Synthesis of Novel Modified Dipeptide Inhibitors of Human Collagenase: β-Mercapto Carboxylic Acid Derivatives

Belle Beszant, John Bird, Laramie M. Gaster, Gregory P. Harper, Ian Hughes, Eric H. Karran, Roger E. Markwell,* Anette J. Miles-Williams, and Stephen A. Smith

SmithKline Beecham Pharmaceuticals, Discovery Research, Coldharbour Road, The Pinnacles, Harlow, Essex, CM19 5AD, U.K.

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The synthesis of a series of thiol-containing, modified dipeptide inhibitors (8) of human collagenase, which incorporate various carboxylic acid derivatives at the presumed P_1 position, β to the thiol group, is described. The compounds were evaluated, *in vitro*, for their ability to inhibit the degradation of rat skin type 1 collagen by purified human lung fibroblast collagenase, and structure–activity relationship studies are described. Optimum potency (IC_{50} values in the nanomolar range) was achieved by incorporating methyl (compounds **43a**, **56a**, and **57ab**) or benzyl esters (**44a**) at the P_1 position. Small amides were also accommodated (e.g. primary amide **47a**), but in general, increasing the size of the P_1 amide substituent lowered potency. PheNHMe, TrpNHMe, and Tyr(Me)NHMe substituents were found to be approximately equipotent P_2'-residues. The results of testing all four diastereoisomers **56a–d** of the compound with (*S*)-TrpNHMe at the P_2' position indicated that the *S,S,S* diastereoisomer **56a** possessed highest potency (IC_{50} 2.5 nM) and that the second most potent diastereoisomer was **56d** (IC_{50} 12 nM) with the *R,R,S* configuration. It appeared that the orientation of the P_1' and the thiol-bearing centers to each other is a more critical influence on potency than any absolute stereochemical requirements. It is suggested that the high potency of the β-mercapto carboxylic acid derivatives may be a consequence of bidentate coordination of the thiol and carbonyl groups to the active-site zinc ion in the collagenase enzyme.

Introduction

The matrix metalloproteinases (MMPs) are a family of zinc-containing enzymes that are capable of degrading all proteinaceous components of the extracellular matrix.[1] An important member of this family is mammalian collagenase (MMP-1),[2] which cleaves all three α-chains of native interstitial collagens at a unique cleavage site (either a Gly–Leu or Gly–Ile bond) to give characteristic one-quarter and three-quarter fragments.[3] The family also includes 72-kDa gelatinase (MMP-2)[4] and 92-kDa neutrophil gelatinase (MMP-9),[5] which cleave denatured collagen (gelatin) and basement membrane, and stromelysin/proteoglycanase (MMP-3),[6] which degrades a wider variety of protein substrates including gelatin, fibronectin, and laminin as well as the core protein of cartilage proteoglycans.

Matrix metalloproteinases are involved in tissue remodeling and connective tissue turnover. The two most important physiological inhibitors of this class of proteinases are believed to be the α_2-macroglobulins[7,8] and a family of tissue inhibitors of metalloproteinases (TIMPs).[9,10] An imbalance between proteinase synthesis and activation, on the one hand, and the local synthesis of endogenous inhibitors such as TIMP, on the other, is believed to be responsible for cartilage destruction in diseases such as rheumatoid arthritis and osteoarthritis.[11] As the loss of the collagen framework in bone and cartilage may be essentially irreversible, and the degradation of native collagen is believed to be mediated exclusively by collagenase, this latter MMP is likely to be the critical enzymic mediator of connective tissue destruction in the arthritides.

Administration of proteinaceous inhibitors such as TIMP may restore the imbalance resulting from overproduction of the proteolytic MMPs, but low molecular weight (MW) inhibitors are substantially more effective in penetrating cartilage than high MW proteinaceous inhibitors, which are excluded by virtue of their size.[12] Synthetic inhibitors of the tissue metalloproteinases, especially of collagenase, could therefore form a new class of antiarthritic agents that actually combat the destructive disease process itself. In addition, such inhibitors could be useful in other diseases in which excessive collagenolytic activity is a causative or contributory factor, such as tumor proliferation, corneal ulceration, and periodontal disease.[13]

The rational design of low MW collagenase inhibitors, based on the structure of the substrate cleavage site, has been reviewed,[11,13–15] the approach being similar to that used in the design of inhibitors of the zinc metalloenzyme angiotensin-converting enzyme (ACE). Previous studies have established that the preferred substituent at the P_1' position is an isobutyl group, whereas substituents with either moderately bulky alkyl, or arylalkyl, side chains are favored at the P_2' position. The addition of P_3' or P_4' residues has little beneficial effect on poteny *in vitro* and increases the MW of the inhibitors. Compounds with MW much above 500 often possess low oral bioavailability due to factors such as poor absorption in the gastrointestinal tract and extensive biliary excretion.[16]

Several potent collagenase inhibitors have been reported that contain a hydroxamic acid moiety, which is believed to bind in a bidentate manner to the active-site zinc ion. For example, the modified tripeptide 1 has an IC_{50} value of 8 nM against human synovial collagenase *in vitro*.[13] Various inhibitors possessing a thiol ligand have also been reported, but generally, these compounds are less potent. For example, compound 2 has a reported IC_{50} value of 360 nM.[17] Potency is increased by including bicyclic aromatic residues, such as tryptophan or 2'-naphthylalanine, at the

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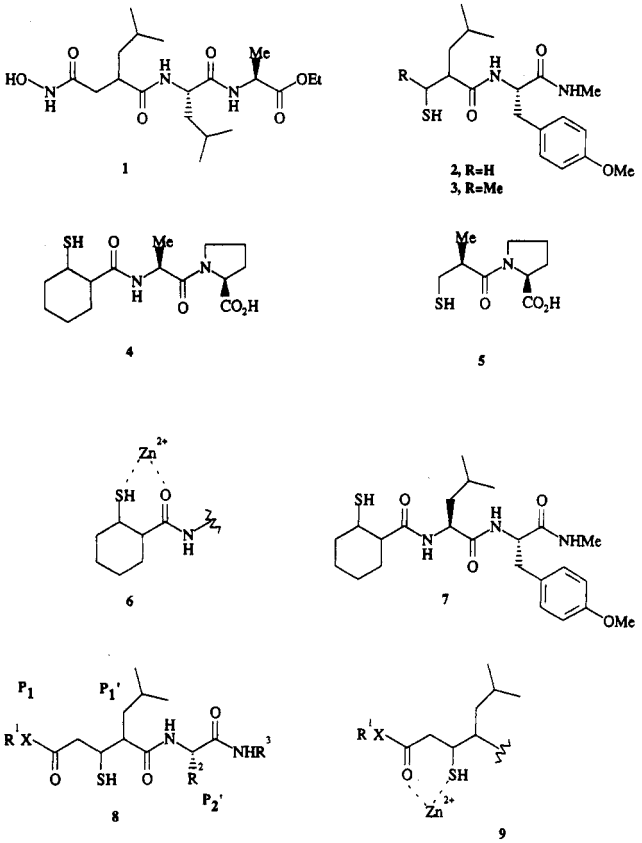
P_2' position.[18] Introduction of a methyl group α to the thiol ligand, as in compound **3**, also increases potency.[17]



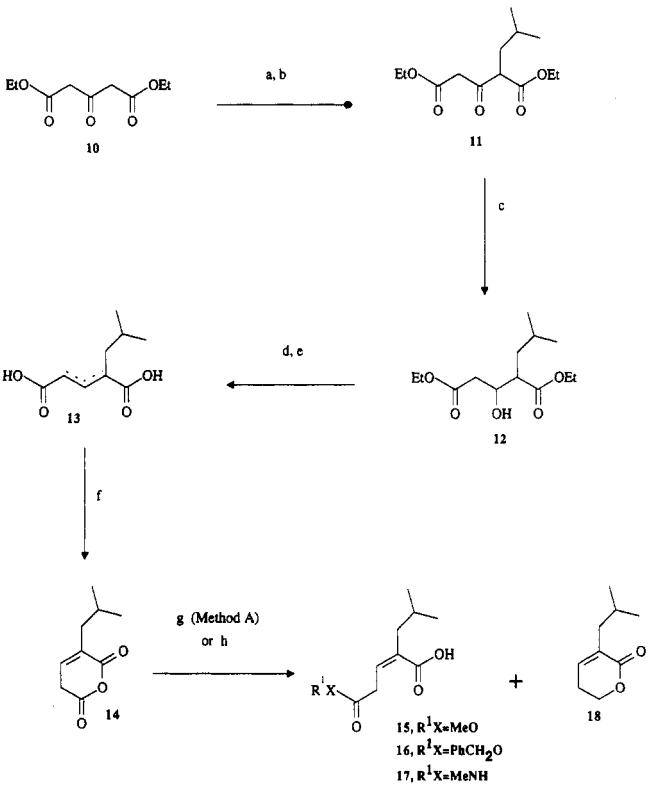
R^1X=RO, RNH, piperazinyl, RCOCHRNH (R=H or alkyl)
R^2=$ArCH_2$, (3-indolyl)CH_2, ($PhCH_2O$)CHMe
R^3=H, Me

Cyclic acyl thiol-containing dipeptides (**4**) have been designed[19] with *in vitro* ACE inhibitory potency nearly 10 times that of the first thiol-based inhibitor, captopril (**5**). The increase in potency observed for dipeptides **4** was hypothesized to result from bidentate binding, involving both thiol and amide carbonyl moieties (see structure **6**). Unfortunately, applying an identical approach to the design of collagenase inhibitors, i.e. thiol **7**, resulted in almost complete loss of activity,[20] and we therefore investigated alternative strategies to obtain thiol ligands with the potential for bidentate binding. One of our approaches was to add a carbonyl functionality to the methyl side chain of compound **3**,[21] and in this paper we describe the synthesis of a series of β-mercapto carboxylic acid derivatives **8**. The compounds were evaluated *in vitro* for their ability to inhibit the degradation of radiolabeled collagen by purified human lung fibroblast collagenase. Several compounds possess enzyme inhibitory potency in the nanomolar range and are considerably more potent than previously reported thiols, such as **3**. This increase in potency may be the result of bidentate coordination of the thiol and carbonyl groups to the active site zinc ion (see structure **9**) in a manner that is similar to that adopted by the hydroxamic acid ligand.

Chemistry

The pentenedioic acid monoesters **15** and **16** were key intermediates in the preparation of all the β-mercapto carboxylic acid derivatives described in this paper, and their synthesis is shown in Scheme I. Monoalkylation of

Scheme I[a]



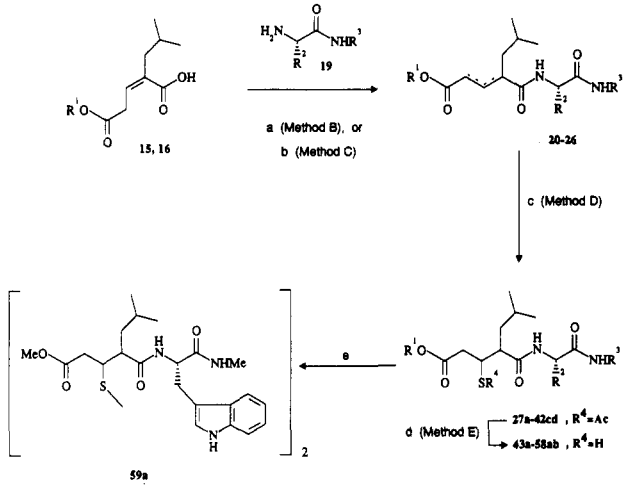
[a] (a) $Mg(OEt)_2$; (b) i-BuBr; (c) $NaBH_4$; (d) P_2O_5; (e) KOH; (f) Ac_2O; (g) MeOH or $PhCH_2OH$; (h) $MeNH_2$.

diethyl acetonedicarboxylate (**10**) was achieved using magnesium ethoxide as base,[22] giving ketone **11**, which was reduced with sodium borohydride to an inseparable mixture of diastereomeric alcohols **12**. Subsequent dehydration and hydrolysis gave the unsaturated diacid **13** as a mixture of regioisomers, which was cyclized to a single anhydride **14**.

The anhydride **14** was always accompanied by a smaller amount of the unsaturated lactone **18**, presumed to arise from overreduction of the keto diester **11**, a process that could not be fully suppressed. The unwanted lactone was most readily removed after reacting the mixture with an alcohol such as methanol or benzyl alcohol (method A), when the desired unsaturated acids **15** and **16** could be isolated by a base extraction. Similarly, the anhydride could be opened with methylamine to give amide **17**. However, the anhydride failed to react with the hindered alcohol *tert*-butyl alcohol.

The β-mercapto ester series was prepared from the acids **15** and **16** as shown in Scheme II. Coupling with a range of amino acid derivatives of natural *S* stereochemistry (**19**) (methods B and C) gave the unsaturated amides **20–25** (Table I), usually as mixtures of double-bond regioisomers. The *tert*-butyl ester **26** was prepared from the methyl ester **20** by base hydrolysis to the carboxylic acid, followed by reaction with isobutylene/sulfuric acid. Treatment of the unsaturated amides **20–26** with thiolacetic acid at room temperature for 20 days (method D) gave thioesters, e.g. **28** (Table II), as mixtures of four diastereoisomers in approximately 2:1:1:2 ratio, as shown by NMR. When the crude reaction mixture was evaporated to dryness and then subjected to silica gel column chromatography, it was found that the thioester corresponding to the most potent thiol diastereoisomer (see below) always eluted first. Although it was usually

Scheme II[a]

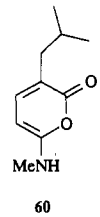


[a] (a) DCC; (b) Im_2CO; (c) AcSH; (d) NH_3/MeOH; (e) I_2/MeOH.

contaminated with a small quantity of one of the other diastereoisomers, recrystallization or trituration of this material usually gave a single diastereoisomer with purity >95% by NMR analysis. In some cases, it was possible to isolate one or more of the other diastereoisomers by chromatography (sometimes HPLC). In the case of thioester **40**, the diastereoisomer **40d** was shown[23] by X-ray crystallography to have *R,R* stereochemistry at the newly created chiral centers.

The free thiols (**43a–58cd**) (Table III) were generated by treatment of the corresponding thioesters (**27a–42cd**) with ammonia in methanol (method E) and were found to be stable toward air oxidation at neutral pH during preparation and storage, although the symmetrical disulfide **59a** could be prepared by treatment of thiol **56a** with iodine in methanol.[24] It was usually possible to obtain thiols as single diastereoisomers (>98% purity by NMR and HPLC analysis) following recrystallization or trituration of the impure reaction product. In the case of thiol **56**, diastereoisomer **56a** was shown[23] by X-ray crystallography to have the *S,S,S* stereochemistry. Single diastereoisomers could be readily distinguished from one another by ^{1}H-NMR. In particular, the SH proton doublets ($CDCl_3$; J = 9–10 Hz) of individual isomers were well separated in the NMR spectra, with the SH signal of the most potent diastereoisomer appearing at highest field.

It was hoped to prepare β-mercapto amides (e.g. **48a**) from the monoamide **17** in an analogous manner to the ester series. However, activation of the acid group prior to the coupling reaction caused an intramolecular cyclization reaction, giving the unstable aminopyrone **60**.



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An alternative route (Scheme III) was developed from the isomerically pure benzyl ester **44a**. Reacylation, followed by catalytic transfer hydrogenation over palladium black,[25] gave the carboxylic acid **30a**, which could be coupled to a variety of amines to give, after removal of the *S*-acetyl group of compounds (**31a–38a**), a series of β-mercapto amides (**47a–54a**). The β-mercapto carboxylic acid **46a** was obtained directly from the thioester **30a**. The carboxylic acids **50a** and **53a** were prepared from the corresponding *tert*-butyl esters **33a** and **36a** by treatment with trifluoroacetic acid (method H), followed by removal of the *S*-acetyl protecting groups with ammonia/methanol.

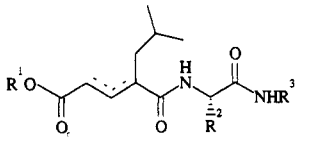
Results and Discussion

Compounds were evaluated for their ability to inhibit the degradation of radiolabeled rat skin type I collagen by purified human lung fibroblast collagenase. The inhibitory activities (IC_{50} values) for the test compounds are shown in Table III. When potent thiol-based inhibitors were tested at low concentrations in the *in vitro* assay, which involved incubations for 1000 min at 37 °C, we were concerned that oxidation of the thiols to inactive disulfides could occur, leading to erroneously low values for inhibitor potency. This problem has been discussed previously for a related series of thiol-based collagenase inhibitors, which had half-lives of only 30 min in a similar assay system.[18] We found that β-mercaptoethanol (at 10^{-4} M or less) had only a minimal effect on the degradation of collagen by collagenase and could be included in the assay buffers to prevent oxidation of the inhibitors and improve reproducibility. The following discussion refers to IC_{50} values generated in the presence of β-mercaptoethanol. A decrease in potency of up to 30-fold was observed for representative β-mercapto carbonyl compounds when tested in the absence of β-mercaptoethanol (see Table III). The Searle thiol-based collagenase inhibitor **3**[17] containing a P_2' Tyr(Me)NHMe moiety was used as a reference standard in our *in vitro* assay and gave an IC_{50} value of 17 nM.

In the initial series of β-mercapto carboxylic acid derivatives synthesized, the P_2' group was kept constant as Tyr(Me)NHMe, and the effect of varying the P_1 substituent was investigated. The free thiol **43a**, containing a methoxycarbonyl P_1 substituent, had an IC_{50} value of 3.1 nM. This carboxylic ester substituent, therefore, enhanced potency approximately 6-fold over the standard **3**. Its thioester precursor **27a** was about 12-fold less potent, presumably reflecting partial hydrolysis to free thiol during the assay incubation period. We have observed that the thioesters in this study are generally 10–100-fold less potent than their parent free thiols *in vitro* (compare also **40a** with **56a**). Replacement of the methyl ester substituent in **43a** by a benzyl ester (**44a**) enhanced the potency slightly, while the sterically hindered *tert*-butyl ester (**45a**) was somewhat less potent. The results of testing the four diastereoisomers (**44a–d**) of the benzyl ester demonstrated that diastereoisomer **44a**, assumed to have the *S,S,S* configuration by comparison to **56a** (see below), was the most potent. The other diastereoisomers were 20–2500 times less active.

Both the carboxylic acid **46a** and the primary amide **47a** were about 10-fold less potent than the esters **43a** and **44a**. Extension of the P_1 substituent to incorporate larger amide groups (compounds **48a–54a**) resulted in considerable loss of potency. The least potent inhibitor was the amide **52a** (IC_{50} 980 nM), derived from L-Leu(O-t-Bu). It is noteworthy that the primary amide **47a** retained reasonable potency (IC_{50} 23 nM), since inhibitors of this structural type may have enhanced stability *in vivo* over compounds such as **43a** having a potentially more metabolically labile ester functionality.

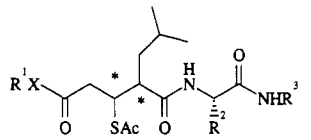
Table I. Physical Data for Unsaturated Amide Intermediates



compd no.	R^1	R^2	R^3	method[a]	yield, %	formula[b]
20	Me	4-MeOPhCH$_2$	Me	B	53	$C_{21}H_{30}N_2O_5$
21	PhCH$_2$	4-MeOPhCH$_2$	Me	C	92	$C_{27}H_{34}N_2O_5$
22	Me	PhCH$_2$	Me	C	69	$C_{20}H_{28}N_2O_4$
23	Me	(*R*)-PhCH$_2$OCHMe	Me	C	59	$C_{22}H_{32}N_2O_5$
24	Me	(3-indolyl)CH$_2$	Me	C	80	$C_{22}H_{29}N_3O_4$
25	Me	PhCH$_2$	H	C	35	$C_{19}H_{26}N_2O_5$
26	t-Bu	4-MeOPhCH$_2$	Me	—[c]	41	$C_{24}H_{36}N_2O_5$

[a] See the text. [b] Elemental analyses for C, H, and N were within 0.4% of the theoretical values. [c] See the Experimental Section.

Table II. Physical Data for Thioesters



compd	R^1X	R^2	R^3	chirality[a]	method[b]	yield, %	mp, °C	crystn solvent	formula[c]
27a	MeO	4-MeOPhCH$_2$	Me	*S,S*	D	HPLC	76–79	EtOAc/hexane	$C_{23}H_{34}N_2O_6S$
28	PhCH$_2$O	4-MeOPhCH$_2$	Me	4 (2:1:1:2)	D	62	foam	–	$C_{29}H_{38}N_2O_6S$
28a	PhCH$_2$O	4-MeOPhCH$_2$	Me	*S,S*	—[d]	65	89–90	Et$_2$O	$C_{29}H_{38}N_2O_6S$
29a	t-BuO	4-MeOPhCH$_2$	Me	*S,S*	D	4	147–151	Et$_2$O/pentane	$C_{26}H_{40}N_2O_6S$
30a	HO	4-MeOPhCH$_2$	Me	*S,S*	—[d]	77	92–95	Et$_2$O[e]	$C_{22}H_{32}N_2O_6S{\cdot}H_2O$
31a	H_2N	4-MeOPhCH$_2$	Me	*S,S*	F	23	235–240	Et$_2$O[e]	$C_{22}H_{33}N_3O_5S$
32a	MeNH	4-MeOPhCH$_2$	Me	*S,S*	F	24	195–199	Et$_2$O[e]	$C_{23}H_{35}N_3O_5S$
33a	t-BuO$_2$CCH$_2$NH	4-MeOPhCH$_2$	Me	*S,S*	G	65	192–194	EtOAc	$C_{28}H_{43}N_3O_7S$
34a	HO$_2$CCH$_2$NH	4-MeOPhCH$_2$	Me	*S,S*	H	89	228–231	Et$_2$O[e]	$C_{24}H_{35}N_3O_7S{\cdot}0.5H_2O$
35a	H$_2$NCOCH$_2$NH	4-MeOPhCH$_2$	Me	*S,S*	G	30	218–219	MeOH/Et$_2$O	$C_{24}H_{36}N_4O_6S{\cdot}0.5H_2O$
36a	t-BuO$_2$CCH(CH$_2$CHMe$_2$)NH	4-MeOPhCH$_2$	Me	*S,S*	G	67	148–157	Et$_2$O[e]	$C_{32}H_{51}N_3O_7S$
37a	HO$_2$CCH(CH$_2$CHMe$_2$)NH	4-MeOPhCH$_2$	Me	*S,S*	H	93	195–200	H$_2$O/MeOH	$C_{28}H_{43}N_3O_7S{\cdot}0.5H_2O$
38a	4-Me-piperazinyl	4-MeOPhCH$_2$	Me	*S,S*	G	37	133–138	EtOAc/hexane	$C_{27}H_{42}N_4O_5S$
39ab	MeO	PhCH$_2$	Me	2 (3:1)	D	25	188–189	EtOAc	$C_{22}H_{32}N_2O_5S$
40a	MeO	(3-indolyl)CH$_2$	Me	*S,S*	D	19	134–136	EtOAc/Et$_2$O	$C_{24}H_{33}N_3O_5S$
40c	MeO	(3-indolyl)CH$_2$	Me	1	D	8.5	197–201	EtOAc	$C_{24}H_{33}N_3O_5S$
40d	MeO	(3-indolyl)CH$_2$	Me	*R,R*	D	7.5	115–117	MeOH/CH$_2$Cl$_2$	$C_{24}H_{33}N_3O_5S$
41ab	MeO	(*R*)-(PhCH$_2$O)CHMe	Me	2 (3:2)	D	33	105–106	Et$_2$O/pentane[e]	$C_{24}H_{36}N_2O_6S$
41cd	MeO	(*R*)-(PhCH$_2$O)CHMe	Me	2 (2:1)	D	40	111–113	Et$_2$O/pentane[e]	$C_{24}H_{36}N_2O_6S$
42ab	MeO	PhCH$_2$	H	2 (4:1)	D	35	160–164	EtOAc/pentane[e]	$C_{21}H_{30}N_2O_5S$
42cd	MeO	PhCH$_2$	H	2 (3:1)	D	24	50–54	Et$_2$O[e]	$C_{21}H_{30}N_2O_5S{\cdot}0.5H_2O$

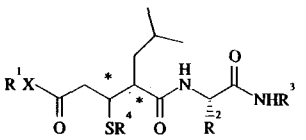
[a] Where known, *S,S* and *R,R* refer to the centers indicated by asterisks, otherwise the number of isomers and their ratio is given. [b] See the text. [c] See Table I, footnote *b*. [d] See the Experimental Section. [e] Trituration solvent.

Aromatic L-amino acids are known to be favored residues at the P_2' position, both of a related series of thiol-based collagenase inhibitors[18] and of collagenase substrates.[3] The effect of replacing the P_2' Tyr(Me)NHMe residue by related aromatic moieties was therefore investigated for the present series of compounds, while the P_1 substituent was kept constant as the methyl ester. Compounds were prepared with PheNHMe (**55a**), TrpNHMe (**56a**), and Thr(OBzl)NHMe (**57ab**; mixture of two diastereoisomers) P_2' residues, and these compounds were approximately equipotent with the original Tyr(Me)NHMe analogue **43a**, with the TrpNHMe analogue **56a** showing slight enhancement of potency when the compounds were tested together in the same assay. In a less potent series of thiol-based collagenase inhibitors, Darlak *et al.*[18] found Trp to be approximately twice as potent as Phe at the P_2' position. Darlak *et al.*[18] also observed that tripeptide inhibitors with P_2'–P_3' substituents (e.g. PheAlaNH$_2$) were up to 10 times more potent than the corresponding dipeptides bearing a primary amido-P_2' moiety (e.g. PheNH$_2$). We found that the P_2' PheNHMe analogue **55a** (IC_{50} 2 nM) was 8 times more potent than the corresponding P_2' PheNH$_2$ compound **58a** (IC_{50} 16 nM).

The disulfide **59a** of the potent free thiol **56a**, with a P_2' TrpNHMe residue, exhibited only weak inhibitory activity (IC_{50} 1400 nM) when tested in the *in vitro* assay in the absence of β-mercaptoethanol, but in the presence of β-mercaptoethanol, potency was considerably enhanced (IC_{50} 22 nM). Although the full potency of the parent thiol was not recovered, this result supports our contention that the increase in *in vitro* potency of the thiol-based inhibitors caused by β-mercaptoethanol is a consequence of the prevention or reversal of oxidation to their much less active disulfides. Formation of disulfides need not be a disadvantage *in vivo* since they are likely to provide a reservoir from which the active drug can be released, thus prolonging duration of action. Indeed, this has been shown to be the case with the ACE inhibitor captopril.[26]

The results of testing all four diastereoisomers **56a–d** of the compound with (*S*)-TrpNHMe at the P_2' position mirrored the data for the series **44a–d**, indicating that the *S,S,S* diastereoisomer **56a** possessed the highest potency

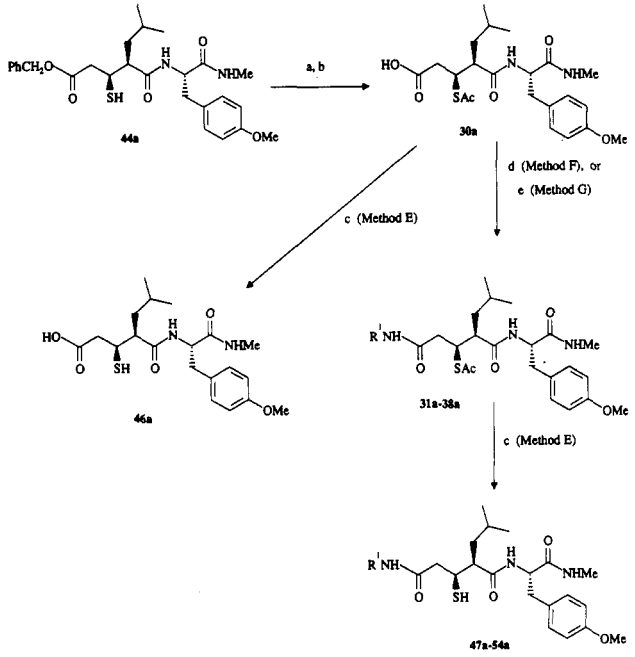
Table III. Physical Data and Inhibitory Potency of Thiols and Related Compounds



compd	R^1X	R^2	R^3	R^4	chirality[a]	yield of thiol, %	SH shift, δ ($CDCl_3$)	mp, °C	crystn solvent	$[\alpha]^{20}_D$, deg (solvent)	formula[b]	IC_{50}, nM (*n*)[c]
43a	MeO	4-MeOPhCH$_2$	Me	H	*S,S*	75	1.67	193–196	H$_2$O[d]	−8 (MeOH)	$C_{21}H_{32}N_2O_6S$	3.1 ± 1.2 (6)
27a	MeO	4-MeOPhCH$_2$	Me	COMe	*S,S*	–	–	76–79	EtOAc/hexane		$C_{23}H_{34}N_2O_6S$	44
44a	PhCH$_2$O	4-MeOPhCH$_2$	Me	H	*S,S*	33	1.66	199–201	MeOH/Et$_2$O	−23 ($CHCl_3$)	$C_{27}H_{32}N_2O_6S$	1.8 ± 0.6 (20) 68 ± 22 (5)[e]
44b	PhCH$_2$O	4-MeOPhCH$_2$	Me	H	1	11[f]	1.99	gum		−3 ($CHCl_3$)	$C_{27}H_{32}N_2O_6S$	4500 ± 3500 (2)
44c	PhCH$_2$O	4-MeOPhCH$_2$	Me	H	1	HPLC[f]	2.09	foam			$C_{27}H_{32}N_2O_6S$[g]	980
44d	PhCH$_2$O	4-MeOPhCH$_2$	Me	H	*R,R*	21[f]	1.90	133–135	Et$_2$O[d]	+53 (MeOH)	$C_{27}H_{32}N_2O_6S$	34 ± 14 (2)
45a	t-BuO	4-MeOPhCH$_2$	Me	H	*S,S*	71	1.65	133–137	Et$_2$O/hexane		$C_{24}H_{32}N_2O_6S$	10
46a	HO	4-MeOPhCH$_2$	Me	H	*S,S*	69	1.79	128–130	EtOAc		$C_{20}H_{28}N_2O_6S$	25
47a	H_2N	4-MeOPhCH$_2$	Me	H	*S,S*	89	–[h]	221–225	hexane[d]		$C_{20}H_{31}N_3O_6S$-0.5H$_2$O	23 ± 4.5 (2)
48a	MeNH	4-MeOPhCH$_2$	Me	H	*S,S*	64	1.74	243–250	H$_2$O[d]		$C_{21}H_{32}N_2O_6S$	145 ± 25 (2)
49a	t-BuO$_2$CCH$_2$NH	4-MeOPhCH$_2$	Me	H	*S,S*	83	2.24	223–226	hexane[d]		$C_{28}H_{41}N_3O_8S$	280
50a	HO$_2$CCH$_2$NH	4-MeOPhCH$_2$	Me	H	*S,S*	80	–[h]	228–231	EtOAc[d]		$C_{23}H_{33}N_3O_8S$[i]	27 ± 15 (2)
51a	H$_2$NCOCH$_2$NH	4-MeOPhCH$_2$	Me	H	*S,S*	70	–[h]	247–252	Et$_2$O[d]		$C_{23}H_{34}N_4O_7S$	32 ± 17 (2)
52a	t-BuO$_2$CCH(CH$_2$CHMe$_2$)NH	4-MeOPhCH$_2$	Me	H	*S,S*	52	2.18	211–215	Et$_2$O/hexane[d]		$C_{30}H_{46}N_2O_8S$	980 ± 130 (2)
53a	HO$_2$CCH(CH$_2$CHMe$_2$)NH	4-MeOPhCH$_2$	Me	H	*S,S*	93	–[h]	216–220	Et$_2$O[d]		$C_{28}H_{41}N_3O_8S$[j]	830 ± 270 (2)
54a	4-Me-piperazinyl	4-MeOPhCH$_2$	Me	H	*S,S*	60	1.85	65–68	hexane[d]		$C_{25}H_{40}N_4O_6S$-H$_2$O	120
55a	MeO	PhCH$_2$	Me	H	*S,S*	28	1.62	169–171	EtOAc/Et$_2$O		$C_{20}H_{30}N_2O_6S$	2.0 ± 0.1 (2)
56a	MeO	(3-indolyl)CH$_2$	Me	H	*S,S*	57	1.67	73–75	Et$_2$O[d]	−33 (MeOH)	$C_{22}H_{31}N_3O_6S$	2.5 ± 0.5 (14) 21 ± 5.8 (3)[e]
56b	MeO	(3-indolyl)CH$_2$	Me	H	1	11[f]	1.98	foam		+6 (MeOH)	$C_{22}H_{31}N_3O_6S$	810 ± 390 (2)
56c	MeO	(3-indolyl)CH$_2$	Me	H	1	91	2.05	130–136	EtOAc/Et$_2$O	−57 (MeOH)	$C_{22}H_{31}N_3O_6S$	730 ± 480 (2)
56d	MeO	(3-indolyl)CH$_2$	Me	H	*R,R*	77	1.92	foam		−19 (MeOH)	$C_{22}H_{31}N_3O_6S$	12 ± 4.2 (4)
40a	MeO	(3-indolyl)CH$_2$	Me	COMe	*S,S*	–	–	134–136	EtOAc/Et$_2$O		$C_{24}H_{33}N_3O_7S$	19 ± 7.5 (4)
59a	MeO	(3-indolyl)CH$_2$	Me	disulfide	*S,S*	–	–	92–98	EtOAc/Et$_2$O		$C_{44}H_{60}N_6O_8S_2$	22 ± 6.6 (3) 1400 ± 660 (3)[e]
57ab	MeO	(*R*)-(PhCH$_2$O)CHMe	Me	H	2 (3:2)	83	1.90, 2.08	113–119	H$_2$O[d]		$C_{22}H_{34}N_2O_6S$-0.5H$_2$O	3.0 ± 0.6 (2)
57cd	MeO	(*R*)-(PhCH$_2$O)CHMe	Me	H	2 (2:1)	72	2.0, 2.2	72–76	Et$_2$O[d]		$C_{22}H_{34}N_2O_6S$	13
58a	MeO	PhCH$_2$	H	H	*S,S*	50	1.62	182–184	EtOAc/Et$_2$O		$C_{19}H_{28}N_2O_6S$-0.5H$_2$O	16 ± 8.5 (2)
58cd	MeO	PhCH$_2$	H	H	2 (2:1)	53	1.91, 2.06	64–66	Et$_2$O[d]		$C_{19}H_{28}N_2O_6S$[k]	190 ± 160 (2)
3	–	–	–	–	–	–	–	–	–	–	–	17 ± 7.5 (2)

[a] See Table II, footnote *a*. [b] Elemental analyses for C, H, and N were within 0.4% of the theoretical values, except where characterized by high resolution MS or otherwise indicated. [c] Values are mean ± SEM of number of experiments indicated in parentheses and were obtained in the presence of β-mercaptoethanol unless indicated. [d] Trituration solvent. [e] Values obtained in the absence of β-mercaptoethanol. [f] See the Experimental Section. [g] C: calcd 64.77; found 64.36. [h] Insoluble in $CDCl_3$. [i] MS (FAB) *m/z* 468.2174 (MH^+ requires 468.2168). [j] MS (FAB) *m/z* 524.2836 (MH^+ requires 524.2794). [k] N: calcd 7.36; found 6.59; MS *m/z* 380.1770 (M^+ requires 380.1770).

Scheme III[a]



[a] (a) Ac_2O; (b) Pd black; (c) NH_3/MeOH; (d) EtO_2CCl/R^1NH_2; (e) EDC/R^1NH_2.

(IC_{50} 2.5 nM) and that the second most potent diastereoisomer was **56d** (IC_{50} 12 nM) with the *R,R,S* configuration. Thus, in this series, there is approximately 5-fold selectivity in favor of the *S,S,S* diastereoisomer possessing the natural configuration at the P_1' Leu center. This is consistent with the findings of the Searle group for isomers of thiol **3**[17] but contrasts with the situation in a series of thiol-based inhibitors lacking a P_1 side chain, in which the unnatural (*R* configuration) is preferred at the P_1' Leu center.[27] In the present series, there appears to be an optimum *relative* stereochemistry at the P_1' and the thiol-bearing centers (i.e. *S,S* and *R,R* are more potent than *R,S* and *S,R*), which dominates any requirement for a specific absolute stereochemistry at the P_1' side chain.

In summary, we have shown that the incorporation of carboxylic acid derivatives (especially esters and small amides) at the P_1-position, β to the thiol group in a series of modified dipeptides, results in collagenase inhibitors with nanomolar potency *in vitro*. This high potency may be a consequence of additional hydrogen bonding of the P_1 carbonyl group to a group situated at the S_1 site of the enzyme, or alternatively, the thiol and carbonyl groups may participate in bidentate coordination to the active-site zinc ion in the collagenase enzyme (**9**), in a similar manner to that postulated for the hydroxamic acid ligand.[13] Several of the compounds of the present series inhibit collagenolysis *in vivo*, and the results will be reported in due course.

Experimental Section

Melting points were determined on a Buchi 510 apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 197 as Nujol mulls unless otherwise stated. Proton magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AC250 or JEOL GX270 at 250 or 270 MHz, respectively, using Me_4Si as an internal standard. Mass spectra (MS) data were obtained on a JEOL DX303 using electrical or chemical (NH_3) ionization procedures, or fast atom bombardment (FAB) using glycerol as the matrix. Elemental analyses were within 0.4% of the theoretical values unless otherwise stated. All evaporations of solvents were carried out under reduced pressure. Unless otherwise stated, organic solutions were dried over Na_2SO_4. For column chromatography, the silica gel used was Merck Kieselgel 60. Brine refers to saturated sodium chloride solution.

Collagenase Inhibitor Assay. The test is essentially as described by Cawston and Barrett.[28] The test compounds were dissolved in MeOH by sonication and then were serially diluted as necessary. Trypsin-activated, semipurified human collagenase was obtained from culture supernatants of the WI-38 human lung fibroblast cell line and was added together with diluent/buffers. In some experiments, to ensure that the thiol compounds remained unoxidized, β-mercaptoethanol was incorporated in the MeOH solvent and/or diluent buffers to give a final concentration of 9.6×10^{-5} M. The minimal direct effect of β-mercaptoethanol at this concentration on the degradation of collagen by human collagenase was controlled for. Assay tubes were cooled to 4 °C, and either ^{3}H- or ^{14}C-acetylated rat skin type 1 collagen (100 μg/tube) was added. The choice of radiolabel did not alter the ability of collagenase to degrade the collagen substrate. Following incubation of the assay tubes at 37 °C for 1000 min, the tubes were centrifuged at 12 000 rpm for 15 min at 4 °C. Undigested radiolabeled collagen was pelleted, while digested radiolabeled collagen fibrils were found as soluble peptides in the supernatant. Aliquots of the supernatant were taken for liquid scintillation counting. A collagenase standard curve demonstrated a linear relationship between enzyme concentration and collagen degradation up to 70% total collagen degraded. For inhibitor assays, an amount of enzyme was added such that 70% of the total collagen would be degraded during the course of the assay. Different preparations of collagenase and collagen were tested to ensure assay comparability. The activity of the test compounds (IC_{50}) is expressed as that concentration of compound that inhibited a known concentration of enzyme by 50%.

Diethyl 2-(2-Methylpropyl)-3-oxopentanedioate (11). Diethyl 3-oxopentanedioate (**10**) (45.5 mL, 0.25 mol) was added to a suspension of magnesium ethoxide [prepared from magnesium turnings (9 g, 0.375 mol) and I_2 (0.1 g) in dry EtOH (275 mL)], and the mixture was heated under reflux for 90 min. 1-Bromo-2-methylpropane (54 mL, 0.5 mol) was added, and the reaction mixture was heated under reflux for 16 h. Further 1-bromo-2-methylpropane (27 mL, 0.25 mol) was added to the boiling mixture, and after 3 h it was evaporated to dryness. The product was partitioned between 2 N HCl and Et_2O. The organic layer was washed successively with water, 5% $NaHCO_3$, water, and brine, dried, and evaporated to dryness. The residue was distilled to yield **11** as a colorless oil (40 g, 62%): bp 118–120 °C (1 mmHg); ^{1}H NMR ($CDCl_3$) δ 0.9 (6 H, d, J = 6 Hz), 1.25 (6 H, t, J = 7 Hz), 1.4–1.9 (3 H, m), 3.5 (2 H, s), 3.7 (1H, d, J = 9 Hz), 4.2 (4 H, q, J = 7 Hz), MS m/z 259 (MH^+). Anal. ($C_{13}H_{22}O_5$) C, H.

Diethyl 3-Hydroxy-2-(2-methylpropyl)pentanedioate (12). Sodium borohydride (1.1 g, 29 mmol) was added to an ice-cold solution of **11** (7.5 g, 29 mmol) in EtOH (75 mL). The mixture was stirred at 5–10 °C for 2 h, and then 2 N HCl (18 mL) was added slowly at 0 °C. The mixture was stirred at room temperature for 45 min, and then it was extracted with EtOAc (4 × 100 mL). The organic fraction was washed with water and brine, dried, and evaporated to dryness to give **12** as an oil (7.5 g, 100%): ^{1}H NMR ($CDCl_3$) δ 0.9 (6 H, d, J = 5 Hz), 1.15 (6 H, t, J = 7 Hz), 1.1–1.8 (3 H, m), 2.2–2.7 (3 H, m), 3.3 (1 H, br s), 3.7–4.1 (1 H, m), 4.1 (4 H, q, J = 7 Hz); MS m/z 261 (MH^+). Anal. ($C_{13}H_{24}O_5$) C, H.

2-(Methylpropyl)pent-2-(and 3-)enedioic Acids (13). Diethyl 3-hydroxy-2-(2-methylpropyl)pentanedioate (**12**) (26 g, 100 mmol) was added to a stirred suspension of P_2O_5 (21.3 g, 150 mmol) in dry benzene (220 mL), and the mixture was heated under reflux for 3 h. Water (200 mL) was added to the cooled mixture, and the aqueous phase was extracted with Et_2O (4 × 150 mL). The organic fraction was washed successively with water and brine and then dried and evaporated to dryness to leave a brown oil. The oil was dissolved in a solution of KOH (16.8 g, 300 mmol) in 80% EtOH (300 mL) and then heated under reflux for 3 h. The EtOH was evaporated, and the residue was diluted with water (400 mL) and washed with Et_2O. The aqueous fraction was acidified with 5 N HCl and extracted with Et_2O (4 × 150 mL). The combined extracts were washed with brine, dried, and evaporated to dryness to afford an approximately

1:1 mixture of acids (13) as an oil (14.3 g, 77%) which slowly solidified and was used in the following stage without separation: ^{1}H NMR (d_6-DMSO) δ 0.9 (6 H, d, J = 6 Hz), 1.3–2.0 (2 H, m), 2.05 (1 H, d, J = 7 Hz), 3.2 (1 H, d, J = 7 Hz), 3.4 (0.5 H, m), 5.85 (0.5 H, d, J = 15 Hz), 6.7 (0.5 H, dd, J = 15, 7 Hz), 6.8 (0.5 H, t, J = 7 Hz); MS m/z 187 (MH^+). Anal. ($C_9H_{14}O_4$) C, H.

2-(2-Methylpropyl)pent-2-enedicarboxylic Anhydride (14). The mixture of carboxylic acids (13) (14.3 g, 77 mmol) in Ac_2O (40 mL) was heated under reflux for 2.5 h and evaporated to dryness. The product was distilled, bp 145–155 °C (5 mmHg) to afford 14 as an unstable low-melting yellow solid (5.8 g, 45%): ^{1}H NMR ($CDCl_3$) δ 0.9 (6 H, d, J = 6 Hz), 1.5–2.4 (3 H, m), 3.5 (2 H, br d, J = 4 Hz), 6.5 (1 H, t, J = 4 Hz); MS m/z 168 (M^+). Anal. ($C_9H_{12}O_3$) C, H. When carried out on a larger scale, this reaction frequently yielded a mixture of the anhydride 14 and lactone 18. The separation of the lactone is described below.

Method A. 4-(Methoxycarbonyl)-2-(2-methylpropyl)but-2-enoic Acid (15). A solution of 14 (30 g, 180 mmol) in MeOH (200 mL) was heated under reflux under N_2 for 2 h and evaporated to dryness. The product was dissolved in Et_2O and extracted with 10% Na_2CO_3. The aqueous fraction was washed with Et_2O, and the combined organic fractions were worked up to give the lactone 18 (14.5 g, 52%): bp 110–120 °C (0.8 mmHg); IR (film) 1725 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.85 (6 H, d, J = 7 Hz), 1.85 (1 H, m), 2.17 (2 H, d, J = 8 Hz), 2.43 (2 H, m), 4.37 (2 H, t, J = 7 Hz), 6.60 (1 H, t, J = 5 Hz); MS m/z 154 (M^+). Anal. ($C_9H_{14}O_2$) C, H. The base extracts were acidified with 5 N HCl and reextracted with Et_2O. The organic fraction was washed with brine, dried, and evaporated to dryness to afford 15 as a pale yellow solid (14.8 g, 41%, 86% based on recovered lactone): mp 40–45 °C (hexane); IR 1735, 1690, 1635 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.9 (6 H, d, J = 6 Hz), 1.4–2.0 (1 H, m), 2.2 (2 H, d, J = 6 Hz), 3.55 (2 H, d, J = 7 Hz), 3.70 (3 H, s), 6.2 (1 H, t, J = 7 Hz), 9.6 (1 H, br s); MS m/z 201 (MH^+). Anal. ($C_{10}H_{16}O_4$) C, H.

4-(Benzyloxycarbonyl)-2-(2-methylpropyl)but-2-enoic Acid (16). A solution of 14 (63.6 g, 380 mmol) and benzyl alcohol (33.1 mL, 320 mmol) in dry toluene (420 mL) was heated under reflux for 2 h and then evaporated to dryness and worked up in a similar manner to 15 to give 16 as a pale yellow solid (70.7 g, 67%): mp 72–74 °C (Et_2O/pentane); ^{1}H NMR ($CDCl_3$) δ 0.88 (6 H, d, J = 7 Hz), 1.80 (1 H, m), 2.18 (2 H, d, J = 8 Hz), 3.70 (2 H, d, J = 8 Hz), 5.15 (2 H, s), 6.30 (1 H, t, J = 6 Hz), 7.34 (5 H, s); MS m/z 277 (MH^+). Anal. ($C_{16}H_{20}O_4$) C, H.

Method B. 6-Methyl-4-[[[1(*S*)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]hept-3-enoic Acid, Methyl Ester (20). *N*,*N*-Dicyclohexylcarbodiimide (0.52 g, 2.5 mmol) was added to a stirred solution of the carboxylic acid 15 (0.5 g, 2.5 mmol) and *O*-methyl-L-tyrosine *N*-methylamide (0.52 g, 2.5 mmol) in dry CH_2Cl_2 (30 mL) at 0 °C, the mixture was allowed to warm up to room temperature, and stirring was continued overnight. The precipitated solid was filtered off, and the filtrate was washed successively with 1 N HCl, 10% $NaHCO_3$, and water. It was dried over $MgSO_4$ and evaporated to dryness. The product was purified by column chromatography on silica gel, eluting with MeOH/CH_2Cl_2 (1:20), to afford 20 which was crystallized from CH_2Cl_2/pentane as a white crystalline solid (0.52 g, 53%): mp 142–144 °C; ^{1}H NMR ($CDCl_3$) δ 0.82 (3 H, d, J = 3 Hz), 0.85 (3 H, d, J = 3 Hz), 1.57 (1 H, m), 2.17 (2 H, d, J = 7 Hz), 2.73 (3 H, d, J = 5 Hz), 3.00 (2 H, m), 3.17 (2 H, d, J = 8 Hz), 3.72 (3 H, s), 3.78 (3 H, s), 3.58 (1 H, q, J = 7 Hz), 5.85 (1 H, br m), 6.32 (1 H, t, J = 8 Hz), 6.45 (1 H, br d), 6.83 (2 H, d, J = 9 Hz), 7.13 (2 H, d, J = 9 Hz); MS m/z 390 (M^+). Anal. ($C_{21}H_{30}N_2O_5$) C, H, N.

Method C. 6-Methyl-4-[[[1(*S*)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]hept-3-enoic Acid, Benzyl Ester (21). A solution of the benzyl ester 16 (26.46 g, 96 mmol) in dry acetonitrile (500 mL) at 0 °C, under N_2, was treated with 1,1′-carbonyldiimidazole (15.69 g, 96 mmol) in one portion. After 1 h at 0 °C, a solution of *O*-methyl-L-tyrosine *N*-methylamide (20 g, 96 mmol) in dry acetonitrile (200 mL) was added dropwise with stirring. After 1 h the mixture was allowed to warm up to room temperature, and stirring was continued overnight. The solution was evaporated to dryness, dissolved in EtOAc, and washed with 1 N HCl. The organic fraction was washed with water, dried over $MgSO_4$, and evaporated to dryness. It was purified by column chromatography on silica gel, eluting with MeOH/$CHCl_3$ (1:50), to afford 21 as a yellow gum (41.01 g, 92%). A portion was recrystallized from EtOAc/pentane to give white crystals: mp 98–101 °C; ^{1}H NMR ($CDCl_3$) δ 0.78 (3 H, d, J = 4 Hz), 0.81 (3 H, d, J = 4 Hz), 1.56 (1 H, m), 2.15 (2 H, d, J = 9 Hz), 2.73 (3 H, d, J = 5 Hz), 3.00 (2 H, m), 3.2 (2 H, d, J = 7 Hz), 3.76 (3 H, s), 4.58 (1 H, m), 5.14 (2 H, s), 5.98 (1 H, br m), 6.35 (1 H, t, J = 7 Hz), 6.44 (1 H, br d), 6.81 (2 H, d, J = 9 Hz), 7.10 (2 H, d, J = 9 Hz), 7.35 (5 H, s); MS m/z 466 (M^+). Anal. ($C_{27}H_{34}N_2O_5$) C, H, N.

6-Methyl-4-[[[1(*S*)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]hept-(2 and 3)-enoic Acids, *tert*-Butyl Esters (26). A solution of 20 (7.2 g, 18 mmol) in MeOH (80 mL) was treated with NaOH (1.1 g, 27 mmol) in water (20 mL) and then was stirred at room temperature overnight. It was diluted with water, the MeOH was evaporated, and the aqueous solution was washed with EtOAc and acidified with 5 N HCl. The solution was extracted with EtOAc, dried, and evaporated to dryness. The crude product (6.6 g) in CH_2Cl_2 (50 mL) was treated with an excess of isobutylene (about 30 mL) and concentrated H_2SO_4 (0.5 mL) for 7 days in a sealed vessel. The solution was washed with 10% Na_2CO_3 solution, dried, and evaporated to dryness to afford a red oil (4.1 g), which was purified by column chromatography on silica gel, eluting with MeOH/$CHCl_3$ (1:9), to give 26 (3.2 g, 41%) as a foam: ^{1}H NMR ($CDCl_3$) δ 0.80 (6 H, d, J = 6 Hz), 1.4 (9 H, s), 1.3–2.3 (3 H, m), 2.65 (3 H, d, J = 4 Hz), 2.7–3.4 (4 H, m), 3.65 (3 H, s), 4.8 (1 H, m), 6.65 (2 H, d, J = 8 Hz), 6.9 (1 H, m), 7.0 (2 H, d, J = 8 Hz); MS (FAB) m/z 433 (MH^+). Anal. ($C_{24}H_{36}N_2O_5$) C, H, N.

Method D. 3-(Acetylthio)-6-methyl-4-[[[1(*S*)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]heptanoic Acid, Benzyl Ester (28). A solution of ester 21 (41 g, 88 mmol) in thiolacetic acid (170 mL, 2.4 mol) was set aside at room temperature for 19 days and then evaporated to dryness. The product was purified by column chromatography on silica gel eluting with a gradient of 0 to 100% EtOAc/Et_2O to give 28 (29.8 g, 62%) as a foam [Anal. ($C_{29}H_{38}N_2O_6S$) C, H, N] containing a mixture of four diastereoisomers (by ^{1}H NMR), which was deprotected with aqueous ammonia and separated into individual thiol diastereoisomers (see below).

Alternatively, a solution of the thiol (44a) (see below) (2 g, 4 mmol) in $CHCl_3$ (100 mL) under N_2 was treated with Ac_2O (2.04 g, 22 mmol) and *N*-methylmorpholine (2.02 g, 18 mmol), and the solution was stirred at room temperature for 3 days. The solution was washed successively with 10% aqueous citric acid and water, dried, and evaporated to dryness to afford a single diastereoisomer 28a (1.4 g, 65%): mp 89–90 °C (Et_2O); ^{1}H NMR ($CDCl_3$) δ 0.84 (6 H, t, J = 7 Hz), 1.17–1.71 (3 H, m), 2.27 (3 H, s), 2.45–2.7 (3 H, m), 2.7 (3 H, d, J = 5 Hz), 2.85–3.04 (2 H, m), 3.78 (3 H, s), 3.92 (1 H, m), 4.52 (1 H, q, J = 8 Hz), 5.12 (2 H, s), 5.52 (1 H, br d), 6.32 (1 H, d, J = 8 Hz), 6.79 (2 H, d, J = 9 Hz), 7.12 (2 H, d, J = 9 Hz), 7.36 (5 H, m); MS m/z 543 (MH^+). Anal. ($C_{29}H_{38}N_2O_6S$) C, H, N.

Method D. 3-(Acetylthio)-6-methyl-4-[[[1(*S*)-[(methylamino)carbonyl]-2-(3-indolyl)ethyl]amino]carbonyl]heptanoic Acid, Methyl Ester (40). A solution of ester 24 (14.8 g, 37 mmol) in thiolacetic acid (65 mL, 0.91 mol) was set aside at room temperature for 21 days and then evaporated to dryness. The product was purified by column chromatography on silica gel eluting with, initially, Et_2O and then Et_2O/$CHCl_3$ (1:1). The first fractions containing product were combined (5.2 g) and recrystallized from EtOAc/Et_2O to afford a single diastereoisomer 40a (3.3 g, 19%): mp 134–136 °C; ^{1}H NMR ($CDCl_3$) δ 0.84 (d, J = 5 Hz) and 0.85 (d, J = 5 Hz) (total 6 H), 1.2–1.75 (2 H, m), 2.29 (3 H, s), 2.5–2.7 (3 H, m), 2.64 (3 H, d, J = 5 Hz), 3.11 (1 H, dd, J = 8, 14 Hz), 3.3 (1 H, dd, J = 6, 14 Hz), 3.62 (3 H, s), 3.94 (1 H, m), 4.72 (1H, q, J = 7 Hz), 5.64 (1 H, br d), 6.52 (1 H, d, J = 8 Hz), 7.05–7.25 (3 H, m), 7.36 (1 H, d, J = 7 Hz), 7.72 (1 H, d, J = 7 Hz), 8.17 (1 H, s); MS m/z 475 (M^+). Anal. ($C_{24}H_{33}N_3O_5S$) C, H, N. The mother liquors contained a 1:1 mixture of 40a and a second diastereoisomer 40b.

Later column fractions were combined (4.3 g) and recrystallized from EtOAc to afford a single diastereoisomer 40c (1.5 g, 8.5%): mp 197–201 °C; ^{1}H NMR ($CDCl_3$) δ 0.70 (d, J = 6 Hz) and 0.77 (d, J = 6 Hz) (total 6 H), 1.15–1.6 (2 H, m), 2.29 (3 H, s), 2.54–2.75 (3 H, m), 2.69 (3 H, d, J = 5 Hz), 3.18 (1 H, dd, J = 7, 15 Hz),

3.2 (1 H, dd, J = 6, 15 Hz), 3.63 (3 H, s), 4.0 (1 H, m), 4.73 (1 H, q, J = 7 Hz), 6.04 (1 H, br d), 6.32 (1 H, d, J = 8 Hz), 7.05–7.25 (3 H, m), 7.37 (1 H, d, J = 7 Hz), 7.67 (1 H, d, J = 7 Hz), 8.17 (1 H, s); MS m/z 475 (M^+). Anal. ($C_{24}H_{33}N_3O_6S$) C, H, N.

The mother liquors, after several recrystallizations from Et_2O/pentane, gave a single diastereoisomer **40d** (1.3 g, 7.5%): mp 94–97 °C; ^1H NMR ($CDCl_3$) δ 0.70 (d, J = 6 Hz) and 0.79 (d, J = 6 Hz) (total 6 H), 1.15–1.7 (2 H, m), 2.27 (3 H, s), 2.52–2.75 (3 H, m), 2.69 (3 H, d, J = 5 Hz), 3.16 (1 H, dd, J = 9, 14 Hz), 3.30 (1 H, dd, J = 6, 14 Hz), 3.65 (3 H, s), 3.92 (1 H, q, J = 6 Hz), 4.72 (1 H, q, J = 7 Hz), 5.96 (1 H, br d), 6.29 (1 H, d, J = 7 Hz), 7.05–7.25 (3 H, m), 7.37 (1 H, d, J = 7 Hz), 7.66 (1 H, d, J = 7 Hz), 8.15 (1 H, s); MS m/z 475 (M^+). Anal. ($C_{24}H_{33}N_3O_5S$), C, H, N. In a later experiment, diastereoisomer **40d** was recrystallized from $MeOH/CH_2Cl_2$ to give a sample suitable for X-ray crystallography, mp 115–117 °C.

Method E. 3-Mercapto-6-methyl-4-[[[1(*S*)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]heptanoic Acid, Benzyl Ester (44). An ice-cooled solution of the mixture of diastereoisomers of **28** (1 g, 1.8 mmol) in N_2-purged MeOH (100 mL) was treated with 35% aqueous ammonia (10 mL), and the mixture was stirred at room temperature under N_2 for 3 h and then filtered. The filtrate was evaporated to dryness, and the residue was recrystallized from Et_2O/MeOH to afford a single diastereoisomer **44a** (0.3 g, 33%): mp 199–201 °C; $[\alpha]^{20}_D$ –23° (c 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.84 (3 H, d, J = 7 Hz), 0.86 (3 H, d, J = 7 Hz), 1.3–1.7 (3 H, m), 1.66 (1 H, d, J = 9 Hz), 2.38 (1 H, dd, J = 9, 15 Hz), 2.41 (1 H, m), 2.66 (1 H, dd, J = 4, 15 Hz), 2.73 (3 H, d, J = 5 Hz), 2.97 (2 H, d, J = 8 Hz), 3.22 (1 H, m), 3.74 (3 H, s), 4.57 (1 H, q, J = 8 Hz), 5.14 (2 H, s), 5.63 (1 H, br d), 6.33 (1 H, d, J = 8 Hz), 6.81 (2 H, d, J = 9 Hz), 7.12 (2 H, d, J = 9 Hz), 7.36 (5 H, m); MS m/z 500 (M^+). Anal. ($C_{27}H_{36}N_2O_5S$) C, H, N.

In a second experiment, after removal of **44a** by crystallization from Et_2O, the mother liquors (10 g) were subjected to column chromatography on silica gel (750 g) eluting with Et_2O followed by $Et_2O/CHCl_3$ (1:1). Diastereoisomer **44b**, the fastest running isomer, was obtained as a gum (1.1 g, 11%): $[\alpha]^{20}_D$ –3° (c 0.95, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.81 (3 H, d, J = 6 Hz), 0.83 (3 H, d, J = 6 Hz), 1.2–1.7 (3 H, m), 1.99 (1 H, d, J = 9 Hz), 2.47 (1 H, m), 2.56 (1 H, dd, J = 8, 15 Hz), 2.72 (1 H, m), 2.72 (3 H, d, J = 6 Hz), 2.96 (1 H, dd, J = 7, 15 Hz), 3.04 (1 H, dd, J = 7, 15 Hz), 3.29 (1 H, m), 3.77 (3 H, s), 4.55 (1 H q, J = 7 Hz), 5.16 (2 H, s), 5.75 (1 H, br d), 6.38 (1 H, d, J = 8 Hz), 6.81 (2 H, d, J = 9 Hz), 7.12 (2 H, d, J = 9 Hz), 7.36 (5 H, m); MS m/z 501 (MH^+). Anal. ($C_{27}H_{36}N_2O_5S$) C, H, N.

Diastereoisomer **44d**, the slowest running isomer, was obtained as a foam (2.1 g, 21%), which solidified on standing: mp 133–135 °C (trituration with Et_2O); $[\alpha]^{20}_D$ +53° (c 0.95, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.73 (3 H, d, J = 7 Hz), 0.79 (3 H, d, J = 7 Hz), 1.2–1.7 (3 H, m), 1.90 (1 H, d, J = 9 Hz), 2.36 (1 H, m), 2.64 (1 H, dd, J = 9, 17 Hz), 2.69 (3 H, d, J = 6 Hz), 2.81 (1 H, dd, J = 4, 17 Hz), 2.94 (1 H, dd, J = 7, 16 Hz), 3.03 (1 H, dd, J = 7, 16 Hz), 3.26 (1 H, m), 3.78 (3 H, s), 4.59 (1 H, q, J = 7 Hz), 5.15 (2 H, m), 5.98 (1 H, br d), 6.16 (1 H, d, J = 8 Hz), 6.82 (2 H, d, J = 9 Hz), 7.10 (2 H, d, J = 9 Hz), 7.36 (5 H, m); MS m/z 501 (MH^+). Anal. ($C_{27}H_{36}N_2O_5S$) C, H, N.

Diastereoisomer **44c** was obtained as a gum from a fraction also containing **44b** and **44d** by preparative HPLC (Lichrosorb diol), eluting with (1% $MeOH/CH_2Cl_2$)/hexane (18:82): ^1H NMR ($CDCl_3$) δ 0.68 (3 H, d, J = 7 Hz), 0.78 (3 H, d, J = 7 Hz), 1.1–1.6 (3 H, m), 2.09 (1 H, d, J = 10 Hz), 2.36 (1 H, m), 2.63 (1 H, dd, J = 6, 17 Hz), 2.74 (3 H, d, J = 6 Hz), 2.85 (1 H, dd, J = 5, 17 Hz), 2.99 (1 H, dd, J = 7, 16 Hz), 3.09 (1 H, dd, J = 7, 16 Hz), 3.29 (1 H, m), 3.79 (3 H, s), 4.68 (1 H, q, J = 7 Hz), 5.91 (1 H, d, J = 8 Hz), 6.24 (1 H, br d), 6.82 (2 H, d, J = 9 Hz), 7.10 (2 H, d, J = 9 Hz), 7.36 (5 H, m); MS m/z 500.2330 (M^+ requires 500.2344). Anal. ($C_{27}H_{36}N_2O_5S$) N, H; C: calcd, 64.77; found, 64.36.

Method E. 3-Mercapto-6-methyl-4-[[[1(*S*)-[(methylamino)carbonyl]-2-(3-indolyl)ethyl]amino]carbonyl]heptanoic Acid, Methyl Ester (56). An ice-cooled solution of **40a** (0.1 g, 0.21 mmol) in N_2-purged MeOH (5 mL) was treated with 35% aqueous ammonia (1.5 mL), and the mixture was stirred at room temperature under N_2 for 2 h and then filtered. The filtrate was evaporated to dryness, and the residue was triturated with cold Et_2O to afford a single diastereoisomer **56a** (52 mg, 57%): mp 73–75 °C; $[\alpha]^{20}_D$ –33° (c 1, MeOH); ^1H NMR ($CDCl_3$) δ 0.87 (6 H, d, J = 6 Hz), 1.4–1.7 (2 H, m), 1.67 (1 H, d, J = 9 Hz), 2.37 (1 H, dd, J = 5, 15 Hz), 2.41 (1 H, m), 2.60 (1 H, dd, J = 3, 15 Hz), 2.65 (3 H, d, J = 5 Hz), 3.13 (1 H, dd, J = 7, 14 Hz), 3.21 (1 H, m), 3.30 (1 H, dd, J = 7, 14 Hz), 3.67 (3 H, s), 4.75 (1 H, q, J = 7 Hz), 5.68 (1 H, br d), 6.53 (1 H, d, J = 8 Hz), 7.05–7.25 (3 H, m), 7.35 (1 H, d, J = 8 Hz), 7.71 (1 H, d, J = 8 Hz), 8.13 (1 H, s); MS m/z 433 (M^+). Anal. ($C_{22}H_{31}N_3O_4S$) C, H, N.

Diastereoisomer **40c** (0.3 g, 0.63 mmol) was treated with 35% aqueous ammonia in the same manner to give **56c** (0.25 g, 91%): mp 130–136 °C (EtOAc/Et_2O); $[\alpha]^{20}_D$ –57° (c 0.95, MeOH); ^1H NMR ($CDCl_3$) δ 0.68 (3 H, d, J = 6 Hz), 0.76 (3 H, d, J = 6 Hz), 1.15–1.7 (3 H, m), 2.05 (1 H, d, J = 9 Hz), 2.37 (1 H, m), 2.56 (1 H, dd, J = 7, 15 Hz), 2.69 (3 H, d, J = 5 Hz), 2.81 (1 H, dd, J = 4, 15 Hz), 3.21 (1 H, dd, J = 7, 15 Hz), 3.28 (1 H, m), 3.37 (1 H, dd, J = 7, 15 Hz), 3.68 (3 H, s), 4.79 (1 H, q, J = 7 Hz), 6.19 (1 H, br d), 6.37 (1 H, d, J = 8 Hz), 7.05–7.25 (3 H, m), 7.38 (1 H, d, J = 8 Hz), 7.67 (1 H, d, J = 8 Hz), 8.13 (1 H, s); MS m/z 433 (M^+). Anal. ($C_{22}H_{31}N_3O_4S$) C, H, N.

Similarly, diastereoisomer **40d** (0.5 g, 1.1 mmol) gave **56d** (0.35 g, 77%) as a foam: $[\alpha]^{20}_D$ –19° (c 1, MeOH); ^1H NMR ($CDCl_3$) δ 0.72 (3 H, d, J = 6 Hz), 0.81 (3 H, d, J = 6 Hz), 1.25–1.7 (3 H, m), 1.92 (1 H, d, J = 9 Hz), 2.38 (1 H, m), 2.60 (1 H, dd, J = 8, 16 Hz), 2.78 (1 H, dd, J = 3, 16 Hz), 2.67 (3 H, d, J = 5 Hz), 3.15 (1 H, dd, J = 7, 14 Hz), 3.23 (1 H, m), 3.32 (1 H, dd, J = 7, 14 Hz), 3.69 (3 H, s), 4.73 (1 H, q, J = 7 Hz), 5.90 (1 H, br d), 6.37 (1 H, d, J = 8 Hz), 7.05–7.25 (3 H, m), 7.38 (1 H, d, J = 8 Hz), 7.68 (1 H, d, J = 8 Hz), 8.12 (1 H, s); MS m/z 433 (M^+). Anal. ($C_{22}H_{31}N_3O_4S$) C, H, N.

A 1:1 mixture of diastereoisomers **40a** and **40b** (0.5 g, 1.1 mmol) was treated with 35% aqueous ammonia in the usual manner. The product was triturated with Et_2O and filtered. The filtrate was purified by preparative HPLC (Lichrosorb diol), eluting with 2-propanol/hexane (1:19), to afford a single diastereoisomer **56b** (50 mg, 11%) as a foam: $[\alpha]^{20}_D$ +6° (c 0.92, MeOH); ^1H NMR ($CDCl_3$) δ 0.87 (6 H, m), 1.25–1.7 (3 H, m), 1.98 (1 H, d, J = 9 Hz), 2.48 (1 H, m), 2.49 (1 H, dd, J = 7, 15 Hz), 2.66 (3 H, d, J = 5 Hz), 2.72 (1 H, dd, J = 5, 15 Hz), 3.13 (1 H, dd, J = 8, 15 Hz), 3.29 (1 H, m), 3.36 (1 H, dd, J = 6, 15 Hz), 3.70 (3 H, s), 4.70 (1 H, q, J = 7 Hz), 5.65 (1 H, br s), 6.47 (1 H, d, J = 8 Hz), 7.05–7.25 (3 H, m), 7.37 (1 H, d, J = 8 Hz), 7.73 (1 H, d, J = 8 Hz), 8.11 (1 H, s); MS m/z 434 (M^+). Anal. ($C_{22}H_{31}N_3O_4S$) C, H, N.

3,3′-Dithiobis[6-methyl-4-[[[1(*S*)-[(methylamino)carbonyl]-2-(3-indolyl)ethyl]amino]carbonyl]heptanoic Acid, Dimethyl Ester] (59a). A solution of I_2 (0.152 g, 0.6 mmol) in MeOH (5 mL) was added dropwise to a stirred solution of thiol **56a** (0.5 g, 1.15 mmol) in MeOH (25 mL) at 0 °C, and the solution was stirred until the color had disappeared (15 min) and then evaporated to dryness. The residue was purified by column chromatography on silica gel, eluting with EtOAc/pentane (1:1). The product was triturated with EtOAc/Et_2O to give **59a** as a white solid (0.46 g, 92%): mp 92–98 °C; ^1H NMR ($CDCl_3$) δ 0.87 (6 H, d, J = 6 Hz), 0.88 (6 H, d, J = 6 Hz), 1.25–1.7 (6 H, m), 2.10 (2 H, dd, J = 10, 15 Hz), 2.36 (2 H, dd, J = 4, 15 Hz), 2.65 (2 H, m), 2.73 (6 H, d, J = 5 Hz), 3.05 (2 H, m), 3.30 (4 H, m), 3.66 (6 H, s), 4.78 (2 H, q, J = 7 Hz), 5.98 (2 H, br d), 7.02 (2 H, d, J = 8 Hz), 7.05–7.25 (6 H, m), 7.35 (2 H, d, J = 8 Hz), 7.71 (2 H, d, J = 8 Hz), 8.78 (2 H, s); MS (FAB) m/z 865 (MH^+). Anal. ($C_{44}H_{60}N_6O_8S_2$) C, H, N.

3-(Acetylthio)-6-methyl-4-[[[1(*S*)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]heptanoic Acid Hydrate (30a). A solution of the single diastereoisomer **28a** (1.4 g, 2.8 mmol) in 4.5% formic acid/MeOH (25 mL) was added, under N_2, to a stirred suspension of palladium black (1.5 g) in the same solvent (25 mL). After 1.5 h, the mixture was filtered through kieselguhr and evaporated to dryness, and the residue was triturated with Et_2O to afford a single diastereoisomer **30a** (0.98 g, 77%), mp 92–95 °C; ^1H NMR ($CDCl_3$) δ 0.85 (3 H, d, J = 6 Hz), 0.91 (3 H, d, J = 6 Hz), 1.2–1.7 (3 H, m), 2.31 (3 H, s), 2.50 (1 H, dd, J = 3, 17 Hz), 2.67 (1 H, dd, J = 7, 17 Hz), 2.68 (3 H, d, J = 5 Hz), 2.85–3.0 (2 H, m), 3.15 (1 H, t, J = 10 Hz), 3.74 (3 H, s), 3.80 (1 H, m), 4.80 (1 H, q, J = 8 Hz), 6.36 (1 H, m), 6.77 (2 H, d, J = 9 Hz), 7.07 (2 H, d, J = 9 Hz),

8.41 (1 H, d, J = 10 Hz); MS m/z 452 (M^+). Anal. ($C_{22}H_{32}N_2O_6S \cdot H_2O$) C, H, N.

4-[(Methylamino)carbonyl]-2-(2-methylpropyl)but-2-enoic Acid (17). A slow stream of methylamine was passed into a solution of impure anhydride **14** (7.5 g; containing about 5.6 g, 33 mmol of pure **14** and contaminated with lactone **18**) in dry Et_2O (40 mL) at 10 °C for 1.5 h. The solution was evaporated to dryness and the product was dissolved in EtOAc and extracted with 10% Na_2CO_3. The aqueous fraction was washed with EtOAc, acidified with 5 N HCl, and reextracted with EtOAc. The organic fraction was washed with brine, dried ($MgSO_4$), evaporated to dryness, and triturated with Et_2O to afford **17** as a white solid (4.6 g, 69%): mp 120–122 °C; IR 3300, 2600 (br), 1690, 1645, 1635 cm^{-1}; ^1H NMR (d_6-DMSO) δ 0.78 (6 H, d, J = 6 Hz), 1.65 (1 H, m), 2.05 (2 H, d, J = 6 Hz), 2.58 (3 H, d, J = 3 Hz), 3.25 (2 H, d, J = 6 Hz), 5.95 (1 H, t, J = 7 Hz), 7.7 (1 H, br s), 12.4 (1 H, br s); MS m/z 200 (MH^+). Anal. ($C_{10}H_{17}NO_3$) C, H, N.

6-(Methylamino)-3-(2-methylpropyl)pyran-2-one (60). *N,N*-Dicyclohexylcarbodiimide (1.6 g, 7.8 mmol) was added to a stirred solution of the carboxylic acid **17** (1.4 g, 7.0 mmol) and *O*-methyl-L-tyrosine *N*-methylamide (1.47 g, 7.1 mmol) in dry CH_2Cl_2 (30 mL) at 0 °C, the mixture was allowed to warm up to room temperature, and stirring was continued overnight. The precipitated solid was filtered off, and the filtrate was diluted with EtOAc and washed successively with 10% Na_2CO_3, 10% citric acid, and water, dried ($MgSO_4$), and evaporated to dryness. The product was purified by column chromatography on silica gel, eluting with EtOAc/pentane (1:1), to afford **60** as an unstable yellow solid (0.85 g, 67%): IR 3250, 1695, 1600, 1575 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (6 H, d, J = 7 Hz), 1.88 (1 H, m), 2.18 (2 H, d, J = 7 Hz), 2.82 (3 H, d, J = 6 Hz), 4.97 (1 H, d, J = 8 Hz), 5.42 (1 H, br s), 7.09 (1 H, d, J = 8 Hz); MS m/z 181.1102 (M^+ requires 181.1098). Anal. ($C_{10}H_{15}NO_2$) C, N; H: calcd, 8.34; found, 8.86. No product was isolated containing the *O*-methyl-L-tyrosine *N*-methylamide residue.

Method F. 3-(Acetylthio)-N^5-methyl-N^1-[1(*S*)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]-2-(2-methylpropyl)pentanediamide (32a). Diisopropylethylamine (0.13 mL, 0.7 mmol) and ethyl chloroformate (0.07 mL, 0.7 mmol) were added to a solution of carboxylic acid **30a** (0.3 g, 0.66 mmol) in dry THF (10 mL) at −20 °C. After 15 min a solution of methylamine (20 mg, 0.61 mmol) in THF (0.8 mL) was added dropwise, and the mixture was stirred at 0 °C for 3 h. The reaction mixture was evaporated to dryness, dissolved in CH_2Cl_2, and washed successively with water, 1 N HCl, water, saturated $NaHCO_3$, and brine. The solution was dried ($MgSO_4$) and evaporated to dryness, and the product was purified by column chromatography on silica gel, eluting with MeOH/EtOAc (1:19), followed by trituration with Et_2O to afford **32a** (75 mg, 24%): mp 195–199 °C; ^1H NMR ($CDCl_3$) δ 0.84 (6 H, q, J = 3 Hz), 1.2–1.7 (3 H, m), 2.10 (1 H, dd, J = 7, 15 Hz), 2.30 (3 H, s), 2.31 (1 H, dd, J = 5, 15 Hz), 2.68 (1 H, m), 2.74 (3 H, d, J = 5 Hz), 2.76 (3 H, d, J = 5 Hz), 2.95 (1 H, dd, J = 6, 14 Hz), 3.08 (1 H, dd, J = 6, 14 Hz), 3.78 (3 H, s), 3.83 (1 H, m), 4.63 (1 H, q, J = 7 Hz), 5.49 (1 H, br s), 5.88 (1 H, br s), 6.68 (1 H, d, J = 7 Hz), 6.82 (2 H, d, J = 8 Hz), 7.14 (2 H, d, J = 8 Hz); MS m/z 465 (M^+). Anal. ($C_{23}H_{35}N_3O_5S$) C, H, N.

Method G. 2-[[3-(Acetylthio)-6-methyl-4-[[[1(*S*)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]heptanoyl]amino]ethanoic Acid, *tert*-Butyl Ester (33a). A solution of carboxylic acid **30a** (90 mg, 0.2 mmol), *N*-ethyl-*N'*-[(dimethylamino)propyl]carbodiimide hydrochloride (EDC) (42 mg, 0.22 mmol), 1-hydroxybenzotriazole (34 mg, 0.25 mmol), glycine, *tert*-butyl ester hydrochloride (37 mg, 0.22 mmol), and diisopropylethylamine (0.07 mL, 0.4 mmol) in CH_2Cl_2 (2 mL) was stirred at room temperature for 18 h. The mixture was diluted with CH_2Cl_2, washed successively with saturated $NaHCO_3$, water, 10% citric acid, and brine, and then evaporated to dryness to afford **33a** (74 mg, 65%): mp 192–194 °C (EtOAc); ^1H NMR ($CDCl_3$) δ 0.77 (3 H, d, J = 6 Hz), 0.83 (3 H, d, J = 6 Hz), 1.2–1.7 (3 H, m), 1.47 (9 H, s), 2.30 (3 H, s), 2.35 (2 H, m), 2.56 (1 H, m), 2.77 (3 H, d, J = 5 Hz), 3.0 (1 H, dd, J = 10, 15 Hz), 3.10 (1 H, dd, J = 7, 15 Hz), 3.70 (1 H, dd, J = 6, 19 Hz), 3.78 (3 H, s), 3.95 (1 H, dd, J = 6, 19 Hz), 4.0 (1 H, m), 4.64 (1 H, q, J = 7 Hz), 6.05 (1 H, m), 6.33 (1 H, br t), 6.83 (2 H, d, J = 9 Hz), 7.14 (2 H, d, J = 9 Hz), 7.43 (1 H, d, J = 7 Hz); MS m/z 565 (M^+). Anal. ($C_{28}H_{43}N_3O_7S$) C, H, N.

Method H. 2-[[3-(Acetylthio)-6-methyl-4-[[[1(*S*)-[(methylamino)carbonyl]-2-(4-methoxyphenyl)ethyl]amino]carbonyl]heptanoyl]amino]ethanoic Acid (34a). A solution of ester **33a** (420 mg, 0.74 mmol) in CH_2Cl_2 (5 mL) and water (0.5 mL) was cooled in an ice bath, and then TFA (5 mL) was added. The solution was stirred at 0–5 °C for 3 h and then at room temperature for 2 h. The solvents were evaporated, and the residue was azeotroped with dry toluene then triturated with dry Et_2O to give the single diastereoisomer **34a** (335 mg, 89%): mp 188–192 °C; ^1H NMR (d_6-DMSO) δ 0.78 (6 H, t, J = 6 Hz), 1.1–1.5 (3 H, m), 2.1–2.3 (3 H, m), 2.23 (3 H, s), 2.54 (3 H, d, J = 5 Hz), 2.73 (1 H, dd, J = 9, 12 Hz), 2.85 (1 H, dd, J = 6, 12 Hz), 3.68 (3 H, s), 3.70 (2 H, s), 3.78 (1 H, m), 4.43 (1 H, m), 6.80 (2 H, d, J = 9 Hz), 7.12 (2 H, d, J = 9 Hz), 7.77 (1 H, br m), 8.08 (1 H, br d, J = 8 Hz), 8.14 (1 H, br t, J = 7 Hz); MS (FAB) m/z 510 (MH^+). Anal. ($C_{24}H_{35}N_3O_7S \cdot 0.5H_2O$) C, H, N.

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