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Investigation of the Inhibition of Leukotriene A_4 Hydrolase

Ian R. Ollmann,[a] J. Heather Hogg,[a] Benito Muñoz,[a] Jesper Z. Haeggström,[b] Bengt Samuelsson[b] and Chi-Huey Wong,*[a]

[a]*Department of Chemistry, The Scripps Research Institute, La Jolla, CA 92037, U.S.A.*
[b]*Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden*

Abstract—In an effort to better understand the favorable binding interactions between the reversible picomolar inhibitor 3-(4-benzyloxyphenyl)-2-(*R*)-amino-1-propanethiol (**1**) and leukotriene A_4 (LTA_4) hydrolase (EC 3.3.2.6), we prepared a number of derivatives of **1**-L and other related structures, and assayed their inhibition of LTA_4 hydrolase-catalyzed hydrolysis of L-alanine-*p*-nitroanilide. The inhibition data was analyzed using a weighted non-linear least-squares curve fitting computer program developed for this purpose to fit data derived under the non-Michaelis–Menten condition of $[I]_t < [E]_t$. The free thiol is necessary for sub-micromolar binding and the enzyme prefers the *R* enantiomer over the *S* enantiomer, in contrast to the stereoselectivity displayed towards bestatin, an inhibitor of somewhat similar structure. Substitution of acid moieties around the periphery of the benzyloxyphenyl portion of **1**-L leads to substantially decreased binding, suggesting that this group resides within a large hydrophobic pocket when bound to the enzyme. Possible LTA_4 binding modes in the active site of LTA_4 hydrolase, including a possible direct role for the carboxylic acid of LTA_4 in the enzyme-catalyzed hydrolysis of leukotriene A_4, are discussed.

Introduction

Leukotriene A_4 (LTA_4) hydrolase (EC 3.3.2.6)[1,2,3] is a zinc containing,[4] monomeric enzyme (M_r ~ 70 kDa)[5] which catalyzes the vinylogous hydrolysis of the unstable epoxide, LTA_4 (5(*S*)-5,6-oxido-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid), to form leukotriene B_4 (LTB_4, 5(*S*),12(*R*)-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid). LTB_4 is a proinflammatory substance which has been shown to stimulate the release of superoxide radicals and to activate polymorphonuclear leukocytes (PMNLs), triggering their chemotaxis, aggregation on the vascular endothelium and degranulation.[2,6,7] Compounds which inhibit the LTA_4 hydrolase-catalyzed transformation of LTA_4 to LTB_4 may prove to be highly potent and selective anti-inflammatory drugs.

LTA_4 hydrolase has been purified to homogeneity from a number of sources, yielding a soluble protein. Its natural substrate, LTA_4, has been prepared by a variety of methods.[8–15] The protein has been crystallized in the presence of bestatin, but no structure has yet been published.[16] The cDNA from human placenta and spleen have been cloned and sequenced,[17] allowing for the identification of a region homologous to the endopeptidase, thermolysin. This led to the prediction of a zinc binding region within LTA_4 hydrolase and suggested a previously unrecognized peptidase activity,[18] which has since been shown to include enkephalins and dynorphins as substrates,[19] as well as small peptides containing N-terminal arginine.[20]

Though the detailed mechanism of either activity has not been fully elucidated, we speculate that in both cases the zinc binds the incident water molecule, facilitating general base catalysis, (Fig. 1), as this is the observed role of the zinc in many other homologous peptidases.[4,21,22] However, there still exists the possibility that in the case of the epoxide hydrolase activity, the zinc is bound to the epoxide. Replacement of glutamic acid-296, sequentially adjacent to one of the zinc binding ligands of LTA_4 hydrolase, with glutamine or alanine has been shown to abrogate the peptidase activity but not the hydrolase activity.[23,24] This suggests that Glu-296 plays an intimate role in the peptidase activity of the enzyme, either as a specific base in catalysis of substrate hydrolysis, or in amino terminal recognition of peptidic substrates. Alteration of any of the zinc binding ligands deprives the enzyme of both epoxide hydrolase and aminopeptidase activities.[25]

We were able to take advantage of the identification of an aminopeptidase activity within LTA_4 hydrolase to produce a series of tight binding inhibitors that also inhibited the epoxide hydrolase activity of this enzyme.[26,27,28] Some of these inhibitors (such as α-ketoesters, β-mercaptoamines and aminohydroxamic acids shown in Fig. 2) bound sufficiently tightly to LTA_4 hydrolase that the standard Michaelis–Menten assumptions were found to be no longer valid. Though a mathematical treatment[29,30] and a method of linear graphical analysis[31] are available for such cases wherein the concentration of inhibitor is no longer much greater than the concentration of enzyme, the graphical method of analysis can be difficult to use as duplicate data points can disperse widely on the plot and proper weighting of data is by no means straightforward.[31]

We have done further work with the tight-binding[32] β-mercaptoamine **1**-L previously presented to better characterize its binding interactions with LTA_4

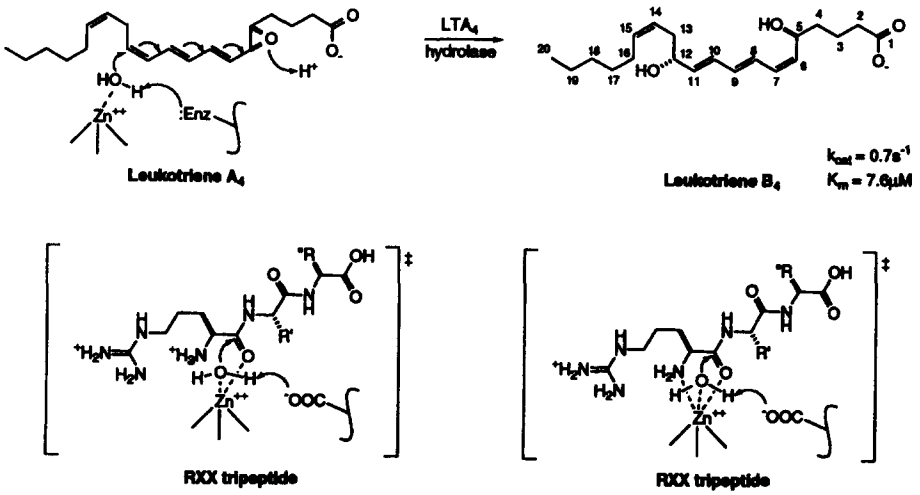


Figure 1. A hypothesized mechanism of LTA_4 hydolysis in the active site of LTA_4 hydrolase and two possible transition states for the aminopeptidase activity, showing different zinc coordination motifs. LTA_4 hydrolysis catalyzed by an electrophilic zinc bound to the epoxide instead is also a possibility.

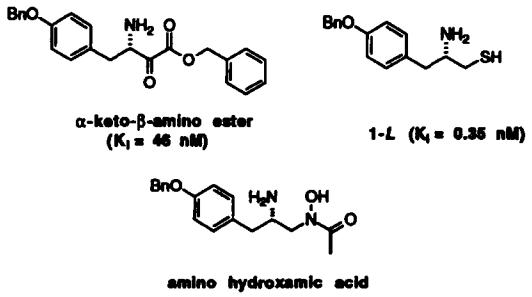


Figure 2. Thiolamine 1-L, α-ketoester and amino hydroxamic acid inhibitors of LTA_4 hydrolase.

hydrolase and have developed a series of new inhibitors of LTA_4 hydrolase using it as a core structure. In order to more accurately characterize the very tight binding behavior of some of these compounds, we have developed a computer program to fit the non-linear equation of Morrison[29] to our inhibition data. These new compounds will hopefully further illuminate the nature of guest–enzyme interactions in the active site of LTA_4 hydrolase. We also present an improved synthesis of LTA_4 methyl ester.

Results and Discussion

In the double reciprocal Lineweaver–Burke plot for the *in vitro* K_i determination of 1-L against the peptidase activity of LTA_4 hydrolase, lines corresponding to $[I]_t < [E]_t$ (total inhibitor concentration is less than total enzyme concentration) intersect on the 1/[S] axis. The lines where $[I]_t > [E]_t$ and $[I]_t = 0$ cross at the 1/v axis, (Fig. 3). Similar $[I]_t$-dependent behavior appears in Dixon plots and other graphical methods of data analysis, wherein at low inhibitor concentrations, the inhibitor appears to be non-competitive and at high inhibitor concentrations ($[I]_t > [E]_t$), the inhibitor appears to be competitive. Data where $[I]_t > [E]_t$, when taken alone, predict a K_i much lower than that predicted by the data taken as a whole. The transition from nearly 100% enzyme activity to less than 1% activity occurs over a comparatively very narrow range of inhibitor concentrations, $0.1[E]_t < [I]_t < 2[E]_t$. All of this behavior is characteristic of a tight binding inhibitor whose K_i is significantly below the total concentration of the enzyme used in the assay. In such cases, because the usual Michaelis–Menten assumption that $[I]_t >> [E]_t$ and thus $[I] \approx [I]_t$ fails, these graphical methods give results which behave like non-competitive inhibition when $[I]_t < [E]_t$, though the actual mode of inhibition may be of a competitive, uncompetitive, non-competitive or mixed non-competitive type.[31] To resolve this ambiguity, the data were fit with a non-Michaelis–Menton rate equation[29]

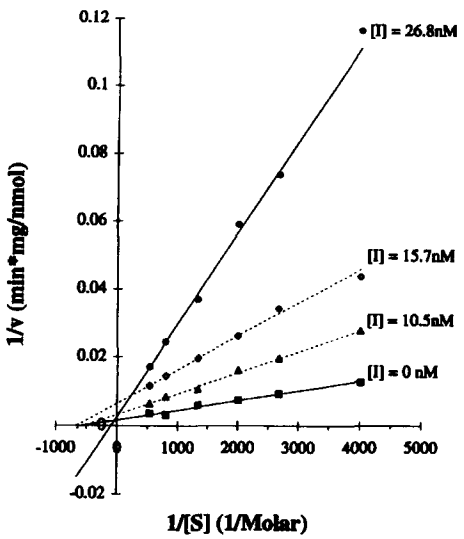


Figure 3. A double reciprocal Lineweaver–Burke plot illustrating the unusual inhibition data for 1-L against the aminopeptidase activity of LTA_4 hydrolase obtained because $K_i < [E]$. Dotted lines are weighted linear least-squares fits of data at inhibitor concentrations where the Michaelis–Menten assumption $[I] > [E]$ fails. Solid lines represent concentrations where these assumptions are more valid. The inhibitor *appears* to be 'non-competitive' at low inhibitor concentrations and competitive at high inhibitor concentrations, consistent with a tight binding competitive inhibitor with $K_i < [E]$. $[E] = 20$ nM.

which corrects for inhibitor depletion in solution resulting from having a significant fraction of the inhibitor bound to the enzyme. This was achieved using a computer program written in Excel to fit the equation using the Newton–Gauss method of non-linear least-squares error minimization. The data from the K_i determination of 1-L were fit well by curves corresponding to competitive inhibition of the aminopeptidase activity of LTA_4 hydrolase (Fig. 4), with a K_i equal to 0.35 nM.

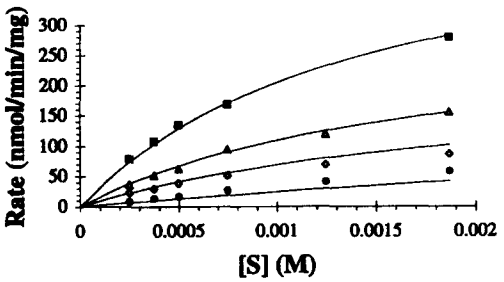
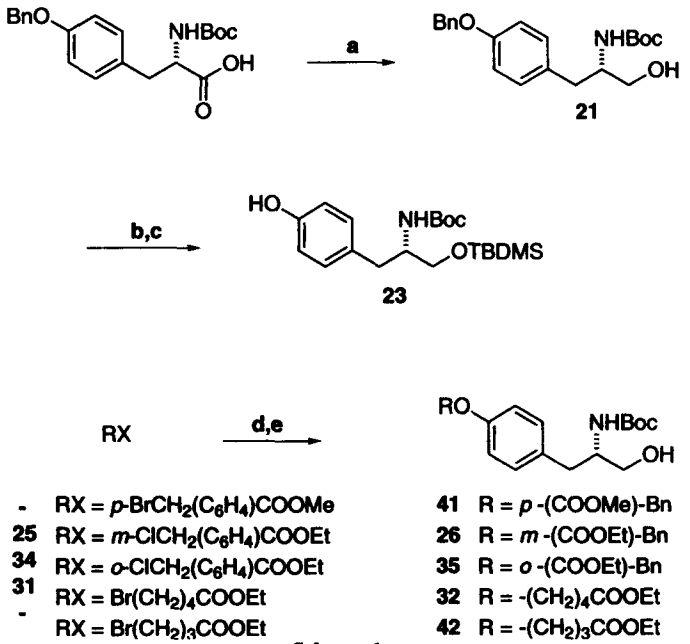


Figure 4. A weighted non-linear least squares fit of the equation of Morrison for mixed non-competitive tight-binding inhibitors to the K_i determination data for inhibition by **1** against LTA_4 hydrolase-catalyzed hydrolysis of alanine-*p*-nitroanilide. In this case, K_{ii} was found to converge to infinity, suggesting that **1** is a competitive inhibitor against the peptidase activity with K_{ii} = 0.35 nM.

The β-mercaptoamine inhibitor 1-L and its disulfide dimer were further assayed to determine their inhibitory potency against the formation of thromboxane B_2 and leukotriene B_4 in human whole blood. Both were found to inhibit LTB_4 production by 50% at concentrations of 1 μM; 100% inhibition was achieved at 10 μM. The similarity between the activity of these two compounds might be explained by simple *in vivo* reductive cleavage of the disulfide bond within the dimer to yield two molecules of 1-L, as the disulfide is a very weak inhibitor *in vitro* of LTA_4 hydrolase. However, at 10 μM, 1-L was also found to inhibit 100% of the thromboxane B_2 production but the disulfide dimer of 1-L only inhibited thromboxane B_2 production by 24%. Due to its structural similarity to many of the compounds in the arachidonic acid metabolic pathway, 1-L may be a good inhibitor of many of the enzymes therein, so this measurement does not necessarily reflect inhibition specific to LTA_4 hydrolase, but rather inhibition of perhaps more than one of the enzymes in the arachidonic acid metabolic pathway.

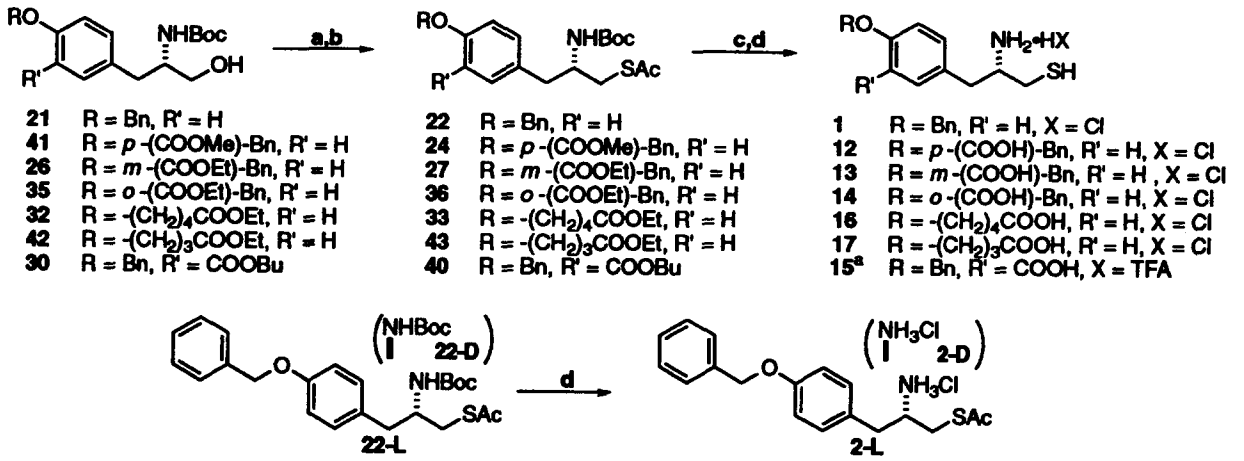
Our attempts to understand the binding mode of 1-L to LTA_4 hydrolase led us to explore enzyme–inhibitor interactions over varied portions of the molecule by subtly adding to, or modifying, its structure. Initially, we investigated the relevance of the stereochemistry at the amine. The *S* enantiomer of 1-L was synthesized by the same procedure as the *R* enantiomer, starting from N-Boc O-benzyl-D-tyrosine, (Schemes 1 and 2.) It inhibited LTA_4 hydrolase-catalyzed hydrolysis of alanine-*p*-nitroanilide competitively with an inhibition constant (K_i) approximately two orders of magnitude greater than the L-tyrosine derived enantiomer, (K_i 1-L: 0.35 nM, K_i 1-D: 25 nM). Likewise, LTA_4 hydrolase displayed similar preferential inhibition by the *R* enantiomer of the S-acetylated derivatives of these two compounds, as determined by their relative IC_{50}s (2-L: 1 μM and 2-D: 8 μM). Both of these cases stand in marked contrast to the stereochemical discrimination exhibited towards bestatin and its diastereomer.[28] In that example, the *S* enantiomer at the amine (IC_{50} = 0.2 μM) binds more tightly by two orders of magnitude than the other diastereomer (IC_{50} = 20 μM).

Acetylation of the thiol appears to have triggered a marked decrease in binding. Similarly, replacement of the mercapto functionality with a hydroxyl to give the



Scheme 1.

(a) BH_3·THF, 0 °C; (b) TBDMS–Cl, DMAP, CH_2Cl_2, TEA; (c) Pd/C, H_2, EtOAc; (d) **23**, K_2CO_3, TBAI, acetone reflux; (e) TBAF, THF.



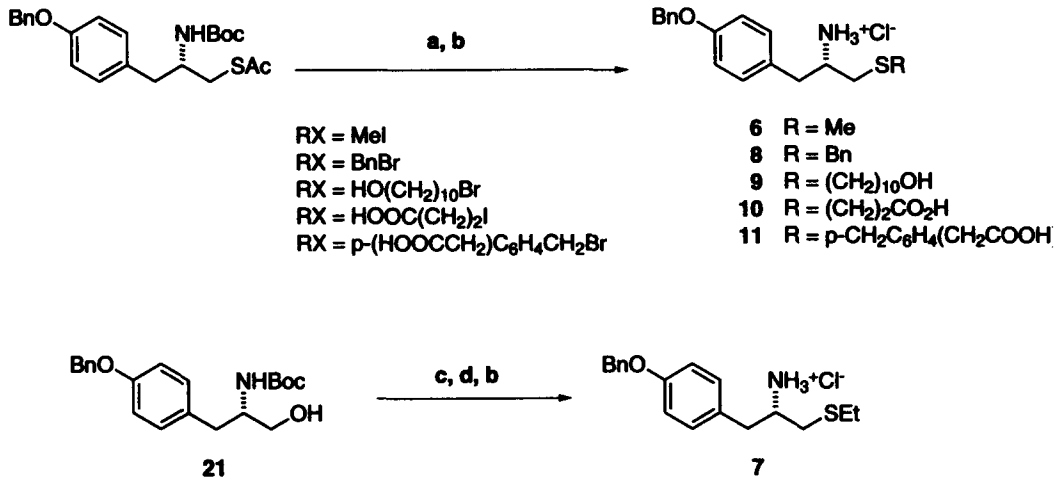
Scheme 2.
(a) MsCl, CH_2Cl_2, 4 h; (b) KSAc, DMF or refluxing acetone; (c) EtOH, $NaOH/H_2O$; (d) sat'd ethereal HCl or 20% TFA/CH_2Cl_2.
[a]15 was obtained as its disulfide dimer.

β-aminoalcohol **3** caused a large decrease in binding (IC_{50} = 14 μM), despite the obvious steric similarities between **1**-L and the β-aminoalcohol **3**. Replacement with an amine functionality as in the case of **53** (IC_{50} = 0.5 μM) may also have triggered a large reduction in binding. Thiols are not known for forming strong hydrogen bonds. The presence or absence of 50 mM dithiothreitol during the assay had little or no effect on binding, and the absence of time-dependent inhibition makes the formation of an enzyme–inhibitor disulfide bond an improbable explanation. For these reasons, it appears likely that this exquisite selectivity for the free thiol arises from the formation of a highly favorable zinc–sulfide bond. When the sulfur moiety is replaced by another metallophile such as a hydroxamic acid, binding (Fig. 2) again becomes tight enough to produce nanomolar binding constants.[33]

The assumption that compounds containing free and acetylated thiols bind in a similar manner places the thioacetate moiety in the vicinity of the zinc catalytic unit, though perhaps not within the reach of the active site water, if still present. We investigated the possibility that the thioester would be sufficiently active to allow for cleavage by LTA_4 hydrolase, yielding the appropriate enantiomer of the tight binding inhibitor, **1**-L. Pre-incubation of the S-acetylated inhibitors, **2**-L and **2**-D, with the enzyme for 30 min prior to addition of alanine-*p*-nitroanilide substrate yields no measurable change in inhibition for either enantiomer, indicating that these two are not substrates for the enzyme, nor are the conditions of the assay (50 mM Tris, pH 8.0) sufficiently basic for uncatalyzed saponification of the thioester to occur in measurable quantities over this time period.

The next compounds to be examined were a series of thioethers. LTA_4 hydrolase displayed a remarkable lack of selectivity among the various thioethers tested, failing to discriminate between rigid and flaccid groups as well as between hydrophilic and hydrophobic moieties. Such failure of the enzyme to display any selectivity between substituents bound to sulfur may be indicative that in the case of thioethers, this portion extends out into solvent. The loss in inhibitory potency due to S-alkylation was similar to that observed for S-acetylation. It appears that modification of the free thiol severely curtails interactions between sulfur and zinc, possibly eliminating them completely. Thioethers are known to be poor metal ligands.[34] There are few examples in nature of thioethers, such as methionine, bound to metal centers. A notable exception is the class of blue copper proteins, which incorporates methionine into its unusual metal coordination sphere.[35,36] Even crown thioethers are poor ligands compared to their oxygen containing cousins, though such structures incorporating zinc are known.[37] Diminished binding in the case of thioethers is not, then, unexpected.

The thioethers were synthesized from the N-Boc-S-acetyl protected derivative of **1**-L, as shown in Scheme 3. Saponification of the thioester and subsequent alkylation of the liberated thiol was generally accomplished in one pot in good yield,[38] providing compounds **6**, **8**, **9**, **10** and **11**. We were concerned that this method might allow some of the highly potent inhibitor **1**-L to be produced as a co-product in the synthesis of these compounds. Contamination of only 0.01% by the free thiol inhibitor **1**-L would be sufficient for the inhibition of LTA_4 hydrolase that we measured. In order to obtain a sample of thioether very likely free of contamination by **1**-L, the ethyl thioether **7** was synthesized by an alternative route. The addition of ethanethiol to the mesylate of N-Boc-O-Bn-L-tyrosinol gave nucleophilic displacement of the mesylate functionality, providing N-Boc protected **7**. The Boc protecting group was removed as for the other thioethers. The inhibition of the aminopeptidase activity of LTA_4 hydrolase displayed by the ethyl thioether **7** was consistent with that displayed by other analogous compounds prepared by the other method. For this reason, we felt that free thiol contamination did not occur in sufficient quantities to significantly bias our results towards greater inhibition.



Scheme 3.
(a) i. NaOH, ii. RX; (b) HCl, ether; (c) MsCl, TEA, DMAP; (d) CH_3CH_2SH, NaOH (aq.)

Table 1. Binding constants for inhibition of LTA_4 hydrolase-catalyzed cleavage of L-alanine-*p*-nitroanilide. The enzyme concentration for both IC_{50}s and K_is was 20 nM. (Naph = naphthyl; Bn = benzyl)

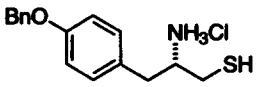
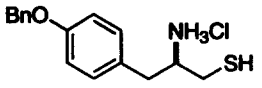
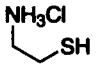
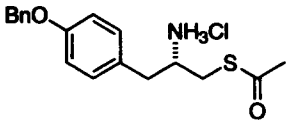
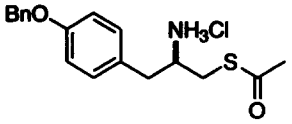
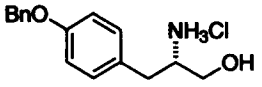
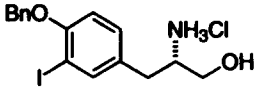
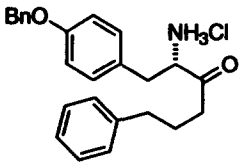
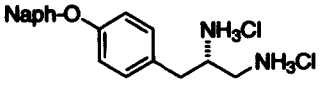
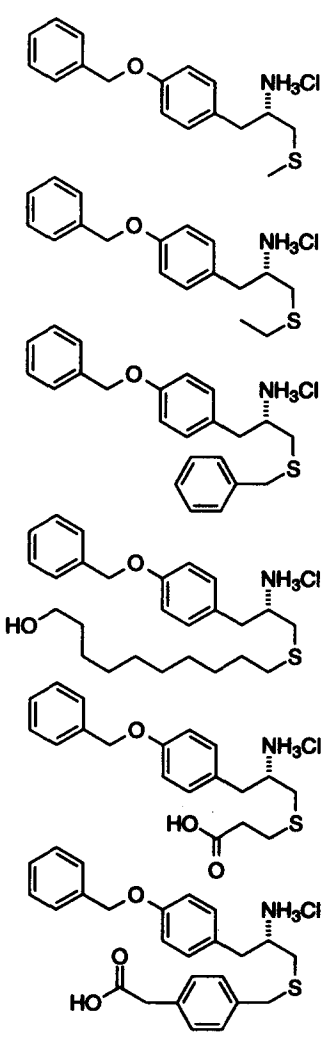
	#	K_i	IC_{50}
	**1-*L***	0.35 nM	15 nM
	**1-*D***	25 nM	27 nM
	**5**	—	27 μM
	**2-*L***	—	1 μM
	**2-*D***	—	8 μM
	**3**	—	14 μM
	**4**	—	18 μM
	**47**	—	0.8 μM
	**53**	—	0.5 μM

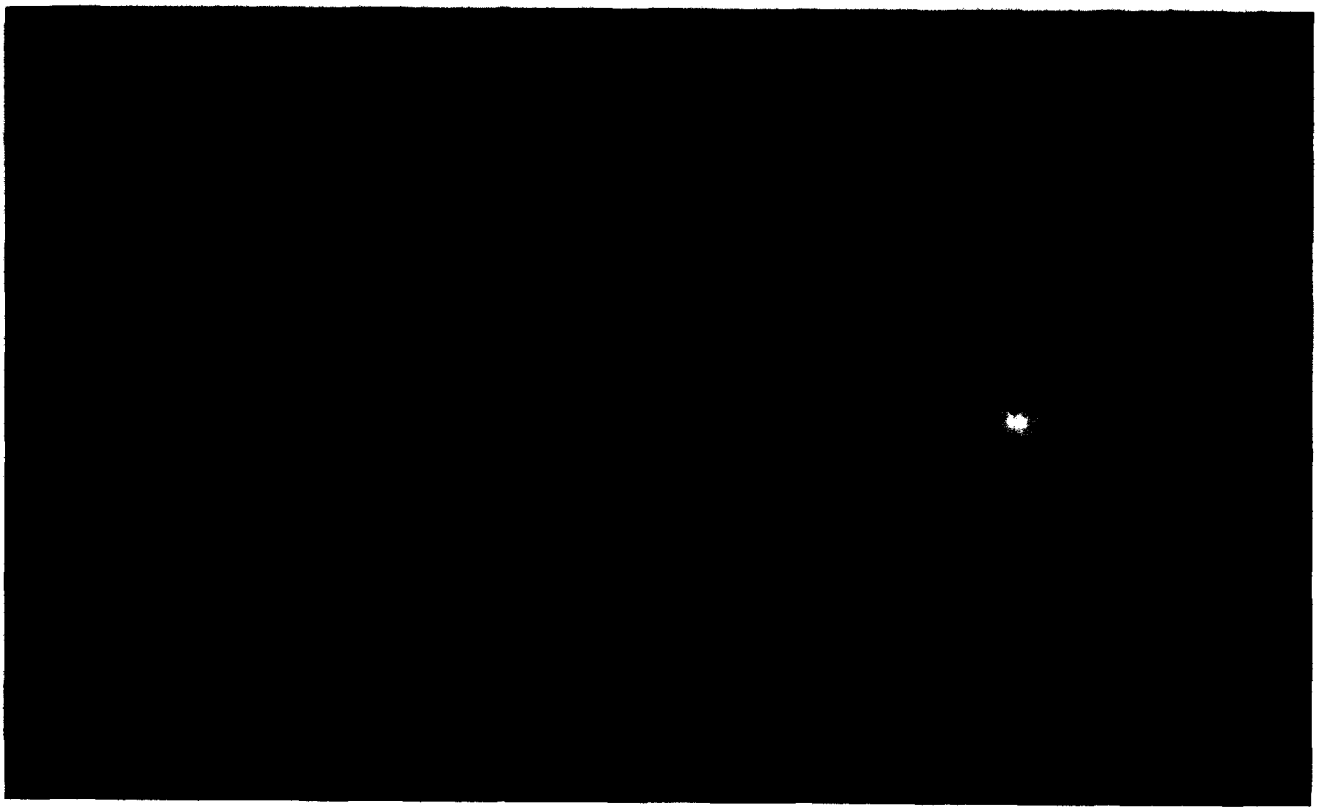
Table 2. Binding constants for inhibition of LTA_4 hydrolase-catalyzed cleavage of L-alanine-*p*-nitroanilide by various thioethers. The enzyme concentration for these assays was 20 nM



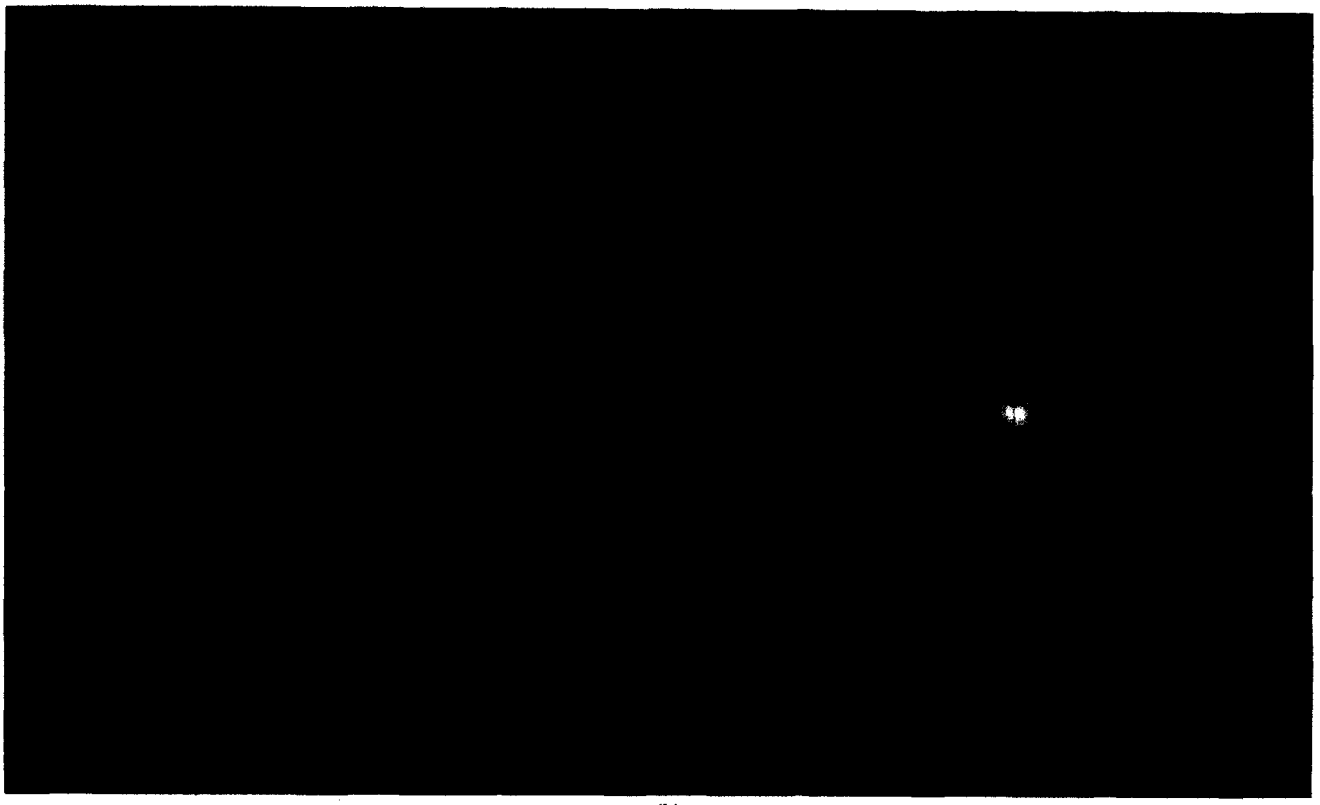
#	IC_{50}
6	4 μM
7	4 μM
8	4 μM
9	1.5 μM
10	3 μM
11	1.9 μM

Analysis of the structures of inhibitor 1-L and the hypothesized transition state of LTA_4 hydrolysis reveals some structural similarities. If the enzyme-catalyzed LTA_4 hydrolysis goes by a concerted mechanism then the conformation of the C-5 to C-13 region of LTA_4 must s-*cis* about the C6–C7 bond and s-*trans* about the C8–C9 and C10–C11 bonds, barring any *cis–trans* isomerase activity by the enzyme. This defines a large region of rigid hydrophobics similar to the benzyloxyphenyl portion of 1-L. When orienting a minimized structure of LTA_4 (Insight/Discover) such that the zinc and incident water act at C-12, and overlaying a similarly minimized 1-L with sulfur bound at zinc, displacing water, one finds that the oxygen of the aforementioned benzyloxyphenyl is proximal to the epoxide oxygen of LTA_4 and the benzyl group can be positioned to overlay with the C-1 to C-4 region of LTA_4 (Fig. 5d). The rest of 1-L, with exception of the sulfur and amine, fits nicely into the region of space carved out by LTA_4. Leukotriene A_4 hydrolase is very specific with regards to the nature of the substitution at C-1 of LTA_4. Though LTA_4 itself is a substrate, LTA_4 hydrolase does not catalyze epoxide hydrolysis of LTA_4 methyl ester.[39,40] Presumably, there must be a potent carboxylic acid recognition element within the enzyme. We therefore decided to investigate the location of LTA_4's acid terminus when bound to the hydrolase's active site by placing acids around the presumably rigid periphery of 1-L, and assaying these derivatives for inhibition.

The carboxylated derivatives of 1-L were prepared from N-Boc-L-tyrosinol *tert*-butyldimethylsilyl ether. The free phenol was alkylated with the appropriate alkyl halide (see Scheme 3) after which the silyl protecting group was removed, the resultant free alcohol converted to thiol and the Boc group removed as shown in Scheme 2. This procedure was relatively straightforward and even potentially troublesome alkylations such as that for the preparation of inhibitor **14** were achieved in good yields. The synthesis of the novel 3'-carboxytyrosine derivative **15** differed from the others as necessitated by its differing substitution pattern (Scheme 4). 3'-Iodotyrosine was N-Boc protected and then dibenzylated. The benzyl ester was selectively reduced to the free alcohol using lithium aluminum



(a)

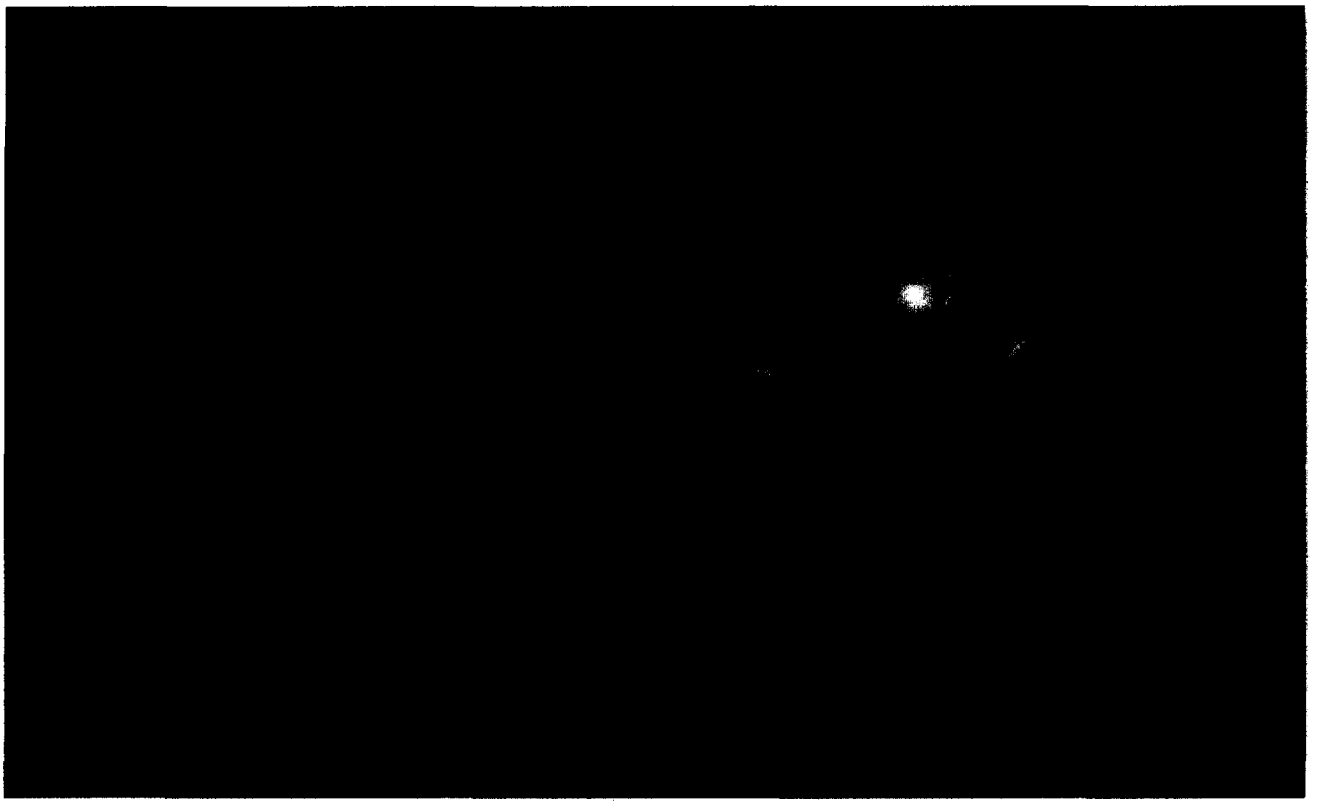


(b)

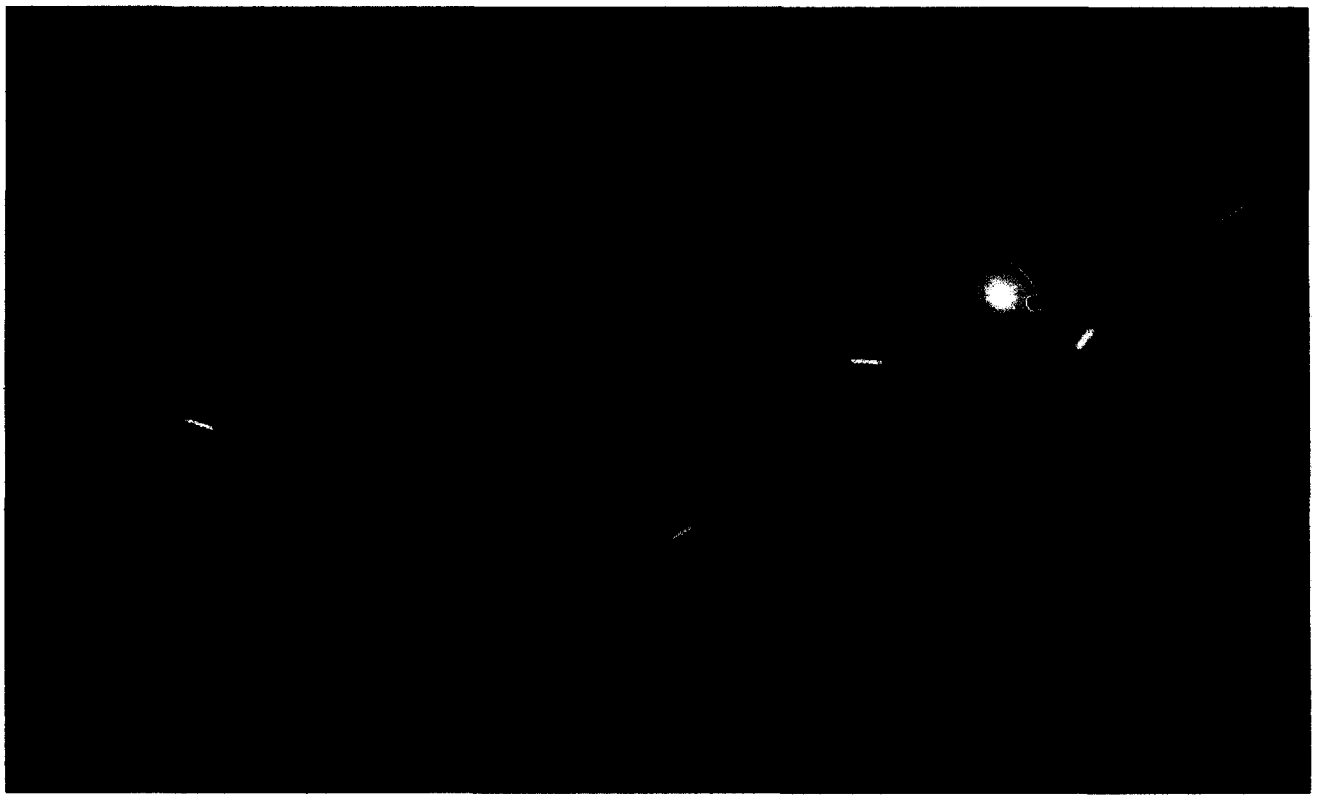
Figure 5. Some possible alternative modes of binding of **1**-L shown with hypothesized binding modes of LTA_4 to explain the results obtained from the series of acid substituted derivatives of **1**-L.

(a) The benzyloxy tail of **1**-L may bend away from the C_1–C_5 binding area, possibly into the region of the substrate binding pocket responsible for binding the C_{15}–C_{20} region of LTA_4 or some other hydrophobic pocket, placing the benzyloxy tail away from the putative carboxylic acid binding site.

(b) **1**-L may bind in a 'reversed' binding mode, again removing the benzyl from the vicinity of the putative acid binding site.



(c)

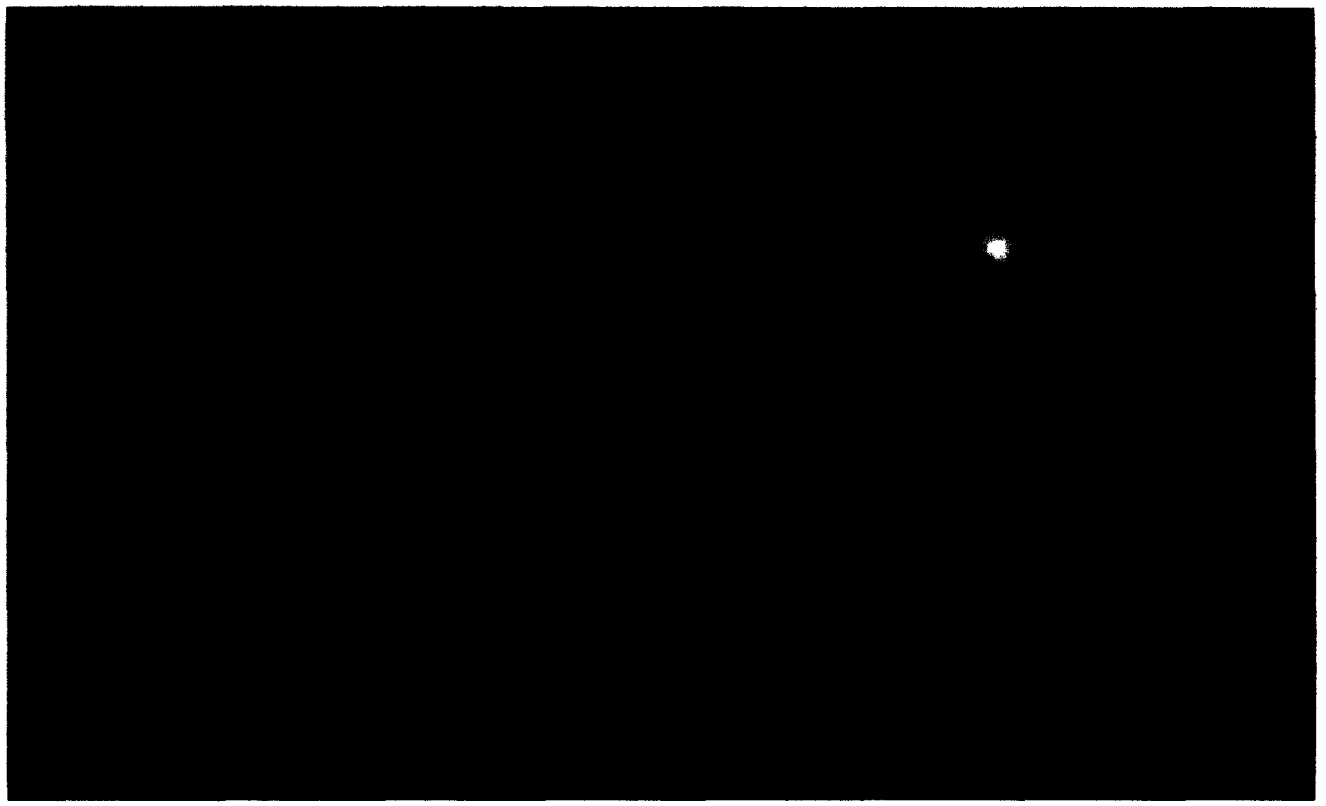


(d)

Figure 5. *Continued*

(c) LTA_4 shown with the catalytic zinc atom activating the epoxide of LTA_4, rather than the incident water molecule.

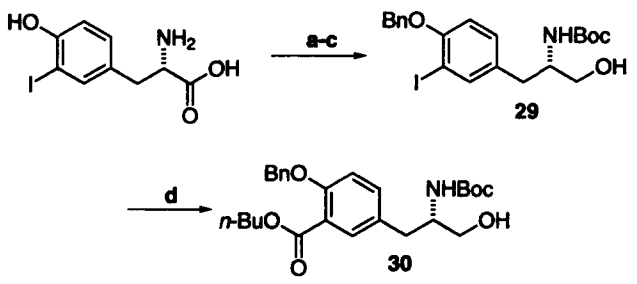
(d) One of the original overlays of **1**-L with LTA_4 which led to the speculation that an acid binding site resided in the vicinity of the benzyloxyphenyl portion of **1**-L. In this picture, LTA_4 is shown with its carboxylic acid participating in the catalysis of epoxide ring opening.



(e)

Figure 5. *Continued*
(e) A possible binding mode for a competitive 'reversed' hydroxamic acid inhibitor.[33]

hydride at 0 °C. The iodine was replaced with a butyl ester using a palladium catalyzed Heck reacton.[41] The free alcohol was then converted to a thiol by the same procedure as for the other mercaptoamine acids. It was found that treatment of the Boc derivative with ethereal HCl in the presence of the free 3' acid, resulted in the cleavage of the benzyl group, probably through a combination of general acid catalysis and inductive effects; 20% trifluoroacetic acid in dichloromethane (50 min) was used instead.



Scheme 4.
(a) $(Boc)_2O$, NaOH, 0 °C; (b) BnBr, NBAI, acetone reflux; (c) $LiAlH_4$, 0 °C; (d) $Pd(OAc)_2$, PPh_3 (2 eq.), $(C_8H_{17})_3N$, BuOH, 100 °C.

Substitution of an acid moiety on the periphery of the benzyl substituent gave a set of relatively poor inhibitors of LTA_4 hydrolase. Inhibitors **12**, **13** and **15** displayed a 1000-fold reduction in binding as compared to the parent compound, (**12**: 12 μM, **13**: 6 μM, **15**: 8 μM). Compound **14** displayed yet less activity against the enzyme. The curve obtained from the assay suggested an IC_{50} for **14** in the range of 100 to 500 μM. However, those concentrations approach the limit of its solubility in Tris buffer at pH 8.0 so a value of greater than 60 μM was assigned. We suspected that the acids might be too rigidly bound to the rings to achieve the proper interaction with the enzyme, so we prepared the more flexible inhibitors, **16** and **17**, which were designed to more accurately mimic the substrate. These oddly gave similar results to **12**, **13** and **15**, suggesting that interactions between the enzyme and either LTA_4 or mercaptoamine **1**-L are not as they originally were hypothesized to be.

We suggest several possible explanations to account for these observations (Fig. 5). The more straightforward set holds that the benzyloxyphenyl portion of **1**-L, **12**, **13**, **14** and **15**, does not overlay with the C-1 to C-12 portion of LTA_4 quite as originally thought. The benzyl moiety either could bend back upon the phenyloxy portion of the members of the mercaptoamine family rather than extend towards the C-1 to C-4 binding space within the active site (Fig. 5a), the entire benzyloxyphenyl group may occupy an entirely separate hydrophobic pocket (Fig. 5b), or the zinc could instead

activate LTA_4 hydrolysis by binding the epoxide rather than the incident water, causing a reversed binding mode for LTA_4 such as that shown in Figure 5c. Each of these modes of deviance from our early hypothesis poses interesting questions. If the former is the case, then it is unexpected that inhibitors **16** and **17** would not find the putative carboxylic acid recognition site, for they are flexible enough not to likely be forced to curl back on themselves. If it is the second, then LTA_4 hydrolase must possess two very large hydrophobic binding pockets in the vicinity of the catalytic zinc atom, one capable of holding most of LTA_4, and the other large enough to allow the hydrophobic tail of inhibitor **1**-L to bind. In the third, the zinc binds at the epoxide. This would help to explain the different roles in catalysis displayed by Glu-296.[23,24] However, it is probably more energetically favorable to employ zinc to activate water for attack than to activate an epoxide to open. Maintaining a catalytic zinc atom in the active site simply to help open the epoxide does not seem necessary from a chemical standpoint.

A fourth very different possible explanation stems from another means of enzymatic selection between LTA_4 and LTA_4 methyl ester. If LTA_4 hydrolase were to force the C-1 to C-5 tail of LTA_4 and derivatives to fold back on the epoxide upon binding in the active site and also encourage its protonation, it might allow for a catalytic role for the free acid in epoxide hydrolysis (Fig. 5d and 6). If this proton is intimately involved in catalysis of epoxide hydrolysis, then its absence as per the case of

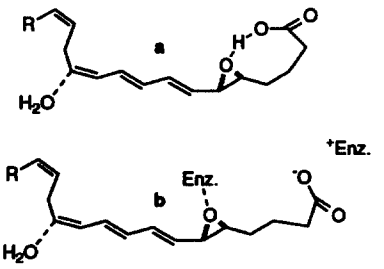
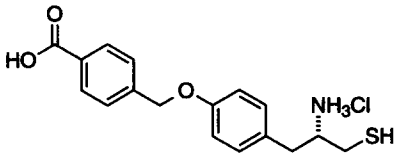
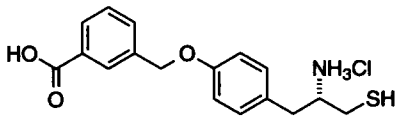
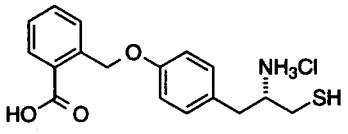
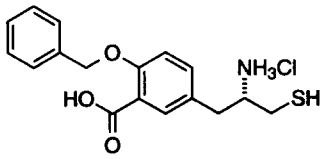
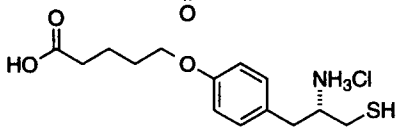
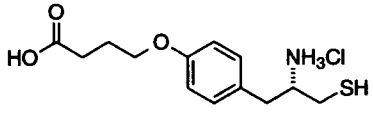
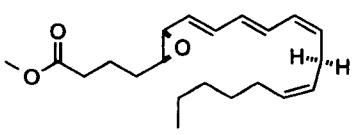


Figure 6. Two possible transition states for LTA_4 hydrolysis in the active site of LTA_4 hydrolase: (a) a possible cyclic transition state in which the protonated acid terminus of LTA_4 aids in epoxide ring opening through the transfer of a hydrogen to the epoxide oxygen; (b) another possible transition state in which the enzyme aids in epoxide ring opening through the transfer of a proton or via ligation to the active site zinc atom.

Table 3. Binding constants for inhibition of LTA_4 hydrolase-catalyzed cleavage of L-alanine-*p*-nitroanilide by various acid substituted thiols and LTA_4 methyl ester. The enzyme concentration used for all IC_{50}s was 20 nM

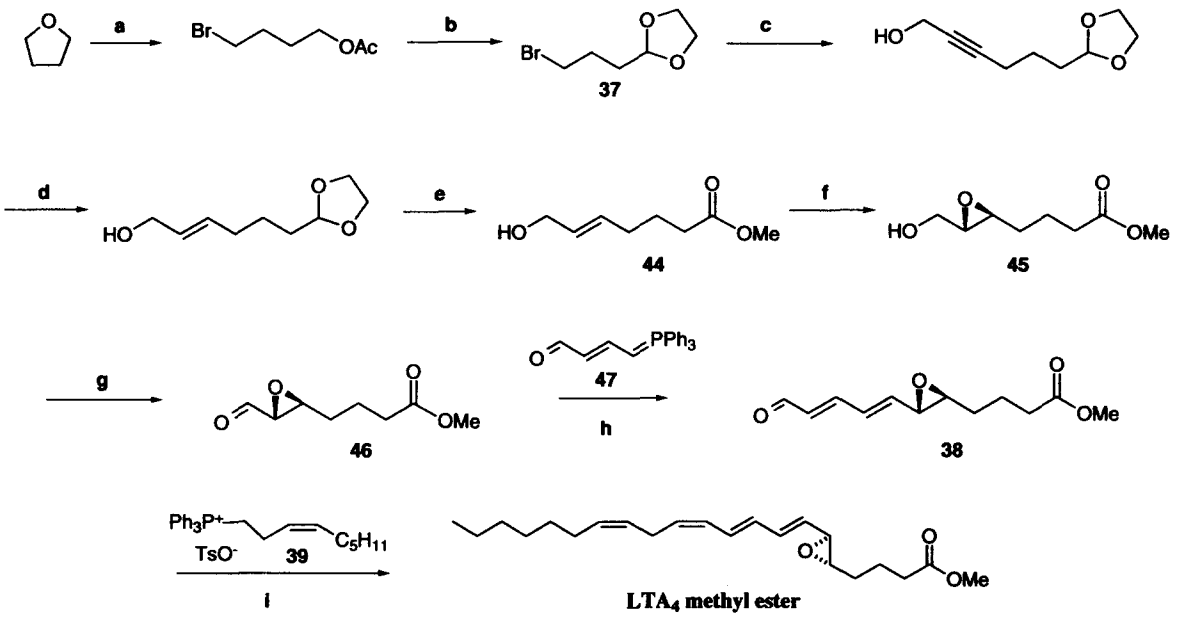
	#	IC_{50}
	**12**	12 μM
	**13**	6 μM
	**14**	> 60 μM
	**15**	8 μM
	**16**	3.0 μM
	**17**	3.3 μM
	**LTA_4 Me Ester**	2 μM

LTA_4 methyl ester would effectively prevent LTA_4 hydrolase from functioning. By some as yet undetermined mechanism, the carboxylic acid accelerates epoxide hydrolysis in water in the absence of enzyme. LTA_4 methyl ester displays a half life of 500 s in buffer at pH 7.4, as compared to 14 s for the hydrolysis of LTA_4.[39] If the acid is truly catalytic for hydrolysis of the epoxide, then an evolving LTA_4 hydrolase might choose to amplify this effect rather than develop a mode of catalysis entirely *de novo*.

For this mechanism to function effectively, the enzyme must be able to promote protonation of the free acid on LTA_4, a chemical group which is normally substantially deprotonated at physiological pH. The binding of LTA_4, or more specifically, the binding of its acid terminus must therefore involve an energetic penalty of some degree to provide for protonation of the acid. Thus, the enzyme would have to make some very favorable and perhaps specific interactions to compensate for this and orchestrate the proper binding and pK_a shift required for LTA_4 autocatalysis. Failure to ensure that the proper specific interactions are made (atoms placed at the right position in the right orientation) might leave an LTA_4 mimic suffering only from the unspecific effects of whatever electrostatic field is used to promote protonation, possibly leading to the results that we have obtained.

These two interpretations pose differing implications for LTA_4 methyl ester inhibition of the aminopeptidase activity of LTA_4 hydrolase. A strong carboxylate binding/recognition region would likely interact much more weakly with the methyl ester than the free acid. However, a selection process based upon catalytic involvement of the protonated acid of LTA_4 might actually favor binding by LTA_4 methyl ester over LTA_4, depending on the degree of unfavorable interaction due to the added steric bulk of the methyl group and associated loss of hydrogen bonds with the enzyme. Finding no report in the literature, we assayed LTA_4 methyl ester for reversible inhibition of the aminopeptidase activity of LTA_4 hydrolase, obtaining an IC_{50} of 2 μM.[42] Orning *et al.*[43] obtained a K_i value of 5.3 μM for aminopeptidase inhibition by free LTA_4, indicating that LTA_4 methyl ester binds to LTA_4 hydrolase as well as does LTA_4. These results agree with analogous data based upon time-dependent inactivation of LTA_4 hydrolase by these two compounds. (Apparent binding constants for suicide inactivation, K_I, were determined to be 28 μM for LTA_4 methyl ester and 25 μM for LTA_4 by Orning *et al.*)[44] The extent of the superimposability of covalent and non-covalent binding modes is not known. In as much as a free carboxylic acid is apparently not necessary for tight (reversible) binding of these leukotrienes to LTA_4 hydrolase, the enzyme must employ some other means to achieve its demonstrated substrate specificity. Two possible such methods are an alternate binding mode for the methyl ester and the above mentioned catalytic involvement of the acid.

The improved synthesis of LTA_4 methyl ester is shown in Scheme 5. The early stages of the synthetic procedure were taken from Bobrova *et al.*[45] Tetrahydrofuran reacted with acetyl bromide to give 4-bromo-1-acetoxybutane. We found that removing the acetate from this product using HCl in methanol, followed by oxidation under Swern conditions, provided the aldehyde in better yield. Successive protection of the aldehyde functionality with ethylene glycol gave the acetal, 4-bromo-1,1-ethylenedioxybutane, **37**.[45] Reaction of the bromoacetal with propargyl alcohol in the presence of lithium amide in liquid ammonia gave



Scheme 5.

(a) AcBr, 88%; (b) i. HCl, MeOH ii. $(CO)_2Cl_2$, TEA iii. ethylene glycol, *p*TsOH 56%; (c) Li, NH_3, propargyl alcohol, 90%; (d) $LiAlH_4$, 92%; (e) i. H_2SO_4, ii. $AgNO_3$, iii. CH_2N_2, 60%; (f) $Ti(OiPr)_4$, (+)-DET, *t*BuOOH, 80%; (g) CrO_3, pyridine, 80%; (h) 70%; (i) 58%.

1,1-ethylenedioxy-5-heptyn-7-ol, and successive reduction of the alkyne with lithium aluminum hydride afforded 1,1-ethylenedioxy-5*E*-hepten-7-ol, both in excellent yield. Deprotection of the aldehyde with sulfuric acid, oxidation to the carboxylic acid using silver nitrate, and esterification with diazomethanegave methyl 7-hydroxy-5*E*-heptenoate, in 60% yield from the aldehyde.[45] Stereoselective epoxidation of the allylic alcohol was achieved essentially under Sharpless conditions[46,47] to give **45** (94% *e.e.*). Collin'sreagent oxidized the terminal alcohol to give methyl 7-oxo-5*S*,6*S*-epoxyheptanoate **46**,[12] setting the stage for addition of the rest of LTA_4 via two Wittig olefinations.[48] Excess triphenylphosphoranylidene-crotonaldehyde, was coupled with aldehyde **46**, followed by conversion of the olefinoid geometry to entirely *trans* using iodine to give **38**. A final Wittig coupling with 1-triphenylphosphoranylidene-3-(*Z*)-nonene tosylate (**39**), provided LTA_4 methyl ester in 6.4% overall yield from THF. This procedure is quite reproducible and useful for the synthesis of the highly unstable material.

Conclusions

It is difficult to conclusively evaluate the data presented here in the absence of a crystal structure for LTA_4 hydrolase. All available evidence suggests that the thiol found on compound **1**-L binds to the catalytic zinc within the hydrolase, most probably displacing the active site water found there. Substitution at sulfur appears to have resulted in a severe curtailment of the sulfur's affinity for the zinc atom due to the prevention of the formation of a thiolate anion, (RS^-), which is a much better zinc ligand than a thioether or ester. Freedom or flexibility within the inhibitor carboskeleton near the active site probably plays a role in the relatively small degree of preference displayed for the *R* enantiomer at the position of the amine. Bestatin displays a larger degree of substitution in the analogous region thought to be responsible for zinc binding. The enantioselectivity displayed by bestatin is opposite to that displayed by the mercaptoamines, suggesting a rather different binding mode between the two classes of compounds.

We have not yet resolved if the amine from compound **1**-L is involved in chelating the zinc atom in partnership with the sulfur or if it makes specific interactions with other groups, possibly the putative amino-recognition element responsible for amino-terminal peptidase activity. It is likely that the proximity of the amine does aid in the loss of a proton from the sulfur. Acetylation of the amine has been shown to diminish binding of similar compounds significantly.[33] Mutation of Glu-296, a residue sequentially adjacent to one of the three zinc binding ligands, to Gln abrogates the peptidase activity but not the epoxide hydrolase activity of LTA_4 hydrolase. We do not know if this residue is responsible for amino-terminal recognition of peptides or is essential to catalysis as is the case with thermolysin, another metalloprotease sharing the HExxH consensus sequence.[4,49] However, the E296Q mutant of LTA_4 hydrolase has been shown to bind just as tightly as the wild-type enzyme to a similar mercaptoamine derived from leucine.[24] Consistent with the possibility that the free amine group of an aminopeptidase substrate coordinates to the zinc,[50] both the mercapto and amino functionalities of **1**-L may coordinate to the Zn of LTA_4 hydrolase. We will attempt to investigate possible interactions between LTA_4 hydrolase and the amine in the future.

We have not yet resolved whether or not the free acid tail of LTA_4 catalyzes epoxide ring opening within the active site of LTA_4 hydrolase. The strong non-covalent inhibition of the LTA_4 hydrolase peptidase activity by the methyl ester of its natural substrate is remarkable considering that the methyl ester of LTA_4 is not a substrate for the enzyme. If the methyl ester of LTA_4 does not adopt a wholly different binding mode from the free acid, then it appears likely that such selectivity may be based upon a mechanistic role for the free acid. This information will color our interpretation of data derived from addition of carboxylic acids around the benzyloxyphenyl portion of compound **1**, as to the reason for the diminished binding of these derivatives.

Experimental

General

Except where noted, all buffers, biologicals, solvents and simple chemicals such as sodium bicarbonate were obtained from Fisher and organic reagents were obtained from Aldrich. Silica gel thin layer chromatography (TLC) plates were purchased from EM industries. Water used for enzymatic assays was purified before use using a Millipore water filtration system. LTA_4 hydrolase was prepared essentially as reported.[51]

Assays for inhibition of leukotriene A_4 hydrolase aminopeptidase activity

Assays for inhibition of the hydrolysis of alanine-*p*-nitro anilide (Ala-*p*NA) were performed in 1 mL cuvettes containing 50 mM Tris buffer and 1.4 μg of enzyme, using a Beckman DU-70 UV-visible multicell spectrophotometer monitoring at 405 nm to determine the rate of formation of *p*-nitroaniline. The estimated error in this rate was used as an estimate of the standard deviation of each rate in the case of K_i determinations. Each run was accompanied by a control lacking both enzyme and inhibitor to correct for the rate of uncatalyzed hydrolysis of Ala-*p*NA in buffer, spectrometer drift and other sytematic errors (if present). The adjusted data was used for all IC_{50} and K_i determinations. Inhibitors were dissolved in methanol and diluted 99:1 or more with Tris buffer. The concentration of methanol in the assays never exceeded 0.2%. IC_{50}s reported here are the concentrations of

inhibitor necessary to achieve half maximal rate of Ala-*p*NA hydrolysis. For all IC_{50} assays, the concentration of Ala-*p*NA was 1.87 mM (3.7 K_m) and the concentration of enzyme was 20 nM.

A value of ε_{405nm} = 10800 M^{-1} cm^{-1} was used for K_i calculations. K_i determinations used Ala-*p*NA concentrations of between 0.2 and 3.7 K_m. K_is were determined by fitting a general rate equation for mixed inhibition using non-linear least squares regression analysis of the rate data using Excel. This choice of programming language allows for its ready modification to accommodate other rate equations corresponding to other mechanistic patterns, and allows for quick creation of additional functions and graphs as desired. We chose to fit the data with an equation for mixed inhibition because it is a more general equation which allows for the possibilities of competitive ($K_{ii} = \infty$), uncompetitive ($K_{is} = \infty$), non-competitive ($K_{ii} = K_{is}$) and mixed-noncompetitive types ($K_{ii} \neq K_{is}$) of inhibition. The rate equation used was the general rate equation of Morrison,[29] for tight binding inhibitors:

$$v = \frac{N}{2}\left[\sqrt{\left(\frac{1}{\sum\left(\frac{N_i}{K_i}\right)} + \frac{[I]_t - [E]_t}{D}\right)^2 + \frac{4[E]_t}{D\sum\left(\frac{N_i}{K_i}\right)}} - \left(\frac{1}{\sum\left(\frac{N_i}{K_i}\right)} + \frac{[I]_t - [E]_t}{D}\right)\right]$$

For the simple case of mixed inhibition of

$$E + S \underset{}{\overset{K_m}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$$

$$\sum\left(\frac{N_i}{K_i}\right) = \frac{K_m}{K_{is}} + \frac{S}{K_{ii}}$$

$$N = \frac{V_{max}S}{E_t} \quad \text{and} \quad D = S + K_m$$

which collapses to the usual Michaelis–Menton equation for such cases wherein $[E]_t << [I]_t$:

$$v = \frac{V_{max}S}{K_m\left(1 + \frac{[I]_t}{K_{is}}\right) + S\left(1 + \frac{[I]_t}{K_{ii}}\right)}$$

$$K_m = \frac{[E][S]}{[ES]} \qquad K_{is} = \frac{[E][I]}{[EI]} \qquad K_{ii} = \frac{[ES][I]}{[ESI]}$$

(S = [S] = initial concentration of substrate.) This was necessary because in some cases K_i was less than or equal to $[E]_t$. ($[E]_t$ was 20 nM in most experiments.) In such cases, the usual Michaelis–Menten assumption that $[I]_t >> [E]_t$ fails for most useful inhibitor concentrations. We could not further lower the enzyme concentration or raise the inhibitor concentration because that would place the rate of *p*-nitroaniline formation below measurable limits. The fit was obtained by the Newton–Gauss iterative method of weighted non-linear least squares regression analysis,[52] using initial estimated values for K_m, V_{max}, K_{ii}, and K_{is} obtained from weighted linear least-squares fits of the usual double-reciprocal (1/v versus 1/[S]) Lineweaver–Burke plots. For this purpose, the weights of the reciprocals are calculated using $\sigma_{1/v} = \sigma_v/v^2$. In our experience, (using both synthetic data and actual data), this method generally converges after only 5–8 iterations, depending on the quality of the data and the initial estimates of the three binding constants and V_{max}. In cases wherein the inhibitor is competitive, K_{ii} will converge to ± infinity. Likewise, for cases wherein the inhibitor is uncompetitive, K_{is} will converge to ± infinity. For strictly non-competitive inhibitors, $K_{is} = K_{ii}$. Depending on the quality of the data and initial estimates, in some simulated cases of mixed inhibition with added random error, the value of the larger K_i (equating to less tightly bound) converges to ± infinity when one of the K_is is smaller by more than two orders of magnitude than the other. As a result, where we report competitive or uncompetitive inhibition, the possibility also exists that the inhibitor is of the mixed non-competitive type, strongly biased towards competitive or uncompetitive binding, respectively. This method assumes that [S] >> $[E]_t$ and that the proportional measurement errors associated with $[I]_t$ and [S], ($\sigma_I/[I]_t$ and $\sigma_S/[S]$), are much less than that of the rate, (σ_v/v).

Assays for inhibition of thromboxane and leukotriene B_4 formation in whole human blood

Compounds were dissolved in dimethyl sulfoxide then diluted in Hank's BSS. Blood was drawn from one human volunteer and to a 1 mL aliquot was added test compound or vehicle. After a 15 min incubation, 14 μg calcium ionophore A-23187 (free acid) was added and incubated for an additional 15 min. The reaction was stopped in slush ice and the samples then centrifuged at > 11,000 *g* to pellet red cells and leukocytes. An aliquot of the plasma was assayed for the presence of thromboxane B_2 or leukotriene B_4 using standard radio immunoassay techniques.

Synthesis of inhibitors

The synthetic procedures presented below are for the L enantiomer. The D-enantiomeric compounds **1**-D and **2**-D were prepared from N-Boc-O-benzyl-D-tyrosine (Bachem California) by the same procedure as the L-enantiomers. Syntheses and spectra for L and D enantiomers of the following compounds are identical, except for optical rotations. Some yields have not been optimized.

N-Boc-O-Benzyl-tyrosinol (21). 12.5 g (371.4 g mol^{-1}, 33.7 mmol) of N-Boc-O-benzyl-tyrosine (Peninsula) were dissolved in 100 mL of dry tetrahydrofuran (THF). The solution was cooled to 0 °C under argon on an ice-salt bath. 60 mL of 1 M BH_3·THF complex (60 mmol, Aldrich) were added slowly over 3 h. The reaction was monitored by TLC (1:1 ethyl acetate:hexanes; product $R_f = 0.6$) until starting material ($R_f = 0.3$) disappeared. The solution was allowed to continue to react for an additional 30 min, gradually warming to room temperature, before it was poured carefully into 600 mL of 1 N HCl. The acidic THF–water mixture was stirred for 30 min until bubbling ceased. This solution was extracted three times with 300 mL of ethyl acetate and the solvent was removed under reduced pressure to yield an off-white solid. This solid was taken up again in 800 mL of ethyl acetate and was washed with 2 × 500 mL 1 N HCl, 1 × 500 mL sat'd $NaHCO_3$ and 1 × sat'd NaCl, filtered through anhydrous $MgSO_4$, and the solvent was removed *in vacuo* to yield 10.7 g (89%) of a white, powdery solid. ^1H NMR ($CDCl_3$, TMS, 500 MHz) and melting point were as reported.[53] ^{13}C NMR ($CDCl_3$, TMS, 125 MHz): 28.3, 36.5, 53.8, 64.4, 70.0, 79.7, 114.8, 114.9, 127.4, 127.9, 128.6, 130.0, 130.2, 137.0, 157.5 ppm; HRMS: 380.1825, calcd for $C_{21}H_{27}NO_4Na^+$: 380.1838.

N-Boc-S-Acetyl-3-(4-benzyloxyphenyl)-2-amino-1-mercapto-propane (22). 8.056 g of N-Boc-O-benzyl-tyrosinol **21** (22.7 mmol) were dissolved in 200 mL of dry CH_2Cl_2. The solution was placed under argon and cooled to 0 °C in an ice bath with stirring. 4.5 mL of methylsulfonyl chloride (Aldrich, 58 mmol) were added via syringe, followed by the dropwise addition of 4.5 mL of triethylamine (32 mmol). Upon completion of addition, a catalytic amount of 4-(dimethylamino)-pyridine (DMAP) dissolved in 0.5 mL of dry THF was added. The reaction progress was monitored by thin layer chromatography (TLC, 1:1 ethyl acetate:hexanes; product $R_f = 0.75$) and the reaction was allowed to proceed to completion over 3 h, during which time it was allowed to warm to 25 °C. The reaction mixture was washed with 2 × 75 mL 1 N HCl, 2 × 75 mL sat'd $NaHCO_3$, 1 × 200 mL sat'd NaCl, filtered through anhydrous $MgSO_4$, and the solvent was removed under reduced pressure, yielding an off-white solid. Crude mesylated **21** (9.231 g) was obtained (FW: 435 g mol^{-1}, 21.2 mmol, 93.6% yield). HRMS (FAB^+, NBA/NaI): 458.1613; calcd for $C_{22}H_{29}NO_6SNa^+$: 458.1595.

9.231 g of crude methylsulfonylated **21** were dissolved in a minimum amount (70 mL) of anhydrous *N,N*-dimethylformamide (DMF) to which was added 2.666 g potassium thioacetate (23.34 mmol, Fluka), also dissolved in a minimum of anhydrous DMF. Reaction was monitored by TLC (1:1 ethyl acetate:hexanes; product $R_f = 0.9$) and allowed to proceed at 25 °C with stirring for 24 h, during which time a white solid (presumably potassium methylsulfonate) precipitated. (Potassium thioacetate from Aldrich gave greater quantities of a red by-product which proved difficult to remove.) The solvent was removed under reduced pressure yielding a reddish solid. This product was taken up in a heterogeneous mixture of 300 mL ethyl acetate/100 mL H_2O and placed in a separatory funnel. The organic phase was further washed with 2 × 150 mL 1N HCl, 2 × 150 mL $NaHCO_3$ and 1 × 150 mL sat'd NaCl, filtered through $MgSO_4$ and the solvent was removed *in vacuo* to yield an orange crystalline solid (97% crude yield). This was then recrystallized three times: the solid was dissolved in 200 mL refluxing diethyl ether with a minimum of CH_2Cl_2 added (~5%) to aid in dissolution. This was allowed to cool slowly and stand for 24 h without removal of the condenser. Within 10 h clear needles began to form. The third recrystallization yielded clear, odor-free needles in 70% yield. Later experiments with analogous compounds revealed that this lengthy purification probably could have been avoided by prior purification of the mesylate by silica gel chromatography and using refluxing acetone as solvent for this reaction. Purification could also be achieved by silica gel chromatography using 1:4 ethyl acetate:hexanes, followed by a second silica gel column using 2% ethyl acetate in chloroform as eluant. ^1H NMR ($CDCl_3$, TMS, 300 MHz) was as reported.[54] ^{13}C NMR (125 MHz, $CDCl_3$, TMS): 28.3, 30.6, 32.7, 39.4, 51.8, 69.9, 79.3, 114.8, 127.9, 128.5, 129.6, 130.3, 155.3, 157.6, 195.8 ppm; HRMS ($LSIMS^+$, NBA/NaI): 438.1730; calcd for $C_{23}H_{29}NO_4S^+$: 438.1715; mp: 120 °C.

S-Acetyl-3-(4-benzyloxyphenyl)-2-amino-1-mercapto-propane, HCl salt (2). 0.53 g of the protected thio-tyrosinol derivative **22**, (1.277 mmol) were dissolved in diethyl ether saturated with HCl. The solution was stirred in a closed flask for 24 h, during which time a white precipitate formed. The solid was collected and recrystallized from methanol/ether. The solvent was removed *in vacuo* to yield 241.6 mg of **2** (FW: 351.5, 0.687 mmol, 53.8%). ^1H NMR: (500 MHz, CD_3OD): 2.32 (*s*, 3H), 2.85 ($Ab_{quartet}$ *dd*, J_{AB} = 15.5 Hz, J_{d1} = 7.5 Hz, J_{d2} = 2.0 Hz, 2H), 2.95 (*ddd*, J_1 = 14.8 Hz, J_2 = 7.0 Hz, J_3 = 2.0 Hz, 1H), 3.16 (*ddd*, J_1 = 14.6 Hz, J_2 = 4.5 Hz, J_3 = 2.0 Hz, 1H), 3.53 (*m*, 1H), 5.01 (*s*, 2H), 6.92 (*dm*, J_d = 8.6 Hz, 2H), 7.17 (*dm*, J_d = 8.6 Hz, 2H), 7.23 (*t*, *J* = 7.5 Hz, 1H), 7.29 (*t*, *J* = 7.5 Hz, 2H), 7.35 ppm (*t*, *J* = 7.5 Hz, 2H); ^{13}C NMR (CD_3OD): 30.4, 31.3, 38.2, 54.3, 70.9, 116.5, 128.4, 128.5, 128.9, 129.5, 131.5, 138.6, 159.7, 196.3 ppm; HRMS ($LSIMS^+$, NBA): 316.1360; calcd for $C_{18}H_{22}NO_2S^+$: 316.1371; mp: 148 °C.

3-(4-Benzyloxyphenyl)-2-amino-1-mercapto-propane, hydrochloride salt (1). 0.503 g of protected thio-tyrosinol derivative **22** (FW: 415 g mol^{-1}, 1.21 mmol) were dissolved in 15 mL of ethanol. Argon was bubbled through the solution for 2 h to remove any oxygen present. 2.50 mL of degassed aqueous 1 N NaOH was added and the reaction was monitored by TLC (1:4 ethyl acetate:hexanes; SM: $R_f = 0.4$, product $R_f = 0.55$). When the reaction appeared to be complete, 50 mL deoxygenated ethyl acetate were added and washed with the following deoxygenated aqueous solutions: 50 mL 1 N HCl, 50 mL sat'd $KHCO_3$, 50 mL sat'd NaCl. The organic phase was removed *in vacuo* to yield a

crude white solid. This material was then dissolved in 50 mL of diethyl ether saturated with anhydrous HCl. The reaction was allowed to proceed for 16 h, after which time it was filtered giving **1** as white, odorless crystals (345 mg, 92%). This compound could be recrystallized from DMF and ether. ^{1}H NMR (500 MHz, DMSO-d_6, TMS): 2.592 (*m*, 1H), 2.726 (*m*, 1H), 2.859 (*dd*, J_1 = 14.0 Hz, J_2 = 8.5 Hz, 1H), 2.949 (*dd*, J_1 = 14 Hz, J_2 = 5.5 Hz, 1H), 3.035 (*m*, 1H), 3.431, (*br s*, 1H), 5.083 (*s*, 2H), 6.988 (*d*, *J* = 8.5 Hz, 2H), 7.209 (*d*, *J* = 8.5 Hz, 2H), 7.333 (*t*, *J* = 7.3 Hz, 1H), 7.396 (*t*, *J* = 7.5 Hz, 2H), 7.449 (*d*, *J* = 7.0 Hz, 2H), 8.325 ppm (*br s*, 3H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS): 25.4, 35.7, 53.8, 69.2, 114.9, 127.7, 127.8, 128.1, 128.4, 130.4, 137.1, 157.3 ppm; HRMS: (FAB$^+$, NBA) 274.1266; calcd for $C_{16}H_{20}NOS^+$: 274.1268; mp: 181 °C.

*N-Boc-1-O-(*tert*-Butyldimethylsilyl)-tyrosinol (23).* 0.501 g of N-Boc-O-benzyl-tyrosinol **21** (1.40 mmol) were dissolved in 20 mL dry CH_2Cl_2. 254 mg (1.69 mmol) of *tert*-butyldimethylsilyl chloride (Aldrich) and 2.4 mL of triethylamine (17.2 mmol) were added, followed by 190 mg of DMAP (1.54 mmol). The reaction was allowed to proceed for 2 h until it was judged to be complete by TLC (1:2 ethyl acetate:hexanes; silylated product R_f = 0.9). The CH_2Cl_2 solution was diluted with 30 mL of CH_2Cl_2 and then washed with 2 × 50 mL 0.5 N HCl, 2 × 50 mL sat'd $NaHCO_3$ and 1 × 50 mL sat'd NaCl. The solvent was removed under reduced pressure to yield a light yellow oil, which was then purified by silica gel chromatography (1:8 ethyl acetate:hexanes), to yield a clear oil. ^{1}H NMR ($CDCl_3$, TMS, 300 MHz): 0.04 (*s*, 3H), 0.10 (*s*, 3H), 0.92 (*s*, 9H), 1.42 (*s*, 9H), 2.77 (*d*, *J* = 6 Hz, 2H), 3.48 (*m*, 2H), 3.70–3.85 (*br s*, 1H), 4.73 (*m*, 1H), 5.04 (*s*, 2H), 6.89 (*d*, *J* = 8.5 Hz, 2H), 7.13 (*d*, *J* = 8.5 Hz, 2H), 7.30–7.45 (*m*, 5H); MS: 604 amu, expected M + Cs$^+$: 604 amu.

The silylation product was taken up in 20 mL of ethyl acetate and combined with 74.5 mg of 10% palladium on carbon (0.07 mmol). Argon was bubbled through the mixture for 30 min followed by hydrogen for 8 h. The reaction was allowed to stir for an additional 10 h after which time it was judged to be complete by TLC (1:4 ethyl acetate:hexanes; SM: R_f = 0.7; product R_f = 0.3). The mixture was filtered through Celite and the solvent removed *in vacuo* to yield a light yellow, highly viscous liquid. Purification by flash chromatography (1:4 ethyl acetate:hexanes) yielded 0.47 g of **23** (1.23 mmol, 88%) as a clear, highly viscous liquid. ^{1}H NMR (DMSO-d_6, TMS, 500 MHz): 0.012 (*s*, 6H), 0.850 (*s*, 9H), 1.313 (*s*, 9H), 2.430 (*dd*, J_1 = 14.0 Hz, J_2 = 8.5 Hz, 1H), 2.652 (*dd*, J_1 = 14.0 Hz, J_2 = 5.5 Hz, 1H), 3.422 (*m*, 2H), 3.512 (*m*, 1H), 6.568 (*d*, *J* = 8.5 Hz, 1H), 6.633 (*d*, *J* = 8.5 Hz, 2H), 6.950 (*d*, *J* = 8.5 Hz, 2H), 9.120 ppm (*s*, 1H); ^{13}C NMR (DMSO-d_6, TMS, 125 Mhz): –5.4, –5.3, 17.9, 25.8, 28.3, 35.6, 53.9, 77.4, 114.9, 129.1, 129.8, 155.1, 155.5 ppm; HRMS (FAB, NBA): 382.2405; calcd for $C_{20}H_{36}NO_4Si^+$: 382.2414 amu.

N-Boc-3-[4-(Methyl 4-carboxybenzyl)-oxyphenyl]-2-amino-1-propanol (41). 0.47 g N-Boc-1-O-TBDMS-tyrosinol **23** (1.234 mmol) were dissolved in 20 mL dry acetone. 275 mg of methyl 4-bromomethylbenzylate (1.20 mmol, Aldrich) and 180 mg (1.3 mmol) of K_2CO_3 were added to the stirring mixture. The reaction was monitored by TLC (1:4 ethyl acetate:hexanes; SM: R_f = 0.3; product R_f = 0.55) and found to be complete after 23 h. It was then diluted with 50 mL diethyl ether and washed with 50 mL water. The water was extracted twice with 50 mL ether. The organic fractions were pooled and the solvent was removed *in vacuo*. The compound was purified by flash chromatography (eluant, 1:6 ethyl acetate:hexanes) to yield a clear oil. ^{1}H NMR ($CDCl_3$, TMS, 300 MHz): 0.04 (*s*, 6H), 0.92 (*s*, 9H), 1.42 (*s*, 9H), 2.77 (*d*, *J* = 7 Hz, 2H), 3.48 (*m*, 2H), 3.77 (*m*, 1H), 3.92 (*s*, 3H), 4.73 (*br d*, 1H), 5.12 (*s*, 2H), 6.89 (*d*, *J* = 8.5 Hz, 2H), 7.13 (*d*, *J* = 8.5 Hz, 2H), 7.50 (*d*, *J* = 8.1 Hz, 2H), 8.06 (*d*, *J* = 8.2, 2H).

This product, N-Boc-O-(*tert*-butyldimethylsilyl)-3-[4-(methyl 4-carboxybenzyl)-oxyphenyl]-2-amino-1-propanol was then dissolved in 50 mL CH_2Cl_2. 0.89 mL of 10% v/v acetic acid in CH_2Cl_2 was added (1.56 mmol AcOH), followed by 18 mL of 1 N NBu_4F (18 mmol) in THF. The reaction was monitored by TLC (1:4 ethyl acetate:hexanes; free alcohol product R_f = 0.05) and allowed to continue for 48 h before the solvent was removed *in vacuo*. The product was purified by flash chromatography (3:4 ethyl acetate:hexanes) to give two products, both of which were white solids. The later eluting band was determined to be the desired product, **41**; yield: 289.6 mg (0.698 mmol, 56.6%). ^{1}H NMR (300 MHz, $CDCl_3$, TMS): 1.43 (*s*, 9H), 2.22 (*br s*, 1H), 2.79 (*d*, *J* = 7.1 Hz), 3.55 (*m*, 1H), 3.66 (*m*, 1H), 3.82 (*m*, 1H), 3.92 (*s*, 3H), 4.70 (*m*, 1H), 5.11 (*s*, 2H), 6.90 (*d*, J = 8.6 Hz, 2H), 7.13 (*d*, *J* = 8.5 Hz, 2H), 7.50 (*d*, *J* = 8.1 Hz, 2H), 8.06 ppm (*d*, *J* = 8.2 Hz, 2H); MS: 416, expected (M + H$^+$): 416 amu. The earlier eluting band was determined to be N-Boc-O-acetyl-3-[4-(methyl benzyloxy-4-carboxylate)-phenyl]-2-amino-1-propanol; yield: 136.0 mg (0.298 mmol, 24.2%). ^{1}H NMR (300 MHz, $CDCl_3$, TMS): 1.42 (*s*, 9H), 2.09 (*s*, 3H), 2.75 (*m*, 2H), 3.92 (*s*, 3H), 4.02 (*s*, 2H), 4.04 (*m*, 1H), 4.63 (*m*, 1H), 5.10 (*s*, 2H), 6.90 (*d*, *J* = 8.5 Hz, 2H), 7.10 (*d*, *J* = 8.4 Hz, 2H), 7.50 (*d*, *J* = 8.1 Hz, 2H), 8.06 (*d*, *J* = 8.2 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): 28.3, 36.5, 52.2, 53.8, 64.5, 69.4, 79.5, 114.9, 127.0, 129.6, 129.9, 130.3, 142.3, 156, 157.2, 166.9; MS: 458, expected (M + H$^+$): 458 amu.

N-Boc-S-Acetyl-3-[4-(methyl 4-carboxybenzyl)-oxyphenyl]-2-amino-1-mercaptopropane (24). N-Boc-3-[4-(methyl 4-carboxybenzyl)-oxyphenyl]-2-amino-1-propanol (289.6 mg, 0.698 mmol) was dissolved in 10 mL dry CH_2Cl_2 and cooled under Ar to 0 °C on an ice-salt bath. 110 µL methylsulfonyl chloride (Aldrich, 1.40 mmol) were added, followed by slow addition of 110 µL triethylamine (0.838 mmol). After 3 h, the reaction was found to be incomplete, as indicated by TLC (3:4 ethyl acetate:hexanes). An additional 110 µL of both methylsulfonyl chloride and triethylamine were added. After an additional hour, the starting material had disappeared and two product spots were visible. The

reaction was diluted with 70 mL ethyl acetate and washed with the following aqueous solutions: 2 × 150 mL 1 N HCl, 1 × 150 mL sat'd $NaHCO_3$ and 1 × 150 mL sat'd NaCl. It was filtered through $MgSO_4$ and the organic solvent was removed under reduced pressure. The resultant solid was purified by flash chromatography (3:4 ethyl acetate:hexanes), to yield 125.2 mg of a white, chalky powder (0.26 mmol, 36.4% yield). ^{1}H NMR (300 MHz, $CDCl_3$, TMS): 1.42 (*s*, 9H), 2.81 (*m*, 2H), 3.01 (*s*, 3H), 3.92 (*s*, 3H), 4.05 (*m*, 1H), 4.11 (*dd*, *J* = 10.0 Hz, *J* = 3.9 Hz, 1H), 4.24 (*dd*, *J* = 9.6 Hz, *J* = 3.3 Hz, 1H), 4.71 (*br d*, *J* = 7.2 Hz, 1H), 5.11 (*s*, 2H), 6.93 (*d*, *J* = 8.6 Hz, 2H), 7.14 (*d*, *J* = 8.5 Hz, 2H), 7.50 (*d*, *J* = 8.3 Hz, 2H), 8.06 (*d*, *J* = 8.3 Hz, 2H).

N-Boc-^{1}O-methanesulfonyl-3-[4-(methyl 4-carboxybenzyl)-oxyphenyl]-2-amino-1-propanol (125.2 mg, 0.254 mmol) was dissolved in 5 mL anhydrous DMF, to which was added 32 mg potassium thioacetate (0.280 mmol). The reaction was monitored by TLC (1:4 ethyl acetate:hexanes) and allowed to continue for 48 h, after which the dimethyl formamide was removed under reduced pressure. The resultant reddish solid was taken up in 70 mL ether and washed with 2 × 50 mL 1 N HCl, 2 × 50 mL sat'd $NaHCO_3$ and 1 × 50 mL sat'd NaCl and the solvent was removed under reduced pressure to yield a very light brown solid which was purified by flash chromatography (1:3 ethyl acetate:hexanes) to yield 106.2 mg (0.214 mmol, 84%) of **24** as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): 1.40 (*s*, 9H), 2.36 (*s*, 3H), 2.71 (*dd*, J_1 = 13.8 Hz, J_2 = 7.5 Hz, 1H), 2.86 (*dd*, J_1 = 13.8 Hz, J_2 = 4.5 Hz), 2.93 (*dd*, J_1 = 13.8 Hz, J_2 = 7.5 Hz, 1H), 3.07 (*dd*, J_1 = 13.8 Hz, J_2 = 4.5 Hz), 3.87–3.97 (*m*, 1H), 3.92 (*s*, 2H), 4.52–4.65 (*m*, 1H), 5.01 (*s*, 2H), 6.90 (*d*, *J* = 8.6 Hz, 2H), 7.10 (*d*, *J* = 8.6 Hz, 2H), 7.50 (*d*, *J* = 8.2 Hz, 2H), 8.05 ppm (*d*, J = 8.3 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): 28.2, 30.5, 32.6, 39.4, 51.7, 69.2, 79.3, 114.8, 126.8, 129.5, 129.7, 129.8, 130.3, 142.2, 155.2, 157.2, 166.7, 195.7 ppm; HRMS (SIMS$^+$, NBA/NaI): 496.1770; calcd for $C_{25}H_{31}NO_6SNa^+$: 496.1770; mp: 122–123 °C; $[\alpha]_D$ = 3.5° (L isomer).

3-[4-(4-Carboxybenzyl)-oxyphenyl]-2-amino-1-mercapto-propane, HCl salt (12). 85.7 mg of **24** (0.173 mmol) was added to 8 mL of ethanol and the oxygen was removed by bubbling nitrogen through the solution in a closed container connected to a bubbler for 1 h. 1.73 mL of deoxygenated 1 N NaOH in water was added via syringe. The reaction was allowed to proceed for 6 h while being monitored by TLC (1:3 ethyl acetate:hexanes). The solvent was removed under reduced pressure, and the white, solid product stored under nitrogen.

25 mL diethyl ether saturated with HCl was added to the basic hydrolysis product of **24** with stirring. Anhydrous HCl gas was bubbled through the solution for 30 min and the reaction was allowed to stir for 24 h. The solid product was collected by filtration, redissolved in ethanol and filtered to remove non-dissolving solid. The ethanolic solution was diluted with diethyl ether and the product, **12** was collected as a white crystalline powder after 24 h. Yield: 54.3 mg (0.171 mmol, 99%). ^{1}H NMR (500 MHz, DMSO-d_6, TMS): 2.595 (*m*, 1H), 2.730 (*m*, 1H), 2.863 (*m*, 1H), 2.970 (*m*, 1H), 3.103 (*m*, 1H), 5.180 (*s*, 2H), 6.994 (*d*, *J* = 8.5 Hz, 2H), 7.217 (*d*, *J* = 8.5 Hz, 2H), 7.565 (*d*, *J* = 8.0 Hz, 2H), 7.963 ppm (*d*, *J* = 8.0 Hz, 2H), 8.5 ppm (br, 3H); ^{13}C NMR (DMSO-d_6, TMS, 125 MHz): 25.4, 35.6, 53.8, 69.2, 115.0, 127.4, 128.4, 129.5, 130.1, 130.5, 142.2, 157.1, 167.0 ppm; HRMS (LSIMS$^+$, NBA): 318.1171; expected for $C_{17}H_{20}NO_3S^+$: 318.1164 amu; mp: 250 °C, decomposition.

Tyrosinol, HCl salt (3). 50 mg of N-Boc tyrosinol, **21**, (M_r 357, 0.42 mmol) were dissolved in 20 mL of anhydrous ether. Anhydrous HCl was bubbled through the stirred solution for 2 h. The reaction was allowed to continue to stir for another 22 h. The product was collected via filtration as a fine white powder. Yield 97.4 mg (0.33 mmol, 78%). ^{1}H NMR (500 MHz, DMSO-d_6, TMS): 2.760 (*dd*, J_1 = 13 Hz, J_2 = 9.5 Hz, 1H), 2.902 (*dd*, J_1 = 13.5 Hz, J_2 = 5.0 Hz, 1H), 3.230 (*m*, 1H), 3.381 (*dd*, J_1 = 11.5 Hz, J_2 = 5.5 Hz, 1H), 3.503 (*dd*, J_1 = 11.5 Hz, J_2 = 3.0 Hz, 1H), 5.083 (*s*, 2H), 5.372 (*br s*, 1H), 6.975 (*d*, *J* = 8.5 Hz, 2H), 7.199 (*d*, *J* = 8.0 Hz, 2H), 7.333 (*t*, *J* = 7.0 Hz, 1H), 7.396 (*t*, *J* = 7.5 Hz, 2H), 7.450 (*d*, *J* = 7.5 Hz, 2H), 8.1–8.3 ppm (*br*, 3H); ^{13}C NMR (DMSO-d_6, TMS, 125 MHz): 33.6, 54.1, 59.5, 69.2, 114.8, 127.7, 127.8, 128.5, 128.8, 130.4, 137.2, 157.2 ppm; HRMS (LSIMS$^+$, NBA/NaI): 258.1495 amu; calcd for $C_{16}H_{20}NO_2^+$: 258.1494 amu; mp: 178 °C.

Ethyl 3-chloromethyl-benzoate (25). 3-Chloromethyl-benzoyl chloride (10 mmol, 1.89 g, 1.42 mL, Aldrich) was added dropwise to 25 mL of absolute ethanol (water and methanol free). The solution was allowed to react for 15 min and then the ethanol was removed *in vacuo* to yield 1.95 g (98%) of **25** as a clear liquid. ^{1}H NMR (500 MHz, $CDCl_3$, TMS): 1.406 (*t*, *J* = 7.0 Hz, 3H), 4.393 (q, *J* = 7.0 Hz, 2H), 4.628 (*s*, 1H), 7.448 (*t*, *J* = 7.5 Hz, 1H), 7.591 (*d*, *J* = 7.5 Hz, 1H), 8.008 (*d*, *J* = 7.5 Hz, 1H), 8.066 (*s*, 1H); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): 14.3, 45.5, 61.1, 128.8, 129.5, 129.6, 131.0, 132.9, 137.7, 166.1 ppm; HRMS (FAB$^+$, NBA/NaI): 221.0355; calcd for $C_{10}H_{11}O_2ClNa^+$: 221.0345 amu.

N-Boc-3-[4-(Ethyl 3-carboxybenzyl)-oxyphenyl]-2-amino-1-propanol (26). Ethyl 3-chloromethyl-benzoate **25** (6.045 mmol, 1.20 g) was added slowly to a mixture of N-Boc-1-O-(*tert*-butyldimethylsilyl)-tyrosinol **23** (2.3 g, 6.04 mmol), anhydrous powdered K_2CO_3 (0.97 g, 7 mmol) and 60 mL anhydrous acetone (Aldrich). NBu_4I was then added (1.5 mmol, 550 mg). The reaction was monitored by TLC (1:2 ethyl acetate:hexanes; phenol R_f = 0.8, product R_f = 0.9). Upon completion, the acetone was removed under reduced pressure to yield a yellow oil.

The oil was dissolved in 20 mL dry THF. 24 mL of 1 N NBu_4F in THF was added and stirring commenced for 3 h at 35 °C. The reaction was monitored by TLC (1:2 ethyl acetate:hexanes; silyl ether R_f = 0.9, product R_f =

0.2) to determine completion status. Upon completion, the THF was removed under reduced pressure and the resultant yellow oil was taken up in 250 mL ethyl acetate. This solution was washed with 2 × 300 mL 1 N HCl, 1 × sat'd $NaHCO_3$ and 1 × sat'd NaCl. The organic phase was then filtered through anhydrous $MgSO_4$ and concentrated *in vacuo* to yield a yellow oil, which was purified by silica gel chromatography (1:1 ethyl acetate:hexanes) to give **26** (1.7 g, 4.0 mmol, 66%) as a clear, highly viscous oil. ^1H NMR (500 MHz, $CDCl_3$, TMS): 1.392 (*t*, *J* = 7.0 Hz, 3H), 1.402 (*s*, 9H), 2.787 (*d*, *J* = 6.5 Hz), 3.2–3.3 (br, 1H), 3.536 (*m*, 1H), 3.618 (*m*, 1H), 3.824 (*m*, 1H), 4.376 (quartet, *J* = 7.0 Hz, 2H), 5.0 (*m*, 1H), 5.051 (*s*, 2H), 6.900 (*d*, *J* = 8.5 Hz, 2H), 7.141 (*d*, *J* = 8.5 Hz, 2H), 7.441 (*t*, *J* = 8.0 Hz, 1H), 7.617 (*d*, *J* = 7.5 Hz, 1H), 7.996 (*d*, *J* = 8.0 Hz, 1H), 8.100 ppm (*s*, 1H); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): 14.2, 20.9, 28.2, 36.3, 53.6, 61.0, 63.6, 69.3, 79.3, 114.6, 128.3, 128.5, 128.9, 130.2, 130.6, 131.7, 137.3, 156.0, 157.0, 166.3 ppm; HRMS (LSIMS$^+$, NBA): 430.2235; calcd for $C_{24}H_{32}NO_6^+$: 430.2230.

N-Boc-S-Acetyl-3-[4-(ethyl 3-carboxybenzyl)-oxyphenyl]-2-amino-1-mercaptopropane (27). The tyrosinol derivative **26** (1.500 g, 3.500 mmol) was dissolved in 50 mL dry CH_2Cl_2. This solution was placed under argon and chilled to 0 °C on an ice bath. Methanesulfonyl chloride (2 eq., 7 mmol, 0.8 g, 0.55 mL) was added by syringe, followed by dropwise addition of triethyl amine (1.5 eq., 0.531 g, 731 μL). The reaction was monitored by TLC (1:1 ethyl acetate:hexanes) and was found to be complete after 1 h. The reaction was diluted with 300 mL of ethyl acetate and washed with 2 × 300 mL 1 N HCl, 2 × 300 mL sat'd $NaHCO_3$ and 1 × 70 mL sat'd NaCl. The organic phase was filtered through anhydrous $MgSO_4$ and the solvent removed under reduced pressure to yield the mesylate of **26** (1.8 g) as a light yellow oil.

The oil so obtained was dissolved in 30 mL anhydrous DMF. Potassium thioacetate (Fluka, 460 mg, 4 mmol) was added. The vessel was sealed with a septum and argon was blown through the vessel to remove the oxygen. The reaction was monitored by TLC (1:1 ethyl acetate: hexanes), and was found to be complete after 3 h, during which time it had turned a dark reddish-brown. The DMF was removed under reduced pressure and the resultant nearly black solid was taken up in a biphasic mixture of 100 mL ethyl acetate and 70 mL sat'd $NaHCO_3$. This mixture was transferred to a separatory funnel, and diluted with 200 mL ethyl acetate and 100 mL sat'd $NaHCO_3$. The organic phase was washed with 2 × sat'd $NaHCO_3$ in water, 1 × 250 mL 1 N HCl and 1 × 100 mL sat'd NaCl, and then filtered through anhydrous $MgSO_4$. The solvent was removed *in vacuo* giving a brown solid. This material was purified by silica gel chromatography (1:4 ethyl acetate:hexanes) to give an orange oil which crystallized upon standing. It was found that refluxing a solution containing this solid in ethyl acetate in the presence of a small amount of decolorizing carbon for 5 min readily removed all contaminants. Unfortunately the yield was also poor: **27** was obtained as 243 mg (0.5 mmol, 14.2%) of a white crystalline solid. ^1H NMR (500 MHz, $CDCl_3$, TMS): 1.404 (*t*, *J* = 7 Hz, 3H), 1.408 (*s*, 12H), 2.359 (*s*, 3H), 2.720 (*dd*, J_1 = 13.5 Hz, J_2 = 7 Hz, 1H), 2.856 (*dd*, J_1 = 14 Hz, J_2 = 5.5 Hz, 1H), 2.929 (*dd*, J_1 = 13.5 Hz, J_2 = 8.0 Hz, 1H), 3.071 (*dd*, J_1 = 13.5 Hz, J_2 = 4.0 Hz, 1H), 3.937 (*m*, 1H), 4.390 (quartet, *J* = 7 Hz, 2H), 4.611 (*br d*, *J* = 8 Hz, 1H), 5.079 (*s*, 2H), 6.914 (*d*, *J* = 8.5 Hz, 2H), 7.112 (*d*, *J* = 8.0 Hz, 2H), 7.462 (*t*, *J* = 8.0 Hz, 1H), 7.635 (*d*, *J* = 8.0 Hz, 1H), 8.007 (*d*, J = 7 Hz, 1H), 8.108 ppm (*s*, 1H); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): 14.3, 28.3, 29.7, 30.6, 32.7, 39.5, 51.8, 61.1, 69.4, 79.4, 114.8, 128.5, 128.6, 129.1, 129.8, 130.4, 130.8, 131.8, 155.3, 157.3, 166.4, 195.8 ppm; HRMS (LSIMS$^+$, NBA/NaI): 510.1923; calcd for $C_{26}H_{33}NO_6SNa^+$: 510.1926 amu.

3-[4-(3-Carboxybenzyl)-oxyphenyl]-2-amino-1-mercaptopropane (13). 190 mg (0.390 mmol) of thioester **27** were dissolved in 10 mL anhydrous ethanol. 1 N NaOH aqueous solution (5 eq., 2 mL), was then added. Argon was bubbled through the stirring solution and the reaction allowed to proceed for 12 h until the starting material disappeared as determined by TLC. The ethanol was removed *in vacuo*. The flask was resealed and purged with argon, followed by the addition of 60 mL of degassed ethyl acetate and 40 mL of degassed 1 N HCl. The mixture was swirled until all solid had dissolved and transferred to a separatory funnel which was also purged with argon. The organic phase was then washed with the following anaerobic aqueous solutions: 2 × 100 mL 1 N HCl and 1 × 80 mL sat'd NaCl. The organic layer was filtered through anhydrous $MgSO_4$ and the solvent removed under reduced pressure to give the saponified free thiol-free acid as a white solid.

This was immediately redissolved in 25 mL of anhydrous sat'd ethereal HCl. A stirring bar was added, the vessel sealed with a septum (and bubbler to relieve pressure) and allowed to stir for 14 h, during which time **13** precipitated from the ether as a white powdery solid. The solid was collected by filtration and allowed to air dry, during which time it was found to be fairly hygroscopic, soon forming a sticky white paste. This material was dried under high vacuum overnight, yielding **13** as a pale yellow glass, containing 10% disulfide by NMR (presence of disulfide confirmed by MS). ^1H NMR (500 MHz, DMSO-d_6, TMS): 2.6–3.1 (*m*, 5H), 3.6 (*br s*, 1H), 5.18 (*s*, 2H), 7.010 (*d*, *J* = 8.0 Hz, 2H), 7.225 (*d*, *J* = 8.0 Hz, 2H), 7.538 (*t*, *J* = 7.5 Hz, 1H), 7.705 (*d*, *J* = 8.0 Hz, 1H), 7.911 (*d*, *J* = 7.5 Hz, 1H), 8.033 (*s*, 1H), 8.3–8.5 (*br m*, 3H), 13.0–13.2 (*br s*, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS): 25.4, 35.7, 53.8, 68.6, 115.0, 128.3, 128.7, 128.8, 130.5, 131.0, 132.0, 137.8, 145.7, 157.2, 167.1; HRMS (FAB$^+$, NBA): 318.1158; calcd for $C_{17}H_{20}NO_3S^+$: 318.1164 amu; mp (glass) 140–160 °C.

N-Boc-O,O'-dibenzyl-3'-iodotyrosine (28). 3-Iodo-L-tyrosine (15 mmol, 4.6 g, Sigma) was dissolved in a solution of 15 mL water, 30 mL dioxane and 15 mL 1 N NaOH. Argon was bubbled through the solution for 30 min before 1.3 eq. of Boc-pyrocarbonate (19.5 mmol,

4.25 g) were added. Stirring was continued under argon for 12 h, after which time 100 mL ethyl acetate was added and the solution was acidified (with vigorous stirring) to pH 3 with 6 N HCl. The aqueous phase was separated and extracted twice with 100 mL of ethyl acetate. The organic fractions were pooled and the solvent removed under reduced pressure to yield a clear oil. This material was then dissolved in 250 mL of dry acetone (dried over anhydrous K_2CO_3). Powdered, anhydrous potassium carbonate (3.5 eq., 52.5 mmol, 7.26 g) and a catalytic amount of Bu_4NI were added. Argon was bubbled through the solution for 30 min before addition of 2.2 eq. benzyl bromide (33 mmol, 5.64 g, 3.93 mL). The reaction was then brought to reflux and allowed to continue to completion by TLC (1:2 ethyl actetate:hexanes, 16 h.) The product was purified by silica gel chromatography and the solvent removed (for the most part) under reduced pressure to yield 7.8 g of **28** as a pale yellow oil containing 13% (by ^{1}H NMR) ethyl acetate by weight, (11 mmol, 76%). Removal of the residual ethyl acetate proved to be difficult by conventional means. The oil was taken on in this form. ^{1}H NMR (500 MHz, $CDCl_3$, TMS): 1.431 (*s*, 9H), 2.988 (ABX, $\Delta\nu$ = 27 Hz, J_{AB} = 14 Hz, J_X = 6 Hz, 2H), 4.561 (*m*, 1H), 5.023 (*d*, J = 8 Hz, 1H), 5.093 (*s*, 2H), 5.128 (AB, $\Delta\nu$ = 26 Hz, J_{AB} = 12 Hz, 2H), 6.677 (*d*, J = 8.5 Hz, 1H), 6.922 (*d*, J = 8.5 Hz, 1H), 7.29–7.41 (*m*, 8H), 7.485 (d, J = 8 Hz, 2H), 7.540 (*s*, 1H); HRMS (FAB$^+$, NBA/NaI): 610.1078; calcd for $C_{28}H_{30}NO_5INa^+$: 610.1066 amu.

N-Boc-O'-benzyl-3'-iodotyrosinol (29). N-Boc-O,O'-dibenzyl-3'-iodotyrosine, **28**, (7.5 g, 12.8 mmol) was dissolved in 150 mL of THF and cooled to 0 °C in an ice-salt bath under argon. Lithium aluminum hydride (LAH) was added in small amounts (approximately 50 mg at a time) at half hour intervals until TLC revealed that the reaction was complete. (Total LAH addition: 250 mg, 6.5 mmol.) The reaction was quenched by dropwise addition of 5 mL 1 N HCl, followed by slow addition of 130 mL of a concentrated aqueous solution of potassium sodium tartrate. This solution was transferred to a separatory funnel and further diluted with 200 mL of concentrated potassium sodium tartrate solution. This solution was extracted with 250 mL ethyl acetate four times until the aqueous phase became clear with only a small grey haze remaining. The organic fractions were pooled and the ethyl acetate removed under reduced pressure. The resultant off-white solid was taken up in 400 mL of ethyl acetate and washed with 2 × 250 mL 1 N HCl, 1 × 250 mL sat'd $NaHCO_3$, and 1 × sat'd NaCl. The organic phase was dried by simple filtration through anhydrous $MgSO_4$ and the solvent removed *in vacuo*. The product was purified by silica gel chromatography (3:4 ethyl acetate: hexanes) to yield 5.1 g of **29** as a powdery white solid (10.5 mmol, 82%). ^{1}H NMR (500 MHz, $CDCl_3$, TMS): 1.423 (*s*, 9H), 2.437 (*br s*, 1H), 2.749 (*d*, J = 7 Hz, 2H), 3.544 (*m*, 1H), 3.652 (*m*, 1H), 3.787 (*m*, 1H), 4.781 (*d*, J = 8 Hz, 1H), 5.125 (*s*, 2H), 6.785 (*d*, J = 8 Hz, 1H), 7.125 (*d*, J = 8 Hz, 1H), 7.323 (*t*, J = 7.5 Hz, 1H), 7.396 (*t*, J = 7.5 Hz, 2H), 7.491 (*d*, J = 7.5 Hz, 2H), 7.657 (*d*, J = 2 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): 28.3, 35.9, 53.6, 64.0, 70.8, 79.8, 86.8, 112.6, 126.9, 127.8, 128.5, 130.2, 132.4, 136.4, 140.0, 155.9 ppm; HRMS (FAB, NBA/H$^+$): 484.0967; calcd for $C_{21}H_{27}NO_4I$: 484.0985 amu; mp: 116 °C.

N-Boc-3-(4-Benzyloxy-3-butoxycarbonyl-phenyl)-2-amino-1-propanol (30). N-Boc-O'-benzyl-3'-iodotyrosinol, **29**, (1.25 g, 2.58 mmol), palladium(II) acetate (65 mg, 0.29 mmol) and triphenylphosphine (128 mg, 0.488 mmol) were placed in a 250 mL three necked flask with a stirring bar and reflux condenser. The flask was evacuated and flushed with nitrogen several times before being flushed once with carbon monoxide. 20 mL of dry *n*-butanol (dried over a liberal amount of anhydrous $MgSO_4$ for 1 h and filtered) were added via syringe. The temperature was brought to 110 °C, with vigorous stirring. After 25 min, a brown color began to evolve quickly. Trioctylamine (1.1 mL, 1.36 g, 3.8 mmol) was added (eliminating some of the brown color) and the reaction was allowed to stir at 110 °C for 6 h, at which point it appeared to be complete by TLC. (1:1 ethyl acetate: hexanes, R_f of starting material and product were 0.65. A co-spot of the two revealed that the product pushed the starting material ahead of it on the plate to an approximate R_f of 0.75. The two could be visually distinguished by their fluorescence on the plate as illuminated on a Fotodyne Fotoprepl gel visualizer. The product fluoresced electric blue while the starting material fluoresced only weakly with a yellow green color.) The *n*-butanol was removed under reduced pressure and the product was taken up in 100 mL of ethyl acetate. This was washed twice with 100 mL of 1 N HCl, 1 × 100 mL sat'd $NaHCO_3$ and 1 × 50 mL sat'd NaCl. It was then dried over anhydrous $MgSO_4$ and the solvent removed *in vacuo*. Further purification was achieved by silica gel chromatography (3:4 ethyl acetate:hexanes), to give **30** as a clear oil which crystallizes upon standing. Yield: 946 mg (2.07 mmol, 80%). ^{1}H NMR (500 MHz, $CDCl_3$, TMS): 0.915 (*t*, J = 7 Hz, 3H), 1.692 (*m*, 2H), 2.3 (*br s*, 1H), 2.806 (*d*, J = 7 Hz, 2H), 3.564 (*m*, 1H), 3.661 (*m*, 1H), 3.820 (*m*, 1H), 4.295 (*t*, J = 7.0 Hz, 2H), 4.73 (*br*, 1H), 5.147 (*s*, 2H), 6.957 (*d*, J = 8.5 Hz, 1H), 7.28 (*m*, 1H), 7.313 (*t*, J = 7.5 Hz, 1H), 7.380 (*t*, J = 7.5 Hz, 2H), 7.477 (*d*, J = 7.5 Hz, 2H), 2.625 (*s*, 1H); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): 13.74, 19.18, 28.29, 30.7, 37.0, 53.8, 63.8, 64.9, 70.7, 79.1, 113.9, 121.8, 127.1, 127.8, 128.5, 129.8, 132.3, 133.9, 136.7, 157.1, 166.3; HRMS (FAB$^+$, NBA/NaI): 480.2383; calcd for $C_{26}H_{35}NO_6Na^+$: 480.2362 amu.

N-Boc-S-Acetyl-3-(butyl 4-benzyloxy-3-carboxyphenyl)-2-amino-1-propanethiol (40). 503 mg (1.10 mmol) N-Boc-3-(4-Benzyloxy-3-butoxycarbonyl-phenyl)-2-amino-1-propanol, **30**, were dissolved in 20 mL of dry CH_2Cl_2 and placed in a 100 mL flask under argon. Methanesulfonyl chloride (130 μL, 1.68 mmol) was added followed by triethylamine (160 μL, 1.15 mmol). The solution was allowed to stir for 2 h, after which time, it was judged to be complete by TLC (1:2 ethyl acetate:hexanes, R_f SM: 0.17, R_f product: 0.31). The reaction was diluted with ethyl acetate (200 mL) and

washed with 2 × 1 N HCl (100 mL), 1 × sat'd $NaHCO_3$ (100 mL) and 1 × 50 mL of sat'd NaCl. The organic phase was dried over anhydrous $MgSO_4$ for 10 min, filtered and then the organic solvent was removed under reduced pressure to give mesylated **30** as a pale yellow gel. This material was purified by silica gel chromatography (7:12 ethyl acetate: hexanes) to give pure mesylated **30** as a clear gel that formed white crystals upon standing. Yield 492.2 mg (0.92 mmol, 84%).

This material was dissolved in 20 mL dry acetone (dried over K_2CO_3) and 250 mg (2.19 mmol) of potassium thioacetate (Fluka) was added. The mixture were brought to reflux under argon with vigorous stirring. After 30 min copious amounts of a white flocculent precipitate began to form. The reaction was monitored by TLC (1:1 ethyl acetate:hexanes, R_f SM: 0.5, R_f product: 0.76) and found to be complete after 3 h. The yellow mixture was filtered to remove the precipitate, washed with acetone and the acetone removed *in vacuo* to give a yellow oil. This was purified by silica gel chromatography (1:4 ethyl acetate: hexanes) and again using a different solvent (1:75 ethyl acetate:chloroform) to yield **40** as a pale yellow oil. Yield: 368 mg (0.715 mmol, 65% from **30**). ^{1}H NMR (500 MHz, $CDCl_3$, TMS): 0.915 (*t*, J = 7.0, 3H), 1.35–1.45 (*m*, 13H), 1.693 (*m*, 2H), 2.365 (*s*, 3H), 2.753 (*dd*, J_1 = 14 Hz, J_2 = 7 Hz, 1H), 2.851 (*dd*, J_1 = 13.5 Hz, J_2 = 7 Hz, 1H), 2.947 (*dd*, J_1 = 14 Hz, J_2 = 7.5 Hz, 1H), 3.073 (*dd*, J_1 = 13.5 Hz, J_2 = 4 Hz, 1H), 3.94 (*m*, 1H), 4.293 (*t*, J = 6.5 Hz, 2H), 4.596 (*br d*, J = 7.5 Hz, 1H), 5.142 (*s*, 2H), 6.952 (*d*, J = 8.5 Hz, 1H), 7.27 (*m*, 1H), 7.310 (*t*, J = 7.5 Hz, 1H), 7.378 (*t*, J = 7.5 Hz, 2H), 7.475 (*d*, J = 7.5 Hz, 2H), 7.602 (*s*, 1H); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): 13.68, 19.1, 28.22, 30.51, 30.63, 32.66, 39.1, 51.7, 64.8, 70.6, 79.3, 113.8, 121.0, 127.0, 127.7, 128.4, 129.4, 132.3, 133.8, 136.6, 155.2, 156.7, 166.5, 195.6 ppm; HRMS (FAB$^+$, NBA/NaI): 538.2245; calcd for $C_{28}H_{37}NO_6SNa^+$: 538.2230 amu.

3-(4-Benzyloxy-3-carboxyphenyl)-2-amino-1-propanethiol, HCl salt, disulfide dimer (15). 60 mg (0.107 mmol) of **40** were dissolved in 5 mL of ethanol and placed in a flask. The flask was flushed with nitrogen and evacuated several times to remove oxygen. 1 mL of 1 N NaOH was added and the reaction allowed to stir until starting material had disappeared and only baseline materials remained by TLC (1:4 ethyl acetate:hexanes, R_f SM: 0.3, R_f product: 0, 3 days). The ethanol and water were removed with a continuous stream of nitrogen leaving a white solid in the flask. The flask was then rinsed three times under nitrogen with alternating washes of 25 mL of ethyl acetate and 25 mL 1 N HCl, both degassed. The washings were diverted to a separatory funnel (filled with nitrogen) and allowed to combine. The resultant mixture was shaken and allowed to separate. The water layer was drained away and the organic phase was washed with 2 × 50 mL 1 N HCl. The organic solvent was removed under reduced pressure. This material was purified by silica gel chromatography (50:50:1 ethyl acetate: hexanes:acetic acid, SM: R_f = 0.8, product: R_f = 0.3) to remove any trace 3'-iodo substituted material which may have survived purification steps during earlier stages of the synthesis due to an R_f similar to the butyl ester. The resultant white residue was dissolved in 5 mL of dichloromethane. TFA (1 mL) was added and the solution was stirred at rt for 50 min. The solvent and trifluoroacetic acid were removed under reduced pressure. Lengthy exposure to high vacuum gave a yellow oil, containing approximately 90% product (NMR). This product was purified by reversed phase HPLC (Dynamax C-8 column, gradient of 0–95% water–acetonitrile with 0.5% TFA present in solutions over a period of 40 min) and lyophilized, yielding **15** as 5.8 mg (0.007 mmol, 13%) of a fine white powder which became sticky on contact with air. Yield was calculated by comparison of ^{1}H NMR integration against a known amount of ethyl acetate. This material was assayed in the presence of 50 mM dithiothreitol, which we have found to cleave disulfide bonds on other α-thiol-β-amines. We have found previously that silica gel chromatography of N-Boc-α-thiol-β-amines results in disulfide formation. Presumably, this is why we obtained **15** as predominantly dimer, while **1**, **13**, **14**, **16** and **17** were obtained as the free thiol monomer. ^{1}H NMR (500 MHz, DMSO-d_6, TMS): 2.824 (*d*, J = 6.0 Hz, 4H), 2.85–2.95 (*m*, 6H), 3.55–3.60 (*m*, 2H), 5.154 (*s*, 4H), 7.151 (*d*, J = 8.5 Hz, 2H), 7.324 (*m*, 4H), 7.388 (*t*, J = 7.0 Hz, 4H), 7.486 (*d*, J = 7.5 Hz, 4H), 7.588 (*s*, 2H), 8.0–8.2 (*br s*, 6H), 12.5–13.0 (*br*, 2H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS): 36.2, 38.3, 50.8, 69.7, 114.2, 121.9, 127.1, 127.4, 127.7, 128.5, 131.8, 133.9, 137.0, 156.3, 158.0, 167.3 ppm; HRMS (FAB, NBA): 633.2070; calcd for $C_{34}H_{39}N_2O_6S_2^+$, 633.2093 amu.

Ethyl 5-bromovaleroate (31). 5-Bromovaleric acid (2.05 g, 11.3 mmol, Aldrich) was dissolved in 10 mL anhydrous ethanol. To this solution was added triethylorthoformate (2.00 ml, 1.78 g, 12.0 mmol) and a catalytic amount of acetyl bromide. The reaction was allowed to stir for 14 h, until it was determined to be complete by TLC (1:4 ethyl acetate:hexanes, SM: R_f = 0.3, product: R_f = 0.7). A few drops of water were added and the solvent was removed *in vacuo* to yield a yellow oil. This oil was taken up in 40 mL of ethyl acetate and washed with 3 × sat'd $NaHCO_3$ and 1 × sat'd NaCl. The organic phase was then filtered through anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. Yield: 2.02 g (9.64 mmol, 85%) of a pale yellow liquid 96% pure with 4% contamination with the methyl ester. ^{1}H NMR (500 MHz, $CDCl_3$, TMS): 1.263 (*t*, J = 7.0 Hz, 3H), 1.786 (quintet, J = 7.5 Hz, 2H), 1.908 (quintet, J = 8.0 Hz, 2H), 2.341 (*t*, J = 7.5 Hz, 2H), 3.421 (*t*, J = 7.0 Hz, 2H), 3.683 (*s*, 0.11H, methyl ester impurity), 4.137 ppm (*q*, J = 7.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): 14.2, 23.5, 32.0, 33.1, 33.3, 60.4, 173.1 ppm; GC/MS (EI$^+$): 208/210 amu (methyl ester: 194/196).

N-Boc-3-[4-(Ethyl 5-valeroate)-oxyphenyl]-2-amino-1-propanol (32). Ethyl 5-bromovaleroate (1.000 g, 4.902 mmol, 96% purity) and **23** (1.45 g, 3.77 mmol) were

dissolved in 20 mL of dry acetone. 4.2 g of powdered K_2CO_3 were added and the reaction was allowed to stir at reflux for 14 h, at which point the reaction appeared to be 95% complete by TLC (1:4 ethyl acetate: hexanes, SM: $R_f = 0.35$, product $R_f = 0.6$). The residual K_2CO_3 was removed by filtration and the acetone removed *in vacuo* to yield a crude product. This material was partially purified by silica gel chromatography (1:7 ethyl acetate:hexanes). The eluant solvent was removed and the resultant clear oil was dissolved in 30 mL dry THF. 15 mL of a 1 M solution of NBu_4F (15 mmol) were added and the reagents allowed to stir until the reaction was complete as judged by TLC (1:4 ethyl acetate:hexanes, product $R_f = 0.05$, 2 h). The THF was removed under reduced pressure and replaced with 100 mL ethyl acetate. This solution was washed with 2 × 1 N HCl, 1 × sat'd $NaHCO_3$ and 1 × sat'd NaCl, filtered through anhydrous $MgSO_4$ and the solvent removed under reduced pressure. The material was purified by silica gel chromatography (2:3 ethyl acetate:hexanes) to yield 1.14 g (2.89 mmol, 76.5%) of **32** as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$, TMS): 1.252 (*t*, J = 7.0 Hz, 3H), 1.406 (*s*, 9H), 1.804 (*m*, 4H), 2.376 (*m*, 2H), 2.775 (*d*, J = 6.5Hz, 2H), 3.393 (*br s*, 1H), 3.510 (*m*, 1H), 3.601 (*dd*, J_1 = 10 Hz, J_2 = 3.5 Hz, 1H), 3.931 (*t*, J = 6.5 Hz, 2H), 4.124 (*q*, J = 7.0 Hz, 2H), 5.045 (*br d*, J = 7.5 Hz, 1H), 6.806 (*d*, J = 8.0 Hz, 2H), 7.118 ppm (*d*, J = 8.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): 14.0, 21.4, 28.2, 28.5, 33.7, 36.2, 53.5, 60.2, 63.5, 67.1, 79.3, 114.2, 130.1, 156.0, 157.4, 173.4 ppm; HRMS (FAB$^+$, NBA/NaI): 418.2218; calcd for $C_{21}H_{33}NO_6Na^+$: 418.2206 amu.

N-Boc-S-Acetyl-3-[4-(ethyl 5-valeroate)-oxyphenyl]-2-amino-1-propanethiol (33). N-Boc-3-[4-(ethyl 5-valeroate)-oxyphenyl]-2-amino-1-propanol, **32**, (1.050 g, 2.655 mmol) was dissolved in 30 mL dry CH_2Cl_2. Methanesulfonyl chloride (5.2 mmol, 595.4 mg, 0.40 mL) and 0.40 mL triethylamine were added followed by a catalytic amount of DMAP. The reaction was allowed to stir until it was observed to be complete by TLC (1:1 ethyl acetate:hexanes, SM: $R_f = 0.25$, product $R_f = 0.80$, 1 h). The reaction was diluted with 50 mL ethyl acetate and washed with 2 × 1 N HCl, 1 × sat'd $NaHCO_3$ and 1 × sat'd NaCl. The solvent was removed under reduced pressure to yield a white crystalline solid, apparently one spot by TLC. This product was purified by silica gel chromatography (4:7 ethyl acetate:hexanes), providing the mesylate as a white crystalline solid.

The mesylate was then dissolved in 30 mL of acetone, to which was added potassium thioacetate (402.5 mg, 3.520 mmol). The mixture was brought to reflux and stirred for 2 h, during which time copious amounts of a white precipitate appeared. The reaction was determined to be complete at the end of this time by TLC (1:2 ethyl acetate:hexanes; SM: $R_f = 0.35$, product $R_f = 0.65$). It was diluted with 50 mL ethyl acetate and washed with 2 × 75 mL sat'd $NaHCO_3$ and 1 × 50 mL sat'd NaCl. It was then filtered through anhydrous $MgSO_4$ and the solvent removed under reduced pressure to give a pale orange, crystalline solid. This product was purified twice by silica gel chromatography, once with 50:1 chloroform:ethyl acetate as the eluant and a second time using 1:4 ethyl acetate:hexanes to elute the product. Removal of the solvent afforded **33** as a white, odorless powder (672 mg, 1.53 mmol, 58%). ^{1}H NMR (500 MHz, $CDCl_3$, TMS): 1.251 (*t*, J = 7 Hz, 3H), 1.407 (*s*, 9H), 1.808 (*m*, 4H), 2.344 (*s*, 3H), 2.376 (*m*, 2H), 2.707 (*dd*, J_1 = 13.5 Hz, J_2 = 7 Hz, 1H), 2.83 (*m*, 1H), 2.916 (*dd*, J_1 = 13.5 Hz, J_2 = 8 Hz, 1H), 3.064 (*dd*, J_1 = 14 Hz, J_2 = 4 Hz, 1H), 3.939 (*m*, 3H), 4.124 (*q*, J = 7 Hz, 2H), 4.696 (*br d*, J = 6 Hz, 1H), 6.812 (*d*, J = 8 Hz, 2H), 7.082 (*d*, J = 8 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): 14.1, 21.5, 28.21, 28.52, 30.45, 32.56, 33.78, 39.34, 51.7, 60.1, 67.2, 79.1, 114.3, 129.1, 130.1, 155.2, 157.6, 173.3, 195.6 ppm; HRMS (LSIMS$^+$, NBA/NaI), (M + Na$^+$): 476.2075; calcd for $C_{23}H_{35}NO_6SNa$: 476.2083 amu; mp: 81 °C.

Ethyl 2-chloromethyl benzoate (34).[55] Phthalide (2.00 g, 14.9 mmol, Aldrich) and dichlorotriphenylphosphine (6.00 g, 14.9 mmol, 80% pure from Aldrich) were placed in a 25 mL round bottom flask with a reflux condenser, stir bar and septum. The flask was evacuated and filled with argon three times before 10 mL of *n*-decane was added. The mixture was brought to reflux (decane, 175–180 °C) and allowed to stir under a slight argon pressure for 5 h, during which time the heterogeneous reaction mixture turned brown and developed a black precipitate. The reaction was allowed to cool to 90 °C and 2.5 mL of ethanol were added dropwise. The reaction was then cooled to room temperature and washed from the flask with 400 mL of CH_2Cl_2. The reaction was found to be nearly complete by TLC (1:10 ethyl acetate:hexanes, phthalide: R_f = 0.2, product $R_f = 0.7$). The CH_2Cl_2 was removed under reduced pressure and the resultant brown material was purified by silica gel chromatography (1:30 ethyl acetate:hexanes) to yield 2.1 g (10.5 mmol, 70%) of a pale yellow liquid consisting of 91.5% ethyl 2-chloromethylbenzoate **34** and 8.5% methyl 2-chloromethylbenzoate, as determined from the ^{1}H NMR spectrum. This material was used in subsequent reactions without further purification. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): 1.42 (*t*, J = 7 Hz, 3H), 3.94 (*s*, 0.25 H, methyl ester impurity), 4.40 (*q*, J = 7 Hz, 2H), 5.05 (*s*, 2H), 7.41 (*m*, 1H), 7.53 (*m*, 2H), 7.98 ppm (*d*, J = 7.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): 14.2, 44.5, 61.3, 128.4, 129.5, 130.8, 131.0, 132.4, 138.6, 166.7 ppm; we were unable to obtain a high resolution mass spectrum for this compound due to its instability.

N-Boc-3-[4-(Ethyl 2-carboxybenzyl)-oxyphenyl]-2-amino-1-propanol (35). N-Boc-1-O-(*tert*-Butyldimethylsilyl)-tyrosinol, **23**, (1.50 g, 3.92 mmol) and ethyl 2-chloromethylbenzoate **34** (1.011 g, 5.10 mmol) were placed in a dry 100 mL flask with 30 mL of dry acetone (dried over K_2CO_3). Powdered K_2CO_3 (13.7 mmol, 1.90 g) and a catalytic amount of Bu_4NI were added. The mixture was brought to reflux with stirring and allowed to continue until the reaction was complete as indicated by TLC (1:4 ethyl acetate:hexanes, **34**: R_f = 0.80, phenol: $R_f = 0.38$, product: $R_f = 0.70$). The K_2CO_3

was then removed by filtration and the acetone removed *in vacuo*. The solvent was replaced by 30 mL of dry THF and NBu_4F, (1 M solution in THF, 12 mL) was added. The solution was stirred for 3 h until the spot at R_f = 0.70 disappeared. The THF was removed under reduced pressure and replaced with 75 mL of ethyl acetate. This product was washed with 2 × 75 mL of sat'd $NaHCO_3$ and 1 × sat'd NaCl. The organic layer was concentrated *in vacuo* and purified by silica gel chromatography (3:4 ethyl acetate:hexanes) to yield 1.16 g (2.77 mmol, 71%) of **35** as a white powdery solid. ^1H NMR (500 MHz, $CDCl_3$, TMS): 1.374 (*t*, *J* = 7 Hz, 3H), 1.417 (*s*, 9H), 2.50–2.55 (*br s*, 1H), 2.777 (*d*, *J* = 7 Hz, 2H), 3.532 (*dd*, J_1 = 11 Hz, J_2 = 5 Hz, 1H), 3.635 (*dd*, J_1 = 11 Hz, J_2 = 3 Hz, 1H), 3.821 (*br s*, 1H), 4.358 (*q*, *J* = 7 Hz, 2H), 4.760 (*br d*, *J* = 8 Hz, 1H), 5.470 (*s*, 2H), 6.926 (*d*, *J* = 8.5 Hz, 2H), 7.123 (*d*, *J* = 8.5 Hz, 2H), 7.374 (*t*, *J* = 7.5 Hz, 1H), 7.545 (*td*, J_1 = 7 Hz, J_2 = 1 Hz, 1H), 7.732 (*d*, *J* = 7 Hz, 1H), 8.032 ppm (*dd*, J_1 = 7.5 Hz, J_2 = 1 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$, TMS): 14.2, 28.3, 36.5, 53.8, 61.1, 64.4, 68.2, 79.7, 114.9, 127.2, 127.4, 128.0, 130.0, 130.2, 130.7, 132.5, 139.4, 156.2, 157.4, 167 ppm; HRMS (FAB^+, NBA/NaI): 452.2059; calcd for $C_{24}H_{31}NO_6Na$: 452.2049.

N-Boc-S-Acetyl 3-[4-(ethyl 2-carboxybenzyl)-oxyphenyl]-2-amino-1-propanethiol (36). N-Boc-3-[4-(Ethyl 2-carboxylbenzyl)-oxyphenyl]-2-amino-1-propanol, **35**, (1.000 g, 2.395 mmol) was dissolved in 30 mL dry CH_2Cl_2. Methanesulfonylchloride (372 µL, 550 mg, 4.8 mmol, Aldrich), triethylamine (434 µL, 315 mg, 3.12 mmol) and a catalytic amount of DMAP were added to this solution and the mixture was allowed to stir for 3 h until the reaction was complete by TLC (1:1 ethyl acetate:hexanes, SM: R_f = 0.50, product: R_f = 0.70). The reaction was diluted with 80 mL of ethyl acetate and washed with 2 × 100 mL 1 N HCl, 1 × 100 mL sat'd $NaCO_3$ and 1 × 50 mL sat'd NaCl. The organic layer was then evaporated *in vacuo* and then further purified by silica gel chromatography (4:7 ethyl acetate:hexanes) providing the mesylate as a white, crystalline solid. This material was then dissolved in 40 mL of dry acetone (HPLC grade over anhydrous K_2CO_3, Aldrich). Potassium thioacetate (400 mg, 3.50 mmol, Fluka) was added and the mixture was brought to reflux with stirring under argon. The reaction was allowed to proceed for 3.5 h, during which time it was monitored by TLC (1:2 ethyl acetate:hexanes, product R_f = 0.65, SM: R_f = 0.30). The mixture, which by the end of this period would be better described as a sandy gel due to the precipitation of unusual volumes of what is supposed to be potassium methanesulfonate, was diluted with 50 mL of ethyl acetate and 50 mL of sat'd $NaHCO_3$. The organic fraction washed with 1 × 75 mL sat'd $NaHCO_3$ and 1 × 50 mL sat'd NaCl, dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to give a light brown crystalline solid. This material was purified by two successive silica gel columns, the first using 1:4 ethyl acetate:hexanes as eluant, the second using 50:1 chloroform:ethyl acetate. Removal of the solvent under reduced pressure gave **36** as an odorless, white crystalline solid (0.565 g, 1.16 mmol, 48.4%). ^1H NMR (500 MHz, $CDCl_3$, TMS): 1.369 (*t*, *J* = 7.0 Hz, 3H), 1.410 (*s*, 9H), 2.348 (*s*, 3H), 2.718 (*dd*, J_1 = 13.5 Hz, J_2 = 7 Hz, 1H), 2.844 (*dd*, J_1 = 13 Hz, J_2 = 5 Hz, 1H), 2.927 (*dd*, J_1 = 14 Hz, J_2 = 7.5 Hz, 1H), 3.076 (*dd*, J_1 = 13.5 Hz, J_2 = 4 Hz, 1H), 3.938 (*m*, 1H), 4.354 (*q*, *J* = 7 Hz, 2H), 4.645 (*d*, *J* = 7 Hz, 1H), 5.466 (*s*, 2H), 6.922 (*d*, *J* = 8.5 Hz, 2H), 7.100 (*d*, *J* = 8 Hz, 2H), 7.366 (*t*, *J* = 7.5 Hz, 1H), 7.538 (*td*, J_t = 7.5 Hz, J_d = 1 Hz, 1H), 7.733 (*d*, *J* = 7.5 Hz, 1H), 8.028 ppm (*dd*, J_1 = 7 Hz, J_2 = 1 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$, TMS) 14.2, 28.2, 30.5, 32.6, 39.4, 51.7, 61.0, 68.1, 79.3, 114.8, 127.1, 127.3, 128.0, 129.5, 130.3, 130.6, 132.4, 139.4, 155.2, 157.4, 166.9, 195.7 ppm; HRMS (FAB^+, NBA/NaI): 510.1938; calcd for $C_{26}H_{33}NO_6SNa^+$: 510.1926 amu.

3-(4-(5-Valeroate)-oxyphenyl)-2-amino-1-mercaptopropane, HCl salt (16). 350 mg (0.773 mmol) of N-Boc-S-acetyl-3-[4-(ethyl 5-valeroate)-oxyphenyl]-2-amino-1-propanethiol, **33**, was dissolved in 10 mL EtOH under argon. Care was taken to remove oxygen. 4 mL of 1 M NaOH were added and the reaction was allowed to stir for 14 h (monitored by TLC, 1:1 ethyl acetate: hexanes), after which time, 3 mL of 1 N HCl were added and the solvent was removed *in vacuo*. The resultant white solid was taken up in 50 mL ethyl acetate (insoluble portion was taken up in 1 N HCl) and was washed twice with 50 mL 1 N HCl under argon. The organic layer was dried overnight under high vacuum to remove all residual water. It was then dissolved in 35 mL of anhydrous ether. HCl was bubbled through this solution for 30 min and it was allowed to stir for 8 h, during which time a fine white precipitate formed. This was collected by filtration,washed with 50 mL of dry ether and the solvent removed under reduced pressure for 30 min to give **16** as a fine white powder, yielding 210 mg (0.739 mmol, 95.6%). ^1H NMR (500 MHz, DMSO-d_6, TMS): 1.624 (*m*, 2H), 1.712 (*m*, 2H), 2.287 (*t*, *J* = 7.5 Hz, 2H), 2.662 (ABX, Δν = 68.5 Hz, J_{AB} = 14.5 Hz, J_X = 4.5 Hz, 2H), 2.903 (ABX, Δν = 46.5 Hz, J_{AB} = 13.5 Hz, J_X = 7 Hz, 2H), 2.8–3.2 (*m*, 1H), 3.424 (*m*, 1H), 3.943 (*t*, *J* = 6.0 Hz, 2H), 6.897 (*d*, *J* = 9 Hz, 2H), 7.189 (*d*, *J* = 9 Hz, 2H), 8.2–8.5 (*br*, 3H), 12.0–12.2 ppm (*br*, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS): 21.3, 25.4, 28.2, 33.3, 35.7, 53.8, 67.1, 114.6, 127.8, 130.4, 157.6, 174.4 ppm; HRMS (FAB^+, NBA): 284.1320 amu; calcd for $C_{14}H_{22}NO_3S^+$: 284.1325 amu; mp: 149 °C.

(2S)-N-Boc-3-[4-(Ethyl 4-butyrate)-oxyphenyl]-2-amino-1-propanol (42). 0.6 g (1.64 mmol) of compound **23** and ethyl 4-bromobutyrate (350 µL, 2.32 mmol) were dissolved in dry acetone (10 mL). Finely ground K_2CO_3 (850 mg, 6 mmol) was added and the mixture was refluxed overnight with rapid stirring (90% complete by TLC, 1:2 ethyl acetate:hexane, SM: R_f = 0.4, product: R_f = 0.6). The reaction mixture was then filtered, the solvent removed *in vacuo*, and the product purified by flash chromatography (10:1 hexane:ethyl acetate) to give 0.8 g of a colorless oil. The oil was dissolved in 6 mL dry THF and 6 mL of a 1 M solution of NBu_4F (6 mmol) were added, and the solution was stirred until

the reaction was complete as judged by TLC (1:1 ethyl acetate:hexane, product R_f = 0.4, 30 min). The THF was removed under reduced pressure and replaced with 100 mL ethyl acetate. This solution was washed (1 N HCl, sat'd $NaHCO_3$, and sat'd NaCl), dried ($MgSO_4$) and the solvent removed. The material was purified by silica gel chromatography (1:2 ethyl acetate:hexane) to yield 0.54 g (1.46 mmol, 89%) of **32** as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): 1.24 (*t*, *J* = 9 Hz, 3H), 1.42 (*s*, 9H), 2.09 (*m*, 2H), 2.50 (*t*, *J* = 7.5 Hz, 2H), 2.76 (*d*, *J* = 8.5 Hz, 2H), 3.49 (*m*, 2H), 3.97 (*t*, *J* = 7.5 Hz, 2H), 4.13 (*q*, *J* = 8 Hz, 2H), 4.74 (*br d*, 1H), 6.81 (*d*, *J* = 8.5 Hz, 2H), 7.09 ppm (*d*, *J* = 8.5 Hz, 2H); HRMS: 382.2221; calcd for $C_{20}H_{32}NO_6^+$: 382.2229.

(2S)-N-Boc-S-Acetyl-3-[4-(ethyl 4-butyrate)-oxyphenyl]-2-amino-1-propanethiol (43). Compound **42** (0.52 g, 1.41 mmol) was dissolved in 10 mL of dry CH_2Cl_2. Methanesulfonyl chloride (3.6 mmol, 280 μL) and 290 μL of triethylamine were added followed by a catalytic amount of DMAP. The reaction was allowed to stir until it was observed to be complete by TLC (1:1 ethyl acetate:hexanes, SM: R_f = 0.4, product R_f = 0.6, 30 min). 40 mL ethyl acetate were added and the solution was washed (1 N HCl, sat'd $NaHCO_3$, sat'd NaCl) and dried ($MgSO_4$). The solvent was then removed under reduced pressure to yield the mesylate as a pale yellow solid (0.60 g, 94%) which was used without further purification.

The mesylate (6.0 g, 1.31 mmol) was dissolved in 100 mL dry acetone and potassium thioacetate (270 mg, 2.36 mmol) was added. The mixture was brought to reflux and stirred for 6 h. The mixture was filtered and the acetone removed *in vacuo* to give a red oil/solid mixture. Water (50 mL) was added and the aqueous solution extracted with ether. The ethereal layers were then washed (1 N HCl, sat'd $NaHCO_3$, sat'd NaCl) and dried ($MgSO_4$) and the solvent removed under reduced pressure to give an orange solid. This was purified by two flash columns (50:1 CH_2Cl_2:ethyl acetate, then 4:1 hexane:ethyl acetate). The product was finally recrystallized from ether/hexane to give 330 mg (57%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): 1.25 (*t*, *J* = 8.5 Hz, 3H), 1.41 (*s*, 9H), 2.09 (*m*, 2H), 2.36 (*s*, 3H), 2.51 (*t*, *J* = 7.5 Hz, 2H), 2.71 (*dd*, J_1 = 9 Hz, J_2 = 15 Hz, 1H), 2.86 (*m*, 2H), 3.17 (*dd*, J_1 = 4 Hz, J_2 = 12 Hz, 1H), 3.94 (*m*, 1H), 3.98 (*t*, *J* = 7.5 Hz, 2H), 4.15 (*q*, *J* = 8 Hz, 2H), 4.58 (*bs*, 1H), 6.81 (*d*, *J* = 9.5 Hz, 2H), 7.09 ppm (*d*, *J* = 9.5 Hz, 2H); HRMS: 440.2115; calcd for $C_{22}H_{34}NO_6S^+$: 440.2107.

(2S)-3-[4-(4-Butyrate)oxyphenyl]-2-amino-1-mercaptopropane, HCl salt (17). All solvents and solutions used here were de-oxygenated to avoid formation of disulfide. 300 mg (0.68 mmol) of compound **43** was dissolved in 20 mL EtOH. 6 mL of 1 N NaOH were added and the reaction was allowed to stir for 5 h (monitored by TLC, 1:1 ethyl acetate:hexanes), after which time it was acidified to pH 3 with 1 N HCl. The aqueous solution was extracted with ethyl acetate and the organic layers washed (1 N HCl, sat'd NaCl), dried ($MgSO_4$), and the solvent removed *in vacuo* to give a white solid. It was then dissolved in 50 mL concentrated ethereal HCl and stirred overnight to give **17** (5% molar contamination by disulfide) as a white solid (166 mg, 91%). ^{1}H NMR (500 MHz, CD_3OD, TMS): 2.05 (*m*, 2H), 2.48 (*t*, *J* = 7.5 Hz, 2H), 2.64 (*dd*, J_1 = 6.5 Hz, J_2 = 15 Hz, 1H), 2.64 (*dd*, J_1 = 4.5 Hz, J_2 = 14.5 Hz, 1H), 2.94 (*m*, 2H), 3.54 (*m*, 1H), 4.01 (*t*, *J* = 6.5 Hz, 2H), 6.91 (*d*, *J* = 7 Hz, 2H), 7.19 ppm (*d*, *J* = 7 Hz, 2H); ^{13}C NMR (125 MHz, CD_3OD, TMS): 25.8, 26.6, 31.3, 37.5, 55.8, 68.0, 116.1, 128.4, 131.4, 159.8, 177.0 ppm; HRMS: 270.1160; calcd for $C_{13}H_{20}NO_3S^+$: 270.1164; mp 148–156 °C (dec.).

3-(4-(2-Carboxylbenzyl)-oxyphenyl)-2-amino-1-mercaptopropane, HCl salt (14). 240 mg of **36** (0.492 mmol) were dissolved in 15 mL of ethanol. Oxygen was removed by bubbling argon through for 30 min. 0.5 mL of 10 N NaOH were added and the reaction allowed to stir until completion as indicated by TLC (1:1 ethyl acetate:hexanes, 2 days, SM: R_f = 0.9, product R_f = 0.4). The ethanol was removed under reduced pressure and the white solid was taken up by successive washing with ethyl acetate and 1 N HCl. The organic layer was washed twice more with 1 N HCl and then the solvent removed overnight under vacuum. The resultant white solid was dissolved in 30 mL dry ether. Anhydrous HCl was bubbled through this solution for 30 min and then it was allowed to stir for 14 h, after which time, the product was collected by filtration. The solid was washed twice with ether and then the solvent was removed *in vacuo* for 3 h to remove residual water acquired during the filtration process. Yield: 96.4 mg (0.272 mmol, 55%) of **14**, containing 10% (molar) disulfide. ^{1}H NMR (500 MHz, DMSO-d_6, TMS): 2.6–3.1 (*m*, 5H), 3.6 (*m*, 1H), 5.43 (*s*, 2H), 6.961 (*d*, *J* = 8.0 Hz, 2H), 7.225 (*d*, *J* = 8.0 Hz, 2H), 7.446 (*t*, *J* = 7.5 Hz, 1H), 7.589 (*t*, *J* = 8.0 Hz, 1H), 7.638 (*d*, *J* = 7.5 Hz, 1H), 7.940 (*d*, *J* = 8.0 Hz, 1H), 8.3–8.5 (*br m*, 3H), 13.0–13.2 (*br s*, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, TMS): 25.4, 36.7, 52.1, 67.6, 114.9, 127.7, 127.9, 128.0, 129.4, 130.5, 130.7, 132.2, 138.4, 157.4, 168.1 ppm; HRMS (FAB$^+$, NBA): 318.1159; calcd for $C_{17}H_{20}NO_3S^+$: 318.1164 amu; mp 207 °C.

(2S)-S-Benzyl-3-(4-benzyloxyphenyl)-2-amino-1-propanethiol, HCl salt (8). To a deoxygenated solution of **22** (250 mg, 0.602 mmol) in 7 mL of EtOH was added 1.2 mL deoxygenated 1 N NaOH, and the reaction stirred at rt for 1 h. An additional 0.6 mL 1 N NaOH were then added followed by benzyl bromide (71 μL, 0.60 mmol). The reaction was stirred for 2 h, 50 mL ethyl acetate added, and the solution washed with 1 × 1 N HCl, 1 × sat'd $NaHCO_3$ and 1 × sat'd NaCl. Removal of the solvent *in vacuo* gave N-Boc protected **8** as a colorless oil. Deprotection with ethereal HCl gave **8** (205 mg, 86%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): 2.51 (*m*, 2H), 2.75 (*dd*, J_1 = 8.5 Hz, J_2 = 15 Hz, 1H), 2.97 (*dd*, J_1 = 6.5 Hz, J_2 = 14 Hz, 1H), 3.45 (*bs*, 1H), 3.71 (AB, *J* = 13.5 Hz, $\Delta\upsilon$ = 21.1, 2H), 5.10 (*s*, 2H), 6.97 (*d*, *J* = 8.5 Hz, 2H), 7.12 (*m*, 4H), 7.21 (*m*, 2H), 7.34 (*m*, 1H), 7.42 (*t*, *J* = 8 Hz, 2H), 7.46 (*d*, *J* = 7 Hz,

2H) 8.21 ppm (*bs*, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): 31.9, 34.9, 36.7, 51.1, 69.2, 114.9, 126.9, 127.7, 127.9, 128.4, 128.5, 128.9, 130.5, 137.1, 138.0, 157.4 ppm; HRMS: 364.1730; calcd for $C_{23}H_{26}NOS^+$: 364.1735; mp 182 °C.

(2S)-S-(4-Carboxymethylbenzyl)-3-(4-benzyloxyphenyl)-2-amino-1-propanethiol, HCl salt (11). To a deoxygenated solution of **22** (250 mg, 0.602 mmol) in 7 mL EtOH was added 1.2 mL deoxygenated 1 N NaOH and the reaction stirred at rt for 1 h. An additional 1.2 mL of 1 N NaOH was then added followed by 4-(bromomethyl)phenyl acetic acid (137 mg, 0.60 mmol). The reaction was stirred for 30 min (product began to precipitate out). The mixture was acidified to pH 2 and worked up/deprotected as for **8** to give **11** (200 mg, 73%) as a white solid. ^1H NMR (500 MHz, DMSO-d_6): 2.56 (*m*, 1H), 2.64 (*dd*, J_1 = 7.5 Hz, J_2 = 14 Hz, 1H), 2.82 (*dd*, J_1 = 8.5 Hz, J_2 = 13.5 Hz, 1H), 2.97 (*dd*, J_1 = 6.5 Hz, J_2 = 14 Hz, 1H), 3.45 (*m*, 1H), 3.71 (AB, J = 13.5 Hz, $\Delta\upsilon$ = 21.1, 2H), 5.10 (*s*, 2H), 7.01 (*d*, J = 8.5 Hz, 2H), 7.15 (*m*, 6H), 7.38 (*t*, J = 7 Hz, 1H), 7.44 (*t*, J = 8 Hz, 2H), 7.50 (*d*, J = 7.5 Hz, 2H), 8.36 (*bs*, 3H), 12.4 ppm (*bs*, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): 32.0, 34.7, 36.6, 51.3, 69.2, 114.9, 127.8, 127.9, 128.0, 128.5, 128.8, 129.4, 130.5, 133.6, 136.4, 137.1, 157.4, 172.7 ppm; HRMS: 422.1807; calcd for $C_{25}H_{28}NO_3S^+$: 422.1790; mp 196 °C.

(2S)-S-Methyl-3-(4-benzyloxyphenyl)-2-amino-1-propanethiol, HCl salt (6). To a deoxygenated solution of **22** (167 mg, 0.40 mmol) in 7 mL EtOH was added 0.8 mL deoxygenated 1 N NaOH and the reaction stirred at room temperature for 1 h. An additional 0.4 mL 1 N NaOH was then added followed by methyl iodide (25 µL, 0.40 mmol). The reaction was stirred for 1 h (product began to precipitate out). Workup and deprotection as for **8** gave **6** (84 mg, 65%) as a white solid. ^1H NMR (300 MHz, CD_3OD): 2.13 (*s*, 3H), 2.64 (*dd*, J_1 = 7.5 Hz, J_2 = 14 Hz, 1H), 2.76 (*dd*, J_1 = 3.5 Hz, J_2 = 15 Hz, 1H), 2.9 (*d*, J_1 = 6 Hz, 2H), 3.55 (*m*, 1H), 5.10 (*s*, 2H), 7.01 (*d*, J = 8.5 Hz, 2H), 7.18 (*d*, J = 8.5 Hz, 2H), 7.5–7.25 ppm (*m*, 5H); ^{13}C NMR (125 MHz, DMSO-d_6): 15.3, 35.1, 36.4, 51.1, 69.2, 114.9, 127.7, 127.9, 128.0, 128.5, 130.5, 137.1, 157.4 ppm; HRMS: 288.1413; calcd for $C_{17}H_{22}NOS^+$: 288.1422; mp 165 °C.

(2S)-S-(10-Hydroxydecyl)-3-(4-benzyloxyphenyl)-2-amino-1-propanethiol, HCl salt (9). To a deoxygenated solution of **22** (167 mg, 0.40 mmol) in 7 mL EtOH was added 0.8 mL deoxygenated 1 N NaOH and the reaction stirred overnight. An additional 0.4 mL 1 N NaOH was then added followed by 95 µL (0.43 mmol) of 10-bromo-1-decanol (90% tech grade). The reaction was stirred for 4 h (product began to precipitate out). The mixture was worked up as for **8** to give N-Boc protected **9**. Due to the impurities in the bromo-alcohol, flash column chromatography (1:1 ethyl acetate:hexane) was necessary to purify the product. Deprotection with ethereal HCl gave **9** (100 mg, 58%) as a gummy white solid. ^1H NMR (500 MHz, DMSO-d_6): 1.22 (*m*, 10H), 1.28 (*m*, 2H), 1.38 (*m*, 2H), 1.43 (*m*, 2H), 2.32 (*dd*, J_1 = 7.5 Hz, J_2 = 13 Hz, 1H), 2.47 (*m*, 2H), 2.64 (*dd*, J_1 = 6 Hz, J_2 = 13.5 Hz, 1H), 2.93 (*m*, 1H), 3.35 (*t*, J = 7 Hz, 2H), 5.05 (*s*, 2H), 6.91 (*d*, J = 8.5 Hz, 2H), 7.10 (*d*, J = 8.5 Hz, 2H), 7.32 (*t*, J = 6 Hz, 1H), 7.38 (*t*, J = 8 Hz, 1H), 7.43 ppm (*d*, J = 7 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): 25.5, 28.2, 28.6, 29.0, 29.1, 29.2, 31.6, 32.6, 41.5, 52.3, 60.7, 69.1, 114.5, 127.6, 127.8, 128.4, 130.2, 131.4, 137.2, 156.7 ppm; HRMS: 430.2801; calcd for $C_{26}H_{40}NO_2S^+$: 430.278; mp 74–77 °C.

(2S)-S-(2-Carboxyethyl)-3-(4-benzyloxyphenyl)-2-amino-1-propanethiol, HCl salt (10). To a deoxygenated solution of **22** (167 mg, 0.40 mmol) in 7 mL EtOH was added 0.8 mL deoxygenated 1 N NaOH and the reaction stirred at room temperature for 1 h. An additional 0.8 mL 1 N NaOH was then added followed by 3-iodopropionic acid (90 mg, 0.41 mmol). The reaction was stirred overnight, but some free thiol remained. The mixture was acidified to pH 2, and worked up as for **8** to give N-Boc protected **10**. Residual free thiol was removed by flash chromatography (1:2 ethyl acetate: hexane, followed by ethyl acetate) and deprotection with HCl gave **10** (92 mg, 60%) as a white solid. ^1H NMR (500 MHz, DMSO-d_6): 2.46 (*t*, J = 7 Hz, 2H), 2.68 (*m*, 4H), 2.84 (*dd*, J_1 = 7.5 Hz, J_2 = 14 Hz, 1H), 2.92 (*dd*, J_1 = 6.5 Hz, J_2 = 14 Hz, 1H), 3.45 (*m*, 1H), 3.71 (AB, J = 13.5 Hz, $\Delta\upsilon$ = 21.1, 2H), 5.07 (*s*, 2H), 6.97 (*d*, J = 8.5 Hz, 2H), 7.18 (*d*, J = 8.5 Hz, 2H), 7.38 (*m*, 1H), 7.39 (*t*, J = 7.5 Hz, 2H), 7.44 (*d*, J = 7.5 Hz, 2H), 8.19 (*bs*, 3H), 12.35 ppm (*bs*, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): 25.6, 31.5, 32.9, 35.0, 50.2, 67.9, 113.6, 126.4, 126.6, 127.2, 129.2, 135.8, 156.1, 171.6 ppm; HRMS: 346.1486; calcd for $C_{19}H_{24}NO_3S^+$: 346.1477.

(2S)-S-Ethyl-3-(4-benzyloxyphenyl)-2-amino-1-propanethiol, HCl salt (7). 440 mg (1 mmol) of the mesylate of N-Boc-O-Bn-tyrosinol (prepared as for **22**) were dissolved in 3 mL of EtOH. Ethanethiol (740 µL, 10 mmol) and 1 N NaOH (1 mL) were then added and the solution stirred for 4 days. The reaction did not go to completion, giving a mixture of mesylate, alcohol, and the ethyl thioether which was purified using flash chromatography (3:1 ethyl acetate:hexane). The Boc protecting group was then removed with ethereal HCl to give **7** (30 mg, 10%) as a white solid. ^1H NMR (500 MHz, DMSO-d_6) 1.10 (*t*, J = 5 Hz, 3H), 2.50 (*m*, 4H), 2.81 (*dd*, J_1 = 7.5 Hz, J_2 = 13.5 Hz, 1H), 2.94 (*dd*, J_1 = 5.5 Hz, J_2 = 14 Hz, 1H), 3.43 (*m*, 1H), 5.08 (*s*, 2H), 6.98 (*d*, J = 8.5 Hz, 2H), 7.18 (*d*, J = 8.5 Hz, 2H), 7.31 (*t*, J = 7 Hz, 1H), 7.38 (*t*, J = 8 Hz, 2H), 7.42 (*d*, J = 7.5 Hz, 2H), 8.16 ppm (*bs*, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): 14.5, 25.4, 32.2, 36.4, 51.5, 69.2, 114.9, 127.7, 127.9, 128.0, 128.5, 130.5, 137.1, 157.4 ppm; HRMS: 302.1570; calcd for $C_{18}H_{24}NOS^+$: 302.1579; mp 168–171°C.

(2S)-1-[4-(Benzyloxy)phenyl]-6-phenyl-2-amino-3-oxo-N-Boc-hexane (46). To a stirred solution of N-Boc-O-benzyl-L-tyrosine-*N*-methoxy-*N*-methylamide (1.5 g, 3.62 mmol) in anhydrous THF (10 mL) under N_2 at rt was added freshly prepared 1-phenyl-propane magnes-

ium bromide (10.9 mmol) in anhydrous THF (10 mL). Stirring was continued for 3 h after which time the reaction was poured onto 1 N HCl (30 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (4 × 25 mL). The combined organics were washed with 3 × 25 mL 1 N HCl, 3 × 25 mL sat'd $NaHCO_3$ and 1 × 25 mL sat'd NaCl. The organic layer was dried over anhydrous $MgSO_4$ and concentrated to give a crude yellowish solid. Recrystallization from ethyl acetate:hexanes gave the **46** as a white crystalline solid (1.35 g, 80%). R_f = 0.44 (1:4 ethyl acetate:hexanes). ^1H NMR (500 MHz, $CDCl_3$): 1.40 (*s*, 9H), 1.78–1.95 (*m*, 2H), 2.25–2.48 (*m*, 2H), 2.56 (*t*, *J* = 14.0 Hz, 2H), 2.96 (*dd*, *J* = 6.8, 14.0 Hz, 1H), 4.46 (*q*, *J* = 7.5 Hz, 1H), 5.05 (*s*, 2H), 5.10 (*d*, *J* = 7.5 Hz, 1H), 6.88–7.13 (AB, 4H), 7.2–7.4 (*m*, 10H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.7, 28.3, 34.9, 37.0, 40.0, 60.1, 70.0, 79.0, 114.9, 125.9, 127.4, 128.4, 128.5, 128.6, 130.2, 136.9, 141.3, 155.15, 157.77, 209.16 ppm; HRMS (FAB$^+$, NBA): 472.2490; calcd for $C_{30}H_{36}NO_4^+$: 472.2488. Anal. calcd for $C_{30}H_{35}NO_4$: C 76.08%, H 7.45%, N 2.96%; found C 76%, H 7.45%, N 2.97%; mp 128–129 °C.

(2S)-1-[4-(Benzyloxy)phenyl]-6-phenyl-2-amino-3-oxo-hexane HCl (47). To a stirred solution of (2*S*)-1-[4-(benzyloxy)phenyl]-6-phenyl-2-amino-3-oxo-N-Boc-hexane (**3**) (0.1 g, 0.21 mmol) in ether (5 mL) at room temperature was added a saturated HCl/ether solution (20 mL). After 24 h of stirring, the resulting precipitate was isolated by filtration. The crude product was recrystallized from MeOH/ether to give **47** as hard white crystals (0.065 g, 75%); mp 147–148 °C; ^1H NMR (300 MHz, DMSO-d_6): 1.65–1.78 (*m*, 2H), 2.49–2.55 (*m*, 4H), 3.0 (*d*, *J* = 6.5 Hz, 2H), 4.31 (*t*, *J* = 6.5 Hz, 1H), 5.07 (*s*, 2H), 6.92–7.8 (*m*, 14H), 8.2 (*br s*, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): 24.4, 34.6, 34.8, 58.7, 68.2, 115.0, 125.9, 126.8, 127.7, 127.8, 128.3, 128.4, 128.5, 130.6, 137.1, 157.8, 208.3 ppm; HRMS (FAB$^+$, NBA): 374.2106; calcd for $C_{25}H_{29}NO_2^+$: 374.2120. Anal. calcd for $C_{25}H_{28}NO_2Cl$: C 73.25%, H 6.88%, N 3.42%; found C 73.25%, H 6.81%, N 3.40%.

O-(2-Naphthylmethyl)-L-tyrosine-N-Boc-(2-naphthylmethyl) ester (48). To a stirred solution of N-Boc-L-tyrosine (6.1 g, 21.4 mmol) in DMF (30 mL) at room temperature was added 2-(bromomethyl)naphthalene (10.4 g, 47.0 mmol), Cs_2CO_3 (17.4 g, 53.4 mmol) and TBAI (25 mg, 0.07 mmol). Stirring was continued for 48 h after which the reaction was taken up in ethyl acetate (200 mL). The organic layer was washed with 3 × 240 mL 1 N HCl, 3 × 40 mL sat'd $NaHCO_3$ and 1 × 40 mL sat'd NaCl. The organic layer was dried over anhydrous $MgSO_4$ and concentrated to give a crude brown oil. Recrystallization from MeOH afforded the title compound (**48**) as a white solid (5.0 g, 42%). R_f = 0.35 (1:4 ethyl acetate:hexanes). ^1H NMR (300 MHz, $CDCl_3$): 1.41 (*s*, 9H), 3.02 (*d*, *J* = 5.7 Hz, 2H), 4.62 (*m*, 1H), 5.01 (*brd*, *J* = 7 Hz, 1H), 5.07 (*s*, 2H), 5.23 (*d*, *J* = 12.2 Hz, 1H), 5.34 (*d*, *J* = 12.3 Hz, 1H), 6.83 (AB, *J* = 8.4, 43.2 Hz, 4H), 7.4–7.53 (*m*, 6H), 7.8–7.99 (*m*, 8H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): 28.3, 38.3, 55.0, 67.2, 70.2, 78.2, 125.3, 126.1, 126.2, 126.3, 126.4, 126.5, 127.7, 127.8, 127.9, 128.1, 128.4, 130.4, 132.6, 133.07, 133.13, 133.19, 133.29, 134.48, 157.85, 171.57 ppm; HRMS (FAB$^+$, NBA/CsI): 694.1570; calcd for $C_{36}H_{35}NO_5Cs^+$: 694.1570; mp 99.5–101 °C; $[\alpha]^{25}_D$ –16.96° (*c* 2.24, CH_2Cl_2).

(2S)-3-[4-(2-Naphthylmethyloxy)phenyl]-2-amino-N-Boc-propanol (49). To a stirred solution of O-(2-naphthylmethyl)-L-tyrosine-N-Boc-(2-naphthylmethyl) ester, **48**, (1.5 g, 2.6 mmol) in anhydrous THF (25 mL) at rt under N_2 was added $LiBH_4$ (0.058 g, 2.67 mmol). After 18 h the reaction mixture was poured onto 1 N HCl (10 mL). The solvent was reduced and the resulting slurry was taken up in ethyl acetate (50 mL). The organic layer was washed with 3 × 25 mL 1 N HCl, 3 × 25 mL sat'd $NaHCO_3$ and 1 × 25 mL sat'd NaCl, dried over anhydrous $MgSO_4$ and concentrated to give a crude white solid. Purification by flash chromatography (1:4 ethyl acetate:hexanes) afforded **49** as a white solid (1.0 g, 96%). R_f = 0.44 (1:1 ethyl acetate:hexanes). ^1H NMR (300 MHz, $CDCl_3$): 1.40 (*s*, 9H), 2.76 (*d*, *J* = 7.2 Hz, 2H), 3.51 (*dd*, *J* = 5.1, 10.9 Hz, 1H), 3.63 (*dd*, *J* = 3.6, 11.2 Hz, 1H), 3.8 (*bs*, 1H), 4.78 (*bd*, *J* = 7.8 Hz, 1H), 5.18 (*s*, 2H), 7.0 (AB, *J* = 8.4, 53.1 Hz, 4H), 7.4–7.6 (*m*, 3H), 7.7–7.9 (*m*, 4H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): 28.3, 36.5, 53.7, 64.4, 70.0, 79.7, 115.0, 125.2, 126.0, 126.2, 126.3, 127.7, 127.9, 128.4, 130.2, 130.3, 133.0, 133.3, 134.4, 157.3 ppm; HRMS (FAB$^+$, NBA/CsI): 540.1153; calcd for $C_{25}H_{29}NO_4Cs^+$: 540.1151. Anal. calcd for $C_{25}H_{29}NO_4$: C 73.68%, H 7.17%, N 3.43%; found C 73.60%, H 7.21%, N 3.61%; mp 138–139 °C; $[\alpha]^{25}_D$: –13.89° (*c* 1.8, CH_2Cl_2).

(2S)-O-Mesyl-3-[4-(2-naphthylmethyloxy)phenyl]-2-amino-N-Boc-propanol (50). To a stirred solution of (2*S*)-3-[4-(2-naphthylmethyloxy)phenyl]-2-amino-N-Boc-propanol, **49**, (1.0 g, 2.45 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0 °C under N_2 was added triethylamine (0.52 mL, 3.7 mmol), DMAP (cat.) and methanesulfonyl chloride (0.29 mL, 3.7 mmol). After 1 h the reaction was quenched by addition of H_2O (5 mL). The organic layer was separated and the aqueous layer was extracted with 3 × 10 mL CH_2Cl_2. The combined organics were washed with 2 × 20 mL 1 N HCl, 2 × 20 mL sat'd $NaHCO_3$ and 1 × 20 mL sat'd NaCl, dried over anhydrous $MgSO_4$ and concentrated to give a white solid. Purification by flash chromatography (1:2 ethyl acetate:hexanes) afforded **50** as a white solid (0.92 g, 78%). R_f = 0.68 (1:4 ethyl acetate:hexanes). ^1H NMR (300 MHz, $CDCl_3$): 1.41 (*s*, 9H), 2.78–2.82 (*m*, 2H), 2.99 (*s*, 3H), 4.0–4.25 (*m*, 3H), 4.6–4.65 (*bd*, 1H), 5.2 (*s*, 2H), 6.9–7.2 (*m*, 4H), 7.45–7.08 (*m*, 3H), 7.8–7.9 (*m*, 4H) ppm; ^{13}C (125 MHz, $CDCl_3$): 28.3, 37.2, 36.25, 50.9, 69.7, 70.2, 79.9, 115.2, 125.2, 126.3, 126.4, 127.7, 127.9, 128.4, 128.9, 130.3, 133.1, 133.3, 134.4, 157.8 ppm; HRMS (FAB$^+$, NBA): 485.1870; calcd for $C_{26}H_{31}NSO_6^+$: 485.1872 amu; mp 112.5–113.5 °C; $[\alpha]^{25}_D$ –12.03° (*c* 1.33, CH_2Cl_2).

(2S)-3-[4-(2-Naphthylmethyloxy)phenyl]-2-amino-N-Boc-azido-propane (51). To a stirred solution of the above mesylate (**50**) (0.9 g, 1.85 mmol) in DMF (10 mL) at

60 °C was added NaN_3 (0.72 g, 11.1 mmol). After 3 h the reaction mixture was taken up in 50 mL of ethyl acetate and then washed with 2 × 20 mL 1 N HCl, 2 × 20 mL sat'd $NaHCO_3$ and 1 × 20 mL sat'd NaCl. The organic layer was dried over anhydrous $MgSO_4$ and concentrated. The crude white solid was purified by flash chromatography to give the title compound (**51**) as a white solid (0.6 g, 76%). R_f = 0.39 (1:4 ethyl acetate:hexanes). ^1H NMR (500 MHz, $CDCl_3$): 1.44 (*s*, 9H), 2.7–2.78 (*dd*, *J* = 8.0, 14.0 Hz, 1H), 2.8–2.9 (*d*, *J* = 6.0 Hz, 1H), 3.28–3.33 (*dd*, *J* = 4.0, 12.0 Hz, 1H), 3.41–3.49 (*m*, 1H), 3.94 (*s*, 1H), 4.68 (*bd*, *J* = 7.0 Hz, 1H,), 5.22 (*s*, 3H), 6.95–7.2 (AB, *J* = 8.0, 75.0 Hz, 4H), 7.48–7.76 (*m*, 3H), 7.8–7.9 (*m*, 4H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): 28.3, 37.2, 51.4, 53.0, 70.1, 79.68, 115.0, 125.2, 126.0, 126.2, 126.3, 127.7, 127.9, 128.3, 129.4, 130.3, 133.0, 133.2, 134.4, 155.0, 157.6 ppm; IR (KBr) 3373, 2981, 2943, 2861, 2361, 2103, 1683, 1611, 1510, 1288, 1242, 1167 cm^{-1}; HRMS (FAB^+, NBA/NaI): 429.2139; calcd for $C_{25}H_{30}N_2O_3Na^+$: 429.2154 amu. Anal. calcd for $C_{25}H_{28}N_4O_3$: C 69.40%, H 6.53%, N 12.95%; found C 69.12%, H 6.86%, N 13.20%; mp 141–142 °C; $[\alpha]^{25}_D$ –5.8° (*c* 1.72, CH_2Cl_2).

(2S)-3-[4-(2-Naphthylmethyloxy)phenyl]-1,2-diamino-N-Boc-propane (52). To a stirred solution of (2*S*)-3-[4-(2-naphthylmethyloxy)phenyl]-2-amino-N-Boc-azidopropane, **51**, (0.11 g, 0.27 mmol) in 5 mL anhydrous THF at rt under N_2 was added LAH (0.010 g, 0.26 mmol). After 30 min 1 N HCl (1 mL) was added to the reaction mixture. The reaction mixture was concentrated and then taken up in 1 N NaOH (3 mL). The aqueous layer was extracted with 4 × 10 mL CH_2Cl_2. The combined organics were washed with 2 × 10 mL sat'd NaCl, dried over anhydrous $MgSO_4$ and concentrated to give a white solid. Purification by flash chromatography (20:1 CH_2Cl_2:Et_3N) afforded **52** as a white solid (0.07 g, 63%). R_f = 0.4 (20:1 CH_2Cl_2:Et_3N). ^1H NMR (300 MHz, $CDCl_3$): 1.41 (*s*, 9H), 2.58 (*dd*, *J* = 6.8, 13.1 Hz, 2H), 2.72 (*dt*, *J* = 8.6, 13.2 Hz, 2H), 3.75 (*bs*, 1H), 4.73 (*bd*, *J* = 8.6 Hz, 1H), 5.19 (*s*, 2H), 6.9–7.2 (AB, 4H), 7.4–7.6 (*m*, 3H), 7.8–7.9 ppm (*m*, 4H); ^{13}C NMR (500 MHz, $CDCl_3$): 28.3, 37.9, 44.5, 54.0, 70.1, 79.9, 114.8, 125.2, 126.0, 126.2, 126.3, 127.7, 127.9, 128.3, 130.2, 133.0, 133.2, 134.5, 155.8, 157.4 ppm; HRMS: 429.2139; calcd for $C_{25}H_{30}N_2O_3Na^+$: 429.2154 amu. Anal. calcd for $C_{25}H_{30}N_2O_3$: C 73.865%, H 7.43%, N 6.98%; found C 73.84%, H 7.51%, N 6.83%; mp 81.5–83.0 °C; $[\alpha]^{25}_D$ –7.75° (*c* 1.16 methanol).

(2S)-3-[4-(2-Naphthylmethyloxy)phenyl]-1,2-diamino-propane HCl (53). To (2*S*)-3-[4-(2-naphthylmethyloxy)phenyl]-1,2-diamino-N-Boc-propane, **52**, (0.06 g, 0.15 mmol) suspended in ether (1 mL) was add a saturated solution of HCl in ether (5 mL). After 24 h the reaction mixture was concentrated and the crude solid recrystallized from MeOH/ether to give **53** as a white solid (0.02 g, 34%). ^1H NMR (500 MHz, CD_3OD): 2.92 (*dd*, *J* = 8.1, 14.7 Hz, 1H), 3.03 (*dd*, *J* = 7.0, 14.8 Hz, 1H), 3.19 (*dd*, *J* = 5.6, 14.0 Hz, 1H), 3.27 (*dd*, *J* = 7.1, 13.9 Hz, 1H), 3.75 (*p*, *J* = 7.0 Hz, 1H), 5.27 (*s*, 2H), 7.12 (AB, *J* = 9.0, 103.3 Hz, 4H), 7.42–7.61 (*m*, 3H), 7.82–7.90 (*m*, 4H) ppm; ^{13}C NMR (125 MHz, $CDOD_3$) δ 36.8, 49.5, 52.3, 71.9, 116.8, 126.3, 127.1, 127.3, 127.5, 128.9, 129.3, 131.6, 134.5, 134.7, 136.1, 159.9 ppm; HRMS (FAB, NBA): 307.1810, calcd for $C_{20}H_{25}N_2O^+$: 307.1810 amu. Anal. calcd for $C_{20}H_{24}N_2OCl_2$: C 63.33%, H 6.38%, N 7.39%; found C 63.45%, H 6.41%, N 7.20%.

4-Bromo-1,1-ethylenedioxybutane (37). To a methanolic solution of HCl (40 mL) was added 5.0 g (25.6 mmol) of 4-bromo-1-acetoxybutane.[45] The solution was stirred for 2 h at which point no more starting material was seen by TLC. The methanol was removed *in vacuo*, the residue taken up in CH_2Cl_2, and solid $NaHCO_3$ added and swirled briefly to remove HCl. Rapid filtration and removal of CH_2Cl_2 gave 3.45 g (88%) of 4-bromo-1-butanol. ^1H NMR (300 MHz, $CDCl_3$) matches that in literature.[45]

To a solution of 4.5 mL (9 mmol, 2.0 M in CH_2Cl_2) of oxalyl chloride in 35 mL CH_2Cl_2 (–78 °C, argon) was added 1.3 mL (15 mmol) of dimethyl sulfoxide (dropwise, rapidly). The solution was stirred for 10 min and then 1.0 g (6.5 mmol) of 4-bromo-1-butanol in 5 mL CH_2Cl_2 was added over 10 min. After stirring an additional 45 min, 5.2 mL (71 mmol) of triethylamine were added, forming a dense precipitate. After 10 min, the reaction mixture was allowed to warm to room temperature and quenched with 30 mL water. The aqueous layer was extracted (2 × 30 mL CH_2Cl_2) and the combined organic layers washed (1 × 30 mL sat'd NaCl, 2 × 30 mL sat'd NH_4Cl, 3 × 30 mL H_2O, 1 × 30 mL sat'd NaCl) and dried ($MgSO_4$). Removal of the solvent gave 1.0 g (99%) of product. ^1H NMR (300 MHz, $CDCl_3$) matches that published in the literature.[45]

Protection of the aldehyde functionality with ethylene glycol was done essentially as reported by Bobrova, *et al.* to give **37**. ^1H NMR (300 MHz, $CDCl_3$) matches that in literature.[45] There was a small amount of contamination by 4-(4-bromobutyloxy)-1,1-ethylenedioxybutane, a result of the treatment of the 4-bromo-1-butanol with base. This was easily removed by flash chromatography.

Methyl 7-hydroxy-(5S,6S)-epoxyheptanoate (45). A solution of allylic alcohol **44**, (4.0 g, 25.3 mmol) in 15 mL of CH_2Cl_2 was added to a mixture of (+)-diisopropyl tartrate (0.85 g, 3.6 mmol) and 800 mg of 4 Å powdered molecular sieves in 30 mL CH_2Cl_2. The reaction mixture was stirred for 20 min at room temperature, cooled to –30 °C, and $Ti(O_iPr)_4$ (0.75 g, 3.0 mmol) in 5 mL CH_2Cl_2 added over 5 min. The reaction was allowed to stir for 30 min at –23 °C at which time *t*BuOOH (4.7 M in CH_2Cl_2, 11.2 mL, 52 mmol) was added dropwise over 20 min. The reaction was complete after 3 h at –10 °C. 15.2 mL dimethyl sulfide was added and the reaction mixture allowed to warm to room temperature over 1 h. 100 mL of aqueous sat'd NaF solution was added, the mixture stirred 20 min, and then NaCl was added to saturate the aqueous layer. The mixture was then filtered through Celite, the layers separated, and the aqueous layer extracted with CH_2Cl_2. Purification

by flash chromatography gave 3.75 g (85%) of **45** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) 1.60 (*m*, 2H), 1.80 (*m*, 2H), 2.0 (*bs*, 1H), 2.37 (*t*, *J* = 9 Hz, 2H), 2.95 (*m*, 2H), 3.64 (*dd*, *J* = 5 and 14 Hz, 1H), 3.78 (*s*, 3H), 3.88 ppm (*dd*, *J* = 3 and 14 Hz, 1H). The % *e.e.* for this compound was determined to be 94% *e.e.* based on ^{1}H NMR analysis of the Mosher ester. [46]

Methyl 11-oxo-(5S,6S,7E,9E)-epoxyundecadienoate (38). 420 mg (2.44 mmol) of methyl 7-oxo-5*S*,6*S*-epoxyhexanoate (**46**) in 5 mL dry CH_2Cl_2 were added over 15 min to a solution of 968 mg (2.93 mmol) of unpurified ylid (**47**)[56,57] in 5 mL CH_2Cl_2 at room temperature. The reaction was stirred for 1.5 h at which point the reaction was complete as indicated by TLC (2:1 Et_2O:hexanes, SM: R_f = 0.3, *cis* R_f = 0.45, *trans* R_f = 0.4). [Note, if reaction does not proceed to completion, additional ylid is added (in CH_2Cl_2 soln) to the reaction mixture until no more initial aldehyde remains. Also, the number of mmol reported here for the ylid are based on the ylid being pure, which is probably not the case.] The reaction mixture was then diluted with 15 mL 2:1 hexanes:EtOAc and filtered through a plug of silica (8 mL, packed with 1:1 hexanes:EtOAc, 2% triethylamine), which was then washed with 2:1 hexanes:EtOAc (2 × 50 mL). The solvent was removed *in vacuo*, taking care to keep the resulting residue under argon, and purified by chromatography (packed: hexanes, 2% triethylamine, run: 2:1 hexanes:EtOAc, 0.1% triethylamine). The solvent was removed *in vacuo* and the residue was dried under high vacuum to remove the final traces of triethylamine (will cause problems in isomerization if not removed). The yellow oil was then dissolved in 20 mL of CH_2Cl_2 and I_2 (20 mg in 6 mL) added and the reaction run under the light in the fumehood. This was stirred for 2 h (monitored by TLC, solvent as above) at which time little change was seen (still small amount of *cis* isomer). The reaction mixture was washed with $Na_2S_2O_3$ to remove the I_2 and then dried quickly with $MgSO_4$. A flash silica gel column was used to purify the *trans,trans* from the *trans,cis* isomer (7:3 hexanes:EtOAc, 2% triethylamine), yielding 265 mg (1.22 mmol, 50%). ^{1}H NMR agrees with that in the literature.[12]

(Z)-(3-Nonen-1-yl)triphenylphosphonium tosylate (39).[48] A solution of 2.0 g (6.7 mmol) of (*Z*)-3-nonenyl tosylate and 1.76 g (6.7 mmol) of triphenylphospine in 2 mL toluene was heated at 110–120 °C for 28 h. The resulting thick oil was then extracted with petroleum ether to remove trace impurities. The product was purified by flash chromatography (19:1 $CCHCl_3$:MeOH) to give a clear oil which solidified on standing (90%). ^{1}H NMR (300 MHz, $CDCl_3$): 0.85 (*t*, *J* = 10 Hz, 3H), 1.3–1.1 (*m*, 6H), 1.7 (*m*, 2H), 2.3 (*s*, 3H), 2.35 (*m*, 2H), 3.70 (*m*, 2H), 5.5–5.3 (*m*, 2H), 7.05 (*d*, *J* = 9 Hz, 2H), 7.75–7.5 ppm (*m*, 17H).

LTA_4 methyl ester.[12] To a solution of 1.0 g (1.79 mmol) phosphonium tosylate (**39**) in 15 mL of anhydrous THF was added dropwise 2.45 mL (1.53 mmol) of *n*-BuLi (1.6 N in hexanes) (–78 °C, argon). The resulting orange solution was stirred for 45 min, 2.6 mL (15.34 mmol) of HMPA were added and the mixture stirred for 15 min. Aldehyde **38** (265 mg, 1.18 mmol) in 10 mL dry THF was then added dropwise over 10 min. The reaction was stirred a further 15 min and was then placed in an ice bath and allowed to warm to 0 °C over 45 min. 10 mL of water were added and the reaction worked up with ether (water layer washed 3 × 10 mL ether, organic layers combined). Drying with Na_2SO_4 proved to be ineffective and so the oil obtained after ether evaporation was taken up in a small amount of EtOAc and *quickly* dried (less than 2 min) with $MgSO_4$. The crude mixture was then purified by column chromatography (19:1 hexanes:ethyl acetate, 2% triethylamine—column packed with 5% triethylamine in hexane) to give 220 mg (58%) product. ^{1}H NMR agrees with that published.[12] ^{1}H NMR (300 MHz, C_6D_6): 0.95 (*t*, *J* = 8.0 Hz, 3H), 1.4–1.25 (*m*, 8H), 1.65 (*m*, 2H), 2.08 (*m*, 2H), 2.12 (*t*, *J* = 8.5 Hz, 2H), 2.60 (*dt*, J_1 = 2 Hz, J_2 = 5.5 Hz, 1H), 2.96 (*dd*, J_1 = 8 Hz, J_2 = 2 Hz, 1H), 3.0 (*m*, 2H), 5.35 (*dd*, J_1 = 16 Hz, J_2 = 7.5 Hz, 1H), 5.50 (*m*, 3H), 6.10 (*t*, *J* = 11 Hz, 1H), 6.15 (*dd*, J_1 = 11 Hz, J_2 = 15 Hz, 1H), 6.39 (*dd*, J_1 = 11 Hz, J_2 = 15 Hz, 1H), 6.60 (*dd*, J_1 =11.5 Hz, J_2 = 14.5 Hz, 1H). HRMS (FAB$^+$, NBA): 355.2246; calcd for $C_{21}H_{32}O_3^+$: 355.2249 amu.

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