22.34, 22.76, 24.97, 28.19, 38.14, 39.09, 40.82, 41.71, 52.50, 55.71, 79.79, 80.57, 126.63, 128.48, 129.25, 137.12, 154.08, 168.53, 172.40, 197.07.

(2(*S*)-Hydroxy-3(*R*)-amino-4-phenylthiobutanoyl)-L-leucine Trifluoroacetate (49). The protected thiodipeptide **48** (0.17 mmol) was saponified with 0.4 mL of 1 N NaOH, which removed both the acetyl group and the methyl ester as shown by ^{1}H NMR spectroscopy. The reaction was worked up as described for compound **6** to yield a white solid (0.16 mmol) in 94% yield: ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.98 (d, 6 H, J = 6 Hz), 1.26 (s, 9 H), 1.81 (br, 3 H), 2.97 (br, 2 H), 4.30–4.74 (m, 2 H), 5.18 (br, 2 H), 6.61 (br, 1 H), 7.31 (s, 5 H), 9.51 (br, 1 H). The Boc free acid (0.13 mmol) was used directly and deprotected with 4 N HCl/dioxane. Trituration with ether yielded a yellow oil, which was subjected to semipreparative HPLC for purification with a 20–40% gradient of CH_3CN/H_2O, both containing 0.1% TFA. Lyophilization yielded a white solid, which was extremely sensitive to moisture (0.094 mmol, 72%): $[\alpha]^{23}_D$ −22.2° (c 0.12, CH_3OH); ^{1}H NMR (90 MHz, MeOH-d_4 + $CDCl_3$) δ 0.97 (d, 3 H, J = 6 Hz), 1.02 (d, 3 H, J = 6 Hz), 1.02, (d, 3 H, J = 6 Hz), 1.59–2.01 (m, 3 H), 3.02 (d, 2 H, J = 8 Hz), 3.10 (d, 2 H, J = 8 Hz), 3.57–3.84 (m, 1 H), 3.95–4.24 (m, 1 H), 4.87–5.09 (m, 1 H), 7.34 (s, 5 H); ^{13}C NMR (MeOH-d_4) 20.32, 20.75, 24.16, 34.73, 39.11, 56.07, 56.45, 73.41, 126.33, 127.85, 128.18, 134.52, 172.76 (broadened), 200.39.

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Registry No. 1, 58970-76-6; **4**, 88264-65-7; **4a**, 7533-40-6; **5**, 110675-37-1; **5a**, 110690-36-3; **6**, 114886-46-3; **8**, 77171-42-7; **9**, 114886-47-4; **10**, 114886-48-5; **11**, 114886-49-6; **12**, 114886-50-9; **13**, 109687-65-2; **14**, 112157-31-0; **15**, 112157-32-1; **16**, 112157-33-2; **17**, 114886-51-0; **18**, 114907-39-0; **19**, 114886-52-1; **20**, 114886-53-2; **21**, 114886-54-3; **22**, 114886-55-4; **23**, 114886-56-5; **24**, 114886-57-6; **25**, 114886-58-7; (2*R*,3*R*)-**26**, 114886-59-8; (2*S*,3*R*)-**26**, 114886-86-1; (2*R*,3*R*)-**27**, 114886-60-1; (2*S*,3*R*)-**27**, 114886-87-2; (2*R*,3*R*)-**28**, 114886-61-2; (2*S*,3*R*)-**28**, 114886-88-3; **28a**, 114886-83-8; (2*R*,3*S*)-**29**, 114886-62-3; (2*S*,3*S*)-**29**, 114886-81-6; (2*R*,3*S*)-**30**, 114886-63-4; (2*S*,3*S*)-**30**, 114886-82-7; (2*R*,3*S*)-**31**, 114886-64-5; (2*S*,3*S*)-**31**, 114976-87-3; (2*R*,3*S*)-**32**, 114886-65-6; (2*S*,3*S*)-**32**, 114926-91-9; (2*S*,3*S*)-**32a**, 111061-11-1; (2*R*,3*S*)-**32a**, 111061-12-2; **33**, 114886-66-7; **34**, 114886-67-8; **35**, 114886-68-9; **36**, 114886-70-3; **37**, 114926-84-0; **37a**, 88826-02-2; **38**, 114886-71-4; **39**, 114886-72-5; **40**, 114926-85-1; **41**, 114976-69-1; **42**, 114926-87-3; (2*S*,3*R*)-**43**, 114926-88-4; (2*R*,3*R*)-**44**, 114976-70-4; (2*R*,3*R*)-**45**, 114926-89-5; (2*R*,3*R*)-**46**, 114926-90-8; **47**, 114886-73-6; **48**, 114886-74-7; **48** (saponified), 114886-89-4; **49**, 114886-76-9; **50**, 114886-77-0; **51**, 114886-78-1; **52**, 114886-79-2; **53**, 114886-80-5; AP-M, 9054-63-1; LAP, 9001-61-0; AP-B, 9073-92-1; (2*R*,3*R*)-$PhCH_2CH(NH(BOC))CH(SH)CONH(i\text{-}C_5H_{11})$, 114886-84-9; (2*S*,3*R*)-$PhCH_2CH(NH(BOC))CH(SH)CONH(i\text{-}C_5H_{11})$, 114886-85-0.

Design of Novel Inhibitors of Aminopeptidases. Synthesis of Peptide-Derived Diamino Thiols and Sulfur Replacement Analogues of Bestatin

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Investigations were directed toward inhibition of an aminopeptidase, isolated from rat brain, which has been implicated in the metabolic inactivation of enkephalins. The design rationale and synthesis of novel peptidyl diamino thiol inhibitors of rat brain aminopeptidase are presented, along with accompanying structure–activity analysis. Some of the reported compounds are highly active aminopeptidase inhibitors and possess enzyme inhibitory potency in the nanomolar range (**62**; I_{50} = 1 nM). Analysis of the data permits speculations on possible modes of binding of diamino thiols to aminopeptidase. Other investigations were directed toward understanding the mode of enzyme binding of the naturally occurring aminopeptidase inhibitor bestatin. On the basis of published models of enzyme binding, replacement of the C-2 hydroxyl group of bestatin by a sulfhydryl group was anticipated to lead to enhanced inhibition due to a strengthened interaction of this group with enzymic zinc. Contrary to expectations, "thiobestatin" inhibited rat brain aminopeptidase with only the same degree of effectiveness as the corresponding alcohol. Speculations on the possible mode of enzyme–inhibitor binding of bestatin are offered.

The aminopeptidases (APASE) are a group of exopeptidases that specifically cleave polypeptide chains at the amino terminus. These enzymes are ubiquitous in nature and are of biochemical and medicinal importance due to their key role in the metabolism of numerous biologically active peptides, for example the enkephalins. It is well established that the weak and short-lasting biological activity of the enkephalins can be attributed to their rapid inactivation. Enkephalins are metabolized by several hydrolytic enzymes present in brain: (1) aminopeptidases release Tyr^1, (2) a dipeptidyl aminopeptidase releases the Tyr^1–Gly^2 fragment, and (3) two enzymes cleave the penultimate Gly^3–Phe^4 bond to release the C-terminal dipeptide; angiotensin converting enzyme (ACE) and neutral endopeptidase 24.11, often commonly designated "enkephalinase" (ENKASE)[1,2] (see Figure 1). Cleavage at the Tyr^1–Gly^2 bond may be physiologically important in light of the analgesic effects induced by the aminopeptidase inhibitor bestatin (**1**),[3–6] and because of the complete protection of endogenous enkephalins released from K^+ depolarized brain slices in the presence of **1** together with thiorphan, an enkephalinase inhibitor.[6] A further point bearing on the pharmacological significance of aminopeptidase degradation is that enkephalin analogues fortified against APASE action elicited enhanced analgesic activity.[8] In view of our earlier work directed

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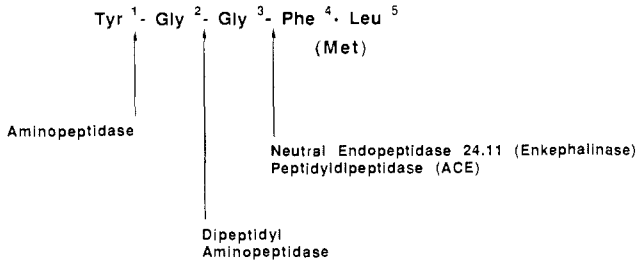

Figure 1. Sites of enkephalin hydrolysis by brain peptidases.

at exploring the inhibition of ENKASE by mercaptopropanoyl amino acid analogues,[9-11] we were intrigued by the possibility of identifying agents for inhibiting the cleavage catalyzed by APASE, and ultimately using both types of inhibitors to potentiate the action of endogenous enkephalins in vivo.

The cleavage of peptides by many aminopeptidases is believed to proceed via interaction of an enzyme-bound zinc atom with the scissile amide carbonyl group of the peptide substrate. Further mechanistic analogy for the mode of action of zinc-containing aminopeptidases may be drawn from the more thoroughly studied zinc metallopeptidases: carboxypeptidase A and B, thermolysin, and angiotensin converting enzyme.[12] A major difference between the aminopeptidases and these other enzymes is that, in the former, a specific amino group recognition site on APASE likely participates in the proper alignment of substrates with the active-site functionality, whereas the other enzymes index substrate via different key interactions. Such an amino group recognition site might be a negatively charged enzymic carboxyl group, which would interact with the positively charged amino group of substrate via an electrostatic attraction, or it might be a hydrogen-bond acceptor which could interact with the unprotonated amino group. Hence, the design of APASE inhibitors should reflect the need for a free amino terminus in the inhibitor molecule.

A number of aminopeptidase inhibitors are known in the literature, but only a few relatively simple compounds have been designed as inhibitors. These include amino acid hydroxamic acids,[13-17] α-mercapto ketones derived from amino acids,[13] boronic acids,[18-20] α-amino aldehydes,[21]

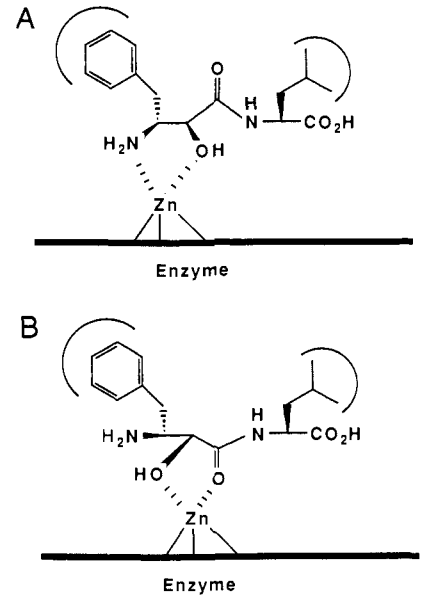

Figure 2. (A) Hypothetical model of bestatin binding to the active site of aminopeptidases as proposed by Nishizawa et al. (B) Hypothetical model of bestatin binding to the active site of aminopeptidase as proposed by Nishino and Powers.

simple amino acid substitutions in puromycin,[22] and some novel phosphorus-based inhibitors.[23,24] To date, among the most intriguing inhibitors of aminopeptidases have been bestatin, amastatin,[25,26] the arphamenines,[27-31] and other natural products.[32-35] Bestatin has been the most frequently utilized APASE inhibitor in published enkephalin metabolism studies; its chemistry,[36-38] pharmacol-

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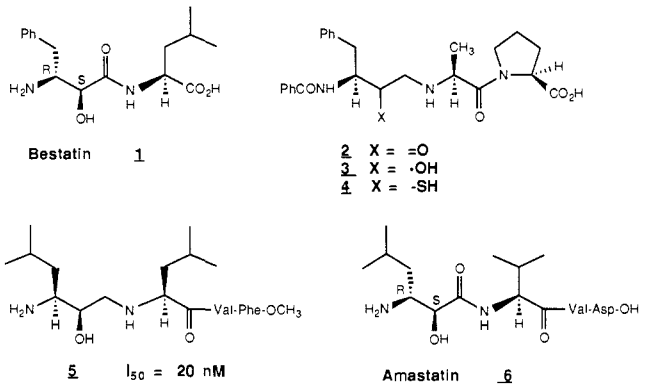
ogy,[39-44] and structure-activity relationships (SAR)[45-48] have been well documented, and a number of mode of enzyme binding studies have been reported.[49-52] However, there is little reason to believe that this material has been optimized for enkephalin degrading APASE. In previous investigations with angiotensin converting enzyme we designed several series of potent tripeptide-based ACE inhibitors[53-56] (**2–4**). We reasoned that with proper modification of the peripheral binding functionality and inclusion of an unblocked amino terminus, the design elements present in **2–4** (diamino thiols) might also be applicable to the preparation of inhibitors of the enkephalin degrading aminopeptidase, an enzyme that possesses properties that suggest it to be a zinc metallopeptidase.



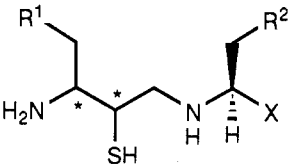
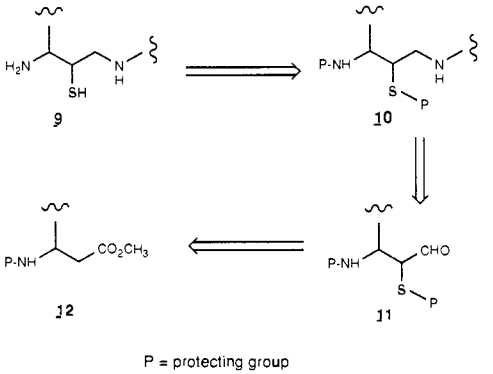


Figure 3. General structure of proposed aminopeptidase inhibitors.

Scheme I. Retrosynthetic Route to Diamino Thiols



"Thiobestatin". The presence and stereochemistry of the 2(*S*)-hydroxyl group of the novel amino acids, 2(*S*)-hydroxy-3(*R*)-amino-4-phenylbutanoic acid (AHPBA), of bestatin and AHMHA of amastatin have been shown to be critical for tight binding to aminopeptidases.[46] The sp^3 geometry of the C-2 alcohol group is reminiscent of the tetrahedral intermediate of amide bond hydrolysis and suggests that bestatin may function as a transition-state inhibitor. Two mechanisms of binding have been proposed that implicate the C-2 alcohol and other inhibitor functionality in bidentate binding to the active-site zinc of metal-containing aminopeptidases (Figure 2A,B). Nishizawa et al.[45] proposed a model in which enzymic zinc is chelated by the 2(*S*)-hydroxyl and the 3-amino groups of AHPBA. Alternatively, Nishino and Powers[57] suggested that the 2(*S*)-hydroxyl and its neighboring carbonyl group are operative zinc ligands. Both models place the aromatic side chain of AHPBA at P_1 for binding to the S_1 enzyme site.

In light of these postulates, and the likelihood that rat brain APASE is a zinc metallopeptidase, it was of interest to prepare an analogue of bestatin in which all of the molecular features were conserved, with the exception that the hydroxyl moiety had been replaced by a sulfhydryl group. The gross backbone structure of "thiobestatin" resembles the diamino thiols but differs in the absolute configuration of the N-terminal amino acid derived component and in the presence of an "inner amide" linkage rather than an amine function. If a sulfur–zinc inhibitor–enzyme interaction is important, then the proposed "thiobestatin" compound would be expected to possess enhanced enzyme affinity with respect to the parent natural product.

Chemistry

(A) Diamino Thiols. L-Leucinethiol (**67**) is a potent inhibitor of leucine aminopeptidase (LAP), a zinc metallopeptidase, whereas the corresponding alcohol (L-leucinol) is a much poorer inhibitor.[58] Similar results have been reported by Rich,[59] who determined that L-lysinethiol

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competitively inhibited aminopeptidase B with a K_i and 9.1×10^{-10} M; a binding constant 100000 times lower than that found for L-lysinol. Our observation that amino thiols of type 4[60] could cause profound ACE inhibition suggested that the diamino thiol framework (general structure shown in Figure 3) might allow us to design potent inhibitors of enkephalin degrading APASE, which incorporate additional subsite interactions. Several series of compounds that address the following parameters were synthesized and tested for inhibition of rat brain APASE: (1) importance of absolute configuration (i.e. asterisk in Figure 3); (2) optimal R^1, R^2; (3) optimal peptide backbone length (X).

In considering the synthesis of the diamino thiols, we wished to develop a convergent approach that would lend itself to the preparation of a number of analogues. In principle it should be possible to construct the 1,3-diamino-2-propanethiol unit shown in general structure 7 from a suitably protected 1,3-diamino-2-propanol 8 by activation of the hydroxyl group (e.g. mesylate), followed by displacement with a thiol nucleophile. However we felt such a displacement might prove difficult due to steric encumberance and the high risk of unwanted intramolecular reactions (such as aziridine or oxazoline formation).

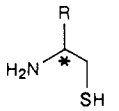
7 8

P = Protecting group

Alternatively, retrosynthetic analysis of the diamino thiol subunit suggested that the CH_2–N bond of **10** might be formed by reductive amination of an aldehyde having the general structure **11**, with a suitably protected amine component. Aldehydes of type **11** should be available from a protected β-amino ester by sulfenylation[61] followed by reduction (Scheme I).

Chiral β-amino esters are readily available by the Arndt–Eistert homologation of the corresponding amino acid derivatives.[62,63] *N*-BOC-L-phenylalanine (**13**) was converted to the corresponding diazo ketone **17** followed by Wolff rearrangement to afford the protected β-amino ester **21** (Scheme II). Treatment of the dianion[64] of **21** with *p*-methoxybenzyl disulfide gave the desired α-sulfenylated ester **25**. Reduction of **25** with lithium borohydride gave the mixture of diastereomeric alcohols **29** and **30**, which were conveniently separated by chromatography. Oxidation of alcohols **29** and **30** to the corresponding aldehydes **37** and **38** was readily accomplished with SO_3/pyridine/DMSO[65] or, more conveniently, with Dess–Martin periodinane.[66] With this methodology, the analogous α-sulfenylated aldehydes derived from *N*-BOC-D-phenylalanine, *N*-BOC-L-leucine, and *N*-BOC-D-leucine were prepared.

Table I. In Vitro Rat Brain Aminopeptidase Inhibition by Simple Amino Thiols: Variation of the 2-Substituent and Absolute Configuration



no.	R	*	I_{50}, μM
67	$CH_2CH(CH_3)_2$	*S*	0.010
68	CH_2Ph	*S*	0.039
69	CH_2Ph	*R*	6.9

Reductive amination of the α-sulfenylated aldehydes was best accomplished by condensation to form the intermediate imine followed by subsequent reduction to form the desired protected diamino thiol. For example, treatment of aldehyde **37** with L-phenylalanyl-L-leucine *tert*-butyl ester afforded an imine, which was then reduced with sodium borohydride to yield **61** as a mixture of diastereomers at the carbon bearing sulfur. Unfortunately, the α-sulfenylated aldehydes undergo facile epimerization during imine formation; therefore a mixture of diastereomeric aldehydes was generally used for the reductive amination sequence. The simultaneous removal of all the protecting groups in **61** by the method of Fujino[67] gave the desired amino thiol **62**. All of the thiol compounds shown in Table II–V were prepared in a similar manner.

Amino thiol **66**, which has been previously reported by Roques,[68] was also synthesized by the reductive amination methodology. Alkylation of thiol **63** with 2-chloroethanol afforded alcohol **64**, which upon oxidation gave aldehyde **65**.[69] Reductive amination of aldehyde **65** with L-Phe-L-Leu-O-*t*-Bu followed by deprotection gave amino thiol **66** (Scheme III).

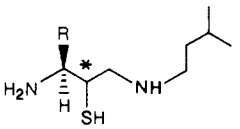
(B) Thiobestatins. Sulfenylated esters **25** and **27**, prepared for the synthesis of the diamino thiols, proved to be excellent starting points for an expeditious synthesis of the sulfur replacement analogues of bestatin. Hydrolysis of **27** afforded the corresponding acid, which was coupled (2-morpholinoethyl isocyanide[70,71]/HOBT) with L-leucine *tert*-butyl ester. Chromatographic separation of the resulting diastereomeric esters **76** and **77** followed by deprotection afforded the isomerically pure C-2 diastereomers of "thiobestatin" **78** and **79** respectively (Scheme IV). Application of the same methodology to the enantiomeric ester **25** afforded a diastereomeric pair of "epithiobestatin" analogues (**81**), which possess the *S* configuration at C-1.

Results and Discussion

Inhibitory activities were measured against a partially purified soluble rat brain aminopeptidase, similar in properties to the Leu-enkephalin degrading, rat brain aminopeptidase designated MII by Hersh.[72] The enzyme cleaves *p*-nitroanilides of neutral (Leu, Ala) and basic (Arg,

(60) Godfrey, J. D.; Gordon, E. M., unpublished observations.
(61) Trost, B. M.; Tamaru, Y. *J. Am. Chem. Soc.* **1977**, *99*, 3101–3113.
(62) From *N*-BOC-L-Phe, see: Ondetti, M. A.; Engel, S. L. *J. Med. Chem.* **1975**, *18*, 761–763.
(63) From *N*-BOC-L-Leu, see: Johnson, R. L.; Verschoor, K. *J. Med. Chem.* **1983**, *26*, 1457–1462.
(64) For a recent report on the α-alkylation of β-aminobutanoates, see: Seebach, D.; Estemann, H. *Tetrahedron Lett.* **1987**, *28*, 3103–3106.
(65) Parikh, J. R.; Doering, W. v. E. *J. Am. Chem. Soc.* **1967**, *89*, 5505–5507.
(66) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155–4156.
(67) Nishimura, O.; Kitada, C.; Fujino, M. *Chem. Pharm. Bull.* **1978**, *26*, 1576–1585.
(68) Roques, B. P.; Schwartz, J-C.; Lecomte, J. M. European Patent Application (1981), Derwent 82006D.
(69) For an alternative method of preparation, see: Lantos, I.; Razgaitis, C.; Sutton, B. M. *J. Heterocycl. Chem.* **1982**, *19*, 1375–1376.
(70) Use of morpholinoethyl isocyanide (sold by Fluka): Aigner, H.; Koch, G.; Marquarding, D. In *Chemistry of Peptides and Proteins, Proceedings of the 3rd USSR–FRG Symposium*; Voelter, W., Wunsch, E., Eds.; de Gryuyter: Berlin, 1982; Vol. 1.
(71) Marquarding, D.; Aigner, H. Ger Offen 2942606; Eur. Pat. 29909.
(72) Hersh, L. B. *J. Neurochem.* **1985**, *44*, 1427–1435.

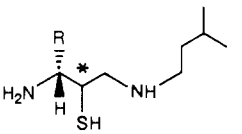
Table II. In Vitro Rat Brain Aminopeptidase Inhibition by Diamino Thiols: Variation of the 2-Substituent and Absolute Configuration at C-2, C-3



no.	R	*[a]	I_{50}, μM
53	CH_2Ph	A	0.30
54	CH_2Ph	B	0.020
55	$CH_2CH(CH_3)_2$	A	0.081
56	$CH_2CH(CH_3)_2$	B	0.029

[a] A or B indicates pure diastereomers of undetermined configuration at the starred center.

Table III. In Vitro Rat Brain Aminopeptidase Inhibition by Diamino Thiols: Variation of the 2-Substituent and Absolute Configuration at C-2, C-3



no.	R	*[a]	I_{50}, μM
57	CH_2Ph	A	3.1
58	CH_2Ph	B	0.60
59	$CH_2CH(CH_3)_2$	A	7.5
60	$CH_2CH(CH_3)_2$	B	2.5

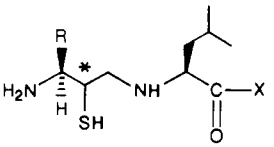
[a] A or B indicates pure diastereomers of undetermined configuration at the starred center.

Lys) amino acids (K_m = 20–80 μM; V_{max} = 50–100 nmol/min^{-1} mg^{-1}) and is inhibited by bestatin (I_{50} = 0.17 μM), amastatin (I_{50} = 0.08 μM), and Leu^5-enkephalin (K_i = 27 μM; K_i for MII = 29 μM). In particular, the rat brain enzyme utilized is differentiated from a membrane-bound enkephalin-hydrolyzing aminopeptidase isolated by Schwartz and co-workers[73] by its puromycin sensitivity (puromycin, I_{50} = 0.42 μM). The enzyme characterized by Schwartz resembles aminopeptidase M (EC 3.4.11.2) purified from rat brush border membranes and is puromycin insensitive (K_i = 100 μM). The fact that MII is inactivated by dialysis against EDTA and that activity can be restored by addition of $CoCl_2$ or $MnCl_2$ suggest that the enzyme is a metallopeptidase.

Tables II–VI summarize inhibitory activities of the various classes of novel materials prepared. In order to gauge the effectiveness of various modifications, we also prepared and tested three simple aminothiols shown in Table I. Since the N-terminal asymmetric centers of bestatin and amastatin are of the D configuration, we synthesized both L and D isomers in several series. Inspection of the data in Table I indicates that the L-phenylalanine-derived simple amino thiol (**68**)[74] is approximately 4 times less potent than the corresponding L-leucine derived inhibitor (**67**).[58] The D-phenylalanine-derived thiol (*R*-**69**)[74] was nearly 200-fold less potent than the opposite (L-Phe-derived) enantiomer **68**.

(A) Diamino Thiols. Tables II and III summarize the inhibitory activities of simple diamino thiols in which the C-2 substituent and the absolute configurations at C-2 and C-3 have been systematically varied. Compounds **53–56** were derived from L-amino acids, whereas compounds **57–60** were synthesized from D amino acid isomers. In each case, the L-derived materials (Table II) expressed a higher degree of inhibitory potency than the corresponding D-derived analogues (Table III). The most active diamino thiol diastereomers in the "L" series (**54**,**56**) proved to be of comparable inhibitory potency, and the degree of inhibition was not significantly different from that in the simple amino thiol case (Table I).

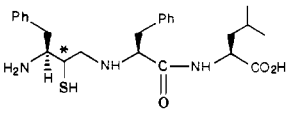
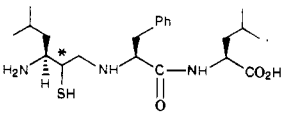
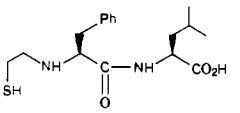
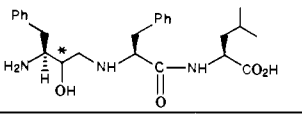
Table IV. In Vitro Rat Brain Aminopeptidase Inhibition by Dipeptidyl Diamino Thiols



no.	R^1	*[a]	X	I_{50}, μM
70	CH_2Ph	A	OH	4.4
71	CH_2Ph	B	OH	0.22
72	CH_2Ph	A, B	NH_2	0.033
73	$CH_2CH(CH_3)_2$	A, B	NH_2	0.034

[a] A or B indicates pure diastereomers of undetermined configuration at the starred center, and A, B indicates a mixture.

Table V. In Vitro Rat Brain Aminopeptidase Inhibition by Tripeptidyl Diamino Thiols and Related Analogues

no.	structure[a]	I_{50}, μM
62		0.001
74		0.004
66		240.0
75		0.58

[a] An asterisk indicates a mixture of diastereomers.

Table IV summarizes the inhibitory activities of a series of dipeptide-like diamino thiols in which the C-terminal carboxyl substituent (X) and the absolute configuration of the carbon bearing sulfur have been varied. Compounds containing a C-terminal carboxylic acid function (**70**, **71**) were found to suffer a significant loss of activity with respect to their C-terminal amide analogues (**72**, **73**). Amides **72** and **73** are diastereomers in which the C-2 stereocenter was uncontrolled. Allowing for the fact that these materials are approximately 1:1 mixtures, substances **72** and **73** were about equal in activity to compounds **54** and **56**, thus indicating that the additional carboxamide function did not lead to enhanced enzyme affinity.

Compounds **62** and **74** (Table V) contain the diamino thiol modification embedded within a tripeptide-like system. Comparison of these materials with **72** and **73** shows that a marked enhancement in inhibitory potency is obtained by the addition of a C-terminal leucine residue to form the tripeptide-like materials. Substance **62**, which possesses an I_{50} = 1 nM, is 33 times more potent than the dipeptide-like **72**, whereas **74** is approximately 8-fold more active than **73**. Deletion of N-terminal functionality up to the sulfhydryl group in **62** or **74** (i.e. **66**)[68] leads to a

(73) Giros, C.; Giros, B.; Schwartz, J-C. *Biochemistry* **1985**, *24*, 2179–2185.
(74) Yankeelov, J. A., Jr.; Fok, K. F. U.S. Patent 4 631 270, 1986.

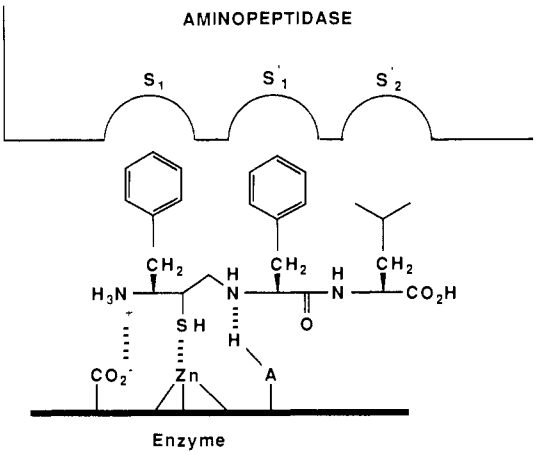
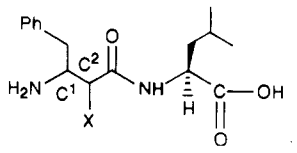


Figure 4. Proposed binding model of tripeptidyl diamino thiols to rat brain aminopeptidase.

Table VI. In Vitro Rat Brain Aminopeptidase Inhibition: Comparison of Bestatin and "Thiobestatin"



no.	X	C^1	C^{2a}	I_{50}, μM
1	OH (bestatin)	*R*	S	0.170
78	SH	*R*	A	10.0
79	SH	*R*	B	0.220
81	SH	*S*	A, B	1.6

[a] A or B indicates pure diastereomers of undetermined configuration at this center, and A, B indicates a mixture.

marked decline in inhibitory potency. The results observed in the diamino thiols is mirrored in the corresponding diamino alcohol series,[75] in which the most active inhibitor was found to be a tetrapeptide-like compound (**5**, I_{50} = 20 nM). In general, the diamino thiols were more potent APASE inhibitors than their diamino alcohol counterparts. In one case substance **62** possessed ~600 times the inhibitory potency of the corresponding alcohol analogue **75**. However the corresponding hydroxy analogue of **74** was only 40-fold less potent than the thiol compound.[75] Overall, the results indicate that sulfhydryl likely plays a positive role in the binding of tripeptide-sized diamino thiol inhibitors to rat brain APASE and is suggestive of a direct interaction of inhibitor sulfhydryl with enzymic zinc. In order to achieve inhibition that significantly exceeds that obtained with simple thiols **67** or **68**, the inhibitor molecule must be at least tripeptide sized. This suggests that rat brain APASE contains an extended active site, which may favor the binding of peptides of tripeptide size and perhaps longer. The structure–activity relationships revealed in this study point to a mode of inhibitory–enzyme binding consistent with the depiction in Figure 4.

(B) Thiobestatins. We synthesized analogues of **1** in which the secondary hydroxyl moiety was replaced by a sulfhydryl group. Although the N-terminal chiral center of bestatin is of the *R* configuration (corresponding to a D amino acid), we prepared sulfur-containing analogues

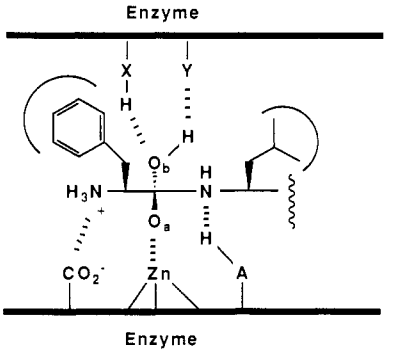


Figure 5. Hypothetical model of substrate hydrolysis by zinc-containing aminopeptidase.

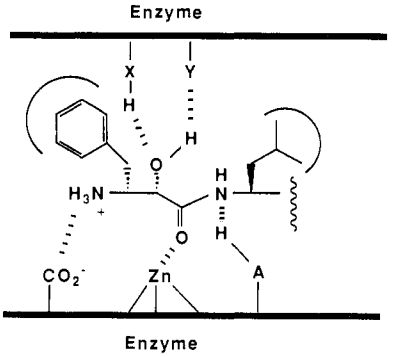


Figure 6. Hypothetical model of bestatin binding to the active site of zinc-containing aminopeptidase.

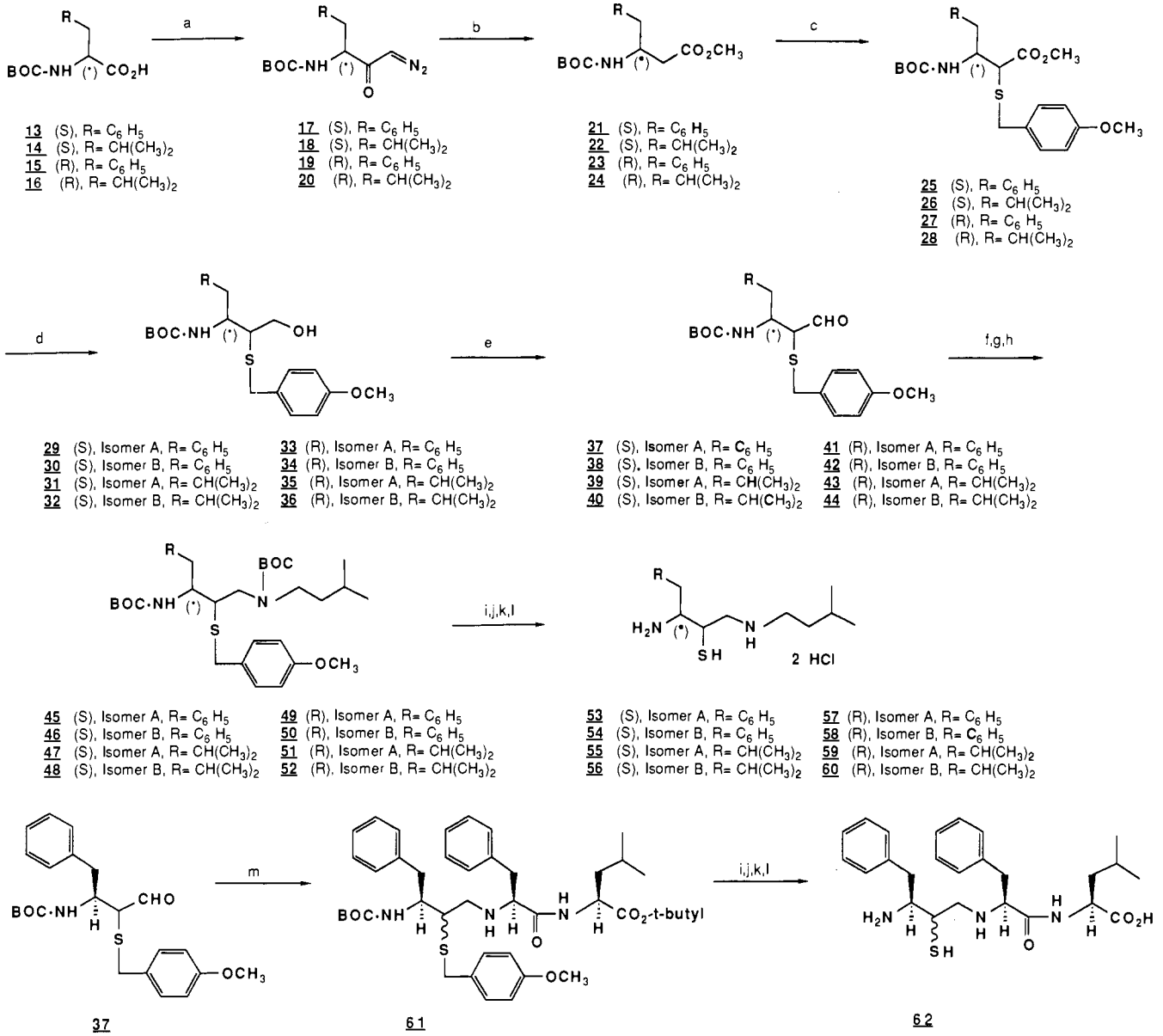
in which C-1 was either *R* or *S*. Introduction of the thiol group at C-2 of bestatin did not lead to more potent inhibitors of rat brain APASE (Table VI). The most active "thiobestatin" analogue proved to be compound **79**, which possessed approximately the same level of inhibitory activity of the rat brain enzyme as did bestatin itself. The "thiobestatin" analogue **81** in which C-1 possessed the *S* configuration (and hence similar to the diamino thiols described above) was weakly inhibitory and about 4–8 times less active than the corresponding diamino thiol **71**.

Contrary to expectations, the data indicate that replacing the hydroxyl in bestatin with sulfhydryl has little effect on the degree of enzyme inhibition. Since a sulfhydryl–zinc interaction would be expected to confer greatly enhanced enzyme–inhibitor binding compared to a hydroxyl–zinc interaction, it is probable that the mode of binding of bestatin (thiobestatin) to rat brain aminopeptidase invovles a role other than metal binding for the OH/SH groups.[76] This is likely provided that the more sterically demanding thiol does not cause a detrimental displacement of other binding functionality. An alternative functional role for the C-2 hydroxyl group of bestatin, which still adheres to the view of **1** as a transition-state analogue, is based on the alcohol serving as a mimic of the incoming nucleophile, i.e. the oxygen of the tetrahedral hydrolysis intermediate *which does not bind to zinc*. A comparison of models of the putative mode of amide bond cleavage, along with possible active-site binding of bestatin to zinc-containing aminopeptidases, is shown in Figures 5 and 6. Inspection of the proposed enzyme–inhibitor binding model shows that the C-2 hydroxyl of bestatin can occupy the same position in space as O_b of the substrate model, with respect to the (indexing) amino terminus. The

(75) Delaney, N. G.; Gordon, E. M.; Asaad, M. M.; Cushman, D. W.; Ryono, D. E.; Neubeck, R.; Natarajan, S. In *Peptides: Chemistry and Biology. Proceedings of the Tenth American Peptide Symposium*; Marshall, G. R., Ed.; ESCOM: Leiden, 1988; pp 120–122.

(76) Ocain, T. D.; Rich, D. H. Presented as a poster at the Ninth American Peptide Symposium, June 23–28, 1985, Toronto, Canada.

Scheme II. Synthesis of Diamino Thiols[a]



[a] (a) Isobutyl chloroformate, *N*-methylmorpholine, CH_2N_2; (b) silver benzoate, methanol; (c) LDA, *p*-methoxybenzyl disulfide; (d) sodium borohydride, lithium chloride; (e) Dess–Martin periodinane, *tert*-butyl alcohol; (f) $H_2NCH_2CH(CH_3)_2$, benzene, molecular sieves; (g) sodium borohydride, methanol; (h) Boc anhydride, diisopropylethylamine, THF; (i) TFA, anisole; (j) mercuric trifluoroacetate; (k) hydrogen sulfide; (l) HCl; (m) L-Phe-L-Leu-o-*t*-Bu benzene, molecular sieves, sodium borohydride, methanol.

inhibitor amide carbonyl, which substantially rigidifies the molecular backbone relative to the diamino thiol (alcohol) case, is displaced by one atom relative to the amino terminus. However, depending on the actual binding conformation, this may not preclude its interaction with enzyme zinc via the usual polarization interaction as occurs with substrate. Implied is that the inhibitor alcohol engages in enzyme–inhibitor interactions that are normally reserved for stabilization of the attacking oxygen atom in formation of the tetrahedral intermediate. Presumably the sulfhydryl group of "thiobestatin" could also satisfactorily participate in similar enzyme–inhibitor binding, especially proton donation (i.e. S–H - - -Y).

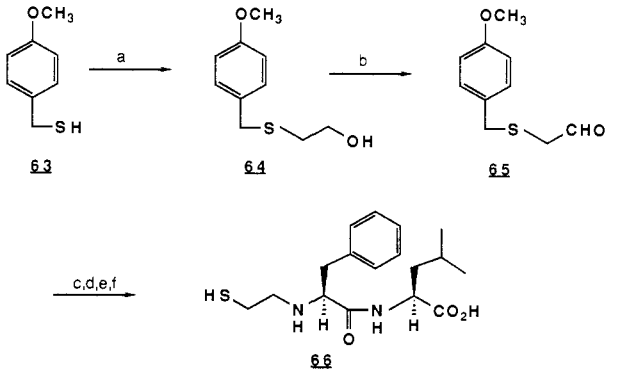
A variant of the proposed inhibitor binding shown in Figure 6 could involve chelation of zinc by amino nitrogen. This mode of interaction would differ from the model of Nishizawa et al.[45] in that the amino and carbonyl groups would participate as zinc ligands, as opposed to the amino and C-2 hydroxyl groups. The fact that the absolute stereochemistry of the amino terminal group in bestatin is of the unnatural D perhaps facilitates an amine–zinc interaction unavailable to normal L substrates, while still permitting the aromatic side chain (P_1) to productively bind to the S_1 APASE pocket.[77]

Experimental Section

Thin-layer chromatograms were run on E. Merck glass plates precoated with silica gel 60 F-254. Melting points were determined on a Thomas-Hoover capillary melting point apparatus in open capillaries and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 983 or a Matteson Sirius 100 spectrometer. ^1H NMR spectra were determined on a JEOL GX-400 (400 MHz), JEOL JNM-GX270 (270 MHz), or JEOL FX-270 (270 MHz) instrument. Chemical shifts are reported (δ) downfield from internal tetramethylsilane. Chemical-ionization (CI) mass spectra were obtained on Finnigan TSQ-4600 mass spectrometer and fast atom bombardment (FAB) mass spectra were obtained on a VG

(77) Portions of this work were presented as a poster at The Tenth American Peptide Symposium, May 23–28, 1987, St. Louis, MO.

Scheme III[a]



[a] (a) 2-Chloroethanol, sodium bicarbonate, sodium iodide; (b) sulfur trioxide/pyridine, DMSO, diisopropylethylamine; (c) L-Phe-L-Leu-o-*t*-Bu, sodium cyanoborohydride; (d) trifluoroacetic acid, anisole; (e) mercuric trifluoroacetate; (f) hydrogen sulfide.

ZAB mass spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter.

Column (flash) chromatographic separations were effected on Whatman LPS-1 silica gel (13–24 μm) with the indicated solvents. Tetrahydrofuran was distilled under argon from potassium benzophenone ketyl prior to use. Dichloromethane was distilled from phosphorus pentoxide. Diisopropylamine and diisopropylethylamine were distilled from calcium hydride. Brine refers to saturated sodium chloride solution.

Preparation of Rat Brain Aminopeptidase. Whole fresh brains of Sprague–Dawley rats were homogenized, by Polytron, in 20 volumes (w/v) of ice-cold 50 mM Tris·HCl buffer, pH 7.4, containing 1 mM dithiothreitol (DTT) and 0.01 mM zinc chloride. The homogenization vessel was kept over ice at all times. After homogenization, the soluble supernatant fraction was collected upon centrifugation at 80000*g* and 4 °C for 2 h. The supernatant was fractionated with solid ammonium sulfate. The pellet obtained between 40 and 70% saturation with ammonium sulfate was redissolved in a minimal amount of 100 mM Tris·HCl buffer, pH 7.4, containing 1 mM DTT and 0.01 mM zinc chloride and dialyzed against 10 volumes of the same buffer.

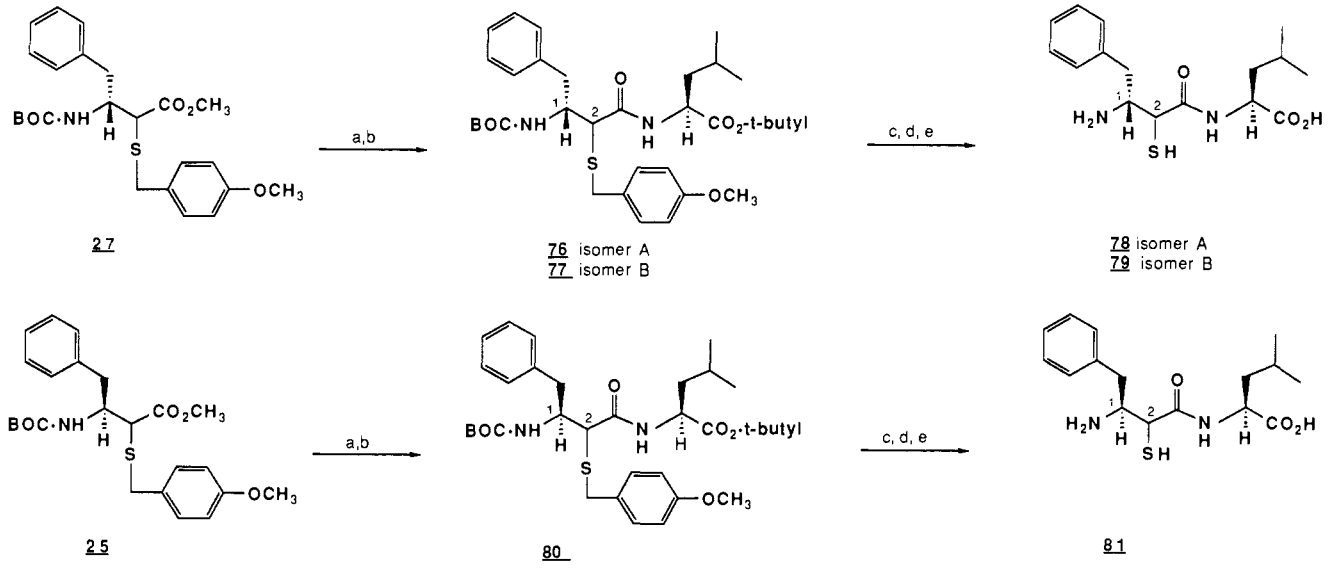
Aminopeptidase Assay. The enzymatic activity of the dialyzed fraction was assayed by the spectrophotometric determination at 405 nm for the rate of *p*-nitroaniline formation upon the hydrolysis of leucine *p*-nitroanilide. The enzymatic reaction was conducted for up to 1 h either at 37 °C or at room temperature and was initiated by adding 100-μL aliquots of the dialyzed enzyme preparation (about 30 μg of protein) to a mixture consisting of 500 μL of leucine *p*-nitroanilide solution (final concentration 100 μM), 50 μL of 50 mM Tris·HCl buffer, pH 7.4, containing 1 mM DTT and 0.01 mM zinc chloride, and inhibitor solution or water to a final volume of 1.5 mL. Control enzyme activity was about 20 nmol/mg of protein per min. In some experiments the *p*-nitroaniline released was reacted to form a purple azo dye by adding to the incubation mixture, after the reaction had been terminated with 100 μL of glacial acetic acid, 25-μL aliquots of 2.7% sodium nitrite, 13.5% ammonium sulfamate, and 2.7% *N*-naphthylethylenediamine dihydrochloride and allowing 10 min for color development. Absorbance was measured at 550 nm; *p*-nitroaniline was used as a standard.

1-Diazo-3(*S*)-[[(1,1-dimethylethoxy)carbonyl]amino]-4-phenyl-2-butanone (17). Compound **17** was prepared by using a modification of the procedure by Ondetti and Engel.[62] To a solution of *N*-BOC-L-phenylalanine (**13**) (47.75 g, 0.18 mol) and *N*-methylmorpholine (19.8 mL, 0.18 mol) in tetrahydrofuran (300 mL) at −20 °C under argon was added, over a 5-min period, isobutyl chloroformate (23.4 mL, 0.18 mol). After the mixture was stirred for 20 min at −20 °C, the *N*-methylmorpholine hydrochloride was removed by filtration and the filter cake was washed with a small portion of cold tetrahydrofuran. The filtrate was treated with a cold (−20 °C), ethereal solution of diazomethane (~270 mmol, prepared from 64.2 g of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide and distilled). After stirring at −20 °C for 30 min, the mixture was warmed to room temperature and stirred for 2.5 h. The excess diazomethane was removed by bubbling a stream of argon through the reaction mixture for 1 h and the solvent was removed at reduced pressure. The residue was dissolved in ethyl acetate and washed with water, 0.25 M citric acid, 1 N $NaHCO_3$, and brine. After drying ($MgSO_4$), the solvent was removed at reduced pressure and the residue was recrystallized from isopropyl ether to afford **17** as a bright yellow solid: 41.4 g (80%); mp 95–97 °C; IR ($CHCl_3$) 3433, 2116, 2113, 1709, 1700, 1642 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.42 (9 H, s), 3.02 (2 H, m), 4.42 (1 H, br s), 5.11 (1 H, br s), 5.22 (1 H, s), 7.15–7.32 (5 H, m); $[\alpha]_D$ −34.8° (*c* 2.61, CH_3OH); R_f 0.29 (hexane–ethyl acetate, 7:3).

1-Diazo-3(*S*)-[[(1,1-dimethylethoxy)carbonyl]amino]-5-methyl-2-hexanone (18). Diazoketone **18** was prepared by the procedure described for the preparation of **17**, starting from *N*-BOC-L-leucine **14** and was recrystallized from petroleum ether: $[\alpha]_D$ −78.8° (*c* 2.83, CH_3OH) (lit.[63] $[\alpha]_D$ −78.3° (*c* 2.51, CH_3OH)).

1-Diazo-3(*R*)-[[(1,1-dimethylethoxy)carbonyl]amino]-4-phenyl-2-butanone (19). Diazo ketone **19** was prepared by the procedure described for the preparation of **17**, starting from *N*-BOC-D-phenylalanine (**15**): $[\alpha]_D$ +35.2° (*c* 2.56, CH_3OH).

Scheme IV. Synthesis of Thiobestatins[a]



[a] (a) Sodium hydroxide, water, THF; (b) 2-morpholinoethyl isocyanide, HOBT, L-leucine *tert*-butyl ester; (c) trifluoroacetic acid, anisole; (d) mercuric trifluoroacetate; (e) hydrogen sulfide.

1-Diazo-3(*R*)-[[(1,1-dimethylethoxy)carbonyl]amino]-5-methyl-2-hexanone (20). Diazoketone **20** was prepared by the procedure described for the preparation of **17**, starting from *N*-BOC-D-leucine (**16**) and was recrystallized from petroleum ether: $[\alpha]_D$ +78.8° (*c* 2.51, CH_3OH).

Methyl 3(*S*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-4-phenylbutyrate (21). Ester **21** was prepared by using a modification of the procedure by Ondetti and Engel.[62] To a solution of diazo ketone **17** (10.0 g, 34.5 mmol) in anhydrous methanol (100 mL) was added 10 mL of a solution of silver benzoate (1.0 g) in triethylamine (20 mL). The solution darkened and rapid gas evolution was noted. After the mixture was stirred for 20 min, an additional 5.0 mL of the silver benzoate–triethylamine solution was added. After the mixture was stirred for an additional 45 min, Celite and decolorizing carbon was added followed by saturated sodium chloride (30 mL). After stirring for several minutes, the mixture was filtered through Celite and the filtrate was concentrated at reduced pressure. The residue was dissolved in ethyl acetate and the resulting solution was washed with water, 1 N $NaHCO_3$, 1 N HCl, 1 N $NaHCO_3$, and brine. After drying ($MgSO_4$), the solvent was removed at reduced pressure and the residue chromatographed (benzene–isopropyl ether, 85:15) to give **21** as a colorless solid: 8.43 g (83%); mp 51–52 °C; IR ($CHCl_3$) 3439, 1725, 1709, 1498 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.40 (9 H, s), 2.47 (2 H, m), 2.86 (2 H, m), 3.68 (3 H, s), 4.14 (1 H, br m), 5.00 (1 H, br s), 7.16–7.32 (5 H, m); $[\alpha]_D$ −20.1° (*c* 1.12, CH_3OH); R_f 0.26 (benzene–isopropyl ether, 85:15). Anal. ($C_{16}H_{23}NO_4$) C, H, N.

Methyl 3(*S*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-5-methylhexanoate (22). Ester **22** was prepared by the procedure described for the preparation of **21**, starting with *S* diazo ketone **18** (35.8 g, 0.14 mol). Chromatography (hexane–ether, 4:1) gave **22**[63] as a nearly colorless oil: 32.3 g (89%); $[\alpha]_D$ −22.8° (*c* 1.47, CH_3OH). Anal. ($C_{13}H_{25}NO_4$) C, H, N.

Methyl 3(*R*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-4-phenylbutyrate (23). Ester **23** was prepared by the procedure described for the preparation of **21**, starting with *R* diazo ketone **19**: $[\alpha]_D$ +19.9° (*c* 1.29, CH_3OH). Anal. ($C_{16}H_{23}NO_4$) C, H, N.

Methyl 3(*R*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-5-methylhexanoate (24). Ester **24** was prepared by the procedure described for the preparation of **21**, starting with *R* diazo ketone **20** (25.1 g, 98 mmol). Chromatography (hexane–ether, 4:1) afforded **24** as a nearly colorless oil: 25.03 g (98%); $[\alpha]_D$ +22.9° (*c* 1.42, CH_3OH). Anal. ($C_{13}H_{25}NO_4$) C, H, N.

4-Methoxybenzyl Disulfide. To a solution of potassium carbonate (44.8 g, 0.32 mol) in water (200 mL) and methanol (200 mL) were added 4-methoxy-α-toluenethiol (25 g, 0.16 mol) and benzene (200 mL). To the resulting mixture, with rapid stirring, was added iodine, portionwise, until the color of iodine persisted (21.6 g of I_2 required, 85 mmol). After the mixture was stirred for 15 min, the excess iodine was destroyed by the addition of sodium thiosulfate. An additional 200 mL of benzene was added, and the aqueous phase was separated and extracted with benzene (100 mL). The organic fractions were combined and washed with water, dilute sodium thiosulfate solution, and brine. After drying ($MgSO_4$), the solvent was removed at reduced pressure and the residue recrystallized from ethyl acetate to afford 4-methoxybenzyl disulfide as a colorless solid: 17.3 g (69.8%); mp 100 °C; ^1H NMR (270 MHz, $CDCl_3$) δ 3.60 (2 H, s), 3.81 (3 H, s), 6.85 (2 H, d, *J* = 8.4 Hz), 7.17 (2 H, d, *J* = 8.4 Hz). Anal. ($C_{16}H_{18}O_2S_2$) C, H, S.

Methyl 3(*S*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-(*R*,*S*)-[[(4-methoxyphenyl)methyl]thio]-5-methylhexanoate (25). To a solution of diisopropylamine (2.10 mL, 15 mmol) in dry tetrahydrofuran (20 mL) at 0 °C under argon was added a hexane solution of *n*-butyllithium (6.10 mL of a 2.40 M solution, 14.65 mmol). After stirring at 0 °C for 30 minutes, the resulting solution of lithium diisopropylamide was cooled to −78 °C and a solution of ester **21** (2.0 g, 6.81 mmol) in tetrahydrofuran (8 mL) was added dropwise over 5 min. After the mixture was stirred for 15 min, a solution of 4-methoxybenzyl disulfide (2.50 g, 8.18 mmol) in tetrahydrofuran (9 mL) was added. After stirring for 5 min at −78 °C, the mixture was warmed to 0 °C and stirred for 45 min. The reaction was then quenched by adding 1 N HCl and diluted with ethyl acetate. The resulting solution was washed with water, 1 N HCl, 1 N $NaHCO_3$, and brine. After drying ($MgSO_4$), the solvent ware removed at reduced pressure and the residue chromatographed (benzene–isopropyl ether, 92:8) to give **25**, a mixture of diastereomers, as a colorless oil: 2.20 g (72%); IR ($CHCl_3$) 3431, 1721, 1709, 1512, 1498 cm^{-1}; R_f 0.40 (benzene–isopropyl ether, 85:15).

Methyl 3(*S*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-(*R*,*S*)-[[(4-methoxyphenyl)methyl]thio]-5-methylhexanoate (26). α-Sulfenylated ester **26** was prepared by the procedure described for the preparation of **25**, starting with ester **22** (2.0 g, 7.71 mmol). Chromatography (benzene–ethyl acetate, 95:5) afforded **26** as a nearly colorless oil: 1.86 g (58%); R_f 0.27 (benzene–ethyl acetate, 95:5).

Methyl 3(*R*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-(*R*,*S*)-[[(4-methoxyphenyl)methyl]thio]-4-phenylbutyrate (27). α-Sulfenylated ester **27** was prepared by the procedure described for the preparation of **25**, starting with ester **23**.

Methyl 3(*R*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-(*R*,*S*)-[[(4-methoxyphenyl)methyl]thio]-5-methylhexanoate (28). α-Sulfenylated ester **28** was prepared by the procedure described for the preparation of **26**, starting with ester **24**.

3(*S*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-[[(4-methoxyphenyl)methyl]thio]-4-phenylbutanol (29, Isomer A; 30, Isomer B). To a solution of ester **25** (2.20 g, 4.94 mmol) in tetrahydrofuran (20 mL) and absolute ethanol (20 mL) were added lithium chloride (0.91 g, 21.4 mmol) and sodium borohydride (0.81 g, 21.4 mmol). After the mixture was stirred for 18 h at room temperature, the reaction was quenched with 1 N HCl and extracted with ethyl acetate. The organic fraction was washed with water, 1 N HCl, 1 N $NaHCO_3$, and brine. After drying ($MgSO_4$), the solvent was removed at reduced pressure and the residue was chromatographed (benzene–acetone, 92:8) to give the separated diastereomers as colorless solids. **29** (isomer A): 0.43 g (21%); mp 93–94 °C; IR ($CHCl_3$) 3435, 1688, 1510, 1505 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.37 (9 H, s), 2.67 (1 H, m), 2.84, 2.95 (2 H, coupled AB q, J_{AB} = 13.7 Hz, J_{HA} = 7.4 Hz, J_{HB} = 8.0 Hz), 3.47 (2 H, m), 3.64 (2 H, s), 3.78 (3 H, s), 3.98 (1 H, dd, *J* = 5.8, 9.0 Hz), 4.40 (1 H, m), 4.54 (1 H, d, *J* = 9.5 Hz), 6.81 (2 H, d, *J* = .0 Hz), 7.10–7.30 (7 H, m); $[\alpha]_D$ −14.9° (*c* 1.35, CH_3OH); R_f 0.28 (benzene–ethyl acetate, 92:8). Anal. ($C_{23}H_{31}NO_4S$) C, H, N, S. **30** (isomer B): 1.17 g (57%); mp 73–74 °C; IR ($CHCl_3$) 3430, 1702, 1511, 1503 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.35 (9 H, s), 2.55–3.05 (4 H, m), 3.65 (2 H, m), 3.73 (2 H, s), 3.79 (3 H, s), 4.09 (1 H, br), 4.79 (1 H, d, *J* = 8.4 Hz), 6.82 (2 H, d, *J* = 8.4 Hz), 7.10–7.27 (7 H, m); $[\alpha]_D$ +9.20° (*c* 1.25, CH_3OH); R_f 0.20 (benzene–ethyl acetate, 92:8). Anal. ($C_{23}H_{31}NO_4S$) C, H, N, S.

3(*S*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-[[(4-methoxyphenyl)methyl]thio]-5-methylhexanol (31, Isomer A; 32, Isomer B). Alcohols **31** and **32** were pepared by the procedure described for the preparation of **29** and **30**, starting with ester **26** (1.38 g, 3.35 mmol). Chromatography (toluene–ethyl acetate, 4:1) afforded the separated diastereomers. **31** (isomer A): 0.13 g (10%); mp 121–123 °C; IR ($CHCl_3$) 3436, 1686, 1510, 1506 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.85 (6 H, m), 1.10 (1 H, m), 1.25–1.58 (11 H, m with singlet at 1.42), 2.61 (1 H, m), 3.40 (1 H, m), 3.56 (1 H, m), 3.64, 3.70 (2 H, AB q, *J* = 13.5 Hz), 3.79 (3 H, s), 4.10 (1 H, br), 4.21 (1 H, m), 4.44 (1 H, d, *J* = 9.5 Hz), 6.84 (2 H, d, *J* = 8.4 Hz), 7.20 (2 H, d, *J* = 8.4 Hz); MS (CI) $(M + H)^+$ *m/e* 384; R_f 0.41 (toluene–ethyl acetate, 4:1). Anal. ($C_{20}H_{33}NO_4S$) C, H, N, S. **32** (isomer B): colorless oil, 0.52 g (40%); IR ($CHCl_3$) 3435, 1702, 1510 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.88 (6 H, d, *J* = 7 Hz), 1.17–1.72 (12 H, m, singlet at 1.43), 2.53 (1 H, br), 2.65 (1 H, m), 3.60–3.70 (2 H, m), 3.72 (2 H, s), 3.78 (3 H, m), 3.82–3.97 (1 H, m), 4.65 (1 H, br d, *J* = 9.4 Hz), 6.83 (2 H, d, *J* = 8.8 Hz), 7.22 (2 H, d, *J* = 8.8 Hz); MS (FAB), $(M + H)^+$ *m/e* 384, $(M - H)^-$ *m/e* 382; R_f 0.22 (toluene–ethyl acetate, 4:1).

3(*R*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-[[(4-methoxyphenyl)methyl]thio]-4-phenylbutanol (33, Isomer A; 34, Isomer B). Alcohols **33** and **34** were prepared by the procedure described for the preparation of **29** and **30**, starting with ester **27**. **33**, (isomer A): mp 94–95 °C, $[\alpha]_D$ +14.7° (*c* 1.08, CH_3OH). **34** (isomer B): mp 73–74 °C, $[\alpha]_D$ −8.5° (*c* 1.15, CH_3OH). Anal. ($C_{23}H_{31}NO_4S$) C, H, N, S.

3(*R*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-[[(4-methoxyphenyl)methyl]thio]-5-methylhexanol (35, Isomer

A; 36, Isomer B). Alcohols 35 and 36 were prepared by the procedure described for the preparation of 31 and 32, starting with ester 28.

3(*S*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-[[(4-methoxyphenyl)methyl]thio]-4-phenylbutanal (37, Isomer A; 38, Isomer B). To a stirred suspension of Dess–Martin periodinane[66] (1.34 g, 3.16 mmol) in anhydrous dichloromethane (20 mL) at room temperature under argon was added *tert*-butyl alcohol (0.30 mL, 3.19 mmol). After the mixture was stirred for 20 min, a slightly hazy solution was obtained. To this solution was added a solution of alcohol **29** (1.25 g, 3.01 mmol) in dichloromethane (8 mL). After 20 min the reaction mixture was diluted with ether and then added to a stired solution of sodium thiosulfate pentahydrate (5.20 g, 21.0 mmol) in 1 N $NaHCO_3$ (50 mL). After the mixture was stirred for 20 min, the organic fraction was washed with 1 N $NaHCO_3$, water, and brine. After drying ($MgSO_4$), the solvent was removed at reduced pressure to give **37** as a pale yellow solid which was used without further purification: 1.24 g (99%); IR ($CHCl_3$) 1708, 1510, 1496 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.39 (9 H, s), 2.85 (2 H, m), 3.13 (1 H, br), 3.50 (3.67 (2 H, AB q, J = 13.2 Hz), 3.79 (3 H, s), 4.27 (1 H, br), 4.59 (1 H, br), 6.82 (2 H, d, J = 8.8 Hz), 7.02–7.25 (7 H, m), 9.32 (1 H, d, J = 4.1 Hz); R_f 0.53 (benzene–ethyl acetate, 4:1).

Aldehyde **38**, a pale yellow solid, was prepared by the procedure described for the preparation of **37**, starting with alcohol **30**: mp 128 °C dec; IR ($CHCl_3$) 1709, 1512, 1496 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.37 (9 H, s), 2.87 (2 H, d, J = 5.8 Hz), 3.01 (1 H, dd, J = 4.7, 8.9 Hz), 3.51, 3.66 (2 H, AB q, J = 13.6 Hz), 3.82 (3 H, s), 4.23 (1 H, br), 4.60 (1 H, d, J = 8.9 Hz), 6.85 (2 H, d, J = 8.4 Hz), 6.91 (1 H, m), 7.16–7.25 (6 H, m), 9.20 (1 H, d, J = 4.7 Hz); R_f 0.53 (benzene–ethyl acetate, 4:1). Anal. ($C_{23}H_{29}NO_4S$) C, H, N, S.

3(*S*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-[[(4-methoxyphenyl)methyl]thio]-5-methylhexanal (39, Isomer A; 40, Isomer B). Oxidation of alcohols **31** and **32**, by the procedure described for the preparation of **37**, afforded aldehydes **39** and **40**, respectively, as colorless solids which were used without further purification. **39**: IR ($CHCl_3$) 1708, 1510 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.86 (6 H, d, J = 6.5 Hz), 1.10–1.70 (12 H, m with singlet at 1.43), 3.09 (1 H, br), 3.54, 3.69 (2 H, AB q, J = 13.2 Hz), 3.79 (3 H, s), 4.08 (1 H, m), 4.45 (1 H, br), 6.84 (2 H, d, J = 8.8 Hz), 7.22 (2 H, d, J = 8.8 Hz), 9.33 (1 H, d, J = 4.1 Hz); R_f 0.39 (toluene–ethyl acetate, 9:1). **40**: IR ($CHCl_3$) 1708, 1510, 1501 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.87 (3 H, d, J = 7.0 Hz), 0.88 (3 H, d, J = 6.5 Hz), 1.15–1.70 (12 H, m with singlet at 1.43), 3.04 (1 H, dd, J = 4.1, 7.3 Hz), 3.56, 3.67 (2 H, AB q, J = 13.5 Hz), 3.79 (3 H, s), 4.05 (1 H, m), 4.54 (1 H, br), 6.84 (2 H, d, J = 8.8 Hz), 7.22 (2 H, d, J = 8.8 Hz), 9.28 (1 H, d, J = 4.1 Hz); R_f 0.39 (toluene–ethyl acetate, 9:1).

3(*R*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-[[(4-methoxyphenyl)methyl]thio]-4-phenylbutanal (41, Isomer A; 42, Isomer B). Aldehydes **41** and **42** were prepared by the oxidation of alcohols **33** and **34**, respectively, by the procedure described for the preparation of **37**.

3(*R*)-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-[[(4-methoxyphenyl)methyl]thio]-5-methylhexanal (43, Isomer A; 44, Isomer B). Oxidation of alcohols **35** and **36**, by the procedure described for the preparation of **37**, afforded aldehydes **43** and **44**, respectively, as colorless solids which were used without further purification.

General Procedure for Reductive Amination of the Aldehydes. A mixture of the aldehyde (1.3–3.4 mmol), the amine component (2–4 equiv), and crushed 3-Å molecular sieves (~2 g) in anhydrous benzene (10–20 mL) was stirred at room temperature under argon for 0.75–2.5 h. The mixture was then filtered through Celite and the filtrate was concentrated at reduced pressure to give the crude imine.

The crude imine was dissolved in ice cold anhydrous methanol (10–30 mL) and then treated with sodium borohydride (2 equiv). After the mixture was stirred for 15–30 min at 0 °C, the reaction was quenched by the careful addition of 1 N HCl and then extracted with ethyl acetate. The organic fraction was washed with water, 1 N HCl, 1 N $NaHCO_3$, and brine. After drying ($MgSO_4$), the solvent was removed at reduced pressure to give the crude product.

General Method for Deprotection. A solution of distilled trifluoroacetic acid (14 mL) and anisole (1.0 mL) was added to the compound to be deprotected (0.68–1.86 mmol), and the resulting solution was stirred at room temperature under argon for 1–1.5 h. After the mixture was cooled to 0 °C, mercuric trifluoroacetate (1.1 equiv), was added and stirring continued for 1–1.25 h. The resulting solution was concentrated at reduced pressure and the residue was triturated with ether–hexane to afford a solid which was collected by filtration, washed with hexane, and dried under vacuum.

The solid from above was dissolved in HOAc–H_2O (4:1, 20 mL) and hydrogen sulfide was bubbled through the solution for 30 min. The resulting mercuric sulfide was removed by filtration through Celite and the colorless filtrate was concentrated at reduced pressure. The residue was dissolved in degassed distilled water (20 mL) and 1 N HCl (1.5 equiv for each amino group present) and the resulting solution was filtered (0.45-μm membrane filter). The filtrate was lyophilized and the residue was dissolved in degassed distilled water and relyophilized (repeated twice) to afford the desired compounds as hydrochloride salts.

β(*S*)-Amino-α-[[(3-methylbutyl)amino]methyl]benzenepropanethiol Dihydrochloride (53, Isomer A; 54, Isomer B). Reductive amination of a mixture of aldehydes **37** and **38** (1.0 g, 2.41 mmol) with isoamylamine (0.86 mL, 7.3 mmol) using the standard conditions for the reductive amination afforded the intermediate amine as a yellow oil which could not be purified by chromatography. This material was dissolved in anhydrous tetrahydrofuran (20 mL) and then treated with di-*tert*-butyl dicarbonate (0.68 g, 3.12 mmol) followed by diisopropylethylamine (0.57 mL, 3.27 mmol). After stirring at room temperature for 2 h, the mixture was diluted with ethyl acetate and washed with water, 1 N HCl, 1 N $NaHCO_3$, and brine. After drying ($MgSO_4$), the solvent was removed at reduced pressure and the residue chromatographed (cyclohexane–ether, 4:1) to give the separated diastereomers as colorless oils. **45** (Isomer A): 0.43 g (31%); MS (FAB), $(M + H)^+$ m/e 587, $(M - H)^-$ m/e 585; R_f 0.32 (cyclohexane–ether, 4:1). **46** (isomer B): 0.54 g (38%); MS (FAB), $(M + H)^+$ m/e 587, $(M - H)^-$ m/e 585; R_f 0.23 (cyclohexane–ether, 4:1).

Compound **45** from above (0.40 g, 0.68 mmol) was deprotected to give **53** as a colorless solid: 0.22 g (88%); mp 83–95 °C; ^1H NMR (270 MHz, CD_3OD) δ 0.95 (6 H, d, J = 7 Hz), 1.56–1.73 (3 H, m), 3.02–3.50 (7 H, m), 3.91 (1 H, m), 7.28–7.43 (5 H, m); MS (CI), $(M + H)^+$ m/e 267; $[\alpha]_D$ −42.4° (c 1.04, pyridine); R_f 0.62 (n-BuOH–HOAc–H_2O, 3:1:1). Anal. ($C_{15}H_{26}N_2S$·2.12HCl·1.16H_2O) C, H, N, S, SH, Cl.

Compound **46** from above (0.45 g, 0.77 mmol) was deprotected to give **54** as a colorless solid: 0.26 g (96%); mp 108–115 °C; ^1H NMR (270 MHz, CD_3OD) δ 0.98 (6 H, d, J = 7 Hz), 1.59–1.67 (3 H, m), 2.85–3.15 (5 H, m), 3.49 (1 H, dd, J = 5.4, 13.2 Hz), 3.69 (1 H, dt, J = 2.4, 10.8 Hz), 3.99 (1 H, m), 7.30–7.50 (5 H, m); MS (CI), $(M + H)^+$ m/e 267; $[\alpha]_D$ −26.9° (c 1.37, pyridine); R_f 0.62 (n-BuOH–HOAc–H_2O, 3:1:1). Anal. ($C_{15}H_{26}N_2S$·2.07HCl·0.58H_2O) C, H, N, S, SH, Cl.

3(*S*)-Amino-5-methyl-1-[(3-methylbutyl)amino]-2-hexanethiol Dihydrochloride (55, Isomer A; 56, Isomer B). Reductive amination of a mixture of aldehydes **39** and **40** (1.89 g, 4.95 mmol) with isoamylamine (1.72 mL, 14.9 mmol) afforded the intermediate amine as a nearly colorless oil. The crude amine was dissolved in anhydrous tetrahydrofuran (20 mL) and then treated with di-*tert*-butyldicarbonate (1.13 g, 5.2 mmol) followed by diisopropylethylamine (1.29 mL, 7.43 mmol). After stirring at room temperature for 1 **h**, the mixture was diluted with ethyl acetate and washed with water, 1 N HCl, 1 N $NaHCO_3$, and brine. After drying ($MgSO_4$), the solvent was removed at reduced pressure and the residue chromatographed (cyclohexane–ether, 85:15) to give the separated diastereomers as colorless oils. **47** (isomer A): 1.12 g (41%), R_f 0.35 (cyclohexane–ether, 4:1). **48** (isomer B): 0.93 g (34%), R_f 0.28 (cyclohexane–ether, 4:1).

Compound **47** from above (1.03 g, 1.86 mmol) was deprotected to give **55** as a tan solid: 0.33 g (55%); mp 75–80 °C; ^1H NMR (270 MHz, CD_3OD) δ 1.0 (12 H, m), 1.58–1.80 (6 H, m), 3.05–4.12 (6 H, m); MS (CI), $(M + H)^+$ m/e 233; $[\alpha]_D$ +16.1°, $[\alpha]_{365}$ +55.8° (c 0.52, pyridine); R_f 0.52 (n-BuOH–HOAc–H_2O, 4:1:1). Anal. ($C_{12}H_{28}N_2S$·2.0HCl·0.83H_2O) C, H, N, S, SH, Cl.

Compound **48** from above (0.85 g, 1.53 mmol) was deprotected to give **56** as a nearly colorless solid: 0.41 g (84%); mp 221 °C dec; ^{1}H NMR (270 MHz, CD_3OD) δ 1.02 (12 H, m), 1.30 (1 H, m), 1.60–1.78 (5 H, m), 3.08–3.72 (6 H, m); MS (CI), $(M + H)^+$ *m/e* 233; $[\alpha]_D$ +2.04° (*c* 0.54, pyridine); R_f 0.52 (*n*-BuOH–HOAc–H_2O, 4:1:1). Anal. ($C_{12}H_{28}N_2S$·2.0HCl·0.60H_2O) C, H, N, S, SH, Cl.

β(*R*)-Amino-α-[[(3-methylbutyl)amino]methyl]benzenepropanethiol Dihydrochloride (57, Isomer A; 58, Isomer B). Reductive amination of a mixture of aldehydes **41** and **42** with isoamylamine followed by treatment with di-*tert*-butyl dicarbonate, as described for the preparation of **45** and **46**, afforded the separated diastereomers, **49** (isomer A) and **50** (isomer B), as colorless oils. Deprotection of **49** and **50** afforded **57** and **58**, respectively. **57** (isomer A): $[\alpha]_D$ +44.7° (*c* 1.02, pyridine). Anal. ($C_{15}H_{26}N_2S$·2.05HCl·0.72H_2O) C, H, N, S, SH, Cl. **58** (isomer B): $[\alpha]_D$ +26.7° (*c* 1.13, pyridine). Anal. ($C_{15}H_{26}N_2S$·2.05HCl·0.55H_2O) C, H, N, S, SH, Cl.

3(*R*)-Amino-5-methyl-1-[(3-methylbutyl)amino]-2-hexanethiol Dihydrochloride (59, Isomer A; 60, Isomer B). Reductive amination of a mixture of aldehydes **43** and **44** with isoamylamine followed by treatment with di-*tert*-butyl dicarbonate, as described for the preparation of **47** and **48**, afforded the separated diastereomers, **51** (isomer A) and **52** (isomer B), as colorless oils. Deprotection of **51** and **52** afforded **59** and **60**, respectively. **59** (isomer A): $[\alpha]_D$ −15.5°, $[\alpha]_{365}$ −56.1° (*c* 0.51, pyridine). Anal. ($C_{12}H_{28}N_2S$·2.0HCl·0.85H_2O) C, H, N, S, SH, Cl. **60** (isomer B): $[\alpha]_D$ −1.93° (*c* 0.57, pyridine). Anal. ($C_{12}H_{28}N_2S$·2.0HCl·0.50H_2O) C, H, N, S, SH, Cl.

***N*-[*N*-[3(*S*)-Amino-2(*R*,*S*)-mercapto-4-phenylbutyl]-L-phenylalanyl]-L-leucine Dihydrochloride (62).** Reductive amination of aldehyde **37** (0.55 g, 1.32 mmol) with L-phenylalanyl-L-leucine *tert*-butyl ester (0.88 g, 2.63 mmol) followed by chromatography (benzene–acetone, 95:5) afforded the ester **61**, a mixture of diastereomers, as a colorless oil: 0.78 g (80%), R_f 0.33, 0.38 (benzene–ethyl acetate, 4:1).

Ester **61** (0.78 g, 1.06 mmol) was deprotected to give **62**, a 4:1 mixture of diastereomers, as a colorless solid: 0.49 g (82%); mp 123–160 °C; IR (KBr) 3407, 1722, 1673 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 0.88 (3 H, d, J = 7 Hz), 0.94 (3 H, d, J = 7 Hz), 1.50–1.73 (3 H, m), 2.85, 3.02 (2 H, coupled AB q, J_{AB} = 16 Hz, J_{HA} = 10 Hz, J_{HB} = 7 Hz), 3.09–3.41 (5 H, m), 3.62 (1 H, m), 3.82 (0.5 H, m), 3.93 (1 H, m), 4.10 (0.5 H, m), 4.22 (1 H, t, J = 7 Hz), 4.32 (1 H, dd, J = 7, 10 Hz), 7.26–7.41 (10 H, m); MS (FAB), $(M + H)^+$ *m/e* 458, $(M - H)^-$ *m/e* 456; $[\alpha]_D$ −12.8° (*c* 1.23, CH_3OH); R_f 0.70 (*n*-BuOH–HOAc–H_2O, 4:1:1). Anal. ($C_{25}H_{35}N_3O_3S$·1.8HCl·1.2H_2O) C, H, N, S, SH, Cl.

2-[[(4-Methoxyphenyl)methyl]thio]ethanol (64). A mixture of 4-methoxy-α-toluenethiol (25.5 g, 0.165 mol), 2-chloroethanol (13.3 g, 0.165 mol), sodium bicarbonate (16.7 g, 0.198 mol), and sodium iodide (4.96 g, 33 mmol) in acetone (250 mL) was heated at reflux under argon. After 3 days, TLC analysis showed the conversion to be very slow. An additional portion of sodium iodide (4.96 g, 33 mmol) was added along with water (20 mL). After the mixture was heated for 24 h, an additional portion of sodium iodide (5.0 g, 33 mmol) and sodium bicarbonate (8.0 g, 95 mmol) was added and heating continued. After an additional 48 h at reflux, the mixture was filtered and the filtrate was concentrated at reduced pressure. The residue was dissolved in ethyl acetate and the resulting solution was washed with water, 1 N $NaHCO_3$, and brine. After drying ($MgSO_4$), the solvent was removed at reduced pressure to give a nearly colorless oil which, upon standing, deposited crystals. The crystals were collected, and the mother liquor was concentrated and treated with hexane to afford a second crop of crystals. The material was then recrystallized twice from isopropyl ether to afford **64** as a colorless solid: 15.71 g (48%); mp 48.9–49.2 °C; IR ($CHCl_3$) 3617, 3487, 1610, 1511, 1251 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.09 (1 H, br s), 2.62 (2 H, t, J = 6 Hz), 3.66 (2 H, t, J = 6 Hz), 3.68 (2 H, s), 3.78 (3 H, s), 6.84 (2 H, m), 7.22 (2 H, m); R_f 0.26 (benzene–acetone, 9:1).

[[(4-Methoxyphenyl)methyl]thio]acetaldehyde (65). A solution of sulfur trioxide–pyridine complex (3.60 g, 22.6 mmol) in anhydrous dimethyl sulfoxide (24 mL) was stirred at room temperature under argon for 15 min and then diluted with dichloromethane (12 mL). To the resulting solution were added, in one portion, a solution of alcohol **64** (1.50 g, 7.56 mmol) and diisopropylethylamine (7.90 mL, 45.3 mmol) in dichloromethane (18 mL). After stirring for 15 min, the mixture was diluted wth ethyl acetate and washed with water, 1 N HCl, 1 N $NaHCO_3$, and brine. After drying ($MgSO_4$), the solvent was removed at reduced pressure and the residue chromatographed (hexane–ether, 7:3) to afford aldehyde **65**[69] as a pale yellow oil: 0.92 g (62%); IR ($CHCl_3$) 1719 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.07 (2 H, d, J = 3.7 Hz), 3.59 (2 H, s), 3.79 (3 H, s), 6.85 (2 H, d, J = 8.4 Hz), 7.21 (2 H, d, J = 8.4 Hz), 9.41 (1 H, t, J = 3.7 Hz); R_f 0.53 (benzene–acetone, 9:1).

***N*-[*N*-(2-Mercaptoethyl)-L-phenylalanyl]-L-leucine Monohydrochloride (66).** A mixture of aldehyde **65** (0.92 g, 4.68 mmol), L-phenylalanyl-L-leucine *tert*-butyl ester (3.13 g, 9.35 mmol), and crushed 3-Å sieves (6 g) in tetrahydrofuran (15 mL) and absolute ethanol (15 mL) was stirred at room temperature under argon. After the mixture was stirred for 2 h, sodium cyanoborohydride (0.88 g, 14 mmol) was added. After the mixture was stirred for 16 h, the reaction mixture was filtered through Celite and the filtrate was diluted with ethyl acetate and washed with water, 1 N HCl, 1 N $NaHCO_3$, and brine. After drying ($MgSO_4$), the solvent was removed at reduced pressure and the residue chromatographed [benzene–ethyl acetate (85:15) followed by rechromatography using hexane–ethyl acetate (65:35)] to give the intermediate amine as a colorless oil: 0.90 g (37%); MS (FAB), $(M + H)^+$ *m/e* 515, $(M - H)^-$ 513; R_f 0.26 (hexane–ethyl acetate, 65:35).

The intermediate from above (0.58 g, 1.13 mmol) was deprotected to give **66**[68] as a colorless solid: 0.28 g (66%); mp 158–164 °C; IR (KBr) 3397, 3209, 1724, 1675 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 1.92 (3 H, d, J = 7 Hz), 1.95 (3 H, d, J = 7 Hz), 1.53–1.67 (3 H, m), 2.78 (2 H, m), 3.10 (2 H, m), 3.22 (2 H, m), 4.15 (1 H, t, J = 7 Hz), 1.53–1.67 (3 H, m), 2.78 (2 H, m), 3.10 (2 H, m), 3.22 (2 H, m), 4.15 (1 H, t, J = 7 Hz), 4.39 (1 H, t, J = 7 Hz), 7.33 (5 H, m); $[\alpha]_D$ −7.1° (*c* 1.0, CH_3OH); MS (FAB), $(M + H)^+$ *m/e* 339, $(M - H)^-$ *m/e* 337; R_f 0.26 ($CHCl_3$–CH_3OH–HOAc, 18:1:1); R_f 0.75 (*n*-BuOH–HOAc–H_2O, 4:1:1). Anal. ($C_{17}H_{26}N_2O_3S$·HCl·0.65H_2O) C, H, N, S, Cl, SH.

***N*-[3(*S*)-Amino-2-mercapto-4-phenylbutyl]-L-leucine Dihydrochloride (70, Isomer A; 71, Isomer B).** Reductive amination of mxture of aldehydes **37** and **38** (1.43 g, 3.44 mmol) with L-leucine *tert*-butyl ester (1.93 g, 10.3 mmol) followed by chromatography (silica gel, cyclohexane–ether, 6:4) afforded the intermediate as a mixture of diastereomers. Rechromatography (toluene–ethyl acetate, 9:1) afforded the separate diastereomers as colorless oils. Isomer A: 0.89 g (44%); MS (FAB), $(M + H)^+$ *m/e* 587, $(M - H)^-$ *m/e* 585; R_f 0.43 (toluene–ethyl acetate, 9:1). Isomer B: 0.61 g (30%); MS (FAB), $(M + H)^+$ *m/e* 587, $(M - H)^-$ *m/e* 585; R_f 0.28 (toluene–ethyl acetate, 9:1).

Isomer A (0.71 g, 1.21 mmol) was deprotected to afford material which, by ^{1}H NMR, still contained some unprotected *tert*-butyl ester. In order to remove the remaining *tert*-butyl ester, this material was dissolved in 3 N HCl–HOAc (15 mL). The mixture was stirred for 4 h at room temperature, the addition of ether afforded a precipitate which was collected and dissolved in degassed water (15 mL) containing 1 N HCl (0.5 mL). Lyophilization afforded **70** as a colorless solid: 0.34 g (72%); mp 126–134 °C; IR (KBr) 3405, 1732, 1603 cm^{-1}; ^{1}H NMR (400 Mz, CD_3OD) δ 1.01 (3 H, d, J = 7.0 Hz), 1.05 (3 H, d, J = 7.0 Hz), 1.73 (1 H, m), 1.88 (2 H, m), 2.93 (3.12 (2 H, coupled AB q, J_{AB} = 14.5 Hz, J_{HA} = 5.3 Hz, J_{HB} = 9.3 Hz), 3.18, 3.42 (2 H, coupled AB q, J_{AB} = 13 Hz, J_{HA} = 4.9 Hz, J_{HB} = 9.3 Hz), 3.97 (1 H, dd, J = 6.0, 8.0 Hz), 4.03 (1 H, m), 7.30–7.41 (5 H, m); MS (FAB), $(M + J)^+$ *m/e* 311, $(M - H)^-$ *m/e* 309; $[\alpha]_D$ −41.7°, $[\alpha]_{365}$ −138.5° (*c* 1.24, pyridine); R_f 0.53 (*n*-BuOH–HOAc–H_2O, 4:1:1). Anal. ($C_{16}H_{26}N_2O_2S$·1.8HCl·0.8H_2O) C, H, N, S, SH, Cl.

Isomer B (0.51 g, 0.87 mmol) was deprotected under the conditions described for the preparation of **70** to give **71** as a colorless solid: 0.21 g (61%); mp 132–144 °C; IR ($CHCl_3$) 3421, 1732, 1603 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 1.02 (3 H, d, J = 7 Hz), 1.05 (3 H, d, J = 7 Hz), 1.78 (1 H, m), 1.85 (2 H, m), 3.08, 3.18 (2 H, coupled AB q, J_{AB} = 15 Hz, J_{HA} = 6 Hz, J_{HB} = 8 Hz), 3.52 (1 H, m), 3.90 (2 H, m), 7.31–7.42 (5 H, m); MS (FAB), $(M + H)^+$ *m/e* 311, $(M - H)^-$ *m/e* 309; [α]*D* −12.4°, $[\alpha]_{365}$ −31.5° (*c* 1.2, pyridin)); R_f 0.60 (*n*-BuOH–HOAc–H_2O, 4:1:1). Anal. ($C_{16}H_{26}N_2O_2S$·1.78HCl·1.18H_2O) C, H, N, S, SH, Cl.

***N*-[3(*S*)-Amino-2(*R*,*S*)-mercapto-4-phenylbutyl]-L-leucinamide Dihydrochloride (72).** A mixture of aldehydes

37 and 38 (0.96 g, 2.32 mmol), L-leucine amide (0.90 g, 6.92 mmol), crushed 3-Å molecular sieves, anhydrous benzene (16 mL), and anhydrous methanol (5 mL) was stirred at room temperature under argon for 3 h. The mixture was filtered through Celite and the filtrate concentrated at reduced pressure to yield the crude imine. The crude imine was dissolved in ice cold methanol (30 mL) and then treated with sodium borohydride (0.17 g, 4.5 mmol). After the mixture was stirred for 20 min at 0 °C, the reaction was quenched by careful addition of 1 N HCl and extracted with ethyl acetate. The organic fraction was washed with water, 1 N HCl, 1 N $NaHCO_3$, and brine. After drying ($MgSO_4$), the solvent was removed at reduced pressure and the residue chromatographed (toluene–2-butanone, 65:35) to give the intermediate, a 1:1 mixture of diastereomers, as a colorless solid: 0.57 g (46%); MS (FAB), $(M + H)^+$ m/e 530; R_f 0.21 (toluene–2-butanone, 65:35).

The intermediate from above (0.55 g, 1.04 mmol) was deprotected to give **72** as a pale yellow solid: 0.33 g (79%); IR (KBr) 3387, 1686 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 1.01 (6 H, m), 1.73–1.87 (3 H, m), 2.93 (0.5 H, dd, J = 6.6, 10.8 Hz), 3.05–3.40 (3.5 H, m), 3.59 (0.5 H, dd, J = 2.5, 9.0 Hz), 3.71 (0.5 H, m), 3.91–4.08 (2 H, m), 7.31–7.42 (5 H, m); MS (FAB), $(M + H)^+$ m/e 310, $(M - H)^-$ m/e 308; $[\alpha]_D$ −25.2° (c 1.3, pyridine); R_f 0.51 ($CHCl_3$–CH_3OH–HOAc, 3:1:1). Anal. ($C_{16}H_{27}N_3OS \cdot 2.0HCl \cdot 1.0H_2O$) C, H, N, S, SH, Cl.

***N*-[3(*S*)-Amino-2(*R*,*S*)-mercapto-5-methylhexyl]-L-leucinamide Dihydrochloride (73).** A mixture of aldehydes **39** and **40** (0.58 g, 1.52 mmol), L-leucine amide (0.79 g, 6.07 mmol), crushed 3-Å molecular sieves (2.0 g), anhydrous benzene (12 mL), and absolute ethanol (4 mL) was stirred at room temperature under argon for 6 h. The mixture was filtered through Celite and the filtrate concentrated at reduced pressure to yield the crude imine. The crude imine was dissolved in ice cold methanol (20 mL) and then treated with sodium borohydride (0.12 g, 3.17 mmol). After the mixture was stirred for 15 min at 0 °C; the reaction was quenched by careful addition of 1 N HCl and extracted with ethyl acetate. The organic fraction was washed with water, 1 N HCl, 1 N $NaHCO_3$, and brine. After drying ($MgSO_4$), the solvent was removed at reduced pressure and the residue chromatographed (toluene–2-butanone, 6:4) to give the intermediate, a 1:1 mixture of diastereomers, as a colorless oil: 0.43 g (57%); R_f 0.24 (toluene–2-butanone, 6:4).

The intermediate from above (0.43 g, 0.87 mmol) was deprotected to give **73** as a pale purple solid: 0.27 g (85%); mp 128–145 °C; IR (KBr) 3379, 1688 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 1.01–1.08 (12 H, m), 1.29–1.38 (1 H, m), 1.60–1.88 (6 H, m)8 3.45 (0.5 H, m), 3.58 (2 H, m), 3.72 (0.5 H, m), 3.98 (1 H, m); MS (FAB), $(M + H)^+$ m/e 276, $(M - H)^-$ m/e 274; $[\alpha]_D$ −16.3°, $[\alpha]_{365}$ −43.8° (c 0.52, pyridine); R_f 0.52 (n-BuOH–HOAc–H_2O, 3:1:1). Anal. ($C_{13}H_{29}N_3OS \cdot 2.0HCl \cdot 0.84H_2O$) C, H, N, S, SH, Cl.

***N*-[*N*-[3(*S*)-Amino-2(*R*,*S*)-mercapto-5-methylhexyl]-L-phenylalanyl]-L-leucine Dihydrochloride (74).** Reductive amination of aldehyde **40** (0.71 g, 1.86 mmol) with L-phenylalanyl-L-leucine *tert*-butyl ester (1.56 g, 4.66 mmol) using the standard conditions for the reductive amination and chromatography (benzene–acetone, 95:5) afforded the intermediate, a mixture of diastereomers, as a yellow foam: 0.76 g (58%); MS (FAB), $(M + H)^+$ m/e 700, $(M - H)^-$ m/e 698; R_f 0.34 (benzene–acetone, 9:1).

The intermediate from above (0.76 g, 1.09 mmol) was deprotected to give **74**, a mixture of diastereomers, as a colorless solid: 0.50 g (86%); mp 134–146 °C; IR (KBr) 3411, 1724, 1675 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 0.90–1.02 (12 H, m), 1.53–1.75 (6 H, m), 3.07–3.42 (m, includes solvent peak), 3.50–3.62 (2 H, m), 4.15 (0.5 H, br), 4.24 (0.5 H, t, J = 7 Hz), 4.35 (1 H, m), 7.28–7.39 (5 H, m); MS (FAB), $(M + H)^+$ m/e 424, $(M - H)^-$ m/e 422; $[\alpha]_D$ −15.1°, $[\alpha]_{365}$ −32.7° (c 1.06, CH_3OH); R_f 0.68 (n-BuOH–HOAc–H_2O, 4:1:1). Anal. ($C_{22}H_{37}N_3O_3S \cdot 1.7HCl \cdot 0.3CF_3CO_2H \cdot 0.75H_2O$) C, H, N, S, SH, Cl, F.

***N*-[3(*R*)-Amino-2-mercapto-1-oxo-4-phenylbutyl]-L-leucine Hydrochloride (78, Isomer A; 79, Isomer B).** To a solution of ester **27** (2.34 g, 5.25 mmol) in water (4.3 mL) and tetrahydrofuran (20 mL) was added 1 N NaOH (15.8 mL, 15.8 mmol). After the mixture was stirred at room temperature for 18 h, an additional portion of 1 N NaOH (5.0 mL, 5.0 mmol) was added. After stirring for 12 h, the reaction mixture was acidified with 1 N HCl and extracted with ethyl acetate. The organic fraction was washed with water and brine. After drying ($MgSO_4$), the solvent was removed at reduced pressure to afford the corresponding acid (1.98 g, 87%) as an amber oil which was used without purification.

A solution of crude acid from above (1.98 g, 4.59 mmol), 2-morpholinoethyl isocyanide[70,71] (0.63 mL, 4.57 mmol), and 1-hydroxybenzotriazole monohydrate (0.62 g, 4.59 mmol) in anhydrous dichloromethane (20 mL) was stirred at room temperature under argon. After 45 min, the solution was treated with a solution of L-leucine *tert*-butyl ester (0.86 g, 4.59 mmol) in dichloromethane (10 mL) followed by the addition of diisopropylethylamine (0.96 mL, 5.51 mmol). After stirring for 20 h, the mixture was diluted with ethyl acetate and washed with water, 1 N HCl, 1 N $NaHCO_3$, and brine. After drying ($MgSO_4$), the solvent was removed at reduced pressure and the residue chromatographed (benzene–ethyl acetate, 92:8) to afford the separated diastereomers as colorless solids. **76** (isomer A): 0.55 g (20%); MS (FAB), $(M + H)^+$ m/e 601, $(M - H)^-$ m/e 599; R_f 0.35 (benznene–ethyl acetate, 92:8). Anal. ($C_{33}H_{48}N_2O_6S$) C, H, N, S. **77** (isomer B): 0.55 g (20%); MS (FAB), $(M + H)^+$ m/e 601, $(M - H)^-$ m/e 599; R_f 0.25 (benzene–ethyl acetate, 92:8). Anal. ($C_{33}H_{48}N_2O_6S$) C, H, N, S.

Ester **76** (0.48 g, 0.80 mmol) was deprotected to afford **78** as a colorless solid: 0.28 g (92%); mp 94–106 °C; IR (KBr) 1730, 1654 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 1.01 (3 H, d, J = 7 Hz), 1.68–1.71 (3 H, m), 3.0, 3.12 (2 H, coupled AB q, J_{AB} = 14.6 Hz, J_{HA} = 8 Hz, J_{HB} = 7 Hz), 3.58 (1 H, d, J = 4 Hz), 3.79 (1 H, m), 4.41 (1 H, t, J = 7 Hz), 7.25–7.39 (5 H, m); MS (FAB), $(M + H)^+$ m/e 325, $(M - H)^-$ m/e 323; R_f 0.73 (n-BuOH–HOAc–H_2O, 3:1:1). Upon dissolution of **78** in pyridine a purple color formed which interfered with the determination of the specific rotation. Anal. ($C_{16}H_{24}N_2O_3S \cdot 1.0HCl \cdot 1.0H_2O$) C, H, N, S, SH, Cl.

Ester **77** (0.50 g, 0.83 mmol) was deprotected to afford **79** as a colorless solid: 0.25 g (77%); mp 89–97 °C; IR (KBr) 1725, 1659 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 0.94 (3 H, d, J = 7 Hz) 0.98 (3 H, d, J = 7 Hz), 1.60–1.81 (3 H, m), 2.99, 3.14 (2 H, coupled AB q, J_{AB} = 14 Hz, J_{HA} = 7 Hz, J_{HB} = 9 Hz), 3.55 (1 H, d, J = 6 Hz), 3.90 (1 H, m), 4.39 (1 H, dd, J = 6, 9 Hz), 7.30–7.41 (5 H, m); MS (FAB), $(M + H)^+$ m/e 325, $(M - H)^-$ m/e 323; R_f 0.81 (n-BuOH–HOAc–H_2O, 3:1:1). Upon dissolution of **79** in pyriding a purple color formed which interfered with the determination of the specific rotation. Anal. ($C_{16}H_{24}N_2O_3S \cdot 0.90HCl \cdot 0.15CF_3CO_2H \cdot 0.90H_2O$) C, H, N, S, SH, Cl, F.

***N*-[3(*S*)-Amino-2(*R*,*S*)-mercapto-1-oxo-4-phenylbutyl]-L-leucine Hydrochloride (81).** Hydrolysis of ester **25** (1.24 g, 2.78 mmol) followed by coupling with L-leucine *tert*-butyl ester, using the procedure described for the preparation of **76** and **77**, afforded ester **80**, a mixture of diastereomers, as a colorless solid: 0.99 g (53%); MS (FAB), $(M + H)^+$ m/e 601, $(M - H)^-$ m/e 599; IR ($CHCl_3$) 1722, 1707, 1667, 1512, 1496 cm^{-1}; R_f 0.31, 0.34 (benzene–ethyl acetate, 9:1). Anal. ($C_{33}H_{48}N_2O_6S$) C, H, N, S.

Ester **80** (0.64 g, 1.07 mmol) was deprotected to give **81**, a mixture of diastereomers, as a colorless solid: 0.35 g (89%); mp 96–105 °C; IR (KBr) 3391, 1727, 1659 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 0.88–1.10 (6 H, m), 1.58–1.81 (3 H, m), 3.07–3.17 (2 H, m), 3.61 (0.5 H, d, J = 6 Hz), 3.68 (0.5 H, d, J = 6 Hz), 3.74 (0.5 H, m), 3.92 (0.5 H, m), 4.37 (0.5 H, m), 4.48 (0.5 H, dd, J = 4.0, 8.8 Hz), 7.25–7.42 (5 H, m); MS (FAB), $(M + H)^+$ m/e 325, $(M - H)^-$ m/e 323; $[\alpha]_D$ −20.4°, $[\alpha]_{365}$ −76.8° (c 1.08, pyridine); R_f 0.61, 0.72 ($CHCl_3$–CH_3OH–HOAc, 4:1:1). Anal. ($C_{16}H_{24}N_2O_3S \cdot 0.95HCl \cdot 0.09CF_3CO_2H$) Calcd: C, 52.52; H, 6.83; N, 7.58; S, 8.68; SH, 8.95; Cl, 9.12; F, 1.39. Found: C, 52.47; H, 6.79; N, 7.05; S, 8.24; SH, 8.66; Cl, 9.04; F, 1.00.

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Registry No. **13**, 13734-34-4; **14**, 13139-15-6; **15**, 18942-49-9; **16**, 16937-99-8; **17**, 60398-41-6; **18**, 52716-48-0; **19**, 115313-19-4; **20**, 116300-00-6; **21**, 60398-42-7; **22**, 86834-94-8; **23**, 115313-20-7; **24**, 116263-98-0; (2*R*,3*S*)-**25**, 116263-99-1; (2*S*,3*S*)-**25**, 116264-35-8;

(2*R*,3*S*)-**26**, 116264-00-7; (2*S*,3*S*)-**26**, 116264-36-9; (2*R*,3*R*)-**27**, 116264-01-8; (2*S*,3*R*)-**27** (free acid), 116264-45-0; (2*S*,3*R*)-**27**, 116264-37-0; (2*S*,3*R*)-**27** (free acid), 116264-45-0; (2*R*,3*R*)-**28**, 116264-02-9; (2*S*,3*R*)-**28**, 116264-38-1; (2*R*,3*S*)-**29**, 103542-90-1; (2*S*,3*S*)-**29**, 103542-91-2; (2*R*,3*S*)-**31**, 116264-03-0; (2*S*,3*S*)-**31**, 116264-04-1; (2*R*,3*R*)-**33**, 116264-05-2; (2*S*,3*R*)-**33**, 116264-06-3; (2*R*,3*R*)-**35**, 116264-07-4; (2*S*,3*R*)-**35**, 116264-08-5; (2*R*,3*S*)-**37**, 103542-92-3; (2*S*,3*S*)-**37**, 103542-93-4; (2*R*,3*S*)-**39**, 116264-09-6; (2*S*,3*S*)-**39**, 116264-10-9; (2*R*,3*R*)-41, 116264-11-0; (2*S*,3*R*)-**41**, 116264-12-1; (2*R*,3*R*)-**43**, 116264-13-2; (2*S*,3*R*)-**43**, 116264-16-5; (2*R*,3*S*)-**47**, 116264-17-6; (2*S*,3*S*)-**47**, 116264-18-7; (2*R*,3*R*)-**49**, 116264-19-8; (2*S*,3*R*)-**49**, 116264-20-1; (2*R*,3*R*)-**51**, 116264-21-2; (2*S*,3*R*)-**51**, 116264-22-3; (2*R*,3*S*)-**53**·2HCl, 116278-41-2; (2*R*,3*S*)-**53** (free base), 116264-46-1; (2*S*,3*S*)-**53**·2HCl, 116264-23-4; (2*S*,3*S*)-**53** (free base), 116264-47-2; (2*R*,3*S*)-**55**·2HCl, 116264-24-5; (2*R*,3*S*)-**55** (free base), 116264-48-3; (2*S*,3*S*)-**55**·2HCl, 116264-25-6; (2*S*,3*S*)-**55** (free base), 116264-49-4; (2*R*,3*R*)-**57**·2HCl, 116264-26-7; (2*R*,3*R*)-**57** (free base), 116264-50-7; (2*S*,3*R*)-**57**·2HiCl, 116264-27-8; (2*S*,3*R*)-**57** (free base), 116264-51-8; (2*R*,3*R*)-**59**·2HCl, 116264-28-9; (2*R*,3*R*)-**59** (free base), 116264-52-9; (2*S*,3*R*)-**59**·2HCl, 116264-29-0; (2*S*,3*R*)-**59** (free base), 116264-53-0; (2*R*)-**61**, 103542-94-5; (2*S*)-**61**, 103618-11-7; (2*R*)-**62**·2HCl, 116300-01-7; (2*R*)-**62** (free base), 103542-95-6; 3-(2*S*)-**62**·2HCl, 116300-20-0; (2*S*)-**62** (free base), 103618-12-8; **63**, 6258-60-2; **64**, 35378-93-9; **65**, 85301-93-5; **66**·HCl, 116264-30-3; **66** (free base), 81110-01-2; **67**, 116264-31-4; **68**, 116264-32-5; **69**, 116264-33-6; (2*R*)-**70**·2HCl, 116346-47-5; (2*R*)-**70** (free base), 116300-11-9; (2*S*)-**70**·2HCl, 116346-48-6; (2*S*[-**70** (free base), 116300-12-0; (2*R*)-**72**·2HCl, 116346-49-7; (2*R*)-**72** (free base), 116300-13-1; (2*R*)-**72** (*N*-BOC-protected), 116264-41-6; (2*S*)-**72**·2HCl, 116346-51-1; (2*S*)-**72** (free base), 116300-14-2; 1(2*S*)-**72** (*N*-BOC-protected), 116300-06-2; (2*R*)-**73**·2HCl, 116264-34-7; (2*R*)-**73** (free base), 116300-15-3; (2*R*)-**73** (*N*-BOC-protected), 116264-42-7; (2*S*)-**73**·2HCl, 116300-09-5; (2*S*)-**73** (free base), 116346-53-3; (2*S*)-**73** (*N*-BOC-protected), 116300-07-3; (2*R*)-**74**·2HCl, 116346-50-0; (2*R*)-**74** (free base), 116300-16-4; (2*R*)-**74** (*N*-BOC-protected), 116264-43-8; (2*S*)-**74**·2HCl, 116346-52-2; (2*S*)-**74** (free base), 116300-17-5; (2*S*)-**74** (*N*-BOC-protected), 116300-08-4; (2*R*)-**75**, 116300-02-8; (2*S*)-**75**, 116300-19-7; (2*R*)-**76**, 115362-76-0; (2*S*)-**76**, 115362-75-9; (2*R*)-**78**·HCl, 114926-90-8; (2*R*)-**78** (free base), 116346-77-1; (2*S*)-**78**·HCl, 114886-46-3; (2*S*)-**78** (free base), 116300-18-6; (2*R*)-**80**, 116300-03-9; (2*S*)-**80**, 116300-10-8; (2*R*)-**81**·HCl, 116300-04-0; (2*R*)-**81** (free base), 116346-54-4; (2*S*)-**81**·HCl, 116403-21-5; (2*S*)-**81** (free base), 116346-55-5; [(4-MeO)$C_6H_4CH_2S]_2$, 17004-42-1; $H_2NCH_2CH_2CH_2CH(CH_3)_2$, 107-85-7; H-Phe-Leu-OBu-*t*, 28635-78-1; $ClCH_2CH_2OH$, 107-07-3; (4-MeO)$C_6H_4CH_2SCH_2CH_2$-Phe-Leu-OBu-*t*, 116264-39-2; H-Leu-OBu-*t*, 21691-53-2; (2*R*,3*S*)-(BOC)NHCH(CH_2Ph)CH($SCH_2C_6H_4$-*p*-OMe)CH_2-Leu-OBu-*t*, 116264-40-5; (2*S*,3*S*)-(BOC)NHCH(CH_2Ph)CHu($SCH_2C_6H_4$-*p*-OMe)CH_2-Leu-OBu-*t*, 116300-05-1; H-Leu-NHz, 687-51-4; aminopeptidase, 9031-94-1.

Structure–Activity Relationships among Analogues of Pemedolac, *cis*-1-Ethyl-1,3,4,9-tetrahydro-4-(phenylmethyl)pyrano[3,4-*b*]indole-1-acetic acid, a Potent Analgesic Agent

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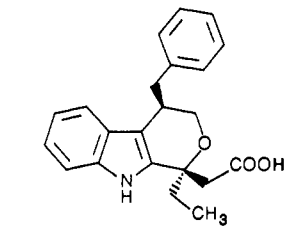
The syntheses of analogues of pemedolac (*cis*-1-ethyl-1,3,4,9-tetrahydro-4-(phenylmethyl)pyrano[3,4-*b*]indole-1-acetic acid), a potent analgesic, are described. They were tested for analgesic and antiinflammatory effects in vivo and for inhibition of prostaglandin production in vitro. Analysis of structure–activity relationships shows that analgesic activity in this series is associated with 1*S*-cis stereochemistry, the presence of a π-system (allyl or benzyl) at position 4, and a log *P* value greater than 4.0.

Recent reports from our laboratories[1,2] describe the synthesis, structure, and pharmacological properties of *cis*-1-ethyl-1,3,4,9-tetrahydro-4-(phenylmethyl)pyrano-[3,4-*b*]indole-1-acetic acid, AY-30,715, pemedolac (USAN), **1**, a new potent analgesic agent that is currently being evaluated in humans. Herein we describe the syntheses, analgesic, and antiinflammatory screening data and structure–activity relationships for a series of pemedolac analogues.

The choice of targets for synthesis was dictated by two independent observations made during the study of structure–activity relationships among analogues of the antiinflammatory–analgesic agent etodolac, **5**.[3,4] First, of the diastereomeric pair of 4-methylpyrano[3,4-*b*]indole-1-acetic acids **2** and **3**, the cis diastereomer **2**[5] is twice as potent as the corresponding 4-desmethyl analogue, while the trans diastereomer **3**, as well as the 4,4-dimethyl analogue **4**, was almost devoid of activity.[6] Second, replacing the pyrano oxygen of etodolac by a methylene group gives the tetrahydrocarbazole **6**, which is almost equipotent to etodolac in the rat adjuvant arthritis model.[7]



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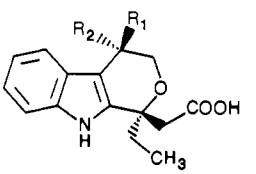
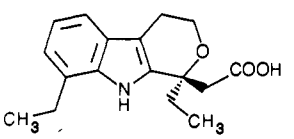
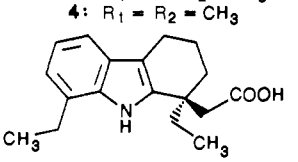


2: $R_1 = CH_3$; $R_2 = H$
3: $R_1 = H$; $R_2 = CH_3$
4: $R_1 = R_2 = CH_3$



5



6

These observations suggested that it might be profitable to synthesize and test a series of 4-substituted pyrano-

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