Probing the Activities and Mechanisms of Leukotriene A_4 Hydrolase with Synthetic Inhibitors

J. Heather Hogg, Ian R. Ollmann, Anders Wetterholm, Martina Blomster Andberg, Jesper Haeggström, Bengt Samuelsson, and Chi-Huey Wong*

Abstract: Leukotriene (LT) A_4 hydrolase catalyzes the hydrolysis of leukotriene A_4 to form leukotriene B_4, a potent inflammatory mediator. Recently, the synthesis and evaluation of the highly effective competitive LTA_4 hydrolase inhibitor **2** (K_i = 1.6 nM) was described.[25] In the present study, we describe the biological activity of **2** against LTB_4 biosynthesis, as well as the design, synthesis, and evaluation of a new series of inhibitors intended to probe the active site of the enzyme. On the basis of these results and of previously reported site-directed mutagenesis and inhibition studies, the mechanisms of peptide and epoxide hydrolysis catalyzed by LTA_4 hydrolase are discussed.

Keywords: enzyme inhibitors · hydrolases · hydroxamates · metalloenzymes · peptides

Introduction

Leukotriene (LT) A_4 hydrolase (EC 3.3.2.6) catalyzes the hydrolysis of LTA_4 [(5*S*,6*S*)-5,6-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid] to LTB_4 [(5*S*,12*R*)-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid], which acts as a chemokine for neutrophils.[1–3] As high levels of LTB_4 have been detected in many sites of inflammation,[4, 5] LTB_4 has been implicated in a number of inflammatory diseases including gout,[6, 7] psoriasis,[8] inflammatory bowel disease,[9] bronchial asthma,[10, 11] and rheumatoid arthritis.[12]

The enzyme also exhibits an intrinsic aminopeptidase activity (Figure 1) which is selective for *N*-terminal arginine-containing tripeptides,[13] but accepts dynorphins and enkephalins as substrates too.[14] The active site contains a single zinc atom, and site-directed mutagenesis indicates that each of the three putative zinc ligands is required for both the peptide and epoxide hydrolase activities.[15] In addition, Tyr^{383} plays critical roles in both activities.[16, 17] These results suggest that the two activities occur at a common or overlapping site.[14, 18–20] Indeed, a series of tight-binding peptide isosteres synthesized as inhibitors of the peptidase activity are also competitive inhibitors of the hydrolase activity,[21–25] including for example mercaptoamine **1**[26] and hydroxamate **2**[25] (Figure 2), which have K_i values of 0.35 nM and 1.6 nM, respectively, measured against the peptidase activity of the enzyme. It has been found that there is a strong linear correlation between the potencies of competitive inhibitors against the two activities.[27] Thus, if a competitive inhibitor is strongly active against the peptidase activity, it will also, in general, be a strongly active competitive inhibitor of the hydrolase activity.

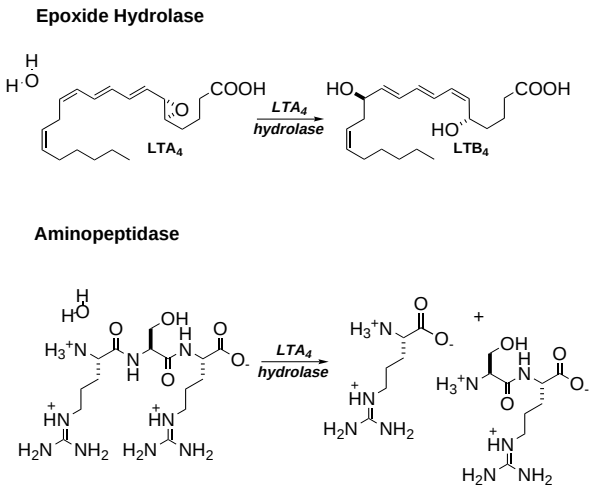

Figure 1. The epoxide hydrolase and aminopeptidase activities of LTA_4 hydrolase.

While the mechanism of the aminopeptidase activity is comparatively well understood as LTA_4 hydrolase is a member of the superfamily of zinc metalloproteases, which

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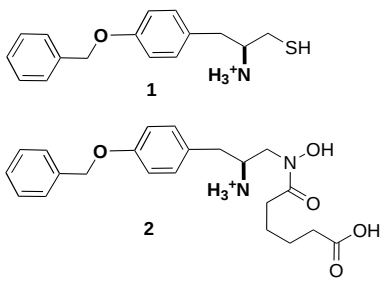


Figure 2. A competitive inhibitor of LTA_4 hydrolase **1** ($K_i = 0.35$ nM) and a hydroxamate-type inhibitor **2** ($K_i = 1.6$ nM).

includes thermolysin and aminopeptidase M, the mechanism of the hydrolase activity remains unclear. The enzyme has very little sequence similarity with other epoxide hydrolases and also differs from them in several other respects.[28, 29] Thus, no analogies between its mode of action and that of the other enzymes can be drawn. Following the discovery that LTA_4 can irreversibly inhibit the enzyme, it was proposed that the epoxide hydrolase mechanism occurred in two distinct steps.[30] Tyrosine 378 was found to be the nucleophile responsible for the irreversible inactivation.[31] Interestingly, the Y378F mutant has enhanced specific activity (k_{cat}) over the wild-type enzyme. However, it produces large quantities of the Δ^6-*trans*-Δ^8-*cis* isomer of LTA_4, suggesting its role is to hold the substrate in the correct geometry in the active site rather than act as a proton donor or reactive intermediate of some kind.[32, 33] A recent finding that the Y383F mutant produces substantial quantities of an isomer of LTB_4, characterized by a *syn*-5,6 diol produced by a formal *syn*-facial attack on the epoxide, resulted in the proposal that the hydrolase activity operated by a two-stage mechanism characterized by an S_N1 opening of the epoxide to form a trienyl cation followed by nucleophilic attack either by water[17] in the case of LTB_4 biosynthesis, or Tyr^{378} in the case of irreversible inactivation.[31]

The single active-site zinc ion may participate in the catalysis in one of two ways. It may act as a Lewis acid on the epoxide, thus facilitating its opening, or it may coordinate the reacting water molecule, activating it toward general-base catalysis, as is thought to occur in the aminopeptidase mechanism.[34] Because of the geometry of the transition state (as suggested by the stereochemistry of the double bonds in the substrate and product) it is apparent that the reacting water molecule and scissile epoxide residue are on opposite faces of the triene, making it unlikely that the zinc acts at both oxygens at the same time. Which mode of action is actually involved has still not been determined, and remains an important question to be answered.

Here we report on a new series of hydroxamate inhibitors of LTA_4 hydrolase and their implications on the mechanism of epoxide hydrolysis.[16, 17, 31, 32, 35]

Results and Discussion

Inhibition of the peptidase activity: The extremely tight interaction between inhibitor **1** and LTA_4 hydrolase was posited to arise from a high-affinity contact between the potent metal ligands at the polar head of these molecules accompanied by a substantial contribution arising from the long hydrophobic benzyloxyphenyl tail, presumably in some part of the same large hydrophobic pocket normally occupied by LTA_4. It is known that LTA_4 methyl ester is not a substrate for this enzyme[36] (though it is a suicide inhibitor); this suggests the importance of the acid moiety in molecular recognition and the likelihood of a highly selective carboxylate recognition site within the enzyme active site.

With derivatives of metallophilic aminopeptidase inhibitor **1**, several possible binding arrangements of these inhibitors relative to the epoxide hydrolysis transition state were examined.[24] Because the zinc is thought to activate water in the aminopeptidase activity, this same role was examined in the epoxide hydrolase activity. A superimposition of a model of **1** over a model of the transition state with the zinc at the reacting water molecule (Figure 3A) suggested that (if this overlapped model is correct) the enzyme's carboxylate-recognition element would be in the vicinity of the benzyl group of **1**. On the basis of this hypothesis, a series of derivatives of **1** with an additional carboxylate substituent were prepared; each derivative resulted in a minimum of a 10000-fold loss of affinity, suggesting that this model is unlikely to be correct. An alternative binding mode as shown in Figure 3B may occur.

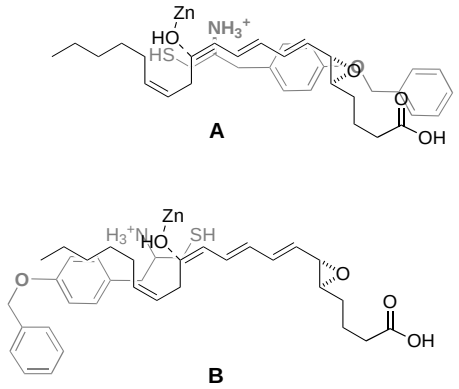


Figure 3. A and B show the overlay of **1** and LTA_4 in the binding site as possible modes of inhibition. Current data supports model B.

A second model which placed the zinc at the scissile epoxide was then considered. Modeling the metallophilic head of the inhibitor to lie at, or near, the position of the scissile epoxide suggested that the addition of a pentanoic acid substituent to the metallophilic head group of the inhibitor would strongly mimic the C1–C5 region of the substrate (Figure 4). Hydroxamate **2** was designed to have a spacer with four methylene groups between the hydroxamate and carboxylate moieties. This modification resulted in a dramatic increase in binding potency (K_i now 1.6 nM) over the parent hydroxamate (Table 1).[25] Hydroxamate **4**, with a shorter linker, binds slightly more weakly ($K_i = 3.4$ nM). The corresponding methyl esters of these compounds, **3** ($K_i = 28$ nM) and **5** ($K_i = 11$ nM), both show an order of magnitude decrease in binding, demonstrating that this binding pocket prefers the

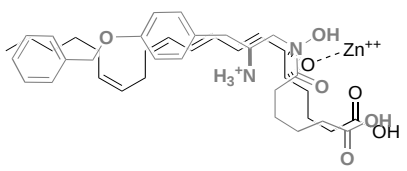
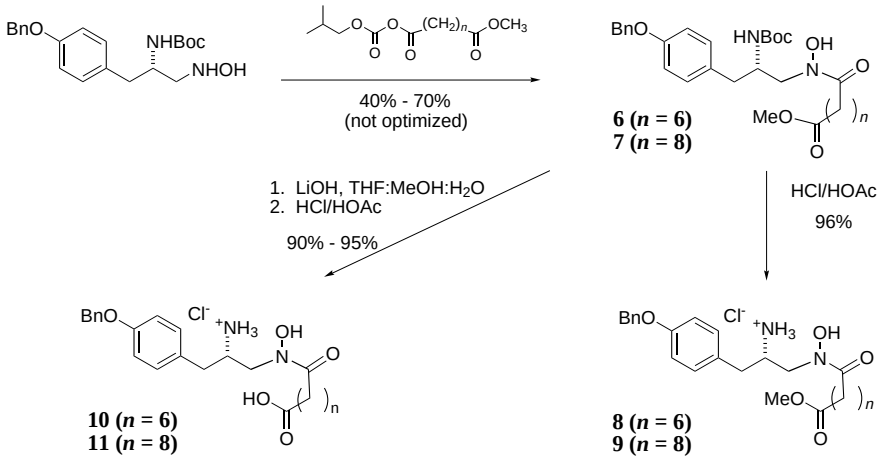


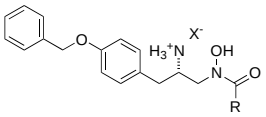
Figure 4. A possible binding mode of **2** with the hydroxamate moiety located at the epoxide binding site and coordinated to the zinc. The two carboxylates are overlapped. Current data supports this binding mode.

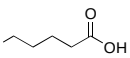
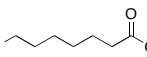
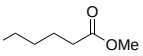
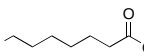
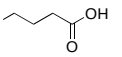
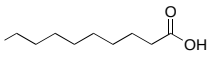
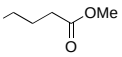
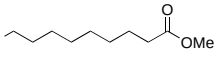
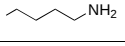
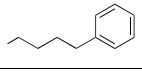
free carboxylate. As the chain which links the carboxylate gets longer (**10** and **11**, the synthesis of which is shown in Scheme 1) the binding potencies decrease, with the corresponding esters again about ten times less active than the acids.



Scheme 1. Synthesis of carboxylate chain links from (2*S*)-2-*N*′-Boc-amino-3-(4-benzyloxyphenyl)-*N*-hydroxypropylamine.

Table 1. IC_{50} and K_i values for hydroxamates containing a carboxylate moiety (against the peptidase activity of LTA_4 hydrolase).



	R	IC_{50} (μM)		R	IC_{50} (μM)
2		0.011 (K_i = 1.6 nM)	**10**		0.018 (K_i = 35 nM)
3		0.055 (K_i = 28 nM)	**8**		0.40
4		0.011 (K_i = 3.4 nM)	**11**		0.025
5		0.025 (K_i = 11 nM)	**9**		0.16
15		0.40	**13**		0.11

Incorporation of groups such as an amine **15** (IC_{50} = 0.40 μM) or a phenyl group **13** (IC_{50} = 0.11 μM) in the place of the carboxylate again results in a decrease in activity (the synthesis of **13** and **15** is shown in Scheme 2). While the methyl esters **3** and **5** are not as potent as their corresponding acids, they are significantly better inhibitors than the others in this series. This may suggest that there is a hydrogen-bonding interaction responsible for the change in the binding free energy rather than a charge–charge interaction.

There still remained a third reasonable alternative binding model for these compounds, which again placed the zinc adjacent to the reacting water molecule during the epoxide hydrolase reaction. This model orients the hydrophobic portion of the inhibitor in the opposite direction from that in the first model (Figure 5). Here, the hydroxamate lies proximal to the end of the triene and the carboxylate lies near the epoxide, perhaps interacting with the same Lewis acid as the opening epoxide. If this is the case, one would predict that by extending our inhibitor to incorporate a second carboxylate which would overlay that of LTA_4, we should again see an increase in potency as the result of finally achieving the putative carboxylate recognition site (Figure 6).

To this end, we studied a final set of hydroxamic acids (**32**–**41**). Initially these compounds were synthesized using an amine as a linker (Schemes 3 and 4). In this way, simply by varying the amino acid used, a number of compounds may be quickly synthesised with a defined stereochemistry at the position alpha to the amine. It was also hypothesized that having a positively charged amine in the vicinity of the carboxylate might help mimic the charge distribution of an opening epoxide, a feature of this model.

An initial exploration of the preferred stereochemistry using L- (**37**, IC_{50} = 0.031 μM) and D-alanine (**38**, IC_{50} = 0.041 μM) derivatives showed very little preference for a single diastereomer (Table 2). As L-amino acids are more readily available and the L-isomer appeared to be slightly more potent, the structure of **37** was chosen as a template for further development. Both compounds, however, were less potent than the original compound **2**. Addition of a second carboxylate on a linker of the appropriate length, in **40**, resulted in a small increase in potency (K_i = 20 nM), but nowhere near the two orders of magnitude we saw with the incorporation of the first carboxylate. The K_i value for **40** (20 nM) compares some-

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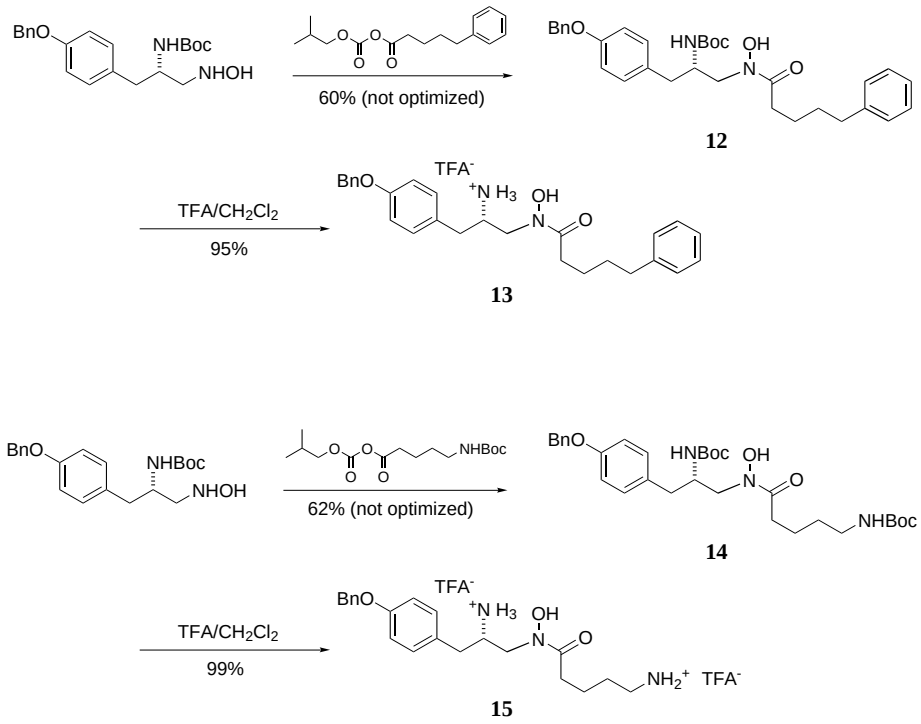


Scheme 2. Synthesis of amine and phenyl derivatives of (2*S*)-2-*N*′-Boc-amino-3-(4-benzyloxyphenyl)-*N*-hydroxypropylamine.

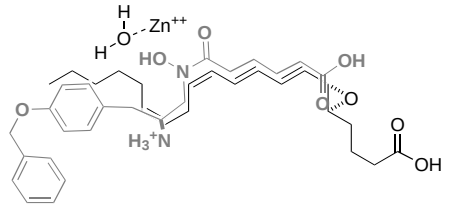


Figure 5. Another possible binding mode of **2** with the carboxylate moiety located at the epoxide binding site and the zinc ion coordinated to a water molecule.

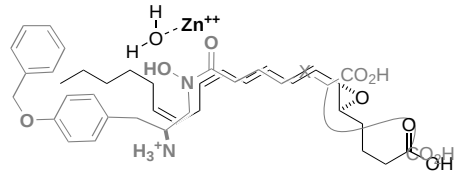
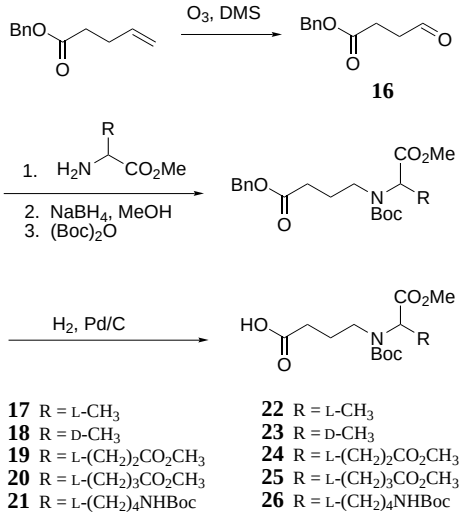


Figure 6. Design of new inhibitors based on the binding mode shown in Figure 5.

in hydrogen-bonding interactions. Use of a thioether as a linker rather than an amine led to inhibitor **46** ($IC_{50} = 0.02\,\mu M$, $K_i = 14\,nM$) which appears to be more potent than its amine-containing analog **37** ($K_i = 78\,nM$), but still not as good as the parent compound **2** ($K_i = 1.6\,nM$). This may be a better choice for a linker for further inhibitors.

The failure to identify any compelling increases in potency on adding a second carboxylate in this series of molecules indicates these modifications contribute only weak additional interactions, if any, with the enzyme. The only binding model to date with good predictive capability has been model two, on which **2** was based (Figure 4). It is of note that this model predicts that the active-site zinc operates at the epoxide, suggesting that its role in epoxide hydrolysis may be as a Lewis acid, facilitating the initial stage of the S_N1 conversion of LTA_4 to LTB_4.



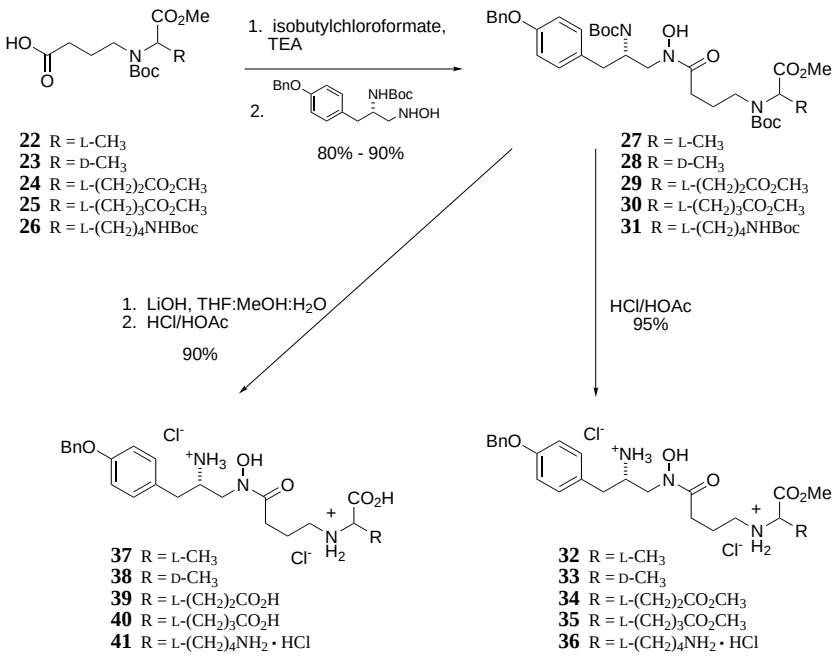
17 R = L-CH_3
18 R = D-CH_3
19 R = L-$(CH_2)_2CO_2CH_3$
20 R = L-$(CH_2)_3CO_2CH_3$
21 R = L-$(CH_2)_4$NHBoc
22 R = L-CH_3
23 R = D-CH_3
24 R = L-$(CH_2)_2CO_2CH_3$
25 R = L-$(CH_2)_3CO_2CH_3$
26 R = L-$(CH_2)_4$NHBoc

Scheme 3. Synthesis of hydroxamic acids **17**–**26**.

what favorably against the shorter **39** ($K_i = 78\,nM$), but the difference is not great. An amino group rather than a carboxylate leads to a tenfold reduction in potency (**41**, $IC_{50} = 0.20$). As observed in the earlier series of carboxylate-containing inhibitors, the corresponding methyl esters (**32**–**36**) were all less potent than their analogous acids.

As the presence of the charged amino linker led to decreased potency (**2** vs. **37**), a final compound which uses a thioether as a linker was synthesized (Scheme 5). The thioether moiety is perhaps a better mimic of a methylene group as it has no associated charge and does not participate

Though it is far from certain that the zinc atom acts as a Lewis acid at the epoxide, a growing body of indirect evidence supports this role. There appears to be a consistent substrate-design motif which specifies a preferred distance between the carboxylate termini of the substrates and the sites of zinc-catalyzed chemistry. The distance between the epoxide and carboxylic acid in LTA_4 is very similar to that between the scissile amide bond and the *C*-terminus of a tripeptide, the preferred substrate size for the enzyme's aminopeptidase activity,[13] and also the length of a series of known peptidase inhibitors[37, 38] (Figure 7).



22 R = L-CH_3
23 R = D-CH_3
24 R = L-$(CH_2)_2CO_2CH_3$
25 R = L-$(CH_2)_3CO_2CH_3$
26 R = L-$(CH_2)_4NHBoc$

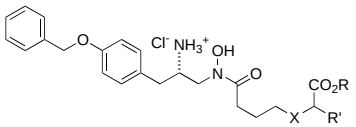
27 R = L-CH_3
28 R = D-CH_3
29 R = L-$(CH_2)_2CO_2CH_3$
30 R = L-$(CH_2)_3CO_2CH_3$
31 R = L-$(CH_2)_4NHBoc$

37 R = L-CH_3
38 R = D-CH_3
39 R = L-$(CH_2)_2CO_2H$
40 R = L-$(CH_2)_3CO_2H$
41 R = L-$(CH_2)_4NH_2 \cdot HCl$

32 R = L-CH_3
33 R = D-CH_3
34 R = L-$(CH_2)_2CO_2CH_3$
35 R = L-$(CH_2)_3CO_2CH_3$
36 R = L-$(CH_2)_4NH_2 \cdot HCl$

Scheme 4. Synthesis of hydroxamic acids **27**–**41**.

Table 2. IC_{50} and K_i values for extended hydroxamates **6–54** and **6–63** (against the peptidase activity of LTA_4 hydrolase).



		R' (R = H)		IC_{50} (μM)		R' (R = Me)		IC_{50} (μM)
X = NH_2^+ Cl^-	**37**	CH_3	*(L)*	0.03	**32**	CH_3	*(L)*	0.4
	38	CH_3	*(D)*	0.04	**33**	CH_3	*(D)*	0.2
	39	CO_2H	*(L)*	0.08 (K_i = 0.07)	**34**	CO_2Me	*(L)*	0.1
	40	CO_2H	*(L)*	0.02 (K_i = 0.02)	**35**	CO_2Me	*(L)*	0.2
	41	NH_2	*(L)*	0.20	**36**	NH_2	*(L)*	1.4
X = S	**46**	CH_3	*(L)*	0.03 (K_i = 0.014)	**45**	CH_3	*(L)*	0.4

Site-directed mutagenesis studies published to date further corroborate this view. Glu^{296} (sequentially adjacent to His^{295}, one of the zinc ligands) is required for the aminopeptidase activity, acting as the general base in the deprotonation of water. However, it does not play this role during epoxide hydrolysis; the E296A mutant displays full epoxide hydrolase activity.[35] This behavior would support the theory that the zinc atom may act as a Lewis acid for the epoxide opening as no general base would be expected to be needed in the vicinity of the zinc ion in such a mechanism. In addition, Tyr^{383} is also required for the aminopeptidase reaction but not for the epoxide hydrolase activity. On the basis of homology with thermolysin, it is thought to be a proton source for the S1 amine during peptide hydrolysis.[16] The Y383F, Y383Q, and Y383H mutants of LTA_4 hydrolase have severely diminished aminopeptidase activities. In the epoxide hydrolysis case, these mutations allow water to attack at the C6 end of the trienyl cation in a *syn*-facial sense at the site of the broken epoxide C–O bond, rather than the usual C12 position.[17] If this is to be explained simply as the appearance of a hole in the enzyme adjacent to residue 383, as the result of the reduction of size, then it appears that Tyr^{383}, and by inference the zinc atom, are adjacent to the epoxide.

Finally, it should be noted that the attack of water on LTA_4 in the active site occurs from the energetically disfavored face of the molecule as predicted by molecular orbital theory. If the zinc atom activates water for a general base catalysis, the epoxide may open concurrently with a general acid catalysis as part of an S_N2' mechanism. However, at least in the case of mutants at position 383, it seems fairly clear that the mechanism is more S_N1 in character. Furthermore, if the conserved zinc atom is to act directly (as opposed to allosterically) to accelerate the reaction as part of an S_N1 mechanism, then it should act to accelerate the rate-determining step, the opening of the epoxide.

For the sake of exploring all possibilities, one could argue that even if the epoxide hydrolysis reaction is S_N2 in nature, if the zinc operated as a water activator and Glu^{296} as a base in this reaction, the absence of Glu^{296} would not be observed in the overall enzymatic rates as it would act after the rate-determining step in the reaction. Similarly, one could argue that the observed distance similarities between the lengths of tripeptides and the C1–C6 region of LTA_4 is a coincidence, and the observation of the 5,6-isomer of LTB_4 as a result of mutations at position 383 arises from some remote allosteric effect. It is clear that there still remains much to be understood about the structure of LTA_4 hydrolase and the epoxide hydrolysis mechanism.

The degree to which this model is in agreement with an earlier model proposed by Orning, Gierse, and Fitzpatrick,[13] which places LTA_4 in the S1′ pocket, remains unclear. In that work, it was originally proposed that the S1′ pocket bound LTA_4 on the basis of its observed preference for hydrophobic substituents as well as some data derived from the inhibitory behavior of arphamenine. As the S1 pocket prefers arginine, whose delocalized positive charge might be a good mimic of the developing delocalized trienyl cation in a S_N1-like LTA_4 hydrolysis transition state, there remains some basis upon

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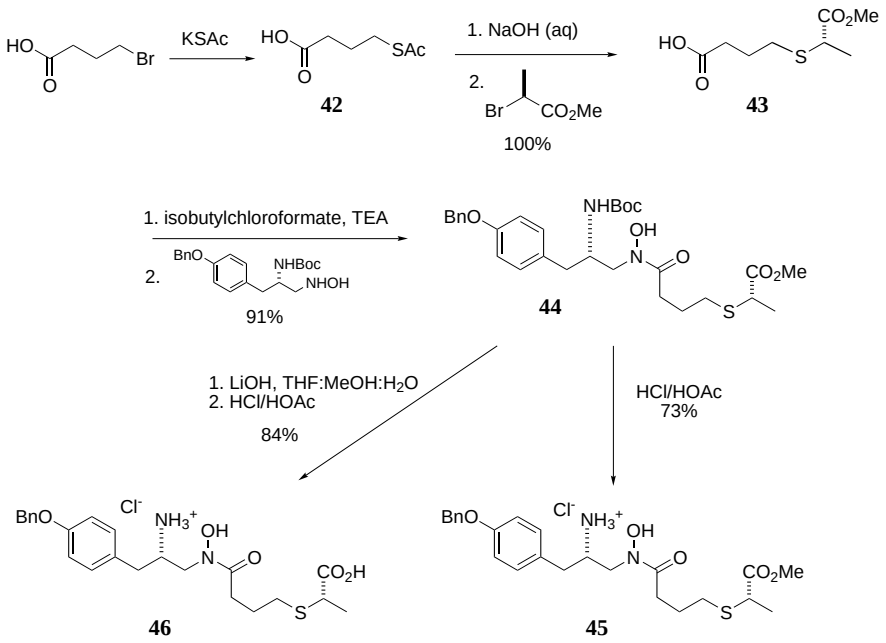


Scheme 5. Synthesis of compounds **45** and **46**, which contain a thioether linker.

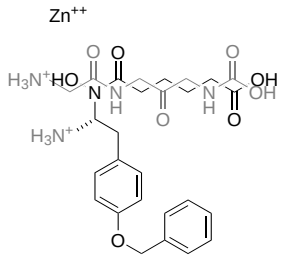
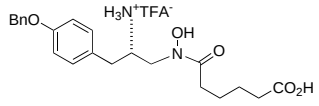
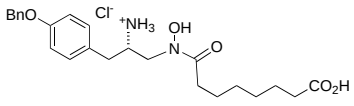
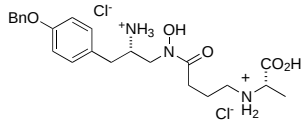
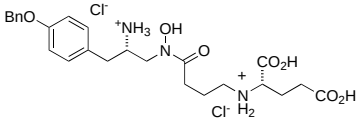
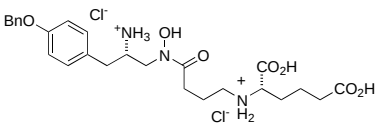
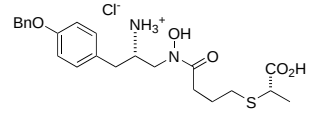


Figure 7. Overlay of **2** and a tripeptide.

which to suspect that the pocket plays host to LTA_4. It appears reasonable that the benzyloxyphenyl portion of the inhibitors discussed here and in previous works binds in either the S1 or S1′ subsites, because of their likely proximity to the zinc atom. Some evidence, including a strong requirement for a free amine adjacent to the zinc ligand[24] and the efficacy of a series of peptide transition-state isosteres,[21–23] suggests that these inhibitors bind S1 despite its preference for arginine. The identification of the subsite which binds the large, rigid, hydrophobic benzyloxyphenyl group might help elucidate a likely home for LTA_4 in the active site, therefore *O*-benzyl-L-tyrosine *p*-nitroanilide hydrochloride **47** was prepared by Tesser's method.[39] Unfortunately, it was found that this material was highly insoluble in water and could not be evaluated as a substrate for the enzyme. *O*-Benzyl-L-serine *p*-nitroanilide **48** was also prepared and found not to be a substrate at 10 mM. Larger, more soluble, *O*-benzyl tyrosine containing peptidic substrates or chimeric peptides containing LTA_4-like structures may help resolve this issue.

Inhibition of the epoxide hydrolase activity: The IC_{50} and K_i values of several of the hydroxamates have been determined against the epoxide hydrolase activity of purified enzyme (Table 3). These values are all consistently an order of magnitude higher than those against the peptidase activity. This reflects the fact that these assays are run at much higher enzyme concentrations, necessary for the detection of the product (see experimental). In these assays, the enzyme concentration is 360 nM; thus the IC_{50} value for **2** (0.14 μM) is an upper limit rather than a close approximation of the K_i. The K_i values for our two best inhibitors, **2** and **46**, were not determined as their inhibition curves were too steep under these high enzyme concentrations to gather meaningful and reproducible data.

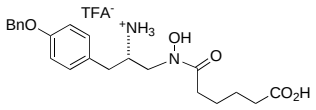
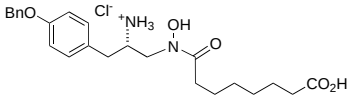
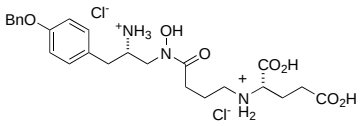
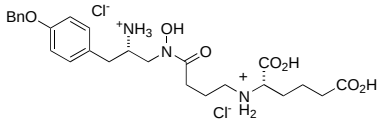
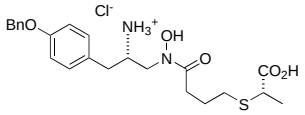
Table 3. IC_{50} and K_i values for selected hydroxamates (against the epoxide hydrolase activity of LTA_4 hydrolase).

	Compound	IC_{50} (μM)	K_i (μM)
2		0.14	N.D.[a]
10		1.1	0.7
37		6.9	N.D.
39		1.2	0.8
40		0.5	0.2
46		0.4	N.D.

[a] N.D.: not determined.

Whole cell assays: A number of these hydroxamates were also shown to be effective inhibitors of LTB_4 synthesis in polymorphonuclear leukocytes stimulated with the ionophore A23187 or incubated with LTA_4 (Table 4). Further experiments with **2** also showed it to be selective for LTA_4 hydrolase (Figure 8). At approximately 1 μM, the production of the nonenzymatic hydrolysis products of LTA_4 has doubled while the production of LTB_4 has essentially been stopped, indicating that while LTA_4 hydrolase has been inhibited, the enzymes leading up to the production of LTA_4 have remained unaffected. However, at higher concentrations we also see a fall in the production of the nonenzymatic hydrolysis products, suggesting that our inhibitor is now interfering with other processes in this pathway. There is, however, a window of opportunity at 1 μM, where hydroxamate **2** is selective for LTA_4 hydrolase. Mercaptoamine **1** has a similar profile; however, it shows optimal selectivity at a concentration of 10 μM rather than at 1 μM.[40]

Table 4. IC_{50} values for selected hydroxamates (against the epoxide hydrolase activity of LTA_4 hydrolase in isolated human polymorphonuclear leukocytes).

	Compound	Stimulation with ionophor A23187 IC_{50} (μM)	Incubation with LTA_4 IC_{50} (μM)
2		0.2	0.2
10		0.3	0.2
39		N.I.[a]	N.D.[a]
40		N.I.	N.D.
46		0.5	0.8

[a] N.I.: no inhibition; N.D.: not determined.

Addition of exogenous arachidonic acid to the assays of hydroxamate **2** fully restores the production of the nonenzymatic hydrolysis products; however, the inhibition of LTB_4 production by LTA_4 hydrolase is still complete (Table 5). Again, a similar result is seen in the case of mercaptoamine **1**.[40]

Conclusions

With compounds **2** and **4**, we have demonstrated that the inclusion of a free carboxylate moiety in our hydroxamate class of inhibitors is crucial for tight binding to LTA_4 hydrolase. When this carboxylate is placed in a position more distal from the hydroxamate moiety we see a decrease in activity, and an even greater decrease is seen when the carboxylate is replaced by another moiety such as an amine or a hydrophobic group. Incorporation of a second carboxylate, as suggested by one of the binding modes shown in Figure 5B, did show a small increase in the potency of the inhibitor when viewed in context (**37** vs. **40**), but it was not as significant as that seen with the incorporation of the first carboxylate.[25] This behavior, in conjunction with previously published mutagenesis results and enzyme-kinetic and inhibitory studies, suggests the possible modes of action of the enzyme (Figures 9 and 10). While in the amide-

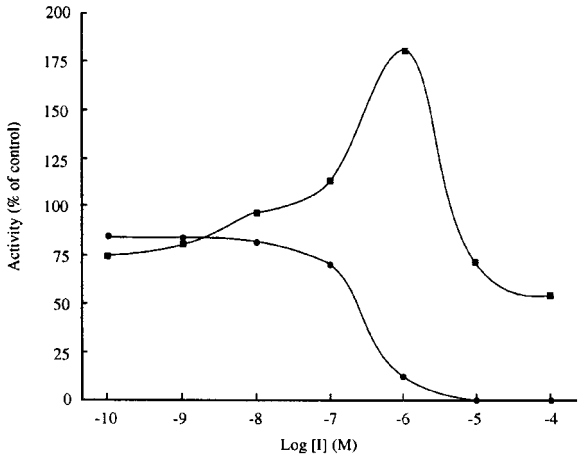


Figure 8. Effects of **6–26** on the formation of LTB_4 (●), and the nonenzymatic hydrolysis products of LTA_4 (all-*trans* isomers of LTB_4, □) in isolated human polymorphonuclear leukocytes.

Table 5. Effects of hydroxamic acid **2** on LTB_4 biosynthesis in intact polymorphonuclear lymphocytes (PMNL). PMNL (20×10^6 in 1 mL) were preincubated with or without inhibitor for 10 min on ice followed by 15 min at 37 °C and further incubated with 2 μM ionophore A23187 ± 30 μM arachidonic acid (AA) for 5 min at 37 °C. Reactions were quenched with MeOH and samples processed and analyzed as described in the experimental section. The all-*trans* isomers of LTB_4 are Δ^6-*trans*- and 12-epi-Δ^6-*trans*-LTB_4. Formation of the respective compounds are expressed in % of the control samples assayed in the absence of inhibitor.

	% of the control		
	all-*trans* isomers of LTB_4	LTB_4	5-HETE
Control	100	100	100
[I] = 100 μM	60	0	0
[I] = 500 μM	0	0	0
Control + AA	100	100	100
[I] = 100 μM + AA	132	0	135
[I] = 500 μM + AA	96	0	100

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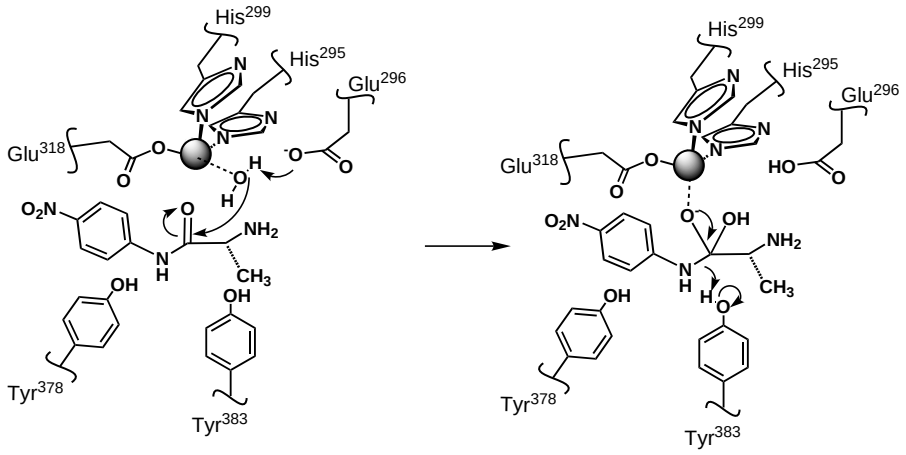

Figure 9. Proposed mechanism of the amidase activity.

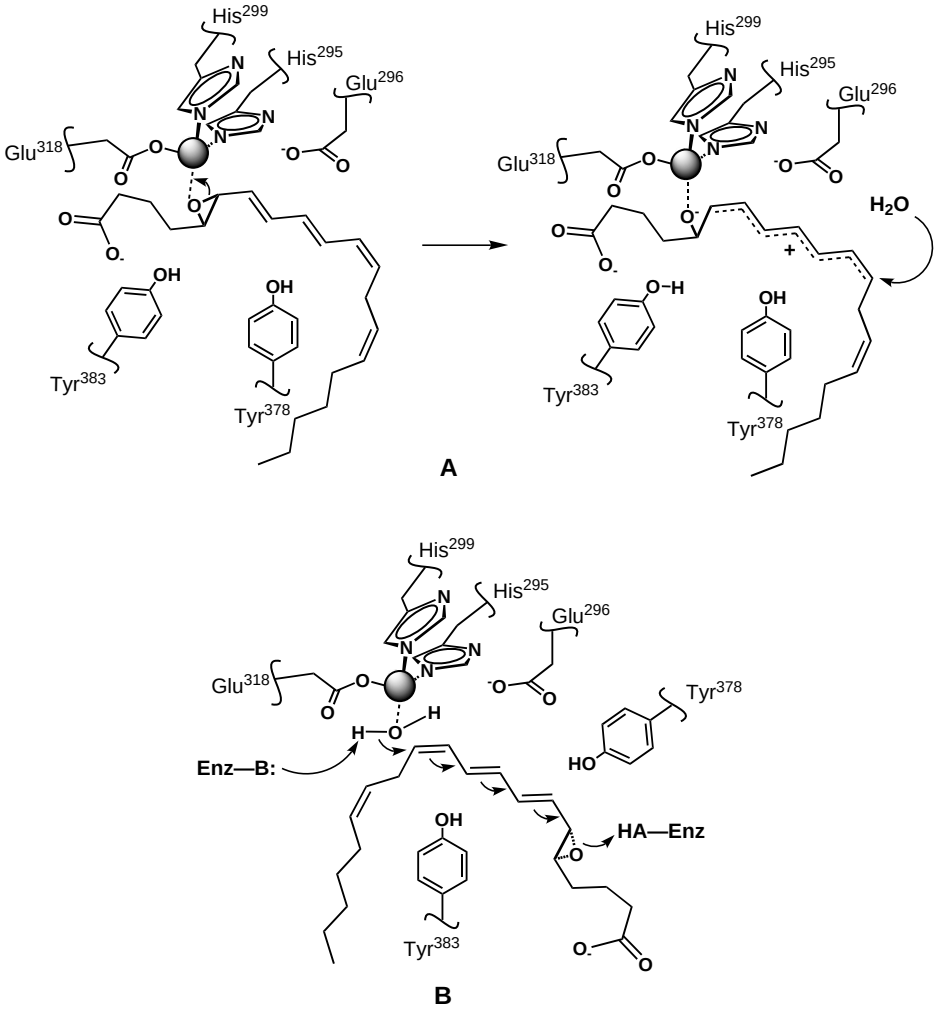

Figure 10. Proposed mechanisms A and B for the epoxide hydrolase activity.

cleavage reaction, the zinc ion appears to be close to the scissile bond, two possible modes of action are proposed for the epoxide hydrolase activity. The active-site zinc atom may act as a Lewis acid on the epoxide during LTA_4 hydrolysis, though no direct evidence of such a role has yet been found, or it may activate the reacting water molecule, as in the amide cleavage reaction, with the involvement of a different general base and a different general acid.

Experimental Section

General: The reagents used were commercially available and used without further purification. Methylene chloride (CH_2Cl_2) was distilled from CaH_2, and tetrahydrofuran (THF) was distilled from sodium and benzophenone prior to use. Anhydrous methanol was purchased from Aldrich. All reactions were run under an inert atmosphere of argon unless otherwise noted. The HCl/acetic acid solution used for Boc deprotections was made by bubbling HCl gas through glacial acetic acid for 5 min.

^1H NMR chemical shifts (δ) are reported relative to tetramethylsilane ($CDCl_3$), the solvent peak at $\delta = 2.49$ ([D_6]DMSO), or the solvent peak at $\delta = 3.30$ ([D_4]methanol). ^{13}C NMR spectra reported in $CDCl_3$ are referenced to the solvent peak at $\delta = 77.0$, those in [D_4]methanol are referenced to the solvent peak at $\delta = 49.0$, and those in [D_6]DMSO are referenced to the solvent peak at $\delta = 39.5$. Thin-layer chromatography was performed on silica-gel plates (0.25 mm, Merck) and flash chromatography was performed on 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.

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 ($[E]_t \approx [I]_t$) was accounted for by using the appropriate kinetic equations for tight-binding inhibitors and the K_i values were determined using nonlinear regression methods.[24]

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 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES; 50 mM), pH 8, (100 μL) with LTA_4 (67 μM) at room temperature. Reactions were quenched with 2 vols of MeOH. PGB_1 was added as internal standard, and samples were extracted and analyzed by RP-HPLC, as previously described.[41] Enzyme and inhibitor were preincubated for 45 min at room temperature 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 the remaining erythrocytes as previously described.[42] The cells were resuspended at a concentration of

20×10^6 cells mL^{-1} 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 with 1 vol of MeOH and subjected to solid-phase extraction and RP-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.

Cell culture: Whole blood was obtained from healthy volunteers by venepuncture and collected into EDTA-treated Vacutainers (Becton Dickinson, Rutherford, NJ). After mixing for 45–50 min, the blood was transferred to LeukoPREP tubes (Becton Dickinson, Lincoln Park, NJ) and the peripheral blood mononuclear cells (PBMC) were separated according to the manufacturer's instructions. The isolated PBMC were washed with two 15 mL volumes of Dulbecco's phosphate-buffered saline (D-PBS) and the cell pellet was resuspended at a concentration of 2×10^6 cells mL^{-1} in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS), 50 μM betamercaptoethanol, 1 mM sodium pyruvate, 50 μg mL^{-1} gentamycin, and 0.1 mM nonessential amino acids. Cells were added to a 96-well plate precoated with OKT3 (Ortho Biotech, Raritan, NJ). Plates were coated as previously described by Ritchie et al.[43] 100 μL (2×10^5 cells) of the cell suspension were added to each well followed by the addition of sufficient phorbol myristate acetate (PMA) to achieve a final concentration of 50 ng mL^{-1}. Drugs or vehicles were then added to cultures for a final well volume of 200 μL. Cultures were maintained in a humid atmosphere of 95% air and 5% CO_2 at 37 °C for 72 h. 6 h prior to harvest, each well was pulsed with 1 μCi of ^{3}H thymidine (6.7 Ci mmol^{-1}, New England Nuclear, Boston, MA). Cells were harvested onto glass-fiber filters with a Tomtec-96 cell harvester (Tomtec, Orange CT) and the radioactivity incorporated was measured with a Packard Matrix 9600 direct beta counter (Packard, Meridian, CT).

Relative binding model generation: The models of the binding orientation of the substrate LTA_4 relative to the inhibitors discussed here were obtained as a result of a combination of model overlay with plastic molecular models (Maruzen, Japan) augmented by computational molecular modeling and minimization techniques using Insight 95 and Discover 2.98 (MSI) on a Silicon Graphics Octane workstation. Preliminary computational experiments revealed that the compounds discussed in this work were found to exhibit a large number of conformers which corresponded to local energetic minima. In each proposed model, it was verified computationally that conformers similar to those shown in Schemes 4, 5, 6, and 7 were energetically reasonable. The compounds were aligned in such a way that portions of the molecule posited to interact with the zinc atom were in spacially similar areas. When possible, hydrophobic regions of inhibitors were paired with hydrophobic regions of the substrate, transition-state complex and hydrogen-bond acceptors and charged regions on the inhibitor were paired with similar moieties in the substrate transition-state complex.

(2*S*)-2-*N*′-Boc-amino-3-(4-benzyloxyphenyl)-*N*-hydroxypropylamine: This compound was synthesized as previously described.[25]

***N*-Hydroxy-*N*-(2*S*)-*N*′-Boc-2-amino-3-phenylpropyl-7-carboxymethylheptanamide** (**6**): Isobutyl chloroformate (76 μL, 0.59 mmol) was added to a solution of suberic acid monomethyl ester (128 μL, 0.71 mmol), and triethylamine (104 μL, 0.59 mmol) in THF (10 mL) at 0 °C. The resulting slurry was stirred for 30 min and then (2*S*)-2-*N*′-Boc-amino-3-(4-benzyloxyphenyl)-*N*-hydroxypropylamine (200 mg, 0.54 mmol) was added. The cooling bath was removed and the reaction mixture stirred for 30 min. The reaction mixture was then poured into CH_2Cl_2 (100 mL), washed (1N HCl, saturated $NaHCO_3$, saturated NaCl), and dried ($MgSO_4$). Purification by flash chromatography (1:3 EtOAc/hexanes) and recrystallization (THF/hexanes) yielded compound **6** as a white solid (217 mg, 74%). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 8.61$ (s, 1H), 7.43 (d, $J = 7$ Hz, 2H), 7.39 (t, $J = 7.5$ Hz, 2H), 7.33 (t, $J = 7.0$ Hz, 1H), 7.10 (d, $J = 8.5$ Hz, 2H), 6.93 (d, $J = 8.5$ Hz, 2H), 5.05 (s, 2H), 4.65 (d, $J = 9$ Hz, 1H), 4.22 (dd, $J = 13.5$ and 13.5 Hz, 1H), 4.13 (m, 1H), 3.66 (s, 3H), 3.03 (dd, $J = 13.5$ and 2.5 Hz, 1H), 2.77 (m, 2H), 2.53 (m, 1H), 2.29 (m, 3H), 1.65–1.52 (m, 4H), 1.39 (s, 9H), 1.32 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 174.7$, 174.2, 158.2, 157.8, 136.9, 130.1, 128.6, 128.0, 127.4, 115.1, 80.9, 70.0, 51.4, 50.4, 48.1, 37.3, 34.0, 32.4, 29.0, 28.9, 28.2, 24.8, 24.4; HRMS $[M+Cs]^+$ calcd for $C_{30}H_{42}N_2O_7Cs$: 675.2046, found: 675.2058.

***N*-Hydroxy-*N*-[(2*S*)-*N*′-Boc-2-amino-3-phenylpropyl]-7-carboxymethylnonanamide** (**7**): Isobutyl chloroformate (73 μL, 0.56 mmol) was added to a solution of sebacic acid monomethyl ester (121 mg, 0.56 mmol) and triethylamine (78 μL, 0.56 mmol) in THF (5 mL) at 0 °C. The resulting slurry was stirred for 30 min and then (2*S*)-2-*N*′-Boc-amino-3-(4-benzyloxyphenyl)-*N*-hydroxypropylamine (140 mg, 0.37 mmol) in THF (5 mL) was added. The reaction mixture was stirred for 30 min and then poured into CH_2Cl_2 (50 mL), washed (1N HCl, saturated $NaHCO_3$, saturated NaCl), and dried ($MgSO_4$). Purification by flash chromatography (1:3 EtOAc/hexanes) and recrystallization (THF/hexanes) yielded compound **7** as a white solid (87 mg, 41%). The major by-product was *iso*-butyl carbamate. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 8.61$ (s, 1H), 7.43 (d, $J = 7$ Hz, 2H), 7.39 (t, $J = 7.5$ Hz, 2H), 7.33 (t, $J = 7.0$ Hz, 1H), 7.10 (d, $J = 8.5$ Hz, 2H), 6.92 (d, $J = 8.5$ Hz, 2H), 5.05 (s, 2H), 4.67 (m, 1H), 4.22 (dd, $J = 13.5$ and 13.5 Hz, 1H), 4.13 (m, 1H), 3.66 (s, 3H), 3.03 (dd, $J = 13.5$ and 2.5 Hz, 1H), 2.77 (m, 2H), 2.53 (m, 1H), 2.29 (m, 3H), 1.65–1.52 (m, 4H), 1.39 (s, 9H), 1.28 (m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 174.8$, 174.3, 158.2, 157.8, 136.9, 130.0, 128.6, 127.9, 127.4, 115.1, 80.9, 70.0, 51.4, 50.4, 48.1, 37.2, 34.1, 32.5, 29.4, 29.2, 29.1, 28.2, 24.9, 24.6; HRMS $[M+Cs]^+$ calcd for $C_{32}H_{46}N_2O_7Cs$: 703.2359, found: 703.2344.

***N*-Hydroxy-*N*-[(2*S*)-2-amino-3-(4-benzyloxyphenyl)propyl]-7-carboxymethylheptanamide** (**8**): Hydroxamate **6** (20 mg, 0.037 mmol) was treated with a solution of HCl/acetic acid (1 mL) for 30 min. The reaction mixture was diluted with toluene (10 mL) and the solvent removed from the resulting mixture. The resulting solid was rinsed with ether and then azeotroped with toluene to give **8** as a white solid (17.0 mg, 96%). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 10.11$ (s, 1H), 8.11 (br s, 3H), 7.43 (d, $J = 7.0$ Hz, 2H), 7.39 (t, $J = 7.0$ Hz, 2H), 7.33 (t, $J = 7.0$ Hz, 1H), 7.21 (d, $J = 8.5$ Hz, 2H), 6.97 (d, $J = 8.5$ Hz, 2H), 5.07 (s, 2H), 3.81 (dd, $J = 8.0$ and 14.5 Hz, 1H), 3.56 (s, 3H), 3.55 (m, 1H), 3.47 (dd, $J = 4.0$ and 14.0 Hz, 1H), 2.89 (dd, $J = 5.5$ and 14.0 Hz, 1H), 2.75 (dd, $J = 8.5$ and 14.0 Hz, 1H), 2.36 (t, $J = 7.0$ Hz, 2H), 2.27 (t, $J = 7.5$ Hz, 2H), 1.47 (m, 4H), 1.24 (m, 4H); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): $\delta = 174.3$, 173.4, 157.4, 137.1, 130.5, 128.5, 128.0, 127.9, 127.7, 114.9, 69.2, 51.2, 50.2, 48.7, 35.1, 33.2, 31.7, 28.5, 28.3, 24.3, 23.7; HRMS $[M+Cs]^+$ calcd for $C_{25}H_{34}N_2O_5Cs$: 575.1522, found: 575.1538.

***N*-Hydroxy-*N*-[(2*S*)-2-amino-3-(4-benzyloxyphenyl)propyl]-9-carboxymethylnonanamide** (**9**): Hydroxamate **7** (14.1 mg, 0.025 mmol) was treated with a solution of HCl/acetic acid (1 mL) for 30 min. The reaction mixture was diluted with toluene (10 mL) and the solvent removed from the resulting mixture. The resulting solid was rinsed with ether and then azeotroped with toluene to give **9** as a white solid (12.0 mg, 97%). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 10.08$ (s, 1H), 8.08 (br s, 3H), 7.43 (d, $J = 7.0$ Hz, 2H), 7.39 (t, $J = 7.0$ Hz, 2H), 7.33 (t, $J = 7.0$ Hz, 1H), 7.21 (d, $J = 8.5$ Hz, 2H), 6.97 (d, $J = 8.5$ Hz, 2H), 5.07 (s, 2H), 3.81 (dd, $J = 8.0$ and 14.5 Hz, 1H), 3.56 (s, 3H), 3.54 (m, 1H), 3.47 (dd, $J = 4.5$ and 14.5 Hz, 1H), 2.88 (dd, $J = 5.5$ and 14.0 Hz, 1H), 2.75 (dd, $J = 8.0$ and 14.0 Hz, 1H), 2.36 (t, $J = 7.0$ Hz, 2H), 2.26 (t, $J = 7.0$ Hz, 2H), 1.47 (m, 4H), 1.22 (m, 8H); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): $\delta = 174.4$, 173.4, 157.4, 137.1, 130.5, 128.5, 128.0, 127.9, 127.7, 114.9, 69.2, 51.2, 50.2, 48.7, 35.1, 33.3, 31.7, 28.8, 28.6, 28.5, 24.4, 23.9; HRMS $[M+Cs]^+$ calcd for $C_{27}H_{38}N_2O_5Cs$: 603.1835, found: 603.1855.

***N*-Hydroxy-*N*-[(2*S*)-2-amino-3-(4-benzyloxyphenyl)propyl]-7-carboxyheptanamide** (**10**): LiOH (1.0M, 2.5 mL) was added to hydroxamate **6** (50 mg, 0.092 mmol) in THF/MeOH (2:1, 7.5 mL), and the biphasic mixture was stirred vigorously for 30 min. The mixture was poured into HCl (1N, 20 mL) and extracted with EtOAc. The organic layer was dried ($MgSO_4$) and the solvent removed to give a pale yellow oil (46 mg, 94%). The oil (16 mg, 0.030 mmol) was then treated with a solution of HCl/acetic acid (1 mL) for 30 min. The reaction mixture was diluted with toluene (10 mL) and the solvent removed from the resulting mixture. The resulting solid was rinsed with ether and then azeotroped with toluene to give an oil. CH_2Cl_2 (5 mL) was added and **10** precipitated out as a white solid (12.3 mg, 87%). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 11.87$ (br s, 1H), 10.07 (s, 1H), 8.06 (br s, 3H), 7.43 (d, $J = 7.0$ Hz, 2H), 7.39 (t, $J = 7.0$ Hz, 2H), 7.33 (t, $J = 7.0$ Hz, 1H), 7.21 (d, $J = 8.5$ Hz, 2H), 6.97 (d, $J = 8.5$ Hz, 2H), 5.07 (s, 2H), 3.81 (dd, $J = 8.0$ and 14.0 Hz, 1H), 3.53 (m, 1H), 3.46 (dd, $J = 4.0$ and 14.0 Hz, 1H), 2.87 (dd, $J = 5.5$ and 13.5 Hz, 1H), 2.75 (dd, $J = 8.0$ and 14.0 Hz, 1H), 2.36 (t, $J = 7.0$ Hz, 2H), 2.17 (t, $J = 7.5$ Hz, 2H), 1.46 (m, 4H), 1.24 (m, 4H); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): $\delta = 174.5$, 174.3, 157.4, 137.1, 130.5, 128.5, 128.0, 127.9, 127.7, 114.9, 69.2, 50.2, 48.7, 35.1, 33.6,

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31.7, 28.5, 28.4, 24.4, 23.8; HRMS $[M+Cs]^+$ calcd for $C_{24}H_{32}N_2O_5Cs$: 561.1366, found: 561.1379.

***N*-Hydroxy-*N*-[(2*S*)-2-amino-3-phenylpropyl]-9-carboxynonanamide (11)**: LiOH (1.0 M, 2.0 mL) was added to hydroxamate **7** (50 mg, 0.088 mmol) in THF/MeOH (2:1, 6.0 mL), and the biphasic mixture was stirred vigorously for 30 min. The mixture was poured into HCl (1N, 30 mL) and extracted with EtOAc. The organic layer was dried ($MgSO_4$) and the solvent removed to give a pale yellow oil (49 mg, 99%). The oil (16 mg, 0.029 mmol) was then treated with a solution of HCl/acetic acid (1 mL) for 30 min. The reaction mixture was diluted with toluene (10 mL) and the solvent removed from the resulting mixture. The resulting solid was rinsed with ether and then azeotroped with toluene to give **11** as a white solid (13.4 mg, 94%). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 11.96$ (br s, 1H), 10.08 (s, 1H), 8.08 (br s, 3H), 7.43 (d, $J = 7.0$ Hz, 2H), 7.39 (t, $J = 7.0$ Hz, 2H), 7.33 (t, $J = 7.0$ Hz, 1H), 7.21 (d, $J = 8.5$ Hz, 2H), 6.97 (d, $J = 8.5$ Hz, 2H), 5.07 (s, 2H), 3.81 (dd, $J = 8.0$ and 14.0 Hz, 1H), 3.53 (m, 1H), 3.47 (dd, $J = 4.5$ and 14.5 Hz, 1H), 2.88 (dd, $J = 5.5$ and 14.0 Hz, 1H), 2.75 (dd, $J = 8.0$ and 14.0 Hz, 1H), 2.35 (t, $J = 7.0$ Hz, 2H), 2.17 (t, $J = 7.0$ Hz, 2H), 1.46 (m, 4H), 1.23 (m, 8H); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): $\delta = 174.5$, 174.3, 157.4, 137.1, 130.5, 128.5, 128.0, 127.9, 127.7, 114.9, 69.2, 50.2, 48.7, 35.1, 33.7, 31.8, 28.8, 28.7, 28.6, 24.5, 23.9; HRMS $[M+Cs]^+$ calcd for $C_{26}H_{36}N_2O_5Cs$: 589.1679, found: 589.1695.

***N*-Hydroxy-*N*-[(2*S*)-*N*′-Boc-2-amino-3-(4-benzyloxyphenyl)propyl]-5-phenylpentanamide (12)**: Isobutyl chloroformate (76 μL, 0.59 mmol) was added to a solution of 5-phenylvaleric acid (127 mg, 0.71 mmol) and triethylamine (104 μL, 0.75 mmol) in THF (10 mL) at 0 °C. The resulting slurry was stirred for 30 min and then (2*S*)-2-*N*′-Boc-amino-3-(4-benzyloxyphenyl)-*N*-hydroxypropylamine (200 mg, 0.54 mmol) was added. The cooling bath was removed and the reaction mixture was stirred for 30 min. The mixture was then poured into CH_2Cl_2 (100 mL), washed (1N HCl, saturated $NaHCO_3$, saturated NaCl), and dried ($MgSO_4$). Purification by flash chromatography (1:3 EtOAc/hexanes) and recrystallization (THF/hexanes) yielded compound **12** as a white solid (172 mg, 60%). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 8.62$ (s, 1H), 7.43 (d, $J = 7$ Hz, 2H), 7.39 (t, $J = 7.5$ Hz, 2H), 7.33 (t, $J = 7.0$ Hz, 1H), 7.25 (dd, $J = 6.5$ and 7.5 Hz, 2H), 7.16 (d, $J = 7$ Hz, 3H), 7.10 (d, $J = 8.5$ Hz, 2H), 6.92 (d, $J = 8.5$ Hz, 2H), 5.05 (s, 2H), 4.65 (m, 1H), 4.22 (dd, $J = 13.5$ and 13.5 Hz, 1H), 4.13 (m, 1H), 3.03 (dd, $J = 13.5$ and 2.5 Hz, 1H), 2.77 (m, 2H), 2.61 (m, 2H), 2.57 (m, 1H), 2.32 (m, 1H), 1.64 (m, 4H), 1.36 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 174.6$, 158.2, 157.8, 142.4, 136.9, 130.1, 128.6, 128.4, 128.2, 128.0, 127.5, 125.6, 115.2, 80.9, 70.0, 50.5, 48.1, 37.3, 35.7, 32.3, 31.3, 28.2, 24.4; HRMS $[M+Cs]^+$ calcd for $C_{32}H_{40}N_2O_5Cs$: 665.1992, found: 665.1979.

***N*-Hydroxy-*N*-[(2*S*)-2-amino-3-(4-benzyloxyphenyl)propyl]-5-phenylpentanamide (13)**: Hydroxamate **12** (20 mg, 0.038 mmol) was treated with a solution of HCl/acetic acid (1 mL) for 30 min. The reaction mixture was diluted with toluene (10 mL) and the solvent removed from the resulting mixture. The resulting solid was rinsed with ether and then dissolved in CH_2Cl_2. Removal of the solvent gave **13** as a white solid (17.6 mg, 99%). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 10.09$ (s, 1H), 8.07 (br s, 3H), 7.43 (d, $J = 7.0$ Hz, 2H), 7.36 (t, $J = 7.0$ Hz, 2H), 7.32 (t, $J = 7.0$ Hz, 1H), 7.25 (t, $J = 7.0$ Hz, 2H), 7.21–7.13 (m, 3H), 7.19 (d, $J = 8.5$ Hz, 2H), 6.96 (d, $J = 8.5$ Hz, 2H), 5.06 (s, 2H), 3.80 (dd, $J = 7.0$ and 14.5 Hz, 1H), 3.52 (br, 1H), 3.45 (dd, $J = 4.5$ and 14.5 Hz, 1H), 2.87 (dd, $J = 4.5$ and 14.0 Hz, 1H), 2.75 (dd, $J = 8.0$ and 14.0 Hz, 1H), 2.55 (t, $J = 7.0$ Hz, 2H), 2.40 (br t, $J = 7.0$ Hz, 2H), 1.51 (m, 4H); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): $\delta = 174.2$, 157.4, 142.1, 137.1, 130.5, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.7, 125.6, 114.9, 69.2, 50.2, 48.7, 41.9, 35.11, 35.0, 31.6, 30.7, 23.6; HRMS $[M+Cs]^+$ calcd for $C_{27}H_{32}N_2O_3Cs$: 565.1467, found: 565.1478.

***N*-Hydroxy-*N*-[(2*S*)-2-amino-3-(4-benzyloxyphenyl)propyl]-5-aminopentanamide (15)**: Hydroxamate **14** (19.1 mg, 0.033 mmol) was treated with a solution of HCl/acetic acid (1 mL) for 30 min. The reaction mixture was diluted with toluene (10 mL) and the solvent removed from the resulting mixture. The resulting solid was rinsed with ether and then dissolved in CH_2Cl_2. Removal of the solvent gave **15** as a white solid (14.7 mg, 99%). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 10.25$ (s, 1H), 8.19 (br s, 3H), 7.93 (br s, 3H), 7.43 (d, $J = 7.5$ Hz, 2H), 7.39 (t, $J = 7.5$ Hz, 2H), 7.33 (t, $J = 7.5$ Hz, 1H), 7.21 (d, $J = 8.5$ Hz, 2H), 6.96 (d, $J = 8.5$ Hz, 2H), 5.07 (s, 2H), 3.81 (dd, $J = 7.0$ and 14.5 Hz, 1H), 3.53 (br m, 1H), 3.49 (dd, $J = 4.5$ and 14.5 Hz, 1H), 2.90 (dd, $J = 4.5$ and 14.0 Hz, 1H), 2.76 (m, 1H), 2.74 (m, 2H), 2.42 (br t, 2H), 1.53 (m, 4H); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): $\delta = 173.9$, 157.4, 137.1, 128.5, 128.1 127.9, 127.7, 114.9, 69.2, 50.2, 48.6, 38.6, 35.1, 31.2, 26.6, 20.8; HRMS $[M+Cs]^+$ calcd for $C_{21}H_{29}N_3O_3Cs$: 504.1263, found: 504.1277.

Benzyl 4-pentenoate: 4-Pentenoic acid (2.0 g, 20 mmol), benzyl bromide (2.8 mL, 24 mmol), anhydrous potassium carbonate (13.8 g, 100 mmol), and tetrabutylammonium iodide (500 mg) were combined in anhydrous acetone (40 mL) and stirred overnight at room temperature. The reaction mixture was filtered and the solvent removed. The residue was taken up in EtOAc, washed (1N HCl, saturated $NaHCO_3$, saturated NaCl) and dried ($MgSO_4$). Purification by flash chromatography (1:30 EtOAc/hexanes then 1:1 EtOAc/hexanes) gave compound benzyl 4-pentenoate as a yellow liquid (3.6 g, 96%). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 7.36$ (m, 5H), 5.84 (ddt, $J = 6.0$, 10.5 and 17 Hz, 1H), 5.12 (s, 2H), 5.05 (ddt, $J = 17$, 1.5, and 1.5 Hz, 2H), 5.00 (ddt, $J = 10$, 1.5, and 1.5 Hz, 2H), 2.47 (m, 2H), 2.40 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 172.9$, 159.8, 136.5, 135.9, 128.5, 128.2, 115.5, 66.2, 33.5, 28.8; HRMS $[M+H]^+$ calcd for $C_{12}H_{15}O_2$: 191.1071, found: 191.1062.

Benzyl 4-oxobutyrate (16): Ozone was bubbled through a solution of benzyl 4-pentenoate (3.6 g, 19.0 mmol) in a 3:1 mixture of CH_2Cl_2 and MeOH (100 mL) at −78 °C until a blue color persisted. Oxygen was then bubbled through until the solution was colorless again and then excess dimethyl sulfide (4.4 mL) was added. The reaction mixture was stirred for 30 min, before $NaHCO_3$ (5%, 100 mL) was added and the cooling bath removed. The layers were separated and the aqueous layer extracted with CH_2Cl_2. TLC analysis showed two overlapping spots. After leaving to stand in CH_2Cl_2 for 2 days, TLC analysis showed only one spot, namely the aldehyde, suggesting that the other spot was the ozonide. Purification by flash chromatography (1:3 EtOAc/hexanes) yielded pure aldehyde **16** (3.36 g, 92%). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 9.80$ (s, 1H), 7.35 (m, 5H), 5.13 (s, 2H), 2.81 (t, $J = 6.5$ Hz, 2H), 2.68 (t, $J = 6.5$ Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 199.9$, 172.0, 135.6, 128.5, 128.2, 128.2, 66.6, 38.4, 26.5; HRMS $[M+H]^+$ calcd for $C_{11}H_{13}O_3$: 193.0865, found: 193.0860.

Benzyl *N*-Boc-*N*-[(1*S*)-1-carboxymethylethyl]-4-aminobutyrate (17): A solution of L-alanine methyl ester hydrogen chloride (394 mg, 2.8 mmol) in CH_2Cl_2 (10 mL) was treated with triethylamine (390 μL, 2.8 mmol) and the resulting salts were filtered off to give a solution of the free amine. This was added to a solution of aldehyde **16** (500 mg, 2.6 mmol) in CH_2Cl_2 (20 mL), and $MgSO_4$ (500 mg) was added. The mixture was stirred at room temperature for 2 h. The reaction mixture was then filtered and the solvent removed to give the imine. This was taken up in MeOH (20 mL, 0 °C) and sodium borohydride (295 mg, 6.5 mmol) was then added in one portion and the resulting mixture was stirred for 30 min. Saturated NH_4Cl (20 mL) was added and the methanol removed in vacuo. CH_2Cl_2 (40 mL) was added and the layers separated. The aqueous layer was extracted twice more with CH_2Cl_2 and the combined organic layers washed (saturated $NaHCO_3$, saturated NaCl) and dried ($MgSO_4$). Removal of the solvent gave the secondary amine (0.65 g, 88%) which was used without further purification.

Di-*tert*-butyl pyrocarbonate (550 mg, 2.53 mmol) was added to a solution of the amine (0.65 g, 2.3 mmol) in dioxane (2 mL), THF (2 mL), and water (2 mL) under argon at 0 °C and stirring was continued at room temperature. The reaction was complete after 30 min (TLC, 1:1 EtOAc/hexanes). Water (10 mL) was added and the product was extracted with EtOAc. The organic layer was dried ($MgSO_4$) and the solvent removed in vacuo to give compound **17** as a colorless oil (784 mg, 90%). This product was used without further purification.

Benzyl *N*-Boc-*N*-[(1*R*)-1-carboxymethylethyl]-4-aminobutyrate (18): A solution of D-alanine methyl ester hydrogen chloride (394 mg, 2.8 mmol) in CH_2Cl_2 (10 mL) was treated with triethylamine (390 μL, 2.8 mmol) and the resulting salts were filtered off to give a solution of the free amine. This was added to a solution of aldehyde **16** (500 mg, 2.6 mmol) in CH_2Cl_2 (20 mL), and $MgSO_4$ (500 mg) was added. The mixture was stirred at room temperature for 2 h and then filtered, and the solvent removed to give the imine. This was taken directly up in MeOH (20 mL) at 0 °C, and sodium borohydride (295 mg, 6.5 mmol) was added in one portion and the mixture was stirred for 30 min. Saturated NH_4Cl (20 mL) was added and the methanol removed in vacuo. CH_2Cl_2 (40 mL) was added and the layers separated. The aqueous layer was extracted twice more with CH_2Cl_2 and the combined organic layers washed (saturated $NaHCO_3$, saturated NaCl)

and dried ($MgSO_4$). Removal of the solvent gave the secondary amine (0.65 g, 88%) which was used without further purification.

Di-*tert*-butyl pyrocarbonate (550 mg, 2.53 mmol) was added to a solution of the amine (0.65 g, 2.3 mmol) in CH_2Cl_2 (10 mL) under argon at 0 °C and stirring was continued overnight at room temperature. The solvent was removed in vacuo and purification by flash chromatography gave compound **18** as a colorless oil (680 mg, 83%). This compound exists as two conformers in $CDCl_3$. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 7.32$ (m, 5H), 5.12 (s, 2H), 4.39 (m, 0.5H), 3.93 (m, 0.5H), 3.69 (s, 3H), 3.43 (m, 0.5H), 3.34 (m, 0.5H), 3.17 (m, 0.5H), 3.08 (m, 0.5H), 2.39 (m, 2H), 1.91 (m, 2H), 1.44 (d, $J = 7.0$ Hz, 3H) 1.42 (s, 4H), 1.41 (s, 5H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 173.1$, 172.9, 172.6, 155.4, 154.8, 135.9, 128.5, 128.2, 80.4, 80.4, 66.2, 55.9, 54.7, 53.4, 52.0, 46.5, 45.4, 31.4, 31.2, 28.3, 24.9, 24.0, 15.9, 15.3; HRMS $[M+H]^+$ calcd for $C_{20}H_{30}NO_6$: 380.2073, found: 380.2079.

Benzyl *N*-Boc-*N*-[(1*S*)-1,3-dicarboxymethylpropyl]-4-aminobutyrate (19): A solution of L-glutamine dimethyl ester hydrogen chloride (548 mg, 2.6 mmol) in CH_2Cl_2 (20 mL) was treated with triethylamine (365 μL, 2.6 mmol) and the resulting salts were filtered off to give a solution of the free amine. This was added to a solution of aldehyde **16** (500 mg, 2.6 mmol) in CH_2Cl_2 (20 mL), and $MgSO_4$ (500 mg) was added. The mixture was stirred at room temperature for 2 h. The reaction mixture was then filtered and the solvent removed to give the imine. This was taken directly up in MeOH (10 mL) at 0 °C, sodium borohydride (295 mg, 6.5 mmol) was added in one portion, and the reaction mixture stirred for 30 min. The reaction mixture was worked up as for **18** to yield the secondary amine (0.75 g, 82%) which was used immediately without further purification.

Di-*tert*-butyl pyrocarbonate (545 mg, 2.5 mmol) was added to a solution of the amine (0.75 g, 2.14 mmol) in CH_2Cl_2 (5 mL) under argon at 0 ° C and stirring was continued overnight at room temperature. Additional di-*tert*-butyl pyrocarbonate (150 mg, 0.69 mmol) was added and stirring continued for 24 h. The solvent was removed in vacuo and purification by flash chromatography gave compound **19** as a colorless oil (844 mg, 72% from the aldehyde). This compound exists as two conformers in $CDCl_3$. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 7.35$ (m, 5H), 5.12 (s, 2H), 4.23 (br, 0.5H), 3.92 (br, 0.5H), 3.69 (s, 3H), 3.68 (s, 3H), 3.52 (br, 0.5H), 3.34 (br, 0.5H), 3.03 (m, 1H), 2.42–2.33 (m, 5H), 2.09 (m, 1H), 1.90 (m, 2H), 1.44 (s, 4H), 1.40 (s, 5H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 173.3$, 173.0, 172.6, 171.6, 155.4, 154.7, 136.0, 128.5, 128.2, 80.7, 80.5, 66.2, 59.5, 58.8, 52.1, 51.7, 47.8, 46.7, 31.4, 31.3, 30.4, 28.2, 25.5, 24.7, 24.3, 23.6; HRMS $[M+Cs]^+$ calcd for $C_{23}H_{33}NO_8Cs$: 584.1261, found: 584.1277.

Benzyl *N*-Boc-*N*-[(1*S*)-1,4-dicarboxymethylbutyl]-4-aminobutyrate (20): A solution of 2-L-aminoadipic acid dimethyl ester hydrogen chloride (450 mg, 2.0 mmol) in CH_2Cl_2 (20 mL) was treated with triethylamine (300 μL, 2.16 mmol) and the resulting salts were filtered off to give a solution of the free amine. This was added to a solution of aldehyde **16** (500 mg, 2.6 mmol) in CH_2Cl_2 (20 mL) and $MgSO_4$ (500 mg) was added. The mixture was stirred at room temperature for 4 h. The reaction mixture was then filtered and the solvent removed to give the imine. This was taken directly up in MeOH (10 mL) at 0 °C), sodium borohydride (295 mg, 6.5 mmol) was added in one portion, and the reaction mixture stirred for 30 min. The resulting mixture was worked up as for **18** to yield the secondary amine (0.72 g, 98%) which was used immediately without further purification.

Di-*tert*-butyl pyrocarbonate (500 mg, 2.3 mmol) was added to a solution of the crude amine (0.72 g, 2.0 mmol) in CH_2Cl_2 (5 mL) under argon at 0 °C and stirring was continued overnight at room temperature. Additional di-*tert*-butyl pyrocarbonate (200 mg, 0.92 mmol) was added and stirring continued for 48 h. The solvent was removed in vacuo and purification by flash chromatography gave compound **20** as a colorless oil (580 mg, 62% from the amine). This compound exists as two conformers in $CDCl_3$. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 7.35$ (m, 5H), 5.11 (s, 2H), 4.29 (br, 0.5H), 3.86 (br, 0.5H), 3.67 (s, 3H), 3.66 (s, 3H), 3.50 (br, 0.5H), 3.31 (br, 0.5H), 3.03 (m, 1H), 2.42–2.33 (m, 4H), 2.00–1.78 (m, 4H), 1.64 (m, 2H), 1.44 (s, 4H), 1.40 (s, 5H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 173.5$, 173.0, 172.8, 172.1, 171.8, 155.5, 154.8, 135.9, 135.9, 128.5, 128.2, 80.5, 80.5, 66.2, 60.2, 59.0, 52.0, 51.5, 47.4, 46.0, 33.6, 33.5, 31.5, 31.3, 29.7, 28.7, 28.2, 24.5, 23.7, 21.9, 21.8; HRMS $[M+Cs]^+$ calcd for $C_{24}H_{35}NO_8Cs$: 598.1417, found: 598.1431.

Benzyl *N*-Boc-*N*-[(1S)-1-carboxymethyl-5-*N'*-Boc-aminopentyl]-4-aminobutyrate (21): A solution of ε-*N*-Boc-L-lysine methyl ester hydrogen chloride (775 mg, 2.6 mmol) in CH_2Cl_2 (20 mL) was treated with triethylamine (365 μL, 2.6 mmol) and the resulting salts were filtered off to give a solution of the free amine. This was added to a solution of aldehyde **16** (500 mg, 2.6 mmol) in CH_2Cl_2 (20 mL), and $MgSO_4$ (500 mg) was added. The mixture was stirred at room temperature for 2 h. The reaction mixture was then filtered and the solvent removed to give the imine. This was taken up directly in MeOH (10 mL) at 0 °C, sodium borohydride (295 mg, 6.5 mmol) was added in one portion, and the reaction mixture stirred for 30 min. The resulting mixture was worked up as for **18** to yield the secondary amine which was used immediately without further purification.

Di-*tert*-butyl pyrocarbonate (625 mg, 2.89 mmol) was added to a solution of the amine in CH_2Cl_2 (10 mL) under argon at 0 °C and stirring was continued overnight at room temperature. Additional di-*tert*-butyl pyrocarbonate (200 mg) was added and stirring continued for 24 h. The solvent was removed in vacuo and purification by flash chromatography gave compound **21** as a colorless oil (1.28 g, 83%). This compound exists as two conformers in $CDCl_3$. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 7.35$ (m, 5H), 5.12 (s, 2H), 4.58 (br, 1H), 4.33 (m, 0.5H), 3.85 (br, 0.5H), 3.68 (s, 3H), 3.49 (br, 0.5H), 3.29 (br, 0.5H), 3.11 (m, 2H), 3.05 (m, 1H), 2.40 (m, 2H), 2.1–1.7 (m, 4H), 1.49 (m, 2H), 1.45 (s, 5H), 1.44 (s, 9H), 1.40 (s, 4H), 1.35 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 172.9$, 172.1, 155.9, 128.5, 128.2, 80.5, 79.1, 66.2, 60.4, 59.0, 52.0, 47.3, 45.7, 40.3, 31.6, 31.3, 30.0, 29.8, 29.5, 29.0, 28.4, 28.3, 24.6, 23.8, 23.5; HRMS $[M+Cs]^+$ calcd for $C_{28}H_{44}N_2O_8Cs$: 669.2152, found: 669.2166.

***N*-Boc-*N*-[(1*S*)-1-carboxymethylethyl]-4-aminobutyric acid (22)**: A solution of benzyl ester **17** (660 mg, 1.74 mmol) in EtOAc (10 mL) was stirred vigorously in the presence of Pd/C (10% on carbon, 30 mg) under a hydrogen atmosphere until no ester remained (2 h). The hydrogen was then evacuated and replaced with argon. The reaction mixture was filtered through Celite and the solvent removed to give **22** as a colorless oil (499 mg, 99%) which was used without further purification. This compound exists as two conformers in $CDCl_3$. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 4.15$ (m, 0.5H), 3.96 (m, 0.5H), 3.72 (s, 3H), 3.50 (m, 0.5H), 3.38 (m, 0.5H), 3.20 (m, 0.5H), 3.11 (m, 0.5H), 3.03 (m, 1H), 2.42–2.33 (m, 4H), 2.00–1.78 (m, 4H), 1.64 (m, 2H), 1.44 (s, 4H), 1.40 (s, 5H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 178.6$, 178.2, 172.9, 172.5, 155.1, 80.8, 80.4, 55.9, 54.7, 52.1, 46.3, 45.2, 31.1, 28.2, 24.6, 24.0, 15.8, 15.3; HRMS $[M+Cs]^+$ calcd for $C_{13}H_{23}NO_6Cs$: 422.0580, found: 422.0590.

***N*-Boc-*N*-[(1*R*)-1-carboxymethylethyl]-4-aminobutyric acid (23)**: A solution of benzyl ester **18** (660 mg, 1.74 mmol) in EtOAc (10 mL) was stirred vigorously in the presence of Pd/C (10% on carbon, 30 mg) under a hydrogen atmosphere until no ester remained (2 h). The hydrogen was then evacuated and replaced with argon. The reaction mixture was filtered through Celite and the solvent removed to give **23** as a colorless oil (499 mg, 99%) which was used without further purification. This compound exists as two conformers in $CDCl_3$. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 4.15$ (m, 0.5H), 3.96 (m, 0.5H), 3.72 (s, 3H), 3.50 (m, 0.5H), 3.38 (m, 0.5H), 3.20 (m, 0.5H), 3.11 (m, 0.5H), 3.03 (m, 1H), 2.42–2.33 (m, 4H), 2.00–1.78 (m, 4H), 1.64 (m, 2H), 1.44 (s, 4H), 1.40 (s, 5H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 178.6$, 178.2, 172.9, 172.5, 155.1, 80.8, 80.4, 55.9, 54.7, 52.1, 46.3, 45.2, 31.1, 28.2, 24.6, 24.0, 15.8, 15.3; HRMS $[M+Cs]^+$ calcd for $C_{13}H_{23}NO_6Cs$: 422.0580, found: 422.0590.

***N*-Boc-*N*-[(1*S*)-1,3-dicarboxymethylpropyl]-4-aminobutyric acid (24)**: A solution of benzyl ester **19** (820 mg, 1.8 mmol) in EtOAc (15 mL) was stirred vigorously in the presence of Pd/C (10% on carbon, 35 mg) under a hydrogen atmosphere until no ester remained (2 h). The hydrogen was then evacuated and replaced with argon. The reaction mixture was filtered through Celite and the solvent removed. Purification by flash chromatography (1:1 EtOAc/hexanes then EtOAc) yielded **24** as a colorless oil (494 mg, 76%). This compound exists as two conformers in $CDCl_3$. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 4.24$ (m, 0.5H), 3.95 (m, 0.5H), 3.73 (s, 3H), 3.69 (s, 3H), 3.57 (m, 0.5H), 3.38 (m, 0.5H), 3.06 (m, 1H), 2.47–2.36 (m, 5H), 2.11 (m, 1H), 1.89 (m, 2H), 1.46 (s, 4H), 1.41 (s, 5H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): 178.8, 178.5, 173.4, 171.7, 171.6, 155.4, 155.0, 81.0, 80.6, 59.5, 58.8, 52.2, 51.7, 47.7, 46.6, 31.2, 30.5, 30.4, 28.2, 25.5, 24.7, 24.0, 23.5; HRMS $[M+Na]^+$ calcd for $C_{16}H_{27}NO_8Na$: 384.1634, found: 384.1645.

***N*-Boc-*N*-[(1*S*)-1,4-dicarboxymethylbutyl]-4-aminobutyric acid (25)**: A solution of benzyl ester **20** (560 mg, 1.2 mmol) in EtOAc (10 mL) was

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stirred vigorously with Pd/C (10% on carbon, 25 mg) under a hydrogen atmosphere until no ester remained (2 h). The reaction mixture was filtered through Celite and the solvent removed. Purification by flash chromatography (1:1 EtOAc/hexanes, then EtOAc) gave **25** as a colorless oil (395 mg, 90%). This compound exists as two conformers in $CDCl_3$. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 4.32$ (m, 0.5H), 3.90 (m, 0.5H), 3.72 (s, 3H), 3.68 (s, 3H), 3.55 (m, 0.5H), 3.35 (m, 0.5H), 3.08 (m, 1H), 2.48–2.31 (m, 2H), 2.37 (t, $J = 7.0$ Hz, 2H), 2.02 (m, 1H), 1.96–1.80 (m, 3H), 1.69 (m, 2H), 1.46 (s, 4H), 1.42 (s, 5H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta =$ 178.8, 178.5, 173.7, 173.5, 172.1, 171.8, 155.6, 155.1, 80.9, 80.5, 60.3, 59.0, 52.1, 51.6, 47.2, 45.8, 33.6, 33.5, 31.2, 29.7, 28.7, 28.2, 24.2, 23.6, 21.9, 21.8; HRMS $[M+Na]^+$ calcd for $C_{17}H_{29}NO_8Na$: 398.1791, found: 398.1776.

***N*-Boc-*N*-[(1*S*)-1-carboxymethyl-5-*N*′-Boc-aminopentyl]-4-aminobutyric acid** (**26**): A solution of benzyl ester **21** (1.28 g, 2.39 mmol) in EtOAc (15 mL) was stirred vigorously in the presence of Pd/C (10% on carbon, 45 mg) under a hydrogen atmosphere until no ester remained (2 h). The hydrogen was then evacuated and replaced with argon. The reaction mixture was filtered through Celite and the solvent removed. Purification by flash chromatography (1:1 EtOAc/hexanes then EtOAc) yielded **26** as a colorless oil (946 mg, 89%). This compound exists as two conformers in $CDCl_3$. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 5.71$ (m, 0.5H), 4.65 (m, 1H), 4.33 (m, 0.5H), 3.87 (m, 0.5H), 3.70 (s, 3H), 3.53 (m, 0.5H), 3.31 (m, 0.5H), 3.11–3.04 (m, 3H), 2.40–2.30 (m, 2H), 2.10–1.70 (m, 4H), 1.53 (m, 2H), 1.46 (s, 4H), 1.43 (s, 9H), 1.40 (s, 5H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 178.0$, 177.9, 172.3, 172.0, 156.0, 155.7, 155.1, 80.7, 80.3, 79.1, 60.3, 58.9, 52.0, 47.0, 45.5, 40.2, 31.1, 29.8, 29.7, 29.5, 28.9, 28.3, 28.2, 24.3, 23.6, 23.4; HRMS $[M+Cs]^+$ calcd for $C_{21}H_{38}N_2O_8Cs$: 579.1682, found: 579.1664.

***N*-Hydroxy-*N*-[(2*S*)-2-*N*″-Boc-amino-3-(4-benzyloxyphenyl)propyl]-*N*′-Boc-*N*′-[(1*S*)-1-carboxymethylethyl]-4-aminobutanamide** (**27**): Isobutyl chloroformate (39 μL, 0.30 mmol) was added to a solution of acid **22** (87 mg, 0.30 mmol) and triethylamine (42 μL, 0.30 mmol) in THF (5 mL, 0 °C). The resulting slurry was stirred for 30 min and then hydroxylamine (2*S*)-2-*N*′-Boc-amino-3-(4-benzyloxyphenyl)-*N*-hydroxypropylamine (100 mg, 0.27 mmol) was added. The cooling bath was removed and the reaction mixture stirred for 30 min. The reaction mixture was then poured into CH_2Cl_2 (50 mL), washed (1N HCl, saturated $NaHCO_3$, saturated NaCl), and dried ($MgSO_4$). Purification by flash chromatography (1:2 EtOAc/hexanes) yielded compound **27** as a colorless oil (109 mg, 63%). This compound exists as two conformers in $CDCl_3$. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 8.66$ (s, 1H), 7.43 (d, $J = 7$ Hz, 2H), 7.39 (t, $J = 7.5$ Hz, 2H), 7.33 (t, $J = 7.0$ Hz, 1H), 7.10 (d, $J = 8.5$ Hz, 2H), 6.93 (d, $J = 8.5$ Hz, 2H), 5.05 (s, 2H), 4.62 (m, 1H), 4.45 (m, 0.5H), 4.19 (m, 2H), 3.96 (m, 0.5H), 3.71 (s, 3H), 3.38–3.15 (m, 2H), 3.09 (m, 1H), 2.81 (m, 2H), 2.62 (m, 1H), 2.31 (m, 1H), 1.87 (m, 2H), 1.45 (s, 9H), 1.40 (s, 3H), 1.38 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 174.1$, 173.9, 172.9, 172.6, 158.2, 157.8, 155.4, 154.8, 136.9, 130.0, 128.7, 128.5, 127.9, 127.4, 115.1, 80.9, 80.7, 80.3, 80.0, 70.0, 55.9, 54.7, 52.0, 50.51, 48.4, 48.1, 47.1, 45.9, 37.3, 37.2, 34.6, 31.5, 30.0, 29.1, 28.3, 24.7, 23.7, 22.6, 16.0, 15.9, 15.4, 14.1; HRMS $[M+Cs]^+$ calcd for $C_{34}H_{49}N_3O_9Cs$: 776.2523, found: 776.2505.

***N*-Hydroxy-*N*-[(2*S*)-2-*N*″-Boc-amino-3-(4-benzyloxyphenyl)propyl]-*N*′-Boc-*N*′-[(1*R*)-1-carboxymethylethyl]-4-aminobutanamide** (**28**): Isobutyl chloroformate (77 μL, 0.59 mmol) was added to a solution of acid **23** (189 mg, 0.65 mmol) and triethylamine (105 μL, 0.75 mmol) in THF (10 mL) at 0 °C. The resulting slurry was stirred for 30 min and then (2*S*)-2-*N*′-Boc-amino-3-(4-benzyloxyphenyl)-*N*-hydroxypropylamine (200 mg, 0.54 mmol) was added. The cooling bath was removed and the reaction stirred for 30 min. The reaction was then quenched by addition of 3-dimethylaminopropylamine and poured into CH_2Cl_2 (60 mL), washed (1N HCl, saturated $NaHCO_3$, saturated NaCl), and dried ($MgSO_4$). Purification by flash chromatography (1:2 EtOAc/hexanes) yielded compound **28** as a colorless oil (285 mg, 82%). This compound exists as two conformers in $CDCl_3$. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 8.65$ (d, $J = 12.0$ Hz, 1H), 7.43 (d, $J = 7.0$ Hz, 2H), 7.39 (t, $J = 7.0$ Hz, 2H), 7.33 (t, $J = 7.0$ Hz, 1H), 7.10 (d, $J = 8.5$ Hz, 2H), 6.93 (d, $J = 8.5$ Hz, 2H), 5.05 (s, 2H), 4.65 (m, 1H), 4.44 (m, 0.5H), 4.18 (m, 1H), 4.15 (m, 1H), 3.96 (m, 0.5H), 3.69 (s, 3H), 3.35 (m, 0.5H), 3.31 (m, 0.5H), 2.76 (m, 2H), 2.59 (m, 0.5H), 2.31 (m, 1H), 1.85 (m, 2H), 1.45 (s, 4H), 1.40 (s, 5H), 1.38 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 174.1$, 173.9, 172.9, 172.6, 158.2, 157.8, 155.5, 154.8, 136.9, 130.1, 128.6, 128.0, 127.5, 115.1, 81.0, 80.8, 80.4, 80.0, 70.0, 55.9, 54.7, 52.0, 50.5, 48.3, 48.1, 47.2, 45.9, 37.3, 37.2, 30.0, 29.2, 28.3, 28.2, 24.7, 23.7, 16.0, 16.0, 15.4; HRMS $[M+Cs]^+$ calcd for $C_{34}H_{49}N_3O_9Cs$: 776.2523, found: 776.2544.

***N*-Hydroxy-*N*-[(2*S*)-2-*N*″-Boc-amino-3-(4-benzyloxyphenyl)propyl]-*N*′-Boc-*N*′-[(1*S*)-1,3-dicarboxymethylpropyl]-4-aminobutanamide** (**29**): Isobutyl chloroformate (77 μL, 0.59 mmol) was added to a solution of acid **24** (234 mg, 0.65 mmol) and triethylamine (105 μL, 0.75 mmol) in THF (10 mL) at 0 °C. The resulting slurry was stirred for 30 min and then (2*S*)-2-*N*′-Boc-amino-3-(4-benzyloxyphenyl)-*N*-hydroxypropylamine (200 mg, 0.54 mmol) was added. The cooling bath was removed and the reaction stirred for 30 min. The reaction was then quenched by addition of 3-dimethylaminopropylamine and poured into CH_2Cl_2 (60 mL), washed (1N HCl, saturated $NaHCO_3$, saturated NaCl), and dried ($MgSO_4$). Purification by flash chromatography (1:2 EtOAc/hexanes) yielded compound **29** as a colorless oil (293 mg, 76%). This compound exists as two conformers in $CDCl_3$. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 8.65$ (br s, 0.5H), 8.61 (br s, 0.5H), 7.43 (d, $J = 7.0$ Hz, 2H), 7.39 (t, $J = 7.0$ Hz, 2H), 7.33 (t, $J = 7.0$ Hz, 1H), 7.10 (d, $J = 8.5$ Hz, 2H), 6.93 (d, $J = 8.5$ Hz, 2H), 5.05 (s, 2H), 4.66 (m, 1H), 4.28 (m, 0.5H), 4.18 (m, 1H), 4.15 (m, 1H), 3.96 (m, 0.5H), 3.70 (s, 3H), 3.67 (s, 3H), 3.48 (m, 0.5H), 3.30 (m, 0.5H), 3.09–2.95 (m, 2H), 2.77 (m, 2H), 2.61 (m, 1H), 2.41 (m, 2H), 2.41–2.20 (m, 2H), 2.11 (br, 1H), 1.85 (m, 2H), 1.45 (s, 4H), 1.40 (s, 5H), 1.38 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 174.0$, 173.9, 173.8, 173.4, 171.8, 171.7, 158.2, 157.9, 157.8, 155.5, 154.8, 136.9, 130.1, 128.7, 128.6, 128.0, 127.4, 115.1, 80.8, 80.7, 80.4, 80.1, 70.0, 59.6, 59.5, 52.1, 51.7, 50.5, 48.3, 48.1, 47.1, 37.3, 37.2, 30.6, 30.6, 30.0, 29.9, 29.3, 29.2, 28.2, 28.2, 25.6, 24.7, 24.1, 23.3; HRMS $[M+Cs]^+$ calcd for $C_{37}H_{53}N_3O_{11}Cs$: 848.2734, found: 848.2755.

***N*-Hydroxy-*N*-[(2*S*)-2-*N*″-Boc-amino-3-(4-benzyloxyphenyl)propyl]-*N*′-Boc-*N*′-[(1*S*)-1,4-dicarboxymethylbutyl]-4-aminobutanamide** (**30**): Isobutyl chloroformate (75 μL, 0.59 mmol) was added to a solution of acid **25** (215 mg, 0.57 mmol) and triethylamine (110 μL, 0.76 mmol) in THF (10 mL) at 0 °C. The resulting slurry was stirred for 30 min and then (2*S*)-2-*N*′-Boc-amino-3-(4-benzyloxyphenyl)-*N*-hydroxypropylamine (200 mg, 0.54 mmol) was added. The cooling bath was removed and the reaction mixture stirred for 30 min. The reaction was then quenched by addition of 3-dimethylaminopropylamine and poured into CH_2Cl_2 (60 mL), washed (1N HCl, saturated $NaHCO_3$, saturated NaCl), and dried ($MgSO_4$). Purification by flash chromatography (1:2 EtOAc/hexanes) yielded compound **30** as a colorless oil (292 mg, 75%). This compound exists as two conformers in $CDCl_3$. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 8.68$ (br s, 0.5H), 8.65 (br s, 0.5H), 7.43 (d, $J = 7.0$ Hz, 2H), 7.39 (t, $J = 7.0$ Hz, 2H), 7.33 (t, $J = 7.0$ Hz, 1H), 7.10 (d, $J = 8.5$ Hz, 2H), 6.93 (d, $J = 8.5$ Hz, 2H), 5.03 (s, 2H), 4.80 (m, 0.5H), 4.76 (br d, J = 9.0 Hz, 0.5H), 4.31 (m, 0.5H), 4.20–4.05 (m, 2H), 3.89 (m, 0.5H), 3.68 (s, 3H), 3.65 (s, 3H), 3.45 (m, 0.5H), 3.27 (m, 0.5H), 3.09–2.96 (m, 2H), 2.76 (m, 2H), 2.60 (m, 1H), 2.41–2.20 (m, 3H), 1.98 (br, 1H), 1.83 (m, 3H), 1.67 (m, 2H), 1.45 (s, 4H), 1.39 (s, 5H), 1.37 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 173.9$, 173.8, 173.7, 173.4, 172.1, 171.9, 158.1, 157.9, 157.7, 155.6, 154.8, 136.8, 130.0, 128.7, 128.5, 127.9, 127.4, 115.0, 80.8, 80.6, 80.4, 80.1, 70.0, 60.2, 60.1, 58.9, 51.9, 51.5, 50.5, 48.3, 48.1, 47.9, 46.5, 37.2, 37.2, 33.7, 33.5, 30.0, 29.7, 29.6, 29.2, 28.8, 28.2, 28.1, 24.2, 23.3, 21.9, 21.8; HRMS $[M+Cs]^+$ calcd for $C_{38}H_{55}N_3O_{11}Cs$: 862.2891, found: 862.2868.

***N*-Hydroxy-*N*-[(2*S*)-2-*N*″-Boc-amino-3-(4-benzyloxyphenyl)propyl]-*N*′-Boc-*N*′-[(1*S*)-1-carboxymethyl-5-*N*′-Boc-aminopentyl]-4-aminobutanamide** (**31**): Isobutyl chloroformate (77 μL, 0.59 mmol) was added to a solution of acid **26** (290 mg, 0.65 mmol) and triethylamine (105 μL, 0.75 mmol) in THF (10 mL) at 0 °C. The resulting slurry was stirred for 30 min and then (2*S*)-2-*N*′-Boc-amino-3-(4-benzyloxyphenyl)-*N*-hydroxypropylamine (200 mg, 0.54 mmol) was added. The cooling bath was removed and the reaction mixture stirred for 30 min. The reaction was then quenched by addition of 3-dimethylaminopropylamine and poured into CH_2Cl_2 (60 mL), washed (1N HCl, saturated $NaHCO_3$, saturated NaCl), and dried ($MgSO_4$). Purification by flash chromatography (1:2 EtOAc/hexanes) yielded compound **31** as a colorless oil (389 mg, 90%). This compound exists as two conformers in $CDCl_3$. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): $\delta = 8.67$ (br d, $J = 14.0$ Hz, 1H), 7.43 (d, $J = 7.0$ Hz, 2H), 7.39 (t, $J = 7.0$ Hz, 2H), 7.33 (t, $J = 7.0$ Hz, 1H), 7.10 (d, $J = 8.5$ Hz, 2H), 6.93 (d, $J = 8.5$ Hz, 2H), 5.05 (s, 2H), 4.79 (m, 1H), 4.65 (m, 1H), 4.38 (m, 0.5H), 4.19 (m, 1H), 4.15 (m, 1H), 3.88 (m, 0.5H), 3.69 (s, 3H), 3.42 (m, 0.5H), 3.25 (m, 0.5H), 3.13 (m, 1H), 3.06 (m, 1H), 2.78 (m, 2H), 2.59 (m, 0.5H), 2.31 (m, 1H), 1.95 (m, 1H), 1.85 (br, 2H), 1.51 (m, 2H), 1.45 (s, 3H), 1.43 (s, 6H), 1.40 (s, 3H), 1.38 (s, 6H), 1.38 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): $\delta = 174.0$, 173.8, 172.4, 172.2, 158.2, 157.9, 157.8, 156.0, 155.0, 136.9, 130.1, 128.6, 128.0, 127.4, 115.1, 80.8, 80.7, 80.4, 80.1, 79.0, 70.0, 60.4, 60.3, 59.0, 52.0, 50.5, 48.4, 48.2,

47.8, 46.3, 46.2, 40.3, 37.2, 30.0, 29.8, 29.7, 29.5, 29.2, 29.0, 28.4, 28.3, 28.2, 24.3, 23.9, 23.6, 23.3; HRMS $[M+Cs]^+$ calcd for $C_{42}H_{64}N_4O_{11}Cs$: 933.3626, found: 933.3649.

***N*-Hydroxy-*N*-[(2*S*)-2-amino-3-(4-benzyloxyphenyl)propyl]-*N*′-[(1*S*)-1-carboxymethylethyl]-4-aminobutanamide, HCl salt (32)**: Hydroxamate **27** (44 mg, 0.068 mmol) was treated with a solution of HCl/acetic acid (1 mL) for 30 min. The reaction mixture was diluted with toluene (10 mL) and the solvent removed from the resulting mixture. The resulting solid was rinsed with ether and then azeotroped with toluene to give **32** as a white solid (28.1 mg, 94 %). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 10.31$ (s, 1H), 9.64 (brs, 1H), 9.27 (brs, 1H), 8.22 (brs, 3H), 7.43 (d, $J = 7.0$ Hz, 2H), 7.39 (t, $J = 7.0$ Hz, 2H), 7.33 (t, $J = 7.0$ Hz, 1H), 7.21 (d, $J = 8.5$ Hz, 2H), 6.97 (d, $J = 8.5$ Hz, 2H), 5.07 (s, 2H), 4.12 (m, 1H), 3.82 (dd, $J = 8.0$ and 14.0 Hz, 1H), 3.74 (s, 3H), 3.54 (br, 1H), 3.47 (dd, $J = 4.0$ and 14.5 Hz, 1H), 3.00–2.88 (m, 2H), 2.93 (dd, $J = 6.0$ and 13.5 Hz, 1H), 2.76 (dd, $J = 7.5$ and 13.5 Hz, 1H), 2.52 (t, $J = 7.0$ Hz, 2H), 1.85 (m, 2H), 1.45 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): $\delta = 173.3$, 169.9, 157.4, 137.1, 130.5, 128.5, 128.1, 127.9, 127.7, 114.9, 69.2, 54.2, 53.0, 50.2, 48.7, 44.8, 35.1, 29.2, 22.5, 20.6, 14.4; HRMS $[M+H]^+$ calcd for $C_{24}H_{34}N_3O_5$: 444.2498, found: 444.1339.

***N*-Hydroxy-*N*-[(2*S*)-2-amino-3-(4-benzyloxyphenyl)propyl]-N′-[(1*R*)-1-carboxymethylethyl]-4-aminobutanamide, HCl salt (33)**: Hydroxamate **28** (58 mg, 0.090 mmol) was treated with a solution of HCl/acetic acid (1.5 mL) for 30 min. The reaction mixture was diluted with toluene (10 mL) and the solvent removed from the resulting mixture. The resulting solid was rinsed with ether and then azeotroped with toluene to give **33** as a white solid (42.8 mg, 92 %). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 10.32$ (s, 1H), 9.68 (brs, 1H), 9.31 (brs, 1H), 8.23 (brs, 3H), 7.43 (d, $J = 7.0$ Hz, 2H), 7.39 (t, $J = 7.0$ Hz, 2H), 7.33 (t, $J = 7.0$ Hz, 1H), 7.21 (d, $J = 8.5$ Hz, 2H), 6.97 (d, $J = 8.5$ Hz, 2H), 5.07 (s, 2H), 4.13 (m, 1H), 3.83 (dd, $J = 8.0$ and 14.5 Hz, 1H), 3.74 (s, 3H), 3.55 (br, 1H), 3.48 (dd, $J = 4.0$ and 14.5 Hz, 1H), 2.95 (m, 3H), 2.77 (dd, $J = 8.0$ and 14.0 Hz, 1H), 2.52 (t, $J = 7.5$ Hz, 2H), 1.88 (m, 2H), 1.45 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): $\delta =$ 173.2, 169.9, 157.3, 137.1, 130.5, 128.5, 128.1, 127.9, 127.7, 114.9, 69.2, 54.2, 53.0, 50.2, 48.7, 44.8, 35.0, 29.0, 20.6, 14.4; HRMS $[M+Cs]^+$ calcd for $C_{24}H_{33}N_3O_5Cs$: 576.1475, found: 576.1489.

***N*-Hydroxy-*N*-[(2*S*)-2-amino-3-(4-benzyloxyphenyl)propyl]-*N*′-[(1*S*)-1,3-dicarboxymethylpropyl]-4-aminobutanamide, HCl salt (34)**: Hydroxamate **29** (75 mg, 0.091 mmol) was treated with a solution of HCl/acetic acid (1 mL) for 30 min. The reaction mixture was diluted with toluene (10 mL) and the solvent removed from the resulting mixture. The resulting solid was rinsed with ether and then azeotroped with toluene to give **34** as a white solid (52.7 mg, 99 %). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 10.30$ (s, 1H), 9.75 (brs, 1H), 9.39 (brs, 1H), 8.22 (brs, 3H), 7.43 (d, $J = 7.0$ Hz, 2H), 7.39 (t, $J = 7.0$ Hz, 2H), 7.33 (t, $J = 7.0$ Hz, 1H), 7.21 (d, $J = 8.5$ Hz, 2H), 6.97 (d, $J = 8.5$ Hz, 2H), 5.07 (s, 2H), 4.09 (br, 1H), 3.82 (dd, $J = 8.0$ and 14.0 Hz, 1H), 3.72 (s, 3H), 3.59 (s, 3H), 3.55 (br, 1H), 3.48 (dd, $J = 3.5$ and 14.0 Hz, 1H), 2.93 (dd, $J = 5.5$ and 14.0 Hz, 1H), 2.93 (m, 2H), 2.77 (dd, $J = 8.0$ and 14.0 Hz, 1H), 2.50 (m, 4H), 2.17 (m, 1H), 2.10 (m, 1H), 1.88 (m, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): $\delta = 173.2$, 172.1, 168.8, 157.3, 137.1, 130.5, 128.5, 128.1, 127.9, 127.7, 114.9, 69.2, 57.9, 53.0, 51.6, 50.2, 48.7, 45.5, 35.0, 29.0, 28.8, 23.9, 20.5; HRMS $[M+Cs]^+$ calcd for $C_{27}H_{37}N_3O_7Cs$: 648.1686, found: 648.1699.

***N*-Hydroxy-*N*-[(2*S*)-2-amino-3-(4-benzyloxyphenyl)propyl]-*N*′-[(1*S*)-1,4-dicarboxymethylbutyl]-4-aminobutanamide, HCl salt (35)**: Hydroxamate **30** (46 mg, 0.063 mmol) was treated with a solution of HCl/acetic acid (1 mL) for 30 min. The reaction mixture was diluted with toluene (10 mL) and the solvent removed from the resulting mixture. The resulting solid was rinsed with ether and then azeotroped with toluene to give **35** as a white solid (27.4 mg, 71 %). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 10.31$ (s, 1H), 9.55 (brs, 1H), 9.32 (brs, 1H), 8.21 (brs, 3H), 7.43 (d, $J = 7.0$ Hz, 2H), 7.39 (t, $J = 7.0$ Hz, 2H), 7.33 (t, $J = 7.0$ Hz, 1H), 7.21 (d, $J = 8.5$ Hz, 2H), 6.97 (d, $J = 8.5$ Hz, 2H), 5.07 (s, 2H), 4.09 (br, 1H), 3.80 (dd, $J = 8.5$ and 14.0 Hz, 1H), 3.75 (s, 3H), 3.58 (s, 3H), 3.55 (br, 1H), 3.48 (dd, $J = 4.0$ and 14.0 Hz, 1H), 2.92 (dd, $J = 5.0$ and 14.0 Hz, 1H), 2.90 (m, 2H), 2.78 (dd, $J = 8.5$ and 14.0 Hz, 1H), 2.51 (t, $J = 7.0$ Hz, 2H), 2.34 (t, $J = 7.0$ Hz, 2H), 1.86 (m, 4H), 1.62 (m, 1H), 1.46 (m, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): $\delta =$ 173.2, 172.8, 169.2, 157.4, 137.1, 130.5, 128.5, 128.1, 127.9, 127.7, 114.9, 69.2, 58.4, 53.0, 51.4, 50.2, 48.7, 45.5, 35.0, 32.5, 29.0, 28.0, 20.5, 19.8; HRMS $[M+Cs]^+$ calcd for $C_{28}H_{39}N_3O_7Cs$: 662.1842, found: 662.1852.

***N*-Hydroxy-*N*-[(2*S*)-2-amino-3-(4-benzyloxyphenyl)propyl]-*N*′-[(1*S*)-1-carboxymethyl-5-aminopentyl]-4-aminobutanamide, HCl salt (36)**: Hydroxamate **31** (50 mg, 0.063 mmol) was treated with a solution of HCl/acetic acid (1 mL) for 30 min. The reaction mixture was diluted with toluene (10 mL) and the solvent removed from the resulting mixture. The resulting solid was rinsed with ether and then azeotroped with toluene to give **36** as a pale yellow solid (38 mg, 99 %). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 10.25$ (s, 1H), 9.75 (brs, 1H), 9.30 (brs, 1H), 8.20 (brs, 3H), 8.04 (brs, 3H), 7.43 (d, $J = 7.0$ Hz, 2H), 7.36 (t, $J = 7.0$ Hz, 2H), 7.32 (t, $J = 7.0$ Hz, 1H), 7.19 (d, $J = 8.5$ Hz, 2H), 6.96 (d, $J = 8.5$ Hz, 2H), 5.06 (s, 2H), 4.03 (br, 1H), 3.83 (dd, $J = 7.0$ and 14.0 Hz, 1H), 3.76 (s, 3H), 3.55 (br, 1H), 3.48 (dd, $J =$ 3.0 and 14.0 Hz, 1H), 2.96 (br, 2H), 2.94 (dd, $J = 3.0$ and 14.0 Hz, 1H), 2.80–2.69 (m, 3H), 2.53 (t, $J = 7.0$ Hz, 2H), 1.96–1.79 (m, 4H), 1.55 (m, 2H), 1.42 (m, 1H), 1.29 (m, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): $\delta = 173.2$, 169.2, 157.2, 130.5, 128.5, 128.1, 127.9, 127.7, 114.9, 89.4, 69.2, 58.5, 53.0, 48.7, 46.0, 38.2, 35.0, 29.0, 26.3, 21.2, 20.5; HRMS $[M+H]^+$ calcd for $C_{27}H_{41}N_4O_5$: 501.3077, found: 501.3087.

***N*-Hydroxy-*N*-[(2*S*)-2-amino-3-(4-benzyloxyphenyl)propyl]-*N*′-[(1*S*)-1-carboxyethyl]-4-aminobutanamide, HCl salt (37)**: LiOH (1.0 M, 2.5 mL) was added to hydroxamate **27** (73 mg, 0.11 mmol) in THF/MeOH (2:17, 5 mL) and the biphasic mixture was stirred vigorously for 30 min. The mixture was poured into HCl (1 N, 30 mL) and extracted with EtOAc. The organic layer was dried ($MgSO_4$) and the solvent removed to give a pale yellow oil (70 mg, 99 %). The oil (26 mg, 0.041 mmol) was then dissolved in HCl/acetic acid (1 mL) and stirred for 30 min. The reaction mixture was diluted with toluene (10 mL) and the solvent removed from the resulting mixture. The resulting solid was rinsed with ether and then azeotroped with toluene to give **37** as a white solid (18.5 mg, 89 %). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 10.26$ (s, 1H), 9.13 (brs, 2H), 8.18 (brs, 3H), 7.43 (d, $J = 7.0$ Hz, 2H), 7.39 (t, $J = 7.0$ Hz, 2H), 7.33 (t, $J = 7.0$ Hz, 1H), 7.21 (d, $J =$ 8.5 Hz, 2H), 6.97 (d, $J = 8.5$ Hz, 2H), 5.07 (s, 2H), 3.96 (m, 1H), 3.84 (dd, $J = 8.0$ and 15.0 Hz, 1H), 3.55 (br, 1H), 3.46 (dd, $J = 4.5$ and 14.5 Hz, 1H), 2.92 (m, 3H), 2.76 (dd, $J = 8.5$ and 14.0 Hz, 1H), 2.52 (t, $J = 7.0$ Hz, 2H), 1.84 (m, 2H), 1.43 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): $\delta = 173.4$, 171.2, 157.6, 137.3, 130.7, 128.7, 128.3, 128.1, 128.0, 115.1, 69.4, 54.6, 50.4, 48.9, 45.0, 35.3, 29.2, 20.8, 14.7; HRMS $[M+Cs]^+$ calcd for $C_{23}H_{31}N_3O_2Cs$: 562.1318, found: 562.1335.

***N*-Hydroxy-*N*-[(2*R*)-2-amino-3-(4-benzyloxyphenyl)propyl]-N′-[(1*R*)-1-carboxyethyl]-4-aminobutanamide, HCl salt (38)**: LiOH (1.0 M, 2.5 mL) was added to hydroxamate **28** (90 mg, 0.13 mmol) in THF/MeOH (2:1, 7.5 mL) and the biphasic mixture was stirred vigorously for 1 h. The mixture was poured into HCl (1 N, 50 mL) and extracted with EtOAc. The organic layer was dried ($MgSO_4$) and the solvent removed to give a pale yellow oil (84 mg, 95 %). The oil (30 mg, 0.048 mmol) was then dissolved in HCl/acetic acid (1 mL) and stirred for 30 min. The reaction mixture was diluted with toluene (10 mL) and the solvent removed from the resulting mixture. The resulting solid was rinsed with ether and then azeotroped with toluene to give **38** as a white solid (22.3 mg, 93 %). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 10.28$ (s, 1H), 9.11 (brs, 2H), 8.19 (brs, 3H), 7.43 (d, $J = 7.0$ Hz, 2H), 7.39 (t, $J = 7.0$ Hz, 2H), 7.33 (t, $J = 7.0$ Hz, 1H), 7.21 (d, $J = 8.5$ Hz, 2H), 6.97 (d, $J = 8.5$ Hz, 2H), 5.07 (s, 2H), 3.92 (m, 1H), 3.84 (m, 1H), 3.55 (br, 1H), 3.46 (dt, $J = 3.0$ and 14.5 Hz, 1H), 2.92 (m, 3H), 2.76 (dd, $J = 8.5$ and 14.0 Hz, 1H), 2.52 (t, 2H), 1.84 (m, 2H), 1.42 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): $\delta = 173.2$, 171.0, 157.2, 137.1, 130.2, 128.5, 128.1, 127.9, 127.7, 114.9, 69.2, 54.5, 50.1, 48.7, 44.8, 35.1, 28.9, 20.6, 14.5; HRMS $[M+Cs]^+$ calcd for $C_{23}H_{31}N_3O_2Cs$: 562.1318, found: 562.1328.

***N*-Hydroxy-*N*-[(2*S*)-2-amino-3-(4-benzyloxyphenyl)propyl]-*N*′-[(1*S*)-1,3-dicarboxypropyl]-4-aminobutanamide, HCl salt (39)**: LiOH (1.0 M, 2.5 mL) was added to hydroxamate **29** (114 mg, 0.16 mmol) in THF/MeOH (2:1, 7.5 mL) and the biphasic mixture stirred vigorously for 1 h. The mixture was poured into HCl (1 N, 30 mL) and extracted with EtOAc. The organic layer was dried ($MgSO_4$) and the solvent removed to give a pale yellow oil (106 mg, 96 %). The oil (37 mg, 0.054 mmol) was then dissolved in HCl/acetic acid (1 mL) and stirred for 30 min. The reaction mixture was diluted with toluene (10 mL) and the solvent removed from the resulting mixture. The resulting solid was rinsed with ether and then azeotroped with toluene to give **39** as a white solid (28.1 mg, 94 %). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 10.27$ (s, 1H), 9.20 (v brs, 2H), 8.19 (brs, 3H), 7.43 (d, $J = 7.0$ Hz, 2H), 7.39 (t, $J = 7.0$ Hz, 2H), 7.33 (t, $J = 7.0$ Hz, 1H), 7.21 (d, $J = 8.5$ Hz, 2H), 6.97 (d, $J = 8.5$ Hz, 2H), 5.07 (s, 2H), 3.92 (m, 1H), 3.84 (m, 1H), 3.55 (br, 1H), 3.46 (m, 1H), 3.00–2.86 (m, 3H), 2.75 (dd, $J = 8.5$ and 14.0 Hz,

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1H), 2.52 (m, 2H), 2.46 (m, 1H), 2.32 (m, 1H), 2.11 (m, 1H), 2.02 (m, 1H), 1.85 (m, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): δ = 173.3, 173.1, 170.0, 157.4, 137.1, 130.5, 128.5, 128.1, 127.9, 127.7, 114.9, 69.2, 58.2, 50.1, 48.7, 45.5, 35.1, 29.3, 29.0, 24.1, 22.5, 20.6; HRMS [M+Cs]$^+$ calcd for $C_{25}H_{33}N_3O_7Cs$: 620.1373, found: 620.1398.

***N*-Hydroxy-*N*-[(2*S*)-2-amino-3-(4-benzyloxyphenyl)propyl]-N′-[(1*S*)-1,4-dicarboxybutyl]-4-aminobutanamide, HCl salt** (**40**): LiOH (1.0 M, 2.5 mL) was added to hydroxamate **30** (86 mg, 0.12 mmol) in THF/MeOH (2:1, 7.5 mL) and the biphasic mixture was stirred vigorously for 30 min. The mixture was poured into a mixture of HCl (1N, 10 mL) and saturated NaCl (10 mL) and extracted with EtOAc. The organic layer was dried ($MgSO_4$) and the solvent removed to give a pale yellow oil (81 mg, 98 %). The oil (29 mg, 0.041 mmol) was then dissolved in HCl/acetic acid (1 mL) and stirred for 30 min. The reaction mixture was diluted with toluene (10 mL) and the solvent removed from the resulting mixture. The resulting solid was rinsed with ether and then azeotroped with toluene to give **40** as a white solid (22.5 mg, 95 %). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): δ = 10.25 (s, 1H), 9.10 (br s, 2H), 8.20 (br s, 3H), 7.43 (d, J = 7.0 Hz, 2H), 7.39 (t, J = 7.0 Hz, 2H), 7.33 (t, J = 7.0 Hz, 1H), 7.21 (d, J = 8.5 Hz, 2H), 6.97 (d, J = 8.5 Hz, 2H), 5.07 (s, 2H), 3.88 (m, 1H), 3.83 (m, 1H), 3.54 (br, 1H), 3.45 (dt, J = 4.5 and 14.0 Hz, 1H), 2.92 (m, 2H), 2.92 (dd, J = 4.5 and 13.5 Hz, 1H), 2.76 (dd, J = 8.0 and 13.5 Hz, 1H), 2.52 (t, 2H), 2.25 (t, J = 7.0 Hz, 2H), 1.84 (m, 2H), 1.64 (m, 1H), 1.46 (m, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): δ = 174.2, 173.5, 170.5, 157.6, 137.3, 130.7, 128.7, 128.3, 128.1, 128.0, 115.1, 69.4, 68.3, 59.1, 50.3, 48.9, 45.7, 35.3, 33.23, 29.2, 28.3, 20.8, 20.1; HRMS [M+Cs]$^+$ calcd for $C_{26}H_{35}N_3O_7Cs$: 634.1529, found: 634.1538.

***N*-Hydroxy-*N*-[(2*S*)-2-amino-3-(4-benzyloxyphenyl)propyl]-N′-[(1*S*)-1-carboxy-5-aminopentyl]-4-aminobutanamide, HCl salt** (**41**): LiOH (1.0 M, 2.5 mL) was added to hydroxamate **31** (93 mg, 0.12 mmol) in THF/MeOH (2:1, 7.5 mL) and the biphasic mixture was stirred vigorously for 1 h. The mixture was poured into HCl (1N, 50 mL) and extracted with EtOAc. The organic layer was dried ($MgSO_4$) and the solvent removed to give a pale yellow oil (87 mg, 96 %). The oil (31 mg, 0.039 mmol) was then dissolved in HCl/acetic acid (1 mL) and stirred for 30 min. The reaction mixture was diluted with toluene (10 mL) and the solvent removed from the resulting mixture. The resulting solid was rinsed with ether and then azeotroped with toluene to give **41** as a white solid (20.1 mg, 86 %). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): δ = 10.29 (s, 1H), 9.23 (br s, 2H), 8.20 (br s, 3H), 8.04 (br s, 3H), 7.43 (d, J = 7.0 Hz, 2H), 7.39 (t, J = 7.0 Hz, 2H), 7.33 (t, J = 7.0 Hz, 1H), 7.21 (d, J = 8.5 Hz, 2H), 6.97 (d, J = 8.5 Hz, 2H), 5.07 (s, 2H), 3.86 (m, 1H), 3.84 (dd, J = 8.0 and 14.5 Hz, 1H), 3.55 (br, 1H), 3.47 (dd, J = 2.0 and 14.5 Hz, 1H), 2.92 (m, 3H), 2.80–2.71 (m, 3H), 2.52 (t, 2H), 1.89 (m, 2H), 1.58 (m, 2H), 1.47 (m, 1H), 1.32 (m, 1H); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): δ = 173.2, 170.2, 157.4, 137.1, 130.5, 128.5, 128.1, 127.9, 127.7, 114.9, 69.2, 58.8, 50.1, 48.7, 45.6, 38.2, 35.1, 29.0, 28.2, 26.4, 21.3, 20.6; HRMS [M+Cs]$^+$ calcd for $C_{26}H_{38}N_4O_5Cs$: 619.1897, found: 619.1892.

***S*-Acetyl-4-mercaptobutyric acid** (**42**): Potassium thioacetate (1.0 g, 9.0 mmol) was added in one portion to a solution of 4-bromobutyric acid (1.0 g, 6.0 mmol) in anhydrous DMF (5 mL) under argon at room temperature. The reaction was difficult to monitor by TLC as the starting material was hard to detect (2:1 hexanes/EtOAc). The reaction mixture was stirred for a total of 1 h then the solvent removed in vacuo. The residue was taken up in ether and washed (0.1N HCl, brine). Purification by flash chromatography yielded VI-58 as a colorless oil (0.75 g, 78 %). ^{1}H NMR (400 MHz, CDCl$_3$, 25 °C): δ = 2.94 (t, J = 7.0 Hz, 2H), 2.45 (t, J = 7.0 Hz, 2H), 2.34 (s, 3H), 1.92 (dt, J = 7.0 and 7.0 Hz, 2H); ^{13}C NMR (100 MHz, CDCl$_3$, 25 °C): δ = 195.7, 178.9, 32.6, 30.6, 28.2, 24.5.

Methyl (*R*)-(+)-2-bromopropionate. (*R*)-(+)-2-bromopropionic acid (1.0 g, 590 μL, 6.5 mmol) was treated with ethereal diazomethane to give the methyl ester. Acetic acid was added to destroy any excess diazomethane and the ethereal solution was concentrated by distillation to give the product in ether (10 mL, approximately 0.65 mM). This solution was used directly in the following reaction (compound **43**).

***S*-[(1*S*)-1-carboxymethylethyl]-4-mercaptobutyric acid** (**43**): NaOH (1N, 1.2 mL, 1.2 mmol, deoxygenated) was added dropwise to thioacetate **42** (300 mg, 1.8 mmol) in methanol (7 mL, deoxygenated). The resulting solution was stirred for 15 min. Additional NaOH (1N, 0.6 mL, 0.6 mmol) was added followed by methyl (*R*)-(+)-2-bromopropionate (5 mL of ≈ 0.65 mM solution, see preceding description). Stirring was continued for 45 min (TLC 1:1 EtOAc/hexanes). The reaction mixture was poured into EtOAc (100 mL), and the layers were separated and the organic layer washed (1N HCl, brine). Purification by flash chromatography (5 % acetone/toluene) gave the desired product **43** as a colorless oil (0.37 g, 100 %). ^{1}H NMR (400 MHz, CDCl$_3$, 25 °C): δ = 3.75 (s, 3H), 3.43 (q, J = 7.0 Hz, 1H), 2.71 (m, 1H), 2.67 (m, 1H), 2.50 (t, J = 7.0 Hz, 2H), 1.92 (m, 2H), 1.45 (d, J = 7.0 Hz, 3H). HRMS [M+H]$^+$ calcd for $C_8H_{15}O_4S$: 207.0691, found: 207.0695.

***N*-Hydroxy-*N*-[(2*S*)-2-*N*′-Boc-amino-3-(4-benzyloxyphenyl)propyl]-*S*-[(1-*S*)-1-carboxymethylethyl]-4-mercaptobutanamide** (**44**): Isobutyl chloroformate (75 μL, 0.59 mmol) was added to a solution of acid **43** (121 mg, 0.59 mmol) and triethylamine (105 μL, 0.75 mmol) in THF (10 mL) at 0 °C. The resulting slurry was stirred for 30 min and then (2*S*)-2-*N*′-Boc-amino-3-(4-benzyloxyphenyl)-*N*-hydroxypropylamine (200 mg, 0.54 mmol) was added. The cooling bath was removed and the reaction mixture stirred for 30 min. The reaction was then quenched by addition of 3-dimethylaminopropylamine and poured into CH_2Cl_2 (60 mL), washed (1N HCl, saturated $NaHCO_3$, saturated NaCl), and dried ($MgSO_4$). Purification by flash chromatography (1:2 EtOAc/hexanes, then 1:1 EtOAc/hexanes) yielded compound **44** as a colorless oil (275 mg, 91 %). ^{1}H NMR (400 MHz, CDCl$_3$, 25 °C): δ = 8.65 (s, 1H), 7.45–7.30 (m, 5H), 7.10 (d, J = 8.5 Hz, 2H), 6.93 (d, J = 8.5 Hz, 2H), 5.05 (s, 2H), 4.65 (d, J = 8.5 Hz, 1H), 4.19 (q, J = 11.5 Hz, 1H), 4.14 (m, 1H), 3.73 (s, 3H), 3.41 (q, J = 7.0 Hz, 1H), 3.04 (dd, J = 1.5 and 11.0 Hz, 1H), 2.77 (m, 2H), 2.72–2.56 (m, 3H), 1.94–1.82 (m, 1H), 1.43 (dd, J = 0.6 and 7.0 Hz, 3H), 1.39 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$, 25 °C): δ = 173.7, 158.2, 157.8, 136.9, 130.0, 128.6, 128.0, 127.5, 115.2, 81.0, 70.0, 52.2, 50.5, 48.1, 40.7, 37.2, 31.4, 31.1, 28.2, 24.2, 17.2; HRMS [M+H]$^+$ calcd for $C_{29}H_{40}N_2O_7SCs$: 693.1611, found: 693.1625.

***N*-Hydroxy-N-[(2*S*)-2-amino-3-(4-benzyloxyphenyl)propyl]-*S*-[(1*S*)-1-carboxymethylethyl]-4-mercaptobutanamide, HCl salt** (**45**): Hydroxamate **44** (80 mg, 0.14 mmol) was treated with a solution of HCl/acetic acid (2 mL) for 30 min. The reaction mixture was diluted with toluene (10 mL) and the solvent was removed from the resulting mixture. The resulting solid was rinsed with ether and then azeotroped with toluene to give **45** as a white solid (51 mg, 73 %). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): δ = 10.06 (br s, 1H), 8.03 (br s, 3H), 7.43 (d, J = 7.0 Hz, 2H), 7.39 (t, J = 7.0 Hz, 2H), 7.33 (t, J = 7.0 Hz, 1H), 7.21 (d, J = 8.5 Hz, 2H), 6.97 (d, J = 8.5 Hz, 2H), 5.07 (s, 2H), 3.81 (dd, J = 8.0 and 14.0 Hz, 1H), 3.62 (s, 3H), 3.58–3.44 (m, 3H), 2.89 (dd, J = 5.5 and 14.0 Hz, 1H), 2.76 (dd, J = 8.0 and 14.0 Hz, 1H), 2.60 (m, 2H), 2.46 (m, 2H), 1.74 (m, 2H), 1.28 (d, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25 °C): δ = 173.6, 173.1, 157.4, 137.1, 130.5, 128.4, 127.9, 127.8, 127.7, 115.1, 69.2, 52.0, 50.1, 48.7, 35.1, 30.7, 30.3, 23.8, 17.2; HRMS [M+Cs]$^+$ calcd for $C_{24}H_{32}N_2O_5SCs$: 593.1086, found: 593.1074.

***N*-Hydroxy-*N*-[(2*S*)-2-amino-3-(4-benzyloxyphenyl)propyl]-*S*-[(1*S*)-1-carboxyethyl]-4-mercaptobutanamide, HCl salt** (**46**): LiOH (1.0 M, 2.5 mL) was added to hydroxamate **44** (90 mg, 0.16 mmol) in THF/MeOH (2:1, 7.5 mL) and the biphasic mixture was stirred vigorously for 30 min. The mixture was poured into HCl(1N 30 mL) and extracted with EtOAc. The organic layer was dried ($MgSO_4$) and the solvent removed to give a pale yellow oil (85 mg, 97 %). The oil was then treated with a solution of HCl/acetic acid (2 mL) for 30 min. The reaction mixture was diluted with toluene (10 mL) and the solvent removed from the resulting mixture. The resulting solid was rinsed with ether, azeotroped with toluene, then taken up in CH_2Cl_2 and allowed to crystallize overnight to give **46** as a white solid (67 mg, 84 %). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): δ = 10.13 (br s, 1H), 8.03 (br s, 3H), 7.43 (d, J = 7.0 Hz, 2H), 7.39 (t, J = 7.0 Hz, 2H), 7.33 (t, J = 7.0 Hz, 1H), 7.21 (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), 3.54 (m, 1H), 3.46 (dd, J = 4.0 and 14.5 Hz, 1H), 2.88 (q, J = 7.0 Hz, 1H), 2.90 (dd, J = 5.5 and 14.0 Hz, 1H), 2.75 (dd, J = 7.0 and 13.5 Hz, 1H), 2.61 (m, 2H), 2.46 (m, 2H), 1.73 (m, 2H), 1.28 (d, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25 °C): δ = 173.6, 173.1, 157.4, 137.1, 130.5, 128.4, 128.0, 127.9, 127.7, 115.1, 69.2, 50.1, 48.7, 35.1, 30.8, 30.3, 23.8, 17.4; HRMS [M+Cs]$^+$ calcd for $C_{23}H_{30}N_2O_5SCs$: 579.0930, found: 579.0944.

***O*-Benzyl-L-tyrosine *p*-nitroanilide, HCl salt** (**47**)**:** *N*-Boc-*O*-benzyl-L-tyrosine (10 mmol, Advanced Chemtech) and *p*-nitroaniline (10 mmol) were dissolved in dry pyridine (40 mL). The solution was chilled to between −15 °C and −20 °C on a carefully monitored acetone/dry-ice bath. After 15 minutes, phosphorus oxychloride (11 mmol, 1.0 mL) was added dropwise over a period of five minutes. In contrast to the reaction reported by Tesser, from which this synthetic route was derived, no red by-product was

observed.[39] After 45 minutes, the color was observed to have lightened considerably and the reaction was judged complete by TLC (1:1 EtOAc/hexanes). Crushed ice (50 mL) was then added and the mixture was transferred to a separating funnel. Upon melting of the ice, the organic phase was diluted with ethyl acetate (200 mL) and washed 4×100 mL HCl (0.1N), 1×100 mL saturated $CuSO_4$, 1×100 mL saturated $NaHCO_3$, and 1×50 mL saturated NaCl. The organic phase was dried under reduced pressure, and the resultant yellow solid recrystallized from hot isopropyl alcohol (300 mL), to give *p*-nitroanilide (3.83 g, 78%) as pale yellow crystals. ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 10.66$ (s, 1H), 8.03 (d, $J = 7.2$ Hz, 2H), 7.84 (d, $J = 7.2$ Hz, 2H), 7.42 (d, $J = 6.8$ Hz, 2H), 7.37 (t, $J = 6.8$ Hz, 2H), 7.31 (t, $J = 6.8$ Hz, 1H), 7.23 (d, $J = 7.2$ Hz, 2H), 6.90 (d, $J = 7.2$ Hz, 2H), 5.05 (s, 2H), 4.3 (m, 1H), 3.35 (m, 1H), 2.7–3.0 (m, 2H), 1.32 (s, 9H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25 °C): $\delta = 172.1$, 157.0, 155.5, 145.1, 142.2, 137.2, 130.2, 129.8, 128.4, 127.8, 127.6, 125.0, 118.9, 114.4, 78.2, 69.1, 57.1, 36.2, 28.2; HRMS $[M+Na]^+$ calcd for $C_{27}H_{29}N_3O_6Na$: 514.1954, found: 514.1938.

p-Nitroanilide (492 mg, 1 mmol) was dissolved in conc. HCl/AcOH (30 mL), and stirred for 45 minutes at room temperature. The acetic acid was removed under reduced pressure followed by coevaporation with toluene (3×30 mL). The resultant yellow solid was used without further purification. Yield: 410 mg (96%). ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 11.9$ (brs, 1H), 8.5 (brs, 3H), 8.236 (d, $J = 9.0$ Hz, 2H), 7.911 (d, $J = 9.0$ Hz, 2H), 7.409 (d, $J = 5.5$ Hz, 2H), 7.361 (t, $J = 7.5$ Hz, 2H), 7.301 (t, $J = 7.0$ Hz, 1H), 7.269 (d, $J = 8.0$ Hz, 2H), 6.934 (s, $J = 8.5$ Hz, 2H), 5.041 (s, 2H), 4.40 (m, 1H), 3.0–3.3 (m, 2H), 1.32 (s, 9H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25 °C): $\delta = 168.3$, 158.2, 144.9, 143.4, 137.7, 131.4, 129.5, 129.0, 128.8, 128.4, 128.3, 127.3, 125.6, 119.9, 115.4, 69.7, 55.1, 33.6; HRMS $[M+Na]^+$ calcd for $C_{22}H_{22}N_3O_4$: 392.1610, found: 392.1621.

***O*-Benzyl-L-serine *p*-nitroanilide (48):** *N*-Boc-*O*-benzyl-L-serine (10 mmol, Peninsula) and *p*-nitroaniline (10 mmol) were dissolved in dry pyridine (40 mL). The solution was chilled to between −15 °C and −20 °C on a carefully monitored acetone/dry-ice bath. After 15 minutes, phosphorus oxychloride (11 mmol, 1.0 mL) was added dropwise over a period of five minutes. After 45 minutes, the color was observed to have lightened considerably and the reaction was judged complete by TLC (1:1 EtOAc/hexanes). Crushed ice (50 mL) was then added and the mixture transferred to a separating funnel. Upon melting of the ice, the organic phase was diluted with ethyl acetate (200 mL) and washed 4×100 mL HCl (0.1N), 1×100 mL saturated $CuSO_4$, 1×100 mL saturated $NaHCO_3$, and 1×50 mL saturated NaCl. The organic phase was dried under reduced pressure. This material was highly soluble in isopropyl alcohol, so it was purified by silica-gel chromatography (10% acetone in toluene) instead, to give *p*-nitroanilide (3.68 g, 75%) as a yellow oil. ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C): $\delta = 9.05$ (brs, 1H), 8.122 (d, $J = 8.5$ Hz, 2H), 7.578 (d, $J = 8$ Hz, 2H), 7.3 (m, 5H), 5.555 (d, $J = 7.0$ Hz, 1H), 4.567 (m 2H), 4.48 (m, 1H), 3.815 (m 2H), 1.45 (s, 9H); ^{13}C NMR (125 MHz, [D$_6$]DMSO, 25 °C): $\delta = 169.2$, 155.9, 143.4, 143.3, 136.9, 128.6, 128.2, 127.9, 124.9, 119.1, 80.9, 73.6, 69.3, 54.6, 28.2.

p-Nitroanilide (421 mg, 1.0 mmol) was dissolved in conc. HCl/AcOH (30 mL), followed by stirring for 45 minutes at room temperature. The acetic acid was removed under reduced pressure followed by coevaporation with toluene (3×30 mL). The resultant yellow oil was purified by silica-gel chromatography (5% MeOH, 1% triethylamine in dichloromethane). Yield: 266 mg (84%). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): $\delta = 8.23$ (d, $J = 7.5$ Hz, 2H), 7.92 (d, $J = 7.5$ Hz, 2H), 7.3 (m, 5H), 5.0 (vbrs, 2H), 4.50 (s, 2H), 3.62 (m, 2H), 3.59 (m, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25 °C): $\delta = 173.5$, 145.0, 142.2, 138.2, 128.2, 127.4, 125.0, 118.9, 72.5, 72.1, 55.7; HRMS $[M+Na]^+$ calcd for $C_{16}H_{17}N_3O_4$: 316.1297, found: 316.1307.

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