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Synthesis of glutamic acid analogs as potent inhibitors of leukotriene A_4 hydrolase☆

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Abstract—Leukotriene B_4 (LTB_4) is a potent pro-inflammatory mediator that has been implicated in the pathogenesis of multiple diseases, including psoriasis, inflammatory bowel disease, multiple sclerosis and asthma. As a method to decrease the level of LTB_4 and possibly identify novel treatments, inhibitors of the LTB_4 biosynthetic enzyme, leukotriene A_4 hydrolase (LTA_4-h), have been explored. Here we describe the discovery of a potent inhibitor of LTA_4-h, arylamide of glutamic acid **4f**, starting from the corresponding glycinamide **2**. Analogs of **4f** are then described, focusing on compounds that are both active and stable in whole blood. This effort culminated in the identification of amino alcohol **12a** and amino ester **6b** which meet these criteria.

1. Introduction

Leukotriene B_4 (LTB_4) is a potent pro-inflammatory activator of inflammatory cells, including neutrophils, eosinophils, monocytes, macrophages, T cells and B cells that is a functional link between early innate and late adaptive immune responses.[1] There is substantial evidence that LTB_4 plays a significant role in the amplification of many inflammatory disease states[2] including asthma,[3] inflammatory bowel disease (IBD),[4] chronic obstructive pulmonary disease (COPD),[5] arthritis,[6] psoriasis,[7] and atherosclerosis.[8] LTB_4 also stimulates the production of various cytokines and may play a role in immunoregulation.[9] Therefore, a therapeutic agent that inhibits the response of cells to LTB_4 or inhibits the biosynthesis of LTB_4 may be useful for the treatment of these inflammatory conditions.

Two pharmacologically distinct human LTB_4 receptors, BLT1[10] and BLT2,[11] have recently been described. BLT1 is a high affinity LTB_4 receptor (K_d = 0.1–0.7 nM) with a restricted expression in inflammatory cells, for example, neutrophils, monocytes, thymus, and spleen. BLT2 has a wider tissue expression profile but a 20-fold lower affinity for LTB_4 (K_d = 23 nM) than BLT1. Although the presence of two receptors can provide for a graded immune response, it makes antagonism of a LTB_4 receptor a more difficult therapeutic target. Therefore, we focused on the reduction of LTB_4 production as a therapeutic approach.

The biosynthesis of LTB_4 from arachidonic acid (AA) involves the action of three enzymes: phospholipase A_2 (PLA_2), to release AA from the membrane lipids; 5-lipoxygenase (5-LO), to form the unstable epoxide leukotriene A_4 (LTA_4); and leukotriene A_4 hydrolase (LTA_4-h), to form LTB_4.[12] In addition to being the

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☆ *Note to the editor:* The atomic coordinates described in this paper have been deposited with the Protein Data Bank.
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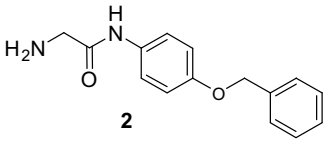
precursor to LTB_4, LTA_4 is a precursor of the pro-inflammatory cysteinyl leukotrienes LTC_4, LTD_4, and LTE_4[13] via LTC_4 synthase and a precursor of lipoxygenases to give the anti-inflammatory mediators, lipoxins A_4 (LXA_4) and B_4 (LXB_4).[14] Thus the targeting of LTA_4-h would leave other immune response pathways intact.

LTA_4-h is a monomeric, soluble 69 kD enzyme.[15] LTA_4-h is a bifunctional zinc-dependent metalloenzyme of the M1 class of metallohydrolases which catalyzes two reactions at the same active site: a stereospecific epoxide hydrolase reaction and a non-specific peptidase reaction. Based on biochemical studies, mutational analysis, and X-ray crystallography,[16] the two enzyme-catalyzed reactions are exerted via distinct and yet overlapping active sites.[17] The bifunctional nature of LTA_4-h allows for the determination of inhibition constants using either the hydrolase or peptidase activity using either LTA_4 or L-alanine-*p*-nitroanilide as substrates, respectively. The differences in the binding and kinetic constants for the two substrates[18] make direct comparison of inhibitor data from the hydrolase and peptidase assays problematic, but comparison of inhibition data within either assay can be used to rank compounds.

High resolution crystal structures of recombinant human LTA_4-h with bound inhibitors have been reported in the literature.[19] The hydroxamic acid **1** (K_i = 1.6 nM) bound in a LTA_4-h crystal structure is believed to make many of the same interactions as LTA_4 (Fig. 1). As can be seen in Figure 1, the interaction between LTA_4-h and molecule **1** can be divided into four distinct regions. A hydrophobic pocket containing the two aromatic rings of inhibitor **1** in Figure 1 is the proposed binding site of the long alkyl tail of LTA_4. Just outside the hydrophobic pocket are Glu271 and Gln136 which form a salt bridge with the free amine on **1**. The zinc atom which chelates to the hydroxamic acid would bind to the epoxide of LTA_4. Basic residue Arg563, which forms a salt bridge with the terminal carboxylic acid of **1**, could make a similar interaction with LTA_4.

Since LTA_4-h is considered a drugg able target with available functional and PD assays, and plays a role in inflammatory diseases,[20] several companies have reported programs designed to discover a potent and selective LTA_4-h inhibitor.[20b,21] Our goal at the start of the Berlex program was to identify a non-hydroxamic acid containing inhibitor using the peptidase assay. The peptidase assay is more suitable for high-throughput screening than the hydrolase assay and was used during the early stage of the project described here. Compound **2** was identified through high-throughput screening as an inhibitor of the peptidase activity of recombinant human LTA_4-h (peptidase assay)[22] with an IC_{50} of 280 nM. The secondary assay for this project was inhibition of LTB_4 synthesis in human whole blood (the whole blood assay),[23] and compound **2** was weakly active in this assay with a potency of 7 μM. The whole blood assay gave insights into cell permeability and protein binding, and high potency in this assay was the ultimate goal for inhibitors.

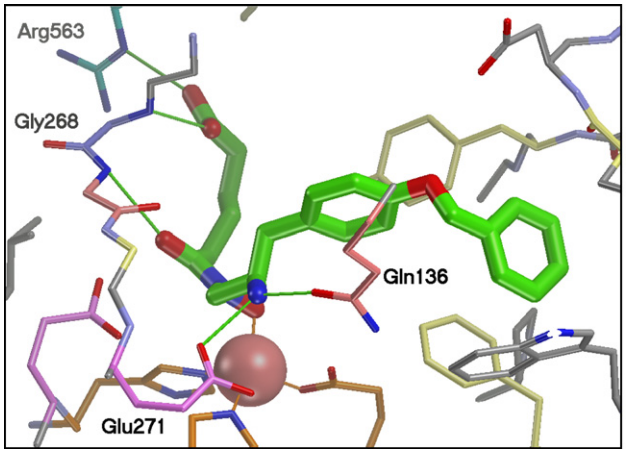


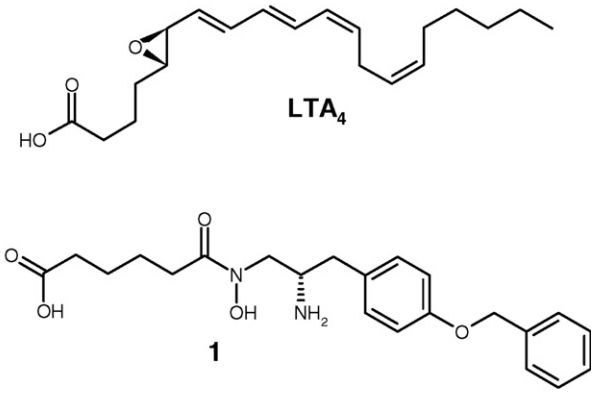
2

Compound **2** was chemically simple and easy to derivatize, and thus was an attractive lead compound. Following is a description of the efforts to optimize this lead into novel, potent and cell-permeable inhibitors of LTA_4-h.

2. Chemistry

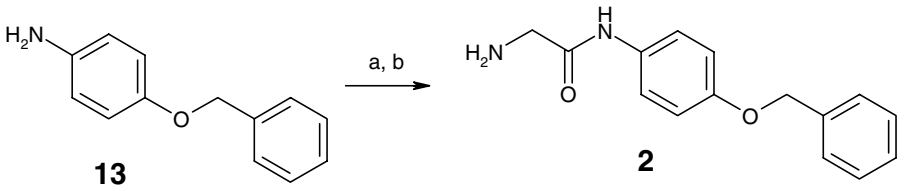
The synthesis of compounds **2**–**12** was carried out in the following manner. Compound **2** was synthesized through carbonyl diimidazole (CDI) mediated coupling of **13** and Boc-glycine followed by acidic deprotection (Scheme 1). Coupling of other simple amino acids to benzyloxyaniline was done in a similar manner.





1

Figure 1. A hydroxamic based inhibitor bond to rhLTA4-h (PDB entry 2VJB). Green is used for the inhibitor's carbon atoms. H-bonds are depicted as green lines. The zinc is displayed as a peach colored sphere that is chelated to His295, His299, and Glu318 (gold atoms). The hydroxamic acid also chelates the zinc and the carboxylic acid forms H-bond with Arg563 (2.6 Å) and Gly268 (2.4 Å).

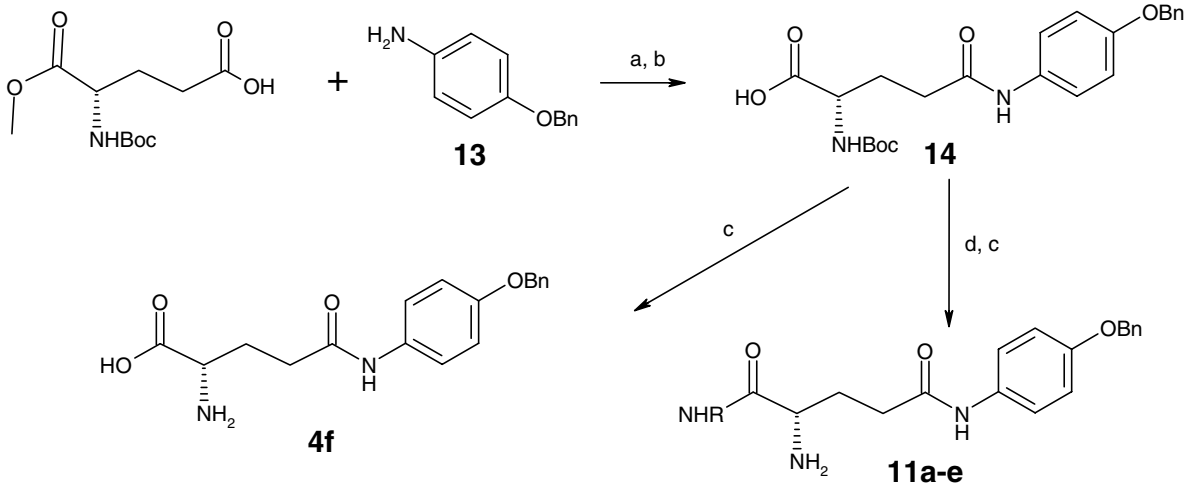


Scheme 1. General synthesis of amino acid analogs. Reagents and condition: (a) CDI, Boc-Gly, CH_2Cl_2, reflux; (b) TFA, CH_2Cl_2.

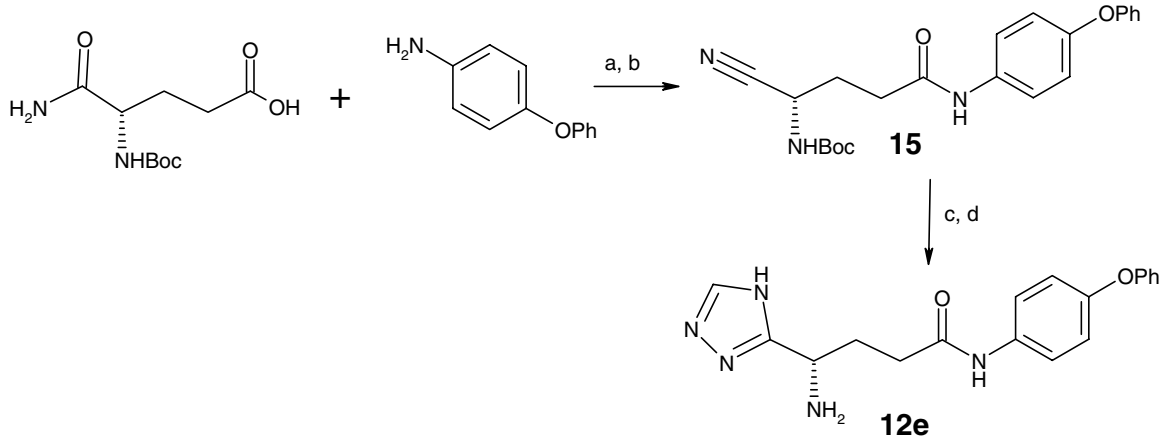
Synthesis of aspartic acid and glutamic acid derivatives was accomplished using isobutyl chloroformate (IBC) coupling conditions followed by sequential deprotection as shown for the synthesis of compound **4f** (Scheme 2). The same conditions were used successfully to couple anilines with both the alpha carboxylic acid and the side-chain carboxylic acid. During the process of varying the aniline portion of the lead series, it was found that standard peptide coupling agents such as HATU or EDC/HOBt were also effective in coupling to glutamic acid derivatives.

Initial modification of the glutamic acid portion of **4f** focused on ester and amide derivatives. Some of these derivatives could be synthesized simply by beginning with the appropriate glutamic esters and glutamines (Scheme 2). Amides **11a–e** were synthesized through coupling the appropriate amine with intermediate **14** using EDC/HOBt conditions, followed by deprotection if necessary.

Replacement of the carboxylic acid of **4f** with a heterocycle was accomplished through the following sequence (Scheme 3). Aminonitrile **15** was synthesized through



Scheme 2. Synthesis of γ-glutamic acid analogs. Reagents and condition: (a) IBC, *N*-methylmorpholine, THF, −20 °C; (b) NaOH, MeOH; (c) TFA, CH_2Cl_2; (d) NH_2R, EDC, HOBt, DMF.



Scheme 3. Synthesis of acid replacement analogs of **4f**. Reagents and condition: (a) IBC, *N*-methylmorpholine, THF, −20 °C; (b) cyanuric chloride, DMF; (c) formic hydrazide, K_2CO_3, EtOH; (d) TFA, CH_2Cl_2.

dehydration of the glutamide formed through coupling 4-phenoxyaniline and Boc-Gln, and then conversion to the triazole and deprotection led to compound **12e**.

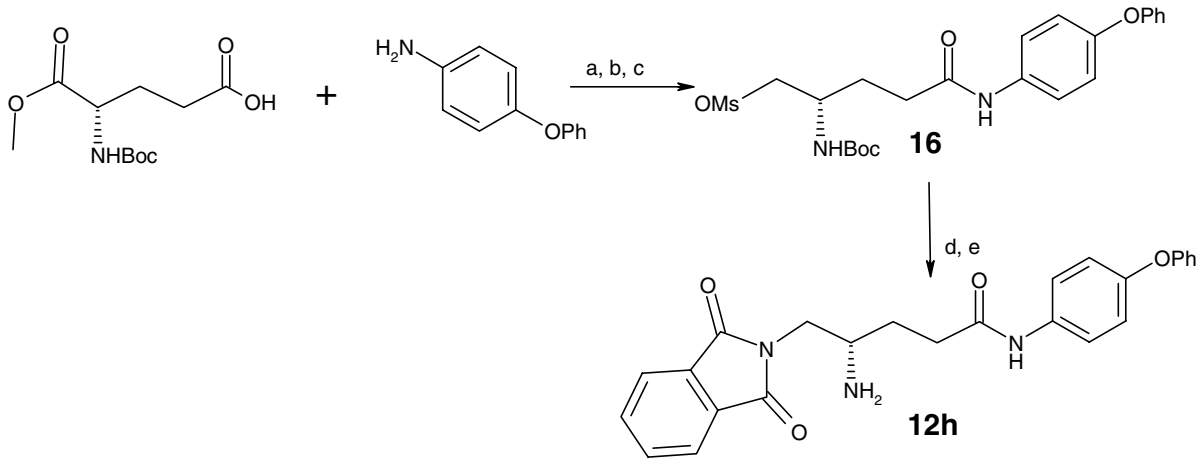
Other intermediates were used to synthesize extended inhibitors. Reduction of the protected amino ester and sulfonylation provided the versatile mesylate **16** (Scheme 4). This mesylate could be displaced by a variety of heterocycles using similar conditions to those shown for the phthalimide for compound **12h**. Deprotection of the amine provided the final class of inhibitors in this series.

3. Results and discussion

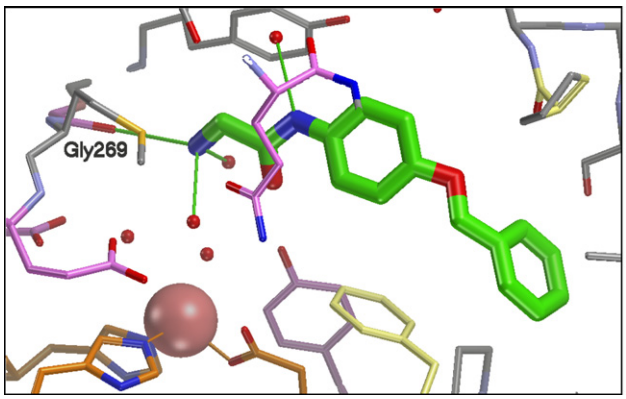
The strategy for altering **2** was based on interactions observed in the crystal structure (Fig. 2). The interaction of this compound with the enzyme appears to be mostly hydrophobic, with the two phenyl rings extending into the presumed binding site for the aliphatic chain of LTA_4.[16] The amine of **2** forms the only direct hydrogen bond (H-bond) to the protein through the carbonyl of Gly269 (2.9 Å). The amine is also the closest inhibitor atom to the active site zinc (5.5 Å) and there are several waters that fill the gap between the two atoms. The initial plan was to probe the binding site through the use of amino acids having hydrophobic, basic, and acidic side chains with the ultimate goal of making a positive interaction with the zinc ion by displacing the water molecules.

Substitution on the glycine portion of **2** with hydrophobic groups was explored first (Table 1). The decrease in the inhibitory activity of analogs with an increase in the size of the hydrophobic group, analogs **3a**–**c**, confirmed the prediction based on the analysis of Figure 2 that the binding pocket is compact and disfavors non-polar groups. The lack of inhibitory activity with the proline analog **3d** can be explained by both the increase in non-polar groups and the loss of a hydrogen bond between LTA_4-h and the secondary amine. Next we explored the glycine analogs with polar side chains with the anticipation of filling the polar pocket and having a positive interaction with the zinc. However, substitution of glycine with an amino acid having a polar side chain, glutamine (Gln, **3e**), or a basic side chain, lysine and histamine (Lys and His, **3f**–**g**), or acidic side chain, glutamic acid or aspartic acid (Glu or Asp, **4a**–**b**) all showed reduced activity.

A crystal structure of **4b** bound to the enzyme (Fig. 3) shows the side-chain carboxylic acid interacting with the zinc (2.8 Å), although not within the chelation



Scheme 4. Synthesis of extended analogs of **4f**. Reagents and condition: (a) IBC, *N*-methylmorpholine, THF, −20 °C; (b) $NaBH_4$, MeOH/THF; (c) MsCl, pyridine; (d) potassium phthalimide, DMF; (e) TFA, CH_2Cl_2.



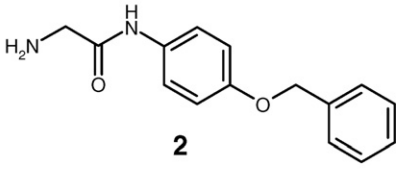
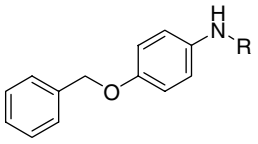
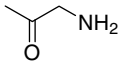
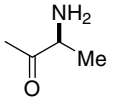
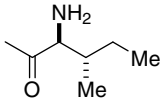
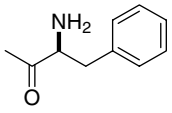
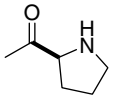
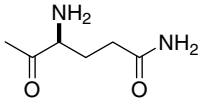
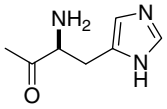
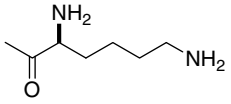
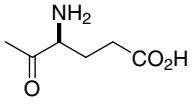
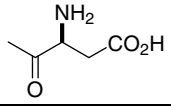


Figure 2. Compound **2** bound to $rhLTA_4$ (PDB entry 3CHO).

Table 1. Amino acid analogs



Number	R	Peptidase IC_{50}	WBA IC_{50}
2		280	7000
3a		150	>10,000
3b		>2000	ND
3c		>2000	ND
3d		>2000	ND
3e		>2000	ND
3f		10,000	ND
3g		5800	ND
4a		680	>10,000
4b		5400	ND

IC_{50} values (nM) are means of at least two independent determinations. ND = Not determined.

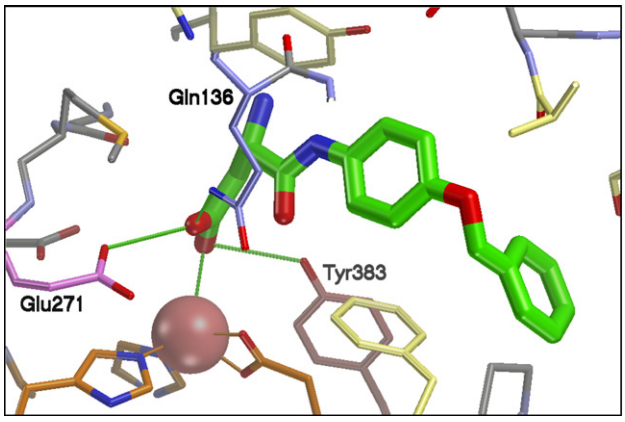
sphere (<2.4 Å). The loss of potency may be explained by observation that the amine does not form any H-bonds with the protein. This implies that there is an energetic penalty for desolvating the basic amine when it binds to the protein. It was anticipated that simple modifications of these Glu and Asp derivatives would improve interactions with the protein and increase the binding affinity.

Following this logic, we explored the orientation of the amine group using D-Glu and D-Asp (**4c**–**d**) without improvement in potency (Table 2). Shifting the position of the amino substitution on the chain by coupling to the aniline through the side-chain carboxylic acid provided a breakthrough. Compound **4f** was 40 times more potent than **4a**, and over 10 times more potent than our lead compound **2**. The specificity of the binding was shown with the decrease in potency by shortening the carbon chain, **4e**, or inverting the configuration at the amino group, **4g**.

Examining the crystal structure of **4f** gives some rationale for this potency increase (Fig. 4). The carboxylic acid binds to the zinc (2.1 Å) and becomes the fourth member of the chelation sphere. The carboxylic acid also forms H-bonds to the side chains of Glu296 (2.6 Å) and Tyr383 (2.8 Å), two residues that have been shown to be catalytically active.[16] In addition, the amine forms H-bonds to Gln136 (2.7 Å) and Glu271 (2.9 Å). A similar interaction was observed with the free amine in the hydroxamic acid based inhibitor of LTA4-h (Fig. 1). Based on the proposed model of the enzyme's peptidase activity,[17] the glutamic acid portion of **4f** fills the same pocket as the N-terminal residue of the substrate peptide.

However, compound **4f** was 400-fold less active in the whole blood assay than in the peptidase assay. There are many possible reasons for this significant drop in activity, but lack of cell permeability caused by the zwitterionic nature of the alpha amino acid was considered the most likely. With the interaction between the zinc and carboxylic acid observed in the crystal structure, we felt the amino group might not be critical for binding of the inhibitor. However, the simple carboxylic acid, **5**, was completely inactive in the peptidase assay (Table 3). Analogs of **4f**, both ester **6a** or **6b** and amide **6c** have similar activity to **4f** in the peptidase assay but are significantly more potent than **4f** in the whole blood activity. The hydroxamic acid **6d** has a similar profile. The potency difference in the whole blood assay can be attributed to an improvement in cell permeability of the ester and amide analogs over the zwitterion **4f**, but the similar potency in the peptidase assay suggests that the amides and esters are cleaved by LTA_4-h during binding. Unfortunately due to technical difficulties, we were unable to confirm that analogs **6a**–**d** are acting as pro-drugs and more importantly if binding of the acid to the zinc is critical for potency.

Removal of the carboxylic acid from **4f** yielded **7a** which is threefold less potent in the peptidase assay, but 20-fold more potent in the whole blood assay than **4f**. As can be seen in Figure 5, the crystal structure **7a** with LTA4-h can be superimposed on the corresponding structure with **4f** with the exception for chelation to the zinc. In retrospect, the minimal increase in binding affinity associated with the carboxylic acid to zinc interaction is consistent with the fact that a free carboxylic acid is the product of LTA_4-h acting as a peptidase. According to the Circe effect,[24] the enzyme is designed to have reduced affinity for the products versus the reactants. The relative unimportance of the carboxylic acid to binding affinity has been observed earlier.[25] For our



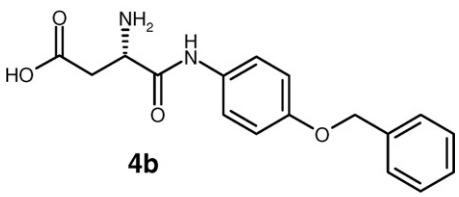
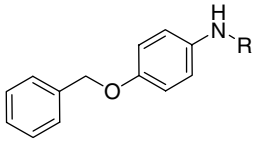
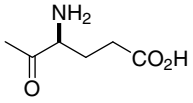
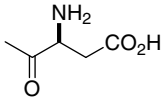
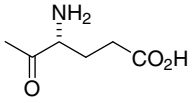
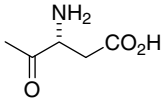
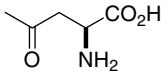
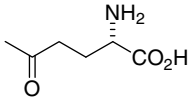
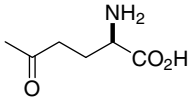


Figure 3. Compound **4b** bound to rhLTA4-h (PDB entry 3CHP).

Table 2. Glu/Asp analogs



Number	R	Peptidase IC_{50}	WBA IC_{50}
4a		680	>10,000
4b		5400	ND
4c		7200	ND
4d		9300	ND
4e		1100	>10,000
4f		20	8000
4g		1600	ND

IC_{50} values (nM) are means of at least two independent determinations. ND = Not determined.

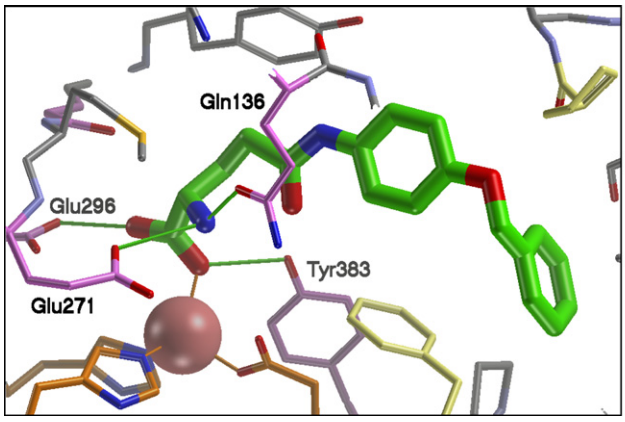
inhibitors, the carboxylic acid of **4f** appears to be tolerated, but it does not noticeably improve binding. The significant decrease in affinity upon the addition of one or two methyl groups to the amine (**7b–c**) can be explained by the loss of a hydrogen bond, but modeling studies based on the LTA_4-h/**4f** complex indicate that steric interactions are also a factor.

With the glutamic acid on the right side of the molecule, we explored modification to the aryl region (Table 4). For this set of analogs, no significant difference in potency in the peptidase assay was observed between the corresponding primary amide, the ester or the carboxylic acid. Removal of the benzyl group, **8a**, or removal of a flexible tether, **8b**, gave inactive analogs. Substitution of pyridine for benzene, **8d**, or changing the substitution pattern on the central ring to meta, **8c**, resulted in a significant loss in potency. The linker between the two aryl groups could be shortened to an oxygen, **8e**, or carbon, **8f**, without loss in potency. A secondary amine, **8g**, was tolerated as a linker but not a tertiary amine, **8h**. Lengthening the tether to a three or four atom chain (**8i–j**) or placing substituents on the tether (**8k–l**) was tolerated.

The deep, relatively narrow pocket accessed by these flexible, hydrophobic moieties was predicted from the crystal structure. As shown in the structure of **8l** (Fig. 6), the second aromatic unit simply extends down into the pocket of the enzyme. This pocket is believed to bind the long hydrophobic tail of the natural substrate LTA_4,[17] and the best binding results were always achieved with hydrophobic residues in this region that possessed some flexibility.

For analogs of the terminal aryl group, the simple ether linkage was preferred for its potency and ease of synthesis (Table 5). Changing the terminal aryl group to an alkyl group such as methyl or cyclohexyl (**9a–b**) removed potency altogether. A methyl scan on the terminal phenyl ring (**9c–e**) showed that ortho and meta substituents reduced activity, while the para substituent was well tolerated. Introduction of heterocyclic substituents in the para position such as pyrrole or furan (**9f–g**) is tolerated, but does not improve the potency relative to the simple phenoxyphenyl substitution (**8e**). There was a potency difference in the WBA.

With the selection of the phenoxyphenyl group on the left-hand side, modification of the glutamic acid portion



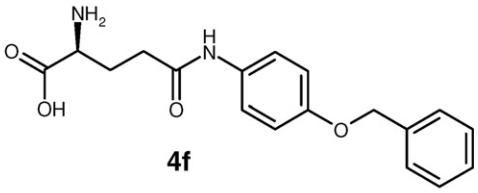
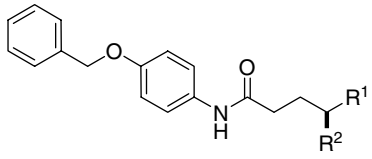
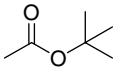
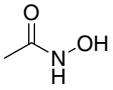


Figure 4. The rhLTA4/**4f** crystal structure (PDB entry 3CHQ).

Table 3. Initial acid modification

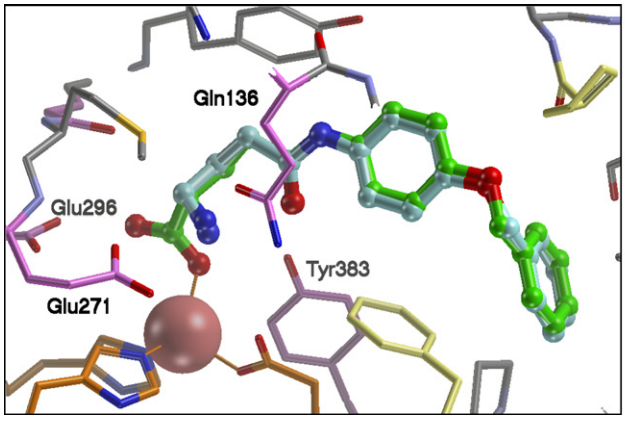


Number	R^1	R^2	Peptidase IC_{50}	WBA IC_{50}
4f	CO_2H	NH_2	20	8000
5	CO_2H	H	>18,000	ND
6a	CO_2Me	NH_2	39	48
6b		NH_2	60	27
6c		NH_2	23	85
6d		NH_2	25	130
7a	H	NH_2	61	380
7b	H	NHMe	11,000	>10,000
7c	H	NMe_2	9400	ND

IC_{50} values (nM) are means of at least two independent determinations. ND = Not determined.

of **8e** was investigated. The initial goal was to extend the inhibitor in order to make a strong interaction with Arg563. This interaction was intended to mimic the natural interaction of LTA_4 with this amino acid, and the interaction is shown clearly with the structure of hydroxamic acid **1** with LTA_4-h in Figure 1. Modifying compound **8e** by extending the acid one carbon gave **10**, the most potent compound in the peptidase assay (Table 6). However, the carboxylic acid is still interacting with the zinc rather than the arginine according to the crystal structure (not shown PDB entry XXX7).

Seeking a simple way to further separate the carboxylic acid from the hydrophobic tail, a series of amido acids was synthesized (**11a**–**c**). Although these compounds were all potent in the primary assay, they were uniformly weak in the whole blood assay. In addition, no change in potency was observed on variation of the chain length, a clear sign that no specific interaction was being made with the terminal acid. Furthermore, none of these compounds were more potent than our benchmark compound, **8e**, in either assay. Even more compelling, replacing the carboxylic acid with a hydroxyl group (**11d**) or an amine (**11e**) provided compounds that were equipotent. Once again, these compounds could all be pro-drugs, in which the amide bond is



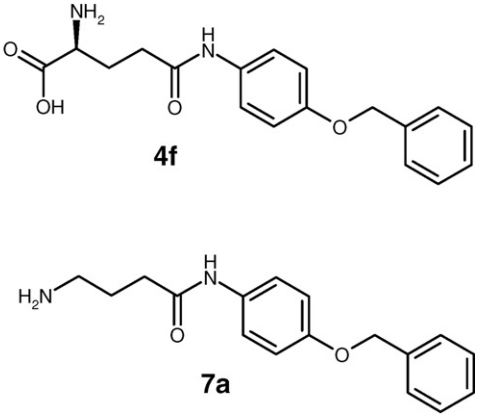
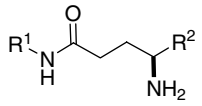
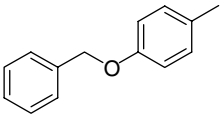
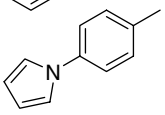
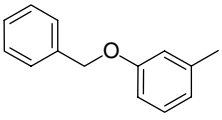
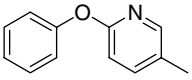
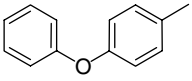
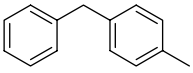
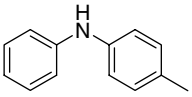
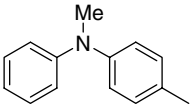
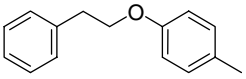
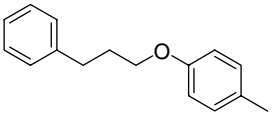
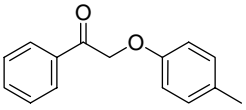
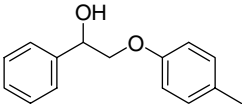


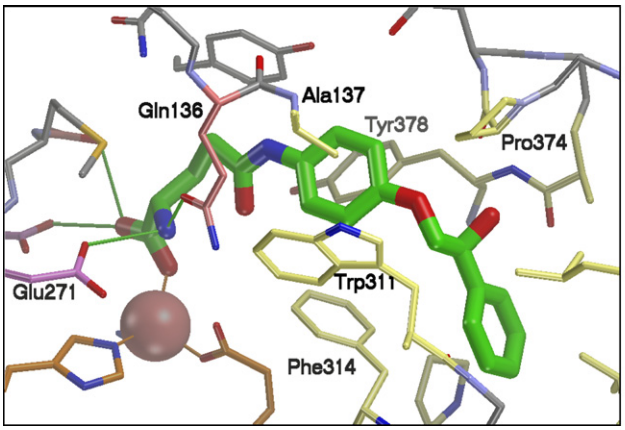
Figure 5. 7a (cyan atoms) superimposed on the crystal structure of **4f** (green atoms) bound to rhLTA4-h (PDB entries 3CHR and 3CHQ, respectively).

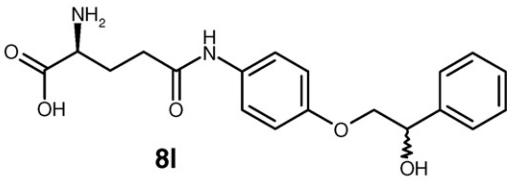
Table 4. Central ring and aryl tether modifications



Number	R^1	R^2	Peptidase IC_{50}	WBA IC_{50}
4f		CO_2H	20	8000
8a			>18,000	ND
8b			9700	ND
8c		CO_2Me	730	1300
8d			2600	ND
8e		CO_2H	19	3300
8f			21	110
8g		CO_2H	210	180
8h		CO_2Me	8000	ND
8i		CO_2H	46	>10,000
8j		CO_2H	31	>10,000
8k		CO_2H	18	>10,000
8l		CO_2H	17	>10,000

IC_{50} values (nM) are means of at least two independent determinations. ND = Not determined.

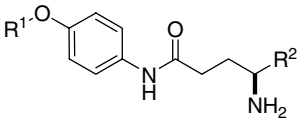


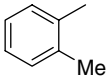
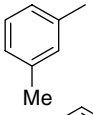
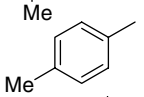
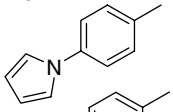
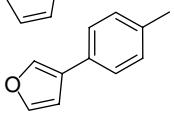


8l

Figure 6. The rhLTA4-h/**8l** complex (PDB entry 3CHS). The view emphasizes the hydrophobic pocket that binds the aromatic rings of the inhibitors. The figure includes several residues that were omitted from previous figures in order to simplify the view. These include Ala137, Trp311, and Tyr378.

Table 5. Terminal aromatic ring modifications



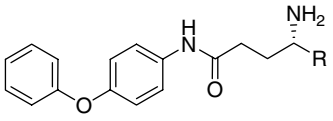
Number	R^1	R^2	Peptidase IC_{50}	WBA IC_{50}
8e		CO_2H	19	3300
9a	Me	CO_2Me	>18,000	ND
9b			>18,000	ND
9c			2800	ND
9d			150	570
9e			13	110
9f		CO_2H	22	4700
9g			29	92

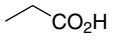
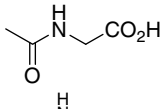
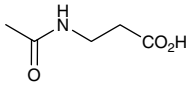
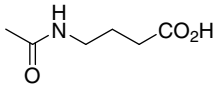
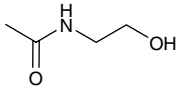
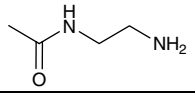
IC_{50} values (nM) are means of at least two independent determinations. ND = Not determined.

cleaved by LTA_4-h resulting in the formation of **8e**. Compound **11d** even had excellent whole blood potency, a property not shared by any of the extended acids. Therefore, attempts to make a positive interaction with Arg563 with a terminal carboxylic acid were abandoned.

Instead, attention was turned to replacing the carboxylic acid in order to improve the whole blood potency. It was

Table 6. Attempts to extend the acid

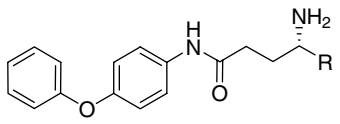


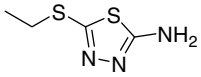
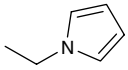
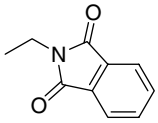
ZK Number	R	Peptidase IC_{50}	WBA IC_{50}
8e	CO_2H	19	3300
10		6	7000
11a		22	>1000
11b		17	3400
11c		19	>1000
11d		20	72
11e		22	3100

IC_{50} values (nM) are means of at least two independent determinations. ND = Not determined.

felt that removing the zwitterionic character of these compounds would improve their cell permeability, and thus bring the whole blood potency closer to the peptidase potency. This theory was supported by the high whole blood activity of esters and amides such as compound **6b**. Converting the carboxylic acid to a primary alcohol (**12a**) or an amine (**12b**) provided some of the most potent compounds in the whole blood assay (Table 7). However, a compound with a methoxy group in this position (**12c**) had low activity in the peptidase assay, perhaps due to the lack of a hydrogen donating group. The sulfonic acid analog of compound **10** (**12d**) was a

Table 7. Attempts to replace the acid



Number	R	Peptidase IC_{50}	WBA IC_{50}
12a		14	110
12b		21	440
12c		5600	ND
12d		130	>10,000
12e		740	2000
12f		350	ND
12g		390	ND
12h		85	7000

IC_{50} values (nM) are means of at least two independent determinations. ND = Not determined.

relatively potent compound, but had no activity in whole blood. A number of heterocyclic replacements for the acid were also synthesized (**12e**–**h**). However, all of these compounds had lower activity than the simple hydroxyl or amine containing compounds.

4. Conclusion

A series of glutamic acid based compounds that are potent inhibitors of LTA_4-h has been identified. Starting from the weakly active HTS hit **2**; compound **4f** was identified with excellent activity in the primary peptidase assay. The poor activity of this compound in the secondary whole blood assay was believed to be caused by poor cell permeability. Several derivatives of this compound were quite active in suppressing LTB_4 production in whole blood, particularly the amino alcohol **12a** and the *tert*-butyl ester **6b**.

5. Experimental

5.1. Biological methods

5.1.1. Peptidase assay. This assay was modified from the previously described protocol[20] as described: Compound potency against the peptidase activity of LTA4 hydrolase was measured by inhibition of the hydrolysis of L-alanine-*p*-nitroanilide to L-alanine and highly colored nitroaniline. In brief, LTA_4-h (29 nM) was incubated with L-alanine-*p*-nitroanilide (1 mM = K_m) in 50 mM HEPES (pH 7.5), 100 mM KCl, 1% DMSO in the absence or presence of test compound for 1 h at ambient temperature. Eight concentrations (at 1:3.16 dilution) of test compound were run for IC_{50} determination. Reaction was terminated by addition of acetic acid (final concentration 1%). Formation of colored nitroaniline was measured by the increase in absorbance at 405 nm in a Victor 2 plate reader (Wallac). Spontaneous hydrolysis of the substrate was corrected for by subtracting the absorbance of control incubations without enzyme. A standard compound was included in each assay plate. The IC_{50} values were determined from dose–response curves by non-linear fit of data to the 4-parameter fit equation. At least two independent determinations were made for each IC_{50} value.

5.1.2. Whole blood assay. The whole blood assay was performed as described previously.[21]

5.1.3. Crystallography. The protein was purified and crystallized according to the previously established protocols.[16] Protein crystallization was performed in melting point capillaries using liquid–liquid diffusion. Shortly for this 5 μL protein 5 mg/mL in 10 mm Tris buffer, pH 7.5 was layered on top of 5 μL precipitate solution containing 28% (w/v) polyethylene glycol (MW 8000), 100 mM imidazole pH 7.2 and 5 mM YbCl3. Capillaries were closed and equilibrated at room temperature. Plate-like crystals appear within one to three weeks. The space group of these crystals is $P2_12_12$ with cell dimensions $a = 67$ Å, $b = 133$ Å, and $c = 84$ Å. The inhibitors were introduced to the crystals by using soaking experiments. The synthesized compounds were dissolved in 100% DMSO to make stock solutions with a concentration of 10 mM or 20 mM. The crystals were soaked overnight in 1 mM inhibitor, 10 or 5% DMSO, 50 mM imidazole, pH 7.2 and 2.5 mM YbCl3. The soaking solution was refreshed three times.

Data for the several inhibitor-LTA_4h complexes were collected at stations I711 or I911-2 at the MaxLab synchrotron, Lund Sweden. Prior to data collection crystals were briefly soaked in 1 mM inhibitor, 10 or 5% DMSO, 50 mM imidazole pH 7.2, 2.5 mM $YbCl_3$ and 25% glycerol and flash cooled in a cooled nitrogen gas-stream at 100 K. Data were collected on a 165 mm Mar-research CCD detector at 100 K. Data processing was done by using Mosflm[26] and subsequent scaling and merging of the data were performed by using programs from the CCP4 software package.[27] Coordinates were refined using Refmac[28] using the LTA4-h bestatin complex (PDB entry 1HS6) as a starting model with water molecules and inhibitor removed but not the coordinates for the co-crystallized Yb ions or a bound imidazole molecule. In each case about 2000 reflections were used for monitoring (R_{free}) during the refinement cycles. Dictionaries for the different inhibitors were created using the 'monomer library sketcher' in the CCP4 suite. Water

Table 8. Crystallographic data and refinement statistics

Compound	2	4b	4f	7a	8i
A (Å)	67.37	67.77	67.23	67.35	67.62
B (Å)	133.18	133.57	133.26	133.21	133.27
C (Å)	84.18	83.63	84.59	83.10	83.64
Resolution (Å)	1.8	2.1	2.09	2.2	2.55
Completeness %	99.1 (99.6)	99.8 (99.8)	99.7 (99.8)	99.6 (99.6)	99.9 (100)
R_{merge} (%)	5.8 (25.2)	5.1 (27.5)	8.2 (30.6)	7.1 (33.2)	6.9 (30.9)
$I/\sigma I$	10.4 (2.9)	11.0 (2.4)	13.1 (4.4)	6.9 (2.0)	
Multiplicity	3.9 (3.7)	4 (3.8)	3.6 (3.7)	4.1 (3.9)	
R_{factor} (%)	18.81	23.2	20.6	20.68	21.12
R_{free} (%)	22.32	27.8	24.0	26.69	24.75
Rsmd bonds (Å)	0.014	0.007	0.005	0.015	0.008
Rsmd angles (°)	1.68	0.80	0.80	1.41	1.05
No. of water molecules	541	430	344	360	68

molecules were added by using Arp/wArp.[29] Manual model building as well as interpretation of electron density maps was performed using the program XtalView (McRee, 1999).[30] Details on the processing and refinement of the data can be found in Table 8. The occupancies of the various inhibitors were judged by a comparison between the B-factors of the inhibitors and those of the surrounding protein atoms.

5.2. Chemistry

5.2.1. 2-Amino-*N*-[4-(phenylmethoxy)phenyl]-acetamide (2). To a solution of Boc-Gly (6.7 g, 29 mmol) in CH_2Cl_2 was added carbonyl diimidazole (4.6 g, 1 equiv). After stirring for 1 h, 4-benzyloxyaniline hydrochloride (5 g, 1 equiv) was added and the reaction was heated to reflux. After 16 h, the reaction was brought to room temperature, washed with K_2CO_3 (satd aq), dried and concentrated to give a brown solid. This crude product was dissolved in CH_2Cl_2 and TFA (10 equiv) was added. After stirring for 16 h, solvent was removed and the residue was partitioned between NaOH (1 M aq) and CH_2Cl_2. The layers were separated, the aqueous layers were extracted with 2× CH_2Cl_2, and the combined organic layers were dried and concentrated. The resulting brown solid was recrystallized from EtOAc/hexanes to provide **2** (2.1 g, 29%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.23 (s, 1 H), 7.49 (dt, J_1 = 9.2 Hz, J_2 = 2.4 Hz, 2H), 7.42–7.30 (m, 5H), 6.93 (dt, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 2H), 5.03 (s, 2H), 3.44 (s, 2H); Anal. ($C_{15}H_{16}N_2O_2$) C, H, N.

5.2.2. 2*S*-2-Amino-*N*-[4-(phenylmethoxy)phenyl]-propanamide hydrochloride (3a). The synthesis of **3a** was carried out in an analogous manner to **2** using Boc-Ala and deprotection with 4 M hydrogen chloride in dioxane: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.51 (s, 1H), 8.25 (br s, 2H), 7.51 (dt, J_1 = 9.1 Hz, J_2 = 3.3 Hz, 2H), 7.41 (dd, J_1 = 8.5 Hz, J_2 = 1.9 Hz, 2H), 7.36 (t, J = 7.0 Hz, 2H), 7.29 (tt, J_1 = 7.0 Hz, J_2 = 1.4 Hz, 1H), 6.97 (dt, J_1 = 8.8 Hz, J_2 = 3.7 Hz, 2H), 5.04 (s, 2H), 3.98 (q, J = 7.3 Hz, 1H), 1.42 (d, J = 6.9 Hz, 3H); Anal. ($C_{16}H_{18}N_2O_2$·1HCl) C, H, N, Cl.

5.2.3. 2*S*,3*S*-2-Amino-3-methyl-*N*-[4-(phenylmethoxy)-phenyl]pentanamide (3b). The synthesis of **3b** was carried out in an analogous manner to **2** using Boc-Ile: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.50 (dt, J_1 = 9.1 Hz, J_2 = 3.7 Hz, 2 H), 7.40 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 2H), 7.35 (t, J = 6.9 Hz, 2H), 7.29 (tt, J_1 = 6.9 Hz, J_2 = 2.9 Hz, 1 H), 6.92 (dt, J_1 = 9.2 Hz, J_2 = 3.3 Hz, 2 H), 5.03 (s, 2H), 3.06 (d, J = 5.8 Hz, 1H), 1.75 (br s, 1H), 1.63–1.58 (m, 1H), 1.49–1.41 (m, 1H), 1.12–1.06 (m, 1H), 0.84 (d, J = 6.9 Hz, 3H), 0.80 (t, J = 7.3 Hz, 3H); Anal. ($C_{19}H_{24}N_2O_2$) C, H, N.

5.2.4. 2*S*-2-Amino-*N*-[4-(phenylmethoxy)phenyl]benzene-propanamide hydrochloride (3c). The synthesis of **3c** was carried out in an analogous manner to **2** using Boc-Phe and deprotection with 4 M hydrogen chloride in dioxane: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.41 (s, 1H), 8.01 (br s, 2 H), 7.41 (dt, J_1 = 8.8 Hz, J_2 = 3.7 Hz, 4H), 7.36 (tt, J_1 = 7.0 Hz, J_2 = 1.8 Hz, 2H), 7.32–7.19 (m, 6H), 6.95 (dt, J_1 = 9.1 Hz, J_2 = 2.2 Hz, 2H), 5.03 (s, 2H), 4.09 (t, J = 7.3 Hz, 1H), 3.12 (dd, J_1 = 13.6 Hz, J_2 = 6.2 Hz, 1H), 3.04 (dd, J_1 = 13.5 Hz, J_2 = 7.3 Hz, 1H); Anal. ($C_{22}H_{22}N_2O_2$·0.4 H_2O·1HCl) C, H, N, Cl.

5.2.5. 2*S*-*N*-[4-(Phenylmethoxy)phenyl]-2-pyrrolidinecarb-oxamide hydrochloride (3d). The synthesis of **3d** was carried out in an analogous manner to **2** using Boc-Pro and deprotection with 4 M hydrogen chloride in dioxane: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1H), 7.50 (dt, J_1 = 9.1 Hz, J_2 = 3.3 Hz, 2H), 7.41 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 2H), 7.36 (t, J = 7.0 Hz, 2H), 7.29 (tt, J_1 = 7.0 Hz, J_2 = 2.9 Hz, 1H), 6.98 (dt, J_1 = 9.1 Hz, J_2 = 3.3 Hz, 2H), 5.05 (s, 2H), 4.29 (t, J = 7.0 Hz, 1H), 3.26–3.17 (m, 1H), 2.39–2.32 (m, 1H), 1.94–1.86 (m, 4H); Anal. ($C_{18}H_{20}N_2O_2$·1HCl) C, H, N, Cl.

5.2.6. N^1-[4-(Phenylmethoxy)phenyl]-L-glutamamide hydrochloride (3e). The synthesis of **3e** was carried out in an analogous manner to **2** using Boc-Gln and deprotection with 4 M hydrogen chloride in dioxane: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1H), 8.36 (br s, 2H), 7.52 (dt, J_1 = 9.2 Hz, J_2 = 3.3 Hz, 2 H), 7.46 (br s, 1H), 7.42 (d, J = 6.6 Hz, 2H), 7.36 (t, J = 7.3 Hz, 2H), 7.30 (tt, J_1 = 7.4 Hz, J_2 = 2.6 Hz, 1H), 6.98 (dt, J_1 = 9.1 Hz, J_2 = 3.3 Hz, 2H), 6.91, (br s, 1H), 5.06 (s, 2H), 3.96 (br s, 1H), 2.21 (t, J = 7.7 Hz, 2H), 2.06–1.96 (m, 2H); Anal. ($C_{18}H_{21}N_3O_3$·0.9H_2O·1.5HCl) C, H, N, Cl.

5.2.7. 2*S*-2-Amino-*N*-[4-(phenylmethoxy)phenyl]-3-[4-(1H-imidazole)]propanamide dihydrochloride (3f). The synthesis of **3f** was carried out in an analogous manner to **2**

using Boc-His and deprotection with 4 M hydrogen chloride in dioxane: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.81 (s, 1H), 8.99 (s, 1H), 8.55 (br s, 2H), 7.49 (d, J = 8.8 Hz, 2H), 7.41 (dt, J_1 = 6.8 Hz, J_2 = 1.6 Hz, 2H), 7.37 (tt, J_1 = 7.2 Hz, J_2 = 1.6 Hz, 2H), 7.30 (tt, J_1 = 7.2 Hz, J_2 = 2.8 Hz, 1H), 6.98 (dt, J_1 = 9.2 Hz, J_2 = 2.4 Hz, 2H), 5.05 (s, 2H), 4.37 (br s, 1H), 3.32–3.20 (m, 2H); Anal. ($C_{19}H_{20}N_4O_2\cdot0.4H_2O\cdot2HCl$) C, H, N, Cl.

5.2.8. 2*S*-2,6-Diamino-*N*-[4-(phenylmethoxy)phenyl]hexanamide dihydrochloride (3g). To a solution of Diboc-Lys (1.7 g, 5 mmol) and *N*-methylmorpholine (1.1 mL, 2 equiv) in THF (10 mL) at −20 °C was added isobutyl chloroformate (0.65 mL, 1 equiv). After stirring at this temperature for 30 min, 4-benzyloxyaniline (1 g, 1 equiv) was added and the turbid reaction was allowed to warm to room temperature over 16 h. The reaction was filtered and concentrated. The residue was dissolved in EtOAc, washed with $NaHSO_4$ (satd aq), $NaHCO_3$ (satd aq) and brine, dried and concentrated. The crude coupled product was dissolved in CH_2Cl_2 and HCl (4 M in dioxane, 10 equiv) was added. After stirring for 24 h, solvent was removed. The resulting pink solid was washed with EtOAc/MeOH (4:1) to provide pure **3g** (10%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.76 (s, 1H), 8.36 (br s, 2H), 7.92 (br s, 2H), 7.55 (d, d, J = 8.8 Hz, 2H), 7.41 (d, J = 6.8 Hz, 2H), 7.37 (tt, J_1 = 6.8 Hz, J_2 = 1.2 Hz, 2H), 7.30 (tt, J_1 = 7.2 Hz, J_2 = 1.6 Hz, 1H), 6.98 (d, J = 9.2 Hz, 2H), 5.05 (s, 2H), 3.97 (br s, 1H), 2.74 (br s, 2H), 1.87–1.75 (m, 2H), 1.60–1.53 (m, 2H), 1.43–1.35 (m, 2H); Anal. ($C_{19}H_{25}N_3O_2\cdot0.6$ EtOAc·2HCl) C, H, N, Cl.

5.2.9. N^1-[4-(Phenylmethoxy)phenyl]-L-glutamine trifluoroacetate (4a). To a solution of Boc-Glu(OBn)-OH (1.7 g, 5 mmol) and *N*-methylmorpholine (1.1 mL, 2 equiv) in THF (10 mL) at −20 °C was added isobutyl chloroformate (0.65 mL, 1 equiv). After stirring at this temperature for 30 min, 4-benzyloxyaniline (1 g, 1 equiv) was added and the turbid reaction was allowed to warm to room temperature over 16 h. The reaction was filtered and concentrated. The residue was dissolved in EtOAc, washed with $NaHSO_4$ (satd aq), $NaHCO_3$ (satd aq) and brine, dried and concentrated. The coupled product was dissolved in MeOH and NaOH (1 M, aq) was added. After stirring for 24 h, the solvent was removed and the residue was partitioned between CH_2Cl_2 and $NaHSO_4$ (satd aq) and the layers were separated. The aqueous layers were extracted with 2× CH_2Cl_2 and the combined organic layers were dried ($MgSO_4$) and concentrated to give the free carboxylic acid. This acid was dissolved in CH_2Cl_2 and TFA (10 equiv) was added. After stirring for 4 days, solvent was removed. The resulting residue was purified by reverse phase HPLC using a gradient of MeCN in water to provide pure **4a** (13%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.66 (d, J = 7,6 Hz, 2H), 6.62–6.58 (m, 2H), 6.54 (t, J = 7.6 Hz, 2H), 6.50–6.46 (m, 1H), 6.16 (d, J = 8.0 Hz, 2H), 4.26 (s, 2H), 3.19 (t, J = 6.4 Hz, 1H), 1.71 (t, J = 7.2 Hz, 2H), 1.40(hp, J = 7.2 Hz, 2H); Anal. ($C_{18}H_{20}N_2O_4\cdot0.22H_2O\cdot1.1CF_3CO_2H$) C, H, N, F.

5.2.10. N^1-[4-(Phenylmethoxy)phenyl]-L-aspartamine trifluoroacetate (4b). Synthesis of **4b** was carried out in an analogous manner to **4a** using Boc-Asp(OMe)-OH: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (s, 1H), 8.21 (br s, 2H), 7.45 (d, J = 9.2 Hz, 2H), 7.42–7.34 (m, 4H), 7.30 (t, J = 6.8 Hz, 1H), 6.95 (d, J = 9.2 Hz, 2H), 5.04 (s, 2H), 4.24 (br s, 1H), 2.96–2.82 (m, 2H); Anal. ($C_{17}H_{18}N_2O_4\cdot1.05CF_3CO_2H$) C, H, N, F.

5.2.11. N^1-[4-(Phenylmethoxy)phenyl]-D-glutamine trifluoroacetate (4c). Synthesis of **4c** was carried out in an analogous manner to **4a** using D-Boc-Glu(OBn)-OH: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.31 (s, 1H), 8.20 (br s, 2H), 7.47 (d, J = 8.8 Hz, 2H), 7.43 (t, J = 7.2 Hz, 2H), 7.37 (t, J = 8.0 Hz, 2H), 7.31 (t, J = 6.8 Hz, 1H), 6.99 (d, J = 8.8 Hz, 2H), 5.06 (s, 2H), 3.89 (br s, 1H), 2.34 (t, J = 6.4 Hz, 2H), 2.05–2.00 (m, 2H); Anal. ($C_{18}H_{20}N_2O_4\cdot0.5H_2O\cdot1.2CF_3CO_2H$) C, H, N, F.

5.2.12. N^1-[4-(Phenylmethoxy)phenyl]-D-aspartamine (4d). Synthesis of **4d** was carried out in an analogous manner to **4a** using Boc-Asp(OBn)-OH: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.04 (s, 1H), 8.19 (br s, 2H), 7.48–7.36 (m, 4H), 7.28 (t, J = 7.6 Hz, 2H), 7.23 (t, J = 8.4 Hz, 1H), 6.93 (d, J = 7.6 Hz, 2H), 5.02 (s, 2H), 4.24–4.20 (m, 1H), 2.93–2.88 (m, 2H); Anal. ($C_{17}H_{18}N_2O_4\cdot0.2CF_3CO_2H$) C, H, N, F.

5.2.13. N^4-[4-(Phenylmethoxy)phenyl]-L-aspartamine trifluoroacetate (4e). Synthesis of **4e** was carried out in an analogous manner to **4a** using Boc-Asp(OH)-OBn: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (s, 1H), 8.22 (br s, 2H), 7.45 (d, J = 8.8 Hz, 2H), 7.40 (t, J = 6.8 Hz, 2H), 7.36 (t, J = 7.2 Hz, 2H), 7.30 (t, J = 6.8 Hz, 1H), 6.95 (d, J = 8.8 Hz, 2H), 5.04 (s, 2H), 4.24 (br s, 1H), 2.96–2.85 (m, 2H); Anal. ($C_{17}H_{18}N_2O_4\cdot1.15CF_3CO_2H$) C, H, N, F.

5.2.14. N^5-[4-(Phenylmethoxy)phenyl]-L-glutamine hemitrifluoroacetate (4f)

5.2.14.1. Step 1: Preparation of N^2-[(1,1-dimethylethoxy)carbonyl]-N^5-[4-(phenylmethoxy)phenyl]-L-glutamine (14). To a solution of Boc-Glu(OH)-OBn (3.5 g, 10 mmol) and *N*-methylmorpholine (2.2 mL, 2 equiv) in THF (20 mL) at −20 °C was added isobutyl chloroformate (1.2 mL, 1 equiv). After stirring at this temperature for 30 min, 4-benzyloxyaniline (2 g, 1 equiv) was added and the turbid reaction was allowed to warm to room temperature for over 16 h. The reaction was filtered and concentrated. The residue was dissolved in EtOAc, washed with $NaHSO_4$ (satd aq), $NaHCO_3$ (satd aq) and brine, dried, and concentrated. The coupled product was dissolved in MeOH and NaOH (1 M, aq) was added. After stirring for 24 h, the solvent was removed and the residue was partitioned between CH_2Cl_2 and $NaHSO_4$ (satd aq) and the layers were separated. The aqueous layers were extracted with 2× CH_2Cl_2 and the combined organic layers were dried ($MgSO_4$) and concentrated. The residue was recrystallized from EtOAc/hexanes to provide compound **14** (56%) as a pale pink solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.73 (s, 1H), 7.45 (d, J = 9.1 Hz, 2H), 7.41 (d, J = 6.6 Hz, 2H), 7.36 (t, J = 7.0 Hz, 2H), 7.29 (tt, J_1 = 6.9 Hz,

J_2 = 2.5 Hz, 1H), 7.08 (d, J = 8.1 Hz, 1H), 6.91 (dt, J_1 = 9.1 Hz, J_2 = 2.2 Hz, 2H), 5.03 (s, 2H), 3.92–3.86 (m, 1H), 2.33 (t, J = 7.6 Hz, 2H), 2.03–1.95 (m, 1H), 1.82–1.74 (m, 1H), 1.35 (s, 9H).

5.2.14.2. Step 2: Preparation of N^5-[4-(phenylmethoxy)phenyl]-L-glutamine hemitrifluoroacetate (4f). Compound **14** was slurried in CH_2Cl_2 and TFA (10 equiv) was added to give a clear solution. After stirring for 16 h, solvent was removed. The resulting oil was triturated with EtOAc/hexanes and washed with MeOH to provide pure **4f** (69%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.82 (s, 1H), 8.22 (d, J = 4.4 Hz, 2H), 7.44 (dt, J_1 = 9.2 Hz, J_2 = 2.0 Hz, 2H), 7.37 (d, J = 6.8 Hz, 2H), 7.31 (t, J = 6.8 Hz, 2H), 7.24 (t, J = 7.2 Hz, 1H), 6.88 (d, J = 8.8 Hz, 2H), 5.00 (s, 2H), 3.96–3.90 (m, 1H), 2.54–2.39 (m, 2H), 2.06 (hp, J = 7.6 Hz, 2H); Anal. ($C_{18}H_{20}N_2O_4 \cdot 1H_2O \cdot 0.5CF_3CO_2H$) C, H, N, F.

5.2.15. N^5-[4-(Phenylmethoxy)phenyl]-D-glutamine hydrochloride (4g). Synthesis of **4g** was carried out in an analogous manner to **4a** using D-Boc-Glu(OH)-OBn and deprotection with 4 M hydrogen chloride in dioxane: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.95 (s, 1H), 8.33 (s, 2H), 7.46 (dt, J_1 = 9.2 Hz, J_2 = 2.0 Hz, 2H), 7.42–7.4 (m, 2H), 7.38–7.34 (m, 2H), 7.29 (tt, J_1 = 7.6 Hz, J_2 = 1.2 Hz, 1H), 6.92 (dt, J_1 = 8.8 Hz, J_2 = 3.6 Hz, 2H), 5.03 (s, 2H), 3.91 (t, J = 6.4 Hz, 1H), 2.56–2.39 (m, 2H), 2.11–1.96 (m, 2H); Anal. ($C_{18}H_{20}N_2O_4 \cdot 0.5H_2O \cdot 0.9HCl$) C, H, N, Cl.

5.2.16. 5-Oxo-5-[[4-(phenylmethoxy)phenyl]amino]pentanoic acid (5). Synthesis of **5** was carried out in an analogous manner to **14** using monomethyl glutaric acid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (s, 1H), 7.45 (d, J = 9.2 Hz, 2H), 7.41 (d, J = 6.8 Hz, 2H), 7.36 (tt, J_1 = 6.8 Hz, J_2 = 1.2 Hz, 2H), 7.30 (tt, J_1 = 7.2 Hz, J_2 = 1.6 Hz, 1H), 6.91 (d, J = 9.2 Hz, 2H), 2.28 (t, J = 7.2 Hz, 2H), 2.24 (t, J = 7.6 Hz, 2H), 1.76 (tt, J_1 = 8.0 Hz, J_2 = 7.2 Hz, 2H); Anal. ($C_{18}H_{19}NO_4 \cdot 0.1H_2O$) C, H, N.

5.2.17. N^5-[4-(Phenylmethoxy)phenyl]-L-glutamine methyl ester hydrochloride (6a)

5.2.17.1. Step 1: Synthesis of N^2-[(1,1-dimethylethoxy)carbonyl]-N^5-[4-(phenylmethoxy)phenyl]-L-glutamine methyl ester (17). To a solution of Boc-Glu(OH)-OMe (1.7 g, 5 mmol) and *N*-methylmorpholine (1.1 mL, 2 equiv) in THF (20 mL) at −20 °C was added isobutyl chloroformate (0.65 mL, 1 equiv). After stirring at this temperature for 30 min, 4-benzyloxyaniline (1 g, 1 equiv) was added and the turbid reaction was allowed to warm to room temperature for over 16 h. The reaction was filtered and concentrated. The residue was dissolved in EtOAc, washed with $NaHSO_4$ (satd aq), $NaHCO_3$ (satd aq) and brine, dried and concentrated. The resulting solid was recrystallized from EtOAc/hexanes to provide compound **17** (54%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) 9.73 (s, 1H), 7.45 (d, J = 9.2 Hz, 2H), 7.41 (d, J = 7.0 Hz, 2H), 7.36 (t, J = 7.3 Hz, 2H), 7.31–7.26 (m, 1H), 6.91 (dt, J_1 = 9.2 Hz, J_2 = 3.3 Hz, 2H), 5.03 (s, 2H), 4.00–3.95 (m, 1H), 3.60 (s, 3H), 2.33 (t, J = 7.3 Hz, 2H), 2.03–1.96 (m, 1H), 1.83–1.75 (m, 1H), 1.35 (s, 9H).

5.2.17.2. Step 2: Synthesis of N^5-[4-(phenylmethoxy)phenyl]-L-glutamine methyl ester hydrochloride (6a). Compound **17** was slurried in CH_2Cl_2 and HCl (4 M in dioxane, 10 equiv) was added to give a clear solution. After stirring for 16 h, solvent was removed. The resulting solid was washed with EtOAc/MeOH (4:1) to provide pure **6a** (59%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.95 (s, 1H), 8.51 (s, 2H), 7.47 (dt, J_1 = 9.2 Hz, J_2 = 2 Hz, 2H), 7.42–7.34 (m, 4H), 7.29 (tt, J_1 = 7.6 Hz, J_2 = 1.2 Hz, 1H), 6.92 (dt, J_1 = 6.8 Hz, J_2 = 2 Hz, 2H), 5.03 (s, 2H), 4.05 (t, J = 6.4 Hz, 1H), 3.71 (s, 3H), 2.55–2.39 (m, 2H), 2.12–2.01 (m, 2H); Anal. ($C_{19}H_{22}N_2O_4 \cdot 1HCl$) C, H, N, Cl.

5.2.18. N^5-[4-(Phenylmethoxy)phenyl]-L-glutamine 1,1-dimethylethyl ester (6b). Synthesis of **6b** was carried out in an analogous manner to **3g** using Boc-Glu(OH)-OtBu: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.44–7.30 (m, 6H), 7.25 (d, J = 4.4 Hz, 1H), 6.91–6.87 (m, 2H), 5.01 (d, J = 3.2 Hz, 2H), 3.52–3.49 (m, 1H), 2.58- 2.48 (m, 2H), 1.93–1.89 (m, 2H), 1.44 (d, J = 3.6 Hz, 9H); Anal. ($C_{22}H_{28}N_2O_4$) C, H, N.

5.2.19. N^5-[4-(Phenylmethoxy)phenyl]-L-glutamamide trifluoroacetate (6c). To a solution of **14** (0.3 g) in acetonitrile (10 mL) were added Boc anhydride (0.18 g, 1.2 equiv), pyridine (0.05 mL, 1 equiv), and $(NH_4)_2CO_3$ (0.2 g, 3 equiv). After stirring for 3 h, the reaction was partitioned between EtOAc and water and the layers were separated. The aqueous layer was extracted with 2× EtOAc and the combined organic layers were washed with HCl (1N, aq), water, and brine, then dried ($MgSO_4$), and concentrated. This product was dissolved in CH_2Cl_2 and TFA (10 equiv) was added. After stirring for 2 h, solvent was removed. The resulting white solid was recrystallized from MeOH/Et_2O to provide **6c** (0.2g, 62%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1H), 8.06 (s, 2H), 7.85 (s, 1H), 7.62 (s, 1H), 7.45 (dt, J_1 = 8.8 Hz, J_2 = 2.0 Hz, 2H), 7.40 (dt, J_1= 6.4 Hz, J_2 = 2.0 Hz, 2H), 7.38–7.34 (m, 2H), 7.29 (tt, J_1 = 7.6 Hz, J_2 = 1.2 Hz, 1H), 6.92 (dt, J_1 = 8.8 Hz, J_2 = 2.0 Hz, 2H), 5.03 (s, 2H), 3.75 (t, J = 6.4 Hz, 1H), 2.24–2.30 (m, 2H), 2.03–1.97 (m, 2H); Anal. ($C_{18}H_{21}N_3O_3 \cdot 0.5H_2O \cdot 1.1CF_3CO_2H$) C, H, N, F.

5.2.20. N^1-Hydroxy-N^5-[4-(phenylmethoxy)phenyl]-L-glutamamide trifluoroacetate (6d). To a solution of **17** (1 g) in MeOH (25 mL) was added hydroxylamine (50% in water, 4 mL, 20 equiv) over four days of stirring. Solvent was removed, and the resulting white solid was washed with EtOAc, water, and $NaHSO_4$ (satd aq) and dried to provide the desired product as a mixture with 4-benzyloxyaniline sulfate. This mixture was slurried in CH_2Cl_2 and TFA (10 equiv) was added. After stirring for 4 h, the solid was filtered off and the mother liquor was concentrated. The resulting white solid was purified by reverse phase HPLC using a gradient of MeCN in water to provide **6d** (1%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 7.44–7.40 (m, 4H),

7.34 (t, J = 7.2 Hz, 2H), 7.30–7.27 (m, 1H), 6.93 (d, J = 7.6 Hz, 2H), 5.04 (s, 2H), 3.84 (t, J = 5.2 Hz, 1H), 2.63–2.49 (m, 2H), 2.25–2.13 (m, 2H); Anal. ($C_{18}H_{21}N_3O_4 \cdot 1.1H_2O \cdot 1CF_3CO_2H$) C, H, N, F.

5.2.21. 4-Amino-*N*-[4-(phenylmethoxy)phenyl]butanamide hydrochloride (7a). Synthesis of **7a** was carried out in an analogous manner to **3g** using *N*-Boc-4-aminobutanoic acid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.00 (s, 1H), 7.98 (s, 2H), 7.49 (dt, J_1 = 9.2 Hz, J_2 = 2 Hz, 2H), 7.42–7.33 (m, 4H), 7.29 (tt, J_1 = 7.2 Hz, J_2 = 1.6 Hz, 1H), 6.91 (dt, J_1 = 9.2 Hz, J_2 = 2.4 Hz, 2H), 5.02 (s, 2H), 2.79 (t, J = 7.6 Hz, 2H), 2.38 (t, J = 7.6 Hz, 2H), 1.87–1.79 (m, 2H); Anal. ($C_{17}H_{20}N_2O_2 \cdot 1HCl$) C, H, N, Cl.

5.2.22. 4-Methylamino-*N*-[4-(phenylmethoxy)phenyl]butanamide trifluoroacetate (7b). Synthesis of **7b** was carried out in an analogous manner to **3g** using *N*-Boc-*N*-methyl-4-aminobutanoic acid with purification by preparative HPLC: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.84 (s, 1H), 8.38 (s, 2H), 7.46 (d, J = 7.6 Hz, 2H), 7.40 (d, J = 7.2 Hz, 2H), 7.36 (tt, J_1 = 6.0 Hz, J_2 = 1.6 Hz, 2H), 7.30 (tt, J_1 = 5.6 Hz, J_2 = 2.4 Hz, 1H), 6.92 (dt, J_1 = 6.4 Hz, J_2 = 2.8 Hz, 2H), 5.03 (s, 2H), 2.90 (t, J = 6.4 Hz, 2H), 2.54 (d, J = 3.2 Hz, 3H), 2.36 (t, J = 5.6 Hz, 2H), 1.87–1.79 (m, 2H); Anal. ($C_{18}H_{22}N_2O_2 \cdot 1CF_3CO_2H$) C, H, N, F.

5.2.23. 4-Dimethylamino-*N*-[4-(phenylmethoxy)phenyl]-butanamide trifluoroacetate (7c). Synthesis of **7c** was carried out in an analogous manner to **17** using *N*,*N*-dimethyl-4-aminobutanoic acid hydrochloride and purification by preparative HPLC: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.87 (s, 1H), 9.50 (br s, 1H), 7.46 (d, J = 5.2 Hz, 2H), 7.40 (d, J = 6.4 Hz, 2H), 7.36 (tt, J_1 = 6.0 Hz, J_2 = 1.6 Hz, 2H), 7.30 (t, 5.6 Hz, 1H), 6.93 (d, J = 5.6 Hz, 2H), 5.03 (s, 2H), 3.08–3.02 (m, 2H), 2.76 (s, 6H), 2.38–2.32 (m, 2H), 1.93–1.85 (m, 2H); Anal. ($C_{19}H_{24}N_2O_2 \cdot 0.2H_2O \cdot 0.1CH_2Cl_2 \cdot 1.4CF_3CO_2H$) C, H, N, F.

5.2.24. N^5-phenyl-L-glutamamide dihydrochloride (8a). To a solution of Boc-Gln-OH (246 mg, 1 mmol) and HATU (456 mg, 1.2 mmol) in DMF (10 mL) was added Et3N (152 mg, 1.5 mmol) at rt. After 30 min, aniline (93 mg, 1 equiv) was added. The reaction was stirred at rt overnight, and quenched with water. The reaction mixture was extracted with EtOAc, washed with brine, dried and concentrated. To a solution of this residue in CH_2Cl_2 was added HCl (4 M in dioxane, 10 equiv). After stirring for 16 h, the resulting white solid was filtered off, washing with water, to provide **8a** (87%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.18 (s, 1H), 8.29 (br s, 3H), 7.99 (s, 1H), 7.60–7.56 (m, 3H), 7.26 (t, J = 7.3 Hz, 2H), 7.00 (t, J = 7.7 Hz, 1H), 3.77 (br s, 1H), 2.51–2.40 (m, 2H), 2.10–1.99 (m, 2H); Anal. ($C_{11}H_{15}N_3O_2 \cdot 0.07\ H_2O \cdot 2.1HCl$) C, H, N.

5.2.25. N^5-[4-(1H-pyrrol-1-yl)phenyl]-L-glutamamide hydrochloride (8b). Synthesis of **8b** was carried out in an analogous manner to **8a** using 4-(1H-pyrrol-1-yl)-aniline: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.17 (s, 1H), 8.17 (br s, 3H), 7.92 (br s, 1H), 7.67–7.63 (m, 3H), 7.48 (d, J = 8.8 Hz, 2H), 7.27 (t, J = 2.1 Hz, 2H), 6.21 (t, J = 2.2 Hz, 1H), 3.77 (br s, 1H), 2.46–2.38 (m, 2H), 2.07–2.01 (m, 2H); Anal. ($C_{15}H_{18}N_4O_2 \cdot 1.45HCl$) C, N, H.

5.2.26. N^5-[3-(phenylmethoxy)phenyl]-L-glutamine, methyl ester (8c). Synthesis of **8c** was carried out in an analogous manner to **3g** using Boc-Glu(OH)-OMe and 3-benzyloxyaniline: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.43–7.39 (m, 4H), 7.37 (t, J = 8.0 Hz, 2H), 7.31 (tt, J_1 = 7.2 Hz, J_2 = 2.8 Hz, 1H), 7.18 (t, J = 8.0 Hz, 1H), 6.99 (d, J = 8.0 Hz, 1H), 6.70 (dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 1H), 5.29 (s, 2H), 3.71 (s, 3H), 3.57 (dd, J_1 = 8.8 Hz, J_2 = 4.8 Hz, 1H), 2.61–2.45 (m, 2H), 2.27–2.19 (m, 1H), 1.96–1.87 (m, 1H); Anal. ($C_{19}H_{22}N_2O_4 \cdot 0.8H_2O$) C, H, N.

5.2.27. N^5-[(4-Phenoxy)-3-pyridyl]-L-glutamamide tristrifluoroacetate (8d)

5.2.27.1. Step 1: Synthesis of 2-Phenyloxy-5-nitropyridine (18). To a solution of phenol (2 g, 21.2 mmol) in DMSO (20 mL) was added KOtBu (2.4 g, 21.2 mmol) at rt. After 30 min, 2-chloro-5-nitropyridine (2.1 g, 19.3 mmol) was added. The reaction was heated to 120 °C, then after 2 h of stirring the heat was removed. The reaction mixture was poured into ice water and the resulting solid was collected by filtration. This solid was purified by column chromatography using a gradient of MeOH in CH_2Cl_2 to afford **18** (96%) as a yellow solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (1H, s), 8.45 (d, J = 3.2 Hz, 1H), 7.54 (m, 2H), 7.36 (m, 1H), 7.18 (m, 2H), 7.06 (d, J = 8.8 Hz, 1H).

5.2.27.2. Step 2: Synthesis of N^5-[(4-phenoxy)-3-pyridyl]-L-glutamamide (8d). To a solution of $SnCl_2$ H_2O (16.7 g, 74 mmol) in EtOAc (250 mL) was added **19** (4 g, 8.1 mmol) in EtOAc (10 mL). The reaction mixture was kept at reflux for 3 h, and cooled. The reaction mixture was quenched with 50% NaOH to pH 12, and resulting solid was filtered off. The solution phase was extracted with ether, dried, and concentrated to provide the aminopyridine. To a solution of Boc-Gln (505 mg, 3 mmol) and HOBt (460 mg, 3 mmol) in DMF (10 mL) was added EDC (593 mg, 3 mmol) at rt. After 30 min, the aminopyridine (465.2 mg, 2.5 mmol) was added. The reaction mixture was stirred at rt overnight, and quenched with water. The reaction mixture was extracted with EtOAc, washed with brine, dried, and concentrated. The residue was slurried in CH_2Cl_2 and TFA (10 equiv) was added to give a clear solution. After stirring for 16 h, solvent was removed. The resulting solid was purified by reverse phase HPLC using a gradient of MeCN in water to provide pure **8d** (33%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.23 (s, 1H), 8.32 (d, J = 2.7 Hz, 1H), 8.17 (br s, 3H), 8.03 (dd, J_1 = 9.2 Hz, J_2 = 2.9 Hz, 1H), 7.91 (s, 1H), 7.62 (s, 1H), 7.37 (t, J = 7.4 Hz, 2H), 7.15 (t, J = 7.4 Hz, 1H), 7.05 (d, J = 7.7 Hz, 2H), 6.98 (d, J = 9.1 Hz, 1H), 3.79 (q, J = 5.5 Hz, 1H), 2.50–2.36 (m, 2H), 2.07–1.98 (m, 2H); Anal. ($C_{16}H_{18}N_4O_3 \cdot 2.65CF_3CO_2H$) C, H, N.

5.2.28. N^5-(4-Phenoxyphenyl)-L-glutamine (8e)

5.2.28.1. Step 1: N^2-[(1,1-dimethylethoxy)carbonyl]-N^5-(4-phenoxyphenyl)-L-glutamine (19). Synthesis of **19** was carried out in an analogous manner to **14** using Boc-Glu(OH)-OMe and 4-phenoxyaniline: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.90 (s, 1H), 7.56 (d,

J = 8.8 Hz, 2H), 7.32 (tt, J_1 = 7.3 Hz, J_2 = 2.0 Hz, 2H), 7.05 (tt, J_1 = 7.3 Hz, J_2 = 1.1 Hz, 1H), 6.92 (t, J = 8.9 Hz, 4H), 3.91–3.86 (m, 1H), 2.35 (t, 7.5 Hz, 2H), 2.06–1.96 (m, 1H), 1.83–1.72 (m, 1H), 1.34 (s, 9H).

5.2.28.2. Step 2: N^5-(4-Phenoxyphenyl)-L-glutamine trifluoroacetate (8e). Compound **19** was dissolved in CH_2Cl_2 and TFA (10 equiv) was added. After stirring for 16 h the reaction was concentrated. The residue was purified by reverse phase HPLC using a gradient of MeCN in water to provide **8e** (29%) as a white solid: ^{1}H NMR (400 MHz, DMSO) δ 10.04 (s, 1H), 8.26 (s, 2H), 7.57 (dt, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 2H), 7.36–7.31 (m, 2H), 7.09–7.05 (m, 1H), 6.98–6.90 (m, 2H), 3.95 (t, J = 6 Hz, 1H), 2.57–2.42 (m, 2H), 2.13–1.99 (m, 2H); Anal. ($C_{17}H_{18}N_2O_4 \cdot 1.05CF_3CO_2H$) C, H, N, F.

5.2.29. N^5-[4-Benzylphenyl]-L-glutamamide trifluoroacetate (8f). To a solution of Boc-Gln-OH (784 mg, 3 mmol) and HOBt (552 mg, 1.2 equiv) in DMF (10 mL) was added EDC (712 mg, 1.2 equiv) at rt. After 30 min, 4-benzyl aniline (550 mg, 1 equiv) was added. The reaction was stirred at rt overnight, and quenched with water. The reaction mixture was extracted with EtOAc, washed with 0.1N HCl, $NaHCO_3$ (satd aq), and brine. The organic layer was dried and concentrated. To a solution of this residue in CH_2Cl_2 was added TFA (10 equiv). After stirring for 16 h, reaction was concentrated. The residue was suspended in cold ether and filtered to provide **8f** (76%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 8.13 (br s, 3H), 7.87 (s, 1H), 7.61 (s, 1H), 7.47 (d, J = 8.4 Hz, 2H), 7.25 (t, J = 7.3 Hz, 2H), 7.18–7.11 (m, 5H), 3.85 (s, 2H), 3.76 (t, J = 6.2 Hz, 1H), 2.44–2.34 (m, 2H), 2.01 (q, J = 7.0 Hz, 2H); Anal. ($C_{18}H_{21}N_3O_2 \cdot 0.5H_2O \cdot 1.23CF_3CO_2H$) C, H, N.

5.2.30. N^5-[4-(N-Phenylamino)phenyl]-L-glutamine bistrifluoroacetate (8g). Synthesis of **8g** was carried out in an analogous manner to **8f** using N-phenyl-p-phenylenediamine: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.84 (s, 1H), 8.27 (br s, 3H), 7.47–7.39 (m, 2H), 7.18–7.12 (m, 2H), 7.01–6.93 (m, 5H), 6.76–6.69 (m, 1H), 3.95 (br s, 1H), 2.49–2.36 (m, 2H), 2.06–2.02 (m, 2H); Anal. ($C_{17}H_{19}N_3O_3 \cdot 1.9CF_3CO_2H$) C, H, N.

5.2.31. N^5-[4-(N-Methyl-N-phenylamino)phenyl]-L-glutamine methyl ester trifluoroacetate (8h). To a solution of Boc-Glu(OH)-OMe (260 mg, 1 mmol) and HOBt (200 mg, 1 equiv) in DMF (10 mL) was added EDC (210 mg, 1.1 equiv). After stirring for 30 min, N-phenyl-p-phenylenediamine (184 mg, 1 equiv) was added. The reaction was stirred for 16 h and then quenched with water. The reaction mixture was extracted with EtOAc, washed with 0.1 N HCl, $NaHCO_3$ (satd aq), and brine. The organic layer was dried and concentrated. To a solution of the coupled product in CH_2Cl_2/MeOH/AcOH (10 mL, 5:1:0.2) were added formaldehyde (40% in water, 10 equiv) and $NaBH_3CN$ (4 equiv). The cloudy reaction was stirred for 1 h, then diluted with EtOAc, and washed with brine. The organic layer was dried and concentrated. To a solution of this residue in CH_2Cl_2 was added HCl (4 M in dioxane, 10 equiv). After stirring for 16 h, reaction was concentrated. The resulting solid was purified by reverse phase HPLC using a gradient of MeCN in water to provide **8h** (40%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.94 (s, 1H), 8.38 (br s, 3H), 7.50 (d, J = 8.8 Hz, 2H), 7.19 (t, J = 8.8 Hz, 2H), 7.00 (d, J = 8.8 Hz, 2H), 6.84 (d, J = 7.7 Hz, 2H), 6.81 (t, J = 7.3 Hz, 1H), 4.10 (br s, 1H), 3.74 (s, 3H), 3.19 (s, 3H), 2.53–2.40 (m, 2H), 2.10–2.03 (m, 2H); Anal. ($C_{19}H_{23}N_3O_3 \cdot 1.35CF_3CO_2H$) C, N, H.

5.2.32. N^5-[4-(2-Phenylethoxy)phenyl]-L-glutamine (8i)

5.2.32.1. Step 1: Synthesis of N^2-[(1,1-dimethylethoxy) carbonyl]-N^5-(4-hydroxyphenyl)-L-glutamine, 1,1-dimethylethyl ester (20). To a solution of Boc-Glu(OH)-OtBu (1.7 g, 5 mmol) and N-methylmorpholine (1.1 mL, 2 equiv) in THF (20 mL) at −20 °C was added isobutyl chloroformate (0.65 mL, 1 equiv). After stirring at this temperature for 30 min, 4-benzyloxyaniline (1 g, 1 equiv) was added and the turbid reaction was allowed to warm to room temperature over 16 h. The reaction was filtered and concentrated. The residue was dissolved in EtOAc, washed with $NaHSO_4$ (satd aq), $NaHCO_3$ (satd aq), and brine, dried and concentrated. The coupled product was dissolved in THF, 10% Pd/C (5 mol%) was added, and the reaction mixture stirred under 40 psi H_2 overnight. The reaction mixture was then filtered over Celite and concentrated. The resulting white solid was purified by silica gel chromatography using a gradient of EtOAc in hexanes to provide **20** (27%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (s, 1 H), 9.10 (s, 1H), 7.34 (d, J = 9.0 Hz, 2H), 7.15 (d, J = 6.4 Hz, 1H), 6.65 (d, J = 7.0 Hz, 2H), 3.85–3.76 (m, 1H), 2.34 (t, J = 8.1 Hz, 2H), 1.98–1.90 (m, 1H), 1.81–1.74 (m, 1H), 1.41 (s, 9H), 1.39 (s, 9H).

5.2.32.2. Step 2: Synthesis of N^5-[4-(2-phenylethoxy)phenyl]-L-glutamine (8i). To a solution of **20** (600 mg, 1.52 mmol) in DMF (10 mL) was added K_2CO_3 (525 mg, 4 equiv). After stirring for 30 min phenethyl bromide (310 mg, 1.67 mmol) was added. The reaction mixture was kept at rt overnight, and quenched with water. The reaction mixture was extracted with EtOAc, washed with brine, and dried and concentrated. The residue was dissolved in CH_2Cl_2 and HCl (4 M in dioxane, 10 equiv) was added. After stirring for 16 h, solvent was removed. The resulting solid was purified by reverse phase HPLC using a gradient of MeCN in water to provide pure **8i** (21%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.89 (s, 1H), 7.44 (d, J = 8.8 Hz, 2H), 7.38–7.30 (m, 5H), 6.85 (d, J = 8.5 Hz, 2H), 4.12 (t, J = 6.3 Hz, 2H), 3.79 (br s, 1H), 2.97 (t, J = 6.5 Hz, 2H), 2.38–2.33 (m, 2H), 2.06–1.95 (m, 2H); Anal. ($C_{19}H_{22}N_2O_4 \cdot 0.4CF_3CO_2H$) C, H, N.

5.2.33. N^5-[4-(3-Phenylpropoxy)phenyl]-L-glutamine (8j). Synthesis of **8j** was carried out in an analogous manner to **8i** using 1-phenyl-3-bromopropane: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.91 (s, 1H), 7.46 (d, J = 8.8 Hz, 2H), 7.28–7.13 (m, 5H), 6.82 (d, J = 8.7 Hz, 2H), 3.86 (t, J = 7.0 Hz, 2H), 3.76 (br s, 1H), 2.71 (t, J = 6.2 Hz, 2H), 2.30–2.21 (m, 2H), 2.04–1.95 (m, 4H); Anal. ($C_{20}H_{24}N_2O_4 \cdot 0.18CF_3CO_2H$) C, H, N.

5.2.34. N^5-[4-(2-Oxo-3-phenylpropoxy)phenyl]-L-glutamine trifluoroacetate (8k). Synthesis of **8k** was carried out in an analogous manner to **8i** using phenacetyl bromide: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.89 (s, 1H), 8.27 (br s, 3H), 7.93 (d, J = 7.2 Hz, 2H), 7.67 (t, J = 7.0 Hz, 1H), 7.55 (t, J = 7.2 Hz, 2H), 7.46 (d, J = 8.8 Hz, 2H), 6.88 (d, J = 9.1 Hz, 2H), 5.51 (s, 2H), 3.94 (br s, 1H), 2.54–2.41 (m, 2H), 2.09–2.00 (m, 2H); Anal. ($C_{19}H_{20}N_2O_5 \cdot 1.4CF_3CO_2H$) C, H, N.

5.2.35. N^5-[4-(2-Hydroxy-3-phenylpropoxy)phenyl]-L-glutamine trifluoroacetate (8l). To a solution of **20** (3 g, 7.6 mmol) in DMF (40 mL) was added K_2CO_3 (2.6 g, 2.5 equiv). After stirring for 30 min, phenacetyl bromide (1.7 g, 1.1 equiv) was added. The reaction mixture was kept at rt overnight, and quenched with water. The reaction mixture was extracted with EtOAc, washed with brine, and dried and concentrated. The residue was dissolved in MeOH, cooled to 0 °C, and $NaBH_4$ was added. After stirring for 30 min the reaction was quenched with brine, extracted with EtOAc, and the organic layer was dried and concentrated. The residue was dissolved in CH_2Cl_2 and HCl (4 M in dioxane, 10 equiv) was added. After stirring for 16 h, solvent was removed. The resulting solid was purified by reverse phase HPLC using a gradient of MeCN in water to provide pure **8l** (30%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1H), 8.33 (br s, 3H), 7.48–7.40 (m, 4H), 7.36 (t, J = 7.4 Hz, 2H), 7.26 (t, J = 7.1 Hz, 1H), 6.85 (d, J = 8.2 Hz, 2H), 4.86 (t, J = 2.6 Hz, 1H), 3.99–3.92 (m, 3H), 2.55–2.42 (m, 2H), 2.12–2.03 (m, 2H); Anal. ($C_{19}H_{22}N_2O_5 \cdot 0.6H_2O \cdot 1.3CF_3CO_2H$) C, H, N.

5.2.36. N^5-[4-Methoxyphenyl]-L-glutamine methyl ester trifluoroacetate (9a). To a solution of Boc-Glu(OH)-OMe (940 mg, 1.2 equiv) and HOBt (552 mg, 1.2 equiv) in DMF (10 mL) was added EDC (710 mg, 1.2 equiv) at rt. After 30 min, *p*-methoxyaniline (370 mg, 3 mmol) was added. The reaction was stirred at rt for 16 h, and then quenched with water. The reaction mixture was extracted with EtOAc, washed with 0.1 N HCl, $NaHCO_3$ (satd aq), and brine. The organic layer was dried and concentrated. To a solution of this residue in CH_2Cl_2 was added TFA (10 equiv). After stirring for 1 h, the reaction mixture was concentrated. The residue was purified by reverse phase HPLC using a gradient of MeCN in water to provide **9a** (92%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.85 (s, 1 H), 8.42 (br s, 3H), 7.46 (d, J = 9.2 Hz, 2H), 6.84 (d, J = 9.1 Hz, 2H), 4.08 (br s, 1H), 3.72 (s, 3H), 3.68 (s, 3H), 2.52–2.40 (m, 2H), 2.10–2.03 (m, 2H); Anal. ($C_{13}H_{18}N_2O_4 \cdot 1.32CF_3CO_2H$) C, H, N.

5.2.37. N^5-[4-(Cyclohexyloxy)phenyl]-L-glutamamide (9b). To a solution of cyclohexylamine (2.1 g, 21.2 mmol) and 4-fluoronitrobenzene (3 g, 1 equiv) in DMSO (30 mL) was added K_2CO_3 (5.8 g, 2 equiv). The reaction mixture was heated to 110 °C, then after 3 h of stirring the heat was removed. The reaction mixture was poured into ice water and the water was extracted with EtOAc. The organic layer was dried and concentrated to provide the crude nitro compound. This product was dissolved in MeOH/EtOAc (60 mL, 2/1), 10% Pd/C (5 mol%) was added, and the reaction mixture stirred under 40 psi H_2 for 3 h. The reaction mixture was then filtered over Celite and concentrated to provide the desired 4-cyclohexyloxyaniline. To a solution of Cbz-Gln (505 mg, 1.2 equiv) and HOBt (280 mg, 1.2 equiv) in DMF (10 mL) was added EDC (360 mg, 1.2 equiv) at rt. After 30 min, the cyclohexyloxyaniline (287 mg, 1.5 mmol) was added. The reaction mixture was stirred at rt for 16 h, and quenched with water. The reaction mixture was extracted with EtOAc, washed with 0.1 N HCl, $NaHCO_3$ (satd aq) and brine. The organic layer was dried and concentrated. This product was dissolved in MeOH (10 mL), 10% Pd/C (5 mol%) was added and the reaction stirred under 40 psi H_2 for 2 h. The reaction mixture was then filtered over Celite and concentrated to provide pure **9b** (65%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (s, 1H), 7.43 (d, J = 9.2 Hz, 2H), 7.38 (s, 1H), 7.00 (s, 1H), 6.82 (d, J = 9.2 Hz, 2H), 4.23–4.17 (m, 1H), 3.01 (t, J = 7.7 Hz, 1H), 2.65–2.56 (m, 1H), 2.39–2.23 (m, 2H), 1.88–1.61 (m, 3H), 1.50–1.30 (m, 3H), 0.94–0.88 (m, 5H); Anal. ($C_{17}H_{25}N_3O_3 \cdot 0.8H_2O$) C, H, N.

5.2.38. N^5-[4-(2-methylphenoxy)phenyl]-L-glutamamide hydrochloride (9c). Synthesis of **9c** was carried out in an analogous manner to **9b** using *o*-cresol: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 1H), 8.20 (br s, 3H), 7.94 (s, 1H), 7.60 (s, 1H), 7.55 (dt, J_1 = 9.2 Hz, J_2 = 2.8 Hz, 2H), 7.27 (d, J = 7.6 Hz, 1H), 7.16 (td, J_1 = 7.6 Hz, J_2 = 1.6 Hz, 1H), 7.04 (td, J_1 = 7.2 Hz, J_2 = 1.2 Hz, 1H), 6.84 (dt, J_1 = 9.2 Hz, J_2 = 2.4 Hz, 2H), 6.78 (d, J = 7.2 Hz, 1H), 3.79–3.74 (m, 1H), 2.45–2.34 (m, 2H), 2.16 (s, 3H), 2.06–2.00 (m, 2H); Anal. ($C_{18}H_{21}N_3O_3 \cdot 0.6H_2O \cdot 1HCl$) C, H, N.

5.2.39. N^5-[4-(3-methylphenoxy)phenyl]-L-glutamamide hydrochloride (9d). Synthesis of **9d** was carried out in an analogous manner to **9b** using *m*-cresol: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.31 (s, 1H), 8.37 (br s, 3H), 8.06 (s, 1H), 7.66–7.54 (m, 3H), 7.19 (s, 1H), 6.94–6.72 (m, 5H), 3.79 (br s, 1H), 2.54–2.40 (m, 2H), 2.23 (s, 3H), 2.10–2.00 (m, 2H); Anal. ($C_{18}H_{21}N_3O_3 \cdot 0.6H_2O \cdot 1HCl \cdot 0.25Et_2O$) C, H, N.

5.2.40. N^5-[4-(4-methylphenoxy)phenyl]-L-glutamamide hydrochloride (9e). Synthesis of **9e** was carried out in an analogous manner to **9b** using *p*-cresol: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.12 (s, 1H), 8.20 (br s, 3H), 7.93 (s, 1H), 7.61 (s, 1H), 7.57 (d, J = 9.2 Hz, 2H), 7.14 (d, J = 8.0 Hz, 2H), 6.91 (d, J = 9.2 Hz, 2H), 6.83 (d, J = 8.4 Hz, 2H), 3.76 (br s, 1H), 2.46–2.39 (m, 2H), 2.25 (s, 3H), 2.06–2.00 (m, 2H); Anal. ($C_{18}H_{21}N_3O_3 \cdot 0.7H_2O \cdot 1HCl \cdot 0.25Et_2O$) C, H, N.

5.2.41. N^5-[4-[4-(1H-pyrrol-1-yl)phenoxy]phenyl]-)-L-glutamine hemitrifluoroacetate (9f). Synthesis of **9f** was carried out in an analogous manner to **9b** using 4-(1H-pyrrol-1-yl)-phenol: ^{1}H NMR (400 MHz, DMSO) δ 10.10 (s, 1H), 8.11 (s, 2H), 7.59–7.51 (m, 4H), 7.26 (t, J = 2 Hz, 2H), 7.00 (t, J = 9.2 Hz, 4H), 6.22 (t, J = 2 Hz, 2H), 3.78 (s, 1H), 2.48–2.46 (m, 2H), 2.04–2.02 (m, 2H); Anal. ($C_{21}H_{21}N_3O_4 \cdot 0.5H_2O \cdot 0.5CF_3CO_2H$) C, H, N, F.

5.2.42. N^5-[4-(4-(3-Furyl)phenoxy)phenyl]-L-glutamamide trifluoroacetate (9g). A mixture of 4-(4-bromophenoxy)-aniline (2.2 g, 8.2 mmol), 3-furylboronic acid (915 mg, 1

equiv), $Pd(PPh_3)_2Cl_2$ (576 mg, 10 mol%), and Na_2CO_3 (2N aq, 9 mL) in *i*-PrOH (30 mL) was kept at 80 °C overnight. The reaction mixture was cooled to rt, diluted with water, and extracted with EtOAc. The organic phase was washed with brine, dried, and concentrated to provide crude aniline which was carried on to the next step. To a solution of Boc-Gln-OH (296 mg, 1.2 equiv) and HOBt (184 mg, 1.2 equiv) in DMF (5 mL) was added EDC (238 mg, 1.2 equiv) at rt. After 30 min, the aniline (250 mg, 1 mmol) was added. The reaction mixture was stirred at rt overnight, and quenched with water. The reaction mixture was extracted with EtOAc, and the organic layer was washed with brine. The organic layer was dried and concentrated. To a solution of this residue in CH_2Cl_2 was added HCl (4 M in dioxane, 10 equiv). After stirring for 16 h, reaction mixture was concentrated. The resulting solid was purified by reverse phase HPLC using a gradient of MeCN in water to provide **9g** (36%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (s, 1H), 8.14 (br s, 3H), 7.89 (s, 1H), 7.70–7.57 (m, 5H, 6.99–6.77 (m, 6H), 3.77 (br s, 1H), 2.45–2.33 (m, 2H), 2.05–1.98 (m, 2H); Anal. ($C_{21}H_{21}N_3O_4 \cdot 1.48CF_3CO_2H$) C, H, N.

5.2.43. N^6-[4-(4-methylphenoxy)phenyl]-L-homoglutamine trifluoroacetate (10). To a solution of Boc-homoglu(OBz)-OH (1 g, 2.8 mmol) in MeOH/$PhCH_3$ (1/1, 30 mL) was added $TMSCHN_2$ (2 M in hexanes, 10 mL total) dropwise until bubbling was not observed and the yellow color persisted for more than 1 minute. The solution was then concentrated. A slurry of this product and Pd/C (0.2 g, 20 w/w%) in MeOH (100 mL) was stirred under 40 psi H_2 for 24 h. The reaction mixture was filtered over Celite and the mother liquor was concentrated to give the desired acid as a yellow oil. To a solution of this acid (0.8 g, 2.5 mmol) and *N*-methylmorpholine (0.7 mL, 2 equiv) in THF (10 mL) at −20 °C was added isobutyl chloroformate (0.33 mL, 1 equiv). After stirring at this temperature for 30 min, 4-phenoxyaniline (0.5 g, 1 equiv) was added and the turbid reaction mixture was allowed to warm to room temperature for over 16 h. The reaction mixture was filtered and concentrated. The residue was dissolved in EtOAc, washed with $NaHSO_4$ (satd aq), $NaHCO_3$ (satd aq) and brine, dried and concentrated. The coupled product was dissolved in MeOH and NaOH (1 M, aq) was added. After stirring for 24 h, the solvent was removed and the residue was partitioned between CH_2Cl_2 and $NaHSO_4$ (satd aq), and the layers were separated. The aqueous layers were extracted with 2× CH_2Cl_2 and the combined organic layers were dried ($MgSO_4$) and concentrated to give the free carboxylic acid. This acid was slurried in CH_2Cl_2 and HCl (4 M in dioxane, 10 equiv) was added to give a clear solution. After stirring for 16 h, solvent was removed. The resulting solid was purified by reverse phase HPLC using a gradient of MeCN in water to provide pure **10** (22%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.03 (s, 1H), 7.91 (br s, 3H), 7.58 (d, J = 8.8 Hz, 2H), 7.34 (t, J = 7.3 Hz, 2H), 7.08 (t, J = 7.3 Hz, 1H), 6.97 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 8.4 Hz, 2H), 3.64–3.58 (m, 1H), 2.68–2.41 (m, 4H), 1.92–1.83 (m, 2H); Anal. ($C_{18}H_{20}N_2O_4 \cdot 1H_2O \cdot 1.1CF_3CO_2H$) C, H, N, F.

5.2.44. N^1-(2-Carboxyethyl)-N^5-(4-Phenoxyphenyl)-L-glutamamide trifluoroacetate (11a). To a solution of **18** (0.83 g, 2.0 mmol) and HOBt hydrate (0.4 g, 1.5 equiv) in DMF (10 mL) were added EDC (0.4 g, 1.1 equiv) and triethylamine (1 mL, 1.5 equiv). After stirring for 30 min, H-Gly-OMe (0.18 g, 1 equiv) was added and the reaction mixture was stirred overnight. Partitioned the reaction between EtOAc and water, separated the layers, washed the organic layer with $NaHSO_4$ (satd aq), $NaHCO_3$ (satd aq) and brine, dried ($MgSO_4$) and concentrated. The residue was dissolved in MeOH and NaOH (1 M, aq) was added. After stirring for 24 h, the solvent was removed, the residue was partitioned between CH_2Cl_2 and $NaHSO_4$ (satd aq), and the layers were separated. The aqueous layers were extracted with 2× CH_2Cl_2 and the combined organic layers were dried ($MgSO_4$) and concentrated to give the free carboxylic acid This acid was slurried in CH_2Cl_2 and HCl (4 M in dioxane, 10 equiv) was added to give a clear solution. After stirring for 16 h, solvent was removed. The resulting solid was purified by reverse phase HPLC using a gradient of MeCN in water to provide pure **11a** (36%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.02 (s, 1H), 8.80 (t, J = 5.9 Hz, 1H), 8.18 (br s, 3H), 7.58 (d, J = 9.1 Hz, 2H), 7.34 (t, J = 8.8 Hz, 2H), 7.08 (t, J = 7.3 Hz, 1H), 6.96 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 7.7 Hz, 2H), 3.93–3.86 (m, 3H), 2.50–2.43 (m, 2H), 2.04 (q, J = 6.6 Hz, 2H); Anal. ($C_{19}H_{21}N_3O_5 \cdot 0.5H_2O \cdot 1.4CF_3CO_2H$) C, H, N, F.

5.2.45. N^1-(3-Carboxypropyl)-N^5-(4-phenoxyphenyl)-L-glutamamide trifluoroacetate (11b). Synthesis of **11b** was carried out in an analogous manner to **11a** using β-alanine ethyl ester: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.96 (s, 1H), 8.54 (s, 1H), 8.20–8.08 (m, 2H), 7.56 (d, J = 7.2 Hz, 2H), 7.34 (t, J = 8.4 Hz, 2H), 7.08 (t, J = 6.8 Hz, 1H), 6.97 (d, J = 8.0 Hz, 2H), 6.92 (d, J = 7.2 Hz, 2H), 3.77 (br s, 1H), 3.55–3.45 (m, 4H), 2.38–2.33 (m, 2H), 2.06–1.95 (m, 2H); Anal. ($C_{20}H_{23}N_3O_5 \cdot 1H_2O \cdot 1.25CF_3CO_2H$) C, H, N, F.

5.2.46. N^1-(4-Carboxybutyl)-N^5-(4-phenoxyphenyl)-L-glutamamide bistrifluoroacetate (11c). Synthesis of **11c** was carried out in an analogous manner to **11a** using ethyl 3-aminopropionate: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.04 (s, 1H), 8.48 (t, J = 5.5 Hz, 1H), 8.17 (br s, 3H), 7.58 (d, J = 9.2 Hz, 2H), 7.34 (t, J = 7.3 Hz, 2H), 7.07 (t, J = 7.3 Hz, 1H), 6.96 (d, J = 9.1 Hz, 2H), 6.92 (d, J = 7.7 Hz, 2H), 3.76 (br s, 1H), 3.17–3.11 (m, 2H), 2.37 (q, J = 7.7 Hz, 2H), 2.25 (t, J = 7.4 Hz, 2H), 2.03–1.97 (m, 2H), 1.66 (t, J = 7.3 Hz, 2H); Anal. ($C_{21}H_{25}N_3O_5 \cdot 0.3H_2O \cdot 1.6CF_3CO_2H$) C, H, N, F.

5.2.47. N^1-(2-Hydroxyethyl)-N^5-(4-phenoxyphenyl)-L-glutamamide trifluoroacetate (11d). A solution of **18** (2.7 g, 6.5 mmol) and HOBt hydrate (1.3 g, 1.5 equiv) in DMF (30 mL) was cooled to 0 °C and EDC (1.4 g, 1.1 equiv) and triethylamine (1 mL, 1.1 equiv) were added. After stirring for 30 min, 2-aminoethanol (0.4 g, 1 equiv) was added and the reaction mixture was allowed to warm to rt overnight. Partitioned the reaction mixture between EtOAc and water, separated the layers, washed the

organic layer with $NaHSO_4$ (satd aq), $NaHCO_3$ (satd aq) and brine, dried ($MgSO_4$) and concentrated. The resulting solid was purified by reverse phase HPLC using a gradient of MeCN in water to provide pure **11d** (16%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.00 (s, 1H), 8.47 (t, J = 5.6 Hz, 1H), 8.18 (d, J = 10.8 Hz, 2H), 7.58–7.56 (m, 2H), 7.36–7.32 (m, 2H), 7.07 (t, J = 7.6 Hz, 1H), 6.97–6.91 (m, 4H), 3.79 (s, 1H), 3.45–3.42 (m, 2H), 3.24–3.17 (m, 2H), 2.40–2.33 (m, 2H), 2.00–1.98 (m, 2H); Anal. ($C_{19}H_{23}N_3O_4 \cdot 1.5CF_3CO_2H$) C, H, N, F.

5.2.48. N^1-(2-Aminoethyl)-N^5-(4-phenoxyphenyl)-L-glutamamide hydrochloride (11e). A solution of **18** (0.52 g, 1.2 mmol) and HOBt hydrate (0.25 g, 1.5 equiv) in DMF (10 mL) was cooled to 0 °C and EDC (0.26 g, 1.1 equiv) and triethylamine (0.19 mL, 1.1 equiv) were added. After stirring for 30 min, *N*-Boc ethylenediamine (0.2 g, 1 equiv) was added and the reaction was allowed to warm to rt overnight. Partitioned the reaction mixture between EtOAc and water, separated the layers, washed the organic layer with $NaHSO_4$ (satd aq), $NaHCO_3$ (satd aq) and brine, dried ($MgSO_4$) and concentrated. The residue was dissolved in CH_2Cl_2 and HCl (4 M in dioxane, 10 equiv) was added to give a clear solution. After stirring for 48 h, the precipitated solid was filtered to provide pure **11 e** (2%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.01 (s, 1H), 8.60 (t, J = 3.2 Hz, 1H), 8.17 (br s, 3H), 7.75 (br s, 2H), 7.58 (d, J = 7.2 Hz, 2H), 7.28 (t, J = 8.0 Hz, 2H), 7.03 (t, J = 7.2 Hz, 1H), 6.95 (d, J = 8.4 Hz, 2H), 6.89 (d, J = 7.6 Hz, 2H), 3.82–3.77 (m, 1H), 3.41–3.34 (m, 2H), 2.94–2.85 (m, 2H), 2.42–2.39 (m, 2H), 2.08–2.01 (m, 2H); Anal. ($C_{19}H_{24}N_4O_3 \cdot 0.2$EtOAc$\cdot 0.8$HCl) C, H, N.

5.2.49. 4*S*-4-Amino-5-hydroxy-*N*-(4-phenoxyphenyl)pentanamide (12a)

5.2.49.1. Step 1: Synthesis of *S*-4-(1,1-dimethylethylcarbamoylamino)-5-hydroxy-*N*-(4-phenoxyphenyl)pentanamide (21). To a solution of Boc-Glu(OH)-OBn (8 g, 25 mmol) and *N*-methylmorpholine (7 mL, 2 equiv) in THF (100 mL) at −20 °C was added isobutyl chloroformate (3.3 mL, 1 equiv). After stirring at this temperature for 30 min, 4-phenoxyaniline (5 g, 1 equiv) was added and the turbid reaction mixture was allowed to warm to room temperature for over 16 h. The reaction mixture was filtered and concentrated. The residue was dissolved in EtOAc, washed with $NaHSO_4$ (satd aq), $NaHCO_3$ (satd aq) and brine, dried and concentrated. This product was dissolved in MeOH/THF (3/1) and heated to 50 °C. $NaBH_4$ (4 equiv) was added and the reaction was allowed to self-reflux for 20 min. The reaction was stirred for another 4 h, then it was poured into iced HCl (0.5 M, aq). The resulting solid was filtered, then recrystallized from MeOH/water to provide compound **21** (80%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1 H), 7.58 (d, J = 8.8 Hz, 2H), 7.33 (tt, J_1 = 8.8 Hz, J_2 = 2.2 Hz, 2H), 7.08 (t, J = 7.3 Hz, 1H), 6.96–6.90 (m, 4H), 6.48 (d, J = 8.5 Hz, 1H), 4.61 (t, J = 5.9 Hz, 1H), 3.38–3.28 (m, 1H), 3.25–3.19 (m, 1H), 2.31–2.25 (m, 2H), 1.86–1.80 (m, 1H), 1.55–1.50 (m, 1H), 1.35 (s, 9H).

5.2.49.2. Step 2: Synthesis of 4*S*-4-amino-5-hydroxy-*N*-(4-phenoxyphenyl)pentanamide (12a). Compound **21** was slurried in CH_2Cl_2 and HCl (4 M in dioxane, 10 equiv) was added to give a clear solution. After stirring for 16 h, solvent was removed. The resulting solid was partitioned between EtOAc and NaOH (1M aq), the layers were separated, the aqueous layer was extracted with EtOAc, and the combined organic layers were dried ($MgSO_4$) and concentrated. The resulting solid was purified by silica gel chromatography using a gradient of MeOH in CH_2Cl_2 to provide pure **12a** (30%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.02 (s, 1H), 7.82 (br s, 2H), 7.58 (d, J = 9.2 Hz, 2H), 7.33 (tt, J_1 = 8.7 Hz, J_2 = 2.2 Hz, 2H), 7.07 (t, J = 7.4 Hz, 1H), 6.96 (d, J = 9.2 Hz, 2H), 6.92 (d, J = 7.7 Hz, 2H), 3.60 (dd, J_1 = 11.3 Hz, J_2 = 3.6 Hz, 1H), 3.44 (dd, J_1 = 11.4 Hz, J_2 = 6.2 Hz, 1H), 3.17–3.11 (m, 1H), 2.42 (q, J = 7.7 Hz, 2H), 1.84–1.78 (m, 2H); Anal. ($C_{17}H_{20}N_2O_3 \cdot 0.6CH_2Cl_2$) C, H, N.

5.2.50. 4*S*-4,5-Diamino-*N*-(4-phenoxyphenyl)pentanamide (12b)

5.2.50.1. Step 1: Synthesis of 4*S*-4-(1,1-dimethylethylcarbamoylamino)-5-methansulfonyloxy-*N*-(4-phenoxyphenyl)pentanamide (16). To a solution of **21** (3.6 g, 9 mmol) in pyridine (60 mL) was added MsCl (3.5 mL, 5 equiv) which caused a substantial exotherm and a precipitate formed. The reaction mixture was stirred for 1 h, then MeOH (20 mL) was added until all of the solid dissolved. The reaction mixture was poured into iced HCl (0.5 M aq) and the resulting solid was filtered to provide pure **16** (99%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (br s, 1H), 7.54 (d, J = 8.9 Hz, 2H), 7.33 (tt, J_1 = 8.8 Hz, J_2 = 3.1 Hz, 2H), 7.09 (t, J = 7.8 Hz, 1H), 6.99–6.94 (m, 4H), 5.01 (d, J = 8.1 Hz, 1H), 4.27 (dd, J_1 = 11.0 Hz, J_2 = 3.2 Hz, 1H), 4.22 (dd, J_1 = 11.5 Hz, J_2 = 4.9 Hz, 1H), 4.01–3.96 (m, 1H), 3.04 (s, 3H), 2.45 (t, J = 8.1 Hz, 2H), 1.97–1.91 (m, 2H), 1.43 (s, 9H).

5.2.50.2. Step 2: Synthesis of 4*S*-4-(1,1-dimethylethylcarbamoylamino)-5-amino-*N*-(4-phenoxyphenyl)pentanamide trifluoroacetate (22). To a solution of **16** (0.48 g, 1.0 mmol) in DMF (5 mL) was added NaN_3 (0.2 g, 3 equiv). This reaction mixture was stirred at 60 °C for 6 h, and then poured into ice water. The water was extracted with 2× EtOAc and the combined organic layers were dried ($MgSO_4$) and concentrated to give the azide as a white solid. This azide (0.1 g) was dissolved in MeOH/EtOAc (1/1, 10 mL) and Pd/C was added. The black suspension was agitated under 50 psi H_2 pressure for 2.5 h. The reaction mixture was filtered over Celite and concentrated. This residue was purified by reverse phase HPLC using a gradient of MeCN in H_2O to provide **22** (60%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (br s, 1 H), 7.47 (d, J = 9.1 Hz, 2H), 7.29 (tt, J_1 = 8.7 Hz, J_2 = 2.3 Hz, 2H), 7.06 (t, J = 7.6 Hz, 1H), 6.96–6.91 (m, 4H), 5.86 (d, J = 8.1 Hz, 1H), 3.88–3.81 (m, 1H), 3.18–3.02 (m, 2H), 2.44 (t, J = 9.1 Hz, 2H), 2.01–1.94 (m, 2H), 1.37 (s, 9H).

5.2.50.3. Step 3: Synthesis of 4*S*-4,5-diamino-*N*-(4-phenoxyphenyl)pentanamide bistrifluoroacetate (12b). Compound **22** was dissolved in CH_2Cl_2 and TFA (10

equiv) was added. After stirring for 4 h the reaction mixture was concentrated. The resulting yellow oil was purified by reverse phase HPLC using a gradient of MeCN in water to provide **12b** (16%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 7.55–7.53 (m, 2H), 7.32 (t, J = 7.6 Hz, 2H), 7.07 (t, J = 7.6 Hz, 1H), 6.94 (d, J = 8.8 Hz, 4H), 3.687–3.624 (m, 1H), 3.30–3.26 (m, 2H), 2.70–2.66 (m, 2H), 2.18–1.98 (m, 2H); Anal. ($C_{17}H_{21}N_3O_2{\cdot}2.3CF_3CO_2H$) C, N, F. H: Calcd, 4.18, found, 3.50.

5.2.51. 4*S*-4-Amino-5-methoxy-*N*-(4-phenoxyphenyl)-pentanamide (12c). Compound **21** (0.5 g, 1.3 mmol) was dissolved in MeCN/THF (3/1, 20 mL) and Ag_2O (1.5 g, 5 equiv) and MeI (1.2 mL, 10 equiv) were added to give a black suspension. After stirring for 7 days, the reaction was filtered over Celite and the mother liquor was concentrated. This product was dissolved in CH_2Cl_2 and TFA (10 equiv) was added. After stirring for 2 h, solvent was removed. The residue was partitioned between NaOH (1 M aq) and CH_2Cl_2. The layers were separated, the aqueous layers were extracted with 2× CH_2Cl_2, and the combined organic layers were dried and concentrated. The resulting solid was purified by silica gel chromatography using a gradient of MeOH in CH_2Cl_2 to provide pure **12c** (16%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.93 (s, 1H), 7.58 (d, J = 9.1 Hz, 2H), 7.33 (tt, J_1 = 7.3 Hz, J_2 = 2.2 Hz, 2H), 7.06 (t, J = 7.3 Hz, 1H), 6.97–6.91 (m, 4H), 3.23 (s, 3H), 3.02 (dd, J_1 = 9.2 Hz, J_2 = 5.2 Hz, 1H), 3,13 (dd, J_1 = 9.5 Hz, J_2 = 6.6 Hz, 1H), 2.81–2.75 (m, 1H), 2.44–2.28 (m, 2H), 1.74–1.65 (m, 1H), 1.47–1.38 (m, 1H); Anal. ($C_{18}H_{22}N_2O_3{\cdot}0.1CH_2Cl_2$) C, H, N.

5.2.52. 2*S*-2-Amino-5-oxo-5-[(4-phenoxyphenyl)amino]-1-pentanesulfonic acid (12d). A solution of **16** (0.4 g, 0.8 mmol) and potassium thioacetate (0.2 g, 2 equiv) in acetone (10 mL) was stirred for 48 h. The suspension was filtered over Celite and concentrated to provide the thioacetate. To a solution of this product in AcOH (1 mL) was added H_2O_2 (30% in water, 0.5 mL) in AcOH (1 mL). After stirring for 24 h, the reaction mixture was diluted with Et_2O and the resulting precipitate was filtered and washed with MeOH and water to provide **12d** (28%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 7.84 (s, 2H), 7.56 (dt, J_1 = 8.8 Hz, J_2 = 2 Hz, 2H), 7.36–7.31 (m, 2H), 7.07 (tt, J_1 = 7.2 Hz, J_2 = 1.2 Hz, 1H), 6.97–6.91 (m, 4 H), 3.38 (s, 1H), 2.84 (dd, J_1 = 12 Hz, J_2 = 2.4 Hz, 1H), 2.65–2.59 (m, 1H), 2.48–2.29 (m, 2H), 1.99–1.79 (m, 2H); Anal. ($C_{17}H_{20}N_2O_5S{\cdot}0.15H_2O{\cdot}0.1\ CH_2Cl_2$) C, H, N, S.

5.2.53. 4*S*-4-Amino-4-(2-(1,3,4-triazolyl))-*N*-(4-phenoxyphenyl)butanamide (12e)

5.2.53.1. Step 1: Synthesis of 4*S*-4-(1,1-dimethylethylcarbamoylamino)-4-cyano-*N*-(4-phenoxyphenyl)butanamide (15). To a solution of Boc-Gln (8 g, 25 mmol) and *N*-methylmorpholine (7 mL, 2 equiv) in THF (100 mL) at −20 °C was added isobutyl chloroformate (3.3 mL, 1 equiv). After stirring at this temperature for 30 min, 4-phenoxyaniline (5 g, 1 equiv) was added and the turbid reaction mixture was allowed to warm to room temperature for over 16 h. The reaction mixture was filtered and concentrated. The residue was dissolved in EtOAc, washed with $NaHSO_4$ (satd aq), $NaHCO_3$ (satd aq) and brine, dried and concentrated. A solution of the resulting amide (6 g, 13 mmol) in DMF (250 mL) was cooled to 0 °C and cyanuric chloride (2.5 g, 1 equiv) was added. The reaction mixture was allowed to warm to room temperature and stirred for 16 h, and then the reaction was poured into water. The solid was filtered off to provide **15** (66%) as a pale yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (br s, 1H), 7.45 (d, J = 9.0 Hz, 2H), 7.26 (tt, J_1 = 8.4 Hz, J_2 = 2.1 Hz, 2H), 7.05 (t, J = 7.8 Hz, 1H), 6.96–6.91 (m, 4H), 5.6 (br s, 1H), 4.64 (br s, 1H), 2.65–2.53 (m, 2H), 2.30–2.23 (m, 2H), 1.43 (s, 9H).

5.2.53.2. Step 2: Synthesis of 4*S*-4-amino-4-(2-(1,3,4-triazolyl))-*N*-(4-phenoxyphenyl)butanamide trifluoroacetate (12e). A slurry of cyanide (2 g, 4.7 mmol), formic hydrazide (0.56 g, 2 equiv) and K_2CO_3 (0.14 g, 0.2 equiv) in EtOH was stirred at reflux for 24 h. The reaction mixture was then poured into water and the resulting solid was isolated by filtration. This product was slurried in CH_2Cl_2 and TFA (10 equiv) was added to give a solution. After stirring for 2 h, solvent was removed. The residue was purified by reverse phase HPLC using a gradient of MeCN in H_2O to provide **12e** (12%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.93 (s, 1H), 8.70 (d, J = 2.5 Hz, 1H), 8.50 (br s, 2H), 7.55 (d, J = 9.1 Hz, 2H), 7.34 (t, J = 7.3 Hz, 2H), 7.07 (t, J = 7.3 Hz, 1H), 6.95 (d, J = 9.2 Hz, 2H), 6.92 (d, J = 7.7 Hz, 2H), 4.47 (br s, 1H), 2.35 (t, J = 6.9 Hz, 2H), 2.27–2.11 (m, 2H); Anal. ($C_{18}H_{19}N_5O_2{\cdot}0.2H_2O{\cdot}1CF_3CO_2H$) C, H, N, F.

5.2.54. 4*S*-4-Amino-5-(2-thio-5-amino-1,3,4-thiadiazol-2-yl)-*N*-(4-phenoxyphenyl)pentanamide bistrifluoroacetate (12f). To a solution of 5-amino-1,3,4-thiadiazole-2-thiol (0.06 g, 1.1 equiv) in DMF (5 mL) was added NaH (0.02 g, 1.1 equiv) giving a strong exotherm. After stirring for 3 h, compound **16** (0.2 g, 0.4 mmol) was added. This reaction mixture was stirred for 16 h, and then poured into ice water. The solid was filtered off and the mother liquor was concentrated to provide crude product. This product was dissolved in CH_2Cl_2 and HCl (4 M in dioxane, 10 equiv) was added. After stirring for 24 h the reaction mixture was concentrated. The resulting yellow oil was purified by reverse phase HPLC using a gradient of MeCN in water to provide **12f** (11%) as a yellow oil: ^{1}H NMR (400 MHz, CD_3OD) δ 7.52 (dd, J_1 = 8.8 Hz, J_2 = 2.2 Hz, 2H), 7.32 (tt, J_1 = 7.3 Hz, J_2 = 2.2 Hz, 2H), 7.08 (t, J = 7.4 Hz, 1H), 6.96–6.92 (m, 4H), 3.68–3.64 (m, 3H), 3.56 (dd, J_1 = 14.6 Hz, J_2 = 4.8 Hz, 1H), 3.34 (J_1 = 15.0 Hz, J_2 = 9.8 Hz, 1H), 2.68–2.57 (m, 2H), 2.13–2.08 (m, 2H); Anal. ($C_{19}H_{21}N_5O_2S_2{\cdot}2.5CF_3CO_2H$) C, H, N, S.

5.2.55. 4*S*-4-Amino-5-(1H-pyrrol-1-yl)-*N*-(4-phenoxyphenyl)pentanamide bistrifluoroacetate (12g). A solution of compound **22** (0.3 g, 0.75 mmol) and NaOAc (0.06 g, 1 equiv) in AcOH (250 mL) was heated to 80 °C and 2,5-dimethoxytetrahydrofuran (0.13 mL, 1.3 equiv) was added dropwise to the solution. After stirring for 2 h, the reaction was diluted with water and washed with 2× CH_2Cl_2. The water layer was then brought to pH

13 with K_2CO_3 (satd aq) and extracted with 2× CH_2Cl_2. The organic fractions were dried and concentrated to provide crude pyrrole. This pyrrole was dissolved in CH_2Cl_2 and HCl (4 M in dioxane, 10 equiv) was added. After stirring for 6 h the reaction mixture was concentrated. The residue was purified by reverse phase HPLC using a gradient of MeCN in water to provide **12g** (1%) as a yellow oil: ^{1}H NMR (400 MHz, CD_3OD) δ 7.53 (d, J = 8.8 Hz, 2 H), 7.32 (t, J = 7.3 Hz, 2H), 7.08 (t, J = 7.4 Hz, 1H), 6.94 (d, J = 8.8 Hz, 4H), 6.76 (t, J = 1.8 Hz, 2H), 6.16 (t, J = 1.8 Hz, 2H), 4.22 (dd, J_1 = 14.6 Hz, J_2 = 4.8 Hz, 1H), 4.12 (J_1 = 14.7 Hz, J_2 = 7.3 Hz, 1H), 2.66–2.51 (m, 2H), 1.96 (q, J = 6.6 Hz, 2H); Anal. ($C_{21}H_{23}N_3O_2 \cdot 2.1CF_3CO_2H$) C, N. H: Calcd, 4.30, found, 3.83.

5.2.56. 4*S*-4-Amino-5-(1-phthalimido)-*N*-(4-phenoxyphenyl)pentanamide trifluoroacetate (12h). To a solution of **16** (1.2 g, 2.5 mmol) in DMF (25 mL) was added potassium phthalimidate (0.5 g, 1.1 equiv). This slurry was stirred at 100 °C for 4 h, and then poured into $NaHCO_3$ (satd aq). The water layer was extracted with 2× EtOAc and the combined organic layers were dried ($MgSO_4$) and concentrated to give the phthalimide as a white solid. This product was dissolved in CH_2Cl_2 and TFA (10 equiv) was added. After stirring for 16 h the reaction was concentrated. The residue was purified by reverse phase HPLC using a gradient of MeCN in water to provide **12h** (14%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.93 (s, 1H), 7.86–7.80 (m, 4H), 7.56 (d, J = 9.1 Hz, 2H), 7.33 (t, J = 8.8 Hz, 2H), 7.06 (t, J = 7.3 Hz, 1H), 6.96–6.91 (m, 4H), 3.50 (dd, J_1 = 7.4 Hz, J_2 = 2.2 Hz, 2H), 2.98–2.91 (m, 1H), 2.45–2.33 (m, 2H), 1.77–1.68 (m, 1H), 1.51–1.44 (m, 1H); Anal. ($C_{25}H_{23}N_3O_4 \cdot 0.51CF_3CO_2H$) C, H, N, F.

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