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Amino Hydroxamic Acids as Potent Inhibitors of Leukotriene A_4 Hydrolase

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Abstract—Leukotriene A_4 hydrolase is a zinc-containing enzyme which catalyzes the hydrolysis of LTA_4 to LTB_4, a proinflammatory mediator. The enzyme also exhibits an aminopeptidase activity. Due to its biological importance, it is of considerable interest to develop selective inhibitors of this enzyme. The design and synthesis of a number of potent β-amino hydroxylamine and amino hydroxamic acid inhibitors are described here. It was found that having a free amine was essential for high activity. Hydroxylamines were found to be about an order of magnitude less potent than their analogous hydroxamic acids. Our investigation of amino hydroxamic acids as inhibitors of leukotriene A_4 hydrolase has led to the development of hydroxamates **16** and **17**, which are among the most potent inhibitors found to date. These, compounds were found to be competitive inhibitors with K_i values of 1.6 nM and 3.4 nM respectively, against the peptidase activity. Inhibitor **16** has an IC_{50} value of ≤ 0.15 μM against the epoxide hydrolase activity and is also potent against the production of LTB_4 by isolated polymorphonuclear leukocytes (PMNL) activated with ionophore A23187 (IC_{50} ≈ 0.3 μM).

Introduction

Leukotriene (LT) A_4 hydrolase (EC 3.3.2.6) is an important zinc-containing enzyme in the arachidonic acid metabolic pathway, that catalyzes the hydrolysis of LTA_4 (5*S*-5,6-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid) into LTB_4 (5*S*,12*R*-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid).[1] LTB_4 is a potent chemotactic factor, thought to be a strong proinflammatory mediator, stimulating the adhesion of circulating neutrophils to vascular endothelium and directing their migration towards inflammation sites.[1-3] The enzyme also exhibits an intrinsic aminopeptidase activity which occurs at common or overlapping sites.[4-8]

Although the mechanisms of the enzyme's activities have not been elucidated, we speculate that it may occur as shown in Figure 1. The zinc(II) ion coordinates to the nucleophilic water molecule, thus activating it to general base catalysis and, in the peptidase activity, it may also act concurrently on the carbonyl as a Lewis acid.[9] In the epoxide hydrolase activity, the zinc ion may alternatively act as a Lewis acid on the epoxide, activating its opening.

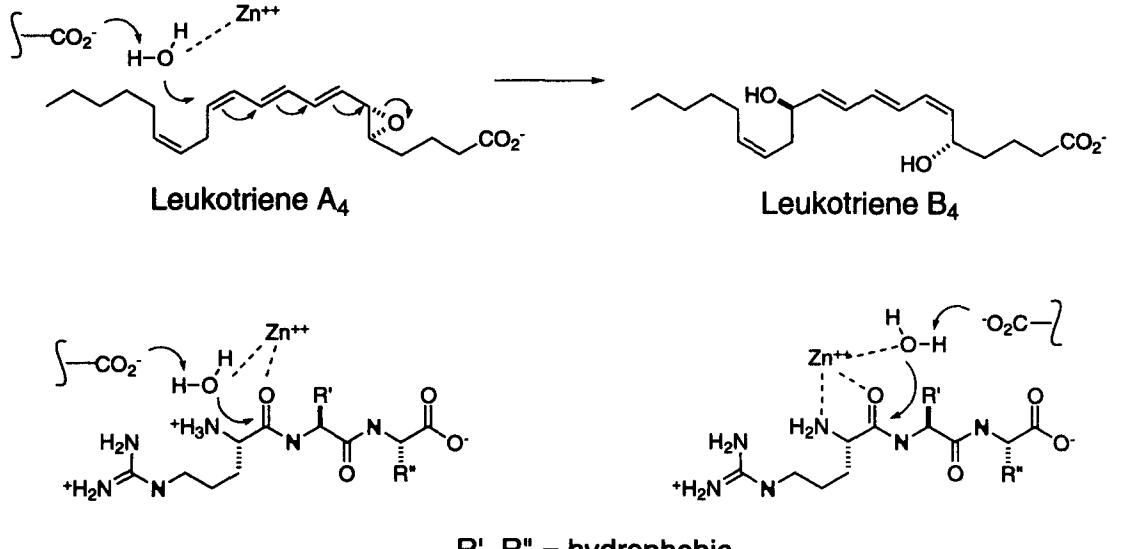


Figure 1. Proposed mechanisms for LTA_4 hydrolase-catalyzed hydrolysis of LTA_4 and RXX.

†Scholarship from NSERC Canada.

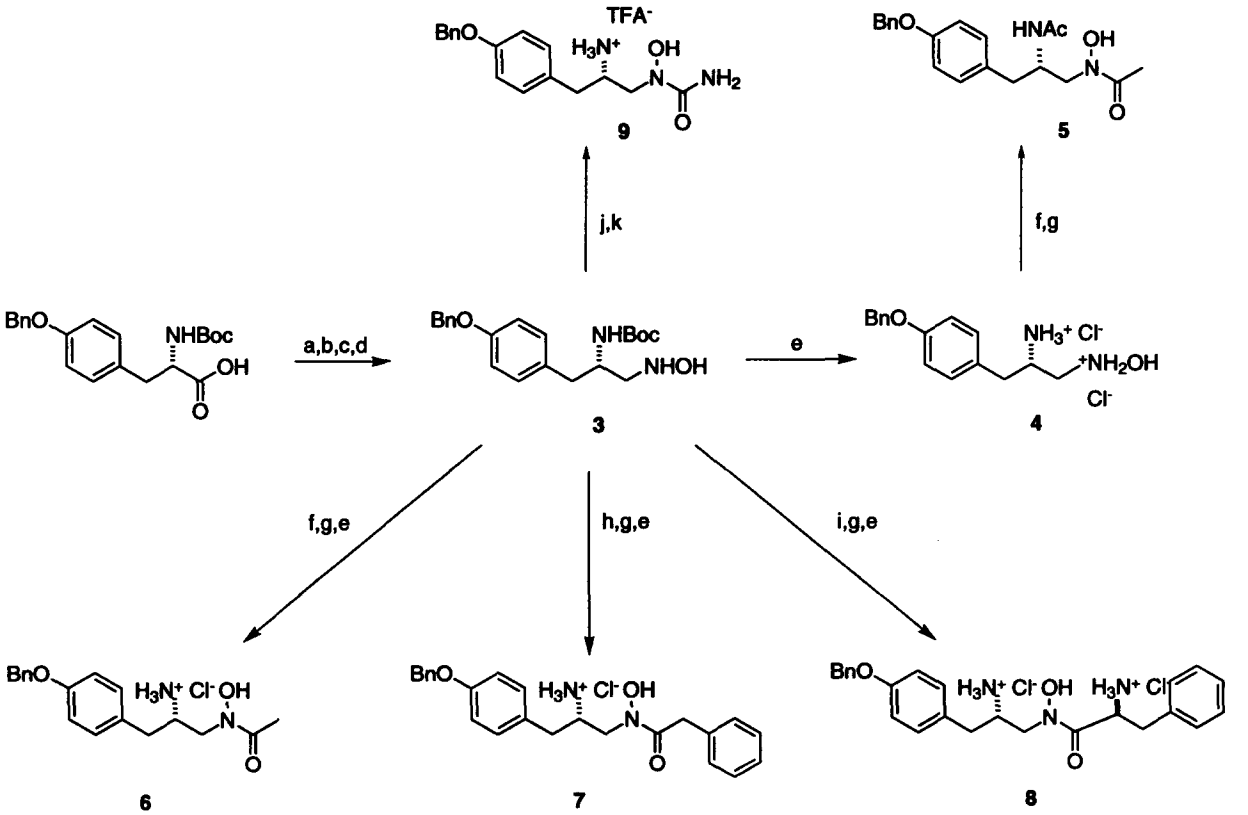
Since LTB_4 has an important biological role in the inflammatory response, it is of great interest to develop selective inhibitors of LTA_4 hydrolase. Inhibitors of this enzyme reported to date include LTA_4 and its methyl ester,[10,11] LTA_3,[12] LTA_5,[13] bestatin, captopril[5,14] and a series of ω-[(ω-arylalkyl)thienyl] alkanoic acids.[15,16] An extensive and systematic study of a variety of synthetic inhibitors by our group[17–20] has led to the development of two of the most potent inhibitors to date, a mercaptoamine (K_i = 0.35 nM against the peptidase activity)[20] and an α-keto-β-amino ester (K_i = 46 nM).[19] Attempts to use the mercaptoamine core to explore additional binding pockets were unsuccessful because any modification of the zinc-binding free-thiol moiety resulted in at least a 10,000-fold reduction in potency. Here we report the development of a new series of potent LTA_4 hydrolase inhibitors which incorporate a hydroxamic acid as a zinc-chelating moiety. This type of core structure allows us to easily incorporate a variety of additional complimentarity groups which enable us to further characterize the active site of LTA_4 hydrolase.

Discussion

O-Benzyl-tyrosine hydroxamic acid (*O*-Bn-TyrNHOH, **1**) has no activity against the peptidase activity at concentrations up to 1 mM, which is rather interesting given the recent report that both TyrNHOH and PheNHOH are good inhibitors, with K_i values of 50 μM and 32 μM, respectively.[8]

A second generation of hydroxamates (Scheme 1) was designed to incorporate a methylene spacer between the amine and the hydroxamate moiety, making these inhibitors more flexible and thus perhaps allowing for a better fit in the active site. The homotyrosine derived hydroxamic acid **2**, is a good inhibitor of LTA_4 hydrolase (Table 1). Furthermore, **6**, an 'inverted' hydroxamic acid[21] of the parent *O*-Bn-tyrosine template binds even more tightly. Replacement of the hydroxamate moiety with the hydroxy urea **9** gives no improvement in binding, though this type of modification improved binding to other metalloenzyme inhibitors.[21a] Comparison of the IC_{50} values of **6**, its corresponding hydroxylamine **4**, and the N-acetylated **5** reveals that both the free amine and a good zinc-binding group are important features for inhibition. The greater potency of these inhibitors as compared to **1** may be attributed to their increased flexibility, combined with a more optimal distance between the amine and the zinc-binding moiety.[22]

The addition of hydrophobic moieties in the vicinity of the hydroxamic acid gave no significant increase in binding in contrast to our experience with α-keto-β-aminoesters.[19] Hydroxamate **7**, incorporating an additional phenyl group, is only slightly better than parent compound **6**. Compound **8**, which combined structural



Scheme 1. General synthesis of inhibitors

(a) BH_3·THF; (b) $(COCl)_2$, DMSO, TEA; (c) NH_2OH·HCl, TEA, MeOH; (d) $NaCNBH_3$, MeOH, pH 3; (e) HCl/ether; (f) acetyl chloride, TEA; (g) NaOMe, MeOH; (h) phenylacetyl chloride, TEA; (i) *N*-Boc-Phe, EDC; (j) TMS-NCO; (k) TFA/CH_2Cl_2.

Table 1. Inhibitors of LTA_4 hydrolase

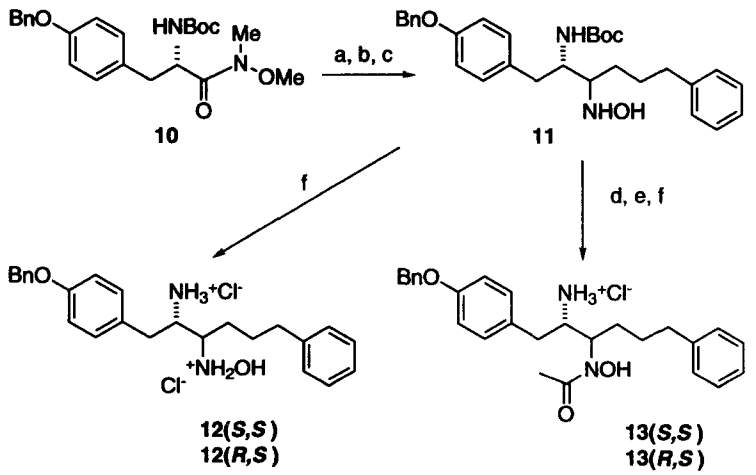
Compound	IC_{50} (μM) (K_i)[a]	Compound	IC_{50} (μM) (K_i)[a]
1[c]	N.I.[b]	12(*S,S*)[d]	2.2
2[c]	4.6	12(*R,S*)[d]	15
4[d]	4.0	13(*S,S*)[d]	N.I.[b]
5	42	13(*R,S*)[d]	4.1
6[d]	0.53 (220 nM)	16[c]	0.011 (1.6 nM)
7[d]	0.38	17[c]	0.011 (3.4 nM)
8[d]	4.0	18[d]	0.055 (28 nM)
9[c]	2.0	19[d]	0.025 (11 nM)

[a]All assays were preformed in Tris–HCl buffer (50 mN, pH 8.0) with L-alanyl-*p*-nitroanilide (1.87 mM) as substrate. LTA_4 hydrolase (1.4 μg) purified from human leukocytes was added for each assay (final volume = 1.0 mL, [E] = 20 nM). The rate of formation of *p*-nitroaniline was spectrophotometrically monitored at 405 nM. K_i values were determined using nonlinear regression methods and the inhibitors shown to be competitive.[20]
[b]No inhibition seen at 0.1 mM.
[c]TFA salt.
[d]HCl salt.

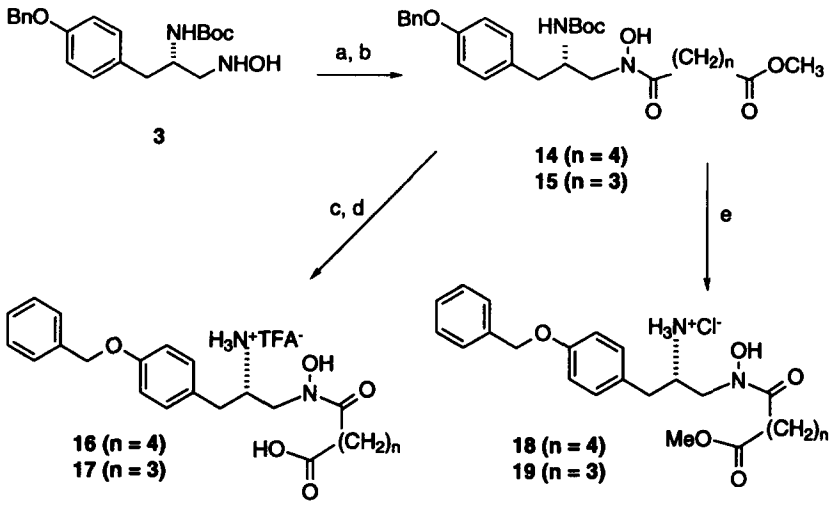
elements of **6** and PheNHOH, is 10 times less active than **6**, but 10 times more active than PheNHOH. Compound **13**(*R,S*) (Scheme 2) is a potent inhibitor of the peptidase activity and its corresponding hydroxylamine **12**(*R,S*) is four times less active, a result consistent with the trend seen for **6** and **4**. Interestingly, while hydroxylamine **12**(*S,S*) is even more potent than its (*R,S*) counterpart, its corresponding hydroxamate **13**(*S,S*) shows no inhibition activity at concentrations up to 0.1 mM. A possible cause for the lack of activity of **13**(*S,S*) may be an unfavorable steric interaction between the methyl group of the acetate and the α-methylene of the 3-phenylpropyl group, giving some insight into the binding conformation of this type of inhibitor.

As it has been shown that LTA_4 hydrolase/aminopeptidase favors tripeptides as substrates, a model which places these inverted hydroxamic acid inhibitors in S1 predicts a carboxylic acid binding site on the far side of S2'. A dramatic increase in binding was found with compound **16**, (K_i = 1.6 nM) which contains an extra carboxylate attached to the inhibitor by an alkyl linker of the appropriate length (Scheme 3). Compound **17**, with a slightly shorter linker binds more weakly, (K_i = 3.4 nM). Compounds **18** and **19**, the methyl esters of **16** and **17**, both show decreased binding, demonstrating that this binding pocket prefers the free carboxylate. Whether this binding pocket is also responsible for the strong preference for LTA_4 over LTA_4 methyl ester displayed by LTA_4 hydrolase[23] is still unclear.

Hydroxamate **16** is also very active against the epoxide hydrolase activity, with an IC_{50} of 0.15 μM. It should be noted here that the enzyme concentration in this assay was 0.36 μM (in order to facilitate the kinetic analysis as LTA_4 is very unstable with a half-life of 15 s under assay conditions); therefore, our value of 0.15 μM for the IC_{50} is an upper limit, rather than a close approximation of the K_i. Compound **16** is a potent inhibitor of LTB_4 synthesis in polymorphonuclear leukocytes stimulated with the ionophore A23187 (IC_{50} ≈ 0.3 μM). While hydroxamic acids have been used extensively in the development of inhibitors for 5-lipoxygenase, the iron-containing enzyme which catalyzes the synthesis of LTA_4 from arachidonic acid,[21,24] **16** is selective for LTA_4 hydrolase and does not inhibit 5-lipoxygenase.



Scheme 2. General synthesis of inhibitors **12** and **13**.
(a) 3-phenylmagnesium bromide, ether; (b) NH_2OH·HCl, TEA, MeOH; (c) i) $NaCNBH_3$, MeOH, pH 3; ii) separation of diastereomers by recrystallization (THF/hexane); (d) acetyl chloride, TEA; (e) NaOMe, MeOH; (f) HCl, ether.



Scheme 3. General synthesis of inhibitors **16–19**.
(a) $CH_3O_2C(CH_2)_nCOCl$, pyridine; (b) NaOMe, MeOH; (c) 0.8 M LiOH, MeOH:H_2O (2:1); (d) TFA, CH_2Cl_2· (e) HCl, ether.

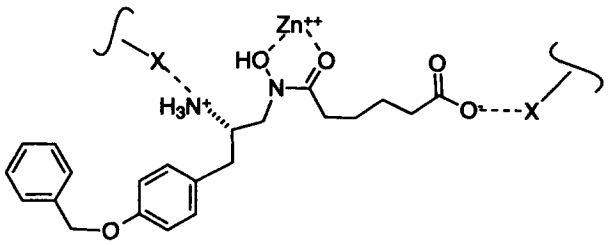


Figure 2. Proposed binding mode of inhibitor **16**.

Conclusion

The class of inverted hydroxamic acids are potent and selective inhibitors of LTA_4 hydrolase. The presence of a free amine proximal to the hydroxamic acid appears necessary for potency. Whether the amine makes a specific interaction with Glu-296, the zinc ion, or the putative amino-terminal recognition site is still not clear. The ability to add additional complimentarity groups to the metal binding ligand lead to the discovery of a nearby carboxylate binding region within the active site which might be responsible for the enzyme's demonstrated preference for tripeptides and for its selection of LTA_4 over LTA_4 methyl ester. This information coupled with further structural studies on LTA_4 hydrolase will hopefully yield better inhibitors of LTB_4 biosynthesis.

Experimental

General

The reagents used were commercially available and used without further purification. ^1H NMR data chemical shifts are reported in ppm relative to tetramethylsilane. ^{13}C NMR were obtained at 125 MHz. Thin-layer chromatography was performed on silica gel plates (0.25 mm, Merck) and flash chromatography was performed using silica gel (230–400 mesh, Merck). The known reaction of hydroxamic acids with ferric chloride to give a red color was used systematically to further confirm the presence of the hydroxamate moiety. All yields are unoptimized.

O-Benzyl-L-tyrosine hydroxamic acid (1).[27] Hydroxylamine hydrochloride (360 mg, 5.2 mmol) was dissolved in boiling methanol and then cooled to 30–40 °C. A solution of KOH (440 mg, 7.8 mmol) in warm methanol (1 mL, anhydrous) was added and the mixture allowed to stand in an ice bath for 10 min to ensure complete precipitation of the KCl. *N*-Boc-*O*-Bn-tyrosine methyl ester (1.0 g, 2.6 mmol) in 1 mL MeOH was added, and the mixture immediately filtered. The precipitate was washed with 1 mL MeOH and the combined filtrates allowed to stand for 48 h during which time *N*-Boc-*O*-benzyl-L-tyrosine hydroxamic acid (*N*-Boc-*O*-Bn-TyrNHOH) precipitated out as the potassium salt. The crystals were gathered, washed with EtOH, then dissolved in 1 N HCl. Extraction with EtOAc gave *N*-Boc-*O*-Bn-TyrNHOH as the free acid (530 mg, 53%). *N*-Boc-*O*-Bn-TyrNHOH (30 mg, 0.078 mmol) was treated with 20% TFA in CH_2Cl_2 to remove the Boc protecting group, giving **1** as a pale-yellow solid (25 mg, 80%). ^1H NMR (300 MHz, CD_3OD) δ 7.4–7.2 (*m*, 5H), 7.12 (*d*, J = 8.5 Hz, 2H), 6.95 (*d*, J = 8.5 Hz, 2H), 5.05 (*s*, 2H), 3.75 (*t*, J = 6.5 Hz, 1H), 2.99 (dAB, J = 7 Hz, J_{AB} = 14 Hz, Δυ = 33, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.3, 157.6, 137.1, 130.5, 128.5, 127.9, 127.7, 127.0, 114.8, 69.2, 51.9, 36.2.

HRMS (M + H)$^+$ calcd for $C_{16}H_{19}N_2O_3$: 287.1390; found: 287.1398.

O-Benzyl-L-homotyrosine hydroxamic acid (2). *N*-Boc-*O*-Bn-homotyrosine ethyl ester was prepared from *N*-Boc-*O*-Bn-tyrosine,[28] using flash chromatography (4:1 hexane:EtOAc, then 10:10:1 hexane:CH_2Cl_2:ether) to remove the trace amounts of *N*-Boc-*O*-Bn-tyrosine methyl ester. The potassium salt of the hydroxamic acid was then synthesized in a 71% yield as described for **1**. The potassium salt (200 mg, 0.44 mmol), was then suspended in 5 mL water and the mixture acidified to pH 2 with acetic acid. EtOAc (10 mL) was then added and the mixture stirred until all the solid had dissolved. The layers were separated and the organic layer dried with $MgSO_4$. The solvent was removed and the residue recrystallized (THF:hexane) to give *N*-Boc-*O*-Bn-HTyrNHOH as the free acid (150 mg, 82%). *N*-Boc-*O*-Bn-HTyrNHOH (15 mg, 0.036 mmol) was treated with 20% TFA in CH_2Cl_2 (1 mL) to remove the Boc protecting group, giving **2** as a pale-yellow oily solid (14 mg, 90%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.69 (*s*, 1H), 8.95 (*bs*, 1H), 7.97 (*bs*, 3H), 7.43 (*d*, *J* = 7.5 Hz, 2H), 7.38 (*t*, *J* = 7.5 Hz, 2H), 7.32 (*t*, *J* = 7.5 Hz, 1H), 7.13 (*d*, *J* = 8.5 Hz, 2H), 6.98 (*d*, *J* = 8.5 Hz, 2H), 5.07 (*s*, 2H), 3.59 (*m*, 1H), 2.85 (*dd*, *J* = 8 and 6 Hz, 1H), 2.67 (*dd*, *J* = 8 and 13.5 Hz, 1H), 2.21 (*m*, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.0, 158.8, 158.5, 158.2, 157.9, 157.4, 137.1, 130.5, 128.5, 127.9, 127.7, 115.0, 69.2, 49.3, 37.1, 33.1, 26.3. HRMS (M + H)$^+$ calcd for $C_{17}H_{21}N_2O_3$: 301.1552; found: 301.1564.

(2S)-2-N-Boc-amino-3-(4-benzyloxyphenyl)propanol. To a solution of *N*-Boc-*O*-Bn-tyrosine (12.36 g, 33.3 mmol) in 100 mL anhydrous THF (ice bath, under argon) was added BH_3·THF (1.0 M in THF, 60 mL, 60 mmol) dropwise over 2 h. The reaction mixture was allowed to warm to room temperature (rt) and stirred an additional hour at which time no starting material remained (TLC, 1:1 EtOAc:hexane). The reaction was quenched by pouring the solution into 600 mL 1 N HCl and this mixture was then extracted with EtOAc (4 × 200 mL) and the solvent removed to give a white slurry. An additional 300 mL EtOAc was then added and the mixture washed (3 × 150 mL 1 N HCl, 1 × 100 mL NaCl (satd), 3 × 100 mL $NaHCO_3$ (satd), 1 × 100 mL NaCl (satd)). Removal of the solvent *in vacuo* yielded 10.1 g (85%) of the alcohol as a white powdery solid. ^{1}H NMR matches that previously reported.[20]

(2S)-2-N-Boc-amino-3-(4-benzyloxyphenyl)propanal. To a solution of oxalyl chloride (2.0 M in CH_2Cl_2, 1.6 mL, 3.2 mmol) in 5 mL CH_2Cl_2 (–78 °C, under argon) was added DMSO (420 μL, 5.84 mmol). The solution was stirred for 15 min and a solution of *N*-Boc-*O*-Bzl-tyrosinol (1.0 g, 2.8 mmol) in 1 mL CH_2Cl_2 was added dropwise over 20 min. The cloudy mixture was stirred for an additional 45 min and then triethylamine (2.0 mL, 14.3 mmol) was added dropwise. After 10 min, the reaction mixture was allowed to warm to rt. Water (8 mL) and 20 mL EtOAc were added, the layers separated, and the organic layer washed (1 N HCl, $NaHCO_3$ (satd), NaCl (satd)). Drying ($MgSO_4$) and removal of the solvent *in vacuo* yielded 960 mg (96%) of a pale-yellow solid which was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.60 (*s*, 1H), 7.42 (*d*, *J* = 7.5 Hz, 2H), 7.38 (*t*, *J* = 7.5 Hz, 2H), 7.31 (*t*, *J* = 7.5 Hz, 1H), 7.06 (*d*, *J* = 8.5 Hz, 2H), 6.90 (*d*, *J* = 8.5 Hz, 2H), 5.04 (*d*, *J* = 7 Hz, 1H), 5.02 (*s*, 2H), 4.38 (*q*, *J* = 6.5 Hz, 1H), 3.04 (*d*, *J* = 6.5 Hz, 2H), 1.42 (*s*, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.6, 157.9, 155.4, 136.8, 130.4, 128.6, 128.0, 127.9, 127.4, 115.0, 80.2, 70.0, 60.8, 34.5, 28.2. HRMS (M + H)$^+$ calcd for $C_{21}H_{26}NO_4$: 356.1862; found: 356.1869.

(2S)-2-N'-Boc-amino-3-(4-benzyloxyphenyl)-N-hydroxypropylamine (3). *N*-Boc-*O*-Bn-tyrosinal (680 mg, 1.9 mmol) was dissolved in 5 mL THF. Methanol (40 mL) was then added followed by hydroxylamine hydrochloride (740 mg, 10.6 mmol) and the pH adjusted to 5 with triethylamine (~ 0.5 mL). The reaction was stirred for 30 min at which time no aldehyde remained (TLC, 1:1 EtOAc:hexane). The pH was then lowered to 3 (as indicated by methyl orange) with ethereal HCl and $NaCNBH_3$ (100 mg, 1.6 mmol) added.[29] The reaction was stirred for 1.5 h, with occasional additions of ethereal HCl to maintain this pH. The reaction mixture was then concentrated to approximately 10 mL, 30 mL water was added, and the pH adjusted to > 10 with 6 N NaOH. The resulting mixture was then extracted with EtOAc (3 × 20 mL) and the organic layers washed with NaCl (satd), dried ($MgSO_4$), and the solvent removed *in vacuo* to give a pale-yellow solid. Flash chromatography (1:1 EtOAc:hexane, then EtOAc) yielded 570 mg (81%) of **3** as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (*d*, *J* = 7.5 Hz, 2H), 7.36 (*t*, *J* = 7.5 Hz, 2H), 7.32 (*t*, *J* = 7.5 Hz, 1H), 7.11 (*d*, *J* = 8.5 Hz, 2H), 6.91 (*d*, *J* = 8.5 Hz, 2H), 5.04 (*s*, 2H), 4.62 (*d*, *J* = 8.5 Hz, 2H), 4.13 (*m*, 1H), 3.10 (*d*, *J* = 13 Hz, 1H), 2.77 (*d*, *J* = 6.5 Hz, 2H), 2.64 (*t*, *J* = 5 Hz, 1H), 1.43 (*s*, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.4, 156.9, 136.9, 130.2, 129.5, 128.5, 127.8, 127.3, 114.8, 79.6, 69.9, 56.9, 49.0, 37.8, 28.3. HRMS (M + H)$^+$ calcd for $C_{21}H_{29}N_2O_4$: 373.2127; found: 373.2119.

(2S)-2-Amino-3-(4-benzyloxyphenyl)-N-hydroxypropylamine, HCl salt (4). Hydroxylamine **3** (50 mg, 0.13 mmol) was treated with ethereal HCl for 4 h to give **4** (35 mg, 78%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.1 (*bs*, 1.5 H), 11.18 (*bs*, 0.5H), 8.60 (*bs*, 3H), 7.43 (*d*, *J* = 7 Hz, 2H), 7.37 (*t*, *J* = 7 Hz, 2H), 7.31 (*t*, *J* = 7 Hz, 1H), 7.22 (*d*, *J* = 8.5 Hz, 2H), 6.98 (*d*, *J* = 8.5 Hz, 2H), 5.06 (*s*, 2H), 3.8 (*bs*, 1H), 3.46 (*dd*, *J* = 8.5 and 14.5 Hz, 1H), 3.13 (*dd*, *J* = 3 and 14.5 Hz, 1H), 3.03 (*dd*, *J* = 6 and 14 Hz, 1H), 2.88 (*dd*, *J* = 8.5 and 14 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 157.6, 137.1, 130.6, 128.5, 127.9, 127.8, 127.4, 115.0, 69.2, 50.9, 47.6, 35.4. HRMS (M + H)$^+$ calcd for $C_{16}H_{21}N_2O_2$: 273.1603; found: 273.1611.

N-Hydroxy-N-[(2S)-2-amino-3-(4-benzyloxyphenyl)propyl]acetamide, HCl salt (6). To hydroxylamine **3** (100 mg, 0.27 mmol) in 5 mL THF was added triethylamine (140 μL, 1.0 mmol) followed by acetyl chloride (60 μL,

0.81 mmol). The reaction was exothermic and complete in 5 min. The reaction mixture was diluted with 15 mL hexane and filtered through Celite. Removal of the solvent gave the diacetate as a colorless oil. This oil was then taken up in 10 mL anhydrous methanol and the pH adjusted to 10 with NaOMe. The solution was stirred at rt for 10 min, at which time all the diacetate was converted to the hydroxamic acid, as seen by TLC (1:1 EtOAc:hexane, R_f (diacetate) = 0.5, R_f (acid) = 0.45). The solution was neutralized with Dowex 50 H^+ resin, the solvent removed, the residue taken up in THF, dried ($MgSO_4$), and recrystallized (THF:hexane) to give 85 mg (76%) of *N*-Boc protected **6**.

Removal of the Boc protecting group with HCl–ether gave 75 mg (89%, 68% from the hydroxylamine) of **6** as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 7.45–7.2 (*m*, 5H), 7.21 (*d*, *J* = 10 Hz, 2H), 6.98 (*d*, *J* = 10 Hz, 2H), 5.07 (*s*, 2H), 3.95 (*m*, 1H), 3.7 (*m*, 2H) 2.91 (*d*, *J* = 8.5 Hz, 2H), 2.14 (*s*, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ 171.0, 159.7, 139.5, 135.0, 131.5, 129.5, 128.9, 128.5, 116.5, 70.9, 53.0, 51.0, 37.4, 20.3. HRMS (M + H)$^+$ calcd for $C_{18}H_{23}N_2O_3$: 315.1709; found: 315.1718.

N-*Hydroxy*-N-*[(2S)-2-amino-3-(4-benzyloxyphenyl)propyl]phenylacetamide, HCl salt (7).* To hydroxylamine **3** (100 mg, 0.27 mmol) in 5 mL THF was added triethylamine (140 μL, 1.0 mmol) followed by phenylacetyl chloride (80 μL, 0.54 mmol). The reaction was exothermic and complete in 5 min. Workup as described for **6** gave a mixture of the diphenylacetate and hydroxamic acid as a colorless oil (TLC, 1:1 EtOAc:hexane, R_f (diacetate) = 0.6, R_f (acid) = 0.55). Treatment with NaOMe/MeOH (30 min) as described for **6** and recrystallization (THF:hexane) gave 94 mg (71%) of the *N*-Boc protected **7**. Removal of the Boc protecting group with HCl–ether gave 71 mg (87%, 62% from hydroxylamine) of **7** as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.41 (*s*, 1H), 8.19 (*m*, 3H), 7.42 (*d*, *J* = 7 Hz, 2H), 7.38 (*t*, *J* = 7 Hz, 2H), 7.31 (*t*, *J* = 7 Hz, 1H), 7.26 (*t*, *J* = 7 Hz, 2H), 7.20 (*m*, 5H), 6.96 (*d*, *J* = 8.0 Hz, 2H), 5.06 (*s*, 2H), 3.94 (*m*, 1H), 3.86 (AB, J_{AB} = 15.5 Hz, Δυ = 25, 2H), 3.57 (*bs*, 1H), 3.51 (*dd*, *J* = 3.3 and 14 Hz, 1H), 3.37 (*m*, 1H), 2.92 (*dd*, *J* = 5 and 14.5 Hz, 1H), 2.76 (*dd*, *J* = 9.5 and 14 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.3, 157.3, 137.1, 135.6, 130.5,129.7, 128.5, 128.1, 127.8, 127.7, 126.3, 114.9, 69.1, 50.1, 48.7, 38.5, 35.0. HRMS (M + H)$^+$ calcd for $C_{24}H_{27}N_2O_3$: 391.2022; found: 391.2037.

N-*Hydroxy*-N-*[(2S)-2-amino-3-(4-benzyloxyphenyl)propyl]-2-amino-3-phenylpropionamide, HCl salt (8).* To a solution of *N*-Boc-phenylalanine (79 mg, 0.30 mmol), EDC (62 mg, 0.32 mmol), and triethylamine (37 μL, 0.27 mmol) in 5 mL CH_2Cl_2 was added hydroxylamine **3** (100 mg, 0.27 mmol) and the reaction allowed to stir at rt for 2 h. EtOAc (15 mL) was then added, the mixture washed (1 N HCl, $NaHCO_3$ (satd), NaCl (satd)), and the solvent removed to yield a glassy solid. Treatment with NaOMe/MeOH (1 h) as described for **6** and removal of the Boc protecting groups with ethereal HCl yielded 15 mg (11%) of **8** as a pale-yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.2 (*s*, 1H), 8.30 (*m*, 3H), 7.45 (*d*, *J* = 7 Hz, 2H), 7.39 (*t*, *J* = 7 Hz, 2H), 7.34–7.29 (*m*, 6H), 7.25 (*d*, *J* = 8.5 Hz, 2H), 6.98 (*d*, *J* = 8.5 Hz, 2H), 5.08 (*s*, 2H), 4.49 (*m*, 1H), 3.75 (*d*, *J* = 8.0 Hz, 1H), 3.64 (*m*, 1H), 3.40 (*dd*, *J* = 5 and 15 Hz, 1H), 3.01 (*dd*, *J* = 10 and 15 Hz, 1H), 2.89 (*m*, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.6, 157.4, 137.1, 135.3, 130.6, 129.6, 128.6, 128.5, 128.0, 127.9, 127.8, 127.0, 114.9, 69.2, 51.6, 50.3, 49.3, 34.8. HRMS (M + H)$^+$ calcd for $C_{25}H_{30}N_3O_3$: 420.2287; found: 420.2299.

N-*Hydroxy*-N-*[(2S)-2-amino-3-(4-benzyloxyphenyl)propyl]urea, TFA salt (9).* Hydroxylamine **3** (200 mg, 0.54 mmol) was added to a solution of trimethylsilyl isocyanate (103 mg, 0.89 mmol) in 5 mL of anhydrous dioxane under argon.[30] After refluxing the solution for 30 min, it was cooled in an ice bath until solid started forming and then 10 mL NH_4Cl (satd) was added and the mixture was stirred at rt for 5 h. Water (50 mL) was added and the mixture extracted with EtOAc. The organic layer was dried ($MgSO_4$) and the solvent removed to give the Boc-protected **9** as a white solid (190 mg, 85%). Boc-protected **9** (51 mg) was then treated with 4 mL TFA:CH_2Cl_2:H_2O (20:80:1), stirred for 30 min, and the solvent removed *in vacuo*. The residue was triturated with ether to give 40 mg (73%) of **9** as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.68 (*s*, 1H), 7.89 (*bs*, 3H), 7.43 (*d*, *J* = 7 Hz, 2H), 7.38 (*t*, *J* = 7 Hz, 2H), 7.32 (*t*, *J* = 7 Hz, 1H), 7.18 (*d*, *J* = 8.0 Hz, 2H), 6.97 (*d*, *J* = 8.0 Hz, 2H), 6.58 (*s*, 2H), 5.07 (*s*, 2H), 3.60 (*dd*, *J* = 8.5 Hz and 14.5 Hz, 1H), 3.44 (*m*, 1H), 3.31 (*dd*, *J* = 4.5 and 14.5 Hz, 1H), 2.81 (*m*, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.9, 157.4, 130.5, 128.5, 128.2, 127.9, 127.7, 115.0, 69.2, 51.5, 50.9, 35.1. HRMS (M + H)$^+$ calcd for $C_{17}H_{22}N_3O_3$: 316.1662; found: 316.1665.

N-*Hydroxy*-N-*[(2S)*-N′-*acetyl-2-amino-3-(4-benzyloxyphenyl)propyl] acetamide (5).* Hydroxylamine **4** (52 mg, 0.15 mmol) in 5 mL THF was treated with triethylamine (180 μL, 1.3 mmol) and acetyl chloride (80 μL, 1.1 mmol) to give the triacetate. Treatment with NaOMe/MeOH as for **6** gave **5** (50 mg, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.05 (*bs*, 1H), 7.44–7.26 (*m*, 5H), 7.09 (*d*, *J* = 8.5 Hz, 2H), 6.90 (*d*, *J* = 8.5 Hz, 2H), 6.70 (*t*, *J* = 13.5 Hz, 1H), 5.02 (*s*, 2H), 4.35 (*m*, 1H), 4.21 (*t*, *J* = 7.5 Hz, 1H), 3.09 (*d*, *J* = 12 Hz, 1H), 2.77 (*d*, *J* = 7 Hz, 2H), 2.08 (*s*, 3H), 1.89 (*s*, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.2, 172.4, 157.7, 136.8, 129.9, 128.9, 128.6, 128.0, 127.4, 115.1, 70.0, 50.2, 48.3, 36.6, 22.9, 22.9, 20.3. HRMS (M + H)$^+$ calcd for $C_{20}H_{25}N_2O_4$: 357.1815; found: 357.1820.

Weinreb amide (10). *N*-Boc-*O*-Bn-tyrosine (3.7 g, 10 mmol) and *N,O*-dimethylhydroxylamine hydrochloride (1.45 g, 15 mmol) (both dried *in vacuo* over P_2O_5 overnight) were combined with triethylamine (3.6 mL, 26 mmol) and HBTU (4.9 g, 13 mmol) in DMF (20 mL). The reaction was stirred at rt for 2 h, the DMF removed, and the residue taken up in CH_2Cl_2. This

solution was then washed (1 N HCl, $NaHCO_3$ (satd)) and the solvent removed. Flash chromatography (2:1 hexane:EtOAc) gave 3.5 g (81%) of **10** as a white solid. ^1H NMR (500 MHz, $CDCl_3$) δ 7.42 (*d*, *J* = 7 Hz, 2H), 7.37 (*t*, *J* = 7 Hz, 2H), 7.31 (*t*, *J* = 7 Hz, 1H), 7.08 (*d*, *J* = 8.5 Hz, 2H), 6.90 (*d*, *J* = 8.5 Hz, 2H), 5.16 (*br d*, *J* = 8.5 Hz, 1H), 5.03 (*s*, 2H), 4.91 (*m*, 1H), 3.65 (*s*, 3H), 3.16 (*s*, 3H), 2.99 (*dd*, *J* = 6 and 14 Hz, 1H), 2.82 (*dd*, *J* = 7 and 13.5 Hz, 1H), 1.39 (*s*, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 157.6, 155.2, 137.0, 130.4, 128.8, 128.5, 127.9, 127.4, 114.7, 79.5, 69.9, 61.5, 51.5, 37.9, 32.0, 28.3. HRMS $(M + H)^+$ calcd for $C_{23}H_{31}N_2O_5$: 415.2233; found: 415.2224.

(2S)-N-Boc-1-(4-benzyloxyphenyl)-2-amino-6-phenyl-3-hexanone (20).[31] To magnesium turnings (600 mg, 25 mmol) in 20 mL anhydrous ether (under argon) was added 0.5 mL of a solution of 3-bromo-1-phenylpropane (4.9 g, 25 mmol) in 10 mL ether. A small crystal of I_2 was added to initiate the reaction and the remaining solution of bromide was added at a rate to maintain reflux. The reaction was then refluxed for an additional 30 min. Amide **10** (2.0 g, 4.8 mmol) in 5 mL THF was then added and the mixture stirred for 3 h at reflux at which point the reaction was seen to be done by TLC (2:1 hexane:EtOAc). The reaction mixture was then cooled and poured into 100 mL 1 N HCl and extracted with EtOAc. Flash chromatography (4:1 hexane:EtOAc) yielded 2.2 g (96%) of the ketone as a white solid. ^1H NMR (500 MHz, $CDCl_3$) δ 7.43 (*d*, *J* = 7 Hz, 2H), 7.40 (*t*, *J* = 7 Hz, 2H), 7.35 (*t*, *J* = 7 Hz, 1H), 7.28 (*t*, *J* = 7.5 Hz, 2H), 7.20 (*m*, 1H), 7.14 (*d*, *J* = 7.5 Hz, 2H), 7.00 (*d*, *J* = 8.5 Hz, 2H), 6.90 (*d*, *J* = 8.5 Hz, 2H), 5.22 (*d*, *J* = 7.5 Hz, 1H), 5.04 (*s*, 2H), 4.48 (*q*, *J* = 7.0 Hz, 1H), 2.96 (*dd*, *J* = 7.0 and 14.0 Hz, 1H), 2.89 (*dd*, *J* = 7.0 and 14.0 Hz, 1H), 2.56 (*t*, *J* = 7.5 Hz, 2H), 2.38 (*m*, 2H), 1.86 (*m*, 2H), 1.42 (*s*, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 209.2, 157.8, 155.2, 141.4, 136.9, 130.2, 128.6, 128.4, 128.4, 128.0, 127.4, 125.9, 114.9, 79.8, 69.9, 60.1, 40.0, 37.0, 34.9, 28.3, 24.7. HRMS $(M + Cs)^+$ calcd for $C_{30}H_{35}NO_4Cs$: 606.1620; found: 606.1640.

(1S,2S)-1-(3-Phenylpropyl)-2-N′-Boc-amino-3-(4-benzyloxyphenyl)-N-hydroxypropylamine (11(S,S)) and (1R,2S)-1-(3-phenylpropyl)-2-N′-Boc-amino-3-(4-benzyloxyphenyl)-N-hydroxypropylamine (11(R,S)). To a solution of hydroxylamine hydrochloride (1.0 g, 14.3 mmol) in 40 mL of methanol was added triethylamine to adjust the pH to 5. Ketone **20** (1.0 g, 2.1 mmol) in 5 mL THF was then added and the reaction stirred at rt for 1 h until no more ketone remained by TLC (2:1 hexane:EtOAc). The pH of the solution was then reduced to 3 with ethereal HCl, using methyl orange as an indicator. $NaCNBH_3$ (100 mg, 1.6 mmol) was added and the pH maintained at 3 by occasional additions of ethereal HCl. After 1 h, a second portion of $NaCNBH_3$ (100 mg, 1.6 mmol) was added and the pH maintained in the same way. Reaction was complete after an additional hour. The solvent was removed, the residue taken up in water and the pH adjusted to > 10 with the addition of 6 N NaOH. Extraction with EtOAc and removal of the solvent gave 900 mg (90%) of a pale-yellow solid which consisted of a 1:1 mixture of diastereomers which were separated in the following way. The yellow solid was taken up in a minimum of THF (~ 5 mL) and then hexane was added (20 mL) to crash out **11**(*R,S*). Collection of the precipitate followed by a second recrystallization (THF:hexane) gave **11**(*R,S*) as a diastereomerically pure white solid (320 mg). The mother liquor from the first recrystallization was concentrated and an overnight recrystallization (THF:hexane) of the residue afforded **11**(*S,S*) as a white solid (550 mg, still about a 5% contamination by **11**(***R,S***)). The assignments of the stereochemistries of these two compounds were accomplished through NOE experiments on the cyclic *N*-hydroxyurea derivatives of these compounds (**21**(*R,S*) and **21**(*S,S*)).

11(*S,S*): ^1H NMR (500 MHz, $CDCl_3$) δ 7.43 (*d*, *J* = 7 Hz, 2H), 7.39 (*t*, *J* = 7 Hz, 2H), 7.33 (*t*, *J* = 7 Hz, 1H), 7.27–7.14 (*m*, 5H), 7.05 (*d*, *J* = 7.5 Hz, 2H), 6.88 (*d*, *J* = 8.5 Hz, 2H), 5.02 (*s*, 2H), 4.97 (*d*, *J* = 9.5 Hz, 1H), 3.92 (*m*, 1H), 2.87 (*dd*, *J* = 8.0 and 15.0 Hz, 1H), 2.80 (*dd*, *J* = 8.0 and 15.0 Hz, 1H), 2.63 (*m*, 4H), 1.78–1.45 (*m*, 4H), 1.41 (*s*, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.4, 156.4, 142.1, 137.0, 130.4, 130.2, 128.5, 128.4, 128.3, 127.9, 127.4, 125.8, 114.8, 79.4, 70.0, 61.8, 53.6, 37.7, 35.7, 29.7, 28.4, 28.4, 27.9. HRMS $(M + H)^+$ calcd for $C_{30}H_{39}N_2O_4$: 491.2910; found: 491.2921.

11(*R,S*): ^1H NMR (500 MHz, $CDCl_3$) δ 7.43 (*d*, *J* = 7 Hz, 2H), 7.39 (*t*, *J* = 7 Hz, 2H), 7.34–7.28 (*m*, 3H), 7.22–7.16 (*m*, 3H), 7.06 (*d*, *J* = 8.5 Hz, 2H), 6.89 (*d*, *J* = 8.5 Hz, 2H), 7.06 (*d*, *J* = 7.5 Hz, 2H), 6.88 (*d*, *J* = 7.5 Hz, 2H), 5.03 (*s*, 2H), 4.73 (*d*, *J* = 9.5 Hz, 1H), 4.25 (*m*, 1H), 2.91 (*m*, 1H), 2.63 (*dt*, *J* = 3.0 and 9.5 Hz, 4H), 1.78–1.48 (*m*, 4H), 1.44 (*s*, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.3, 141.7, 137.0, 130.4, 130.3, 128.5, 128.4, 128.3, 127.9, 127.4, 125.9, 114.8, 79.7, 70.0, 63.6, 61.7, 53.6, 51.6, 36.5, 35.8, 28.3, 25.5. HRMS $(M + H)^+$ calcd for $C_{30}H_{39}N_2O_4$: 491.2910; found: 491.2922.

N-Hydroxy-N-[(1S,2S)-1-(3-phenylpropyl)-2-N′-Boc-amino-3-(4-benzyloxyphenyl)propyl]acetamide (22(S,S)). To a solution of the diastereomerically enriched hydroxylamine **11**(*S,S*) (200 mg, 0.41 mmol) in 5 mL THF was added triethylamine (240 μL, 1.72 mmol) and then acetyl chloride (87 μL, 1.23 mmol). The reaction was exothermic and complete in 5 min. The reaction mixture was diluted with 25 mL hexane and filtered through Celite. The solvent was removed *in vacuo* and the resulting oil (diacetate) was taken up in anhydrous methanol. Treatment with NaOMe/MeOH as described for **6** gave **22**(*S,S*) as a pale-yellow oil (200 mg, 92%) which resisted attempts at recrystallization. The undesired diastereomer **22**(*R,S*) was, however, easily removed at this point as it crystallized out (THF:hexane), leaving only the desired diastereomer **22**(*S,S*) in solution. ^1H NMR (500 MHz, $CDCl_3$) δ 8.43 (*s*, 1H), 7.43 (*d*, *J* = 7 Hz, 2H), 7.39 (*t*, *J* = 7 Hz, 2H), 7.33 (*t*, *J* = 7 Hz, 1H), 7.29 (*t*, *J* = 7.5 Hz, 2H), 7.19 (*m*, 3H), 7.00 (*d*, *J* = 8.5 Hz, 2H), 6.91 (*d*, *J* = 8.5 Hz, 2H), 5.05 (*s*, 2H), 4.65 (*d*, *J* = 9.5 Hz, 1H), 4.42 (*dt*, *J* = 3.5 and 11.0 Hz, 1H), 3.75 (*dq*, *J* = 4.0 and 9.0 Hz, 1H), 2.94

(*dd*, *J* = 3.5 and 14.5 Hz, 1H), 2.62 (*m*, 2H), 2.56 (*dd*, *J* = 9.0 and 14.5 Hz, 1H), 2.11 (*s*, 3H), 1.82–1.60 (*m*, 4H), 1.39 (*s*, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 158.4, 157.7, 142.0, 137.5, 136.9, 129.9, 128.8, 128.6, 128.4, 128.3, 128.0, 127.4, 125.7, 115.1, 80.9, 70.0, 57.8, 52.4, 35.9, 35.3, 28.1, 27.9, 27.2, 20.4. HRMS (M + H)$^+$ calcd for $C_{32}H_{41}N_2O_5$: 533.3015; found: 533.3027.

N-Hydroxy-N-[(1S,2S)-1-(3-phenylpropyl)-2-amino-3-(4-benzyloxyphenyl)propyl]acetamide, HCl salt (13(S,S)). *N*-Boc-hydroxamate **22**(*S,S*) (200 mg, 0.38 mmol) was dissolved in 20 mL ether and HCl gas bubbled through for 30 min. The reaction was allowed to stir overnight and the product **13**(*S,S*) precipitated out as a white solid (100 mg, 61%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.00 (*s*, 1H), 8.06 (*br s*, 3H), 7.43 (*d*, *J* = 7 Hz, 2H), 7.39 (*t*, *J* = 7 Hz, 2H), 7.33 (*t*, *J* = 7 Hz, 1H), 7.25 (*t*, *J* = 7 Hz, 2H), 7.17 (*d*, *J* = 7 Hz, 2H), 7.15 (*t*, *J* = 7.5 Hz, 1H), 7.11 (*d*, *J* = 8 Hz, 2H), 6.95 (*d*, *J* = 9.0 Hz, 2H), 5.07 (*s*, 2H), 4.49 (*m*, 1H), 3.34 (*m*, 1H), 2.84 (*m*, 2H), 2.55–2.42 (*m*, 2H), 2.07 (*s*, 3H), 1.73 (*m*, 1H), 1.49 (*m*, 2H), 1.34 (*m*, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.6, 157.3, 141.9, 137.1, 130.7, 128.5, 128.3, 128.1, 127.9, 127.7, 125.7, 69.2, 54.4, 53.8, 34.6, 34.5, 27.4, 27.1, 20.9. HRMS (M + H)$^+$ calcd for $C_{27}H_{33}N_2O_2$: 433.2491; found: 433.2498.

N-Hydroxy-N-[(1R,2S)-1-(3-phenylpropyl)-2-N′-Boc-amino-3-(4-benzyloxyphenyl)propyl]acetamide (22(R,S)). To a solution of hydroxylamine **11**(*R,S*) (50 mg, 0.10 mmol) in 1 mL THF was added triethylamine (80 μL, 0.57 mmol) and then acetyl chloride (500 μL, 7.0 mmol). The reaction was exothermic and complete in 5 min. Workup and treatment with NaOMe/MeOH as for **6** gave **22**(*R,S*) as a white solid which was recrystallized from THF:hexane (44 mg, 81%). Analysis by NMR in $CDCl_3$ showed this compound exists as two conformers (a 1:1 mixture); neither set of signals match those for **22**(*S,S*). Running the ^{1}H NMR in CD_3OD gives a single set of peaks, suggesting that the two conformers are a result of a hydrogen bond interaction. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.71 (*bs*, 0.5H), 7.95 (*s*, 0.5H), 7.44–7.16 (*m*, 10H), 7.067 (*d*, *J* = 8.5 Hz, 1H), 7.063 (*d*, *J* = 8.5 Hz, 1H), 6.912 (*d*, *J* = 8.5 Hz, 1H), 6.906 (*d*, *J* = 8.5 Hz, 1H), 5.04 (*s*, 2H), 4.68 (*m*, 0.5H), 4.60 (*d*, *J* = 7.5 Hz, 0.5H), 4.52 (*d*, *J* = 8 Hz, 0.5H), 4.24 (*m*, 0.5H), 3.80 (*m*, 1H), 2.92 (*m*, 1H), 2.75 (*dd*, *J* = 11 and 14 Hz, 0.5H), 2.64 (*m*, 2.5H), 2.15 (*s*, 1.5H), 2.08 (*s*, 1.5H), 1.85–1.5 (*m*, 4H), 1.37 (*s*, 4.5H), 1.35 (*s*, 4.5H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6, 165.4, 157.3, 155.3, 141.9, 141.5, 136.9, 136.8, 129.8, 128.5, 128.3, 128.3, 128.2, 127.9, 127.4, 125.9, 125.6, 114.9, 114.8, 80.8, 79.6, 69.9, 61.1, 56.9, 55.8, 55.4, 38.0, 35.5, 35.3, 34.9, 28.2, 28.1, 27.8, 27.2, 24.7, 20.6, 18.5. HRMS (M + H)$^+$ calcd for $C_{32}H_{41}N_2O_5$: 533.3015; found: 533.3027.

N-Hydroxy-N-[(1R,2S)-1-(3-phenylpropyl)-2-amino-3-(4-benzyloxyphenyl)propyl]acetamide, HCl salt (13(R,S)). Compound **22**(*R,S*) (40 mg, 0.81 mmol) was stirred in 5 mL ether and HCl gas bubbled through for 30 min. The reaction was allowed to stir overnight. The product failed to precipitate out and so the solvent was removed and the resulting residue washed with ether to give **13**(*R,S*) as a pale-yellow oil (31 mg, 90%). ^{1}H NMR data again indicated that this was a mixture of two conformers; however, this time, neither running it in CD_3OD nor at a higher temperature managed to resolve the signals into a single set of peaks. No (*S,S*) diastereomer was seen. ^{1}H NMR (500 MHz, CD_3OD) δ 7.4–7.15 (*m*, 12H), 6.97 (*d*, *J* = 8.5 Hz, 1H), 6.91 (*d*, *J* = 8.5 Hz, 1H), 5.06 (*s*, 1H), 5.03 (*s*, 1H), 4.64 (*dt*, *J* = 4 and 11 Hz, 0.5H), 4.48 (*dt*, *J* = 1 and 7 Hz, 0.5H), 3.62 (*m*, 0.5H), 3.57 (*dt*, *J* = 1, 6.5 Hz, 0.5H), 3.32 (*m*, 0.5H), 2.97 (*dd*, *J* = 6.5 and 14.5 Hz, 0.5H), 2.83 (*dd*, *J* = 8 and 14.5 Hz, 0.5H), 2.76 (*d*, *J* = 7.5 Hz, 1H), 2.70 (*m*, 1H), 2.61 (*m*, 1H), 2.16 (*s*, 1.5H), 1.93 (*s*, 1.5H), 1.86 (*m*, 1.5H), 1.72 (*m*, 2H), 1.52 (*m*, 0.5H). ^{13}C NMR (125 MHz, CD_3OD) δ 175.6, 175.5, 159.5, 159.2, 143.1, 142.5, 138.6, 131.5, 131.0, 129.6, 129.5, 129.4, 129.4, 128.9, 128.6, 128.6, 128.5, 128.5, 127.1, 126.9, 116.5, 116.1, 70.9, 70.9, 66.4, 57.2, 57.1, 36.4, 36.1, 36.1, 29.5, 29.1, 26.8, 25.5, 22.3. HRMS (M + H)$^+$ calcd for $C_{27}H_{33}N_2O_3$: 433.2491; found: 433.2499.

(1S,2S)-1-(3-Phenylpropyl)-2-amino-3-(4-benzyloxylphenyl)-N-hydroxypropylamine, HCl salt (12(S,S)). Hydroxylamine **11**(*S,S*) (80 mg, 0.16 mmol) was treated with ethereal HCl for 4 h to give **12**(*S,S*) (55 mg, 86%) (contaminated by 5% **12**(*R,S*)). ^{1}H NMR (500 MHz, CD_3OD) δ 7.42 (*d*, *J* = 7 Hz, 2H), 7.34 (*t*, *J* = 7 Hz, 2H), 7.30–7.10 (*m*, 8H), 7.01 (*d*, *J* = 8.5 Hz, 2H), 5.09 (*s*, 2H), 3.90 (*dt*, *J* = 4.5 and 10 Hz, 1H), 3.71 (*m*, 1H), 3.17 (*dd*, *J* = 4.5 and 14.5 Hz, 1H), 2.83 (*dd*, *J* = 10 and 14.5 Hz, 1H), 2.71 (*m*, 1H), 2.65 (*m*, 1H), 1.91–1.73 (*m*, 4H). ^{13}C NMR (125 MHz, CD_3OD) δ 159.9, 142.4, 138.6, 131.5, 129.5, 128.9, 128.5, 128.0, 127.2, 116.7, 70.9, 62.4, 53.9, 36.3, 35.2, 28.7, 26.3. HRMS (M + H)$^+$ calcd for $C_{25}H_{31}N_2O_2$: 391.2386; found: 391.2380.

(1R,2S)-1-(3-Phenylpropyl)-2-amino-3-(4-benzyloxylphenyl)-N-hydroxypropylamine, HCl salt (12(R,S)). Hydroxylamine **11**(*R,S*) (45 mg, 0.16 mmol) was treated with ethereal HCl for 4 h to give **12**(*R,S*) (30 mg, 84%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.42 (*d*, *J* = 7 Hz, 2H), 7.34 (*t*, *J* = 7 Hz, 2H), 7.30 (*m*, 3H), 7.24 (*d*, *J* = 8 Hz, 2H), 7.21 (*t*, *J* = 7.5 Hz, 1H), 7.14 (*d*, *J* = 8.5 Hz, 2H), 7.00 (*d*, *J* = 8.5 Hz, 2H), 5.09 (*s*, 2H), 3.90 (*dt*, *J* = 1.5 and 8 Hz, 1H), 3.56 (*t*, *J* = 6.5 Hz, 1H), 2.93 (*m*, 2H), 2.72 (*m*, 2H), 1.91 (*m*, 1H), 1.80 (*m*, 2H), 1.73 (*m*, 1H). ^{13}C NMR (125 MHz, CD_3OD) δ 159.6, 142.4, 138.6, 131.4, 129.6, 129.0, 128.5, 127.7, 127.3, 116.8, 70.9, 62.1, 54.6, 36.2, 35.3, 28.9, 24.8. HRMS (M + H)$^+$ calcd for $C_{25}H_{31}N_2O_2$: 391.2386; found: 391.2381.

Cyclic N-hydroxyurea (21(S,S)). Hydroxylamine **12**(*S,S*) (22 mg, 0.048 mmol) in 2 mL THF was treated with K_2CO_3 (50 mg) and triphosgene (10 mg) and the reaction mixture stirred for 30 min. The mixture was then filtered through Celite, and the product purified by flash chromatography (2:1 EtOAC:hexane) to give **21**(*S,S*) (10 mg, 50%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (*bs*, 1H), 7.44–7.15 (*m*, 10H), 7.04 (*d*, *J* = 8.5 Hz, 2H), 6.91 (*d*, *J* = 8.5 Hz, 2H), 5.05 (*s*, 2H), 4.78 (*s*,

1H), 3.37 (*m*, 1H, C_3), 3.35 (*m*, 1H, C_2), 2.82 (*dd*, *J* = 4 and 13.5 Hz, 1H, C_1), 2.62 (*m*, 2H, C_6), 2.58 (*m*, 1H, C_1), 1.72 (*m*, 2H, C_5), 1.68 (*m*, 2H, C_4). HRMS (M + H)$^+$ calcd for $C_{26}H_{29}N_2O_2$: 417.2178; found: 417.2165. ROESY experiments reveal NOEs between C_1 and C_2, C_3 and C_4, but not between C_1 and C_4, leading to the assignment of *S,S*.

Cyclic N-*hydroxyurea (21(*R,S*)).* Hydroxylamine **12**(*R,S*) (17 mg, 0.037 mmol) in 2 mL THF was treated with K_2CO_3 (100 mg) and triphosgene (10 mg) and the reaction mixture stirred for 30 min. The mixture was then filtered through Celite, and the product purified by flash chromatography (4:1 EtOAC:hexane) to give **21**(*R,S*) (12 mg, 60%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (*bs*, 1H), 7.42–7.25 (*m*, 8H), 7.18 (*d*, *J* = 7 Hz, 2H) 6.95 (*d*, *J* = 8.5 Hz, 2H), 6.89 (*d*, *J* = 8.5 Hz, 2H), 5.02 (*s*, 2H), 4.75 (*s*, 1H), 3.66 (*m*, 1H), 3.63 (*m*, 1H), 2.75 (*m*, 2H, C_1 and C_6), 2.66 (*m*, 1H, C_6), 2.51 (*dd*, *J* = 11 and 13.5 Hz, 1H, C_1), 1.96 (*m*, 1H, C_5), 1.83 (*m*, 1H, C_5), 1.71 (*m*, 2H, C_4). HRMS (M + H)$^+$ calcd for $C_{26}H_{29}N_2O_2$: 417.2178; found: 417.2167. ROESY experiments reveal NOEs between C_1 and C_2, C_3 and C_4, and between C_1 and C_4, leading to the assignment of *R,S*.

N-*Hydroxy*-N-*[(2S)*-N′-*Boc-2-amino-3-(4-benzyloxyphenyl)propyl]-5-carboxymethylpentanamide (14).* Adipic acid monomethyl ester (120 μL, 0.81 mmol) was added to a solution of oxalyl chloride (70 μL, 0.8 mmol) and DMF (10 μL) in 5 mL CH_2Cl_2. Pyridine (500 μL) was added followed by hydroxylamine **3** (100 mg, 0.27 mmol) and the reaction stirred for 1 h at which time no hydroxylamine remained. 3-Dimethylaminopropylamine (100 μL) was added, the reaction mixture diluted with 20 mL CH_2Cl_2, washed (1 N HCl, $NaHCO_3$ (satd), NaCl (satd)) and dried ($MgSO_4$). Removal of the solvent gave a mixture of *N*-acyl and *N,O*-diacyl products. Treatment with NaOMe/MeOH as described for **6** and recrystallization from THF:hexane gave **14** (122 mg, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.62 (*s*, 1H), 7.43 (*d*, *J* = 7 Hz, 2H), 7.39 (*t*, *J* = 7 Hz, 2H), 7.33 (*t*, *J* = 7 Hz, 1H), 7.07 (*d*, *J* = 8.5 Hz, 2H), 6.90 (*d*, *J* = 8.5 Hz, 2H), 5.03 (*s*, 2H), 4.62 (*d*, *J* = 9 Hz, 1H), 4.17 (*q*, *J* = 12.5 Hz, 1H), 4.11 (*m*, 1H), 3.63 (*s*, 3H), 3.01 (*dd*, *J* = 2 and 13 Hz, 1H), 2.74 (*m*, 2H), 2.54 (*m*, 1H), 2.30 (*m*, 3H), 1.60 (*m*, 4H), 1.36 (*s*, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.2, 158.2, 157.8, 136.9, 130.1, 128.6, 128.0, 127.5, 115.2, 81.0, 70.0, 51.5, 50.5, 48.1, 37.3, 33.9, 32.1, 28.2, 24.7, 24.1. HRMS (M + Cs)$^+$ calcd for $C_{28}H_{38}N_2O_7Cs$: 647.1733; found: 647.1748.

N-*Hydroxy*-N-*[(2S)-2-amino-3-(4-benzyloxyphenyl)propyl]-5-carboxypentanamide, TFA salt (16).* To a solution of hydroxamate **14** (10 mg, 0.02 mmol) in 1 mL MeOH was added LiOH (0.5 mL, 2.0 M) and the solution stirred 10 min. The solution was poured into 1 N HCl (8 mL) and extracted with EtOAc. The organic layer was dried ($MgSO_4$) and the solvent removed to give a pale-yellow oil. The oil was then dissolved in 1 mL TFA:CH_2Cl_2:H_2O (20:80:1), stirred for 30 min, and the solvent removed *in vacuo*. The residue was washed with ether to give 6.9 mg (69%) of **16** as a pale-yellow oil. ^{1}H NMR (500 MHz, CD_3OD) δ 9.90 (*bs*, 1H), 7.85 (*bs*, 3H), 7.44 (*d*, *J* = 7 Hz, 2H), 7.39 (*t*, *J* = 7 Hz, 2H), 7.32 (*t*, *J* = 7 Hz, 1H), 7.18 (*d*, *J* = 8.5 Hz, 2H), 6.98 (*d*, *J* = 8.5 Hz, 2H), 5.07 (*s*, 2H), 3.79 (*dd*, *J* = 7.5 and 14 Hz, 1H), 3.71 (*m*, 2H), 2.90 (*d*, *J* = 7 Hz, 2H), 2.29 (*bs*, 2H), 2.20 (*m*, 2H), 1.48 (*m*, 4H). ^{13}C NMR (125 MHz, DMSO-d_6): 174.4, 173.7, 157.4, 137.1, 130.4, 128.5, 127.9, 127.7, 114.9. 69.2, 50.3, 35.1, 33.5, 31.3, 24.3, 23.5. HRMS (M + H)$^+$ calcd for $C_{22}H_{29}N_2O_5$: 401.2076; found: 401.2064.

N-*Hydroxy*-N-*[(2S)-2-amino-3-(4-benzyloxyphenyl)propyl]-5-carboxymethylpentanamide, HCl salt (18).* Hydroxamate **14** (24 mg, 0.047 mmol) was dissolved in 10 mL ethereal HCl and the mixture stirred overnight. The solvent was then removed *in vacuo* to give **18** as a white solid (19 mg, 91%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.11 (*s*, 1H), 8.12 (*bs*, 3H), 7.43 (*d*, *J* = 7 Hz, 2H), 7.39 (*t*, *J* = 7 Hz, 2H), 7.32 (*t*, *J* = 7 Hz, 1H), 7.20 (*d*, *J* = 8.5 Hz, 2H), 6.97 (*d*, *J* = 8.5 Hz, 2H), 5.07 (*s*, 2H), 3.81 (*dd*, *J* = 8.5 and 14.5 Hz, 1H), 3.56 (*s*, 3H), 3.55 (*m*, 1H), 3.47 (*dd*, *J* = 4 and 14.5 Hz, 1H), 2.89 (*dd*, *J* = 5.5 and 14 Hz, 1H), 2.75 (*dd*, *J* = 8 and 14 Hz, 1H), 2.38 (*t*, *J* = 7 Hz, 2H), 2.28 (*t*, *J* = 7 Hz, 2H), 1.48 (*m*, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 174.1, 173.3, 157.4, 137.1, 128.4, 128.0, 127.8, 127.7, 114.9, 69.2, 51.2, 50.2, 48.7, 35.1, 33.2, 31.4, 24.2, 23.3. HRMS (M + H)$^+$ calcd for $C_{23}H_{31}N_2O_5$: 415.2233; found: 415.2248.

N-*Hydroxy*-N-*[(2S)*-N′-*Boc-2-amino-3-(4-benzyloxyphenyl)propyl]-5-carboxymethylbutanamide (15).* Glutamic acid monomethyl ester (200 mg, 1.37 mmol) was added to a solution of oxalyl chloride (150 μL, 1.7 mmol) and DMF (20 μL) in 10 mL CH_2Cl_2. Pyridine (900 μL) was added followed by hydroxylamine **3** (250 mg, 0.67 mmol) and the reaction stirred for 6 h at which time no hydroxylamine remained. 3-Dimethylaminopropylamine (200 μL) was added, the reaction mixture diluted with 60 mL CH_2Cl_2, washed (1 N HCl, $NaHCO_3$ (satd), NaCl (satd)) and dried ($MgSO_4$). Removal of the solvent gave a mixture of *N*-acyl and *N,O*-diacyl products. Treatment with NaOMe/MeOH as described for **6** and recrystallization from THF:hexane gave **15** (298 mg, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64 (*s*, 1H), 7.44 (*d*, *J* = 7 Hz, 2H), 7.39 (*t*, *J* = 7 Hz, 2H), 7.33 (*t*, *J* = 7 Hz, 1H), 7.1 (*d*, *J* = 8.5 Hz, 2H), 6.93 (*d*, *J* = 8.5 Hz, 2H), 5.03 (*s*, 2H), 4.62 (*d*, *J* = 9 Hz, 1H), 4.17 (*q*, *J* = 13.5 Hz, 1H), 4.12 (*m*, 1H), 3.66 (*s*, 3H), 3.04 (*dd*, *J* = 2 and 13 Hz, 1H), 2.74 (*m*, 2H), 2.61 (*m*, 1H), 2.36 (*m*, 3H), 1.91 (*m*, 2H), 1.39 (*s*, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.7, 158.2, 157.8, 136.9, 130.1, 128.6, 128.0, 127.4, 115.1, 81.0, 70.0, 51.5, 50.5, 48.1, 37.2, 33.4, 31.5, 29.7, 28.2, 19.8. HRMS (M + Cs)$^+$ calcd for $C_{27}H_{36}N_2O_7Cs$: 633.1577; found: 633.1549.

N-*Hydroxy*-N-*[(2S)-2-amino-3-(4-benzyloxyphenyl)propyl]-5-carboxybutanamide, TFA salt (17).* To a solution of hydroxamate **15** (200 mg, 0.40 mmol) in 14 mL MeOH was added LiOH (1.0 g in 14 mL MeOH:H_2O (1:1)) and the solution stirred 30 min. The solution was poured into

1 N HCl (50 mL) and extracted with EtOAc. The organic layer was dried ($MgSO_4$) and the solvent removed to give *N*-Boc protected **17** as a pale-yellow solid which was then recrystallized from THF:hexane (160 mg, 82%). This compound (10.7 mg) was dissolved in 1 mL TFA:CH_2Cl_2:H_2O (20:80:1), the reaction stirred for 30 min, and the solvent removed *in vacuo*. The residue was washed with ether to give 10.9 mg (100%) of **17** as a pale-yellow oil. ^{1}H NMR (500 MHz, CD_3OD) δ 7.42 (*d*, *J* = 7 Hz, 2H), 7.35 (*t*, *J* = 7 Hz, 2H), 7.29 (*t*, *J* = 7 Hz, 1H), 7.20 (*d*, *J* = 8.5 Hz, 2H), 6.99 (*d*, *J* = 8.5 Hz, 2H), 5.08 (*s*, 2H), 3.79 (*dd*, *J* = 8.5 and 15.5 Hz, 1H), 3.69 (*m*, 2H), 2.89 (*d*, *J* = 7 Hz, 2H), 2.56 (*t*, *J* = 7 Hz, 2H), 2.36 (*t*, *J* = 7 Hz, 2H), 1.88 (*tt*, *J* = 7 and 7 Hz, 2H). ^{13}C NMR (125 MHz, CD_3OD) δ 177.1, 176.9, 159.2, 138.7, 131.5, 129.5, 128.9, 128.5, 116.5, 70.9, 53.0, 50.8, 36.9, 34.1, 32.3, 20.8. HRMS (M + H)$^+$ calcd for $C_{21}H_{27}N_2O_5$: 387.1920; found: 387.1907.

N-Hydroxy-N-[(2S)-2-amino-3-(4-benzyloxyphenyl)propyl]-5-carboxymethylbutanamide, HCl salt (19). Hydroxamate **15** (24 mg, 0.048 mmol) was dissolved in 6 mL ethereal HCl and the mixture stirred overnight. The solvent was then removed *in vacuo* and the residue washed with ether to give **19** as a white solid (18.9 mg, 90%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.11 (*s*, 1H), 8.07 (*bs*, 3H), 7.43 (*d*, *J* = 7 Hz, 2H), 7.38 (*t*, *J* = 7 Hz, 2H), 7.32 (*t*, *J* = 7 Hz, 1H), 7.20 (*d*, *J* = 8.5 Hz, 2H), 6.97 (*d*, *J* = 8.5 Hz, 2H), 5.07 (s, 2H), 3.81 (dd, *J* = 8 and 14.5 Hz, 1H), 3.56 (s, 3H), 3.54 (m, 1H), 3.45 (dd, *J* = 4 and 14.5 Hz, 1H), 2.88 (*dd*, *J* = 6.5 and 14 Hz, 1H), 2.75 (*dd*, *J* = 8.5 and 14 Hz, 1H), 2.41 (*t*, *J* = 7 Hz, 2H), 2.32 (*t*, *J* = 7.5 Hz, 2H), 1.71 (*tt*, *J* = 7.5 and 7.5 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.8, 173.2, 157.3, 137.1, 128.4, 128.0, 127.8, 127.7, 114.9, 69.2, 51.2, 50.1, 48.7, 35.1, 32.7, 30.8, 19.3. HRMS (M + H)$^+$ calcd for $C_{22}H_{29}N_2O_5$: 401.2076; found: 401.2058.

Inhibition studies of the peptidase activity of LTA_4 hydrolase

All assays were performed in Tris–HCl buffer (50 mM, pH 8.0) with L-alanyl-*p*-nitroanilide (1.87 mM) as substrate. LTA_4 hydrolase (1.4 μg) purified from human leukocytes was added for each assay (final volume = 1.0 mL, [E] = 20 nM). The rate of formation of *p*-nitroaniline was spectrophotometrically monitored at 405 nM. The high enzyme concentration as compared to inhibitor concentration ($[E]_t \approx [I]_t$ for compounds **16–19**) was accounted for by using the appropriate kinetic equations for tight-binding inhibitors and the K_i values were determined using non-linear regression methods.[20]

Inhibition studies of the epoxide hydrolase activity of LTA_4

The epoxide hydrolase activity was determined from short-time (15 s) incubations of enzyme (2.5 μg = 360 nM) and inhibitor (0.01–10 μM) dissolved in DMSO (final conc = 0.5%; v/v) in 50 mM Hepes, pH 8, (100 μL) with LTA_4 (67 μM) at rt. Reactions were quenched with 2 vol of MeOH. PGB_1 was added as internal standard, and samples were extracted and analyzed by reversed-phase HPLC, essentially as described.[32] Enzyme and inhibitor were preincubated 45 min at rt prior to activity determinations.

Preparation and incubation of granulocytes

Human granulocytes were prepared from buffycoat by dextran sedimentation, centrifugation on Lymfoprep™ and hypotonic lysis of remaining erythrocytes as described.[33] The cells were resuspended at a concentration of 20 × 10^6 per mL in Dulbecco's phosphate-buffered saline (pH 7.4). Aliquots (1 mL) were preincubated with or without various concentrations of inhibitor for 10 min on ice followed by 15 min at 37 °C prior to the addition of A23187 (2 μM) ± arachidonic acid (30 μM). After 5 min, the incubations were quenched by 1 vol of MeOH and subjected to solid phase extraction and reversed-phase HPLC. The eluate was monitored at 270 nm and 235 nm, for the detection and quantitation of LTB_4 and 5-hydroxyeicosatetraenoic acid (5-HETE), respectively.

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22. It has also been shown that amino hydroxamic acids bind in a reversed mode to thermolysin;[25] that is, they bind in the S1' subsite rather than the expected S1 subsite (S1 and S1' being defined as by the notation of Schechter and Berger).[26] Perhaps then, as **2** is an inverted hydroxamic acid, its binding is now in the usual S1 subsite. Thus, while PheNHOH may be binding in S1', **2** may be binding in S1, resulting in the observed difference in inhibition potencies and suggesting a significant difference in size requirements between P1 and P1'.

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