	human plasma renin IC50, nM
5	0	H	OH	G	53	178–179 dec	$C_{32}H_{47}N_5O_7S\cdot0.25H_2O$	646	904
6a	0	H	OEt	G	22	97–99	$C_{34}H_{51}N_5O_7S$	674	58
6b	0	*i*-Bu (*R*)	OEt	H	64	>80 (gradual)	$C_{38}H_{59}N_5O_7S\cdot0.4C_2H_6O$[c,d]	730.4217[e]	288
6c	0	*i*-Bu (*S*)	OEt	H	59	>82 (gradual)	$C_{38}H_{59}N_5O_7S\cdot0.67H_2O$	730	305
11a	0	H		I	42	185–187 dec	$C_{38}H_{53}N_7O_6S\cdot0.5H_2O$	736	92
11b	0	H	$NH(CH_2)_2NMe_2$	J	85	160–164	$C_{36}H_{57}N_7O_6S\cdot0.5H_2O$	716	161
11c	0	H		I	76	>155 dec (gradual)	$C_{38}H_{59}N_7O_7S$	758.4268[f]	40
11d	0	H		I	32	>140 dec (gradual)	$C_{39}H_{59}N_7O_6S$	754.4376[g]	29
11e	0	H		K	68	>106 dec (gradual)	$C_{37}H_{57}N_7O_6S\cdot H_2O$	728	29
11f	1	H		J	24	188–190 dec	$C_{38}H_{59}N_7O_8S\cdot1.75H_2O$	774	19
11g	2	H		J	43	>110 dec (gradual)	$C_{38}H_{59}N_7O_9S\cdot2H_2O$[c]	790.4207[h]	9.4
11h	2	H		L	28	209–210 dec	$C_{37}H_{57}N_7O_8S$	760.4042[i]	20
11i	2	H		J	46	139–140 dec	$C_{44}H_{63}N_7O_8S$	850.4600[j]	34
11j	2	H		K	24	219–220 dec	$C_{39}H_{59}N_7O_9S$	802.4181[k]	4.2
11k	0	*i*-Bu (*RS*)		L	47[l]	105–106	$C_{42}H_{67}N_7O_7S\cdot0.25CH_2Cl_2$	814	73
11l	1	*i*-Bu (*RS*)		L	44	93–95 dec	$C_{42}H_{67}N_7O_8S$	830.4905[m]	88
11m	2	*i*-Bu (*RS*)		L	29	>100 (gradual)	$C_{42}H_{67}N_7O_9S$	846.4831[n]	77
19	2	H		M	42	>120 (gradual)	$C_{38}H_{60}N_6O_9S_2\cdot H_2O$	809	7.2
20[p]									31

[a] See Experimental Section for description of general methods. [b] Analyses for C, H, and N within ±0.4% except where characterized by high resolution mass spectrum or as otherwise indicated. [c] Characterized by elemental analysis as well as high-resolution FAB-MS. [d] Solvated with EtOH. [e] Calcd for $C_{38}H_{60}N_5O_7S$ (M + H)+: 730.4213. [f] Calcd for $C_{38}H_{60}N_7O_7S$ (M + H)+: 758.4276. [g] Calcd for $C_{39}H_{60}N_7O_6S$ (M + H)+: 754.4327. [h] Calcd for $C_{38}H_{60}N_7O_9S$ (M + H)+: 790.4174. [i] Calcd for $C_{37}H_{58}N_7O_8S$ (M + H)+: 760.4068. [j] Calcd for $C_{44}H_{64}N_7O_8S$ (M + H)+: 850.4538. [k] Calcd for $C_{39}H_{60}N_7O_9S$ (M + H)+: 802.4174. [l] From **4c**; $[\alpha]^{20}_D$ −17.0° (*c* 2, CH_2Cl_2). Similarly obtained in 52% yield from **4b** with identical NMR, $[\alpha]^{20}_D$ −16.5° (*c* 2, CH_2Cl_2). [m] Calcd for $C_{42}H_{68}N_7O_8S$ (M + H)+: 830.4850. [n] Calcd for $C_{42}H_{68}N_7O_9S$ (M + H)+: 846.4800. [o] BocPhe replaced by (*S*)-2-benzyl-3-(*tert*-butylsulfonyl)propionyl. [p] Data from ref 14.

group but led to a mixture of the free acid **5** and the ester **6a**. In addition, the 7-membered lactam **7** was isolated as a byproduct from this reaction. For the branched esters **4b,c** it was essential to avoid very basic conditions for the deprotection in order not to epimerize the mercapto ester. The use of the weakly basic hydrazine hydrate at room temperature effectively deblocked the imidazole and left the ester intact, affording **6b,c**.

Treatment of **3a** with 3 equiv of *m*-chloroperbenzoic acid furnished the sulfone **8**, which was further converted to the blocked dipeptidyl derivative **9a** as above (Scheme II). The oxidation of the thioether could also be accomplished after coupling with BocPhe(N^{im}-BocHis)-OH, as indicated in the conversion of **4b** to **9b**. Similarly, the use of 1.1 equiv of *m*-chloroperbenzoic acid transformed **4a,c** to the corresponding sulfoxides **10a,b**. No separation of sulfoxide

Scheme III[a]



[a] (a) HNR"R"', Δ; (b) HCl/MeOH; (c) BocPheHis(N^{im}-Boc)-OH, BOP, Et_3N; (d) NH_3/MeOH; (e) MCPBA (3 equiv).

diastereomers was feasible at this stage or subsequently. Several amides **11** were obtained by direct reaction of **4**, **9**, and **10** with primary or secondary amines. Under these conditions, the imidazole Boc group was removed simultaneously. In the case of **11k–m** the chirality α to the ester was lost in the process. This was shown definitively for **11k**, as the use of either the *R* (**4b**) or *S* (**4c**) mercapto ester precursor yielded material with the same optical rotation. An alternative route to amides **11a,c,d** entailed coupling the acid **5** with the appropriate amine in the presence of BOP reagent.

The C-terminal amide linkage could also be formed at an early stage of the synthesis (Scheme III). Amides **12a,b** were made by direct reaction of **3a** with amines. Conversion to the fully protected dipeptidyl derivatives **13a,b** proceeded as in Scheme I. The 3-aminoquinuclidine derivative **13b** yielded the trioxo species **14** upon oxidation with 3 equiv of *m*-chloroperbenzoic acid. Deprotection of **13a** and **14** with methanolic ammonia gave the target products **11e** and **11j**, respectively.

We were also interested in the incorporation of the *tert*-butyl sulfone BocPhe mimic developed by the Ciba-Geigy group[22] and used to good effect in the potent renin inhibitor CGP 38 560.[23] This offered the possibility of providing increased proteolytic stability and enhanced water solubility. (*S*)-(+)-2-Benzyl-3-(*tert*-butylsulfonyl)-propionic acid (**15**)[22] was converted to its *N*-hydroxysuccinimide ester **16** in the presence of DCC (Scheme IV). The sulfone ester **8** was treated with neat *N*-(2-aminoethyl)morpholine to yield **17**, which upon deblocking with TFA and coupling with N^{α},N^{im}-bis(*tert*-butoxycarbonyl)-L-histidine[24,25] gave **18**. Full deprotection with TFA followed by treatment with **16** in the presence of triethylamine provided **19**.

Biological Results and Discussion

In Vitro Renin Inhibition. Compounds of structures **5**, **6**, **11**, and **19** were evaluated against human plasma renin at pH 7.4 (Table III). Although the mercaptoacetic acid derivative **5** was a weak inhibitor, the corresponding ethyl ester **6a** was approximately 15-fold more potent, indicating a possible hydrophobic binding effect. However, introduction of an isobutyl side chain α to the carbonyl of **6a** led to about a 5-fold decrease in potency for **6b,c**, which was essentially independent of stereochemistry. Some of the N-substituted mercaptoacetamide derivatives **11a–e**, including those containing a solubilizing tertiary amine group, were relatively potent inhibitors. Although the 2-(dimethylamino)ethyl amide **11b** was not particularly effective, the more hydrophobic analogues **11c–e** had IC_{50} values in the 30–40 nM range, similar to **20**, the most

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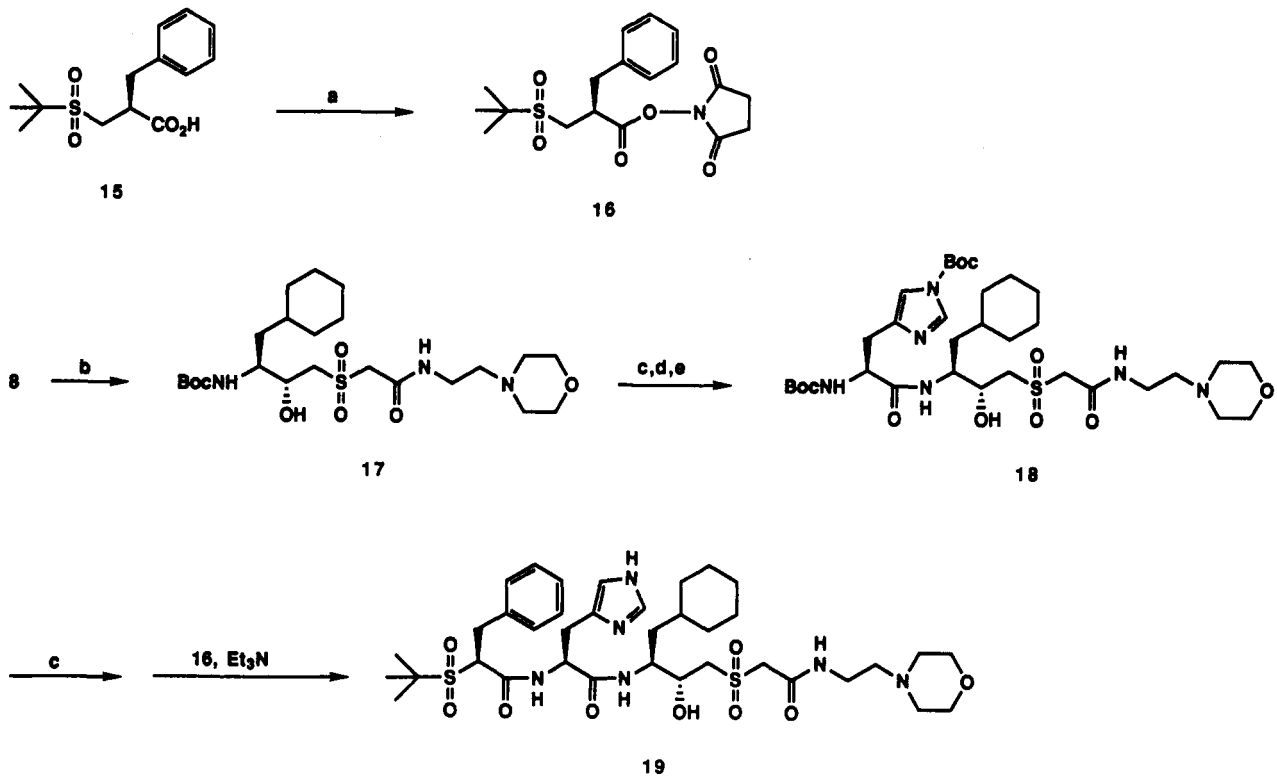
(23) Wood, J. M.; Criscione, L.; de Gasparo, M.; Bühlmayer, P.; Rüeger, H.; Stanton, J. L.; Jupp, R. A.; Kay, J. CGP 38 560: Orally Active, Low-Molecular-Weight Renin Inhibitor with High Potency and Specificity. *J. Cardiovasc. Pharmacol.* **1989**, *14*, 221–226.

(24) Keller, O.; Keller, W. E.; Van Look, G.; Wersin, G. *tert*-Butoxycarbonylation of Amino Acids and Their Derivatives: N-*tert*-Butoxycarbonyl-L-phenylalanine (L-Phenylalanine, N-[(1,1-Dimethylethoxy)carbonyl]-). *Org. Synth.* **1985**, *63*, 160–170.

(25) Le Nguyen, D.; Seyer, R.; Heitz, A.; Castro, B. Renin Substrates. Part 1. Liquid-phase Synthesis of the Equine Sequence with Benzotriazolyloxytris(dimethylamino)-phosphonium Hexafluorophosphate (BOP). *J. Chem. Soc., Perkin Trans. 1* **1985**, 1025–1031.

Scheme IV[a]



[a] (a) N-hydroxysuccinimide, DCC, MeCN; (b) 4-(2-aminoethyl)morpholine; (c) TFA; (d) aqueous Na_2CO_3; (e) $(Boc)_2$His-OH, BOP, Et_3N.

potent of our earlier series of inhibitors containing heterocyclylthio C-termini.[14] Compared to the sulfide **11c**, the sufoxide **11f** and sulfone **11g** analogues were progressively more potent. The sulfone **11h** was modestly more potent than the corresponding thioether **11e**. The most active compound in the series was **11j**, which contains both the sulfone and quinuclidine *N*-oxide moieties. With an IC_{50} value of 4.2 nM, **11j** was about 7-fold more potent than its unoxidized parent **11d**. The epimerized, α-branched amides **11k–m** were only fair inhibitors (IC_{50} 70–90 nM), and there was little difference in activity among thioether, sufoxide, and sulfone. As in the case of the esters **6b,c**, the presence of the isobutyl substituent α to the carbonyl group was clearly unfavorable. Replacement of the BocPhe moiety in **11g** by (*S*)-2-benzyl-3-(*tert*-butylsulfonyl)propionyl[22] in **19** yielded an inhibitor of at least equal potency (IC_{50} 7.2 nM).

In Vivo Pharmacology. Selected compounds were administered intravenously to conscious, sodium-deficient rhesus monkeys in order to determine their effects on in vivo plasma renin activity (PRA), blood pressure, and heart rate (Table IV). At doses (0.06–1 mg/kg) proportional to in vitro inhibitory potency, each compound reduced mean arterial pressure by 15–25 mmHg. This was comparable to **20**, the most active of our series of analogues with C-termini derived from mercaptoheterocycles.[14] As in that series, the compounds exhibited a short half-life of hypotensive activity, <10 min in each case (borderline for **11e**). At the time of peak hypotensive effect, PRA was inhibited by 86–94%. Two analogues (**11d** and **19**) still inhibited PRA by approximately 60% after 45 min. Compounds **11e** (9 mg/kg) and **19** (10 mg/kg) were tested orally in this monkey model and found to be inactive, as had been the case for **20** (50 mg/kg).[14]

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 activity, % inhibn[d]
6a	0.63	−15	<10	−23	87
11a	0.99	−16	<10	−26	88
11c	0.43	−20.5	<10	−26	90
11d	0.26	−20	<10	−18	93[e]
11e	0.31	−25	<10, 14	+4	86
11g	0.10	−15	<10	−18	88
19	0.063	−20.5	<10	−13	94[f]
20[g]	0.34	−22	<10	+1	95

[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. [e] 61% inhibition at 45 min. [f] 59% inhibition at 45 min. [g] Data from ref 14.

Conclusions

Epoxide adducts of α-mercaptoalkanoate esters have been elaborated into transition-state analogue renin inhibitors. Several of the resulting C-terminal amide derivatives containing solubilizing tertiary amine groups proved to be potent inhibitors of human plasma renin, with IC_{50} values as low as 4.2 nM for **11j** and 7.2 nM for **19**, a compound which contains a single aminoacyl residue. Where direct comparisons could be made in the $-SCH_2CO-$ series, the order of potency followed the trend sulfone > sulfoxide > sulfide. The hope of enhancing binding affinity by introduction of an alkyl branch at the mercaptoacetyl moiety was not realized. An isobutyl substituent at this position is apparently unable to achieve favorable contact with the presumed hydrophobic pocket at the $S_{1'}$ site[7b] of renin responsible for binding the Val[11] side chain of angiotensinogen. In fact, the presence of such a substituent was unfavorable regardless of stereochemistry. Some of the more potent members of this series strongly inhibited plasma renin activity and produced significant but short-lived reduction in blood pressure when administered intravenously to sodium-deficient monkeys. Oral bioavailability could not be demonstrated,

even for analogue 19, which contained a stable BocPhe replacement. Subsequent to the completion of this work, some similar renin inhibitors have been described in a recent patent application from the E. Merck group.[26]

Experimental Section

Melting points (uncorrected) were determined in open capillary tubes with a Thomas-Hoover apparatus. ^{1}H 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 designations "br" or "v br" used alone indicate a broad or very 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.

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.

Ethyl (*R*)-2-Mercapto-4-methylpentanoate (2b). A solution of 5.70 g (38.5 mmol) of (*R*)-2-mercapto-4-methylpentanoic acid, $[\alpha]^{20}_D$ +24.5° (*c* 2.9, Et_2O), prepared from L-leucine by a literature route,[18,19] and 173 mg (0.91 mmol) of *p*-toluenesulfonic acid monohydrate in 10 mL of absolute EtOH and 10 mL of $CHCl_3$ was stirred at reflux under a Dean–Stark trap for 4 h. The cooled solution was diluted with an equal volume of $CHCl_3$ and washed successively with H_2O, saturated aqueous $NaHCO_3$, and H_2O. The organic phase was dried over $MgSO_4$, filtered, and concentrated in vacuo to give 4.02 g (59%) of **2b** as an oil: $[\alpha]^{20}_D$ +19.1° (*c* 2, Et_2O); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.92 (t, *J* = 7 Hz, 6 H), 1.28 (t, *J* = 7 Hz, 3 H), 1.5–1.9 (complex m, 3 H), 2.02 (d, *J* = 9 Hz, 1 H), 3.38 (m, 1 H), 4.21 (q, *J* = 7 Hz, 2 H). Anal. ($C_8H_{16}O_2S$) C, H.

Ethyl (*S*)-2-Mercapto-4-methylpentanoate (2c). By the procedure used for **2b**, (*S*)-2-mercapto-4-methylpentanoic acid,[18,19] $[\alpha]^{20}_D$ −25.3° (*c* 2.2, Et_2O), was converted in 81% yield to **2c** as an oil: $[\alpha]^{20}_D$ −19.0° (*c* 2, Et_2O); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.90 (t, *J* = 7 Hz, 6 H), 1.28 (t, *J* = 7 Hz, 3 H), 1.5–1.9 (complex m, 3 H), 2.02 (d, *J* = 9 Hz, 1 H), 3.38 (m, 1 H), 4.20 (q, *J* = 7 Hz, 2 H); EI-MS *m/e* 176 (M^+). Anal. ($C_8H_{16}O_2S$) C, H.

Method A. Ethyl 2-[[(2*R*,3*S*)-3-[(*tert*-Butoxycarbonyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]acetate (3a). A solution of 1.41 g (5.25 mmol) of **1**, 0.55 mL (0.60 g, 5 mmol) of ethyl 2-mercaptoacetate, and 0.70 mL (0.51 g, 5 mmol) of triethylamine in 15 mL of absolute EtOH was stirred overnight under N_2. The resulting solution was concentrated in vacuo at room temperature. Chromatography of the golden-yellow residual oil on a column of silica gel (60 × 3.5 cm) packed in hexane (elution with 7:1 and then 4:1 hexane–EtOAc) provided 1.64 g (84%) of colorless, viscous oil: $[\alpha]^{20}_D$ −43.8° (*c* 2, $CHCl_3$); TLC in 4:1 hexane–EtOAc; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.7–1.9 (complex m, 25 H) including 1.28 (t, *J* = 7 Hz, 3 H) and 1.32 (s, 9 H), 2.64 (dd, *J* = 9.5 Hz, 14 Hz, 1 H), 2.86 (dd, *J* = 3.5 Hz, 14 Hz, 1 H), 3.29 (d, *J* = 2 Hz, 2 H), 3.67 (br m, 2 H), 4.22 (q, *J* = 7 Hz, 2 H), 4.66 (br d, *J* = 9 Hz, 1 H); FAB-MS *m/e* 390 $(M + H)^+$. Anal. ($C_{19}H_{35}NO_5S$) C, H, N.

Method B. Ethyl 2-[[(2*R*,3*S*)-3-[(*tert*-Butoxycarbonyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]sulfonyl]acetate (8). A solution of 389 mg (1 mmol) of **3a** in 4 mL of CH_2Cl_2 stirred at room temperature was treated with 518 mg (2.4 mmol based on 80% purity) of *m*-chloroperbenzoic acid. A thick precipitate soon separated. After 1.5 h the mixture was partitioned between 50 mL of EtOAc and 50 mL of saturated aqueous Na_2CO_3. The organic layer was washed with an additional portion of saturated Na_2CO_3 solution, then dried (Na_2SO_4), filtered, and concentrated to yield 373 mg (89%) of white solid: mp 102–104 °C; TLC in 95:5 CH_2Cl_2–MeOH; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.75–1.85 (complex m, 25 H) including 1.33 (t, *J* = 7 Hz, 3 H) and 1.45 (s, 9 H), 3.29 (d, *J* = 15 Hz, 1 H), 3.49 (br, 1 H), 3.64 (dd, *J* = 10.5 Hz, 15 Hz, 2 H), 4.01 (d, *J* = 15 Hz, 1 H), 4.25 (d, *J* = 15 Hz, 1 H) overlapping 4.27 (q, *J* = 7 Hz, 2 H), 4.68 (d, *J* = 9 Hz, 1 H); FAB-MS *m/e* 422 $(M + H)^+$. Anal. ($C_{19}H_{35}NO_7S$) C, H, N.

Method C. 3-[2-[[(2*R*,3*S*)-3-[(*tert*-Butoxycarbonyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]acetamido]quinuclidine (12b). To 1.96 g (5.04 mmol) of **3a** was added a solution of 3.7 g (29.4 mmol) of 3-aminoquinuclidine (freshly generated from the dihydrochloride by partitioning between CH_2Cl_2 and 2.5 N NaOH followed by Na_2SO_4-drying and concentration of the organic fractions) in 9 mL of EtOH. The resulting yellow solution was stirred under N_2 at 90 °C for 3 h and then concentrated in vacuo. The residual oil was partitioned between 150 mL of EtOAc and 100 mL of saturated aqueous NH_4Cl. The organic layer was washed again with saturated NH_4Cl and finally with saturated NaCl solution. The EtOAc phase was dried over $MgSO_4$, filtered, and concentrated in vacuo to afford 1.90 g (75%) of a white solid: mp 105–106 °C; $[\alpha]^{20}_D$ −20.7° (*c* 2, $CHCl_3$); TLC in 80:20:2 $CHCl_3$–MeOH-concentrated NH_4OH; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.75–1.0 (complex m, 2 H), 1.1–1.75 (complex m, 19 H) including 1.41 (s, 9 H), 1.81 (br d, *J* = 13 Hz, 1 H), 1.91 (br m, 2 H), 2.1–2.4 (complex m, 2 H), 2.5–3.8 (complex m, 12 H) including 3.30 (t, *J* = 7 Hz, 1 H), 4.25 (br m, 1 H), 4.75 (t, *J* = 10 Hz, 1 H), 8.25–8.45 (br m, 1 H); FAB-MS *m/e* 470 $(M + H)^+$. Anal. ($C_{24}H_{43}N_3O_4S \cdot 1.75H_2O$) C, H, N.

Method D. 4-[2-[2-[[(2*R*,3*S*)-3-[(*tert*-Butoxycarbonyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]acetamido]ethyl]morpholine (17). A mixture of 1.98 g (4.7 mmol) of **3a** and 2.3 mL of 4-(2-aminoethyl)morpholine was stirred under N_2 at room temperature for 2 days. The light amber syrup was worked up as in method C to yield 2.19 g (92%) of a nearly colorless, stiff foam: $[\alpha]^{20}_D$ −16.9° (*c* 2, $CHCl_3$); TLC in 9:1 $CHCl_3$–MeOH; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.75–1.05 (complex m, 2 H), 1.1–1.9 (complex m, 20 H) including 1.44 (s, 9 H), 2.4–2.6 (m, 6 H), 3.2–3.7 (complex m, 4 H), 4.73 (t, *J* = 5 Hz, 4 H), 4.00 (AB q, *J* = 14 Hz, 2 H), 4.21 (d, *J* = 10 Hz, 1 H), 4.60 (d, *J* = 9 Hz, 1 H), 7.09 (br m, 1 H); FAB-MS *m/e* 506 $(M + H)^+$. Anal. ($C_{23}H_{43}N_3O_7S$) C, H, N.

Method E. Ethyl 2-[[(2*R*,3*S*)-3-[(Boc-L-phenylalanyl-N^{im}-Boc-L-histidyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]acetate (4a). To 1.56 g (4 mmol) of **3a** was added 15 mL of absolute EtOH, freshly saturated with HCl gas at room temperature. The resulting solution was stirred under a drying tube. Gas evolution, initially vigorous, subsided within a few minutes. After about 1 h, the solution was evaporated under a stream of N_2 while being stirred at 40 °C. The residue was dried in vacuo (oil pump, KOH trap) and then dissolved in 20 mL of dry CH_2Cl_2, and treated with 1.11 mL (808 mg, 8 mmol) of triethylamine. The solution was reconcentrated and again dried in vacuo (oil pump) with only slight warming. The residual gum was treated successively with 1.89 g (4 mmol) of Boc-L-phenylalanyl-N^{im}-Boc-L-histidine,[20] 1.77 g (4 mmol) of (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent), 25 mL of dry CH_2Cl_2, and 0.56 mL (404 mg, 4 mmol) of triethylamine. The resulting solution was stirred under N_2 at room temperature for 17 h and then concentrated in vacuo at room temperature. The oily residue was dissolved in EtOAc (125 mL), filtered, and washed successively with 1 × 25 mL of H_2O, 3 × 25 mL of saturated aqueous $NaHCO_3$, and finally 1 × 25 mL of saturated aqueous NaCl. The organic phase was dried ($MgSO_4$), filtered, and concentrated. Chromatography of the residual oil on a column of silica gel (64 × 3.5 cm; elution with 99:1 and then 98:2 $CHCl_3$–iPrOH) yielded 1.45 g (47%) of a colorless, hard glass, which was pulverized to a powder: mp >60 °C (gradual); TLC in 97:3 $CHCl_3$–iPrOH; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.73 (br m, 1 H), 0.86 (br m, 1 H), 1.0–1.7 (complex m, 31 H) including 1.17 (t, *J* = 7 Hz, 3 H), 1.27 (s, 9 H), and 1.53 (s, 9 H), 2.40 (d, *J* = 6.7 Hz, 2 H), 2.5–3.0 (complex m, 4 H), 3.4–3.7 (complex m, 2 H), 3.93 (br m, 1 H), 4.0–4.2 (complex m, 3 H)

(26) Raddatz, P.; Minck, K.-O.; Schmitges, C. J. European Patent Appl. 464,517, 1992.

including 4.06 (q, J = 7 Hz, 2 H), 4.55 (q, J = 7 Hz, 1 H), 5.02 (d, J = 5.8 Hz, 1 H), 7.01 (d, J = 8.3 Hz, 1 H), 7.1–7.3 (complex m, 6 H), 7.37 (d, J = 9.4 Hz, 1 H), 8.06 (s, 1 H), 8.12 (d, J = 8.0 Hz, 1 H); FAB-MS m/e 774 $(M + H)^+$. Anal. ($C_{39}H_{59}N_5O_9S \cdot 0.5H_2O$) C, H, N.

Method F. Ethyl 2-[[(2*R*,3*S*)-3-[(Boc-L-phenylalanyl-N^{im}-Boc-L-histidyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]sulfinyl]acetate (10a). A solution of 150 mg (0.194 mmol) of **4a** and 44 mg (approximately 0.21 mmol) of 80–85% *m*-chloroperbenzoic acid in 3 mL of CH_2Cl_2 was stirred at room temperature for 4.5 h and then partitioned between EtOAc (20 mL) and saturated aqueous Na_2CO_3 (20 mL). The EtOAc layer was washed twice more with saturated Na_2CO_3, then dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was chromatographed on a column of silica gel (37 × 2.1 cm; elution with a gradient of 2–8% iPrOH in CH_2Cl_2) to give an oil, which solidified upon drying in vacuo (oil pump). There was obtained 104 mg (68%) of **10a**: mp 140–142 °C dec; TLC in 9:1 CH_2Cl_2–MeOH; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.7–1.8 (complex m, 34 H) including 1.31 (dt, J = 3 Hz, 7 Hz, 3 H), 1.38 (s, 9 H), and 1.60 (s, 9 H), 2.8–3.2 (complex m, 4 H), 3.24 (m, 2 H), 3.70 (d, J = 13 Hz, 1 H), 3.86 (m, 1 H), 3.93 (d, J = 13 Hz, 1 H), 4.04 (m, 2 H), 4.15–4.3 (complex m, 3 H), 4.57 (m, 1 H), 4.98 (m, 1 H), 6.67 (m, 1 H), 7.18 (s, 1 H), 7.2–7.4 (m, 5 H), 7.95 (s, 1 H), 8.53 (d, J = 7 Hz, 1 H), 8.64 (d, J = 7 Hz, 1 H); FAB-MS m/e 790 $(M + H)^+$. Anal. ($C_{39}H_{59}N_5O_{10}S \cdot 0.67H_2O$) C, H; N: calcd, 8.73; found, 8.01.

Method G. 2-[[(2*R*,3*S*)-3-[(Boc-L-phenylalanyl-L-histidyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]acetic Acid (5), Its Ethyl Ester (6a), and (5*S*,6*R*)-5-(Cyclohexylmethyl)-6-hydroxy-4,5,6,7-tetrahydro-1,4-thiazepin-3(2*H*)-one (7). A mixture of 1.39 g (1.8 mmol) of **4a**, 1.38 g (10 mmol) of pulverized anhydrous K_2CO_3, and 70 mL of absolute EtOH was stirred vigorously at room temperature for 3 h and then concentrated in vacuo (oil pump) at ≤20 °C. The residue was treated with 100 mL of EtOAc, and the mixture was stirred vigorously for 15 min. The filtered solution was shaken with 20 mL of H_2O. The gelatinous aqueous layer was separated and acidified with glacial AcOH, giving a voluminous precipitate, which was collected on a filter and washed with H_2O. Drying in vacuo at 60 °C in the presence of P_2O_5 afforded 622 mg (53%) of **5** as a white powder: mp 178–179 °C (preliminary softening); TLC in 80:20:2 $CHCl_3$–MeOH–H_2O containing a trace of AcOH; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.73 (br m, 1 H), 0.86 (br m, 1 H), 1.0–1.4 (complex m, 15 H) including 1.28 (s, 9 H), 1.56 (br m, 4 H), 1.74 (br d, J = 12 Hz, 1 H), 2.42 (m, 2 H), 2.71 (dd, J = 11 Hz, 14 Hz, 1 H), 2.84 (dd, J = 6.7 Hz, 14.7 Hz, 1 H), 2.94 (m, 2 H), 3.21 (AB q, J = 15 Hz, 2 H), 3.48 (m, 1 H), 3.93 (br m, 1 H), 4.13 (m, 1 H), 4.47 (q, J = 7 Hz, 1 H), 6.83 (s, 1 H), 7.05 (d, J = 8.2 Hz, 1 H), 7.17 (m, 1 H), 7.25 (m, 4 H), 7.30 (d, J = 9.2 Hz, 1 H), 7.55 (s, 1 H), 8.19 (d, J = 8.0 Hz, 1 H); FAB-MS m/e 646 $(M + H)^+$. Anal. ($C_{32}H_{47}N_5O_7S \cdot 0.25H_2O$) C, H, N.

The EtOAc phase from the above separation was washed further with 2 × 20 mL of H_2O, then dried (Na_2SO_4), filtered, and concentrated in vacuo. The glassy residue was chromatographed on a column of silica gel (48 × 2.4 cm). Initially eluted with 97:3 CH_2Cl_2–MeOH was a material isolated as a gum, but which solidified upon trituration with petroleum ether to give 118 mg of **7** as a cream-colored powder: mp 149–151 °C (remained cloudy up to 159 °C); TLC in 95:5 $CHCl_3$–MeOH; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.8–1.0 (complex m, 2 H), 1.1–1.7 (complex m, 11 H), 2.91 (dd, J = 4.7 Hz, 14.5 Hz, 1 H), 3.04 (apparent t, J = 15 Hz, 2 H), 3.34 (d, J = 14.2 Hz, 1 H), 3.50 (m, 1 H), 3.73 (m, 1 H), 5.17 (m, 1 H); FAB-MS m/e 244 $(M + H)^+$. Anal. ($C_{12}H_{21}NO_2S$) C, H, N.

Further elution of the column with 96:4 CH_2Cl_2–MeOH yielded 265 mg (22%) of **6a** as a white powder: mp 97–99 °C; TLC in 9:1 $CHCl_3$–MeOH; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.74 (br m, 1 H), 0.87 (br m, 1 H), 1.0–1.6 (complex m, 18 H) including 1.18 (t, J = 7.1 Hz, 3 H) and 1.28 (s, 9 H), 1.5–1.6 (m, 4 H), 1.75 (br d, J = 13 Hz), 2.41 (m, 2 H), 2.70 (m, 1 H), 2.91 (m, 2 H), 3.01 (dd, J = 5.9 Hz, 11.3 Hz, 1 H), 3.30 (m, 2 H), 3.50 (m, 1 H), 3.94 (br m, 1 H), 4.07 (q, J = 7.1 Hz, 2 H), 4.13 (m, 1 H), 4.56 (m, 1 H), 7.02 (d, J = 8.3 Hz, 1 H), 7.09 (s, 1 H), 7.18 (m, 1 H), 7.2–7.3 (m, 5 H), 7.36 (d, J = 9.4 Hz, 1 H), 8.29 (d, J = 7.7 Hz, 1 H); FAB-MS m/e 674 $(M + H)^+$. Anal. ($C_{34}H_{51}N_5O_7S$) C, H, N.

Method H. Ethyl (2*S*)-2-[[(2*R*,3*S*)-3-[(Boc-L-phenylalanyl-L-histidyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]-4-methylpentanoate (6c). A solution of 86 mg (0.1 mmol) of **4c** in 4 drops of EtOH was treated with 2 drops of hydrazine hydrate and stirred under N_2 at room temperature for 1 h. The solution was partitioned between ethyl acetate (50 mL) and 0.2 N HCl (50 mL). The EtOAc phase was washed with 50 mL of saturated NaCl solution, then dried over $MgSO_4$, filtered, and concentrated to dryness. Chromatography of the residue on a column of silica gel (18 × 1.6 cm; elution with 97:3 and then 95:5 CH_2Cl_2–EtOH) provided 44 mg (59%) of **6c** as a solid: mp >82 °C (gradual); TLC in 9:1 CH_2Cl_2–EtOH; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.7–0.95 (complex m, 8 H) including 0.85, 0.86 (overlapping d, J = 7 Hz, each 3 H), 1.0–1.7 (complex m, 24 H) including 1.18 (t, J = 7.1 Hz, 3 H) and 1.28 (s, 9 H), 1.74 (br d, J = 12 Hz, 1 H), 2.43 (m, 2 H), 2.69 (dd, J = 11 Hz, 14 Hz, 1 H), 2.91 (m, 2 H), 3.07 (dd, J = 5.5 Hz, 15.2 Hz, 1 H), 3.47 (m, 1 H), 3.94 (br m, 1 H), 4.0–4.2 (complex m, 3 H) including 4.08 (q, J = 7.1 Hz, 2 H), 4.61 (m, 1 H), 7.01 (d, J = 8.4 Hz, 1 H), 7.1–7.3 (complex m, 6 H), 7.39 (d, J = 9.2 Hz, 1 H), 8.32 (d, J = 8.4 Hz, 1 H), 8.64 (br s, 1 H); FAB-MS m/e 730 $(M + H)^+$. Anal. ($C_{38}H_{59}N_5O_7S \cdot 0.67H_2O$) C, H, N.

Method I. 4-[[2-[[(2*R*,3*S*)-3-[(Boc-L-phenylalanyl-L-histidyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]acetamido]methyl]pyridine (11a). A gelatinous suspension of 65 mg (0.1 mmol) of **5** and 49 mg (0.11 mmol) of (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) in 1.5 mL of dry CH_2Cl_2 was stirred at room temperature as 15.3 μL (11.1 mg, 0.11 mmol) of triethylamine was added, resulting in a nearly clear solution. To this was added 11.2 μL (11.9 mg, 0.11 mmol) of 4-(aminomethyl)pyridine. Stirring was continued at room temperature in a stoppered flask. After approximately 15 h, the gelatinous mixture was rotary-evaporated. Thorough trituration of the residue with ether gave a solid, which was collected on a filter and washed successively with ether, H_2O, half-saturated aqueous $NaHCO_3$ (accompanied by vigorous trituration), H_2O, and finally ether. The solid was dissolved in MeOH and filtered. After evaporation of the filtrate, the residue was leached with 10 mL of hot acetone containing 2% triethylamine and then allowed to stand at room temperature. The solid was collected on a filter and washed with 98:2 acetone–triethylamine, followed by ether, to yield 31 mg (42%) of white powder: mp 185–187 °C dec (slight preliminary softening); TLC in 80:20:2 $CHCl_3$–MeOH–concentrated NH_4OH; ^1H NMR (DMSO-d_6, 200 MHz) δ 0.6–1.9 (complex m, 22 H) including 1.28 (s, 9 H) and 1.75 (br d, J = 12 Hz, 1 H), 2.6–3.1 (complex m, 4 H), 3.22 (s, 2 H), 3.95 (br m, 1 H), 4.15 (br m, 1 H), 4.32 (d, J = 6.5 Hz, 2 H, collapsing to s upon D_2O exchange), 4.48 (br m, 1 H), 5.28 (v br, 1 H), 6.83 (s, 1 H), 7.06 (d, J = 8 Hz, 1 H), 7.1–7.4 (m, 8 H), 7.52 (s, 1 H), 8.20 (d, J = 8 Hz, 1 H), 8.49 (d, J = 5.5 Hz, 2 H), 8.62 (br m, 1 H); FAB-MS m/e 736 $(M + H)^+$. Anal. ($C_{38}H_{53}N_7O_6S \cdot 0.5H_2O$) C, H, N.

Method J. *N*-[2-[[(2*R*,3*S*)-3-[(Boc-L-phenylalanyl-L-histidyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]-acetyl]-*N'*,*N'*-dimethylethylenediamine (11b). To 74 mg (0.11 mmol) of **6a** was added 100 μL of *N*,*N*-dimethylethylenediamine. The mixture was stirred at room temperature in a stoppered flask and became homogeneous within a few minutes. After 18 h, the reaction mixture, which had gelled, was triturated thoroughly with hot ether. The resulting solid was collected on a filter and washed several times with ether, triturating thoroughly each time, to give 68 mg (85%) of white powder: mp 160–164 °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), 1.0–1.4 (complex m, 15 H) including 1.28 (s, 9 H), 1.57 (br m, 4 H), 1.74 (br d, J = 10 Hz, 1 H), 2.12 (s, 6 H), 2.27 (t, J = 6.6 Hz, 2 H), 2.3–2.45 (m, 2 H), 2.71 (dd, J = 11 Hz, 14 Hz, 1 H), 2.8–3.0 (complex m, 3 H), 3.05–3.2 (complex m, 4 H), 3.46 (br m, 1 H), 3.91 (br m, 1 H), 4.12 (br m, 1 H), 4.45 (br m, 1 H), 5.39 (v br, 1 H), 6.83 (br m, 1 H), 7.07 (br m, 1 H), 7.1–7.3 (complex m, 6 H), 7.51 (s, 1 H), 7.91 (br m, 1 H), 8.21 (d, J = 7.8 Hz, 1 H); FAB-MS m/e 716 $(M + H)^+$. Anal. ($C_{36}H_{57}N_7O_6S \cdot 0.5H_2O$) C, H, N.

Method K. 1-[2-[[(2*R*,3*S*)-3-[(Boc-L-phenylalanyl-L-histidyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]-acetyl]-4-methylpiperazine (11e). A filtered solution of 245 mg (0.3 mmol) of **13a** in 4 mL of MeOH was treated with a stream

of NH_3 gas for several minutes. The solution was stirred at room temperature in a stoppered flask for 3 h and then evaporated under a stream of N_2 at 35 °C. Trituration of the residue with hot ether gave a solid, which was collected on a filter and washed with ether. This material was dried to yield 153 mg (68%) of **11e** as a white solid: mp >106 °C; TLC in 90:10:1 $CHCl_3$–MeOH–concentrated NH_4OH; ^1H NMR (DMSO-d_6, 200 MHz) δ 0.6–1.9 (complex m, 22 H) including 1.27 (s, 9 H), 2.1–2.5 (complex m, 11 H) including 2.16 (s, 3 H), 2.6–3.0 (complex m, 4 H), 3.1–3.6 (complex m, 5 H, partially obscured by H_2O peak), 3.93 (br m, 1 H), 4.12 (br m, 1 H), 4.45 (br m, 1 H), 5.15 (v br, 1 H), 6.81 (s, 1 H), 7.03 (d, J = 9 Hz, 1 H), 7.1–7.3 (m, 6 H), 7.50 (s, 1 H), 8.18 (d, J = 8 Hz, 1 H); FAB-MS m/e 728 $(M + H)^+$. Anal. ($C_{37}H_{57}N_7O_6S·H_2O$) C, H, N.

Method L. 4-[2-[[(2*RS*)-[[(2*R*,3*S*)-3-[(Boc-L-phenylalanyl-L-histidyl)amino]-4-cyclohexyl-2-hydroxy-1-butyl]thio]-4-methylpentanoyl]amino]ethyl]morpholine (11k). A solution of 105 mg (0.125 mmol) of **4c** in 4 mL of 4-(2-aminoethyl)morpholine was stirred under N_2 at 80 °C for 4 days. The cooled solution was diluted with 40 mL of EtOAc and washed with 40 mL of saturated aqueous NH_4Cl followed by 40 mL of saturated NaCl solution. The organic phase was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was chromatographed on a silica gel column (21 × 2 cm; elution with a gradient of 4–8% MeOH in CH_2Cl_2) to afford 49 mg (47%) of white solid: mp 105–106 °C; $[\alpha]^{20}_D$ −17.0° (*c* 2, CH_2Cl_2); TLC in 9:1 $CHCl_3$–MeOH; ^1H NMR (DMSO-d_6, 200 MHz) δ 0.7–1.9 (complex m, 31 H) including 0.88 (overlapping d, J = 7 Hz, 6 H) and 1.31 (s, 9 H), 2.3–2.5 (complex m, 6 H), 2.6–3.1 (complex m, 4 H), 3.1–3.7 (complex m, 10 H, partially obscured by H_2O peak) including 3.58 (t, J = 5 Hz, 4 H), 3.97 (br m, 1 H), 4.17 (br m, 1 H), 4.49 (br m, 1 H), 6.86 (s, 1 H), 7.08 (d, J = 8 Hz, 1 H), 7.1–7.4 (m, 6 H), 7.56 (s, 1 H), 7.99 (br m, 1 H), 8.24 (br m, 1 H); FAB-MS m/e 814 $(M + H)^+$. Anal. ($C_{42}H_{67}N_7O_7S·0.25CH_2Cl_2$) C, H, N. This material was obtained similarly from **4b** in 52% yield with identical NMR and nearly identical rotation, $[\alpha]^{20}_D$ −16.5° (*c* 2, CH_2Cl_2), indicating that epimerization α to the C-terminal carbonyl occurs during the reaction.

(*S*)-2-[(*tert*-Butylsulfonyl)methyl]-3-phenylpropionic Acid Succinimido Ester (16). A mixture of 511 mg (1.8 mmol) of (*S*)-2-[(*tert*-butylsulfonyl)methyl]-3-phenylpropionic acid (**15**),[22] 207 mg (1.8 mmol) of *N*-hydroxysuccinimide, 371 mg (1.8 mmol) of *N*,*N*′-dicyclohexylcarbodiimide, and 3.6 mL of dry acetonitrile was stirred at room temperature in a stoppered flask for 3 days. The thick mixture was diluted with 25 mL of EtOAc, allowed to stand for a few minutes, and then filtered to remove *N*,*N*′-dicyclohexylurea. The filtrate was washed with 25 mL of saturated aqueous $NaHCO_3$, then dried ($MgSO_4$), filtered, and concentrated in vacuo. Crystallization of the residue from a small volume of EtOAc yielded 429 mg of colorless crystals: mp 154–155.5 °C; TLC in 95:5 CH_2Cl_2–MeOH; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.38 (s, 9 H), 2.84 (s, 4 H), 3.05 (dd, J = 6.5 Hz, 13.5 Hz, 1 H), 3.26 (dd, J = 6.5 Hz, 14 Hz, 1 H), 3.40 (m, 2 H), 3.79 (apparent quint, J = 6.5 Hz, 1 H); FAB-MS m/e 382 $(M + H)^+$. Anal. ($C_{18}H_{23}NO_6S$) C, H, N. Evaporation of the mother liquor gave a solid, which was triturated and washed with ether. Recrystallization of this material from a small volume of EtOAc provided a usable second crop of colorless crystals, mp 151–155 °C. The total yield of **16** was 522 mg (76%).

4-[2-[2-[[(2*R*,3*S*)-3-[[N^{α},N^{im}-Bis(*tert*-butoxycarbonyl)-L-histidyl]amino]-4-cyclohexyl-2-hydroxy-1-butyl]sulfonyl]acetamido]ethyl]morpholine (18). To 1.01 g (2 mmol) of **17** was added 4 mL of anhydrous trifluoroacetic acid, and the mixture was stirred at room temperature under N_2. Vigorous gas evolution occurred initially but subsided within a few minutes. After 1.5 h, the solution was evaporated under a stream of N_2 and then dried further in vacuo (oil pump, KOH trap) at room temperature. The viscous residual oil was dissolved in 70 mL of EtOAc and washed with 2 × 50 mL of saturated aqueous Na_2CO_3. The EtOAc phase was dried over Na_2SO_4, filtered, and concentrated in vacuo at room temperature. To the light amber residual glass were added 710 mg (2 mmol) of N^{α},N^{im}-bis(*tert*-butoxycarbonyl)-L-histidine[24] (freshly prepared from the corresponding dicyclohexylamine salt[25] by partitioning between CH_2Cl_2 and 5% aqueous citric acid), 884 mg (2 mmol) of (benzotriazol-1-yloxy)-tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent), 10 mL of dry CH_2Cl_2, and 278 μL (202 mg, 2 mmol) of triethylamine. The resulting solution was stirred at room temperature in a stoppered flask for 3 days and then concentrated in vacuo. Chromatography of the residue on a silica gel column (65 × 3.5 cm; elution with a gradient of 3–7% MeOH in CH_2Cl_2) yielded material which still contained some triethylamine. Consequently, it was dissolved in 30 mL of EtOAc and washed with 2 × 30 mL of saturated aqueous NH_4Cl. The EtOAc fraction was dried over Na_2SO_4, filtered, and concentrated in vacuo (finally on oil pump at 85 °C) to give 895 mg (60%) of a cream-colored, stiff foam: TLC in 9:1 $CHCl_3$–MeOH; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.7–1.75 (complex m, 31 H) including 1.46 (s, 9 H) and 1.60 (s, 9 H), 2.4–2.7 (m, 6 H), 2.96 (dd, J = 6 Hz, 14 Hz, 1 H), 3.0–3.15 (m, 2 H), 3.31 (m, 1 H), 3.55 (m, 2 H), 3.72 (t, J = 5 Hz, 4 H), 3.9–4.1 (complex m, 3 H) including 4.00 (AB q, J = 14 Hz, 2 H), 4.14 (br d, J = 9 Hz, 1 H), 4.32 (apparent q, J = 6 Hz, 1 H), 6.28 (br m, 1 H), 6.48 (d, J = 8.5 Hz, 1 H), 7.21 (s, 1 H), 8.02 (s, 1 H); high-resolution FAB-MS m/e 743.4009 [calcd for $C_{34}H_{59}N_6O_{10}S$ $(M + H)^+$: 743.4013].

Method M. 4-[2-[2-[[(2*R*,3*S*)-3-[[N^{α}-[(*S*)-2-[(*tert*-Butylsulfonyl)methyl]-3-phenylpropionyl]-L-histidyl]amino]-4-cyclohexyl-2-hydroxy-1-butyl]sulfonyl]acetamido]ethyl]morpholine (19). To 802 mg (1.08 mmol) of **18** was added 3.2 mL of anhydrous trifluoroacetic acid, and the mixture was stirred under N_2 at room temperature. Gas evolution was observed initially, and a clear solution was obtained within a few minutes. After 4 h, the solution was evaporated under a stream of N_2. The residual oil was reconcentrated three times from EtOH and dried in vacuo (oil pump, KOH trap). The resulting tan, stiff foam was treated with 412 mg (1.08 mmol) of **16**, 902 μL (654 mg, 6.48 mmol) of triethylamine, and 3.2 mL of dry CH_2Cl_2. The mixture was stirred at room temperature in a stoppered flask, and a clear solution was soon obtained. Additional triethylamine (151 μL, 1.08 mmol) was added at 15.5 h. After 39 h, the solution was concentrated in vacuo at room temperature to give a viscous oil. This was dissolved in a mixture of 30 mL of EtOAc and 20 mL of THF and washed with 2 × 20 mL of saturated aqueous $NaHCO_3$ followed by 3 × 20 mL of saturated aqueous NH_4Cl. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The residual foam was chromatographed first on a column of silica gel (64.5 × 3.5 cm; elution with a gradient of 95:5:0.5 to 92.5:7.5:0.5 CH_2Cl_2–MeOH–concentrated NH_4OH) and then on twenty 1000-μm preparative silica gel TLC plates (20 × 20 cm; developed in 85:15:1.5 CH_2Cl_2–MeOH–concentrated NH_4OH, product bands extracted with the same solvent) to yield 373 mg (42%) of a glass, which was pulverized to a powder: mp >120 °C (gradual, with preliminary softening); TLC in 90:10:1 $CHCl_3$–MeOH–concentrated NH_4OH; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.7–1.0 (m, 2 H), 1.1–1.45 (complex m, 15 H) including 1.35 (s, 9 H), 1.55–1.75 (m, 5 H), 2.4–2.6 (m, 6 H), 2.85–3.65 (complex m, 12 H), 3.71 (t, J = 5 Hz, 4 H), 4.08 (AB q, J = 14 Hz, 2 H) overlapping 4.11 (m, 1 H), 4.63 (m, 1 H), 6.65 (m, 1 H), 6.99 (s, 1 H), 7.15–7.35 (m, 5 H), 7.52 (br m, 1 H), 7.67 (br m, 1 H) overlapping 7.70 (s, 1 H); FAB-MS m/e 809 $(M + H)^+$. Anal. ($C_{38}H_{60}N_6O_9S_2·H_2O$) C, H, N.

In Vitro Plasma Renin Assay. The inhibition of human plasma renin was assayed essentially under the conditions previously described.[10] Briefly, lyophilized human plasma was reconstituted with distilled H_2O on the day of the assay. The radioimmunoassay[27] 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 three to five 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.

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In Vivo Evaluation of Renin Inhibitors. Following the previously described protocol,[28] male and female rhesus monkeys (*Macaca mulatta*) 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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New Potent Mitomycin Derivatives: Synthesis and Antitumor Activity of 7,7-(Ethylenedioxy)mitomycins

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A series of 6,7-dihydro-7,7-(ethylenedioxy)mitomycins was synthesized and evaluated for antitumor and anticellular activities. These compounds were prepared by basic treatment of 7-methoxymitomycins with ethylene glycol, and were structurally novel mitomycin derivatives containing a masked quinone moiety. 5,6-Enol or 6-chloro derivatives of 6,7-dihydro-7,7-(ethylenedioxy)mitomycins were also prepared and the (allyloxy)carbonyl group at the aziridine nitrogen has proved to be an efficient protecting group in chemical modification of mitomycins. Most of these mitomycin derivatives displayed potent antitumor activity against P388 leukemia in mice and anticellular activity against HeLa S_3 cells.

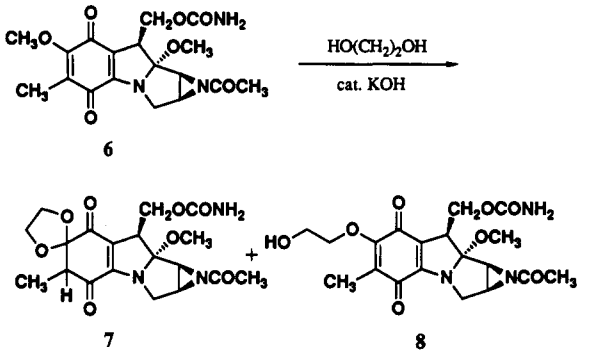
Mitomycin C (1) is one of the most potent antitumor antibiotics which have been clinically used in cancer chemotherapy, but its use is limited by side effects, such as severe bone marrow suppression or gastrointestinal damage.[1,2] Hundreds of compounds targeting less toxicity or more effective activity have been derived from natural mitomycins (Figure 1). Among these derivatives, some of 7-substituted mitomycins[2,3,4] have been reported to possess superior activity to mitomycin C against experimental tumors. On the basis of the recent understanding of detailed mode of action of mitomycins,[5] extensive studies have been underway to develop more useful mitomycin analogues.[3,6,7] Since the reductive activation is believed to be essential for antitumor activity,[5] modification of the quinone might be an interesting approach to develop novel mitomycin analogues which have different chemical and biological property from those of known mitomycin analogues. During the course of our synthesis of 7-substituted mitomycins, we recently reported that a reaction of 1a-acetylmitomycin A (6) with ethylene glycol under basic condition gave 7,7-(ethylenedioxy)mitomycin 7, a novel mitomycin derivative containing an acetal group at the C7 position and lacking the quinone moiety, as a major product along with its isomer 8 (Scheme I).[8a]

In addition to the usefulness of 7 as an intermediate for isotopically labeled mitomycins,[8] we found that this series of compounds showed potent antitumor activity. We now wish to report the synthesis and antitumor activity of these structurally unique mitomycin derivatives.

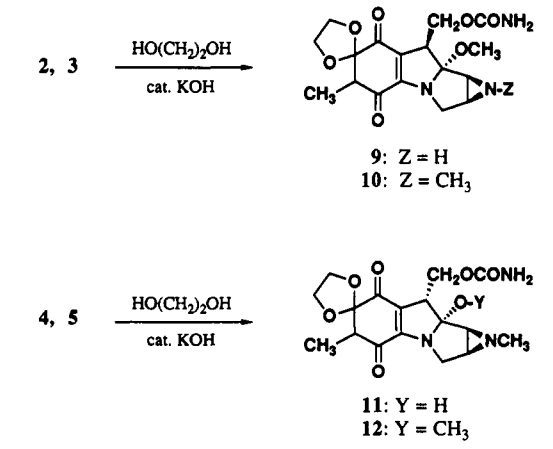
Chemistry

As mentioned above, 7,7-(ethylenedioxy)mitomycins 9–12 were prepared by reactions of natural 7-methoxymitomycins, such as mitomycins A (2), F (3), B (4), and J (5), with ethylene glycol in the presence of KOH in yields

Scheme I



Scheme II



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‡ Pharmaceutical Research Laboratories.

(1) Carter, S. K.; Crooke, S. T. *Mitomycin C: Current Status and New Developments*; Academic Press: New York, 1979.

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