Development of Selective Tight-Binding Inhibitors of Leukotriene A_4 Hydrolase

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Leukotriene A_4 hydrolase is a zinc-containing enzyme which exhibits both epoxide hydrolase and aminopeptidase activities. Since the enzyme product leukotriene B_4 is an inflammatory mediator, it is of interest to develop selective inhibitors of leukotriene A_4 hydrolase as potential antiinflammatory agents and as mechanistic probes. A systematic study on the enzyme specificity and the inhibition of its amidase activity with more than 30 synthetic inhibitors has led to the development of an α-keto-β-amino ester (**26**) and a thioamine (**27**) as tight-binding, competitive type transition-state analog inhibitors of the aminopeptidase activity, with K_i values of 46 and 18 nM, respectively. Both compounds also inhibit the epoxide hydrolase activity, with the IC_{50} values of 1 μM and 0.1 μM for **26** and **27**, respectively.

Leukotriene (LT) A_4 hydrolase (EC 3.3.2.6)[1] is a zinc-containing monomeric enzyme (MW ~ 70 kDa),[2] which catalyzes the formation of leukotriene (LT) B_4 (5*S*,12*R*-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid) from its natural substrate LTA_4 ((5*S*)-5,6-oxido-7,9-*trans*-11,-14-*cis*-eicosatetraenoic acid), one of the physiologically important processes in the arachidonic acid metabolic pathway.[1] Like aminopeptidases, the enzyme also catalyzes the hydrolysis of some amino acid amides.[3,4]

The enzyme has been purified to homogeneity from various sources as a soluble protein,[1] and the cDNAs coding for the human enzyme from placenta and spleen have been cloned and sequenced.[5] Though the detailed mechanism of the enzyme has not been elucidated, it is speculated that addition of a water molecule to its substrate is general base-assisted, and the role of the zinc ion is to serve as a Lewis acid to polarize the epoxide ring or the carbonyl of amide (Schemes I and II) and to stabilize the negative charge developed during the reaction. The zinc ion may also bind to the nucleophilic water molecule to facilitate the general-base catalysis. The peptidase and epoxide hydrolase activities seen to occur at closely related overlapping active sites but use different general bases as indicated in the recent site-directed mutagenesis studies.[6a] It has been demonstrated that the aminopeptidase and epoxide hydrolase activities are activated by albumin. A characteristic which seems to distinguish this enzyme from other Zn^{++} metallohydrolases.[6b]

It is of great interest to develop selective inhibitors of LTA_4 hydrolase as mechanistic probes and as potential antiinflammatory agents since LTB_4 is a strong proinflammatory mediator which stimulates adhesion of circulating neutrophils to vascular endothelium[7a] and directs their migration toward sites of inflammation.[7b] LTA_4 hydrolase was irreversibly inhibited by its substrate LTA_4[8a] and substrate analogues LTA_3 and LTA_5,[8b-d] and ω-[(ω-arylalkyl)thienyl]alkanoic acids.[9a,b] Some inhibitors of Zn^{++}-containing amino peptidases (e.g. Bestatin) and angiotensin converting enzyme (e.g. Captopril) are reversible inhibitors of LTA_4 hydrolase.[9c]

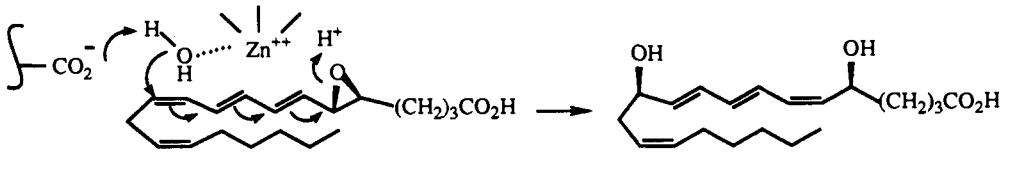
We report here our study on the specificity of the amidase activities of LTA_4 hydrolase and the synthesis of a series of α-hydroxy β-amino acids and peptide isosteres as inhibitors to probe the active site of the enzyme. This study has led to the development of non-peptide, transition-state analog inhibitors based on the proposed mechanism of the aminopeptidase activity and the natural substrate structure of the enzyme.[10]

Results and Discussion

Several amino acid amides were tested as substrates for LTA_4 hydrolase. It was found that only L-enantiomers were substrates, and L-alanine *p*-nitroanilide showed the highest k_{cat} and K_m values. Considering the specificity constant k_{cat}/K_m, L-Pro-*p*-nitroanilide was, however, slightly better than the Ala derivative as a substrate (Table I).

The inhibition of LTA_4 hydrolase with Bestatin prompted us to study the other L- and D-phenylalanine-derived norstatine-type of inhibitors. We speculate that the aminopeptidase activity of LTA_4 hydrolase is mechanistically like that of Zn^{++}-containing thermolysin or aminopeptidase. The norstatine type of peptide isosteres, therefore, should be good inhibitors. The inhibition by Captopril may be due to a strong interaction between the SH group and the zinc ion at the enzyme's active site. We

Scheme I



Leukotriene A_4 Leukotriene B_4

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Scheme II

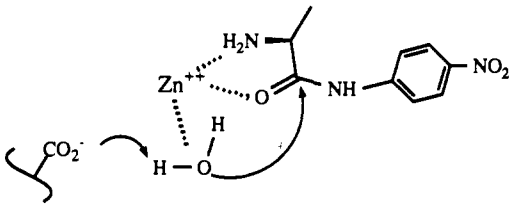


Table I. Comparison of Kinetic Parameters for LTA_4 Hydrolase-Catalyzed Hydrolysis of LTA_4 and Amide Substrates[a]

substrate	K_m (μM)	k_{cat} (s^{-1})	V_{max} (nmol/min per mg)	k_{cat}/K_m ($M^{-1}\,s^{-1}$)
LTA_4	7.6[b]	$6.8^b \times 10^{-1}$	572[b]	9.0×10^4
L-Lysine *p*-nitroanilide	100	3.5×10^{-2}	30	3.5×10^2
L-Ala *p*-nitroanilide	500	6.0×10^{-1}	530	1.2×10^3
D-Ala *p*-nitroanilide	-	-	0	-
L-Arg *p*-nitroanilide	200	1.5×10^{-1}	135	7.5×10^2
L-Pro *p*-nitroanilide	100	1.5×10^{-1}	135	1.5×10^3
L-Leu *p*-nitroanilide	300	1.5×10^{-1}	135	5.0×10^2

[a] Determined in 50 mM Tris-Cl, pH 7.6, in the presence of 0.1 M NaCl, $\epsilon_{410\ nm}$ for *p*-nitroaniline = 8850 $M^{-1}\,cm^{-1}$. [b] See ref 14 for ranges.

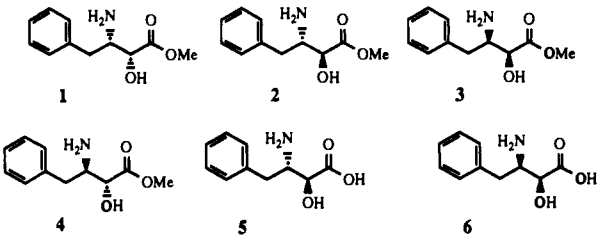
Table II. Inhibition Constants of the Listed Compounds for LTA_4 Hydrolase[a]

compound	inhibition constant	compound	inhibition constant
1	$IC_{50} > 0.5$ mM[b]	17	$IC_{50} > 5$ mM[e]
2	$I_{i,app} = 50\ \mu M$[c]	18	$IC_{50} > 1$ mM[e]
3	NI[d]	19	$IC_{50} > 100\ \mu M$
4	NI	20	$IC_{50} = 20\ \mu M$
5	$IC_{50} > 1$ mM	21	$IC_{50} = 80\ \mu M$
6	NI	22	$IC_{50} = 0.5\ \mu M$
7	$IC_{50} = 20\ \mu M$	23	$IC_{50} = 20\ \mu M$
8	$K_i = 15\ \mu M$	24	$IC_{50} = 0.8\ \mu M$
9	$IC_{50} = 100\ \mu M$	25	$IC_{50} = 0.6\ \mu M$
10	$IC_{50} = 80\ \mu M$	26	$K_i = 46$ nM
11	$IC_{50} = 250\ \mu M$	27	$K_i = 18$ nM
12	$IC_{50} > 250\ \mu M$	28	$IC_{50} = 3\ \mu M$
13	$IC_{50} > 0.1$ mM[e]	29	$IC_{50} = 10\ \mu M$
14	$IC_{50} > 0.1$ mM[e]	30	$K_i = 14\ \mu M$
15	$IC_{50} > 0.1$ mM[e]	31	$IC_{50} = 140\ \mu M$
16	$IC_{50} > 0.1$ mM[e]	32	$IC_{50} = 20\ \mu M$

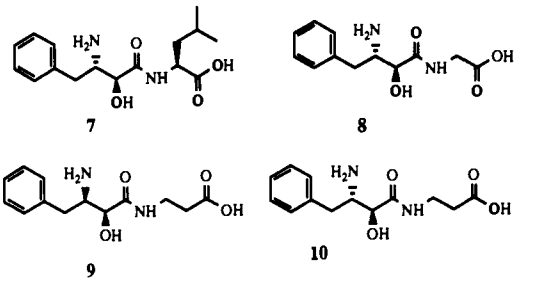
[a] All assays were performed in Tris-HCl buffer (0.05 M, pH 8.0) with L-alanyl *p*-nitroanilide (1.5 mM) as substrate unless otherwise indicated. LTA_4 hydrolase (1.4 μg) purified from human leukocytes was added for each assay (final volume = 1.0 mL). *p*-Nitroaniline formation was monitored spectrophotometrically at 405 nm. Thiol compounds were assayed with the presence of 5 mM DTT. The values are within ±5% accuracy. The IC_{50} value is the concentration of inhibitor which inhibits 50% of the enzyme activity under these conditions. Lineweaver–Burk plots were conducted at various substrate and inhibitor concentrations to determine the inhibition constant K_i. [b] Less than 50% inhibition was observed at this concentration. [c] Dixon plot was used to determine the $K_{i,app}$ value. [d] NI, no inhibition observed with 1 mM inhibitor in the assay. [e] Less than 10% inhibition was observed at this concentration.

believe better inhibitors can be developed if this binding/coordination interaction can be optimized.

A series of norstatine-related compounds were synthesized and evaluated for ability to inhibit the amidase activity of LTA_4 hydrolase, purified from human leukocytes, with L-alanyl *p*-nitroanilide as substrate. Initial studies[10a] of four isomeric 3-amino-2-hydroxy-4-phenylbutanoic acid (AHPA) methyl esters 1–4 reveal that the configuration at both 2- and 3-positions are important for inhibitory activity, and the isomer (2*S*,3*S*)-AHPA methyl ester **2** is the most potent with an inhibition constant $K_i = 50\ \mu M$. The other three isomers (2*R*,3*S*), (2*R*,3*R*), and (2*R*,3*R*)-AHPA methyl esters **1**, **3**, and **4**, showed poor or no inhibition. A free amino group is necessary as *N*-Boc-

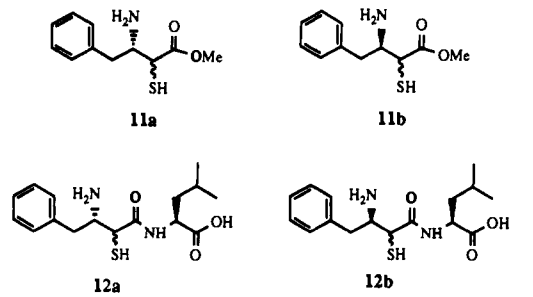


(2*S*,3*S*)-AHPA methyl ester shows no inhibition activity. Hydrolysis of the methyl ester to free acids (see compounds **5** and **6**) lost their inhibition potency. The inhibition activity was improved in the case where the C-terminus of (2*S*,3*S*)-AHPA was coupled to L-leucine or glycine ($IC_{50} = 20\ \mu M$ for **7** and $K_i = 15\ \mu M$ for **8**), but became worse

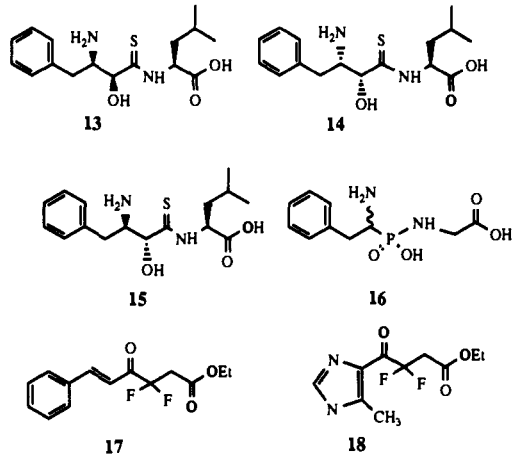


when coupled to β-alanine (**10**, $IC_{50} = 80\ \mu M$). It is interesting that though (2*S*,3*R*)-AHPA methyl ester **3** shows no activity to this enzyme,[10a] its amide derivative of L-leucine (Bestatin) is a potent inhibitor ($IC_{50} = 4\ \mu M$).[9] The β-alanine derivative of **3** (compound **9**), however, is a weaker inhibitor ($IC_{50} = 100\ \mu M$) than Bestatin, indicating the importance of P′ moiety in binding. Coupling of **2** with D-leucine did not improve the inhibitory potency.

At this point, we speculate that the mode of inhibition of these AHPA derivatives is similar to that of aminopeptidase with Bestatin where the free amine and the OH group coordinate to the zinc ion.[11] We therefore replaced the OH group with the SH group because it is a better ligand for Zn^{++}. Surprisingly, the inhibition potency was not improved for the four thiol compounds[12] **11a,b** and **12a,b**. Similar results were observed in the previous study

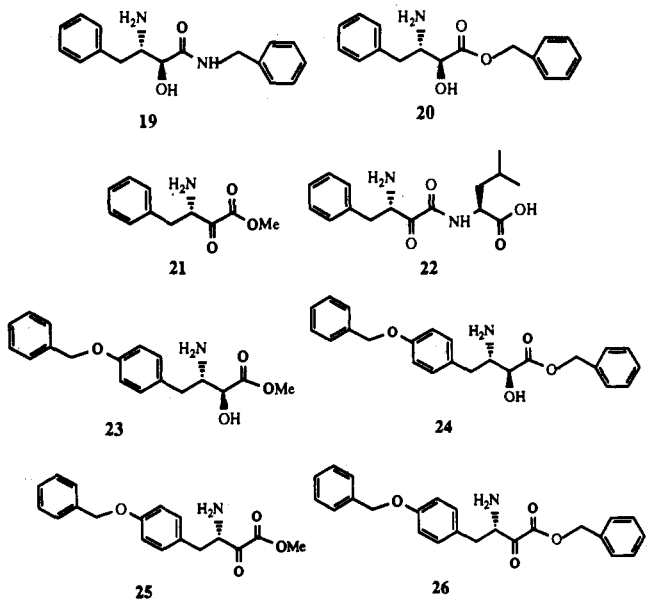


of aminopeptidase.[12] The thioamide analogs **13–15** are also poor inhibitors. We then synthesized phosphoramidate **16**, which resembled the transition state of the amide cleavage and therefore was expected to be a potent inhibitor. It was, however, a much weaker inhibitor than **8**. A similar situation was also reported in the inhibition of aminopeptidase.[13] The fluoro ketones **17** and **18**, though hydrated to form a tetrahedral *gem*-diol to mimic the transition state of the amide cleavage, do not inhibit the enzyme at 1 mM concentration of the fluoro compounds.



13 14 15 16 17 18

After considering the natural substrate structure and the mechanism of aminopeptidase inhibition,[11] another class of compounds (19–32) were developed and proven to be better inhibitors. These inhibitors contain a transition-state mimic of the enzyme-catalyzed amide cleavage as a "core" and additional complementary components (the aromatic moieties) which resemble the hydrophobic nature of the conjugated polyene system of the natural substrate LTA_4, which binds to the enzyme more tightly than the amide substrates.[14] The reason to choose α-keto esters instead of α-keto amides[15] is based on the inhibition results of compounds 19 and 20, where the ester derivative binds to the enzyme more tightly than the amide. The α-keto amide with a free carboxyl group (compound 22) is, however, a good inhibitor (IC_{50} = 0.5 μM) and the inhibition potency is better than the corresponding α-(*S*)-OH derivative 7. Interestingly, though compound 21 is comparable with 2, compound 25 (IC_{50} = 0.6 μM) binds to the



19 20 21 22 23 24 25 26

enzyme 30 times more tightly than 23. Further adjustment of the inhibitor structure at the P1′ and P1–P3 sites led to the development of the β-amino α-keto ester 26 as an inhibitor of the aminopeptidase with K_i = 0.046 μM (Figure 1). According to the ^{1}H-NMR and ^{13}C-NMR spectra, the β-amino α-keto esters and amides are completely hydrated,[16] indicating that the inhibitors exist as a *gem*-diol in solution, then bind to the enzyme active site as shown in Scheme III. The free amino group and one of the

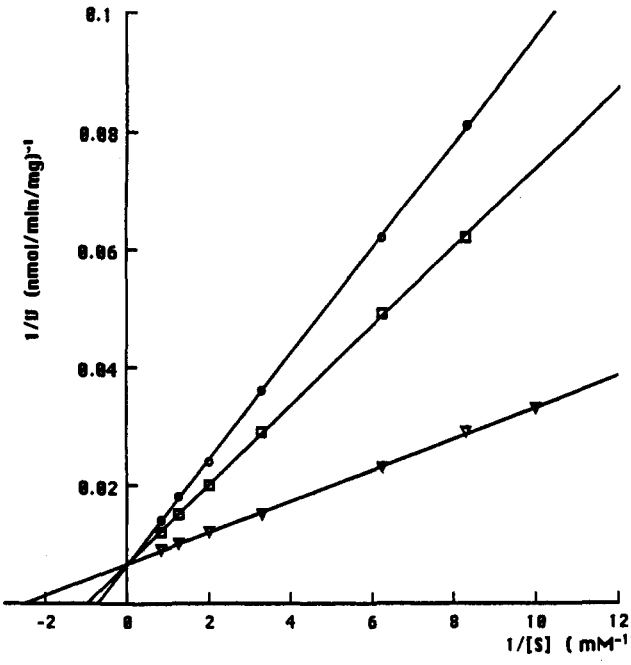
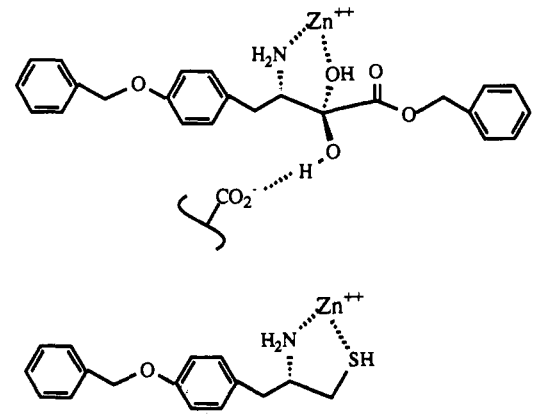
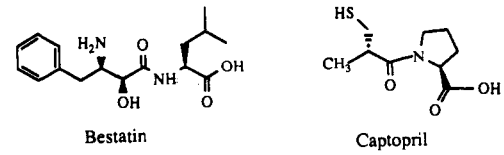


Figure 1. Double reciprocal plot for the inhibition of LTA_4 hydrolase. Concentrations of inhibitors are 0 mM (△), 30 nM (□) for 27, and 0.1 μM (○) for 26. The assays were done in Tris-HCl buffer (50 mM), pH 8.0, 25 °C with L-Ala-*p*-nitroalinide as substrate (with the presence of 5 mM DTT for inhibitor 27). *p*-Nitroanilide (ϵ = 10800 cm^{-1} M^{-1}) formation was monitored at 405 nm. The amount of enzyme used was 1.4 μg for all experiments. K_m = 420 μM, apparent K_m values are 1.1 mM (□) and 1.3 mM (○) and K_i values are 18 nM (□) and 46 nM (○), respectively.

Scheme III



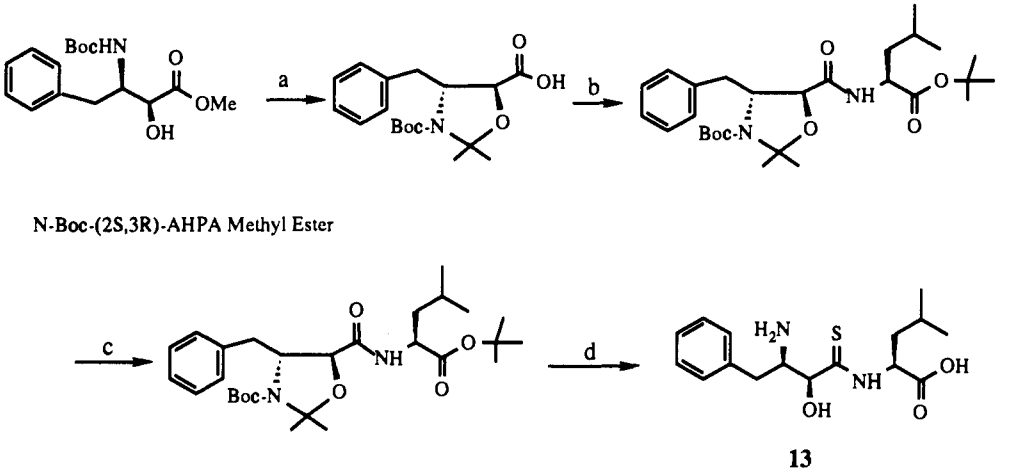
hydroxyl groups may coordinate to the Zn^{++} (as *N*-Boc and *N*-Cbz derivatives are not inhibitors) and the other hydroxyl group interacts with the general base ($-CO_2^-$) via H-bonding, a complex possibly resembling the transition-state structure of the enzymatic amide cleavage[11] and LTA_4 binding. Unlike Bestatin and Captopril, compounds 24–



Bestatin Captopril

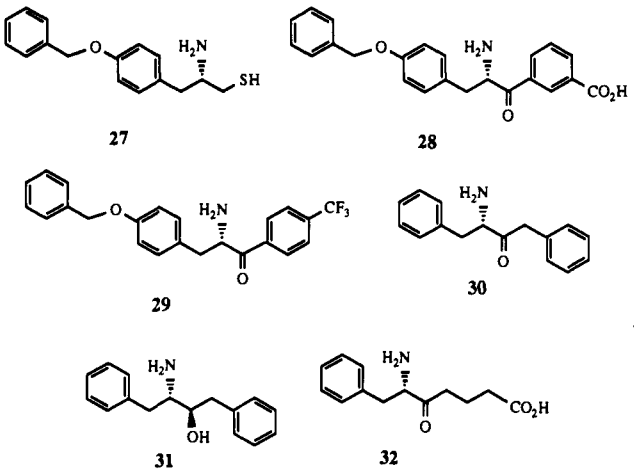
26 are quite selective for LTA_4 hydrolase. They are very weak inhibitors of other aminopeptidases; the IC_{50} values are >100, 80, and >100 μM for aminopeptidase M, and 80, 50, and >100 μM for cytosolic leucine aminopeptidase, respectively.[17]

Scheme IV[a]



[a] (a) (1) 2,2-Dimethoxypropane, TsOH, rt, (2) LiOH, 10% THF in MeOH, 88%; (b) DCC, L-leucine-*tert*-butyl ester, 79%; (c) Lawesson's reagent, benzene, 88 °C, 96%; (d) (1) TFA, rt, (2) 10% MeOH in TFA, 70 °C, 77%.

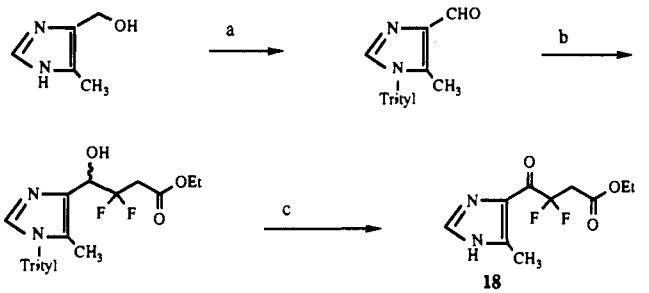
At this point, we turned our attention to the synthesis of more stable, non-peptide transition-state analog inhibitors **27–32**. These compounds, except **27**, are, however,



not as potent as **26**, probably due to the lack of complete hydration of the carbonyl group. Compound **27** is so far the best inhibitor of LTA_4 hydrolase. It inhibits the aminopeptidase activity with K_i = 18 nM and the epoxide hydrolase activity with K_i = 0.1 μM. It is a weaker inhibitor of aminopeptidase M and cytosolic leucine aminopeptidase with IC_{50} = 0.5 and >50 μM, respectively. Compound **27** probably forms a tight-binding complex in which the Zn^{++} coordinates to the amine and the thiol groups (Scheme III). Further investigation on the mechanisms of inhibition with **26** and **27** is in progress.

The syntheses of inhibitors described in this report are straightforward (Schemes IV–VIII). α-Hydroxy β-amino acids were prepared from the corresponding α-amino acids.[18] *N*-(*tert*-Butyloxycarbonyl)-(2*RS*,3*S*)-3-amino-2-hydroxyphenylbutanoic acid (abbreviated as *N*-Boc-(2*RS*,3*S*)-AHPA) methyl esters were separated on silica gel to give *N*-Boc-(2*R*,3*S*)-AHPA and *N*-Boc-(2*S*,3*S*)-AHPA methyl esters. The stereochemistry at the 2-position was assigned by comparison of (2*S*,3*R*)-AHPA isopropyl ester to the published data.[18] Similar procedures were followed to prepare **34*S*** and **34*R*** as shown in Scheme VI. The dipeptides **7–10** were made by DCC-mediated coupling followed by deprotection. The α-keto esters **21**, **22**, **25**, and **26** were prepared from the corresponding

Scheme V[a]



[a] (a) (1) Trityl-Cl, Et_3N, 88%, (2) MnO_2, dioxane, 67%; (b) zinc, $BrCF_2CO_2Et$, 84%; (c) (1) DCC, DMSO, H_3PO_4, 24 h, rt, 53% (2) TFA, rt, 82%.

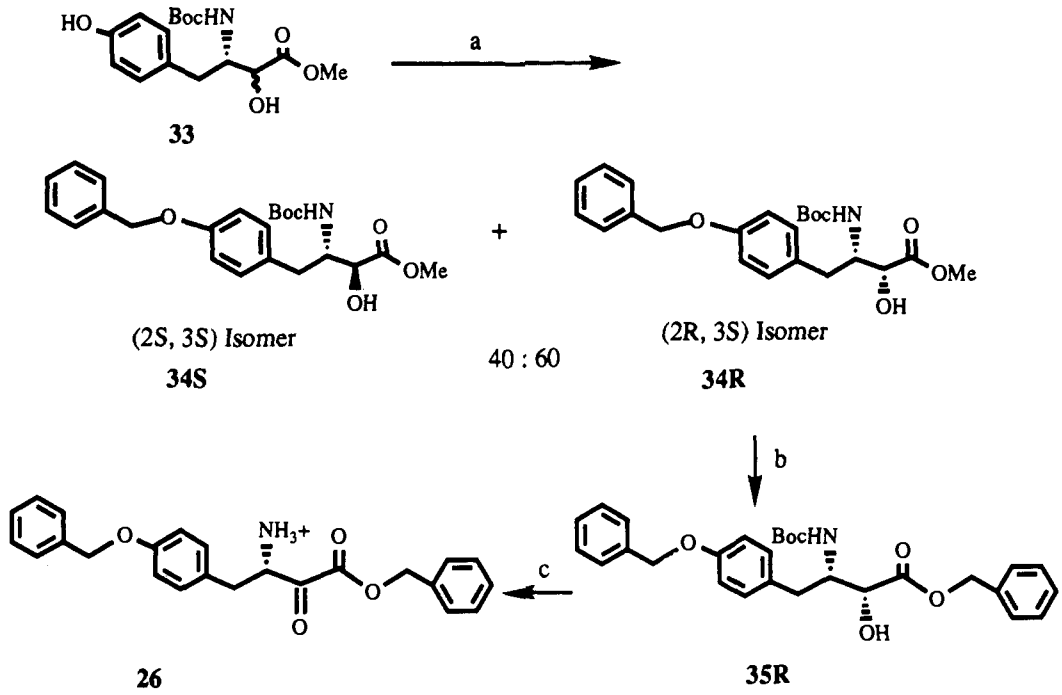
alcohols via Swern oxidation[19a] or treatment with Dess–Martin reagent[19b,c] followed by deprotection. Other inhibitors were prepared via Weinreb amides[20] (Scheme VII and VIII), unless otherwise indicated.

Experimental Section

General Methods. ^{1}H-NMR spectra were obtained at 300 or 400 MHz, and ^{13}C-NMR spectra were obtained at 100 or 125 MHz. All chemical shifts are reported in δ units (ppm) relative to tetramethylsilane (assigned to 0.0 ppm). Thin-layer chromatography (TLC) was performed on silica gel plates (0.25 mm, Merck) and using the following detection methods: UV, visualized under an ultraviolet lamp; I_2 on silica; AMA, dipped into a solution containing 5% ammonium molybdate, 4.2% H_2SO_4, and 0.6% sodium arsenate and heated on a hot plate. Flash chromatography was performed with silica gel (230–400 mesh, Merck). The following solvents were used: chloroform (C), ethyl acetate (EA), hexane (H), methanol (M). Elemental analyses were reported only by symbols of the elements; results were within 0.4% of the theoretical values. Leukotriene A_4 ethyl ester was saponified in tetrahydrofuran containing 6% 1 M LiOH, at 4 °C for 56 h.

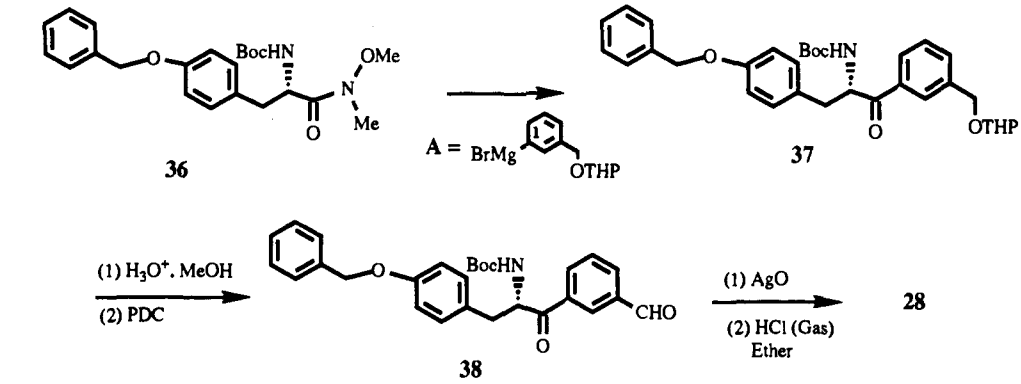
Method A: General Procedure for Peptide-Bond Formation. To a solution containing an N-protected amino acid (1 equiv, 0.1 M) in DMF was added a C-protected compound (1 equiv, usually as a HCl salt) and HOBt (1.5-2 equiv), followed by *N*-methylmorpholine (1 equiv) and DCC (1 equiv). The mixture was stirred overnight at 25 °C. The reaction mixture was diluted with ethyl acetate and the solid was removed by filtration. The filtrate was washed with 10% citric acid (2×), saturated sodium bicarbonate (2×), and brine. The organic layer was dried over $MgSO_4$ and concentrated to dryness in vacuo. The final product was either directly used in the next step or purified by flash chromatography depending on the purity as judged by NMR spectra or TLC.

Scheme VI[a]

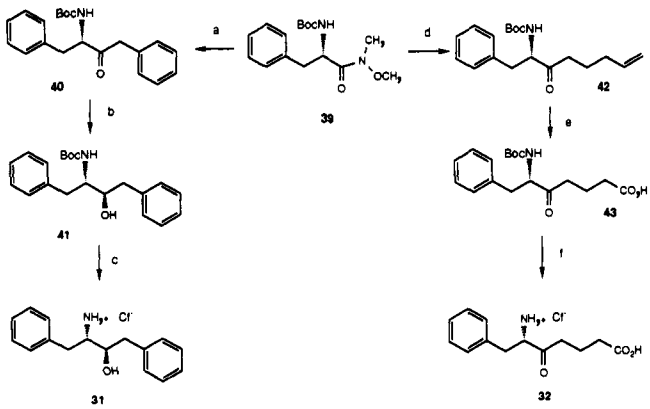


[a] (a) (1) Benzyl bromide, Cs_2CO_3, $(n\text{-butyl})_4NI$, (2) silica G column; (b) (1) LiOH, (2) benzyl bromide, Cs_2CO_3, $(n\text{-butyl})_4NI$; (c) (1) $(COCl)_2$, DMSO, TEA or Dess–Martin reagent, (2) HCl (gas), ether.

Scheme VII



Scheme VIII[a]



[a] (a) 2.0 M Benzylmagnesium chloride, 98%; (b) $NaBH_4$, −23 °C, 80%; (c) TFA, then 1 N HCl, 85%; (d) pentenylmagnesium bromide, 97%; (e) $NaIO_4$, $KMnO_4$, 78%; (f) TFA, then 1 N HCl, 88%.

Method B: General Deprotection Procedure.

(1) The protected peptide (0.5 M) dissolved in 50% trifluoroacetic acid in dry dichloromethane was stirred at room temperature for 3 h. The solvent was removed in vacuo and the remaining trifluoroacetic acid was removed by repeated evaporation from dichloromethane in vacuo.

(2) The protected peptide was dissolved in ether (0.2–0.5 mM) and dry HCl (gas) was introduced until saturated. The reaction mixture was stirred at room temperature overnight. The solid was triturated with ether and collected by filtration.

(3) The methyl ester was dissolved in 20% methanol in THF to a concentration of about 0.1 M, and 1.1 equiv of lithium hydroxide dissolved in 0.5–1 mL water was added. The reaction progress was monitored by TLC. The reaction took about 1–3 h to complete. Most solvents were removed in vacuo and the residue was dissolved in ethyl acetate. The organic layer was washed with 10% citric acid and then water and dried over $MgSO_4$. The free acid was obtained after removal of the solvent in vacuo.

Method C: Preparation of *N*-(*tert*-Butyloxycarbonyl)-AHPA (3-Amino-2-Hydroxy-4-Phenylbutanoic Acid) Methyl Ester. To a stirred solution of L-phenylalanine methyl ester (10.8 g, 50 mmol, hydrochloride salt) in 150 mL of 20% THF in water was added sodium bicarbonate (8.4 g, 100 mmol) at room temperature, followed by di-*tert*-butyl dicarbonate (10.9 g, 50 mmol). The mixture was stirred at room temperature overnight, then extracted with ether (2 × 100 mL), and the ether layers were combined and dried over $MgSO_4$. Boc-phenylalanine methyl ester was obtained as a pale yellow oil. The oil was dissolved in 70 mL dry toluene, cooled to −77 °C under nitrogen, and 125 mmol diisobutylaluminum hydride (1.0 M in hexane, 125 mL, 2.25 equiv) was added in 15 min. Methanol (10 mL) was added immediately to quench the reaction. The reaction mixture was poured into a solution of 100 mL of 20% sodium potassium

tartrate (Rochelle salt) and stirred at room temperature until the two layers separated. The organic layer was taken and concentrated in vacuo. The aldehyde was treated with a solution of sodium metabisulfite (14.5 g, 75 mmol) in 50 mL water and then to the emulsion was added a solution of potassium cyanide (4.9 g, 75 mmol) in 50 mL of water with stirring. The reaction was stirred at room temperature for about 5 h, the cyanohydrin was extracted twice with ether, and the ether layer was combined and concentrated in vacuo to dryness. The residue was treated with 25% hydrochloric acid and the solution was stirred at 80 °C for 12 h. The water was removed in vacuo to give crude (2*RS*,3*S*)-AHPA as a brown solid, which was dissolved in 100 mL of 20% THF in water, and the pH was adjusted to about 8 by addition of 10 N NaOH solution. To the solution was added sodium carbonate (8.3 g, 100 mmol) and di-*tert*-butyl dicarbonate (21.8 g, 100 mmol). The mixture was stirred at room temperature overnight, and the pH of the reaction was adjusted to 2 with 6 N HCl and extracted with ethyl acetate. The organic layer was taken and washed with 10% citric acid once. The product, *N*-(*tert*-butyloxycarbonyl)-AHPA, was again extracted by 1 N NaOH to the aqueous phase. The side products and other impurities were left in the organic layer. The aqueous phase was acidified to pH 2 and extracted by ethyl acetate. The ethyl acetate was removed to obtain *N*-BOC-AHPA as pale yellow solid. The solid was dissolved in 15 mL of DMF and methylated by adding potassium bicarbonate (8 g, 80 mmol) and iodomethane (11.4 g, 80 mmol). After 12 h, the reaction was diluted with ethyl acetate and washed with 10% citric acid twice and then water once. Concentration of the organic layer afforded a mixture of two diastereomers (2*R*:2*S* ~ 7:3) (8.5 g, 55% overall yield), which were separated by preparative TLC (2 mm, Merck about 200 mg each) by using 35% EA in H as solvent or by flash column chromatography (5% EA in H, 10% EA in H then 20% EA in H). *N*-Boc-(2*R*,3*S*)-AHPA methyl ester: TLC (R_f 0.33, 25% EA in H, UV, or AMA); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.39 (s, 9 H), 2.9 (m, 2 H), 3.75 (s, 3 H), 4.05 (d, J = 4.0 Hz, 1 H), 4.25 (m, 1 H), 4.78 (d, J = 9.0 Hz, 1 H), 7.15–7.35 (m, 5 H); $[\alpha]^{24}_D$ = −78.5° (c = 1.0, $CHCl_3$); *N*-Boc-(2*S*,3*S*)-AHPA methyl ester: TLC (R_f 0.21, 25% EA in H, UV, or AMA); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.39 (s, 9 H), 2.8 (m, 2 H), 3.75 (s, 3 H), 4.33 (m, two overlapped protons), 4.85 (d, J = 8.0 Hz, 1 H), 7.15–7.35 (m, 5 H); $[\alpha]^{24}_D$ = −15.5 (c = 1.0, $CHCl_3$); HRMS: 310.1657 (M + H)$^+$, calcd for $(C_{18}H_{23}NO_5 + H)$ 310.1654.

(2*R*,3*S*)-, (2*S*,3*S*)-, (2*S*,3*R*)-, (2*R*,3*R*)-AHPA Methyl Esters 1-4. The four isomers were made in the same way as free bases. A representative procedure is given below. 100 mg of *N*-Boc-(2*S*,3*S*)-AHPA methyl ester was deprotected by following the general deprotecting procedure B(1). After removal of solvent, the residue was treated with 10 mL of saturated sodium bicarbonate solution and extracted with ethyl acetate (2 × 100 mL). The organic layers were combined and dried over $MgSO_4$. The product was obtained as a pale yellow oil (solidified at room temperature in a few days) after removal of the solvent (60 mg, 90%): ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 2.58 (m, 1 H), 2.80 (m, 1 H), 3.38 (m, 1 H), 3.80 (s, 3 H), 4.24 (d, J = 3.0 Hz, 1 H), 7.15–7.35 (m, 5 H); $[\alpha]^{24}_D$ = −3.3° (c = 1.2, 1 N HCl). (2*S*,3*R*)-AHPA methyl ester: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 2.74 (m, 1 H), 2.92 (m, 1 H), 3.35 (m, 1 H), 3.80 (s, 3 H), 4.08 (d, J = 3.5 Hz, 1 H), 7.2–7.35 (m, 5 H); $[\alpha]^{24}_D$ = +19.6° (c = 0.84, 1 N HCl); HRMS 210.1132 (M + H)$^+$, calcd for $(C_{11}H_{15}NO_3 + H)$ 210.1130.

(2*S*,3*S*)- and (2*S*,3*R*)-AHPA (5, 6). The two compounds were made in the same manner as trifluoroacetic acid salt as described previously.[21] *N*-Boc-(2*S*,3*S*)-AHPA methyl ester (200 mg, 0.65 mmol) was demethylated according to method B(3) to give the corresponding free acid. The acid was deprotected by following method B(1) to give (2*S*,3*S*)-AHPA as a trifluoroacetic acid salt (160 mg, 80%): ^{1}H-NMR (D_2O, 400 MHz) δ 2.98 (m, 2 H), 4.0 (m, 1 H), 4.50 (d, J = 3.0 Hz, 1 H), 7.20–7.40 (m, 5 H); $[\alpha]^{24}_D$ = −2.2° (c = 0.67, 1 N HCl). (2*S*,3*R*)-AHPA: ^{1}H-NMR (D_2O, 300 MHz) δ 3.0 (m, 2 H), 3.88 (m, 1 H), 4.25 (d, J = 3.0 Hz, 1 H), 7.20–7.40 (m, 5 H); $[\alpha]^{24}_D$ = +11.8° (c = 1.02, 1 N HCl). The following compounds were prepared by coupling appropriate *N*-Boc amino acids and amino acid *tert*-butyl esters according to method A and deprotected by following method B(1).

(2*S*,3*S*)-AHPA-L-Leu (7)[21] (trifluoroacetic acid salt): ^{1}H-NMR (D_2O, 400 MHz) δ 0.90 (m, 6 H), 1.6–1.8 (m, 3 H), 2.95 (d, J = 13 Hz, 2 H), 4.02 (m, 1 H), 4.25 (m, 1 H), 4.56 (d, J = 3.0 Hz, 1 H), 7.25–7.40 (m, 5 H); $[\alpha]^{24}_D$ = −26.5° (c = 1.0, H_2O).

(2*S*,3*S*)-AHPA-Gly (8) (trifluoroacetic acid salt): NMR (D_2O, 400 MHz) δ 3.0 (m, 2 H), 3.89 (s, 2 H), 4.02 (m, 1 H), 4.55 (d, J = 3.0 Hz, 1 H), 7.15–7.35 (m, 5 H); $[\alpha]^{24}_D$ = −19.0° (c = 0.1, H_2O); HRMS: 253.1183 (M + H)$^+$, calcd for $(C_{12}H_{16}N_2O_4 + H)$ 253.1188.

(2*S*,3*S*)-AHPA-β-Ala (10) (trifluoroacetic acid salt): ^{1}H-NMR (D_2O, 300 MHz) δ 2.45 (t, J = 7.8 Hz, 2 H), 2.88 (m, 2 H), 3.25 (t, J = 8.0 Hz, 2 H), 3.95 (m, 1 H), 4.38 (d, J = 3.0 Hz, 1 H), 7.2–7.4 (m, 5 H); $[\alpha]^{24}_D$ = −16.7° (c = 1.02, H_2O); HRMS 267.1345 (M + 1)$^+$, calcd for $(C_{13}H_{16}N_2O_4 + H)$ 267.1345.

(2*RS*,3*S*)- and (2*RS*,3*R*)-3-Amino-2-mercapto-4-phenylbutanoic Methyl Ester (11a, 11b). Compounds 11a and 11b were made using the procedure of Rich and Ocain[12] with some modifications. *N*-Boc-(2*R*,3*S*)-AHPA methyl ester (840 mg, 2.72 mmol) was dissolved in 15 mL of dry CH_2Cl_2, and triethylamine (404 mg, 4.0 mmol) and methanesulfonyl chloride (458 mg, 4.0 mmol) were added dropwise at 0 °C with stirring. The reaction was stirred at room temperature for 3–4 h. TLC (50% H in EA, AMA) showed a complete reaction. The solvent was removed in vacuo and the residue was dissolved in ethyl acetate. The organic layer was washed with 10% citric acid (2×), saturated $NaHCO_3$ (2×), and water, and dried over $MgSO_4$. The organic solvent was removed in vacuo to give a white solid. The white solid was dissolved in 10 mL of dry DMF, and potassium thioacetate (1.2 g, 8 mmol) was added under nitrogen. The reaction was stirred at 25 °C until the starting material disappeared (about 10–12 h). The reaction was diluted with ethyl acetate, and the solution was washed with water (3×). The organic layer was concentrated and the residue purified on silica gel to give the protected thiol compound as a pale yellow solid (650 mg, 65% for two steps): TLC (R_f 0.33 and 0.37, two diastereomers, 25% EA in H, UV or AMA); NMR ($CDCl_3$, 300 MHz) δ 1.33 (s, 9 H), 2.35 and 2.40 (two s, 3 H), 2.84 (m, 2 H), 3.72 and 3.73 (two s, 3 H), 4.28 (m, 0.5 H), 4.36 (d, 0.5 H, J = 4.3 Hz), 4.45 (d, 0.5 H, J = 3.9 Hz), 4.50 (m, 0.5 H), 7.15–7.30 (m, 5 H). The protected thiol compound (150 mg, 0.41 mmol) was dissolved in 10 mL of 50% methanol in THF (nitrogen saturated). To this solution was added a solution of lithium hydroxide (21 mg, monohydrate, 0.5 mmol) in 0.5 mL of H_2O. The reaction was stirred at 25 °C for 5 min. TLC (25% EA in H, product R_f 0.44) showed a complete reaction. The solvent was removed in vacuo and the residue was dissolved in ethyl acetate and washed with 10% citric acid and water (all solvents were nitrogen saturated). The organic layer was concentrated to dryness, the residue was dissolved in ether (10 mL), and HCl (gas) was bubbled in for about 30 min. The solvent was removed and the residue was dissolved in 4 mL H_2O and lyophilized overnight to give the final product (65 mg, 61% for two steps) as a pale yellow solid: NMR (D_2O, 300 MHz), δ 3.05 (m, 2 H), 3.62 and 3.64 (two s, 3 H), 3.73 (d, 0.5 H, J = 7.0 Hz), 4.0 (d, 0.5 H, J = 5.5 Hz), 4.08 (m, 1 H), 7.2–7.4 (m, 5 H). Characterization is consistent with previous reported synthesis.[12]

Endothiodipeptides 13 and 15. The endothiopeptides 13 and 15 were prepared by modification of Rich and Ocain procedure for 13.[12] *t*-Boc-(2*S*,3*R*)-AHPA methyl ester (500 mg, 1.6 mmol) was dissolved in a solution of 2,2-dimethoxypropane (15 mL) and *p*-toluenesulfonic acid (100 mg, dried azeotropically from benzene). After being stirred at room temperature for 36 h, the reaction mixture was diluted with ethyl acetate and the organic layer was washed with saturated $NaHCO_3$ (2×) and water and concentrated in vacuo to dryness. Purification of the residue on silica gel (5% EA, 45% H in C, then 10% EA in H) afforded the isopropylidene methyl ester as a pale yellow oil which was hydrolyzed by following the general deprotection procedure to afford an acid as a white solid (470 mg, 88% for two steps). The general procedure for peptide formation was followed to effect the coupling of the acid (425 mg, 1.27 mmol) and L-leucine *tert*-butyl ester (HCl salt, 310 mg, 1.5 mmol), giving a product as a white solid (505 mg, 79%) after purification on silica gel (5% EA, 20% H in C). The protected dipeptide product (150 mg, 0.3 mmol) was dissolved in 7 mL of dry benzene and the Lawesson's reagent (75 mg, 0.18 mmol, Aldrich) was added. The solution was stirred at 88 °C (bath temperature) for 1.5 h in a capped flask. TLC showed the reaction was complete (starting material R_f = 0.61, product R_f = 0.71, 25% ethyl acetate in hexane, UV or AMA). The reaction mixture was concentrated in vacuo, and

the residue was purified on preparative TLC (2 mm, Merck, 30% EA in H) to afford the protected endothiodipeptide **26** as a pale yellow oil (150 mg, 96%, MS 512 $(M + 1)^+$). The thio compound was treated with 10 mL trifluoroacetic acid and stirred at room temperature for 5 h, and then 2 mL of methanol was added to the solution and stirred at 70 °C (bath temperature) for 6 h. The solvent was removed in vacuo and the residue lyophilized to give product **13** as a white solid (75 mg, 77%). NMR and TLC showed the existence of diastereomer **15**, perhaps racemization took place at α-carbon during the thiolation. The product (35 mg) was further purified by silica gel preparative TLC (2 mm, Merck, 30% methanol in chloroform, developed twice) to give pure compounds **13** (20 mg) and **15** (8 mg). Compound **13** (TFA salt): TLC (R_f 0.36, 25% M in C, UV); ^{1}H-NMR (D_2O, 400 MHz) δ 0.85 (d, 3 H, J = 6.4 Hz), 0.88 (d, 3 H, J = 6.4 Hz), 1.60–1.89 (m, 3 H), 2.78 (m, 1 H), 2.97 (m, 1 H), 3.82 (m, 1 H), 4.35 (d, 1 H, J = 3.0 Hz), 4.60 (m, 1 H), 7.23–7.38 (m, 5 H); $[\alpha]^{22}_D$ = −17.2° (c = 0.87, 1 N HCl); MS: 325 $(M + 1)^+$. Compound **15** (TFA salt): TLC (R_f 0.28, 25% M in C, UV); ^{1}H-NMR (D_2O, 400 MHz) δ 0.90 (d, 3 H, J = 6.4 Hz), 0.94 (d, 3 H, J = 6.4 Hz), 1.66–1.89 (m, 3 H), 2.62 (m, 1 H), 2.94 (m, 1 H), 3.91 (m, 1 H), 4.62 (d, 1 H, J = 3.0 Hz), 4.68 (m, 1 H), 7.26–7.38 (m, 5 H); $[\alpha]^{22}_D$ = +15.6° (c = 0.15, 1 N HCl), MS 325 $(M + 1)^+$.

Endothiodipeptide 14. Compound 14 was prepared as outlined above for **13** and **15** TLC (R_f 0.42, 25% M in C, UV or AMA); ^{1}H-NMR (D_2O, 400 MHz) δ 0.81 (d, 3 H, J = 6.5 Hz), 0.86 (d, 3 H, J = 6.5 Hz), 1.57 (m, 1 H), 1.65 (m, 1 H), 1.69 (m, 1), 2.88 (m, 2 H), 3.81 (m, 1 H), 4.29 (d, 1 H, J = 2.4 Hz), 4.65 (m, 1 H), 7.20–7.35 (m, 5 H); $[\alpha]^{22}_D$ = +11.1° (c = 0.57, 1 N HCl).

Preparation of 18 (Scheme V). 4-Methyl-5-imidazolemethanol hydrochloride (1.5 g, 10 mmol, Aldrich) was dissolved in a solution of triethylamine (2.8 mL, 20 mmol), DMF (10 mL) and dichloromethane (20 mL) at room temperature and triphenylmethyl chloride (2.76 g, 9.9 mmol) was then added. The solution was stirred at room temperature overnight and the solid formed was collected by filtration, washed with H_2O and ethyl acetate, and air-dried to give the protected imidazole alcohol as two isomers (3.1 g, 88%). The protected imidazole alcohol (1.5 g, 4.2 mmol) was suspended in 30 mL of dry 1,4-dioxane, and the solution was heated with a hair-dryer to dissolve most of the solid. Activated MnO_2 (1.5 g, 4 × 4.2 mmol, Aldrich) was added, and the reaction was stirred at room temperature for 24 h. The dark reaction solution was filtered through a Celite pad, and the filtrate was concentrated in vacuo to dryness. The residue was purified on silica gel (5% EA, 5% H in C) to give 1-(triphenylmethyl)-4(5)-methylimidazole-5(4)-carboxyaldehyde as a white solid (1.0 g, 67%): TLC (R_f 0.5, 50% EA in H); NMR ($CDCl_3$, 300 MHz) δ 1.85 (s, 3 H, CH_3), 9.9 (s, 1 H, CHO); MS 353 $(M + H)^+$.

The aldehyde was coupled with ethyl bromodifluoroacetate according to a published procedure.[22] An amount of 262 mg (4.0 mmol) of zinc (activated) and 10 mL of THF in a two-neck flask was refluxed at 75–80 °C and 0.52 mL (4.0 mmol) of ethyl bromodifluoroacetate was added through a syringe over 10 s. Aldehyde **28** (0.71 g, 2.0 mmol) dissolved in 5 mL of CH_2Cl_2, and 5 mL of THF was added over 1 min. The solution was refluxed for 15 min. The solvent was removed in vacuo and the residue dissolved in chloroform was extracted with 5% EDTA solution (pH = 10) twice and dried over $MgSO_4$. Removal of chloroform gave the crude product as a white solid (0.8 g, 84%). The compound was used in the next oxidation step without further purification: TLC (R_f 0.62, 10% M in C, UV or AMA).

The oxidation procedure was described before.[23] The crude compound (200 mg, 0.42 mmol) was dissolved in dry benzene (3 mL) and DMSO (0.15 mL, 5 × 0.42 mmol), and DCC (435 mg, 5 × 0.42 mmol) and anhydrous phosphoric acid (25 mg, 0.25 mmol) were added. The mixture was stirred at room temperature for 24 h. The solid was removed by filtration and the filtrate was concentrated in vacuo. The residue was purified on silica gel preparative TLC (2 mm, Merck, 30% ethyl acetate in hexane) to give the triethyl-protected difluoro ketone as a white solid (105 mg, 53%): TLC (R_f's 0.35 and 0.30, two isomers, 25% ethyl acetate in hexane, UV or AMA). Deprotection was done by dissolving the compound in 5 mL of trifluoroacetic acid with stirring for 4 h at room temperature. The acid was removed in vacuo and the residue was dissolved in 4 mL H_2O (2 × 2 mL) and filtered to remove the solid. The filtrate was lyophilized to give the final product **18** as a white solid (42 mg, 82%): NMR (300 MHz, D_2O, hydrated and ketone forms) δ 1.20 (two t, 3 H, J = 7.2 Hz), 2.33 and 2.52 (two s, 3 H, CH_3), 4.29 (two q, 2 H, J = 7.2 Hz), 8.53 and 8.54 (two s, 1 H, aromatic H); MS (methanol as solvent) 233 $(M + 1)^+$ and 251 $(M + 18 + H)^+$, hydrated form, 265 $(M + 32 + H)^+$, hemiketal with methanol.

(2*S*,3*S*)-AHPA Benzyl Amide (19). *N*-Boc-(2*S*,3*S*)-AHPA (90 mg, 0.31 mmol) was coupled with benzyl amine (70 mg, 0.65 mmol) according to method A and deprotected by following method B(1) to give the title compound as a white solid (101 mg, overall 82% in two steps, trifluoroacetic acid salt): ^{1}H-NMR (DMSO, 300 MHz) δ 2.78 (d, J = 6.8 Hz, 2 H), 3.68 (m, 1 H), 4.20 (d, J = 8.1 Hz, 2 H), 4.28 (d, J = 3 Hz, 1 H), 7.15–7.35 (m, 5 H); MS 285 $(M + 1)^+$; $[\alpha]^{24}_D$ = −24.8° (c = 0.70, AcOH); HRMS: 285.1603, calcd for $(C_{17}H_{21}N_2O_2 + H)$ 285.1603.

Method D: General Procedure for the Preparation of Phenol Benzyl Ether or Benzyl Ester. Representative syntheses of (2*RS*,3*S*)-3-*N*-(*tert*-butyloxycarbonyl)amino-2-hydroxy-4-(4-benzyloxyphenyl)butanoic acid methyl ester (**34*S***) and its diastereomer (**34*R***) are presented. Compound **33** (prepared from Boc-Tyr(benzyl) methyl ester according to method C, 1.2 g, 3.7 mmol) was dissolved in 5 mL of dry DMF, and Cs_2CO_3 (2.4 g, 7.4 mmol, Aldrich), tetrabutylammonium iodide (50 mg, Aldrich), and benzyl bromide (1.2 g, 1.8 × 3.7 mmol) were added sequentially. The disappearance of the starting material was followed by TLC (usually took 2–5 h). The reaction mixture was diluted with ethyl acetate, and the organic layer was washed with water (2×) and concentrated in vacuo to dryness. The residue was purified on silica G (20% EA in H) to give the (2*R*,3*S*) isomer (680 mg, 44%, white wax), the (2*S*,3*S*) isomer (450 mg, 29%, white wax), and a mixture of the two (260 mg, 17%). (2*R*,3*S*)-**34*R***: TLC (R_f 0.58, 50% EA in H, UV or AMA); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.38 (s, 9 H), 2.84 (m, 2 H), 3.73 (s, 3 H), 4.05 (br s, 1 H), 4.18 (m, 1 H), 4.77 (d, J = 10.0 Hz, 1 H), 5.03 (s, 2 H), 6.91 (d, J = 6.8 Hz, 2 H), 7.18 (d, J = 8.4 Hz, 2 H), 7.30–7.43 (m, 5 H). (2*S*,3*S*)-**34*S***: TLC (R_f 0.44, 50% EA in H, UV or AMA); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.38 (s, 9 H), 2.73 (m, 2 H), 3.55 (s, 3 H), 4.24 (br s, 1 H), 4.30 (d, J = 4.8 Hz, 1 H), 4.82 (d, J = 6.8 Hz, 2 H), 5.03 (s, 2 H), 6.87 (d, J = 6.4, 2 H), 7.10 (d, J = 8.4 Hz, 2 H), 7.25–7.42 (m, 5 H).

Method E: Preparation of Compound 25. DMSO (256 mg, 3.28 mmol) was added to a solution of $(COCl)_2$ (211 mg, 1.64 mmol) in 5 mL of dry CH_2Cl_2 at −77 °C and the mixture was stirred at this temperature for 10 min. To the solution, compound **34*R*** (170 mg, 0.41 mmol) in 5 mL of dry CH_2Cl_2 was added dropwise. After stirring at −77 °C for 20 min, the reaction mixture was treated with triethylamine (331 mg, 3.28 mmol) and stirred at the same temperature for 10 min before warming up to room temperature. The reaction mixture was diluted with 20 mL of hexane and filtered to remove the salt. The filtrate was concentrated and the residue purified on silica G (25% EA in H) to obtain the Boc-protected **25** (clear oil, 150 mg, 89%). The Boc group was removed by following method B(2) to give compound **25** as a pale yellow solid (85 mg, 67%, HCl salt): ^{1}H-NMR (D_2O, 400 MHz) δ 2.85 (dd, J = 8.1, 14.3 Hz, 1 H), 3.00 (dd, J = 6.8, 14.7 Hz, 1 H), 3.49 (s, 3 H), 3.84 (t, J = 7.04 Hz, 1 H), 5.18 (s, 2 H), 7.03 (d, J = 8.6 Hz, 2 H), 7.21 (d, J = 8.6 Hz, 2 H), 7.37–7.50 (m, 5 H); ^{13}C-NMR (10% D_2O in DMSO, 100 MHz) δ 32.90, 52.33, 57.59, 69.10, 91.74, 114.81, 127.62, 127.69, 127.83, 127.90, 128.51, 130.55, 137.10, 157.16, 169.77; HRMS 314.1386 $(M+H)^+$, calcd for $(C_{18}H_{19}NO_4 + H)$ 314.1392.

Compound 21. The compound was prepared by oxidation of the corresponding alcohol *N*-Boc-(2*RS*,3*S*)-AHPA methyl ester according to method E and deprotected according to method B(2) to give the title compound (HCl salt, pale yellow powder): ^{1}H-NMR (D_2O, 400 MHz) δ 2.88 (dd, J = 8.9, 14.5 Hz, 1 H), 3.12 (dd, J = 6.0, 14.4 Hz, 1 H), 3.65 (s, 3 H), 3.89 (dd, J = 6.0, 8.8 Hz, 1 H), 7.30–7.43 (m, 5 H); MS: 208 $(M + 1)^+$, 226 $(M + H_2O + 1)^+$ (hydrate form).

Compound 22. The title compound was prepared by oxidizing the corresponding alcohol *N*-Boc-(2*S*,3*S*)-AHPA-L-Leu-*tert*-butyl ester according to method E and deprotected according to method B(1) (trifluoroacetic acid salt, pale yellow powder): ^{1}H-NMR (D_2O, 400 MHz) δ 0.91 (m, 6 H), 1.65–1.78 (m, 3 H), 2.78 (m, 1

H), 3.10 (m, 1 H), 3.75 (m, 1 H), 4.37 (m, 1 H), 7.18–7.42 (m, 5 H); MS (FAB⁻): 305 (M − 1)⁻. Anal. ($C_{18}H_{22}N_2O_6F_3$) C, H, N.

Compound 23. The title compound was made via deprotection of the corresponding *N*-Boc-protected compound (**34S**) according to method B(2) (HCl salt, white powder): NMR (D_2O, 400 MHz) δ 2.93 (m, 2 H), 4.08 (br m, 1 H), 4.57 (d, *J* = 2.2 Hz, 1 H), 5.18 (s, 2 H), 7.02 (d, *J* = 8.4 Hz, 2 H), 7.21 (d, *J* = 8.4 Hz, 2 H), 7.39–7.50 (m, 5 H); $[\alpha]^{24}_D$ = +3.5° (c = 1.0, AcOH); HRMS 316.1550, calcd for ($C_{18}H_{21}NO_4$ + H) 316.1548. Anal. ($C_{18}H_{22}NO_4Cl$) C, H, N.

Compound 24. Compound (**34S**) (400 mg, 0.96 mmol) was demethylated according to method B(3) to give the corresponding free acid (380 mg, 99%), which (150 mg) was benzylated by following method D to give **35S** as a white solid (150 mg, 81%) after purification on silica G column (30% EA in H). The benzyl ester (100 mg) was deprotected according to method B(2) to give the title compound (HCl salt, white powder, 75 mg, 86%): ^{1}H-NMR (D_2O, 400 MHz) δ 2.85 (d, *J* = 6.9 Hz, 2 H), 4.05 (br m, 1 H), 4.42 (d, *J* = 11.9 Hz, 1 H), 4.57 (br s, 1 H), 4.71 (d, *J* = 12.0 Hz, 1 H), 5.12 (s, 2 H), 6.94 (d, *J* = 7.8 Hz, 2 H), 7.10 (d, *J* = 8.2 Hz, 2 H), 7.25–7.44 (m, 10 H); $[\alpha]^{24}_D$ = +3.0° (c = 1.0, AcOH); HRMS 392.1858, calcd for ($C_{24}H_{25}NO_4$ + H) 392.1861. Anal. ($C_{24}H_{26}NO_4Cl$) C, H, N.

Method F: Preparation of Compound 26. The benzyl ester (*RS*)-**35** (180 mg, 0.37 mmol) was dissolved in 8 mL of dry CH_2Cl_2 under argon, and to this solution was added Dess–Martin reagent[19b,c] (400 mg, 0.94 mmol) portionwise at room temperature with stirring. After stirring at room temperature for 1.2 h, the reaction mixture was diluted with ether (30 mL), followed by saturated $NaHCO_3$ (10 mL) and $Na_2S_2O_3$ (1.0 g in 10 mL of H_2O) and stirred until two layers became clear. The organic layer was separated and washed with H_2O and dried over $MgSO_4$. The Boc-protected **26** was obtained as a pale yellow solid after removal of solvent and lyophilization. The compound was pure as judged by TLC and ^{1}H-NMR and was deprotected according to method B(2) to give **26** as a white powder (HCl salt, 120 mg, 77% for two steps): ^{1}H-NMR (20% D_2O in DMSO, 400 MHz) δ 2.71 (d, *J* = 6.9 Hz, 2 H), 3.49 (t, *J* = 6.8 Hz, 1 H), 4.67 (d, *J* = 12 Hz, 1 H), 4.86 (d, *J* = 12 Hz, 1 H), 5.03 (s, 2 H), 6.86 (d, *J* = 8.4 Hz, 2 H), 7.01 (d, *J* = 8.8 Hz, 2 H), 7.25–7.38 (m, 10 H); ^{13}C-NMR (20% D_2O in DMSO, 100 MHz) δ 32.85, 57.65, 66.97, 69.12, 91.77, 114.84, 114.81, 127.57, 127.63, 127.71, 127.92, 128.30, 128.47, 128.53, 130.14, 130.52, 135.00, 137.02, 157.19, 169.23; HRMS 390.1709 (M + H)⁺, calcd for ($C_{24}H_{23}NO_4$ + H) 390.1705. Anal. ($C_{24}H_{24}NO_4Cl$) C, H, N.

Compound 27. The title compound was prepared first as a Boc-protected disulfide dimer by following the same procedures as described[24] and then deprotected according to the method B(2) to give the compound as a white solid HCl salt): ^{1}H-NMR (d_4-MeOH, 400 MHz) δ 2.80 (m, 4 H), 2.89 (m, 2 H), 3.02 (m, 2 H), 3.77 (br s, 2 H), 5.06 (s, 4 H), 6.99 (d, *J* = 8.6 Hz, 4 H), 7.16 (d, *J* = 8.6 Hz, 4 H), 7.32–7.45 (m, 10 H); HRMS 545.2305, calcd for ($C_{32}H_{36}N_2O_2S_2$ + H) 545.2296. Anal. ($C_{32}H_{38}N_2O_2S_2Cl$) C, H, N.

Method G: Preparation of 37. THP-protected 3-bromobenzyl alcohol (2.71 g, 10 mmol) was added to a suspension of Mg turnings (270 mg, 11 mmol) in 20 mL of dry THF under argon. The resulting solution was refluxed for 3 h under argon, and the solution was cooled to room temperature. To this freshly prepared Grignard reagent solution was added Weinreb amide[20] (1.65 g, 4.0 mmol) portionwise under a positive argon pressure and stirred at room temperature for 3 h before pouring into a saturated NH_4Cl solution at 0 °C. The product was extracted with ethyl acetate (2×) and purified on silica gel column (20% EA in H) to afford compound **37** as a pale yellow oil (2.0 g, 92%): TLC (R_f 0.35, 25% EA in H, UV or AMA); NMR ($CDCl_3$, 400 MHz) δ 1.42 (s, 9 H), 1.54–1.85 (m, 6 H), 2.86 (dd, *J* = 5.6, 14 Hz, 1 H), 3.12 (dd, *J* = 5.6, 14 Hz, 1 H), 3.57 (m, 1 H), 3.91 (m, 1 H), 4.53 (m, 1 H), 4.72 (m, 1 H), 4.81 (m, 1 H), 5.0 (s, 2 H), 5.39 (d, *J* = 8 Hz, 1 H), 5.48 (m, 1 H), 6.81 (d, *J* = 8.4 Hz, 2 H), 6.90 (d, *J* = 8.4 Hz, 2 H), 7.25–7.39 (m, 5 H), 7.42 (t, *J* = 8 Hz, 1 H), 7.52 (d, *J* = 8 Hz, 1 H), 7.80 (d, *J* = 8 Hz), 7.92 (s, 1 H).

Compound 38. Compound **37** (0.6 g, 1.1 mmol) in 10 mL of MeOH was treated with 1 drop of 6 N HCl and stirred at room temperature for 2 h. The reaction was diluted with ethyl acetate and washed with 5% $NaHCO_3$ (2×) and dried over $MgSO_4$. The corresponding alcohol was obtained as a white wax (500 mg, 98%) after removal of the solvent: TLC (R_f 0.55, 50% EA in H, UV or AMA). The benzylic alcohol (210 mg, 0.46 mmol) was oxidized to aldehyde **38** (195 mg, 93%) with pyridinium dichromate[25] (400 mg, 1.1 mmol) in dry DMF (4 mL) and purified by silica G column (35% EA in H): TLC (R_f 0.3, 25% EAZ in H, UV or AMA); NMR ($CDCl_3$, 400 MHz) δ 1.42 (s, 9 H), 2.97 (dd, *J* = 5.6, 14 Hz, 1 H), 3.12 (dd, *J* = 6.4, 13.6 Hz, 1 H), 4.98 (s, 2 H), 5.36 (d, *J* = 8 Hz, 1 H), 5.49 (m, 1 H), 6.80 (d, *J* = 8.4 Hz, 2 H), 6.92 (d, *J* = 8.8 Hz, 2 H), 7.31–7.38 (m, 5 H), 7.61 (t, *J* = 8 Hz, 1 H), 8.06 (d, *J* = 8 Hz, 1 H), 8.14 (d, *J* = 8 Hz, 1 H), 8.34 (s, 1 H), 10.03 (s, 1 H).

Compound 28. To a heterogeneous solution of AgO (124 mg, 1 mmol) in 2 mL of 1 N NaOH was added aldehyde **38** in 4 mL of MeOH. The reaction mixture was stirred at room temperature for 30 min. TLC showed disappearance of the starting material. The reaction mixture was diluted with ethyl acetate and the organic layer was washed with 1 N HCl and dried over $MgSO_4$. The Boc-protected **28** was obtained as a pale yellow powder (160 mg, 86%) after removal of the solvent and lyophilization. The protected **28** (160 mg, 0.34 mmol) was dissolved in 15 mL of dry ether and deprotected according to the method B(2) to give compound **28** as a white powder (110 mg, 80%, HCl salt): NMR (DMSO, 500 MHz) δ 3.10 (m, 2 H), 5.03 (s, 2 H), 5.45 (br s, 1 H), 6.86 (d, *J* = 8.7 Hz, 2 H), 7.03 (d, *J* = 8.6 Hz, 2 H), 7.34–7.43 (m, 5 H), 7.64 (t, *J* = 7.7 Hz, 1 H), 8.21 (m, 2 H), 8.38 (s, 1 H), 8.48 (br m, 3 H); HSMS 376.1549 (M + H)⁺, calcd for $C_{23}H_{21}NO_4$ + H) 376.1549. Anal. ($C_{23}H_{22}NO_4Cl$) C, H, N.

Preparation of Compound 29. The title compound was prepared by first coupling Weinreb amide[20] with 4-bromobenzotrifluoride according to method G and deprotected according to method B (2) to give compound **29** as a pale yellow solid (HCl salt): NMR (DMSO, 400 MHz) δ 3.13 (t, *J* = 6.8 Hz, 2 H), 5.02 (s, 2 H), 5.44 (br s, 1 H), 6.85 (d, *J* = 8.6 Hz, 2 H), 7.04 (d, *J* = 8.6 Hz, 2 H), 7.33–7.41 (m, 5 H), 7.89 (d, *J* = 8.2 Hz, 2 H), 8.13 (d, *J* = 8.2 Hz, 2 H), 8.61 (br s, 3 H); HRMS 400.1540 (M + H)⁺, calcd for ($C_{23}H_{20}F_3NO_2$ + H⁺) 400.1524. Anal. ($C_{23}H_{21}F_3NO_2Cl$) C, H, N.

(3*S*)-1,4-Diphenyl-2-oxo-3-amino-*N*-Boc-butane (40): To a stirred solution of *N*-Boc-L-phenylalanine-*N*-methoxy-*N*-methylamide[26] (**39**) (5.0 g, 14.5 mmol) in anhydrous THF (50 mL) under N_2 at 0 °C was added 2.0 M benzylmagnesium chloride in THF (21.7 mL, 43.5 mmol). The mixture was gradually warmed to room temperature and stirred for an additional 3 h. The reaction mixture was then poured onto 1 N HCl (25 mL). The organic layer was separated and the aqueous layer was extracted with ether (3 × 35 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to give a crude product. Purification of the crude material by flash chromatography (EA/H; 1:4) afforded **40** as a white solid (4.8 g, 98%): R_f 0.3 (EA/H; 1:4); mp 86–87 °C; $[\alpha]^{25}_D$ +31.22° (c 2.21, CH_2Cl_2); IR 3485, 2978, 1709, 1704, 1490, 1363, 1250 cm⁻¹; ^{1}H-NMR ($CDCl_3$) δ 1.14 (s, 9 H), 2.9–3.15 (m, 2 H), 3.65 (q, 2 H, *J* = 11.6 Hz), 4.61 (d, 1 H, *J* = 6.9 Hz), 5.1 (bs, 1 H), 7.0–7.2 (m, 10 H) ppm; ^{13}C ($CDCl_3$) δ 28.3, 37.8, 47.8, 59.5, 79.9, 127.0, 127.1, 128.8, 129.2, 129.6, 133.1, 135.2, 155.1, 206.5 ppm; HRMS 472.0880, calcd for $C_{21}H_{25}NO_3$ + Cs^+ 472.0889.

(2*R*,3*S*)-1,4-Diphenyl-2-hydroxy-3-amino-*N*-Boc-butane (41): To a stirred solution of **40** (0.49 g, 1.4 mmol) in anhydrous MeOH (1 mL) under N_2 at −22 °C was added $NaBH_4$ (0.16 g, 4.4 mmol). After 30 min the mixture was gradually warmed to room temperature and then poured onto saturated NH_4Cl (10 mL). The mixture was extracted with ether (5 × 25 mL). The combined organic phase was washed with 1 N HCl (2 × 25 mL), saturated $NaHCO_3$ (3 × 25 mL), dried ($MgSO_4$), and concentrated to give a crude product as a 9:1 mixture of diastereomers as determined by ^{1}H-NMR. Recrystallization from benzene afforded **41** as a crystalline white solid (0.42 g, 80%): R_f 0.26 (EA/H, 1:4); mp 157–158 °C; $[\alpha]^{25}_D$ −10.12° (c 0.5, CH_2Cl_2); IR 3691, 3155, 2927, 1794, 1706, 1471, 1381, 1166, 1096 cm⁻¹; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.35 (s, 9 H), 2.55–3.05 (m, 4 H), 3.83–4.0 (m, 2 H), 4.65 (bs, 1 H), 7.05–7.2 (m, 10 H) ppm; ^{13}C ($CDCl_3$, 125 MHz) δ 28.3, 35.4, 40.3, 56.1, 74.8, 77.3, 126.4, 126.6, 128.4, 128.7, 129.3, 129.4, 138.1, 193.2 ppm; HRMS 364.1902, calcd for $C_{23}H_{25}NO_3$ + H⁺ 364.1913.

The product was derivatized by treatment with $SOCl_2$ to give the 3,4-dibenzyloxazolidinone.[27] The assignment of stereochem-

istry was based on the vicinal coupling, J_{34} = 5.0 Hz, consistent with that of the *trans*-oxazolidinone.[28,29]

(2*R*,3*S*)-1,4-Diphenyl-2-hydroxy-3-aminobutane HCl (31): To a stirred solution of **41** (0.20 g, 0.58 mmol) in CH_2Cl_2 (5 mL) was added TFA (0.23 mL, 2.9 mmol). After 12 h, the mixture was concentrated to give a crude white solid, which was taken up in 1 N HCl (1 mL) and concentrated to give a crude product. This procedure was repeated three times. Recrystallization from ether/MeOH afforded **31** as a white crystalline solid (0.14 g, 87%): mp 158–159 °C; $[\alpha]^{25}_D$ −11.83° (c 3.21, MeOH); ^{1}H-NMR (CD_3OD, 300 MHz) δ 2.6–2.8 (m, 3 H), 3.05 (dd, 2 H, J = 10.0, 15.3 Hz), 3.2–3.3 (m, 1 H), 3.43 (q, 2 H, J = 7.0 Hz), 3.88 (sept, 1 H, J = 3 Hz), 7.0–7.2 (m, 10 H) ppm; ^{13}C-NMR (CD_3OD, 125 MHz) δ 33.9, 40.0, 58.1, 72.9, 127.7, 128.5, 129.7, 130.2, 130.3, 130.4, 137.4, 139.4 ppm; HRMS 264.6601, calcd for $C_{16}H_{20}NO^+$ + Na^+ 264.6611. Anal. ($C_{16}H_{20}NOCl$) C, H, N.

(7*S*)-6-Oxo-7-amino-8-phenyl-*N*-Boc-octene (42): To a stirred solution of **39** (2.3 g, 7.4 mmol) in anhydrous ether (30 mL) under N_2 at 0 °C was added freshly prepared pentylmagnesium bromide (6.5 g, 37.3 mmol) in anhydrous ether (20 mL). The mixture was gradually warmed to room temperature and stirring was continued for 18 h. The reaction mixture was poured onto 1 N HCl (30 mL) at 0 °C. The organic layer was separated and the aqueous layer was extracted with ether (4 × 25 mL). The combined organic phase was washed successively with 1 N HCl × 25 mL, saturated $NaHCO_3$ (2 × 25 mL), and saturated NaCl (1 × 25 mL), dried over ($MgSO_4$), and concentrated to give a crude white solid. Purification by flash chromatography (EA/H; 1:2) gave **42** as a white crystalline solid (2.4 g, 98%): R_f 0.7 (EA/H; 1:1); mp 77.5–79 °C; $[\alpha]^{25}_D$ +50.58° (c 3.16, CH_2Cl_2); IR 3436, 2978, 2932, 1708, 1705, 1490, 1367, 1249, 1171 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.41 (s, 9 H), 1.64 (s, 2 H, J = 6.8 Hz), 2.0 (q, 2 H, J = 7.1 Hz), 2.27–2.47 (m, 2 H), 3.0 (dq, 2 H, J = 6.7, 13.9 Hz), 4.52 (q, 1 H, J = 7.1 Hz), 4.94–5.09 (m, 2 H), 5.1 (bd, 1 H, J = 7.1 Hz), 5.64–5.77 (m, 1 H), 7.0–7.2 (m, 5 H) ppm; ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 22.2, 28.2, 32.8, 37.8, 39.8, 59.9, 79.7, 115.2, 126.9, 128.5, 129.1, 136.2, 137.7, 155.1, 209.1 ppm; HRMS 450.1030, calcd for $C_{19}H_{27}NO_3$ + Cs^+ 450.1045.

(6*S*)-5-Oxo-6-amino-7-phenyl-*N*-Boc-heptanoic Acid (43). To a stirred solution of **42** (0.23 g, 0.73 mmol) in acetone (20 mL) and water (20 mL) at room temperature were added $NaIO_4$ (0.62 g, 2.93 mmol) and $KMnO_4$ (0.07 g, 0.46 mmol). After an additional 12 h of stirring the mixture was filtered and the resulting filtrate was concentrated. The concentrate was treated with 0.01 N NaOH (20 mL) and washed with ether (2 × 10 mL). The aqueous layer was then treated with 1 N HCl until acidic. The aqueous layer was then extracted with ether (4 × 15 mL). These organic layers were combined, dried over ($MgSO_4$), and then concentrated to give a crude oil. Purification by chromatography (EA/H:1:4; 0.1% AcOH) afforded **43** as a white solid (0.19 g, 78%): R_f 0.2 (EA/H/0.1% AcOH); mp 88–89 °C; $[\alpha]^{25}_D$ +8.32° (c 2.88, CH_2Cl_2); IR 3691, 3553, 2983, 1794, 1708, 1472, 1381, 1166, 1097 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.37 (s, 9 H), 1.75–1.90 (m, 2 H), 2.2–2.6 (m, 4 H), 2.9–3.1 (m, 2 H), 4.55 (q, 1 H), 5.1 (bd, 1 H), 7.0–7.3 (m, 5 H) ppm; ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 18.1, 28.3, 32.7, 37.9, 39.5, 60.0, 80.1, 27.1, 128.7, 129.2, 136.1, 155.3, 178.2, 208.7 ppm; HRMS 358.1630, calcd for $C_{18}H_{25}NO_5$ + Na^+ 358.1630.

(6*S*)-5-Oxo-6-amino-7-phenylheptanoic Acid Hydrochloride (32). To a stirred solution of **43** (0.17 g, 0.5 mmol) in CH_2Cl_2 (3 mL) was added TFA (0.4 mL, 5.1 mmol). After 12 h, the mixture was concentrated to give a crude white solid, which was taken up in 1 N HCl (1 mL) and concentrated. This was repeated three times. The crude product was recrystallized from MeOH/EA to give **32** as a white crystalline solid (0.12 g, 88%): mp 154–155 °C; $[\alpha]^{25}_D$ +10.68° (c 1.03, MeOH); ^{1}H-NMR (d_6-DMSO, 300 MHz) δ 1.5–1.8 (m, 2 H), 2.15 (dt, 2 H, J = 2.2, 7.0 Hz), 2.4–2.6 (m, 2 H), 3.1 (d, 2 H, J = 7.0 Hz), 3.34 (s, 4 H), 4.36 (t, 1 H, J = 7.0 Hz), 7.25–7.30 (m, 5 H) ppm; ^{13}C-NMR (DMSO, 125 MHz) δ 18.0, 32.4, 35.3, 38.94, 58.5, 127.3, 128.7, 129.4, 134.8, 174.0, 206.0 ppm; HRMS: 236.2801, calcd for $C_{13}H_{18}NO_3Cl^+$ 236.2870. Anal. ($C_{13}H_{18}NO_3Cl$) C, H, N.

Enzymatic Assays and Inhibition Analysis Based on the Amidase Activity. Procedures and conditions for the enzymatic assays, kinetic analyses, and inhibition studies are essentially the same as previously reported and indicated[10] in Table I and Figure 1.

Inhibition of the Epoxide Hydrolase Activity of LTA_4 Hydrolase. Aliquots of enzyme (2.5 μg) were preincubated for 30–40 min at room temperature in 100 μL of 50 mM HEPES, pH 8, containing various amounts of the respective inhibitor. Each compound was added to the buffer in DMF (final concentration < 0.25%) and in the case of compound **27**, 1 mM DTT was also included in the incubation buffer. Samples were further incubated with 20–25 μM LTA_4 for 15 s at room-temperature and the reactions were terminated by the addition of 2 vols of MeOH. After addition of 200 ng of PGB_1 (internal standard), samples were acidified to pH 3 with 0.1 M HCl, extracted on Chromabond C18 columns (Mackerey & Nagel), and finally analyzed on a computerized Waters HPLC system. The column (Radial-Pak C_{18} cartridge, 100 × 5 mm, Waters) was eluted with a mixture of methanol/water/acetic acid (70:30:0.01, v/v) at a flow rate of 1.2 mL/min. The UV absorbance of the eluate was monitored continuously at 270 nm. Amounts of LTB_4 were computer calculated from peak area ratios between LTB_4 and the internal standard PGB_1. Peak area ratios were converted into molar ratios from comparisons with standard curve constructed from injections of known amounts of the respective compound.

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References

(1) For review, see Samuelsson, B.; Funk, C. D. Enzymes Involved in the Biosynthesis of Leukotriene B_4. *J. Biol. Chem.* **1989**, *264*, 19469–19472.

(2) Haeggström, J. Z.; Wetterholm, A.; Shapiro, R.; Vallee, B. L.; Samuelsson, B. Leukotriene A_4 Hydrolase: A Zinc Metalloenzymes. *Biochem. Biophys. Res. Commun.* **1990**, *172*, 965–970.

(3) Haeggström, J. Z.; Wetterholm, A.; Vallee, B. L.; Samuelsson, B. Leukotriene A_4 Hydrolase: An Epoxide Hydrolase with Peptide Activity. *Biochem. Biophys. Res. Commun.* **1990**, *173*, 431–437.

(4) Minami, M.; Ohishi, N.; Mutoh, H.; Izumi, T.; Bito, H.; Wada, H.; Seyama, Y.; Toh, H.; Shimizu, T. Leukotriene A_4 Hydrolase is a Zinc-Containing Aminopeptidase. *Biochem. Biophys. Res. Commun.* **1990**, *173*, 620–626.

(5) Funk, C. D.; Radmark, O.; Fu, J. Y.; Matsumoto, T.; Jornvall, H.; Shimizu, T.; Samuelsson, B. Molecular Cloning and Amino Acid Sequence of Leukotriene A_4 Hydrolase. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 6671–6681.

(6) (a) Wetterholm, A.; Medina, J. F.; Radmark, O.; Shapiro, R.; Haeggströpom, J. Z.; Vallee, B. L.; Samuelsson, B. Leukotriene A_4 hydrolase: Abrogation of the peptidase activity by mutation of glutamic acid-296. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9141–9145. (b) Oring, L.; Fitzpatrick, F. A. Albumins Activate Peptide Hydrolysis by the Bifunctional Enzyme LTA_4/Aminopeptidase. *Biochemistry* **1992**, *31*, 4218–4223.

(7) (a) McIntyre, T.; Zimmerman, G.; Prescott, S. Leukotrienes C_4 and D_4 Stimulate Human Endothelial Cells to Synthesize Platelet-Activating Factor and Bind Neutrophils. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 2204–2208. (b) Ford-Hutchinson, A. W.; Bray, M. Leukotriene B, A Potent Chemokinetic and Aggregating Substance Released from Polymorphonuclear Leukocytes. *Nature* **1980**, *286*, 264–265.

(8) (a) McGee, J.; Fitzpatrick, F. A. Enzymatic Hydration of Leukotriene A_4: Purification and Characterization of A Novel Epoxide Hydrolase from Human Erythrocytes. *J. Biol. Chem.* **1985**, *260*, 12832–12837. (b) Evans, J. F.; Nathaniel, D. J.; Zamboni, R. J.; Ford-Hutchinson, A. W. Leukotriene A_3 a Poor Substrate but a Potent Inhibitor of Rat and Human Neutrophil Leukotriene A_4 Hydrolase. *J. Biol. Chem.* **1985**, *269*, 10966–10970. (c) Nathaniel, D. J.; Evans, J. F.; Leblanc, Y.; Léveillé, C.; Fitzsimmons, B. J.; Ford-Hutchinson, A. W. Leukotriene A_5 is a Substrate and An Inhibitor of Rat and Human Neutrophil LTA_4 Hydrolase. *Biochem. Biophys. Res. Commun.* **1985**, *131*, 827–835. (d) Ohishi, N.; Izumi, T.; Minami, M.; Kitamura, S.; Seyama, Y.; Ohkawa, S.; Terao, S.; Yotsumoto, H.; Takaku, F.; Shimizu, T. Leukotriene A_4 Hydrolase in the Human Lung, Inactivation of the Enzyme with Leukotriene A_4 Isomer. *J. Biol. Chem.* **1987**, *262*, 10200–10205.

(9) (a) Labaudiniére, G. H.; Hilboll, G.; Leon-Lomeli, A.; Lautenschläger, H.-H.; Parnham, M.; Kuhl, P.; Dereu, N. ω-[(ω-Arylalkyl)-thienyl]alkanoic Acids: A New Class of Specific LTA_4 Hydrolase Inhibitors. *J. Med. Chem.* **1992**, *35*, 3156–3169. (b) Orning, L.; Krivi, G.; Fitzpatrick, F. A. Leukotriene A_4 Hydrolase. Inhibition of Bestatin and Intrinsic Amino Peptidase Activity Establish its Functional Resemblance to Metallohydrolase Enzymes. *J. Biol. Chem.* **1991**, *266*, 1375–1378.

(10) (a) For a preliminary study, see Yuan, W.; Zhong, Z.; Wong, C.-H.; Haeggström, J. Z.; Wetterholm, A.; Samuelsson, B. Probing the Inhibition of Leukotriene A_4 Hydrolase Based on its Aminopeptidase Activity. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 551–556. (b) Yuan, W.; Wong, C.-H.; Haeggström, J. Z.; Wetterholm, A.; Samuelsson, B. Novel Tight-Binding Inhibitors of Leukotriene A_4 Hydrolase. *J. Am. Chem. Soc.* **1992**, *114*, 6552–6553. The K_i of **26** based on a Dixon plot is 0.1 μM. **25** is not very stable at room temperature.

(11) Burley, S. K.; David, P. R.; Lipscomb, W. N. Leucine Aminopeptidase: Bestatin Inhibition and A Model for Enzyme-Catalyzed Peptide Hydrolysis. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6916–6920. For other transition-state structure analyses of Zn^{++} proteases, see Bartlett, P. A.; Marlowe, C. K. Evaluation of Intrinsic Binding Energy from A Hydrogen Bonding Group in an Enzyme Inhibitor. *Science* **1987**, *235*, 569–571. Tronrud, D. E.; Holden, H. M.; Matthews, B. W. Structures of Two Thermolysin-Inhibitor. Complexes that Differ by a Single Hydrogen Bond. *Science* **1987**, *235*, 571–574. Christianson, D. W.; Lipscomb, W. N. Carboxypeptidase A. *Acc. Chem. Res.* **1989**, *22*, 62–69. Breslow, R.; Chin, J.; Hilvert, D.; Trainor, G. Evidence for the General Base Mechanism in Carboxypeptidase A-Catalyzed Reactions: Partitioning Studies on Nucleophiles and H_2O^{18} Kinetic Isotope Effects. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 4585–4589. Izquierdo-Martin, M.; Stein, R. L. Mechanistic Studies on the Inhibition of Thermolysin by a Peptide Hydroxamic Acid. *J. Am. Chem. Soc.* **1992**, *114*, 325–331. Nishizawa, R.; Saino, T.; Takita, T.; Suda, H.; Aoyagi, T.; Umezawa, H. Synthesis and Structure-Activity Relationships of Bestatin Analogues, Inhibitors of Aminopeptidase B. *J. Med. Chem.* **1977**, *20*, 510–515.

(12) Ocain, T. D.; Rich, D. H. Synthesis of Sulfur-Containing Analogues of Bestatin. Inhibition of Aminopeptidases by α-Thiolbestatin Analogues. *J. Med. Chem.* **1988**, *31*, 2193–2199.

(13) Giannousis, P. P.; Bartlett, P. A. Phosphorus Amino Acid Analogues as Inhibitors of Leucine Aminopeptidase. *J. Med. Chem.* **1987**, *30*, 1603–1609.

(14) For human native LTA_4 hydrolase, the values of K_m and V_{max} ranging between 7–30 μM and 1.7–3.0 μmol/mg per min, respectively, have been reported using LTA_4 as a substrate: Radmark, O.; Haeggström, J. Properties of Leukotriene A_4 Hydrolase. *Adv. Prostaglandin, Thromboxane Leukotriene Res.* **1990**, *20*, 35–42.

(15) α-Keto amide inhibitors of aminopeptidase were reported by Ocain, T. D.; Rich, D. H. *J. Med. Chem.* **1992**, *35*, 451–456. For other types of keto ester inhibitors, see Hori, H.; Yasutake, A.; Minematsu, Y.; Powers, J. C. Inhibition of Human Leukocyte Elastase, Porcine Pancreatic Elastase and Cathepsin G by Peptide Ketones. In *Peptides: Structure and Function*, Proceedings of the 9th American Peptide Symposium; Deber, C. M., Hruby, V. J., Kopple, K. E., Eds.; Pierce Chemical Co.: Rockford, IL, 1985, pp 819–822. Angelastroc, M. R.; Mehdi, S.; Burkhart, J. P.; Peet, N. P.; Bey, P. α-Diketones and α-Ketone Ester Derivatives of N-Protected Amino Acids and Peptides as Novel Inhibitors of Cysteine and Serine Proteinases. *J. Med. Chem.* **1990**, *33*, 11–13. Hu, L. Y.; Abeles, R. H. Inhibition of Cathepsin B and Papain by Peptidyl α-Keto Esters, α-Keto Amides, α-Diketones, and α-Keto Acids. *Arch. Biochem. Biophys.* **1990**, *281*, 271–274.

(16) For compounds **25** and **26**, the β-protons in their ^1H-NMR's show up at about 3.8 ppm (hydrated form) in D_2O and about 5.0 ppm (ketone form) in DMSO; the α-carbons in their ^{13}C-NMR's show up at about 92 ppm (hydrated form) in 10% D_2O–DMSO and at about 188 ppm (ketone form) in DMSO.

(17) Aminopeptidase assays were carried out according to ref 12.

(18) Iizuka, K.; Kamijo, T.; Harada, H.; Akahane, K.; Kubota, T.; Umeyama, H.; Ishida, T.; Kiso, Y. Orally Potent Human Renin Inhibitors Derived from Angiotensinogen Transition State: Design, Synthesis, and Mode of Interaction. *J. Med. Chem.* **1990**, *33*, 2707–2714.

(19) (a) Mancuso, A. J.; Huang, S.; Swern, D. Oxidation of Long-Chain and Related Alcohols to Carbonyls by Dimethyl Sulfoxide "Activated" by Oxalyl Chloride. *J. Org. Chem.* **1978**, *43*, 2480–2482. (b) Dess, D. B.; Martin, J. C. Readily Accessible 12-T-5 Oxidant for the Conversion of Primary and Secondary Alcohols to Aldehydes and Ketones. *J. Org. Chem.* **1983**, *48*, 4155–4156. (c) Burkhart, J. P.; Peet, N. P.; Bey, P. Oxidation of α-Hydroxy Esters to α-Keto Esters Using the Dess–Martin Periodinane Reagent. *Tetrahedron Lett.* **1988**, *29*, 3433–3436.

(20) (a) Nahm, S.; Weinreb, S. M. N-Methoxy-N-methyl Amides as Effective Acylating Agents. *Tetrahedron Lett.* **1981**, *22*, 3815–3818. (b) Angelastro, M. R.; Peet, N. P.; Bey, P. An Efficient Synthesis of Novel α-Diketone and α-Keto Ester Derivatives of N-Protected Amino Acids and Peptides. *J. Org. Chem.* **1989**, *54*, 3913–3916.

(21) These compounds were reported before: Nishizawa, R.; Saino, T.; Takita, T.; Suda, H.; Aoyagi, T.; Umezawa, H. Synthesis and Structure-Activity Relationships of Bestain Analogues, Inhibitors of Aminopeptidase B. *J. Med. Chem.* **1977**, *20*, 510–515.

(22) Thaisrivongs, S.; Pals, D. T.; Kati, W. M.; Turner, S. R.; Thomasco, L. M.; Watt, W. Design and Synthesis of Potent and Specific Renin Inhibitors Containing Difluorostatine, Difluorostatone, and Related Analogues. *J. Med. Chem.* **1986**, *29*, 2080–2087.

(23) Pfitzner, K. E.; Moffat, J. G. Sulfoxide-Carbodiimide Reactions. I. A Facile Oxidation of Alcohols. *J. Am. Chem. Soc.* **1965**, *87*, 5661–5670.

(24) Fournié-Zaluski, M. C.; Coric, P.; Turcand, S.; Bruetschy, L.; Lucas, E.; Noble, F.; Roques, B. P. Potent and Systematically Active Aminopeptidase N Inhibitors Designed from Active-Site Investigation. *J. Med. Chem.* **1992**, *35*, 1259–1266.

(25) Corey, E. J.; Schmidt, G. Useful Procedures for the Oxidation of Alcohols Involving Pyridium Dichromate in Aprotic Media. *Tetrahedron Lett.* **1979**, 399–402.

(26) (a) Fehrentz, J.-A.; Castro, B. An Efficient Synthesis of Optically Active α-t-Butoxycarbonyl Aminol-Aldehydes from α-Amino Acids. *Synthesis* **1983**, 676–678. (b) The amino acid derivative *N*-Boc-L-phenylalanine-*N*-methoxy-*N*-methylamide (**39**) was prepared according to the procedure cited in ref 26a with the modification that EDC was substituted for BOP. Spectral data was identical to that reported in ref 26a.

(27) Kano, S.; Yokomatsu, T.; Iwasawa, H.; Shibuya, S. A New Facile Diastereoconversion of 2-Amino Alcohols Involving a Novel Cyclocarbonation. *Tetrahedron Lett.* **1987**, *28*, 6331–6334.

(28) Rich, D. H.; Sun, T. O. S. Synthesis of Analogues of Carboxyl Protease Inhibitor Pepstatin. Effect of Structure on Inhibition of Pepsin and Renin. *J. Med. Chem.* **1980**, *23*, 27–33.

(29) Futagawa, S.; Inui, T.; Shiba, T. Nuclear Magnetic Resonance Study of the Stereoisomeric 2-Oxazolidone and 2-Phenyl-2-oxazoline Derivatives of α-Amino-β-hydroxy Acids. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 3305–3310.