New Inhibitors of Human Renin That Contain Novel Replacements at the P_2 Site[1]

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A series of renin inhibitors with novel modifications at the P_2 site has been prepared. Structure–activity relationships reveal that for a particular P_2 fragment the in vitro potency is highly dependent on the nature of the P_2' portion in addition to the P_1–P_1' group. The length of the P_2 side chain and choice of ϵ-N P_2 substitution have been found to be important for in vitro potency although the degree of unsaturation in the P_2 side chain is not particularly significant. Molecular modeling studies have shown that it is possible for the P_2 side chain to interact unfavorably with the P_2' binding site. It has been possible to control the specificity for renin over cathepsin D by correct modification at the P_2' and P_1–P_1' sites. Variations at the P_4 site have been utilized to lower the log P values of these renin inhibitors while maintaining high potency. Compound **42**, which exhibited an IC_{50} of 3.70 nM, log P of 2.3, and showed high specificity for renin, was selected for further studies. It was found to be very stable under neutral, acidic, and basic conditions. In simulated intestinal juice, compound **42** had a half-life of 37 min while it was virtually unaffected by simulated gastric juice after 4 h. Compound **42** produced a significant hypotensive response upon intravenous administration to the salt-depleted normotensive cynomolgus monkey.

Introduction

Renin is an aspartic proteinase synthesized and stored in the epithelioid cells of the juxtaglomerular apparatus of the kidney. It is a highly specific proteolytic enzyme that cleaves the polypeptide angiotensinogen to produce the decapeptide angiotensin I.[2] Angiotensin I, which has no known biological activity, is subsequently cleaved by angiotensin converting enzyme (ACE) to afford the vasoactive peptide angiotensin II. The renin–angiotensin system plays a central physiological role in the regulation of blood pressure and electrolyte homeostasis. Recent evidence indicates that renin and angiotensinogen genes and their products are expressed at many local tissue sites.[3] The existence of a local renin–angiotensin system (RAS) is thought to contribute to the control of vascular tone.[4,5] The activity of the tissue system under different conditions may influence the pharmacologic response to inhibitors of the RAS.[6]

The design of ACE inhibitors as useful drugs in the treatment of hypertension and heart failure has been reviewed.[7] Renin however possesses much greater substrate specificity than ACE, and this property has led to intense interest in the search for effective inhibitors of renin.[8]

Most of the potent renin inhibitors reported are based upon the natural substrate angiotensinogen sequence, incorporating nonhydrolyzable transition-state moieties at the active site.[9-15] Thus these compounds remain partly peptidic in nature and their therapeutic efficacy is limited due to low oral activity and short duration of action. Several studies of renin inhibitors in animals[16-20] and more recently in humans have been reported.[21,22]

In a search for novel and effective renin inhibitors,[23] we became interested in variation at the P_2 site (nomenclature for renin inhibitors illustrated for compound **17** in Scheme I)[24] where a histidine residue is present in the substrate angiotensinogen and similarly in many of the potent inhibitors synthesized to date.[9-15] It has been reported that the presence of histidine at P_2 is important for potency[11,13] as well as high selectivity[13,14,25] toward renin over related aspartic proteinases. It has been suggested that the protonated state of the imidazole group at P_2 is a contributor to the inhibitor specificity.[26] However Rosenberg et al. have reported that replacements of the P_2 imidazole group with other heterocycles of varying basicity did not ad-

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(1) Abbreviations follow the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature for amino acids and peptides: *Eur. J. Biochem.* **1984**, *158*, 9-31. Additional abbreviations are as follows: ACE, angiotensin converting enzyme; DCC, dicyclohexylcarbodiimide, DMF, dimethylformamide; DMSO, dimethyl sulfoxide; BOC, *tert*-butoxycarbonyl; Z, benzyloxycarbonyl; TFA, trifluoroacetic acid; TEA, triethylamine; HOBT, 1-hydroxybenzotriazole; Sta, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid; ACHPA, (3*S*,4*S*)-4-amino-3-hydroxy-5-cyclohexylpentanoic acid; DFSta, (3*R*,4*S*)-4-amino-2,2-difluoro-3-hydroxy-6-methylheptanoic acid; FCS, (3*S*,4*S*)-4-amino-2,2-difluoro-3*R*-hydroxy-5-cyclohexylpentanoic acid; DFO, (4*S*)-4-amino-2,2-difluoro-3-oxo-6-methylheptanoic acid; AEM, 4-(2-aminoethyl)morpholine.

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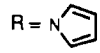
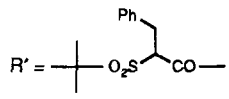
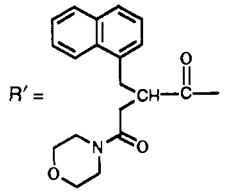
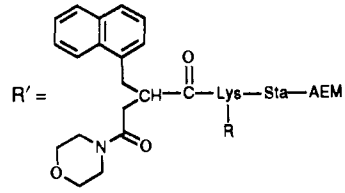
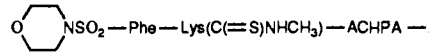
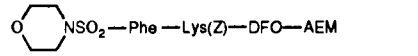
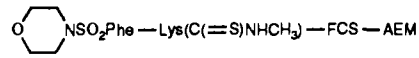
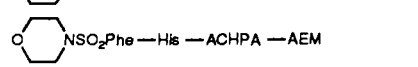
Table I. Lysine Derivatives

compd	structure	IC_{50}, nM monkey plasma	IC_{50}, nM human plasma	log P^a	molecular formula[b]
1	BNMA-His-Sta-$NHCH_2CH(CH_3)CH_2CH_3$	85	61		see ref 30
	BNMA-Lys(R)-Sta-$NHCH_2CH$-$(CH_3)CH_2CH_3$				
5	R = H	3490	2580	5.25	$C_{43}H_{58}N_4O_4 \cdot 1.5H_2O$
17	R = C(=S)$NHCH_3$	23	13	5.71	$C_{45}H_{61}N_5O_4S \cdot 0.25C_4H_8O_2 \cdot 0.5H_2O$
18	R = C(=O)$NHCH_3$	44	29	5.63	$C_{45}H_{66}N_5O_5 \cdot 0.33C_4H_8O_2 \cdot 0.5H_2O$
19	R = C(=S)NHPh	138	58	6.60	$C_{50}H_{63}N_5O_4S \cdot 0.20CH_2Cl_2$
20	R = P(=O)$(OPh)_2$	48% at 10^{-5} M	740	7.88	$C_{55}H_{67}N_4O_7P \cdot H_2O$
21	R = C(=NCN)SCH_3	41	19	5.51	$C_{46}H_{60}N_6O_4S \cdot H_2O$
22	R = 	144	87	7.21	$C_{47}H_{60}N_4O_4$
23	R = C(=O)NH_2	89	50	5.31	$C_{44}H_{59}N_5O_5 \cdot CH_3OH$
24	R = Tos	48% at 10^{-5} M	3000	6.70	$C_{50}H_{64}N_4O_6S \cdot 0.07CHCl_3$
25	R = $COCH_3$	36	6.2	4.38	$C_{45}H_{60}N_4O_6$
	BNMA-Lys(R)-Sta-AEM				
26	R = C(=S)$NHCH_3$	125	120	3.91	$C_{46}H_{62}N_6O_5S \cdot 0.3CHCl_3$
27	R = C(=NH)$NHNO_2$	40	45	4.09	$C_{45}H_{60}N_8O_7 \cdot 0.4CHCl_3$
28	R = C(=NCN)SCH_3	90	*c*	4.67	$C_{47}H_{61}N_7O_5S \cdot 0.5CHCl_3$
	BNMA-Lys(R)-Sta-Leu-$NHCH_2Ph$				
29	R = Z	8000	2900	7.69	$C_{59}H_{71}N_5O_7$
30	R = C(=S)$NHCH_3$	99	60	6.70	$C_{53}H_{68}N_6O_5S \cdot 0.4CHCl_3$
31	R = C(=NH)$NHNO_2$	49	15	5.51	$C_{52}H_{66}N_8O_7 \cdot 0.4CHCl_3$
	R′-Lys(C(=S)$NHCH_3$)-ACHPA-AEM				
32	R′ = BNMA	29	*c*	5.44	$C_{49}H_{66}N_6O_5S$
33	R′ = 	27	*c*	2.64	$C_{39}H_{66}N_6O_7S_2 \cdot C_6H_8O_7 \cdot 1.56H_2O$
34	R′ = 	36	20	1.68	$C_{44}H_{67}N_7O_7S \cdot 0.4CHCl_3$
	R′ = 				
35	R = C(=S)$NHCH_3$	693	108	2.43	$C_{41}H_{63}N_7O_7S \cdot 1.0CHCl_3$
36	R = C(=NCN)SCH_3	350	*c*	1.68	$C_{42}H_{62}N_8O_7S \cdot 1.0CHCl_3$
37	R = C(=NH)$NHNO_2$	395	*c*	1.95	$C_{40}H_{81}N_9O_9 \cdot 2.0H_2O$[e]
38	BNMA-Lys(C(=S)$NHCH_3$)-ACHPA-$NHCH_2$-$CH(CH_3)CH_2CH_3$	47	*c*	6.63	$C_{48}H_{65}N_5O_4S \cdot 0.44C_4H_8O_2$
39	BNMA-Lys(C(=S)$NHCH_3$)-Sta-N$(CH_3)(OCH_3)$	10560	*c*	4.83	$C_{42}H_{55}N_5O_5S \cdot 0.18CHCl_3$[f]
40	BNMA-Lys(C(=S)$NHCH_3$)-DFSta-AEM	350	*c*	5.50	$C_{48}H_{60}N_8O_5SF_2 \cdot 0.15CHCl_3$
41	Me_2NSO_2-Phe-Lys(C(=S)$NHCH_3$)-ACHPA-AEM	15	14	2.41	$C_{38}H_{62}N_8O_7S_2 \cdot 0.15CHCl_3$
42		3.2	*c*	2.26	$C_{38}H_{64}N_8O_2S_2 \cdot 0.50CH_2Cl_2$
43		3.3	*c*	2.32	$C_{41}H_{59}O_{10}N_7SF_2 \cdot 0.038CHCl_3 \cdot 1.5H_2O$
44		2.3	*c*	2.93	$C_{38}H_{62}O_8N_8SF_2 \cdot 0.9CHCl_3$
45		5.0	5.4	2.25	$C_{36}H_{56}N_8O_8S \cdot C_2H_7O_2 \cdot H_2O$

[a] log *P* values measured at pH 7.4. [b] Analysis for C, H, N were within ±40% except where noted. [c] Not measured. [d] Two isomers. [e] N: calcd, 14.87; found 13.81. [f] S: calcd, 4.20; found 4.72.

versely affect the potency of a series of statine-derived renin inhibitors.[27] Moreover, potent renin inhibitors with a β-aspartyl residue at the P_2 site have recently been reported.[28]

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Scheme I

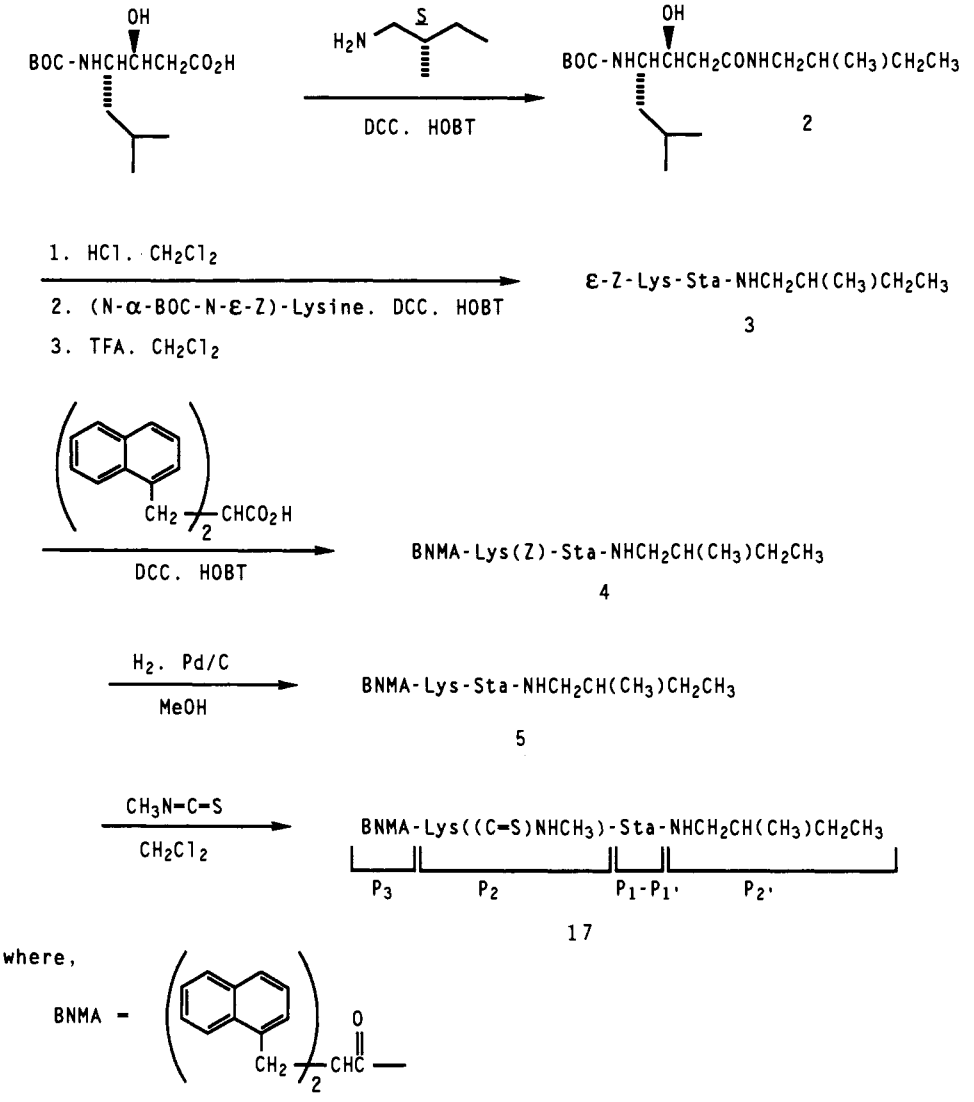

Table II. Chain Length Variation

compd	structure	IC_{50}, nM monkey plasma	IC_{50}, nM human plasma	log P^a	molecular formula[b]
	BNMA-NHCH(R″)CO-Sta-$NHCH_2CH(CH_3)CH_2CH_3$				
46	R″ = $CH_2CH_2NHCOCH_3$	4150	$>10^{-5}$ M	3.6/4.4[c]	$C_{43}H_{56}N_4O_5$
47	R″ = $CH_2CH_2NHC(=S)NHCH_3$	2490	9350	5.5/5.7[c]	$C_{43}H_{57}N_5O_4S\cdot0.35CHCl_3$
48	R″ = $(CH_2)_3$—N⟨ ⟩	390	540	7.09	$C_{46}H_{58}N_4O_4\cdot0.5H_2O$
49	R″ = $(CH_2)_5NHC(=S)NHCH_3$	39	40	5.41	$C_{46}H_{63}N_5O_4S\cdot0.3CH_2Cl_2$
50	R″ = $(CH_2)_5NHC(=NH)NHNO_2$	20	26	5.10	$C_{45}H_{61}N_7O_6\cdot0.3CH_2Cl_2$
51	R″ = $(CH_2)_6NHC(=S)NHCH_3$	44	48	5.64	$C_{47}H_{65}N_5O_4S\cdot0.33C_4H_8O_2$

[a] log *P* values measured at pH 7.4. [b] Analysis for C, H, N were within ±0.4% except where noted. [c] Two isomers.

Our study into the effect of variation at the P_2 site indicates that replacement of histidine with modified lysine derivatives maintains high potency, but often reduces selectivity for renin. However, suitable modifications, par-

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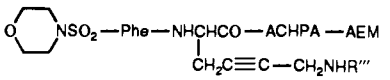
Table III. Unsaturation in the P_2 Lysine Side Chain

compd	structure	IC_{50}, nM monkey plasma	IC_{50}, nM human plasma	log P^a	molecular formula[b]
	BNMA-NHCH(R‴)CO-Sta-$NHCH_2CH(CH_3)CH_2CH_3$				
52	R‴ = $CH_2C{\equiv}C{-}CH_2NHZ$	248	150	7.13/7.37[c]	$C_{51}H_{60}N_4O_8{\cdot}0.33C_4H_8O_2$[d]
53	R‴ = $CH_2CH{=}CHCH_2NHC(=S)NHCH_3$ (*E*)	26	41	5.23	$C_{45}H_{59}N_5O_4S{\cdot}0.07CHCl_3$
54	R‴ = $CH_2CH{=}CHCH_2NHCOCH_3$ (*Z*)	100	*g*	5.56	$C_{45}H_{58}N_4O_5{\cdot}0.5CH_3OH$[e]
55a	R‴ = $CH_2C{\equiv}CCH_2NHCOCH_3$ (isomer A)	4.6	2.0	5.32	$C_{45}H_{56}N_4O_5{\cdot}0.1CHCl_3$
55b	R‴ = $CH_2C{\equiv}CCH_2NHCOCH_3$ (isomer B)	41	29	5.06	$C_{45}H_{56}N_4O_5{\cdot}0.05CHCl_3$
	BNMA-NHC($CH_2C{\equiv}C{-}CH_2NHR‴$)HCO-ACHPA-AEM				
56	R‴ = BOC	700	*g*	6.00	$C_{52}H_{67}N_5O_7{\cdot}0.75CHCl_3$
57	R‴ = $C(=S)NHCH_3$	48	*g*	5.46	$C_{49}H_{62}N_6O_5S{\cdot}C_6H_8O_7{\cdot}3H_2O{\cdot}0.2CHCl_3$
58	R‴ = $C(=NH)NHNO_2$	12	*g*	4.73	$C_{48}H_{60}N_8O_7{\cdot}C_8H_8O_7{\cdot}0.2CHCl_3$
59	R‴ = $COCH_3$	9.0	*g*	4.52	$C_{49}H_{61}N_5O_6{\cdot}C_6H_8O_7{\cdot}2.2H_2O$
60	R‴ = CHO	8.7	*g*	5.44	$C_{48}H_{59}N_5O_8{\cdot}C_6H_8O_7{\cdot}1.5H_2O{\cdot}0.2CHCl_3$[f]
					
61a	R‴ = BOC (isomer A)	11.0	*g*	3.96	$C_{41}H_{65}N_7O_{10}S$
61b	R‴ = BOC (isomer B)	700	*g*	3.13	$C_{41}H_{65}N_7O_7O_{10}S{\cdot}0.8CHCl_3$
62	R‴ = $C(=S)NHCH_3$ (from 61a)	3.3	*g*	2.90	$C_{38}H_{60}N_8O_8S_2{\cdot}C_6H_8O_7{\cdot}3.8H_2O{\cdot}0.11CHCl_3$
63	R‴ = $COCH_3$ (from 61a)	0.5	*g*	2.12	$C_{38}H_{59}N_7O_9S{\cdot}C_6H_8O_7{\cdot}1.4H_2O{\cdot}0.05CHCl_3$

[a] log *P* values measured at pH 7.4. [b] Analysis for C, H, N, were within ±0.4% except where noted. [c] Two isomers. [d] C: calcd, 53.57; found 53.04. [e] N: calcd, 7.46; found 8.00. [f] N: calcd, 6.70; found 6.18. [g] Not measured.

ticularly at the P_2' site in our lysine-type P_2-substituted renin inhibitors restores high selectivity.

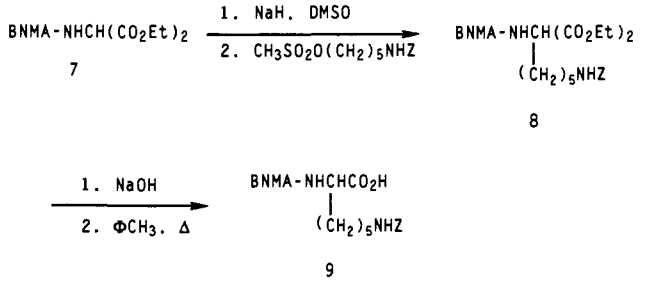
Thus we have prepared a large range of novel compounds (Tables I–III) many of which are highly potent and selective for renin.

Chemistry

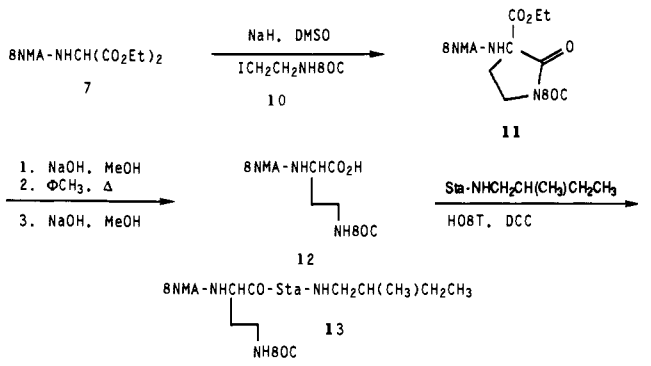
Table I lists compounds (**17–45**) with a lysine P_2 residue, where the ϵ-amine is substituted with a variety of groups. These compounds were prepared by using known synthetic methods.[29] Scheme I illustrates the synthesis of **17**, exemplary of this group. In addition the corresponding histidine analogue **1** is included in Table I for comparison (BNMA-His-Sta-$NHCH_2CH(CH_3)CH_2CH_3$).[30]

Thus BOC-statine was coupled with (*S*)-3-methylbutylamine (MBA),[30] by using the standard DCC/HOBT procedure to afford **2** which was followed by hydrochloric acid deprotection and subsequent coupling with *N*-α-BOC-*N*-ϵ-Z-lysine. Trifluoroacetic acid treatment and neutralization gave **3**. Coupling with bis(1-naphthylmethyl)acetic acid[30] gave intermediate **4**. Hydrogenolysis to remove the Z protecting group gave **5**, which was treated

Scheme II



Scheme III



with methyl isothiocyanate in dichloromethane affording renin inhibitor **17**.

The syntheses of renin inhibitors **40**, **43**, and **44** in Table I began by coupling of *N*-α-BOC-difluorostatine (DFSta),[31] with 4-(2-aminoethyl)morpholine (AEM), deprotection and subsequent coupling with *N*-α-BOC-*N*-ϵ-Z-lysine. Acidic deprotection was followed by standard coupling procedures with either bis(1-naphthylmethyl)acetic acid (BNMA) or

(23) Kaltenbronn, J. S.; Hudspeth, J. P.; Lunney, E. A.; Michniewicz, B. M.; Nicolaides, E. D.; Repine, J. T.; Roark, W. H.; Stier, M. A.; Tinney, F. J.; Woo, P. K. W.; Essenburg, A. D. *J. Med. Chem.* **1990**, *33*, 838.
(24) Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157.
(25) Cumin, F.; Nisato, D.; Gagnol, J.-P.; Corvol, P. *Biochemistry* **1987**, *26*, 7615.
(26) Maibaum, J.; Rich, D. H. *J. Med. Chem.* **1988**, *31*, 625.
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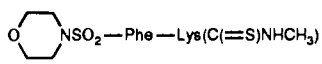
(28) Thaisrivongs, S.; Mao, B.; Pals, D. T.; Turner, S. R.; Kroll, L. T. *J. Med. Chem.* **1990**, *33*, 1337.
(29) (a) Konig, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788. (b) Bodanszky, M. *Principles of Peptide Synthesis*; Springer-Verlag: Berlin, 1984, p 158. (c) Bodansky, M. *Peptide Chemistry-A Practical Textbook*; Springer-Verlag: Berlin, 1988.
(30) (a) Kokubu, T.; Hiwada, K.; Nagae, A.; Murakami, E.; Morisawa, Y.; Yabe, Y.; Koike, H.; Iijima, Y. *Hypertension* **1986**, *8* (Suppl. II), 1. (b) Morisawa, Y.; Yabe, Y.; Kataoka, M.; Iijima, Y.; Kokubu, T.; Hiwada, K.; EP 186,977, 1986.
(31) (a) Thaisrivongs, S.; Pals, D. T.; Kati, W. M.; Turner, S. R.; Thomasco, L. M.; Watt, W. *J. Med. Chem.* **1986**, *29*, 2080. (b) Gelb, M. H.; Svaren, J. P.; Abeles, R. H. *Biochemistry* **1985**, *24*, 1813. (c) Fearon, K.; Spaltenstein, A.; Hopkins, P. B.; Gelb, M. H. *J. Med. Chem.* **1987**, *30*, 1617.

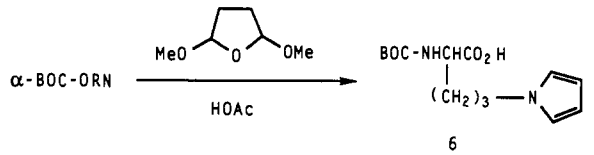
Table IV. Selectivity Data

compd	structure	IC_{50}, nm (and/or % inhibition at 10^{-6} M) cathepsin D (bovine)	renin (monkey plasma)	ratio C/R
17	BNMA-Lys(C(=S)NHCH$_3$)-Sta-NHCH$_2$CH(CH$_3$)CH$_2$CH$_3$	781	23	34
26	BNMA-Lys(C(=S)NHCH$_3$)-Sta-AEM	5403	125	43
32	BNMA-Lys(C(=S)NHCH$_3$)-ACHPA-AEM	9% at 10^{-6} M	29	-
38	BNMA-Lys(C(=S)NHCH$_3$)-ACHPA-NHCH$_2$CH(CH$_3$)CH$_2$CH$_3$	3200	47	68
42		33150	3.4	9750
	BNMA-NHCH(R')-CO-Sta-NHCH$_2$CH(CH$_3$)CH$_2$CH$_3$			
50	R^1 = (CH$_2$)$_5$NHC(=NH)NHNO$_2$	1471	20	74
52	R^1 = CH$_2$CH=CHCH$_2$NHC(=S)NHCH$_3$	1922	41	47
55a	R^1 = CH$_2$C≡C—CH$_2$NHCOCH$_3$	78	4.6	17
	BNMA-NHCH(CH$_2$C≡C—CH$_2$NHR)CO-ACHPA-AEM			
56	R = C(=S)NHCH$_3$	81000	48	1688
58	R = C(=NH)NHNO$_2$	0.7% at 10^{-6} M	11	>10^3
59	R = COCH$_3$	0.3% at 10^{-6} M	9	>10^3

(morpholinosulfonyl)phenylalanine.[32] Compound **43** was obtained by Swern oxidation of the precursor difluorostatine compound.

Renin inhibitors **32**, **33**, **34**, **41**, and **42** were synthesized by using standard chemistry from intermediate Lys(Z)-ACHPA-AEM, by coupling with the requisite P_3–P_4 group, hydrogenolysis, and acylation.

The inhibitors in Table II, **46–51** illustrate the variation in chain length at the P_2 position. The pyrrole analogue **48** (Table II) was prepared from aminoacid **6**.[33,34]



6

The other modified lysine chain length compounds shown in Table II (**46**, **47**, **49–51**) were synthesized in racemic form via alkylation of the diester **7** with the appropriate halide or mesylate (Scheme II).

It should be noted that during the analogous synthesis of the two-carbon homologue **46** from **7** that cyclization to the five-membered ring **11** occurred (Scheme III). However, hydrolysis and decarboxylation to afford **12**, followed by coupling with Sta-NHCH$_2$CH(CH$_3$)CH$_2$CH$_3$ occurred under the usual conditions to afford **13** which was successfully converted to renin inhibitors **46** and **47**.

Compounds in Table III were synthesized in order to investigate the effect of unsaturation in the P_2 lysine side chain. The analogues **52–63** were prepared by using the alkylation chemistry described for some of the compounds in Table II.

Thus, renin inhibitor **52** was prepared via alkylation of BOCNHCH(CO$_2$Et)$_2$ (**10**), with 4-chloro-α-Z-but-2-ynylamine.[35] Due to problems experienced in the selective removal of the Z protecting group, compounds **53** and **55a–60** were prepared by using the alkylation chemistry with BOCNHCH$_2$CH=CH-CH$_2$Br[35] and BOCNHCH$_2$C≡C-CH$_2$Br[35] as the electrophiles, respectively. Compound **54** was obtained by Lindlar reduction of analogue **55a**.

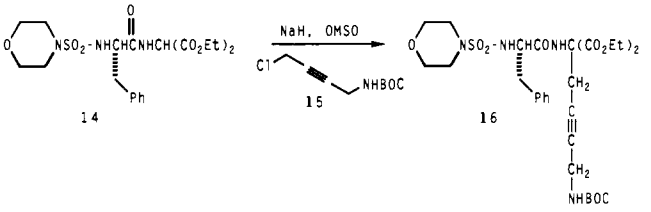
(32) Wegler, R.; Bodenbenner, K. *Justus Liebigs Ann. Chem.* **1959**, *624*, 25.
(33) Josey, A. D.; Jenner, E. L. *J. Org. Chem.* **1962**, *27*, 2466.
(34) Poduska, K.; Rudinger, J.; Gloede, J.; Gross, H. *Collect. Czech. Chem. Commun.* **1969**, *34*, 1002.
(35) The synthesis of these electrophiles was carried out according to the procedure described by Marszak-Fleury: Marszak-Fleury, A. *Ann. Chim.* **1958**, *13*, 656.

Table V. Physical Data for Renin-Inhibiting Compounds

compd	log P^a	solubility,^{b} mg/mL
17	5.25	<0.01
26	3.91	<0.01
30	6.70	<0.01
35	2.43	2.75
41	2.41	2.93
42	2.26	5.0

^{a} pH 7.4. ^{b} pH 7.4 phosphate buffer.

Acetylenic analogues **61–63** were prepared via alkylation of diester **14** with 1-[(*tert*-butoxycarbonyl)amino]-4-chloro-2-butyne (**15**), affording intermediate **16**.



14 15 16

Results and Discussion

1. **Structure–Activity Relationships.** Most of the compounds in Tables I–V have been tested for inhibition of monkey plasma renin activity (PRA). For comparative purposes some of the compounds have also been evaluated for inhibition of human plasma renin activity. Since PRA values are considerably higher in the salt-depleted furosemide-treated monkey than the human, the IC_{50} values for the former are considered to be more accurate. Clearly the observed trends within the monkey plasma IC_{50} values are predictive for results in human plasma.

From Table I it is interesting to note that changes in substitution at the P_2' position influence the resulting potency when the P_2 site contains a lysine-substituted side chain. For example, direct comparison of **17** with **26** and **21** with **28** illustrates that when P_1–P_1' is statine, it is preferable for the P_2' to be substituted with the (2*S*)-methylbutylamine group rather than the (aminoethyl)-morpholine group. However, this same trend is not observed when P_1–P_1' is ACHPA (comparison of **38** with **32**). The inactivity of the *O*,*N*-dimethylamide P_2'-substituted analogue **39** was somewhat surprising in view of the high potency of many of the other thiourea analogues and the good potency of the corresponding analogue with His at P_2 (BNMA-His-Sta-NCH$_3$(OCH$_3$)) (IC_{50} = 62 nm). These results indicate that potency is affected by the combination of P_2 and P_2' substitutions chosen. Molecular modeling

studies with the inactive *O,N*-dimethylamide analogue **39** and the active compound **17** are described in section 3 and provide some explanation for the potencies observed.

At the P_1–P_1' site the expected increase in potency by replacement of statine with ACHPA[11] is illustrated by comparison of **26** with **32** and **35** with **34**.

The statine-containing inhibitor **26** is slightly more potent than the corresponding fluorine-containing analogue **40**, a trend reported previously.[10c] However this same trend is not seen when comparing the ACHPA and corresponding difluoro-substituted ACHPA group in compounds **42** and **44**, where the potencies are very similar.

Comparison of the different acyl groups on the P_2 side chain indicates that the phosphonate, tosylate, and Z groups tend to be detrimental to in vitro potency, i.e., compounds **20**, **24**, and **29**. This may be related to the orientation of the lysine side chain in the flap region of the enzyme cleft. However it should be noted that all of the lysine-derivatized compounds **17**–**25** (with the exception of **20** and **24**) are more potent than **5** which contains a free lysine group at P_2. It can be seen that substitution of histidine at P_2 (compound **1**) does not appear to adversely affect the in vitro potency with many of the compounds studied.

The activity of **43** is somewhat surprising in view of the fact that other ϵ-*N*-Z-lysine compounds showed poor activity, i.e., **29** and **52**. Presumably this is partly a result of the presence of the difluorostatone moiety at P_1–P_1' which is believed to bind in the active site as the hydrated form of the difluoroketone group. Unfortunately, other analogues could not be prepared since attempts to remove this Z group were unsuccessful in the presence of the difluoroketone moiety.

Turning to the effect of the P_4 group in our renin inhibitors, comparison of **32**, **33**, and **34** indicates little change in in vitro potency. However, substitution with (dimethylsulfonyl)phenylalanine and particularly the (morpholinosulfonyl)phenylalanine P_4–P_3 groups, i.e., **41** and **42**, caused a substantial increase in in vitro potency.

Comparison of **26** with **35**, **27** with **37**, and **28** with **36** indicates that the bis(1-naphthylmethyl)acetyl group is preferable to the substituted succinic acid P_3 group in these examples.

More recently, efforts have been directed toward incorporation of polar P_4 groups in an attempt to lower the log *P* values and increase aqueous solubility of our renin inhibitors. Thus, use of the sulfonylphenylalanine P_4–P_3 fragment has given rise to several highly active compounds **41**–**45** with lower log *P* values (see Table I). The aqueous solubility of selected inhibitors over a range of log *P* values is shown in Table V. As the log *P* value is lowered, aqueous solubility is indeed increased.

In general the activity results from the P_2 chain length study indicate that a four-carbon chain, as for lysine, appears to be optimal. However some caution must be exercised when comparing compounds in Table I with those in Table II since those with the unnatural amino acid chain length (i.e., $\neq 4$) are all diastereomeric mixtures at the P_2 site (except **48** which was synthesized from Boc-ornithine) and hence the true IC_{50} values may be lower than those determined for the mixtures.

However some comparisons may still be made with certainty. Thus the fall off in potency observed on shortening the length of the side chain from 4 through 2 carbons is illustrated by comparison of **17** with **47** and **25** with **46**. Comparison of **22** with **48** indicates a slight loss of potency of the three-carbon analogue. Five and six-carbon chain compounds show little difference in potency relative to the natural amino acid four-carbon chain length illustrated by comparison of **17** with **49** and **51** (note **49** and **51** are diastereomeric mixtures).

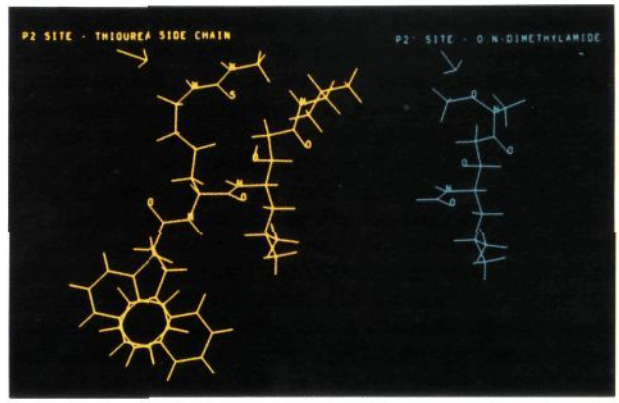


Figure 1. Colors indicate the following: yellow, compound **17**; blue, C terminus of compound **39**.

Table III includes compounds with unsaturation in the lysine side chain. Most of these are mixtures, diastereomeric at the P_2 site (although in a few cases separation has been possible i.e., **55a,b**, **61a,b**, **62**, and **63**). The *E* and *Z* olefinic analogues are of comparable potency to the saturated compounds, i.e., comparison of **53** with **17** and **54** with **25** (note **53** and **54** are diastereomeric mixtures). In general the acetylenic analogues have shown comparable or slightly higher potency than the corresponding saturated analogues: for example, comparison of **55a** with **25**, **57** with **32**, and **62** with **42**.

Many highly potent compounds have been synthesized within this acetylenic series with variation of the N-terminal acyl group, i.e., **55a**, **58**, **59**, **60**, **61a**, **62**, and **63**.

2. Selectivity. Table IV illustrates the specificity of some selected inhibitors of renin (from Tables I–III) over the closely related aspartic proteinase cathepsin D (bovine). When statine is present at P_1–P_1', selectivity for renin over cathepsin D is less than 100 (compounds **17**, **26**, **50**, **52**, and **55a**). Different chain lengths or degree of unsaturation at the P_2 site do not appear to affect the selectivity significantly, illustrated by compounds **50**, **52**, and **55a**. However when statine is replaced by ACHPA and MBA with AEM (simultaneously) there is a substantial increase in selectivity (cf. compound **32** with compounds **17**, **26**, and **38**). Thus compounds **42**, **56**, **58**, and **59** are all highly specific inhibitors of renin.

3. Molecular Modeling. In an attempt to rationalize some of the structure–activity relationships described above, we studied possible orientations for P_2 side chains in the active site of renin. This molecular modeling study involved docking analyses of several renin inhibitors into a model of human renin derived from the crystal structures of various fungal aspartic proteases.[36] The thiourea derivative of lysine, found in compound **17**, (Figure 1), was among the substituents studied. Particular attention was given to the relationship upon binding, between the Lys-(C(=S)NHCH$_3$) side chain and the P_2' substituent which can occupy a vacant S_1' binding site (with statine-type moieties at P_1 and P_1' there is no P_1' side chain). Results indicate that P_2 side chains having a certain length and flexibility, are able to extend into the S_1' binding pocket thus reducing the P_2' binding size availability. In the

(36) Blundell, T. L.; Sibanda, B. L.; Hemmings, A. M.; Foundling, S. F.; Tickle, I. J.; Pearl, L. H.; Wood, S. P. In *Molecular Graphics and Drug Design*; Roberts, G. C. K., Tute, M. S., Eds.; Elsevier Science, B. V. (Biomedical Division): Amsterdam, The Netherlands, 1986; p 324.

Figure 2. Dot VdW surfaces. Colors indicate the following: *yellow, thiourea side chain of compound 17; blue; N,O-dimethylamide of compound* **39**.

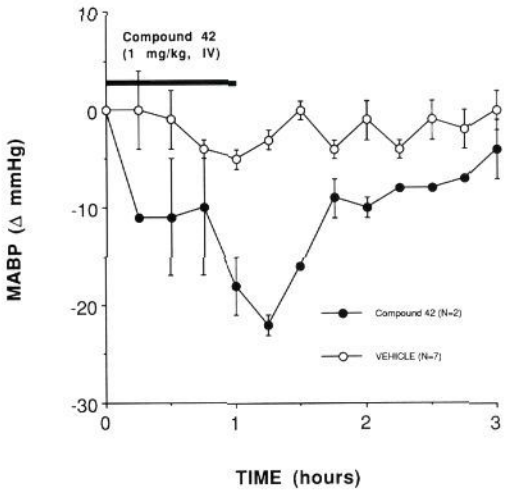


Figure 3. Effect of compound **42** (1 mg/kg iv infusion, 1 h) on blood pressure in salt-depleted normotensive cynomolgus monkeys (n = 2).

example of the inactive analogue **39**, the lack of potency might be due to the interaction of the long P_2 side chain with the *O,N*-dimethylamide at P_2' upon docking into the enzyme (Figures 1 and 2).

Moreover, further modeling analyses supported the premise that the *O,N*-dimethylamide functionality was compatible with the cleft. This was substantiated when the histidine derivative of **39** (BNMA-His-Sta-NCH_3-(OCH_3)) was prepared and found to be active (monkey renin, IC_{50} = 62 nM). With the shorter substituent at P_2, there is no interference between the P_2 and P_2' residues upon binding.

Thus, molecular modeling studies involving docking experiments indicate that a potential unfavorable interaction between the P_2 and P_2' substituents may occur in some cases, resulting in lower than expected potency.

4. Evaluation of Compound 42. Compound **42** was chosen for further evaluation due to its high potency and selectivity for renin. In simulated intestinal juice at 37 °C, **42** possessed a half-life of 37 min. It was virtually unaffected in simulated gastric juice after 4 h. Compound **42** was also found to be very stable in neutral, acidic, and basic aqueous solution, with more than 95% inhibitor remaining after 24 h. Renin inhibitor **42** was selective for renin versus other closely related aspartic proteinases, inhibiting bovine cathepsin D with an IC_{50} = 33 μM and porcine pepsin with an $IC_{50} > 10^{-3}$ M.

Renin inhibitor **42** (IC_{50} = 3.4 nM) was selected for in vivo evaluation. Figure 3 illustrates the blood pressure response to an intravenous infusion of 1 mg/kg of **42** over 1 h to salt-depleted normotensive cynomolgus monkeys (N = 2). Mean arterial pressure fell by a maximum of 22 mmHg. However, compound **42** showed no oral activity upon po administration (30 mg/kg).

Conclusion

Renin inhibitors with novel modifications at the P_2 site were prepared and tested in vitro. A number of conclusions can be drawn from the structure–activity relationships.

First, it is clear that the cleft of the renin enzyme tolerates a wide range of acyl-substituted lysine side chains at the P_2 site. Side chains smaller than the natural lysine C4 chain appear to adversely affect in vitro potency. Unsaturation in the lysine chain or longer carbon side chains showed little effect on potency.

Second, the activity of the P_2-modified renin inhibitors is largely dependent on the nature of the P_2' site. We have shown by modeling that it is possible for the P_2 side chain to interact unfavorably with the P_2' binding site and this may provide some explanation for the low activity of analogue **39**.

Third, variation at the P_4 site has been utilized to lower the log P values of representative renin inhibitors effectively. The stability of the P_2 lysine modified renin inhibitors in gastric and intestinal juices has been found to be fairly high. However, these factors are clearly not the only requirements for oral efficacy and duration of action. Hence, although a reasonable and prolonged drop in blood pressure was noted upon intravenous administration of compound **42**, oral administration showed little effect on blood pressure. Poor oral absorption and/or extensive elimination are possible reasons for this observation.[37,38]

Finally we have shown that it is possible to control the specificity of our inhibitors between renin and cathepsin D by simple modification at the P_2' and P_1–P_1' sites.

Experimental Section

The NMR spectra were recorded on a Varian EM-390, Varian XL-200, or an IBM WP100SY instrument. The FAB-MS was determined on a VG analytical 7070E/HF mass spectrometer in a thioglycerol matrix with xenon used as the target gas. Rotations were recorded on a Perkin-Elmer Model 142 polarimeter. TLC was done on precoated sheets (Silica gel 60F 254, Merck). Silica gel chromatography was done with Kieselgel 60 (70–230 mesh or 230–400 mesh for flash).

All compounds were purified by chromatography on silica gel and were usually obtained as solid foams that often retained solvent, even on prolonged drying under vacuum. Intermediates and the compounds in Tables I–IV all showed the correct molecular ion in the FAB mass spectrum. The NMR was consistent with the assigned structures. The log P values of the compounds in Tables I–III were determined by using a standard HPLC correlation method.[39]

Modeling. Studies were carried out with the VAX version of the SYBYL molecular modeling program[40] with use of an Evans and Sutherland PS390.

A model of human renin derived from the crystal structures of various fungal aspartic proteases including endothiapepsin, penicillopepsin, and rhizopus chinensis[36] were used in this study.

(37) Taylor, D. C. *Pharm. Int.* **1986**, 179.
(38) Humphrey, M. J.; Ringrose, P. S. *Drug Metab. Rev.* **1986**, *17* (3–4), 283.
(39) Haky, J. E.; Young, A. M. *J. Chromatog.* **1984**, *7* (4), 675.
(40) Version 3.5, Tripos Associates, Inc., A subsidiary of Evans and Sutherland, 1699 S. Hanley Rd., Suite 303, St. Louis, Missouri 63144.

The docking experiments were performed within the confines of the active site containing those residues in contact with the particular binding pocket of interest. Initial docking used available data for inhibitors cocrystallized with endothiapepsin.[41] Graphic manipulation combined with standard Van der Waals analyses was then performed to refine the P_2 and P_2' side chain orientations. Final conformations were evaluated through molecular mechanics treatment by using the Tripos force field available with SYBYL.

Biological Methods. Inhibition of renin activity by novel renin inhibitor drugs was determined by a radioimmunoassay for angiotensin I, based on the method of Haber et al.[42] The in vitro angiotensin I generation step utilized 500 μL of monkey plasma (containing native renin and angiotensinogen), 50 μL of maleate buffer (pH 6.0), 5 μL of (phenylmethyl)sulfonyl fluoride (PMSF), and 2 μL of an appropriate concentration of inhibitor in a dimethyl sulfoxide (DMSO) vehicle. Incubation was for 60 min at 37 °C. Following incubation, each mixture was analyzed (in duplicate) for angiotensin I via radioimmunoassay by using ^{125}I-labeled angiotensin I and carried out in tubes coated with rabbit anti-angiotensin I antibody (Gamma Coat RIA Kit, Dade Clinical Assays). Monkey plasma renin activity ranged from 3–8 ng AI/mL per h. Values for inhibitor tubes were compared to vehicle (DMSO) control tubes to estimate percent inhibition. At the concentration used, DMSO inhibits the generation of angiotensin I by <10%. The inhibition results were expressed as IC_{50} values, which were obtained by plotting six inhibitor concentrations and estimating the concentration producing 50% inhibition by using nonlinear regression analysis. IC_{50} values for inhibition of human renin were determined by the method reported previously.[23]

Inhibition of bovine cathepsin D (Sigma) activity was assessed in duplicate by the hydrolysis of bovine hemoglobin (2× crystallized, Sigma) at pH 3.2 and 37 °C (modified from Kokubu et al. and Aoyogi et al.).[30,43] Net absorbance (at 280 nm) was measured in acid-precipitated supernatant fractions of inhibited vs uninhibited control assays. The IC_{50} values were determined as described above.

Intravenous Administration of 42. Male cynomolgus monkeys weighing between 4.9 and 7.7 kg were instrumented with vascular-access ports (Norfolk Medical Products, Skokie, IL) for intraarterial blood pressure monitoring and iv drug administration. The animals had previously been trained to sit quietly in a primate restrainer (Primate Products, Woodside, CA). For normotensive studies, animals were placed on a low sodium diet (Bio-Serv. Inc., Frenchtown, NJ) 7–10 days prior to administration of compound **42**.

Each monkey was treated with furosemide (Lasix, INJ USP 5%, Hoechst Roussel) 2 mg/kg per day im for four consecutive days before testing. Blood pressure was determined by using a stathum pressure transducer, Gould polygraph, and a computer data acquisition system. Compound **42** was given as a solution in a vehicle of ethyl alcohol and 5% dextrose (ET-D5W). The compound was dissolved in ethyl alcohol (200 proof) and an equal volume of 5% dextrose in water was slowly added. The concentration was adjusted such that the total dose was given over 1 hour in a volume of 0.3 mL/kg.

Oral Dosing. Oral studies were carried out in renin-dependent hypertensive cynomolgus monkeys. These were prepared by partial occlusion (ea. 60% reduction in renal blood flow) of the left renal artery by utilizing an adjustable clamp. Two or more weeks later monkeys were challenged with an intravenous influsion (20 μg/mg per min for 30 or 60 min) of the angiotensin II receptor antagonist saralasin (Sar^1-Val^5-Ala^8-All, Bachem Inc., Torrance, CA). Monkeys were included in oral dose studies if (1) renal occlusion raised blood pressure by at least 15 mmHg above preocclusion blood pressure and (2) blood pressure fell by at least 15 mmHg during the saralasin infusion. Blood pressure and heart rate were measured by using a computer data acquisition system. The vehicle consisted of a mixture of 7% dimethylacetamide, 26% Tween 80, and 67% distilled water (added in this order). The concentration was adjusted for a total dose administered in a volume of 2 mL/kg. Compound **42** was given by oral gavage by using a 16-French rectal–colon tube (Davol, Cranston, RI).

Stability Studies. Simulated gastric juice was prepared according to USP XXI (1985). To 24.5 mL of gastric juice at 37 °C was added 0.5 mL of a stock solution of **42** in methanol (5 mg/mL). The solution was placed in a water bath at 37 °C. Samples were withdrawn periodically and analyzed by HPLC (mobile phase of 35:65 acetonitrile/ammonium phosphate monobasic (adjusted to pH 3 with H_3PO_4), flow rate of 1.0 mL/min and wavelength detection at 214 nm).

Stability testing in simulated intestinal juice (USP XXI (1985)) was carried out by using the above experimental conditions.

General Synthetic Procedures. General Procedure A. Coupling Reactions Using Dicyclohexylcarbodiimide and 1-Hydroxybenzotriazole. The amine·HCl component (1.0 mmol), the carboxy component (1.0 mmol), and HOBT (1.0 mmol) were dissolved in DMF (15 mL) and cooled to 0 °C. The solution was treated with DCC (1.0 mmol) in DMF (5 mL) followed by TEA (1.0 mmol). The reaction was stirred for 1 h at 0 °C followed by 16 h at room temperature.

The mixture was then filtered and evaporated to dryness. The residue was taken up in EtOAc (30 mL) and washed with saturated $NaHCO_3$ solution and brine sequentially. The organic extract was dried (Na_2SO_4), evaporated to dryness and the residue chromatographed on silica gel.

General Procedure B. Coupling Reactions Involving Dicyclohexylcarbodiimide and 1-Hydroxybenzotriazole. The carboxy component (1.0 mmol) and HOBT (1.0 mmol) were dissolved in DMF (15 mL) and cooled to 0 °C. The solution was treated with DCC (1.0 mmol) in DMF (5 mL) and stirred for 5–10 min. The amino component (1.0 mmol) was then added. After stirring at 0 °C for 1 h and 16–24 h at room temperature the suspension was filtered and the filtrate evaporated to dryness. The residue was taken up in EtOAc (30 mL) and washed with saturated $NaHCO_3$ solution (10 mL) and brine (10 mL). The organic layer was dried (Na_2SO_4) evaporated to dryness and the residue chromatographed on silica gel.

General Procedure C. Coupling Reactions Involving Dicyclohexylcarbodiimide and 1-Hydroxybenzotriazole. This procedure is similar to general procedure B with the modified workup described. After filtration and evaporation under reduced pressure, the residue was taken up in EtOAc (30 mL) and washed sequentially with 10-mL portions of 1 N citric acid, saturated NaCl, saturated $NaHCO_3$ solution, and saturated NaCl solution. Any precipitate was filtered from the organic layer which was then dried (Na_2SO_4) and evaporated and the residue chromatographed.

General Procedure D. Removal of *tert*-Butoxycarbonyl Group. (a) A solution of the BOC-protected amine (1.0 mmol) in CH_2Cl_2 (10 mL) was treated with TFA (1 mL) and stirred for 2–6 h at room temperature. Reaction completion was assessed by TLC. The solvent was removed under reduced pressure and the residue diluted with EtOAc (30 mL) followed by washing with Na_2CO_3 solution. The organic layer was washed with brine and dried (Na_2SO_4). The solvent was evaporated and the residue dried under high vacuum for several hours.

(b) A solution of the BOC-protected amino component (1.0 mmol) in CH_2Cl_2 (10 mL) or CH_2Cl_2/MeOH (4:1) was cooled to 0 °C and dry HCl gas bubbled into the mixture for 15 min. After the solution was stirred for a further 30 min, the solvent was evaporated under reduced pressure. The residue was taken up in $CHCl_3$ and the solvent removed under reduced pressure. This procedure was repeated twice more. The residue was then dried under high vacuum for several hours and the resulting amine hydrochloride used directly in ensuing reactions.

General Procedure E. Removal of Benzyloxycarbonyl Groups by Catalytic Hydrogenation. A solution of the Z-protected amino component (1.0 mmol) in methanol (15 mL) was treated with 20% palladium on carbon (0.1 mmol) and stirred under a hydrogen atmosphere for 4–8 h. Reaction completion was assessed by TLC. The mixture was filtered and evaporated under reduced pressure.

BNMA-His-Sta-NHCH$_2$CH(CH$_3$)CH$_2$CH$_3$ (1). Title compound 1 was prepared as previously reported.[30b]

ε-Z-Lys-Sta-NHCH$_2$CH(CH$_3$)CH$_2$CH$_3$ (3). α-*N*-BOC-ε-Z-lysine (1.98 g, 5.20 mmol) and Sta-NHCH$_2$CH(CH$_3$)CH$_2$CH$_3$·HCl

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(2)[44-47] (1.50 g, 5.34 mmol) were coupled according to general procedure A with HOBT (0.72 g), TEA (0.74 mL, 5.34 mmol), and DCC (1.10 g, 5.33 mmol) in DMF (20 mL). The product was purified by column chromatography on silica gel eluting with 1:1 EtOAc–hexane to afford the coupled product (2.70 g, 85.7%) as a white foam. Deprotection of BOC-Lys(Z)-Sta-$NHCH_2CH(CH_3)CH_2CH_3$ was carried out according to general procedure D (a) to afford the product **3** (2.10 g, 93.3%) as a white foam, utilized directly in the next reaction.

BNMA-Lys(Z)-Sta-$NHCH_2CH(CH_3)CH_2CH_3$ (4). Compound **3** (2.10 g, 4.14 mmol) and bis(1-naphthylmethyl)acetic acid (1.51 g, 4.21 mmol) were coupled according to general procedure B. The crude product was recrystallized from $CHCl_3$ to afford a white foam (1.95 g, 57.9%). The residue from the mother liquor was purified by column chromatography on silica gel eluting with $CHCl_3$/EtOAc (3:1) to afford additional product (0.50 g, 14.8%). Anal. ($C_{51}H_{64}N_4O_6$) C, H, N; C: calcd C, 73.88; found C, 73.34. FAB MS: [M + H] m/z 829.3 (829).

BNMA-Lys-Sta-$NHCH_2CH(CH_3)CH_2CH_3$ (5). Compound **5** was prepared in 95.9% yield from **4** (1.95 g, 2.40 mmol) by general procedure E. Anal. ($C_{43}H_{58}N_4O_4 \cdot 1.5H_2O$) C, H, N. FAB MS: [M + H] m/z 695.5 (695).

***N*-[(1,1-Dimethylethoxy)carbonyl]-5-(1*H*-pyrrol-1-yl)-L-norvaline (6).** A solution of α-BOC-ornithine (1.20 g, 5.20 mmol) in AcOH (30 mL) was treated with 2,5-dimethoxytetrahydrofuran (0.80 g, 6.10 mmol) and heated to reflux while allowing AcOH (~15 mL) to distill off. The remainder of the acid was removed under reduced pressure. The residue was taken up in dilute NaOH, acidified with 1 N citric acid, and extracted twice with $CHCl_3$. The organic extracts were dried (Na_2SO_4), filtered, and evaporated. The crude product was purified by chromatography on silica gel with EtOAc to afford the product **6** as a yellow oil (0.70 g, 47.7%).[33,34]

Diethyl [[3-(1-Naphthalenyl)-2-(1-naphthalenylmethyl)-1-oxopropyl]amino]propanedioate (7). A suspension of (diethylamino)malonate hydrochloride (4.13 g, 0.0195 mol) in CH_2Cl_2 (100 mL) was treated with bis(1-naphthylmethyl)acetyl chloride (7.0 g, 0.0195 mol) at 0 °C. TEA (5.50 mL, 0.039 mol) was added dropwise and the whole stirred at 0 °C for 30 min and at room temperature overnight. The suspension was filtered and evaporated under reduced pressure.

The residue was taken up in EtOAc and washed with 1 N HCl, H_2O, saturated $NaHCO_3$ solution, and brine sequentially. After the residue was dried (Na_2SO_4) and evaporated, the product **7** was obtained as a white solid (9.26 g, 95.4%); mp 118–120°; MS (EI) [M] m/z 497 (497); ^1H NMR ($CDCl_3$) δ 1.10 (6 H, t, J = 13.3 Hz), 2.95–3.70 (5 H, m), 4.05 (4 H, q, J = 13.3 Hz), 5.85 (1 H, d, J = 14 Hz), 7.15–7.95 (14 H, m). Anal. ($C_{31}H_{31}NO_5$) C, H, N.

Diethyl [[3-(1-Naphthalenyl)-2-(1-naphthalenyl)-1-oxopropyl]amino]-2-[5-[[(phenylmethoxy)carbonyl]amino]pentyl]propanedioate (8). Sodium hydride (50% in oil, washed free of oil with dry petroleum ether, 0.48 g, 9.50 mmol) was suspended in DMSO (30 mL). The malonate **7** (4.74 g, 9.50 mmol) was added in portions and the suspension stirred until evolution of hydrogen had ceased. The solution was then treated with 1-Z-amino-5-[(methylsulfonyl)oxy]pentane (3.10 g, 9.50 mmol) and KI (1.0 g) in DMSO (10 mL) and the mixture stirred for 5 days. The solution was then diluted with EtOAc and washed twice with water, saturated $NaHCO_3$ solution, and saturated NaCl, sequentially. Drying (Na_2SO_4) and removal of the solvent under reduced pressure gave the crude product **8**. Purification by silica gel chromatography (1% MeOH/$CHCl_3$) afforded pure material **8** (1.30 g) and a mixture of starting material **7** and product **8** (2.90 g, ~50:50) and additional **7** (3.00 g): FAB MS (M + H) m/z 717.3 (717).

1-Z-Amino-5-[(methylsulfonyl)oxy]pentane. To 5-aminopentan-1-ol (20 g, 0.19 mol) in dioxane (100 mL) and water (50 mL) at 0 °C was added benzyl chloroformate (27.7 mL, 0.19 mol) dropwise. After half of this reagent had been added NaOH (7.7 h in 50 mL H_2O) was added. After complete addition the reaction was warmed to room temperature and stirred for 48 h. Extraction into EtOAc and washing with H_2O, $NaHCO_3$, and brine gave, after drying (Na_2SO_4) and evaporation, the crude product 5-Z-aminopentan-1-ol (45 g, 99.5%). This material (10.0 g, 0.042 mol) was dissolved in $CHCl_3$ (150 mL) and cooled to −20 °C. TEA (5.90 mL, 0.042 mol) followed by methanesulfonyl chloride (3.30 mL, 0.042 mol) were added. After stirring for 2 h the solution was washed with saturated $NaHCO_3$ and then saturated NaCl. The organic layer was dried (Na_2SO_4) and evaporated. The crude material was filtered through a small plug of silica gel to afford the product 1-Z-amino-[5-(methylsulfonyl)oxy]pentane (12.40 g, 93%) as an oil: ^1H NMR (200 MHz, $CDCl_3$) δ 1.40–1.90 (6 H, m), 3.00 (3 H, s), 3.20 (2 H, dd, J = 6.4, 12.8 Hz), 4.20 (2 H, m), 4.90 (1 H, br s), 5.10 (2 H, s), 7.36 (5 H, s); MS (Cl + CH_4) (M + H) m/z 315.9 (316).

(±)-2-[[3-(1-Naphthalenyl)-2-(1-naphthalenylmethyl)-1-oxopropyl]amino]-7-[[(phenylmethoxy)carbonyl]amino]-heptanoic Acid (9). A solution of the diester **8** (1.30 g, 1.8 mmol) in dioxane (10 mL) and MeOH (20 mL) was treated with NaOH solution (0.20 g in 5 mL H_2O, 5.0 mmol) and the resulting mixture stirred for 3 h at room temperature. The solution was diluted with H_2O and washed with Et_2O. Acidification to congo red with dilute HCl was followed by extraction with ethyl acetate. The combined organic extracts were washed with water and then brine. After drying (Na_2SO_4) and evaporation under reduced pressure the residue was dissolved in toluene (50 mL) and dioxane (10 mL) and heated under reflux for 4 h to affect decarboxylation. The solvent was evaporated under reduced pressure and the crude product **9** (0.70 g) utilized directly in the next reaction: FAB MS (M + H) m/z 617.2 (617).

2-Iodo-1-[(*tert*-butyloxycarbonyl)amino]ethane (10). To 2-bromoethylamine hydrobromide (10.0 g, 48.8 mmol) suspended in CH_2Cl_2 (150 mL) at room temperature was added TEA (7.11 mL, 51.2 mmol) followed by di-BOC dicarbonate (10.64 g, 48.8 mmol) in CH_2Cl_2 (40 mL). After stirring for 16 h the solvent was evaporated under reduced pressure and the residue taken up in EtOAc and washed sequentially with H_2O and brine. Drying ($MgSO_4$) and concentration gave a colorless oil which was purified by column chromatography on silica gel eluting with CH_2Cl_2. The product 2-bromo-1-[(*tert*-butyloxycarbonyl)amino]ethane was obtained as a white solid (5.90 g, 54%); ^1H NMR (90 MHz, $CDCl_3$) δ 1.45 (9 H, s), 3.40 (4 H, m). Anal. ($C_7H_{14}NO_2Br$) C, H, N.

The bromide (5.20 g, 25.0 mmol) was taken up in acetone (20 mL) and treated with excess anhydrous sodium iodide (5.0 g). After stirring for 24 h at room temperature the suspension was diluted with Et_2O and filtered through a small pad of neutral alumina. Evaporation of the solvent gave a crude yellow oil which was purified by column chromatography on silica gel eluting with 40% Et_2O in hexane to afford a colorless oil (4.42 g, 70.2%) identified as the title iodide: ^1H NMR (90 MHz, $CDCl_3$) δ 1.48 (9 H, s), 3.20 (2 H, m), 3.40 (2 H m).

Ethyl 1-[(1,1-Dimethylethoxy)carbonyl]-3-[[3-(1-naphthalenyl)-2-(1-naphthalenylmethyl)-1-oxopropyl]-amino]-2-oxo-3-pyrrolidinecarboxylate (11). To sodium hydride (50% in oil, washed free of oil with dry petroleum ether, 0.70 g, 17.5 mmol) in DMSO (30 mL) was added bis(1-naphthylmethyl) acetimidomalonate (6.89 g, 13.8 mmL) in DMSO (30 mL) portionwise. The mixture was stirred for 1.5 h after which 2-iodo-1-[(*tert*-butyloxycarbonyl)amino]ethane (**10**) (4.42 g, 13.8 mmol) in DMSO (30 mL) was added. After 72 h in the dark the reaction was diluted with EtOAc (1 L), and the layers were separated. The aqueous layer was extracted with EtOAc twice, and the combined organic extracts were washed with H_2O, saturated $NaHCO_3$, and brine. Drying ($MgSO_4$), concentration, and chromatography on silica gel employing 25% EtOAc in hexane afforded recovered malonate (3.25 g, 47%), iodide (0.64 g, 18.3%), and the title compound **11** (2.75 g, 31%): ^1H NMR (200 MHz, $CDCl_3$) δ 1.17 (3 H, t, J = 7 Hz), 1.54 (9 H, s), 1.95 (1 H, m), 2.38 (1 H, ddd, J = 6.7, 2.5 Hz), 3.05 (1 H, m), 3.34 (2 H, m), 3.47 (2 H, m), 3.75 (2 H, m), 4.16 (2 H, m), 6.14 (1 H, s), 7.41 (8 H, m), 7.50–7.92 (6 H, m); FAB MS (M − NBOC) m/z 495.1 (495). Anal. ($C_{36}H_{38}N_2O_6$) C, H, N.

(±)-4-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-[[3-(1-naphthalenyl)-2-(1-naphthalenylmethyl)-1-oxopropyl]-amino]butanoic Acid (12). Treatment of ester **11** (3.22 g, 5.02

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mmol) in MeOH (40 mL) with NaOH solution (0.47 g in 15 mL of H_2O, 11.8 mmol) for 4 h resulted in monohydrolysis only. After dilution with H_2O (30 mL) the pH was adjusted to 2–3 with 1 N HCl. The mixture was extracted with EtOAc three times and the organic layer washed with water and dried ($MgSO_4$). After evaporation of the solvent in vacuo the resulting white foam (2.93 g) was taken up in toluene (50 mL) and refluxed for 2 h. The solvent was evaporated and the residue taken up in MeOH (30 mL). NaOH solution (0.19 g in 5 mL of H_2O, 4.78 mmol) was added and the reaction stirred for 14 h. The pH was once again brought to 2–3 with 1 N HCl and the mixture extracted with several portions of EtOAc. After the mixture was washed with H_2O, the combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The title amino acid (**12**) was obtained as a white foam (2.00 g, 81.3%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.37 (9 H, s), 1.55 (1 H, m), 1.80 (1 H, m), 2.80 (2 H, m), 3.12 (3 H, m), 3.30 (3 H, m), 4.19 (1 H, dd, J = 14, 8 Hz), 6.65 (1 H, br s), 7.29 (2 H, m), 7.42 (6 H, m), 7.65 (2 H, dd, J = 7.8, 5.4 Hz), 7.77 (2 H, d, J = 4.6 Hz), 7.90 (2 H, d, J = 7.6 Hz), 12.42 (1 H, br s); FAB MS [M + H – BOC] m/z 484.2 (484).

1,1-Dimethylethyl [4-[[2-Hydroxy-4-[(2-methylbutyl)amino]-1-(2-methylpropyl)-4-oxobutyl]amino]-3-[[3-(1-naphthalenyl)-2-(1-naphthalenylmethyl)-1-oxopropyl]amino]-4-oxobutyl]carbamate (13). The title compound **13** was obtained by coupling of previous acid **12** (0.50 g, 0.93 mmol) and Sta-NHCH$_2$CH(CH$_3$)CH$_2$CH$_3$ (227 mg, 0.93 mmol), according to general procedure B. The product was purified by column chromatography on silica gel eluting with 2 → 4% MeOH in $CHCl_3$ (0.60 g, 84.3%) obtained as a white foam: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.90 (12 H, m), 1.0–2.40 (12 H, m), 1.40 (9 H, s), 3.10 (2 H, m), 3.35–4.35 (8 H, m), 6.48 (1 H, d, J = 9.8 Hz), 7.25–7.55 (8 H, m), 7.60–8.00 (6 H, m); FAB MS [M + H] m/z 767.6 (767).

Diethyl (*S*)-[[2-[(4-Morpholinylsulfonyl)amino]-3-phenyl-1-oxopropyl]amino]propanedioate (14). A solution of (morpholinosulfonyl)phenylalanine (12.57 g, 0.04 mol), HOBT (5.40 g, 0.04 mol), and (diethylamino)malonate hydrochloride (8.47 g, 0.04 mol) in DMF (200 mL) was cooled in ice, and TEA (5.60 mL, 0.04 mol) was added, followed by a solution of DCC (8.34 g, 0.04 mol) in DMF (25 mL). After stirring at 0 °C for 30 min the mixture was allowed to warm to room temperature and stirred overnight. Filtration and evaporation gave the crude residue which was taken up in EtOAc and washed with 1 N HCl, H_2O, saturated $NaHCO_3$ solution, and brine, respectively. Drying ($MgSO_4$) and removal of the solvent under reduced pressure gave **14** as a viscous oil (18.10 g, 96.3%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.30 (6 H, t, J = 7 Hz), 2.90–3.16 (6 H, m), 3.60 (4 H, t, J = 4.8 Hz), 4.13 (1 H, m), 4.29 (4 H, dd, J = 7, 14 Hz), 4.90 (1 H, d, J = 8.4 Hz), 5.12 (1 H, d, J = 6.6 Hz), 7.02 (1 H, d, J = 6.4 Hz), 7.23–7.41 (5 H, m); MS (Cl + CH_4) [M + H] m/z 472 (472).

Diethyl (*S*)-2-[4-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-butynyl]-2-[[2-[(4-morpholinylsulfonyl)amino]-1-oxo-3-phenylpropyl]amino]propanedioate (16). A suspension of sodium hydride (3.50 g, 0.072 mol) (50% in oil) washed free of oil was suspended in DMSO (75 mL) under nitrogen and treated in portions with a solution of the malonate **14** (17.0 g, 0.036 mol) in DMSO (50 mL). The mixture was stirred for 4 h and then treated with 1-[(*tert*-butoxycarbonyl)amino]-4-chloro-2-butyne (**15**)[32] (7.47 g, 0.036 mol) and KI (1.0 g). After stirring for 40 h the mixture was diluted with EtOAc and washed with 1 N citric acid, H_2O, and saturated NaCl solution, respectively. After drying ($MgSO_4$), the solvent was evaporated under reduced pressure and the residue chromatographed on silica gel, with 1% MeOH in $CHCl_3$ as eluent to afford **15** (14.2 g, 61.9%) as a viscous golden oil: FAB MS (M + H – BOC) m/z 538.9 (539).

BNMA-Lys(C=S(NHCH$_3$))-Sta-NHCH$_2$CH(CH$_3$)CH$_2$CH$_3$ (17). Compound **5** (0.50 g, 0.74 mmol) was dissolved in CH_2Cl_2 (15 mL) and cooled to 0 °C. Methyl isothiocyanate (0.053 g) was added, and the mixture was allowed to warm to 25 °C and stir for 6 h. The solvent was evaporated and the crude product purified by column chromatography on silica gel, with a gradient of EtOAc/hexane (1:1) as eluent to pure EtOAc. The appropriate fractions were combined to afford the product **17** as a white foam (0.30 g, 54.6%): FAB MS [M + H] m/z 768.2 (768). Anal. C, H, N.

BNMA-Lys(C=O)(NHCH$_3$))Sta-NHCH$_2$(CH$_3$)CH$_2$CH$_3$ (18). To compound **5** (0.50 g, 0.74 mmol) in CH_2Cl_2 (15 mL) at 0 °C was added methyl isocyanate (42 μL, 0.71 mmol) and the mixture allowed to warm to room temperature. After 4 h of stirring, EtOAc was added to the white suspension and the mixture filtered. The solid was dried to afford the product **18** (0.30 g, 55%). Anal. C, H, N.

BNMA-Lys(C=S(NHPh))-Sta-NHCH$_2$CH(CH$_3$)CH$_2$CH$_3$ (19). By using the procedure of example 17 phenyl isothiocyanate gave the title compound **19**. Anal. C, H, N.

BNMA-Lys(P=O(OPh)$_2$)-Sta-NHCH$_2$CH(CH$_3$)CH$_2$CH$_3$ (20). To a solution of **5** (0.50 g, 0.72 mmol) in CH_2Cl_2/THF (4/1, 15 mL)) at 0 °C was added TEA, followed by diphenyl phosphorochloridate (0.15 mL, 0.72 mmol). After stirring for 3 h, the solution was evaporated and diluted with EtOAc. Washing with saturated $NaHCO_3$ solution and brine, drying (Na_2SO_4), and evaporation under reduced pressure afforded the crude product **20**. Chromatography on silica gel, eluting with EtOAc/hexane (1/1 → 5/1) afforded the pure product **20** (0.46 g, 70.0%) as a white foam. Anal. C, H, N.

BNMA-Lys(C=NCN(SCH$_3$))-Sta-NHCH$_2$CH(CH$_3$)-CH$_2$CH$_3$ (21). A solution of **5** (0.31 g, 0.44 mmol) in $CHCl_3$ (10 mL) was treated with dimethyl cyanimidodithiocarbonate[48] (70 mg, 0.57 mmol) and stirred at room temperature overnight. After evaporation under reduced pressure, the residue was chromatographed on silica gel, with EtOAc/hexane (1:1) → EtOAc as eluent to afford the product **21** (0.30 g, 87.7%) as a white foam. Anal. C, H, N.

***N*-[2-Hydroxy-4-[(2-methylbutyl)amino]-1-(1-methylpropyl)-4-oxobutyl]-α-[[3-(1-naphthalenyl)-2-(1-naphthalenylmethyl)-1-oxopropyl]amino]-1*H*-pyrrole-1-hexanamide (22).** Compound **22** was prepared from bis(1-naphthylmethyl)acetic acid and (6-pyrrolo-2-aminohexanoyl)-Sta-2(*S*)-methylbutylamine in 34% yield by the methods described for example **48**. Anal. C, H, N.

BNMA-Lys(CONH$_2$)-Sta-NHCH$_2$CH(CH$_3$)CH$_2$CH$_3$ (23). A solution of **5** (0.30 g, 0.40 mmol) in dioxane (15 mL) was treated with AcOH (30 μL) followed by sodium cyanate (30 mg, 0.46 mmol) in H_2O (1 mL). The reaction mixture was warmed to 45 °C for 4 h, then cooled, and partitioned between EtOAc and saturated $NaHCO_3$ solution. The EtOAc layer was washed with brine, dried (Na_2SO_4), filtered, and evaporated. Column chromatography on silica gel eluting with EtOAc, then EtOAc/MeOH (9:1) afforded **23** (0.20 g, 69.2%) as a white foam. Anal. C, H, N.

BNMA-Lys(Tos)-Sta-NHCH$_2$CH(CH$_3$)CH$_2$CH$_3$ (24). Bis-(1-naphthylmethyl)acetic acid (0.44 g, 1.3 mmol) and Lys-(Tos)-Sta-NHCH$_2$CH(CH$_3$)CH$_2$CH$_3$ (0.63 g, 1.22 mmol) (prepared by the method described for **3**) were coupled according to general procedure C, to afford the product **24** (0.99 g, 97.0%) as a white foam after chromatography on silica gel (eluting with 0 → 5% MeOH in $CHCl_3$). Anal. C, H, N, Cl.

BNMA-Lys(COCH$_3$)-Sta-NHCH$_2$CH(CH$_3$)CH$_2$CH$_3$ (25). A solution of *N*-acetylimidazole (0.124 g, 0.72 mmol) and **5** (0.50 g, 0.72 mmol) in DMF (2 mL) was stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue taken up in EtOAc. The solution was washed with 1 N citric acid, saturated $NaHCO_3$, and brine, respectively. After solution was dried (Na_2SO_4) and column chromatographed on silica gel with 0 → 1% MeOH in $CHCl_3$ as eluent, the product **25** was obtained (0.24 g, 46.2%) as a white foam. Anal. C, H, N.

BNMA-Lys(C=S(NHCH$_3$))-Sta-AEM (26). By procedures similar to those used for the synthesis of compound **17**, but using (aminoethyl)morpholine instead of 2(*S*)-methylbutylamine, **26** was obtained as a white foam from BNMA-Lys-Sta-AEM in 67% yield. Anal. C, H, N, S. (HPLC: 70:30 → 0:100 H_2O/CH_3CN, over 20 min, Vydac column, 1.5 mL/min flow rate; 93% purity t_R 8.08 min).

BNMA-Lys(C=NH(NHNO$_2$))-Sta-AEM (27). To a solution of BNMA-Lys-Sta-AEM (synthesized by procedures similar to those for compound **5**) (1.50 g, 2.0 mmol) in EtOH (50 mL) was added 2-methyl-1-nitro-2-thiopseudourea (305 mg, 2.20 mmol) and the mixture stirred at room temperature for 72 h. The solvent

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was evaporated and the residue chromatographed on silica gel by using a gradient elution of 5 → 12% MeOH in $CHCl_3$. The product **27** was obtained as a white foam (0.82 g, 48.8%). Anal. C, H, N, Cl.

BNMA-Lys(C=NCN(SCH$_3$))-Sta-AEM (28). By procedures similar to those used in the synthesis of compound **21**, **28** was obtained as a white foam (0.55 g, 39% final step). Anal. C, H, N.

BNMA-Lys(Z)-Sta-Leu-NHCH$_2$Ph (29). By procedures similar to those used for the synthesis of compound **4** but substituting Leu-$NHCH_2Ph$ instead of 2(*S*)-methylbutylamine, the title compound **29** was obtained as a white foam (5.50 g, 65.7% final step). Anal. C, H, N.

BNMA-Lys(C=S(NHCH$_3$))-Sta-Leu-NHCH$_2$Ph (30). By procedures similar to those for the synthesis of **17**, the title compound **31** was obtained as a white foam (1.20 g, 70.3% final step). Anal. C, H, N.

BNMA-Lys(C=NH(NHNO$_2$))-Sta-Leu-NHCH$_2$Ph (31). By procedures similar to those used for the synthesis of **27**, the title compound **31** was obtained as a white foam (0.50 g, 43.3% final step). Anal. C, H, N.

Preparation of (*S*,*S*)-γ-Amino-β-hydroxy-*N*-[2-(4-morpholinyl)ethyl]cyclohexanepentanamide (ACHPA-AEM). The title compound was prepared according to the literature procedure recently reported.[47]

BNMA-Lys(C=S(NHCH$_3$))-ACHPA-AEM (32). Preparation for BNMA-Lys-ACHPA-AEM as described for **17** and purification by chromatography on silica gel eluting with 3% MeOH in $CHCl_3$ gave the title compound **32** as a white foam (380 mg, 30% final step): FAB MS [M + H] *m/z* 851.6 (852). (HPLC analysis indicated 88% purity.)

BBPS-Lys(C=S(NHCH$_3$))-ACHPA-AEM (33). Substituting (+)-2-benzyl-3-(*tert*-butylsulfonyl)propionic acid[49] for bis(1-naphthylmethyl)acetic acid and ACHPA for Sta, title compound **33** was prepared by methods similar to those used for compound **17**. After purification by chromatography on silica gel, eluting with 2 → 8% gradient of MeOH in $CHCl_3$, the product **33** was obtained as a white foam (1.08 g, 61% final step). The compound was converted to the citrate salt for analysis. Anal. C, H, N.

Compound 34. By using the procedures for the synthesis of **17** substituting 2-(1-naphthylmethyl)-3-(morpholinocarbonyl)-propionic acid[50,51] for bis(1-naphthylmethyl)acetic acid and ACHPA for Sta, title compound **34** was obtained: FAB MS [M + H] *m/z* 838.3 (838). Anal. C, H, N, S.

Compound 35. Title compound **35** was prepared (0.35 g, 41%) as a white foam according to procedures similar to those for **17**, substituting ACHPA-AEM for Sta-$NHCH_2CH(CH_3)CH_2CH_3$. Anal. C, H, N.

Compound 36. Title compound **36** (0.52 g, 77.6% final acylation) was prepared according to procedures described for **17**. Anal. C, H, N, S.

Compound 37. Title compound **37** (1.19 g, 88.5% final acylation) was prepared according to procedures described for **27**. Anal. C, H, N; N: calcd, 14.87; found, 13.81.

BNMA-Lys(C=S(NHCH$_3$))-ACHPA-NHCH$_2$CH(CH$_3$)-CH$_2$CH$_3$ (38). Title compound **38** was prepared in 69.5% yield according to the procedures described for **17**. Anal. C, H, N.

BNMA-Lys(C=S(NHCH$_3$))-Sta-N(OCH$_3$)CH$_3$ (39). Title compound **39** was prepared as a white foam (1.20 g, 95%) by using methods described previously for **17** starting with the *O*,*N*-dimethylamide of BOC-Sta (prepared by reaction of *O*,*N*-dimethylamine hydrochloride salt and BOC-Sta in the presence of carbonyldiimidazole (1 equiv) and Et_3N (1 equiv) at 0 °C): FAB MS [M + H] *m/z* 742.2 (742). Anal. C, H, N, Cl, S; S: calcd, 4.20; found, 4.72. (HPLC: 70:30 → 0:100 H_2O/CH_3CN, 20 min; Vydac column; 1.5 mL/min; t_R 8.04 min).

BNMA-Lys(C=S(NHCH$_3$))DFSta-AEM (40). Compound **40** was obtained in 55% yield by procedures described previously for **17**: FAB MS [M + H] *m/z* 847.3 (847). Anal. C, H, N, F.

Preparation of *N*-[(Dimethylamino)sulfonyl]-L-phenylalanine. A solution of phenylalanine (3.30 g, 0.02 mol) in 1 N NaOH (20 mL) was treated with a solution of *N*,*N*-dimethylsulfonyl chloride (2.30 mL, 0.02 mol) in THF (20 mL) and stirred vigorously at 25 °C for 3 h. The reaction mixture was then treated with additional 1 N NaOH (20 mL) and *N*,*N*-dimethylsulfonyl chloride (2.30 mL, 0.02 mol) and stirred 3 h further at 25 °C. Finally 1 N NaOH (20 mL) and Et_2O (80 mL) were added. The mixture was shaken and the aqueous layer separated and acidified to pH 1 by addition of 1 N HCl (25 mL). The product was extracted into EtOAc, and the solution was dried ($MgSO_4$) and evaporated to a gum which slowly solidified (4.0 g, 69.0%); ^1H NMR (90 MHz, $CDCl_3$) 8.7 (1 H, br), 7.3 (5 H, m), 5.0 (1 H, d), 4.3 (1 H, m), 3.1 (2 H, AB of ABX), 2.6 (6 H, s).

***N*-[(Dimethylamino)sulfonyl]-L-Phe-Lys(C=S-(NHCH$_3$))ACHPA-AEM (41).** By methods described previously for **17**, **41** was obtained as a white foam (0.58 g, 25%) after column chromatography on silica gel eluting with 0 → 6% MeOH in $CHCl_3$: FAB MS [M + H] *m/z* 783.6 (783). Anal. C, H, N, S. (HPLC: 70:30 → 0:100 H_2O/CH_3CN, 20 min; Vydac column RPC18; flow rate 1 mL/min; 100% purity; t_R 12.43 (59%) and t_R 13.22 (41%).

***N*-(4-Morpholinylsulfonyl)-Phe-Lys(C=S(NHCH$_3$))-ACHPA-AEM (42).** By methods described previously for **17**, **42** was obtained as a white foam (0.82 g, 63%) after purification by column chromatography on silica gel eluting with 7% MeOH in CH_2Cl_2. Anal. C, H, N, S.

***N*-(4-Morpholinylsulfonyl)-Phe-Lys(Z)-DFO-AEM (43).** To oxalyl chloride (64.4 μL, 0.74 mmol) in dry CH_2Cl_2 (5 mL) at −70 °C was added DMSO (106 μL, 1.50 mmol) and the solution stirred for 10 min. The difluorostatine analogue (0.50 g, 0.57 mmol) in CH_2Cl_2 (5 mL) was added and the solution stirred at −60 to −50 °C for 30 min following by warming to −20 °C for a further 45 min. After the solution was recooled to −60 °C, dry TEA (394 μL, 2.84 mmol) was added and the reaction warmed to room temperature slowly. Dilution with CH_2Cl_2 and washing with saturated $NaHCO_3$ solution was followed by extraction of the aqueous layer with further portions of CH_2Cl_2. The combined organic extracts were washed with brine and dried ($MgSO_4$). Filtration, concentration under reduced pressure, and chromatography on silica gel (5% → 10% MeOH in $CHCl_3$) gave the product **42** as a mixture of diastereoisomers (378 mg, 75.6%): FAB MS (thioglycerol) [M + H] *m/z* 880.4 (880), [M + 109] 988.3. Anal. C, H, N, F, Cl.

***N*-(4-Morpholinylsulfonyl)-Phe-His-ACHPA-AEM (44).** A mixture of His(trit)-ACHPA-AEM (1.13 g, 3.6 mmol), HOBT·H_2O (0.50 g, 3.6 mmol), and DCC (0.74 g, 3.6 mmol) was dissolved in DMF (15 mL) and stirred at room temperature for ca. 15 min until a precipitate appeared. A solution of *N*-[(4-morpholinylamino)sulfonyl]-L-phenylalanine (2.47 g, 3.5 mmol) in CH_2Cl_2 (15 mL) was then added, and the reaction was stirred for 64 h at room temperature. The resulting suspension was concentrated to remove CH_2Cl_2, and the concentrate was filtered, rinsing the solid with EtOAc (30 mL). The filtrate and washings were evaporated in vacuo. Flash chromatography of the residue on silica gel, eluting with $CHCl_3$/MeOH (98:2), gave the product which was dissolved in CH_2Cl_2, evaporated to a foam, and dried to constant weight at 0.5 mmHg, room temperature to afford 2.5 g (71.3%) of *N*-(4-morpholinylsulfonyl)-Phe-His(trit)-ACHPA-AEM (TLC, $CHCl_3$/MeOH 9:1 R_f 0.55): MS (FAB) [M + H] *m/z* 1003.2 (1003). This material was used without further purification.

A solution of the previous intermediate (2.38 g, 2.37 mmol) in 80% HOAc (20 mL) was heated on a steam bath until the solution temperature was ≥90 °C for 3 min. Distilled H_2O (60 mL) was then added, and the mixture was cooled to room temperature. Solid was removed by filtration, rinsing with 10% HOAc. The combined filtrate and washings were evaporated to a syrup which was redissolved in distilled H_2O (75 mL), filtered, concentrated to 40 mL, frozen, and lyophilized to give the title compound **44** (1.83 g, 87.4%) as a colorless powder: MS (FAB) [M + H] *m/z* 761.4 (761). Anal. C, H, N. (HPLC 99.4% purity).

***N*-(4-Morpholinylsulfonyl)-Phe-Lys(C=SNHCH$_3$)-FCS-AEM (45).** Title compound **45** was prepared by methods similar to those used in the preparation of **40** and **44** but with ethyl 4(*S*)-[(*tert*-butyloxycarbonyl)amino]-5-cyclohexyl-2,2-difluoro-

(49) Bohlmayer, P.; Stanton, J. L.; Fuhrer, W.; Goschke, R.; Rasetti, V.; Rueger, H.; EP 236,734, 1987.
(50) Hirada, H.; Yamaguchi, T.; Iyobe, A.; Tsubaki, A.; Kamijo, T.; Iizuka, K.; Ogura, K.; Kiso, Y. *J. Org. Chem.* **1990**, *55*, 1679.
(51) Inoguchi, K.; Morimoto, T.; Achiwa, K. *J. Organometal. Chem.* **1989**, *370* (1-3), C9.

3(*R*)-hydroxypentanoate[31] used as an intermediate. Anal. C, H, N, S. (HPLC: one peak 95.2% purity.)

BNMA-NHCH($CH_2CH_2NHCOCH_3$)CO-Sta-NHCH$_2$CH(CH_3)CH_2CH_3 (46). Title compound **46** was prepared from **13** by deprotection according to general procedure D (b) (0.42 g, 100%) followed by treatment with Ac_2O (2 mL), H_2O (10 mL), and NaOAc·$3H_2O$ (82 mg, 0.60 mmol) for 1.5 h. Dilution with EtOAc was followed by washing of the organic layer with 5% aqueous NaOH solution. After drying (Na_2SO_4) and evaporation, the crude product was chromatographed on silica gel with 3 → 5% MeOH in $CHCl_3$ as eluent to afford **45** as a white solid (320 mg, 75.6%): FAB MS [M + H] *m/z* 709.4 (709). Anal. C, H, N.

BNMA-NHCH(CH_2CH_2NHC=S($NHCH_3$))CO-Sta-NHCH$_2$CH(CH_3)CH_2CH_3 (47). Title compound **47** was prepared from **13** by deprotection according to general procedure D (b) followed by treatment with methyl isothiocyanate as described previously for the preparation of **17**: FAB MS [M + H] *m/z* 740.3 (740). Anal. C, H, N. (HPLC: 70:30 → 0:100 H_2O/CH_3CN, 20 min; 91% purity two isomers, t_R 12.03 min (47%) and t_R 12.79 min (44%)).

1,1-Dimethylethyl [1*S*-[1*R(*RS*),2*R**(*R**)]]-[1-[[[2-Hydroxy-4-[(2-methylbutyl)amino]-1-(2-methylpropyl)-4-oxobutyl]amino]carbonyl]-4-(1*H*-pyrrol-1-yl)butyl]carbamate.** The title compound was prepared according to procedure A by coupling **6** with Sta-NHCH$_2$CH(CH_3)CH_2CH_3·HCl: $[\alpha]^{23}_D$ = +3.75° (*c* 0.64%, EtOH); mp 136–139 °C.

***N*-[2(*S*)-Hydroxy-4-[(2(*S*)-methylbutyl)amino]-1-(2-methylpropyl)-4-oxobutyl]-α-[[3-(1-naphthalenyl)-2-(1-naphthalenylmethyl)-1-oxopropyl]amino]-1*H*-pyrrole-1-pentanamide (48).** The above compound was deprotected according to procedure D followed by coupling with BNMA-OH according to procedure B.

Purification by chromatography on silica gel, eluting with EtOAc/hexane (1:1), gave the product **48** as a white foam (0.40 g, 50.0%): FAB MS [M + H] *m/z* 730.9 (731). Anal. C, H, N.

BNMA-NHCH((CH_2)$_5$NHC=S(NHMe))-Sta-NHCH$_2$CH(CH_3)CH_2CH_3 (49). Compound **49** (0.30 g, 74.4%) was obtained from **9** by methods described previously for **17** as a white foam after column chromatography on silica gel with 2% MeOH in CH_2Cl_2 as eluent: FAB MS [M + H] *m/z* 782.5 (783). Anal. C, H, N. (HPLC: 70:30 → 0:100 H_2O/CH_3CN, 20 min; 95% purity two isomers, t_R 12.48 min (41%) and t_R 13.16 min (54%).

BNMA-NHCH((CH_2)$_5$NH(C=NH)$NHNO_2$)CO-Sta-NHCH$_2$CH(CH_3)CH_2CH_2 (50). Compound **50** was prepared as a white foam (0.40 g, 81%) from **9** by procedures similar to those described for **49** and **27**. Anal. C, H, N.

BNMA-NHCH((CH_2)$_6$NHC=S($NHCH_3$))-Sta-NHCH$_2$CH(CH_3)CH_2CH_3 (51). Compound **51** was prepared as a white foam (0.34 g, 68.7%) by procedures similar to those described for **49** and **17**. Anal. C, H, N.

Diethyl 2-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-[4-[[(phenylmethoxy)carbonyl]amino]-2-butynyl]propanedioate. [BOCNHC(CH_2C≡CCH_2NHZ)(CO_2Et)$_2$]. Sodium hydride (0.33 g, 6.90 mmol, 50% in mineral oil) was washed free of oil and suspended in DMSO (15 mL). Diester **7** (1.90 g, 6.8 mmol) was added and the suspension stirred until evolution of hydrogen had ceased. 4-Chloro-Z-but-2-ynlamine (1.80 g, 6.60 mmol) and KI (2.0 g) were then added and the reaction stirred overnight at room temperature. The solution was diluted with EtOAc and washed sequentially with H_2O and saturated NaCl solution. Drying (Na_2SO_4) and removal of the solvent under reduced pressure gave the crude product which was chromatographed on silica gel, with hexane/EtOAc (9:1) as eluent. The product was obtained as a viscous oil (2.50 g, 77.2%) used directly in subsequent reactions: ^{1}H NMR ($CDCl_3$, 60 MHz) δ 1.10 (6 H, t, *J* = 6.4 Hz), 1.32 (9 H, s), 3.10 (2 H, m), 3.70–4.10 (2 H, m), 4.15 (4 H, dd, *J* = 6.4, 14 Hz), 5.05 (2 H, s), 5.95 (1 H, br s), 7.25 (5 H, s).

BNMA-NHCH(CH_2C≡CCH_2NHZ)-Sta-NHCH$_2$CH(CH_3)CH_2CH_3 (52). Title compound **52** was prepared from the previous compound BOCNHC(CH_2C≡CCH_2NHZ)(CO_2Et)$_2$ after hydrolysis by using methods similar to those for analogue **49** described previously: FAB MS [M + H] *m/z* 825.7 (826). Anal. C, H, N; C: calcd, 53.57; found, 53.04.

BNMA-NHCH(CH_2C≡CCH_2NHC(=S)$NHCH_3$)-Sta-NHCH$_2$CH(CH_3)CH_2CH_3 (53). Title compound **53** was prepared by treatment of **52** (0.20 g) in AcOH (mL) with 30% HBr/AcOH (0.25 mL). After 2 h the reaction was evaporated and eluted through a pad of silica gel by employing CH_2Cl_2 followed by EtOAc to obtain starting **52** (0.10 g). Further dilution with EtOAc/MeOH/Et_3N (8:1:1) gave the amine (0.10 g). This was dissolved in $CHCl_3$ and treated with methyl isothiocyanate (17 mg). By following the procedure for preparation of **17** compound **53** was obtained as a white solid in 29% overall yield. [HPLC: 70:30 → 0:100 H_2O/CH_3CN, 20 min; mixture of diastereoisomers t_R 12.41 (57%) and t_R 13.20 min (42.3%).] Anal. C, H, N.

BNMA-NHCH(CH_2CH=^{Z}=$CHCH_2NHCOCH_3$)CO-Sta-NHCH$_2$CH(CH_3)CH_2CH_3 (54). A solution of **55a** (0.43 g, 0.59 mmol) in MeOH (70 mL) and pyridine (5 mL) was treated with 5% Pd/$CaCO_3$ (40 mg) and shaken under an atmosphere of hydrogen until the calculated quantity of hydrogen had been taken up. The mixture was then filtered and the solvent removed under reduced pressure. The residue was taken up in EtOAc and washed twice with 1 N HCl, then H_2O, saturated aqueous $NaHCO_3$ solution, and finally saturated NaCl solution. Concentration followed by addition of hexane and filtration gave completely reduced material (203 mg, 46.8%). Stripping the filtrate to dryness and trituration with hexane gave the unsaturated product **54** as a white solid (78 mg, 18.0%). Anal. C, H, N; N: calcd, 7.46; found, 8.00.

BNMA-NHCH(CH_2C≡$CCH_2NHCOCH_3$)CO-Sta-NHCH$_2$CH(CH_3)CH_2CH_3 (55a,b). A solution of DNMA-NHCH(CH_2C≡$CCH_2NHCOCH_3$)CO_2H (1.70 g, 3.4 mmol) was coupled with Sta-NHCH$_2$CH(CH_3)CH_2CH_3 according to general procedure B to afford a crude mixture of **55a** and **55b**. Addition of EtOAc to the residue obtained caused the less soluble isomer to precipitate (**55a**). Filtration and drying of the solid gave **55a**, mp 164–166 °C. Anal. C, H, N. (HPLC: 70:30 → 0:100 H_2O/CH_3CN, 20 min; t_R 12.38 min 89%.) The remaining filtrate was washed with 1 N HCl, H_2O, saturated $NaHCO_3$, and brine, respectively. Drying (Na_2SO_4) and evaporation under reduced pressure gave a yellow foam. Chromatography on silica gel eluting with 5% MeOH in $CHCl_3$ gave the product **55b** as a white foam. Anal. C, H, N. (HPLC: 70:30 → 0:100 H_2O/CH_3CN, 20 min; two isomers; t_R 11.73 min (76%) and t_R 12.40 min (12%).)

BNMA-NHCH(CH_2C≡CCH_2NHBOC)CO-ACHPA-AEM (56). A solution of BNMA-NHCH(CH_2C≡CCH_2NHBOC)CO_2H (prepared by procedures described) (5.00 g, 8.90 mmol) was coupled with ACHPA-AEM (2.90 g, 8.90 mmol) according to general procedure B to afford **56** after chromatography on silica gel eluting with 3% MeOH in $CHCl_3$ (4.50 g, 57.8%). Anal. C, H, N.

BNMA-NHCH(CH_2C≡CCH_2NHC=S($NHCH_3$))CO-ACHPA-AEM (57). Title compound **57** was prepared from **56** by deprotection according to general procedure D followed by treatment with methyl isothiocyanate according to the method described for analogue **17**. The product **57** (0.59 g, 55%) was converted to its citrate salt and lyophilized. Anal. C, H, N, Cl.

BNMA-NHCH(CH_2C≡CCH_2NH(C=NH)($NHNO_2$))CO-ACHPA-AEM (58). Title compound **58** was prepared from **56** by deprotection according to general procedure D followed by treatment with 2-methyl-1-nitro-2-thiopseudourea according to the method described for compound **27**. The compound (0.78 g, 77.5%) was converted to its citrate salt and lyophilized. Anal. C, H, N, Cl.

BNMA-NHCH(CH_2C≡$CCH_2NHCOCH_3$)CO-ACHPA-AEM (59). Compound **59** was prepared from **56** by deprotection according to general procedure D followed by treatment with 1-acetylimidazole (1.1 equiv) and TEA (2 equiv) in CH_2Cl_2. After stirring for 16 h, the solvent was removed under reduced pressure and the residue taken up in EtOAc. Washing with saturated $NaHCO_3$, H_2O, and brine, drying (Na_2SO_4), and evaporation gave the crude product **59**. Chromatography on silica gel, eluting with 5% MeOH in $CHCl_3$, gave pure **59** (0.76 g, 67.2%) as a white foam, converted to its citrate salt and lyophilized. Anal. C, H, N.

BNMA-NHCH(CH_2C≡CCH_2NHCHO)CO-ACHPA-AEM (60). Compound **60** was prepared from **56** by deprotection according to general procedure D followed by treatment with formic acetic anhydride (1 equiv) and TEA (1 equiv) at 0 °C. After stirring overnight the crude product was chromatographed on silica

gel, eluting with 3% MeOH in $CHCl_3$. The product **60** (0.67 g, 65%) was converted to its citrate salt and lyophilized to a white foam. Anal. C, H, N, Cl; N: calcd, 6.70; found, 6.18.

***N*-(4-Morpholinylsulfonyl)-Phe-NHCH($CH_2C{\equiv}CCH_2NHBOC$)CO-ACHPA-AEM (61).** A solution of the diester **15** (9.82 g, 15.4 mmol) in dioxane (45 mL) and ethanol (45 mL) was treated with 2 N NaOH (25 mL, 48.0 mmol) and stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue taken up in H_2O and washed twice with Et_2O. The aqueous phase was brought to pH 2.5 and extracted twice with EtOAc. The combined EtOAc extracts were washed with saturated NaCl solution and dried ($MgSO_4$). Removal of the solvent under reduced pressure left an oil which was dissolved in dioxane (100 mL) and toluene (100 mL) and heated at reflux for 3 h. Evaporation under reduced pressure gave the amino acid as a golden brown oil (7.80 g, 94.2%) used directly in the next reaction: FAB MS [M + H − BOC] m/z 438.8 (439).

A solution of the acid (5.00 g, 9.30 mmol), HOBT (1.26 g, 9.30 mmol), and ACHPA-AEM (3.04 g, 9.30 mmol) in DMF (50 mL) was cooled in ice and treated with a solution of DCC (1.94 g, 9.30 mmol) in DMF (10 mL). After stirring at 0 °C for 0.5 h and at room temperature overnight the mixture was filtered and solvent evaporated under reduced pressure. The residue was taken up in EtOAc and washed with saturated $NaHCO_3$ solution and brine. After drying ($MgSO_4$), the solvent was concentrated. A solid precipitated and was collected by filtration and washed with EtOAc. A single diastereomer **61a** (1.65 g) was identified: FAB MS [M] m/z 848.3 (848). Anal. C, H, N; H: calcd, 7.33; found, 8.00.

The filtrate was evaporated and the residue chromatographed on silica gel with 4% MeOH in $CHCl_3$ as eluent to afford **61b** as a yellowish foam (2.55 g): FAB MS [M] m/z 848.2 (848). Anal. C, H, N.

***N*-(4-Morpholinylsulfonyl)-Phe-NHCH($CH_2C{\equiv}CCH_2NHC{=}S(NHCH_3)$)CO-ACHPA-AEM (62).** Title compound **62** was prepared from **61a** by deprotection according to general procedure D followed by treatment with methyl isothiocyanate according to the method described for **17** (to afford 260 mg, 27%): MS (FAB/thioglycerol) 821.6 (M + 1). The material was converted to the citrate salt and lyophilized. Anal. C, H, N.

***N*-(4-Morpholinylsulfonyl)-Phe-NHCH($CH_2C{\equiv}CCH_2NHCOCH_3$)CO-ACHPA-AEM (63).** Compound **63** (540 mg, 51%) was prepared from **61a** by deprotection according to general procedure D followed by treatment with acetylimidazole according to the method described for **59**: MS (FAB/thioglycerol) 790 (M^+). The material was converted to the citrate salt and lyophilized. Anal. C, H, N.

Ethyl 4(*S*)-[(*tert*-Butyloxycarbonyl)amino]-2,2-difluoro-3(*R*)-hydroxy-5-isopropylpentanoate. Activated zinc dust (325 mesh) was suspended in benzene and dried by distilling off the solvent. Under argon a suspension of the zinc dust (0.35 g, 5.35 mmol) in dioxane (15 mL) was treated with a crystal of iodine and ethyl bromodifluoroacetate (0.10 mL, 0.78 mmol). The mixture was subjected to ultrasonic conditions for 10 min. A solution of ethyl bromodifluoroacetate (0.55 mL, 4.28 mmol) and BOC-leucinal (0.49 g, 1.92 mmol) in dioxane (10 mL) was added over a 30-min period and the mixture subjected to ultrasonic conditions for 45 min. The mixture was poured in CH_2Cl_2 and washed with 1 N sodium bisulfite solution. Drying (Na_2SO_4) and removal of the solvent under reduced pressure gave the crude product. Chromatography on silica gel eluting with a gradient of 10% → 20% EtOAc in hexane gave the desired product (0.50 g, 76.9%) identical with previously reported material.[31a]

4(*S*)-[(*tert*-Butyloxycarbonyl)amino]-2,2-difluoro-3(*R*)-hydroxy-5-isopropylpentanoic Acid. A solution of the ester (5.20 g, 15.3 mmol) in the THF (30 mL) was treated with NaOH (0.62 g, 15.5 mmol in 15 mL of H_2O) and stirred for 4 h. The pH was brought to 2–3 with dilute HCl and the solution extracted three times with EtOAc. The combined organic extracts were washed with H_2O and brine. Drying (Na_2SO_4) and removal of the solvent under reduced pressure gave the acid (4.23 g, 88.7%) as a white foam which was used directly in subsequent reactions.

[4(*S*)-[(*tert*-Butyloxycarbonyl)amino]-2,2-difluoro-3(*R*)-hydroxy-5-isopropylpentanoyl]-2-(aminoethyl)morpholine. A solution of the previous acid (4.23 g, 13.6 mmol) and HOBT (1.84 g, 13.6 mmol) in DMF (50 mL) was cooled to 0 °C. DCC (2.81 g, 13.6 mmol) in DMF (10 mL) was added followed by 4-(2-aminoethyl)morpholine (1.77 g, 13.6 mmol). After stirring for 1 h at 0 °C and room temperature for 16 h, the mixture was filtered and the filtrate evaporated under reduced pressure. The residue was then taken up in EtOAc and washed with 1 N NaOH, followed by brine. Drying (Na_2SO_4) and removal of the solvent under reduced pressure gave the crude product which was chromatographed on silica gel, with a gradient of 5% → 7% MeOH in $CHCl_3$ as eluent. The title compound was obtained as a white solid (3.87 g, 67.5%): MS (Cl + CH_4) [M + H] m/z 424 (424).

Boc-Lys(ε-Z)-DFSta-AEM. To a flask containing [4(*S*)-[(*tert*-butyloxycarbonyl)amino]-2,2-difluoro-3(*R*)-hydroxy-5-isopropylpentanoyl]-2-(aminoethyl)morpholine (3.30 g, 7.80 mmol) in $CHCl_3$ (100 mL) was passed a stream of HCl gas for 15 min. The solution was stirred for a further 20 min and then evaporated to dryness. The residue was redissolved in $CHCl_3$ and reevaporated, this procedure being repeated several times before drying of the foam under high vacuum. This foam (3.05 g) was coupled directly with (*N*-α-BOC-*N*-ε-Z)-lysine (2.96 g, 7.80 mmol) according to general procedure A. Chromatography of the product on silica gel eluting with 2 → 5% MeOH in $CHCl_3$ afforded the product as a white foam (3.82 g, 71.5%): FAB MS [M + H] m/z 686.3 (686). Anal. ($C_{33}H_{53}F_2N_5O_8$) C, H, N, F; F: calcd, 5.50; found, 6.20.

(ε-Z)Lys-DFSta-AEM. The title compound was prepared from (*N*-α-BOC-*N*-ε-Z)-Lys-DFSta-AEM in 92% yield by general procedure D: FAB MS (thioglycerol) [M + H] m/z 586.3 (586).

***N*-(4-Morpholinylsulfonyl)-Phe-Lys(Z)-DFSta-AEM.** The title compound was prepared by coupling (*N*-ε-Z)Lys-DFSta-AEM (1.50 g, 12.56 mmol) with (morpholinylsulfonyl)-L-phenylalanine (0.80 g, 2.56 mmol) according to general procedure A. The product was obtained as a white foam (2.0 g, 88.5%): FAB MS (thioglycerol) [M + H] m/z 882.22 (882). Anal. ($C_{52}H_{63}F_2N_5O_7 \cdot 0.8CHCl_3$) C, H, N, Cl, F; F: calcd, 4.18; found 4.68.

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Registry No. **1**, 104380-56-5; **2**, 100002-50-4; **2** (deprotected)·HCl, 100002-57-1; **3**, 119808-82-1; **4**, 119808-83-2; **5**, 119808-15-0; **6**, 119808-42-3; **7**, 132101-67-8; **8**, 132125-33-8; **9**, 132101-68-9; **10**, 122234-46-2; **11**, 132101-69-0; **12**, 132101-70-3; **13**, 132125-34-9; **14**, 127953-50-8; **15**, 77369-60-9; **16**, 127953-51-9; **17**, 119808-98-9; **18**, 119808-99-0; **19**, 119809-01-7; **20**, 119809-25-5; **21**, 119809-04-0; **22**, 119809-07-3; **23**, 119809-15-3; **24**, 119808-76-3; **25**, 132101-71-4; **26**, 132101-72-5; **27**, 132101-73-6; **28**, 132101-74-7; **29**, 119809-27-7; **30**, 119809-09-5; **31**, 119809-08-4; **32**, 132101-75-8; **33**, 132101-76-9; **34**, 132101-77-0; **35**, 132101-78-1; **36**, 132101-79-2; **37**, 132101-80-5; **38**, 119809-02-8; **39**, 132125-35-0; **40**, 132101-81-6; **41**, 124278-27-9; **42**, 124278-26-8; **43**, 124306-05-4; **44**, 132101-82-7; **45**, 124278-30-4; **46**, 132101-83-8; **47**, 132101-84-9; **48**, 119809-26-6; **49**, 119832-15-4; **50**, 119809-10-8; **51**, 119809-11-9; **52**, 119809-14-2; **53**, 132101-85-0; **54**, 132101-86-1; **55** (diastereomer 1), 132101-87-2; **55** (diastereomer 2), 132199-76-9; **56**, 132125-36-1; **57**, 132101-88-3; **58**, 132101-89-4; **59**, 132101-90-7; **60**, 132101-91-8; **61** (diastereomer 1), 132101-92-9; **61** (diastereomer 2), 132199-78-1; **62**, 132101-93-0; **63**, 132101-94-1; AEM, 2038-03-1; ACHPA-AEM, 124278-65-5; ZCl, 501-53-1; BNMA-OH, 2481-59-6; BNMA-Cl, 119808-26-3; Sta-MBA, 104597-05-9; Boc-DFSta-OEt, 96056-65-4; Boc-DFSta-OH, 108385-36-0; Boc-DFSta-AEM, 132125-38-3; Boc-Lys(Z)-OH, 2389-45-9; Boc-Lys(Z)-Sta-MBA, 119808-81-0; Boc-Orn-OH, 21887-64-9; Lys(Tos)-Sta-MBA, 119808-19-4; BNMA-Lys-Sta-AEM, 132101-96-3; BNMA-Lys-ACHPA-AEM, 132101-97-4; Boc-Sta-N(OMe)Me, 132101-98-5; Phe-OH, 63-91-2; Me_2NSO_2-Phe-OH, 124278-35-9; His(trit)-ACHPA-AEM, 132101-99-6; Boc-Lys(Z)-DFSta-AEM, 132102-01-3; Lys(Z)-DFSta-AEM, 132199-79-2; 2,5-$(MeO)_2$-THF, 696-59-3; (4-morpholinyl)-SO_2-Phe-OH, 124278-39-3; Nle[6-(1*H*-pyrrol-1-yl)]-Sta-MBA, 119808-92-3; (4-morpholinyl)-SO_2-Phe-His(trit)-ACHPA-AEM, 132125-37-2; Boc-Nva[5-(1*H*-pyrrol-1-yl)]-Sta-MBA, 119808-43-4; (4-morpholinyl)-SO_2-Phe-Lys(Z)-DFSta-AEM,

132199-80-5; (±)-BNMA-NHCH(COOH)$CH_2C{\equiv}CH_2$NH-Boc, 132102-00-2; (4-morpholinyl)-SO_2-Phe-(*R*)-NHCH(COOH)-$CH_2C{\equiv}CCH_2$NH-Boc, 132293-94-8; (4-morpholinyl)-SO_2-Phe-(*S*)-NHCH(COOH)$CH_2C{\equiv}CCH_2$NH-Boc, 132199-77-0; H_2NCH-$(COOEt)_2$·HCl, 13433-00-6; $MeSO_3(CH_2)_5NHZ$, 132101-95-2; $H_2N(CH_2)_5OH$, 2508-29-4; $ZNH(CH_2)_5OH$, 87905-98-4; $BrCH_2C$-H_2NH_2·HCl, 58861-74-8; MeNCS, 556-61-6; MeNCO, 624-83-9; PhNCS, 103-72-0; $ClP(O)(OPh)_2$, 2524-64-3; $(MeS)_2C{=}NCN$, 10191-60-3; (+)-(*S*)-*t*-$BuSO_2CH_2CH(CH_2Ph)COOH$, 114530-04-0; Me_2NSO_2Cl, 13360-57-1; $ZNHCH_2C{\equiv}CCH_2Cl$, 119808-58-1; Boc-$NHC(CH_2{\equiv}CCH_2NHZ)(COOEt)_2$, 119808-05-8; $BrCF_2COOEt$, 667-27-6; (*S*)-Boc-NHCH(*i*-Bu)CHO, 58521-45-2; 2-(1-naphthylmethyl)-3-(morpholinocarbonyl)propionic acid, 106868-58-0; renin, 9015-94-5.

Supplementary Material Available: CAS nomenclature for target renin inhibitors (7 pages). Ordering information is given on any current masthead page.