Renin Inhibitors Containing α-Heteroatom Amino Acids as P_2 Residues[1]

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A series of renin inhibitors having α-heteroatom amino acids as P_2 substitutions has been prepared. Examples where the heteroatom is oxygen, sulfur, or nitrogen are described. Many of the compounds exhibit subnanomolar potency when tested in vitro against monkey renin. When selected compounds were tested orally in conscious, salt-depleted, normotensive, Cynomolgus monkeys, low to moderate blood pressure lowering was observed. At an oral dose of 30 mg/kg, compound **53a** lowered blood pressure by a maximum of 18 mmHg at 2.5 h post-dose.

The success of angiotensin-converting enzyme inhibitors has demonstrated that inhibition of the renin–angiotensin system can provide an effective treatment for hypertension.[2a,b] Renin inhibitors, which interrupt the first step in the biochemical cascade leading to the pressor hormone angiotensin II, also produce antihypertensive activity, and the search for therapeutically useful renin inhibitors continues to be an active area of research.[3,4] However limited oral bioavailability and short duration of action continue to be barriers to the development of a therapeutically useful renin inhibitor.[4]

As part of our continued effort in this area,[5] we have explored variations at the P_2 position. The P_2 subsite[6] has been shown to tolerate a wide variety of change in the size and polarity of the side chains of the amino acids in this position while maintaining high potency.[7a-d] Variation of the substituents at the P_2 position can also produce substantial differences in enzyme selectivity for renin vs other aspartic proteinases.[7b]

α-Heteroatom amino acids,[8-11] in which the side chain of the amino acid is attached to the backbone through a heteroatom, attracted our interest as possible P_2 substitutions. It was of interest to see what effect the altered steric requirements around the amino acid backbone and the altered polarities of these amino acids would have on potency when they were incorporated into renin inhibitors.

In this paper we describe compounds having α-heteroatom amino acids, where the heteroatom is oxygen, sulfur, or nitrogen, at the P_2 position. With proper selection of the other subunits of the renin inhibitor molecule, highly potent inhibitors can be prepared.

Chemistry

Schemes I–III, using specific examples, outline the routes used in the preparation of these compounds. The other compounds of Tables I–III were prepared in accordance with these schemes. Scheme I illustrates the preparation of the oxygen and sulfur α-amino acids that are substituted on the amine with the BNMA[12] acyl group. Coupling to Sta-MBA[12] gave the compounds of Table I.

When **1** was treated with glyoxylic acid hydrate in refluxing acetone the hemiaminal **2** was obtained. Treatment of **2** with methanethiol in HOAc with concentrated H_2SO_4 gave the thioether–acid **3**, which was coupled in the usual manner to give **24** as a mixture of diastereomers.

The α-amino derivatives[11] were prepared from the completed renin inhibitors having the α-sulfur moiety at P_2. Treatment of **24** with ethylamine in the presence of $HgCl_2$ gave the renin inhibitors **33** and **34**. These diastereomers were separated by column chromatography on silica gel. The formation of the α-amino derivatives proceeded with racemization of the P_2 amino acid. When a pure diastereomer corresponding to **24** was used in this reaction, the amino analogues corresponding to **33** and **34** were obtained as an approximately 1:1 mixture of diastereomers.

When the hemiaminal **2** was treated with EtOH and concentrated H_2SO_4 and left stirring for two days, the

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(6) The "P" subsite nomenclature relates amino acid residues or mimics in the inhibitor to corresponding residues in the natural substrate angiotensinogen. See: Schecter, I.; Berger, A. On the Size of the Active Site in Proteases. I. Papain. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157–162.

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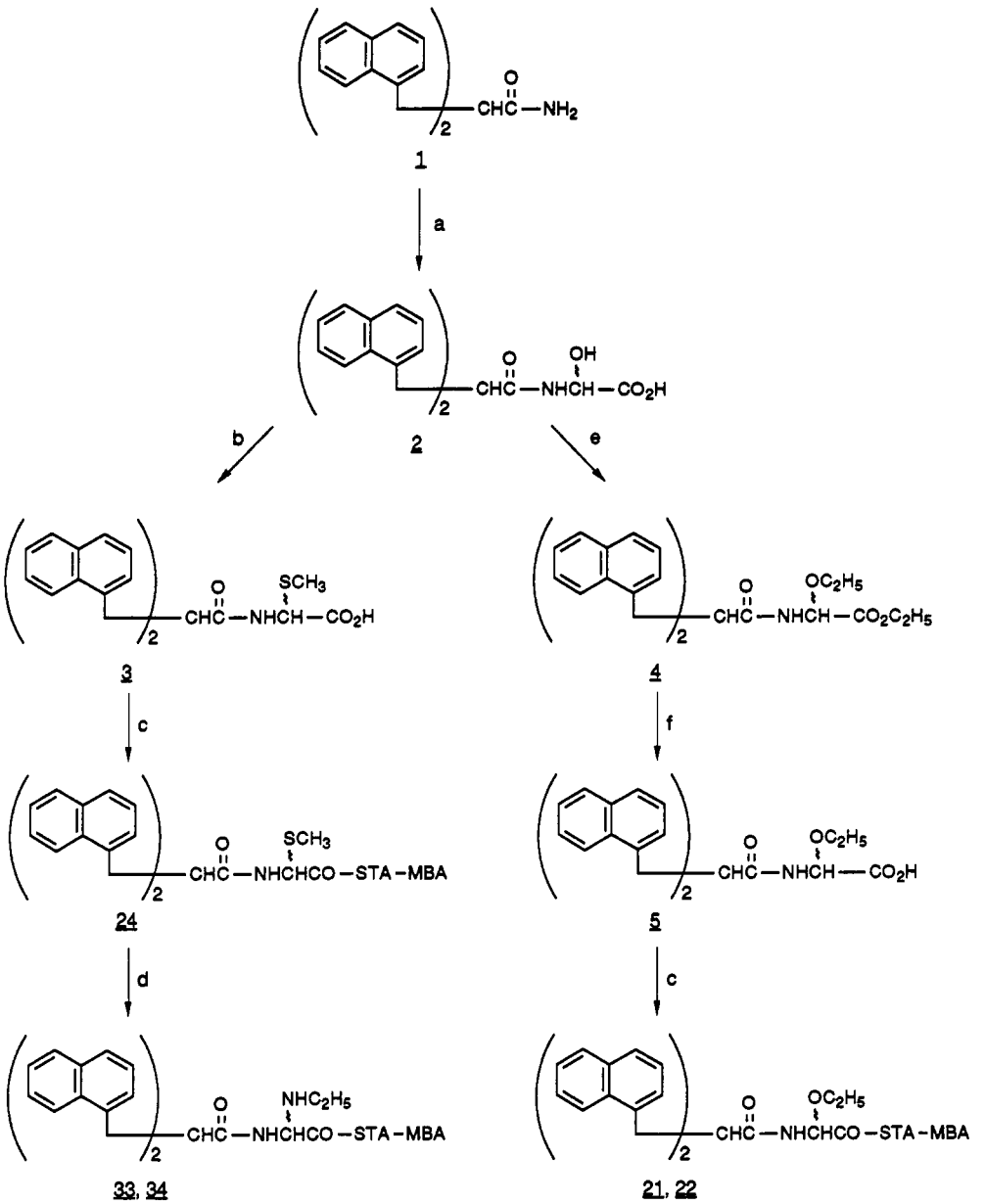
Scheme I[a]



[a] (a) Glyoxylic acid. (b) CH_3SH, H_2SO_4, HOAc. (c) Sta-MBA, DCC, HOBT. (d) $C_2H_5NH_2$, $HgCl_2$. (e) EtOH, H_2SO_4, 2 days. (f) NaOH.

ester–ether **4** was obtained. This ester was hydrolyzed with NaOH to the acid **5** and coupled with Sta-MBA in the usual manner to give the renin inhibitors **21** and **22**. These diastereomers were separated by column chromatography on silica gel.

Scheme II describes the preparation of the oxygen and sulfur α-amino acids having the amine substituted with the SMO group.[12] Coupling to ACHPA[13,14] and the 2,2-difluoro containing P_1–P_1' groups derived from it[15a,b] gave the compounds of Table II. Coupling to ACDMH[16,17] gave the renin inhibitors of Table III.

(12) Sta is 4(*S*)-amino-3(*S*)-hydroxy-6-methylheptanoic acid. MBA is 2(*S*)-methylbutylamine. BNMA is bis(1-naphthylmethyl)-acetic acid. SMO is 4-morpholinesulfonic acid. DFKCYS is 4(*S*)-amino-3-oxo-2,2-difluoro-5-cyclohexanepentanoic acid. AEM is 4-(2-aminoethyl)morpholine.

(13) ACHPA is 4(*S*)-amino-3(*S*)-hydroxy-5-cyclohexylpentanoic acid.

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(16) ACDMH is 2(*S*)-amino-1-cyclohexyl-3(*R*),4(*S*)-dihydroxy-6-methylheptane.

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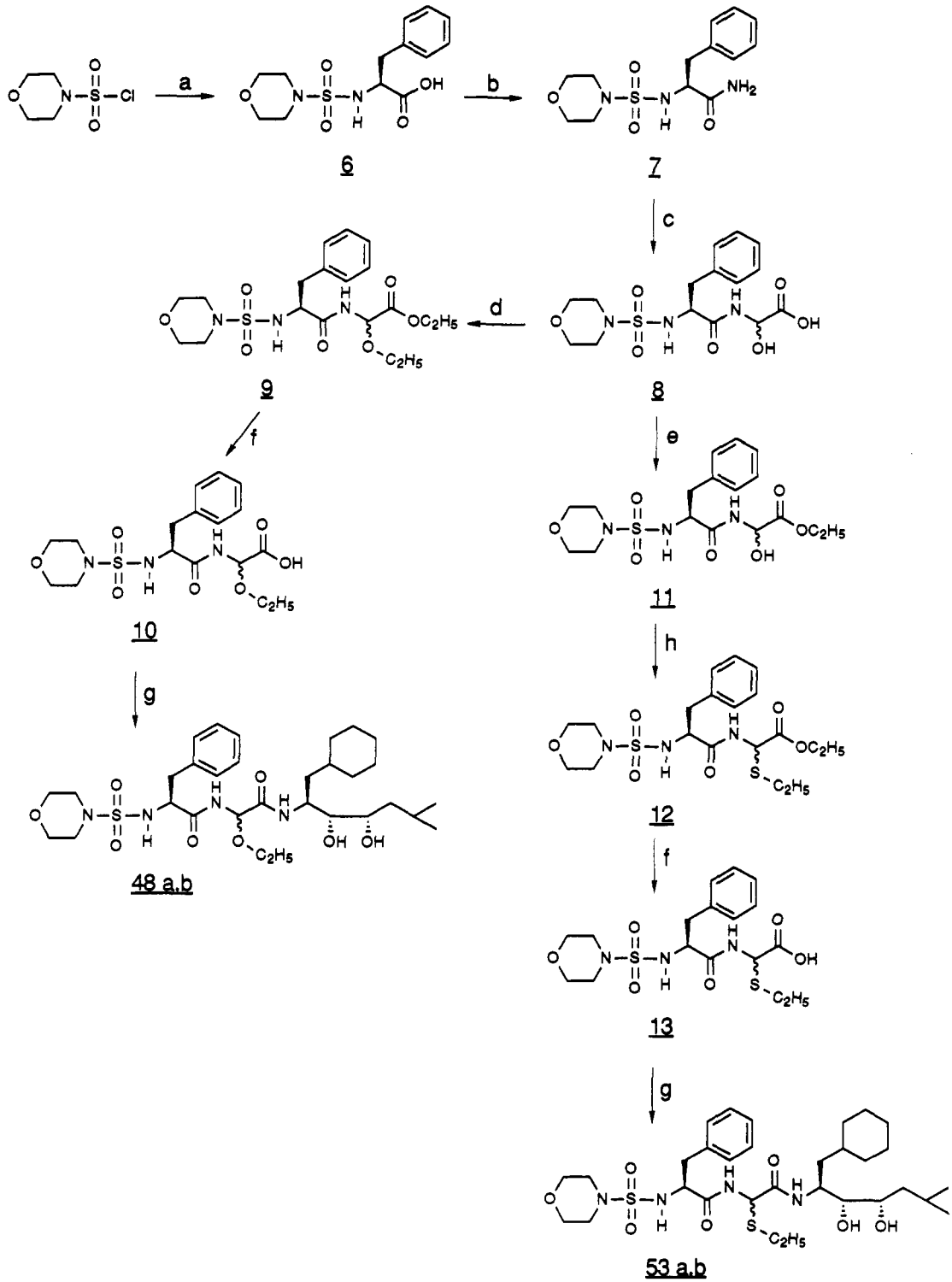
Scheme II[a]



[a] (a) Phe, NaOH. (b) CDI, NH_3. (c) Glyoxylic acid. (d) EtOH, H_2SO_4, 5 days. (e) EtOH, H_2SO_4, overnight. (f) NaOH. (g) DCC, HOBT, ACDMH·HCl, Et_3N. (h) C_2H_5SH, 2-naphthalenesulfonic acid.

4-Morpholinesulfonyl chloride[18] was reacted with 2 equiv of Phe in a Schotten–Baumann reaction to give **6**. Formation of the imidazolide and treatment with NH_3 gave the amide **7**. Refluxing **7** with glyoxylic acid hydrate in acetone for 2 days gave the hemiaminal **8**. This intermediate when stirred in absolute EtOH in the presence of concentrated H_2SO_4 for 5 days gave the ester–ether **9**. This ester was hydrolyzed with NaOH to the acid **10** and coupled with ACDMH in the presence of DCC/HOBT to give the renin inhibitors **48a** and **48b**. These diastereomers were separated by column chromatography on silica gel.

When hemiaminal **8** was dissolved in absolute EtOH in the presence of concentrated H_2SO_4 and stirred overnight rather than 5 days, the major product was the hemiaminal–ester **11**. When **11** was treated with ethanethiol in CH_2Cl_2 in the presence of anhydrous 2-naphthalenesulfonic acid, **12** was formed. The ester **12** was hydrolyzed to the acid **13** and coupled in the usual manner to give the

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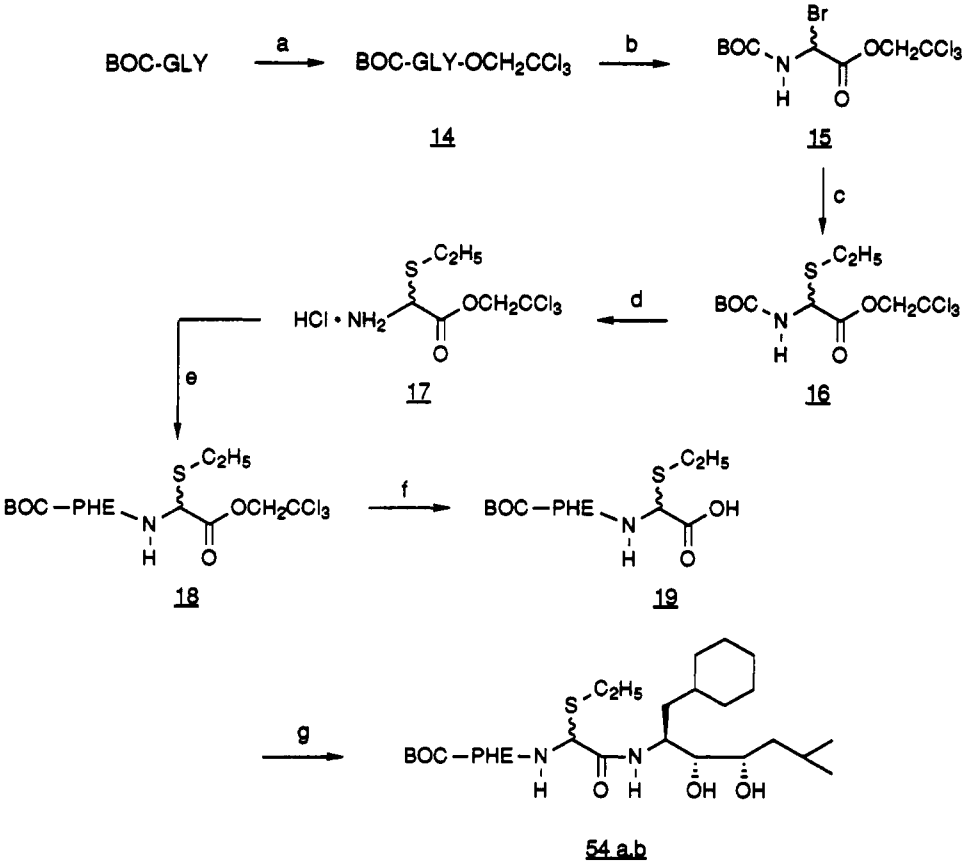
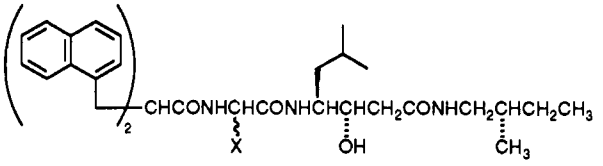
Scheme III[a]



[a] (a) Cl_3CCH_2OH, DCC, DMAP. (b) NBS. (c) C_2H_5SH, NaH. (d) HCl gas. (e) Boc-Phe, DCC, HOBT, Et_3N. (f) NaOH. (g) ACDMH·HCl, DCC, HOBT, Et_3N.

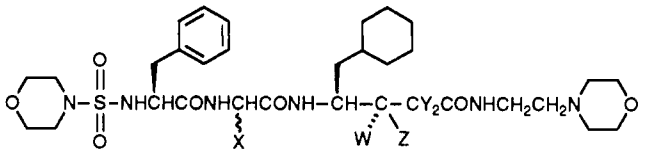
Table I. In Vitro Activity vs Renin of Statine Containing Derivatives Having an α-Heteroatom Amino Acid at the P_2 Position



compd	X	isomer[a,b]	formula[c]	renin (monkey): IC_{50}, nM
20	OCH_3	mixture	$C_{40}H_{51}N_3O_5$	180
21	OC_2H_5	fast	$C_{41}H_{53}N_3O_5$	26
22	OC_2H_5	slow	$C_{41}H_{53}N_3O_5$	>1000
23	OC_6H_5	mixture	$C_{45}H_{53}N_3O_5 \cdot 0.125CHCl_3$[d]	633
24	SCH_3	mixture	$C_{40}H_{51}N_3O_4S \cdot 0.125H_2O$	95
25	$SOCH_3$	mixture	$C_{40}H_{51}N_3O_5S$	615
26	SO_2CH_3	mixture	$C_{40}H_{51}N_3O_6S$	>1000
27	$SCH(CH_3)_2$	fast	$C_{42}H_{55}N_3O_4S \cdot 0.06CHCl_3$	95
28	$SCH(CH_3)_2$	slow	$C_{42}H_{55}N_3O_4S \cdot 0.5H_2O$	>1000
29	$SO_2CH(CH_3)_2$	mixture	$C_{42}H_{55}N_3O_6S \cdot 0.25H_2O$	>1000
30	SC_6H_5	mixture	$C_{45}H_{53}N_3O_4S \cdot 0.25H_2O$	656
31	NH_2	fast	$C_{39}H_{50}N_4O_4 \cdot 1.1HCl \cdot 0.5H_2O$[e]	>1000
32	NH_2	slow	$C_{39}H_{50}N_4O_4 \cdot 1.2HCl \cdot 0.6H_2O$[f]	1000
33	NHC_2H_5	fast	$C_{41}H_{54}N_4O_4$	>1000
34	NHC_2H_5	slow	$C_{41}H_{54}N_4O_4 \cdot 0.15H_2O$	210
35	$NHCH(CH_3)_2$	fast	$C_{42}H_{56}N_4O_4 \cdot 0.05CHCl_3$	>1000
36	$NHCH(CH_3)_2$	slow	$C_{42}H_{56}N_4O_4 \cdot 0.1CHCl_3 \cdot 0.25H_2O$	500
37	$N(CH_3)_2$	fast	$C_{41}H_{54}N_4O_4$	>1000
38	$N(CH_3)_2$	slow	$C_{41}H_{54}N_4O_4$	74
39	NHC_6H_5	mixture	$C_{45}H_{54}N_4O_4 \cdot 0.25C_4H_8O_2 \cdot 0.25H_2O$	310
40	$NHCOCH_3$	slow	$C_{41}H_{52}N_4O_5$	936
41	$NHCO_2C_2H_5$	mixture	$C_{42}H_{54}N_4O_6$	516
42	P_2=His		$C_{43}H_{53}N_5O_4$	85[g]

[a] Mixture refers to a mixture of two diastereomers, isomeric at the P_2 position. Compound **23** in a mixture of four diastereomers, as a consequence of the asymetric sulfoxide group. [b] Fast refers to the faster moving diastereomer on TLC plates. Slow refers to the slower moving diastereomer on these plates. [c] Analyses for the elements and H_2O were witnin ±0.4% except as noted. [d] Cl: calcd, 1.82; found 1.28. [e] C: calcd, 68.09; found 68.50. [f] C: calcd, 67.55; found 68.37. [g] See ref 31. The authors report an IC_{50} value of 9.2 nM against human plasma renin.

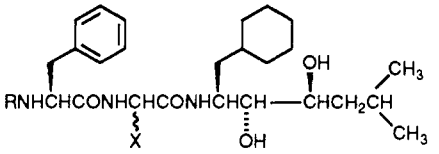
Table II. In Vitro Activity vs Renin and Cathepsin D for ACHPA-Derived Analogues Having an α-Heteroatom Amino Acid at the P_2 Position



compd	X	W	Z	Y	isomer[a]	formula[b]	IC_{50}, nM: renin (monkey)	IC_{50}, nM: cathepsin D (bovine)	selectivity ratio: cathepsin D/renin
43	OC_2H_5	HO	H	H	mixture	$C_{34}H_{56}N_6O_9S\cdot0.2CHCl_3\cdot0.6H_2O$	5.3	>10,000	>2000
44	OC_3H_{7-n}	HO	H	F	fast	$C_{35}H_{56}N_6O_9SF_2$[c]	0.44	698.5	1605
45	OC_3H_{7-n}	–O–		F	*d*	$C_{35}H_{54}N_6O_9SF_2\cdot C_6H_8O_7\cdot1.5H_2O$	2.1	131.0	65.5
46	$SCH_2CH{=}CH_2$	HO	H	H	mixture	$C_{35}H_{56}N_6O_8S_2$	1.5	10.0	6.7
47	P_2=His	HO	H	H		$C_{36}H_{56}N_8O_8S\cdot C_2H_4O\cdot H_2O$	5.0	>10,000	>2000[e]

[a] Mixture refers to a mixture of two diastereomers, isomeric at the P_2 position. Fast refers to the faster moving diastereomer on thin-layer plates. [b] Analyses for the elements and H_2O were within ±0.4% except as noted. [c] N: calcd, 10.85; found 10.35. [d] Obtained by oxidation of fast diastereomer **44**. [e] Reference 7a.

Table III. In Vitro Activity vs Renin and Cathepsin D for ACDMH-Containing Derivatives Having an α-Heteroatom Amino Acid at the P_2 Position



compd	X	R	isomer[a]	formula[b]	IC_{50}, nM: renin (monkey)	IC_{50}, nM: cathepsin D (bovine)	selectivity ratio: cathepsin D/renin
48a	OC_2H_5	SMO	fast	$C_{31}H_{52}N_4O_8S$	0.25	14.0	56
49a	OC_3H_{7-n}	SMO	fast	$C_{32}H_{54}N_4O_8S$	0.21	22.0	101
50a	$OCH_2CH{=}CH_2$	SMO	fast	$C_{32}H_{52}N_4O_8S\cdot0.5H_2O$	0.22	10.5	233
51	$OCH_2C{\equiv}CH$	SMO	mixture	$C_{32}H_{50}N_4O_8S\cdot0.25H_2O$	0.52	80.1	154
52a	OCH_2CF_3	SMO	fast	$C_{31}H_{49}N_4O_8SF_3$	0.18	21.2	115
53a	SC_2H_5	SMO	fast	$C_{31}H_{52}N_4O_7S_2$	0.13	10.0	7.7
54a	SC_2H_5	BOC	fast	$C_{32}H_{53}N_3O_6S$	1.4	28.1	20.1
55a	$SCH_2CH{=}CH_2$	SMO	fast	$C_{32}H_{52}N_4O_7S$	0.17	19.4	118
56		SMO	mixture	$C_{33}H_{50}N_4O_7S_3$	13	470	36.1
57	$N(CH_3)CH_2CH{=}CH_2$	BOC	mixture	$C_{34}H_{56}N_4O_6$	35	60.4	1.7
58	P_2=His	SMO		$C_{33}H_{52}N_6O_7S\cdot0.32CH_2Cl_2$	0.23	>10,000	>2000[c]

[a] Mixture refers to a mixture of two diastereomers, isomeric at the P_2 position. Fast refers to the faster moving diastereomer on TLC plates. [b] Analyses for the elements and H_2O were within ±0.4%. [c] Reference 24.

renin inhibitors **53a** and **53b**. These diastereomers in turn were separated by column chromatography on silica gel.

Scheme III provides an alternate route to the α-sulfur compounds. In this route formation of the α-sulfur derivative occurs under basic conditions and permits the use of acid-sensitive groups such as the Boc group which could not be used under the acidic conditions of Schemes I and II.

Since we had a supply of **14** on hand for other purposes, this β-trichloroethyl ester was used in the sequence outlined in Scheme III. Treatment[19-21] of **14** with *N*-bromosuccinimide and irradiating with a Hanovia mercury lamp gave the bromo derivative **15**. This derivative was treated[10] with the anion of ethanethiol giving **16**. Some replacement of the alcohol from the ester with ethanethiol also occurred, and the resulting mixture could not be separated by chromatography. Consequently, the crude material was carried through the subsequent steps as a mixture of ester and thioester. Treatment of **16** with HCl gas in CH_2Cl_2 gave **17**, which was acylated with Boc-Phe in the presence of DCC/HOBT to give **18**. Treatment with NaOH hydrolyzed both the ester and thioester and gave **19**, which was coupled with ACDMH in the usual manner to give **54a** and **54b**. These diastereomers were separated by column chromatography on silica gel.

Molecular Modeling

Molecular modeling experiments were undertaken to qualitatively study the binding compatibilities of the α-heteroatom analogues in a human renin model. The model was derived from the crystal structures of homologous fungal aspartic acid proteinases.[22] The analogues were

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(22) Blundell, T. L.; Sibanda, B. L.; Hemmings, A. M.; Foundling, S. I.; Tickle, I. J.; Pearl, L. H.; Wood, S. P. A Rational Approach to the Design of Renin Inhibitors. *Molecular Graphics and Drug Design*; Burgen, A. S. V., Roberts, G. C. K., Tute, M. S., Eds.; Elsevier Science, B. V., Biomedical Division: Amsterdam, The Netherlands, 1986; pp 324–334.

docked in the active site of the enzyme following a reference binding mode found in the crystal structures of endothiapepsin[23] (a fungal enzyme homologous to renin) complexed with various inhibitors. This reference binding mode was characterized by an extended conformation between the P_1 and P_3 sites with the side chains alternating above and below the backbone. The hydroxyl at P_1 was always positioned between Asp32 and Asp215 and a general hydrogen bonding scheme was conserved. Compound **48a**, with an *S* configuration at the heteroatom amino acid (analogous to an L-amino acid) could be docked in the enzyme following the binding scheme found in the crystal complexes (Figure 2). The docking of the analogue **48b** with the *R* configuration at P_2 resulted in significantly different binding modes and an overall less favorable interaction with the enzyme. Analogous findings were made in a similar study[24] of renin inhibitor diastereomers containing a malonate derivative at P_2. If the general binding scheme observed with the crystal structures were considered to be the ideal binding mode, the diastereomer corresponding to an L-amino acid for each α-heteroatom analogue can be assigned the stronger potency value.

Analogues with the various heteroatom substitutions at P_2 in the *S* configuration were docked in the renin enzyme cleft. The ethers, as well as the thioethers, were compatible with the binding site and could hydrogen bond to Ser76 in the flap region of the enzyme. These results were in agreement with the strong potencies reported for these compounds. The sulfone **26**, however, was too bulky to fit the S_2 pocket which explains its low binding affinity. It was difficult to rationalize the low potency of the sulfoxide analogue **25** or the structure–activity relationships of the amine derivatives on the basis of steric or electrostatic repulsions. In the latter series, the electropositive nature of the S_2 pocket might be expected to hinder the binding of a positively charged group; however, **38** with the dialkylated amine at P_2 exhibited fairly strong binding affinity.

Recently Blundell and Cooper[25] have determined the X-ray crystal structure of the more potent diastereomer **53a** bound in the endothiapepsin enzyme and indeed found that the side chain of the P_2 residue has the *S* configuration corresponding to that of an L-amino acid.

Discussion

The results of our initial attempts at utilizing the α-heteroatom amino acids as P_2 residues are found in Table I. These derivatives all contained Sta-MBA[12] as the P_1–P_2' fragment and BNMA[12] as the P_3 moiety. Four of the compounds (**21**, **24**, **27**, and **38**) had IC_{50} values of less than 100 nM when tested against monkey renin. Even though this group of compounds exhibited only modest activity, some conclusions concerning the SAR could be drawn from this data. The *O*-alkyl and *S*-alkyl derivatives provided about equal potencies, and despite the good activity shown by **38** it appeared that in general the *N*-alkyl derivatives were less potent. Oxidation of the α-sulfur atom to a sulfoxide (**25**) lowered activity, and this was further lowered by oxidation to a sulfone (**26** and **29**). *O*-, *S*-, and *N*-Aryl derivatives (**23**, **30**, and **39**) gave comparable potencies and in general were less potent than alkyl derivatives. Acylating the α-amine (**40** and **41**) did not improve activity. Many of the compounds could be separated by column chromatography on silica gel into fast- and slow-eluting diastereomers. The highest potency in a pair of diastereomers was uniformly found in the fast-eluting diastereomer for the O- and S-derivatives and in the slower eluting diastereomer for the N-derivatives. Modeling studies (vide supra) suggest that the more potent diastereomer in a pair has the P_2 side chain in a configuration that corresponds to that of the side chain in an L-amino acid.

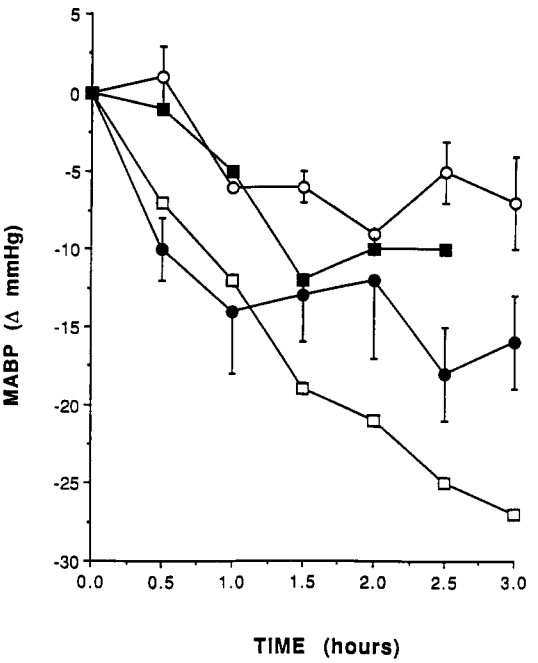

Figure 1. Comparative oral activity of renin inhibitors in conscious, high renin, normotensive Cynomolgus monkeys after an oral dose of 30 mg/kg: vehicle ($N = 4$), 2 mL/kg, ○; CGP-38560[32] ($N = 2$), ■; compound **53a** ($N = 3$), ●; CP-80794[33] ($N = 2$), □.

Encouraged by these results, we decided to concentrate on the α-heteroatom amino acids having *O*- and *S*-alkyl substituents and to use them as P_2 fragments in combination with other potency enhancing groups. Table II shows the results of these substitutions. When combined with the morpholinosulfonyl group at P_4 and the ACHPA and 2,2-difluoro derivatives derived from ACHPA at P_1–P_1', highly potent compounds with nanomolar activity were obtained (**43**–**46**). One of these, **44**, exhibited subnanomolar potency. The hydroxyl group in difluoro derivative **44** has the *R* configuration,[15a] corresponding to the *S* configuration in ACHPA.

Finally, the α-heteroatom amino acids were combined with the ACDMH group.[16,17] The results are found in Table III. Seven compounds (**48a**, **49a**, **50a**, **51**, **52a**, **53a**, and **55a**) showed subnanomolar potency. Again in those cases where diastereomers derived from the O- and S-series could be separated, higher potency resided in the faster eluting diastereomer.

The slower eluting diastereomers could not be obtained diastereomerically pure, but contained from 0.5% to 10.0% of the faster eluting diastereomer. Because of the high potencies exhibited by the fast-eluting diastereomers, even a small amount present in the slow-eluting diastereomers

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(24) Repine, J. T.; Himmelsbach, R. J.; Hodges, J. C.; Kaltenbronn, J. S.; Sircar, I.; Skeen, 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.; Belmont, D. T.; Taylor, M. D. Renin Inhibitors Containing Esters at the P_2 Position. Oral Activity in a Derivative of Methyl Aminomalonate. *J. Med. Chem.* **1991**, *34*, 1935–1943.

(25) Blundell, T. L.; Cooper, J. Unpublished results.

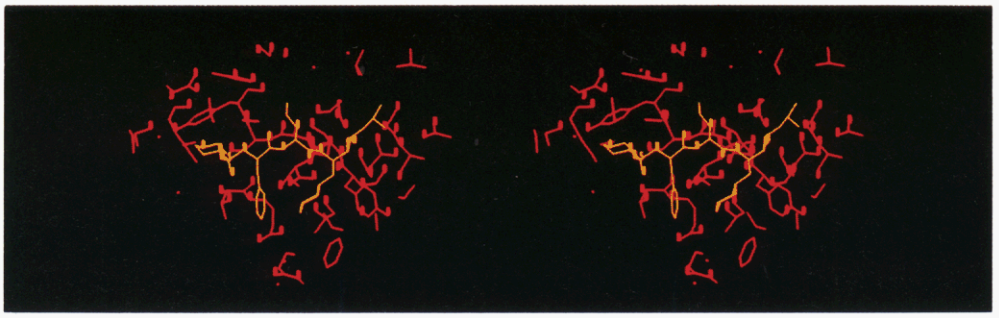

Figure 2. Stereoview of **48a** (*S* configuration at the P_2 α-heteroatom amino acid) bound in the cleft of the human renin model. This isomer binds in the standard mode.

makes estimating the potencies of the slow-eluting diastereomers difficult. Consequently the potencies of the slow-eluting diastereomers have not been included in Table III.

There did not appear to be any notable SAR trends among these highly potent derivatives. Modest chain length variation (**48a** vs **49a**) did not make any appreciable difference in potency. Unsaturation in the side chain did not uniformly increase potency (**51** and **55a** vs **49a**) when compared to the saturated analogue. The β-trifluoroethyl derivative **52a** was also not markedly different from the *O*- and *S*-alkyls (**48a**, **49a**, and **53a**). An *S*-aryl derivative (**56**) showed reduced potency as did the single α-amine example **57**. The morpholinosulfonyl group (SMO) at P_4 enhanced potency 10-fold when compared to the corresponding derivative containing a Boc group at P_4 (**53a** vs **54a**).

Since a high degree of specificity is usually desirable for a potentially useful therapeutic agent, we were interested in comparing the inhibitory activity of these compounds against a closely related aspartic protease, bovine cathepsin D. Tables II and III list the IC_{50} values for compounds when tested against this enzyme as well as the selectivity ratio for renin/cathepsin D. Selectivity ratios ranged from >2000 for **43** to 1.7 for **57**. Several of the compounds with subnanomolar potency had selectivity ratios for renin of >100, namely **44**, **49a**, **50a**, **51**, **52a**, and **55a**. No attempt was made to explore the SAR in an effort to maximize this selectivity.

A series of representative compound types, **44**, **45**, **46**, **52a**, and **53a** was tested orally at 30 mg/kg in Cynomolgus monkeys. The results of these experiments can be found in Table IV. Little or no hypotensive effect was seen with **44**, **45**, **46**, or **52a**. The best compound was **53a** which caused a maximum average blood pressure drop of 18 ± 3 mmHg at 2.5 h (Figure 1).

Conclusions

We have shown that α-heteroatom amino acids, especially those where the heteroatom is oxygen or sulfur, can serve admirably as P_2 fragments, giving renin inhibitors with IC_{50} values in the subnanomolar range. However, the α-heteroatom amino acids conferred no notable positive effects on oral activity. Only **53a** showed moderate hypotensive effects when given orally. Given the excellent renin inhibitory activity of these compounds, the relatively small effects observed in vivo probably reflect the low oral bioavailability of these compounds.

Experimental Section

The NMR spectra were recorded on a Varian EM-390, Varian XL-200, Varian XL-300, or a IBM WP100SY instrument. The FAB-MS was determined on a VG analytical 7070E/HF mass spectrometer or a Finnegan TSQ-70 in a thioglycerol matrix using xenon as the target gas. Chemical-ionization mass spectra were obtained on a Finnegan 4500 using methane as reagent gas at 0.4 Torr. Rotations were recorded on a Perkin-Elmer Model 142 polarimeter. TLC was done on precoated plates (silica gel 60F 254, Merck). Silica gel chromatography was done with Kieselgel 60 (70–230 mesh or 230–400 mesh for flash).

Table IV. Effect on Blood Pressure from Oral Administration of Selected Renin Inhibitors to Conscious, Salt-Depleted, Normotensive, Cynomolgus Monkeys[a]

		change in blood pressure, mmHg		
compd	N^b	1 h	2 h	3 h
vehicle[c]	4	−6 ± 0	−8 ± 1	−7 ± 3
44	2	3 ± 5	−1 ± 5	−3 ± 8
45	3	−10 ± 5	−10 ± 2	−9 ± 4
46	2	−3 ± 3	−7 ± 1	−6 ± 1
52a	2	−1 ± 4	−1 ± 6	−2 ± 1
53a	3	−14 ± 4	−12 ± 5	−16 ± 3

[a] Compounds administered at 30 mg/kg. [b] Number of animals tested. [c] Vehicle is 7.5% DMA, 30% Tween 80, and 62.5% H_2O.

All compounds were purified by chromatography on silica gel and were usually obtained as solid foams that often retained solvent, even on prolonged drying under vacuum. Intermediates and the compounds of Tables I–III all showed the correct molecular ion in the mass spectrum. The NMR spectra were consistent with the assigned structures.

Bis(1-naphthylmethyl)acetamide (BNMA-NH_2, 1). A suspension of 46.8 g (0.137 mol) of bis(1-naphthylmethyl)acetic acid in 120 mL of thionyl chloride was warmed to 30 °C, to effect solution. After the mixture was stirred at room temperature overnight, the solvent was removed under reduced pressure giving an oil. Addition of 400 mL of Et_2O gave a solid. After dilution with 500 mL of hexane and concentrating under reduced pressure the solid was collected and washed with hexane giving 42.9 g of bis(1-naphthylmethyl)acetyl chloride.

A solution of 16.0 g (0.045 mol) of bis(1-naphthylmethyl)acetyl chloride in 125 mL of THF was added dropwise to 150 mL of a THF solution saturated with NH_3 gas at −60 °C over a 15-min period. After the mixture stirred at room temperature overnight, the solvent was removed under reduced pressure. The residue was taken up in EtOAc and washed with 1 N citric acid, saturated NaCl, saturated $NaHCO_3$, and saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure left a solid. Recrystallization from EtOAc/Et_2O gave 13.4 g (88% yield) of the product as a white solid, mp 159–161 °C. Anal. C, H, N.

BNMA-NHCH(OH)CO_2H (2). A solution of 12.9 g (0.038 mol) of **1** in 250 mL of acetone was treated with 4.34 g (0.047 mol) of glyoxylic acid hydrate and heated at reflux for 18 h. The solvent was removed under reduced pressure and the residue taken up in Et_2O and washed with saturated NaCl. After the residue was washed with saturated $NaHCO_3$, a solid precipitated and was collected. The solid was suspended in EtOAc and washed with 1 N citric acid. The EtOAc solution was washed with saturated NaCl and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave 12.7 g (80% yield) of the product as a foam: ^1H NMR (200 MHz, DMSO-d_6, D_2O wash) δ 3.00–3.40 (m, 5 H), 5.46 (d, 1 H), 7.22–7.98 (m, 14 H), 8.92 (d, 1 H); MS (FAB, thioglycerol) m/z 414.2 (M + 1).

BNMA-NHCH(SCH_3)CO_2H (3). A solution of 6.4 g (15.4 mmol) of **2** in 90 mL of HOAc was cooled in ice and 6 mL of

methanethiol added, followed by 10 mL of concentrated H_2SO_4. After stirring at room temperature for 2 days, the mixture was poured onto ice and extracted with EtOAc. The EtOAc solution was washed with saturated NaCl and then with saturated $NaHCO_3$. The organic phase, which still contained the product, was washed with 1 N HCl and then with saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure gave 5.94 g (89% yield) of the product as a white foam. The material was used without further purification.

BNMA-NHCH(OC_2H_5)$CO_2C_2H_5$ (4). A solution of 3.0 g (7.3 mmol) of **2** in 100 mL of absolute EtOH was treated with 1 mL of concentrated H_2SO_4 and stirred at room temperature for 2 days. The solvent was removed under reduced pressure and the residue taken up in Et_2O and washed with saturated $NaHCO_3$, saturated NaCl, 1 N citric acid, and saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure left 2.94 g of the crude product. Chromatography on silica gel, eluting with $CHCl_3$/EtOAc (1:1) gave 1.39 g (40.9% yield) of the product as a white foam: ^1H NMR (200 MHz, DMSO-d_6) δ 0.90 (t, 3 H), 1.09 (t, 3 H), 2.90–3.34 (m, 7 H), 4.02 (q, 2 H), 5.30 (d, 1 H), 7.29–7.58 (m, 8 H), 7.70–7.98 (m, 6 H), 8.86 (d, 1 H); MS (FAB, thioglycerol) m/z 470.2 (M + 1).

BNMA-NHCH(OC_2H_5)CO_2H (5). A solution of 1.27 g (2.7 mmol) of **4** in 45 mL of dioxane was treated with 5.7 mL (5.7 mmol) of 1 N NaOH and stirred at room temperature for 1.5 h. The solution was treated with 6 mL of 1 N HCl and the solvent removed under reduced pressure. The residue was taken up in EtOAc and washed with 1 N citric acid and then saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure left 1.19 g (100% yield) of the product as a white foam: ^1H NMR (200 MHz, DMSO-d_6) δ 0.90 (t, 3 H), 2.88–3.52 (m, 7 H), 5.28 (d, 1 H), 7.20–8.00 (m, 14 H), 8.84 (d, 1 H), 12.88 (broad s, 1 H); MS (FAB, thioglycerol) m/z 442.2 (M + 1).

***N*-(4-Morpholinylsulfonyl)-L-phenylalanine (SMO-Phe, 6).** A solution of 66.0 g (0.4 mol) of Phe in 120 mL of 3.33 N NaOH was treated dropwise over 30 min with a solution of 37.1 g (0.2 mol) of 4-morpholinesulfonyl chloride in 80 mL of THF. The solution was stirred at room temperature for 6 h and then acidified to pH 2 with concentrated HCl. The mixture was extracted with EtOAc, and the EtOAc was washed with 1 N HCl and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a solid. Recrystallization from water gave 27.0 g (43% yield) of the pure product as a white solid, mp 157–158 °C. Anal. C, H, N.

***N*-(4-Morpholinylsulfonyl)-L-phenylalaninamide (SMO-Phe-NH_2, 7).** A solution of 31.4 g (0.1 mol) of **6** in 500 mL of CH_2Cl_2/THF (1:1) was cooled to −10 °C, 17.9 g (0.1 mol) of 1,1′-carbonyldiimidazole was added, and the solution was allowed to stir for 45 min. The solution was cooled to −20 °C, NH_3 gas was bubbled through the solution for 1 h, and the solution was then allowed to stir at room temperature overnight. The solution was concentrated to a gel and was then triturated with an Et_2O and water mixture to afford 23.8 g (76% yield) of a white solid: mp 220–221 °C dec; $[\alpha]^{23}_D$ −40.3° (*c*, 1.35, 1 N NaOH). Anal. C, H, N.

SMO-Phe-NHCH(OH)CO_2H (8). A solution of 5.9 g (18.8 mmol) of **7** and 3.64 g (39.5 mmol) of glyoxylic acid hydrate in 300 mL of acetone was heated at reflux for 2 days. The solvent was removed under reduced pressure and the residue taken up in EtOAc. The EtOAc was washed with saturated NaCl and twice with saturated $NaHCO_3$. The combined $NaHCO_3$ washes were acidified to Congo Red end point with concentrated HCl. The aqueous layer was concentrated and taken up in EtOAc. The solids were filtered off, and the EtOAc layer was washed with saturated NaCl and dried over $MgSO_4$. Removal of the solvent under reduced pressure left 5.27 g (72.3% yield) of the product as a white foam: ^1H NMR (200 MHz, DMSO-d_6, D_2O wash) δ 2.55–2.80 (m, 5 H), 2.82–3.00 (m, 1 H), 3.40 (broad s, 4 H), 4.00 (m, 1 H), 5.55 (m, 1 H), 7.29 (m, 5 H); MS (FAB, thioglycerol) m/z 388.1 (M + 1). Anal. C, H, N.

SMO-Phe-NHCH(OC_2H_5)$CO_2C_2H_5$ (9). A solution of 5.11 g (13.2 mmol) of **8** in 100 mL of absolute EtOH was treated with 1 mL of concentrated H_2SO_4 and stirred at room temperature for 5 days. The solvent was removed under reduced pressure and the residue taken up in EtOAc and washed with saturated $NaHCO_3$ and saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure gave the crude product. Chromatography on silica gel, eluting with $CHCl_3$/EtOAc (1:1), gave 4.22 g (66% yield) of the product as a glass: ^1H NMR (300 MHz, $CDCl_3$) δ 1.24 (m, 3 H), 1.32 (m, 3 H), 2.80–3.24 (m, 6 H), 3.60 (m, 4 H), 3.72 (m, 1 H), 4.12 (m, 1 H), 4.30 (m, 2 H), 5.12 (m, 1 H), 5.56 (m, 1 H), 7.10 (t, 1 H), 7.30 (m, 6 H); MS (CI + CH_4) m/z 444 (M + 1).

SMO-Phe-NHCH(OC_2H_5)CO_2H (10). A solution of 4.11 g (9.3 mmol) of **9** in 50 mL of EtOH was treated with 20 mL (20 mmol) of 1 N NaOH and stirred at room temperature for 1 h. Most of the EtOH was removed under reduced pressure and the residue treated with 23 mL of 1 N HCl and extracted with EtOAc. The EtOAc was washed with saturated NaCl and dried over $MgSO_4$. Removal of the solvent under reduced pressure left 3.77 g (97.9% yield) of the product as a white foam: ^1H NMR (200 MHz, $CDCl_3$) δ 1.24 (m, 3 H), 2.70–3.30 (m, 6 H), 3.40–3.82 (m, 6 H), 4.10 (m, 1 H), 5.60 (m, 1 H), 5.98 (broad s, 1 H), 7.30 (m, 6 H), 7.55 (m, 1 H); MS (CI + CH_4) m/z 416 (M + 1).

SMO-Phe-NHCH(OH)$CO_2C_2H_5$ (11). A solution of 13.0 g (33.5 mmol) of **8** in 200 mL of absolute EtOH was treated with 2.0 mL of concentrated H_2SO_4 and stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue taken up in EtOAc and washed with saturated NaCl, saturated $NaHCO_3$, 1 N citric acid, and saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure gave the crude product. Chromatography on silica gel, eluting with $CHCl_3$/EtOAc (1:1) gave 8.75 g (67.4% yield) of a white foam: ^1H NMR (200 MHz, $CDCl_3$) δ 1.30 (m, 3 H), 2.74–3.22 (m, 6 H), 3.50 (m, 4 H), 4.10 (m, 1 H), 4.28 (m, 2 H), 4.52 (d, 0.5 H), 4.74 (d, 0.5 H), 5.58 (m, 2 H), 7.30 (m, 5 H), 7.60 (m, 1 H); MS (FAB, thioglycerol) m/z 416.0 (M + 1). Anal. C, H, N.

SMO-Phe-NHCH(SC_2H_5)$CO_2C_2H_5$ (12). A solution of 4.4 g (10.6 mmol) of **11** in 50 mL of CH_2Cl_2 was treated with 2.6 mL (29 mmol) of ethanethiol and 0.15 g of anhydrous 2-naphthalenesulfonic acid and heated at reflux for 2 h. The mixture was filtered and the filtrate evaporated to an oil. The oil was taken up in EtOAc and washed with saturated $NaHCO_3$, saturated NaCl, 1 N citric acid, and saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure left the crude product. Chromatography on silica gel, eluting with $CHCl_3$/EtOAc (60/40) gave 3.83 g (83% yield) of the product as a glass: ^1H NMR (200 MHz, $CDCl_3$) δ 1.28 (m, 6 H), 2.64 (m, 2 H), 2.80–3.25 (m, 6 H), 3.60 (m, 4 H), 4.08 (m, 1 H), 4.28 (m, 2 H), 4.90 (m, 1 H), 5.48 (m, 1 H), 6.90 (m, 1 H), 7.28 (m, 5 H); MS (FAB, thioglycerol) m/z 460.0 (M + 1). Anal. C, H, N.

SMO-Phe-NHCH(SC_2H_5)CO_2H (13). A solution of 3.54 g (7.7 mmol) of **12** in 25 mL of dioxane was treated with 15.4 mL (15.4 mmol) of 1 N NaOH, stirred at room temperature for 45 min, and then treated with 7.7 mL of 1 N HCl. The solvent was removed under reduced pressure, an additional 7.7 mL of 1 N HCl was added, and the residue was taken up in EtOAc. The EtOAc was washed with saturated NaCl and dried over $MgSO_4$. Removal of the solvent under reduced pressure left 3.32 g (100% yield) of the product as a foam: ^1H NMR (200 MHz, DMSO-d_6) δ 1.20 (m, 3 H), 2.48–2.80 (m, 6 H), 2.92 (m, 2 H), 3.10–3.70 (broad H_2O s, obscuring 4 morpholine protons), 4.14 (m, 1 H), 5.40 (m, 1 H), 7.30 (m, 5 H), 7.82 (m, 1 H), 9.02 (m, 1 H); MS (FAB, thioglycerol) m/z 432 (M + 1).

Boc-Gly-OCH_2CCl_3 (14). A solution of 26.3 g (0.15 mmol) of Boc-Gly and 27.0 g (0.18 mmol) of 2,2,2-trichloroethanol in 250 mL CH_2Cl_2 was cooled in ice and 0.18 g of 4-(dimethylamino)-pyridine added, followed by 31.6 g (0.153 mol) of DCC. After stirring at room temperature for 3.5 h, the mixture was filtered and the solvent removed under reduced pressure. The residue was taken up in EtOAc and washed with 1 N citric acid, saturated NaCl, saturated $NaHCO_3$, and saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure left the crude product. Chromatography on silica gel, eluting with hexane/EtOAc (9/1) gave 45.0 g (97.8% yield) of the product as an oil which solidified. Anal. C, H, N, Cl; C: calcd, 35.26; found, 35.69.

Boc-NHCH(Br)$CO_2CH_2CCl_3$ (15). A solution of 40.0 g (0.13 mol) of **14** and 23.6 g (0.133 mol) of *N*-bromosuccinimide in 400 mL of CCl_4 in a quartz reaction flask was irradiated with a Corex-filtered 450 watt Hanovia mercury lamp at 40 °C for 2 h. The succinimide was filtered off and the filtrate evaporated giving an oil. Trituration with Et_2O/hexane gave 49.2 g (98% yield) of a

solid. The material was used directly in the following reaction.

Boc-NHCH(SC_2H_5)$CO_2CH_2CCl_3$ (16). A suspension of 1.2 g (25 mmol) of NaH-mineral oil (50%) was washed free of mineral oil with THF, then resuspended in 100 mL THF, and treated with 2.15 mL (29 mmol) of ethanethiol. After 1 h at room temperature, the suspension was cooled to 0 °C and a solution of 9.46 g (25 mmol) of **15** in 50 mL of THF was added dropwise over 15 min, and the mixture then left stirring at room temperature overnight. The solvent was removed under reduced pressure and the residue taken up in EtOAc and washed with 1 N citric acid, saturated NaCl, saturated $NaHCO_3$, and saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure left 7.11 g of the crude product as a red oil. Chromatography on silica gel, eluting with hexane/EtOAc (70/30), gave 7.04 g (76.8% yield) of a yellow oil. NMR and MS revealed that some replacement of trichloroethanol by ethanethiol had occurred. The crude material was used in the following reaction: MS (CI + CH_4) m/z 280.0 (thioester), 366.0, 367.9 (trichloroethyl ester).

H_2NCH(SC_2H_5)$CO_2CH_2CCl_3$HCl (17). A solution of 7.04 g (19.2 mmol) of **16** in 200 mL of CH_2Cl_2 was occasionally purged with HCl gas over 5 h. After standing at room temperature overnight, the mixture was filtered and the solvent removed under reduced pressure. After trituration with Et_2O, the material was stripped under reduced pressure to give 5.47 g (94%) of the product as an orange syrup. The crude material was used directly in the following reaction.

Boc-Phe-NHCH(SC_2H_5)$CO_2CH_2CCl_3$ (18). A solution of 4.71 g (17.7 mmol) of Boc-Phe, 2.47 g (18.3 mmol) of HOBT, and 5.38 g (17.7 mmol) of **17** in 125 mL of CH_2Cl_2 was cooled in ice and 3.78 g (18.3 mmol) of DCC added, followed by 4.1 mL (28.9 mmol) of Et_3N. After stirring at room temperature overnight, the mixture was filtered and the solvent removed under reduced pressure. The residue was taken up in EtOAc, filtered, and washed with 1 N citric acid, saturated NaCl, saturated $NaHCO_3$, and saturated NaCl. Drying over $MgSO_4$, treatment with charcoal, and removal of the solvent under reduced pressure gave 9.43 g of the crude product as a dark red oil. Chromatography on silica gel, eluting with hexane/EtOAc (80/20) gave 6.08 g (71% yield) of the product as a white foam. NMR and MS showed the presence of some thioester formed in the preparation of **16**. The material was used in the next reaction without further purification: MS (FAB, thioglycerol) m/z 427.2 (thioester), 513.1, 515.1 (trichloroethyl ester).

Boc-Phe-NHCH(SC_2H_5)CO_2H (19). A solution of 5.89 g (11.5 mmol) of **18** in 25 mL of dioxane was treated with 20 mL (20.0 mmol) of 1 N NaOH and stirred at room temperature for 2 h. The solution was treated with 12 mL of 1 N HCl and the solvent removed under reduced pressure. The residue was treated with EtOAc and 12 mL of 1 N HCl. The EtOAc was separated and washed with saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure gave 4.3 g (98%) of the product as a foam: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.30 (t, 3 H), 1.40 (s, 9 H), 2.70 (q, 2 H), 3.10 (m, 2 H), 5.20 (m, 1 H), 5.45 (m, 1 H), 7.30 (m, 7 H); MS (FAB, thioglycerol) m/z 383.0 (M + 1).

BNMA-NHCH(OC_2H_5)CO-Sta-MBA (21 and 22). A solution of 1.19 g (2.7 mmol) of **5** and 0.38 g (2.7 mmol) of HOBT in 4 mL of DMF was cooled in ice and treated with a solution of 0.67 g (2.7 mmol) of Sta-MBA in 20 mL of CH_2Cl_2 followed by 0.58 g (2.8 mmol) of DCC. After stirring at room temperature overnight, the mixture was filtered and the solvent removed under reduced pressure. The residue was taken up in EtOAc and washed with 1 N citric acid, saturated NaCl, saturated $NaHCO_3$, and saturated NaCl. Drying over $MgSO_4$ and removel of the solvent under reduced pressure gave 1.86 g of the crude product as a foam. Chromatography on silica gel, eluting with a gradient of 0–2% MeOH in $CHCl_3$/EtOAc (1:1) gave the faster eluting diastereomer. Trituration with Et_2O gave 0.45 g (24.9% yield) of **21** as a white amorphous solid: ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.70–1.10 (m, 14 H), 1.12–1.56 (m, 5 H), 2.10 (m, 2 H), 2.82 (q, 2 H), 2.94–3.18 (m, 4 H), 3.18–3.42 (m, 3 H), 3.68–3.84 (m, 2 H), 4.96 (d, 1 H), 5.30 (d, 1 H), 7.10–7.98 (m, 18 H), 8.66 (d, 1 H); MS (FAB, thioglycerol) m/z 668.5 (M + 1). Anal. C, H, N.

Continued elution from the column gave the slower eluting diastereomer which was triturated with Et_2O to give 0.48 g (26.5% yield) of **22** as a white amorphous solid. ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.70–1.14 (m, 13 H), 1.14–1.70 (m, 5 H), 2.10 (d, 2 H), 2.80–3.20 (m, 6 H), 3.20–3.58 (m, 6 H), 3.68–3.98 (m, 2 H), 5.01 (d, 1 H), 5.41 (d, 1 H), 7.10–7.62 (m, 10 H), 7.62–8.08 (m, 6 H), 8.68 (d, 1 H); MS (FAB, thioglycerol) m/z 668.4 (M + 1). Anal. C, H, N.

BNMA-NHCH(SCH_3)CO-Sta-MBA (24). A solution of 5.86 g (13.2 mmol) of **3** in 100 mL of CH_2Cl_2 was cooled to 0 °C and treated with a solution of 1.84 g (13.6 mmol) of HOBT in 6 mL of DMF. This solution was then treated with 2.81 g (13.6 mmol) of DCC followed by a solution of 3.22 g (13.2 mmol) of Sta-MBA in 25 mL of CH_2Cl_2. After stirring at room temperature overnight, the mixture was filtered and the solvent removed under reduced pressure. The residue was taken up in EtOAc, filtered, and washed with 1 N citric acid, saturated NaCl, saturated $NaHCO_3$, and saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure gave the crude product. Chromatography on silica gel, eluting with $CHCl_3$/EtOAc (1/1) gave 7.37 g (83% yield) of the product as a foam as a mixture of diastereomers: ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.82 (m, 11 H), 0.92–1.70 (m, 7 H), 1.80 (closely separated pair of s, 3 H), 2.12 (m, 2 H), 2.94 (m, 2 H), 3.15 (m, 2 H), 3.48 (broad s, 1 H), 3.82 (m, 2 H), 4.98 (m, 1 H), 5.50 (m, 1 H), 7.38 (m, 9 H), 7.62–8.00 (m, 9 H), 8.68 (d, 1 H); MS (FAB, thioglycerol) m/z 670.4 (M + 1). Anal. C, H, N, S.

BNMA-NHCH($SOCH_3$)CO-Sta-MBA (25). A solution of 1.5 g (2.24 mmol) of **24** in 25 mL of MeOH was treated with a solution of 1.01 g (4.68 mmol) of $NaIO_4$ in 10 mL of H_2O and warmed to 50 °C for 2 h. The solvent was removed under reduced pressure and the residue taken up in EtOAc and washed with H_2O, 10% $NaHSO_3$ solution, saturated $NaHCO_3$, and saturated NaCl. After drying over $MgSO_4$ the solvent was removed under reduced pressure leaving a foam. Chromatography on silica gel, eluting with EtOAc/$CHCl_3$ (3/1) gave 0.85 g (55.3% yield) of the product as a foam as a mixture of diastereomers: MS (FAB, thioglycerol) m/z 686.4 (M + 1). Anal. C, H, N, S.

BNMA-NHCH(SO_2CH_3)CO-Sta-MBA (26). A solution of 1.5 g (2.24 mmol) of **24** in 50 mL of CH_2Cl_2 was treated with 1.16 g (5.7 mmol) of *m*-chloroperbenzoic acid and stirred at room temperature for 3 h. The solvent was removed under reduced pressure and the residue taken up in EtOAc. The EtOAc was washed with 2 N Na_2SO_3, saturated $NaHCO_3$, and saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure left a foam. Trituration with Et_2O gave 1.25 g (79.6% yield) of an amorphous solid as a mixture of diastereomers: MS (FAB, thioglycerol) m/z 702.4 (M + 1). Anal. C, H, N, S.

BNMA-NHCH(NHC_2H_5)CO-Sta-MBA (33 and 34). A solution of 1.54 g (2.3 mmol) of **24** in 50 mL of THF was cooled in ice and 5 mL of ethylamine added followed by 0.94 g of $HgCl_2$. Solution occurred and a precipitate then started to form. After stirring for 1 h at 0 °C, the mixture was allowed to stir at room temperature overnight. The mixture was filtered and the solvent removed under reduced pressure. The residue was taken up in EtOAc, filtered, and washed with saturated NaCl, 1 N NaOH, and saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure left 1.6 g of a white foam. Chromatography on silica gel, eluting with EtOAc/$CHCl_3$/MeOH (55/35/10) gave 0.52 g (33.9% yield) of the faster eluting diastereomer **33** as a solid: mp 143–144 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.82 (m, 14 H), 1.04 (m, 2 H), 1.14–1.68 (m, 5 H), 2.04 (m, 3 H), 2.15 (d, 2 H), 2.80–3.30 (m, 6 H), 3.38–3.52 (m, 2 H), 3.84 (m, 1 H), 4.90 (d, 1 H), 5.00 (d, 1 H), 7.24 (m, 1 H), 7.30–7.60 (m, 9 H), 7.64–8.02 (m, 6 H), 8.26 (d, 1 H); MS (FAB, thioglycerol) m/z 667.3 (M + 1). Anal. C, H, N.

Continued elution from the column gave 0.73 g (47.7% yield) of the slower eluting diastereomer **34** as a solid: mp 124–127 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.82 (m, 14 H), 0.92–1.20 (m, 2 H), 1.20–1.80 (m, 5 H), 1.92–2.30 (m, 5 H), 2.78–3.30 (m, 5 H), 3.40 (m, 2 H), 3.86 (m, 2 H), 4.78 (d, 1 H), 4.98 (d, 1 H), 7.18–8.00 (m, 16 H), 8.34 (d, 1 H); MS (FAB, thioglycerol) m/z 667.2 (M + 1). Anal. C, H, N.

BNMA-NHCH($NHCOCH_3$)CO-Sta-MBA (40). A suspension of 0.73 g (1.05 mmol) of **32** in 25 mL of CH_2Cl_2 was treated with 0.7 mL (2.1 mmol) of Et_3N causing solution. The solution was then treated with 0.09 mL (1.08 mmol) of acetyl chloride and stirred at room temperature for 2.5 h. The mixture was filtered and the solvent removed under reduced pressure. The residue was taken up in EtOAc and washed with H_2O, 1 N citric acid,

and saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure left a gum. Trituration with Et_2O gave 0.62 g (86.3% yield) of the product as a white powder: mp 211–213 °C; MS (FAB, thioglycerol) m/z 681.3 (M + 1). Anal. C, H, N.

BNMA-NHCH(NHCO$_2$Et)CO-Sta-MBA (41). A solution of 0.35 g (0.5 mmol) of BNMA-NHCH(NH_2)CO-Sta-MBA as a mixture of diastereomers in 20 mL of THF was treated with 0.08 mL (0.56 mmol) of Et_3N followed by 0.06 mL (0.56 mmol) of ethyl chloroformate. After stirring at room temperature for 2 h, the mixture was filtered and the filtrate evaporated under reduced pressure. The residue was taken up in EtOAc and washed with 1 N citric acid, saturated NaCl, saturated $NaHCO_3$, and saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure gave 0.38 g (97.4% yield) of the product as a foam as a mixture of diastereomers: MS (FAB, thioglycerol) m/z 711.3 (M + 1). Anal. C, H, N.

SMO-Phe-NHCH(OC$_3$H$_7$-n)CO-DFKCYS-AEM (45). A solution of 1.8 g (2.3 mmol) of **44** in 50 mL of DMSO/toluene (1:1) was cooled in ice and 4.75 g (23 mmol) of DCC added, followed by 0.28 mL (3.45 mmol) of dichloroacetic acid. The solution was kept at 0 °C for 2 h and then allowed to stir at room temperature overnight. The mixture was filtered and the solvent removed under reduced pressure. The residue was taken up in EtOAc and washed with saturated $NaHCO_3$, H_2O, and saturated NaCl. Drying over Na_2SO_4 and removal of the solvent under reduced pressure left the crude product. Chromatography on silica gel, eluting with $CHCl_3$/MeOH (95/5) gave 1.36 g (76.4%) of the pure product. The free base (1.19 g) was converted to the citrate salt by dissolving in a small amount of EtOH, adding an equimolar amount of 1.0 M citric acid, diluting with H_2O, and freeze drying. There was obtained 1.42 g of the product as an amorphous white solid: MS (FAB, thioglycerol) m/z 774 (M + 1). Anal. C, H, N, H_2O.

SMO-Phe-NHCH(OC$_2$H$_5$)CO-ACDMH (48a,b). A solution of 1.6 g (4.17 mmol) of **10**, 1.17 g (4.17 mmol) of ACDMH·HCl, and 0.58 g (4.38 mmol) of HOBT in 25 mL of DMF was cooled in ice and treated with 0.64 mL (4.59 mmol) of Et_3N followed by 0.91 g (4.38 mmol) of DCC. After 0.5 h at 0 °C, the mixture was allowed to stir at room temperature overnight. The mixture was filtered and the filtrate concentrated under high vacuum. The residue was taken up in EtOAc and washed with 1 N HCl, H_2O, saturated $NaHCO_3$, and saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure gave the crude product. Chromatography on silica gel, eluting with a gradient of 0–2% MeOH in $CHCl_3$ gave 0.9 g (33.7% yield) of the fast-eluting diastereomer **48a** as a foam: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.94 (m, 7 H), 1.26 (m, 7 H), 1.48 (m, 3 H), 1.56–2.00 (m, 8 H), 2.68–3.04 (m, 5 H), 3.12–3.88 (m, 9 H), 4.02 (m, 2 H), 4.35 (m, 1 H), 5.30 (d, 1 H), 5.50 (d, 1 H), 6.90 (d, 1 H), 7.24 (m, 6 H), 7.62 (d, 1 H); MS (FAB, thioglycerol) m/z 641.3 (M + 1). Anal. C, H, N.

Continued elution from the column gave 0.8 g (29.9% yield) of the slow-eluting diastereomer **48b** as a white foam: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.96 (m, 7 H), 1.22 (m, 7 H), 1.45 (m, 3 H), 1.60–2.00 (m, 8 H), 2.72–3.06 (m, 5 H), 3.28 (m, 3 H), 3.52 (m, 6 H), 3.78 (m, 1 H), 4.08 (m, 1 H), 4.32 (m, 1 H), 5.46 (d, 1 H), 5.66 (d, 1 H), 6.78 (d, 1 H), 7.30 (m, 7 H); MS (FAB, thioglycerol) m/z 641.4 (M + 1). Anal. C, H, N; C: calcd, 58.10; found, 58.60.

SMO-Phe-NHCH(SC$_2$H$_5$)CO-ACDMH (53a,b). A solution of 3.3 g (7.7 mmol) of **13** and 1.08 g (8.08 mmol) of HOBT in 100 mL CH_2Cl_2 was cooled in ice and 1.67 g (8.08 mmol) of DCC added, followed by a cold solution of 2.15 g (7.7 mmol) of ACDMH·HCl and 1.13 mL (8.08 mmol) of Et_3N in 30 mL of CH_2Cl_2. After stirring at room temperature overnight the mixture was filtered and the filtrate evaporated to an oil. The oil was taken up in EtOAc and washed with saturated NaCl, 1 N citric acid, saturated NaCl, saturated $NaHCO_3$, and saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure gave 5.22 g of the crude product as a foam. Chromatography on silica gel, eluting with $CHCl_3$/EtOAc (50/50) gave the faster eluting diastereomer as a white, amorphous solid. Trituration with Et_2O gave 1.36 g (27% yield) of **53a**: ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.90 (m, 6 H), 1.24 (m, 10 H), 1.40–1.90 (m, 8 H), 2.52–3.10 (m, 10 H), 3.22 (m, 2 H), 3.30–3.50 (broad H_2O s, obscuring 3 of the morpholine protons), 4.12 (m, 2 H), 4.30 (d, 1 H), 4.92 (d, 1 H), 5.62 (d, 1 H), 7.30 (m, 5 H), 7.72 (d, 1 H), 7.96 (d, 1 H), 8.84 (d, 1 H); MS (FAB, thioglycerol) m/z 657.2 (M). Anal. C, H, N, S.

Continued elution from the column gave 1.49 g (29.5% yield) of the slower eluting diastereomer **53b** as a white solid: mp 163–165 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.86 (m, 6 H), 1.18 (m, 8 H), 1.32–1.90 (m, 10 H), 2.48–2.80 (m, 7 H), 2.82–3.24 (m, 4 H), 3.30–3.50 (broad H_2O s, obscuring 4 morpholine protons), 4.16 (m, 2 H), 4.58 (d, 1 H), 4.94 (d, 1 H), 5.80 (d, 1 H), 7.30 (m, 5 H), 7.78 (d, 1 H), 8.10 (d, 1 H), 8.92 (d, 1 H); MS (FAB, thioglycerol) m/z 657.2 (M). Anal. C, H, N, S.

BOC-Phe-NHCH(SC$_2$H$_5$)CO-ACDMH (54a,b). A solution of 5.48 g (14.3 mmol) of **19** and 1.97 g (14.6 mmol) of HOBT in 100 mL of CH_2Cl_2 and 10 mL of DMF was cooled in ice and treated with a cold solution of 4.05 g (14.3 mmol) of ACDMH·HCl and 2.0 mL (14.6 mmol) of Et_3N in 70 mL of CH_2Cl_2 followed by 3.0 g (14.6 mmol) of DCC. After stirring at room temperature overnight, the mixture was filtered and the solvent removed under reduced pressure. The residue was taken up in EtOAc, filtered, and washed with 1 N citric acid, saturated NaCl, saturated $NaHCO_3$, and saturated NaCl. After drying over $MgSO_4$, the solvent was removed under reduced pressure giving 8.5 g of the crude product. Chromatography on silica gel, eluting with $CHCl_3$/EtOAc (75/25) gave the faster eluting diastereomer. Recrystallization from Et_2O/hexane gave 2.26 g (26.0% yield) of **54a** as a white solid: mp 166–168 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.84 (m, 6 H), 1.04–1.36 (m, 18 H), 1.36–1.92 (m, 10 H), 2.66 (m, 3 H), 2.84–3.08 (m, 2 H), 3.20 (m, 1 H), 4.06–4.30 (m, 3 H), 4.94 (d, 1 H), 5.60 (d, 1 H), 7.10 (d, 1 H), 7.28 (m, 5 H), 8.00 (d, 1 H), 8.28 (d, 1 H); MS (FAB, thioglycerol) m/z 608 (M). Anal. C, H, N.

Continued elution from the column gave the slower eluting diastereomer. Recrystallization from Et_2O/hexane gave 2.22 g (25.5% yield) of **54b** as a white solid: mp 180–181 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.82 (m, 7 H), 1.18 (m, 9 H), 1.28 (s, 9 H), 1.36–1.88 (m, 9 H), 2.50–2.82 (m, 3 H), 2.86–3.04 (m, 2 H), 3.16 (m, 1 H), 4.12 (m, 1 H), 4.30 (m, 1 H), 4.54 (d, 1 H), 4.92 (d, 1 H), 5.60 (d, 1 H), 7.02 (d, 1 H), 7.30 (m, 5 H), 8.08 (d, 1 H), 8.62 (d, 1 H); MS (FAB, thioglycerol) m/z 608 (M). Anal. C, H, N.

Modeling Experiments. Experiments were performed with the Sybyl software package[26] operating on a Silicon Graphics 4D/25TG. The renin model was derived from the crystal structures of fungal aspartic proteinases including endothiapepsin, penicillopepsin, and rhizopus chinensis proteinase.[23] All optimizations were carried out using molecular mechanics and the Tripos force field. They were performed within the confines of the enzyme cleft including residues within approximately 8 Å of **48a** with the *S* configuration at P_2. During the optimizations, the cleft was aggregated as were inhibitor atoms near the cleavage site. The default options were taken for the SO_2 torsion parameters, while parameters were adjusted to deal with aromatic ring planarity.[27] The electrostatics of the cleft region were determined using the cavity program.[28] The diastereomers **48a** and **48b** were manually docked in the cleft and optimized. Using the conformation of the *S* diastereomer **48a**; the *S* diastereomers of **24**, **25**, and **26**; and the *R* diastereomer **31** were modeled, docked in the cleft, optimized, and evaluated.

Biological Methods. Inhibition of renin activity was determined by a radioimmunoassay for angiotensin I, based on the method of Haber.[29] The in vitro angiotensin I generation step utilized 500 μL of monkey plasma (containing native renin and

(26) Sybyl software package (Version 5.32), Tripos Associates, Inc., a subsidiary of Evans and Sutherland, 1699 S. Hanley Rd., Suite 303, St. Louis, MO, 63144.
(27) Added taff-tons: Wild, C. ar, C. ar, C. ar, ar 2.35-2 wild. Modified taff-oop: C. ar 630 wild.
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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 a dimethylsulfoxide (DMSO) vehicle. Incubation was for 60 min at 37 °C. Following incubation, each mixture was analyzed (in duplicate) for angiotensin I via radioimmunoassay using ^{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 3–8 ng Al per mL per h. Values for inhibitor tubes were compared to vehicle (DMSO) control tubes to estimate percent inhibition. At the concentration used, DMSO inhibits the generation of angiotensin I by <10%. The inhibition results were expressed as IC_{50} values, which were obtained by plotting six inhibitor concentrations and estimating the concentration producing 50% inhibition using nonlinear regression analysis.

Inhibition of bovine cathepsin D (Sigma) activity was assessed in duplicate by the hydrolysis of bovine hemoglobin (2× crystallized, Sigma) at pH 3.2 and 37 °C (modified from Aoyogi et al.[30] and Kokubu et al.[31] 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.

Conscious, High-Renin, Normotensive Monkey Model. Male Cynomolgus monkeys weighing between 4.9 and 7.7 kg were placed on a low-sodium diet (Bio-Serv Inc., Frenchtown, NJ) 7–10 days prior to testing. Each monkey was then treated with furosemide (Lasix, INJ 5%, Hoechst-Roussel) at 2 mg per kg per day IM for four consecutive days prior to testing.

Solutions were prepared using a vehicle of 7.5% DMA, 30% Tween 80, and 62.5% H_2O. Concentrations were adjusted to allow the total dose to be administered in a volume of 2 mL/kg. The solution was administered by oral gavage using a 16-French rectal-colon tube (Davol, Cranston, RI). The monkeys were instrumented with vascular access ports (Norfolk Medical Products, Skokie, IL) for intraarterial blood pressure monitoring. Blood pressure was measured using a computer data acquisition system. Monkeys selected for these studies had been trained to rest quietly in a basic macaque restrainer (Primate Products, Woodside, CA).

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Hybrid Cholecystokinin-A Antagonists Based on Molecular Modeling of Lorglumide and L-364,718

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A series of novel nonpeptide cholecystokinin-A (CCK-A) antagonists have been synthesized. Designed on the basis of the structural homology between lorglumide and L-364,718, as investigated with molecular modeling, these compounds constitute a link between the *N*-acylglutamic acid and 3-amino-5-phenyl-1,4-benzodiazepin-2-one derived antagonists. The prepared compounds were tested in vitro as antagonists of the binding of [^{3}H]-(±)-L-364,718 and [^{3}H]-CCK-8(S) to rat pancreas and guinea pig brain membranes, respectively. All compounds proved to be selective for the (peripheral) CCK-A receptor, the most potent analogue, **6**, having a K_i value of 90 nM. The structure–activity profile of the series of hybrid compounds relates closest to that of the *N*-acylglutamic acid derived antagonists.

Introduction

Cholecystokinin (CCK) is a gastrointestinal peptide hormone that stimulates biliary and pancreatic secretion, gastrointestinal motility, and gallbladder contraction.[1] The identification of CCK in[2] and isolation from[3] the mammalian brain has increased the interest for this peptide considerably. It is now well-recognized that CCK has a neurotransmitter[4,5] or neuromodulator[6] function in the central nervous system (CNS), especially in the modulation of dopamine-mediated neurotransmission.[7-9] With various radiolabeled probes, at least two CCK receptor subtypes have been characterized.[10,11] Whereas CCK-B receptors are confined to the CNS, CCK-A receptors have been detected in both gastrointestinal[10] and CNS[12,13] tissues. Albeit the density of type A receptors in the CNS is low, their physiological relevance has been demonstrated. For

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