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A Comparison of Cyclohexanone and Tetrahydro-4*H*-thiopyran-4-one 1,1-Dioxide as Pharmacophores for the Design of Peptide-Based Inhibitors of the Serine Protease Plasmin

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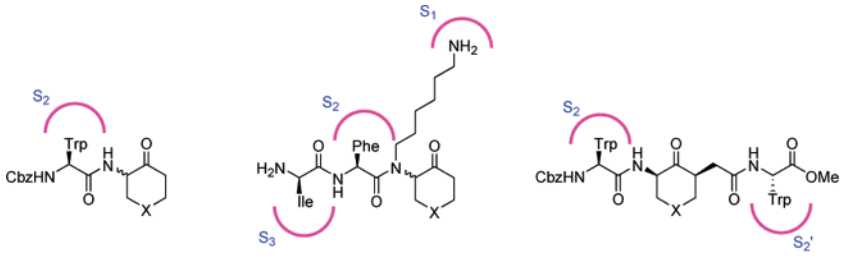

1 X = CH_2 **2** X = SO_2 **3** X = CH_2 **4** X = SO_2 **5** X = CH_2 **6** X = SO_2

The plasminogen system is important in the proteolytic cascade that facilitates angiogenesis, a process that is essential for tumor growth and metastasis. The serine protease plasmin has a central role in the plasminogen system. This protease acts by degrading several components of the basement membrane and by activating other proteases. Therefore, inhibition of plasmin may be an effective method for blocking angiogenesis and, as a result, inhibiting the growth of primary tumors and secondary metastases. Three pairs of plasmin inhibitors were synthesized to compare the relative potency of inhibitors that are based upon a cyclohexanone or a tetrahydro-4*H*-thiopyran-4-one 1,1-dioxide nucleus. Compounds **1**, **3**, and **5** were cyclohexanone-based inhibitors, whereas compounds **2**, **4**, and **6** were tetrahydro-4*H*-thiopyran-4-one 1,1-dioxide-based inhibitors. Compounds **5** and **6** are reasonable inhibitors with IC_{50} values of 25 and 5.5 μM, respectively. Comparisons of the IC_{50} values of the three pairs show that the electron-withdrawing sulfone functional group is a beneficial element for the design of plasmin inhibitors. The presence of the sulfone increases inhibitor potency by a factor of 3−5 when compared to inhibitors that are based upon a simple cyclohexanone core.

Introduction

Angiogenesis, the growth of new blood vessels from pre-existing ones, is critical for the development of cancer.[1,2] Without a blood supply, tumors are usually limited in size to 1−2 mm^3.[3] Growth of a tumor beyond this size is angiogenesis-dependent, since vascularization of the tumor is required to supply it with oxygen and nutrients and to dispose of waste products. As a result, angiogenesis allows the tumor to grow rapidly beyond a microscopic size and also provides cancer cells with a route to spread to other parts of the body.

In recent decades, Folkman and several other research groups have demonstrated that angiogenesis inhibitors can impede both the growth of primary tumors and the formation of secondary metastases.[4−6] Angiogenesis is mediated by a proteolytic cascade that involves a large number of enzymes. Components of the plasminogen activator (PA)-plasmin system and matrix metalloproteases (MMPs) play critical roles in this proteolytic cascade.[7] Hence, therapeutic strategies that are aimed at inhibiting these enzymes may have significant potential for the treatment of cancer and other diseases that are dependent on angiogenesis.

Plasmin is a serine protease that is a primary constituent of the proteolytic cascade. This protease degrades

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a wide variety of substrates and components of the extracellular matrix including fibrin, glycoproteins such as laminin and fibronectin, and proteoglycans. It activates other proteases such as the pro-metalloproteinases MMP-1, MMP-3, and MMP-9.[8] Additionally, plasmin activates or releases a number of growth factors from the extracellular matrix including latent transforming growth factor (TGF-β), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF).[8] Thus, plasmin plays a vital role in the breakdown of the basement membrane and facilitates angiogenesis and the migration of cancer cells. Because of these features, plasmin is a potential target for the development of chemotherapeutic agents that could block degradation of the basement membrane and the activation of other enzymes. Such activity, in turn, could lead to the inhibition of angiogenesis and metastasis.

Considerable advances have been made in the design of inhibitors for plasmin and other related serine proteases. Currently, there are two primary families of inhibitors for plasmin. They are classified by the different domains within the enzyme that are targeted by the inhibitors. The first family targets the lysine binding site of plasmin and includes inhibitors such as ϵ-aminocaproic acid, *p*-aminomethylbenzoic acid, and *trans*-4-aminocyclohexane carboxylic acid, several of which have been used in clinical applications.[9] The lysine binding site mediates the interaction between plasmin and fibrin, which is a key step in the processes of fibrinolysis and the generation of active plasmin from its inactive precursor plasminogen.[10] This family of inhibitors effectively inhibits these processes. However, these inhibitors cannot block the activity of plasmin that has already been activated by cleavage of plasminogen. The second family of inhibitors, which are targeted to the active site of plasmin, can overcome this limitation. They act by blocking the catalytic activity of the serine residue in the active site of the enzyme.[11]

Design of the Inhibitors. Over the last several years, we have been investigating active site-directed inhibitors for plasmin that are based upon a 4-heterocyclohexanone nucleus.[12−14] These inhibitors contain an electrophilic ketone moiety that is designed to form a reversible covalent hemiketal bond with the active site serine residue of the protease. The electrophilicity of the ketone, and thus the potency of the inhibitors, can be enhanced by incorporating an electronegative functional group in place of the C-4 atom of the cyclohexanone ring. The dipole of the electronegative group induces a through-space electrostatic repulsion with the dipole of the ketone.[14−16] This unfavorable electrostatic interaction destabilizes the ketone and makes it more susceptible to attack by nucleophiles, including the serine hydroxyl group in the active site of plasmin (Figure 1). In previous studies, we demonstrated that cyclohexanone-based inhibitors react with the active site cysteine residue of papain to give a reversibly formed hemithioketal linkage.[14b]

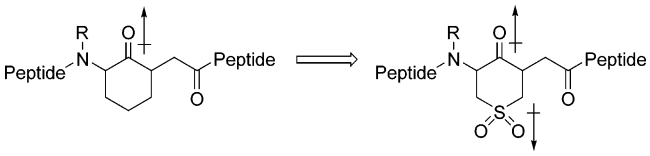

FIGURE 1. Enhancement of the electrophilicity of the ketone by introducing an electronegative sulfone group into the cyclohexanone ring system. R = H or $(CH_2)_6NH_2$.

Herein, we report the synthesis and evaluation of three pairs of inhibitors for plasmin (compounds **1**−**6**, Figure 2). The inhibitors in each pair have identical structures, except that one inhibitor in each pair is based upon a cyclohexanone nucleus, whereas in its partner the C-4 carbon of the cyclohexanone ring has been replaced by an SO_2 group. These pairs of inhibitors were designed to test how effective the sulfone functional group is at enhancing the electrophilicity of the ketone moiety via a through-space electrostatic interaction and to gauge the influence of the sulfone group on the potency of the inhibitors. The cyclohexanone or tetrahydro-4*H*-thiopyran-4-one 1,1-dioxide nucleus of the inhibitors is derivatized with one or two peptide appendages. These peptides target the inhibitors to the enzyme active site and are designed to correctly align the electrophilic ketone moiety of the inhibitor for reaction with the active site serine nucleophile.

Compounds **1** and **2** are the simplest of the three pairs of inhibitors. They incorporate a single Trp residue to bind in the S2 subsite of the enzyme.[17] To increase the number of contacts between the inhibitors and the enzyme, we synthesized a second inhibitor pair, **3** and **4**, which employ two binding elements in their peptide-based side chains. First, the aminohexyl group mimics the side chain of positively charged amino acids such as Lys and Arg. This group is targeted to the S1 subsite, since it is know that trypsin-like serine proteases prefer to bind substrates with positively charged amino acids at the P1 position.[10] Second, the dipeptide D-Ile-Phe was chosen to interact with the S3 and S2 subsites of plasmin, based upon work that has been published by Okada and

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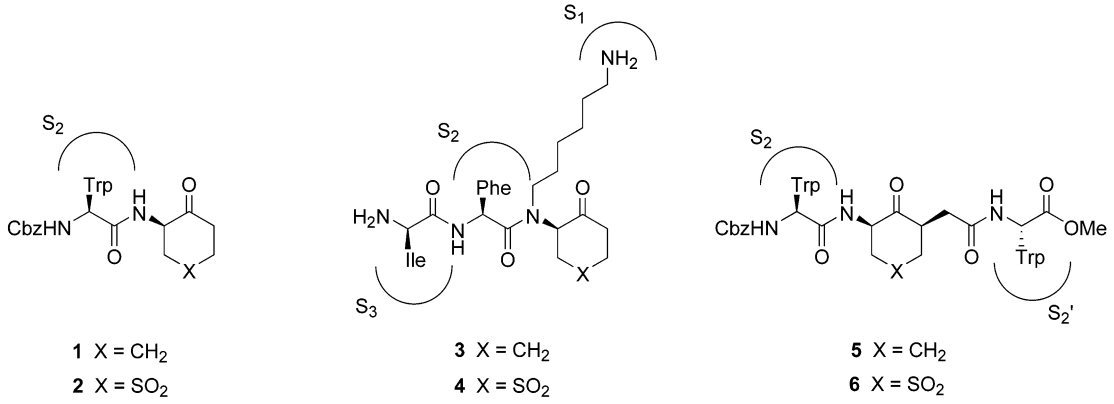
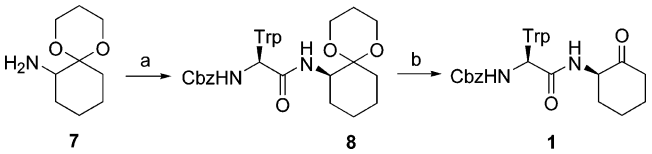


FIGURE 2. Structures of inhibitors **1**−**6**. One of two diastereomers is shown for each inhibitor.

SCHEME 1. Synthesis of Inhibitor 1[a]



[a] Reagents: (a) Cbz-Trp-OH, HBTU, DIEA, 80%; (b) TFA, H_2O, 55%. Only one of the two diastereomers is shown for compounds **8** and **1**.

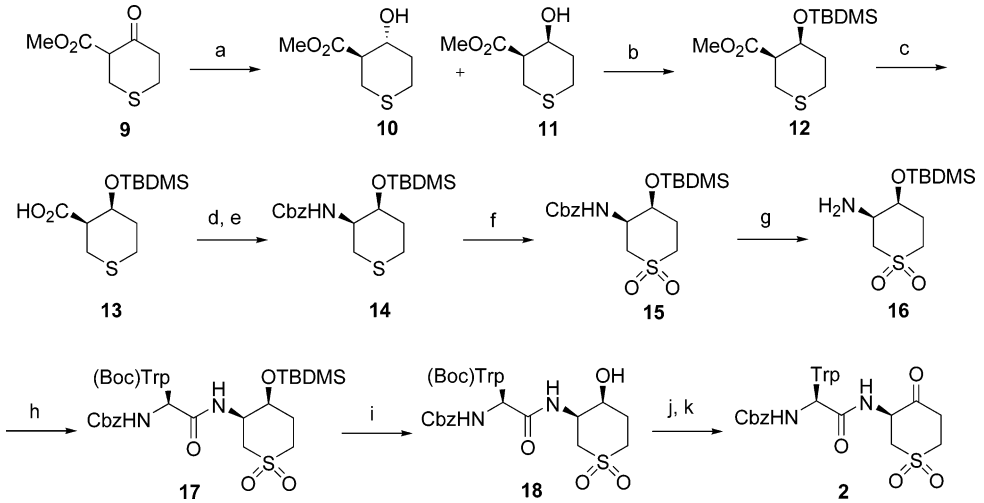
co-workers and work from our own laboratory.[12] The final pair of inhibitors, **5** and **6**, have peptides attached on both sides of the ketone group. One Trp reside is designed to bind in the S2 subsite, similar to inhibitors **1** and **2**. The second Trp residue extends toward the leaving group subsites and binds in the S2′ subsite.[15] The results that are presented in the next section comparing inhibition constants among these three pairs of inhibitors demonstrate that inhibitor potency is increased by a factor of 3−5 when the C-4 atom of a cyclohexanone-based inhibitor is replaced by the more electronegative sulfone functional group.

Results and Discussion

Inhibitor 1. The synthesis of inhibitor **1** (Scheme 1) began with coupling of primary amine **7** with Cbz-Trp-OH employing a standard peptide coupling procedure with *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and *N*,*N*-diisopropylethylamine (DIEA) to give amide **8** as a mixture of two diastereomers. The synthesis of compound **7** has been reported previously.[13] The ketal-protecting group in compound **8** was removed with aqueous TFA to give inhibitor **1**.

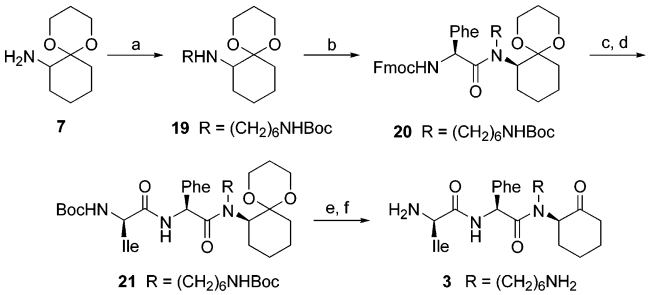
Inhibitor 2. Inhibitor **2** was synthesized according to the procedure shown in Scheme 2. β-Ketoester **9** was reduced with $NaBH_4$ to give a mixture of alcohols **10** and **11**. The synthesis of compound **9** has been reported previously.[13] Compounds **10** and **11** were separated by flash chromatography, and racemic **11** was carried through the remainder of the synthesis. The hydroxyl group in compound **11** was protected with TBDMSCl to give TBDMS ether **12**, which was subsequently saponified with aqueous NaOH in methanol to yield carboxylic acid **13**. Compound **13** was treated with diphenylphosphoryl azide in toluene at 60 °C to first generate the acyl

SCHEME 2. Synthesis of Inhibitor 2[a]



[a] Reagents: (a) $NaBH_4$, 60%; (b) TBDMSCl, imidazole, 86%; (c) NaOH, MeOH, 70%; (d) $(C_6H_5O)_2PON_3$, DIEA; (e) BnOH, *n*-BuLi, 84% (two steps); (f) $NaIO_4$, $KMnO_4$, $NaHCO_3$, 74%; (g) H_2, Pd/C, 100%; (h) Cbz-Trp(Boc)-OH, HBTU, DIEA, 86%; (i) TBAF, 89%; (j) Dess−Martin periodinane; (k) TFA, 34% (two steps). Only one of the two diastereomers is shown for compounds **17**, **18**, and **2**.

SCHEME 3. Synthesis of Inhibitor 3[a]



[a] Reagents: (a) $BocNH(CH_2)_5CHO$, $Ti(O^iPr)_4$, followed by $NaBH_4$; (b) Fmoc-Phe-OH, HATU, DIEA, 31% (two steps); (c) piperidine; (d) Boc-D-Ile-OH, HBTU, DIEA, 78% (two steps); (e) TFA; (f) TFA, H_2O, 59% (two steps). Only one of the two diastereomers is shown for compounds **20**, **21**, and **3**.

azide and then to induce the Curtius rearrangement. The resulting isocyanate was trapped with the anion of benzyl alcohol to generate Cbz-protected amine **14**. Oxidation of the thioether in compound **14** with $NaIO_4$ and $KMnO_4$ gave sulfone **15**. An alternate method for this oxidation employing $NaBO_3$ also gave a reasonable yield of the desired sulfone.[18]

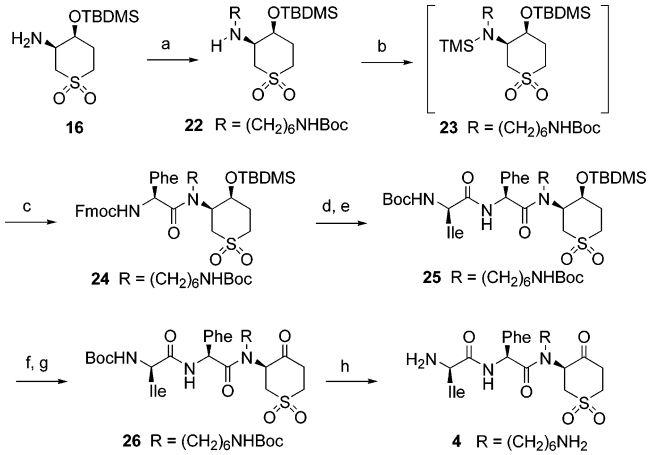
Catalytic hydrogenation of the Cbz-protecting group generated primary amine **16**, which was coupled with Cbz-Trp(Boc)-OH to give amide **17** as a mixture of two diastereomers. The TBDMS-protecting group was removed using tetrabutylammonium fluoride (TBAF) to give alcohol **18**, which was oxidized back to the ketone with Dess−Martin periodinane. Finally, the Boc-protecting group on the side chain of Trp was removed by TFA to give inhibitor **2**.

Inhibitor 3. Reductive alkylation of amine **7** was accomplished using a two-step procedure (Scheme 3). First, the imine was formed between the primary amine and the aldehyde $BocNH(CH_2)_5CHO$. The synthesis of this aldehyde has been reported previously.[10] We examined several dehydrating reagents to promote formation of the imine including $Ti(O^iPr)_4$, 4 Å molecular sieves, and $MgSO_4$. $Ti(O^iPr)_4$ gave the highest yield of imine as determined by ^1H NMR spectroscopy. In a second step, the resulting imine was reduced with $NaBH_4$ to give secondary amine **19**.[19] Other reducing reagents including $NaCNBH_3$ and $NaBH(OAc)_3$ gave lower yields of the desired secondary amine.

Compound **19** was coupled with Fmoc-Phe-OH using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) at 60 °C to generate amide **20** as a mixture of two diastereomers. Substitution of HBTU for HATU in this coupling reaction gave low yields of the desired amide. The Fmoc-protecting group in compound **20** was removed with piperidine, and the resulting amine was coupled with Boc-D-Ile-OH to generate compound **21**. Finally, the two Boc-protecting groups were removed with TFA, and the ketal was hydrolyzed using aqueous TFA to give inhibitor **3**.

Inhibitor 4. In contrast to the reductive alkylation of amine **7** (Scheme 3), amine **16** underwent smooth reductive alkylation with $BocNH(CH_2)_5CHO$[10] without the need for a dehydrating agent. In this case, $NaBH(OAc)_3$ was used as the reducing agent (Scheme 4). The resulting secondary amine **22** was coupled with Fmoc-Phe-F using

SCHEME 4. Synthesis of Inhibitor 4[a]



[a] Reagents: (a) $BocNH(CH_2)_5CHO$; $NaBH(OAc)_3$, 75%; (b) BSA; (c) Fmoc-Phe-F, catalytic TBAF, 65%; (d) piperidine; (e) Boc-D-Ile-OH, HBTU, DIEA, 84% (two steps); (f) TBAF; (g) Dess−Martin periodinane, 69% (two steps); (h) TFA, 70%. Only one of the two diastereomers is shown for compounds **24**, **25**, **26**, and **4**.

a two-step procedure. First, amide **22** was treated with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) to generate the *N*-TMS derivative **23**.[20] This compound was reacted in situ with Fmoc-Phe-F to furnish amide **24** as a mixture of two diastereomers. The synthesis of Fmoc-Phe-F has been reported previously.[11] The coupling reaction between secondary amine **22** and Fmoc-Phe-OH could be accomplished using HATU at 60 °C. However, this method gave a lower yield when compared to the procedure employing the acid fluoride. The Fmoc group in compound **24** was removed, and the resulting amine was coupled with Boc-D-Ile-OH to give dipeptide **25**. The TBDMS ether was cleaved with TBAF, and the resulting alcohol was oxidized to ketone **26** with Dess−Martin periodinane. Finally, the Boc-protecting groups were removed with TFA to give inhibitor **4**.

Inhibitor 5. Unlike inhibitors **1**−**4**, inhibitors **5** and **6** incorporate functionality that extend toward the leaving group subsites of the enzyme active site. This extension was accomplished using the strategy outlined in Scheme 5. Compound **27** was treated with 2 equiv of LDA to generate the corresponding dienolate, which was alkylated with 1 equiv of allyl bromide to give compound **28**.[21] The ketone group in compound **28** was converted to ketal **29** using 1,3-propanediol in the presence of TMSCl. Compound **29** was obtained as a racemic mixture in which the ethoxycarbonyl and the allyl groups are oriented cis to one another.[22] Saponification of the ester group in compound **29**, followed by treatment of the resulting carboxylic acid with diphenylphosphoryl azide, induced the Curtius rearrangement to give the corresponding isocyanate. This isocyanate was treated with *t*-BuOK to generate the Boc-protected amine **30**. Oxidative cleavage of the alkene with $KMnO_4$ and $NaIO_4$ resulted in a carboxylic acid, which was coupled with

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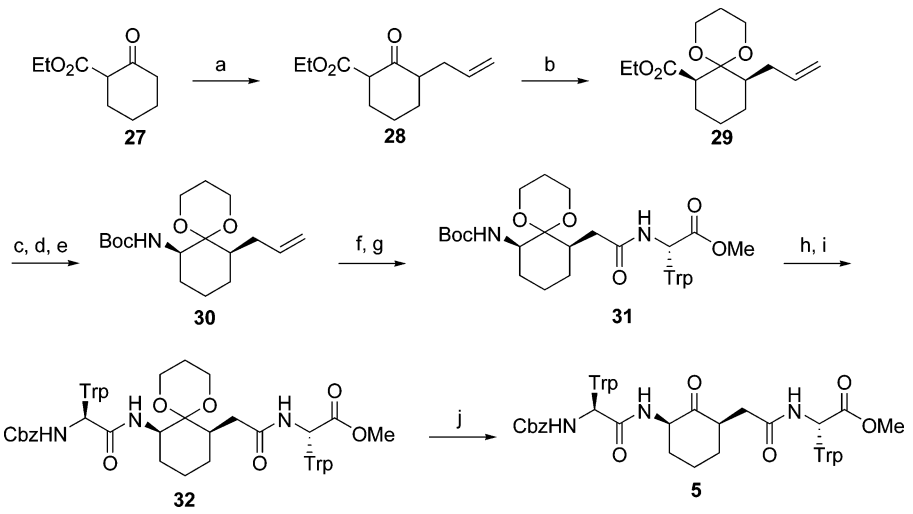
SCHEME 5. Synthesis of Inhibitor 5[a]



[a] Reagents: (a) LDA (2 equiv), allyl bromide, 78%; (b) 1,3-propanediol, TMSCl, 84%; (c) NaOH, MeOH; (d) $(PhO)_2PON_3$, DIEA; (e) *t*-BuOK, 65% (three steps); (f) $NaIO_4$, $KMnO_4$, $NaHCO_3$; (g) H-Trp-OMe, HBTU, DIEA, 80%; (h) TFA; (i) Cbz-Trp-OH, HBTU, DIEA, 77% (two steps); (j) TFA, H_2O, 67%. Only one of the two diastereomers is shown for compounds **31**, **32**, and **5**.

H-Trp-OMe to yield amide **31** as a mixture of two diastereomers. The Boc-protecting group was removed with TFA, and the resulting amine was coupled with Cbz-Trp-OH to give compound **32**. Finally, the ketal-protecting group was removed using aqueous TFA to give inhibitor **5**.

Inhibitor 6. The synthetic strategy that we had employed for the preparation of inhibitor **5** did not work for the preparation of inhibitor **6**. When β-ketoester **9** (Scheme 2) was treated with 2 equiv of LDA it decomposed rather than giving clean formation of the dienolate. This decomposition may have been caused by β-elimination of the thioether, although such elimination reactions are often slow in six-membered rings.[23]

Since our attempts to alkylate compound **9** were unsuccessful, we chose an alternate strategy for the preparation of inhibitor **6** that is shown in Scheme 6. Ketone **33** was converted to its enol ether **34** using allyl alcohol, 2,2-dimethoxypropane, and catalytic *p*-toluenesulfonic acid in benzene at 80 °C. Without isolating the product, the reaction solvent was switched to toluene. It was then heated at reflux to induce a Cope rearrangement to give alkene **35**.[24] We also prepared compound **35** by treating ketone **33** with LDA, followed by alkylation of the enolate with allyl bromide. However, the product of this reaction was contaminated with a significant amount of the dialkylated ketone, and this material was difficult to separate from the desired monoalkylated ketone.

To add the second substituent to the cyclohexanone core, alkene **35** was deprotonated with LDA and the resulting enolate was treated with methyl cyanoformate to give β-ketoester **36** as predominantly the enol tautomer.[25] Other CO_2 equivalents such as dimethyl carbonate and methyl chloroformate were not as satisfactory. Reduction of compound **36** with $NaBH_4$ gave alcohol **37** as a mixture of several diastereomers. Protection of alcohol **37** with TBDMSCl, followed by saponification of the ester generated carboxylic acid **38**. At this point, the diastereomers with the carboxylic acid, OTBDMS, and allyl substituents cis to one another were separated from the other diastereomers by flash chromatography, and these two diastereomers were carried on through the remainder of the synthesis. The relative configuration of the three substituents on the ring in compound **38** was confirmed by 2D-COSY NMR. Compound **38** was converted into Cbz-protected amine **39** using the procedure that was described earlier for the synthesis of compound **14** (Scheme 2). Compound **39** was treated with an excess of $NaIO_4$ in the presence of catalytic $KMnO_4$ to accomplish both oxidative cleavage of the alkene and oxidation of the thioether to give sulfone **40**.

We planned to measure the activity of the two separate diastereomers of inhibitor **6** against plasmin. Thus, after compound **40** was coupled with Trp(Boc)-OMe, the two resulting diastereomers were separated by flash chromatography to give **41a** and **b**, and these two diastereomers were separately carried on through the synthesis. Deprotection of the Cbz group and coupling with Cbz-Trp(Boc)-OH gave compounds **42a** and **b**. The silyl ether was then removed to give alcohols **43a** and **b**. When these two alcohols were oxidized to the corresponding ketone (**44**) with Dess−Martin periodinane, we found that the two reactions gave identical products that were approximately a 1:1 mixture of two diastereomers, even though the starting materials for both reactions were single diastereomers. The arrangement of the sulfone and the ketone in the six-membered ring makes the ketone very prone to enolization. As a result, the two stereocenters α to the ketone racemized under the reaction conditions. The synthesis was completed by removing the two Boc groups in the 1:1 mixture of diastereomers of compound **40** with TFA to yield the desired inhibitor.

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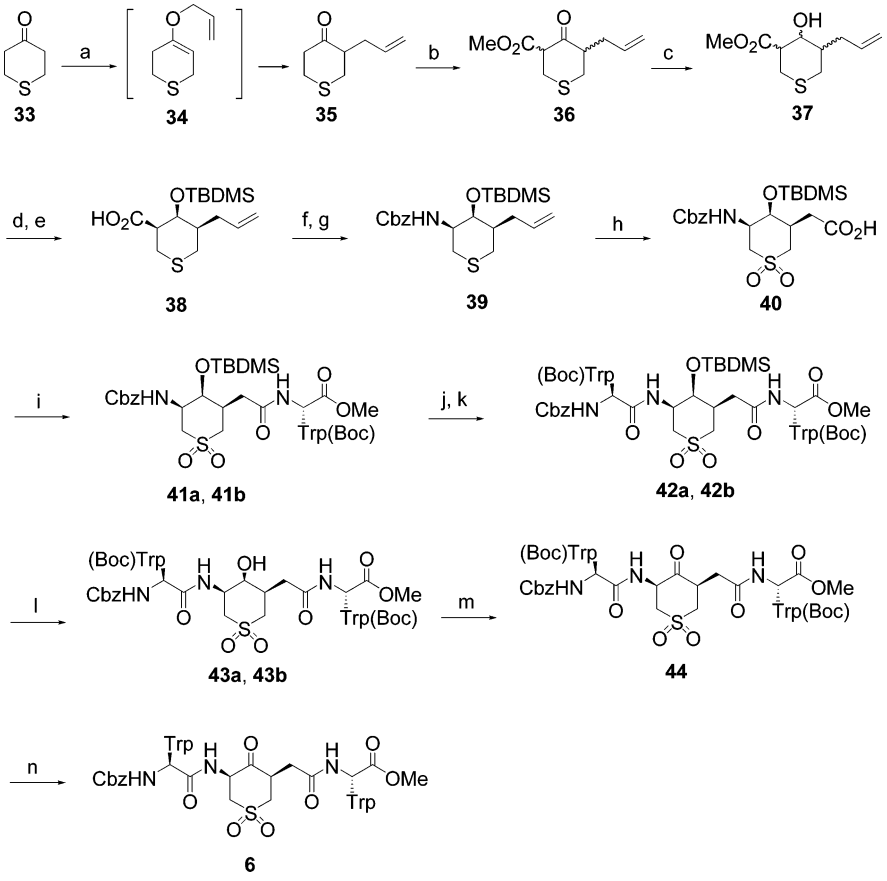
SCHEME 6. Synthesis of Inhibitor 6[a]



[a] Reagents: (a) allyl alcohol, 2,2-dimethoxypropane, *p*-toluenesulfonic acid, 93%; (b) LDA; MeO_2CCN, 64%; (c) $NaBH_4$, 78%; (d) TBDMSCl, imidazole; (e) NaOH, MeOH, 42% (two steps); (f) $(C_6H_5O)_2PON_3$, DIEA; (g) BnOH, *n*-BuLi, 72% (two steps); (h) $NaIO_4$, $KMnO_4$, $NaHCO_3$, 65%; (i) H-Trp(Boc)-OMe, HBTU, DIEA, **41a**: 35%, **41b**: 31%; (j) H_2, Pd/C; (k) Cbz-Trp(Boc)-OH, HBTU, DIEA, **42a**: 70%, **42b**: 76% (two steps); (l) TBAF, **43a**: 81%, **43b**: 80%; (m) Dess−Martin periodinane, 92%; (n) TFA, 76%. For compounds **41**−**43**, **a** and **b** refer to two different diastereomers. Only one of the two diastereomers is shown for compounds **41**−**44** and **6**.

TABLE 1. Inhibition Constants of Compounds 1−6 against Plasmin

inhibitor	IC_{50} (μM)	inhibitor	IC_{50} (μM)
1	800 ± 60	**2**	200 ± 16
3	122 ± 5	**4**	45 ± 5
5	25 ± 3	**6**	5.5 ± 0.5

The enolization of compounds **44** and **6** prevented us from being able to prepare inhibitor **6** as two separate diastereomers. This same behavior is likely to be seen in the other sulfone-containing inhibitors **2** and **4**. Therefore, we elected to measure the activity of all of the inhibitors as mixtures of two diastereomers. In previous studies, we found that the diastereomers of related inhibitors have similar activities; in most cases, the diastereomers have IC_{50} values that are within a factor of 2 of each other.[12,17]

Inhibition Studies. Inhibitors **1**−**6** were assayed against plasmin using D-Val-Ile-Lys-*p*NA (*p*NA = *p*-nitroanilide) as the substrate (Table 1). Initial rates were measured using UV spectroscopy to monitor formation of *p*-nitroaniline. The assay mixture contained 50 mM sodium phosphate buffer at pH 7.4, and 10% DMSO to ensure solubility of the inhibitors. Under these conditions, the K_M value for this substrate was measured to be 170 μM.

Compounds **1** and **2** have similar structures. They both incorporate a simple Trp side chain at the P2 position that binds in the S2 subsite of the enzyme. Inhibitor **1** has low activity with an IC_{50} value of 800 μM. This result is not surprising since the inhibitor has only a single amino acid, in addition to the electrophilic ketone, to make contacts with active site residues. In inhibitor **2**, the C-4 atom of the cyclohexanone ring has been replaced with an SO_2 group. This replacement induces a repulsive electrostatic interaction between the sulfone and the ketone, which increases the electrophilicity of the ketone and enhances its interaction with the active site serine nucleophile. As a result, there is a 4-fold increase in potency when inhibitor **2** is compared with **1**.

Inhibitors **3** and **4** incorporate a dipeptide side chain and are expected to make a greater number of noncovalent contacts with the active site. The aminohexyl group in these inhibitors mimics the side chain of Lys and is designed to bind in the S1 subsite. The aromatic ring of Phe fits into the hydrophobic S2 subsite, whereas D-Ile is aligned with the S3 subsite. The free *N*-terminus of the D-Ile residue is likely to be protonated at physiological pH. This positions a positive charge at the base of the S3 subsite, which has been reported to be beneficial for binding.[11] Comparison of inhibitors **1** and **3** shows that replacement of the Cbz-Trp with D-Ile-L-Phe increases the

potency of the cyclohexanone-based inhibitors by a factor of approximately 6. Inhibitor **4** also incorporates the sulfone functional group and shows a further increase in potency to give an IC_{50} value of 45 μM.

Inhibitors **5** and **6** incorporate peptide side chains that extend into both the nonprimed and the primed subsites. Like inhibitors **1** and **2**, the Cbz-Trp moiety is designed to bind in the S2 subsite. The Trp-OMe group on the C-terminal side of the inhibitors is aligned to bind in the S2′ subsite. We chose Trp for P2′ position because our earlier studies with a combinatorial library of inhibitors demonstrated that Trp is the optimal amino acid for this position of these inhibitors.[17] Inhibitor **5** has an IC_{50} value of 25 μM, which represents a 5-fold increase in potency over inhibitor **3**, and a 32-fold increase over inhibitor **1**. One plausible explanation for the good activity of inhibitor **5** is that this molecule is anchored into the active site on both the S and S′ sides. This set of interactions may be more effective at properly aligning the electrophilic ketone for reaction with the serine nucleophile, when compared to the inhibitors **1**−**4** that anchor the inhibitors only through interactions with the S subsites. Thus, a combination of both favorable noncovalent and covalent interactions between enzyme and inhibitor would lead to better inhibition by compound **5**.

Inhibitor **6**, which is structurally similar to inhibitor **5** but incorporates the sulfone group, has an IC_{50} value of 5.5 μM and is the best inhibitor out of the series. Across all three pairs of inhibitors, we observe that incorporation of the sulfone increases inhibitor potency by a factor of 3−5. This result is consistent with our proposed mechanism of inhibition, which involves reversible formation of a hemiketal linkage between the active site serine reside and the ketone group in the inhibitors. It is also consistent with the trend that we observed in previous studies between inhibitor potency and ketone elecrophilicity.[14] An alternate explanation for the enhanced activity of the sulfone-containing inhibitors is that the sulfone could be forming a hydrogen bond with nearby residues in the active site. However, since we have observed similar increases in potency for a number of inhibitors of both serine and cysteine proteases, we believe that the main role of the sulfone is to increase the electrophilicity of the ketone.

Conclusions

In summary, we synthesized three pairs of compounds (**1**−**6**) as inhibitors against the serine protease plasmin. Compounds **1**, **3**, and **5** are based upon a cyclohexanone pharmacophore, while compounds **2**, **4**, and **6** are based upon the tetrahydro-4*H*-thiopyran-4-one 1,1-dioxide structure. The direct comparisons of the three pairs of inhibitors show that the strongly electronegative sulfone group increases inhibitor potency by a factor of 3−5 when compared to the cyclohexanone analogues. In addition, inhibitors that bind in both the S and S′ subsites have higher activity when compared to inhibitors that contact only half of the active site.

Experimental Section

Enzyme Assays. IC_{50} values for inhibitors **1**−**6** were measured for plasmin using the chromogenic substrate D-Val-Leu-Lys-*p*NA. Enzyme and substrate were used as received. Plasmin was 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 UV−vis spectrometer. When measuring the IC_{50} of inhibitors, the substrate concentration was held constant at its K_M value, which was measured to be 170 μM. Data analysis was performed with the commercial graphing package Grafit (Erithacus Software Ltd.).

Amide 8. Amine **7** (200 mg, 1.18 mmol) was dissolved in DMF (5 mL). To this solution, Cbz-Trp-OH (598 mg, 1.77 mmol), HBTU (671 mg, 1.77 mol), and DIEA (620 μL, 460 mg, 3.54 mmol) were added. After the reaction was stirred for 1 h at room temperature, the reaction mixture was partitioned between EtOAc (200 mL) and 1 N HCl (200 mL). The organic layer was washed with 1 N HCl (150 mL), saturated $NaHCO_3$ (150 mL), and brine (100 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 amide **8** as a mixture of two diastereomers (463 mg, 944 μmol, 80%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00−1.45 (m, 4H), 1.46−1.70 (m, 4H), 1.71−1.95 (m, 1H), 2.40−2.70 (m, 1H), 3.05−3.35 (m, 2H), 3.50−3.95 (m, 5H), 4.45−4.71 (br s, 1H), 5.05−5.25 (m, 2H), 5.40−5.80 (m, 1H), 5.81−6.35 (m, 1H), 6.95−7.25 (m, 3H), 7.29−7.45 (m, 6H), 7.58−7.85 (m, 1H), 8.10−8.30 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.6, 17.0, 21.8, 21.99, 22.05, 23.9, 24.1, 25.6, 25.7, 28.0, 29.0, 29.3, 29.6, 54.3, 55.6, 55.9, 59.5, 59.6, 60.8, 67.2, 69.7, 75.4, 77.7, 97.5, 97.7, 110.6, 111.5, 119.3, 119.5, 120.0, 120.1, 122.4, 122.6, 123.7, 123.8, 128.45, 128.50, 128.90, 128.93, 136.6, 136.7, 156.3, 170.7, 171.1; HRMS-FAB ($M + H^+$) calcd for $C_{28}H_{34}N_3O_5$ 492.2498, found 492.2503.

Inhibitor 1. To compound **8** (200 mg, 407 μmol), an aqueous TFA solution (10 mL, 50%) was added at 0 °C. The reaction was warmed to room temperature, stirred for an additional 12 h, and then concentrated by rotary evaporation. The resulting residue was diluted with EtOAc (50 mL) and washed with saturated aqueous Na_2CO_3 (50 mL) and brine (50 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 inhibitor **1** as a mixture of two diastereomers (97 mg, 224 μmol, 55%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05−1.25 (m, 1H), 1.40−1.92 (m, 5H), 2.10−2.70 (m, 2H), 2.85−3.35 (m, 3H), 3.90−4.60 (m, 1H), 5.00−5.25 (m, 3H), 5.60−6.80 (m, 1H), 6.92−7.21 (m, 4H), 7.28−7.90 (m, 6H), 8.31−8.80 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.0, 21.8, 22.9, 24.3, 24.5, 26.0, 26.6, 28.3, 30.0, 33.0, 33.8, 34.3, 35.5, 41.2, 41.3, 56.1, 56.7, 58.2, 58.5, 59.6, 60.4, 60.5, 67.4, 67.8, 68.0, 69.7, 77.7, 108.8, 110.3, 110.5, 111.4, 111.7, 118.8, 119.2, 119.9, 120.2, 122.5, 122.8, 123.8, 127.1, 127.8, 128.4, 128.5, 128.6, 128.8, 128.90, 128.96, 129.1, 135.2, 136.20, 136.23, 136.3, 136.7, 155.1, 156.3, 170.3, 171.4, 207.2; HRMS-FAB ($M + Na^+$) calcd for $C_{25}H_{27}N_3NaO_4$ 456.1899, found 456.1892.

Alcohol 10. Ketoester **9** (10 g, 57 mmol) was dissolved in dry THF (150 mL) at 0 °C. To this solution, $NaBH_4$ (4.25 g, 115 mmol) was slowly added. After 30 min, the reaction was quenched with 1 N HCl (200 mL), and the THF was removed by rotary evaporation. The resulting material was partitioned between EtOAc (500 mL) and 1 N HCl (300 mL). The organic layer was washed with 1 N HCl (300 mL), saturated aqueous $NaHCO_3$ (300 mL), and brine (500 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, gradient of 1:9 to 1:2) to yield compounds **10** (1.52 g, 8.6 mmol, 15%) and **11** (6.07 g, 34.5 mmol, 60%). **10**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55−1.75 (m, 1H), 2.15−2.32 (dm, J = 13.5 Hz, 1H), 2.49−2.70 (m, 4H), 2.71−2.90 (dm, J = 12.3 Hz, 1H), 3.25 (s, 1H), 3.55−3.65 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.8, 30.0, 35.6, 52.3, 52.5, 70.4,

174.2; HRMS-FAB (M + H$^+$) calcd for $C_7H_{12}O_3S$ 176.0507, found 176.0510. **11**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.85−2.00 (tm, J = 14.5 Hz, 1H), 2.03−2.25 (m, 1H), 2.30−2.40 (dm, J = 15.0 Hz, 1H), 2.55−2.65 (dm, J = 13.5 Hz, 1H), 2.85−2.92 (dt, J = 10.5, 3.0 Hz, 1H), 2.95−3.12 (tm, J = 13.8 Hz, 1H), 3.05−3.25 (m, 2H), 3.78 (s, 3H) 4.15−4.25 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.2, 25.4, 33.7, 47.8, 52.5, 66.2, 174.6; HRMS-FAB (M + H$^+$) calcd for $C_7H_{12}O_3S$ 176.0507, found 176.0512.

TBDMS Ether 12. To a solution of alcohol **11** (6.07 g, 34.5 mmol) in DMF (12 mL), imidazole (3.52 g, 51.7 mmol) and TBDMSCl (12.9 g, 86.3 mmol) were added. The reaction was stirred at 50 °C for 12 h. The mixture was diluted with EtOAc (1 L), and the organic layer was washed with 1 N HCl (2 × 500 mL), saturated aqueous $NaHCO_3$ (500 mL), and brine (500 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, 1:10) to give compound **12** (8.60 g, 29.7 mmol, 86%): ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.04−0.04 (d, J = 19.5 Hz, 6H), 0.85 (s, 9H), 1.83−1.90 (tq, J = 12.3, 1.8 Hz, 1H), 2.02−2.09 (dm, J = 13.9 Hz, 1H), 2.18−2.23 (dm, J = 13.2 Hz, 1H), 2.47−2.52 (dm, J = 13.5 Hz, 1H), 2.64−2.71 (dt, J = 12.0, 3.0 Hz, 1H), 2.96−3.06 (td, J = 13.1, 2.4 Hz, 1H), 3.17−3.26 (dd, J = 13.3, 12.1 Hz, 1H), 3.66 (s, 3H), 4.51−4.54 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.0, −4.0, 18.3, 21.9, 23.1, 26.0, 35.4, 49.6, 52.0, 67.3, 173.2; HRMS-FAB (M + H$^+$) calcd for $C_{13}H_{27}O_3SSi$ 291.1450, found 291.1455.

Carboxylic Acid 13. To compound **12** (6.00 g, 20.7 mmol), MeOH (120 mL) and 1 N aqueous NaOH (120 mL) were added. The mixture was heated at reflux for 24 h. The MeOH was removed by rotary evaporation, and to the aqueous layer were added EtOAc (500 mL) and 1 N HCl (500 mL). The organic layer was washed with brine (300 mL) and dried over $MgSO_4$. The solvent was removed by rotary evaporation. The crude oil was purified by flash chromatography (EtOAc/hexanes, 1:2) to yield carboxylic acid **13** (3.87 g, 14.5 mmol, 70%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.04−0.09 (d, J = 15.0 Hz, 6H), 0.98 (s, 9H), 1.87−1.97 (tt, J = 14.0, 3.4 Hz, 1H), 2.03−2.12 (dm, J = 13.7 Hz, 1H), 2.28−2.33 (dm, J = 13.2 Hz, 1H), 2.52−2.58 (dm, J = 13.4 Hz, 1H), 2.74−2.81 (dt, J = 11.1, 2.8 Hz, 1H), 2.96−3.01 (m, 1H), 3.20−3.25 (m, 1H), 4.48−4.49 (dm, J = 2.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.9, −4.0, 18.4, 22.6, 23.7, 26.1, 35.3, 49.0, 67.8, 177.8; HRMS-FAB (M + Na$^+$) calcd for $C_{12}H_{24}NaO_3SSi$ 299.1113, found 299.1112.

Carbamate 14. To a solution of **13** (3.8 g, 13.8 mmol) in dry toluene (100 mL), DIEA (3.6 mL, 2.69 g, 20.7 mmol) and $(PhO)_2PON_3$ (4.56 mL, 4.57 g, 16.6 mmol) were added. The reaction was heated at 60 °C under nitrogen for 4 h. The formation of the isocyanate was followed by IR spectroscopy at 2257 cm^{-1}. The alkoxide from benzyl alcohol was prepared in a separate flask as follows: Benzyl alcohol (4.50 mL, 4.47 g, 41.4 mmol) was added to dry THF (60 mL) at 0 °C under nitrogen, followed by dropwise addition of *n*-butyllithium (11.0 mL, 2.5 M solution in hexanes, 27.6 mmol) over a period of 5 min. The isocyanate solution was then cooled to room temperature and added dropwise to the alkoxide solution at 0 °C. After the addition was complete, the reaction was allowed to warm to room temperature over 30 min. The reaction was then quenched with 1 N HCl (100 mL), followed by the removal of the THF by rotary evaporation. The remaining solution was partitioned between EtOAc (500 mL) and 1 N HCl (300 mL) and washed with saturated aqueous $NaHCO_3$ (300 mL) and brine (300 mL). It was then dried over $MgSO_4$, and the solvents were removed by rotary evaporation. The crude oil was purified by flash chromatography (EtOAc/hexanes, 1:9) to yield compound **14** (3.60 g, 9.49 mmol, 84%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.08 (s, 6H), 0.93 (s, 9H), 1.95−2.00 (tm, J = 11.6 Hz, 1H), 2.06−2.12 (m, 1H), 2.21−2.26 (dm, J = 13.6 Hz, 1H), 2.41−2.46 (dd, J = 10.0, 2.6 Hz, 1H), 2.84−2.93 (t, J = 11.8 Hz, 2H), 3.87−4.00 (m, 2H), 5.01−5.05 (d, J = 9.2 Hz, 1H), 5.11−5.16 (m, 2H), 7.30−7.38 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.6, −4.1, 18.5, 22.1, 26.1, 26.2, 28.0, 35.4, 53.0, 67.0, 69.3, 128.4, 128.5, 128.9, 129.0, 137.0, 155.8; HRMS-FAB (M + Na$^+$) calcd for $C_{19}H_{31}NNaO_3SSi$ 404.1692, found 404.1680.

Sulfone 15. Compound **14** (2.0 g, 5.25 mmol) was dissolved in acetone (200 mL) and H_2O (100 mL). To this solution, $NaIO_4$ (6.74 g, 31.5 mmol), $KMnO_4$ (620 mg, 3.94 mmol), and $NaHCO_3$ (450 mg, 5.25 mmol) were added. The solution was stirred for 6 h at room temperature. The acetone was removed by rotary evaporation, and the remaining material was partitioned between EtOAc (500 mL) and 1 N HCl (300 mL). The organic layer was washed with 1 N HCl (2 × 300 mL) and brine (300 mL) and then dried over $MgSO_4$. The solvent was removed by rotary evaporation, and the crude oil was purified by flash chromatography (EtOAc/hexanes, 1:2) to yield **15** (1.60 g, 3.88 mmol, 74%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.10 (s, 6H), 0.92 (s, 9H), 2.06−2.15 (m, 1H), 2.15−2.25 (t, J = 13.0 Hz, 1H), 2.83−2.90 (dm, J = 14.0 Hz, 1H), 3.15−3.28 (m, 2H), 4.10−4.15 (m, 1H), 4.28−4.35 (m, 1H), 4.90−4.93 (d, J = 9.2 Hz, 1H), 5.13 (s, 2H), 7.27−7.40 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.6, −4.2, 18.4, 26.1, 29.2, 45.1, 51.4, 51.8, 67.1, 67.6, 128.6, 128.8, 129.0, 136.4, 155.3; HRMS-FAB (M + Na$^+$) calcd for $C_{19}H_{31}NaNO_5SSi$ 436.1590, found 436.1579.

Primary Amine 16. To compound **15** (2.0 g, 4.84 mmol), dry MeOH (60 mL) and 10% Pd on carbon (1.0 g) were added. The reaction flask was purged and kept under 1 atm of hydrogen gas. The reaction was stirred at room temperature for 5 h. The Pd/carbon was then removed by filtration. The clear solution was concentrated by rotary evaporation, and the resulting residue was dried under vacuum for 24 h to give compound **16** (1.35 g, 4.84 mmol, 100%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.14−0.16 (d, J = 9.0 Hz, 6H), 0.94 (s, 9H), 2.10−2.20 (m, 2H), 2.78−2.86 (dq, J = 13.8, 3.7 Hz, 1H), 2.93−3.01 (dt, J = 13.2, 3.6 Hz, 1H), 3.10−3.26 (m, 2H), 3.33−3.40 (dq, J = 11.4, 2.1 Hz, 1H), 4.02−4.04 (t, J = 2.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 0.6, 0.8, 23.4, 31.1, 34.3, 49.8, 57.7, 58.9, 59.9, 74.3; HRMS-FAB (M + Na$^+$) calcd for $C_{11}H_{25}NNaO_3SSi$ 302.1222, found 302.1228.

Amide 17. Compound **16** (200 mg, 0.72 mmol) was dissolved in DMF (2 mL). To this solution, Cbz-Trp(Boc)-OH (346 mg, 0.80 mmol), HBTU (545 mg, 1.44 mmol), and DIEA (375 μL, 281 mg, 2.16 mmol) were added. After 2 h at room temperature, the reaction mixture was partitioned between EtOAc (100 mL) and 1 N HCl (100 mL). The organic layer was then washed with 1 N HCl (50 mL), saturated $NaHCO_3$ (50 mL), and brine (50 mL). It was dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The crude oil was purified by flash chromatography (EtOAc/hexanes, 1:2) to yield compound **17** as a mixture of two diastereomers (433 mg, 0.62 mmol, 86%): ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.4−0.0 (m, 6H), 0.70−0.80 (d, J = 10.0 Hz, 9H), 1.66 (s, 9H), 1.85−2.00 (m, 1H), 2.00−2.16 (m, 1H), 2.50−3.40 (m, 7H), 4.19−4.40 (m, 2H), 5.00−5.20 (m, 2H), 5.25−6.15 (m, 2H), 7.10−7.70 (m, 9H), 8.11−8.21 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.1, −5.0, −4.4, −4.3, 18.0, 18.2, 25.9, 26.0, 28.6, 29.1, 29.2, 29.3, 45.0, 45.1, 50.0, 50.4, 50.6, 50.8, 55.7, 66.7, 67.6, 67.7, 84.3, 84.6, 115.5, 115.8, 116.0, 119.3, 119.5, 123.2, 123.4, 124.8, 124.9, 125.2, 125.6, 128.6, 128.8, 129.0, 130.2, 130.7, 135.8, 135.9, 136.3, 136.4, 170.2, 170.6; HRMS-FAB (M + H$^+$) calcd for $C_{35}H_{49}N_3NaO_8SSi$ 722.2907, found 722.2899.

Alcohol 18. To a solution of compound **17** (100 mg, 0.17 mmol) in dry THF (5 mL), a solution of TBAF (66 mg, 0.25 mmol) in dry THF (5 mL) was added. The reaction was stirred at room temperature for 30 min. The solvents were then removed by rotary evaporation, and the resulting material was purified by flash chromatography (EtOAc/chloroform 2:1) to give compound **18** as a mixture of two diastereomers (87 mg, 0.15 mmol, 89%): ^{1}H NMR (400 MHz, acetone-d_6) δ 0.68 (s, 9H), 2.00−2.35 (m, 2H), 2.75−3.00 (m, 2H), 3.11−3.35 (m, 4H), 3.89−4.11 (m, 1H), 4.30−4.70 (m, 3H), 4.96−5.12 (m, 2H), 6.10−6.75 (m, 1H), 7.15−7.43 (m, 6H), 7.45−7.80 (m, 3H), 8.11−8.21 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 32.8, 33.2, 50.1, 50.2, 55.3, 55.5, 55.7, 60.46, 60.51, 69.9, 70.0, 70.8, 71.4, 88.8, 88.9, 120.4, 121.58, 121.63, 124.6, 127.8, 127.9, 129.65,

129.69, 129.8, 130.1, 133.1, 133.2, 133.8, 136.0, 136.1, 140.8, 142.5, 154.77, 154.81, 161.4, 161.5, 176.0, 176.1; HRMS-FAB ($M + Na^+$) calcd for $C_{29}H_{35}N_3NaO_8S$ 608.2043, found 608.2047.

Inhibitor 2. To a solution of alcohol **18** (50 mg, 85 μmol) in dry CH_2Cl_2 (10 mL), Dess−Martin reagent (55 mg, 128 μmol) was added. The reaction was stirred at room temperature for 24 h. The reaction mixture was then partitioned between EtOAc (30 mL) and saturated Na_2CO_3 (30 mL), and the organic layer was washed with saturated Na_2CO_3 (30 mL) and brine (30 mL). It was then dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The crude ketone was purified by flash chromatography (EtOAc/hexanes, 2:1). The resulting ketone was redissolved in CH_2Cl_2 (5 mL), and to this solution TFA (2.5 mL) was added. The reaction was stirred at room temperature for 1.5 h, the solvents were removed by rotary evaporation, and the resulting material was partitioned between EtOAc (25 mL) and saturated Na_2CO_3 (10 mL). The organic layer was washed with saturated Na_2CO_3 (10 mL) and brine (10 mL) and then dried over $MgSO_4$. The solvent was removed by rotary evaporation, and the crude material was purified by flash chromatography (EtOAc/CH_2Cl_2, 2:1) to yield inhibitor **2** as a mixture of two diastereomers (14 mg, 29 μmol, 34%): ^{1}H NMR (300 MHz, acetone-d_6) δ 2.69−2.89 (m, 1H), 3.11−3.79 (m, 8H), 4.43−4.62 (m, 1H), 4.99−5.15 (m, 3H), 6.96−7.15 (m, 2H), 7.20−7.51 (m, 8H), 7.51−7.72 (m, 1H); ^{13}C NMR (75 MHz, acetone-d_6) δ 14.0, 20.3, 32.7, 36.3, 49.9, 50.0, 54.08, 54.14, 54.2, 54.3, 56.0, 60.0, 66.3, 110.3, 110.5, 111.7, 118.8, 119.1, 121.7, 124.0, 128.0, 128.1, 128.7, 136.2, 136.9, 127.6, 156.3, 170.4, 170.6, 199.9, 200.0; HRMS-FAB ($M + Na^+$) calcd for $C_{24}H_{25}N_3NaO_6S$ 506.1362, found 506.1345.

Amide 20. Primary amine **7** (200 mg, 1.18 mmol) was dissolved in $CDCl_3$ (7 mL). To this solution, a solution of $BocNH(CH)_5CHO$ (216 mg, 1.00 mmol) in $CDCl_3$ (3 mL) and titanium(IV) isopropoxide (585 μL, 568 mg, 2.00 mmol) were added. The reaction was stirred at room temperature, and the formation of the imine was monitored using ^{1}H NMR spectroscopy by following the disappearance of a resonance for the aldehyde at 9.77 ppm. After the formation of the imine was complete, the reaction was added dropwise to a solution of $NaBH_4$ (185 mg, 5.0 mmol) in MeOH (10 mL) at 0 °C. The solution was warmed to room temperature and stirred for an additional 1 h. Water (2 mL) was added with stirring, and the resulting inorganic precipitate was filtrated and washed with EtOAc (50 mL). The filtrate was then partitioned between EtOAc (75 mL) and 1 N HCl (75 mL). The organic layer was washed with 1 N HCl (50 mL), saturated aqueous Na_2CO_3 (50 mL), and brine (50 mL). It was then dried over $MgSO_4$, and the solvents were removed by rotary evaporation. The resulting oil was purified by flash chromatography (0−15% MeOH in CH_2Cl_2) to give a mixture of starting material **7** and secondary amine **19**. To this mixture, Fmoc-Phe-OH (685 mg, 1.77 mmol), HATU (673 mg, 1.77 mmol), DIEA (620 μL, 460 mg, 3.54 mmol), and DMF (800 μL) were added. The reaction was stirred at 60 °C for 3 h. The reaction mixture was cooled to room temperature and partitioned between EtOAc (100 mL) and 1 N HCl (100 mL). The organic layer was washed with 1 N HCl (75 mL), saturated aqueous $NaHCO_3$ (75 mL), and brine (75 mL). It was then dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (0−15% MeOH in CH_2Cl_2) to give compound **20** (229 mg, 310 μmol, 31% for two steps) as a mixture of two diastereomers: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.70−1.00 (m, 1H), 1.11−1.56 (m, 19H), 1.57−2.04 (m, 4H), 2.63−3.22 (m, 6H), 3.43−3.60 (m, 1H), 3.62−3.96 (m, 3H), 3.97−4.29 (m, 3H), 4.31−4.48 (m, 1H), 4.49−4.61 (m, 1H), 4.62−5.15 (m, 2H), 5.34−6.03 (m, 1H), 6.89−7.26 (m, 4H), 7.28−7.47 (m, 5H), 7.47−7.69 (m, 2H), 7.70−7.89 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.0, 21.7, 22.1, 22.3, 25.4, 25.7, 25.9, 26.0, 26.6, 26.87, 26.93, 27.1, 27.5, 28.2, 28.3, 28.4, 28.6, 28.8, 29.0, 29.4, 30.3, 30.5, 38.9, 41.0, 41.7, 45.1, 45.2, 47.4, 47.49, 47.54, 47.7, 52.4, 53.1, 53.3, 58.3, 59.3, 59.4, 59.56, 59.63, 62.4, 67.0, 67.3, 69.7, 75.4, 77.7, 79.4, 98.6, 99.1, 99.5, 100.0, 120.3, 125.5, 125.6, 125.7, 126.8, 127.2, 127.4, 128.0, 128.6, 128.8, 129.9, 130.1, 137.2, 137.4, 138.3, 141.60, 141.62, 141.7, 144.2, 144.35, 144.41, 144.5, 155.6, 155.9, 156.1, 156.4, 171.1, 173.1, 173.3; HRMS-FAB ($M + Na^+$) calcd for $C_{44}H_{57}N_3NaO_7$ 762.4094, found 762.4091.

Dipeptide 21. Compound **20** (200 mg, 271 μmol) was stirred in a 1:1 mixture of piperidine and DMF (10 mL) at room temperature for 1 h. The solvents were then removed by rotary evaporation. The resulting residue was dried under vacuum for 12 h to remove excess piperidine. The white solid was redissolved in DMF (5 mL). To this solution, Boc-D-Ile-OH (126 mg, 542 μmol), HBTU (205 mg, 542 μmol), and DIEA (143 μL, 106 mg, 813 μmol) were added. The reaction was stirred for 2 h at room temperature, and the mixture was partitioned between EtOAc (100 mL) and 1 N HCl (75 mL). The organic layer was washed with 1 N HCl (75 mL), saturated aqueous $NaHCO_3$ (75 mL), and brine (75 mL). It was then dried over $MgSO_4$ and concentrated. The crude oil was purified by flash chromatography (EtOAc/hexanes, 2:3) to yield compound **21** as a mixture of two diastereomers (155 mg, 211 μmol, 78%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.52−1.03 (m, 8H), 1.04−1.35 (m, 8H), 1.36−1.68 (m, 22H), 1.69−2.08 (m, 6H), 2.48−3.00 (m, 3H), 3.01−3.17 (m, 3H), 3.28−3.66 (m, 2H), 3.67−4.10 (m, 4H), 4.11−4.65 (m, 1H), 4.58−5.30 (m, 2H), 6.30−7.00 (m, 1H), 7.00−7.27 (m, 5H), 7.30−7.82 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.0, 12.1, 15.57, 15.63, 15.9, 22.1, 22.3, 22.4, 24.6, 24.7, 24.9, 25.1, 25.3, 25.6, 25.7, 25.9, 26.4, 26.6, 26.7, 26.9, 27.5, 28.2, 28.3, 28.4, 28.7, 28.9, 29.0, 29.4, 30.4, 30.5, 37.9, 38.4, 38.8, 40.7, 40.9, 41.2, 44.5, 45.1, 45.2, 50.6, 51.9, 55.3, 58.0, 59.3, 59.4, 59.5, 62.0, 62.3, 77.7, 79.4, 79.9, 98.5, 99.1, 99.5, 99.9, 125.8, 126.7, 127.2, 128.6, 128.7, 128.8, 129.78, 129.84, 130.1, 137.3, 138.2, 155.8, 156.0, 156.4, 156.5, 170.3, 171.0, 173.0, 173.3; HRMS-FAB ($M + Na^+$) calcd for $C_{40}H_{66}N_4NaO_8$ 753.4778, found 753.4788.

Inhibitor 3. Compound **21** (50 mg, 68 μmol) was dissolved in CH_2Cl_2 (2 mL). To this solution TFA (1 mL) was added. The reaction was stirred at room temperature for 30 min. The solvents were removed by rotary evaporation. To the resulting residue, 50% aqueous TFA (3 mL) was added, and the reaction was stirred at room temperature for an additional 12 h. The solvents were removed by rotary evaporation. The crude material was purified by flash chromatography (5−15% MeOH in CH_2Cl_2) to yield inhibitor **3** as a mixture of two diastereomers (19 mg, 40 μmol, 59%): ^{1}H NMR (300 MHz, MeOH-d_4) δ 0.62−1.09 (m, 7H), 1.18−1.54 (m, 6H), 1.57−1.85 (m, 6H), 1.92−2.23 (m, 4H), 2.36−2.63 (m, 3H), 2.81−3.11 (m, 4H), 3.14−3.49 (m, 2H), 3.57−3.87 (m, 2H), 4.27−4.46 (m, 1H), 4.47−4.59 (m, 1H), 4.70−5.19 (m, 2H), 7.00−7.25 (m, 5H), 7.30−8.00 (m, 1H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 10.60, 10.65, 10.73, 13.6, 13.8, 21.4, 22.5, 23.9, 24.0, 24.4, 24.5, 24.6, 24.9, 25.2, 25.4, 25.5, 26.1, 26.3, 26.6, 27.5, 29.8, 30.1, 30.3, 31.0, 36.6, 36.7, 36.8, 39.6, 40.9, 43.8, 51.6, 51.8, 54.7, 57.7, 58.0, 59.0, 65.0, 65.1, 65.5, 66.3, 120.6, 124.9, 127.0, 127.2, 127.8, 128.67, 128.73, 129.1, 129.27, 129.33, 129.6, 129.7, 136.9, 137.0, 141.4, 142.7, 168.1, 171.8, 206.2, 206.3; HRMS-FAB ($M + Na^+$) calcd for $C_{27}H_{44}N_4NaO_3$ 495.3311, found 495.3318.

Secondary Amine 22. The aldehyde $BocNH(CH_2)_5CHO$ (277 mg, 1.29 mmol) was dissolved in DCE (dichloroethane) (5 mL). It was added to a solution of compound **16** (300 mg, 1.08 mmol) in DCE (5 mL). After 25 min, sodium triacetoxyborohydride (366 mg, 1.73 mmol) was added. The reaction was stirred at room temperature for 5 h. The reaction was then partitioned between saturated aqueous $NaHCO_3$ (200 mL) and EtOAc (200 mL). The organic layer was washed with brine (200 mL) and then dried over $MgSO_4$. It was concentrated by rotary evaporation, and the crude oil was purified by flash chromatography (EtOAc/hexanes, 1:2) to yield the secondary amine **22** (387 mg, 0.81 mmol, 75%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.12−0.13 (d, J = 3.0 Hz, 6H), 0.93 (s, 9H), 1.31−1.34 (m, 5H), 1.33−1.47 (m, 13H), 2.07−2.20 (m, 2H), 2.55−2.65 (m, 2H), 2.75−2.95 (m, 1H), 3.05−3.20 (m, 5H), 3.20−

3.31 (m, 1H), 4.10−4.18 (s, 1H), 4.43−4.60 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.4, −4.1, 18.4, 26.1, 27.0, 27.3, 28.1, 29.6, 30.2, 30.4, 30.7, 40.9, 45.4, 46.6, 51.9, 58.5, 66.7, 69.7, 77.6, 79.4, 102.7, 156.4; HRMS-FAB (M + Na^+) calcd for $C_{22}H_{46}N_2NaO_5SSi$ 501.2794, found 501.2782.

Amide 24. To a solution of compound **22** (200 mg, 418 μmol) in acetonitrile (8 mL), BSA (203 μL, 170 mg, 209 μmol) was added. The reaction was stirred at room temperature under an atmosphere of nitrogen. After 2 h, Fmoc-Phe-F (364 mg, 936 μmol) and catalytic TBAF (5 mg) were added. The reaction was stirred at 60 °C for an additional 4 h, then cooled to room temperature. The reaction was diluted with CH_2Cl_2, and the organic layer was washed with saturated aqueous $NaHCO_3$ (150 mL), 1 N HCl (150 mL), and brine (150 mL). It was then dried over $MgSO_4$, and the solvents were removed by rotary evaporation. The crude oil was purified by flash chromatography (EtOAc/hexanes, 1:2) to give compound **24** as a mixture of two diastereomers (230 mg, 272 μmol, 65%): ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.12−0.08 (m, 6H), 0.72−0.92 (m, 9H), 1.00−1.33 (m, 4H), 1.33−1.58 (m, 11H), 1.92−2.35 (m, 2H), 2.70−3.73 (m, 9H), 4.00−4.48 (m, 5H), 4.49−5.17 (m, 3H), 5.42−5.73 (m, 1H), 7.09−7.48 (m, 9H), 7.48−7.68 (m, 3H), 7.68−7.86 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.9, −4.6, −4.3, −4.2, −3.9, 14.4, 18.2, 18.3, 26.2, 26.4, 26.79, 26.84, 27.1, 27.3, 28.9, 29.56, 29.62, 29.8, 29.9, 30.2, 30.4, 32.0, 32.1, 40.3, 40.5, 40.7, 40.8, 40.9, 45.3, 45.6, 45.8, 46.2, 47.4, 50.2, 50.4, 50.7, 51.6, 52.9, 53.5, 54.0, 56.3, 67.5, 67.65, 67.73, 69.0, 69.7, 77.7, 79.4, 120.4, 125.4, 125.5, 125.6, 127.46, 127.53, 127.7, 128.12, 128.16, 128.20, 129.1, 129.4, 129.6, 129.8, 130.0, 135.5, 136.6, 136.7, 141.67, 141.70, 143.95, 144.08, 144.1, 144.2, 155.9, 156.0, 156.36, 156.40, 171.9, 172.6, 173.6; HRMS-FAB (M + Na^+) calcd for $C_{46}H_{65}NaN_3O_8SSi$ 870.4159, found 870.4129.

Ketone 26. Compound **24** (200 mg, 236 μmol) was stirred in a 1:1 mixture of piperidine and DMF (10 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 excess piperidine. The white solid was then redissolved in DMF (5 mL). To this solution, Boc-D-Ile-OH (110 mg, 472 μmol), HBTU (179 mg, 472 μmol), and DIEA (124 μL, 92 mg, 708 μmol) were added. The reaction was stirred at room temperature for 2 h, and the mixture was partitioned between EtOAc (100 mL) and 1 N HCl (75 mL). The organic layer was washed with 1 N HCl (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine (50 mL). It was then dried over $MgSO_4$ and concentrated. The crude oil was purified by flash chromatography (EtOAc/hexanes, 1:1) to yield compound **25**. To a solution of dipeptide **25** in dry THF (5 mL), a solution of TBAF (92 mg, 354 μmol) in dry THF (5 mL) was added. The reaction was stirred at room temperature for 30 min. The solvents were removed by rotary evaporation, and the resulting material was purified by flash chromatography (EtOAc/chloroform, 2:1) to give the corresponding alcohol. The alcohol was redissolved in CH_2Cl_2 (15 mL). To this solution, Dess−Martin reagent (150 mg, 354 μmol) was added. The reaction was stirred at room temperature for 12 h and then partitioned between EtOAc (150 mL) and saturated Na_2CO_3 (150 mL). The organic layer was washed with saturated Na_2CO_3 (100 mL) and brine (100 mL). It was then dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The crude material was then purified by flash chromatography (EtOAc/hexanes, 2:1) to yield ketone **26** as a mixture of two diastereomers (98 mg, 136 μmol, 58% for three steps): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.72−0.95 (m, 6H), 1.06−1.19 (m, 2H), 1.19−1.32 (m, 4H), 1.32−1.59 (m, 23H), 1.70−1.96 (m, 1H), 2.88−3.03 (m, 3H), 3.03−3.15 (m, 4H), 3.16−3.47 (m, 2H), 3.58−4.10 (m, 4H), 4.58−4.85 (m, 1H), 4.86−5.21 (m, 2H), 6.55−6.86 (m, 1H), 7.11−7.50 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.8, 11.9, 12.0, 14.6, 15.8, 15.9, 16.0, 24.9, 25.0, 26.1, 26.4, 26.5, 26.6, 28.7, 28.8, 28.9, 29.4, 30.0, 30.2, 37.0, 37.2, 37.47, 37.53, 37.7, 39.0, 40.4, 40.8, 48.3, 48.4, 50.2, 50.4, 50.5, 51.2, 51.6, 59.5, 60.8, 62.6, 77.6, 79.5, 80.4, 127.6, 127.9, 129.1, 129.2, 129.8, 129.9, 136.0, 136.1, 156.0, 156.5, 171.3, 171.4, 171.6, 171.7, 171.8, 172.2, 195.6, 196.0; HRMS-FAB (M + Na^+) calcd for $C_{36}H_{58}N_4NaO_9S$ 745.3822, found 745.3833.

Inhibitor 4. Compound **26** (50 mg, 69 μmol) was dissolved in CH_2Cl_2 (2 mL). To this solution TFA (0.5 mL) was added. The reaction was stirred at room temperature for 30 min. The solvents were removed by rotary evaporation. The crude material was purified by flash chromatography (5−15% MeOH in CH_2Cl_2) to yield inhibitor **4** as a mixture of two diastereomers (25 mg, 48 μmol, 70%): ^{1}H NMR (300 MHz, MeOH-d_4) δ 0.72−0.98 (m, 4H), 0.98−1.09 (m, 3H), 1.12−1.57 (m, 7H), 1.58−2.06 (m, 5H), 2.86−3.25 (m, 6H), 2.81−3.11 (m, 3H), 3.32−3.57 (m, 3H), 3.58−3.80 (m, 2H), 3.81−4.08 (m, 1H), 4.09−4.56 (m, 1H), 4.94−5.18 (m, 1H), 7.15−7.35 (m, 5H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 10.7, 10.8, 13.8, 13.9, 14.1, 23.9, 24.1, 24.3, 26.1, 26.2, 27.5, 28.8, 29.1, 29.3, 36.6, 36.8, 37.2, 37.7, 38.4, 39.6, 49.6, 50.5, 51.2, 51.4, 51.5, 51.7, 52.1, 52.5, 57.7, 57.9, 62.1, 115.0, 118.9, 127.2, 127.4, 128.5, 128.77, 128.82, 129.1, 129.3, 129.4, 129.5, 129.6, 129.7, 136.7, 136.8, 161.2, 161.7, 168.1, 168.3, 171.9, 172.2, 197.4, 198.0; HRMS-FAB (M + Na^+) calcd for $C_{26}H_{42}N_4O_5S$ 523.2954, found 523.2966.

Alkene 28. To a solution of diisopropylamine (8.42 mL, 6.06 g, 60.0 mmol) in THF (60 mL), *n*-butyllithium (23.5 mL, 58.8 mmol, 2.5 M in hexanes) was added 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, compound **27** (5.0 g, 29.4 mmol) was slowly added. After 15 min, allyl bromide (2.60 mL, 3.72 g, 31.0 mmol) was added dropwise. The reaction was stirred at 0 °C for 30 min and then quenched with water. The THF was removed by rotary evaporation, and the mixture was partitioned between EtOAc (300 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). 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 **28** as a mixture of ketone and enol tautomers (4.63 g, 22 mmol, 75%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.10−1.30 (m, 3.5H), 1.31−1.55 (m, 1.6H), 1.56−1.77 (m, 2.3H), 1.80−2.05 (m, 1.4H), 2.06−2.28 (m, 2.7H), 2.29−2.45 (m, 1.1H), 2.46−2.66 (m, 1.2H), 3.20−3.40 (m, 0.4H), 4.00−4.35 (m, 2H), 4.80−5.10 (m, 2H), 5.50−5.80 (m, 1H), 14.42 (s, 0.5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.06, 14.13, 14.2, 20.0, 21.6, 21.9, 22.4, 22.8, 24.0, 26.7, 29.0, 30.2, 30.8, 33.0, 33.4, 33.7, 36.3, 38.2, 48.9, 50.5, 56.2, 57.8, 60.0, 60.2, 60.8, 61.2, 98.0, 116.3, 116.6, 128.18, 128.28, 128.32, 135.9, 136.0, 136.4, 169.9, 172.9, 173.7; HRMS-FAB (M + H^+) calcd for $C_{12}H_{19}O_3$ 211.1334, found 211.1338.

Ketal 29. A solution of compound **28** (4.0 g, 19 mmol) in THF (10 mL) was cooled in an ice bath. To this solution, 1,3-propanediol (41 mL, 43.3 g, 570 mmol) and TMSCl (4.77 mL, 4.1 g, 38.0 mmol) were added. The reaction was stirred at room temperature for 48 h and then partitioned between EtOAc (400 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 **29** (4.33 g, 16.2 mmol, 85%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05−1.35 (m, 5H), 1.36−1.55 (m, 2H), 1.56−1.70 (m, 2H), 1.71−1.79 (m, 4H), 2.05−2.32 (m, 1H), 2.40−2.60 (m, 1H), 3.70−3.95 (m, 3H), 4.00−4.25 (m, 3H), 4.80−5.10 (m, 2H), 5.55−5.85 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 20.0, 25.2, 25.5, 26.1, 32.4, 59.1, 59.4, 60.0, 98.4, 115.3, 138.4, 172.4; HRMS-FAB (M + Na^+) calcd for $C_{15}H_{24}NaO_4$ 291.1572, found 291.1575.

Alkene 30. To a solution of compound **29** (5.0 g, 18.7 mmol) in MeOH (50 mL), 2 N aqueous NaOH (50 mL) was added. The reaction was heated at reflux for 48 h, then cooled to room temperature. The MeOH was removed by rotary evaporation, and the resulting solution was partitioned between EtOAc (400 mL) and 1 N HCl (400 mL). The organic layer was washed

with brine (300 mL) and then dried over $MgSO_4$. The solvent was removed by rotary evaporation to yield the corresponding carboxylic acid as a white solid. The carboxylic acid was redissolved in toluene (50 mL). To this solution, DIEA (4.91 mL, 3.65 g, 28.0 mmol) and $(PhO)_2PON_3$ (4.87 mL, 6.2 g, 22.5 mmol) were added. The reaction was heated at 85 °C under nitrogen for 16 h, then cooled to room temperature. To a separated flask containing a solution of potassium *tert*-butoxide (5.24 g, 46.8 mmol) in THF (150 mL) at 0 °C, the isocyanate solution was added 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 (400 mL) and 1 N HCl (400 mL). The organic layer was washed with 1 N HCl (300 mL), saturated $NaHCO_3$ (300 mL), and brine (300 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 **30** (4.54 g, 14.6 mmol, 78%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20−1.38 (m, 2H), 1.39−1.45 (m, 9H), 1.46−1.55 (m, 2H), 1.56−1.80 (m, 4H), 1.85−2.10 (m, 2H), 2.28−2.53 (m, 1H), 3.72−3.98 (m, 4H), 3.99−4.30 (m, 1H), 4.60−4.90 (m, 1H), 4.91−5.12 (m, 2H), 5.60−5.86 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.9, 25.2, 28.4, 31.6, 58.9, 59.0, 79.0, 98.7, 115.7, 137.8, 155.9; HRMS-FAB (M + Na^+) calcd for $C_{19}H_{29}NNaO_4$ 334.1994, found 334.1988.

Amide 31. Alkene **30** (284 mg, 912 μmol) was dissolved in a 2:1 mixture of acetone and water (40 mL). To this solution, $NaIO_4$ (976 mg, 4.56 mmol), $KMnO_4$ (108 mg, 648 μmol), and $NaHCO_3$ (78 mg, 912 μmol) were added. The reaction was stirred at room temperature for 2 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, and the crude oil was purified by flash chromatography (EtOAc/hexanes, 1:2) to yield the corresponding carboxylic acid. The carboxylic acid was redissolved in DMF (5 mL). To this solution, H_2N-Trp-OMe (300 mg, 1.37 mmol), HBTU (519 mg, 1.37 mmol), and DIEA (480 μL, 356 mg, 2.74 mmol) were added. The reaction was stirred at room temperature for 2 h and then partitioned between EtOAc (200 mL) and 1 N HCl (200 mL). The organic layer was washed with 1 N HCl (100 mL), saturated aqueous $NaHCO_3$ (100 mL), and brine (100 mL). It was then dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (EtOAc/hexanes, 2:1) to give **31** as a mixture of two diastereomers (386 mg, 730 μmol, 80%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26−1.38 (m, 5H), 1.38−1.58 (m, 11H), 1.58−1.86 (m, 3H), 1.87−2.00 (m, 1H), 2.42−2.75 (m, 1H), 3.17−3.36 (m, 2H), 3.55−3.70 (s, 3H), 3.70−3.94 (m, 4H), 4.76−5.10 (m, 2H), 6.05−6.36 (m, 1H), 6.95−7.20 (m, 3H), 7.30−7.41 (m, 1H), 7.43−7.62 (m, 1H), 8.30−8.55 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.8, 17.1, 19.7, 21.9, 25.4, 25.5, 28.2, 28.3, 29.0, 52.9, 53.0, 53.4, 59.5, 59.7, 59.8, 61.0, 69.8, 75.6, 77.8, 79.8, 98.7, 110.5, 111.9, 119.0, 119.1, 120.0, 120.1, 122.6, 122.7, 123.4, 128.1, 128.2, 136.7, 156.5, 173.0, 173.1; HRMS-FAB (M + Na^+) calcd for $C_{28}H_{39}N_3NaO_7$ 552.2686, found 552.2698.

Amide 32. Compound **31** (300 mg, 567 μmol) was dissolved in CH_2Cl_2 (8 mL). To this solution, TFA (4 mL) was added. The reaction was stirred at room temperature for 30 min. The solvents were removed by rotary evaporation, and the residue was diluted with EtOAc (150 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, Cbz-Trp-OH (287 mg, 851 μmol), HBTU (323 mg, 851 μmol), and DIEA (298 μL, 221 mg, 1.70 mmol) were added. The reaction was stirred at room temperature for 1 h, and then the reaction mixture was partitioned between EtOAc (150 mL) and 1 N HCl (150 mL). The organic layer was washed with 1 N HCl (100 mL), saturated $NaHCO_3$ (100 mL), and brine (100 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 **32** as a mixture of two diastereomers (296 mg, 327 μmol, 77%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00−1.68 (m, 9H), 1.77−1.97 (m, 1H), 2.83 (s, 2H), 2.99−3.21 (m, 1H), 3.22−3.49 (m, 3H), 3.60−3.90 (m, 7H), 4.42−4.73 (br s, 1H), 4.83−5.06 (m, 1H), 5.07−5.28 (m, 2H), 5.68−6.08 (m, 1H), 6.35−6.72 (m, 1H), 6.85−7.05 (m, 2H), 7.06−7.25 (m, 5H), 7.28−7.46 (m, 7H), 7.47−7.92 (m, 2H), 8.60−9.00 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.0, 19.3, 21.8, 25.0, 25.2, 28.0, 28.8, 39.1, 52.77, 52.82, 53.1, 55.9, 59.3, 59.6, 67.4, 69.7, 75.4, 77.7, 98.3, 110.1, 110.3, 111.8, 111.9, 118.8, 118.9, 119.1, 120.0, 122.47, 122.52, 123.4, 123.8, 127.9, 128.0, 128.5, 128.6, 129.0, 136.59, 136.64, 136.7, 136.9, 156.4, 156.5, 171.2, 171.5, 173.3; HRMS-FAB (M + Na^+) calcd for $C_{42}H_{47}N_5NaO_8$ 772.3322, found 772.3308.

Inhibitor 5. To compound **32** (120 mg, 160 μmol) was added aqueous TFA (10 mL, 50%) at 0 °C. The reaction was warmed to room temperature, stirred for 12 h, and then concentrated by rotary evaporation. The resulting residue was diluted with EtOAc (50 mL) and washed with saturated aqueous Na_2CO_3 (50 mL) and brine (50 mL). It was then dried over $MgSO_4$, the solvent was removed by rotary evaporation, and the crude oil was purified by flash chromatography (EtOAc/hexanes, 2:1) to yield **5** as a mixture of two diastereomers (74 mg, 107 μmol, 67%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32−1.80 (m, 4H), 1.87−2.58 (m, 3H), 2.60−3.46 (m, 5H), 3.58−3.80 (m, 3H), 4.10−4.96 (m, 2H), 5.00−5.87 (m, 3H), 6.27−7.05 (m, 2H), 7.05−7.25 (m, 4H), 7.28−7.90 (m, 13H), 8.31−8.91 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.6, 19.3, 23.1, 23.6, 26.8, 27.6, 27.9, 28.6, 28.9, 29.7, 32.0, 34.8, 35.5, 35.7, 35.8, 36.1, 36.6, 39.6, 46.9, 47.2, 52.87, 52.93, 53.1, 53.3, 55.9, 56.2, 56.6, 58.1, 58.3, 64.7, 67.4, 67.6, 77.7, 108.8, 109.6, 110.1, 110.8, 111.7, 111.8, 111.9, 112.0, 118.81, 118.84, 118.9, 119.0, 119.7, 119.97, 120.05, 120.1, 120.2, 120.6, 122.4, 122.5, 122.6, 122.70, 122.72, 122.9, 123.1, 123.4, 123.7, 126.3, 127.5, 127.9, 128.4, 128.5, 128.6, 128.7, 128.9, 129.0, 132.9, 136.2, 136.5, 136.56, 136.61, 156.3, 156.4, 171.1, 171.2, 171.4, 171.8, 173.1, 173.3, 176.9, 207.3, 208.3; HRMS-FAB (M + Na^+) calcd for $C_{39}H_{41}N_5NaO_7$ 714.2904, found 714.2930.

Alkene 35. To a solution of compound **33** (10.0 g, 86 mmol) in benzene (60 mL), 2,2-dimethoxypropane (12.5 mL, 10.7 g, 103 mmol), allyl alcohol (7.83 mL, 11.0 g, 189 mmol), and *p*-toluenesulfonic acid (35 mg, 0.09 mmol) were added. The reaction was heated at 80−85 °C, and the byproducts (acetone and MeOH) were collected in a Dean−Stark trap. After 4 h, the temperature of the oil bath was increased to distill off most of the benzene. Toluene (100 mL) was added to the solution, and allyl alcohol was distilled out as an azeotropic mixture with toluene. The resulting dark brown oil was purified by distillation (bp 72−76 °C, 1.0−1.5 mmHg) to yield alkene **35** (12.5 g, 80 mmol, 93%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.05−2.22 (m, 1H), 2.46−2.80 (m, 5H), 2.85−3.09 (m, 3H), 4.95−5.15 (m, 2H), 5.54−5.86 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 31.3, 34.0, 35.6, 44.4, 117.8, 135.5, 209.9; HRMS-FAB (M + Na^+) calcd for $C_8H_{12}NaOS$ 179.0507, found 170.0510.

Ketoester 36. To a solution of diisopropylamine (3.0 mL, 21.1 mmol) in dry diethyl ether (150 mL), *n*-butyllithium (8.5 mL, 21.1 mmol, 2.5 M in hexanes) was added at −78 °C under an atmosphere of nitrogen. The temperature of the solution was slowly increased to −20 °C and maintained there for an additional 30 min. The mixture was then cooled to −100 °C, and then compound **35** (3.0 g, 19.2 mmol) was added as a solution in diethyl ether (20 mL). After 1 h, methylcyanoformate (1.71 mL, 1.72 g, 20.2 mmol) was added dropwise. The reaction was stirred at −100 °C for an additional 4 h and then quenched with ice water. The organic layer was washed with saturated Na_2CO_3 (150 mL) and brine (150 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 **36** as a mixture of tautomers (2.63 g, 12.3 mmol, 64%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.98−2.25 (m, 0.6H), 2.38−2.85 (m, 3.8H), 2.85−3.10 (m, 1.4H), 3.15−3.47 (m, 1.5H), 3.61−3.85 (m, 3.6H), 4.95−5.18 (m, 2H), 5.68−5.90 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.1, 29.1, 33.3, 33.5, 33.8, 34.0, 35.4, 36.4, 39.5, 51.3, 52.3, 53.0, 53.4, 57.8, 60.4, 77.7, 98.0, 118.1, 118.2, 135.0, 135.2, 136.0, 169.5, 169.6, 172.5, 174.8, 205.2, 205.4; HRMS-FAB (M + Na^+) calcd for $C_{10}H_{14}NaO_3S$ 237.0561, found 237.0562.

Alcohol 37. Ketoester **36** (2.0 g, 9.4 mmol) was dissolved in dry THF (150 mL) at 0 °C. To this solution, $NaBH_4$ (1.04 g, 28.0 mmol) was added. After 30 min the reaction was quenched with 1 N HCl (200 mL), and the THF was removed by rotary evaporation. The resulting material was partitioned between EtOAc (300 mL) and 1 N HCl (200 mL). The organic layer was washed with 1 N HCl (200 mL), saturated aqueous $NaHCO_3$ (200 mL), and brine (200 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, gradient of 1:9 to 1:4) to yield alcohol **37** as a mixture of diastereomers (1.58 g, 7.3 mmol, 78%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.72−1.85 (m, 1H), 2.09−2.30 (m, 3H), 2.49−2.62 (d, J = 9.8 Hz, 2H), 2.75−2.99 (m, 2H), 3.05−3.25 (t, J = 9.6 Hz, 1H), 3.68−3.80 (s, 3H), 4.23 (s, 1H), 4.98−5.15 (m, 2H), 5.69−5.88 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.7, 26.5, 38.1, 43.3, 49.8, 52.5, 67.6, 117.5, 136.2, 174.7; HRMS-FAB (M + Na^+) calcd for $C_{10}H_{16}NaO_3S$ 239.0718, found 239.0721

Carboxylic Acid 38. To a solution of compound **37** (3.0 g, 13.9 mmol) in DMF (10 mL), imidazole (3.67 g, 20.9 mmol) and TBDMSCl (5.21 g, 34.8 mmol) were added. The reaction was stirred at 50 °C for 12 h and then diluted with EtOAc (500 mL). The organic layer was washed with 1 N HCl (2 × 300 mL), saturated aqueous $NaHCO_3$ (300 mL), and brine (300 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 the corresponding TBDMS ether. The TBDMS ether was dissolved in MeOH (50 mL). To this solution, 1 N aqueous NaOH (50 mL) was added. The mixture was heated at reflux for 24 h, and the MeOH was removed by rotary evaporation. The aqueous layer was partitioned between EtOAc (300 mL) and 1 N HCl (300 mL). The organic layer was washed with brine (200 mL) and then dried over $MgSO_4$. The solvent was removed by rotary evaporation, and the crude oil was purified by flash chromatography (EtOAc/hexanes, gradient of 1:18 to 1:4) to yield carboxylic acid **38** (2.75 g, 8.69 mmol, 42% for two steps): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.06−0.08 (d, J = 19.0 Hz, 6H), 0.89 (s, 9H), 1.94−1.99 (m, 1H), 2.12−2.25 (dm, J = 9.6 Hz, 1H), 2.25−2.39 (m, 1H), 2.40−2.59 (m, 2H), 2.80−3.00 (dm, J = 11.7 Hz, 1H), 3.15−3.28 (t, J = 13.0 Hz, 2H), 4.20−4.25 (s, 1H), 4.96−5.16 (m, 2H), 5.62−5.88 (m, 1H), 9.35−11.43 (br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.9, −4.1, 1.4, 16.9, 18.4, 21.2, 21.7, 23.0, 26.1, 26.4, 33.8, 40.8, 44.4, 59.5, 69.7, 71.1, 75.4, 117.5, 136.2, 177.9, 179.5; HRMS-FAB (M + Na^+) calcd for $C_{15}H_{28}NaO_3SSi$ 339.1426, found 339.1421.

Carbamate 39. To a solution of compound **38** (2.0 g, 6.33 mmol) in dry toluene (50 mL), DIEA (1.66 mL, 1.23 g, 9.49 mmol) and $(PhO)_2PON_3$ (2.05 mL, 2.61 g, 9.49 mmol) were added. The reaction was heated at 80 °C under nitrogen for 16 h. The formation of the isocyanate was followed by IR spectroscopy at 2256 cm^{-1}. The alkoxide from benzyl alcohol was prepared in a separate flask as follows: Benzyl alcohol (2.05 mL, 2.05 g, 19.0 mmol) was added to dry THF (40 mL) at 0 °C under nitrogen. To the same container, *n*-butyllithium (5.06 mL, 2.5 M solution in hexanes, 12.7 mmol) was added dropwise over a period of 5 min. The isocyanate solution was then cooled to room temperature and added slowly to the alkoxide solution at 0 °C. After the addition was complete, the reaction was allowed to warm to room temperature over 30 min. The reaction was quenched with 1 N HCl (100 mL). The THF was removed by rotary evaporation, and the remaining solution was partitioned between EtOAc (300 mL) and 1 N HCl (300 mL) and washed with saturated aqueous $NaHCO_3$ (200 mL) and brine (200 mL). It was then dried over $MgSO_4$, and the solvents were removed by rotary evaporation. The crude oil was purified by flash chromatography (EtOAc/hexanes, 1:9) to yield carbamate **39** (1.92 g, 4.56 mmol, 72%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.08 (s, 6H), 0.94 (s, 9H), 1.91−2.03 (m, 1H), 2.10−2.20 (dd, J = 9.9, 3.1 Hz, 1H), 2.30−2.55 (m, 3H), 2.78−2.92 (dd, J = 9.7, 9.1 Hz, 1H), 3.65−3.75 (s, 1H), 3.95−4.10 (m, 1H), 4.95−5.25 (m, 5H), 5.66−5.85 (m, 1H), 7.30−7.45 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.8, −4.6, 18.1, 25.8, 26.4, 28.2, 33.7, 41.7, 49.1, 66.7, 72.7, 117.3, 128.0, 128.1, 128.5, 135.9, 136.6, 155.5; HRMS-FAB (M + H^+) calcd for $C_{22}H_{35}NNaO_3SSi$ 444.2005, found 444.2003.

Carboxylic Acid 40. Compound **39** (1.00 g, 2.38 mmol) was dissolved in a mixture of acetone (100 mL) and H_2O (40 mL). To this solution, $NaIO_4$ (5.08 g, 23.8 mmol), $KMnO_4$ (282 mg, 1.79 mmol), and $NaHCO_3$ (205 mg, 2.38 mmol) were added. The reaction was stirred at room temperature for 8 h. The acetone was removed by rotary evaporation, and the remaining material was partitioned between EtOAc (200 mL) and 1 N HCl (200 mL). The organic layer was washed with 1 N HCl (2 × 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, gradient of 1:2 to 2:1) to yield acid **40** (730 mg, 1.55 mmol, 65%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.12−0.15 (m, 6H), 0.92 (s, 9H), 2.60−2.75 (m, 2H), 2.90−3.15 (m, 3H), 3.18−3.40 (m, 2H), 3.90−3.96 (s, 1H), 4.30−4.45 (m, 1H), 5.00−5.18 (m, 3H), 7.28−7.41 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.6, −4.3, 14.6, 18.3, 23.1, 26.1, 32.0, 33.5, 36.4, 48.9, 51.4, 67.8, 70.7, 77.6, 128.7, 128.8, 129.0, 136.2, 155.5, 176.2; HRMS-FAB (M + Na^+) calcd for $C_{21}H_{33}NaNO_7SSi$ 494.1645, found 494.1652.

Amides 41a and **41b.** To compound **40** (400 mg, 849 μmol), H_2N-Trp(Boc)-OMe (406 mg, 1.27 mmol), HBTU (481 mg, 1.27 mmol), DIEA (445 μL, 330 mg, 2.54 mmol), and DMF (4 mL) were added. The reaction was stirred at room temperature for 2 h and then partitioned between EtOAc (200 mL) and 1 N HCl (200 mL). The organic layer was washed with 1 N HCl (100 mL), saturated aqueous $NaHCO_3$ (100 mL), and brine (100 mL). It was then dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (EtOAc/hexanes, gradient of 1:4 to 1:1) to give compound **41a** (228 mg, 297 μmol, 35%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.08−0.13 (d, J = 6.8 Hz, 6H), 0.91 (s, 9H), 1.69 (s, 9H), 2.40−2.60 (m, 1H), 2.65−2.85 (m, 3H), 3.00−3.12 (dm, J = 11.8 Hz, 1H), 3.15−3.35 (m, 4H), 3.70 (s, 3H), 3.90−3.95 (s, 1H), 4.30−4.45 (m, 1H), 4.80−5.05 (m, 2H), 5.10 (s, 2H), 6.35−6.45 (d, J = 7.5 Hz, 1H), 7.18−7.27 (m, 2H), 7.29−7.45 (m, 5H), 7.46−7.60 (m, 2H), 8.05−8.20 (d, J = 7.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.7, −4.3, 18.3, 26.1, 27.8, 28.6, 35.8, 37.1, 48.3, 48.7, 51.3, 52.9, 53.1, 67.6, 70.7, 84.2, 115.2, 115.8, 119.1, 123.1, 124.7, 125.0, 128.6, 128.8, 129.0, 130.6, 135.8, 136.3, 150.0, 155.4, 170.4, 172.2; HRMS-FAB (M + Na^+) calcd for $C_{38}H_{53}N_3NaO_{10}SSi$ 794.3119, found 794.3128; and **41b** (202 mg, 263 μmol, 31%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.06−0.16 (d, J = 13.5 Hz, 6H), 0.91 (s, 9H), 1.69 (s, 9H), 2.25−2.41 (m, 1H), 2.65−2.85 (m, 2H), 3.00−3.40 (m, 5H), 3.73 (s, 3H), 3.80−3.90 (s, 1H), 4.30−4.45 (m, 1H), 4.80−5.05 (m, 2H), 5.12 (s, 2H), 6.05−6.15 (d, J = 6.8 Hz, 1H), 7.18−7.27 (m, 2H), 7.29−7.45 (m, 5H), 7.46−7.60 (m, 2H), 8.05−8.20 (d, J = 7.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.6, −4.2, 18.3, 26.1, 27.7, 28.6, 35.8, 37.7, 47.1, 48.8, 51.4, 53.0, 53.1, 67.7, 71.0, 84.2, 115.3, 115.8, 119.1, 123.1, 124.5, 125.0, 128.6, 128.6, 128.8, 129.0, 130.6, 136.3, 149.9, 155.3, 170.6, 172.3; HRMS-FAB (M + Na^+) calcd for $C_{38}H_{53}N_3NaO_{10}SSi$ 794.3119, found 794.3134.

Amide 42a. To a solution of compound **41a** (200 mg, 261 μmol) in dry MeOH (40 mL), 10% Pd on carbon (60 mg) was added. The reaction flask was purged and kept under 1 atm of hydrogen gas. The reaction was stirred at room temperature for 2 h, and then the Pd/carbon was removed by filtration. The

clear solution was concentrated by rotary evaporation, and the residue was dried under vacuum for an additional 24 h to give the corresponding primary amine. The resulting primary amine was dissolved in DMF (3 mL). To this solution, Cbz-Trp(Boc)-OH (320 mg, 467 μmol), HBTU (198 mg, 467 μmol), and DIEA (137 μL, 101 mg, 784 μmol) were added. The reaction was stirred at room temperature for 1 h and then partitioned between EtOAc (60 mL) and 1 N HCl (60 mL). The organic layer was washed with 1 N HCl (40 mL), saturated $NaHCO_3$ (40 mL), and brine (40 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, gradient of 1:4 to 1:2) to yield **42a** (193 mg, 183 μmol, 70%): ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.22 (s, 3H), −0.02 (s, 3H), 0.72 (s, 9H), 1.66 (s, 18H), 2.30−2.50 (m, 1H), 2.50−2.80 (m, 5H), 2.95−3.35 (m, 5H), 3.40−3.55 (s br, 1H), 3.67 (s, 3H), 4.22−4.42 (m, 1H), 4.43−4.67 (m, 1H), 4.80−4.95 (m, 1H), 5.00−5.25 (m, 2H), 5.40−5.65 (s br, 1H), 5.70−5.90 (s br, 1H), 6.50−6.60 (d, J = 7.4 Hz, 1H), 7.15−7.26 (m, 2H), 7.28−7.42 (m, 7H), 7.41−7.56 (m, 3H), 7.57−7.75 (s br, 1H), 8.00−8.25 (s br, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.4, −4.8, 17.7, 25.5, 27.5, 28.1, 28.2, 28.7, 35.4, 36.7, 46.2, 47.7, 50.2, 52.5, 52.7, 55.4, 67.3, 70.0, 83.8, 84.2, 114.8, 115.1, 115.4, 115.6, 118.8, 119.1, 122.7, 123.0, 124.3, 124.6, 125.1, 128.3, 128.6, 129.8, 130.2, 135.5, 135.9, 149.4, 149.6, 155.7, 169.8, 170.0, 172.0; HRMS-FAB (M + Na^+) calcd for $C_{54}H_{71}N_5NaO_{13}SSi$ 1080.4436, found 1080.4405.

Amide 42b. Amide **42b** was synthesized with a procedure similar to that used for amide **42a** (210 mg, 199 μmol, 76%): ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.19 (s, 3H), −0.01 (s, 3H), 0.79 (s, 9H), 1.68 (s, 18H), 2.05−2.15 (m, 1H), 2.50−2.80 (m, 3H), 2.82−3.40 (m, 8H), 3.73 (s, 3H), 4.22−4.42 (m, 2H), 4.80−4.95 (m, 1H), 5.00−5.20 (m, 2H), 5.30−5.45 (d, J = 6.1 Hz, 1H), 5.95−6.05 (d, J = 6.0 Hz, 1H), 6.60−6.70 (d, J = 7.4 Hz, 1H), 7.15−7.26 (m, 1H), 7.28−7.42 (m, 9H), 7.41−7.56 (m, 4H), 8.00−8.25 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.8, −4.7, 14.2, 17.8, 21.1, 25.6, 27.3, 28.0, 28.3, 35.4, 37.4, 46.9, 50.4, 52.6, 52.8, 55.8, 60.4, 67.4, 70.3, 83.8, 84.1, 114.9, 115.4, 115.6, 118.7, 118.9, 119.1, 122.9, 124.1, 124.5, 124.9, 125.1, 128.3, 128.5, 128.7, 130.3, 135.3, 135.8, 149.4, 149.6, 155.9, 169.9, 170.3, 171.9; HRMS-FAB (M + Na^+) calcd for $C_{54}H_{71}N_5NaO_{13}$-SSi 1080.4436, found 1080.4415.

Alcohol 43a. To a solution of compound **42a** (150 mg, 142 μmol) in dry THF (8 mL) was added a solution of TBAF (56 mg, 213 μmol) in dry THF (7 mL). The reaction was stirred at room temperature for 30 min. The solvents were removed by rotary evaporation, and the crude material was purified by flash chromatography (EtOAc/hexanes, gradient of 2:1 to 5:1) to give alcohol **43a** (107 mg, 114 μmol, 81%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55−1.72 (s, 18H), 1.98 (s, 1H), 2.20−2.35 (m, 2H), 2.36−2.57 (m, 2H), 2.58−2.70 (dm, J = 11.0 Hz, 1H), 2.71−2.82 (m, 1H), 3.00−3.22 (m, 4H), 3.23−3.31 (m, 1H), 3.32−3.45 (m, 1H), 3.60−3.75 (m, 4H), 4.28−4.41 (m, 1H), 4.41−4.55 (m, 1H), 4.85−4.95 (m, 1H), 5.00−5.20 (s, 2H), 5.75−5.90 (d, J = 8.1 Hz, 1H), 6.65−6.75 (d, J = 8.1 Hz, 1H), 6.90−7.00 (m, 1H), 7.16−7.26 (m, 2H), 7.28−7.41 (m, 7H), 7.42−7.65 (m, 5H), 8.00−8.25 (s br, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 1.1, 14.2, 27.4, 28.1, 28.2, 36.0, 47.5, 50.4, 52.4, 52.6, 55.7, 67.2, 84.1, 114.9, 115.1, 115.4, 115.6, 118.8, 122.7, 124.3, 124.5, 124.7, 124.8, 128.1, 128.3, 128.6, 130.3, 135.3, 136.0, 149.7, 156.1, 170.4, 172.1; HRMS-FAB (M + Na^+) calcd for $C_{48}H_{57}N_5$-$NaO_{13}S$ 966.3571, found 966.3591.

Alcohol 43b. Alcohol **43b** was synthesized with a procedure similar to that used for amide **43a** (106 mg, 113 μmol, 80%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.60−1.80 (s, 18H), 1.90 (s, 1H), 2.06−2.20 (m, 1H), 2.22−2.65 (m, 3H), 2.70−3.05 (m, 2H), 3.05−3.20 (m, 4H), 3.21−3.33 (m, 2H), 3.34−3.60 (m, 2H), 3.72 (s, 3H), 4.30−4.65 (m, 2H), 4.80−4.95 (m, 1H), 5.00−5.20 (s, 2H), 5.55−5.65 (d, J = 8.1 Hz, 1H), 6.60−6.70 (d, J = 8.1 Hz, 1H), 6.80−7.10 (s br, 1H), 7.15−7.26 (m, 2H), 7.28−7.41 (m, 7H), 7.42−7.65 (m, 4H), 8.00−8.20 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.2, 27.9, 28.1, 28.2, 36.1, 47.4, 50.9, 52.3, 52.6, 55.2, 67.4, 76.7, 84.0, 84.2, 84.3, 114.9, 115.0, 115.4, 115.6, 118.7, 118.8, 119.1, 122.7, 122.9, 124.1, 124.3, 124.5, 124.8, 130.1, 130.3, 135.4, 135.9, 149.6, 149.7, 170.5, 172.9; HRMS-FAB (M + Na^+) calcd for $C_{48}H_{57}N_5NaO_{13}S$ 966.3571, found 966.3595.

Ketone 44. Alcohol **43a** or **43b** (50 mg, 53 μmol) was dissolved in CH_2Cl_2 (5 mL). To this solution, Dess−Martin reagent (33 mg, 79 μmol) was added. The reaction was stirred at room temperature for 12 h and then partitioned between EtOAc (25 mL) and saturated aqueous Na_2CO_3 (20 mL). The organic layer was washed with saturated Na_2CO_3 (20 mL) and brine (25 mL). It was then dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (EtOAc/hexanes, 5:1) to yield ketone **44** as a mixture of two diastereomers (starting from **43a**: 46 mg, 49 μmol, 92%; starting from **43b**: 46 mg, 49 μmol, 92%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.60−1.68 (m, 18H), 2.32−2.78 (m, 3H), 3.00−3.38 (m, 6H), 3.40−3.57 (m, 1H), 3.58−3.70 (m, 1H), 3.70−3.76 (m, 3H), 4.32−4.61 (m, 2H), 4.80−4.94 (m, 1H), 5.00−5.21 (m, 2H), 5.50−5.70 (m, 1H), 6.20−6.80 (m, 1H), 7.05−7.26 (m, 2H), 7.28−7.78 (m, 12H), 7.99−8.22 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.6, 21.5, 27.6, 27.8, 28.6, 33.3, 34.3, 34.4, 36.1, 41.4, 42.1, 42.2, 50.3, 53.0, 53.1, 53.2, 53.3, 54.1, 54.2, 54.6, 54.9, 55.4, 55.6, 56.0, 60.8, 67.5, 68.1, 77.6, 84.4, 84.7, 95.1, 115.0, 115.1, 115.2, 115.4, 115.6, 115.8, 115.9, 119.1, 119.4, 119.5, 123.1, 123.2, 123.3, 124.5, 124.6, 124.8, 125.1, 125.2, 125.5, 128.38, 128.44, 128.5, 128.6, 128.7, 128.8, 128.92, 128.97, 129.03, 130.5, 130.8, 130.9, 131.0, 132.1, 133.6, 135.6, 135.8, 136.4, 142.1, 149.9, 150.0, 156.3, 168.7, 169.1, 169.4, 169.5, 171.3, 171.4, 171.6, 172.2, 172.4, 172.5, 201.0, 201.4, 201.7; HRMS-FAB (M + Na^+) calcd for $C_{48}H_{55}N_5NaO_{13}S$ 964.3415, found 964.3405.

Inhibitor 6. Ketone **44** (50 mg, 47 μmol) was dissolved in CH_2Cl_2 (2 mL). To this solution, TFA (0.5 mL) was added. The reaction was stirred at room temperature for 1.5 h, and then the solvents were removed by rotary evaporation. The crude material was purified by flash chromatography (2−15% MeOH in CH_2Cl_2) to yield inhibitor **6** as a mixture of two diastereomers (26 mg, 36 μmol, 76%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.05−2.45 (m, 2H), 2.60−2.79 (m, 1H), 2.80−3.22 (m, 5H), 3.39−3.48 (s, 2H), 3.49−3.62 (m, 3H), 3.63−3.81 (m, 1H), 4.15−4.49 (m, 3H), 4.87−5.07 (m, 2H), 6.88−7.20 (m, 7H), 7.21−7.39 (m, 7H), 7.40−7.70 (m, 3H), 8.30−8.59 (m, 1H), 10.70−10.95 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 27.9, 28.0, 28.7, 31.3, 33.9, 42.2, 42.3, 52.7, 54.0, 54.1, 54.2, 54.3, 56.2, 66.1, 110.1, 110.3, 110.8, 110.9, 112.2, 112.3, 118.9, 119.1, 119.3, 121.7, 121.8, 124.6, 124.8, 127.9, 128.1, 128.3, 128.4, 128.6, 129.2, 136.9, 137.8, 138.2, 138.4, 139.7, 141.3, 156.7, 168.0, 170.55, 170.61, 170.7, 171.7, 172.2, 172.6, 172.8, 173.1, 173.2, 174.7, 201.4, 201.5; HRMS-FAB (M + Na^+) calcd for $C_{38}H_{39}N_5$-NaO_9S 764.2366, found 764.2379.

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

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