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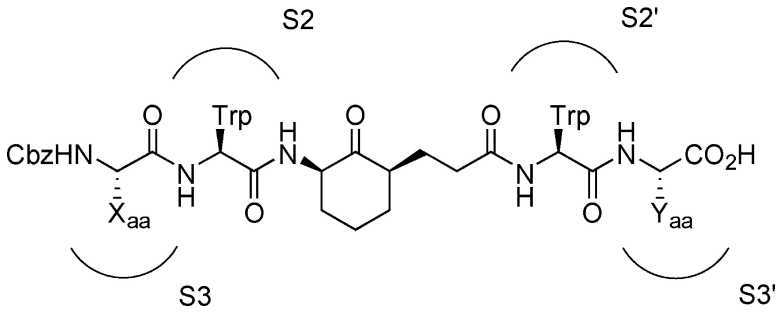

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Selective Inhibitors of the Serine Protease Plasmin: Probing the S3 and S3′ Subsites Using a Combinatorial Library

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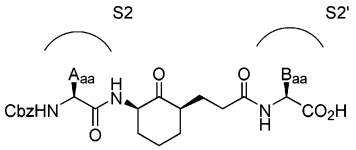
A combinatorial library of 400 serine protease inhibitors with the general structure Cbz-X_{aa}-Trp-cyclohexanone-Trp-Y_{aa}-OH has been constructed. The library was synthesized on the solid phase using mix-and-split synthesis, where 20 different amino acids were incorporated at both the X_{aa} and Y_{aa} positions. These two positions correspond to the S3 and S3′ subsites of the active site. Iterative deconvolution was used to identify hits from the library. The library was screened against four serine proteases: plasmin, kallikrein, thrombin, and trypsin. Seven inhibitors from the library that showed promising activities were resynthesized using solution-phase methods. Four of these compounds were good inhibitors of plasmin with IC_{50} values in the range of 2.7−3.6 μM. The most potent of these inhibitors showed >150-fold selectivity for plasmin when compared to the other three serine proteases.

Introduction

Proteases regulate a host of physiological processes including digestion, fertilization, growth, cellular migration, immunological defense, blood pressure, wound healing, and remodeling of the extracellular matrix (ECM).[1] Plasmin is a serine protease that plays an important role during ECM remodeling by degrading a number of ECM components such as fibrin, fibronectin, laminin, and proteoglycans.[2] In addition to its direct role in this process, plasmin also plays an indirect role by activating the matrix metalloproteases (MMPs) MMP-1, 3, and 9.[3] These MMPs in turn regulate matrix deposition and remodeling and also stimulate the release of several bioactive growth factors. During the past decade, a number of studies have shown that ECM remodeling is one of key processes associated with angiogenesis, tumor growth, and invasion.[4,5] Therefore, potent and selective inhibitors of plasmin may have potential as chemotherapeutic agents that impede angiogenesis and block the rapid growth of primary tumors and the spread of secondary metastases.

There are several endogenous inhibitors of plasmin, including α-macroglobulin[6] and α-antiplasmin,[7] that regulate its biological activity.[8,9] In addition, a number of small molecule inhibitors have been developed. For example, ϵ-aminocaproic acid[10] and *trans*-4-aminocyclohexane carboxylic acid[11] are two inhibitors that have seen use in the clinic. These compounds act by blocking the lysine binding site of plasmin.[12,13] Okada and coworkers have developed a variety of synthetic inhibitors that are targeted to plasmin's active site that show good potency and selectivity.[14−21]

Over the past several years our group has investigated a series of cyclohexanone-based inhibitors **1** that have been targeted to serine and cysteine proteases.[22−24]



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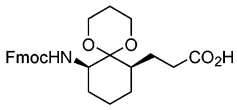

2

The ketone in the cyclohexanone ring is designed to react with the Ser or Cys active site nucleophile to give a reversibly formed hemiketal or hemithioketal linkage. In one study, we demonstrated the formation of such an intermediate in the active site of the cysteine protease papain.[25] The amino acid side chains at the A_{aa} and B_{aa} positions of the inhibitor were designed to bind in the S2 and S2′ subsites of the protease target. We constructed a combinatorial library around the general structure **1** in order to investigate the specificity of these two subsites in several proteases.[26] The library was synthesized using solid-phase methods and made use of compound **2** as a key synthon that incorporated the ketone in protected form and that was amenable to Fmoc-based peptide synthesis. These studies revealed that for plasmin, Trp was preferred at both the S2 and S2′ subsites.

In the present work, we describe the preparation and screening of a second generation library of inhibitors with the general structure **3** (Figure 1). The compounds contain the centrally positioned Trp-cyclohexanone-Trp fragment that emerged from screening the first generation library against plasmin.[26] The X_{aa} and Y_{aa} diversity elements, which are targeted to the S3 and S3′ subsites, are varied among 20 different amino acid side chains. The library has been screened against plasmin and three other related serine proteases: kallikrein, thrombin, and trypsin. This study has three major objectives. They are (1) To improve the potency of the plasmin inhibitors by extending noncovalent binding interactions into the S3 and S3′ subsites; (2) to investigate the specificity of the S3 and S3′ subsites in plasmin; and

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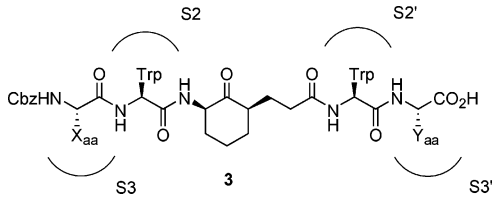


Figure 1. General structure of the compounds in the library. X_{aa} and Y_{aa} are each one of 20 amino acids.

(3) to generate inhibitors that are highly selective for plasmin over other related proteases. To accomplish this third objective, we have focused our attention on portions of the inhibitor that do not interact with the primary recognition element of plasmin, which is the S1 subsite.

Result and Discussion

Synthesis of the Cyclohexanone Fragment. Before we began construction of the library, we first prepared the central cyclohexanone portion of the inhibitors in a form that was suitably protected for solid-phase peptide synthesis. The synthesis of compound **2** is shown in Scheme 1 and began with double deprotonation of ketoester **4** followed by alkylation of the more reactive enolate with 4-bromo-1-butene to give alkene **5** as a mixture of ketone and enol tautomers. Protection of the ketone with 1,3-propanediol in the presence of TMSCl yielded ketal **6**, in which the ester and butene substituents on the cyclohexane ring are oriented in the thermodynamically favored *cis*-1,3-diequitorial arrangement.[27] Saponification of the ethyl ester in **6** gave carboxylic acid **7**. This compound was treated with diphenylphosphoryl azide and diisopropylethylamine (DIEA) to generate the acyl azide, which upon heating underwent the Curtius rearrangement. The resulting isocyanate was trapped with potassium *tert*-butoxide to give the corresponding Boc-protected amine **8**. The Boc protecting group was removed with TFA, and the resulting amine was treated with FmocCl to yield Fmoc-protected amine **9**. Finally, oxidative cleavage of the alkene with $KMnO_4$ and $NaIO_4$ gave protected amino acid **2**. The synthesis and characterization of compounds **2** and **9** have been reported previously.[26]

Design and Synthesis of the Library. We used the mix-and-split strategy to prepare the library and incorporated 20 amino acids at both the X_{aa} and Y_{aa} positions in compound **3** (Figure 1) to give a total library size of 400 compounds.[28,29] The initial library was prepared as 20 pools that each contained 20 inhibitors. The compounds in each pool incorporated a single defined amino acid at the X_{aa} position, and a randomized mixture of all twenty amino acids at the Y_{aa} position. We used the iterative deconvolution strategy, which was developed by Houghten,[30] to identify active compounds.

The solid-phase synthesis of the library is shown in Scheme 2. Twenty batches of Wang resin that each had been preloaded with an Fmoc-protected amino acid were mixed together to randomize the Y_{aa} position of the inhibitors (compound **10**). Three cycles of Fmoc group deprotection and peptide coupling reactions were performed sequentially with Fmoc-Trp-OH, compound **2**, and Fmoc-Trp-OH to yield compound **11**. The resin was then separated into 20 equal portions, the Fmoc group was removed, and each portion was coupled to a different Cbz-protected amino acid to give compound **12**. The inhibitors were cleaved from the resin, and the protecting groups on the amino acid side chains were removed with TFA. Finally, the ketal was hydrolyzed to the corresponding ketone with aqueous TFA to give 20 pools of inhibitors, each containing a mixture of 20 amino acids at the Y_{aa} position, and a single defined amino acid at the X_{aa} position. After biological evaluation of this initial library, we performed a deconvolution of the most active pool by individually synthesizing and evaluating the 20 members in that pool. A similar solid-phase synthesis procedure was used in the deconvolution process, except that the mix-and-spit step was omitted.

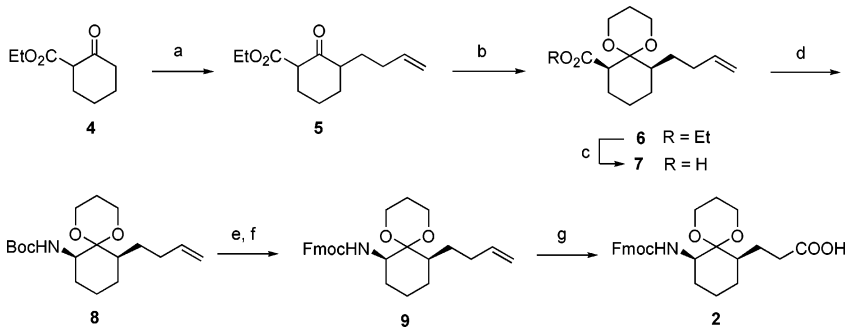
Assay of the Library. Serine proteases are generally classified into three different families based upon their substrate specificity at the P_1 position of peptide substrates. Trypsin-like serine proteases prefer to bind substrates with positively charged amino acids at the P_1 position such as Lys and Arg. Elastase-like enzymes bind small hydrophobic residues such as Ala or Val, while chymotrypsin-like proteases are specific for large hydrophobic residues such as Phe, Tyr, or Leu. Designing inhibitors that are specific for one particular protease within a family can be a challenging prospect since one of the dominant features of substrate specificity for all of the enzymes within that family is the identity of the P_1 residue.

We screened the initial library against four different serine proteases: plasmin, kallikrein, thrombin, and trypsin. All of these enzymes are trypsin-like proteases that prefer to bind positively charged side chains at P_1. We purposely selected four enzymes that are in the same family in order to investigate the potential of this library to generate inhibitors that are specific for plasmin over other closely related enzymes. We had a reasonable hope of discovering inhibitors with high specificity since the library was designed to maximize interactions with the S2, S3, S2′ and S3′ binding sites. Unlike most other protease inhibitors, this library does not have a functional group that is targeted toward the S1 subsite. In our previous studies we have found that, for this class of inhibitors, plasmin prefers to bind Trp at both the S2 and S2′ subsites.[10] Since we have incorporated Trp at both the P2 and P2′ positions in this inhibitor library, we expected that the inhibitors would have better activity against plasmin than the other three protease.

The initial library was screened against the four proteases with a total inhibitor concentration in each pool of 5 μM. This corresponds to a concentration of 250 nM for each of the 20 inhibitors in a pool. The assays were performed in 50 mM phosphate buffer at pH 7.4 using *p*-nitroanilide substrates, and initial rates were measured by UV spectroscopy. The assays contained a DMSO concentration of 10% in order to ensure the solubility of all of the inhibitors.

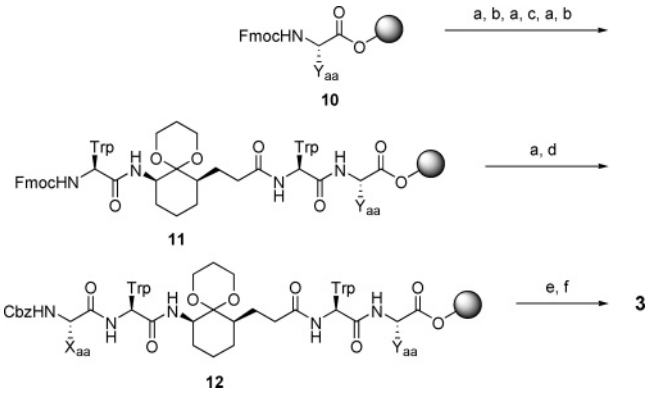
As shown in Figure 2, none of the inhibitor pools showed higher than 20% inhibition against kallikrein, thrombin, or trypsin. By contrast, several of the pools had good activity against plasmin including pools with the X_{aa} position defined as Leu (33% inhibition), Phe (43% inhibition), and Trp (73% inhibition). This result suggests that plasmin prefers to bind aromatic and large

Scheme 1[a]



[a] Reagents: (a) (i) LDA (2 equiv), (ii) 4-bromo-1-butene; (b) 1,3-propanediol, TMSCl; (c) NaOH, MeOH; (d) (i) $(PhO)_2PON_3$, DIEA, (ii) *t*-BuOK; (e) TFA; (f) FmocCl, DIEA; (g) $NaIO_4$, $KMnO_4$, $NaHCO_3$. Only one of the two enantiomers is shown.

Scheme 2[a]



[a] Reagents: (a) piperidine; (b) Fmoc-Trp-OH, HBTU, DIEA; (c) **2**, HBTU, DIEA; (d) Cbz-X_{aa}-OH, HBTU, DIEA; (e) TFA; (f) TFA, H_2O. One of two diastereomers is shown for **11**, **12,** and **3**.

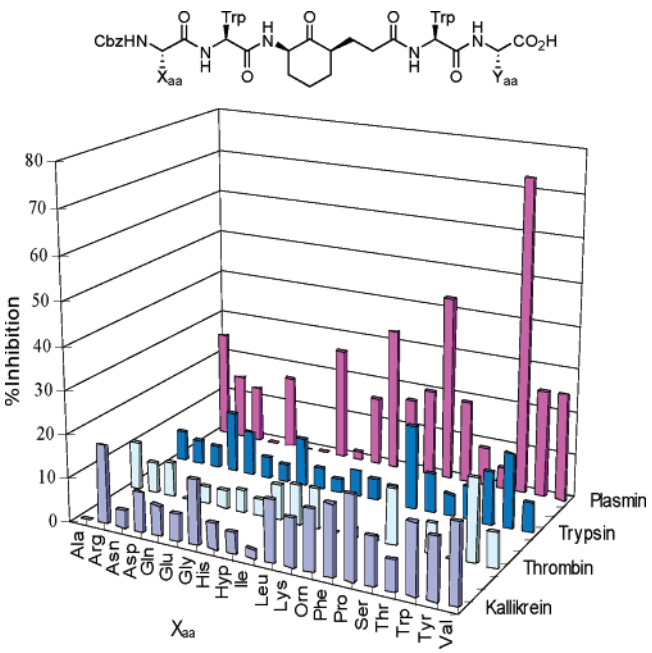


Figure 2. Assay of 20 pools that each contains 20 inhibitors against plasmin, trypsin, thrombin and kallikrein. Each bar represents the activity of a pool that contains inhibitors in which the X_{aa} position is defined by the amino acids on the *x*-axis of the graph, and Y_{aa} is a mixture of 20 different amino acids. The data represent the average of two measurements. The error in the assays is approximately ±5%.

hydrophobic amino acid side chains in the S3 subsite. It also confirms our supposition that the inhibitors would be specific for plasmin over the other three

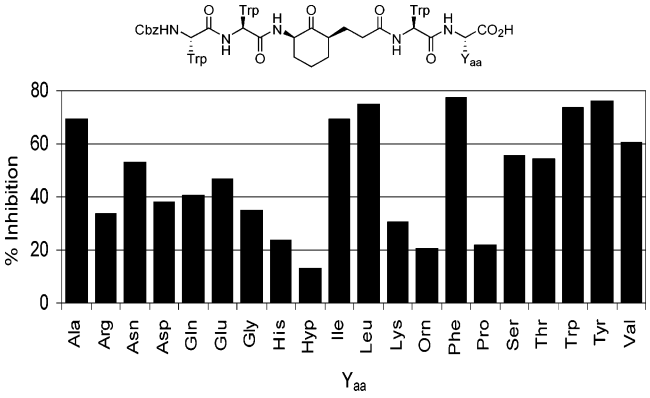


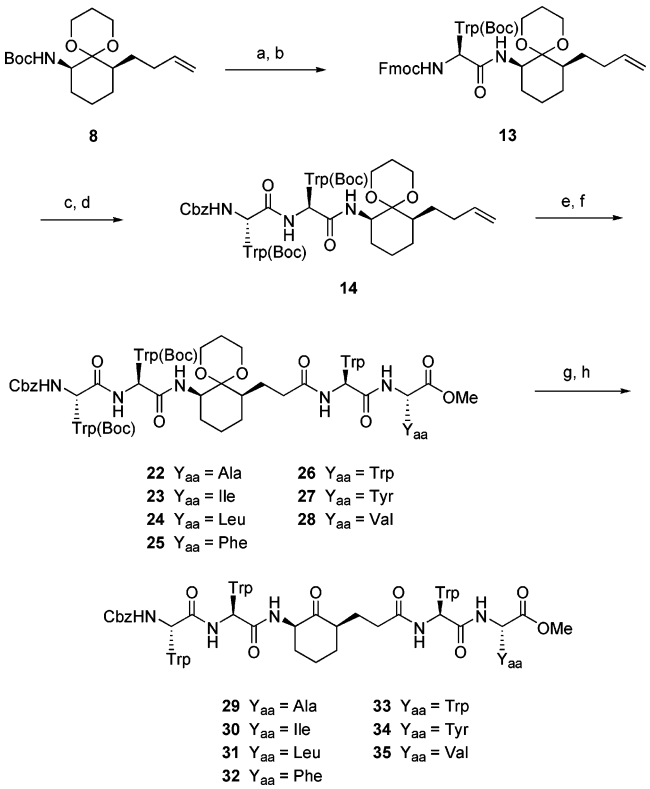
Figure 3. Assay of the X_{aa} = Trp deconvolution against plasmin. Each bar represents the assay of a single compound with the amino acid at the Y_{aa} position defined by the *x*-axis of the plot.

trypsin-like serine proteases. Amino acids that are particularly disfavored at the S3 subsite include Asp, Glu, and Gly.

Upon the basis of the results from screening of the initial library, we chose the X_{aa} = Trp pool for deconvolution. The 20 inhibitors in this pool were each synthesized individually using the solid-phase strategy shown in Scheme 2. The activities of these inhibitors were examined at 5 μM concentration against plasmin (Figure 3). Four of the compounds gave >70% inhibition at this concentration, including those with Leu, Phe, Trp, and Tyr at the Y_{aa} position. This observation is consistent with data reported by Tanaka,[37] and indicates that, within the context of these cyclohexanone-based inhibitors, plasmin prefers to bind hydrophobic and aromatic amino acids at both the S3′ and S3 subsites. Three other compounds gave good activity (>60% inhibition) including those with Ala, Ile, and Val at the Y_{aa} position. Polar amino acids such as Orn and His and conformationally constrained residues such as Pro and Hyp (hydroxyproline) gave low activity and are detrimental to binding in the S3′ subsite.

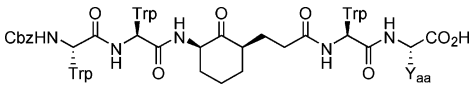
Resynthesis and Evaluation of Inhibitors 29−35. The assay results from our library screens prompted us to select seven inhibitors (compounds **29**−**35**, Scheme 3) to resynthesize and evaluate more completely against the proteases. We began the synthesis by removing the Boc protecting group from ketal **8** and coupling the resulting primary amine with Fmoc-Trp(Boc)-OH to give compound **13** (Scheme 3). Fmoc deprotection and coupling with Cbz-Trp(Boc)-OH yielded intermediate **14**. The alkene group in **14** was subjected to oxidative

Scheme 3[a]



[a] Reagents: (a) TFA; (b) Fmoc-Trp(Boc)-OH, HBTU, DIEA; (c) piperidine; (d) Cbz-Trp(Boc)-OH, HBTU, DIEA; (e) $NaIO_4$, $KMnO_4$, $NaHCO_3$; (f) H_2N-Trp-Y_{aa}-OMe where Y_{aa} = Ala (**15**), Ile (**16**), Leu (**17**), Phe (**18**), Trp (**29**), Tyr(*t*Bu) (**20**), or Val (**21**), HATU, DIEA; (g) TFA; (h) TFA, H_2O. One of two diastereomers is shown for compounds **13**−**14** and **22**−**35**.

Table 1. Inhibition of Plasmin by Inhibitors **29**−**35**



inhibitor[a]	Y_{aa}	IC_{50} (μM)
29	Ala	6.6 ± 0.6
30	Ile	13.5 ± 1.8
31	Leu	3.2 ± 0.3
32	Phe	3.3 ± 0.3
33	Trp	3.6 ± 0.2
34	Tyr	2.7 ± 0.2
35	Val	14.1 ± 1.3

[a] Assayed as a mixture of two diastereomers.

cleavage with $KMnO_4$ and $NaIO_4$, and the resulting carboxylic acid was coupled with seven different dipeptides with the general structure H_2N-Trp-Y_{aa}-OMe (compounds **15**−**21**) using HATU at 60 °C to generate compounds **22**−**28**. Finally, the protecting groups on the amino acid side chains were removed with TFA, and the ketal protecting group on the cyclohexanone core was removed using aqueous TFA to give inhibitors **29**−**35**. These final inhibitors were purified to homogeneity by reverse phase HPLC.

Compounds **29**−**35** were assayed against plasmin, and the results are presented in Table 1. We evaluated the inhibitors as mixtures of the two diastereomers that are generated during their syntheses. In previous studies, we have found that similar pairs of diastereomers have only modest differences in activity. For example, the two diastereomers of compound **1**, where A_{aa} and B_{aa} are both the side chain of Trp, show only a 2-fold difference in potency.[26] Inhibitors **29**, **30**, and **35**, which gave between 60% and 70% inhibition during the deconvolution process (Figure 3), had IC_{50} values in the range of 6.6−14.1 μM. Among the seven inhibitors in this series, these compounds gave the lowest activity in both the deconvolution step and in our examination of the purified inhibitors. This correlation demonstrates that the data that we obtained during the initial library screening and deconvolution procedures using crude inhibitors was reliable enough to predict the activity of the purified compounds. Inhibitors **30** and **35** were the weakest of the seven inhibitors by a significant margin. Both of these compounds incorporate a β-branched amino acid at the Y_{aa} position, suggesting that β-branching is an unfavorable characteristic for binding in the S3′ subsite of plasmin. By contrast, compounds **31**−**34** are all good inhibitors with IC_{50} values that range from 2.7 to 3.6 μM. This observation confirms our conclusion from the deconvolution process that plasmin prefers to bind large hydrophobic and aromatic side chains in the S3′ pocket.

Table 2. Inhibition of Serine Proteases by Inhibitor **34**[a]

serine protease	IC_{50} (μM)
plasmin	2.7 ± 0.2
kallikrein	>400[b]
thrombin	>400[b]
trypsin	>400[b]

[a] Mixture of two diastereomers. [b]No inhibition observed at this concentration.

Compound **34**, with an IC_{50} value of 2.7 μM, is the best plasmin inhibitor that we have identified through this library screening process. To examine the specificity of this compound, we also examined its activity against the other three trypsin-like serine proteases (Table 2). The assay solutions contained 10% DMSO in order to maintain solubility of the inhibitors. Under these conditions the limit of solubility of compound **34** is approximately 400 μM. At this concentration we observed no inhibition of kallikrein, thrombin, and trypsin, showing that compound **34** has >150-fold selectivity for plasmin over the other three enzymes, even though all four of them are members of the same family of serine proteases.

The data presented in Figure 2 show that none of the compounds in the library have high activity against kallikrein, thrombin, or trypsin. This observation is reasonable, since the library is based around a core structure that incorporates Trp at both P2 and P2′. Data from the literature indicate that, in their S2 subsites, kallikrein,[31,32] thrombin,[33,34] and trypsin[35] prefer to bind Phe, Pro, and Asn/Thr/His, respectively. At S2′, kallikrein prefers Arg, trypsin prefers Trp/Phe,[36] while thrombin does not show a strong preference at this position. Therefore, none of the inhibitors provide a complete match to the binding preferences of these proteases.

At first glance, the structure of compound **34** is a little surprising since it incorporates Trp residues in three out of the four amino acid sites, with the fourth being occupied by Tyr. It is a very hydrophobic structure, which might suggest that its binding interactions with an enzyme active site would be predominantly through nonspecific hydrophobic interactions. However, if this

were the case, then compound **34** should have little selectivity for a particular active site and would likely interact similarly with a number of different enzymes. Since we observe high selectivity for plasmin over other similar enzymes, we believe that **34** must be making specific hydrogen bonding, aryl−aryl, and hydrophobic interactions with amino acids that make up the S3, S2, S2′, and S3′ subsites of plasmin.

The inhibitors shown in Table 1 have molecular weights that are in the range of 1000 Da and are peptidic in nature. Thus, it is unlikely that these specific compounds would have significant bioavailability. However, since they are designed to control remodeling and degradation of the extracellular matrix the inhibitors do not need to penetrate through the cell membrane. One way to improve their pharmacokinetic characteristics would be to replace the amide bonds by appropriate nonhydrolyzable analogues to decrease their susceptibility to hydrolysis by proteases.

Conclusions

In summary, we have prepared and screened a library of inhibitors that was designed to probe the specificity of the S3 and S3′ binding sites of plasmin. The central portion of the inhibitors is made up of a Trp-cyclohexanone-Trp core structure, which originated from our previous investigations of the binding preferences of the S2 and S2′ subsites. From our screening studies of this library we conclude that both the S3 and S3′ subsites prefer to bind large hydrophobic and aromatic side chains. For these inhibitors, Trp and Tyr are the preferred amino acids at the P3 and P3′ sites, respectively. Both conformationally constrained and polar amino acids are detrimental to binding at the P3 position, while β-branched amino acids are less favorable at P3′ than residues such as Tyr and Leu. The best inhibitor that we have discovered from this library, compound **34**, has an IC_{50} value of 2.7 μM and very high selectivity for plasmin over other related serine proteases.

This work highlights a strategy for designing inhibitors with high specificity for one particular member of a family of enzymes. We have disregarded the primary recognition element of the trypsin-like serine proteases, which in this example is the S1 subsite that prefers to bind positively charged residues. Instead, we have concentrated our design and optimization efforts on regions of the active site that are usually less important, but also less homologous in their specificity, among this family of enzymes. As a result we have been able to develop an inhibitor with both good activity and high specificity for our chosen target, plasmin.

Experimental Section

Enzyme Assays. IC_{50} values for inhibitors **29**−**35** were measured for the serine proteases plasmin, kallikrein, thrombin, and trypsin using the chromogenic substrates D-Val-Leu-Lys-*p*NA, d-Pro-Phe-Arg-*p*NA, D-Phe-Pip-Arg-*p*NA, and D-Phe-Pip-Arg-*p*NA, respectively (*p*NA = *p*-nitroanilide). Enzymes and substrates were purchased from Sigma or Chromogenix (distributor DiaPharma Group, Inc.) and used as received without further purification. All proteases were assayed at 25 °C in a 50 mM sodium phosphate buffer (pH 7.4) with or without inhibitors. A final concentration of 10% DMSO was used in the assay mixtures to ensure solubility of the inhibitors. Initial rates of the enzymatic reactions were determined by monitoring the formation of *p*-nitroaniline at 405 nm from 30 to 120 s after mixing on a Perkin-Elmer 8452A diode array UV−vis spectrometer. When measuring the IC_{50} of inhibitors, the substrate concentrations were held constant at its K_M values, which were measured to be 167, 135, 45, and 122 μM for the respective substrates plasmin, kallikrein, thrombin, and trypsin. Data analysis was performed with the commercial graphing package Grafit (Erithacus Software Ltd.).

Alkene 5. To a solution of diisopropylamine (8.42 mL, 6.06 g, 60.0 mmol) in THF (60 mL) was added *n*-butyllithium (23.5 mL, 58.8 mmol, 2.5 M in hexanes) at −78 °C under an atmosphere of nitrogen. The temperature of the solution was slowly increased to 0 °C and maintained at that temperature for an additional 10 min. To this solution was slowly added ketoester **4** (5.0 g, 29.4 mmol). After 15 min, 4-bromo-1-butene (3.80 mL, 5.34 g, 44.0 mmol) was added dropwise. The reaction was stirred at room temperature for 30 h and then quenched with water. The THF was removed by rotary evaporation, and the mixture was partitioned between EtOAc (500 mL) and 1 N HCl (250 mL). The organic layer was washed with 1 N HCl (250 mL), saturated $NaHCO_3$ (250 mL), and brine (250 mL). It was then dried over $MgSO_4$, and the resulting solution was concentrated by rotary evaporation. The crude oil was purified by flash chromatography (EtOAc:hexanes 1:18) to yield **5** as a mixture of ketone and enol tautomers (5.16 g, 22.9 mmol, 78%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12−1.34 (m, 3.8H), 1.35−1.55 (m, 2.7H), 1.56−1.83 (m, 2.1H), 1.84−1.99 (m, 1.4H), 2.00−2.24 (m, 4.0H), 2.25−2.43 (m, 1.1H), 4.05−4.33 (m, 2.0H), 4.85−5.10 (m, 2H), 5.60−5.90 (m, 1H), 12.42 (s, 0.7H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.07, 14.12, 14.23, 20.0, 21.7, 22.8, 24.1, 27.1, 28.1, 28.9, 30.3, 30.8, 31.0, 31.1, 31.2, 33.6, 34.2, 37.9, 48.7, 50.1, 56.1, 57.9, 60.1, 60.8, 61.1, 97.7, 114.7, 114.79, 114.81, 138.1, 138.2, 138.4, 169.9, 170.0, 172.9, 174.6; HRMS−FAB ($M + Na^+$) calcd for $C_{13}H_{21}O_3$ 225.1491, found 225.1481.

Ketal 6. A solution of compound **5** (5.0 g, 22.2 mmol) in THF (10 mL) was cooled in an ice bath. To this solution, 1,3-propanediol (30 mL, 31.7 g, 417 mmol) and TMSCl (5.57 mL, 4.8 g, 44.4 mmol) were added. The reaction was stirred at room temperature for 48 h and then partitioned between EtOAc (500 mL) and saturated $NaHCO_3$ (400 mL). The organic layer was washed with saturated $NaHCO_3$ (400 mL) and brine (400 mL). It was then dried over $MgSO_4$, and the resulting solution was concentrated by rotary evaporation. The crude oil was purified by flash chromatography (EtOAc:hexanes 1:18) to yield **6** (6.0 g, 19.8 mmol, 89%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16−1.39 (m, 5H), 1.40−2.06 (m, 10H), 2.07−2.35 (m, 2H), 3.28−3.60 (br s, 1H), 3.75−3.96 (m, 3H), 4.00−4.28 (m, 3H), 4.86−5.10 (m, 2H), 5.60−5.90 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.7, 20.4, 25.6, 25.9, 26.5, 27.1, 32.4, 59.5, 59.7, 60.5, 99.2, 114.5, 139.8, 173.0; HRMS−FAB ($M + Na^+$) calcd for $C_{16}H_{26}NaO_4$ 305.1729, found 305.1740.

Carboxylic Acid 7. To a solution of compound **6** (6.0 g, 19.7 mmol) in MeOH (50 mL) was added 2 N aqueous NaOH (50 mL). The reaction was heated at reflux for 24 h and then cooled to room temperature. The MeOH was removed by rotary evaporation. The resulting aqueous solution was washed with EtOAc (50 mL), the organic phase was discarded, and the aqueous phase was partitioned between EtOAc (500 mL) and 1 N HCl (300 mL). The organic layer was washed with brine (300 mL) and then dried over $MgSO_4$. The solvent was removed by rotary evaporation. The crude product was recrystallized from a mixture of EtOAc and hexanes to yield carboxylic acid **7** as a white solid (4.53 g, 16.4 mmol, 83%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.10−1.61 (m, 7H), 1.61−2.00 (m, 4H), 2.01−2.23 (m, 1H), 2.24−3.08 (m, 2H), 3.68−4.10 (m, 4H), 4.80−5.06 (m, 2H), 5.60−5.87 (m, 1H), 10.35−11.08 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.2, 24.8, 25.2, 25.5, 32.0, 59.5, 59.7, 100.7, 115.4, 138.7, 174.7; HRMS−FAB ($M + Na^+$) calcd for $C_{14}H_{22}NaO_4$ 277.1416, found 277.1410.

Carbamate 8. Carboxylic acid **7** (3.0, 10.8 mmol) was dissolved in toluene (50 mL). To this solution were added DIEA (2.3 mL, 1.7 g, 13.0 mmol) and $(PhO)_2PON_3$ (2.8 mL, 3.6 g, 13.0 mmol). The reaction was heated at 85 °C under nitrogen for 16 h and then cooled to room temperature. To a separated

flask containing a solution of potassium *tert*-butoxide (2.4 g, 21.6 mmol) in THF (150 mL) at 0 °C was added the isocyanate solution dropwise. The reaction was allowed to warm to room temperature over 30 min, and then it was quenched with water (30 mL). The THF was removed by rotary evaporation, and the resulting material was partitioned between EtOAc (300 mL) and 1 N HCl (300 mL). The organic layer was washed with 1 N HCl (200 mL), saturated $NaHCO_3$ (200 mL), and brine (200 mL). It was then dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The crude oil was purified by flash chromatography (EtOAc:hexanes 1:9) to give compound **8** (3.2 g, 9.3 mmol, 86%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20−1.39 (m, 3H), 1.40−1.47 (m, 10H), 1.48−1.63 (m, 3H), 1.64−1.81 (m, 3H), 1.84−2.28 (m, 3H), 3.70−3.95 (m, 4H), 3.96−4.30 (br s, 1H), 4.72−4.92 (br s, 1H), 4.93−5.10 (m, 2H), 5.70−5.95 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.3, 25.5, 28.8, 32.2, 59.26, 59.33, 79.4, 99.4, 115.0, 139.3, 156.4; HRMS−FAB (M + Na^+) calcd for $C_{18}H_{31}NNaO_4$ 348.2151, found 348.2158.

Amide 13. Compound **8** (1.0 g, 2.9 mmol) was dissolved in CH_2Cl_2 (16 mL). To this solution was added TFA (8 mL). The reaction was stirred at room temperature for 30 min. The solvents were removed by rotary evaporation, and the residue was diluted with EtOAc (200 mL). The organic layer was washed with saturated aqueous Na_2CO_3 (100 mL) and brine (100 mL) and then dried over Na_2CO_3. The solvent was removed by rotary evaporation to give the corresponding primary amine. The resulting amine was redissolved in DMF (5 mL). To this solution were added Fmoc-Trp(Boc)-OH (2.3 g, 4.4 mmol), HBTU (1.7 g, 4.4 mmol), and DIEA (1.5 mL, 1.1 g, 8.8 mmol). The reaction was stirred at room temperature for 1 h, and then the reaction mixture was partitioned between EtOAc (250 mL) and 1 N HCl (150 mL). The organic layer was washed with 1 N HCl (150 mL), saturated $NaHCO_3$ (150 mL), and brine (150 mL). It was then dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The crude oil was purified by flash chromatography (EtOAc:hexanes 2:1) to yield **13** as a mixture of two diastereomers (1.5 g, 2.0 mmol, 69%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30−1.50 (m, 7H), 1.51−1.70 (m, 10H), 1.82−2.23 (m, 3H), 2.97−3.50 (m, 2H), 3.52−3.89 (m, 4H), 4.20−4.68 (m, 4H), 4.90−5.15 (m, 2H), 5.60−6.18 (m, 2H), 7.28−7.66 (m, 9H), 7.67−7.92 (m, 3H), 8.04−8.30 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.6, 18.9, 21.1, 23.1, 25.2, 25.4, 25.7, 28.4, 28.58, 28.60, 29.3, 30.0, 32.0, 32.12, 32.15, 35.1, 47.5, 47.6, 55.0, 55.6, 59.08, 59.13, 59.21, 59.24, 67.5, 77.7, 83.9, 99.0, 115.2, 115.7, 116.1, 119.5, 119.8, 120.4, 123.2, 123.3, 124.9, 125.08, 125.13, 125.5, 125.6, 127.5, 128.1, 130.2, 138.97, 139.04, 141.7, 144.18, 144.22, 149.9, 156.3, 170.0, 170.6; HRMS−FAB (M + Na^+) calcd for $C_{44}H_{51}N_3NaO_7$ 756.3625, found 756.3610.

Amide 14. Compound **13** (1.2 g, 1.6 mmol) was stirred in a 1:1 mixture of piperidine and DMF (20 mL) at room temperature for 1 h. The solvents were removed by rotary evaporation. The resulting residue was dried under vacuum for 12 h to remove residual piperidine. The white solid was then redissolved in DMF (5 mL). To this solution were added Cbz-Trp(Boc)-OH (1.1 g, 2.4 mmol), HBTU (900 mg, 2.4 mmol), and DIEA (840 μL, 624 mg, 4.8 mmol). The reaction was stirred at room temperature for 2 h, and the mixture was partitioned between EtOAc (250 mL) and 1 N HCl (150 mL). The organic layer was washed with 1 N HCl (150 mL), saturated aqueous $NaHCO_3$ (150 mL), and brine (150 mL). It was then dried over $MgSO_4$ and concentrated. The crude oil was purified by flash chromatography (EtOAc:hexanes 2:1) to yield compound **14** as a mixture of two diastereomers (1.2 g, 1.28 mmol, 80%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30−1.50 (m, 7H), 1.51−1.79 (m, 21H), 1.80−2.00 (m, 1H), 2.10−2.22 (m, 1H), 2.50−2.90 (m, 1H), 2.91−3.31 (m, 3H), 3.52−3.89 (m, 4H), 4.45−4.80 (m, 2H), 4.90−5.15 (m, 4H), 5.30−5.50 (m, 1H), 5.52−6.10 (m, 2H), 6.70−7.27 (m, 5H), 7.29−7.35 (m, 5H), 7.36−7.80 (m, 5H), 8.04−8.25 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 0.4, 18.9, 25.4, 25.6, 28.3, 28.6, 32.2, 54.1, 55.2, 55.3, 59.1, 59.2, 67.5, 77.7, 83.6, 84.0, 84.1, 99.0, 115.1, 115.3, 115.4, 115.5, 115.6, 115.7, 119.3, 119.5, 120.0, 123.0, 123.1, 124.8, 124.9, 125.1, 125.8, 128.4, 128.6, 128.9, 130.6, 130.7, 135.9, 136.4, 139.1, 149.8, 149.9, 156.2, 169.8, 171.0; HRMS−FAB (M + Na^+) calcd for $C_{53}H_{65}N_5NaO_{10}$ 954.4629, found 954.4650.

Boc-Trp-Ala-OMe 15. Boc-Trp-OH (500 mg, 1.6 mmol) was dissolved in DMF (10 mL). To this solution were added H_2N−Ala-OMe (170 mg, 1.6 mmol), HBTU (758 mg, 2.0 mmol), and DIEA (530 μL, 390 mg, 3.0 mmol). The reaction was stirred at room temperature for 2 h, and then it was partitioned between EtOAc (250 mL) and 1 N HCl (200 mL). The organic layer was washed with 1 N HCl (200 mL), saturated $NaHCO_3$ (200 mL), and brine (200 mL). The organic layer was dried with $MgSO_4$ and concentrated by rotary evaporation. The crude oil was purified by flash chromatography (EtOAc: hexanes 2:1) to yield dipeptide **15** (580 mg, 1.50 mmol, 94%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20−1.30 (d, J = 7.2 Hz, 3H), 1.45 (s, 9H), 3.10−3.40 (m, 2H), 3.67 (s, 3H), 4.30−4.60 (t, J = 6.8 Hz, 2H), 5.15−5.28 (br s, 1H), 6.35−6.58 (d, J = 6.8 Hz, 1H), 7.00−7.16 (m, 2H), 7.17−7.23 (m, 1H), 7.30−7.45 (d, J = 8.0 Hz, 1H), 7.55−7.75 (d, J = 7.6 Hz, 1H), 8.50 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.5, 28.5, 48.4, 52.6, 55.4, 75.3, 80.3, 110.6, 111.5, 119.1, 119.9, 122.4, 123.6, 127.8, 136.5, 155.7, 171.6, 173.7; HRMS−FAB (M + Na^+) calcd for $C_{20}H_{27}N_3NaO_5$ 412.1848, found 412.1829.

Boc-Trp-Ile-OMe 16. Dipeptide **16** was synthesized using a procedure that was similar to that used to prepare dipeptide **15** (635 mg, 1.47 mmol, 92%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.70−0.80 (d, J = 6.8 Hz, 3H), 0.81−0.90 (t, J = 7.2 Hz, 3H), 0.95−1.10 (m, 1H), 1.29−1.40 (m, 1H), 1.46 (s, 9H), 1.70−1.82 (br s, 1H), 3.15−3.38 (m, 2H), 3.64 (s, 3H), 4.30−4.60 (dd, J = 8.4, 5.2 Hz, 2H), 5.05−5.35 (br s, 1H), 6.30−6.41 (d, J = 8.4 Hz, 1H), 7.00−7.18 (m, 2H), 7.17−7.23 (t, J = 7.2 Hz, 1H), 7.30−7.45 (d, J = 8.0 Hz, 1H), 7.55−7.75 (d, J = 8.0 Hz, 1H), 8.37 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.8, 15.4, 25.3, 28.4, 28.6, 38.1, 52.2, 55.5, 56.8, 59.4, 69.6, 75.3, 80.4, 110.8, 111.4, 119.1, 119.9, 122.4, 123.5, 127.7, 136.5, 155.8, 171.7, 172.0; HRMS−FAB (M + Na^+) calcd for $C_{23}H_{33}N_3NaO_5$ 454.2318, found 454.2320.

Boc-Trp-Leu-OMe 17. Dipeptide **17** was synthesized using a procedure that was similar to that used to prepare dipeptide **15** (648 mg, 1.50 mmol, 94%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80−0.90 (m, 6H), 1.30−1.60 (m, 12H), 3.10−3.37 (m, 2H), 3.65 (s, 3H), 4.32−4.62 (m, 2H), 5.10−5.28 (br s, 1H), 6.20−6.30 (d, J = 8.1 Hz, 1H), 7.00−7.22 (m, 3H), 7.32−7.45 (d, J = 7.8 Hz, 1H), 7.55−7.70 (d, J = 7.8 Hz, 1H), 8.37 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.9, 22.7, 24.6, 28.1, 28.3, 41.6, 50.8, 52.2, 80.1, 95.7, 110.5, 111.2, 118.9, 119.7, 122.2, 123.4, 127.5, 136.3, 155.5, 171.5, 172.9; HRMS−FAB (M + Na^+) calcd for $C_{23}H_{33}N_3NaO_5$ 454.2318, found 454.2310.

Boc-Trp-Phe-OMe 18. Dipeptide **18** was synthesized using a procedure that was similar to that used to prepare dipeptide **15** (729 mg, 1.57 mmol, 98%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.90−3.00 (d, J = 5.6 Hz, 2H), 2.62−2.88 (m, 2H), 3.62 (s, 3H), 4.35−4.60 (br s, 1H), 4.65−4.82 (br s, 1H), 5.05−5.32 (br s, 1H), 6.25−6.45 (d, J = 6.0 Hz, 1H), 6.75−6.90 (dd, J = 8.0, 1.2 Hz, 2H), 6.95−7.10 (br s, 1H), 7.12−7.22 (m, 5H), 7.32−7.42 (d, J = 8.0 Hz, 1H), 7.65−7.75 (d, J = 7.6 Hz, 1H), 8.46 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.4, 38.1, 52.4, 53.5, 55.4, 59.4, 69.6, 75.2, 80.3, 110.5, 111.5, 119.1, 119.9, 122.4, 123.6, 127.2, 127.7, 128.7, 129.4, 129.6, 135.8, 136.5, 155.6, 159.7, 171.6, 173.2; HRMS−FAB (M + Na^+) calcd for $C_{26}H_{31}N_3NaO_5$ 488.2161, found 488.2154.

Boc-Trp-Trp-OMe 19. Dipeptide **19** was synthesized using a procedure that was similar to that used to prepare dipeptide **15** (716 mg, 1.42 mmol, 89%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (s, 9H), 1.50−1.70 (br s, 1H), 3.00−3.25 (m, 3H), 3.27−3.40 (m, 1H), 3.62 (s, 3H), 4.35−4.60 (br s, 1H), 4.75−4.92 (br s, 1H), 5.00−5.20 (br s, 1H), 6.15−6.35 (br s, 1H), 6.55−6.70 (s, 1H), 6.85−7.00 (br s, 2H), 7.15−7.21 (m, 2H), 7.22−7.227 (m, 2H), 7.29−7.42 (m, 2H), 7.60−7.80 (d, J = 7.6 Hz, 1H), 7.80−8.00 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.9, 28.6, 52.6, 53.0, 55.4, 60.7, 80.2, 110.0, 110.8, 111.4, 111.5, 118.8, 119.4, 119.9, 120.1, 122.5, 123.2, 123.8, 127.7, 127.9, 136.3, 136.5, 145.6, 155.6, 171.4, 172.0; HRMS−FAB (M + Na^+) calcd for $C_{28}H_{32}N_4NaO_5$ 527.2270, found 527.2268.

Boc-Trp-Tyr(*t*Bu)-OMe 20. Dipeptide **20** was synthesized using a procedure that was similar to that used to prepare dipeptide **15** (790 mg, 1.47 mmol, 92%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (s, 9H), 1.44 (s, 9H), 2.75−2.95 (m, 2H), 3.00−3.20 (m, 1H), 3.27−3.43 (m, 1H), 3.60 (s, 3H), 4.35−4.50 (br s, 1H), 4.60−4.75 (m, 1H), 5.10−5.17 (br s, 1H), 6.20−6.35 (d, J = 7.5 Hz, 1H), 6.60−6.80 (m, 4H), 6.80−7.00 (br s, 1H), 7.09−7.21 (m, 2H), 7.29−7.42 (d, J = 7.8 Hz, 1H), 7.60−7.70 (d, J = 7.7 Hz, 1H), 8.49 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.5, 28.7, 29.2, 37.7, 52.6, 53.7, 55.6, 78.9, 80.5, 110.6, 111.7, 119.2, 120.1, 122.6, 123.9, 124.5, 127.9, 130.0, 130.9, 136.6, 154.6, 155.8, 171.6, 171.8; HRMS−FAB (M + Na^+) calcd for $C_{30}H_{39}N_3NaO_6$ 560.2737, found 560.2720.

Boc-Trp-Val-OMe 21. Dipeptide **21** was synthesized using a procedure that was similar to that used to prepare dipeptide **15** (647 mg, 1.55 mmol, 97%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.70−0.87 (m, 6H), 1.45 (s, 9H), 1.85−2.01 (m, 1H), 3.10−3.40 (m, 2H), 3.64 (s, 3H), 4.35−4.55 (m, 2H), 5.00−5.40 (br s, 1H), 6.25−6.45 (d, J = 8.4 Hz, 1H), 7.00−7.25 (m, 3H), 7.30−7.40 (d, J = 7.8 Hz, 1H), 7.65−7.72 (d, J = 7.8 Hz, 1H), 8.44 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.2, 19.1, 28.5, 28.7, 31.6, 52.4, 55.7, 80.5, 110.8, 111.6, 119.2, 120.0, 122.5, 123.7, 127.9, 136.7, 156.0, 172.1, 172.2; HRMS−FAB (M + Na^+) calcd for $C_{22}H_{31}N_3NaO_5$ 440.2161, found 440.2152.

Compound 22. Compound **14** (100 mg, 107 μmol) was dissolved in a 2:1 mixture of acetone and water (60 mL). To this solution were added $NaIO_4$ (114 mg, 535 μmol), $KMnO_4$ (12 mg, 75 μmol), and $NaHCO_3$ (10 mg, 107 μmol). The reaction was stirred at room temperature for 6 h, and then the acetone was removed by rotary evaporation. The remaining material was partitioned between EtOAc (100 mL) and 1 N HCl (75 mL). The organic layer was washed with 1 N HCl (3 × 75 mL) and brine (75 mL) and dried over $MgSO_4$. The solvent was removed by rotary evaporation to give the crude carboxylic acid.

In a separate flask, dipeptide **15** (Boc-Trp-Ala-OMe, 50 mg, 120 μmol) was dissolved in CH_2Cl_2 (3 mL). To this solution was added TFA (1.5 mL). The reaction was stirred at room temperature for 20 min. The solvents were removed by rotary evaporation, and the resulting residue was partitioned between EtOAc (75 mL) and saturated Na_2CO_3 (75 mL). The organic layer was washed with saturated Na_2CO_3 (75 mL) and then dried with Na_2CO_3. The resulting solution was concentrated by rotary evaporation to yield H_2N-Trp-Ala-OMe as a white solid.

The crude carboxylic acid (95 mg, 100 μmol) was redissolved in DMF (5 mL). To this solution were added H_2N-Trp-Ala-OMe (35 mg, 120 μmol), HATU (57 mg, 150 μmol), and DIEA (55 μL, 39 mg, 300 μmol). The reaction was stirred at 50 °C for 4 h and then partitioned between EtOAc (50 mL) and 1 N HCl (50 mL). The organic layer was washed with 1 N HCl (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine (50 mL). It was dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (EtOAc:hexanes 4:1) to give peptide **22** as a mixture of two diastereomers (54 mg, 45 μmol, 45%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18−1.32 (m, 5H), 1.33−1.39 (m, 4H), 1.40−1.53 (m, 3H), 1.55−1.68 (m, 18H), 1.70−2.00 (m, 2H), 2.05−2.15 (m, 1H), 2.20−2.38 (m, 1H), 2.90−3.30 (m, 7H), 3.46−3.95 (m, 6H), 4.40−4.65 (m, 2H), 4.68−4.85 (m, 3H), 4.90−5.05 (m, 2H), 6.88−7.10 (m, 2H), 7.12−7.40 (m, 11H), 7.45−7.70 (m, 5H), 8.00−8.50 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.4, 16.8, 18.4, 18.5, 21.6, 22.9, 25.2, 28.1, 28.5, 30.0, 30.1, 31.9, 32.3, 34.4, 34.9, 39.0, 48.46, 48.52, 52.7, 53.9, 59.1, 59.4, 62.2, 67.4, 67.5, 69.6, 75.3, 84.0, 84.1, 84.2, 111.5, 115.2, 115.3, 115.6, 115.7, 118.9, 119.1, 119.3, 119.8, 122.3, 122.9, 123.0, 123.9, 124.7, 124.9, 125.0, 127.7, 127.8, 128.2, 128.5, 128.8, 130.5, 135.6, 135.8, 136.1, 136.2, 136.6, 136.7, 149.8, 149.9, 170.2, 170.4, 171.0, 171.2, 171.3, 171.4, 173.20, 173.25, 173.29; HRMS−FAB (M + Na^+) calcd for $C_{67}H_{80}N_8NaO_{14}$ 1243.5692, found 1243.5677.

Compound 23. Compound **23** was synthesized using a procedure that was similar to that used to prepare amide **22** (50 mg, 40 μmol, 40%): ^{1}H NMR (400 MHz, MeOH-d_4) δ 0.78−0.91 (m, 6H), 1.10−1.50 (m, 12H), 1.54−1.66 (m, 18H), 1.70−1.90 (m, 2H), 2.00−2.14 (m, 1H), 2.20−2.40 (m, 1H), 2.85−3.30 (m, 6H), 3.45−4.00 (m, 7H), 4.40−4.68 (m, 2H), 4.70−4.85 (m, 2H), 4.91−5.05 (m, 1H), 6.85−7.10 (m, 3H), 7.11−7.20 (m, 4H), 7.20−7.30 (m, 7H), 7.45−7.68 (m, 5H), 8.00−8.15 (m, 2H), 8.16−8.30 (m, 1H); ^{13}C NMR (75 MHz, MeOH-d_4) δ −1.0, 10.7, 10.8, 13.5, 14.8, 14.9, 18.8, 19.9, 21.4, 22.7, 25.2, 25.3, 27.1, 27.4, 27.7, 27.9, 28.3, 31.7, 34.1, 34.7, 35.2, 37.5, 37.9, 47.1, 51.4, 51.5, 53.7, 54.3, 54.6, 55.5, 57.2, 58.9, 59.0, 60.6, 66.6, 83.8, 98.9, 109.7, 109.8, 111.2, 111.3, 115.1, 115.9, 116.3, 118.3, 118.5, 118.8, 119.1, 119.2, 121.4, 122.7, 123.5, 123.7, 124.5, 127.5, 127.8, 127.9, 128.4, 130.7, 130.9, 135.8, 137.0, 149.9, 150.0, 157.2, 171.5, 172.0, 172.1, 172.2, 172.9, 173.1, 173.2, 175.2; HRMS−FAB (M + Na^+) calcd for $C_{70}H_{86}N_8NaO_{14}$ 1285.6161, found 1285.6126.

Compound 24. Compound **24** was synthesized using a procedure that was similar to that used to prepare amide **22** (50 mg, 40 μmol, 40%): ^{1}H NMR (400 MHz, MeOH-d_4) δ 0.81−0.95 (m, 6H), 1.20−1.38 (m, 6H), 1.40−1.50 (m, 3H), 1.57−1.70 (m, 21H), 1.75−2.00 (m, 2H), 2.01−2.15 (m, 1H), 2.20−2.40 (m, 1H), 2.85−3.05 (m, 1H), 3.05−3.25 (m, 4H), 3.25−3.30 (m, 1H), 3.50−3.90 (m, 7H), 4.50−4.62 (m, 2H), 4.71−4.85 (m, 3H), 4.90−5.00 (m, 2H), 6.90−7.10 (m, 3H), 7.13−7.22 (m, 5H), 7.23−7.35 (m, 6H), 7.45−7.68 (m, 5H), 8.00−8.20 (m, 2H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 1.4, 14.5, 14.6, 18.9, 21.1, 21.5, 22.3, 23.0, 23.1, 23.6, 25.1, 25.3, 25.7, 28.0, 28.1, 28.6, 28.8, 30.1, 30.8, 32.0, 32.2, 32.4, 34.4, 34.9, 35.1, 41.6, 41.7, 51.4, 52.7, 54.0, 55.3, 59.3, 60.8, 62.5, 67.5, 77.6, 84.2, 98.7, 110.5, 111.7, 111.9, 115.3, 115.4, 115.7, 115.8, 119.0, 119.2, 119.5, 119.7, 119.9, 120.1, 122.5, 122.6, 123.1, 123.2, 124.0, 124.9, 125.0, 125.2, 127.7, 127.8, 128.2, 128.3, 128.6, 128.9, 130.5, 135.7, 135.9, 136.3, 136.6, 136.8, 149.9, 150.0, 156.3, 170.3, 171.4, 171.6, 171.8, 173.3, 173.4; HRMS−FAB (M + Na^+) calcd for $C_{70}H_{86}N_8NaO_{14}$ 1285.6161, found 1285.3078.

Compound 25. Compound **25** was synthesized using a procedure that was similar to that used to prepare amide **22** (57 mg, 44 μmol, 44%): ^{1}H NMR (400 MHz, MeOH-d_4) δ 1.20−1.35 (m, 5H), 1.38−1.50 (m, 3H), 1.50−1.70 (m, 18H), 1.78−1.90 (m, 1H), 2.20−2.40 (m, 1H), 2.85−3.30 (m, 8H), 3.35−3.46 (m, 4H), 3.60−3.90 (m, 7H), 4.57−4.77 (m, 3H), 4.90−4.95 (m, 1H), 4.99−5.10 (m, 1H), 6.85−7.12 (m, 6H), 7.13−7.22 (m, 9H), 7.23−7.33 (m, 3H), 7.45−7.65 (m, 5H), 8.00−8.15 (m, 2H), 8.16−8.30 (m, 1H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 0.6, 15.1, 17.3, 20.1, 20.7, 21.7, 23.1, 24.5, 26.6, 28.7, 28.8, 29.1, 29.4, 29.6, 29.8, 30.6, 31.0, 31.3, 33.2, 34.3, 39.0, 39.2, 39.5, 53.3, 53.4, 55.2, 55.7, 55.8, 56.1, 57.0, 57.3, 59.9, 60.5, 60.7, 62.2, 64.9, 68.2, 71.3, 76.6, 85.4, 100.5, 109.7, 111.4, 112.9, 116.7, 117.7, 118.0, 119.9, 120.0, 120.4, 120.7, 120.9, 122.9, 124.3, 125.1, 125.3, 126.0, 126.1, 128.4, 129.0, 129.4, 130.0, 130.9, 131.0, 131.6, 132.3, 132.4, 137.2, 137.3, 138.4, 138.5, 138.6, 151.5, 158.7, 158.8, 173.0, 173.2, 173.6, 174.3, 174.6, 174.7, 176.6; HRMS−FAB (M + Na^+) calcd for $C_{73}H_{84}NaN_8O_{14}$ 1319.6005, found 1319.6030.

Compound 26. Compound **26** was synthesized using a procedure that was similar to that used to prepare amide **22** (61 mg, 46 μmol, 46%): ^{1}H NMR (400 MHz, MeOH-d_4) δ 1.10−1.35 (m, 6H), 1.35−1.50 (m, 3H), 1.53−1.70 (m, 18H), 1.72−1.90 (m, 1H), 1.95−2.05 (m, 1H), 2.10−2.28 (m, 1H), 2.85−3.30 (m, 8H), 3.60−3.90 (m, 7H), 4.47−4.67 (m, 1H), 4.68−4.85 (m, 3H), 4.90−5.02 (m, 2H), 6.85−7.12 (m, 6H), 7.12−7.41 (m, 12H), 7.42−7.70 (m, 6H), 7.90−8.20 (m, 3H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 13.5, 18.7, 19.9, 21.4, 21.5, 22.7, 25.2, 27.4, 27.7, 29.0, 29.4, 31.5, 31.7, 34.0, 34.7, 36.0, 37.9, 49.1, 49.8, 51.8, 53.7, 53.8, 54.0, 54.2, 54.5, 55.5, 55.7, 58.9, 60.6, 66.6, 83.8, 98.9, 109.1, 109.8, 111.4, 113.5, 115.1, 116.0, 116.3, 118.1, 118.3, 118.5, 118.6, 118.9, 119.1, 119.2, 121.5, 121.8, 122.7, 123.7, 123.8, 124.5, 127.5, 127.7, 127.8, 127.9, 128.4, 130.6, 130.9, 135.8, 136.9, 137.0, 149.9, 150.0, 157.2, 166.5, 171.5, 172.1, 172.4, 172.5, 172.7, 172.8, 172.9, 173.0, 175.1; HRMS−FAB (M + Na^+) calcd for $C_{75}H_{85}N_9NaO_{14}$ 1358.6114, found 1358.6161.

Compound 27. Compound **27** was synthesized using a procedure that was similar to that used to prepare amide **22** (53 mg, 40 μmol, 40%): ^{1}H NMR (400 MHz, MeOH-d_4) δ 1.10−

1.49 (m, 10H), 1.55−1.70 (m, 19H), 1.75−1.95 (m, 1H), 2.00−2.16 (m, 1H), 2.20−2.40 (m, 1H), 2.75−3.30 (m, 9H), 3.47−3.91 (m, 7H), 4.48−4.80 (m, 4H), 4.91−5.02 (m, 2H), 6.56−6.71 (m, 1H), 6.87−6.97 (m, 2H), 6.98−7.01 (m, 1H), 7.02−7.08 (m, 1H), 7.09−7.12 (s, 1H), 7.13−7.35 (m, 10H), 7.45−7.65 (m, 5H), 8.00−8.30 (m, 3H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 0.6, 15.1, 20.4, 23.1, 26.8, 29.1, 29.5, 29.8, 30.7, 31.0, 32.3, 33.2, 35.8, 37.6, 38.3, 39.5, 53.2, 53.3, 53.7, 55.3, 55.8, 56.0, 56.1, 57.1, 60.6, 60.7, 62.2, 68.3, 85.5, 100.5, 111.5, 112.9, 113.0, 116.8, 116.9, 117.1, 117.6, 118.0, 119.9, 120.3, 120.4, 120.8, 122.9, 123.0, 124.4, 125.1, 126.1, 129.0, 129.1, 129.2, 129.5, 129.6, 130.1, 132.0, 132.3, 132.5, 137.4, 138.7, 151.6, 151.7, 158.0, 158.1, 158.9, 165.5, 173.2, 173.7, 173.8, 174.4, 174.5, 176.7; HRMS−FAB (M + Na^+) calcd for $C_{77}H_{92}N_8NaO_{15}$ 1335.5954, found 1335.5980.

Compound 28. Compound **28** was synthesized using a procedure that was similar to that used to prepare amide **22** (63 mg, 50 μmol, 50%): ^{1}H NMR (400 MHz, MeOH-d_4) δ 0.80−0.95 (m, 6H), 1.14−1.35 (m, 6H), 1.36−1.97 (m, 4H), 1.52−1.67 (m, 18H), 1.80−1.95 (m, 1H), 2.02−2.18 (m, 2H), 2.22−2.42 (m, 1H), 2.83−3.40 (m, 7H), 3.55−3.87 (m, 7H), 4.21−4.65 (m, 3H), 4.72−4.82 (m, 2H), 4.90−5.02 (m, 2H), 6.90−7.10 (m, 3H), 7.11−7.35 (m, 11H), 7.40−7.70 (m, 6H), 8.00−8.20 (m, 2H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 0.6, 15.1, 19.1, 19.2, 20.0, 20.1, 20.4, 21.5, 23.1, 26.8, 29.1, 29.3, 29.5, 32.3, 32.6, 33.2, 35.7, 37.6, 39.5, 53.0, 53.1, 55.3, 55.9, 56.3, 57.1, 59.8, 59.9, 60.6, 62.2, 68.3, 70.5, 85.4, 100.5, 111.4, 111.5, 112.8, 112.9, 116.8, 117.6, 117.9, 119.9, 120.4, 120.7, 120.8, 123.0, 124.3, 125.2, 126.1, 129.1, 129.4, 129.5, 130.1, 132.3, 132.5, 137.4, 138.6, 151.6, 151.7, 158.8, 165.5, 168.1, 173.1, 173.6, 173.7, 173.8, 173.9, 174.4, 174.5, 174.8, 174.9, 175.1, 176.7; HRMS−FAB (M + Na^+) calcd for $C_{69}H_{84}N_8NaO_{14}$ 1271.6005, found 1271.6041.

Inhibitor 29. To peptide **22** (40 mg, 33 μmol) was added an aqueous TFA solution (4 mL, 70%) at 0 °C. The reaction was warmed to room temperature and stirred for an additional 12 h, and then it was concentrated by rotary evaporation. The resulting residue was dried under vacuum for 24 h. The crude material was purified by flash chromatography (MeOH in CH_2Cl_2 2−6%) to yield inhibitor **29** as a mixture of two diastereomers (24 mg, 25 μmol, 75%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.77−1.40 (m, 7H), 1.41−1.70 (m, 3H), 1.71−1.85 (m, 2H), 1.86−2.36 (m, 4H), 2.72−2.94 (m, 2H), 2.95−3.22 (m, 3H), 3.45−3.55 (m, 3H), 4.15−4.75 (m, 4H), 4.90−5.00 (m, 1H), 6.81−7.12 (m, 7H), 7.13−7.48 (m, 8H), 7.52−7.68 (m, 2H), 7.90−8.55 (m, 3H), 10.60−10.95 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 17.7, 18.0, 21.9, 24.1, 25.5, 26.0, 28.6, 29.0, 30.1, 31.3, 32.8, 33.5, 34.8, 35.2, 35.4, 42.6, 44.0, 48.4, 29.0, 52.7, 53.7, 54.2, 56.5, 58.1, 66.1, 66.4, 66.8, 67.9, 98.5, 110.6, 111.0, 111.1, 112.1, 119.0, 119.3, 119.4, 121.7, 124.5, 124.6, 125.8, 127.5, 128.1, 128.3, 128.5, 128.9, 129.1, 136.8, 136.9, 137.8, 140.1, 156.7, 158.7, 171.7, 172.4, 172.6, 172.7, 173.8, 174.9, 209.0; HRMS−FAB (M + Na^+) calcd for $C_{54}H_{58}N_8NaO_9$ 985.4224, found 985.4205.

Inhibitor 30. Inhibitor **30** was synthesized using a procedure that was similar to that used to prepare inhibitor **29** (23 mg, 23 μmol, 70%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.65−0.95 (m, 6H), 1.05−1.48 (m, 6H), 1.49−1.85 (m, 6H), 1.86−2.36 (m, 4H), 2.76−3.21 (m, 5H), 3.51−3.70 (m, 3H), 4.20−4.40 (m, 3H), 4.55−4.85 (m, 2H), 4.90−5.00 (m, 1H), 6.85−7.01 (m, 3H), 7.02−7.12 (m, 4H), 7.17−7.28 (m, 3H), 7.29−7.37 (m, 5H), 7.50−7.71 (m, 3H), 7.90−8.30 (m, 3H), 10.67−10.95 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 12.0, 16.2, 24.1, 25.5, 25.6, 25.9, 28.5, 28.6, 29.0, 31.3, 33.4, 34.8, 35.2, 35.4, 37.2, 49.1, 52.5, 53.7, 54.2, 56.5, 57.2, 58.1, 66.1, 66.4, 67.9, 110.6, 110.9, 111.1, 112.1, 119.0, 119.3, 119.4, 121.7, 124.4, 124.6, 125.8, 127.5, 128.1, 128.2, 128.3, 128.5, 128.9, 129.1, 136.8, 136.9, 137.0, 137.8, 156.7, 171.7, 172.4, 172.5, 172.7, 172.9, 208.9; HRMS−FAB (M + Na^+) calcd for $C_{57}H_{64}N_8NaO_9$ 1027.4694, found 1027.4670.

Inhibitor 31. Inhibitor **31** was synthesized using a procedure that was similar to that used to prepare inhibitor **29** (24 mg, 24 μmol, 72%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.72−0.97 (m, 6H), 0.98−1.43 (m, 5H), 1.44−1.70 (m, 5H), 1.71−1.85 (m, 2H), 1.86−2.35 (m, 4H), 2.78−3.20 (m, 5H), 3.55−3.65 (m, 3H), 4.20−4.70 (m, 4H), 4.85−5.00 (m, 1H), 6.85−7.12 (m, 7H), 7.13−7.38 (m, 8H), 7.40−7.66 (m, 3H), 7.90−8.40 (m, 3H), 10.65−10.95 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.9, 22.1, 22.2, 23.6, 23.7, 24.1, 25.0, 25.1, 25.5, 25.9, 27.3, 28.6, 28.9, 30.1, 30.6, 31.3, 31.7, 32.8, 33.5, 34.7, 35.2, 35.4, 43.1, 45.6, 49.1, 51.1, 52.7, 53.7, 54.2, 56.5, 58.1, 66.1, 66.4, 67.9, 110.6, 111.0, 111.1, 112.1, 118.1, 118.6, 119.0, 119.3, 119.4, 121.7, 122.0, 124.4, 124.6, 125.4, 125.8, 127.5, 128.1, 128.3, 128.5, 128.8, 128.9, 129.0, 129.1, 136.8, 136.9, 137.0, 137.8, 140.0, 156.6, 171.7, 172.4, 172.6, 172.8, 172.9, 173.2, 173.7, 174.0, 174.9, 208.9; HRMS−FAB (M + Na^+) calcd for $C_{57}H_{64}N_8NaO_9$ 1027.4694, found 1027.4674.

Inhibitor 32. Inhibitor **32** was synthesized using a procedure that was similar to that used to prepare inhibitor **29** (24 mg, 23 μmol, 70%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.00−1.40 (m, 4H), 1.41−1.70 (m, 3H), 1.72−2.35 (m, 6H), 2.72, 3.20 (m, 6H), 3.50−3.67 (m, 3H), 4.18−4.83 (m, 4H), 4.85−4.99 (m, 1H), 6.80−7.12 (m, 7H), 7.13−7.37 (m, 10H), 3.38−7.77 (m, 3H), 7.80−8.50 (m, 3H), 10.65−10.95 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.9, 24.0, 25.5, 25.9, 27.3, 28.6, 29.0, 30.2, 30.6, 31.3, 31.6, 32.6, 32.8, 33.5, 34.8, 35.2, 35.4, 37.4, 42.3, 42.5, 43.3, 43.6, 44.1, 44.5, 49.1, 52.7, 53.2, 53.7, 54.3, 54.5, 56.5, 58.1, 66.2, 66.4, 66.8, 67.9, 70.6, 75.1, 110.6, 111.0, 111.1, 111.5, 112.1, 112.5, 119.0, 119.3, 119.4, 121.7, 124.4, 124.6, 125.8, 127.4, 128.1, 128.3, 128.5, 129.1, 129.7, 129.9, 130.0, 130.5, 130.7, 136.8, 136.9, 137.0, 137.8, 137.9, 138.3, 140.0, 152.3, 156.7, 158.8, 159.3, 171.7, 172.4, 172.5, 172.6, 172.7, 173.6, 209.0; HRMS−FAB (M + Na^+) calcd for $C_{60}H_{62}N_8NaO_9$ 1061.4537, found 1061.4515.

Inhibitor 33. Inhibitor **33** was synthesized using a procedure that was similar to that used to prepare inhibitor **29** (19 mg, 18 μmol, 53%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10−1.28 (m, 2H), 1.29−1.48 (m, 2H), 1.51−1.70 (m, 2H), 1.71−1.85 (m, 3H), 1.87−2.13 (m, 3H), 2.15−2.32 (m, 1H), 2.72−2.96 (m, 2H), 2.97−3.25 (m, 4H), 3.50−3.70 (m, 4H), 4.20−4.45 (m, 2H), 4.46−4.75 (m, 2H), 4.86−4.95 (m, 1H), 6.80−7.13 (m, 9H), 7.14−7.38 (m, 9H), 7.39−7.69 (m, 4H), 7.85−8.08 (m, 2H), 8.09−8.45 (m, 2H), 10.60−10.90 (m, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.0, 25.4, 25.9, 27.3, 27.8, 28.6, 28.9, 30.1, 30.6, 32.7, 33.5, 34.8, 35.4, 43.6, 49.1, 52.7, 53.8, 53.9, 54.3, 56.5, 58.1, 66.1, 67.9, 110.1, 110.6, 111.0, 111.1, 112.1, 112.3, 118.8, 119.0, 119.3, 119.4, 121.7, 121.8, 124.4, 124.6, 127.5, 127.9, 128.1, 128.3, 128.5, 129.1, 130.4, 136.8, 136.9, 137.8, 159.3, 171.7, 172.4, 172.7, 172.8, 173.0, 209.0; HRMS−FAB (M + Na^+) calcd for $C_{62}H_{63}N_9NaO_9$ 1100.4646, found 1100.4619.

Inhibitor 34. Inhibitor **34** was synthesized using a procedure that was similar to that used to prepare inhibitor **29** (21 mg, 20 μmol, 62%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.09−1.45 (m, 3H), 1.46−1.68 (m, 2H), 1.70−1.85 (m, 5H), 1.86−2.10 (m, 3H), 2.15−2.35 (m, 1H), 2.72−2.97 (m, 3H), 2.98−3.16 (m, 3H), 3.50−3.60 (m, 5H), 4.25−4.75 (m, 4H), 4.86−4.99 (m, 1H), 6.50−6.80 (m, 2H), 6.85−7.12 (m, 9H), 7.13−7.38 (m, 8H), 7.50−7.72 (m, 3H), 7.86−8.05 (m, 2H), 8.06−8.20 (m, 1H), 8.25−8.36 (m, 1H), 10.65−10.90 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.0, 25.4, 26.0, 28.6, 29.0, 30.2, 30.6, 33.5, 34.8, 35.4, 36.8, 43.9, 49.1, 52.6, 53.7, 54.3, 54.6, 54.8, 56.5, 58.1, 66.1, 67.9, 110.6, 111.0, 111.1, 112.1, 115.9, 119.0, 119.3, 119.4, 121.7, 122.0, 124.1, 124.3, 124.5, 125.5, 127.5, 127.8, 128.1, 128.3, 128.5, 129.1, 130.9, 136.7, 136.8, 136.9, 137.8, 155.7, 156.7, 156.9, 158.5, 158.9, 171.7, 172.4, 172.6, 172.7, 172.8, 173.8, 208.9; HRMS−FAB (M + Na^+) calcd for $C_{60}H_{62}N_8NaO_{10}$ 1077.4487, found 1077.4452.

Inhibitor 35. Inhibitor **35** was synthesized using a procedure that was similar to that used to prepare inhibitor **29** (22 mg, 22 μmol, 67%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.71−0.99 (m, 6H), 1.00−1.17 (m, 1H), 1.18−1.34 (m, 2H), 1.35−1.45 (m, 1H), 1.48−1.83 (m, 5H), 2.69−2.95 (m, 2H), 2.96−3.22 (m, 3H), 3.50−3.70 (m, 4H), 4.10−4.42 (m, 3H), 4.55−4.95 (m, 3H), 6.80−7.15 (m, 7H), 7.16−7.48 (m, 8H), 7.49−7.70 (m, 3H), 7.89−8.30 (m, 3H), 10.60−10.92 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 19.1, 19.8, 24.1, 25.5, 26.0, 28.5, 28.6, 29.0, 30.6, 30.8, 33.4, 34.7, 35.4, 49.0, 52.5, 53.8, 54.2,

56.5, 58.1, 58.2, 66.1, 67.9, 110.6, 111.0, 111.1, 112.1, 119.0, 119.3, 119.4, 121.7, 124.4, 124.6, 128.1, 128.2, 128.3, 128.5, 129.1, 136.8, 136.9, 137.0, 137.8, 156.7, 171.7, 172.4, 172.7, 173.1, 208.9; HRMS−FAB ($M + Na^+$) calcd for $C_{56}H_{62}N_8NaO_9$ 1013.4537, found 1013.4573.

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Supporting Information Available: ^1H and ^{13}C NMR spectra for all new compounds. HPLC characterization for compounds **29**−**35**. This material is available free of charge via the Internet at http://pubs.acs.org.

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