Mercaptoacyl Amino Acid Inhibitors of Atriopeptidase. 1. Structure–Activity Relationship Studies of Methionine and S-Alkylcysteine Derivatives

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A broad series of *N*-(3-mercaptoacyl) amino acid derivatives was evaluated for their ability to inhibit atriopeptidase (neutral endopeptidase, EC 3.4.24.11) *in vitro* and *in vivo*. Structural parameters studied were *(i)* the substituent on the 2-position of the 3-mercaptopropionyl moiety, *(ii)* the amino acid component, *(iii)* the S-terminal derivative, and *(iv)* the C-terminal derivative. Optimum activity was observed for derivatives of methionine and *S*-alkylcysteines. *N*-[3-Mercapto-2(*S*)-[(2-methylphenyl)methyl]-1-oxopropyl]-L-methionine was identified as a highly effective inhibitor of atriopeptidase meriting evaluation as a potential cardiovascular therapeutic agent.

Introduction

The atrial natriuretic factors (ANF) are peptide hormones first isolated from cardiac atrial tissue.[1] Related peptidic hormones, brain natriuretic peptide[2] and urodilatin,[3] have been identified. Many pharmacological actions have been demonstrated for ANF, most prominently vasorelaxant and natriuretic activities.[4] The half-life of these peptides in circulation is quite short,[5] with proteolytic cleavage occurring in several positions,[6] but predominantly between Cys-7 and Phe-8 in the disulfide ring (see Figure 1). The cleavage products possess little biological activity.[7] Consequently, prolonging the action of ANF may result in useful pharmacological effects.

The enzymes participating in the degradation of ANF have been studied extensively.[6,8] These studies led to the identification of neutral endopeptidase (NEP, EC 3.4.24.11, also termed atriopeptidase) as a critical enzyme in the degradation.[9] This enzyme[10] is a zinc-containing metallo-protease found in the kidney, brain (where it has been termed enkephalinase), and other tissues. It participates in the degradation of numerous peptidic substrates. NEP cleaves ANF *in vitro*,[6] and inhibition of NEP *in vivo* has been shown to produce beneficial vasodilatory and natriuretic responses.[11,12] Thus, development of a practical enzyme inhibitor would permit evaluation of the clinical utility of inhibition of NEP.[13]

Prior to our studies, inhibitors of NEP had been described by several laboratories whose efforts were directed at inhibition of "enkephalinase" to achieve an analgesic effect.[14] These enzyme inhibitors featured a variety of functional groups capable of binding to the active-site zinc atom. Reported enkephalinase inhibitors[15] contained a mercapto,[14,16] carboxy,[17] phosphonic acid,[18] or hydroxamic acid[19] group as the zinc ligand. We began by evaluating the relative utility of the first three ligand types, and some of our results with carboxyalkyl-dipeptides have been reported.[20] This report

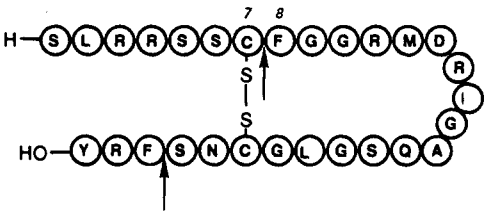
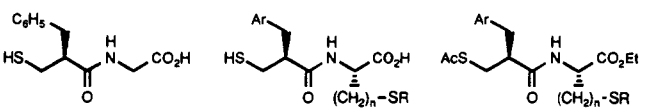


Figure 1. Primary sites for metabolic cleavage of ANF.

describes the first phase of our examination of mercapto compounds.

Since the *in vitro* enzyme inhibition assay was well-established,[21] a critical element in developing an effective agent was the establishment of a relevant *in vivo* assay for cardiovascular activity. Initially, activity of test compounds was determined in spontaneously hypertensive rats by measuring potentiation of the hypotensive response to exogenous ANF.[22] An additional and more useful assay was found to be the hypotensive response produced by test compounds in DOCA-salt rats.[22] This is a volume-dependent hypertensive model in which endogenous plasma ANF is elevated. With these tools in hand, we were equipped to evaluate a series of mercaptoacyl amino acids.

We began our survey of mercapto compounds with a series related to thiorphan (**1**).[14] While exploring a variety of amino acid components, we found encouraging activity in mercaptoacyl derivatives of *S*-alkylcysteines and of methionine. Furthermore, we soon discovered that both the underivatized mercapto acids **2** (*ipso*-drugs) and protected forms **3** (prodrugs) were capable



1 **2** **3**

of expressing good *in vivo* activity. Within both the cysteine and methionine classes, broad structure–activity relationships were established by variation of the following parameters: *(i)* the substituent on the 2-position of the 3-mercaptopropionyl moiety, *(ii)* the amino acid component, *(iii)* the *S*-terminal derivative, and *(iv)* the C-terminal derivative.

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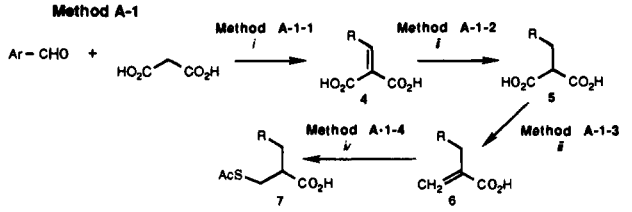
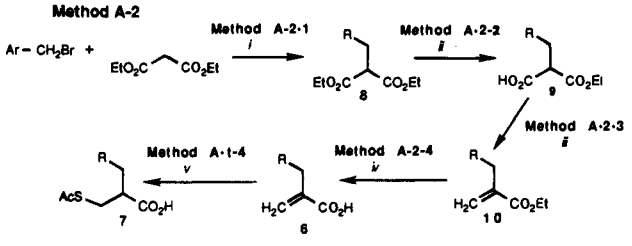
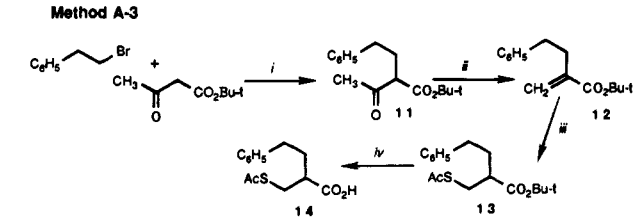
Scheme 1



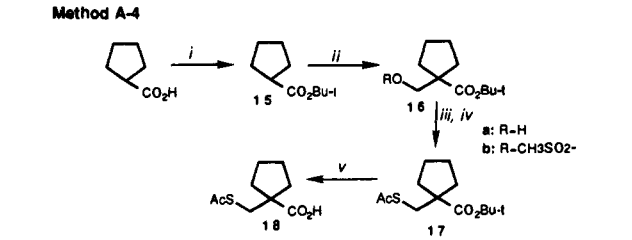
i. Δ neat or gl. AcOH; *ii.* H_2,10% Pd/C, EtOAc; *iii. (a)* 40% aq. Me_2NH, 37% aq. HCHO; *(b)* Δ; *iv.* AcSH, CH_2Cl_2.



i. Na, EtOH; *ii.* KOH, EtOH; *iii.* Et_2NH, 37% aq. HCHO; *iv.* 1N NaOH, dioxane; *v.* AcSH, CH_2Cl_2.



i. Na, EtOH; *ii.* n-BuLi, $IsoPr_2NH$, HCHO; *iii.* AcSH, CH_2Cl_2; *iv.* TFA.

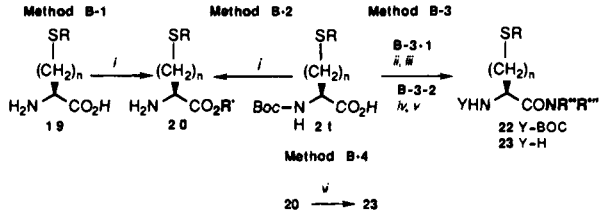


i. isobutylene, t-BuOH, cat. H_2SO_4; *ii.* LDA/THF, CH_2O; iii. MsCl, Et_3N, Et_2O; *iv.* AcSH, K_2CO_3, DMF, 100°C; *v.* TFA/CH_2Cl_2.

Chemistry

For variation of the mercaptoacyl moiety, we prepared the appropriate 2-substituted 3-(acetylthio)propionic acids (**7**) by Michael addition of thiolacetic acid to the respective 2-substituted acrylic acids (**6**) (see Scheme 1, methods A-1 and A-2). These were in turn available by classical malonic acid Mannich reaction of **5** or **9**. The homologous 2-[(acetylthio)methyl]-4-phenylbutyric acid (**14**) was prepared as shown in Scheme 1, method A-3. For 1-[(acetylthio)methyl]cyclopentanecarboxylic acid (**18**) (Scheme 1, method A-4), the *tert*-butyl ester **15** of cyclopentanecarboxylic acid was prepared, and the ester–enolate was treated with formaldehyde to produce the alcohol **16a** in high yield. Mesylation was followed by facile thioacetate displacement at the neopentyl center. Final ester deprotection furnished the desired acid **18**.

Scheme 2



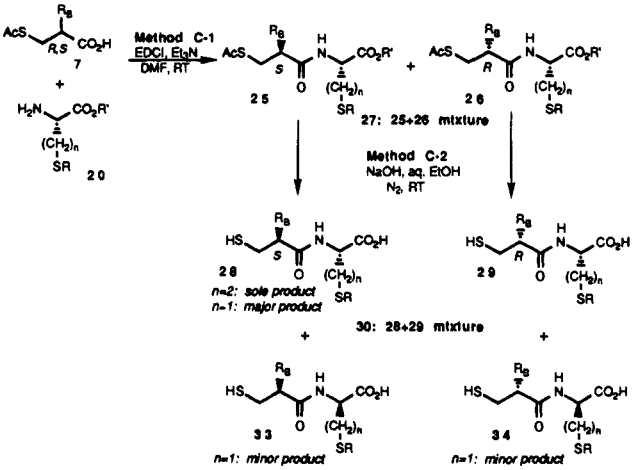
i. $SOCl_2$, R'OH, reflux; *ii.(a)* NEt_3, EtO_2CCl, THF; *(b)* NH_4OH; *iii.* TFA, CH_2Cl_2; *iv.* EDC, R'''R''''NH, DMF; *v.* HCl, dioxane; *vi.* amine, 2 equiv., Δ.

20	n	R	R'	Method
a:	1	$C_6H_5CH_2CH_2$	CH_3CH_2	B-1
b:	1	CH_3	CH_3CH_2	B-1
c:	1	CH_3CH_2	CH_3CH_2	B-1
d:	2	CH_3CH_2	CH_3CH_2	B-1
e:	1	$(C_6H_5)_3CH_2$	CH_3	B-1
f:	1*	$C_6H_5CH_2$	CH_3CH_2	B-2
g:	1	4-$CH_3C_6H_4CH_2$	CH_3	B-2
h:	1	4-$CH_3C_6H_4CH_2$	CH_3CH_2	B-2
j:	1	3,4-$(CH_3)_2C_6H_3CH_2$	CH_3CH_2	B-2
k:	1	4-$CH_3OC_6H_4CH_2$	CH_3CH_2	B-2
m:	1	$(CH_3)_3C$	CH_3	B-2

22/23	n	R	NR''R'''	Method
a:	1	4-$CH_3C_6H_4CH_2$	NH_2	B-3-1
b:	2	CH_3	N(CH2)5	B-3-2
c:	2	CH_3	$N(CH_2)_2O(CH_2)_2$	B-3-2
d:	2	CH_3	$NH(CH_2)_2OH$	B-3-2
e:	2	CH_3	$NH(CH_2)_2CO_2Et$	B-3-2
f:	2	CH_3	$N(CH_2)_4$	B-4

* D-Cysteine

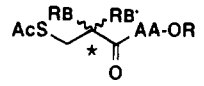
Scheme 3



The amino acid components were generally commercially available. *S*-(2-Phenylethyl)-L-cysteine was prepared by alkylation of L-cysteine with 2-phenylethyl bromide. Methyl and ethyl esters **20** of the amino acids were prepared by reaction of the amino acid **19** or *N*-Boc amino acid **21** with $SOCl_2$ in the corresponding alcohol (Scheme 2, methods B-1 and B-2). Amino acid amides **23** were prepared from the *N*-Boc amino acid **21** and appropriate amine by either mixed anhydride- or carbodiimide-mediated coupling (Scheme 2, methods B-3–1 and B-3–2), with subsequent removal of the BOC group. Amino acid amides **23** were also prepared by heating the amino acid ester **20** with excess amine (Scheme 2, method B-4).

The standard method for acylation of an amino acid ester **20** with the acetylthio acid **7** employed carbodiimide coupling and gave generally very good yields. Reaction of a racemic acetylthio acid **7** with a single enantiomer of the amino ester **20** furnished two diastereomeric amido esters **25** and **26** (Scheme 3, method

Table 1. Physicochemical Data for *N*-[3-(Acetylthio)-2-substituted-1-oxopropyl]amino Acid Esters

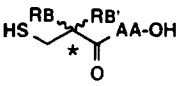


compd	stereo (*)	AA–OR	method[a]	mp, °C[b]	yield (%)[c]	optical rotation $[\alpha]_D$, deg (c, solvent)	NMR, CH δ (solvent)[d]	TLC mobility	formula	anal.
				R_B = Phenylmethyl, $R_{B'}$ = H						
25a	*S*	*S*-benzyl-L-Cys-OEt	C-1[e,f]	83–85	22	−73.5 (0.5, M)	4.72 (C)	faster	$C_{27}H_{29}NO_4S_2$	C, H, N
26a	*R*	*S*-benzyl-L-Cys-OEt	C-1[f]	72–74	16	−9.4 (0.5, M)	4.70 (C)	slower	$C_{27}H_{29}NO_4S_2$	C, H, N
25b	*S*	*S*-benzyl-D-Cys-OEt	C-1[f]	72–73	10	+15.6 (0.65, M)	4.70 (C)	slower	$C_{24}H_{29}NO_4S_2$	C, H, N
26b	*R*	*S*-benzyl-D-Cys-OEt	C-1[f]	84–85	20	+75.9 (0.55, M)	4.66 (C)	faster	$C_{24}H_{29}NO_4S_2$	C, H, N
25c	*S*	*S*-(4-Me-benzyl)-L-Cys-OMe	C-1[g]	oil	18	−76.2 (0.5, M)	4.47 (D)	faster	$C_{24}H_{29}NO_4S_2$	C, H, N
26c	*R*	*S*-(4-Me-benzyl)-L-Cys-OMe	C-1[g]	oil	19	−4.9 (0.2, M)	4.43 (D)	slower	$C_{24}H_{29}NO_4S_2$	C, H, N
25d	*S*	*S*-(4-MeO-benzyl)-L-Cys-OMe	C-1[h]	oil	18	−66.5 (0.1, M)	4.46 (D)	faster	$C_{24}H_{29}NO_5S_2$	C, H, N
26d	*R*	*S*-(4-MeO-benzyl)-L-Cys-OMe	C-1[h]	oil	20	+3.0 (0.1, M)	4.42 (D)	slower	$C_{24}H_{29}NO_5S_2 \cdot 0.5H_2O$	C, H, N[i]
25e	*S*	*S*-(3,4-Me_2-benzyl)-L-Cys-OEt	C-1[j]	90–92	15	−71.1 (0.55, M)	4.67 (C)	faster	$C_{26}H_{33}NO_4S_2$	C, H, N
26e	*R*	*S*-(3,4-Me_2-benzyl)-L-Cys-OEt	C-1[j]	oil	17	−8.4 (0.75, M)	4.71 (C)	slower	$C_{26}H_{33}NO_4S_2$	C, H, N
25f	*S*	*S*-(2-phenylethyl)-L-Cys-OEt	C-1[k]	63–64	28	−51.2 (0.5, M)	4.67 (C)	faster	$C_{25}H_{31}NO_4S_2$	C, H, N
26f	*R*	*S*-(2-phenylethyl)-L-Cys-OEt	C-1[k]	84–86	24	+5.3 (0.5, M)	4.70 (C)	slower	$C_{25}H_{31}NO_4S_2$	C, H, N
27g	*R, S*	*S*-trityl-L-Cys-OMe	C-1a[l,m]	oil	46	+5.9 (0.5, M)	4.39 (C)		$C_{35}H_{35}NO_4S_2$	C, H, N[n]
27h	*S*	*S*-methyl-L-OEt	C-1a[o]	oil	56	−25.9 (0.4, M)			$C_{18}H_{25}NO_4S_2$	C, H, N[p]
25j	*S*	*S*-ethyl-L-Cys-OEt	C-1	foam	8		4.67 (C)			
27k	*R*	*S*-ethyl-L-Cys-OEt	C-1a[q]	oil	14	+8.1 (0.5, M)			$C_{19}H_{26}NO_4S_2$	C, H, N[r]
25l	*S*	*S*-*tert*-butyl-L-Cys-OMe	C-1[s]	oil	47	−44.9 (0.2, M)	4.75 (C)	faster	$C_{20}H_{29}NO_4S_2$	C, H, N
26l	*R*	*S*-*tert*-butyl-L-Cys-OMe	C-1[s]	oil	33	+8.3 (0.14, M)	4.49 (C)	slower	$C_{20}H_{29}NO_4S_2$	C, H, N
27m	*R,S*	L-Met-OMe	C-1a[t,u]	oil	55	−38.9 (0.55, M)	4.38, 4.29 (D)		$C_{18}H_{25}NO_4S_2$	C, H, N[v]
25n	*S*	L-Met-OMe[w]	D[x,y]	78–80	82	−67.9 (0.5, E)	4.60 (C)		$C_{23}H_{27}NO_4S_2$	C, H, N
26n	*R*	L-Met-OMe[w]	D[y]	77–79	89	+39.2 (0.5, E)	4.62 (C)		$C_{23}H_{27}NO_4S_2$	C, H, N
25o	*S*	L-ethionine-OEt	C-1[z]	foam	12	−60.6 (0.6, M)	4.55 (C)	faster	$C_{20}H_{29}NO_4S_2$	C, H, N[aa]
26o	*R*	L-ethionine-OEt	C-1[z]	foam	11	−0.3 (0.4, M)	4.57 (C)	slower	$C_{20}H_{29}NO_4S_2$	C, H, N
36	*S*	*S*-(4-Me-benzyl)-L-Cys-OBu-t	A[bb]	oil	12	−82.1 (0.48, M)	4.56 (C)	faster	$C_{27}H_{36}NO_4S_2$	C, H, N
37	*R*	*S*-(4-Me-benzyl)-L-Cys-OBu-t	A[bb]	oil	14	−23.5 (0.46, M)	4.60 (C)	slower	$C_{27}H_{35}NO_4S_2$	C, H, N
				R_B = Methyl, $R_{B'}$ = H						
25p	*S*	*S*-benzyl-L-Cys-OEt	D[cc,x]	oil	39	−122.4 (0.3, M)	4.75 (C)		$C_{18}H_{25}NO_4S_2$	C, H, N
				R_B = *n*-Propyl, $R_{B'}$ = H						
27q	*RS*	*S*-ethyl-L-Cys-OBu-t	A[dd]	oil	65	−39.4 (0.25, M)	4.69 (C)		$C_{17}H_{31}NO_4S_2$	C, H, N[ee]
				$R_B + R_{B'} = (CH_2)_4$						
27s	–	L-Met-OEt	C-1a[y]	40–42	76	−36.6 (0.50, E)			$C_{16}H_{27}NO_4S_2$	C, H, N
				R_B = 2-Phenylethyl, $R_{B'}$ = H						
25t	*S*	L-Met-OEt	C-1[y]	82–5	17	−53.8 (0.50, E)	4.72 (C)		$C_{20}H_{29}NO_4S_2$	C, H, N
26t	*R*	L-Met-OEt	C-1[y]	100–102	10	−4.2 (0.50, E)	4.70 (C)		$C_{20}H_{29}NO_4S_2$	C, H, N
				R_B = 2-Naphthylmethyl, $R_{B'}$ = H						
25u	*S*	*S*-(4-Me-benzyl)-L-Cys-OEt	C-1[ff]	foam	25	−61.0 (0.5, M)	4.65 (C)	faster	$C_{29}H_{33}NO_4S_2$	C, H, N[gg]
26u	*R*	*S*-(4-Me-benzyl)-L-Cys-OEt	C-1[ff]	foam	23	−20.7 (0.4, M)	4.65 (C)	slower	$C_{29}H_{33}NO_4S_2$	C, H, N[hh]
				R_B = 1-Naphthylmethyl, $R_{B'}$ = H						
25v	*S*	*S*-(4-Me-benzyl)-L-Cys-OEt	C-1[ii]	foam	28	−40.6 (0.5, M)	4.57 (C)	faster	$C_{29}H_{33}NO_4S_2$	C, H, N
26v	*R*	*S*-(4-Me-benzyl)-L-Cys-OEt	C-1[ii]	foam	23	−58.8 (0.8, M)	4.61 (C)	slower	$C_{29}H_{33}NO_4S_2$	C, H, N[jj]
				R_B = (4-Chlorophenyl)methyl, $R_{B'}$ = H						
25w	*S*	*S*-benzyl-L-Cys-OEt	C-1[y]	89–90	17			faster		
26w	*R*	*S*-benzyl-L-Cys-OEt	C-1[y]	103–4	22	−17.9 (0.50, E)	4.69 (C)	slower	$C_{24}H_{28}ClNO_4S_2$	C, H, N
				R_B = (2-Methylphenyl)methyl, $R_{B'}$ = H						
25y	*S*	L-Met-OEt	C-1[y]	91.5–94	19	−62.9 (1.0, M)	4.53 (C)	faster	$C_{20}H_{29}NO_4S_2$	C, H, N
26y	*R*	L-Met-OEt	C-1[y]	foam	14	−11.7 (0.6, M)	4.53 (C)	slower	$C_{20}H_{29}NO_4S_2$	C, H, N

[a] Chromatographic column separations employ Baker silica gel, 60–200 mesh or flash silica gel, 32–64 mesh, with the indicated eluants. [b] White solid unless otherwise stated. [c] Theoretical yield of each diastereomer is 50%. [d] Chemical shift values are for amino acid α-proton. Chloroform-*d* (C); DMSO-*d* (D). [e] See the Experimental Section for this example of method C-1. [f] CH_2Cl_2–EtOAc (49:1). [g] EtOAc–hexane (4:21). [h] EtOAc–hexane (1:4). [i] C: calcd, 59.48; found, 59.04. [j] EtOAc–hexane (25:170); MeOH–hexane (25:170). [k] CH_2Cl_2; CH_2Cl_2–EtOAc (49:1). [l] See the Experimental Section for this example of C-1a. [m] CH_2Cl_2; CH_2Cl_2–EtOAc (1.5:98.5); (1:49). [n] C: calcd, 70.35; found, 69.73. [o] EtOAc–hexane (3:17). [p] C: calcd, 56.37; found, 55.75. [q] EtOAc–hexane (1:3). [r] C: calcd, 57.40; found, 56.99. [s] EtOAc–hexane (3:17). [t] See the Experimental Section for this example of method C-1a. [u] CH_2Cl_2; CH_2Cl_2–EtOAc (49:1). [v] C: calcd, 56.37; found, 55.47. [w] Benzoylthio in place of acetylthio. [x] See the Experimental Section for this example of method D. [y] Et_2O–hexane (1:1). [z] EtOAc–hexane (2.5:17.5). [aa] C: calcd, 58.37; found, 58.86. [bb] Prep 500 silica gel cartridges CH_2Cl_2; CH_2Cl_2–EtOAc (100:1; 200:3; 50:1). [cc] CH_2Cl_2–EtOAc (99:1; 49:1; 9:1). [dd] CH_2Cl_2; CH_2Cl_2–EtOAc (49:1). [ee] C: calcd, 54.08; found, 53.59. [ff] Silica gel, EtOAc–hexane (1:9; 1.1:8.9). [gg] C: calcd, 66.51; found, 66.95. [hh] C: calcd, 66.51; found, 66.99. [ii] EtOAc–hexane (1:9). [jj] C: calcd, 66.51; found, 66.06.

C-1) (Table 1). In the majority of examples, these diastereomers could be separated by column chromatography. The ester and thioester functions of **25** and **26** were saponified[23] with 1 N NaOH in aqueous methanol to give the mercapto acids **28** and **29**, respectively (Scheme 3, method C-2) (Table 2). Problems arose in the *S*-alkylcysteine series during preparation of the mercapto acids **28** (n = **1**) from the precursor ethyl esters **25** (n = 1). In these cases, saponification with NaOH in aqueous ethanol resulted in racemization of the cysteine chiral center to the extent of approximately 15%, yielding diastereomer **33** in addition to **28**. The amount of diastereomer could be determined readily in the NMR spectrum, particularly the signal of the amino acid α-proton. The increased susceptibilty of *S*-alkylated cysteine and *O*-alkylated serine derivatives to racemization is known,[24] and we observed this problem also with *O*-alkylserine examples. Once the ester function has been converted to a carboxylate, no further racemization occurs, since **28** is not converted to **33**

Table 2. Physicochemical Data for *N*-[3-Mercapto-2-substituted-1-oxopropyl]amino Acids



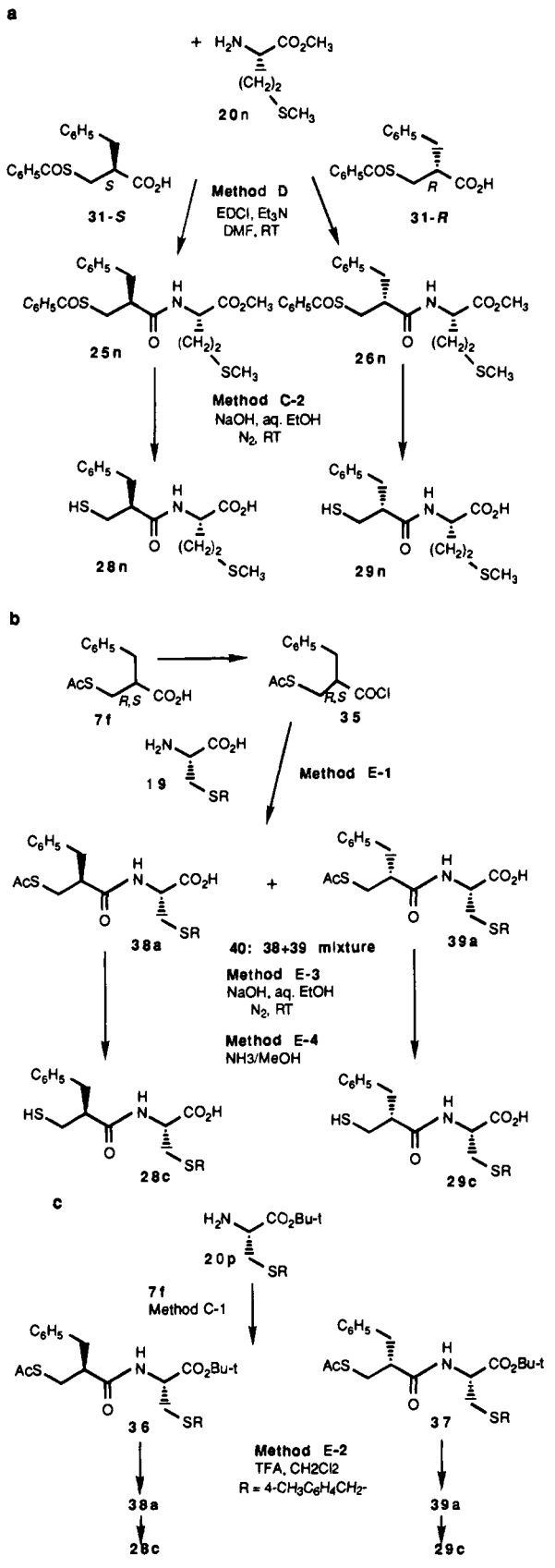
compd	stereo (*)	AA	method	mp, °C[a]	yield (%)	optical rotation $[\alpha]_D$, deg (*c*, solvent)	NMR, CH δ (solvent)[b]	TLC mobility[c]	formula	anal.
					R_B = Phenylmethyl, $R_{B'}$ = H					
28a	*S*	*S*-benzyl-L-Cys	C-2[d]	oil	74	−2.1 (1.0, M)	4.70 (C)	faster	$C_{20}H_{23}NO_3S_2 \cdot 0.1CH_2Cl_2$	C, H, N[e]
29a	*R*	*S*-benzyl-L-Cys	C-2	oil	84	−46.7 (1.1, M)	4.62 (C)	slower	$C_{20}H_{23}NO_3S_2 \cdot 0.2CH_2Cl_2$	C, H, N
28b	*S*	*S*-benzyl-L-Cys	C-2	oil	95	+38.2 (0.6, M)	4.61 (C)	slower	$C_{20}H_{23}NO_3S_2 \cdot 0.75H_2O$	C, H, N
29b	*R*	*S*-benzyl-D-Cys	C-2	oil	85	+13.5 (0.5, M)	4.68 (C)	faster	$C_{20}H_{23}NO_3S_2 \cdot 0.5H_2O$	C, H, N
28c	*S*	*S*-(4-Me-benzyl)-L-Cys	E-4	oil	86	−32.6 (0.4, M)	4.68 (C)	faster	$C_{21}H_{25}NO_3S_2 \cdot 0.5H_2O$	C, H, N[f]
			C-2		78					
29c	*R*	*S*-(4-Me-benzyl)-L-Cys	E-4[g]	oil	84	−60.9 (0.5, M)	4.61 (C)	slower	$C_{21}H_{25}NO_3S_2 \cdot H_2O$	C, H, N[h]
			C-2		58					
28d	*S*	*S*-(4-MeO-benzyl)-L-Cys	C-2	foam	46	−19.3 (0.4, M)	4.69 (C)	faster	$C_{21}H_{25}NO_4S_2 \cdot 0.5H_2O$	C, H, N
29d	*R*	*S*-(4-MeO-benzyl)-L-Cys	C-2	oil	65	−44.2 (0.1, M)	4.41 (D)	slower	$C_{21}H_{25}NO_4S_2 \cdot 0.5H_2O$	C, H, N
28e	*S*	*S*-(3,4-Me_2-benzyl)-L-Cys	C-2	oil	99	−18.0 (0.3, M)	4.70 (C)	faster	$C_{22}H_{27}NO_3S_2$	C, H, N[i]
29e	*R*	*S*-(3,4-Me_2-benzyl)-L-Cys	C-2	oil	69	−56.5 (0.2, M)	4.61 (C)	slower	$C_{22}H_{27}NO_3S_2$	C, H, N[j]
28f	*S*	*S*-(2-phenylethyl)-L-Cys	C-2	oil	92	+4.8 (0.75, M)	4.71 (C)	faster	$C_{21}H_{25}NO_3S_2 \cdot 0.5H_2O$	C, H, N
29f	*R*	*S*-(2-phenylethyl)-L-Cys	C-2	oil	85	−39.7 (0.75, M)	4.65 (C)	slower	$C_{21}H_{25}NO_3S_2 \cdot 0.75H_2O$	C, H, N[k]
30g	*R,S*	*S*-trityl-L-Cys	C-2a[l]	foam	81	+10.5 (0.5, M)	4.21 (C)		$C_{32}H_{31}NO_3S_2 \cdot 0.5H_2O$	C, H, N
30h	*R, S*	*S*-methyl-L-Cys	C-2a	oil	83	−32.1 (0.4, M)	4.74, 4.68 (C)		$C_{14}H_{19}NO_3S_2 \cdot 0.5H_2O$	C, H, N[m]
28j	*S*	*S*-ethyl-L-Cys	C-2	oil	89	−18.6 (0.3, M)	4.73 (C)	faster	$C_{15}H_{21}NO_3S_2 \cdot H_2O$	C, H, N
30k	*S, R*	*S*-ethyl-L-Cys	C-2a	oil	99	−21.9 (0.8, M)	4.73/4.70	–	$C_{15}H_{21}NO_3S_2$	C, H, N[n]
28l	*S*	*S*-*tert*-butyl-L-Cys	C-2	oil	91	+0.4 (0.56, M)	4.80 (C)	faster	$C_{17}H_{26}NO_3S_2 \cdot 0.5H_2O$	C, H, N[o]
29l	*R*	*S*-*tert*-butyl-L-Cys	C-2	foam	97	−32.2 (0.47, M)	4.74 (C)	slower	$C_{17}H_{26}NO_3S_2 \cdot 0.75H_2O$	C, H, N[p]
30m	*R, S*	L-Met	C-2a	132–135	82	−34.6 (0.86, M)	4.34, 4.27 (D)		$C_{15}H_{21}NO_3S_2 \cdot 0.5H_2O$	C, H, N
28n	*S*	L-Met	C-2	oil	99	+4.7 (0.50, E)	4.64 (C)		$C_{15}H_{21}NO_3S_2 \cdot 0.125H_2O$	C, H, N[q]
29n	*R*	L-Met	E-3[r]	foam	92	−38.3 (0.50, E)	4.62 (C)		$C_{15}H_{21}NO_3S_2 \cdot 0.125H_2O$	C, H, N[s]
28o	*S*	L-ethionine	C-2	oil	79	−41.8 (0.2, M)	4.65 (C)	faster	$C_{15}H_{23}NO_3S_2 \cdot 0.5H_2O$	C, H, N
29o	*R*	L-ethionine	C-2	oil	90	−66.0 (0.5, M)	4.60 (C)	slower	$C_{15}H_{23}NO_3S_2$	C, H, N
					R_B = Methyl, $R_{B'}$ = H					
28p	*S*	*S*-Benzyl-L-Cys	C-2	oil	98	−50.1 (0.7, M)	4.80 (C)	–	$C_{14}H_{19}NO_3S_2 \cdot H_2O$	C, H, N
					R_B = n-Propyl, $R_{B'}$ = H					
30q	*R, S*	*S*-Ethyl-L-Cys	E-3[t]	oil	92	−34.1 (0.55, M)	4.80, 4.84 (C)		$C_{11}H_{21}NO_3S_2$	C, H, N
30r	*R, S*	L-Met	C-2a	oil	89	−37.1 (0.9, M)	4.77 (C)		$C_{11}H_{21}NO_3S_2 \cdot 0.7H_2O$	C, H, N
					$R_B + R_{B'} = (CH_2)_4$					
30s		L-Met	C-2	85–87	87	−15.7 (0.50, E)	–	–	$C_{12}H_{21}NO_3S_2$	C, H, N
					R_B = 2-Phenylethyl, $R_{B'}$ = H					
28t	*S*	L-Met	C-2	oil	96	−12.5 (0.5, E)	4.80 (C)		$C_{15}H_{23}NO_3S_2$	C, H, N[u]
29t	*R*	L-Met	C-2	oil	94	−22.9 (0.5, E)	4.69 (C)		$C_{15}H_{23}NO_3S_2 \cdot 0.75H_2O$	C, H, N
					R_B = 2-Naphthylmethyl, $R_{B'}$ = H					
28u	*S*	*S*-(4-Me-benzyl)-L-Cys	C-2	foam	91	+9.9 (0.4, M)	4.65 (C)	faster	$C_{25}H_{27}NO_3S_2$	C, H, N
29u	*R*	*S*-(4-Me-benzyl)-L-Cys	C-2	foam	62	−50.1 (0.25, M)	4.55 (C)	slower	$C_{25}H_{27}NO_3S_2$	C, H, N[v]
					R_B = 1-Naphthylmethyl, $R_{B'}$ = H					
28v	*S*	*S*-(4-Me-benzyl)-L-Cys	C-2	foam	64	+18.3 (0.7, M)	4.64 (C)	faster	$C_{25}H_{27}NO_3S_2 \cdot 0.25H_2O$	C, H, N
29v	*R*	*S*-(4-Me-benzyl)-L-Cys	C-2	foam	59	−102 (0.4, M)	4.54 (C)	slower	$C_{25}H_{27}NO_3S_2$	C, H, N
					R_B = (4-Chlorophenyl)methyl, $R_{B'}$ = H					
28w	*S*	*S*-(4-Me-benzyl)-L-Cys	C-2	film	91	−3.0 (0.50, E)	4.71 (C)	faster	$C_{20}H_{22}ClNO_3S_2$	C, H, N
29w	*R*	*S*-(4-Me-benzyl)-L-Cys	C-2	film	99	−48.7 (0.50, E)	4.63 (C)	slower	$C_{20}H_{22}ClNO_3S_2$	C, H, N[w]
					R_B = [1,1']-Biphenyl]-4-ylmethyl, $R_{B'}$ = H					
28x	*S*	*S*-(4-Me-benzyl)-L-Cys	E-4	foam[x]	71	−2.2 (0.6, M)	4.49 (D)	faster	$C_{27}H_{29}NO_3S_2$	C, H, N
29x	*R*	*S*-(4-Me-benzyl)-L-Cys	E-4	foam[x]	27	−62.2 (0.3, M)	4.45 (D)	slower	$C_{27}H_{29}NO_3S_2$	C, H, N
					R_B = (2-Methylphenyl)methyl, $R_{B'}$ = H					
28y	*S*	L-Met	C-2	114–120	93	+8.6 (0.6, M)			$C_{15}H_{23}NO_3S_2$	C, H, N

[a] White solid unless otherwise stated. [b] Chemical shift values are for amino acid α-proton. Chloroform-*d* (C); DMSO-*d* (D). [c] TLC in (CH_2Cl_2–MeOH–AcOH) system, typically 97.5:2.5:0.25. [d] See the Experimental Section for this example of method C-2. [e] N: calcd, 3.51; found, 3.10. [f] C: calcd, 61.14; found, 61.86. [g] H: calcd, 6.46; found, 5.69. [h] See the Experimental Section for this example of method E-4. [i] C: calcd, 63.28; found, 62.82. [j] C: calcd, 63.28; found, 62.55. [k] H: calcd, 6.40; found, 5.84. [l] See the Experimental Section for an example of method C-2a. [m] C: calcd, 52.15; found, 51.65. H: calcd, 6.26; found, 5.54. [n] C: calcd, 55.02; found, 54.20. [o] C: calcd, 56.01; found, 56.45. [p] H: calcd, 7.24; found, 5.94. [q] H: calcd, 6.53; found, 5.87. [r] See the Experimental Section for this example of method E-3. [s] N: calcd, 4.25; found, 4.71. [t] See the Experimental Section for this example of method E-2. [u] C: calcd, 56.27; found, 55.78. [v] C: calcd, 66.20; found, 65.64. [w] C: calcd, 56.66; found, 55.38. [x] Flash silica gel, CH_2Cl_2–CH_3OH–glacial AcOH (97.5:2.5:0.25).

under the reaction conditions. Changes in reaction conditions did not eliminate the racemization problem, and alternative acid hydrolysis was not a clean process. In general, the diastereomeric contaminant in the product acid **28** was considered tolerable for biological assay. Methionine derivatives were not susecptible to this racemization.

Two approaches were utilized in the preparation of individual diastereomers of the mercaptoacyl amino acids (e.g., **28n** and **29n**). One method (Scheme 4a, method D) employed the resolved *S*-benzoylthio acid **31** reported by Bindra.[25] For methionine, the methyl ester **20n** was acylated with either enantiomer of acid **31**, each furnishing a single amide diastereomer (**25n** and **26n**) of known configuration at the mercaptopropionyl chiral center. These were hydrolyzed respectively to the acids **28n** and **29n**. For *S*-alkylcysteine derivatives, because of the susceptibility to racemization during ester saponification, alternative approaches were employed. In one method (Scheme 4b, method E-1), the

Scheme 4



(acetylthio)propionic acid **7f** was converted into the corresponding chloride **35**, and this was used to acylate the *S*-alkylcysteine acid **19** rather than an ester. Following separation of diastereomers **38a** and **39a**, removal of the *S*-acetyl provided enantiomerically pure acids **28c** and **29c** (Table 3). In a second method (Scheme 4c, method E-2), the amino acid *tert*-butyl ester **20p** was converted to amides **36** and **37**, which were de-esterified to **38a** and **39a** and then deacetylated to **28c** and **29c**, respectively. This approach was limited, since in some cases the diastereomeric *tert*-butyl esters could not be separated by chromatography.

S-Benzoylthio and *S*-acetylthio amides were prepared via carbodiimide coupling with the carboxylic acid (**31** or **7f**) or by use of the acid chloride (**32** or **35**) (Schemes 5 and 6) (Table 4). Hydrolysis of the *S*-protected amides gave the mercapto amides (Table 7).

Assignment of chirality at the mercaptopropionyl center was required in each case where diastereomers were separated. A rigorous method employed correlation with materials derived from the benzoylthio acid enantiomers **31-*R*** or **31-*S***, as shown in Scheme 5. Thus, L-methioninamide **23g** was converted by **31-*R*** or **31-*S*** to mercaptans **47a** and **46a**, respectively. Mercaptan **46a** was identical to the mercaptan obtained from the less polar diastereomer (**48a**) of the *S*-acetyl compounds. This correlation was applied similarly with L-methionine, as described in Scheme 7. A mixture of *S*-acetyl diastereomeric acids (**38b** and **39b**) was prepared from **35** and L-methionine, and the more polar diastereomer **39b** was hydrolyzed to **29n**. Mercapto acid **29n** was identical with the material prepared from *S*-benzoyl acid **31-*R***. This rigorous correlation method was laborious and not applicable to mercaptoacids other than 2-(mercaptomethyl)-3-phenylpropionic acid.

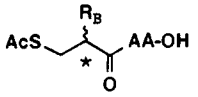
A more general, although less rigorous, method for assigning chirality employed the assignments determined by the rigorous method above and established correlations between stereochemistry and (a) TLC relative polarity, (b) optical rotation, and (c) NMR chemical shift values. These correlations, illustrated in Figure 2, proved quite reliable. The first correlation observed was between the relative TLC polarity of (acetylthio)-acyl L-amino acid ethyl esters (**53/54**) and specific rotations. The (*S*)-diastereomers **53** were less polar and possessed strongly negative rotations, while the (*R*)-diastereomers **54** were more polar and possessed small negative rotations. In general, the difference in specific rotation ranged between 50° and 70°. Hydrolysis to the corresponding mercapto acids gave reversal of these relative rotations, as seen in Table 2. In all pairs of diastereomers separated, the TLC mobilities and relative rotations followed this pattern.

For derivatives of L-methionine, stereochemistry was correlated with NMR chemical shift values. The products with (*S*)-configuration (on the basis of polarity and rotation) at the mercaptopropionyl center showed the SCH_3 singlet at δ 2.10, while those with (*R*)-configuration showed this singlet at δ 2.00. Ultimately, a further verification of stereochemical assignments was secured via the X-ray structure of Sch 42495[22] (**25y**), which agreed with the initial assignment based on the above arguments.

Pharmacology

The *in vitro* assay for NEP inhibition is well-established,[21] as is the assay for ACE inhibition.[26] Initially, potentiation of the hypotensive response to exogenous ANF was employed as the key *in vivo* assay. In this assay, spontaneously hypertensive rats (SHR) are challenged with intravenous ANF, and the acute

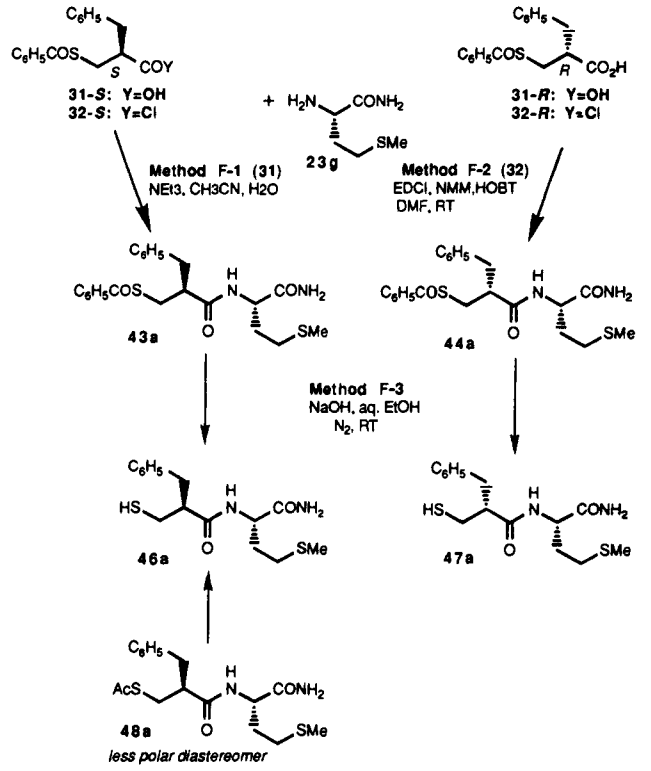
Table 3. Physicochemical Data for *N*-[2-[(Acetylthio)methyl]-3-substituted-1-oxopropyl]amino Acids



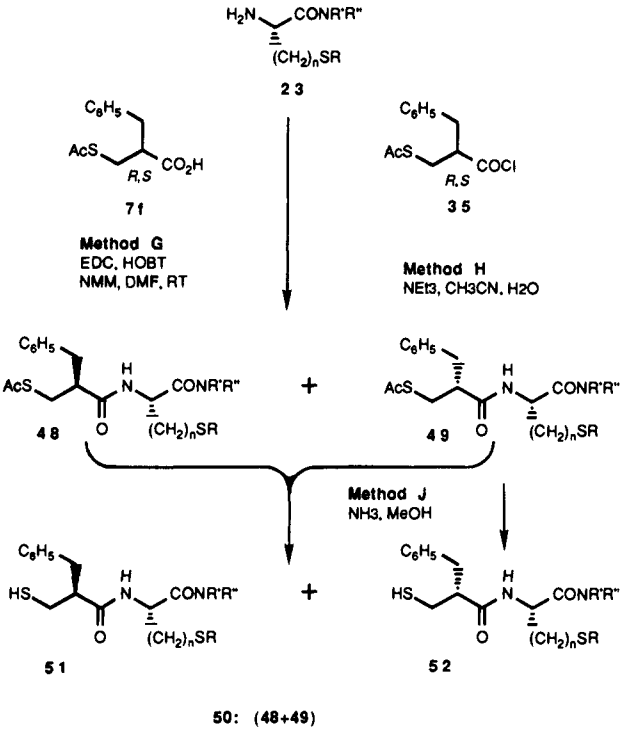
compd	stereo (*)	AA-OH	method[a]	mp, °C[b]	yield (%)	optical rotation $[\alpha]_D$, deg (*c*, solvent)	NMR, CH δ (solvent)[c]	TLC mobility	formula	anal.
					RB = Phenylmethyl					
38a	*S*	*S*-(4-Me-benzyl)-L-Cys-OH	E-1[d,e]	oil	6	−23.0 (1.0, M)	4.65 (C)	faster	$C_{23}H_{27}NO_4S_2 \cdot 0.2CH_2Cl_2$	C, H, N[f]
			E-2[g]		56					
39a	*R*	*S*-(4-Me-benzyl)-L-Cys-OH	E-1[h]	oil	10	−1.3 (1.0, M)	4.58 (C)	slower	$C_{23}H_{27}NO_4S_2 \cdot 0.25$ AcOH	C, H, N
			E-2		59					
38b	*S*	L-Met-OH	E-1	95–98	21	−36.6 (0.5, E)	4.58 (C)	–	$C_{17}H_{23}NO_4S_2$	C, H, N
					RB = [1,1'-Biphenyl]-4-ylmethyl					
38c	*S*	*S*-(4-Me-benzyl)-L-Cys-OH	E-1[i]	123–125	17	−45.5 (0.6, M)	4.59 (C)	faster	$C_{29}H_{31}NO_4S_2$	C, H, N
39c	*R*	*S*-(4-Me-benzyl)-L-Cys-OH	E-1[i]	131–135	17	−7.1 (0.3, M)	4.61 (C)	slower	$C_{29}H_{31}NO_4S_2$	C, H, N
					RB = *n*-Propyl					
40d	*R, S*	*S*-ethyl-L-Cys-OH	E-2	oil	76	−26.9 (0.5, M)	4.79 (C)		$C_{13}H_{23}NO_4S_2 \cdot 0.15\ CF_3CO_2H$	C, H, N

[a] Chromatographic column separations employ Baker silica gel, 60–200 mesh or flash silica gel, 32–64 mesh, with the indicated eluants. [b] White solid unless otherwise stated. [c] Chemical shift values are for amino acid α-proton. Chloroform-*d* (C); DMSO-*d* (D). [d] See the Experimental Section for this example of method E-1. [e] Silica gel, CH_2Cl_2–CH_3OH–glacial AcOH (100:1:0.1), faster moving component. [f] H: calcd, 5.97; found, 5.55. [g] See the Experimental Section for this example of method E-2. [h] CH_2Cl_2–CH_3OH–glacial AcOH (100:1:0.1). [i] CH_2Cl_2–CH_3OH–glacial AcOH (97.5:2.5:0.25).

Scheme 5



Scheme 6

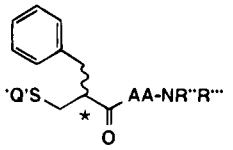


reduction in blood pressure is measured. The rats are treated with the test compound, the challenge is repeated, and the increase in hypotensive response is determined. This assay is directly linked to the desired mechanism of action. While this assay was quite useful, an alternative assay employing DOCA-salt rats proved even more relevant. This is a volume-dependent model of hypertension[27] (in contrast to the SHR, which is a multifaceted genetic model) with elevated levels of plasma ANF,[28] and we have found NEP inhibitors highly effective in reducing blood pressure in this model. This assay can be highly variable, but regular use under the conditions described in the Experimental Section provided reliable results without requiring large groups of animals. The DOCA-rat assay was employed as the primary indicator of compounds with good *in vivo* activity. As further evidence of the mechanism of action, *in vivo* inhibition of ANF degradation has been demonstrated[34] for compounds active in the DOCA-rat assay.

Results and Discussion

The *S*-benzyl-L-cysteine derivative **28a** ((*S,R*)-stereochemistry, as shown), the first member of the series,[29] showed potent inhibitory activity against NEP (IC_{50} = 9 nM) and ACE (IC_{50} = 24 nM) (Table 5). Good *in vivo* activity was observed in both the ANF potentiation and DOCA-rat assays at 30 mg/kg sc. The (*R,R*)-diastereomer **29a** (also derived from L-cysteine) showed a similar profile, but with greatly diminished ACE inhibitory potency (IC_{50} >1000 nM). The known preference[30] of ACE for (*S*)-chirality at the mercaptoacyl center is a further correlation for our stereochemical assignments. The stereochemical tolerance at the 2-position of the

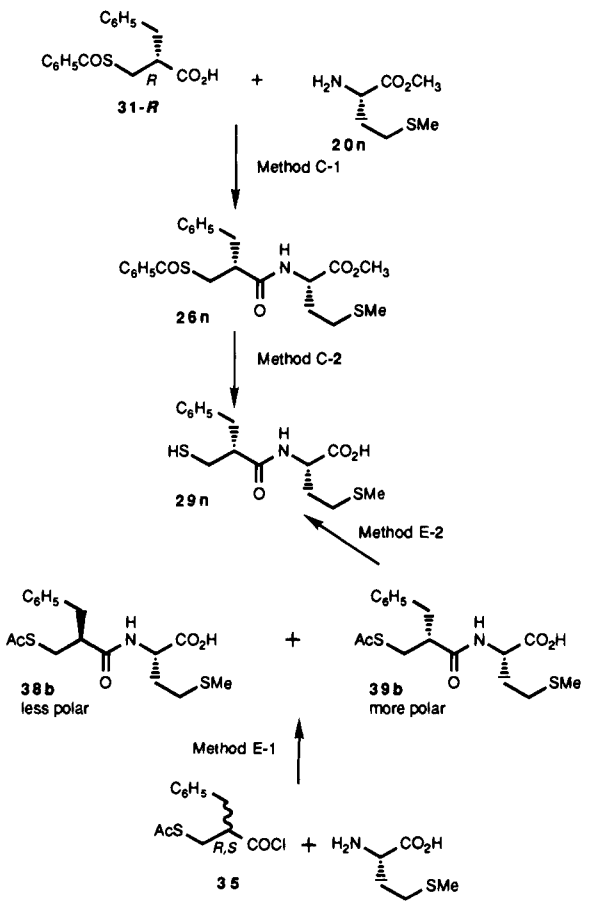
Table 4. Physicochemical Data for *N*-[2-[(Acetylthio)methyl]-; 2-[(Benzylthio)methyl]-; 2-(Mercaptomethyl)-3-phenyl-1-oxopropyl]amino Acid Amides

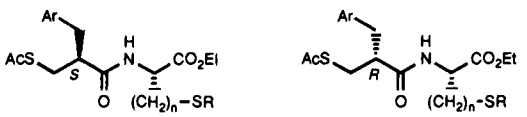


compd	Q	stereo (*)	AA-NR″R‴	method[a]	mp, °C[b]	yield (%)	optical rotation $[\alpha]_D$, deg (*c*, solvent)	NMR, CH δ (solvent)[c]	TLC mobility	formula	anal.
46a	H	*S*	L-Met-NH_2	F-3[d]	133–137	79	−8.4 (0.5, E)	4.60 (C)		$C_{15}H_{22}N_2O_2S_2 \cdot 0.25H_2O$	C, H, N
47a	H	*R*	L-Met-NH_2	F-3	135–136	83	−66.3 (1.0, C)	4.19 (D)		$C_{22}H_{26}N_2O_3S_2$	C, H, N
48a	Ac	*S*	L-Met-NH_2	H[e]	149–151	17	−87.5 (0.5, C)	4.53 (C)		$C_{17}H_{24}N_2O_3S_2$	C, H, N
49c	Ac	*R*	L-Met-NH_2	H	119–121	15	+5.0 (0.5, C)	4.48 (C)		$C_{17}H_{24}N_2O_3S_2$	C, H, N
50c	Ac	*R, S*	L-Met-N$(CH_2)_4$	H[f]	63–5	30	−49.4 (0.5, E)			$C_{21}H_{30}N_2O_3S_2$	C, H, N
50d	Ac	*R, S*	L-Met-N$(CH_2)_5$	H[g]	red oil	59				$C_{22}H_{32}N_2O_3S_2 \cdot H_2O$	C, H, N
43a	Bz	*S*	L-Met-NH_2	F-1[h]	176–180	36	−97.6 (0.5, C)			$C_{22}H_{26}N_2O_3S_2$	C, H, N
44a	Bz	*R*	L-Met-NH_2	F-2[i]	145–149	86	+32.9 (0.5, C)	4.21 (D)		$C_{22}H_{26}N_2O_3S_2$	C, H, N
50e	Ac	*R, S*	L-Met-$NHCH_2CH_2OH$	H[j]	115–118	61	−31.1 (0.5, E)	4.42; 4.48 (C)		$C_{19}H_{28}N_2O_4S_2$	C, H, N
50f	Ac	*R, S*	L-Met-N$(CH_2)_2O(CH_2)_2$	H[k]	oil	75	−30.0 (0.5, E)			$C_{21}H_{30}N_2O_4S_2 \cdot 0.25H_2O$	C, H, N
50g	Ac	*R, S*	L-Met-$NHCH_2CO_2Et$	H[l]	100–103	61	−35.6 (0.5, E)	4.50; 4.58 (C)		$C_{21}H_{30}N_2O_5S_2 \cdot 0.25H_2O$	C, H, N
48h	Ac	*S*	*S*-(4-Me-benzyl)-L-Cys-NH_2	G[o]	foam	7.6	−38.2 (0.6, M)	4.44 (D)	faster	$C_{23}H_{28}N_2O_3S_2$	C, H, N
49h	Ac	*R*	*S*-(4-Me-benzyl)-L-Cys-NH_2	G[m,n]	foam	25	−1.6 (0.5, M)	4.45 (D)	slower	$C_{23}H_{28}N_2O_3S_2$	C, H, N
51h	H	*S*	*S*-(4-Me-benzyl)-L-Cys-NH_2	J[p,q]	130–132	75	−4.2 (0.14, M)	4.53 (D)	faster	$C_{21}H_{26}N_2O_2S_2$	C, H, N
52h	H	*R*	*S*-(4-Me-benzyl)-L-Cys-NH_2	J[q]	foam	65	−29.0 (0.33, M)	4.44 (D)	slower	$C_{21}H_{26}N_2O_2S_2 \cdot 0.5H_2O$	C, H, N

[a] Chromatographic column separations employ Baker silica gel, 60–200 mesh or flash silica gel, 32–64 mesh, with the indicated eluants. [b] White solid unless otherwise stated. [c] Chemical shift values are for amino acid α-proton. Chloroform-*d* (C); DMSO-*d* (D). [d] See the Experimental Section for this example of method F-3. [e] See the Experimental Section for this example of method H. [f] Silica gel column, Et_2O. [g] Silica gel column, MeOH–CH_2Cl_2, 1:49. [h] See the Experimental Section for this example of method F-1. [i] See the Experimental Section for this example of method F-2. [j] MeOH–CH_2Cl_2, 1:19. [k] MeOH–CH_2Cl_2, 3:97. [l] Et_2O–hexane, 1:1. [m] See the Experimental Section for this example of method G. [n] Silica gel (Prep 500, 2 cartridges), CH_2Cl_2–EtOAc (4:1) faster moving component. [o] (Prep 500, 2 cartridges), CH_2Cl_2–EtOAc (4:1). [p] See the Experimental Section for this example of method J. [q] CH_2Cl_2–CH_3OH–glacial AcOH (97.5:2.5:0.25) and (97.5:2.5:0.5).

Scheme 7

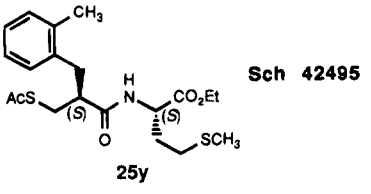


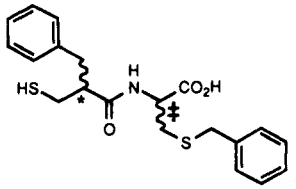


	53	54
TLC mobility:	less polar	more polar
Rotation:	more negative	less negative
NMR, methionine-SMe	δ2.10	δ2.00

Figure 2. Correlation of stereochemistry with TLC mobility, relative optical rotation, and chemical shift.

mercaptopropionyl chain for NEP inhibition is a general feature of the series reported here (with several apparent exceptions). Such a relationship has been reported by others both for inhibitors of NEP[16b,31] and inhibitors of thermolysin.[32] For *S*-benzylcysteine, the L-amino acid confers superior activity relative to the D-amino acid, as seen by the activity of **28b** and **29b**.[33] In general, we have observed greatly diminished NEP and ACE inhibitory activity for mercaptoacyl derivatives of the D-amino acids.[34]

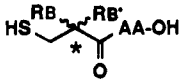




	•	‡
28a	*S*	*R*
29a	*R*	*R*
28b	*S*	*S*
29b	*R*	*S*

In the *S*-alkylcysteine series (Table 5), the influence of the substituent on the sulfur atom was examined. For

Table 5. *In Vitro* and *in Vivo* Biological Activity of *N*-[3-Mercapto-2-substituted-1-oxopropyl]amino Acids



compd	stereo (*)	AA	IC_{50}, nM NEP	IC_{50}, nM ACE	ΔBP, mmHg[a] DOCA	ΔBP, mmHg[a] ANF
		RB = Phenylmethyl, RB' = H				
28a	*S*	*S*-benzyl-L-Cys	9	24	17 (10 sc) 30 (30 sc)	35 (30 sc)
29a	*R*	*S*-benzyl-L-Cys	32	>1000	21 (10 sc) 55 (30 sc)	45 (90 sc)
28b	*S*	*S*-benzyl-D-Cys	19	115		11 (30 sc)
29b	*R*	*S*-benzyl-D-Cys	14	1,250	18 (30 po)	19 (30 po)
28c	*S*	*S*-(4-Me-benzyl)-L-Cys	11	19	17 (30 sc)	48 (30 sc)
29c	*R*	*S*-(4-Me-benzyl)-L-Cys	20	1,200	33 (30 sc) 28 (30 po)	48 (30 sc)
28d	*S*	*S*-(4-MeO-benzyl)-L-Cys	2	95	28 (30 po)	16 (30 sc)
29d	*R*	*S*-(4-MeO-benzyl)-L-Cys	17	290	0 (30 po)	23 (30 sc)
28e	*S*	*S*-(3,4-Me_2-benzyl)-L-Cys	370	460		23 (30 sc)
29e	*R*	*S*-(3,4-Me_2-benzyl)-L-Cys	541	>1000		0 (30 sc)
28f	*S*	*S*-(2-phenylethyl)-L-Cys	110	80	0 (30 po)	37 (30 sc)
29f	*R*	*S*-(2-phenylethyl)-L-Cys	133	>1000	38 (30 po)	23 (30 sc)
30g	*R, S*	*S*-trityl-L-Cys	274	≫1000		0 (30 sc)
30h	*S*	*S*-Me-L-Cys	1.5	77	0 (30 po)	30 (30 sc)
28j	*S*	*S*-ethyl-L-Cys	2.4	28	21 (0.1 sc) 22 (1 sc) 61 (10 po)	19 (30 sc)
30k	*R, S*	*S*-ethyl-L-Cys	6.5	45		25 (30 sc)
28l	*S*	*S*-t-Bu-L-Cys	49	170	17 (30 po)	25 (30 sc)
29l	*R*	*S*-t-Bu-L-Cys	231	640		0 (30 sc)
30m	*R, S*	L-Met	4	120	42 (30 sc) 18 (30 po)	25 (30 sc)
28n	*S*	L-Met	6.5	35	31 (10 po)	23 (30 sc)
29n	*R*	L-Met	3.5	>1000	30 (10 po)	50 (30 sc)
28o	*S*	L-ethionine	5	215		20 (30 sc)
29o	*R*	L-ethionine	31	>1000	0 (30 po)	30 (30 sc)
		RB = Methyl, RB' = H				
28p	*S*	*S*-benzyl-L-Cys	46	58	0 (30 sc)	19 (30 sc)
		RB = *n*-Propyl, RB' = H				
30q	*R, S*	*S*-ethyl-L-Cys	>300	1000		
30r	*R, S*	L-Met	2.5	>1000		
		RB = RB' = $(CH_2)_4$				
30s	–	L-Met	3.5	700	24 (30 sc)	
		RB = Phenethyl, RB' = H				
28t	*S*	L-Met	60	150	18 (10 po)	
29t	*R*	L-Met	>300	>1000	6 (3 po)	
		RB = 2-Naphthylmethyl, RB' = H				
28u	*S*	*S*-(4-Me-benzyl)-L-Cys	>300	460		0 (30 sc)
29u	*R*	*S*-(4-Me-benzyl)-L-Cys		3100		0 (30 sc)
		RB = 1-Naphthylmethyl, RB' = H				
28v	*S*	*S*-(4-Me-benzyl)-L-Cys	2	>1000	47 (1 sc) 26 (3 sc) 8 (10 po) 33 (30 po)	0 (30 sc)
29v	*R*	*S*-(4-Me-benzyl)-L-Cys	39	90		0 (30 sc)
		RB = (4-Chlorophenyl)methyl, RB' = H				
28w	*S*	*S*-benzyl-L-Cys	3	44	20 (30 po)	19 (30 sc)
29w	*R*	*S*-benzyl-L-Cys	147	>1000		0 (30 sc)
		RB = [1,1']-Biphenyl]-4-ylmethyl, RB' = H				
28x	*S*	*S*-(4-Me-benzyl)-L-Cys	180	180	19 (30 po)	0 (30 sc)
29x	*R*	*S*-(4-Me-benzyl)-L-Cys	290	>1000		0 (30 sc)
		RB = (2-Methylphenyl)methyl, RB' = H				
28y	*S*	L-Met	7	100	24 (1 po) 43 (3 sc)	15 (30 po)
25y	*S*	L-Met-OEt	>300	250	27 (1 po) 48 (3 po) 70 (10 po)	36 (30 sc)

[a] The numbers indicating the change in blood pressure in DOCA rats or ANF potentiation represent average values determined in four or more animals. [b] *S*-Acetyl.

the compounds with arylalkyl substituents, the 4-methylbenzyl (**28c, 29c**) and 4-methoxybenzyl (**28d, 29d**) (derivatives showed good activity, while the 3,4-dimethylbenzyl (**28e, 29e**), 2-phenylethyl (**28f, 29f**), and triphenylmethyl (**30g**) derivatives showed reduced potency. For simple alkyl substituents,[35] good activity was

observed for the methyl (**30h**) and ethyl (**28j**) derivatives and somewhat reduced activity for the *tert*-butyl derivatives (**28l, 29l**.). These results serve to establish steric limits for binding in this region of the enzyme. In the methionine series, both methionine (**28n, 29n**) and ethionine (**28o, 29o**) derivatives showed good activity, with particularly good *in vivo* activity for methionine. It is known that NEP shows a moderately broad tolerance for the side-chain on the amino acid moiety of the inhibitor.[10] The *in vivo* activity of this set of compounds parallels most closely the *in vitro* NEP inhibitory activity.

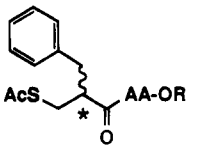
The substituent at the 2-position of the mercaptopropionyl moiety is an important determinant for *in vitro* activity (Table 5). A broad range of substituents was examined. Activity against both NEP and ACE was substantial for the captopril-like derivative with a methyl group (**28p**). With an *n*-propyl substituent (**30r**), NEP inhibitory activity was very good, while ACE inhibitory activity was weak. A similar profile was observed with the α,α-cyclic derivative **30s**. A 2-phenylethyl substituent (**28t, 29t**) led to reduced activity against both enzymes. A 2-naphthylmethyl substituent (**28u, 29u**) produced less activity than the isomeric 1-naphthylmethyl (**28v, 29v**). Substituted benzyl groups were also examined. A 4-chloro substituent (**28w, 29w**) was well tolerated, as was a 2-methyl substituent (**28y**), but a 4-phenyl substituent (**28x, 29x**) less so. We interpret these *in vitro* results as demonstrating a broad structural tolerance in this region of the molecule. This is consistent with the lack of strong *S*/*R* configurational preference at the chiral center and the tolerance for disubstitution. The substituents providing the poorest NEP inhibitory activity all show significant linear extension from the chiral center and indicate a distant spatial intolerance in this region.

Of the compounds in Table 5, six different substituents at the 2-position of the mercaptopropionyl moiety provided derivatives with IC_{50} below 10 nM for inhibition of NEP. *In vivo* activity varies within this group. The greatest activity, especially upon oral administration, was seen with the 2-methylbenzyl derivative (**28y**). Oral bioavailability thus appears to be structure-dependent. The primary objective of our studies was to identify an NEP inhibitor with sufficient *in vivo* activity to permit evaluation of the therapeutic potential of this mechanism. Compound **28y** showed sufficient activity for evaluation in a battery of mechanism-related assays, and detailed SAR was compiled on a set of close analogs of this structure. These studies are described in the succeeding report.[34]

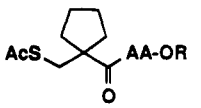
The C-terminal and *S*-terminal groups of these compounds are capable of influencing both *in vitro* and *in vivo* activity (Table 6). Since a free mercapto group is required for inhibition of NEP, an *S*-terminal group can influence the release of the active species. *S*-Acetyl mercaptoacyl amino acids based on methionine (**38b**) and *S*-alkylcysteine (**38a, 39a**) showed good *in vivo* activity, as did two *S*-acylmethionine esters (**26n, 27s**). Thus, both thioester and ester functions are effectively bioactivated.[36] These doubly protected prodrugs serve as excellent precursors to the free mercaptoacids, and many of these are very effective upon oral administration.

Since NEP is an endopeptidase, in contrast to the carboxydipeptidase ACE, substrates (or inhibitors) of the enzyme do not require a C-terminal carboxylic acid.[10] Thus, the mercaptoacyl methioninamides (**46a, 47a**) are moderately active *in vitro* vs NEP, but inactive vs ACE (Table 7). As a group, the *S*-acetylated and *S*-benzoylated amides (particularly **48a, 48h, 49a, 50c, 50d, 50f**) showed generally good *in vivo* activity, although not superior to that of the mercapto acids. The free mercapto acids and *S*-acetyl acid alkyl esters were the focus of subsequent studies, yielding many compounds with excellent *in vivo* activity. Ultimately, Sch 42495 (**25y**) was chosen for clinical evaluation.[34]

Table 6. *In Vivo* Biological Activity of *N*-[3-(Acetylthio)-2-(phenylmethyl)-1-oxopropyl]amino Acids/Esters



Cpd	*	AA-OR	DOCA Δ BP, mm Hg[a]	ANF Δ BP, mm Hg[a]
38b	*S*	L-Met-OH	48 (30 sc)	
39a	*R*	*S*-4-Me-Bn-L-Cys-OH	48 (30 sc)	42 (10 sc)
38a	*S*	*S*-4-Me-Bn-L-Cys-OH		20 (10 po)
26n[b]	*R*	L-Met-OMe	14 (10 po)	45 (30 sc)



27s		L-Met-OEt	27 (10 po)	

[a] The numbers indicating the change in blood pressure in DOCA rats or ANF potentiation represent average values determined in four or more animals. [b] Benzoyl in place of acetyl.

Summary

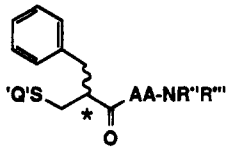
Starting with a hypothesis on the potential utility of inhibitors of neutral endopeptidase in the treatment of cardiovascular diseases, we examined a broad structural range of inhibitors. *In vivo* models relevant to this mechanism of action were employed. In the mercapto class, we developed SAR covering several structural parameters. The results presented here allowed us to focus on specific mercaptoacyl derivatives of methionine, leading ultimately to a potential clinical candidate.

Experimental Section

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. The ^1H NMR spectra were obtained on Varian instruments FT80 H (80 MHz), CFT-20 (80 MHz), and EM-390 (90 MHz) and are reported as ppm downfield from Me_4Si with multiplicity, number of protons, and coupling constants in Hertz indicated parenthetically. NMR solvents: C, chloroform-*d*; D, DMSO-*d*. The EI mass spectra were determined with a Finnigan MAT CH-5 spectrometer. The rotations were determined with a Rudolf Autopol at ambient temperature. Rotations: $[\alpha]^{26}_D$ (solvent, concentration). Solvents: C, chloroform; E, ethanol; H, water; M, methanol. Microanalyses and the above determinations were performed by the Physical-Analytical Chemistry Department, Schering-Plough Research Institute.

Chemistry. Starting materials were purchased or prepared by literature methods: *S*-(Phenylmethyl)-L-cysteine hydro-

Table 7. *In Vitro* and *in Vivo* Biological Activity of *N*-[3-(Acetylthio)-2-(phenylmethyl)-1-oxopropyl]amino Acid Amides



compd	Q	stereo (*)	AA-NR"R"'	IC_{50}, nM		ΔBP, mmHg[a]	
				NEP	ACE	DOCA	ANF
46a	H	*S*	L-Met-NH_2	76	>1000		26 (30 sc)
47a	H	*R*	L-Met-NH_2	94	>1000		
48a	Ac	*S*	L-Met-NH_2			39 (3 po)	23 (30 sc)
49a	Ac	*R*	L-Met-NH_2				27 (30 sc)
50c	Ac	*R, S*	L-Met-$N(CH_2)_4$			24 (10 po)	29 (30 sc)
50d	Ac	*R, S*	L-Met-$N(CH_2)_5$			3 (3 sc) 32 (10 sc)	38 (30 sc)
43a	Bz	*S*	L-Met-NH_2			0 (30 sc)	
44a	Bz	*R*	L-Met-NH_2			0 (30 sc)	19 (3 po) 52 (10 po)
50f	Ac	*R, S*	L-Met-$N(CH_2)_2O(CH_2)_2$	>300		20 (10 po)	24 (30 sc)
50e	Ac	*R, S*	L-Met-$NHCH_2CH_2OH$			24 (10 po)	25 (30 sc)
50g	Ac	*R, S*	L-Met-$NHCH_2CO_2Et$	>300		0 (10 po)	
51h	H	*S*	*S*-(4-Me-benzyl)-L-Cys-NH_2	>300			0 (30 sc)
52h	H	*R*	*S*-(4-Me-benzyl)-L-Cys-NH_2	>300		17 (3 sc) 46 (10 po)	17 (30 sc)
49h	Ac	*R*	*S*-(4-Me-benzyl)-L-Cys-NH_2			0 (10 po)	0 (10 sc)
48h	Ac	*S*	*S*-(4-Me-benzyl)-L-Cys-NH_2			29 (10 po)	12 (30 sc)

[a] The numbers indicating the change in blood pressure in DOCA rats or ANF potentiation represent average values determined in four or more animals.

chloride (Sigma), *S*-(phenylmethyl)-L-cysteine ethyl ester hydrochloride (Sigma), *N*-[(1,1-dimethylethoxycarbonyl]-*S*-(phenylmethyl)-D-cysteine (Bachem), *S*-ethyl-L-cysteine (Chemical Dynamics), *N*-[(1,1-dimethylethoxy)carbonyl]-*S*-[(4-methylphenyl)methyl]-L-cysteine (Bachem), *N*-[(1,1-dimethylethoxy)carbonyl]-*S*-[(3,4-dimethylphenyl)methyl]-L-cysteine (Bachem), *N*-[(1,1-dimethylethoxy)carbonyl]-*S*-[(4-methoxyphenyl)methyl]-L-cysteine (Bachem), *N*-[(1,1-dimethylethoxy)carbonyl]-*S*-(1,1-dimethylethyl)-L-cysteine (Bachem), *S*-(triphenylmethyl)-L-cysteine (Aldrich), L-methionine methyl ester hydrochloride (Sigma), *S*-methyl-L-cysteine (Chemical Dynamics), L-ethionine (Chemical Dynamics), D-(−)-*S*-acetyl-β-mercaptoisobutyric acid (Chemical Dynamics). 2(*R,S*)-[(Benzoylthio)methyl]-3-phenylpropionic acid and the individual enantiomers were prepared by a literature procedure.[25]

Abbreviations: EDC, 3-ethyl-1-[3-(dimethylamino)propyl]-carbodiimide hydrochloride; HOBT, 1-hydroxybenzotriazole hydrate; BOC, (1,1-dimethylethoxy)carbonyl.

Method A-1−1. Preparation of (Arylmethylene)propanedioic Acids. [(4-Chlorophenyl)methylene]propanedioic Acid (4a). A powdered mixture of 4-chlorobenzaldehyde (70.25 g, 500 mmol) and malonic acid (52.1 g, 500 mmol) was heated on a steam bath for 6 h and then cooled to room temperature. The mixture was partitioned between Et_2O (400 mL) and 1 N NaOH (1 L). The aqueous layer was washed with Et_2O (300 mL), cooled, and acidified to pH 1 with concentrated HCl to give a white solid which was filtered, washed with water, and dried to give **4a** as a white solid (79.1 g, 69%): mp 207−209 °C (lit.[25] mp 215 °C dec).

(2-Naphthylmethylene)propanedioic Acid (4b). 2-Naphthaldehyde (15.6 g, 100 mmol) and malonic acid (10.4 g, 100 mmol) in glacial AcOH (6 mL) were heated under reflux at 100 °C for 4 h. The reaction mixture was cooled and diluted with CH_2Cl_2 to give a white precipitate. Filtration gave a white solid A (14.3 g). The filtrate was concentrated *in vacuo*, malonic acid (1.5 g) added, and the mixture treated as above to give solid B (4.82 g). Solids A and B were combined and suspended in water (200 mL), filtered, and washed with water to give **4b** as a white solid (16.6 g, 68%): mp 204−205 °C. Anal. ($C_{14}H_{10}O_4$) C, H.

[[1,1'-Biphenyl]-4-ylmethylene]propanedioic Acid (4c). In a manner similar to that described for **4b**, 4-biphenylcarboxaldehyde (18.0 g, 99 mmol) and malonic acid (10.4 g, 100 mmol) were converted to **4c** as a pale yellow solid (9.56 g, 31%): mp 208−209 °C. Anal. ($C_{16}H_{12}O_4$) C, H.

[(2-Methylphenyl)methylene]propanedioic Acid (4d). o-Tolualdehyde (100 g, 833 mmol) and malonic acid (86 g, 833 mmol) were heated under reflux at 100 °C for 7 h, and the resulting mixture was treated as described in method A-1−1 to give **4d** as a white solid (107.7 g, 62%): mp 198−204 °C dec. Anal. ($C_{11}H_{10}O_4$) C, H.

Method A-1−2. Preparation of (Arylmethyl)propanedioic Acid. [(4-Chlorophenyl)methyl]propanedioic Acid (5a).[25] Diacid **4a** (79 g, 348 mmol) was hydrogenated at 50 psi in EtOAc (1 L) with 10% Pd/C (3.4 g). After filtration and washing with EtOAc (300 mL), concentration *in vacuo* gave **5a** as a white solid (77.9 g, 97.7%): mp 162−164 °C.

(2-Naphthylmethyl)propanedioic Acid (5b). In a manner similar to that described for **5a**, diacid **4b** (16.5 g, 68 mmol) was converted to **5b** as a white solid (16.0 g, 99%): mp 150−152 °C. Anal. ($C_{14}H_{12}O_4$) C, H.

[[1,1'-Biphenyl]-4-ylmethyl]propanedioic Acid (5c). In a manner similar to that described for **5a**, diacid **4c** (1.44 g, 8.6 mmol) was converted to **5c** as a white solid (1.40 g, 96%): mp 180−181 °C. Anal. ($C_{16}H_{14}O_4$) C, H.

[(2-Methylphenyl)methyl]propanedioic Acid (5d). Diacid **4d** (107 g, 519 mmol) in absolute EtOH (300 mL) and EtOAc (300 mL) was hydrogenated with 10% Pd/C (4.0 g) as described in method A-1−2 to give **5d** as a white solid (97.5 g, 90%): mp 137−140 °C. Anal. ($C_{11}H_{12}O_4$) C: calcd, 63.46; found, 63.97; H: calcd, 5.81; found, 6.50.

Method A-1−3. Preparation of α-Methylenearenepropanoic Acids. 4-Chloro-α-methylenebenzenepropanoic Acid (6a). Diacid **5a** (39 g, 171 mmol) in distilled water (50 mL) was cooled to 0−5 °C in a salt−ice bath. To this was added 40% aqueous dimethylamine (43.2 mL) to pH 7.5. Additional **5a** (38.8 g, 170 mmol) was added, followed by sufficient distilled water to give a clear solution. A 37% aqueous formaldehde solution (40 mL) was added, and the mixture was allowed to reach room temperature and then stirred for 20 h. The solid was filtered off and washed with water, suspended in water (250 mL), and heated on a steam bath 2 h. The solution was cooled and acidified with concentrated HCl. The solid was filtered, washed with water, and dried to give **6a** as a white solid (36.1 g, 54%): mp 93-95 °C (lit.[25] mp 95−96 °C).

α-Methylene-2-naphthalenepropanoic Acid (6b). In a manner similar to that described for **6a**, diacid **5b** (16.0 g, 66 mmol) was converted to **6b** as a white solid (9.73 g, 70%): mp 83−84 °C. Anal. ($C_{14}H_{12}O_4 \cdot 0.12H_2O$) C, H.

α-Methylene-[1,1'-biphenyl]-4-propanoic Acid (6c). In a manner similar to that described for **6a**, diacid **5c** (9.10 g, 53.5 mmol) was converted to **6c** as a white solid (6.68 g, 83%): mp 168–170 °C. Anal. ($C_{16}H_{14}O_4$·0.1H_2O) C, H.

2-Methyl-α-methylenebenzenepropanoic Acid (6d). Diacid **5d** (50 g, 240 mmol) in water (100 mL) was cooled in an ice bath and treated with 40% dimethylamine solution (52.2 mL) (mechanical stirring used). After 10 min, additional **5d** (47 g, 226 mmol) and water (200 mL) were added. After 30 min, 37% formaldehyde solution (100 mL) was added. The resulting mixture was warmed to room temperature, stirred for 18 h, and filtered. The white solid (98.5 g) was suspended in water (500 mL) and heated under reflux for 2.5 h. The reaction mixture was cooled, extracted with diethyl ether, acidified with concentrated HCl to pH 2, and filtered to give **6d** as a white solid (36.2 g, 44%): mp 82–85 °C. Anal. ($C_{11}H_{12}O_2$) C, H.

2-Methylenepentanoic Acid (6e). Diethyl *n*-propylmalonate (40 g, 198 mmol) in EtOH (100 mL) was added dropwise to KOH (26 g, 464 mmol) in EtOH (200 mL) and water (50 mL), and the mixture was heated under reflux 6 h. The reaction mixture was cooled, concentrated *in vacuo*, diluted with water (500 mL), acidified to pH 2 with concentrated hydrochloric acid, and extracted with Et_2O. The dried ($MgSO_4$) Et_2O was concentrated *in vacuo* to give *n*-propylmalonic acid, a white solid (26.7 g, 92%), mp 92–95 °C. Anal. ($C_6H_{10}O_4$) C, H. In a manner similar to that described for **6a**, *n*-propylmalonic acid (26.0 g, 178 mmol) was converted to **6e**, a clear oil (5.33 g, 26%) which was used in the next step.

Method A-1–4. Preparation of α-[(*R,S*)-acetylthio)methyl]benzenepropanoic Acids. 4-Chloro-α-[(*R,S*)-acetylthio)methyl]benzenepropanoic Acid (7a). Acid **6a** (8.0 g, 40.7 mmol) in CH_2Cl_2 (100 mL) was treated with thiolacetic acid (20.9 g, 276 mmol) in CH_2Cl_2 (40 mL) for 48 h and concentrated *in vacuo* to give **7a**, a yellow oil (10.4 g, 94%) which was suitable for further use.

α-[(*R,S*)-Acetylthio)methyl]2-naphthalenepropanoic Acid (7b). In a manner similar to that described for **7a**, acid **6b** (9.37g, 44 mmol) was converted to **7b**, a light tan solid (5.27 g, 41%; contains 13% starting material by NMR); mp 103–106 °C.

α-[(*R,S*)-Acetylthio)methyl][1,1'-biphenyl]-4-propanoic Acid (7c). In a manner similar to that described for **7a**, acid **6c** (6.60g, 27.7 mmol) was converted to **7c** as a white solid (3.44 g, 40%): mp 101–103 °C. Anal. ($C_{18}H_{18}O_3S$) C, H.

2-Methyl-α-[(*R,S*)-acetylthio)methyl]benzenepropanoic Acid (7d).[37] Acid **6d** (36.1 g, 205 mmol) and thiolacetic acid (22.4 mL, 430 mmol) were stirred for 3 days. Toluene (200 mL) was added, and the mixture was concentrated *in vacuo* (3 times). The residue was triturated with hexane, filtered, and dried to give **7d** as a white waxy solid (44.2 g, 86%); mp 75–76 °C. Anal. ($C_{13}H_{16}O_3S$) C, H.

α-[(*R,S*)-Acetylthio)methyl]pentanoic Acid (7e). In a manner similar to that described for **7a**, **6e** (5.33 g, 47 mmol) was converted to **7e** as an oil (6.45 g, 73%). Anal. ($C_8H_{14}O_3S$) H; C: calcd, 50.50; found, 49.15.

α-[(*R,S*)-Acetylthio)methyl]1-naphthalenepropanoic Acid (7g). In a manner similar to that described for **7a**, acid **6g** (9.5 g, 46 mmol) was converted to **7g** as a white solid (11.95 g, 92%): mp 94–95 °C. Anal. ($C_{16}H_{16}O_3S$) H; C: calcd, 66.65; found, 66.09.

α-[(*R,S*)-Acetylthio)methyl]benzenepropionic Acid (7f). In a manner similar to that described for **6a**, acid **6f** (105 g, 649 mmol) was converted to **7f**, a white wax (used in next step) (153 g, 99%).

Method A-2–1. Preparation of Diethyl (1-Naphthylmethyl)propanedioate (8g). Sodium metal (11.0 g, 478 mmol) was added with cooling to absolute EtOH (650 mL). To the solution was added diethyl malonate (75.6 g, 472 mmol), followed by 1-(bromomethyl)naphthalene (100.0 g, 452 mmol). After 3 h, the mixture was concentrated *in vacuo*, diluted with water, and extracted with Et_2O. The dried ($MgSO_4$) Et_2O was concentrated *in vacuo* to give **8g** as an oil (133.7 g, 98%) which was used in the next step.

Method A-2–2. Preparation of Monoethyl (Arylmethyl)propanedioic Acids. Monoethyl (1-Naphthylmethyl)-propanedioic Acid (9g). To **8g** (133.7 g, 446 mmol) in EtOH (400 mL) was added a solution of potassium hydroxide (24.9 g, 445 mmol) in ethanol (400 mL), and the mixture was stirred 18 h, concentrated *in vacuo*, diluted with ice–water, and extracted with Et_2O. The aqueous solution was cooled, acidified to pH 2 with concentrated HCl, and extracted with Et_2O. The dried ($MgSO_4$) Et_2O was concentrated *in vacuo* to give **9g** as an oil (used in next step) (100.0 g, 82%).

Monoethyl (Phenylmethyl)propanedioic Acid (9f). In a manner similar to that described for **9g**, diethyl benzylmalonate (300 g, 1.2 mol) was converted to **9f** as a yellow oil (266 g, 99%) which was used in the next step.

Method A-2–3. Ethyl α-Methylenearenepropanoates. Ethyl α-Methylene-1-naphthalenepropanoate (10g). To **9g** (100.0 g, 367 mmol) and diethylamine (39 mL) was added 37% aqueous formaldehyde solution (38 mL) over 30 min at 0–5 °C. The mixture was stirred for 7 h at room temperature and extracted with Et_2O (3 × 500 mL). The Et_2O extracts were washed with 2 N HCl (2 × 500 mL), saturated NaHCO3 (500 mL), and brine (500 mL). The dried ($MgSO_4$) Et_2O was concentrated *in vacuo* to give **10g**, an oil (58.6 g, 66%) which was used in the next step.

Ethyl α-Methylenebenzenepropanoate (10f). In a manner similar to that described for **10g**, **9f** (266 g, 1.2 mol) was converted to **10f** as an oil (165 g, 72%) which was used in the next step.

Method A-2–4. α-Methylenearenepropanoic Acids via Ethyl α-Methylenearenepropanoates. α-Methylene-1-naphthalenepropanoic Acid (6g). Ester **10g** (12.0 g, 50 mmol) in dioxane (50 mL) was treated with 1 N NaOH (60 mL) and stirred at room temperature 20 h. The reaction mixture was concentrated *in vacuo*, diluted with water, and extracted with EtOAc. The aqueous solution was cooled to 0–5 °C, and concentrated HCl was added slowly to give a precipitate which was filtered off, washed with water, and dried to give **6g** as a white solid (9.62 g, 90%), mp 115–117 °C. Anal. ($C_{14}H_{12}O_2$·0.1H_2O) C, H.

α-Methylenebenzenepropanoic Acid (6f). In a manner similar to that described for **6g**, **10f** (165 g, 868 mmol) was converted to **6f** as a white solid (105 g, 75%), mp 67–68 °C.

Method A-3. Preparation of α-[(*R,S*)-Acetylthio)methyl]benzenebutanoic Acid. 1,1-Dimethylethyl 2-(2-Phenylethyl)-3-oxobutanoate (11).[38] Sodium (2.3 g, 100 mmol) was added to absolute EtOH (50 mL). The solution was cooled to 0–5 °C, and *tert*-butyl acetoacetate (15.8 g, 100 mmol) was added. After 10 min, 2-(bromoethyl)benzene (18.5 g, 100 mmol) was added and the mixture heated under reflux 4 h. The mixture was cooled and filtered, the filtrate concentrated *in vacuo*, and the residual oil distilled to give **11** as a colorless oil (12.85 g, 49%): bp 100-118 °C (0.1 mm); NMR (C) 3.32 (t,1H), 2.62 (m, 2H), 2.17 (m, 2H). This contains approximately 0.25 equiv of ethyl ester and was used in the next step.

1,1-Dimethylethyl α-Methylenebenzenebutanoate (12).[38] At −60 °C, 2.5 M *n*-butyllithium in hexane (18.75 mL, 47 mmol) was added dropwise to a solution of diisopropylamine (4.75 g, 47 mmol) in dry THF (30 mL). The mixture was warmed to 0 °C and then cooled to −60 °C, and **11** (12.0 g, 45.8 mmol) in THF (20 mL) was added. After 10 min, paraformaldehyde (6.87 g) was added, and the reaction mixture was allowed to warm to room temperature, stirred at that temperature 1 h, heated under reflux 1 h, cooled, and filtered. The filtrate was concentrated *in vacuo* and the residue partitioned between water and Et_2O. The Et_2O solution was washed sequentially with 1 N HCl, water, and 1 N $NaHCO_3$. The dried ($MgSO_4$) Et_2O was concentrated *in vacuo* to give an oil (10.03 g). Distillation (Kugelrohr at 0.1 mm between 100–150 °C) gave **12** as a colorless oil (3.58 g, 34%): NMR (C) δ 5.42 + 6.07 (C=CH2). This contains approximatey 0.25 equiv of ethyl ester and was used in the next step.

1,1-Dimethylethyl α-[(*R,S*)-Acetylthio)methyl]benzenebutanoate (13). Ester **12** (1.5 g, 6.5 mmol) and thiolacetic acid (0.98 g, 12.9 mmol) were stirred for 3 days and concentrated under high vacuum. The residue was partitioned

between Et_2O and 1 N $NaHCO_3$. The dried ($MgSO_4$) Et_2O was concentrated *in vacuo* to give an oil (1.91 g) which was chromatographed on silica gel (300 g) (hexane–Et_2O, 9:1) to give **13** as an oil (0.92 g, 47%): NMR (C) δ 3.10 (m, 2H), 2.65 (t, 3H), 2.52 (m, 1H), 1.91 (m, 2H).

α-[((*R,S*)-Acetylthio)methyl]benzenebutanoic Acid (14). Ester **13** (0.60 g, 1.9 mmol) and trifluoroacetic acid (10 mL) were stirred for 30 min and then concentrated *in vacuo* to give **14** as an orange oil (0.49 g, 52%): NMR (C) δ 3.17 (m, 2H), 2.71 (t, 2H), 2.70 (m, 1H), 2.01 (m, 2H).

Method A-4. Preparation of 1-[(Acetylthio)methyl]-cyclopentanecarboxylic Acid. 1,1-Dimethylethyl Cyclopentanecarboxylate (15). At −20 to −30 °C, isobutylene (100 mL) was condensed in a threaded tube. *tert*-Butyl alcohol (4 mL), cyclopentanecarboxylic acid (25 g, 219 mmol), and concentrated sulfuric acid (1 mL) were added. The tube was sealed and stirred for 4 days at room temperature, cooled, vented, and purged with nitrogen. The mixture was partitioned with Et_2O and 1 N $NaHCO_3$. The dried ($MgSO_4$) Et_2O was concentrated *in vacuo* to give a pale yellow oil (25.05 g). Distillation gave **15**, a colorless oil (17.7 g, 47%): $bp_{0.1}$ 55–60 °C; NMR (C) 2.63 (quint, 1H), 1.80–1.90 (m, 8H), 1.44 (s, 9H).

1,1-Dimethylethyl 1-(Hydroxymethyl)cyclopentanecarboxylate (16a). *n*-Butyllithium (2.5 M in hexane, 8.3 mL, 21 mmol) was added to a −78 °C solution of diisopropylamine (2.12 g, 21 mmol) in THF (50 mL). This mixture was warmed to 0 °C, stirred for 30 min, cooled to −78 °C, and treated dropwise with **15** (3.00 g, 17.6 mmol) in THF (50 mL) and dipyridyl (0.002 g). Paraformaldehyde (2.58 g) in a separate flask was heated to 160 °C and introduced via a stream of nitrogen into the reaction mixture. After 45 min, the reaction mixture was warmed to room temperature and stirred for 18 h. The mixture was quenched with saturated NH_4Cl solution (100 mL) and then brine (100 mL) and extracted with CH_2Cl_2 (4 × 100 mL). The dried ($MgSO_4$) CH_2Cl_2 was concentrated *in vacuo* to give **16a** as a yellow oil (2.98 g, 84%): NMR (C) δ 3.53 (s, 2H), 1.92, (m, 2H), 1.50–1.80 (m, 8H), 1.46 (s, 9H).

1,1-Dimethylethyl 1-[[(Methylsulfonyl)oxy]methyl]cyclopentanecarboxylate (16b). Methanesulfonyl chloride (0.98 g, 8.30 mmol) was added to **16a** (1.5 g, 7.5 mmol) and triethylamine (0.83 g, 8.8 mmol) in Et_2O (20 mL), and the mixture was stirred at room temperature for 18 h. The reaction mixture was washed with water, and the dried ($MgSO_4$) Et_2O was concentrated *in vacuo* to give **16b** as a yellow oil (2.02 g, 97%): NMR (C) δ 4.24 (s, 2H), 3.02 (t, 3H), 2.07 (m, 2H), 1.50–1.80 (m, 3H),1.46 (s, 9H). This contains 0.15 equiv of methanesulfonyl chloride.

1,1-Dimethylethyl 1-[(Acetylthio)methyl]cyclopentanecarboxylate (17). Ester **16b** (1.85 g, 6.65 mmol), thiolacetic acid (1.11 g, 14.6 mmol), and potassium carbonate (0.92 g, 6.6 mmol) in anhydrous DMF (30 mL) were heated under reflux at 100 °C for 4.5 h. The reaction mixture was partitioned between EtOAc and 1 N HCl. The dried ($MgSO_4$) EtOAc was concentrated *in vacuo* to give a red oil (1.64 g) which was chromatographed on a column of silica gel (300 g) (hexane–Et_2O, 19:1) to give **17** as a red oil (1.25 g, 73%): NMR (C) δ 3.18 (s, 2H), 2.33 (s, 3H), 2.04 (m, 2H), 1.50–1.80 (m, 6H), 1.44 (s, 9H).

1-[(Acetylthio)methyl]cyclopentanecarboxylic Acid (18). Ester **17** (1.25 g, 4.84 mmol) was dissolved in CH_2Cl_2 (40 mL) and TFA (40 mL). After 18 h, the solvent was removed *in vacuo* and the residual solid dried at 50 °C (0.1 mm) to give **18** as a brown solid (0.96 g, 98%): NMR (C) δ 3.23 (s, 2H), 2.35 (s, 3H), 2.12 (m, 2H), 1.60–1.90 (m, 6H).

***S*-(2-Phenylethyl)-L-cysteine (19a).** At 0 °C, nitrogen was bubbled through a mixture of L-cysteine (45.5 g, 376 mmol) in water (180 mL) during the addition of 2 N NaOH (187 mL, 376 mmol), followed by addition of 2-phenylethyl bromide (75.9 g, 410 mmol) in MeOH (800 mL). After 1 h, the mixture was warmed to room temperature and kept for 20 h. The mixture was made acidic to pH 6–7 with glacial AcOH (15 mL) and the white precipitate collected and washed with water to give a white solid (40.8 g), mp 217–219 °C dec. This solid (34 g) was boiled in a mixture of MeOH (180 mL), glacial AcOH (340 mL), and water (700 mL), filtered, concentrated to 500 mL, and chilled to give **19a** as a white solid (22.5 g): mp 224-225 °C dec. Second crop of **19a** as a white solid (5.2 g): mp 221–223 °C.

Method B-1. Preparation of Amino Acid Ester Hydrochlorides from Amino Acids. *S*-(2-Phenylethyl)-L-cysteine Ethyl Ester Hydrochloride (20a).[37] *S*-(2-Phenylethyl)-L-cysteine (**19a**) (2.50 g, 1.11 mmol) was added to a solution resulting from the addition of thionyl chloride (2.0 mL) to absolute EtOH (25 mL). The mixture was heated under reflux for 5 h, cooled, and concentrated *in vacuo*. The residue was dissolved in absolute EtOH, treated with Darco, filtered, and concentrated *in vacuo* to give **20a** as a white solid (2.90 g, 90%): mp 155–156 °C; $[\alpha]^{26}_D$ −1.5° (0.52, M). Anal. ($C_{13}H_{19}NO_2S$·HCl) C, H, N.

***S*-Methyl-L-cysteine Ethyl Ester (20b).** Similarly, *S*-methyl-L-cysteine was converted to crude **20b** as a white solid which was chromatographed on a column of silica gel using CH_2Cl_2–MeOH–NH_4OH, 170:27:3, as eluant to give **20b** as an amber oil (2.20 g, 61%): $[\alpha]^{26}_D$ +25.1° (0.53, M). Anal. ($C_6H_{13}NO_2S$·H_2O) C, N. H: calcd, 8.28; found, 7.10.

***S*-Ethyl-L-cysteine Ethyl Ester Hydrochloride (20c).** Similarly to **20a**, *S*-ethyl-L-cysteine was converted to **20c** as a white solid (99%): mp 130–133 °C (lit.[39] mp 137 °C); $[\alpha]^{26}_D$ −11.0° (0.50, M). Anal. ($C_7H_{15}NO_2S$·HCl) C, H, N.

L-Ethionine Ethyl Ester Hydrochloride (20d).[38] Similarly, L-ethionine was converted to **20d** as a white foam (99%): $[\alpha]^{26}_D$ +15.5° (0.51, M) which was used without further purification.

***S*-(Triphenylmethyl)-L-cysteine Methyl Ester Hydrochloride (20e).** Similarly, *S*-(triphenylmethyl)-L-cysteine in MeOH was converted to **20e** as an off-white foam (95%) which was used in the next step.

Method B-2. Preparation of Amino Acid Ester Hydrochlorides from *N*-[(1,1-Dimethylethoxy)carbonyl]amino Acids. *S*-(Phenylmethyl)-D-cysteine Ethyl Ester Hydrochloride (20f).[38] *N*-Boc-*S*-(phenylmethyl)-D-cysteine (2.50 g, 8.0 mmol) was added to a solution resulting from the addition of thionyl chloride (1.2 mL) to absolute EtOH (30 mL) at 0 °C. After 18 h at room temperature, thionyl chloride (0.60 mL) was added. After 3 h, the mixture was concentrated *in vacuo* and the residue washed with Et_2O to give **20f** as a white solid (2.05 g, 95%): mp 149-151 °C (lit.[40] mp 148 °C); $[\alpha]^{26}_D$ −15.5° (0.51, M). Anal. ($C_{12}H_{17}NO_2S$·HCl) C, H, N.

***S*-[(4-Methylphenyl)methyl]-L-cysteine Methyl Ester Hydrochloride (20g).** Similarly, *N*-Boc-*S*-[(4-methylphenyl)-methyl]-L-cysteine was converted to **20g** as a white foam (94%): $[\alpha]^{26}_D$ −22.9° (0.41, M). Anal. ($C_{12}H_{17}NO_2S$·HCl) H, N; C: calcd, 52.26; found, 51.62.

***S*-[(4-Methylphenyl)methyl]-L-cysteine Ethyl Ester Hydrochloride (20h).** Similarly, *N*-Boc-*S*-[(4-methylphenyl)-methyl]-L-cysteine was converted to **20h** as a white solid (94%): mp 156–160 °C; $[\alpha]^{26}_D$ −28.3° (0.87, M). Anal. ($C_{13}H_{19}NO_2S$·HCl) H, N; C: calcd, 53.88; found, 52.65.

***S*-[(3,4-Dimethylphenyl)methyl]-L-cysteine Ethyl Ester Hydrochloride (20j).** Similarly, *N*-Boc-*S*-[(3,4-dimethylphenyl)methyl]-L-cysteine was converted to **20j** as a white foam (98%): $[\alpha]^{26}_D$ −26.6° (0.37, M). Anal. ($C_{14}H_{21}$ NO_2S·HCl) H, N; C: calcd, 55.34; found, 54.16.

***S*-[(4-Methoxyphenyl)methyl]-L-cysteine Methyl Ester Hydrochloride (20k).** Similarly, *N*-Boc-*S*-[(4-methoxyphenyl)methyl]-L-cysteine in MeOH was converted to **20k** as a white foam (97%): $[\alpha]^{26}_D$ −23.2° (0.82, M). Anal. ($C_{12}H_{17}NO_3S$·HCl) H, N; C: calcd, 49.40; found, 48.73.

***S*-(1,1-Dimethylethyl)-L-cysteine Methyl Ester Hydrochloride (20m).** Similarly, *N*-Boc-*S*-(1,1-dimethylethyl)-L-cysteine in MeOH was converted to **20m** as an oil (75%), which was used in the next step.

Method B-3–1-1. *N*-[(1,1-Dimethylethoxy)carbonyl]-*S*-[(4-methylphenyl)methyl]-L-cysteinamide (22a). *N*-Boc-*S*-[(4-methylphenyl)methyl]-L-cysteine (6.15 g, 19.2 mmol) in THF (60 mL) was treated with triethylamine (4.44 g, 44 mmol) and cooled to 0–5 °C. Ethyl chloroformate (4.77 g, 44 mmol) in THF (5 mL) was added and the mixture stirred 15 min. Concentrated ammonium hydroxide (2 mL) in THF (5 mL) was added dropwise over 5 min. After 15 min, the reaction mixture was warmed to room temperature, stirred for 18 h, and

filtered. The filtrate was concentrated *in vacuo* to give a pale yellow solid (8.0 g) which was dissolved in CH_2Cl_2 and washed with water. The dried ($MgSO_4$) CH_2Cl_2 solution was concentrated *in vacuo* to give **22a** as a white solid (6.4 g, 99%): mp 140-142 °C; $[\alpha]^{26}_D$ −7.5 ° (1.0, M). Anal. ($C_{16}H_{24}N_2O_3S$) C, H, N.

Method B-3−1-2. *S*-[(4-Methylphenyl)methyl]-L-cysteinamide (23a). Amide **22a** (6.15 g, 19.2 mmol) in CH_2Cl_2 (40 mL) was treated with trifluoroacetic acid (10mL). After 44 h, the reaction mixture was concentrated *in vacuo*, and the residue was diluted with CH_2Cl_2, concentrated *in vacuo*, dissolved in EtOAc and washed with saturated $NaHCO_3$. The dried ($MgSO_4$) EtOAc was concentrated *in vacuo* to give **23a** as a white solid (1.53 g, 79%): mp 94−95 °C; $[\alpha]^{26}_D$ −1.3° (0.55, M). Anal. ($C_{11}H_{16}N_2OS$) C, H, N.

Method B-3−2-1. Preparation of 1-[*N*-[(1,1-Dimethylethoxy)carbonyl]amino] Acid Amides. 1-[2(*S*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-4-(methylthio)-1-oxobutyl]piperidine (22b). *N*-Boc-L-methionine (1.50 g, 6 mmol), piperidine (0.51 g, 6 mmol), HOBT (0.91 g, 6 mmol), and EDC (1.15 g, 6.1 mmol) in anhydrous DMF (40 mL) were stirred for 5 h, concentrated *in vacuo*, and partitioned between EtOAc and water. The EtOAc extract was washed with water, 1 N $NaHCO_3$, and brine. The dried ($MgSO_4$) EtOAc was concentrated *in vacuo* to give a colorless oil (1.96 g) which was chromatographed on a column of silica gel (300 g) (CH_2Cl_2−MeOH, 24:1) to give **22b** as a colorless oil (1.84 g, 96%). NMR (C) consistent.

1-[2(*S*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-4-(methylthio)-1-oxobutyl]morpholine (22c). Similarly, *N*-Boc-L-methionine (1.20 g, 4.81 mmol) was converted to **22c** as an oil (1.27 g, 82%). Et_2O was used as eluant: R_f 0.5 (Et_2O); NMR (C) consistent.

2-[[2(*S*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-4-(methylthio)butanoyl]amino]ethanol (22d). Similarly, *N*-Boc-L-methionine (1.50 g, 6.0 mmol) was converted to **22d** as an oil (1.43 g, 81%) (CH_2Cl_2−MeOH 19:1): R_f 0.7 (CH_2Cl_2−MeOH, 17:3); NMR (C) consistent.

***N*-[2(*S*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-4-(methylthio)-1-oxobutyl]-β-alanine Ethyl Ester (22e).** Similarly, *N*-Boc-L-methionine (1.50 g, 6.0 mmol) was converted to **22e** as an oil (1.90 g, 94%) (CH_2Cl_2−MeOH, 19:1): R_f 0.7 (CH_2Cl_2−MeOH, 19:1); NMR (C) consistent.

Method B-3−2-2. Preparation of Amino Acid Amide Hydrochlorides. *N*-[2(*S*)-Amino-4-(methylthio)-1-oxobutyl]piperidine Hydrochloride (23b). Amide **22b** (1.70 g, 5.37 mmol) was treated with 6 M HCl in dioxane (25 mL). After 30 min, the reaction mixture was concentrated *in vacuo* to give **23b** as a foam (1.60 g, crude, suitable for further use).

***N*-[2(*S*)-Amino-4-(methylthio)-1-oxobutyl]morpholine Hydrochloride (23c).** Similarly, amide **22c** (1.25 g, 3.9 mmol) was converted to **23c** as a solid (1.20 g, crude, suitable for further use).

2-[[2(*S*)-Amino-4-(methylthio)butanoyl]amino]ethanol Hydrochloride (23d). Similarly, amide **22d** (1.33 g, 4.55 mmol) was converted to **23d** as an oil (1.05 g, crude, suitable for further use).

***N*-[2(*S*)-Amino-4-(methylthio)-1-oxobutyl]-β-alanine Ethyl Ester Hydrochloride (23e).** Using the method described above, amide **22e** (1.90 g, 5.68 mmol) was converted to **23e** as an oil (1.68 g, crude, suitable for further use).

Method B-4. 1-[2(*S*)-Amino-4-(methylthio)-1-oxobutyl]-pyrrolidine Hydrochloride (23f). L-Methionine methyl ester hydrochloride (4.00 g, 20 mmol) was dissolved in pyrrolidine (10.0 g, 141 mmol) (exotherm). After 18 h, the mixture was concentrated *in vacuo* at 50 °C and partitioned with EtOAc (100 mL), 1 N $NaHCO_3$ (100 mL), and brine (25 mL). The aqueous solution was treated with NaCl (20 g) and 50% NaOH (10 mL) and extracted with a mixture of EtOAc (100 mL) and EtOH (10 mL). The organic extracts were dried ($MgSO_4$) and concentrated *in vacuo*. The residue was dissolved in Et_2O, filtered, and concentrated *in vacuo* to give an oil (1.6 g). The oil was taken up in Et_2O and treated with 2 N HCl/Et_2O (5 mL). The solid was filtered off and recrystallized from MeOH−EtOAc to give **23f** as needles (1.50 g, 31%): mp 124−125 °C. Anal. ($C_9H_{18}N_2OS$·HCl) C, H, N.

Method C-1. *N*-[3-(Acetylthio)-2(*S*)-(phenylmethyl)-1-oxopropyl]-*S*-(phenylmethyl)-L-cysteine Ethyl Ester (25a) and *N*-[3-(Acetylthio)-2(*R*)-(phenylmethyl)-1-oxopropyl]-*S*-(phenylmethyl)-L-cysteine Ethyl Ester (26a). EDC (1.05 g, 5.5 mmol) was added to acid **7f** (1.19 g, 5.0 mmol), *S*-(phenylmethyl)-L-cysteine ethyl ester hydrochloride (**20f**) (1.36 g, 5.0 mmol), HOBT (0.75 g, 5.5 mmol), and *N*-methylmorpholine (1.1 mL, 10 mmol) in DMF (5 mL). The reaction mixture was stirred for 20 h, concentrated *in vacuo*, and partitioned between CH_2Cl_2 and water. The dried ($MgSO_4$) CH_2Cl_2 solution was concentrated *in vacuo* to give a residue which was chromatographed on flash silica gel (300 mL) (CH_2Cl_2−EtOAc, 49:1) to give fraction 1: *N*-[3-(acetylthio)-2(*S*)-(phenylmethyl)-1-oxopropyl]-*S*-(phenylmethyl)-L-cysteine ethyl ester **25a** as a white solid (0.49 g, 22%): mp 83−85 °C; $[\alpha]^{26}_D$ −73.5° (0.28, M). Anal. ($C_{27}H_{29}NO_4S_2$) C, H, N; fraction 2: (0.15 g); fraction 3 (0.50 g); and fraction 4: *N*-[3-(acetylthio)-2(*R*)-(phenylmethyl)-1-oxopropyl]-*S*-(phenylmethyl)-L-cysteine ethyl ester **26a** as a white solid (0.37 g, 16%): $[\alpha]^{26}_D$ −9.4° (0.51, M). Anal. ($C_{27}H_{29}NO_4S_2$) C, H, N.

The *N*-[3-(acetylthio)-2(*S*)-[(substituted)methyl]-1-oxopropyl]-L-amino acid esters (**25c-f,l,n,o,t-w,y, 36**); *N*-[3-(-acetylthio)-2(*S*)-(phenylmethyl)-1-oxopropyl]-*S*-(phenylmethyl)-D-cysteine ethyl ester (**26b**) (Table 1); *N*-[3-(acetylthio)-2(*R*)-[(substituted)methyl]-1-oxopropyl]-L-amino acid esters (**26c-f,l,o,t-w,y, 37**); and *N*-[3-(acetylthio)-2(*R*)-(phenylmethyl)-1-oxopropyl]-*S*-(phenylmethyl)-D-cysteine ethyl ester (**26b**) (Table 1) were prepared using the procedure described above.

Method C-1a. *N*-[3-(Acetylthio)-2(*R,S*)-(phenylmethyl)-1-oxopropyl]-L-methionine Methyl Ester (27m). In a manner similar to that described in method C-1, acid **7f** (3.01 g, 12.6 mmol) and L-methionine methyl ester (2.00 g, 12.3 mmol) were converted to *N*-[3-(acetylthio)-2(*R,S*)-(phenylmethyl)-1-oxopropyl]-L-methionine methyl ester (**27m**) (2.61 g, 55%): $[\alpha]^{26}_D$ −38.9° (0.55, M). Anal. ($C_{18}H_{25}NO_4S_2$) H, N; C: calcd, 56.37; found, 55.47 (Table 1).

The *N*-[3-(acetylthio)-2(*R,S*)-[(substituted)methyl]propyl]-L-amino acid esters (**27g,h,k,q,s**) (Table 1) were prepared using the procedure described above.

Method C-2. *N*-[3-Mercapto-2(*S*)-(phenylmethyl)-1-oxopropyl]-*S*-(phenylmethyl)-L-cysteine (28a). Under a nitrogen atmosphere, ester **25a** (0.48 g, 0.97 mmol) in MeOH[23,41] (20 mL) was treated with 1 N NaOH (3.1 mL, 3 equiv). After 1 h, 1 N HCl (4 mL) and then water (50 mL) were added. The mixture was extracted with EtOAc (500 mL), and the dried ($MgSO_4$) EtOAc was concentrated *in vacuo* to give **28a** as a viscous oil (0.30 g, 74%): $[\alpha]^{26}_D$ −2.1° (1.0, M). Anal. ($C_{20}H_{23}NO_3S_2$·0.1CH_2Cl_2) C, H; N: calcd, 3.51; found, 3.10. This material contains 15% D-cysteine diastereomer, as seen in the NMR.

The *N*-[3-mercapto-2(*S*)-(arylmethyl)-1-oxopropyl]-L-amino acids (**28c-f,l,n,o,t-w,y**); *N*-[3-mercapto-2(*S*)-(phenylmethyl)-1-oxopropyl]-*S*-(phenylmethyl)-D-cysteine (**28b**) (Table 2); *N*-[3-mercapto-2(*R*)-(arylmethyl)propyl]-L-amino acids (**29c-f,l,n,o,t-x**); and *N*-[3-mercapto-2(*R*)-(phenylmethyl)-1-oxopropyl]-*S*-(phenylmethyl)-D-cysteine (**29b**) (Table 2) were prepared using the procedure described above.

Method C-2a. *N*-[3-Mercapto-2(*R,S*)-(arylmethyl)-1-oxopropyl]-L-amino Acids. The *N*-[3-mercapto-2(*R,S*)-(arylmethyl)-1-oxopropyl]-L-amino acids (**30g,h,k,m,r,s**) (Table 2) were prepared using method C-2.

Method D. *N*-[3-(Benzoylthio)-2(*S*)-(phenylmethyl)-1-oxopropyl]-L-methionine Methyl Ester (25n). In a manner similar to that described in method C-1, α(*S*)-[(benzoylthio)-methyl]benzenepropanoic acid (**31-*S***) (1.27 g, 4.0 mmol) and L-methionine methyl ester hydrochloride (0.79 g, 4.0 mmol) were converted to *N*-[3-(benzoylthio)-2(*S*)-(phenylmethyl)-1-oxopropyl]-L-methionine methyl ester (**25n**) (1.45 g, 86%): $[\alpha]^{26}_D$ −67.9° (0.5, E). Anal. ($C_{23}H_{27}NO_4S_2$) C, H, N (Table 1).

***N*-[3-(Benzoylthio)-2(*R*)-(phenylmethyl)-1-oxopropyl]-L-methionine methyl ester (26n)** was prepared in a manner similar to that described above.

***N*-[3-(Acetylthio)-2(*S*)-methyl-1-oxopropyl]-*S*-(phenylmethyl)-L-cysteine Ethyl Ester (25p).** In a manner similar to that described in method C-1, D-(-)-*S*-acetyl-β-mercaptoisobutyric acid (1.46 g, 9 mmol) and *S*-(phenylmethyl)-L-

cysteine ethyl ester hydrochloride (2.76 g, 1 mmol) were converted to *N*-[3-(acetylthio)-2(*S*)-methyl-1-oxopropyl]-*S*-(phenylmethyl)-L-cysteine ethyl ester (**25p**) (1.37 g, 39%): $[\alpha]^{26}_D$ −122.4° (0.3, M). Anal. ($C_{18}H_{25}NO_4S_2$) C, H, N (Table 1).

Method E-1. α-[(*R,S*)-(Acetylthio)methyl]benzenepropanoyl Chloride (35). Acid **7f** (7.10 g, 30 mmol) in toluene (30 mL) was treated with thionyl chloride (3.3 mL). The resulting mixture was heated under reflux for 3 h and concentrated *in vacuo* to give **35**, an amber oil (6.94 g) which was used in the next step.

***N*-[3-(Acetylthio)-2(*S*)-(phenylmethyl)-1-oxopropyl]-*S*-[(4-methylphenyl)methyl]-L-cysteine (38a) and *N*-[3-(Acetylthio)-2(*R*)-(phenylmethyl)-1-oxopropyl]-*S*-[(4-methylphenyl)methyl]-L-cysteine (39a).** *S*-[(4-Methylphenyl)methyl]-L-cysteine (6.10 g, 27 mmol) in acetonitrile (50 mL) and water (25 mL) was treated with triethylamine (3.80 mL), followed by dropwise addition of acid chloride **35** (7.0 g, 25 mmol) in acetonitrile (30 mL). The resulting mixture was stirred at room temperature 20 h, filtered, concentrated *in vacuo*, diluted with water (100 mL), acidified with 1 N hydrochloric acid to pH 2−4, and extracted with EtOAc. The dried ($MgSO_4$) EtOAc was concentrated *in vacuo* to give a brown oil (12.82 g). Column chromatography on silica gel (3 L) (CH_2Cl_2−MeOH, 100:1; 7 L); (CH_2Cl_2−MeOH−glacial AcOH, 400:4:0.1; 2.5 L); then (CH_2Cl_2−MeOH−glacial AcOH, 400:5:0.1) gave fraction 1 (1.00 g); fraction 2 (2.38 g); fraction 3 (1.00 g); and fraction 4 (1.56 g). Fraction 1 was dissolved in EtOAc, treated with Darco, filtered, and then chromatographed (with fraction 2−2, see below) on a column of silica gel (2 L) (CH_2Cl_2-MeOH, 100:1, 1.5 L); (CH_2Cl_2−MeOH−glacial AcOH, 400:4:0.1) to give fraction 1−3, *N*-[3-(acetylthio)-2(*S*)-(phenylmethyl)-1-oxopropyl]-*S*-[(4-methylphenyl)methyl]-L-cysteine (**38a**) an oil (0.34 g, 6.3%): $[\alpha]^{26}_D$ −23.0° (1.0, M). Anal. ($C_{23}H_{27}NO_4S_2$·0.2CH_2Cl_2) C, N; H: calcd, 5.97; found, 5.55. Fraction 2 was chromatographed on a column of silica gel (2 L), (CH_2Cl_2−MeOH, 100:1, 2 L); (CH_2Cl_2−MeOH−glacial AcOH, 100:1:0.1) to give fraction 2−1, (0.1 g); fraction 2−2 (0.54 g); fraction 2−3 (0.41 g); fraction 2−4 (0.10 g): fraction 2−5 (0.28 g); fraction 2−6 (0.12 g); and fraction 2−7 (0.10 g). Fraction 3 and fractions 2−3 to 2−7 were combined and chromatographed on a column of silica gel (2 L), (CH_2Cl_2−MeOH, 100:1, 2 L); (CH_2Cl_2−MeOH−glacial AcOH, 100:1:0.1) to give fraction 3−4, *N*-[3-(acetylthio)-2(*R*)-(phenylmethyl)-1-oxopropyl]-*S*-[(4-methylphenyl)methyl]-L-cysteine (**39a**) an oil (0.52 g, 9.5%): $[\alpha]^{26}_D$ −1.3° (1.0, M). Anal. ($C_{23}H_{27}NO_4S_2$·0.25 CH_3CO_2H) C, H, N.

The *N*-[3-(acetylthio)-2(*S*)-(arylmethyl)-1-oxopropyl]-L-amino acids **38b** and **38c** (Table 3) and *N*-[3-(acetylthio)-2(*R*)-(arylmethyl)-1-oxopropyl]-L-amino acid **39c** (Table 3) were prepared using the procedure described above.

Method E-2. *N*-[3-(Acetylthio)-2(*S*)-(phenylmethyl)-1-oxopropyl]-*S*-[(4-methylphenyl)methyl]-L-cysteine (38a). 1,1-Dimethylethyl ester **36** (0.47 g, 9.5 mmol) in CH_2Cl_2 (10 mL) was treated with trifluoroacetic acid (5 mL) and the mixture stirred 18 h. The reaction mixture was concentrated *in vacuo*, the residue was dissolved in CH_2Cl_2 (25 mL) and concentrated *in vacuo* (twice) and then dissolved in Et_2O (100 mL) and concentrated *in vacuo* (twice) to yield **38a** as a colorless oil (0.24 g, 56%): $[\alpha]^{26}_D$ −58.8° (0.58, M). Anal. ($C_{23}H_{27}NO_4S_2$) C, H, N.

N-[3-(Acetylthio)-2(*R*)-(phenylmethyl)-1-oxopropyl]-*S*-[(4-methylphenyl)methyl]-L-cysteine (**39a**) and *N*-[2(*R,S*)-[(acetylthio)methyl]-1-oxopentyl]-*S*-ethyl-L-cysteine (**40d**) were prepared by the procedure above (Table 3).

Method E-3. Preparation of *N*-[3-Mercapto-2-(alkyl or arylmethyl)-1-oxopropyl] Amino Acids. *N*-[2(*R,S*)-(Mercaptomethyl)-1-oxopentyl]-*S*-ethyl-L-cysteine (30q). Under a nitrogen atmosphere at 0 °C, to acid **40d** (0.72 g, 2.24 mmol) in MeOH[41] (20 mL) was added 1 N NaOH (7 mL) and the mixture was stirred at that temperature for 20 h. The mixture was partitioned between EtOAc and 1 N HCl. The dried ($MgSO_4$) EtOAc solution was concentrated *in vacuo* to give **30q** as a yellow oil (0.58 g, 92%): $[\alpha]^{26}_D$ −34.1° (0.54, M). Anal. ($C_{11}H_{21}NO_3S_2$) C, H, N.

***N*-[3-Mercapto-2(*R*)-(phenylmethyl)-1-oxopropy]-L-methionine (29n).** Acetylthio acid **39b** (0.50 g, 1.35 mmol) was treated as described above to give **29n** as a white foam: $[\alpha]^{26}_D$ −38.3° (0.50, E). Anal. ($C_{15}H_{21}NO_3S_2$·0.125H_2O) C, H, N.

Method E-4. *N*-[3-Mercapto-2(*S*)-(phenylmethyl)-1-oxopropyl]-*S*-[(4-methylphenyl)methyl]-L-cysteine (28c). Under a nitrogen atmosphere, acetylthio acid **38a** (0.217 g, 0.48 mmol) was treated with ammonia in absolute MeOH (37% wt/wt, 100 mL). After 1.5 h, 1 N HCl was added to pH 2. Water (200 mL) was added, and the mixture was extracted with EtOAc. The dried ($MgSO_4$) EtOAc was concentrated *in vacuo* to give **28c** as a colorless oil (0.17 g, 86%): $[\alpha]^{26}_D$ −32.6° (0.4, M). Anal. ($C_{21}H_{25}NO_3S_2$·0.5H_2O) C, H, N.

N-[3-Mercapto-2(*R*)-(phenylmethyl)-1-oxopropyl]-*S*-[(4-methylphenyl)methyl]-L-cysteine (**29c**); *N*-[3-mercapto-2(*S*)-[[1,1'-biphenyl]-4-ylmethyl]-1-oxopropyl]-*S*-[(4-methylphenyl)methyl]-L-cysteine (**28x**); and *N*-[3-mercapto-2(*R*)-[[1,1'-biphenyl]-4-ylmethyl]-1-oxopropyl]-*S*-[(4-methylphenyl)methyl]-L-cysteine (**29x**) were prepared using the procedure described in method **E-4** (Table 3).

Method F-1. *N*-[3-(Benzoylthio)-2(*S*)-(phenylmethyl)-1-oxopropyl]-L-methioninamide (43a). (*S*)-Benzoylthio acid **31-*S*** (4.50 g, 15.0 mmol) was converted to acid chloride **32-*S*** (4.75g, 99%) using the procedure described in method **E-1**. This acid chloride (1.90 g, 5.97 mmol) was added dropwise to L-methioninamide (1.00 g, 5.43 mmol) and triethylamine (1.20 g, 11.94 mmol) in acetonitrile (40 mL) and water (20 mL). After 1 h, the reaction mixture was concentrated *in vacuo* and the residue partitioned between 1 N HCl and EtOAc. The dried ($MgSO_4$) EtOAc solution was concentrated *in vacuo* to give a white solid (2.33 g), mp 130−144 °C. This solid was recrystallized from EtOAc and dried to give **43a** as a white solid (0.84 g, 36%): mp 176−180 °C; $[\alpha]^{26}_D$ −97.6° (0.5, C). Anal. ($C_{22}H_{26}N_2O_3S_2$) C, H, N.

Method F-2. *N*-[3-(Benzoylthio)-2(*R*)-(phenylmethyl)-1-oxopropyl]-L-methioninamide (44a). (*R*)-Benzoylthio acid **31-*R*** (0.51 g, 1.7 mmol), L-methioninamide hydrochloride (0.31 g, 1.7 mmol), EDC (0.32 g, 1.7 mmol), HOBT (0.23 g, 1.7 mmol), and *N*-methylmorpholine (0.72 g, 1.7 mmol) in DMF (5 mL) were stirred at room temperature for 18 h. Dilution with water gave a precipitate which was collected, washed with water, and dried to give **44a** as a white solid, (0.62 g, 86%): mp 145−149 °C; $[\alpha]^{26}_D$ +32.9° (1.0, C). Anal. ($C_{22}H_{26}N_2O_3S_2$) C, H, N.

Method F-3. *N*-[3-Mercapto-2(*S*)-(phenylmethyl)-1-oxopropyl]-L-methioninamide (46a). Benzylthio amide **43a** (0.30 g, 0.69 mmol) in methanol (25 mL) (purged with nitrogen) was treated with 1 N NaOH (3 mL) (purged with nitrogen). After 1 h, the reaction mixture was concentrated *in vacuo* and treated with 1 N HCl (3 mL). The white precipitate was washed with water to give **46a** as a white solid (0.19 g, 79%): mp 133−137 °C; $[\alpha]^{26}_D$ −8.4° (0.13, E). Anal. ($C_{15}H_{22}N_2O_2S_2$) C, H, N.

***N*-[3-Mercapto-2(*R*)-(phenylmethyl)-1-oxopropyl]-L-methioninamide (47a).** Benzoylthio amide **44a** (0.46 g, 1.1 mmol) was converted by the above method to **47a**, a white solid (0.30 g, 83%): mp 135−136 °C; $[\alpha]^{26}_D$ −66.3° (1.0, C). Anal. ($C_{15}H_{22}N_2O_2S_2$) C, H, N.

Method G. *N*-[3-(Acetylthio)-2(*S*)-(phenylmethyl)-1-oxopropyl]-*S*-[(4-methylphenyl)methyl]-L-cysteinamide (48h) and *N*-[3-(Acetylthio)-2(*R*)-(phenylmethyl)-1-oxopropyl]-*S*-[(4-methylphenyl)methyl]-L-cysteinamide (49h). A solution of acid **7f** (1.50 g, 6.3 mmol), HOBT (0.92 g, 6.8 mmol), and EDC (1.30 g, 6.8 mmol) in DMF (10 mL) was treated with *S*-[(4-methylphenyl)methyl]-L-cysteinamide (1.50 g, 6.7 mmol) and *N*-methylmorpholine (2.20 mL, 20 mmol). The mixture was stirred 20 h and concentrated *in vacuo*. The residue was partitioned between EtOAc and water. The dried ($MgSO_4$) EtOAc solution was concentrated *in vacuo* and chromatographed on Prep 500 silica gel (2 cartridges) (CH_2Cl_2−EtOAc, 9:1 (4 L); then 4:1) to give fraction 1, (0.02 g); fraction 2 (0.76 g); fraction 3 (0.20 g); and fraction 4, *N*-[3-(acetylthio)-2(*R*)-(phenylmethyl)-1-oxopropyl]-*S*-[(4-methylphenyl)methyl]-L-cysteinamide (**49h**) as an oil (0.76 g, 26%): $[\alpha]^{26}_D$ −1.6° (0.5, M). Anal. ($C_{23}H_{28}N_2O_3S_2$) C, H, N. Fraction 2 was chromatographed on silica gel to give fraction 2−5, *N*-[3-(acetylthio)-2(*S*)-(phenylmethyl)-1-oxopropyl]*S*-[(4-methylphe-

nyl)methyl]-L-cysteinamide (**48h**) as an oil (0.23 g, 7.6%): $[\alpha]^{26}_D$ −4.2° (0.13, M). Anal. ($C_{23}H_{28}N_2O_3S_2$) C, H, N.

Method H. *N*-[3-(Acetylthio)-2(*S*)-(phenylmethyl)-1-oxopropyl]-L-methioninamide (48a) and *N*-[3-(Acetylthio)-2(*R*)-(phenylmethyl)-1-oxopropyl]-L-methioininamide (49a). Acid chloride **35** (1.53 g, 5.97 mmol) was added dropwise to a solution of L-methioninamide (1.00 g, 5.43 mmol) and triethylamine (1.15 g, 11.40mmol) in acetonitrile (40 mL) and water (20 mL). After 1 h, 1 N hydrochloric acid (13 mL) was added, and the mixture was extracted with Et_2O. The dried ($MgSO_4$) Et_2O solution was concentrated *in vacuo* to give a yellow oil (1.98 g). Chromatography on a silica gel column (500 mL) (MeOH-CH_2Cl_2, 1:24) gave *N*-[3-(acetylthio)-2(*S*)-(phenylmethyl)-1-oxopropyl]-L-methioninamide (**48a**) as a white solid: (0.33 g, 17%) mp 149−151 °C; $[\alpha]^{26}_D$ -87.5° (0.5, C). Anal. ($C_{17}H_{24}N_2O_3S_2$) C, H, N] and *N*-[3-(acetylthio)-2(*R*)-(phenylmethyl)-1-oxopropyl]-L-methioninamide (**49a**) as a white solid (0.30 g, 15%): mp 119−121 °C; $[\alpha]^{26}_D$ +5.0° (0.5, C). Anal. ($C_{17}H_{24}N_2O_3S_2$) C, H, N.

The *N*-[2(*R*,*S*)-[(acetylthio)methyl-3-arylpropanoyl]-L-amino acid amides (**50c-g**) (Table 4) were prepared using method H.

Method J. *N*-[3-Mercapto-2(*S*)-(phenylmethyl)-1-oxopropyl]-L-methioninamide (51h). Amide **48h** (0.23 g, 0.6 mmol) was treated with methanol saturated with ammonia (100 mL) for 1 h. The reaction mixture was purged with nitrogen for 5 min, treated with 0.1 N HCl (200 mL), and extracted with EtOAc. The dried ($MgSO_4$) EtOAc was concentrated *in vacuo* to give **51h** as a white solid (0.18 g, 75%): mp 130−132 °C; $[\alpha]^{26}_D$ −4.2° (0.13, M). Anal. ($C_{21}H_{26}N_2O_2S_2$) C, H, N.

N-[3-Mercapto-2(*R*)-(phenylmethyl)-1-oxopropyl]-L-methioninamide (**52h**) (Table 4) was prepared similarly.

Biological Assays. All procedures in this study were preformed under protocols approved by the Schering-Plough Research Institute's Animal Care and Use Committee. Animals were treated as recommended by the *NIH Guide for the Care and Use of Laboratory Animals* and the Animal Welfare Act in a vivarium certified by the American Association of the Accredidation of Laboratory Animal Care.

***In Vitro* ACE-Inhibitory Activity.** The *in vitro* inhibitory activity was determined by the method of Cushman and Cheung.[26] The crude ACE was prepared as described.[42]

***In Vitro* NEP-Inhibitory Activity.** The effects of compounds were evaluated on purified rabbit kidney NEP EC 24.11 kindly provided by Dr. Phillip Crine (University of Montreal) using 10 mM [^{3}H]Leu-enkaphalin as a substrate according to the method decribed by Chipkin et al.[43] and Sybertz et al.[21]

Antihypertensive Activity in DOCA Rat. Male Spague−Dawley rats weighing 100−150 g were prepared, and DOCA-Na hypertension was induced by methods described by Sybertz *et al.*[21,22] Animals were studied 17−21 days after induction of the hypertension, a time at which the hypertension is well established. Animals with mean blood pressure >150 mmHg were used. Mean blood pressure was recorded after a 90-min equilibration period by the method of Baum.[44] Compounds for testing were administered subcutaneously in TRIS buffer (2 mL/kg) or orally as a solution or suspension in 0.4% aqueous methylcellulose solution (volume 4 mL/kg). Groups of four animals were employed, and maximum reduction in blood pressure over 4-h period was determined relative to vehicle-treated controls. DOCA-Na rats were nonfasted.

Potentiation of ANF Induced Hypotension. Male SHR (270−300 g) were anesthetized with ether, and the caudal artery was cannulated for direct measurement of blood pressure. A jugular vein was cannulated for iv administration of drugs. After a stabilization period of at least 90 min, animals had regained consciousness and were challenged with ANF 103−125 or 99−126, injected iv as a bolus at a dose of 30 mg/kg, both of which are submaximum with respect to lowering blood pressure in the SHR. At 60−90 min after this first challenge with ANF, the animals were dosed sc with drug or vehicle (0.2% aqueous methylcellulose with 20% Tris-HCl) and rechallenged with ANF 15 min later. Responses to this second challenge with ANF were compared in drug-treated and vehicle control animals according to the methods of Sybertz et al.[21] and references cited therein.

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