Articles

Renin Inhibitors Containing Esters at the P_2-Position. Oral Activity in a Derivative of Methyl Aminomalonate

Joseph T. Repine,† Richard J. Himmelsbach,† John C. Hodges,† James S. Kaltenbronn,† Ila Sircar,† Richard W. Skeean,† Sean T. Brennan,† Timothy R. Hurley,† Elizabeth Lunney,† Christine C. Humblet,† Ronald E. Weishaar,‡ Stephen Rapundalo,‡ Michael J. Ryan,‡ David G. Taylor, Jr.,‡ Stephen C. Olson,§ Barbara M. Michniewicz,§ Brian E. Kornberg,† Daniel T. Belmont,‖ and Michael D. Taylor*,†

Departments of Chemistry, Pharmacology, Chemical Development, and Pharmacokinetics/Drug Metabolism, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48106-1047.
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A series of renin inhibitors containing ester side chains at the P_2 subsite are potent inhibitors of primate renin. Derivatives containing the diol isostere (ACDMH) at P_1–P_1' were the most potent inhibitors. Moderate selectivity for renin was observed relative to the closely related aspartic proteinase cathepsin D. The prototype compound, 4 (PD 132002), inhibited pepsin only weakly. In both high-renin normotensive and high-renin renal hypertensive monkeys, 4 produced substantial reductions in blood pressure after oral administration of 30 mg/kg. The maximum drop in blood pressure observed (24 ± 4 mmHg) in the renal hypertensive monkey model was comparable to the drop produced by an intravenous infusion of saralasin at a maximally effective dose. Both the magnitude and duration of the oral antihypertensive effect of 4 is greater than that produced by enalkiren, CGP-38560, or CP-80794 by direct comparison in the same hypertensive monkey model. The malonate ester derivatives were prepared as ca. 65:35 mixtures of epimers. The kinetics of epimerization of 4 were investigated in detail, and it was shown to equilibrate rapidly at physiological pH ($t_{1/2}$ < 2 min). Fractional crystallization was employed to obtain the individual diastereomers in >98% purity, which were indistinguishable in terms of their activity in vitro or in vivo, presumably due to rapid epimerization under the testing conditions.

Introduction

Inhibition of the renin–angiotensin system has proven to be a powerful tool in the treatment of hypertension. In addition to angiotensin converting enzyme inhibitors (CEI),[1] which have gained broad clinical acceptance, renin inhibitors[2] and angiotensin II receptor antagonists[3] also produce effective antihypertensive activity. Interest in renin inhibitors is based, in part, on an expectation that they may have advantages in selectivity relative to CEI.[4] Although many potent inhibitors of renin have been reported,[5] poor oral bioavailability remains an obstacle to their successful development. Despite the comparatively large size and difficult chemical synthesis of renin inhibitors, the combined efforts of numerous groups have resulted in subtantial improvements in potency, selectivity, and oral activity.[2]

As part of our ongoing effort in this area,[6] we investigated structure–activity relationships for several subsites of the renin inhibitor pharmacophore. The P_2 subsite,[7] in particular, has been shown to tolerate a wide variety of substitution while inhibitory activity was maintained.[8] At the same time, P_2-substitutions may produce substantial differences in enzyme selectivity.[8a] This report details a series of amino acid derivatives with ester-containing side chains at the P_2-position. These derivatives were designed to include polar functionality at P_2 to further define structure–activity relationships at this site. Derivatives containing aminomalonate esters at P_2, when combined with a suitable P_1–P_1' isostere, showed an unexpected enhancement of oral activity relative to the homologous aspartic and glutamic acid derivatives as well as the histidine derivative.

Chemistry

The compounds, listed in Table I and including the

† Department of Chemistry.
‡ Department of Pharmacology.
§ Department of Pharmacokinetics/Drug Metabolism.
‖ Department of Chemical Development.

(1) Brunner, H. R.; Nussberger, J.; Waeber, B. *J. Cardiovasc. Pharmacol.* **1985**, *7* (*Suppl. 1*), 2.
(2) Greenlee, W. J. *Med. Res. Rev.* **1990**, *10*, 173.
(3) (a) Duncia, J. V.; Chiu, A. T.; Carini, D. J.; Gregory, G. B.; Johnson, A. L.; Price, W. A.; Wells, G. J.; Wong, P. C.; Calabrese, J. C.; Timmermans, P. B. M. W. *J. Med. Chem.* **1990**, *33*, 1312. (b) Carini, D. J.; Duncia, J. V.; Johnson, A. L.; Chiu, A. T.; Price, W. A.; Wong, P. C.; Timmermans, P. B. M. W. *J. Med. Chem.* **1990**, *33*, 1330.
(4) Haber, E. *J. Hypertens.* **1989**, *7*, S81.
(5) Kleinert, H. D. *Am. J. Hypertens.* **1989**, *2*, 800.
(6) Kaltenbronn, J. S.; Hudspeth, J. P.; Lunney, E. A.; Michniewicz, B. M.; Nicolaides, E. D.; Repine, J. T.; Roark, W. H.; Steir, M. A.; Tinney, F. J.; Woo, P. K. W.; Essenberg, A. D. *J. Med. Chem.* **1990**, *33*, 838.
(7) The "P" subsite nomenclature relates amino acid residues or mimics in the inhibitor to corresponding residues in the natural substrate angiotensinogen. See: Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157.
(8) (a) Jupp, R. A.; Dunn, B. M.; Jacobs, J. W.; Vlasuk, G.; Arcuri, K. E.; Veber, D. F.; Perlow, D. S.; Payne, L. S.; Boger, J.; de Laszlo, S.; Chakravarty, P. K.; tenBroeke, J.; Hangauer, D. G.; Ondeyka, D.; Greenlee, W. J.; Kay, J. *Biochem. J.* **1990**, *265*, 871. (b) Thaisrivongs, S.; Mao, B.; Pals, D. T.; Turner, S. R.; Kroll, L. T. *J. Med. Chem.* **1990**, *33*, 1337. (c) Doherty, A. M.; Kaltenbronn, J. S.; Hudspeth, J. P.; Repine, J. T.; Roark, W. H.; Sircar, I.; Tinney, F. J.; Connolly, C. J.; Hodges, J. C.; Taylor, M. D.; Batley, B. L.; Ryan, M. J.; Essenburg, A. D.; Rapundalo, S. T.; Weishaar, R. E.; Humblet, C.; Lunney, E. A. *J. Med. Chem.* **1991**, *34*, 1258. (d) DeIlaria, J. F.; Maki, R. G.; Bopp, B. A.; Cohen, J.; Kleinert, H. D.; Luly, J. R.; Merits, I.; Plattner, J. J.; Stein, H. H. *J. Med. Chem.* **1987**, *30*, 2137. (e) Hanson, G. J.; Baran, J. S.; Lowrie, H. S.; Sarussi, S. J.; Yang, P.; Babler, M.; Bittner, S. E.; Papaioannou, S. E.; Walsh, G. M. *Biochem. Biophys. Res. Commun.* **1987**, *146*, 959.

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Chart I. Reference Renin Inhibitors

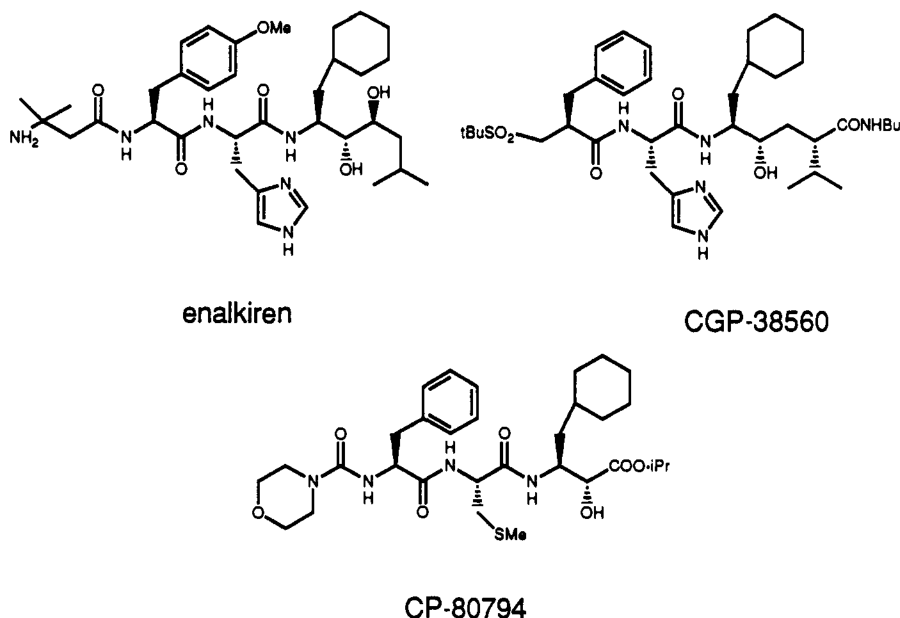
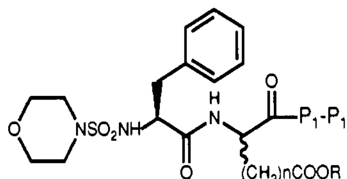


Table I. Biological Activity in Vitro and in Normotensive High-Renin Monkeys



compd	n	R	P_1–P_1'[a]	IC_{50}, nM renin[b]	cathepsin D[c]	oral activity[d]
1	0	Me	STA-AEM	130.0[e]	>1000	NT
2	0	Me	CST-AEM	4.0	>1000	NT[f]
3	0	Me	DFS-AEM	0.79	515	16 ± 5 (2)
4	0	Me	ACDMH	0.28[g]	36	22 ± 7 (4)
4A	0	Me	ACDMH	0.19	46	18 ± 2 (3)
4B	0	Me	ACDMH	0.14	77	14 ± 2 (4)
5	1	Me	ACDMH	0.18	27	3 ± 1 (2)
6	2	Me	ACDMH	1.40	30	12 ± 0 (2)
7	0	H	ACDMH	27.0	310	NT[h]
8	0	*i*-Pr	ACDMH	0.68	43	16 ± 4 (2)
9	0	allyl	ACDMH	0.95	>100	NT
10	0	*n*-Hx	ACDMH	15.0	100	NT
15	(P_2 = His)		ACDMH	0.23	51800	11 ± 1 (2)
vehicle						6 ± 1 (6)

[a] Structures of P_1–P_1' isosteres are shown in Chart II. [b] Monkey plasma renin. [c] Bovine (Sigma). [d] Maximum fall in mean arterial blood pressure expressed in mmHg following a 30 mg/kg oral dose in high-renin normotensive monkeys. Number of observations given in parentheses. [e] Human renin IC_{50} = 130 nM. [f] Not tested orally, but reduced blood pressure by 16 mmHg (N = 1) after iv administration (3 mg/kg). [g] Human renin IC_{50} = 0.28 nM. [h] Not tested orally, but reduced blood pressure by 12 mmHg (N = 2) after iv administration (3 mg/kg).

reference inhibitors enalkiren,[9] CGP-38560,[10] and CP-80794[11] (Chart I), were prepared using standard peptide coupling methods. As an illustration, the synthesis of 4 (PD 132002) and derivatives is shown in Scheme I. The methyl aminomalonate derivatives were prepared from methyl benzyl aminomalonate, whereas the other esters were prepared from the methyl ester via carboxylic acid 7, which was prepared by saponification of 4. The P_1–P_1' isosteres (Chart II) statine (STA) and its cyclohexyl ana-

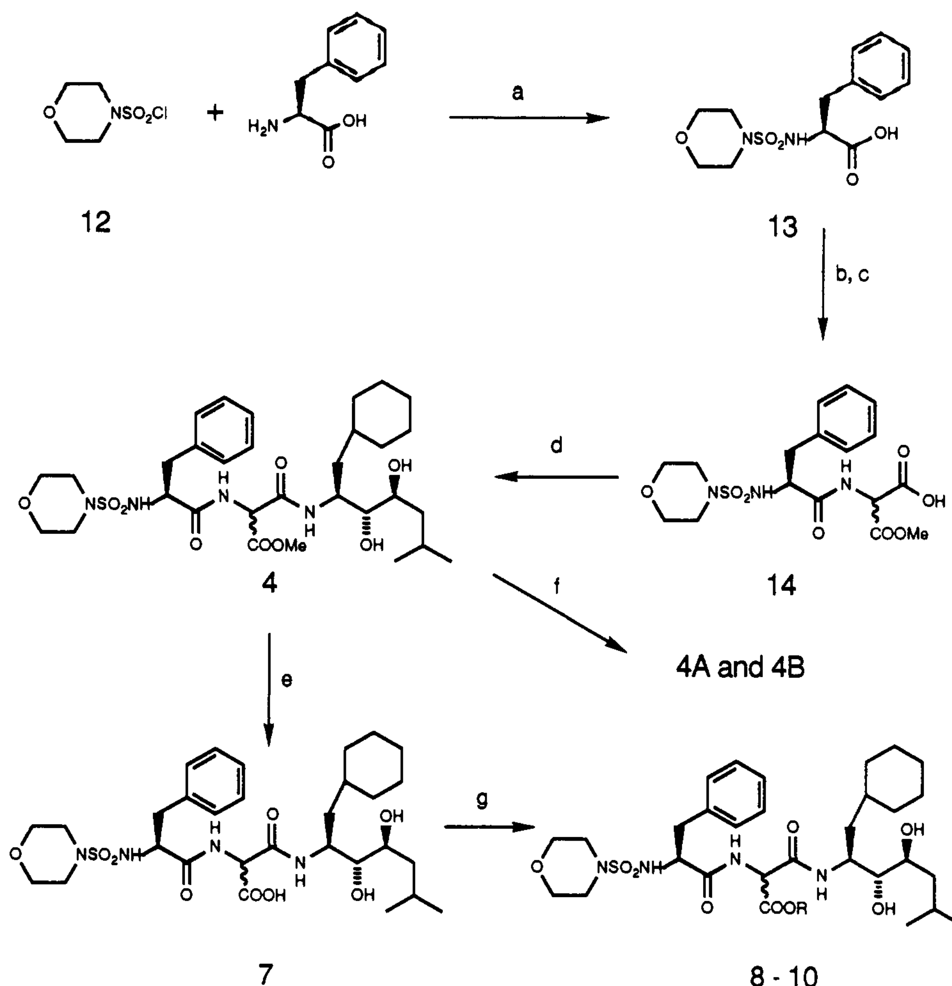
Table II. Isolation of Individual Epimers **4A** and **4B** by Fractional Recrystallization[a]

solvent	% yield	assay[b] %	ratio 4A/4B
4 (starting mixture)		96.0	65/35
methanol	69	96.3	04/96
ethanol	92	98.0	98/02
2-propanol	63	96.0	96/04
1-butanol	79	97.4	99/01
ethyl acetate (5 °C)	80	96.3	04/96
ethyl acetate	66	97.4	02/98

[a] Conditions: 10 mL of solvent and 1 g of 4 stirred at 25 °C for 40 h. [b] Purity of isolated product determined by combined integration of HPLC peaks of **4A** and **4B**.

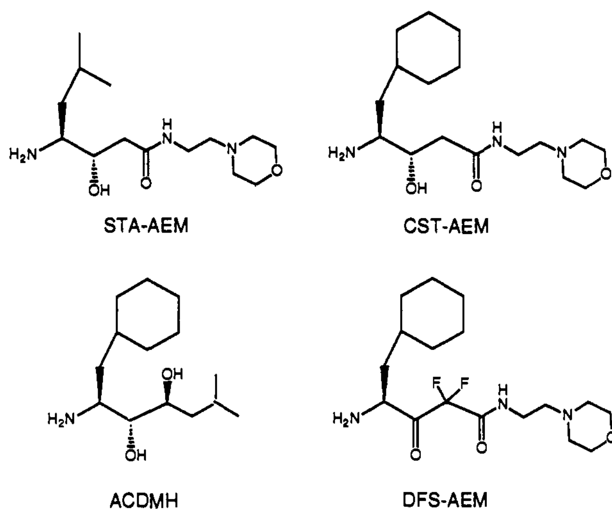
(9) Bursztyn, M.; Gavras, I.; Tifft, C. P.; Luther, R.; Boger, R.; Gavras, H. *J. Cardiovasc. Pharmacol.* **1990**, *15*, 493.
(10) Nussberger, J.; Delabays, A.; De Gasparo, M.; Cumin, F.; Waeber, B.; Brunner, H.; Menard, J. *Hypertension* **1989**, *13*, 948.
(11) Wester, R. T.; Hoover, D. J.; Murphy, W. R.; Holt, W. F.; Rosati, R. L.; Dominy, B. W. The Renin Angiotensin System as a Therapeutic Target (Conference); October 29–31, 1989, Basel; Abstract 16.

Scheme I[a]



[a] (a) NaOH, 2 equiv of Phe; (b) DCC, HOBT, $H_2NCH(COOMe)COOBn$; (c) H_2, Pd/C; (d) ACDMH, DCC, HOBT; (e) NaOH, MeOH; (f) recrystallization from EtOH (**4A**) or EtOAc/TEA (**4B**); (g) ROH, DCC, HOBT.

Chart II. P_1-P_1' Isosteres



logue (CST)[12] difluorocyclostatone (DFS)[13] were prepared as the 2-(4-morpholinyl)ethylamides. The ACDMH ((2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane)[14] fragment was prepared as previously described. The *N*-(4-morpholinylsulfonyl)phenylalanine moiety at P_4–P_3 was used in all of the compounds. This group has been shown to reduce lipophilicity relative to other substitutions, such as isovalerylphenylalanine or bis(naphthylmethyl)acetamide, while in vitro potency and in vivo pharmacologic activity was maintained.[15]

The target esters were isolated as mixtures of epimeric diastereomers, generally in a ratio of about 65:35 as determined by reverse-phase HPLC,[16] except for the aspartic and glutamic acid esters, which were isolated as single stereoisomers. As shown in Table II, either of the individual epimers of **4** (**4A**:**4B**) could be obtained by fractional crystallization using an appropriately selected solvent. That an equilibration is indeed operational is shown by the yield of the pure epimers obtained (up to 92% from the mixture). With methanol or ethyl acetate, pure **4B** was obtained; **4A** was isolated from ethanol, 2-propanol,

(12) Klutchko, S.; O'Brien, P.; Hodges, J. C. *Synth. Commun.* **1989**, *19*, 2573–2583.
(13) Thaisrivongs, S.; Pals, D. T.; Kati, W. M.; Turner, S. R.; Thomasco, L. S.; Watt, W. J. *J. Med. Chem.* **1986**, *29*, 2080.
(14) Luly, J. R.; Bamaung, N.; Soderquist, J.; Fung, A. K. L.; Stein, H.; Kleinert, H. D.; Marcotte, P. A.; Egan, D. A.; Bopp, B.; Merits, I.; Bolis, G.; Greer, J.; Perun, T. J.; Plattner, J. J. *J. Med. Chem.* **1988**, *31*, 2264.
(15) Hodges, J. C.; Connolly, C. J.; Kornberg, B. E.; Repine, J. T.; Klinkefus, B. A.; Batley, B. L.; Painchaud, C. A.; Bernabe, A. A.; Ryan, M. J. American Chemical Society 199th National Meeting; April 22-27, 1990, Boston, MEDI 129.
(16) The **A** and **B** designations were assigned originally based on order of elution on normal-phase HPLC. Later analytical methods employed reverse-phase HPLC and the order of elution is reversed; i.e. **4B** elutes first.

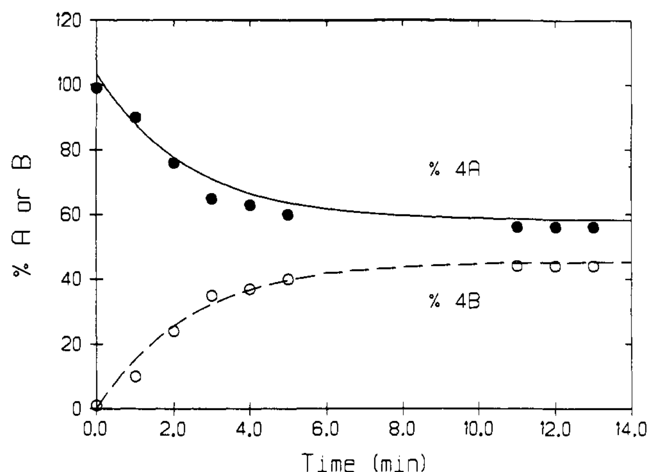


Figure 1. Kinetics of epimerization at pH 7.4 in 1:1 acetonitrile/1.0 M phosphate buffer. Shown are actual data for percent 4A (closed circles) and **4B** (open circles) fitted to the model of opposing first-order reactions.

or 1-butanol. Assignment of the absolute configuration of the two epimers could not be made due to facile interconversion and the unsuitability of crystals of the pure epimers for X-ray crystallographic analysis.

The P_2 α-carbon of the aminomalonate derivatives undergoes rapid epimerization in solution at neutral pH. The rate of epimerization is much slower at acid pH. Epimerization also occurred in some organic solvents and was facilitated by the presence of base. With individual isomers in hand the epimerization of **4** could be studied in detail. The rates of epimerization were determined at various pHs[17] up to pH 7.4 at room temperature in a 50:50 mixture of acetonitrile and 1.0 M aqueous buffer beginning with either pure **4A** or **4B**. The results at pH 7.4 are shown in Figure 1. The epimerization is very rapid, reaching equilibrium in about 6 min. At lower pH the rates decrease significantly such that equilibrium is not reached in 26 h at pH 1.2. At a given pH, the epimerization follows a model composed of opposing first-order reactions.[18]

Biological Results and Discussion

In Vitro Enzyme Inhibition. All of the compounds were evaluated for their ability to inhibit monkey renin and bovine cathepsin D, and the results are shown in Table I. The relative inhibitory activity for renin versus the closely related aspartic proteinase cathepsin D provided an indication of the selectivity of the inhibitors for renin.[8a]

Compounds containing the methyl aminomalonate residue at P_2 exhibited moderate to extremely potent inhibition of renin, depending on the nature of the P_1–P_1' substitution. Statine derivative **1** was the least potent. Nanomolar potency was achieved with cyclohexylstatine derivative **2**, and subnanomolar potency was observed for the difluorocyclostatone (**3**) and ACDMH (**4**) derivatives. Selectivity for renin versus cathepsin D was moderate for the most potent inhibitors (e.g. 130-fold for **4**). Based on the relative potencies, the ACDMH fragment was employed as the optimum P_1–P_1' substitution for incorporation into additional derivatives. Lengthening the side chain maintained potency for the aspartic acid ester derivative **5**, but potency decreased nearly 10-fold for glutamic acid ester **6**. Interestingly, inhibition of bovine cathepsin D remained unchanged through these changes. The aminomalonic acid derivative was much less active than the parent ester.[19] Larger ester groups, such as those contained in **8** and **9**, were consistent with potent renin inhibition, except for *n*-hexyl ester **10**, which was less effective. Except for **10**, all of the aminomalonate ester–ACDMH derivatives were approximately as potent (IC_{50} < 1 nM) for inhibition of renin as the corresponding histidine derivative **15**.

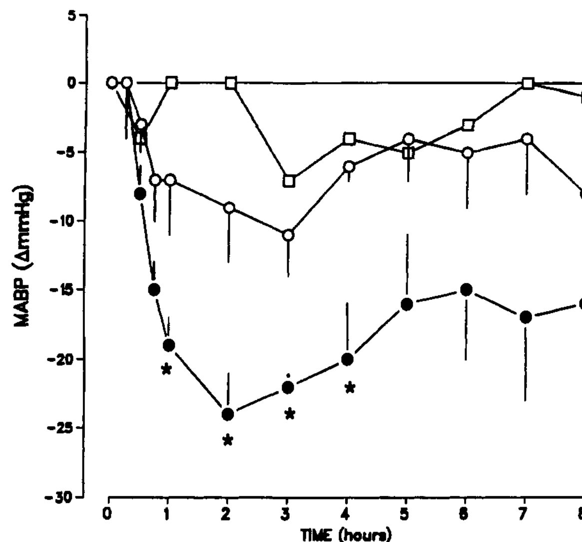


Figure 2. Time course of action of **4** in renal hypertensive cynomolgus monkeys (N = 4 for all) after oral dosing: 10 mg/kg, ○; 30 mg/kg, ●; vehicle, □.

The individual diastereomers of **4** (**4A** and **4B**) were evaluated for renin and cathepsin D inhibitory activity and were found to be indistinguishable. On the basis of molecular modeling predictions (vide infra) and structure–activity relationships,[20] this result is believed to be due to epimerization occurring under the assay conditions.

In Vivo Pharmacology. Two primate models were employed: a high-renin normotensive monkey model in which plasma renin activity (PRA) was raised by sodium restriction and furosemide treatment[21] and a chronic pathological model in which both PRA and mean arterial blood pressure were chronically elevated by renal artery clipping.[22] Several of the compounds listed in Table I were evaluated for oral activity in the normotensive model. Good oral activity was observed for malonate **4**, with somewhat lower activity observed for **3** and **8**. Aspartic acid derivative **5** was not active, and the glutamic acid (**6**) and the difluorostatone (**3**) derivatives showed weak activity. Even though weak, the oral activity of **6** was surprising, given that it is 10-fold less effective as a renin inhibitor, and could be due to greater relative bioavailability compared to **15**.

Compound **4** reduced blood pressure when infused intravenously to conscious high-renin normotensive monkeys.

(17) Since the epimerization was studied in a mixture of aqueous and organic solution, the pH indicated is an apparent pH and was not corrected. Due to presence of organic solvent as well as differences in buffers used at various pH, the kinetics did not follow strictly second order base-catalyzed kinetics across the range of apparent pH studied.

(18) Laidler, K. J. *Chemical Kinetics*; McGraw-Hill: New York, 1950; pp 118–22.

(19) Since the acid was prepared by saponification of the parent methyl ester, the moderate renin inhibition activity observed for **7** could be due, at least in part, to residual contamination (≤1%) by the parent compound.

(20) Routine separation of diastereomers differing in configuration at P_2 results in differences in potency ranging from 10 to 1000-fold, Taylor, M.; Rapundalo, S. unpublished results.

(21) Painchaud, C.; Ryan, M.; Hicks, G.; Fettig, M.; Rapundalo, S.; Taylor, D. *FASEB J.* **1990**, *4*, A748.

(22) Panek, R.; Ryan, M.; Rapundalo, S.; Weishaar, R.; Taylor, D. *J. Mol. Cell. Cardiol.* **1989**, *21* (*Suppl. II*), S106.

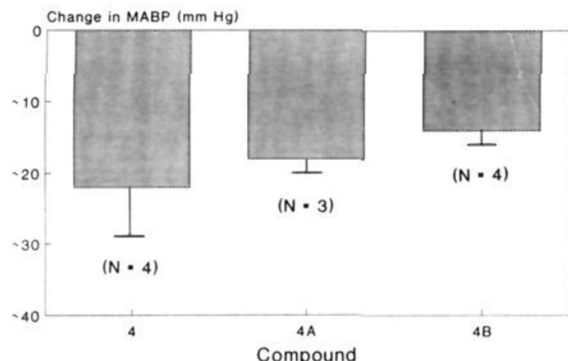


Figure 3. Comparative oral activity of 4, **4A**, and **4B** in high-renin normotensive cynomolgus monkey model. All three compounds were administered at a dose of 30 mg/kg.

Table III. Comparison of 4 with Reference Renin Inhibitors in Vitro and in Renal Hypertensive Monkeys

compd	IC_{50}, nM		oral activity[c] max drop in BP, mmHg (±SEM (N))
	renin[a]	cathepsin D[b]	
4	0.28	36	24 ± 4 (4)
enalkiren	0.75	>1000	8 ± 4 (3)
CGP-38560	0.83	1300	16 ± 3 (3)
CP-80794	0.20	24	9 ± 2 (4)
vehicle			6 ± 7 (4)

[a] Monkey plasma renin. [b] Bovine (Sigma). [c] Maximum fall in mean arterial blood pressure expressed in mmHg following a 30 mg/kg oral dose to renal hypertensive monkeys.

The magnitude of blood pressure reduction, 25 mmHg (not shown), was comparable to that observed with other inhibitors of the renin–angiotensin system including captopril (CEI) and saralasin (angiotensin II receptor antagonist).[21] Following oral administration, 4 produced a 22 mmHg reduction in blood pressure in high-renin normotensive monkeys at a dose of 30 mg/kg (Figure 2). This drop was twice the effect observed for histidine derivative **15**. In this model, a marked fall in blood pressure was noted within 30 min after administration and the hypotensive response was maintained for the entire 3-h observation period. Smaller, statistically nonsignificant, reductions in blood pressure were observed after a 10 mg/kg dose.

The hypotensive activities of the individual epimers **4A** and **4B** were compared with 4 in the high-renin normotensive monkey model. Analogous to the results of the enzyme inhibition assays, both epimers were effective in lowering blood pressure and, although the peak reductions in blood pressure produced by both epimers were smaller than that produced by the mixture, the differences were not statistically significant[23] (Figure 3). This result again was attributed to epimerization under physiological conditions. However, since intrinsic activities for each epimer could not be directly determined, the possibility that both epimers contribute to the activity could not be strictly ruled out.

Compound 4 (PD 132002) was the most active member of this series. It was compared directly with several other reported renin inhibitors in the renal hypertensive monkey model of hypertension. All compounds were administered under identical conditions. Compared to enalkiren (A-64662), CGP-38560, and CP-80794, 4 showed greater and more long-lasting reductions in blood pressure following oral administration of 30 mg/kg (Table III and Figure 4). The blood pressure lowering effects of all four compounds

(23) Group t test.

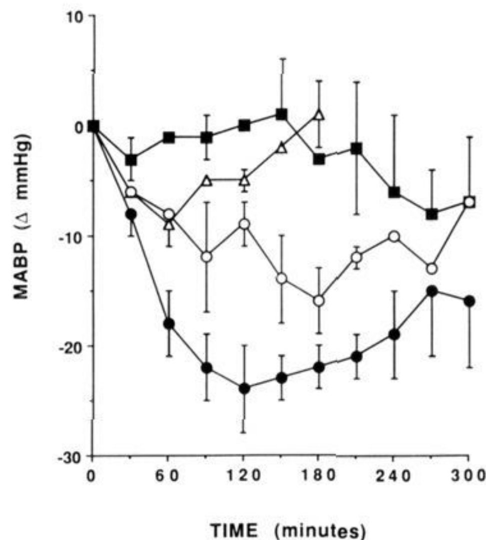


Figure 4. Comparative oral activity of renin inhibitors in renal hypertensive cynomolgus monkeys after an oral dose of 30 mg/kg; CP-80794 (N = 4), △; enalkiren (N = 3), ■; CGP-38560 (N = 3), ○; 4 (N = 4), ●.

Table IV. Chymotrypsin Proteolysis of Aminomalonate Ester Derivatives

compound	R	% degradation at 30 min
4	Me	100[a]
8	*i*-Pr	70
9	allyl	65
10	*n*-Hx	15

[a] At 15 min, 89% of 4 had been degraded.

were comparable when administered intravenously (data not shown).

Susceptibility to Chymotrypsin Proteolysis. One reason for low oral activity of peptide-like renin inhibitors may be their susceptibility to degradation by digestive enzymes present in the gut.[2] To evaluate this possibility, the stability of several of the aminomalonate esters to chymotrypsin treatment was determined following incubation of the test compound in a buffered solution (pH 6.9) of bovine chymotrypsin by using a previously established method.[6] The results are summarized in Table IV. Methyl ester 4 was rapidly degraded such that none of the parent remained after a 30-min digestion. Degradation was shown to involve cleavage of the P_3–P_2 peptide bond to yield smaller fragments, neither of which inhibit renin activity. The larger isopropyl (**8**) and allyl esters (**9**) exhibited greater stability with about 30–35% of the compounds intact after 30 min. The best stability was seen with the *n*-hexyl ester, the bulk of which was intact after 2 h. However, this derivative was also the least effective as a renin inhibitor. The lack of correlation between oral activity and stability to chymotrypsin suggests that oral activity can be observed even in the absence of substantial stability to digestive enzymes. Two possible explanations are raised, one is that the relatively small amount of compound that escapes digestion is sufficient to produce the observed activity or, alternatively, that digestion does not occur in vivo to the extent suggested by in vitro studies.

Molecular Modeling

Molecular modeling methods were used to qualitatively evaluate the relative binding compatibility of R and S epimers of 4 in the active site of a human renin model, which was derived from homologous crystal structures of fungal aspartic proteinases.[24] The binding conformations

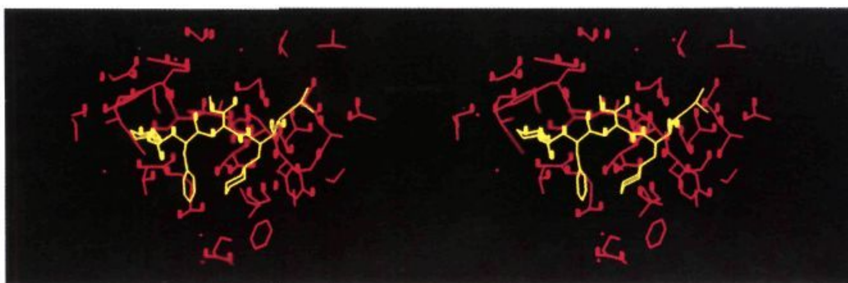


Figure 5. Stereoview of (*R*)-**4** bound to the cleft of a model of human renin. This isomer binds in the normal mode with the P_2 side chain filling the S_2 subsite.

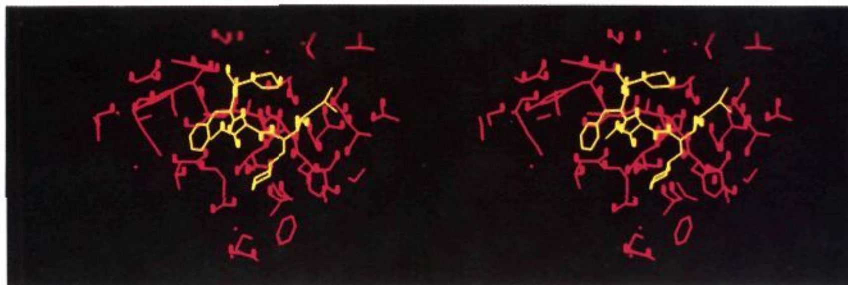


Figure 6. Stereoview of (*S*)-**4** bound to the cleft of a model of human renin. This isomer binds in an abnormal mode with the P_2 side chain unable to bind in the S_2 subsite.

of inhibitors at the active sites of aspartic proteinases have now been described in crystal structures of endothiapepsin (a fungal enzyme homologous with renin) cocrystallized with various inhibitors.[25] The bound conformations of the inhibitors uniformly involve an extended structure from the P_3 to P_1 sites, with the side chains occupying pockets alternating on opposite sides of the backbone. The hydroxyl group at the P_1 residue is always positioned between Asp215 and Asp32 at the active site, and a general hydrogen-bonding pattern involving the amide bonds of the ligands is conserved. With this binding scheme as a template, each epimer of **4** was docked in the renin cleft. The docked conformation of the *R* epimer, which corresponds to an L-amino acid configuration, adopts the binding pattern described above for the crystal structures (Figure 5). However, in the case of the *S* diastereomer favorable bound conformations differ considerably from the crystal-based one. For example, as shown in Figure 6, the *S* diastereomer can position the P_2 side chain into the previously defined backbone area extending to the upper S_3 site. Simultaneously, it projects the P_3 and P_4 side chains within the S_2 site and further into the S_4 region. Although this binding mode allows for particular H-bonding interactions, these are not viewed as compensating for the absence of a large hydrophobic P_3 moiety. This prediction is based on the literature precedent indicating that highly potent renin inhibitors generally contain a large hydrophobic P_3 moiety.[8a] Binding modes for the *S* diastereomer differ significantly from that conserved in the crystal structures and determined for the *R* diastereomer. It is unlikely therefore that both the *R* and *S* epimers would have comparable inhibitory potencies. This analysis supports the premise that the near equipotent activities measured for the individual epimers result from rapid epimerization under the assay conditions, and do not reflect intrinsic relative potencies.

Summary. Moderate oral activity was observed for a renin inhibitor (**4**) containing methyl aminomalonate at P_2. This activity was observed in spite of a high degree of susceptibility to degradation by digestive enzymes, specifically chymotrypsin. The magnitude and duration of the activity was superior to other reported inhibitors of similar in vitro potency. The significance of the epimerization and relevance of enzymatic stability for the oral activity deserve further study.

Experimental Section

General. The NMR spectra were obtained in $CDCl_3$ solution with tetramethylsilane as an internal standard and recorded on Varian EM-390, IBM WP100SY, Varian XL-200, or Bruker 250 spectrometers. Copies of the NMR spectra of the compounds in Table I are included in the supplemental material. The mass spectral data are FAB mass spectra determined on a VG analytical 7070E/HF mass spectrometer in a thioglycerol matrix using xenon as the target gas unless otherwise indicated. Spectra and microanalyses were performed by the Parke-Davis Analytical Chemistry Section. Precoated plates (silica gel 60F 254, Merck) were used for TLC. Column chromatography was performed with flash grade (230–400 mesh) silica gel.

Benzyl Methyl Aminomalonate Oxime. Benzyl methyl malonate (183.7 g, 0.88 mol) was placed in a 2-L flask and cooled to 5 °C. A solution of acetic acid (174 mL) and water (250 mL) was added to the flask. Sodium nitrite (182.16 g, 2.64 mol) was added in small portions over 3 h (yellow gas is evolved). After the addition was over the solution was stirred at room temperature overnight. Diethyl ether (700 mL) was added. The aqueous layer was separated and the organic layer was washed successively with water (250 mL, twice), saturated aqueous sodium bicarbonate (250 mL, twice), brine (200 mL, twice), and finally dried over anhydrous magnesium sulfate. The solvent was removed in vacuo and the yellow oil was dried in vacuo to remove any residual solvent. The isolated material was used directly in the next step: yield 199 g (95.4%); NMR (90 MHz) reveals a 54:46 mixture of cis and trans isomers; δ 7.28 and 7.25 (s, combined 5 H), 5.20 and 5.25 (s, combined 2 H), 3.78 and 3.73 (s, combined 3 H).

Benzyl Methyl Aminomalonate Hydrochloride. Benzyl methyl aminomalonate oxime (119 g, 0.84 mol), glacial acetic acid (55.4 g, 0.92 mol), aluminum (8–20 mesh, 56 g), mercuric chloride (2.0 g), and diethyl ether (1750 mL) were added to a reaction flask.

(24) 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*; Burgen, A. S. V.; Roberts, G. C. K.; Tite, M. S., Eds., Elsevier Science: Amsterdam, 1986; p 323.

(25) Cooper, J. B.; Foundling, S. I.; Blundell, T. L.; Boger, J. *Biochemistry* **1989**, *28*, 8596.

A small amount of water (1–2 mL) was added to start the reaction (**Caution:** As the reaction proceeds it refluxes vigorously at times and an ice/water bath is needed to cool the reaction mixture.) Water (250 mL) was added over a period of 3–4 h, and the reaction mixture was stirred at room temperature overnight. The amalgam was removed by vacuum filtration and the solution was treated with 1 N HCl (700 mL). The aqueous layer was separated, and the organic layer was treated with additional 1 N HCl (200 mL). The combined aqueous fractions were extracted with diethyl ether (2×) and placed in an ice/water bath. It was neutralized to pH 7 with solid sodium bicarbonate. The aqueous solution was extracted with 700 mL of methylene chloride, which was then dried over anhydrous magnesium sulfate. A saturated solution of anhydrous hydrogen chloride in methylene chloride (200 mL) was added and the solution was concentrated to yield 50 g (27%) of a highly viscous gum: NMR (250 MHz) δ 8.24 (br s, 2 H), 7.30 (m, 5 H), 5.8–5.2 (m, 2 H), 5.1 (m, 1 H), 3.73 (s, 3 H). The amine hydrochloride, which was not easily purified, was converted to the BOC-amide derivative for analysis: NMR (90 MHz) δ 7.33 (s, 5 H), 5.65 (br d, J = 8 Hz, 1 H), 5.30 (d, J = 11 Hz, 1 H), 5.13 (d, J = 11 Hz, 1 H), 5.05 (d, J = 8 Hz, 1 H), 3.78 (s, 3 H), 1.47 (s, 9 H). Anal. Calcd for $C_{16}H_{21}NO_6$: C, N; H: calcd 6.55, found 6.97.

[*S*-(*R,*R**)]-*N*-[*N*-(4-Morpholinosulfonyl)-L-phenylalanyl]-3-[[2-hydroxy-1-(2-methylpropyl)-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]amino]-3-oxo-DL-alanine Methyl Ester (1).** A solution of 1.50 g (3.5 mmol) 14 in 45 mL of CH_2Cl_2 was cooled up 0 °C and 0.49 g (3.6 mmol) of HOBT in 6 mL of DMF was added. A solution of 1.0 g (3.5 mmol) of statine morpholinylethylamide[12] in 15 mL of methylene chloride was then added, followed by 0.74 g (3.6 mmol) of dicyclohexylcarbodiimide. The mixture was allowed to warm to room temperature over 3 h, then stirred overnight. The mixture was filtered and concentrated to yield an oil that was dissolved in ethyl acetate, filtered, and extracted with 1 N citric acid. The acid washes were combined, neutralized with solid sodium bicarbonate, and extracted with ethyl acetate. The ethyl acetate solution was washed with brine, dried (magnesium sulfate), and concentrated to yield a pink foam 1.67 g (67%). This material was combined with 1.49 g (76%) from a second run and purified by flash chromatography (100 g of silica gel, 0–10% methanol in 1:1 ethyl acetate/chloroform) to yield 2.63 g (60% overall yield): MS m/e 699.2 (M + 1). Anal. Calcd for $C_{31}H_{50}N_6O_{10}S{\cdot}0.10CHCl_3{\cdot}0.25H_2O$: C, H, N, S, Cl, H_2O.

[*S*-(*R,*R**)]-3-[[1-(Cyclohexylmethyl)-2-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]amino]-*N*-[*N*-(4-morpholinosulfonyl)-L-phenylalanyl]-3-oxo-DL-alanine Methyl Ester (2).** A solution of 1.20 g (2.8 mmol) of 14, 0.91 g (2.8 mmol) of cyclostatine-morpholinylethylamide,[12] and 0.40 g (2.9 mmol) of HOBT in 80 mL of methylene chloride was cooled to 0 °C and 0.6 g (2.9 mmol) of dicyclohexylcarbodiimide was added. The mixture was allowed to warm to room temperature over 3 h, then stirred overnight. The mixture was filtered and concentrated to yield an oil that was dissolved in ethyl acetate, filtered, and extracted with 1 N citric acid. The acid washes were combined, neutralized with solid sodium bicarbonate, and extracted with ethyl acetate. The ethyl acetate solution was washed with brine, dried (magnesium sulfate), and concentrated to yield a pink foam (2.04 g), which was purified by flash chromatography (100 g of silica gel, 0–5% methanol in chloroform) to yield 1.78 g (88%) as a white foam. From this material, 1.61 g (2.18 mmol) was added to 0.42 g (2.18 mmol) of citric acid and dissolved in 20 mL of water. The mixture was filtered and lyophilized to yield a white solid (1.84 g, 91%): MS m/e 739.3 (M + 1); NMR consistent with structure. Anal. Calcd for $C_{34}H_{54}N_6O_{10}S{\cdot}1.0C_6H_8O_7{\cdot}1.5H_2O$: C, H, N, S, Cl, H_2O.

(*S*)-3-[[1-(Cyclohexylmethyl)-3,3-difluoro-4-[[2-(4-morpholino)ethyl]amino]-2,4-dioxobutyl]amino]-*N*-[*N*-(4-morpholinosulfonyl)-L-phenylalanyl]-3-oxo-DL-alanine Methyl Ester Methanesulfonate (3). A solution of 5.15 g (12 mmol) of 14, 4.31 g (12 mmol) of (2*S*,3*R*)-[1-(cyclohexylmethyl)-3,3-difluoro-4-[[2-(4-morpholino)ethyl]amino]-2-hydroxy-4-oxobutyl]amine (prepared analogously to examples described by Thaisrivongs et al.[13]) and 3.22 g (14 mmol) of HOBT in 150 mL of methylene chloride was chilled to 0 °C and 3.03 g (14 mmol) of dicyclohexylcarbodiimide was added. The mixture was kept cold for 2–3 h, then allowed to warm to room temperature overnight. After stirring for 38 h, the reaction mixture was filtered and the filtrate was washed with saturated sodium bicarbonate, dried, filtered, and concentrated. Flash chromatography of the residue (5% methanol in chloroform) gave 3.9 g of product which was rechromatographed on a second column (5% methanol in ethyl acetate) to yield 3.17 g (34%) of a white foam. This adduct was used directly in the next step as described below: IR (KBr) 3001, 1734, 1695, 1575 cm^{-1}; NMR consistent with structure; MS m/e 775.3 (M + 1). Anal. Calcd for $C_{34}H_{52}F_2N_6O_{10}S{\cdot}0.6C_3H_8O_2$: C, H, N.

A solution of 1.0 g (1.3 mmol) of the adduct, thus prepared, in 10 mL of methylene chloride at 0 °C was treated with 0.17 mL (2.0 mmol) of dichloroacetic acid followed by 1.5 mL of dimethyl sulfoxide and 2.68 g (1.3 mmol) of dicyclohexylcarbodiimide. The mixture was kept cold for 2–3 h, then allowed to warm to room temperature overnight. After stirring for 24 h, a solution of 1.89 g (1.5 mmol) of oxalic acid in 42 mL (14.7 mmol) of methanol was slowly added, the mixture was stirred for 30 min, followed by addition of ether (12 mL). The final solution was stirred an additional 15 min, the precipitate removed by filtration, and the filtrate was concentrated. The residue was dissolved in 120 mL of 1:5 H_3PO_4/water and extracted with 120 mL of ethyl acetate. The emulsion that formed was diluted with a second portion (180 mL) of acid solution. The aqueous layer was separated, and the pH adjusted gradually to 4.5–5.0 (pH meter) with ammonium hydroxide while cold (0 °C). The solution was washed three times with ethyl acetate and the combined organic fractions were washed with brine, dried, filtered, and concentrated to 1.04 g of a white foam. The foam was dissolved in 20 mL of methanol and 0.12 mL (1.9 mmol) of methanesulfonic acid was added. After stirring for 15 min, the solution was filtered and concentrated. The residue was dissolved in 15 mL of water and lyophilized. A white solid (**3**, 1.15 g, 86%) was isolated: HPLC ratio of diastereomers 76:24, assay 94.2%; NMR was consistent with the structure; IR (KBr) 3370 (br), 2950, 1772, 1690; MS m/e 773.4 (M + 1). Anal. Calcd for $C_{34}H_{50}F_2N_6O_{10}S{\cdot}0.7H_2O{\cdot}2.5CH_3SO_3H$: C, H, N, S, H_2O.

[1*S*-(1*R,2*S**,3*R**)]-3-[[1-(Cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]amino]-*N*-[*N*-(4-morpholinosulfonyl)-L-phenylalanyl]-3-oxo-DL-alanine Methyl Ester (4).** HOBT (5.2 g, 38.5 mmol) was added to a solution of 14 (15 g, 35 mmol) in methylene chloride (350 mL). DMF (210 mL) was added with stirring to dissolve HOBT. ACDMH[14] (as the hydrochloride salt; 10.28 g, 37 mmol) was added followed by triethylamine (5.36 mL, 38.5 mmol). The reaction mixture was cooled to 0 °C, at which point dicyclohexylcarbodiimide (7.94 g, 38.5 mmol) was added. The reaction mixture was allowed to warm up to room temperature and stirred overnight under nitrogen. The reaction mixture was filtered and concentrated, and the residue was taken up in ethyl acetate. The solution was washed successively with 1 N citric acid, saturated aqueous sodium bicarbonate, and brine, and finally dried over anhydrous magnesium sulfate. The mixture was concentrated, and the crude product was purified via flash chromatography (2–5% methanol in chloroform) to yield 11.9 g (52%) of the product (93% pure, 34:66 ratio of epimers by HPLC). An additional 3 g of slightly impure product (85% by HPLC) was also obtained. NMR was consistent with the structure. Anal. Calcd for $C_{31}H_{50}N_4O_9S$: C, H, N, S.

Fractional Crystallization of 4A. Compound 4 (42.6 g) was stirred in absolute ethanol (250 mL) at 25 °C for 72 h. The slurry was filtered, and the cake was washed with 50 mL of water. The white solid was dried under vacuum at 40 °C to give 28.1 g (66%), which was 96.7% 4A and 0.81% 4B (HPLC). Anal. Calcd for $C_{31}H_{50}N_4O_9S$: C, H, N, S.

Fractional Crystallization of 4B. Compound 4 (50.0 g) was added to 500 mL of ethyl acetate and 0.5 mL of triethylamine. The mixture was heated to reflux to achieve solution. The solution was cooled to 25 °C and stirred for 72 h. The resulting slurry was filtered and the cake washed with 15 mL of ethyl acetate. The white solid was dried under vacuum at 40 °C to give 33.2 g (66%), which was 98.3% **4B** and 0.4% 4A (HPLC). Anal. Calcd for $C_{31}H_{50}N_4O_9S$: C, H, N, S.

[1*S*-(1*R,2*S**,3*R**)]-3-[[1-(Cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]amino]-*N*-[*N*-(4-morpholinosulfonyl)-L-phenylalanyl]-3-oxo-DL-alanine (7).** A solution of 2.49 g 4 (3.8 mmol) in 45 mL of methanol and 3.8 mL of 1 N

sodium hydroxide was stirred overnight at room temperature under nitrogen. The solution was concentrated (bath temperature < 30 °C) and extracted with ethyl acetate. The extracts were combined, dried, and concentrated to yield 0.35 g of an unidentified white foam (mass spectral analysis showed this material was not the starting ester). The aqueous extract was acidified with 3 N HCl to pH 4 and extracted with ethyl acetate. The extracts were combined, dried, and concentrated to yield 2.1 g (83%) of a white foam (43:57 mixture of epimers): MS m/e 641 (M^+). NMR consistent with structure. Anal. Calcd for $C_{30}H_{48}N_4O_9S$: C, H, N.

[1*S*-(1*R,2*S**,3*R**)]-3-[[1-(Cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]amino]-*N*-[*N*-(4-morpholinosulfonyl)-L-phenylalanyl]-3-oxo-DL-alanine Allyl Ester (9), Isopropyl Ester (8), and *n*-Hexyl Ester (10).** To 0.64 g of 7 in 20 mL of ether was added 0.1 mL (1.5 mmol) of allyl alcohol. The solution was chilled in an ice bath and 0.2 g (1.0 mmol) of dicyclohexylcarbodiimide was added. A yellow color was immediately produced, and a white precipitate began to appear. The mixture was stirred overnight at room temperature, then filtered, and the cake washed thoroughly with ether. The filtrate was concentrated to yield 0.6 g (88%) of allyl ester **9** as a white foam (33:67 mixture of epimers). NMR was consistent with structure. Anal. Calcd for $C_{33}H_{52}N_4O_9S$: C, H, N. ***n*-Hexyl ester (10)** (35:65 mixture of epimers) was prepared from 0.73 g of the acid: NMR consistent with structure. Anal. Calcd for $C_{36}H_{60}N_4O_9S\cdot 0.15CHCl_3$: C, H, N. **Isopropyl ester (8)**: NMR consistent with structure; IR (KBr) 3400 (br), 2926, 2856, 1747, 1661 cm^{-1}; MS (FAB) 269, 387, 440, 516, 596, 683 (M + 1). Anal. Calcd for $C_{33}H_{54}N_4O_9S$: C, H, N.

4-Morpholinesulfonyl Chloride (12). The procedure employed is a modification of that reported by Wegler and Bodenbenner.[26] The reaction was run in a 22-L, five-necked flask fitted with a condenser, stirrer, addition funnel, temperature probe, and stopper. The flask was cooled in an ice/water bath. To a stirred, cooled (13 °C) solution of concentrated hydrochloric acid (803 g, 8.15 mol) and water (804 g) was added morpholine (710 g, 8.15 mol) in one portion, which caused the temperature to rise to 54 °C. When the solution had cooled to 5 °C dichloromethane (4 L) was added followed by the dropwise addition of a 5.25% solution of sodium hypochlorite in water (12 kg) over 60 min. The addition rate was controlled to keep the temperature below 11 °C. After the addition was complete the cooling bath was removed, and the mixture allowed to warm to 16 °C over 45 min. The dichloromethane layer was separated, dried (magnesium sulfate), and returned to the reactor after the reactor had been washed with water and acetone and dried. To the stirred dichloromethane solution was added, in one portion, a freshly prepared, cold (-70 °C) solution of sulfur dioxide (1135 g, 17.7 mol) in dichloromethane (2.5 L). The addition funnel was replaced with a dry ice/acetone cold-finger condenser, and the stopper with a gas inlet adapter was connected to a lecture bottle of chlorine. To the stirred solution was condensed 500–650 drops of chlorine followed by addition of dichloromethane (1.5 L), and the mixture was allowed to warm to 25 °C over 25 h. The mixture was washed with water followed by a pH 7.0 phosphate buffer (0.25 M) until the pH of the washes remained 7.0. The dichloromethane layer was dried (magnesium sulfate) and concentrated to an oil (yield 1.04 kg). The oil was combined with another lot (56.6 g) and distilled in vacuo (bp 97–106 °C/2.0–3.5 mmHg, bath temperature 138 °C); yield 1.00 kg. The distillate was filtered to remove a dark, finely divided particulate, and the filtrate stored in a brown plastic bottle; yield 985 g. Before the material was used in subsequent reactions it was freshly purified as follows: a portion (793.0 g) was dissolved in dichloromethane (2 L), and the solution washed with saturated sodium bicarbonate (1 L). The organic layer was dried (magnesium sulfate) and evaporated to an oil; yield 785 g. The oil was distilled in vacuo (bp 91–95 °C/1.5–2.0 mmHg) to give a clear, light yellow oil: yield 779 g (60.7% extrapolated yield); NMR (90 MHz) δ 3.9 (t, 4 H), 3.3 (t, 4 H); GC (column, DB-17 FSOT, 0.52 mm × 15 cm; solvent, CH_2Cl_2; injection volume, 10 μL; temperature, 250 °C) 93% pure. Anal. Calcd for $C_{13}H_{18}N_2O_5S$: C, H, N, S.

(26) Wegler, R.; Bodenbenner, K. *Ann. Chem.* **1959**, *624*, 25.

***N*-(4-Morpholinylsulfonyl)-L-phenylalanine (13).** The reaction was run in a 12 L, five-necked flask fitted with a condenser, stirrer, addition funnel, and two stoppers. To a stirred, 25 °C solution of L-phenylalanine (1416 g, 8.57 mol) and sodium hydroxide (342 g, 8.56 mol) in water (2.5 L) was added dropwise a solution of freshly distilled 4-morpholinesulfonyl chloride (779 g, 4.19 mol) in dry tetrahydrofuran (1.7 L) over 3 h. The mixture was stirred for 70 h before it was acidified to pH 2 with concentrated hydrochloric acid (289 mL). The mixture was partitioned between water (5 L) and ethyl acetate (5 L) and filtered. The organic layer was washed with 1 M hydrochloric acid (4 L), dried (magnesium sulfate), and concentrated to a pale yellow solid; yield 814 g (61.7% crude yield). The solid was triturated with ether (2.5 L) to remove a contaminating yellow oil and redried; yield 765 g. This material was added to hot (99 °C) water (9.9 L) to give a clear water layer and a small amount of denser yellow oil. The aqueous phase was withdrawn by suction filtration (candle filter) and the filtrate allowed to cool. The product crystallized to give a solid bottom layer of material that was yellow with an oily impurity and a free-floating suspension of off-white crystals on top. The lighter solids were collected on a filter, pressed dry, and recrystallized from water (5 L). The recrystallized product was filtered off and dried under steam-jet vacuo (47 °C, nitrogen stream): yield 257 g; mp 155–157 °C. The bottom layer of solids from the first crystallization was filtered off, pressed dry, and triturated with ether (500 mL). The triturated material was recrystallized from water (2.6 L). The recrystallized product was filtered off, pressed dry, and triturated again with ether (200 mL). The material was dried under steam-jet vacuo (47 °C, nitrogen stream): yield 194.1 g; mp 157–158 °C; Total yield 42.9%; IR (KBr) 3314, 2000, 1753, 1353 cm^{-1}; NMR (200 MHz) δ 8.0 (d, 1 H), 7.2 (s, 5 H), 3.8 (m, 1 H), 3.3 (t, 4 H), 3.0 (dd, 1 H), 2.9 (dd, 1 H), 2.7 (t, 4 H); the COO*H* proton was not observed. Anal. Calcd for $C_{13}H_{18}N_2O_5S$: C, H, N, S.

***N*-(4-Morpholinylsulfonyl)-L-phenylalanyl-DL-2-(methoxycarbonyl)glycine Methyl Ester (14).** (Morpholinosulfonyl)phenylalanine (29.4 g, 93 mmol) was dissolved in methylene chloride (800 mL) by adding *N*,*N*-dimethylformamide (25 mL). The flask was cooled to 5 °C and placed under nitrogen. Dicyclohexylcarbodiimide (20.2 g, 98 mmol) and HOBT (13.2 g, 98 mmol) were added to the solution and allowed to stir for 10 to 15 min. Methyl benzyl aminomalonate hydrochloride (23.2 g, 59 mmol) was added followed by triethylamine (13.6 mL, 98 mmol). The reaction mixture was allowed to warm to room temperature and stirred overnight under nitrogen. The solution was filtered and concentrated. It was then dissolved in ethyl acetate (600 mL) and washed with water (4 × 100 mL), saturated aqueous sodium bicarbonate (2 × 100 mL), and brine (2 × 100 mL) and dried over magnesium sulfate. The solvent was removed in vacuo and the residue was chromatographed by eluting with ethyl acetate/hexane (1:4)–ethyl acetate/hexane (1:1) to provide 23 g of product. A second fraction (5 g) was obtained which was slightly impure. Total yield 67%. The diester (23.0 g, 0.044 mol) was dissolved in methanol (200 mL), concentrated, and redissolved in methanol (500 mL) under N_2. Pd/C (20%) was added and stirred vigorously under an atmosphere of H_2 gas (hydrogen balloon). After 2 h the reaction was complete (TLC). The mixture was filtered through a bed of Celite and the filtrate was concentrated to yield 18.5 g (97.4%) of the desired product. NMR was consistent with the desired product. This material was used in the next step without further purification to avoid potential decarboxylation: NMR (250 MHz) δ 7.5–7.2 (m, 5 H), 6.53 (br s, 1 H), 5.54 (m, 1 H), 5.15 (m, 1 H), 4.15 (m, 1 H), 3.85 and 3.82 (2 s, combined 3 H), 3.53 (m, 4 H), 3.23–3.11 (m, 1 H), 3.11–2.80 (m, 5 H); COO*H* was not observed; IR (KBr) 3300 (br), 2862, 1755, 1669 cm^{-1}. Anal. Calcd for $C_{16}H_{21}NO_6$: C, H, N.

[1*S*-(1*R,2*S**,3*R**)]-3-[[1-(Cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]amino]-*N*-[*N*-(4-morpholinosulfonyl)-L-phenylalanyl]histidine (15).** A solution of the *N*-tritylated precursor (0.77 g, 0.84 mmol), prepared by standard coupling methods, in 7 mL of 80% acetic acid was heated on a steam bath for 3–5 min. Water (7 mL) was added to precipitate the triphenylcarbinol, which was isolated by vacuum filtration. The acetic acid filtrate was concentrated and then carefully partitioned between methylene chloride and 5% aqueous sodium carbonate solution. The organic fraction was concentrated and

the residue was dried in vacuo. The crude material was purified by flash chromatography eluting with chloroform containing 0–4% methanol to yield 0.20 g (32%) of a white foam: NMR consistent with structure; MS m/e 677 (M + 1, 100). Anal. Calcd for $C_{33}H_{51}N_6O_7S{\cdot}0.45CHCl_3$: C, H, N.

Kinetics of Epimerization of 4. Kinetic studies at pH 1.2, 3.0, 5.0, and 7.4 were conducted with 1.0 mg/mL solutions of the purified epimers **4A** and **4B**. The pH 1.2 diluent was a 1:1 mixture of acetonitrile and 1.0 N HCl. The pH 3.0, 5.0, and 7.4 diluents were 1:1 mixtures of acetonitrile and 1.0 M $NH_4H_2PO_4$ premixed and subsequently adjusted to the desired pH with ammonia or phosphoric acid. The use of acetonitrile as cosolvent was required because of the poor aqueous solubility of the compounds. After addition of the compound, each solution was kept at 37 °C in separate screw-capped reaction vials. The solutions were sampled at the time intervals shown in Figure 1 with an HPLC syringe and directly injected onto an HPLC under the following conditions: Column, Altex Ultrasphere 5 μm C_{18}, 250 mm × 4.6 mm i.d.; mobile phase, 55:45 0.005 M $NH_4H_2PO_4$ (pH 3.0)/acetonitrile; flow rate, 2.0 mL/min; detection, 214 nm; loop size, 10 μL; t_R, **4A**, 6.4 min; **4B**, 6.7 min.

The percentage of each epimer at each injection was determined from the integrals. Injections were made at the indicated intervals until equilibrium had been achieved. The data were fit to a model of opposing first-order reactions as described, with a nonlinear curve fitting program (MINSQ). The rate constants for reactions starting with 4A were (×10^3): 1.67 (pH 1.2), 1.98 (pH 3), 8.73 (pH 5.0), and 407 min^{-1} (pH 7.4). Comparable constants were determined for the reverse reaction (1.42, 1.93, 8.41, and 330 min^{-1}, respectively).

Enzyme Inhibition. Inhibition of renin activity was determined by the method of Haber et al.[27] 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 anti-angiotensin 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.

Inhibition of bovine cathepsin D (Sigma) activity was assessed in duplicate by the hydrolysis of bovine hemoglobin (twice crystallized, Sigma) at pH 3.2 and 37 °C (modified from Aoyogi et al.[28] and Kokubu et al.[29]). 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.

In Vivo Models. Details of the protocols for both the high-renin normotensive[21] and high-renin hypertensive monkey[25] models have been reported. Compounds were administered by oral gavage as a solution in a vehicle composed of water, dimethylacetamide, and Tween 80 (62.5:7.5:30). Volume of vehicle was 2 mL/kg.

Molecular Modeling Methods. Studies were performed with the Sybyl software package[30] operating on either a Silicon Graphics 4D/220 or a Vax 6430. All optimizations were carried out with molecular mechanics and the Tripos force field. The default options were used for the SO_2 (sulfone) torsion parameters, while parameters were adjusted to deal with aromatic ring planarity (add taff_tors: * car, car, car, ar, 2.35, −2 and modify taff_oop car to 630). The renin model was derived from the crystal structures of fungal aspartic proteinases including endothiapepsin, penicillopepsin, and *Rhizopus chinensis* proteinase. Optimizations performed within the confines of the enzyme included residues within 8 Å of the bound *R* epimer of 4. The *R* epimer of **4** was built with a crystal structure from the Cambridge Structural Database[31] for the morpholinylsulfonamide moiety. The analogue was manually docked in the binding site of renin as has been described with various endothiapepsin complexes. The *R* epimer was then optimized without the cleft. By placing the resulting structure back into the cleft and aggregating the enzyme, and separately inhibitor atoms near the cleavage site, the analogue was again optimized. This resulted in the structure shown in Figure 5. The P_2 chiral center was inverted to give the *S* configuration. This analogue was manually docked in the cleft and then optimized within the confines of the cleft as described for the *R* epimer (Figure 6). A SEARCH[32] conformational analysis of the P_2 to P_4 residues was carried out within the confines of the cleft, to determine possible binding modes for the analogue. Energy evaluation (cutoff energy = 10 kcal/mol) was included and van der Waals factors (general and 1,4) were reduced by a factor of 0.8 to adjust for the rigidity of the cleft residues. The resulting conformations were evaluated for their binding compatibility with the enzyme.

Supplementary Material Available: ^{1}H NMR spectra for **1–10** and **15** (11 pages). Ordering information is given on any current masthead page.

(27) Haber, E.; Koerner, T.; Page, L. B.; Kliman, B.; Purnode, A. *J. Clin. Endocrinol. Metab.* **1969**, *29*, 1349.
(28) Aoyagi, T.; Morishima, H.; Nishizawa, R.; Kunimoto, S.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1972**, *25*, 689.
(29) Kokubu, T.; Hiwada, K.; Murakami, E.; Imamura, Y.; Matsueda, R.; Yabe, Y.; Koike, H.; Iijima, Y. *Hypertension* **1985**, *7 (Suppl. I)*, 18.
(30) Sybyl, Versions 5.3 and 5.32; Tripos Associates, Inc., A subsidiary of Evans and Sutherland, 1699 S. Hanley Rd., Suite 303, St. Louis, Missouri 63144.
(31) (a) Allen, F. H.; Kennard, O.; Taylor, R. *Acc. Chem. Res.* **1983**, *16*, 146. (b) Allen, F. H.; Bellard, S.; Brice, M. D.; Cartwright, B. A.; Doubleday, A.; Higgs, H.; Hummelink, T.; Hummelink-Peters, B. G.; Kennard, O.; Motherwell, W. D. S.; Rodgers, J. R.; Watson, D. G. *Acta Crystallogr.* **1979**, *B35*, 2331.
(32) Motoc, I.; Dammkoehler, R. A.; Mayer, D.; Labanowski, J. *Quant. Struct.-Act. Relat.* **1986**, *5*, 99.