Combinatorial Library of Serine and Cysteine Protease Inhibitors That Interact with Both the S and S′ Binding Sites

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A combinatorial library of 400 inhibitors has been synthesized and screened against several serine and cysteine proteases including plasmin, cathepsin B, and papain. The inhibitors are based upon a cyclohexanone nucleus and are designed to probe binding interactions in the S2 and S2′ binding sites. This methodology has led to the discovery of inhibitor **15A**, which incorporates Trp at both the P2 and P2′ positions and has an inhibition constant against plasmin of 5 μM. Data from screening of the library shows that plasmin has a strong specificity for Trp at the S2 subsite and prefers to bind hydrophobic and aromatic amino acids such as Ile, Phe, Trp, and Tyr at the S2′ subsite. In contrast, the S2′ subsites of cathepsin B and papain do not show a strong preference for any particular amino acid.

Introduction

Combinatorial chemistry has emerged as a powerful method for generating lead compounds for drug discovery and for optimizing the biological activity of those leads.[1] This technique has been used to develop new ligands for a variety of biological targets including proteases, kinases, various receptors, and antibodies, among others. Proteases are particularly interesting targets because they are involved with a wide variety of important diseases that include AIDS, cancer, and malaria. Many of the libraries that have been generated for screening against proteases incorporate a chemical functionality that mimics the tetrahedral intermediate that occurs during enzyme-catalyzed peptide hydrolysis. For example, phosphonic acids have been screened against the metalloprotease thermolysin,[2] and statine,[3] (hydroxyethyl)amine,[4] and diamino diol[5] isosteres have been used to synthesize libraries against the aspartic protease HIV protease. In addition, a peptide aldehyde library has been targeted against the cysteine protease interleukin-1β converting enzyme.[6]

We have recently designed a new class of inhibitors for serine and cysteine proteases that are based upon a 4-heterocyclohexanone pharmacophore.[7,8] These inhibitors react with the enzyme active site nucleophile to generate a reversibly formed hemiketal or hemithioketal adduct that also mimics the tetrahedral intermediate.[9] One attractive feature of the 4-heterocyclohexanone-based inhibitors is that they can be derivatized in two directions, allowing them to make contacts with both the S and S′ subsites.[10−12] Thus, the 4-heterocyclohexanone pharmacophore, with its bidirectional nature, can easily be incorporated into a combinatorial synthesis of inhibitors. In this paper we describe the synthesis and screening of a 400-membered library of inhibitors that are based upon a cyclohexanone nucleus (Figure 1). The X_{aa} position in compound **1** is designed to fit into the S2 specificity pocket, the Y_{aa} position will fit into the S2′ site, and the carbonyl moiety of the cyclohexanone ring is designed to react with the active site nucleophile to give a reversibly formed covalent adduct.

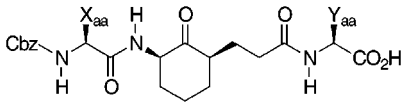

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Figure 1. General structure of the compounds that are present in the 400-member combinatorial library of inhibitors. X_{aa} and Y_{aa} are each one of 20 different amino acids.

This work has three objectives. First, we demonstrate that the cyclohexanone nucleus can be a useful platform for developing protease inhibitors that interact with both the S and S′ binding sites using combinatorial chemistry. Second, the library is screened against several medicinally relevant serine and cysteine proteases in order to discover potential leads. Third, we explore the S2′ specificity of these proteases. For many of these enzymes the specificity of this site has not been well defined.

Results and Discussion

Design and Synthesis of the Library. Before we began constructing the library, we needed to devise an efficient synthesis of a building block such as compound **4** (Scheme 1). This molecule incorporates the cyclohexanone nucleus, is amenable to solid-phase peptide synthesis, and carries the ketone functionality in a suitably protected form. We have reported previously that compound **2** can be converted to **4** by oxidative cleavage of the double bond and replacement of the Boc protecting group with Fmoc.[10] However, we have found that on larger scale, reaction of the amino acid with Fmoc chloride or Fmoc *N*-hydroxysuccinimide ester under a variety of conditions gave relatively low and inconsistent yields of **4**. This problem can be circumvented by switching the protecting groups first and then oxidizing the alkene to the acid as shown in Scheme 1. Compound **4** is a mixture of two diastereomers, each of which has the substituents on the cyclohexanone ring in the 2,6-*cis* configuration.[10]

We have chosen the "split synthesis" strategy, first described by Furka,[13] for constructing the library and

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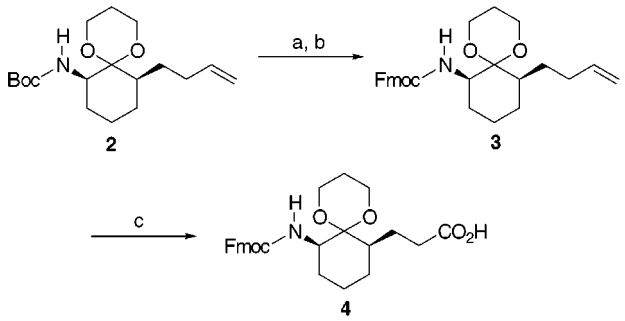
Scheme 1[a]



[a] Reagents: (a) TFA, CH_2Cl_2; (b) FmocCl, DIEA; (c) $NaIO_4$, $KMnO_4$, $NaHCO_3$. One of two enantiomers is shown.

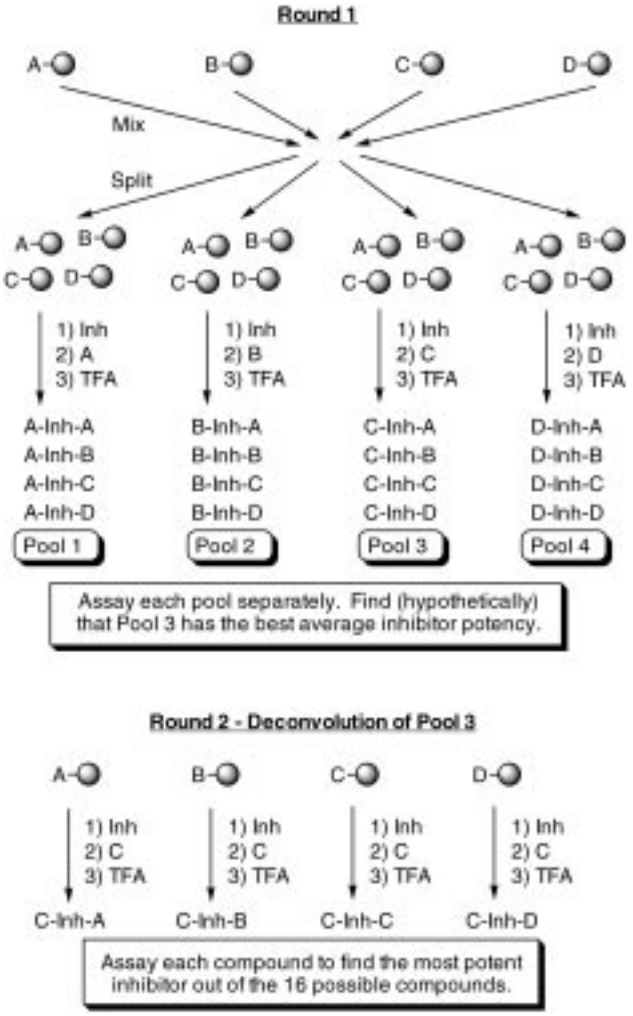
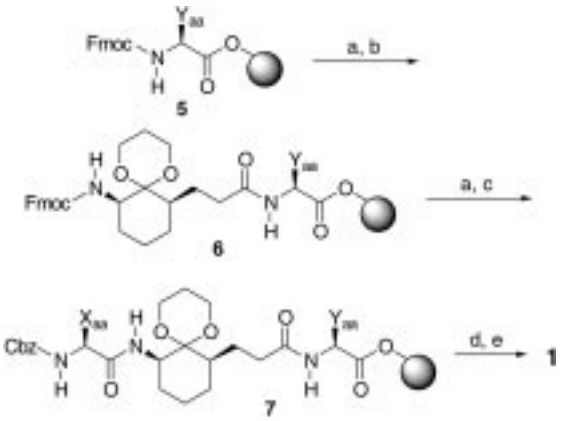


Figure 2. Schematic diagram of the deconvolution strategy used in the synthesis and screening of the combinatorial library of protease inhibitors. **A**−**D** correspond to four different amino acids. In the actual library 20 amino acids were used, but the figure has been limited to 4 amino acids for the sake of clarity. Inh represents the cyclohexanone pharmacophore, and TFA is F_3CCO_2H.

the iterative deconvolution strategy, developed by Houghten, for assaying its biological activity.[14] These techniques, as applied to the cyclohexanone-based inhibitor library, are outlined in Figure 2. Synthesis of the library began with 20 batches of peptide synthesis resin, each with a different amino acid attached. The resin was mixed and then split into 20 pools that contained a mixture of all 20 amino acids. The inhibitor

Scheme 2[a]



[a] Reagents: (a) piperidine, DMF; (b) **4**, HBTU, DIEA; (c) Cbz-X_{aa}-OH, HBTU, DIEA; (d) TFA; (e) TFA, H_2O. One of two diastereomers is shown.

core was attached to all of the pools, followed by a second amino acid, each pool receiving a different amino acid. Finally, the inhibitors were cleaved from the beads, and the protecting groups were removed. This resulted in 20 pools of inhibitors, each containing 20 different compounds. The inhibitors in an individual pool all have the same amino acid on their N-terminus, but a mixture of the 20 different amino acids on the C-terminus. The pools were assayed for inhibitory activity, and one or several of the pools which showed the highest activity were for chosen for deconvolution.

The second round of synthesis again began with 20 pools of resin, each with a different amino acid attached. These were coupled to the inhibitor core; then every compound was coupled to the N-terminal amino acid that corresponded to the pool from the first round of synthesis that was chosen for deconvolution. After cleavage from the solid support and removal of the protecting groups, these pools were assayed to determine which amino acid was optimal for the C-terminal side of the inhibitor. Although the iterative deconvolution strategy does not always result in identification of the best inhibitor in the library,[1e] it is a straightforward and reliable method for determining the structure of molecules that have high activity compared to the other members of the library.

The details of the solid-phase synthesis are shown in Scheme 2. Resin was purchased preloaded with the Fmoc-protected amino acids. The N-terminus was deprotected with piperidine and then coupled to inhibitor core **4** using HBTU to give **6**. Piperidine deprotection followed by coupling to an N-Cbz amino acid gave compound **7**. The side chains of the amino acids were then deprotected using TFA, which also released the inhibitors from the solid support. Finally, the ketal protecting group was removed by adding H_2O to the cleavage cocktail from the previous reaction and allowing the solution to stand at room temperature overnight. Using this same chemistry, we have reported previously the solid-phase synthesis of an N-acetylated inhibitor that contained Orn and Pro at the X_{aa} and Y_{aa} positions, respectively.[10] This inhibitor was fully characterized, and the overall yield of the isolated and HPLC-purified compound was 50%.

Eighteen of the 20 common amino acids were incorporated into the library. Cysteine and methionine were

omitted to avoid problems associated with sulfur oxidation, and they were replaced with hydroxyproline (Hyp) and ornithine (Orn).

Assay of the Library. The initial library of inhibitors was assayed against five proteases: cathepsin B, plasmin, urokinase, kallikrein, and papain. The first four of these enzymes have all been implicated in the progression of cancer.[15] These enzymes promote the processes of angiogenesis and metastasis, either directly by degrading components of the basement membrane which surround blood vessels or indirectly by activating other proteases which in turn attack the basement membrane.[16] Compounds which inhibit these proteases may have potential as anticancer chemotherapeutic agents.[17] Papain was chosen as a control protease since it is well-established that this enzyme prefers aromatic amino acids such as phenylalanine at the P2 position.[18] Thus we expected that in the screening of the initial library against papain, the pools which incorporated aromatic amino acids at the X_{aa} position would show good activity against this protease.

Figure 3 shows the results of the solution-phase assays of the library against plasmin, cathepsin B, and papain. The assays were performed using *p*-nitroanilide substrates and were monitored by UV spectroscopy. A single concentration of the inhibitors was used, and the runs were performed in duplicate or triplicate. The concentration of each individual inhibitor in these assays was 50 μM, giving a total inhibitor concentration for all 20 inhibitors in each pool of 1 mM. The inhibitors in several of the pools were not soluble at this concentration, so the assays were performed in more dilute solution as noted in the captions to Figures 3, 5, and 6.

For papain the three best pools of inhibitors incorporate Phe, Trp, and Tyr at the X_{aa} position as expected, based upon the known specificity of the S2 subsite of this protease.[18] We infer from these results that for papain, the inhibitors are binding in the active site in the anticipated manner with X_{aa} in the S2 subsite.

Cathepsin B displays relatively small differences in activity between the various pools, with most showing between 10% and 30% inhibition. Ile and Leu are the only pools that show significantly better activity. These data are consistent with information from previous inhibition studies, which suggest that cathepsin B prefers hydrophobic amino acids such as Leu, Ile, and Phe at the P2 position.[16]

Unlike papain and cathepsin B, plasmin shows very high selectivity for one particular pool within the library. This pool contains Trp at the X_{aa} position. Thus plasmin prefers the extended aromatic side chain of Trp at the P2 position to the exclusion of all other amino acids, including those with smaller aromatic groups such as Phe and Tyr and those with simple hydrophobic side chains such as Ile and Leu. The X-ray crystal structure of the active site portion of plasmin has not been reported. However it will be interesting to see, once the structure has been solved, if this selectivity is caused by specific aromatic stacking interactions between the inhibitor and aromatic side chains in the S2 subsite. Alternately, S2 could comprise a simple hydrophobic pocket that has good shape complementarity to extended aromatic rings, or there may be a specific hydrogen-bonding interaction that occurs with the N-H functionality of Trp.

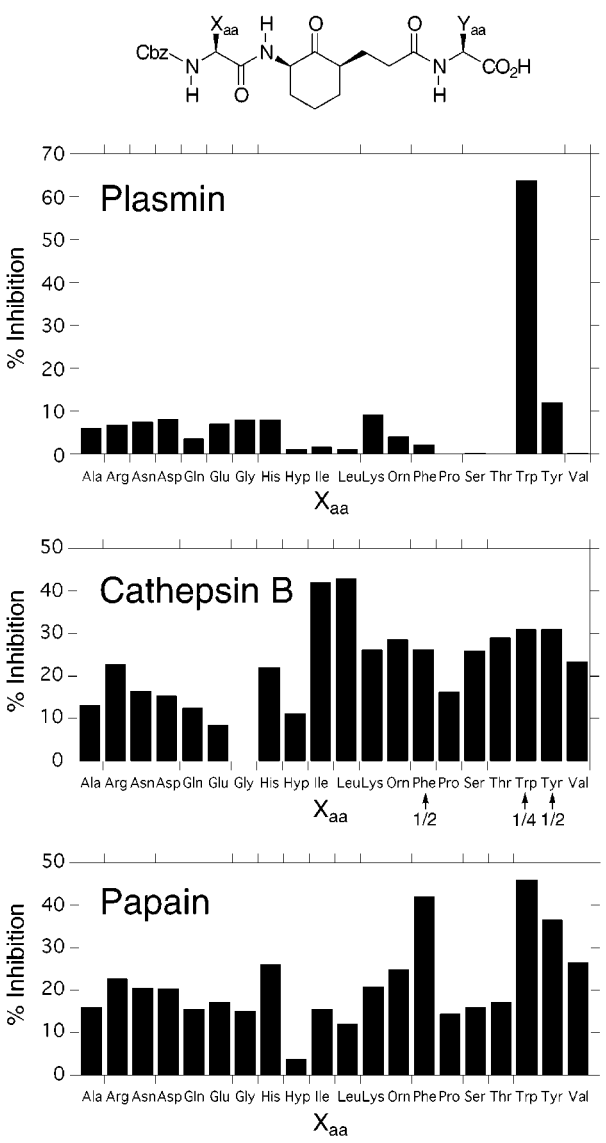


Figure 3. Assay of 20 pools of 20 compounds each against plasmin, cathepsin B, and papain. Each pool contains compounds in which the X_{aa} position is defined by the amino acids on the *x*-axes of the graphs, and Y_{aa} is a mixture of all 20 amino acids. The error in these measurements is approximately ±5%. For cathepsin B the pools in which X_{aa} = Phe and Tyr were assayed at 1/2 concentration and for X_{aa} = Trp at 1/4 concentration, compared to the other pools in the library due to low solubility of the inhibitors in the assay solution.

We have also assayed the inhibitor library against urokinase and kallikrein. With these two proteases only low levels of inhibition were observed, and the variations between the pools were similar to the approximate ±5% error in the assays (data not shown). There are several possible explanations for this lack of inhibition. First, the structure or conformation of the inhibitors in the library may not be sterically compatible with the active sites of kallikrein and urokinase. Second, the inhibitors may bind to the enzyme through weak noncovalent interactions but not react with the active site nucleophile to give the reversibly formed covalent adduct. In the 4-heterocyclohexanone series of inhibitors, such compounds have inhibition constants in the millimolar range against papain. Thus inhibitors of this type are

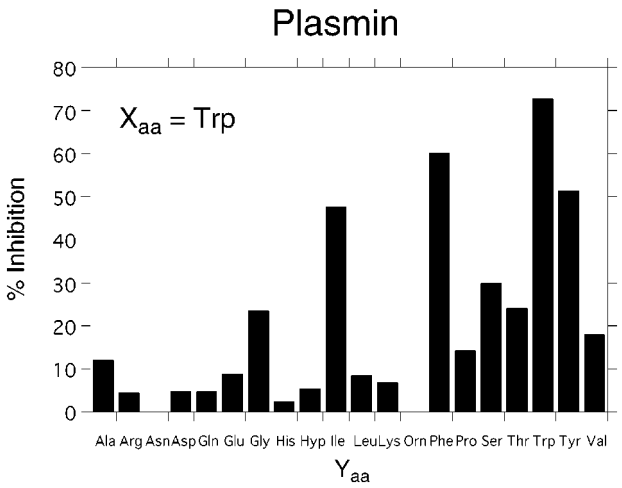


Figure 4. Assay of the Trp deconvolution against plasmin. Each bar represents the assay of one compound which has Trp at the X_{aa} position and an amino acid at the Y_{aa} position that is indicated by the *x*-axis of the graph.

not likely to give significant activity under our assay conditions.[7,9]

On the basis of the results of the initial assays, we chose several pools for deconvolution. For plasmin, only the Trp pool was worth exploring further. For cathepsin B, we chose to deconvolute the Ile pool and the three pools that had aromatic amino acids at the X_{aa} position, Phe, Trp, and Tyr. The later three pools had activities comparable to that of several other pools in the library, but they were assayed at lower concentrations and thus should contain better inhibitors on average when compared to the other pools. For papain, the Phe and Trp pools showed the best activity and were thus chosen for deconvolution.

The results from the assays of the deconvolutions are shown in Figures 4−6. Plasmin (Figure 4) clearly prefers hydrophobic amino acids at the Y_{aa} position of these inhibitors, with Ile, Phe, Trp, and Tyr showing significantly higher activities than the other compounds in the deconvolution. Of the three enzymes, plasmin again shows the largest variation in activity among the 20 pools of the library.

Cathepsin B displays limited variations in activity among the four different deconvolutions, as shown in Figure 5. In general, we observe that this protease has a small preference for amino acids such as Arg, Gln, His, Ile, Leu, Phe, and Trp at the Y_{aa} position of the inhibitors. In addition, the inhibitors with Gly, Hyp, and Ser at this position all have low activity.

One of the assumptions that we make in using the iterative deconvolution strategy is that binding interactions at one part of the inhibitor do not significantly perturb binding at another part. If this assumption were strictly true, then binding of the inhibitors to the S2 and S2′ subsites should be completely decoupled from one other, with the result that all four of the deconvolutions in Figure 5 would have identical binding profiles and differ only in magnitude. For cathepsin B, this does not appear to be the case. However, on the basis of the combined data from the four deconvolutions, we conclude that this protease has a limited preference for hydrophobic amino acids at the S2′ subsite. When a binding site has low specificity as we observed for the

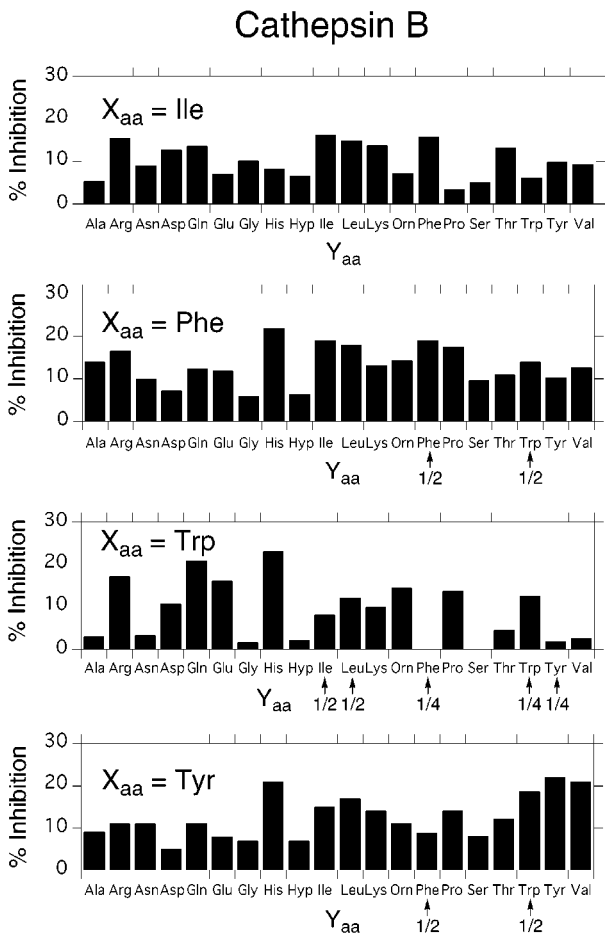


Figure 5. Assay of the Ile, Tyr, and Phe deconvolutions against cathepsin B. Several of the assays were performed at 1/2 or 1/4 concentration as noted on the *x*-axes.

S2′ site of cathepsin B, the specificity of the site only becomes visible by comparing the results of multiple deconvolutions and is not easily apparent from a single deconvolution.

For papain, hydrophobic amino acids including Ile, Leu, Phe, Trp, and Tyr are preferred at the Y_{aa} position of the inhibitors. In addition, when X_{aa} = Trp, residues such as Glu, Pro, and Val at the Y_{aa} position have good activity. For this protease, comparison of the deconvolutions shown in Figure 6 indicates that the specificity patterns at the Y_{aa} positions for the two sets of inhibitors have some similarity. This similarity suggests that binding interactions at the P2′ position are not significantly perturbed by differences that may occur when Phe or Trp is present at P2.

Resynthesis and Evaluation of Inhibitors 15−18. After evaluating all of the data from the assays of the combinatorial library, we have chosen to resynthesize four of the inhibitors (**15**−**18**, Scheme 3) using solution-phase methods and to examine the biological activity of these compounds in greater detail. The four inhibitors were selected based upon a combination of their activity against the individual proteases and the presence of overlapping activity among the three enzymes. The solution-phase synthesis of inhibitors **15**−**18**, shown in Scheme 3, follows closely the solid-phase synthesis that was used to construct the library. The only significant differences in the solution-phase chemistry were that Boc protecting groups were used for

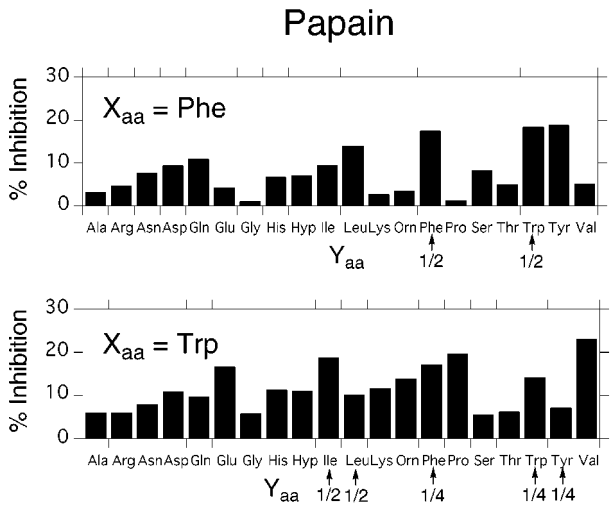
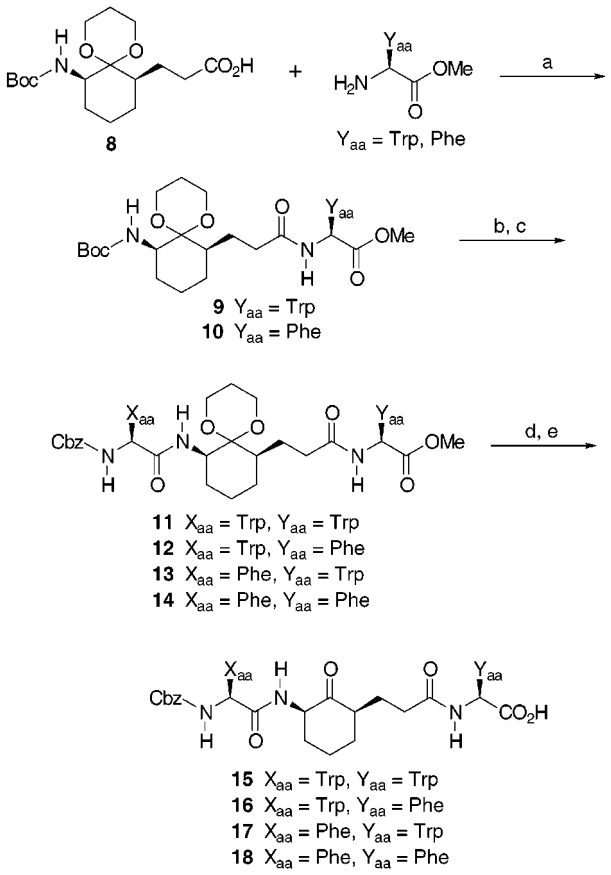


Figure 6. Assay of the Phe and Trp deconvolutions against papain. Several of the assays were performed at 1/2 or 1/4 concentration as noted on the *x*-axes.

Scheme 3[a]



[a] Reagents: (a) HBTU, DIEA; (b) TFA; (c) Cbz-X_{aa}-OH where X_{aa} = Trp and Phe, HBTU, DIEA; (d) LiOH; (e) TFA, H_2O. One of two diastereomers is shown.

amines and the C-termini of the inhibitors were protected as methyl esters. The esters were removed using basic hydrolysis near the end of the synthesis.

Compounds **15**−**18** were assayed against plasmin, cathepsin B, and papain, and the resulting inhibition data are shown in Table 1. Compound **18** has low activity against cathepsin B, with a K_i value of 1.1 mM. This result is expected based upon the low activity of the compound during the library screening (Figure 5).

Table 1. Inhibition of Proteases by Inhibitors **15**−**18**

			K_i (μM)		
compd[a]	X_{aa}	Y_{aa}	plasmin	papain	cathepsin B
15A	Trp	Trp	5 ± 0.6	380[b] ± 60	
15B			10 ± 2		
16A	Trp	Phe	100 ± 20	250[b] ± 80	
16B			150 ± 25		
17[b]	Phe	Trp	420 ± 40	490 ± 70	
18[b]	Phe	Phe	3000 ± 1000	870 ± 90	1100 ± 200

[a] **A** and **B** represent two different diastereomers. [b] Assayed as a mixture of two diastereomers.

Compounds **15**−**18** all have moderate activity against papain with inhibition constants that range from 250 to 870 μM. These values are also consistent with the assays of the library against papain (Figure 6).

Compounds **17** and **18**, both of which have Phe at the X_{aa} position, are expected to be weak inhibitors of plasmin as indicated by the low activity of the X_{aa} = Phe pool against this protease (Figure 3). Analysis of the inhibitors that have been resynthesized by solution-phase methods gives inhibition constants of 420 and 3000 μM, respectively, for these two compounds. In contrast inhibitors **15** and **16**, which have Trp at the X_{aa} position, should have good activity against plasmin as indicated by the data in Figure 4. We have used HPLC to separate the two diastereomers of each of these compounds, although we have not determined their absolute configurations. Compounds **16A**,**B** have K_i values of 100 and 150 μM, while compounds **15A**,**B** have values of 5 and 10 μM, respectively. These results demonstrate that we have been able to generate an inhibitor with good activity against plasmin, such as **15A**, by synthesizing a combinatorial library of inhibitors around a cyclohexanone nucleus that take advantage of binding interactions in both the S2 and S2′ subsites.

We have recently reported the synthesis and evaluation of a "rationally designed" inhibitor of plasmin that is based upon a tetrahydrothiopyranone ring system.[19] This inhibitor incorporates an aminoalkyl side chain at the P1 position that is designed to interact with an aspartic acid residue at the base of the S1 subsite. Plasmin is specific for substrates that have a positively charged amino acid at the P1 position, and interactions with the S1 subsite are critical for recognition and binding of both substrates and inhibitors.[20] In the tetrahydrothiopyranone-based inhibitors, we have found that if the aminoalkyl side chain at the P1 position is removed, there is a 200−300-fold decrease in potency.[19] In light of these observations, it is interesting to note that compound **15A** has significant affinity for plasmin with an inhibition constant of 5 μM, although it does not incorporate a positively charged P1-like side chain.

Using the combinatorial library described in this paper, we have discovered **15A** as a potential lead compound for developing high-affinity inhibitors of plasmin. Two modifications to the structure of **15A** are likely to increase its potency. First, we can incorporate an aminoalkyl side chain that is positioned to bind in the S1 subsite. The amide nitrogen at the 2-position of the cyclohexanone ring should be an appropriate place to attach such functionality.[19] Second, we can substitute the methylene group at the 4-position of the cyclohexanone ring with an electronegative functionality such

as S, O, or SO_2. This electronegative functionality will increase the electrophilicity of the ketone by inducing an unfavorable through-space electrostatic repulsion between the electronegative group and the dipole of the ketone.[7] We have demonstrated previously that there is a good correlation between ketone electrophilicity and inhibition constants in 4-heterocyclohexanone-based inhibitors.[7]

Conclusions

In this paper we have described the construction and screening of a combinatorial library of protease inhibitors that extend into both the S and S′ specificity sites. Using this method we have found an inhibitor that has significant affinity for the serine protease plasmin. In addition, this work has shown that combinatorial chemistry is an efficient method for probing the specificity of the S2′ subsite. Currently there is little information available concerning the binding specificity of this position for many proteases.

Our data have shown that for plasmin, the S2′ subsite prefers to bind hydrophobic and especially aromatic amino acids. Furthermore, binding of such hydrophobic residues in this site significantly increases the affinity of the inhibitor for the enzyme. On the other hand, the S2′ subsites of cathepsin B and papain do not appear to have strong preferences for any particular amino acid, and binding in this position leads to only incremental increases in affinity. Concerning the S2 subsites, the data that we have presented are consistent with the known specificity of cathepsin B and papain, which prefer hydrophobic amino acids at this position.[18] For plasmin, it has been believed that Phe binds well in the S2 subsite. However our results show that Trp, with its larger aromatic surface and potential for hydrogen bonding, provides up to an 80-fold increase in affinity when compared to Phe. Clearly, combinatorial chemistry has provided useful information concerning the binding interactions at the S2 and S2′ subsites of several proteases. A similar approach should be equally amenable to exploring the specificities of the other leaving group subsites and for incorporating nonpeptidic functionality into the inhibitors.

Experimental Section

General Methods. NMR spectra were recorded on Bruker Avance-300 or Avance-400 instruments. Spectra were calibrated using TMS ($\delta = 0.00$ ppm) for ^{1}H NMR. Mass spectra were recorded on a Kratos MS 80 under fast-atom bombardment (FAB) conditions or were performed using electrospray ionization at the Harvard University Chemistry Department Mass Spectrometry Facility. HPLC analyses were performed on a Rainin HPLC system with Rainin Microsorb silica or C18 columns and UV detection. Semipreparative HPLC was performed on the same system using semipreparative columns (21.4 × 250 mm). UV spectra were recorded on a Perkin-Elmer 8452A diode array UV−vis spectrophotometer.

Reactions were conducted under an atmosphere of dry nitrogen in oven-dried glassware. Anhydrous procedures were conducted using standard syringe and cannula transfer techniques. THF and toluene were distilled from sodium and benzophenone. Piperidine was distilled from KOH. Other solvents were of reagent grade and were stored over 4 Å molecular sieves. All other reagents were used as received. Organic solutions were dried over $MgSO_4$ unless otherwise noted. Solvent removal was performed by rotary evaporation at water aspirator pressure. Wang resins that were prederivatized with Fmoc amino acids were purchased from Calbiochem-Novabiochem Corp. Amino acids and their side chain protecting groups were used as follows: Ala, Arg(PMC), Asn(Trt), Asp(*t*-Bu), Gln(Trt), Glu(*t*-Bu), Gly, His(Trt), Hyp(*t*-Bu), Ile, Leu, Lys(Boc), Orn(Boc), Phe, Pro, Ser(*t*-Bu), Thr(*t*-Bu), Trp(Boc), Tyr(*t*-Bu), and Val.

Synthesis of the Library. Dry Wang resin that was prederivatized with the 20 Fmoc amino acids (0.2 mmol each) was combined in a flask and shaken with 125 mL of methylene chloride. The solvent was removed; the beads were dried under vacuum and then split into 20 even batches. The beads were placed into 20 1- × 10-cm Econo-Columns (Biorad) which served as synthesis vessels. The columns were fitted with Teflon stopcocks and connected to a 24-port vacuum manifold (Burdick and Jackson) that was used to drain solvents and reagents from the columns. The resin in each column was swelled in DMF; then the Fmoc protecting groups were removed by treatment with 2 mL of a 1:1 solution of DMF and piperidine for 10 min. After washing with 5 × 5 mL of DMF, a positive Kaiser test indicated the presence of free amines on the resin.

Coupling of Compound 4. To couple compound **4** to the resin, a stock solution was prepared that contained compound **4** (5.6 g, 12 mmol, 3 equiv), HBTU (4.54 g, 12 mmol, 3 equiv), diisopropylethylamine (DIEA; 2.76 mL, 16 mmol, 4 equiv), and DMF solvent in a total volume of 40 mL. Aliquots (2 mL) of this stock solution were added to each of the synthesis vessels and the reactions were gently agitated for 1.5 h. A negative Kaiser test indicated that the reaction had gone to completion. The resin was washed with 5 × 5 mL of DMF; then any unreacted amino groups were capped by treating the resin for 10 min with 2 mL of a freshly prepared stock solution that contained acetic anhydride (1.5 mL, 20 mmol, 5 equiv), DIEA (2.76 mL, 16 mmol, 4 equiv), and DMF in a total volume of 40 mL. After capping, the resin was then washed with 5 × 5 mL of DMF, and the N-terminal Fmoc group was deprotected as described above.

Coupling of the Second Amino Acid. To couple the second amino acid to the resin, the 20 synthesis vessels were treated with 3 equiv of an N-Cbz amino acid, each vessel receiving a different amino acid. The reactions also contained 3 equiv of HBTU and 4 equiv of DIEA in 2 mL of DMF and were agitated for 1.5 h. After the reactions were complete as judged by a negative Kaiser test, the resin was washed with 5 × 5 mL of DMF, 5 × 10 mL of methylene chloride, and 5 × 10 mL of MeOH, and dried under vacuum overnight.

Cleavage from the Resin and Removal of Protecting Groups. The inhibitors were cleaved from the resin and the protecting groups on the amino acid side chains were removed by treating each batch of resin with 3 mL of cleavage cocktail for 2 h. The cleavage cocktail contained 95% TFA, 2.5% water, and 2.5% triisopropylsilane. The solutions were filtered to remove the resin, and the beads were washed with 3 × 1 mL of fresh TFA. The ketal protecting groups on the cyclohexanone rings were removed by adding 3 mL of water to the TFA solutions from the previous reactions and agitating the solutions for 30 h at room temperature. After the initial addition of water to the TFA solutions, the reactions became cloudy. However, after several hours at room temperature, the reactions became homogeneous. After the reactions were complete, the TFA and water were removed and the residual material was dried at 0.02 mmHg for 48 h. The 20 batches of inhibitors were each dissolved in 1 mL of DMSO, filtered, and stored in a freezer at −48 °C. If a total yield of 50% is assumed for the synthesis, then each inhibitor stock solution contained 20 different compounds, with a concentration of 5 mM for each individual compound in the mixtures.

Deconvolution Syntheses. The solid-phase syntheses of the various deconvolutions were performed using a similar procedure as described above. The only changes that were made were that the mix and split step at the beginning was omitted and the syntheses were performed on a 0.04-mmol scale per inhibitor.

Papain Assays. Papain (EC 3.4.22.2; purchased from Sigma) was assayed using L-BAPNA as the substrate, and the reaction progress was monitored by UV spectroscopy.[11a] Initial rates were determined by monitoring the formation of *p*-nitroaniline at 412 nm from 30 to 120 s after mixing. Assay solutions contained 1.5 mM substrate, 5 mM EDTA, 5 mM cysteine, 50 mM sodium phosphate at pH 7.5, and 15% DMSO. Assays of the initial library, which constituted 20 pools of inhibitors with 20 compounds per pool, also contained a total inhibitor concentration of 1 mM (50 μM of each individual inhibitor) and were performed in triplicate. Assays of the deconvolutions contained an inhibitor concentration of 200 μM. Assays of compounds **15**−**18** contained 1.7 mM substrate and inhibitor concentrations that ranged from 2 to 500 μM. K_i values were calculated using a Dixon analysis. Data analysis was performed with the commercial graphing package Grafit (Erithacus Software Ltd.). The K_m value under these conditions was measure to be 6.8 mM. In the absence of DMSO, the K_m value has been reported as 3.2 mM in 100 mM phosphate buffer at pH 7.5.[21]

Cathepsin B Assays. Cathepsin B (EC 3.4.22.1; purchased from Sigma) was assayed using L-BAPNA as the substrate.[22] Assay solutions contained 1.5 mM substrate, 1.5 mM EDTA, 3 mM DTT, 50 mM sodium phosphate buffer at pH 7.4, and 15% DMSO. Assays of compounds **15**−**18** contained 0.7 mM substrate. All other conditions were the same as indicated for the assays of papain. The K_m value under these conditions was measure to be 3.3 mM. The K_m value in the absence of DMSO and in sodium acetate buffer at pH 5.1 is reported to be 1.3 mM.[22]

Plasmin Assays. Plasmin (EC 3.4.21.7; purchased from Sigma) was assayed using D-Val-Leu-Lys-*p*-nitroanilide as substrate.[23] Assay solutions contained 250 μM substrate, 50 mM sodium phosphate buffer at pH 7.4, and 10% DMSO. Assays of compounds **15**−**18** contained 180 μM substrate. All other conditions were the same as indicated for the assays of papain. The K_m value under these conditions was measure to be 180 μM. The K_m value in the absence of DMSO and in Tris-HCl buffer at pH 8.3 is reported to be 270 μM.[24]

Fmoc Alkene 3. To a solution of **2**[10] (9.9 g, 30.5 mmol) in CH_2Cl_2 (75 mL) was added TFA (25 mL) at 0 °C. The solution was warmed to room temperature, stirred for 1 h, and concentrated to remove the TFA. The resulting oil was redissolved in 20 mL of CH_2Cl_2 and washed with saturated Na_2CO_3 and brine (200 mL). The solution was dried over Na_2CO_3 and filtered, and then DIEA (6 mL) and FmocCl (7.89 g, 30.5 mmol) were added. This solution was stirred for 3 h, then washed with 1 N HCl, saturated Na_2CO_3, and brine, and dried. The solution was then reduced to approximately 20 mL, diluted with hexanes (200 mL), and placed in a refrigerator overnight to allow crystallization. White crystals of **3** (9.22 g, 67%) were isolated by vacuum filtration and dried in vacuo: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26−1.30 (m, 1H), 1.42−1.62 (br m, 7H), 1.62−1.90 (br m, 3H), 1.93−2.02 (m, 1H), 2.14−2.23 (m, 1H), 3.85−3.90 (m, 4H), 4.25−4.44 (m, 3H), 4.97−5.07 (m, 2H), 5.15 (br s, 1H), 5.78−5.88 (m, 1H), 7.31 (t, J = 7.4 Hz, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.62 (t, J = 6.8 Hz, 2H), 7.76 (d, J = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 18.8, 22.6, 25.1, 28.8, 31.6, 31.8, 47.3, 58.8, 59.0, 66.5, 99.1, 114.8, 119.9, 125.1, 127.0, 127.6, 138.7, 141.3, 156.4; HRMS-FAB (M + Na^+) calcd for $C_{28}H_{33}NNaO_4$ 470.2307, found 470.2322.

Fmoc Carboxylic Acid 4. To compound **3** (0.46 g, 1.1 mmol) were added 25 mL of 7:3 acetone/H_2O, $NaIO_4$ (1.1 g, 5.2 mmol), $KMnO_4$ (32 mg, 0.20 mmol), and $NaHCO_3$ (0.10 g, 1.0 mmol) and the reaction was stirred at room temperature for 7 h. The reaction was diluted with EtOAc (150 mL) and washed with 100 mL of 1 N HCl and 100 mL of brine, and the organic layer was dried over $MgSO_4$. The crude material was purified by flash chromatography (3:2 EtOAc/hexanes) to yield compound **4** (308 mg, 0.66 mmol, 63%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43−1.71 (m, 10H), 2.07−2.12 (m, 3H), 2.35−2.53 (m, 2H), 3.91 (br s, 4H), 4.14−4.30 (m, 2H), 4.37−4.48 (m, 2H), 5.17 (br s, 1H), 7.31 (t, J = 7.4 Hz, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.62 (m, 2H), 7.78 (d, J = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.8, 22.0, 25.0, 28.4, 32.1, 47.3, 59.0, 66.7, 98.8, 119.9, 125.1, 127.0 127.6, 141.3, 144.0, 156.5, 179.0; HRMS-FAB (M + Na^+) calcd for $C_{27}H_{31}NNaO_6$ 488.2049, found 488.2041.

Tryptophan Methyl Ester 9. To carboxylic acid **8** (250 mg, 0.73 mmol) were added H-Trp-OMe (250 mg, 0.87 mmol), HBTU (398 mg, 0.88 mmol), and 2 mL of DMF, followed by DIEA (0.40 mL, 1.75 mmol). The reaction was stirred for 3 h at room temperature, then diluted with 50 mL of EtOAc. The organic layer was washed with 35 mL of 1 N $KHSO_4$, 35 mL of saturated $NaHCO_3$, and 35 mL of brine and dried over $MgSO_4$. The resulting solution was concentrated by rotary evaporation, and the residual material was purified by flash chromatography (EtOAc) to yield methyl ester **9** (400 mg, 0.68 mmol, 74%): ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.30−1.75 (m, 19H), 1.76−2.00 (m, 1H), 2.05−2.10 (m, 2H), 2.15−2.40 (m, 2H), 3.12−3.21 (m, 1H), 3.62−3.70 (m, 3H), 3.72−3.95 (m, 4H), 4.72−4.79 (m, 1H), 6.89−7.21 (m, 3H), 7.35 (d, J = 8.0 Hz, 1H), 7.53−7.56 (d, J = 7.5 Hz, 1H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 18.9, 25.2, 27.4, 29.7, 33.8, 51.7, 53.7, 58.9, 79.1, 99.2, 109.8, 111.4, 118.1, 118.8, 121.4, 123.5, 127.6, 137.1, 157.2, 173.2, 175.2, 209.2; HRMS-ESI (M + Na^+) calcd for $C_{29}H_{41}N_3NaO_7$ 566.2840, found 566.2858.

Phenylalanine Methyl Ester 10. To carboxylic acid **8** (500 mg, 1.46 mmol) were added H-Phe-OMe (312 mg, 1.75 mmol), HBTU (796 mg, 1.75 mmol), and 4 mL of DMF, followed by DIEA (0.8 mL, 3.5 mmol). The procedure was identical to that used for the preparation of compound **9**. The crude material was purified by flash chromatography (2:3 hexanes/EtOAc) to yield compound **10** (1.10 g, 2.14 mmol, 74%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.20−2.83 (m, 19H), 1.77−2.00 (m, 2H), 2.01−2.34 (m, 2H), 2.93−3.02 (m, 1H), 3.22−3.14 (m, 1H), 3.55−3.71 (m, 3H), 3.74−3.86 (m, 4H), 4.66−4.74 (m, 1H), 7.22−7.33 (m, 5H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 19.0, 25.3, 27.8, 28.7, 33.8, 37.4, 51.8, 54.2, 59.0, 17.2, 99.2, 126.9, 128.5, 129.2, 137.2, 157.1, 172.7, 175.3; HRMS-ESI (M + Na^+) calcd for $C_{41}H_{48}N_4NaO_8$ 527.2732, found 527.2751.

Trp-X-Trp Ester 11. To compound **9** (370 mg, 0.68 mmol) was added a solution of CH_2Cl_2 (11.5 mL), TFA (4.5 mL), and triisopropylsilane (0.38 mL). The reaction was stirred at room temperature for 1 h; then the solvents were removed under vacuum. The crude amine was dissolved in 2 mL of DMF, and DIEA (∼0.5 mL) was added to neutralize the residual TFA and to raise the pH to 8 (as measured with moist pH paper). Cbz-Trp-OH (276 mg, 0.816 mmol), HBTU (309 mg, 0.816 mmol), and DIEA (0.28 mL, 1.6 mmol) were added to the flask and the reaction was allowed to stir for 2 h at room temperature. The workup was identical to the procedure used in the preparation of compound **9**. The crude material was purified by flash chromatography (1:5 hexanes/EtOAc) to yield **11** (0.40 g, 0.52 mmol, 76%): ^{1}H NMR (300 MHz, MeOH-d_4) δ 0.94 (t, J = 6.9 Hz, 1H), 1.12−1.65 (m, 10H), 1.75−1.90 (m, 2H), 2.10−2.14 (m, 1H), 2.20−2.29 (m, 1H), 3.11−3.20 (m, 3H), 3.28−3.30 (m, 1H), 3.50−3.70 (m, 6H), 4.50−4.58 (m, 1H), 4.75−4.85 (m, 1H), 4.98−5.08 (m, 2H), 7.01−7.35 (m, 13H), 7.51−7.61 (m, 2H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 13.5, 18.3, 18.7, 19.9, 24.9, 25.2, 27.4, 28.3, 29.1, 33.7, 51.9, 53.7, 56.4, 58.9, 60.5, 66.6, 99.8, 109.8, 111.0, 118.1, 118.4, 118.8, 121.4, 121.5, 127.7, 128.5, 137.2, 157.1, 172.9, 173.2, 173.5, 175.2; HRMS−FAB (M + H^+) calcd for $C_{43}H_{50}N_5O_8$ 764.3659, found 764.3647.

Trp-X-Phe Ester 12. Compound **10** (387 mg, 0.54 mmol) was deprotected and coupled to Cbz-Trp-OH using a procedure that was similar to that used for the preparation of compound **11**. The crude material was purified by flash chromatography (1:5 hexanes/EtOAc) to give compound **12** (255 mg, 0.391 mmol, 73%): ^{1}H NMR (300 MHz, MeOH-d_4) δ 0.82−0.91 (t, J = 6.3 Hz, 1H), 1.21−1.60 (m, 10H), 1.90−1.96 (m, 1H), 1.97−2.03 (m, 1H), 2.05−2.30 (m, 2H), 2.90−2.99 (m, 1H), 3.07−3.30 (m, 3H), 3.60−3.85 (m, 6H), 4.53 (t, J = 7.3 Hz, 1H), 4.66−4.72 (m, 1H), 5.01−5.12 (m, 2H), 7.00−7.35 (m, 14H), 7.63 (d, J = 7.7 Hz, 1H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 13.5, 18.8, 19.8, 25.3, 28.3, 33.7, 37.3, 51.8, 54.2, 59.0, 60.5, 66.6, 98.8, 110.0, 111.3, 118.4, 118.5, 121.4, 123.7, 126.9, 127.7, 128.5,

129.3, 137.1, 137.4, 172.0, 172.7, 175.3; HRMS-ESI ($M + Na^+$) calcd for $C_{41}H_{48}N_4NaO_8$ 747.4932, found 747.4967.

Phe-X-Trp Ester 13. Compound **9** (450 mg, 0.83 mmol) was deprotected and coupled to Cbz-Phe-OH (297 mg, 1.0 mmol) using a procedure that was similar to that used for the preparation of compound **11**. The crude material was purified by flash chromatography (1:4 hexanes/EtOAc) to yield **13** (280 mg, 0.38 mmol, 46%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.15−1.55 (m, 10H), 1.63−1.66 (m, 1H), 1.70−1.90 (m, 1H), 1.96−2.00 (m, 2H), 2.14−2.22 (m, 1H), 2.70−2.78 (m, 1H), 2.90−3.19 (m, 3H), 3.40 (s, 3H), 3.56−3.60 (m, 4H), 3.74 (m, 3H), 4.32−4.40 (m, 1H), 4.47−4.55 (m, 1H), 4.93−4.97 (m, 2H), 6.97−7.02 (t, J = 7.3 Hz, 1H), 7.05−7.10 (t, J = 7.9 Hz, 1H), 7.15−7.35 (m, 10H), 7.46−7.52 (m, 2H), 8.23−8.28 (m, 1H), 10.9 (s, 1H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 15.8, 20.3, 22.4, 26.4, 28.7, 35.0, 39.3, 53.4, 54.8, 57.7, 60.0, 61.4, 66.8, 100.0, 111.3, 113.1, 119.7, 120.1, 122.6, 125.2, 127.8, 128.7, 129.1, 129.4, 129.6, 129.7, 129.9, 130.0, 130.9, 137.8, 138.7, 139.9, 157.5, 172.9, 174.3; HRMS-ESI ($M + Na^+$) calcd for $C_{41}H_{48}N_4NaO_8$ 747.3367, found 747.3386.

Phe-X-Phe Ester 14. Compound **10** (302 mg, 0.60 mmol) was deprotected and coupled to Cbz-Phe-OH (244 mg, 0.82 mmol) using a procedure that was similar to that used for the preparation of compound **11**. The crude material was purified by flash chromatography (1:4 hexanes/EtOAc) to yield **14** (267 mg, 0.39 mmol, 65%): ^{1}H NMR (300 MHz, MeOH-d_4) δ 0.86−0.95 (m, 1H), 1.20−1.55 (m, 11H), 1.82−1.90 (m, 1H), 2.03−2.30 (m, 2H), 2.82−3.02 (m, 2H), 3.14−3.22 (m, 2H), 3.68−3.85 (m, 6H), 4.44−4.49 (q, J = 5.1 Hz, 1H), 4.67−4.70 (m, 1H), 5.04 (s, 2H), 7.21−7.32 (m, 13H), 7.51 (d, J = 8.3 Hz, 1H), 8.30 (d, J = 7.6 Hz, 1H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 13.5, 18.8, 24.6, 25.3, 33.7, 38.2, 51.7, 54.2, 56.9, 59.0, 59.1, 60.6, 66.6, 99.0, 126.7, 126.8, 126.9, 127.7, 128.0, 128.4, 128.5, 129.2, 129.3, 129.4, 137.2, 137.6, 157.1, 172.8, 175.2, 175.3; HRMS-ESI ($M + Na^+$) calcd for $C_{39}H_{47}N_3NaO_8$ 708.3259, found 708.3251.

Inhibitor 15. Compound **11** (0.39 g, 0.511 mmol) was dissolved in 20 mL of MeOH. To this solution was added a solution of LiOH (80 mg, 3.3 mmol) dissolved in 5 mL of water, and the reaction was stirred for 12 h at room temperature. The basic solution was neutralized with 1 N HCl to pH 7, and the solvents were removed under vacuum at 25 °C. The crude carboxylic acid was dissolved in 8 mL of TFA and 3 mL of water and the solution was stirred for an additional 12 h at room temperature. The solvents were again removed under vacuum at 25 °C and the crude material was purified by flash chromatography (7% MeOH in CH_2Cl_2). Diastereomers **15A**,**B** were separated by preparative HPLC on a silica column (96.5% CH_2Cl_2, 3.4% MeOH, 0.1% TFA). The compound had a low solubility in this solvent system, so the crude material was suspended in solvent and filtered to remove the product that did not dissolve, and the portion that remained in solution was purified by HPLC. This procedure gave **15A** (6 mg, 0.009 mmol, 2%) and **15B** (2 mg, 0.003 mmol, 1%). The large majority of the material was left in crude form and not purified. **15A**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.25−1.75 (m, 2H), 1.81−2.05 (m, 3H), 2.13−2.45 (m, 4H), 3.06−3.23 (m, 2H), 3.34−3.42 (m, 2H), 4.42−4.65 (m, 3H), 5.03−5.13 (m, 2H), 7.10−7.56 (m, 12H), 7.68 (d, J = 7.6 Hz, 1H), 7.81 (d, J = 7.8 Hz, 1H), 8.17−8.31 (m, 1H), 10.97 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_4) δ 24.1, 25.4, 27.9, 28.8, 33.2, 34.7, 49.0, 53.5, 56.3, 58.2, 65.4, 111.1, 112.2, 119.0, 121.7, 124.3, 124.7, 128.3, 129.2, 136.9, 137.9, 156.6, 172.4, 172.8, 209.1. **15B**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.92−1.22 (m, 1H), 1.20−1.29 (m, 3H), 1.52−1.60 (m, 2H), 1.63−1.70 (m, 1H) 1.91−2.07 (m, 4H), 2.25−2.34 (m, 1H) 2.74−3.08 (m, 4H), 4.22−4.39 (m, 6H), 4.84 (s, 2H), 6.83−7.31 (m, 11H), 7.42 (d, J = 7.6 Hz, 1H), 7.52 (d, J = 7.7 Hz, 1H), 7.92 (d, J = 7.4 Hz, 1H), 7.98 (d, J = 8.0 Hz, 1H), 10.70 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_4) δ 24.1, 25.5, 27.9, 28.9, 33.3, 34.8, 35.5, 46.9, 48.2, 49.1, 53.7, 56.4, 58.2, 66.1, 110.9, 112.2, 119.0, 119.4, 121.6, 124.4, 124.7, 128.0, 128.2, 128.5, 129.1, 136.9, 137.9, 156.6, 172.3, 172.9, 174.5, 194.4, 209.0; HRMS-ESI ($M + Na^+$) calcd for $C_{39}H_{41}N_5NaO_7$ 714.2900, found 714.2883 for the mixture of diastereomers **15A**,**B**.

Inhibitor 16. Compound **12** (291 mg, 0.40 mmol) was deprotected using the procedure outlined for the preparation of inhibitor **15**. The diastereomers were separated using preparative reverse-phase HPLC (40% MeCN, 60% H_2O, 0.1% TFA) to give **16A** (25 mg, 0.04 mmol, 10%) and **16B** (25 mg, 0.04 mmol, 10%). Similar to inhibitor **15**, inhibitor **16** was not very soluble in the solvent used during the purification so that a majority of the material was left in crude form and was not purified. **16A**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.15−1.20 (m, 1H), 1.25−1.35 (m, 1H), 1.36−1.60 (m, 1H), 1.65−1.90 (m, 3H), 1.97−2.13 (m, 3H), 2.13−2.25 (m, 1H), 2.35−2.40 (m, 1H), 2.80−2.96 (m, 2H), 3.05−3.21 (m, 2H), 3.34 (s, 3H), 4.32−4.50 (m, 3H), 4.95 (s, 2H), 6.95−7.47 (m, 12H), 7.64−7.67 (d, J = 7.4 Hz, 1H), 8.05 (d, J = 7.1 Hz, 1H), 8.14 (d, J = 8.0 Hz, 1H), 10.82 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.1, 25.5, 28.9, 33.3, 34.9, 35.6, 49.0, 54.2, 56.4, 58.2, 66.0, 111.0, 112.1, 119.0, 119.4, 121.7, 124.7, 127.3, 127.8, 128.2, 128.5, 129.0, 129.2, 130.0, 136.8, 138.0, 138.7, 156.6, 172.2, 172.8, 174.1, 209.0. **16B**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10−1.21 (m, 1H), 1.25−1.34 (m, 2H), 1.65−1.80 (m, 3H), 1.91−2.11 (m, 4H), 2.25−2.35 (m, 1H), 2.80−2.97 (m, 2H), 3.05−3.12 (m, 2H), 3.30 (s, 3H), 4.29−4.42 (m, 3H), 4.89−4.99 (m, 2H), 6.95−7.42 (m, 12H), 7.67 (d, J = 7.5 Hz, 1H), 8.05 (d, J = 7.4 Hz, 1H), 8.16 (d, J = 8.2 Hz, 1H), 10.82 (s, 1H), 12.61 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.2, 25.5, 28.9, 33.2, 34.6, 25.3, 37.5, 48.9, 54.1, 56.4, 58.1, 66.0, 111.1, 112.1, 119.0, 119.4, 121.7, 124.7, 127.2, 128.3, 128.5, 129.0, 129.9, 136.9, 137.8, 138.7, 156.7, 172.4, 172.9, 174.1, 209.1; HRMS-ESI ($M + Na^+$) calcd for $C_{37}H_{40}N_4NaO_7$ 675.2792, found 675.2784 for the mixture of diastereomers **16A**,**B**.

Inhibitor 17. Compound **13** (240 mg, 0.33 mmol) was deprotected using the procedure outlined for the preparation of inhibitor **15**. The purification was accomplished using preparative reverse-phase HPLC (40% MeCN, 60% H_2O, 0.1% TFA) to yield **17** (99 mg, 0.15 mmol, 45%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10−1.20 (m, 1H), 1.25−1.35 (m, 2H), 1.67−1.72 (m, 2H), 1.80−1.87 (m, 2H), 2.00−2.15 (m, 4H), 2.35−2.38 (m, 2H), 2.72−2.79 (m, 2H), 2.94−3.01 (m, 2H), 3.14 (d, J = 4.6 Hz, 1H), 3.18−3.20 (m, 1H), 4.35−4.49 (m, 2H), 4.95 (s, 1H), 6.95−7.35 (m, 11H), 7.50−7.55 (m, 2H), 8.10 (q, J = 3.4 Hz, 1H), 8.82 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 23.1, 24.1, 25.5, 27.9, 33.3, 34.7, 35.3, 35.6, 49.1, 53.7, 57.0, 58.2, 66.0, 110.9, 112.2, 119.0, 121.8, 124.3, 127.1, 128.1, 128.2, 128.8, 129.1, 130.1, 136.9, 137.9, 138.8, 139.1, 156.6, 156.7, 171.8, 172.2, 172.9, 174.4, 207.3, 209.1; HRMS-ESI ($M + Na^+$) calcd for $C_{37}H_{40}N_4NaO_7$ 675.2792, found 675.2797.

Inhibitor 18. Compound **14** (271 mg, 0.40 mmol) was deprotected using the procedure outlined for the preparation of inhibitor **15**. The purification was accomplished using preparative reverse-phase HPLC (40% MeCN, 60% H_2O, 0.1% TFA) to yield **18** (100 mg, 0.15 mmol, 39%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.65−1.80 (m, 3H), 2.00−2.10 (m, 3H), 2.35−2.42 (m, 3H), 2.95−3.10 (m, 2H), 4.30−4.44 (m, 2H), 4.95 (s, 2H), 7.20−7.30 (m, 13H), 7.56 (d, J = 8.3 Hz, 1H), 8.03−8.16 (m, 1H), 12.40 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.2, 25.5, 33.3, 33.4, 33.7, 34.9, 35.7, 38.6, 49.0, 54.1, 57.0, 58.1, 66.0, 71.0, 127.1, 127.2, 128.2, 128.4, 129.0, 130.1, 137.9, 138.7, 138.9, 139.1, 156.7, 164.2, 171.8, 172.1, 172.7, 209.0; HRMS-ESI ($M + Na^+$) calcd for $C_{35}H_{39}N_3NaO_8$ 636.2684, found 636.2654.

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Supporting Information Available: HPLC characterization for compounds **15**−**18**. This material is available free of charge via the Internet at http://pubs.cas.org.

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