Inhibitors of Plasmin that Extend into Both the S and S′ Binding Sites: Cooperative Interactions between S1 and S2

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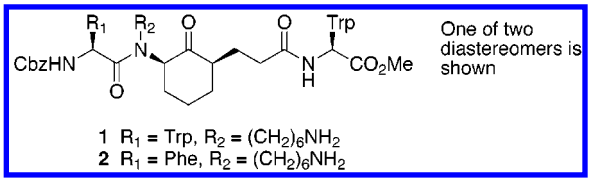
A new procedure for the synthesis of cyclohexanone-based inhibitors of serine proteases is reported. In this procedure the reactive ketone functionality is carried through the synthesis in masked form as a TBDMS-protected alcohol. Deprotection followed by oxidation of the alcohol generates the final form of the inhibitor. Two new inhibitors, which interact with the S1−S3 and S2′ subsites of plasmin, are synthesized using this procedure. Inhibitors **1** and **2** have IC_{50} values against plasmin of 20 and 24 μM, respectively. The inhibition studies show that cooperative binding of inhibitors in the S1 and S2 subsites of plasmin is important for determining inhibitor selectivity.

Introduction

Plasmin is a serine protease that is central to a number of normal physiological processes such as lysis of fibrin clots, tissue remodeling, and cell migration.[1] There is now mounting evidence that also implicates plasmin in the processes of angiogenesis and metastasis during the progression of cancer.[2] Plasmin acts by hydrolyzing components of the basement membrane including fibrin, type IV collagen, fibronectin, and laminin. It also has an indirect mode of action through hydrolytic activation of matrix metalloproteases.[2] During angiogenesis, degradation of the basement membrane allows epithelial cells to migrate into the extracellular matrix and form new blood vessels. Lesions in the basement membrane also promote metastasis by allowing cancer cells to penetrate into the underlying tissues and form secondary tumors.[3] Thus, plasmin is a potential target for the development of new chemotherapeutic agents that could act by inhibiting degradation of the basement membrane, and as a result, inhibiting angiogenesis and metastasis.[2]

Most of the current pharmaceutical agents that inhibit plasmin, such as ϵ-aminocaproic acid and *trans*-4-aminomethylcyclohexanecarboxylic acid, are targeted to the lysine binding site.[4] This binding site anchors plasminogen, which is the inactive precursor to plasmin, to fibrin.[5] Therefore, the compounds are good inhibitors of fibrinolysis. However, they do not affect the active site of the protease, which is separate from the lysine binding site. Inhibitors that are targeted directly to the active site of plasmin may be useful as potential anticancer agents. In addition, they may lead to a better understanding of the diverse roles that are played by this enzyme in both normal and pathological processes.[6] In this paper we report on two new inhibitors (compounds **1** and **2**) that are designed to make a variety of specific contacts with the active site of plasmin. The aminohexyl, R_1, and Cbz groups are designed to bind in the S1−S3 subsites, respectively, while the C-terminal Trp residue will bind in the S2′ subsite. These multiple noncovalent interactions position the ketone moiety of the inhibitors so that it can react with the active site serine nucleophile. The inhibitors show reasonable activity and moderate specificity for plasmin when compared to other related serine proteases including trypsin, thrombin, and kallikrein.



Design of Inhibitors. Over the past several years we have been investigating 4-heterocyclohexanone derivatives as inhibitors for serine and cysteine proteases.[7] These compounds are reversible inhibitors of the proteases and are designed to give a reversibly formed covalent bond between the enzyme active site nucleophile and the electrophilic ketone functionality of the inhibitor.[8] In previous studies we have synthesized inhibitors **3**−**5** (Table 1) that interact with the S1−S3 subsites of plasmin.[9] The aminohexyl side chains in compounds **3** and **4** are designed to form an electrostatic interaction

(1) (a) Dano, K.; Andreasen, P. A.; Grondahl-Hansen, J.; Kristensen, P. L.; Nielsen, L. S.; Skriver, L. *Adv. Cancer Res.* **1985**, *44*, 139. (b) Tryggvason, K.; Hoyhtya, M.; Salo, T. *Biochim. Biophys. Acta* **1987**, *907*, 191.

(2) (a) Pepper, J. S.; Montesano, R.; Mandriots, S. J.; Orci, L.; Vassalli, J. *Enzyme Protein* **1996**, *49*, 138. (b) Kobayashi, H.; Shinohara, H.; Takeuchi, K.; Itoh, M.; Fujie, M.; Saitoh, M.; Terao, T. *Cancer Res.* **1994**, *54*, 844.

(3) Liotta, L. A. *Sci. Am.* **1992**, *Feb.*, 54.

(4) Okada, Y.; Tsuda, Y.; Teno, N.; Wanaka, K.; Bohgaki, M.; Hijikata-Okunomiya, A.; Naito, T.; Okamoto, S. *Chem. Pharm. Bull.* **1988**, *36*, 1289.

(5) Sherry, S. *Fibrinolysis, Thrombosis, and Hemostasis*; Lea & Febiger: Philadelphia, 1992; chapter 1.

(6) For other active site directed inhibitors of plasmin, see: (a) Teno, N.; Wanaka, K.; Okada, Y.; Tsuda, Y.; Okamoto, U.; Hijikata-Okunomiya, A.; Naito, T.; Okamoto, S. *Chem. Pharm. Bull.* **1991**, *39*, 2340. (b) Teno, N.; Wanaka, K.; Okada, Y.; Taguchi, H.; Okamoto, U.; Hijikata-Okunomiya, A.; Okamoto, S. *Chem. Pharm. Bull.* **1993**, *41*, 1079. (c) Wanaka, K.; Okamoto, S.; Horie, N.; Hijikata-Okunomiya, A.; Okamoto, U.; Naito, T.; Ohno, N.; Bohgaki, M.; Tsuda, Y.; Okada, Y. *Thrombosis Res.* **1996**, *82*, 79. (d) Tamura, S. Y.; Goldman, E. A.; Brunck, T. K.; Ripka, W. C.; Semple, J. E. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 331. See also ref 4.

(7) Conroy, J. L.; Sanders, T. C.; Seto, C. T. *J. Am. Chem. Soc.* **1997**, *119*, 4285.

(8) Conroy, J. L.; Seto, C. T. *J. Org. Chem.* **1998**, *63*, 2367.

(9) Sanders, C. T.; Seto, C. T. *J. Med. Chem.* **1999**, *42*, 2969.

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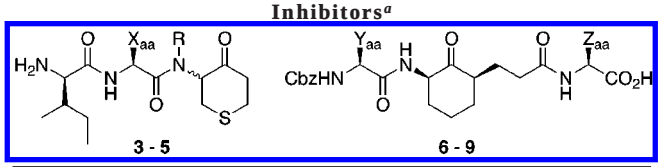
Table 1. Inhibition of Plasmin by Cyclohexanone-Based Inhibitors[a]



compd	X_{aa}	R	Y_{aa}	Z_{aa}	K_i (μM)[b]
3[c]	Phe	$(CH_2)_6NH_2$			50 ± 5, 130 ± 10
4[c]	Trp	$(CH_2)_6NH_2$			240, >1000
5[c]	Phe	H			9000 ± 1000, 16000 ± 1300
6[d]			Trp	Trp	5 ± 0.6, 10 ± 2
7[d]			Phe	Trp	420 ± 40[e]
8[d]			Trp	Phe	100 ± 20, 150 ± 25
9[d]			Phe	Phe	3000 ± 1000[e]

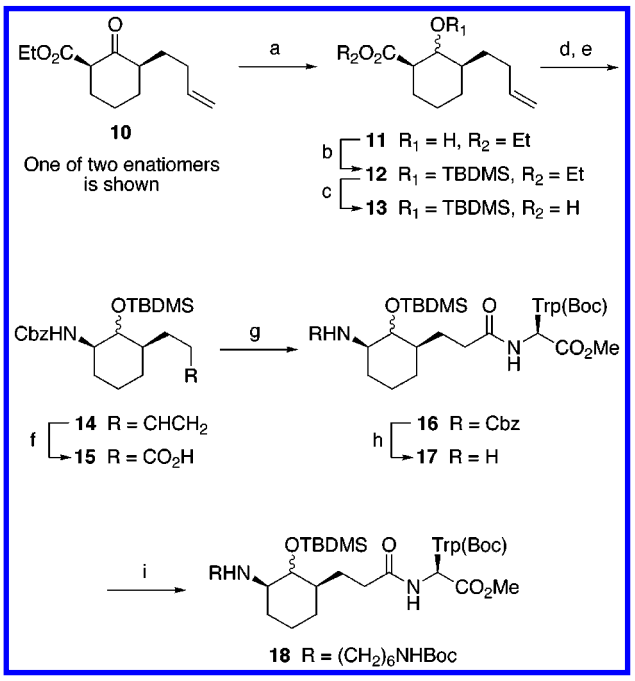
[a] One of two diastereomers of compounds **6**–**9** are shown. [b] The two values correspond to two separate diastereomers. [c] Data taken from refs 9 and 11. [d] Data take from ref 12. [e] Mixture of two diastereomers.

with the aspartic acid residue at the base of the S1 subsite. This side chain is attached to the inhibitor via the exocyclic amide nitrogen atom in a "peptoid-like" arrangement;[10] an arrangement that avoids formation of a quaternary center α to the ketone functionality. The length of the side chain was chosen based upon molecular modeling studies. A comparison of the inhibition constants for compounds **3** and **5** shows that binding of this side chain into the S1 subsite is critical for good affinity in this series of inhibitors. The X_{aa} and the D-isoleucine side chains of the inhibitors are designed to bind in S2 and S3, respectively. Comparison of compounds **3** and **4** shows that, in this series, Phe is moderately preferred over Trp at the P2 position. The difference in inhibition constants between these two compounds is a factor of approximately five.[9,11]

Compounds **6**–**9** represent a second series of inhibitors that were synthesized using combinatorial methods.[12] The Y_{aa} and Z_{aa} groups in these inhibitors are designed to bind in the P2 and P2′ subsites of plasmin. Comparison of compound **6** with **7**, and **8** with **9**, shows that, in contrast to what we observed for inhibitors **3**–**5**, in this series Trp is strongly preferred over Phe at the P2 (Y_{aa}) position. It is important to note that, unlike inhibitors **3** and **4**, compounds **6**–**9** do not incorporate a functional group that interacts with the S1 subsite of the enzyme.

To further explore the specificity of the S2 subsite of plasmin in the context of cyclohexanone-based inhibitors, we have synthesized compounds **1** and **2**. These inhibitors are similar in structure to compounds **6** and **7**, but they also incorporate an aminohexyl group that can interact with the S1 subsite of the protease. This work has three major objectives. First, we have developed a revised strategy for synthesizing the inhibitors. This new protocol avoids one of the shortcomings of our previous synthetic route (see below). Second, we have synthesized inhibitors that are capable of forming multiple interactions with the active site of serine proteases in an attempt to increase their potency and specificity for plasmin over other related proteases. Finally, we have explored the possible role that cooperative binding with the S1 and S2 subsites of plasmin plays in determining the relative binding affinity and selectivity of the inhibitors.

Scheme 1[a]



[a] Reagents: (a) $NaBH_4$, 60%; (b) TBDMSCl, 80%; (c) NaOH, MeOH, 68%; (d) $(C_6H_5O)_2PON_3$; (e) BnOH, n-BuLi; (f) $KMnO_4$, $NaIO_4$, 75% (three steps); (g) H_2NTrp(Boc)OMe, HBTU, 84%; (h) H_2, Pd/C, 62%; (i) BocNH$(CH_2)_5$CHO, AcOH; $NaBH_4$, 30%.

In this work we do not address the specific question of whether the ketones in inhibitors **1** and **2** react with the active site serine of plasmin to give a reversibly formed hemiketal adduct. However, in other related systems we have unambiguously demonstrated the formation of this type of adduct.[7–9] As a result, we expect that the inhibitors reported herein will react in an analogous fashion.

Results and Discussion

Synthesis of Inhibitors. In the syntheses of cyclohexanone-based inhibitors that we have reported previously, the ketone moiety has been carried through the syntheses as a protected cyclic acetal or thioacetal.[7,9,12,13] The acetal is then removed at the end of the synthesis to unmask the ketone using aqueous trifluoroacetic acid, while the thioacetal can be deprotected using aqueous *N*-bromosuccinimide. However, we have found that when the inhibitors incorporate a tertiary amide functional group as do compounds **1** and **2**, this final deprotection proceeds in poor yield and is accompanied by significant hydrolysis of the acid labile tertiary amide. To avoid this problem we have developed a modified synthetic strategy in which the ketone is first reduced to the corresponding alcohol, and then it is carried through the synthesis in protected form as the TBDMS ether. Near the end of the synthesis the TBDMS protecting group is removed and the alcohol is subsequently reoxidized to give back the ketone. This strategy is outlined in Schemes 1 and 2.

The synthesis began with reduction of β-ketoester **10** to give the corresponding alcohol **11** as a mixture of

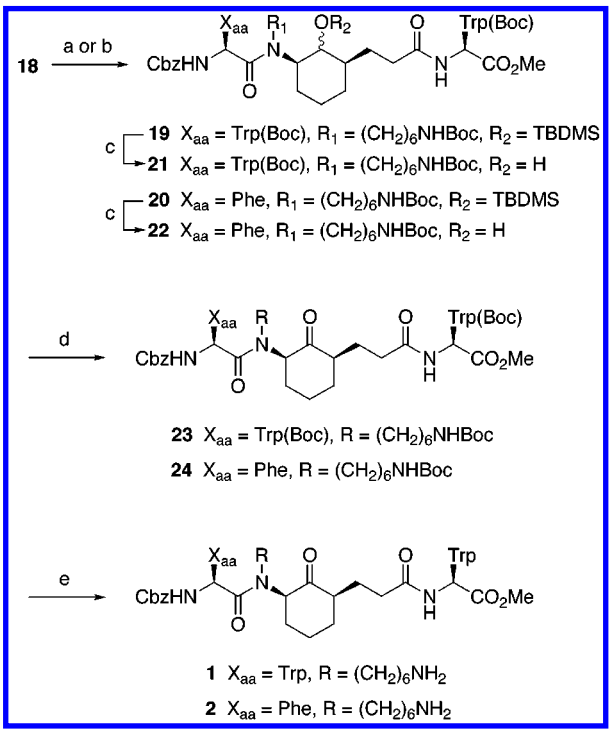
(10) Simon, R. J.; Kania, R. S.; Zuchermann, R. N.; Huebner, V. D.; Jewell, D. A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9367.

(11) Sanders, T. C. Ph.D. Dissertation, Brown University, Providence, RI, 2000.

(12) Abato, P.; Conroy, J. L.; Seto, C. T. *J. Med. Chem.* **1999**, *42*, 4001.

(13) Conroy, J. L.; Abato, P.; Ghosh, M.; Austermuhle, M. I.; Kiefer, M. R.; Seto, C. T. *Tetrahedron Lett.* **1998**, *39*, 8253.

Scheme 2[a]



[a] Reagents: (a) CbzTrp(Boc)OH, HBTU, 69%; (b) CbzPheOH, HBTU, 81%; (c) $Zn(BF_4)_2$ **21**: 79%, **22**: 35%; (d) pyridinium chlorochromate **23**: 78%, **24**: 45%; (e) TFA, **1**: 67%, **2**: 40%. One of two diastereomers of compounds **1** and **2** are shown.

diastereomers, followed by protection of the alcohol with TBDMSCl to give ester **12** (Scheme 1). The synthesis of compound **10** has been reported previously.[13] Ester **12** was saponified with NaOH to give carboxylic acid **13**, which was then treated with diphenylphosphoryl azide in refluxing benzene to induce the Curtius rearrangement. The resulting isocyanate was trapped with the anion of benzyl alcohol to generate the Cbz-protected amine **14**. The alkene in compound **14** was then oxidatively cleaved with potassium permanganate and sodium periodate to give carboxylic acid **15**, which was coupled with the methyl ester of Trp using HBTU to give the corresponding amide **16**. The indole nitrogen atom of Trp was protected as the *tert*-butyl carbamate. Catalytic hydrogenation of the Cbz protecting group gave amine **17**, which was subjected to a two-step reductive alkylation procedure. First, the imine was formed between compound **17** and *N*-Boc-6-aminohexanal using acetic acid as a catalyst.[14] Second, this imine was reduced with sodium borohydride to give secondary amine **18** in modest yield. Other reductive alkylation procedures using sodium cyanoborohydride or sodium triacetoxyborohydride gave lower yields of the desired secondary amine.

The secondary amine **18** was next coupled with CbzTrp(Boc)OH or CbzPheOH using HBTU to give compounds **19** and **20** (Scheme 2). These two compounds were subjected to the same three-step procedure to give inhibitors **1** and **2**. First, the TBDMS protecting group was removed using zinc tetrafluoroborate to give alcohols **21** and **22**.[15] Second, the resulting alcohol was oxidized with pyridinium chlorochromate to give the corresponding ketones **23** and **24**. Finally, the Boc protecting groups on the tryptophan and aminohexyl side chains were removed with trifluoroacetic acid. We were unable to separate the two diastereomers of each of the inhibitors, which were present in approximately a 1:1 mixture.

Table 2. Inhibition of Four Serine Proteases by Inhibitors 1 and 2

serine protease	IC_{50} (μM)[a]	
	1	**2**
plasmin	20 ± 7	24 ± 1
trypsin	160 ± 40	140 ± 30
thrombin	80 ± 60	40 ± 10
kallikrein	36 ± 6	130 ± 30

[a] Mixture of two diastereomers.

Inhibition Studies. Inhibitors **1** and **2** were assayed against four related serine proteases: plasmin, trypsin, thrombin, and kallikrein (Table 2). All four of these proteases have a specificity for substrates that incorporate a positively charged amino acid (either Lys or Arg) at the P1 position. The proteases were assayed using UV spectroscopy to monitor the hydrolysis of the *p*-nitroanilide substrates D-Val-Leu-Lys-pNA, D-Phe-Pip-Arg-pNA, D-Phe-Pip-Arg-pNA, and D-Pro-Phe-Arg-pNA, respectively. The assay mixtures contained 50 mM sodium phosphate buffer at pH 7.4, and 10% DMSO to maintain solubility of the inhibitors. Under these conditions, the K_M values for the four substrates were measured to be 180, 120, 30, and 60 μM, respectively.

Compounds **1** and **2** are both reasonable inhibitors of plasmin with IC_{50} values of 20 and 24 μM. These values are the same within experimental error. Examination of the inhibition constants of compounds **1**−**4** shows that plasmin does not exhibit a strong bias for Phe over Trp at the P2 position. For compounds **3** and **4** the preference is approximately 5-fold for Phe, while for compounds **1** and **2** there is no preference. These observations are consistent with data from the recent literature concerning the substrate specificity of plasmin. Using combinatorial methods, Ellman, Craik, and co-workers have constructed libraries of peptide−aminomethylcoumarin (AMC) substrates and screened them against a variety of proteases.[16] They found that plasmin prefers Lys and Arg at P1, the aromatic amino acids Phe, Trp, and Tyr at P2, and shows broad substrate specificity at P3. Substrates containing Phe, Trp, and Tyr at the P2 position all had similar activity, and there was no strong preference among the three amino acids. In complementary studies, Corey, Madison, and co-workers have examined the substrate specificity of plasmin using phage display.[17] They also found that the P2 position is specific for aromatic amino acids, with no strong preference among Phe, Trp, and Tyr. Thus, the results from studies with synthetic inhibitors, AMC-based substrates, and the purely peptide-based substrates are all in agreement and show that plasmin prefers aromatic amino acids in general at P2, but it does not discriminate among Phe, Trp, and Tyr. All of these inhibitors and substrates have one structural feature in common: they all possess Lys-

(14) The synthesis of the requisite $BocNH(CH_2)_5CHO$ has been reported previously. See ref 9.

(15) Ranu, B. C.; Jana, U.; Majee, A. *Tetrahedron Lett.* **1999**, *40*, 1985.

(16) (a) Backes, B. J.; Harris, J. L.; Leonetti, F.; Craik, C. S.; Ellman, J. A. *Nat. Biotechnol.* **2000**, *18*, 187. (b) Harris, J. L.; Backes, B. J.; Leonetti, F.; Mahrus, S.; Ellman, J. A.; Craik, C. S. *Proc. Natl. Acad. Sci, USA* **2000**, *97*, 7754.

(17) Hervio, L. S.; Coombs, G. S.; Bergstrom, R. C.; Trivedi, K.; Corey, D. R.; Madison, E. L. *Chem. Biol.* **2000**, *7*, 443.

or Arg-type side chains that extend into the S1 subsite of the protease.

The results summarized above can be contrasted with what we observe for inhibitors **6**−**9**. These inhibitors do not incorporate a functional group that interacts with the S1 subsite. Comparison of the inhibition constants for compounds **6** and **7** shows an approximate 80-fold selectivity for Trp over Phe at the P2 (Y_{aa}) position. Comparison of compounds **8** and **9** demonstrates a similar high preference (approximately 30-fold) for Trp at P2. What accounts for the apparent change in selectivity of the S2 subsite in the different studies?

One reasonable explanation for these observations is the presence of cooperative binding between the S1 and S2 sites. Inhibitors **1**−**5**, the AMC-based substrates, and the purely peptide substrates from phage display all incorporate a positively charged side chain that binds in the S1 subsite. This binding interaction could position the molecules in the active site so that Phe, Trp, or Tyr at the P2 position are all bound similarly in the S2 subsite. Thus, there is little discrimination among the three aromatic amino acids. For compounds **6**−**9** that do not interact with S1, the inhibitors may bind in a more relaxed conformation that allows Trp to form more favorable van der Waals or hydrogen bonding contacts with residues in the S2 subsite, when compared to Phe. This would explain the high selectivity for Trp at the P2 position that is observed for inhibitors **6**−**9**. Since binding in the S1 and S2 subsites is linked, the substrate specificity of S2 can be altered depending on whether the S1 subsite is occupied or not.

Inhibitors **6**−**9** and **1** and **2** are different in two respects. First, compounds **1** and **2** incorporate an aminohexyl side chain at the P1 position. This group is not present in **6**−**9**, and we have attributed the differences in specificity at the P2 position that we observe among the different inhibitors to this structural feature. Second, compounds **6**−**9** are C-terminal carboxylic acids, while **1** and **2** are methyl esters. If this second difference plays an important role in determining inhibitor specificity, we would expect inhibitor **1** to have a significantly different inhibition constant than its corresponding carboxylic acid. However, **1** and its free acid derivative have IC_{50} values against plasmin that are the same within experimental error.[18] Thus, the difference between the C-terminal carboxylic acid and methyl ester groups does not play an important role in determining inhibitor selectivity.

Plasmin, trypsin, thrombin, and kallikrein all prefer to cleave substrates that incorporate Lys or Arg at P1. Thus, it is reasonable to expect that inhibitors **1** and **2** would have similar activity against all four proteases. The inhibition data shown in Table 1 demonstrates that we have been at least partly successful in our goal of developing inhibitors that are specific for plasmin. Compound **1** has 8- and 4-fold selectivity for plasmin when compared to trypsin and thrombin, respectively. However, the selectivity between plasmin and kallikrein is less than a factor of 2. Inhibitor **2** has reasonable selectivity for plasmin when compared to trypsin and kallikrein, but its selectivity against thrombin is less than a factor of 2. By contrast, compound **3** has a greater than 10-fold selectivity for plasmin when compared to the other three serine proteases.[9] The differences in specificity that are observed between compounds **2** and **3** may be caused by the D-Ile residue with the free N-terminus in compound **3**. This residue may impart significant selectivity for plasmin compared to the other serine proteases. Replacing this residue with the simple Cbz group that is present in compound **2** results in lower specificity.

In summary, we have developed a new procedure for the synthesis of cyclohexanone-based inhibitors of serine proteases. This procedure avoids the removal of a ketone protecting group during the late stages of the synthesis under conditions that are not compatible with other functional groups in the molecule. Inhibitors **1** and **2**, which result from this synthesis, are reasonable inhibitors of plasmin and show modest selectivity when compared to other related serine proteases. Comparison of the IC_{50} values for these inhibitors with results from previous studies highlight the fact that inhibitor selectivity can be dependent on cooperative binding interactions between the S1 and S2 subsites.

Experimental Section

General Methods. NMR spectra were recorded on Bruker Avance-300 or Avance-400 instruments. Spectra were calibrated using TMS ($\delta = 0.00$ ppm) for ^{1}H NMR and $CDCl_3$ ($\delta = 77.0$ ppm) or CD_3OD ($\delta = 49.0$ ppm) for ^{13}C NMR. IR spectra were recorded on a Perkin-Elmer 1700 series FT-IR spectrometer. Mass spectra were recorded on a Kratos MS 80 under electron impact (EI), chemical ionization (CI), or fast-atom bombardment (FAB) conditions. HPLC analyses were performed on a Rainin HPLC system with Rainin Microsorb silica or C18 columns, and UV detection. Semipreparative HPLC was performed on the same system using a semipreparative column (21.4 × 250 mm).

Reactions were conducted under an atmosphere of dry nitrogen in oven-dried glassware. Anhydrous procedures were conducted using standard syringe and cannula transfer techniques. THF was distilled from sodium and benzophenone. Other solvents were of reagent grade and were stored over 4 Å molecular sieves. All reagents were used as received. Organic solutions were dried over $MgSO_4$ unless otherwise noted. Solvent removal was performed by rotary evaporation at water aspirator pressure.

Alcohol 11. Ketoester **10** (12.9 g, 57.6 mmol) was dissolved in 20 mL of MeOH and cooled in an ice bath. To this solution was added $NaBH_4$ (4.40 g, 115.2 mmol) that was also dissolved in 20 mL of ice cold MeOH. After 1 h the reaction was quenched with 300 mL of 1 N HCl, and the methanol was removed by rotary evaporation. The resulting material was dissolved in 1 L of EtOAc, and the organic layer was washed with 600 mL of 1 N HCl, 600 mL of saturated aqueous $NaHCO_3$, 500 mL of brine, and dried over $MgSO_4$. The resulting solution was concentrated by rotary evaporation yielding 9.87 g of a clear oil. The crude material was purified by flash chromatography (7−9% EtOAc in hexanes) to yield **11** (6.13 g, 27.1 mmol, 47%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (t, $J = 7.1$ Hz, 5H), 1.24−1.33 (m, 3H), 1.37−1.46 (m, 1H), 1.48−1.60 (m, 1H), 1.59−1.74 (m, 2H), 1.94−2.19 (m, 2H), 2.29 (ddd, $J = 12.2, 4.1, 2.3$ Hz, 1H), 3.97−4.12 (m, 3H), 4.25−4.61 (br s, 1H), 4.94 (dddd, $J = 10.2, 2.3, 1.1, 1.1$ Hz, 1H), 5.01 (ddd, $J = 17.1, 3.6, 1.5$ Hz, 2H), 5.80 (dddd, $J = 16.9, 10.2, 6.6, 6.5$ Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 175.0, 140.0, 115.3, 68.6, 60.3, 48.9, 41.9, 32.5, 31.4, 25.6, 22.2, 15.0; HRMS−FAB ($M + Na^+$) calculated for $C_{13}H_{22}NaO_3$ 249.1467, found 249.1466.

TBDMS Ether 12. To the alcohol **11** (4.64 g, 20.5 mmol) was added 10 mL of DMF followed by DIEA (2.54 mL, 1.88 g, 24.6 mmol), imidazole (2.09 g, 31.0 mmol), and TBDMSCl (7.74 g, 51.3 mmol). The reaction was stirred at 50 °C for 12 h. The mixture was diluted with 600 mL of EtOAc, and the organic layer was washed with 2 × 500 mL of 1 N HCl, 500 mL of saturated aqueous $NaHCO_3$, and 500 mL of brine. The organic layer was dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The crude oil was purified by flash

(18) Abato, P.; Seto, C. Unpublished results.

chromatography (3−9% EtOAc in hexanes) to give **12** (6.05 g, 17.8 mmol, 87%): ^{1}H NMR (300 MHz, DMSO-d_6) δ −0.10 (d, J = 14.8 Hz, 6H), 0.82 (s, 9H), 1.14 (t, J = 6.2 Hz, 3H), 1.25−1.35 (m, 2H), 1.35−1.58 (m, 4H), 1.61−1.77 (m, 3H), 1.94−2.15 (m, 2H), 2.48−2.58 (m, 1H), 3.89−4.11 (m, 3H), 4.94−5.06 (m, 2H), 5.80 (dddd, J = 16.9, 10.2, 6.6, 6.5 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.1, 139.5, 115.8, 73.1, 60.5, 44.3, 39.9, 32.0, 29.7, 26.4, 24.7, 23.3, 20.0, 18.5, 14.9, −3.6, −4.4; HRMS−FAB (M + H$^+$) calculated for $C_{19}H_{37}O_3Si$ 341.2512, found 341.2517.

Alkene Acid 13. To compound **12** (5.50 g, 16.2 mmol) were added 300 mL of MeOH and 300 mL of 66.7 mM aqueous NaOH. The reaction was stirred at 85 °C for 72 h. The methanol was removed by rotary evaporation, and the aqueous layer was acidified with concentrated HCl to pH 2. The volume of the aqueous layer was increased to 500 mL with 1 N HCl and extracted with 3 × 400 mL of EtOAc. The organic layers were combined, washed with 500 mL of brine, and dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (2−50% EtOAc in hexanes) to yield **13** (3.53 g, 11.3 mmol, 70%): ^{1}H NMR (300 MHz, DMSO-d_6) δ −0.01(d, J = 5.0 Hz, 6H), 0.83 (s, 9H), 1.13−1.27 (m, 2H), 1.26−1.56 (m, 4H), 1.56−1.72 (m, 4H), 1.95−2.19 (m, 2H), 2.33−2.46 (m, 1H), 3.95−4.06 (m, 1H), 4.89−5.09 (m, 2H), 5.78 (dddd, J = 16.9, 10.2, 6.6, 6.5 Hz, 1H), 11.0−12.5 (br s, 1H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 177.1, 138.8, 114.2, 73.0, 44.0, 40.3, 31.9, 29.8, 25.4, 24.1, 22.7, 19.7, 17.9, −5.2, −5.8; HRMS−FAB (M + H$^+$) calculated for $C_{17}H_{33}O_3Si$ 313.2199, found 313.2194.

Carbamate 14. To a solution of **13** (3.00 g, 9.62 mmol) in 15 mL of toluene were added DIEA (2.01 mL, 1.49 g, 11.5 mmol) and diphenylphosphoryl azide (1.81 mL, 2.32 g, 11.5 mmol). The reaction was heated to 50 °C under nitrogen for 5 h. The formation of the isocyanate was monitored by the increase in IR intensity at 2256 cm^{-1}. The alkoxide from benzyl alcohol was prepared in a separate flask as follows. Benzyl alcohol (5.00 mL, 5.23 g, 48.1 mmol) was added to 30 mL of THF at 0 °C under nitrogen, followed by dropwise addition of *n*-butyllithium (11.6 mL of a 2.5 M solution in hexanes, 28.9 mmol) over a period of 10 min. The isocyanate solution was then 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 quenched by slow addition of 100 mL of 1 N HCl followed by removal of the THF by rotary evaporation. The remaining solution was diluted with 300 mL of EtOAc and washed with 300 mL of 1 N HCl, 300 mL of saturated aqueous Na_2CO_3, and 300 mL of brine solution. The organic layer was dried over $MgSO_4$, and the solvents were removed by rotary evaporation. The crude material was purified by flash chromatography (1:19 EtOAc/hexanes) to yield **14** (2.21 g, 5.27 mmol, 55%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.00 (s, 6H), 0.85 (s, 9H), 0.98−1.10 (m, 1H), 1.13−1.31 (m, 1H), 1.33−1.44 (m, 3H), 1.50−1.74 (m, 4H), 3.55−3.69 (m, 2H), 4.91−5.06 (m, 4H), 5.70−5.88 (m, 1H), 6.81 (d, J = 7.4 Hz, 1H), 7.27−7.38 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.2, 138.9, 128.9, 128.4, 115.1, 74.0, 66.8, 50.7, 40.3, 32.0, 30.0, 28.2, 26.2, 25.1, 20.1, 18.5, 11.8, −4.3, −4.4; HRMS−FAB (M + H$^+$) calculated for $C_{24}H_{40}NO_3Si$ 418.2778, found 418.2797.

Carboxylic Acid 15. To compound **14** (2.20 g, 5.28 mmol) was added 200 mL of acetone followed by 66 mL of an aqueous solution containing the following: $NaIO_4$ (5.64 g, 26.4 mmol), $KMnO_4$ (0.835 g, 5.28 mmol), and $NaHCO_3$ (0.488 g, 5.81 mmol). The solution was stirred for 18 h at room temperature. The acetone was removed by rotary evaporation, and the remaining material was diluted with 600 mL of EtOAc and washed with 3 × 500 mL of 1 N HCl and 400 mL of brine. The organic layer was dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (10−50% EtOAc in hexanes) to yield **15** (1.61 g, 3.95 mmol, 75%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.01 (s, 6H), 0.85 (s, 9H), 0.98−1.10 (m, 1H), 1.30−1.45 (m, 4H), 1.55−1.80 (m, 4H), 2.10−2.30 (m, 2H), 3.56−3.70 (m, 2H), 4.99 (d, J = 12.7 Hz, 2H), 5.04 (d, J = 12.4 Hz, 1H), 7.35−7.40 (m, 5H), 12.00 (br s, 1H); ^{13}C NMR (75 MHz $CDCl_3$) δ 179.3, 156.4, 137.0, 128.9, 128.7, 128.5, 73.8, 67.0, 50.7, 40.5, 23.5, 29.1, 28.1, 26.2, 20.0, 18.4, −4.4; HRMS−FAB (M + Na$^+$) calculated for $C_{23}H_{37}NaNO_5Si$ 458.2339, found 458.2327.

Fmoc-Trp(Boc)-OMe. To commercially available Fmoc-Trp(Boc)-OH (3.00 g, 5.69 mmol) were added 15 mL of methanol, DMAP (69.5 mg, 0.569 mmol), and EDC (2.18 g, 11.4 mmol). The reaction was allowed to stir at room temperature for 1 h. The reaction was diluted with 500 mL of EtOAc and washed with 500 mL of 1 N HCl, 500 mL of saturated aqueous Na_2CO_3, and 500 mL of brine. The organic layer was dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The resulting material was purified by flash chromatography (3:7 EtOAc/hexanes) to yield compound Fmoc-Trp(Boc)-OMe (2.56 g, 4.74 mmol, 83%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.69 (s, 9H), 3.27−3.33 (m, 2H), 3.74 (s, 3H), 4.24 (t, J = 7.1 Hz, 1H), 4.35−4.47 (m, 2H), 4.76−4.83 (m, 1H), 5.45 (d, J = 7.4 Hz, 1H), 7.23−7.35 (m, 4H), 7.37−7.45 (m, 3H), 7.56 (dd, J = 15.1, 7.6, Hz, 3H), 7.79 (d, J = 8.5 Hz, 2H), 8.15 (d, J = 8.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 156.2, 150.0, 144.2, 141.7, 135.8, 130.9, 128.1, 127.5, 125.6, 125.0, 124.6, 123.1, 120.4, 119.2, 115.8, 115.3, 84.2, 67.6, 54.6, 52.9, 47.6, 28.6, 28.3; HRMS−FAB (M + Na$^+$) calculated for $C_{32}H_{32}NaN_2O_6$ 563.2158, found 563.2153.

H_2N-Trp(Boc)-OMe. To compound Fmoc-Trp(Boc)-OMe (2.50 g, 4.62 mmol) was added 15 mL of a 1:1 solution of piperidine:DMF. The reaction was stirred at room temperature for 30 min, after which time it was diluted with 500 mL of EtOAc. The reaction was washed with 2 × 500 mL of saturated aqueous Na_2CO_3 and 500 mL of brine. The organic layer was dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (1:1 EtOAc/hexanes) to yield H_2N-Trp(Boc)-OMe (0.995 g, 3.13 mmol, 67%): ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.67 (s, 9H), 3.06 (ddd, J = 14.4, 6.7, 0.8 Hz, 1H), 3.16 (ddd, J = 14.3, 6.0, 0.9 Hz, 1H), 3.67 (s, 3H), 3.85 (t, J = 6.2 Hz, 1H), 7.22−7.34 (m, 2H), 7.50 (s, 1H), 7.56−7.58 (m, 1H), 8.12 (d, J = 8.1 Hz, 1H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 175.4, 149.9, 136.0, 130.7, 124.5, 124.3, 122.7, 119.0, 116.2, 115.2, 83.8, 54.3, 51.6, 30.1, 27.5; HRMS−FAB (M + H$^+$) calculated for $C_{17}H_{22}N_2O_4$ 319.1658, found 319.1666.

Amide 16. To compound **15** (0.921 g, 2.12 mmol) were added H_2N-Trp(Boc)-OMe (0.875 g, 2.73 mmol), HBTU (1.61 g, 4.24 mmol), DIEA (1.48 mL, 1.10 g, 8.47 mmol), and 4 mL of DMF. The reaction was stirred for 2.5 h at room temperature and diluted with 500 mL of EtOAc. The organic layer was washed with 2 × 400 mL of 1 N HCl, 400 mL of saturated aqueous Na_2CO_3, and 400 mL of brine. The organic layer was dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (1:2 EtOAc/hexanes) to give **16** (1.31 g, 1.78 mmol, 84%): ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.12 to −0.05 (m, 6H), 0.86−0.93 (m, 9H), 1.04−1.16 (m, 1H), 1.35−1.54 (m, 6H), 1.72 (s, 9H), 1.81−1.90 (m, 3H), 2.10−2.23 (m, 2H), 3.21−3.38 (m, 2H) 3.80−3.91 (m, 4H), 4.85−5.00 (m, 2H), 5.09 (s, 2H), 6.01 (t, J = 8.9 Hz, 1H), 7.15−7.45 (m, 8H), 7.49 (d, J = 7.5 Hz, 1H) 8.12 (d, J = 7.9 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.2, 153.9, 147.5, 134.8, 133.3, 128.6, 126.5, 126.0, 122.6, 120.0, 116.8, 113.3, 113.1, 81.7, 71.6. 64.4, 50.5, 48.4, 38.2, 38.0, 32.3, 26.7, 26.2, 25.8, 24.2, 17.6, 16.0, 12.2, −6.7, −6.8; HRMS−FAB (M + H$^+$) calculated for $C_{40}H_{58}N_3O_8Si$ 736.3993, found 736.3992.

Amine 17. To compound **16** (2.73 g, 3.71 mmol) were added 20 mL of MeOH and 10% Pd on carbon (1.0 g). The reaction flask was then purged with and kept under 1 atm of hydrogen gas while stirring for 3 h at room temperature. The reaction was then filtered through a pad of Celite, and the solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (5−10% MeOH in CH_2Cl_2) to give **17** (2.84 g, 3.07 mmol, 83%): ^{1}H NMR (300 MHz, MeOH-d_4) δ −0.04−0.13 (m, 6H), 0.87−0.94 (m, 9H), 0.99−1.12 (m, 1H), 1.20−1.36 (m, 2H), 1.36−1.49 (m, 3H), 1.51−1.66 (m, 3H), 1.69 (s, 9H), 1.73−1.94 (m, 2H), 2.06−2.34 (m, 2H), 2.80−2.90 (m, 1H), 3.05−3.17 (m, 1H), 3.26 (d, J = 5.3 Hz, 1H), 3.43−3.48 (m, 1H), 3.72 (s, 3H), 4.76−4.88 (m, 1H),

7.23−7.35 (m, 2H), 7.50 (s, 1H), 7.59 (d, J = 7.7 Hz, 1H), 8.11 (d, J = 8.1 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.7, 170.3, 147.0, 133.3, 128.6, 122.6, 122.0, 120.6, 116.8, 113.3, 81.7, 50.5, 50.4, 48.6, 36.7, 32.3, 28.8, 26.2, 25.4, 25.1, 24.0, 17.4, 12.2, −6.4, −6.6; HRMS−FAB (M + H$^+$) calculated for $C_{32}H_{52}N_3O_6Si$ 602.3625, found 602.3648.

Secondary Amine 18. To compound **17** (1.39 g, 2.32 mmol) were added 15 mL of $CDCl_3$, N−Boc-6-aminohexaldehyde[14] (2.50 g, 11.6 mmol), AcOH (155 μL, 163 mg, 2.58 mmol) and 1.0 g of 4 Å molecular sieves, and the reaction was heated to 50 °C. Formation of the imine was monitored using ^{1}H NMR spectroscopy by following the appearance of a resonance for the imine at 9.36 ppm and disappearance of a resonance for the aldehyde at 9.77 ppm. After formation of the imine was complete, the reaction was poured into a solution of $NaBH_4$ (5.00 g, 135 mmol) in 200 mL of methanol at 0 °C, and the solution was allowed to warm to room temperature over 30 min. The volume of the solution was then reduced to 50 mL by rotary evaporation. This solution was diluted with 500 mL of EtOAc, washed with 500 mL of saturated Na_2CO_3 and 500 mL of brine. The organic layer was dried over $MgSO_4$ and the solvents were removed by rotary evaporation. The resulting material was purified by flash chromatography (1:1 EtOAc/hexanes to elute the N−Boc-6-aminohexanol, followed by a gradient of 0.1:0.9:99 to 1:9:90 concentrated aqueous NH_4OH:MeOH:CH_2Cl_2) to give compound **18** (0.574 g, 0.835 mmol, 36%): ^{1}H NMR (300 MHz, DMSO-d_6) δ −0.1−0.5 (m, 6H), 0.81−0.84 (m, 9H), 0.84−0.87 (m, 1H), 1.10−1.30 (m, 7H), 1.30−1.35 (m, 1H), 1.37 (s, 9H), 1.39−1.60 (m, 4H), 1.62 (s, 9), 1.90−2.15 (m, 3H), 2.25−2.38 (m, 1H), 2.50−2.61 (m, 0.5H), 2.75−2.81 (m, 0.5H), 2.85−2.91 (m, 2H), 2.95−3.04 (m, 1H), 3.13 (dd, J = 14.9, 5.0 Hz, 1H), 3.36−3.46 (m, 1H), 3.61 (s, 3H), 4.50−4.60 (m, 1H), 6.65−6.80 (m, 1H), 7.29 (ddd, J = 15.2, 7.2, 7.3 Hz, 2H), 7.51 (s, 1H), 7.58 (d, J = 7.5 Hz, 1H), 8.03 (d, J = 8.1 Hz, 1H), 8.27 (dd, J = 7.7, 3.1 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.1, 172.6, 156.4, 149.9, 135.7, 131.0, 125.0, 124.4, 123.0, 119.2, 115.7, 115.4, 84.1, 79.4, 77.9, 77.7, 77.5, 77.0, 58.0, 52.9, 47.6, 41.0, 39.6, 34.9, 34.8, 30.9, 39.5, 30.1, 29.6, 28.8, 28.1, 27.8, 27.2, 26.9, 26.2, 19.8, 18.4, −3.0, −4.0, −4.2; HRMS−FAB (M + H$^+$) calculated for $C_{43}H_{73}N_4O_8Si$ 801.5198, found 801.5225.

Cbz-Trp(Boc)-OH. To commercially available H_2N-Trp(Boc)-OH (1.48 g, 5.14 mmol) were added 25 mL of DMF, DIEA (1.5 mL, 1.13 g, 11.3 mmol) and 1-benzyloxycarbonyl-benzotriazole (1.95 g, 7.71 mmol). The reaction was allowed to stir for 3 h at room temperature. The reaction was then diluted with 1 L of EtOAc and washed with 3 × 600 mL of 1 N HCl and 400 mL of brine. The organic layer was dried over $MgSO_4$ and the solvent removed by rotary evaporation. The crude material was purified by flash chromatography (1−5% MeOH in CH_2Cl_2) to yield compound Cbz-Trp(Boc)-OH (2.02 g, 4.61 mmol, 90%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.67 (s, 9H), 3.27 (dd, J = 14.9, 6.2 Hz, 1H), 3.37 (dd, J = 14.9, 5.2 Hz, 1H), 4.80 (dd, J = 13.2, 6.0 Hz, 1H), 5.12 (d, J = 3.8 Hz, 2H), 5.50 (d, J = 7.9 Hz, 1H), 7.20 (t, J = 7.3 Hz, 1H), 7.29−7.33 (m, 2H), 7.35−7.37 (m, 4H), 7.50 (s, 1H), 7.56 (d, J = 7.8 Hz, 1H), 8.12 (d, J = 8.1 Hz, 1H), 9.37 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.9, 156.6, 150.1, 136.5, 136.0, 131.0, 129.5, 129.0, 128.9, 125.0, 124.8, 123.1, 119.4, 115.7, 115.4, 84.3, 67.6, 61.1, 54.3, 28.6, 28.0, 21.5, 14.6; HRMS−FAB (M + H$^+$) calculated for $C_{24}H_{27}NaN_2O_6$ 461.1689, found 461.1702.

Amide 19. To compound **18** (132 mg, 165 μmol) were added 410 μL of DMF, DIEA (147 μL, 107 mg, 825 μmol), Cbz-Trp(Boc)-OH (223 mg, 494 μmol), and HBTU (250 mg, 660 μmol). The reaction was stirred at 40 °C for 6 h followed by the addition of 100 mL of methylene chloride. The organic layer was washed with 50 mL of 0.25 N HCl, 100 mL of saturated aqueous $NaHCO_3$, and 100 mL of brine. The organic layer was then dried over $MgSO_4$. After the solvents were removed by rotary evaporation, the crude material was purified by flash chromatography (2% MeOH in methylene chloride) to give compound **19** as a mixture of diastereomers (140 mg, 114 μmol, 69%): ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.19 (s, 3H), −0.12 (s, 3H), −0.01−0.16 (m, 1H), 0.82 (s, 9H), 0.87−1.01 (m, 2H), 1.03−1.21 (m, 3H), 1.22−1.37 (m, 5H), 1.46 (s, 13H), 1.63 (s, 9H), 1.64 (s, 9H), 1.73−1.87 (m, 2H), 2.00 (dt, J = 11.7, 3.1 Hz, 1H), 2.54 (dt, J = 13.3, 4.3 Hz, 1H), 2.72−2.85 (m, 1H), 2.89−3.05 (m, 1H), 3.05−3.15 (m, 3H), 3.18−3.40 (m, 3H), 3.55 (s, 2H), 3.72 (s, 3H), 4.58 (br s, 1H), 4.76−4.94 (m, 3H), 5.12 (dd, J = 14.1, 6.6 Hz, 1H), 5.31 (s, 1H), 5.97 (d, J = 8.9 Hz, 1H), 7.15−7.26 (m, 5H), 7.31−7.41 (m, 3H), 7.49 (s, 1H), 7.59 (d, J = 7.0 Hz, 1H), 7.79 (d, J = 7.6 Hz, 1H), 7.94 (d, J = 7.8 Hz, 1H), 8.05 (d, J = 7.7 Hz, 1H), 8.14 (d, J = 8.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 172.9, 171.3, 156.4, 156.2, 150.0, 149.7, 136.4, 135.8, 135.7, 131.0, 130.3, 128.8, 128.5, 128.3, 125.6, 124.9, 124.2, 124.0, 122.9, 119.4, 119.3, 116.2, 115.8, 115.7, 115.1, 84.0, 83.9, 79.4, 73.9, 67.3, 54.9, 53.8, 52.9, 52.7, 50.9, 45.9, 45.5, 41.0, 35.1, 32.3, 31.2, 30.1, 30.0, 29.8, 29.6, 28.9, 28.6, 27.9, 27.5, 26.8, 26.2, 25.7, 25.7, 25.1, 23.1, 20.3, 18.1, −4.4, −4.7; HRMS−FAB (M + Na$^+$) calculated for $C_{67}H_{95}NaN_6O_{13}Si$ 1243.6703, found 1243.6693.

Alcohol 21. To compound **19** (35 mg, 29 μmol) was added 1.3 mL of MeCN followed by 1.56 mL of a 92 mM solution of $Zn(BF_4)_2$ in 1:9 water:MeCN. The reaction was allowed to stir at room temperature for 7 h. The solvents were then removed by rotary evaporation, and the crude material was diluted with 15 mL of EtOAc and washed with 10 mL of saturated aqueous Na_2CO_3 and 10 mL of brine. The organic layer was dried over $MgSO_4$, and the solvents were removed by rotary evaporation. The resulting material was purified by flash chromatography (1.5% MeOH in methylene chloride) to give compound **21** as a mixture of diastereomers (25 mg, 23 μmol, 79%): ^{1}H NMR (300 MHz, MeOH-d_4) δ 0.10−0.30 (m, 1H), 0.73−0.83 (m, 1H), 0.85−1.07 (m, 4H), 1.13 (br s, 1H), 1.20−1.39 (m, 9H), 1.48−1.52 (m, 2H), 1.53−1.58 (m, 2H), 1.63 (s, 10H), 1.66−1.71 (m, 13H), 2.02−2.23 (m, 1H), 2.17−2.34 (m, 1H), 2.35−2.60 (m, 1H), 2.85−2.97 (m, 1H), 3.03 (t, J = 6.9 Hz, 3H), 3.06−3.14 (m, 2H), 3.15−3.24 (m, 2H), 3.24−3.31 (m, 1H), 3.41−3.59 (m, 1H), 3.74 (s, 3H), 3.76−3.83 (m, 1H), 4.79 (s, 2H), 4.94−4.99 (m, 1H), 5.00−5.19 (m, 1H), 7.10−7.21 (m, 3H), 7.23−7.40 (m, 9H), 7.45 (s, 1H), 7.51 (s, 1H), 7.54−7.81 (m, 1H), 7.67 (d, J = 7.7 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 8.13 (d, J = 8.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9, 172.3, 171.9, 156.4, 156.1, 150.6, 150.0, 149.7, 136.6, 136.3, 135.9, 135.6, 135.2, 131.0, 130.3, 128.9, 128.7, 128.6, 128.3, 128.2, 128.9, 125.7, 125.2, 125.0, 124.8, 124.7, 124.4, 123.7, 123.2, 122.9, 119.3, 119.2, 119.1, 115.9, 115.7, 115.5, 115.3, 84.9, 84.2, 84.0, 79.4, 77.7, 73.2, 72.0, 67.4, 67.2, 60.8, 54.7, 53.8, 53.0, 52.9, 52.3, 51.9, 51.8, 45.2, 41.8, 40.9, 40.7, 35.2, 35.0, 33.7, 32.3, 31.0, 30.2, 29.5, 28.9, 28.5, 28.1, 27.8, 27.4, 26.4, 26.2, 25.4, 25.0, 24.5, 24.1, 23.1, 21.5, 20.2, 14.6; HRMS−FAB (M + Na$^+$) calculated for $C_{61}H_{82}NaN_6O_{13}$ 1129.5838, found 1129.5867.

Ketone 23. Compound **21** (25 mg, 23 μmol) was treated with a solution of PCC (15 mg, 70 μmol) and Al_2O_3 (90 mg, 883 μmol) in 2.3 mL of methylene chloride. The reaction was allowed to stir for 5 h at room temperature. The solvent was then removed by rotary evaporation, and the crude material was diluted with 15 mL of EtOAc, washed with 10 mL of saturated aqueous Na_2CO_3, and 10 mL of brine. The organic layer was dried over $MgSO_4$ and the solvent removed by rotary evaporation. The resulting material was purified by flash chromatography (gradient of 1−2% MeOH in methylene chloride) to give compound **23** as a mixture of two diastereomers (20 mg, 18 μmol, 78%): ^{1}H NMR (300 MHz, MeOH-d_4) δ 0.80−0.97 (m, 2H), 1.07 (s, 3H), 1.20−1.36 (m, 6H), 1.45 (s, 12H), 1.62 (s, 4H), 1.63 (s, 3H), 1.65 (s, 9H), 1.67 (s, 1H), 1.91−2.12 (m, 2H), 2.41−2.63 (m, 1H), 2.85−3.08 (m, 4H), 3.08−3.20 (m, 2H), 3.25 (d, J = 5.2 Hz, 1H), 3.27−3.40 (m, 2H), 3.68−3.73 (m, 3H), 4.20−4.35 (m, 1H), 4.77 (s, 1H), 5.04−5.15 (m, 2H), 6.35−6.56 (m, 1H), 7.69 (d, J = 7.5 Hz, 0.5H), 7.75 (d, J = 7.9 Hz, 0.5H), 7.95−8.04 (m, 0.5H), 7.43 (s, 1H), 7.46−7.51 (m, 2H), 7.53−7.58 (m, 1H), 7.64 (d, J = 8.5 Hz, 0.5H), 7.14−7.38 (m, 10H), 8.04−8.15 (m, 1H); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 209.2, 208.9, 172.9, 172.5, 172.3, 172.2, 156.4, 156.1, 156.0, 155.9, 150.1, 149.9, 149.7, 136.7, 136.2, 136.0, 135.7, 131.0, 130.3, 128.8, 128.6, 128.5, 128.2, 126.0, 125.1, 124.9, 124.5, 123.0, 119.3, 119.2, 116.2, 116.0, 115.8, 115.7, 115.4, 84.3, 84.1, 84.0, 79.4, 77.7, 67.4, 67.2, 62.7, 52.8, 52.7, 51.4, 51.1, 50.7, 50.5, 48.8, 48.4, 47.0, 44.7, 40.7, 34.2, 32.3, 31.6, 30.6, 30.3, 30.0, 29.5, 28.8, 28.6, 28.2, 27.9, 27.6, 27.1, 26.4, 20.3, 19.8; HRMS−FAB

($M + Na^+$) calculated for $C_{61}H_{80}NaN_6O_{13}$ 1127.5681, found 1127.6714.

Inhibitor 1. To compound **23** (20 mg, 18 μmol) was added 1 mL of a 1:1 solution of TFA:methylene chloride. The reaction was allowed to stir at room temperature for 20 min, the solvent was removed by rotary evaporation, and the residue was dried under vacuum. The crude material was diluted with 15 mL of EtOAc and washed with 10 mL of saturated aqueous Na_2CO_3 and 10 mL of brine. The organic layer was then dried over $MgSO_4$ and the solvent removed by rotary evaporation. The resulting material was purified by flash chromatography (5% MeOH in methylene chloride) to give inhibitor **1** as a mixture of two diastereomers (10 mg, 12 μmol, 67%): ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.00−1.15 (m, 2H), 1.19−1.35 (m, 3H), 1.61−1.82 (m, 18H), 1.82−1.95 (m, 1H), 1.96−2.20 (m, 1H), 2.25−2.39 (m, 2H), 2.56−2.78 (m, 1H), 2.82−3.19 (m, 3H), 3.19−3.23 (m, 1H), 3.25 (d, J = 5.2 Hz, 1H), 3.48−3.59 (m, 1H), 3.67−3.74 (m, 3H), 4.20−4.35 (m, 1H), 4.63−4.73 (m, 1H), 4.78 (s, 1H), 4.92−4.96 (m, 1H), 5.08 (d, J = 4.0 Hz, 1H), 7.10−7.20 (m, 1H), 7.22−7.33 (m, 8H), 7.35−7.38 (m, 1H), 7.41−7.43 (m, 1H), 7.48−7.53 (m, 1H), 7.53−7.58 (m, 1H), 7.69 (d, J = 7.4 Hz, 0.5H), 7.75 (d, J = 7.7 Hz, 0.5H), 8.00 (d, J = 7.7 Hz, 0.5H), 8.13 (q, J = 8.1 Hz, 1H), 8.20 (d, J = 8.2 Hz, 0.5H); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 209.7, 209.4, 173.2, 173.0, 172.4, 172.1, 156.0, 137.1, 136.9, 136.6, 128.8, 128.7, 128.3, 128.2, 127.9, 124.3, 124.1, 124.0, 123.7, 123.0, 122.2, 120.7, 119.6, 118.8, 118.7, 112.1, 111.7, 110.7, 110.3, 110.2, 67.1, 63.0, 52.6, 52.5, 51.8, 50.1, 46.1, 44.7, 41.9, 34.2, 33.8, 33.6, 32.9, 32.6, 32.1, 31.0, 30.4, 30.1, 29.4, 29.0, 28.3, 28.0, 27.1, 26.6, 26.5, 25.6, 24.4, 20.3, 19.7; HRMS−FAB ($M + Na^+$) calculated for $C_{46}H_{56}NaN_6O_7$ 827.4109, found 827.4099.

Amide 20. To the compound **18** (464 mg, 579 μmol) were added 410 μL of DMF, DIEA (515 μL, 374 mg, 2.90 mmol), commercially available Cbz-Phe-OH (520 mg, 1.74 mmol), and HBTU (878 mg, 2.32 mmol). The reaction was stirred at 40 °C for 6 h, and then 150 mL of methylene chloride was added. The organic layer was washed with 150 mL of 0.25 N HCl, 150 mL of saturated aqueous $NaHCO_3$, and 150 mL of brine and then dried over $MgSO_4$. After the solvents were removed by rotary evaporation, the crude material was purified by flash chromatography (2% MeOH in methylene chloride) to give compound **20** as a mixture of diastereomers (507 mg, 469 μmol, 81%): ^{1}H NMR (300 MHz, DMSO-d_6) δ −0.40−0.10 (m, 6H), 0.74−0.87 (m, 9H), 0.91−1.04 (m, 1H), 1.07−1.27 (m, 6H), 1.37 (br s, 10H), 1.43−1.53 (m, 4H), 1.61 (s, 5H), 1.62 (s, 4H), 2.10−2.25 (m, 2H), 2.69 (s, 3H), 2.74 (s, 1H), 2.80−2.95 (m, 5H), 2.98−3.07 (m, 2H), 3.09−3.21 (m, 2H), 3.57−3.70 (m, 3H), 4.47−4.61 (m, 2H), 4.86−4.95 (m, 2H), 4.95−5.08 (m, 1H), 6.67−6.80 (m, 1H), 7.15−7.35 (m, 12H), 7.49−7.70 (m, 2H), 7.75−7.89 (m, 1H), 7.98−8.05 (m, 1H), 8.28−8.40 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6, 173.5, 173.2, 172.8, 172.7, 172.6, 171.2, 170.4, 163.0, 156.4, 156.1, 156.0, 155.9, 150.0, 137.5, 136.8, 136.7, 136.5, 136.4, 135.7, 131.0, 130.0, 129.8, 129.7, 129.6, 129.3, 129.1, 129.0, 128.9, 128.8, 128.5, 128.4, 128.2, 128.1, 127.7, 127.5, 127.4, 125.0, 124.5, 124.4, 124.1, 123.0, 122.9, 119.3, 119.2, 116.2, 115.7, 84.1, 84.0, 79.4, 77.7, 73.7, 72.4, 67.4, 67.2, 60.8, 54.9, 53.0, 52.9, 52.8, 52.7, 52.6, 45.9, 45.7, 42.5, 42.1, 41.0, 40.9, 40.6, 40.5, 39.0, 36.9, 35.3, 34.7, 34.4, 32.1, 31.8, 30.4, 30.1, 29.5, 28.8, 28.6, 28.3, 28.2, 27.8, 27.4, 26.9, 26.8, 26.5, 26.3, 26.2, 26.1, 26.0, 24.8, 24.3, 24.1, 21.0, 20.8, 19.8, 18.3, 18.1, 14.6, −4.2, −4.3, −4.5, −4.7, −4.9; HRMS−FAB ($M + Na^+$) calculated for $C_{60}H_{87}NaN_5O_{11}$-Si 1104.6069, found 1104.6048.

Alcohol 22. To compound **20** (428 mg, 396 μmol) was added 20.1 mL of MeCN followed by 21.4 mL of a 92 mM solution of $Zn(BF_4)_2$ in 1:9 water:MeCN. The reaction was allowed to stir at room temperature for 7 h. The solvents were removed by rotary evaporation, and the residue was dissolved in 250 mL of EtOAc and washed with 250 mL of saturated aqueous Na_2CO_3 and 250 mL of brine. The organic layer was then dried over $MgSO_4$ and the solvent removed by rotary evaporation. The resulting material was purified by flash chromatography (1.5% MeOH in methylene chloride) to give compound **22** as a mixture of diastereomers (135 mg, 140 μmol, 35%): ^{1}H NMR (300 MHz,$CDCl_3$) δ 0.67−0.79 (m, 1H), 0.80−0.96 (m, 3H), 1.06−1.18 (m, 2H), 1.28 (br s, 12H), 1.44 (s, 10H), 1.51−1.59 (m, 3H), 1.68 (s, 9H), 1.91−2.02 (m, 1H), 2.10−2.28 (m, 2H), 2.45−2.68 (m, 1H), 2.85−2.96 (m, 1H), 2.97−2.16 (m, 3H), 3.16−3.35 (m, 2H), 3.60−3.75 (m, 3H), 4.49−4.67 (m, 1H), 4.87−4.99 (m, 2H), 5.01−5.12 (m, 2H), 5.45−5.57 (m, 1H), 6.40−6.55 (m, 1H), 7.10−7.37 (m, 10H), 7.38−7.44 (m, 1H), 7.50 (d, J = 7.3 Hz, 1H), 8.00−8.16 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.0, 172.7, 171.8, 171.5, 156.6, 156.4, 150.0, 136.5, 136.2, 135.7, 131.0, 130.0, 129.8, 129.6, 129.2, 129.1, 129.0, 128.9, 128.6, 128.5, 128.3, 127.6, 127.5, 124.9, 124.5, 123.0, 119.2, 115.7, 115.5, 84.1, 79.4, 77.7, 67.5, 60.8, 55.7, 55.1, 53.0, 52.8, 47.1, 40.9, 38.1, 36.6, 34.3, 32.3, 30.4, 30.1, 28.8, 28.6, 27.8, 27.3, 27.2, 27.0, 26.9, 23.0, 21.5, 19.5, 14.6, 14.5; HRMS-ESI ($M + H^+$) calculated for $C_{54}H_{74}N_5O_{11}$ 968.5385, found 968.5408.

Ketone 24. Alcohol **22** (125 mg, 129 μmol) was subject to a solution containing PCC (109 mg, 505 μmol) and Al_2O_3 (375 mg, 3.68 mmol) in 6.25 mL of methylene chloride. The reaction was allowed to stir for 8 h at room temperature. The solvent was removed by rotary evaporation, and the crude material was diluted with 150 mL of EtOAc and washed with 100 mL of saturated aqueous Na_2CO_3 and 100 mL of brine. The organic layer was dried over $MgSO_4$ and the solvent removed by rotary evaporation. The resulting material was purified by flash chromatography (gradient of 1−2% MeOH in methylene chloride) to give compound ketone **24** as a mixture of two diastereomers (56 mg, 58 μmol, 45%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80−1.01 (m, 1H), 1.05−1.35 (m, 6H), 1.45 (s, 9H), 1.54−1.59 (m, 2H), 1.60−1.63 (m, 10H), 1.75−1.94 (m, 5H), 1.96−2.12 (m, 3H), 2.18−2.29 (m, 1H), 2.33−2.65 (m, 2H), 2.76−2.94 (m, 1H), 2.95−3.16 (m, 4H), 3.16−3.31 (m, 2H), 3.60−3.77 (m, 3H), 4.36−4.54 (m, 1H), 4.64−4.83 (m, 1H), 4.86−4.99 (m, 1H), 5.00−5.15 (m, 2H), 6.17 (t, J = 8.0 Hz, 1H), 6.50−6.67 (m, 1H), 7.10−7.36 (m, 13H), 7.40−7.60 (m, 2H), 7.95−8.16 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 210.4, 210.0, 209.2, 208.6, 173.0, 172.7, 172.6, 172.7, 172.4, 172.2, 172.0, 171.8, 171.7, 171.5, 170.9, 170.7, 156.4, 156.1, 156.0, 150.0, 137.1, 136.8, 136.6, 135.7, 131.0, 130.0, 129.7, 129.5, 129.1, 129.0, 128.8, 128.5, 128.4, 128.2, 128.1, 127.5, 127.4, 127.2, 125.0, 124.9, 124.6, 123.0, 119.3, 119.2, 116.3, 115.7, 115.4, 84.2, 84.1, 84.0, 79.4, 77.6, 67.4, 67.2, 67.1, 62.5, 60.8, 56.5, 55.6, 53.0, 52.9, 52.7, 50.6, 49.5, 49.4, 48.3, 40.8, 40.4, 40.3, 39.2, 35.0, 34.7, 34.4, 34.1, 32.8, 32.5, 30.3, 30.2, 29.7, 29.3, 28.8, 28.6, 28.2, 27.8, 27.1, 27.0, 26.7, 26.7, 26.6, 21.4, 20.5, 20.3, 20.0, 19.8, 14.6; HRMS−FAB ($M + Na^+$) calculated for $C_{54}H_{71}NaN_5O_{11}$ 988.5048, found 988.5077.

Inhibitor 2. To ketone **24** (24 mg, 25 μmol) was added 1 mL of a 1:1 solution of TFA:methylene chloride. The reaction was allowed to stir at room temperature for 20 min, the solvent was removed by rotary evaporation, and the residue was dried under vacuum. The resulting crude material was dissolved in 15 mL of EtOAc and washed with 10 mL of saturated aqueous Na_2CO_3 and 10 mL of brine. The organic layer was dried over $MgSO_4$ and the solvent removed by rotary evaporation. The resulting material was purified by flash chromatography (5% MeOH in methylene chloride) to give inhibitor **2** as a mixture of two diastereomers (6 mg, 8 μmol, 25%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.61−1.05 (m, 3H), 1.10−1.42 (m, 8H), 1.43−1.92 (m, 8H), 1.93−2.10 (m, 3H), 2.14−2.25 (m, 1H), 2.26−2.70 (m, 3H), 2.80−3.29 (m, 2H), 3.25−3.55 (m, 1H), 3.61−3.85 (m, 3H), 4.25−4.56 (m, 2H), 4.80−5.00 (m, 1H), 5.01−5.20 (m, 2H), 5.21−5.50 (m, 1), 5.90−6.25 (m, 1H), 6.45−6.85 (m, 1H), 7.05−7.45 (m, 13H), 7.46−7.75 (m, 1H), 8.30−6.65 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 213.5, 210.5, 210.2, 208.6, 208.4, 173.1, 172.8, 172.7, 172.5, 171.6, 171.4, 171.0, 170.9, 170.7, 156.4, 136.7, 136.5, 129.7, 129.2, 129.0, 128.6, 128.4, 128.0, 127.7, 127.6, 123.7, 123.5, 123.3, 123.1, 122.7, 122.6, 120.1, 120.0, 118.9, 111.9, 111.7, 110.2, 110.1, 77.6, 67.6, 60.8, 58.5, 58.3, 56.5, 55.5, 53.0, 52.8, 52.7, 49.5, 49.3, 49.0, 39.1, 39.0, 36.2, 36.0, 35.5, 35.1, 34.8, 34.3, 34.1, 34.0, 33.9, 32.5, 32.3, 30.1, 29.8, 28.2, 28.0, 27.8, 27.3, 26.9, 25.4, 25.3, 23.8, 23.7, 23.1, 21.5, 19.8, 14.6; HRMS−FAB ($M + Na^+$) calculated for $C_{44}H_{55}$-NaN_5O_7 788.4000, found 788.3964.

Enzyme Assays. IC_{50} values for inhibitors **1** and **2** were determined for plasmin, kallikrein, thrombin, and trypsin

using the chromogenic substrates D-Val-Leu-Lys-pNA, d-Pro-Phe-Arg-pNA, D-Phe-Pip-Arg-pNA, and D-Phe-Pip-Arg-pNA, respectively. Enzymes and substrates were purchased from Sigma or Chromogenix (distributor: DiaPharma Group, Inc.). Initial rates were determined by monitoring the formation of *p*-nitroaniline at 405 nm using a Fluostar Galaxy UV/fluorescence plate reader from BMG Labtechnologies, Inc. in a 384-well format (plate purchased from Nalge Nunc International). Rate data was collected from 3 to 15 min after preparation of the assay mixtures. In all cases the amount of enzyme used was adjusted to give rates of approximately 3×10^{-4} au/s, and in all cases conversion was kept to less than 10% of substrate. All assay mixtures contained 50 mM sodium phosphate buffer adjusted to pH 7.4. DMSO (10%) was needed in the assay mixtures to keep the inhibitors soluble. During the determinations of IC_{50} values the substrates were kept at a concentration of K_M. The K_M values for the respective substrates of plasmin, kallikrein, thrombin, and trypsin were measured to be 180, 60, 30, and 120 μM. The absorbance data was analyzed using Microsoft Excel and IC_{50} values were determined using a Dixon analysis with the commercial graphing package Grafit (Erithacus Software Ltd.).

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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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