the bonds $P(sp^3)$–$C(sp^2)$ and $S(sp^3)$–$O(sp^3)$ are not defined in this force field. We assigned the values for the corresponding stretching, bending, and torsional force constants by using an iterative minimization procedure in which the values for the force constants were adjusted so as to reproduce the crystallographic structures of related molecules (values available upon request). (iv) A multimolecule structural fitting was done by using the MAXIMIN MULTIFIT option of SYBYL. The MULTIFIT program performs a flexible fit between two or more molecules by selecting the atoms to be fitted. The fit is done by minimization of an energy expression that contains additional spring constants (20 kcal/mol Å²) between the fitted points, thus forcing atoms of different molecules to occupy the same place, while the geometry

of each molecule is simultaneously adjusted in order to relieve any strain. (v) The van der Waals surface and corresponding molecular volumes of the molecules were computed. Logical operations between volumes such as union, subtraction, and intersection were then performed in order to obtain certain regions of interest at the level of the receptor. (vi) The net atomic charges generating the electrostatic charge distribution of the molecules was obtained with the PM3 method[53] of the AMPAC package, which resulted in atomic charge values more in line with chemical intuition, as compared to other methods such as MNDO. The molecular electrostatic potential surfaces in the monopole–monopole approximation were then calculated as implemented in SYBYL according to the approach of Weiner et al.[52] and partitioned into three intervals as follows: −10 kcal/mol and down, from +10 to −10 kcal/mol, and +10 kcal/mol and up.

(59) Motoc, I.; Dammkoehler, R. A.; Mayer, D.; Labanowski, J. Three-dimensional quantitative structure–activity relationships. I. General approach to the pharmacophore model validation. *Quant. Struct.-Act. Relat.* 1986, 5, 99–105.

(60) Labanowski, J.; Motoc, I.; Naylor, C. B.; Mayer, D.; Dammkoehler, R. A. Three-dimensional quantitative structure–activity relationships. 2. Conformational mimicry and topographical similarity of flexible molecules. *Quant. Struct.-Act. Relat.* 1986, 5, 138–152.

(61) Vinter, J. G.; Davis, A.; Saunders, M. R. Strategic approaches to drug design. I. An integrated software framework for molecular modelling. *J. Comput.-Aided Mol. Des. Res.* 1987, 1, 31–51.

Structure–Activity Relationships of a Series of 2-Amino-4-thiazole-Containing Renin Inhibitors

William C. Patt,[*,†] Harriet W. Hamilton,[†] Michael D. Taylor,[†] Michael J. Ryan,[‡] David G. Taylor, Jr.,[‡] Cleo J. C. Connolly,[†] Annette M. Doherty,[†] Sylvester R. Klutchko,[†] Ila Sircar,[†] Bruce A. Steinbaugh,[†] Brian L. Batley,[‡] Christopher A. Painchaud,[‡] Stephen T. Rapundalo,[‡] Barbara M. Michniewicz,[§] and Stephen C. Olson[§]

Departments of Medicinal Chemistry, Pharmacology, and Pharmacokinetics and Drug Metabolism, Parke-Davis Pharmaceutical Research Division of Warner-Lambert Co., 2800 Plymouth Road, Ann Arbor, Michigan 48105. Received January 13, 1992

A series of renin inhibitors was synthesized that contained a 2-amino-4-thiazolyl moiety at the P₂ position. These derivatives are potent inhibitors of monkey renin in vitro and are selective in that they only weakly inhibit the closely related aspartic proteinase, bovine cathepsin D. Four compounds exhibited oral blood pressure lowering activity in high-renin normotensive monkeys. One of these compounds, 22 (PD 134672), was selected for further evaluation in renal hypertensive monkeys, on the basis of its superior efficacy and duration of action in the in vitro assays and the normotensive primate model.

Introduction

The renin–angiotensin system (RAS) has long been a target of the medicinal chemist for the treatment of hypertension in man.[1] The first event in this cascade is the cleavage of angiotensinogen by the aspartic proteinase renin to yield the decapeptide angiotensin I (AI). AI is then transformed by angiotensin converting enzyme (ACE) to produce the extremely potent vasoconstrictor angiotensin II (AII). Inhibitors of ACE are effective agents for treatment of hypertension and congestive heart failure.[2–4] More recently, selective AII receptor antagonists have been reported that may potentially be useful as antihypertensive agents.[5]

Each point of attack for inhibition of the renin–angiotensin system has advantages and disadvantages. For example, angiotensinogen is the only known natural substrate for renin. Thus, renin inhibitors may produce a highly selective inhibition of the RAS, leading to an improved side-effect profile for therapeutic agents.[6,7] Nu-

(1) For recent review, see: Greenlee, W. J. Renin Inhibitors. *Med. Res. Rev.* 1990, 10, 173–236.

(2) Kostis, J. B. Angiotensin-Converting Enzyme Inhibitors. *Am. J. Hypertens.* 1989, 2, 57–64.

(3) Brunner, H. R.; Nussberger, J.; Waeber, B. The Present Molecules of Converting Enzyme Inhibitors. *J. Cardiovasc. Pharmacol.* 1985, 7 (Suppl. 1), S2–S11.

(4) Johnston, C. I. Angiotensin Converting Enzyme Inhibitors. In *Handbook of Hypertension Vol. 11 Antihypertensive Drugs*; Doyle, A. E., Ed.; Elsevier: Amsterdam, 1988; pp 301–326.

(5) Wong, P. C.; Chiu, A. T.; Price, W. A.; Thoolen, M. J. M. C.; Carini, D. J.; Johnson, A. L.; Taber, R. I.; Timmermans, P. B. M. W. M. Nonpeptide Angiotensin II Receptor Antagonists. I. Pharmacological Characterization of 2-n-butyl-4-chloro-1-(2-chlorobenzyl)imidazole-5-acetic acid, Sodium Salt (S-8307). *J. Pharmacol. Exp. Ther.* 1988, 247, 1–7.

---

†Department of Medicinal Chemistry.
‡Department of Pharmacology.
§Department of Pharmacokinetics and Drug Metabolism.

(R,S)-ATG **1**   (S)-ATM **2**   (S)-ATE **3**   SMO-PHE **4**

SMO-TYR(ME) **5**   SPI-PHE **6**   SMO-THIQ **7**   SMO-DMTQ **8**

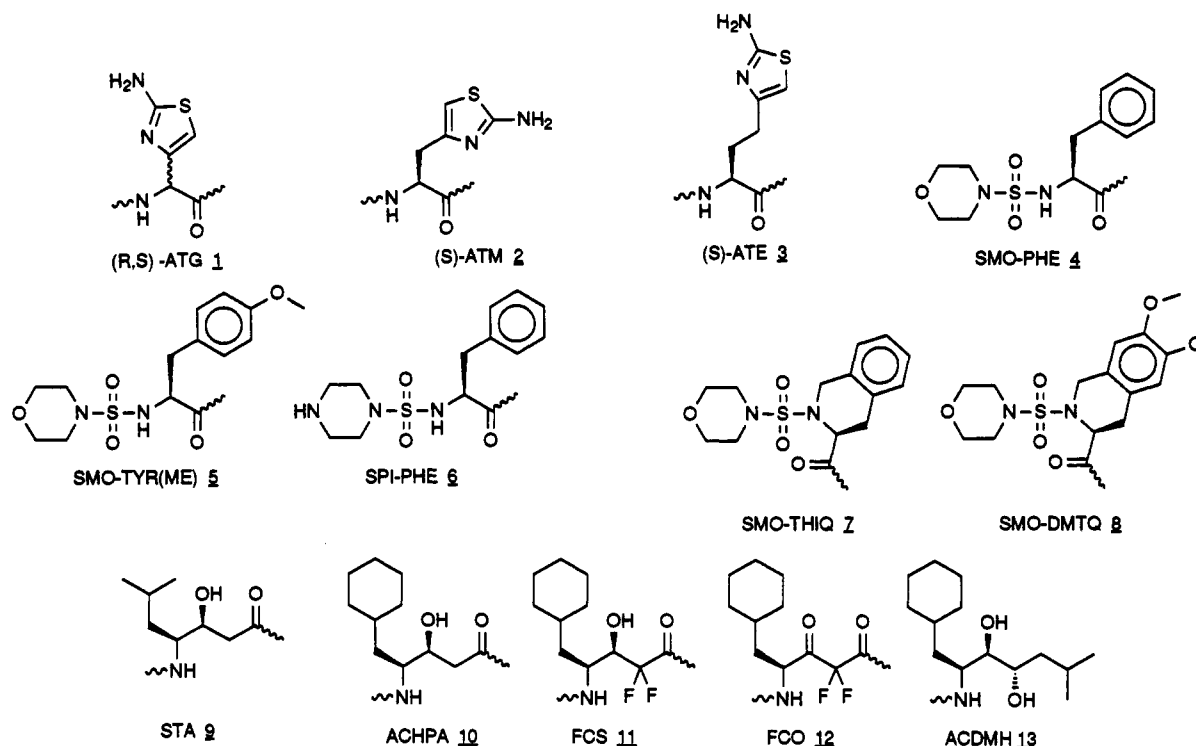STA **9**   ACHPA **10**   FCS **11**   FCO **12**   ACDMH **13**

Figure 1.

merous groups have reported renin inhibitors with substantial in vitro potency, enzyme selectivity, and a degree of oral activity.[8–17] However, the clinical utility of these

(6) Haber, E. Why Renin Inhibitors? *J. Hypertens.* **1989**, 7 (Suppl. 2), S81–S86.

(7) Kokubu, T.; Hiwada, K. Human Renin Inhibitors. *Drugs Today* **1987**, *23* (2), 101–108.

(8) Klienert, H. D. Renin Inhibitors: Discovery and Development (an overview and perspective). *Am. J. Hypertens.* **1989**, *2*, 800–808.

(9) 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, Tsau-Yen; 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.

(10) Johnson, R. L. Inhibition of Renin by Substrate Analogue Inhibitors Containing the Olefinic Amino Acid 5(S)-amino-7-methyl-3(E)-octenoic Acid. *J. Med. Chem.* **1984**, *27*, 1351–1354.

(11) Luly, J. R.; Bolis, G.; BaMaung, N.; Soderquist, J.; Dellaria, J. F.; Stein, H.; Cohen, J.; Perun, T. J.; Greer, J.; Plattner, J. J. New Inhibitors of Human Renin That Contain Novel Lue-Val Replacements. Examination of the P1 Site. *J. Med. Chem.* **1988**, *31*, 532–539.

(12) Thaisrivongs, S.; Pals, D. T.; Kroll, L. T.; Turner, S. R.; Han, F.-S. Renin Inhibitors. Design of Angiotensinogen Transition-State Analogues Containing Novel (2R,3R,4R,5S)-5-amino-3,4-dihydroxy-2-isopropyl-7-methyl-octanoic acid. *J. Med. Chem.* **1987**, *30*, 976–982.

(13) Hui, K. Y.; Carlson, W. D.; Bernatowicz, M. S.; Haber, E. Analysis of Structure–Activity Relationships in Renin Substrate Analogue Inhibitory Peptides. *J. Med. Chem.* **1987**, *30*, 1287–1295.

(14) Allen, M. C.; Fuhrer, W.; Tuck, B.; Wade, R.; Wood, J. M. Renin Inhibitors. Synthesis of Transition-State Analogue Inhibitors Containing Phosphorus Acid Derivatives at the Scissile Bond. *J. Med. Chem.* **1989**, *32*, 1652–1661.

(15) Luly, J. R.; BaMaung, N.; Soderquist, J.; Fung, A. K. L.; Stein, H.; Klienert, H. D.; Marcotte, P. A.; Egan, D. A.; Bopp, B.; Merits, I.; Bolis, G.; Greer, J.; Perun, T. J.; Plattner, J. J. Renin Inhibitors. Dipeptide Analogues of Angiotensinogen Utilizing a Dihydroxyethylene Transition-State Mimic at the Scissle Bond to Impart Greater Inhibitory Potency. *J. Med. Chem.* **1988**, *31*, 2264–2276.

Scheme I

14   15

16
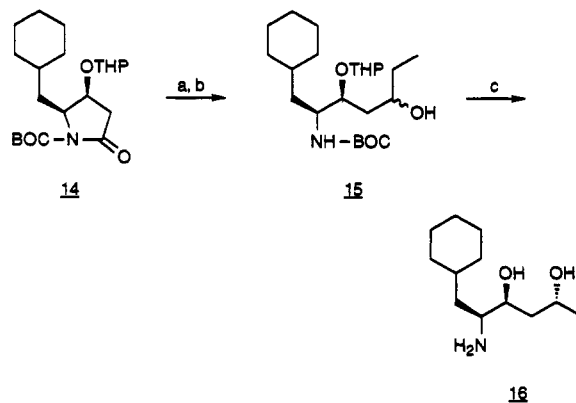
a = EtMgBr; b = KBH₄; c = TsOH, chromatog., HCl

agents has not yet been fully demonstrated. The principal obstacle to development of renin inhibitors is poor oral activity due to very low oral bioavailability.

Much of our synthetic effort in this area has focused on modifications at the P_2 position, which accommodates a wide range of substitutions while retaining in vitro potency.[18] A large number of potent and selective compounds contain histidine (HIS) at the P_2 position, which

(16) Luther, R. R.; Stein, H. H.; Glassman, H. N.; Klienert, H. D. Renin Inhibitors: Specific Modulators of the Renin-Angiotensin System. *Drug Res.* **1989**, *39*, 1–5.

(17) Repine, J. T.; Himmelsbach, R. J.; Hodges, J. C.; Kaltenbronn, J. S.; Sircar, I.; Skeean, R. W.; Brennan, S. T.; Hurley, T. R.; Lunney, E.; Humblet, C. C.; Weishaar, R. E.; Rapundalo, S.; Ryan, M. J.; Taylor, D. G., Jr.; Olsen, S. C.; Michniewicz, B. M.; Kornberg, B. E.; Belmont, D. T.; Taylor, M. D. Renin Inhibitors Containing Esters at the P₂-position. Oral activity in a Derivative of Methyl Aminomalonate. *J. Med. Chem.* **1991**, *34*, 1935–1943.

(18) The P site nomenclature refers to the amino acid residues or mimics in the inhibitor to corresponding amino acid residues in the natural substrate angiotensinogen. Schechter, I.; Berger, A. On the Size of the Active Site in Proteases. I. Papain. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157–162.

Scheme II
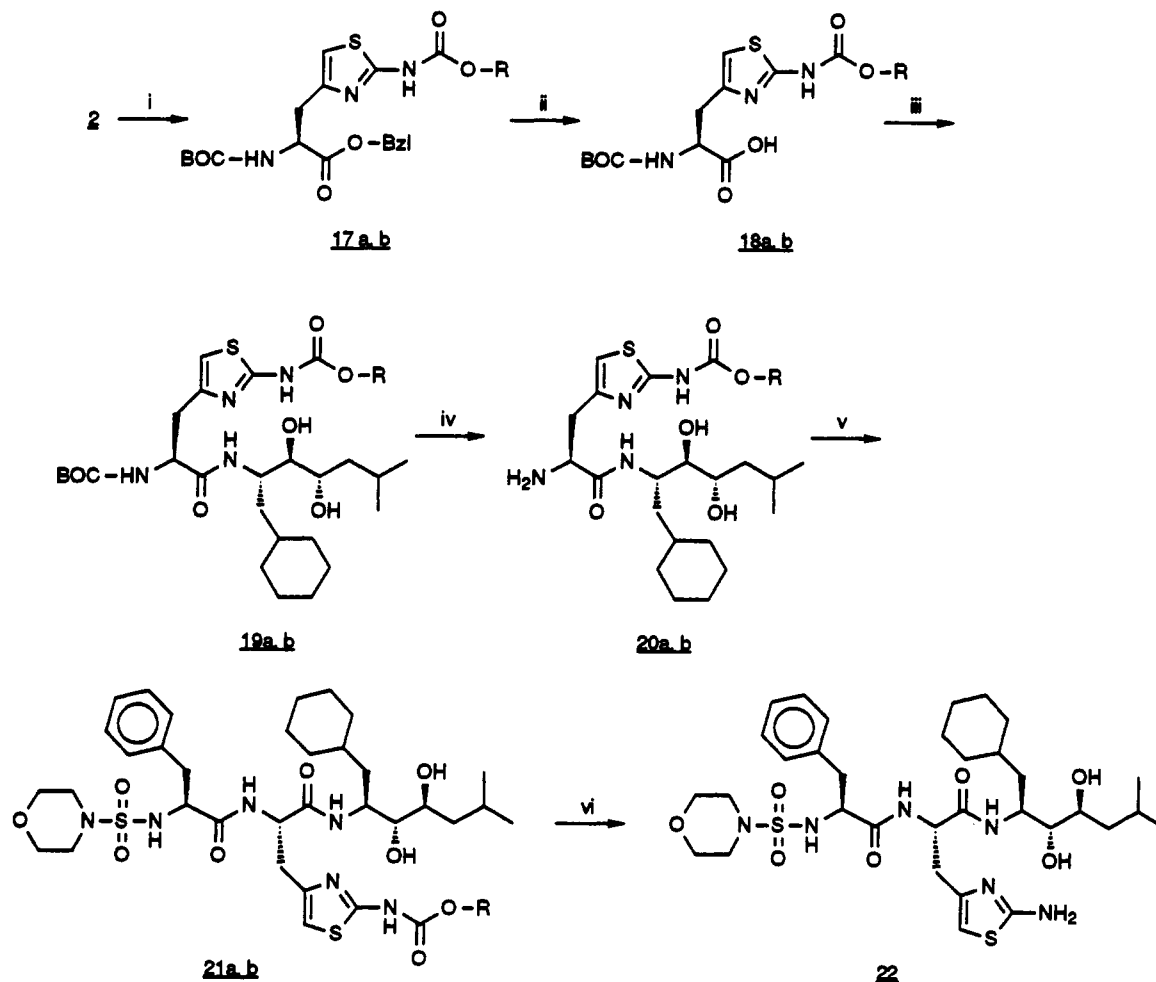
*R = CH$_2$C$_6$H$_5$; b, R = CH$_2$CCl$_3$; i = ROCOCl; ii = KOH; iii = ACDMH, HOBT, DCC; iv = HCl(g); v = SMO-PHE, HOBT, DCC; vi = a, Pd/C, H$_2$, p-TsOH·H$_2$O; b, Zn, NH$_4$Cl.

generally has led to compounds that were susceptible to proteolysis and were problematic to prepare due to the propensity of histidine to epimerize during coupling reactions. We and others observed that an aryl or heteroaryl group in the P$_2$ position maintained enzyme selectivity and increased stability.[19-23] Therefore, we investigated a variety of novel non-HIS "aryl" groups at the P$_2$ position.

One modification that showed promise was the weakly basic 2-amino-4-thiazolyl moiety. This group, which has been used extensively in the antiinfective field, is similar in size to the imidazole of histidine. Many compounds containing this substitution demonstrated both highly active and selective inhibition of primate renin. Several derivatives also exhibited blood pressure lowering activity following oral administration in high-renin normotensive monkeys. One derivative was further examined in renal hypertensive monkeys, and its efficacy was compared to several known renin inhibitor clinical candidates.

Chemistry

The synthesis of the 2-amino-4-thiazolyl containing amino acids 1–3 (Figure 1) were accomplished according to two published accounts.[24,25]

The literature preparation of N-(morpholinosulfonyl)-phenylalanine (4, SMO-PHE)[17] was modified for the synthesis of several novel P$_3$ groups which are shown in Figure 1: N-(morpholinosulfonyl)-O-methyltyrosine (5, SMO-TYR(Me)),[27] N-(piperazinylsulfonyl)phenylalanine (6,

(19) 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 Lue-Val Replacements. *J. Med. Chem.* **1987**, *30*, 1609–1616.

(20) Cumin, F.; Nisato, D.; Gagnol, J.-P.; Corvol, P. A Potent Radiolabeled Human Renin Inhibitor, [^{3}H]SR42128: Enzymatic, Kinetic, and Binding Studies to Renin and Other Aspartic Proteases. *Biochemistry* **1987**, *26*, 7615–7621.

(21) Rosenberg, S. H.; Plattner, J. J.; Woods, K. W.; Stein, H. H.; Marcotte, P. A.; Cohen, J.; Perun, T. J. Novel Renin Inhibitors Containing Analogues of Statine Retro-Inverted at the C-Termini: Specifically at the P$_2$ Histidine Site. *J. Med. Chem.* **1987**, *30*, 1224–1228.

(22) Doherty, A. M.; Kaltenbronn, J. S.; Hudspeth, J. P.; Repine, J. T.; Roark, W. H.; Sircar, I.; Tinney, F. J.; Connolly, C. J. C.; Hodges, J. C.; Taylor, M. D.; Batley, B. L.; Ryan, M. J.; Essenburg, A. D.; Rapundalo, S. T.; Weishaar, R. E.; Humblet, C. C.; Lunney, E. A. New Inhibitors of Human Renin that Contain Novel Replacements at the P$_2$ Site. *J. Med. Chem.* **1991**, *34*, 1258–1271.

(23) Thaisrivongs, S.; Mao, B.; Pals, D. T.; Turner, S. R.; Kroll, L. T. Renin Inhibitory Peptides. A β-aspartyl Residue as a Replacement for the Histidyl Residue at the P-2 Site. *J. Med. Chem.* **1990**, *33*, 1337–1343.

(24) Patt, W. C.; Skeean, R. W.; Steinbaugh, B. A. Synthesis of Enantiomerically Pure Amino Acids Containing a 2-Amino-4-thiazolyl Side Chain. *Synth. Commun.* **1990**, *20*, 3097–3102.

(25) Silberg, A.; Ruse, M.; Mantsch, H.; Csontos, S. Thiazolyl-aminosäuren, *Chem. Ber.* **1964**, *97*, 1767–1769.

(26) Klutchko, S.; O'Brien, P.; Hodges, J. C. Stereospecific Synthesis of S,S-Statine and Its Congeners. *Synth. Commun.* **1989**, *19*, 2573–2583.

Table I. IC$_{50}$ Values (nM) or Percent Inhibition at 10^{-x}

no.	(X)-(P$_3$)-(P$_2$)-(P$_1$P$_1'$)^{a,b}	renin^c	cathepsin D^d	cathepsin D/renin
22	SMO-PHE-ATA-ACDMH	0.36	1250	3472
23	SMO-PHE-ATE-ACDMH	4.10	834	203
24	SMO-PHE-(R,S)ATG-ACDMH	0.581	85.5	147
25	SMO-PHE-(R)ATA-ACDMH	33.0	14500	439
26	SMO-TYR(OME)-ATA-ACDMH	0.350	2170	6200
27	BOC-PHE-ATA-ACDMH	2.34	763	326
28	SPI-PHE-ATA-ACDMH	0.160	8900	55600
29	SMO-THIQ-ATA-ACDMH	2.70	30000	11100
30	IPP-PHE-ATA-ACDMH	0.91	33	36
31	SMO-DMTQ-ATA-ACDMH	40% at 10^{-5}	-2% at 10^{-4}	
32	SMO-PHE-ATA-ACHPA-AEM	3.40	37% at 10^{-4}	
33	SMO-PHE-ATA-ACHPA-BHEA	3.61	25000	6940
34	SMO-PHE-ATA-STA-MBA	13.7	1% at 10^{-7}	
35	SMO-PHE-ATA-CDH	0.27	N at 10^{-7}	
36	SMO-PHE-ATA-FCS-AEM	0.59	95000	161000
37	SMO-PHE-ATA-FCO-AEM	0.50	3700	7400
38	SMO-PHE-ATA-FCO-OET	0.54	6400	12000
39	SMO-PHE-HIS-ACDMH	0.23	51800	225000
40	SMO-PHE-TZA-ACDMH	0.20	130	650
41	SMO-PHE-PHE-ACDMH	0.48	270	560
42	SMO-PHE-AMA-ACDMH	0.28	35	130

^{a} IPP = ... AEM = ... BHEA = ... MBA = ... AMA = ...
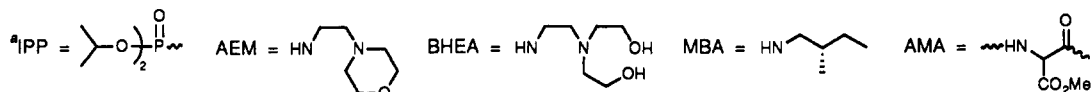
^{b} All fragments are the S configuration, as drawn in the text or as designated in table. ^{c} Monkey. ^{d} Bovine.

SPI-PHE),[27] N-(morpholinosulfonyl)-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid (7, SMO-THIQ), and the 6,7-dimethoxy derivative of SMO-THIQ (8, SMO-DMTQ).

The following P$_1$-P$_1'$ groups, also shown in Figure 1, were synthesized according to published reports: statine (9, STA),[26] cyclohexylstatine (10, ACHPA),[26] difluoro-cyclohexylstatine (11, FCS),[27] difluorocyclohexylstatone (12, FCO),[27] and (S)-2-amino-1-cyclohexyl-(R)-3(S),4-di-hydroxy-6-methylheptane (13, ACDMH).[28] A novel P$_1$-P$_1'$ group, 2-amino-3,5-dihydroxy-1-cyclohexylheptane (16, CDH), was synthesized as shown in Scheme I. The previously disclosed lactam intermediate,[26] 14, was reacted with ethylmagnesium bromide followed by reduction with potassium borohydride to give a doubly protected alcohol 15. This was in turn deprotected and purified by column chromatography to give a diastereomerically pure amine, 16.[43]

A general synthetic pathway to give compounds **22–38** (Table I) is shown in Scheme II and employs methods common to solution peptide synthesis. The exocyclic nitrogen of 2 was protected as either a benzyloxycarbonyl (Z) or trichloroethoxycarbonyl (Troc) group and the corresponding fully protected amino acids 17a,b were hydrolyzed with alcoholic hydroxide to give the intermediate acids, 18a,b. The acids 18a,b were coupled to a transition state mimetic amine, 13 (ACDMH) in this example, using N-hydroxybenzotriazole/dicyclohexylcarbodiimide (HOBT/DCC), yielding amides 19a,b. Amides 19a,b were then deprotected at the N-terminus with dry hydrochloric acid in an inert solvent to give the free amines, or salts, 20a,b, and coupled using HOBT/DCC to the acidic

function of the P$_3$ N-terminal group 4 (SMO-PHE). This gave the P$_2$ protected compounds 21a,b. The remaining protecting groups were then removed with either hydrogen and palladium on carbon (21a) or zinc/NH$_4$Cl (21b) to afford the compounds of interest **20–36**. The order in which the P site fragments were coupled could be reversed in many instances with little or no effect upon overall yield.

Results and Discussion

The first compounds synthesized in this series were prepared to determine which chain length and optical isomer of the 2-amino-4-thiazolyl amino acids would lead to the most potent and selective inhibitors of renin. In order to accomplish this, the P$_1$-P$_1'$ and the P$_3$ groups were kept constant and identical to those present in our renin inhibitor 42 (PD 132002). The P$_2$ position was then varied as (R,S)-2-amino-4-thiazolylglycine (ATG), (S)-2-amino-4-thiazolylalanine ((S)-ATA), (R)-2-amino-4-thiazolyl-alanine ((R)-ATA), and (S)-2-amino-4-(2-thiazolylethyl)-glycine (ATE). This effort produced compounds **22–25**. The (S)-ATA containing compound 22 was essentially equipotent with the ATG compound 24, with IC$_{50}$ values of 0.37 and 0.58 nM. Both 22 and 24 were superior to the (R)-ATA compound 25 (IC$_{50}$ = 33 nM) and 10-fold more potent than the homolog (S)-ATE compound 23 (IC$_{50}$ = 4.1 nM). Further examination of these compounds for selectivity against a similar aspartic proteinase, (bovine) cathepsin D,[29-31] revealed that 22 was 23-fold more selective

(27) Doherty, A. M.; Sircar, I.; Kornberg, B. E.; Quin, J., III; Winters, R. T.; Kaltenbronn, J. S.; Taylor, M. D.; Batley, B. L.; Rapundalo, S. R.; Ryan, M. J.; Painchaud, C. A. Design and Synthesis of Potent, Selective and Orally Active Fluorine-Containing Renin Inhibitors. *J. Med. Chem.* **1992**, *35*, 2–14.

(28) Luly, J. R.; Hsiao, C.-N.; BaMaung, N.; Plattner, J. J. A Convenient Synthesis of 1,2,3-Aminodiols from α-amino acids. *J. Org. Chem.* **1988**, *53*, 6109–6112.

(29) Aoyagi, T.; Morishima, Y.; Nishizawa, R.; Kunimoto, S.; Takeuchi, T.; Umezawa, H.; Ikezawa, H. Biological Activity of Pepstatins, Pepstanone A and Partial Peptides on Pepsin, Cathepsin D, and Renin. *J. Antibiot.* **1972**, *25*, 689–694.

(30) Kokubu, T.; Hiwada, K.; Nagae, A.; Murakami, E.; Morisaw, Y.; Yabe, Y.; Koike, H.; Iijima, Y. Statine-Containing Dipeptide and Tripeptide Inhibitors of Human Renin. *Hypertension* **1986**, *8* (Suppl. II), II-1 to II-5.

(31) Morisawa, Y.; Yabe, Y.; Kataoka, M.; Iijima, Y.; Kokubu, T.; Hiwada, K. New dipeptides useful for diagnosing and treating renin-angiotensin induced hypertension. EP 186,977, 1986.

Table II. Comparison of Orally Active Compounds Identified from SAR Studies, Normotensive Monkeys

no.	dose (mg/kg)	N	decrease in MABP (mmHg) at the time postdose		
			1 h	2 h	3 h
22	10	5	9 ± 3	18 ± 5	16 ± 5
24	30	2	2 ± 1	7 ± 7	12 ± 1
29	30	6	2 ± 3	10 ± 4	9 ± 3
37	10	3	11 ± 6	15 ± 8	15 ± 8

Table III. Comparison of P2 Substituted Analogues, Oral Data Normotensive Monkeys

no.	dose (mg/kg)	N	decrease in MABP (mmHg) at the time postdose		
			1 h	2 h	3 h
22	10	5	9 ± 3	18 ± 5	16 ± 5
39	30	2	7 ± 2	11 ± 1	8 ± 1
40	10	5	6 ± 1	9 ± 3	11 ± 4
41	10	2	5 ± 2	2 ± 1	1 ± 2

Table IV. Inhibition of Aspartic Proteases by Selected Renin Inhibitors

	IC_{50}, nM		
	renin inhibn[41]	cathepsin D inhibn[29]	pepsin inhibn[42]
22	0.36 ± 0.03	1250 ± 87	202000 ± 2000
enalkiren	0.76 ± 0.11	>100000	>100000
CGP-38560	0.83 ± 0.08	1380 ± 90	32700 ± 4400
CP-80794	0.26 ± 0.02	22.0 ± 6.5	170 ± 17
RO 42-5892	0.22 ± 0.04	22200 ± 1660	>10000
PD 132002	0.28 ± 0.02	35.9 ± 6.8	8000 ± 2900

Table V. Plasma Renin Inhibition by 22 in Different Species

species	inhibn of plasma renin activity (IC_{50}, nM ● SEM)
human	0.57 ● 0.06
monkey	0.37 ± 0.03
dog	1.96 ± 0.73
rat	199 ± 36.2

than 24 for renin in this screen. Bovine cathepsin D is employed here to estimate the potential selectivity of renin inhibitors for the human enzyme. Caution should be employed in interpreting the results since significant differences in inhibitor potency has been observed between bovine and human cathepsin D for some compounds.[45] The two most potent compounds, 22 and 24, were then tested in conscious high-renin normotensive cynomolgus monkeys.[22,32] Oral administration of 22 at a dose of 10 mg/kg lowered mean arterial blood pressure (MABP) by 18 mmHg, whereas 24 elicited a similar, albeit lower, response of 14 mmHg only when dosed at 30 mg/kg (Table II). Due to the greater selectivity of 22 for renin versus cathepsin D, an inability to easily synthesize 24 as a single diastereomer and most importantly the enhanced oral activity of 22 versus 24, the (S)-ATA moiety present in 22 was identified as the preferred P_2 fragment of choice.

In executing a search for the optimum P_3 fragment the P_2 and P_1-P_1' sections were held constant as (S)-ATA and ACDMH. Several highly potent compounds 26–30 were identified with IC_{50} values for renin inhibition ranging from 0.16 nM to 2.70 nM. All of these derivatives displayed over 300-fold selectivity for renin versus cathepsin D with one exception, the phosphonamide 30, in which only 36-fold selectivity was attained. From these derivatives a third orally active compound, 29, was identified (Table II). Compound 29 differs from 22 in that the SMO-PHE present at the P_4-P_3 position in 22 was replaced with the constrained analog SMO-THIQ.

During the investigation of the P_1-P_1' area we kept the P_3 and P_2 fragments constant as SMO-PHE and (S)-ATA. Derivatives 32–38 were synthesized and also found to be potent inhibitors of renin with IC_{50} values from 0.27 nM to 13.7 nM. This range of activities followed the normal trend for P_1-P_1' substitutions, ACDMH > ACHPA > STA. These derivatives were also selective for renin relative to cathepsin D. In vivo examination of these analogues yielded a fourth orally active compound, 37. Compound 37 contains a difluorocyclohexylstatine amide (FCO-AEM) at P_1-P_1' replacing the ACDMH group found in both 22 and 29.

All four orally active compounds, 22, 24, 29, and 37, were potent inhibitors of renin in vitro with IC_{50} values of 0.37–2.7 nM. Three of the derivatives were also over 1000-fold selective for renin over cathepsin D, compound 24 was 146-fold selective. Table II contains the oral blood pressure lowering activity data, at the lowest active dose, for these four derivatives. The compounds were dosed at either 10 or 30 mg/kg and the effects on blood pressure followed for 3 h. All four novel compounds elicited a decrease in MABP after oral administration. However, compounds 24 and 29 were active only when dosed at 30 mg/kg. Compounds 22 and 37 were active at the lower dose of 10 mg/kg. Compound 22 was selected for further

biological evaluation on the basis of a consistently more efficacious in vivo profile and practicality of synthesis.[33]

Compound 22 and in particular the P_2 (S)-ATA moiety was compared to several other P_2 aryl substituted compounds (39–41). All of these compounds were potent inhibitors of renin in vitro and were greater than 129-fold selective for renin over cathepsin D (Table I). The HIS derivative 39 displayed the greatest enzyme selectivity, 225000-fold. The (S)-ATA analog 22 imparted a medium degree of selectivity, 3380-fold, and the PHE compound 41 gave the lowest degree of selectivity, 129-fold. These data show that the (S)-ATA group is a viable replacement for HIS at the P_2 site in terms of enzyme selectivity. Table III displays the oral activity data for these derivatives in normotensive monkeys. In this screen the HIS derivative 39 produced only a modest degree of oral activity when dosed at 30 mg/kg and the PHE derivative 41 shows no oral activity at the same dose. The (S)-ATA compound 22 was significantly more potent than the 4-thiazolyl-alanine (TZA) compound 40 although both gave a good oral response at the lower dose of 10 mg/kg.

As illustrated in Table IV, 22 is a potent inhibitor of monkey renin (IC_{50} = 0.37 nM) and selectively inhibits renin versus cathepsin D and pepsin. Several compounds reported previously to have intravenous or oral activity in man or monkey, including 42,[17] were prepared and tested as reference agents. These compounds also are potent inhibitors of renin and exhibit a range of selectivity toward other aspartic proteinases. Table V compares the inhibitory activity of 22 against renin from several species, including human. The compound is a potent inhibitor of monkey and human renin, somewhat less potent with dog

(32) Painchaud, C.; Ryan, M. J.; Hicks, G.; Fettig, M.; Rapundalo, S.; Taylor, D. Effects of CGP-38560 on blood pressure (BP) and plasma renin activity (PRA) on high renin normotensive cynomolgus monkeys. *FASEB J.* 1990, 4, A748.

(33) Derivative 35, which contains the transition state mimetic FCO, is inherently diastereomerically unstable. Epimerization is known to occur at the α-amino site adjacent to the ketone functionality. The synthesis of 35 is consequently longer and more difficult.[14]
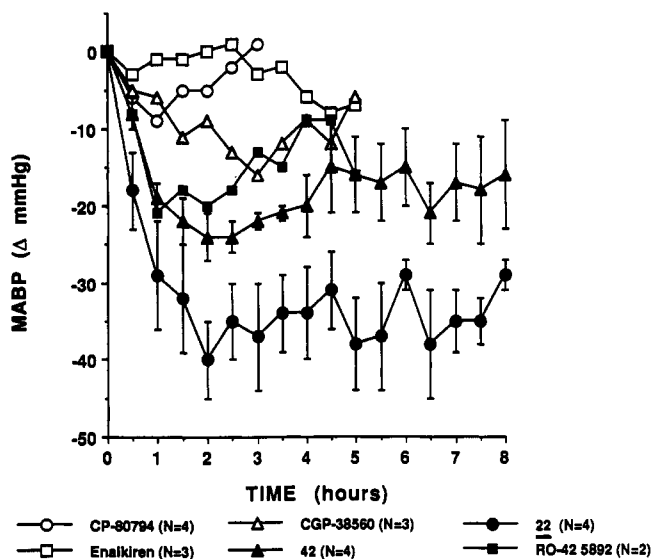
Figure 2. Comparative oral activity in renal hypertensive monkeys after a dose of 30 mg/kg.

Table VI. Chymotrypsin Stability

no.	% parent remaining at time
42	<11% at 20 min
22	88% at 90 min

renin, and much less with the rat renin.

Derivative **22** was then compared to **42**[17] and several reported renin inhibitor clinical candidates, enalkiren,[34] CP-80794,[35] CGP-38560,[36] and RO 42-5892,[37] in high-renin renal hypertensive cynomolgus monkeys.[17,38] The results shown in Figure 2 illustrate that at an oral dose of 30 mg/kg **22** lowered MABP 40 mmHg at 2 h postdose and retained a >30 mmHg drop out to at least 8 h postdose. In this model, **22** was superior in blood pressure reduction and duration of action, as compared to the other compounds tested.

The dose–response for the blood pressure lowering activity of **22** following oral administration to renin-dependent hypertensive monkeys is shown in Figure 3. Compound **22** produced a dose-dependent reduction in MABP; a single oral dose of 3 mg/kg reduced MABP as much as 16 mmHg, and a maximal drop of 43 ± 6 mmHg was produced at 30 mg/kg. This occurred around 2 h postdose for each dose. Plasma renin activity (PRA) was inhibited 86% with the 3 mg/kg dose up to 98% with the 30 mg/kg dose; PRA was measured at 1 h postdose.
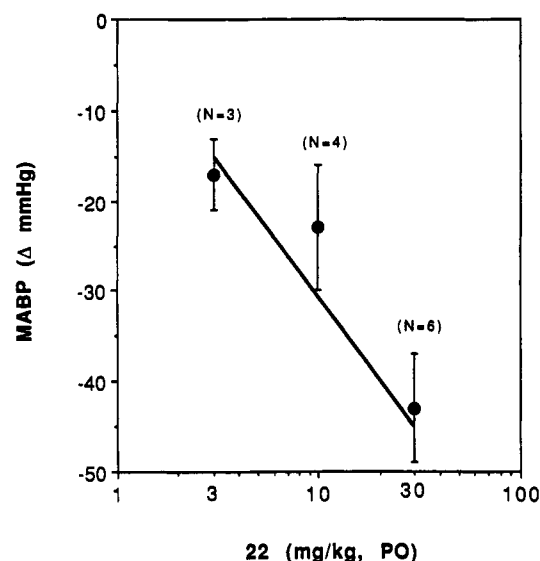
Figure 3. Oral dose–response in renal hypertensive monkeys. Dose response effect of **22** on blood pressure after oral administration to renal hypertensive monkeys. Plotted points are mean ± SEM of number of monkeys indicated in parentheses. The solid line is the dose–response regression line calculated by the method of least squares. The regression line for **22** was $Y = -26.9 \log (\text{dose}) - 1$. The slope ($26.9 \pm 9.3$) was significantly different from zero ($p = 0.0144$). This indicates that as the dose of **22** is increased there is an increase in the blood pressure response.

Enzyme Stability

The degradation of peptidomimetic enzyme inhibitors by gut enzymes has been suggested as a possible contributing factor to their low bioavailability.[39] The enzymatic stability of **22** was therefore compared to **42** in a chymotrypsin preparation.[39] The incubation was performed in a buffered solution (pH 6.5) of bovine chymotrypsin at 25 μg/mL. Table VI shows that **42** was degraded rapidly in this assay, with only 11% parents (compound is an equilibrium mixture of two diastereomers) remained after a 20-min incubation period. Compound **22** was significantly more stable, showing 88% parent remaining after a 90-min incubation period. Since **42** and **22** have similar in vitro potencies, this greater stability to chymotrypsin may in part explain the greater oral potency of **22** compared to **42**.

Summary

A series of renin inhibitors was synthesized that contained 2-amino-4-thiazolyl residues at the P_2 position. These compounds are highly potent in vitro inhibitors of primate renin. The (S)-ATA residue at P_2 imparts selectivity for renin over cathepsin D and also allows for enhancement of stability of these agents toward chymotrypsin degradation. From the structure–activity studies performed at P_3, P_2, and P_1–P_1', four orally active compounds **22, 24, 29,** and **37** were identified. From these **22** was chosen for a more thorough evaluation on the basis

(34) Delabays, A.; Nussberger, J.; Porchet, M.; Waeber, B.; Hoyos, P.; Boger, R.; Glasman, H.; Kleinert, H.; Luther, R.; Brunner, H. R. Hemodynamic and Humoral Effects of the New Renin Inhibitor Enalkiren in Normal Humans. *Hypertension* **1989**, *13*, 941–947.

(35) Abernethy, D. R.; Wilner, K. D.; Lazar, J. D.; Wilkes, B. M. Inhibition of Furosemide-Stimulated Plasma Renin Activity After Oral Administration CP-80,794, a Putative Renin Inhibitor. *Clin. Pharmacol. Ther.* **1990**, *47*, 141.

(36) De Gasparo, M.; Cumin, F.; Nussberger, J.; Guyenne, T. T.; Wood, J. M.; Menard, J. Pharmacological investigations of a New Renin Inhibitor in Normal Sodium-Unrestricted Volunteers. *Br. J. Clin. Pharmacol.* **1989**, *27*, 587–596.

(37) Camenzind, E.; Nussberger, J.; Waeber, B.; van Brummelen, P.; Brunner, H. Oral Renin Inhibition by Ro 42-5892 Reduces Plasma Angiotensin II Levels in Man. *Hypertension* **1989**, *14*, 349.

(38) Cooper, J. B.; Foundling, S. I.; Blundell, T. L.; Boger, J.; Jupp, R. A.; Kay, J. X-ray Studies of Aspartic Proteinase-Statine Inhibitor Complexes. *Biochemistry* **1989**, *28*, 8596–8603.

(39) 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. W. K.; Essenberg, A. D. Renin Inhibitors Containing Isosteric Replacements of the Amide Bond Connecting the P_3 and P_2 Sites. *J. Med. Chem.* **1990**, *33*, 838–845.

of its in vitro and in vivo profile. Compound **22** is a potent inhibitor of primate and human renin and is stable to a 90-min chymotrypsin incubation. Compound **22** is selective for renin over cathepsin D and is a potent, orally active blood pressure lowering agent. In high-renin normotensive monkeys MABP was lowered >15 mmHg at a 10 mg/kg dose. At a dose of 30 mg/kg **22** lowered MABP in high-renin renal hypertensive monkeys from 30 to 40 mmHg for a period of time greater than 8 h. Compound **22** was also found to be superior, in this model, to the currently known renin inhibitor clinical candidates (Figure 2) with statistically greater potency and duration of action.

Experimental Section

Chemistry. All spectra and microanalysis data were obtained by the Parke-Davis Analytical Chemistry section. ¹H NMR spectra were obtained on either a Bruker AM250 or a Varian XL300 NMR spectrometer and were run in either $CDCl_3$ or d_6-DMSO. IR spectra were obtained from a Nicolet MX-1 FTIR. Mass spectra were obtained on (a) Finnigan 4500 MS; (b) VG Analytical 7070E/HF MS, (c) VG Masslab Trio-2A MS, or (d) Finnigan TSQ-70 MS instruments. All mass spectra are FAB unless otherwise noted. Flash chromatography was performed using E. Merck silica gel (230–400 mesh). Thin-layer chromatography was performed using E. Merck 60 F_{254} precoated silica gel plates (0.25 mm × 5 cm × 10 cm). Visualization was obtained by UV light, I_2 staining, or phosphomolybdic acid staining. Solvents were interchangeably dried according to Perrin and Armarego[40] or purchased dry and used fresh from suppliers.

General Methods. General Method A: Dicyclohexylcarbodiimide and 1-Hydroxybenzotriazole Coupling Procedure. The acidic component (1 mmol) was dissolved in a suitable solvent, usually DMF (5–15 mL), and cooled to 0 °C. The solution was then treated sequentially with HOBT (1 mmol) and DCC (1 mmol) and then treated with either the amine component (1 mmol) as a free base or with triethylamine (1–2 mmol) and the amine component (1 mmol) as an acid addition salt. The mixture was stirred for 1 h at 0 °C and then a further 2–24 h at room temperature. The mixture was filtered free of solids and evaporated at reduced pressure to dryness. The residue was dissolved in sufficient ethyl acetate and partially purified via an extractive workup. The resultant organic phase was dried over magnesium sulfate and evaporated at reduced pressure to dryness. This residue was generally purified by chromatography over silica gel.

General Method B: Removal of BOC Protection. The BOC-protected amine component (1 mmol) was dissolved in dichloromethane containing 5–10% methanol (20 mL). The solution was saturated by bubbling into the solution HCl gas. The solution was then stirred for 2–5 h at room temperature. The solvents were removed at reduced pressure and the residue either (1) dried at room temperature at reduced pressure to give an HCl addition salt, or (2) dissolved in ethyl acetate (25 mL) and washed sequentially with saturated sodium bicarbonate (25 mL) and brine (25 mL), followed by drying over magnesium sulfate and evaporation at reduced pressure to give the free base.

(S)-1,2,3,4-Tetrahydro-2-(4-morpholinylsulfonyl)-3-isoquinolinecarboxylic Acid (SMO-THIQ, 7). (S)-1,2,3,4-Tetrahydro-3-isoquinolinecarboxylic acid (10 g, 56.4 mmol) was stirred in methanol (30 mL) with tetramethylammonium hydroxide (25.7 g of a 20% solution in methanol, 56.4 mmol). After 1 h the solution was diluted with toluene (80 mL) and i-PrOH (80 mL) and stirred. The solution was evaporated to dryness, giving a paste. The paste was redissolved in i-PrOH (80 mL) and toluene (80 mL) and again evaporated to dryness, giving a thick paste. The paste was dissolved in THF (120 mL) and i-PrOH (120 mL) and treated with 4-morpholinesulfonyl chloride[17] (5.2 g, 28 mmol) and stirred at room temperature for 16 h. The solution was evaporated at reduced pressure and the residue was partitioned between CH_2Cl_2 (300 mL) and 1 N HCl (300 mL). The organic phase was extracted with 1 N NaOH (3 × 150 mL)

and discarded. The base layers were combined and made acidic with concentrated HCl. This aqueous layer was extracted with CH_2Cl_2 (3 × 150 mL) and the organic phases combined. The organics were washed with brine (150 mL) and dried over $MgSO_4$. The solvents were evaporated at reduced pressure to give 3.65 g (40%) of pure **7**: MS(FAB) *m/e* 327 (M + 1); NMR (DMSO-d_6) δ 3.1 (m, 4 H), 3.2 (m, 2 H), 3.6 (m, 4 H), 4.5 (s, 2 H), 4.7 (m, 1 H), 7.2 (s, 4 H), 12 (br ex, 1 H). Anal. Calcd for $C_{14}H_{18}N_2O_5S$: C, H, N.

(S)-1,2,3,4-Tetrahydro-6,7-dimethoxy-2-(4-morpholinylsulfonyl)-3-isoquinolinecarboxylic Acid (SMO-DMTQ, 8). The compound was synthesized in the same manner as **7**, from 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (5.5 g, 20 mmol). This gave 2.2 g (58%) of pure **8**: MS(FAB) *m/e* 386.3 (M + 1); NMR (DMSO-d_6) δ 3.1 (m, 6 H), 3.6 (m, 4 H), 3.7 (s, 3 H), 3.75 (s, 3 H), 4.5 (s, 2 H), 4.65 (m, 1 H), 6.8 (s, 2 H), 12.8 (br ex, 1 H). Anal. Calcd for $C_{16}H_{22}N_2O_7S$: C, H, N.

[2S-(2R*,3R*,5S*)]-2-Amino-1-cyclohexyl-3,5-heptanediol (CDH, 16). A solution of **14** (15.16 g, 40 mmol) in dry THF (200 mL) at 5 °C was treated over 5 min with a 2 M solution of ethylmagnesium bromide (in THF). The new solution was stirred for 30 min and poured over ice water (300 g). This was treated with a solution of saturated citric acid (200 mL) and extracted with ether (300 mL). The organic phase separated, washed with water, dried over magnesium sulfate, and concentrated in vacuo to give an oil. The oil was chromatographed (silica gel, Hex/EtOAc) to give the pure ketone, 9.4 g (57%).

A sample of the ketone (9.3 g, 23 mmol) was dissolved in ethanol (100 mL) and treated with potassium borohydride (1.24 g, 23 mmol). The mixture stirred for 30 min and then quenched with acetone (10 mL). The mixture was concentrated at reduced pressure and diluted with water. An oil separated and was extracted into petroleum ether (200 mL). The organic phase was dried over potassium carbonate and evaporated at reduced pressure to give 7.9 g (83%) of a mixture of diasteromers (at 5-OH position).

From two runs of above, the diasteromers (14.1 g, 34.1 mmol) was dissolved in ethanol (140 mL) and treated with pyridinium p-toluenesulfonate (0.86 g, 3.41 mmol). The solution was heated to 55 °C for 3 h and evaporated at reduced pressure. The residue was taken up in ethyl acetate and washed twice with water. The organic phase dried over magnesium sulfate, filtered, and evaporated at reduced pressure to give 13.74 g of diasteromers. These were separated by flash chromatography (silica gel, 5–40% EtOAc/Hex) to give 5.72 g of a fast isomer and 4.72 g of a slow isomer.

The slow isomer (2.25 g, 6.83 mmol) was dissolved in methylene chloride (25 mL) and MeOH/HCl (0.029 g/mL HCl, 20.14 mmol) (25 mL). The mixture was stirred at ambient temperature for 23 h. The solvents were evaporated at reduced pressure, and the residue was triturated with ether. The solid collected and dried to give 1.86 g (100%) of the (5-S) isomer. The assignment of absolute configuration is the basis of a manuscript in preparation.[43]

The free base (1.86 g, 6.8 mmol) was made by partitioning the HCl salt between ethyl acetate and saturated sodium bicarbonate. This gave 1.5 g of pure free base (100%): mp 92–94 °C; MS(EI) *m/e* 230 (M + 1); NMR ($CDCl_3$) δ 0.8–1.8 (m, 24 H), 2.69–2.76 (m, 1 H), 3.47–3.55 (m, 1 H), 3.74–3.84 (m, 1 H). Anal. Calcd for $C_{13}H_{27}NO_2$: C, H, N.

Phenylmethyl N-[(1,1-Dimethylethoxy)carbonyl]-3-[2-[[(phenylmethoxy)carbonyl]amino]-4-thiazolyl]-L-alaninate (BOC-ATA(Z)-OBZL, 17a). Compound **2**[24] (10.3 g, 25 mmol) was dissolved in a mixture of THF (50 mL), CH_2Cl_2 (80 mL), and saturated sodium bicarbonate (160 mL). To this solution was

(40) Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*; 3rd ed.; Pergamon Press: New York, 1966.

(41) Haber, E.; Koerner, T.; Page, L. B.; Kliman, B.; Purnode, A. Application of a Radioimmunoassay for Angiotensin I to the Physiologic Measurements of Plasma Renin Activity in Normal Human Subjects. *J. Clin. Endocrinol.* **1969**, *29*, 1349–1355.

(42) Kokubu, T.; Hiwada, K.; Murakami, E.; Imamura, Y.; Matsueda, R.; Yabe, Y.; Kioke, H.; Iijima, Y. Highly Potent and Specific Inhibitors of Human Renin. *Hypertension* **1985**, *7* (Suppl. I), I-8 to I-11.

(43) Klutchko, S.; Hodges, J. C.; Reily, M. Unpublished results. See also Patent WO9007-521-A.

added benzyl chloroformate (10.3 g, 62.5 mmol) and the mixture vigorously stirred at room temperature overnight. The mixture was treated with ethyl acetate (150 mL) and the organic phase separated. The organic phase was washed sequentially with water (200 mL) and brine (2 × 200 mL) and dried over magnesium sulfate. The solution was evaporated in vacuo to dryness, giving **17a** as a crude oil which was used as is.

N-[(1,1-Dimethylethoxy)carbonyl]-3-[2-[[(phenylmethoxy)carbonyl]amino]-4-thiazolyl]-L-alanine (BOC-ATA(Z), 18a). The oil **17a** was dissolved in ethanol (75 mL) and treated with a solution of KOH (7.0 g, 125 mmol) in water (70 mL). The solution was stirred at room temperature for 4 h. The solution was partitioned between ether (400 mL) and water (200 mL) and the organic phase separated and discarded. The aqueous phase was made acidic (pH = 2, wet litmus) with concentrated HCl and then extracted with ethyl acetate (3 × 100 mL). The organic washes were combined, washed with brine (200 mL), and dried over magnesium sulfate. Evaporation of the solvents gave 8.4 g (80%) of the acid **18a**: MS(EI) m/e 422 (M + 1); IR 1232, 1560, 1719, 1723; NMR (CDCl₃) [contains residual EtOAc—not included in the following] δ 1.47 (s, 9 H), 3.31 (m, 2 H), 4.56 (m, 1 H), 5.2 (br, 1 H), 5.25 (m, 2 H), 6.55 (s, 1 H), 7.25 (m, 1 H), 7.3 (m, 5 H), 12.3 (br, 1 H).

Phenylmethyl [1S-[1R*(R*),2S*,3R*]]-[4-[3-[[1-(Cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]amino]-2-[[(1,1-dimethylethoxy)carbonyl]amino]-3-oxopropyl]-2-thiazolyl]carbamate (BOC-ATA(Z)-ACDMH, 19a). The acid **18a** (8.4 g, 210 mmol) was then coupled as in general method A to ACDMH (4.8 g, 20 mmol). The crude residue was purified by chromatography over silica gel (EtOAc/Hex) to give 9.5 g of **19a** (73%): MS(FAB) m/e 647.3 (M + 1); IR 1226, 1558, 1695, 1699, 1718; NMR (CDCl₃) δ 0.7–0.95 (m, 8 H), 1.0–2.0 (m, 14 H), 1.4 (s, 9 H), 2.95 (m, 1 H), 3.2 (m, 2 H), 3.35 (m, 1 H), 4.0 (m, 1 H), 4.2 (m, 1 H), 4.5 (m, 1 H), 5.2 (br, 1 H), 5.2 (s, 2 H), 5.5 (br d, 1 H), 6.8 (s, 1 H), 6.8 (br, 1 H), 7.2 (br ex, 1 H), 7.3 (m, 5 H). Anal. Calcd for C₃₃H₅₀N₄O₇S·0.1EtOAc: C, H, N.

[1S-(1R*,2S*,3R*)]-N-(4-Morpholinylsulfonyl)-L-phenylalanyl-N-[1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]-3-[2-[[(phenylmethoxy)carbonyl]amino]-4-thiazolyl]-L-alaninamide (SMO-PHE-ATA(Z)-ACDMH), 21a). The BOC group was removed as in general method B, using **19a** (9.0 g, 13.9 mmol). This gave 7.3 g of the free amine **20a** as a foam. The amine **20a** (7.3 g, 13.4 mmol) was then coupled as in general method A to **4** (4.22 g, 13.5 mmol). After chromatography (silica gel, MeOH/CHCl₃) 6.8 g (75%) of **21a** was obtained as a white foam: NMR (CDCl₃) δ 0.6–2.0 (m, 22 H), 2.4–3.6 (br m, 12 H), 3.9 (m, 1 H), 4.1–4.3 (m, 2 H), 4.6–4.8 (m, 1 H), 5.2 (s, 2 H), 5.25 (m, 1 H), 5.7 (s, 1 H), 6.7 (m, 1 H), 6.8 (m, 1 H), 6.9 (m, 1 H), 7.2–7.4 (m, 11 H), 8.0 (m, 1 H), 9.9 (m, 1 H).

Phenylmethyl N-[(1,1-Dimethylethoxy)carbonyl]-3-[2-[[(2,2,2-trichloroethoxy)carbonyl]amino]-4-thiazolyl]-L-alaninate (BOC-ATA(TROC)-OBZL, 17b). The free base of **2** (9.56 g, 25 mmol) was suspended in dichloromethane (100 mL) and cooled to (<–20 °C). To this was added DMAP (7.74 g, 62.5 mmol) and a solution resulted. 2,2,2-Trichloroethoxy chloroformate (TROC-Cl) (12.34 g, 57.5 mmol) was then added dropwise and the temperature kept (<–20 °C). The solution stirred for 4 h and then sequentially washed with water, 0.1 N HCl, and brine. The organic phase dried over magnesium sulfate and evaporated at reduced pressure to dryness. This was purified by chromatography (400 g of silica gel, eluting with 1:1 hexane/ethyl acetate), giving 12.8 g (98%) of **17b**: MS(FAB) m/e 553.9 (M + 1); NMR (CDCl₃) δ 1.4 (s, 9 H), 3.2 (m, 2 H), 4.6 (m, 1 H), 4.8 (s, 2 H), 5.15 (m, 2 H), 5.5 (m, 1 H), 6.4 (s, 1 H), 7.3 (s, 5 H), 9.8 (s, 1 H). Anal. Calcd for C₂₁H₂₄N₃O₆S: C, H, N.

2,2,2-Trichloroethyl [1S-[1R*(R*),2S*,3R*]]-[4-[3-[[1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]amino]-2-[[(1,1-dimethylethoxy)carbonyl]amino]-3-oxopropyl]-2-thiazolyl]carbamate (BOC-ATA(TROC)-ACDMH, 19b). Compound **17b** (3.6 g, 6.5 mmol) was dissolved in methanol (100 mL) and treated with a solution of KOH (1.16 g, 47.5 mmol) in water (10 mL) and stirred at room temperature for 3.5 h. The solution was diluted with water (50 mL) and extracted with ethyl acetate (100 mL). The aqueous phase made acidic with HCl and again extracted with ethyl acetate (3 × 100 mL). These organic washes were combined and washed with brine and dried over

magnesium sulfate. The solution was concentrated at reduced pressure to give 2.54 g (84%) of the acid **18b** used as is.

The acid **18b** (27.25 g, 58.9 mmol) from several runs was coupled to **13** (14.33 g, 58.9 mmol) as in method A, giving 29.5 g of **19b** (73%) from recrystallization from ethyl acetate: MS(FAB) m/e 688.9 (M + 1); NMR (CDCl₃) δ 0.9 (m, 8 H), 1.1–1.4 (m, 8 H), 1.4 (s, 9 H), 1.6–2.0 (m, 8 H), 3.0–3.3 (m, 4 H), 4.2 (br, 1 H), 4.4 (br 1, H), 4.5 (br, 1 H), 4.9 (br, 2 H), 5.8 (br d, 1 H), 6.2 (s, 1 H), 6.6 (br d, 1 H). Anal. Calcd for C₂₈H₄₅Cl₃N₄O₇S: C, H, N.

[1S-(1R*,2S*,3R*)]-N-(4-Morpholinylsulfonyl)-L-phenylalanyl-N-[1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]-3-[2-[[(2,2,2-trichloroethoxy)carbonyl]amino]-4-thiazolyl]-L-alaninamide (SMO-PHE-ATA-(TROC)-ACDMH, 21b). Compound **19b** (32.93 g, 47.8 mmol) was suspended in ether/methanol (300 mL/20 mL) and cooled to 0 °C. The cold solution was treated with HCl by bubbling the gas into the solution for 20 min. The mixture was stirred cold overnight and concentrated at reduced pressure to give a foam. The foam was triturated with ether to give a white powder. The powder was collected, giving 27.5 g (92%) of the HCl salt of the amine **20b** and used as is.

The amine **20b** (27.5 g, 44.0 mmol) was coupled to **4** (13.83 g, 44 mmol) as in method A, giving 11.38 g (38%) of **21b** after chromatography: MS(FAB) m/e 885.2 (M + 1); NMR (CDCl₃) δ 0.5–0.95 (m, 3 H), 0.95 (m, 6 H), 1.0–1.2 (m, 5 H), 1.3–1.8 (m, 11 H), 1.8–2.0 (m, 1 H), 2.4–2.5 (m, 2 H), 2.7 (m, 1 H), 2.8–3.1 (m, 4 H), 3.2–3.35 (m, 1 H), 3.35–3.6 (m, 5 H), 3.9 (br d, 1 H), 4.2 (d, 1 H), 4.75 (m, 2 H), 5.05 (m, 1 H), 6.5 (d, 1 H), 6.65 (s, 1 H), 7.3–7.5 (m, 5 H), 9.8–9.9 (m, 2 H). Anal. Calcd for C₃₆H₅₃Cl₃N₆O₉S₂: C, H, N.

[1S-(1R*,2S*,3R*)]-N-(4-Morpholinylsulfonyl)-L-phenylalanyl-3-(2-amino-4-thiazolyl)-N-[1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]-L-alaninamide (SMO-PHE-ATA-ACDMH, 22). Method 1. p-Toluenesulfonic acid hydrate (3.6 g, 19 mmol) and **21a** (6.4 g, 7.6 mmol) were dissolved in methanol (150 mL), treated with 20% palladium on charcoal, and stirred under a hydrogen atmosphere at room temperature for 6 h. The mixture was filtered and evaporated at reduced pressure to dryness. The residue was dissolved in ethyl acetate and washed sequentially with saturated sodium bicarbonate (100 mL) and brine (100 mL). The organic phase was dried over magnesium sulfate and evaporated at reduced pressure to give the crude material. The product was purified by chromatography to give 1.0 g of pure **22** (19%): MS(FAB) m/e 709 (M + 1); NMR (CDCl₃) δ 0.75–0.9 (m, 2 H), 0.95 (d, 6 H), 1.1–1.65 (m, 15 H), 1.90 (m, 1 H), 2.4–2.9 (m, 6 H), 2.9–3.6 (m, 8 H), 3.9 (m, 1 H), 4.2 (m, 1 H), 4.6 (m, 1 H), 5.35 (br, 1 H), 5.6 (br, 2 H), 6.2 (s, 1 H), 6.65 (br, 1 H), 7.35 (m, 5 H), 9.9 (br, 1 H). Anal. Calcd for C₃₃H₅₂N₆O₇S₂: C, H, N.

[1S-(1R*,2S*,3R*)]-N-(4-Morpholinylsulfonyl)-L-phenylalanyl-3-(2-amino-4-thiazolyl)-N-[1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]-L-alaninamide (SMO-PHE-ATA-ACDMH, 22). Method 2. Compound **21b** (10.6 g, 12.0 mmol) was dissolved in THF/methanol (4:1) (250 mL), under nitrogen. To this was added ammonium chloride (6.41 g, 120 mmol) and zinc dust (1.57 g, 24 mmol). The mixture was vigorously stirred at room temperature for 2 h and then treated with an additional 2 equiv of zinc (1.57 g). This was repeated one additional time for a total of 6 equiv of zinc dust. After an additional 5.5 h the reaction mixture was filtered and concentrated to afford 10.96 g of a white foam. This foam (9.76 g) was purified by chromatography, giving 6.47 g of **22** (84% adjusted for sample removal), with spectral and analytical data identical to those of **22** formed by method 1.

[1S-[1R*[R*(R*)],2S*,3R*]]-2-Amino-N-[1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]-α-[[2-[(4-morpholinylsulfonyl)amino]-1-oxo-3-phenylpropyl]amino]-5-thiazolebutanamide (SMO-PHE-ATE-ACDMH, 23). Compound **3** (14.17 g, 36 mmol) was protected in the same manner as **17a**, giving BOC-ATE(Z)-OBZL. The crude exocyclic Z derivative was hydrolyzed with NaOH (7.0 g, 175 mmol) in a manner similar to that used for **18a**. This gave 9.3 g (60%, two steps) of the acid BOC-ATE(Z). The acid (9.3 g, 21 mmol) was coupled to **13** (4.9 g, 20 mmol) as in method A. The crude product was purified by flash chromatography over silica gel (500 g), eluting with ethyl acetate, giving 7.6 g (58%) of pure BOC-ATE(Z)-

ACDMH. This compound (2.38 g, 3.6 mmol) was further deprotected at the α amine as in general method B to give the crude amine as an HCl salt. This was coupled as in method A to 4 (1.13 g, 3.6 mmol), giving 2.97 g of SMO-PHE-ATE(Z)-ACDMH. This compound (2.05 g, 2.4 mmol) was dissolved in methanol (50 mL) and treated with 20% Pd/C (0.4 g) and p-toluenesulfonic acid hydrate (1.18 g, 6.3 mmol). The mixture was stirred under a hydrogen atmosphere for 24 h. The material was filtered free of catalyst and evaporated at reduced pressure to give an oily mass. This was dissolved in ethyl acetate and washed once with saturated NaHCO₃ and evaporated in vacuo to give a foam. The material was purified by flash column chromatography (150 g silica gel) eluting with ethyl acetate to give 0.51 g (35%) of pure 23: MS-(FAB) *m/e* 723.3 (M + 1); NMR (CDCl₃) δ 0.9 (d, 6 H), 1.0–1.5 (m, 9 H), 1.5–1.8 (8 H), 1.8–2.4 (m, 6 H), 2.5–2.7 (m, 3 H), 2.9–3.0 (m, 2 H), 3.1–3.4 (m, 2 H), 3.4–3.6 (m, 4 H), 4.0 (m, 1 H), 4.2 (m, 1 H), 4.35 (m, 1 H), 4.6 (m, 1 H), 5.0 (m, 2 H), 6.2 (s, 1 H), 7.0 (m, 1 H), 7.2–7.4 (m, 5 H), 9.15 (d, 1 H). Anal. Calcd for C₃₄H₅₄N₆O₇S₂: C, H, N.

[1S-[1R*[RS(R*)],2S*,3R*]]-2-Amino-N-[1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]-α-[[2-[(4-morpholinylsulfonyl)amino]-1-oxo-3-phenylpropyl]amino]-3-thiazoleacetamide (SMO-PHE-(R,S)ATG-ACDMH, 24). A solution of ethyl 2-amino-α-(hydroxyimino)-4-thiazoleacetate (22.9 g, 0.1 mmol) in ethanol (275 mL) containing HCl was subjected to catalytic reduction in the presence of 10% Pd/C. Upon usual workup the dihydrochloride addition salt of the amino acid ester (ATG-OEt) was obtained[25] and used as is. The salt was dissolved in water (275 mL) and the solution was cooled to 0 °C. The pH was adjusted to 9 with K₂CO₃. To this solution was added a solution of (BOC)₂O (26 g, 0.12 mol) in THF (150 mL) at 0 °C while the pH was maintained at 9 as before. The mixture was warmed to room temperature and allowed to stir for 24 h. The mixture was extracted with ethyl acetate (2 × 400 mL), and the organic layer was washed with brine, dried over magnesium sulfate, and evaporated at reduced pressure to give a semisolid. This was recrystallized from ethyl acetate to give 23.5 g (78%) of the α-BOC amino acid ester (BOC-(R,S)ATG-OEt). The exocyclic amine of the thiazole ring was protected as a Z in the same manner as 17a from the above ester (15.07 g, 50 mmol) and Z-chloride (21.3 g, 125 mmol), giving a crude oil. This oil was then hydrolyzed with KOH (14 g, 250 mmol) in ethanol/water (1.5:1) (250 mL). The solution was stirred at room temperature for 1.5 h and then diluted with water (350 mL). The solution was extracted with ether (2 × 250 mL) and the aqueous solution was made acidic with 1 N citric acid (pH = 3). The solid was collected by filtration, washed with water, and dried at 65 °C at reduced pressure to give 16.8 g (83%) of the acid BOC-(R,S)ATG(Z), mp 172–173 °C. Anal. Calcd for C₁₈H₂₁N₃O₆S: C, H, N. This acid (3.3 g, 8.0 mmol) was coupled, according to general method A, to ACDMH·HCl (2.2 g, 8.0 mmol), giving after flash chromatography (CHCl₃/MeOH (10%)) 6.0 g of a yellow diastereomeric mixture of BOC-(R,S)ATG(Z)-ACDMH, which was used as is. The BOC group was removed as in general method B from BOC-(R,S)ATG(Z)-ACDMH (6.0 g), giving after evaporation of solvent 5.5 g of the HCl addition salt of the amine (R,S)ATG-(Z)-ACDMH·HCl. This amine (2.4 g, 4.5 mmol) was coupled, according to general method A, to 4 (1.41 g, 4.5 mmol) using HOBT (0.62 g, 4.5 mmol), DCC (0.97 g, 4.5 mmol), and 1 equiv of triethylamine. After chromatography (diastereomers inseparable) (flash silica gel, CHCl₃/MeOH) 3.4 g of the Z-protected compound was obtained, SMO-PHE-(R,S)ATG(Z)-ACDMH. This (2.0 g, 2.4 mmol) was deprotected as in the synthesis of 22 method 1, using p-TsOH·H₂O (0.9 g, 5 mmol) and 20% Pd/C (0.3 g). This gave after chromatography (flash silica gel, CH₂Cl₂/MeOH) 0.7 g (42%) of the 1:1 mixture of diastereomers of 24: MS(FAB) *m/e* 695 (M + 1); NMR (CDCl₃) δ 0.7–1.0 (m, 7 H), 1.0–1.5 (m, 6 H), 1.5–2.0 (m, 9 H), 2.6–3.0 (m, 5 H), 3.0–3.3 (m, 3 H), 3.3–3.6 (m, 4 H), 3.9–4.2 (m, 1 H), 4.2–4.4 (m, 1 H), 5.3 (s, 1 H), 5.4 (m, 1 H), 5.7 (d, 1 H), 5.9 (m, 1 H), 6.3 (s, 1 H), 6.6 (s, 1 H), 6.9–7.6 (m, 8 H). Anal. Calcd for C₃₂H₅₀N₆O₇S₂·0.8H₂O: C, H, N, S, H₂O.

[1S-(1R*,2S*,3R*)]-N-(4-Morpholinylsulfonyl)-L-phenylalanyl-3-(2-amino-4-thiazolyl)-N-[2,3-dihydroxy-5-methyl-1-(cyclohexylmethyl)hexyl]-D-alaninamide (SMO-PHE-(R)ATA-ACDMH, 25). The compound was synthesized in the same manner as 22 method 1 (17a–21a), from BOC-(R)-

ATA-OBZL, giving 0.625 g (14% over six steps) of pure 25: MS(FAB) *m/e* 709 (M + 1); NMR (CDCl₃) δ 0.8–2.0 (br m, 24 H), 2.65–3.0 (m, 4 H), 3.0–3.3 (m, 4 H), 3.4–3.6 (m, 4 H), 3.9 (m, 1 H), 4.2 (m, 1 H), 4.6 (m, 1 H), 5.3 (m, 3 H), 6.2 (s, 1 H), 6.7 (m, 1 H), 7.2–7.5 (m, 7 H), 8.1 (m, 1 H). Anal. Calcd for C₃₃H₅₂N₆O₇S₂·1.1H₂O: C, H, N, H₂O.

[1S-(1R*,2S*,3R*)]-O-Methyl-N-(4-morpholinylsulfonyl)-L-tyrosyl-3-(2-amino-4-thiazolyl)-N-[1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]alaninamide (SMO-TYR(OME)-ATA-ACDMH, 26). The compound was synthesized in two steps from 20a. The first step was a coupling as in general method A from 20a (0.7 g, 1.25 mmol), HOBT (0.169 g, 1.25 mmol), DCC (0.258 g, 1.25 mmol), and 5 (0.430 g, 1.25 mmol). This gave 0.65 g (44%) of the Z-protected compound which was used, as is, in step two. Step two was a hydrogenolysis as in the synthesis of 22 method 1, using SMO-TYR(ME)-ATA-(Z)-ACDMH (0.65 g, 0.74 mmol), p-TsOH (0.305 g, 1.6 mmol), and 20% Pd/C (0.125 g). This gave after chromatography (flash silica gel, CHCl₃/MeOH) 0.33 g (60%) of pure 26: mp 110–120 °C; MS(FAB) *m/e* 739.3 (M + 1); NMR (DMSO-d₆) δ 0.7–1.0 (m, 8 H), 1.0–1.3 (m, 7 H), 1.4–1.9 (m, 9 H), 2.5–3.2 (m, 8 H), 3.4 (m, 4 H), 3.75 (s, 3 H), 3.9 (m, 1 H), 4.15 (m, 1 H), 4.4 (d, 1 H), 4.6 (m, 1 H), 4.8 (d, 1 H), 6.2 (s, 1 H), 6.8 (s, 2 H), 6.9 (d, 2 H), 7.2 (d, 2 H), 7.5 (d, 1 H), 7.8 (d, 1 H), 8.6 (d, 1 H). Anal. Calcd for C₃₄H₅₄N₆O₈S₂: C, H, N.

[1S-(1R*,2S*,3R*)]-N-[(1,1-Dimethylethoxy)-carbonyl]-L-phenylalanyl-3-(2-amino-4-thiazolyl)-N-[1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]-L-alaninamide (BOC-PHE-ATA-ACDMH, 27). The compound was synthesized in two steps from ATA(Z)-ACDMH. In the first step 20a (1.4 g, 2.56 mmol), BOC-PHE (0.68 g, 2.56 mmol), and carbonyldiimidazole (0.415 g, 2.56 mmol) were dissolved in DMF (15 mL), and the mixture was stirred for 72 h. The DMF was evaporated at reduced pressure and the residue was partitioned between ethyl acetate (50 mL) and 1 N HCl (25 mL). The organic phase was separated and washed successively with saturated NaHCO₃ (25 mL) and brine (25 mL). The organic phase was dried over MgSO₄ and purified by chromatography (flash silica gel, 1:1 EtOAc/CH₂Cl₂) to give 0.85 g (43%) of Z-protected compound which was used as is. The Z compound (0.64 g, 0.81 mmol) was subjected to hydrogenolysis in the same manner as 22 method 1, using p-TsOH (0.38 g, 2.0 mmol) and 20% Pd/C (0.15 g). This gave after chromatography (flash silica gel, 1% MeOH in 1:1 EtOAc/CH₂Cl₂) 0.254 g (48%) of pure 27: mp indefinite (<150 °C); MS(FAB) *m/e* 661 (M + 1); NMR (CDCl₃) δ 0.7–1.3 (m, 14 H), 1.4 (s, 9 H), 1.5–2.0 (m, 8 H), 2.8–3.4 (br m, 6 H), 4.1 (m, 1 H), 4.2–4.4 (m, 2 H), 4.5–4.6 (m, 1 H), 4.9 (m, 1 H), 5.0–5.2 (m, 3 H), 6.2 (s, 1 H), 6.45 (d, 1 H), 7.1–7.4 (m, 5 H), 8.35 (m, 1 H). Anal. Calcd for C₃₄H₅₃N₅O₆S: C, H, N.

[1S-(1R*,2S*,3R*)]-N-(1-Piperazinylsulfonyl)-L-phenylalanyl-3-(2-amino-4-thiazolyl)-N-[1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]-L-alaninamide (SPI-PHE-ATA-ACDMH, 28). The compound was synthesized in two steps from TROC-SPI-PHE.[27] In the first step TROC-SPI-PHE (2.1 g, 4.3 mmol) was coupled to 20b (2.5 g, 4.25 mmol) in a manner similar to that used in general method A. This gave after chromatography (flash silica gel, CHCl₃/MeOH) 3.95 g (88%) of the diprotected compound TROC-SPI-PHE-ATA(TROC)-ACDMH. This compound (2.0 g, 1.89 mmol) was deprotected in a manner similar to that used for 22 method 2, using zinc dust (3.5 g). This gave after chromatography (flash silica gel, CHCl₃/MeOH) 0.96 g (72%) of pure 28: MS(FAB) *m/e* 708.4 (M + 1); NMR (DMSO-d₆) δ 0.85 (m, 6 H), 1.0–1.35 (m, 6 H), 1.35–1.85 (m, 9 H), 2.35–2.8 (m, 12 H), 2.8–3.05 (m, 3 H), 3.25–3.45 (m, 2 H), 3.82–3.95 (m, 1 H), 4.05–4.20 (m, 1 H), 4.43 (d, 1 H), 4.5–4.65 (m, 1 H), 4.78 (d, 1 H), 6.20 (s, 1 H), 6.81 (s, 2 H), 7.15–7.35 (m, 5 H), 7.45 (d, 1 H), 8.56 (d, 1 H). Anal. Calcd for C₃₃H₅₃N₇O₆S₂: C, H, N.

[3S-[3R*[R*(1R*,2S*,3R*)]]]-N-[1-[(2-Amino-thiazolyl)methyl]-2-[[1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]amino]-2-oxoethyl]-1,2,3,4-tetrahydro-2-(4-morpholinylsulfonyl)-3-isoquinolinecarboxamide (SMO-THIQ-ATA-ACDMH, 29). The compound was synthesized in two steps in the same manner as 26. In step 1, 7 (2.45 g, 7.5 mmol) was coupled to 20a (2.73 g, 5 mmol) using HOBT (0.95 g, 7 mmol) and DCC (1.0 g, 5 mmol). This gave after chromatography (flash

silica gel, 8:2 EtOAc/CH$_2$Cl$_2$) 1.7 g (40%) of pure Z-protected compound. This Z derivative (1.6 g, 1.9 mmol) was hydrogenolyzed using p-TsOH·H$_2$O (0.89 g, 4.6 mmol) and 20% Pd/C (0.2 g). This gave after chromatography (flash silica gel, 8:2 EtOAc/CH$_2$Cl$_2$) 1.04 g (77%) of pure 29: mp 99–104 °C; MS(FAB) m/e 722 (M + 1); NMR (CDCl$_3$) δ 0.7–2.0 (m, 24 H), 2.55–3.6 (m, 14 H), 4.1–4.25 (m, 2 H), 4.3 (t, 1 H), 4.5 (m, 1 H), 4.7 (s, 2 H), 5.5 (ex s, 2 H), 6.15 (s, 1 H), 6.55 (d, 1 H), 7.25 (m, 4 H), 9.9 (d, 1 H). Anal. Calcd for C$_{34}$H$_{52}$N$_6$O$_7$S$_2$·0.6H$_2$O: C, H, N, H$_2$O.

[1S-(1R*,2S*,4R*)]-N-[Bis(1-methylethoxy)phosphinyl]-L-phenylalanyl-3-[(2-amino-4-thiazolyl)methyl]-N-[1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]-L-alaninamide (IPP-PHE-ATA-ACDMH, 30). An ice-cold solution of phenylalanine (6.61 g, 40 mmol) in triethylamine (20 mL), water (12 mL), and ethanol (8 mL) was treated with diisopropyl phosphite (6.64 mL, 40 mmol) in CCl$_4$ (16 mL). The solution was stirred overnight at room temperature and acidified with 1 N HCl (pH = 2, wet litmus). This solution was extracted with ethyl acetate and the organic phase dried over sodium sulfate. The solvents were evaporated at reduced pressure to give 13.3 g of crude IPP-PHE used as is [MS(EI) m/e 330 (M + 1)]. IPP-PHE (1.27 g, 3.85 mmol) was coupled to 20b (2.26 g, 3.85 mmol) as in general method A using HOBT (0.55 g, 4.0 mmol) and DCC (0.84 g, 4.0 mmol). This gave after chromatography (flash silica gel, EtOAc) 1.90 g (55%) of the TROC-protected compound IPP-PHE-ATA(TROC)-ACDMH [MS(FAB) m/e 900.2 (M + 1)]. This compound (1.76 g, 1.96 mmol) was deprotected in the same manner as in the synthesis of 20 method 2. This gave after chromatography (flash silica gel, CHCl$_3$/MeOH) 0.75 g (53%) of pure 30: MS(FAB) m/e 724.5 (M + 1); NMR (CDCl$_3$) δ 0.9 (m, 11 H), 1.30 (m, 21 H), 1.65 (m, 7 H), 2.8 (m, 2 H), 3.20 (m, 4 H), 3.90 (m, 1 H), 4.37 (m, 1 H), 4.50 (m, 2 H), 5.40 (m, 1 H), 6.25 (s, 1 H), 6.70 (m, 1 H), 7.20 (m, 5 H), 9.13 (m, 1 H). Anal. Calcd for C$_{35}$H$_{58}$N$_5$O$_7$PS: C, H, N.

[3S-[3R*[R*(1R*,2S*,3R*)]]]-N-[1-[(2-Aminothiazolyl)methyl]-2-[[1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]amino]-2-oxoethyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-2-(4-morpholinylsulfonyl)-3-isoquinolinecarboxamide (SMO-DMTQ-ATA-ACDMH, 31). The compound was synthesized in two steps from 20a. Compound 20a (1.27 g, 2.3 mmol) was coupled to 8 (0.9 g, 2.3 mmol) in the same manner as in general method A, using HOBT (0.315 g, 2.3 mmol) and DCC (0.481 g, 2.3 mmol). This gave 0.53 g (25%) of the pure Z-protected compound. The Z protection was removed by hydrogenolysis as in the synthesis of 22 method 1. This gave 0.295 g (84%) of pure 31: MS(FAB) m/e 781 (M⁺); NMR (CDCl$_3$) δ 0.7–2.0 (m, 24 H), 2.5–3.7 (m, 14 H), 3.8 (s, 3 H), 3.9 (s, 3 H), 4.0–4.2 (m, 2 H), 4.4–4.6 (m, 1 H), 4.6–4.8 (m, 2 H), 5.55 (ex s, 2 H), 6.2 (s, 1 H), 6.5 (d, 1 H), 6.75 (s, 1 H), 6.85 (s, 1 H), 9.8 (s, 1 H). Anal. Calcd for C$_{36}$H$_{56}$N$_6$O$_9$S$_2$: C, H, N.

[S-(R*,R*)]-N-(4-Morpholinylsulfonyl)-L-phenylalanyl-3-(2-amino-4-thiazolyl)-N-[1-(cyclohexylmethyl)-2-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]-alaninamide (SMO-PHE-ATA-ACHPA-AEM, 32). The compound was synthesized in four steps from ACHPA-AEM.[22] Compound 18a (2.1 g, 5.0 mmol) was coupled to ACHPA-AEM (1.63 g, 5.0 mmol) as in general method A, using HOBT (0.67 g, 5.0 mmol) and DCC (1.03 g, 5.0 mmol). This gave 2.3 g (63%) of BOC-ATA(Z)-ACHPA-AEM. The BOC group was removed from BOC-ATA(Z)-ACHPA-AEM (2.2 g, 3.0 mmol) as in general method B to give 1.55 g (82%) of the free amine ATA(Z)-ACHPA-AEM. This material (0.85 g, 1.35 mmol) was coupled to 7 (0.41 g, 1.3 mmol) as in general method A, using HOBT (0.175 g, 1.3 mmol) and DCC (0.268 g, 1.3 mmol). This gave 0.99 g (42%) of the Z-protected compound SMO-PHE-ATA(Z)-ACHPA-AEM. This compound (0.88 g, 0.95 mmol) was deprotected as in the synthesis of 22 method 1, using 20% Pd/C (0.4 g) and p-TsOH·H$_2$O (0.453 g, 2.4 mmol). This gave 0.544 g of pure 32: MS(FAB) m/e 793.3 (M + 1); NMR (DMSO-d$_6$) δ 0.7–1.0 (m, 2 H), 1.0–1.4 (m, 7 H), 1.5–1.8 (m, 6 H), 2.1 (m, 2 H), 2.3 (m, 5 H), 2.4–2.8 (m, 5 H), 2.9 (m, 2 H), 3.2 (m, 2 H), 3.4 (m, 4 H), 3.6 (m, 4 H), 3.8 (m, 2 H), 3.9 (m, 1 H), 4.5 (m, 1 H), 5.0 (m, 1 H), 6.2 (s, 1 H), 6.8 (s, 2 H), 7.2–7.4 (m, 6 H), 7.6 (m, 1 H), 7.8 (d, 1 H), 8.6 (d, 1 H). Anal. Calcd for C$_{36}$H$_{56}$N$_8$O$_8$S$_2$·0.8H$_2$O: C, H, N, H$_2$O.

[S-(R*,R*)]-N-(4-Morpholinylsulfonyl)-L-phenylalanyl-3-(2-amino-4-thiazolyl)-N-[4-[[2-[bis(2-hydroxy-ethyl)amino]ethyl]amino]-1-(cyclohexylmethyl)-2-hydroxy-4-oxobutyl]-L-alaninamide (SMO-PHE-ATA-ACHPA-BHEA, 33). The synthesis was performed in the same manner as for the synthesis of 32. The compound was synthesized in four steps from ACHPA-BHEA,[22] giving 0.34 g of 33: MS(FAB) m/e 812.4 (M + 1); NMR (CDCl$_3$) δ 0.8–1.0 (m, 2 H), 1.0–1.3 (m, 5 H), 1.4–1.7 (m, 6 H), 2.3 (d, 2 H), 2.4–2.5 (m, 2 H), 2.65 (m, 7 H), 2.7–2.8 (m, 1 H), 2.8–3.0 (m, 3 H), 3.15–3.55 (m, 9 H), 3.65 (m, 4 H), 3.8–4.0 (m, 1 H), 4.0–4.1 (m, 1 H), 4.1–4.3 (m, 1 H), 4.65 (m, 1 H), 5.5 (m, 3 H), 6.3 (s, 1 H), 6.65 (d, 1 H), 7.4 (m, 7 H), 10.1 (d, 1 H). Anal. Calcd for C$_{36}$H$_{58}$N$_8$O$_9$S$_2$·0.08CH$_2$Cl$_2$: C, H, N, Cl.

[1S-[1R*,2R*,4(R*)]]-N-(4-Morpholinylsulfonyl)-L-phenylalanyl-3-(2-amino-4-thiazolyl)-N-[2-hydroxy-4-[(2-methylbutyl)amino]-1-(2-methylpropyl)-4-oxobutyl]-L-alaninamide (SMO-PHE-ATA-STA-MBA, 34). The compound was synthesized in the same manner as 32. This gave 185 mg of pure 34: MS(FAB) m/e 710.5 (M + 1); NMR (CDCl$_3$) δ 0.75 (d, 3 H), 0.85 (d, 3 H), 0.9 (m, 6 H), 1.0–2.0 (m, 6 H), 2.2 (d, 2 H), 2.4–2.5 (m, 2 H), 2.7–2.9 (m, 4 H), 3.0–3.6 (m, 9 H), 3.7–3.8 (m, 2 H), 3.9–4.0 (m, 1 H), 4.6 (m, 1 H), 5.5 (ex s, 1 H), 5.6 (ex d, 1 H), 6.2 (s, 1 H), 6.75 (m, 1 H), 6.85 (m, 1 H), 7.3–7.5 (m, 5 H), 9.9 (m, 1 H). Anal. Calcd for C$_{32}$H$_{51}$N$_7$O$_7$S$_2$: C, H, N.

[1S-(1R*,2R*,4S*)]-N-(4-Morpholinylsulfonyl)-L-phenylalanyl-3-(2-amino-4-thiazolyl)-N-[1-(cyclohexylmethyl)-2,4-dihydroxyhexyl]-L-alaninamide (SMO-PHE-ATA-CDH, 35). The compound was synthesized in four steps from 18a. CDH (0.18 g, 0.68 mmol) was coupled to 18a (0.29 g, 0.69 mmol) as in general method A using HOBT (90 mg, 0.68 mmol) and DCC (0.14 g, 0.68 mmol). This gave 0.14 g (33%) BOC-ATA(Z)-CDH as a white foam, used as is. The BOC group was removed from BOC-ATA(Z)-CDH (0.17 g, 0.26 mmol) as in general method B. This gave 0.155 g (100%) of the hydrochloride salt which was coupled to 4 (0.08 g, 0.27 mmol) as in general method A using HOBT (40 mg, 0.29 mmol), DCC (50 mg, 0.26 mmol), and DMAP (70 mg, 0.55 mmol). This gave 0.29 g (74%) of SMO-PHE-ATA(Z)-CDH as a white foam. The Z group was removed from this using the same procedure used for the synthesis of 20 method 1. This gave 70 mg (30%) of pure 35: MS(FAB) m/e 695 (M + 1); NMR (CDCl$_3$) δ 0.8 (t, 3 H), 1.0–1.4 (m, 8 H), 1.6–1.95 (m, 7 H), 2.4 (m, 2 H), 2.7 (m, 1 H), 2.85 (m, 6 H), 3.2–3.5 (m, 6 H), 3.7–4.0 (m, 6 H), 4.6 (m, 1 H), 5.45 (s, 1 H), 5.5–5.6 (m, 1 H), 6.2 (s, 1 H), 6.65 (d, 1 H), 7.25–7.4 (m, 5 H), 10.0 (d, 1 H). Anal. Calcd for C$_{32}$H$_{50}$N$_6$O$_7$S$_2$·0.55H$_2$O: C, H, N, H$_2$O.

[S-(R*,S*)]-N-(4-Morpholinylsulfonyl)-L-phenylalanyl-3-(2-amino-4-thiazolyl)-N-[1-(cyclohexylmethyl)-3,3-difluoro-2-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]-L-alaninamide (SMO-PHE-ATA-FCS-AEM, 36).[27] The compound has appeared in the literature.

(S)-N-(4-Morpholinylsulfonyl)-L-phenylalanyl-3-(2-amino-4-thiazolyl)-N-[1-(cyclohexylmethyl)-3,3-difluoro-4-[[2-(4-morpholinyl)ethyl]amino]-2,4-dioxobutyl]-L-alaninamide (SMO-PHE-ATA-FCO-AEM, 37).[27] The compound has appeared in the literature.

(S)-N-(4-Morpholinylsulfonyl)-L-phenylalanyl-3-(2-amino-4-thiazolyl)-N-[1-(cyclohexylmethyl)-4-ethoxy-3,3-difluoro-2,4-dioxobutyl]-L-alaninamide (SMO-PHE-ATA-FCO-OET, 38). The compound was synthesized in five steps from 18b and FCS-OEt.[27] In the first step 18b (5.9 g, 12.75 mmol) was coupled to FCS-OEt·HCl (4.03 g, 12.75 mmol) in a manner similar to general method A, using HOBT (1.73 g, 12.75 mmol), DCC (2.64 g, 12.75 mmol), and Et$_3$N (1.43 g, 12.75 mmol). This gave 2.55 g (28%) of BOC-ATA(TROC)-FCS-OEt which was used without further purification. The BOC group was removed from BOC-ATA(TROC)-FCS-OEt (2.54 g, 3.5 mmol) as in general method B, giving 1.97 g (91%) of the free base ATA(TROC)-FCS-OEt. This amine (1.97 g, 3.1 mmol) was then coupled to 7 (0.99 g, 3.1 mmol) as in general method A, using HOBT (0.43 g, 3.1 mmol) and DCC (0.65 g, 3.1 mmol). This gave 1.53 g (54%) of the TROC-protected compound, SMO-PHE-ATA(TROC)-FCS-OEt, after chromatography (flash silica gel, EtOAc/hexane). The alcohol (1.53 g, 1.8 mmol) was then dissolved in a mixture of Cl$_2$HCCO$_2$H (73 μL), CH$_2$Cl$_2$ (35 mL), and DMSO (3.5 mL) at 0 °C and treated with DCC (3.65 g, 18 mmol). The mixture was stirred for 18 h at room temperature and quenched with a solution of oxalic acid (2 g) in methanol (5 mL). The mixture

was filtered free of solids, and the filtrate was evaporated at reduced pressure to dryness. The residue was treated with EtOAc (25 mL) and filtered again. The filtrate was evaporated to dryness and the residue chromatographed (flash silica gel, EtOAc/hexane (3:1-1:1)) to give 1.4 g (84%) of the ketone SMO-PHE-ATA-(TROC)-FCO-OEt. This (1.32 g, 1.4 mmol) was then deprotected in the same manner as that used for **20** method 2, giving after chromatography (flash silica gel, EtOAc/hexane (1:1)) 0.7 g (67%) of pure **38**: MS(FAB) m/e 744 (M + 1); NMR (CDCl$_3$) δ 0.6-1.0 (m, 3 H), 1.0-1.3 (m, 7 H), 1.3-1.8 (m, 7 H), 2.2-2.5 (m, 2 H), 2.5-3.0 (m, 4 H), 3.1-3.7 (m, 6 H), 3.9-4.2 (m, 1 H), 4.3 (q, 2 H), 4.6-5.1 (m, 3 H), 5.4 (s, 2 H), 6.2 (s, 1 H), 7.1 (d, 1 H), 7.2-7.5 (m, 5 H), 10.1 (d, 1 H). Anal. Calcd for C$_{32}$H$_{44}$F$_2$N$_6$O$_8$S$_2$: C, H, N.

[1S-(1R*,2S*,3R*)]-N-(4-Morpholinylsulfonyl)-L-phenylalanyl-N-[1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]-L-histidinamide (SMO-PHE-HIS-ACDMH, 39).[17] The compound has appeared in the literature.

[1S-(1R*,2S*,3R*)-N-(4-Morpholinylsulfonyl)-L-phenylalanyl-N-[1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]-3-(4-thiazolyl)-L-alaninamide (SMO-PHE-TZA-ACDMH, 40). The compound was synthesized in several steps from BOC-TZA-OBZL. The synthesis of BOC-TZA-OBZL was accomplished using the same procedure as that used for the synthesis of compounds **2** and **3**,[24] using thioformamide[44] instead of thiourea. This gave 14.7 g (50%) pure BOC-TZA-OBZL. The BOC was removed from BOC-TZA-OBZL (7.75 g, 21.4 mmol) in the same manner as general method B. This gave 6.0 g (84%) of pure TZA-OBZL·2HCl (3.69 g, 11 mmol), which was then coupled to **4** (3.46 g, 11 mmol) as in general method A using HOBT (1.49 g, 11 mmol), DCC (2.27 g, 11 mol), and triethylamine (2.53 g, 25 mmol). This gave 4.5 g (73%) of pure SMO-PHE-TZA-OBZL. This ester (4.2 g, 7.5 mmol) was saponified with sodium hydroxide (0.6 g, 15 mmol) in a mixture of methanol (50 mL) and water (20 mL). The mixture was worked up in the usual manner to give 2.8 g (80%) of the pure acid SMO-PHE-TZA. ACDMH (0.338 g, 2.5 mmol) was then coupled to SMO-PHE-TZA (1.20 g, 2.56 mmol) as in general method A using HOBT (0.338 g, 2.5 mmol) and DCC (0.516 g, 2.5 mmol). This gave after chromatography (flash silica gel, 8:2 EtOAc/CH$_2$Cl$_2$) 1.195 g (69%) pure **40**: MS(FAB) m/e 694 (M$^+$); NMR (CDCl$_3$) δ 0.6-0.9 (m, 2 H), 0.95 (m, 6 H), 1.0-2.0 (br m, 10 H), 2.4-2.5 (2 H), 2.6-2.8 (m, 1 H), 2.8-2.9 (m, 2 H), 3.0-3.1 (m, 1 H), 3.2-3.35 (m, 2 H), 3.35-3.65 (m, 6 H), 3.9-4.0 (m, 1 H), 4.1-4.25 (m, 2 H), 4.7-4.8 (m, 1 H), 4.95 (d, 1 H), 6.5 (d, 1 H), 7.15 (s, 1 H), 7.2-7.4 (m, 6 H), 8.9 (s, 1 H), 9.4 (d, 1 H). Anal. Calcd for C$_{33}$H$_{51}$N$_5$O$_7$S$_2$: C, H, N.

[1S-(1R*,2S*,3R*)]-N-(4-Morpholinylsulfonyl)-L-phenylalanyl-N-[1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]-L-phenylalaninamide (SMO-PHE-PHE-ACDMH, 41). Phenylalanine methyl ester hydrochloride (3.23 g, 15 mmol) was coupled to **4** (4.7 g, 15 mmol) in the same manner as general method A using HOBT (2.03 g, 15 mmol) and DCC (3.10 g, 15 mmol) and triethylamine (1.51 g, 15 mmol). This gave

6.71 g (94%) of pure SMO-PHE-PHE-OMe after an extractive workup. SMO-PHE-PHE-OMe (2.38 g, 5 mmol) was saponified with sodium hydroxide (0.6 g, 15 mmol) in a mixture of water (10 mL) and ethanol (20 mL). The mixture was stirred for 3 h at room temperature and then evaporated at reduced pressure to dryness. The residue was partitioned between 0.02 N sodium hydroxide (50 mL) and ether (100 mL). The ether was discarded and the base layer acidified with HCl. This was extracted with EtOAc (100 mL). The organic layer was dried over magnesium sulfate and evaporated at reduced pressure to give 2.2 g (99%) of SMO-PHE-PHE which was used as is. SMO-PHE-PHE (2.1 g, 4.6 mmol) was then coupled to ACDMH (1.12 g, 4.6 mmol) as in general method A using HOBT (0.622 g, 4.6 mmol) and DCC (0.95 g, 4.6 mmol). This gave after chromatography (flash silica gel, 1:1 EtOAc:CH$_2$Cl$_2$) 0.81 g (26%) of pure **41**: MS(FAB) m/e 687.5 (M + 1); NMR (CDCl$_3$) δ 0.7-0.95 (m, 8 H), 1.1-2.0 (m, 14 H), 2.4-2.6 (m, 3 H), 2.8-2.9 (m, 2 H), 3.0-3.3 (m, 5 H), 3.4-3.5 (m, 4 H), 3.8-3.9 (m, 1 H), 4.2 (d, 1 H), 4.3-4.5 (m, 1 H), 4.7-4.8 (q, 1 H), 5.0 (d, 1 H), 6.5 (d, 1 H), 6.85 (d, 1 H), 7.1-7.4 (m, 11 H). Anal. Calcd for C$_{36}$H$_{54}$N$_4$O$_7$S: C, H, N.

Methyl [1S-(1R*,2S*,3R*)]-3-[[1-(Cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]amino]-N-[N-(4-morpholinylsulfonyl)-L-phenylalanyl]-3-oxo-DL-alaninate (SMO-PHE-AMA-ACDMH, 42).[17] The compound has appeared in the literature.

Biological Methods. In vitro renin inhibition was measured according to the method of Haber et al.[41] The in vitro angiotensin I generation step utilized 550 μL of monkey plasma (containing native renin and angiotensinogen), 50 μL of maleate buffer (pH 6.0), 5 μL of phenylmethanesulfonyl fluoride (PMSF), and 2 μL of an appropriate concentration of inhibitor in dimethyl sulfoxide (DMSO) solution. Incubation was for 60 min at 37 °C. Following incubation, each mixture was analyzed with ^{125}I-labeled angiotensin I and carried out in tubes coated with rabbit antiangiotensin I antibody (Gamma Coat RIA Kit, Dade Clinical Assays). Monkey plasma renin activity ranged from 3 to 8 ng/mL per h. Values for inhibitor tubes were compared to vehicle 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 using nonlinear regression analysis.

In vitro bovine cathepsin D inhibition was determined in duplicate according to a modified procedure of Aoyogi and Kokubu.[29] The hydrolysis was performed at 37 °C and at pH 3.2. Net absorbances at 280 nM was measured in acid-precipitated supernatant fractions of inhibited versus uninhibited control assays. The IC$_{50}$ values were determined as described above.

In vitro porcine gastric pepsin inhibition was assayed in duplicate by hydrolysis of bovine hemoglobin at pH 1.7.[42] The percent inhibition was estimated from the net absorbance (280 nm) of acid-precipitated supernatant fractions of inhibited versus uninhibited control assays.

In vivo oral activity was determined using either a conscious, high renin, normotensive, male cynomolgus monkey model that has been previously reported[32] or a renal hypertensive monkey model which has also been previously reported.[38] The compounds were administered orally by gavage as a solution in a mixture of water, dimethylacetamide, and Tween 80 (62.5:7.5:30). Volume of vehicle was 2 mL/kg. The results are listed as the observed drop in the mean arterial blood prssure.

(44) Schmitz, W. R. Preparation of Thioformamide; US Patent 2,682,558, 1954.

(45) Rao, C.; Scarborough, P. E.; Lowther, W. T.; Kay, J.; Batley, B. L.; Rapundalo, S. T.; Klutchko, S. R.; Taylor, M. D.; Dunn, B. M. Structure-function database for active site binding to the aspartic proteinases: Genetics, Structures, and Mechanisms; Dunn, B. M., Ed.; Plenum Press: New York, 1992; pp 143-147.