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# Structure–activity studies of cyclic ketone inhibitors of the serine protease plasmin: Design, synthesis, and biological activity

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**Abstract**—Three series of cyclic ketone inhibitors were synthesized and evaluated against the serine protease plasmin. Peptide inhibitors that incorporated 3-oxotetrahydrofuran and 3-oxotetrahydrothiophene 1,1-dioxide groups had the highest activities. Alkylamino substituents, which were designed to bind in the S1 subsite of plasmin, were attached to the inhibitors. Compounds **5c** and **5g**, which incorporated 6-aminohexyl substituents, were found to be optimal and demonstrated $IC_{50}$ values in the low micromolar range. Incorporating conformationally constrained peptide segments into the inhibitors did not improve their activities.


## 1. Introduction

Proteases catalyze the hydrolysis of amide bonds in peptides and proteins. They play pivotal regulatory roles in many biological processes such as fertilization, digestion, growth, maturation, and death by controlling the activation, synthesis, and turnover of proteins.[1] The uncontrolled activities of proteases contribute to a large number of diseases including cardiovascular diseases,[2] neurodegenerative disorders,[3] parasitic, fungal, and viral infections,[4–6] and cancer.[7,8] For example, many tumors have elevated levels of proteases at all stages of cancer progression and metastasis, which make these enzymes attractive targets for anticancer therapies. Plasmin, a trypsin-like serine protease, is implicated in the proteolytic modification of the extracellular matrix (ECM). During the ECM remodeling process that occurs during cancer-induced angiogenesis, proteases break down the ECM and basement membrane, allowing cancer cells to migrate to other organs and generate secondary metastases. Plasmin processes a broad spectrum of substrates including fibrin, which is a component of the ECM, glycoproteins such as laminin and fibronectin, and proteoglycans. It also activates other proteases including the pro-metalloproteinases MMP-1, MMP-3, and MMP-9. Furthermore, plasmin activates or releases a number of growth factors from the ECM including latent transforming growth factor (TGF-β), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF).[9] Thus, inhibition of plasmin provides a promising mechanism for controlling angiogenesis and metastasis by blocking degradation of the basement membrane and the activation of other enzymes.

Plasmin inhibitors can be divided into two classes. The first class constitutes inhibitors that target the lysine binding site of the enzyme. Notable examples include ϵ-aminocaproic acid[10] and *trans*-4-aminomethylcyclohexane carboxylic acid, both of which have been used in the clinic.[11] The second class of inhibitors target the active site of plasmin. For example, Okada and coworkers have developed a variety of synthetic inhibitors of this type that show good potency and selectivity.[12–19]

Our efforts have been focused on the development of active-site directed, reversible inhibitors of plasmin.[20–26] Structure **1** represents a series of 4-heterocyclohexanone-based inhibitors. The cyclic ketone in inhibitor **1** is designed to react with the nucleophilic Ser residue in the active site of enzyme, while the attached peptide binds in the S1–S3 subsites. In previous work, we have shown that replacement of the C4 carbon atom of the cyclohexanone with electron-withdrawing groups such as O, S or $SO_2$ improves the activity of inhibitors. However, the strategy that we used to synthesize the inhibitors was inefficient. Inhibitor **2**, which has an $IC_{50}$ value of 2.7 μM, was discovered using two positional scanning combinatorial libraries based around the

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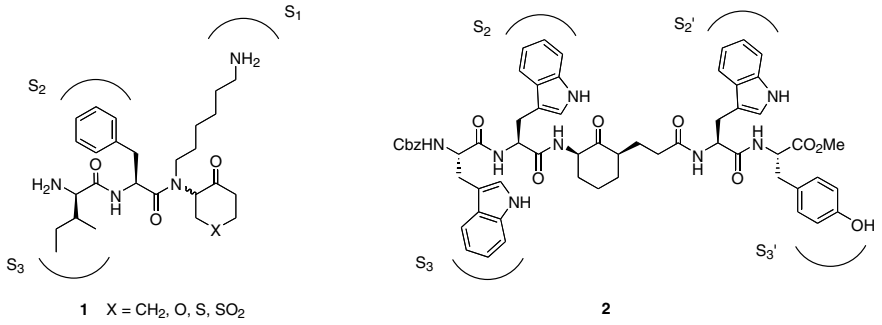



cyclohexanone nucleus. In the context of these inhibitors, we found that plasmin prefers to bind compounds with Trp at the P3, P2, and P2′ positions, and Tyr at the P3′ position. A number of other laboratories have reported cyclic ketone inhibitors for several serine and cysteine proteases including cathepsins K and L.[27–32]

In this article, we report an improved strategy for the preparation of cyclic ketone-containing inhibitors that is significantly more efficient than our previously reported route. Using this strategy, we prepared several series of inhibitors **3**–**5** (Fig. 1) to further explore the structure–activity relationships of these compounds. Three issues that we wanted to investigate included optimal ring size of the cyclic ketone, the effect of electronegative substituents on cyclopentanone-containing inhibitors, and the optimal length and rigidity of alkylamino substituents that are targeted to the S1 subsite. We also prepared inhibitors **6a**–**c** that incorporate conformationally constrained peptide subunits (Fig. 2). There are many examples of conformationally constrained protease inhibitors published in the literature, and Fairlie has recently reviewed this area.[33–37] Conformational constraint provides two major advantages. First, it decreases the entropic penalty for binding by lowering the flexibility of the inhibitor in the unbound state. Second, conformationally constrained analogs of peptidic inhibitors often show enhanced bioavailability because they are less susceptible to proteolytic degradation. Inspired by Fairlie's work, we prepared conformationally constrained inhibitors **6a**–**c** in an attempt to improve both potency and stability.

## 2. Results and discussion

### 2.1. Chemistry

The syntheses of the inhibitors described in this article are outlined in Schemes 1–7. All inhibitors were constructed in two parts: a linear or macrocyclic dipeptide and a cyclic α-aminoketone core. Dipeptides **8a**–**c** were synthesized using a two-step procedure (Scheme 1). First, compounds **7a**–**c** were prepared by coupling two

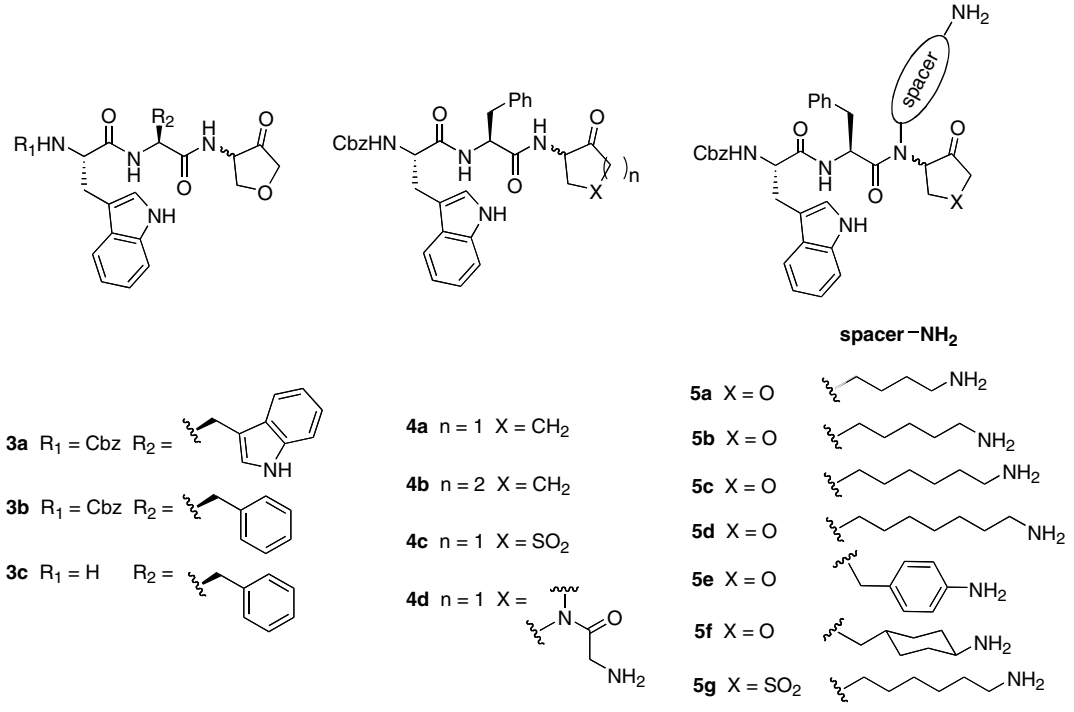
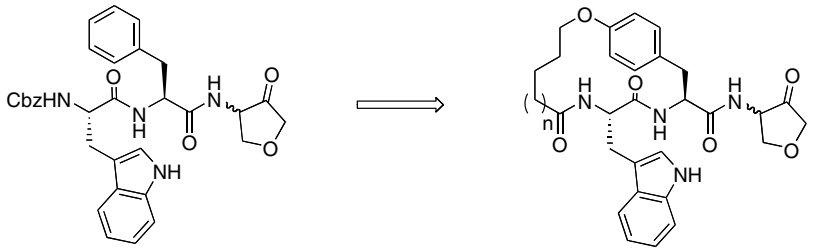


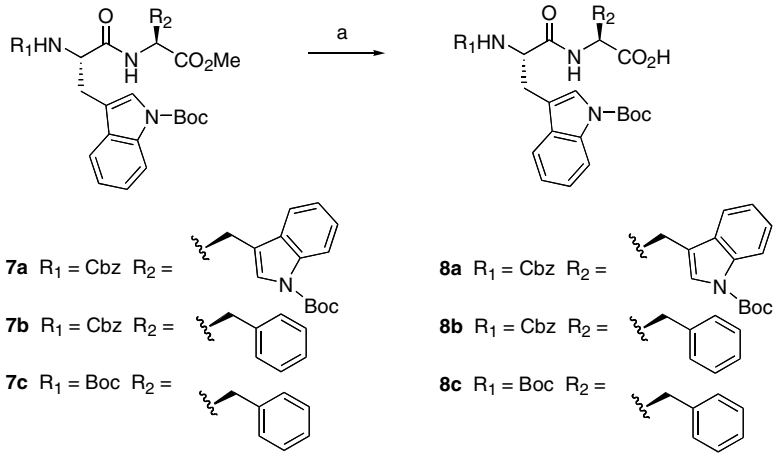

**Figure 1.** Structures of inhibitors **3**–**5**.



**5c**

**6a** n = 1
**6b** n = 2
**6c** n = 4

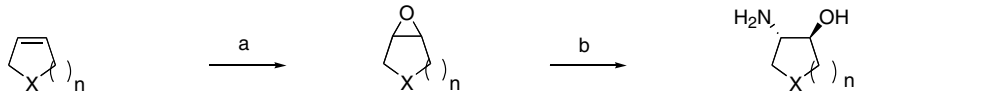
**Figure 2.** Design of conformationally constrained macrocyclic inhibitors **6a**–**c**.



**7a** $R_1$ = Cbz $R_2$ =
**7b** $R_1$ = Cbz $R_2$ =
**7c** $R_1$ = Boc $R_2$ =

**8a** $R_1$ = Cbz $R_2$ =
**8b** $R_1$ = Cbz $R_2$ =
**8c** $R_1$ = Boc $R_2$ =

**Scheme 1.** Reagents and conditions: (a) 2 M NaOH, MeOH, rt, 1–2 h (98–100%).



**9a** n = 1 X = O
**9b** n = 1 X = $SO_2$
**9c** n = 1 X =

**10a** n = 1 X = O
**10b** n = 1 X = $SO_2$
**10c** n = 1 X =
**10d** n = 1 X = $CH_2$
**10e** n = 2 X = $CH_2$

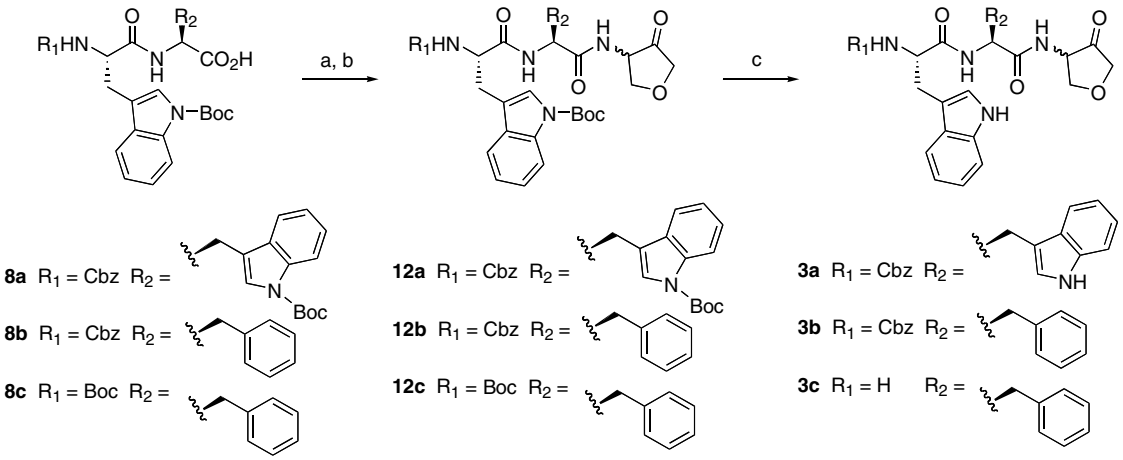
**11a** n = 1 X = O
**11b** n = 1 X = $SO_2$
**11c** n = 1 X =
**11d** n = 1 X = $CH_2$
**11e** n = 2 X = $CH_2$

**Scheme 2.** Reagents and conditions: (a) *m*-CPBA, $CH_2Cl_2$, reflux, 24–48 h (64–90%); (b) $NH_4OH$ (30% in $H_2O$), microwave irradiation, 85 °C, 30 min (65–100%).

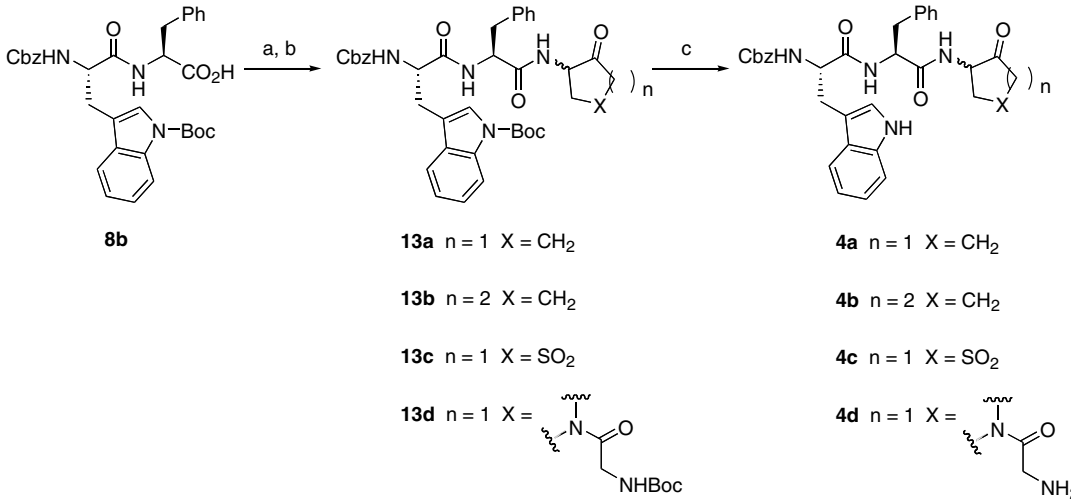
appropriately protected amino acids using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and *N*,*N*-diisopropylethylamine (DIEA). Second, the methyl esters were saponified to give carboxylic acids **8a**–**c**.[38]

The syntheses of β-aminoalcohols **11a**–**e** started with epoxidation of alkenes **9a**–**c** using *m*-CPBA to give compounds **10a**–**c** (Scheme 2). The epoxides were subsequently opened with aqueous ammonium hydroxide under microwave irradiation to cleanly afford β-aminoalcohols **11a**–**e** in good to excellent yields.

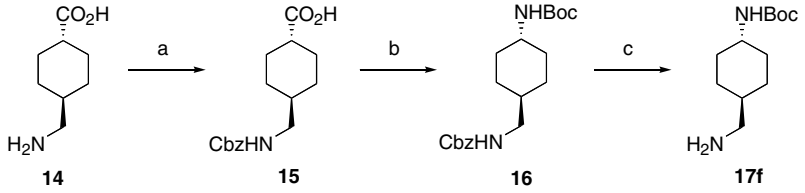
As shown in Scheme 3, inhibitors **3a**–**c** were prepared by coupling aminoalcohol **11a** with dipeptides **8a**–**c** in the presence of 1-hydroxybenzotriazole (HOBt) and 1-[3-(dimethyl-amino)propyl]-3-ethylcarbodiimide hydrochloride (EDC). The resulting secondary alco-




**Scheme 3.** Reagents and conditions: (a) **11a**, HOBt, EDC, rt, 12 h; (b) Dess–Martin periodinane, rt, 0.5–4 h (55–62% for two steps); (c) TFA, $CH_2Cl_2$, rt, 2 h (88–90%).



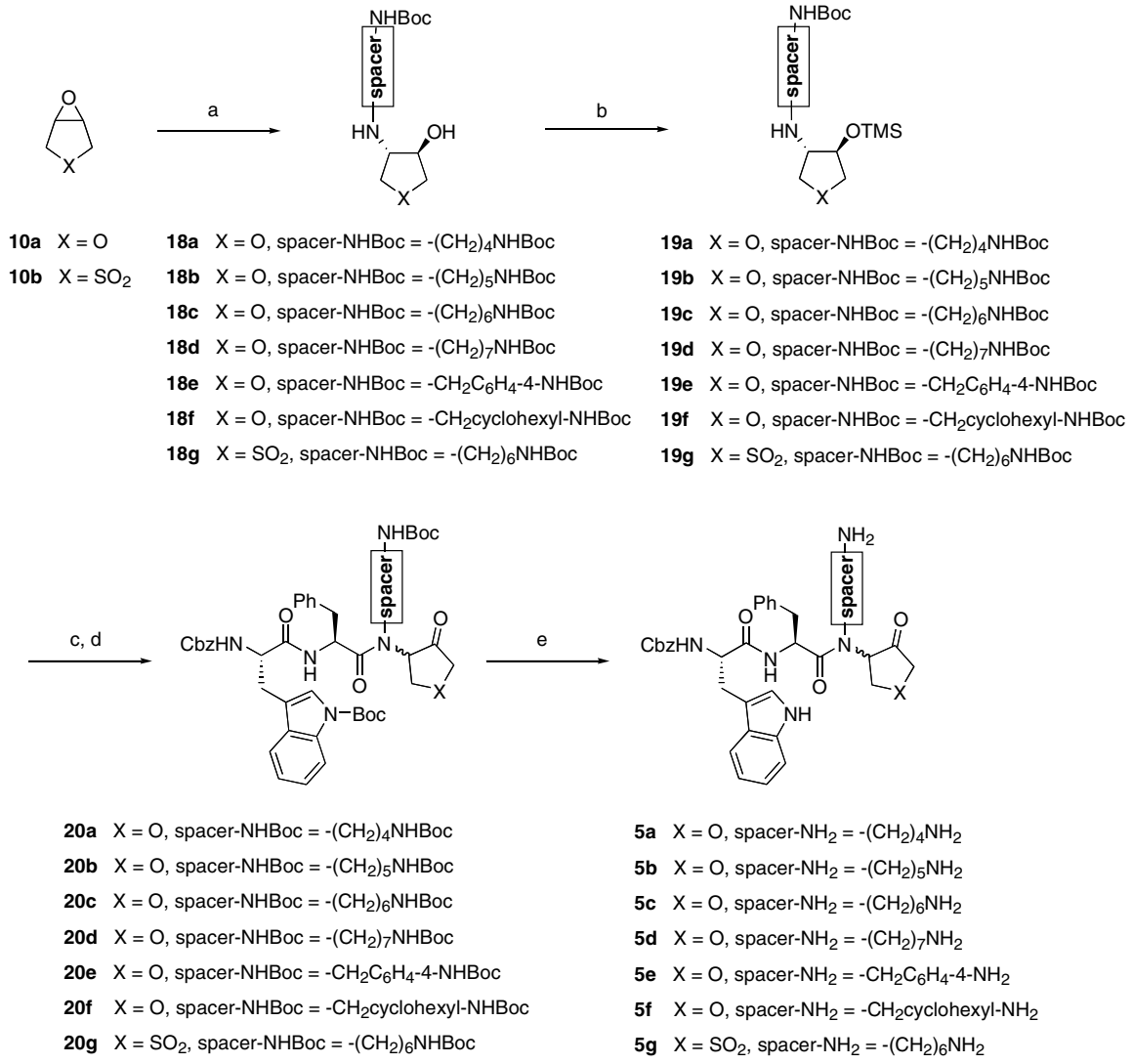

**Scheme 4.** Reagents and conditions: (a) **11b–e**, HOBt, EDC, rt, 12 h (73–80%); (b) Dess–Martin periodinane, rt, 0.5–4 h (65–87%); (c) TFA, $CH_2Cl_2$, rt, 2 h (80–92%).




**Scheme 5.** Reagents and conditions: (a) CbzCl, 2 M NaOH, THF/$H_2O$, rt, 2 h (97%); (b) i—$(PhO)_2PON_3$, DIEA, 85 °C, 20 h; ii—*t*-BuOK (91%); (c) $H_2$, $Pd(OH)_2$/C, rt, 3 h (100%).

hols were then oxidized using Dess–Martin periodinane to generate cyclic ketones **12a–c**. Finally, the Boc-protecting groups were removed with TFA to give inhibitors **3a–c** as mixtures of two diastereomers. In a similar fashion, inhibitors **4a–c** were prepared starting from dipeptide **8b** and aminoalcohols **11b–e** (Scheme 4).

To prepare inhibitors **5a–g**, we required the series of mono-Boc-protected diamines shown in Figure 3. Compounds **17a–c** and **17e** are commercially available, while **17d** was prepared following a procedure from the literature.[39] The synthesis of diamine **17f**, shown in Scheme 5, started with reaction of *trans*-4-aminomethylcyclohexanecarboxylic acid **14** with CbzCl to give carboxylic acid **15**. Curtius rearrangement of this acid and trapping of the resulting isocyanate with *t*-BuOK gave differentially protected diamine **16**. Finally, the Cbz group was removed by catalytic hydrogenation to yield compound **17f**.




**10a** X = O
**10b** X = $SO_2$

**18a** X = O, spacer-NHBoc = -$(CH_2)_4$NHBoc
**18b** X = O, spacer-NHBoc = -$(CH_2)_5$NHBoc
**18c** X = O, spacer-NHBoc = -$(CH_2)_6$NHBoc
**18d** X = O, spacer-NHBoc = -$(CH_2)_7$NHBoc
**18e** X = O, spacer-NHBoc = -$CH_2C_6H_4$-4-NHBoc
**18f** X = O, spacer-NHBoc = -$CH_2$cyclohexyl-NHBoc
**18g** X = $SO_2$, spacer-NHBoc = -$(CH_2)_6$NHBoc

**19a** X = O, spacer-NHBoc = -$(CH_2)_4$NHBoc
**19b** X = O, spacer-NHBoc = -$(CH_2)_5$NHBoc
**19c** X = O, spacer-NHBoc = -$(CH_2)_6$NHBoc
**19d** X = O, spacer-NHBoc = -$(CH_2)_7$NHBoc
**19e** X = O, spacer-NHBoc = -$CH_2C_6H_4$-4-NHBoc
**19f** X = O, spacer-NHBoc = -$CH_2$cyclohexyl-NHBoc
**19g** X = $SO_2$, spacer-NHBoc = -$(CH_2)_6$NHBoc

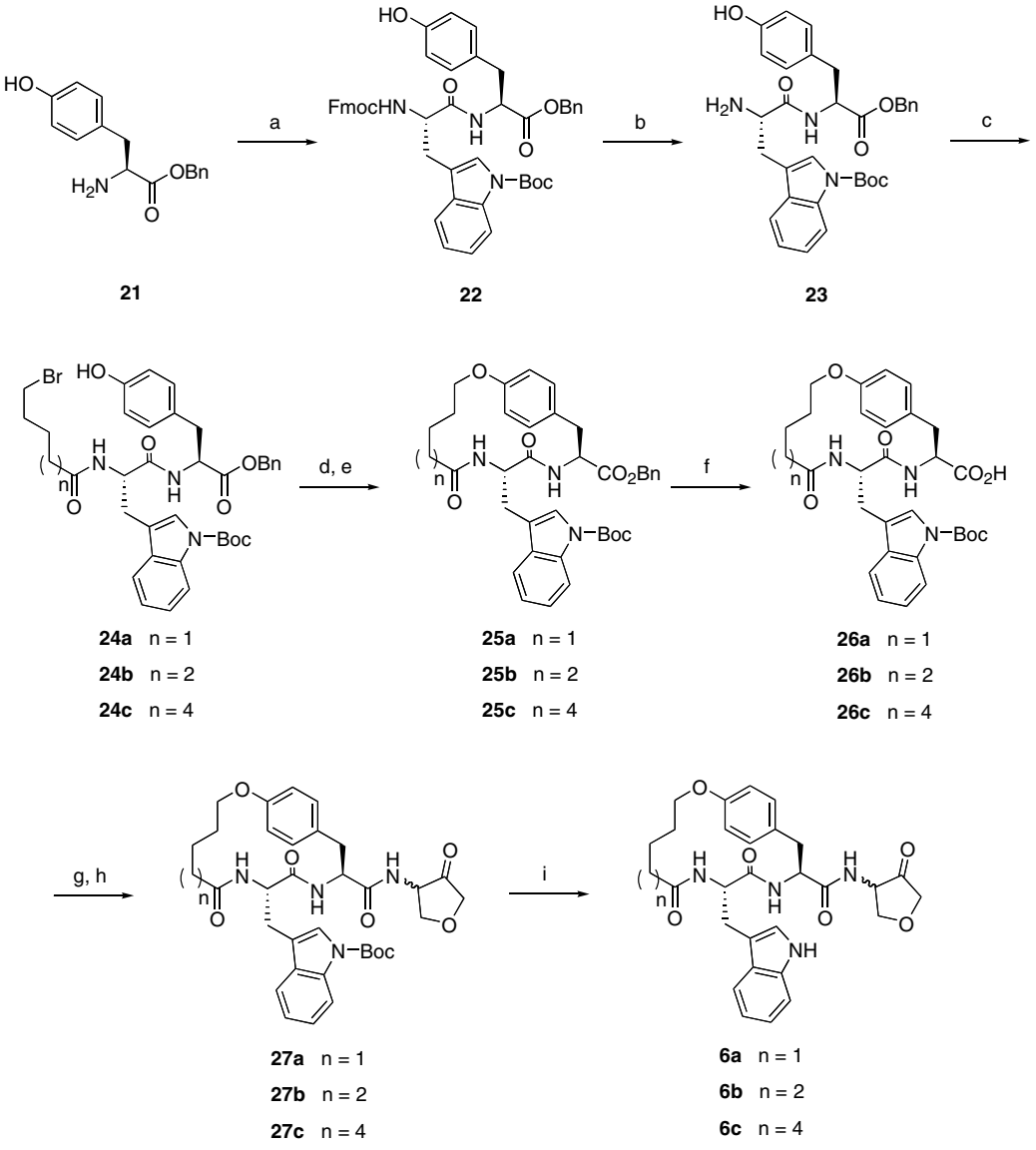
**20a** X = O, spacer-NHBoc = -$(CH_2)_4$NHBoc
**20b** X = O, spacer-NHBoc = -$(CH_2)_5$NHBoc
**20c** X = O, spacer-NHBoc = -$(CH_2)_6$NHBoc
**20d** X = O, spacer-NHBoc = -$(CH_2)_7$NHBoc
**20e** X = O, spacer-NHBoc = -$CH_2C_6H_4$-4-NHBoc
**20f** X = O, spacer-NHBoc = -$CH_2$cyclohexyl-NHBoc
**20g** X = $SO_2$, spacer-NHBoc = -$(CH_2)_6$NHBoc

**5a** X = O, spacer-$NH_2$ = -$(CH_2)_4NH_2$
**5b** X = O, spacer-$NH_2$ = -$(CH_2)_5NH_2$
**5c** X = O, spacer-$NH_2$ = -$(CH_2)_6NH_2$
**5d** X = O, spacer-$NH_2$ = -$(CH_2)_7NH_2$
**5e** X = O, spacer-$NH_2$ = -$CH_2C_6H_4$-4-$NH_2$
**5f** X = O, spacer-$NH_2$ = -$CH_2$cyclohexyl-$NH_2$
**5g** X = $SO_2$, spacer-$NH_2$ = -$(CH_2)_6NH_2$

**Scheme 6.** Reagents and conditions: (a) *N*-Boc-protected diamine, $H_2O$, microwave irradiation, 105 °C, 0.5–1 h (85–92%); (b) i—TMSCl (5 equiv), DIEA (5 equiv), rt, 15 min; ii—MeOH, rt, 12 h (97–100%); (c) **8b**, HATU, DIEA, 45 °C, 16 h, then 1 N HCl, 30 min; (d) Dess–Martin periodinane, rt, 2–4 h (50–81% for two steps); (e) TFA, $CH_2Cl_2$, rt, 2.5 h (59–81%).

With diamines **17a**–**f** in hand, we needed to react them with epoxides **10a** and **10b**. After examining a number of conditions, we found that aminolysis of these epoxides with the diamines proceeded smoothly in $H_2O$ under microwave irradiation at 105 °C to give β-aminoalcohols **18a**–**g** (Scheme 6).[40] We were unable to achieve selective O-silylation of the hydroxyl group in these compounds. However, we found that reaction of the aminoalcohols with an excess of TMSCl gave the *N*,*O*-bis-TMS derivative, which could be cleanly mono-desilylated with methanol to give the desired products **19a**–**g**. Acylation of **19a**–**g** with dipeptide **8b** using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and DIEA, followed by removal of the TMS group and oxidation of the resulting secondary alcohol with Dess–Martin periodinane generated ketones **20a**–**g**. Finally, the Boc-protecting groups were removed with TFA to provide inhibitors **5a**–**g** as mixtures of two diastereomers.

The syntheses of conformationally constrained inhibitors **6a**–**c** are detailed in Scheme 7.[31] Fmoc-Trp(Boc)-OH was coupled with $H_2N$-Tyr-OBn (**21**) using HBTU and DIEA to give dipeptide **22**. Attempts to deprotect the Fmoc group with piperidine in DMF generated the diketopiperazine as the major product. However, we found that the Fmoc group could be removed cleanly using a 1:2 mixture of piperidine and $CH_2Cl_2$ to generate amine **23**.[41,42] Acylation of **23** with 5-bromopentanoyl chloride, 6-bromohexanoyl chloride, or 8-bromooctanoyl chloride under Schotten–Baumann conditions provided compounds **24a**–**c** in excellent yields. These primary alkyl bromides (**24a**–**c**) were treated with NaI in acetone to generate the corresponding iodides, which underwent ring closure in the presence of $K_2CO_3$ to generate macrocycles **25a**–**c**. The benzyl ester in compounds **25a**–**c** was removed by catalytic hydrogenation, and the resulting carboxylic acids **26a**–**c** were coupled with aminoalcohol **11a** using HOBt and EDC. Oxidation of the secondary alcohols with Dess–Martin periodinane




**Scheme 7.** Reagents and conditions: (a) Fmoc-Trp(Boc)-OH, HBTU, DIEA, rt, 2 h (97%); (b) piperidine/$CH_2Cl_2$ (1:2), rt, 35 min (84%); (c) bromoalkanoyl chloride, aqueous $K_2CO_3$ (20%), THF/$H_2O$ (1:1), rt, 5–10 min (95–100%); (d) NaI, acetone, reflux, 2 h; (e) $K_2CO_3$, DMF, rt, 10 h (70–76%); (f) $H_2$, $Pd(OH)_2$/C, rt, 3–4 h, (75–80%); (g) i—HOBt, EDC, rt, 30 min; ii—**11a**, rt, 12 h; (h) Dess–Martin periodinane, rt, 2–4 h (48–61% for two steps); (i) TFA, rt, 2 h (64–78%).

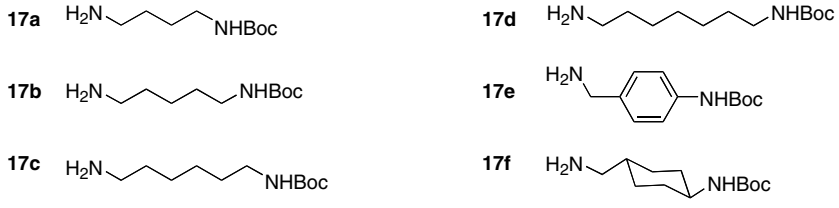



**Figure 3.** *N*-Boc-protected diamines.

provided ketones **27a**–**c**. Finally, the Boc-protecting group was removed to give inhibitors **6a**–**c** as mixtures of two diastereomers.

## 2.2. Plasmin inhibition studies

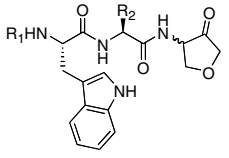
$IC_{50}$ values of the inhibitors were measured for plasmin using the chromogenic substrate D-Val-Leu-Lys-*p*-nitroanilide. Plasmin was assayed at 25 °C in a 50 mM sodium phosphate buffer (pH 7.4). A final concentration of 10% DMSO was used in the assay mixtures to ensure solubility of the inhibitors. When measuring the $IC_{50}$ values, the substrate concentration was held constant at $K_M$, which was measured to be 168 μM. We elected to evaluate the inhibitors as mixtures of two diastereomers. Our own group[25] and others[43] have reported that,

while heteroatom-containing cyclic ketone inhibitors can be separated into diastereomerically pure compounds, they enolize and thus epimerize under aqueous conditions to reform the original mixture of two diastereomers. Thus, it does not make sense to separate the diastereomers since they epimerize under the assay conditions.

Inhibitors **3a–c** were assayed against the serine protease plasmin (Table 1). These three inhibitors incorporate the dipeptides Cbz-Trp-Trp-, Cbz-Trp-Phe-, and H-Trp-Phe- attached to the same 3-amino-4-oxotetrahydrofuran core. Previous studies from our group showed that plasmin typically prefers to bind inhibitors with Trp at the P2 and P3 positions. However, this preference is dependent on the structure of the particular inhibitor, and in some cases Trp and Phe are accommodated equally well at P2.[44] In the 4-oxotetrahydrofuran series, inhibitor **3a**, which incorporates Trp at both P2 and P3, has an $IC_{50}$ value of 30 μM. Inhibitor **3b**, which substitutes Phe in place of Trp at the P2 position, leads to a small improvement in activity with an $IC_{50}$ of 22 μM. Removal of the N-terminal Cbz group of inhibitor **3b** gave **3c**, which is a much weaker inhibitor with an $IC_{50}$ value of 375 μM. These results indicate that, within the context of the 4-oxotetrahydrofuran inhibitors, plasmin displays a small preference for Phe over Trp at the P2 position. The N-terminal Cbz group is important for activity, and may extend interactions of the inhibitor into the S4 subsite of the enzyme.

We next investigated the influence of the cyclic ketone core on inhibitor potency (Table 2). For inhibitor **4a**, the ether oxygen atom in the 3-amino-4-oxotetrahydrofuran core of **3b** has been replaced by a methylene group to give a cyclopentanone ring system. This simple change results in a dramatic reduction in activity; the cyclopentanone-based inhibitor displays no inhibition at concentrations up to 1 mM. The ether oxygen atom in **3b** enhances the electrophilicity of the ketone carbonyl group through a combination of inductive and field effects. As a result, this ketone is more susceptible to nucleophilic attack by the serine active site nucleophile to give a reversibly formed hemiacetal. For

**Table 1.** Inhibition of plasmin by inhibitors **3a–c**



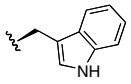
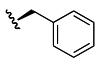
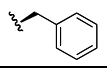
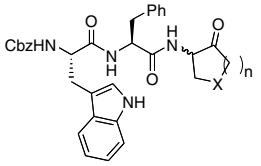

| Inhibitors | $R_1$ | $R_2$ | $IC_{50}$ (μM) |
|---|---|---|---|
| **3a** | Cbz |  | 30 ± 3 |
| **3b** | Cbz |  | 22 ± 1 |
| **3c** | H |  | 375 ± 40 |

**Table 2.** Inhibition of plasmin by inhibitors **3b** and **4a–d**




| Inhibitors | *n* | X | $IC_{50}$ (μM) |
|---|---|---|---|
| **3b** | 1 | O | 22 ± 1 |
| **4a** | 1 | $CH_2$ | >1000 |
| **4b** | 2 | $CH_2$ | >1000 |
| **4c** | 1 | $SO_2$ | 13 ± 1 |
| **4d** | 1 | N-Gly-$NH_2$ | 350 ± 22 |

inhibitor **4a**, these electronic effects are no longer present, which results in decreased electrophilicity of the ketone and reduced inhibitor potency. Compound **4b**, which is the cyclohexanone analog of **4a**, is also a poor inhibitor. This observation underscores the importance of the electronegative ether oxygen for controlling the activity of the inhibitors.

Since inhibitors **4a** and **4b** both have low activities, we cannot draw conclusions about the effect of ring size on potency from these compounds. However, Marquis and coworkers have investigated the effects of ring size and heteroatom position in a series of cyclic ketone inhibitors of cathepsin K.[29,43] These investigators found that inhibitors containing tetrahydrofuran-3-one and tetrahydropyran-3-one groups had good activities, and were significantly more potent than an analogue containing a tetrahydropyran-4-one group. We chose the five-membered ring ketones for further investigation.

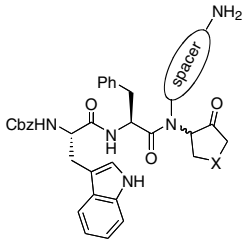
For inhibitor **4c**, we replaced the ether oxygen atom in **3b** with a sulfone group. The sulfone serves to enhance the electrophilicity of the neighboring ketone to a greater extent than the ether, and thus should lead to an improvement in activity. This effect is reflected in the inhibition results with **4c** showing an approximate 2-fold improvement ($IC_{50}$ = 13 μM) compared to inhibitor **3b** ($IC_{50}$ = 22 μM). Finally, we synthesized inhibitor **4d** that incorporates a nitrogen atom into the five-membered ring. Attached to this nitrogen is a glycine residue, which was added in an attempt to extend the inhibitor so that it makes favorable contacts with other regions of the active site. This compound was a modest inhibitor with an $IC_{50}$ value of 350 μM. There are two possible explanations for this inhibitor's low activity. First, since the nitrogen atom in the ring is involved with amide resonance, it is only a weak electron-withdrawing group by induction and does not enhance the reactivity of the neighboring ketone to a large extent. Second, the attached glycine reside may extend out of the active site pocket into solution and thus not make favorable contacts with the surface of the enzyme.

The S1 subsite of plasmin is specific for the positively charged side chains of Lys and Arg.[44] These side chains extend deep into the subsite and form a salt bridge with

an Asp residue at the bottom of the pocket.[44] Inhibitors **3a–c** and **4a–d** contain amino acids that are designed to interact with the S2 and S3 subsites, but they lack interactions with S1. To address this issue we designed a third series of inhibitors **5a–g** that incorporate an alkylamino group targeted toward S1. The alkylamino groups are attached to the inhibitors through a nitrogen atom, rather than being attached directly to the cyclic ketone. We used this 'peptoid'-like arrangement for two reasons.[45] First, it avoids formation of a quaternary center alpha to the electrophilic ketone. Such a quaternary center would likely hinder addition of the active site nucleophile to the ketone due to unfavorable steric interactions, and thus decrease inhibitor potency. Second, from a synthetic perspective, it provides a simple route for making a number of variations to the alkylamino group.

In compounds **5a–d** the spacer between the peptide and the amino group has been varied from four to seven methylene units to optimize its length. Since we attach the alkylamino groups via an amide nitrogen atom, they may need to be longer than the side chains of Lys and Arg to make good contacts with the Asp residue at the base of the S1 pocket. As shown in Table 3, inhibitor **5c** with a six methylene unit spacer appears to be optimal with an $IC_{50}$ value of 9 μM. Inhibitors with a spacer that is one methylene unit too short (**5b**) or too long (**5d**) show a marked decrease in potency. We also prepared inhibitors **5e** and **5f** that incorporate more rigid spacers. Between these two compounds, the aromatic ring in **5e** provides a better match with the S1 subsite. However, neither compound is an improvement over **5c**. Finally, we prepared inhibitor **5g**, which is identical to **5c** except that the ether oxygen atom has been replaced by an $SO_2$ group. This change results in a 1.6-fold improvement in activity ($IC_{50}$ = 9 μM for **5c** vs 5.7 μM for **5g**), and is consistent with the 1.7-fold difference in potencies observed between the similar pair of inhibitors, **3b** and **4c** ($IC_{50}$ = 22 μM vs 13 μM).

**Table 3.** Inhibition of plasmin by inhibitors **5a–g**



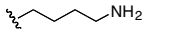
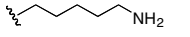
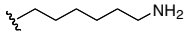
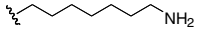
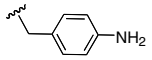
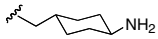
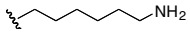
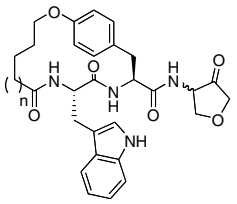

| Inhibitors | X | Spacers-$NH_2$ | $IC_{50}$ (μM) |
|---|---|---|---|
| **5a** | O |  | 140 ± 11 |
| **5b** | O |  | 100 ± 11 |
| **5c** | O |  | 9 ± 2 |
| **5d** | O |  | 51 ± 6 |
| **5e** | O |  | 19 ± 3 |
| **5f** | O |  | 54 ± 7 |
| **5g** | $SO_2$ |  | 5.7 ± 1.0 |

**Table 4.** Inhibition of plasmin by inhibitors **6a–c**




| Inhibitors | *n* | $IC_{50}$ (μM) |
|---|---|---|
| **6a** | 1 | >300 |
| **6b** | 2 | 62 ± 5 |
| **6c** | 4 | 25 ± 2 |

Table 4 summarizes the data we obtained for the conformationally constrained inhibitors **6a–c**. Among these compounds inhibitor **6a**, which incorporates the smallest macrocycle, has the weakest activity ($IC_{50}$ > 300 μM). The macrocyclic ring likely constrains this compound into a conformation that is not compatible with the active site of plasmin. Compound **6b**, which has a slightly larger macrocycle, shows some improvement in activity. By comparison inhibitor **6c**, which incorporates the largest ring size, has the highest potency in the series with an $IC_{50}$ value of 25 μM. The benchmark for the macrocyclic inhibitors is compound **3b**, which is the closest linear analog and has an $IC_{50}$ value of 22 μM. The comparable activities of inhibitors **3b** and **6c** suggest that they can adopt similar conformations when bound to the enzyme. However, no entropic advantage is gained from the macrocyclic structure of **6c** since the macrocycle is formed using a long and flexible seven carbon linker.

## 3. Conclusion

We have described a new procedure for the synthesis of cyclic ketone-based inhibitors of the serine protease plasmin. Using this procedure we synthesized three families of inhibitors, compounds **3–5**. SAR studies of these inhibitors led to the discovery of two inhibitors, **5c** and **5g**, with significant activity against plasmin. Comparison of inhibitors **3b**, **4a**, **4c**, and **4d** showed that for cyclopentanone-based inhibitors, incorporation of electron-withdrawing groups such as O and $SO_2$ into the ring improves their activities. Alkylamino substituents, with an optimal spacer length of 6 carbon atoms, can be added to the inhibitors to bind in the S1 subsite. Conformationally constrained inhibitors **6a–c** were also evaluated, but macrocyclization did not lead to compounds with improved potencies.

## 4. Experimental

### 4.1. General methods

All experiments were conducted using anhydrous conditions under an atmosphere of nitrogen, except where stated, with oven-dried apparatus and employing standard techniques for handling air-sensitive materials. All solvents were distilled and stored under argon before use. All reagents were used as received. Aqueous solutions of sodium bicarbonate and sodium chloride (brine) were saturated. Analytical thin-layer chromatography (TLC) plates were visualized by ultraviolet irradiation, ninhydrin or phosphomolybdic acid (PMA) staining solutions. Flash column chromatography was carried out under a positive pressure of nitrogen. $^1$H NMR spectra were recorded on 300 MHz or 400 MHz spectrometers. Data are presented as follows: chemical shift (in ppm on the $\delta$ scale relative to $\delta = 0.00$ ppm for the protons in TMS), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), coupling constant ($J$/Hz), which was taken directly from the spectra and are uncorrected, and integration. $^{13}$C NMR spectra were recorded at 75 or 100 MHz, and all chemical shift values are reported in ppm on the $\delta$ scale, with an internal reference of $\delta$ 77.0 or 49.0 for $CDCl_3$ or $CD_3OD$, respectively. High-resolution mass spectra were measured using electron impact (EI) or fast atom bombardment (FAB) ionization.

### 4.2. Enzyme assays

$IC_{50}$ values of inhibitors were measured for plasmin using the chromogenic substrate D-Val-Leu-Lys-*p*-nitroanilide. Plasmin (P1867 from Sigma) 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. When measuring the $IC_{50}$ of inhibitors, the substrate concentration was held constant at its $K_M$ value, which was measured to be 168 μM. Data analysis was performed using Grafit (Erithacus Software Ltd).

### 4.3. General methods for synthesis

**4.3.1. General method A: synthesis of dipeptides (7a–c, 22).** Cbz-Trp(Boc)-OH, Boc-Trp(Boc)-OH or Fmoc-Trp(Boc)-OH (1.5 mmol) was dissolved in DMF (10 mL). To this solution were added $H_2$N-Trp(Boc)-OMe, $H_2$N-Phe-OMe, or $H_2$N-Tyr-OBn (1.5 mmol), HBTU (758 mg, 2.0 mmol), and DIEA (530 μL, 390 mg, 3.0 mmol). The reaction mixture was stirred at room temperature for 2 h and then partitioned between EtOAc (250 mL) and 1 N HCl (200 mL). The organic layer was washed with 1 N HCl (200 mL), saturated $NaHCO_3$ (200 mL), and brine (150 mL). The organic layer was dried over $MgSO_4$ and concentrated by rotary evaporation. The crude material was purified by flash chromatography (EtOAc/hexanes 1:1) to yield dipeptides **7a**–**c** and **22**.

**4.3.2. General method B: hydrolysis of methyl esters of dipeptides 7a–c.** To a solution of compounds **7a**–**c** (1.0 mmol) in MeOH (25 mL), 2 N aqueous NaOH (3.5 mL) was added. The reaction mixture was stirred at room temperature for 1–2 h. The solution was partitioned between EtOAc (300 mL) and 1 N HCl (200 mL). The aqueous layer was extracted once with EtOAc (100 mL), and the organic layers were combined, washed with brine (200 mL), and dried over $MgSO_4$. The solvent was removed by rotary evaporation to yield carboxylic acids **8a**–**c**.

**4.3.3. General method C: synthesis of epoxides 10a–c.** To a solution of alkenes **9a**–**c** (28.6 mmol) in $CH_2Cl_2$ (60 mL) was added *m*-CPBA (13 g). The reaction mixture was heated under reflux for 24–48 h and then cooled to room temperature. The white slurry was filtered. The filtrate was diluted with $CH_2Cl_2$ (250 mL) and then washed with saturated $Na_2SO_3$ (300 mL), saturated $NaHCO_3$ (300 mL), and brine (250 mL). The organic layer was dried over $MgSO_4$. The crude product was purified by distillation (**10a**) or flash chromatography (**10b**–**c**).

**4.3.4. General method D: synthesis of aminoalcohols 11a–e.** To a microwave reaction tube were added epoxides **10a**–**e** (3.0 mmol) and aqueous $NH_4OH$ (30%, 4 mL). The tube was sealed and irradiated under microwave at 85 °C for 30 min. The reaction was cooled to room temperature and the solvent was removed by rotary evaporation. The resulting material was dried under vacuum to give aminoalcohols **11a**–**e** (compound **11b** was further purified by recrystallization from diethyl ether).

**4.3.5. General method E: synthesis of ketones 12a–c and 13a–d.** To a solution of carboxylic acids **8a**–**c** (0.17 mmol) in $CH_2Cl_2$ (5 mL) were added HOBt (69 mg, 0.51 mmol), EDC (98 mg, 0.51 mmol), and DIEA (119 μL, 88.4 mg, 0.68 mmol). After stirring the reaction mixture for 30 min, aminoalcohols **11a**–**e** (0.34 mmol) were added dropwise as a solution in DMF (100 μL). The reaction mixture was stirred at room temperature for 12 h and then partitioned between $CH_2Cl_2$ (100 mL) and 1 N HCl (100 mL). The organic layer was washed with saturated $NaHCO_3$ (75 mL) and brine (75 mL), and dried over $MgSO_4$. The solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (EtOAc/hexanes) to yield the product alcohol as a mixture of two diastereomers. The resulting alcohol (90 μmol) was dissolved in $CH_2Cl_2$. To this solution was added Dess–Martin periodinane (58 mg, 130 μmol). The reaction mixture was stirred at room temperature and monitored by TLC. After the reaction was complete (0.5–4 h), the solution was partitioned between $CH_2Cl_2$ (50 mL) and saturated $Na_2S_2O_3$ (30 mL). The organic layer was washed with saturated $NaHCO_3$ (30 mL) and brine (20 mL), and dried over $MgSO_4$. The solvent was removed by rotary evaporation, and the crude material

was purified by flash chromatography (EtOAc/hexanes) to yield ketones **12a–c** and **13a–d** as mixtures of two diastereomers.

**4.3.6. General method F: synthesis of aminoalcohols 18a–g.** To compound **10a** or**10b** (1.5 mmol) were added compounds **17a–f** (1.8 mmol) and $H_2O$ (4 mL). The reaction mixture was sealed and stirred with microwave irradiation at 105 °C for 0.5–1 h. The reaction mixture was cooled to room temperature and partitioned between $CH_2Cl_2$ (8 mL) and saturated $Na_2CO_3$ (8 mL). The aqueous layer was extracted once with $CH_2Cl_2$ (8 mL) and the combined organic layers were dried over $Na_2CO_3$. The solvent was removed by rotary evaporation and the crude material was purified by flash chromatography (5–15% MeOH/$CH_2Cl_2$) to yield compounds **18a–g**.

**4.3.7. General method G: synthesis of TMS ethers 19a–e.** Compounds **18a–e** (1.1 mmol) were dissolved in $CH_2Cl_2$ (20 mL). To this solution TMSCl (900 μL, 545 mg, 5.0 mmol) and DIEA (880 μL, 650 mg, 5.0 mmol) were added. The reaction mixture was stirred at room temperature for 15 min and then partitioned between $CH_2Cl_2$ (200 mL) and $H_2O$ (200 mL). The organic layer was washed twice with $H_2O$ (200 mL) and brine (100 mL), and the solvent was removed by rotary evaporation to yield the *N*,*O*-bis-TMS-protected aminoalcohol. This compound was dissolved in dry MeOH (20 mL) and the reaction mixture was stirred at room temperature for 12 h. The solvents were removed by rotary evaporation, and the crude material was purified by flash chromatography (5% MeOH/$CH_2Cl_2$) to yield TMS ethers **19a–e**.

**4.3.8. General method H: synthesis of ketones 20a–g.** To a solution of acid **8b** (100 mg, 0.17 mmol) in DMF (1 mL) were added HATU (129 mg, 0.34 mmol), DIEA (90 μL, 67 mg, 0.51 mmol), and **19a–g** (0.34 mmol) as a solution in DMF (100 μL). The reaction mixture was stirred at 45 °C for 16 h, cooled to room temperature, and partitioned between $CH_2Cl_2$ (100 mL) and 1 N HCl (100 mL). The organic layer was washed with saturated $NaHCO_3$ (75 mL) and brine (75 mL), dried over $MgSO_4$ and concentrated. The crude material was purified by flash chromatography (EtOAc/hexanes) to yield the corresponding alcohols as mixtures of two diastereomers. To a solution of the resulting alcohols (60 μmol) in $CH_2Cl_2$ (10 mL) was added Dess–Martin periodinane (51 mg, 0.12 mmol). The reaction mixture was stirred at room temperature for 2–4 h, then partitioned between $CH_2Cl_2$ (30 mL) and 10% $Na_2S_2O_3$ (30 mL). The organic layer was washed with saturated $NaHCO_3$ (30 mL) and brine (30 mL), dried over $MgSO_4$, and concentrated. The crude material was purified by flash chromatography (EtOAc/hexanes) to yield ketones **20a–g** as mixtures of two diastereomers.

**4.3.9. General method I: deprotection of Boc groups to give inhibitors 3a–c, 4a–d, 5a–g and 6a–c.** To solutions of ketones **12a–c**, **13a–d**, **20a–g**, and **27a–c** (30 μmmol) in $CH_2Cl_2$ (1.5 mL) was slowly added TFA (1 mL). The reaction mixture was stirred at room temperature for 2.5 h. The solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (5–15% MeOH/$CH_2Cl_2$) to yield inhibitors **3a–c**, **4a–d**, **5a–g**, and **6a–c**.

**4.3.10. Characterization of new compounds**

**4.3.10.1. Cbz-Trp(Boc)-Trp(Boc)-OMe (7a).** Compound **7a** was synthesized starting with Cbz-Trp(Boc)-OH and $H_2N$-Trp(Boc)-OMe[25] using general method A (1.07 g, 0.97 mmol, 97%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.66 (s, 18H), 3.10–3.20 (m, 3H), 3.20–3.30 (m, 1H), 3.59 (s, 3H), 4.40–4.55 (m, 1H), 4.75–4.85 (dd, $J$ = 8.0, 12.0 Hz, 1H), 5.05–5.15 (dd, $J$ = 12.0, 16.0 Hz, 2H), 5.35–5.45 (d, $J$ = 8.0 Hz, 1H), 6.25–6.35 (d, $J$ = 4.0 Hz, 1H), 7.05–7.15 (m, 1H), 7.15–7.27 (m, 2H), 7.28–7.40 (m, 7H), 7.48 (s, 1H), 7.50–7.60 (d, $J$ = 6.0 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 27.8, 28.5, 52.7, 53.1, 67.4, 83.9, 114.8, 115.4, 115.6, 118.9, 119.3, 122.8, 123.0, 124.4, 124.8, 124.9, 128.3, 128.5, 128.8, 149.8, 170.8, 171.4; HRMS-FAB (M+$Na^+$) calcd for $C_{41}H_{46}NaN_4O_9$ 761.3163, found 761.3185.

**4.3.10.2. Cbz-Trp(Boc)-Phe-OMe (7b).** Compound **7b** was synthesized starting with Cbz-Trp(Boc)-OH and $H_2N$-Phe-OMe using general method A (850 mg, 1.42 mmol, 95%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.65 (s, 9H), 2.90–3.10 (m, 2H), 3.10–3.20 (dd, $J$ = 6.0, 11.2 Hz, 1H), 3.20–3.3 (dd, $J$ = 4.8, 12.0 Hz, 1H), 3.63 (s, 3H), 4.40–4.60 (m, 1H), 4.70–4.80 (dd, $J$ = 4.0, 12.0 Hz, 1H), 5.05–5.20 (d, $J$ = 2.0 Hz, 2H), 5.40–5.60 (d, $J$ = 4.0 Hz, 1H), 6.30–6.40 (d, $J$ = 4.0 Hz, 1H), 6.85–6.95 (d, $J$ = 8.0 Hz, 2H), 7.10–7.20 (m, 3H), 7.21–7.27 (m, 1H), 7.30–7.42 (m, 6H), 7.40–7.50 (m, 1H), 7.55–7.65 (m, 1H), 8.10–8.20 (m, $J$ = 6.0 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 28.2, 28.3, 37.9, 52.3, 53.3, 54.9, 60.4, 67.1, 83.7, 115.1, 115.3, 119.1, 122.8, 124.6, 124.7, 127.1, 128.1, 128.3, 128.5, 128.6, 129.1, 130.1, 135.4, 136.1, 149.5, 155.9, 170.3, 171.1; HRMS-FAB (M+$Na^+$) calcd for $C_{34}H_{37}NaN_3O_7$ 622.2529, found 622.2540.

**4.3.10.3. Boc-Trp(Boc)-Phe-OMe (7c).** Compound **7c** was synthesized starting with Boc-Trp(Boc)-OH and $H_2N$-Phe-OMe using general method A (885 mg, 1.56 mmol, 97%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.44 (s, 9H), 1.66 (s, 9H), 2.90–3.00 (d, $J$ = 8.0, 2H), 3.00–3.30 (m, 2H), 3.64 (s, 3H), 4.40–4.50 (br s, 1H), 4.70–4.80 (dd, $J$ = 4.0, 11.2 Hz, 1H), 5.05–5.20 (br s, 1H), 6.30–6.50 (d, $J$ = 4.0 Hz, 1H), 6.80–6.90 (d, $J$ = 4.0 Hz, 2H), 7.10–7.20 (m, 3H), 7.20–7.30 (m, 1H), 7.30–7.35 (m, 1H), 7.46 (s, 1H), 7.55–7.65 (d, $J$ = 8.0 Hz, 1H), 8.10–8.20 (d, $J$ = 8.0 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 28.1, 28.2, 37.9, 52.3, 53.3, 54.6, 80.2, 83.6, 115.3, 115.4, 119.1, 122.7, 124.4, 124.7, 127.1, 128.5, 129.2, 130.2, 135.6, 149.5, 155.3, 170.8, 171.2; HRMS-FAB (M+$Na^+$) calcd for $C_{31}H_{39}NaN_3O_7$ 588.2686, found 588.2695.

**4.3.10.4. Cbz-Trp(Boc)-Trp(Boc)-OH (8a).** Compound **8a** was synthesized starting with **7a** using general method B (722 mg, 1.0 mmol, 100%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.63 (s, 18H), 2.80–3.40 (m, 4H), 4.35–4.75 (m, 1H), 4.76–5.25 (m, 3H), 5.60–5.90 (d,

$J$ = 6.0 Hz, 1H), 6.80–7.00 (d, $J$ = 6.0 Hz, 1H), 7.01–7.35 (m, 9H), 7.36–7.70 (m, 4H), 7.95–8.25 (m, 2H), 10.00–10.50 (br s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 21.2, 27.6, 28.5, 53.1, 55.3, 67.5, 77.7, 84.2, 115.3, 115.7, 119.2, 119.4, 123.0, 123.1, 124.8, 124.9, 125.0, 128.4, 128.5, 128.9, 130.6, 130.9, 135.6, 135.8, 136.4, 150.1, 156.6, 171.8, 174.7, 177.1; HRMS-FAB ($M+Na^+$) calcd for $C_{40}H_{44}NaN_4O_9$ 747.3006, found 747.3023.

**4.3.10.5. Cbz-Trp(Boc)-Phe-OH (8b).** Compound **8b** was synthesized starting with **7b** using general method B (585 mg, 1.0 mmol, 100%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.63 (s, 9H), 2.85–3.00 (dd, $J$ = 6.0, 15.0 Hz, 1H), 3.05–3.25 (m, 3H), 4.50–4.70 (m, 1H), 4.70–4.85 (m, 1H), 4.95–5.15 (m, 2H), 5.60–5.80 (d, $J$ = 9.0 Hz, 1H), 6.60–6.75 (d, $J$ = 6.0 Hz, 1H), 6.80–7.00 (m, 2H), 7.00–7.27 (m, 5H), 7.30–7.40 (m, 5H), 7.41–7.60 (m, 2H), 8.00–8.20 (d, $J$ = 9.0 Hz, 1H), 9.20–9.80 (br s, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 28.4, 28.6, 37.7, 53.7, 55.1, 67.6, 77.7, 84.2, 115.6, 115.7, 119.4, 123.2, 125.0, 125.1, 127.5, 128.4, 128.7, 128.9, 129.0, 129.7, 130.6, 135.8, 135.9, 150.0, 156.6, 171.5, 174.4; HRMS-FAB ($M+Na^+$) calcd for $C_{33}H_{35}NaN_3O_7$ 608.2373, found 608.2385.

**4.3.10.6. Boc-Trp(Boc)-Phe-OH (8c).** Compound **8c** was synthesized starting with **7c** using general method B (665 mg, 1.0 mmol, 98%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.42 (s, 9H), 1.65 (s, 9H), 2.80–3.35 (m, 4H), 4.30–4.70 (m, 1H), 4.71–4.98 (m, 1H), 5.30–5.50 (d, $J$ = 4.0 Hz, 1H), 6.70–6.90 (d, $J$ = 4.0 Hz, 1H), 6.91–7.12 (s, 2H), 7.13–7.38 (m, 5H), 7.39–7.55 (s, 1H), 7.56–7.75 (d, $J$ = 8.0 Hz, 1H), 8.00–8.20 (d, $J$ = 6.0 Hz, 1H), 10.00–10.50 (br s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 21.0, 28.1, 28.4, 37.7, 53.5, 54.5, 60.8, 80.7, 83.9, 115.5, 115.7, 119.3, 122.9, 124.5, 124.8, 127.3, 128.7, 129.6, 130.5, 135.7, 135.9, 149.9, 155.9, 171.6, 174.5, 176.8; HRMS-FAB ($M+Na^+$) calcd for $C_{30}H_{37}NaN_3O_7$ 574.2529, found 574.2536.

**4.3.10.7. Epoxide 10a.** Compound **10a** was synthesized starting with **9a** using general method C (1.7 g, 20 mmol, 71%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.60–3.70 (d, $J$ = 8.4 Hz, 2H), 3.81 (s, 2H), 4.00–4.10 (d, $J$ = 8.4 Hz, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 50.9, 62.4; HRMS-ESI ($M+H^+$) calcd for $C_4H_7O_2$ 87.0446, found 87.0444.

**4.3.10.8. Epoxide 10b.** Compound **10b** was synthesized starting with **9b** using general method C (2.4 g, 16.3 mmol, 64%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.30–3.40 (d, $J$ = 12 Hz, 2H), 3.41–3.55 (dd, $J$ = 3, 12 Hz, 2H), 3.90–3.90 (d, $J$ = 3 Hz, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 51.8, 53.6; HRMS-ESI ($M+Na^+$) calcd for $C_4H_6NaO_3S$ 156.9935 found 156.9915.

**4.3.10.9. Alkene 9c.** To a solution of 3-pyrroline (500 mg, 7.2 mmol) in DMF (20 mL) were added Boc-Gly-OH (1.4 g, 8 mmol), HBTU (4.1 g, 10.9 mmol), and DIEA (2.5 mL, 1.9 g, 14.4 mmol). The reaction mixture was stirred at room temperature for 2 h and then partitioned between EtOAc (500 mL) and 1 N HCl (500 mL). The organic layer was washed with 1 N HCl (300 mL), saturated $NaHCO_3$ (300 mL), and brine (300 mL). It was dried over $MgSO_4$, and concentrated. The crude material was purified by flash chromatography (EtOAc/hexanes 1:2–1:1) to yield compound **9c** (1.5 g, 6.5 mmol, 90%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.40 (s, 9H), 3.75–3.90 (d, $J$ = 4.0 Hz, 2H), 4.10–4.30 (m, 4H), 5.40–5.60 (br s, 1H), 5.70–5.80 (m, 1H), 5.81–6.00 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 28.3, 42.8, 52.2, 53.2, 79.6, 124.7, 126.0, 155.9, 166.8; HRMS-ESI ($M+Na^+$) calcd for $C_{11}H_{18}NaN_2O_3$ 249.1215, found 249.1223.

**4.3.10.10. Epoxide 10c.** The epoxidation of alkene **9c** was accomplished using general method C (1.4 g, 5.4 mmol, 90%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.44 (s, 9H), 3.35–3.60 (m, 2H), 3.65–3.95 (m, 5H), 3.96–4.05 (d, $J$ = 15 Hz, 1H), 5.30–5.50 (br s, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 28.7, 43.4, 47.1, 47.5, 54.8, 55.9, 77.7, 80.1, 156.2, 168.4; HRMS-ESI ($M+Na^+$) calcd for $C_{11}H_{18}NaN_2O_4$ 256.1164, found 256.1169.

**4.3.10.11. Aminoalcohol 11a.** Compound **11a** was synthesized starting from **10a** using general method D (310 mg, 3.0 mmol, 100%): $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 3.30–3.40 (m, 1H), 3.55–3.62 (dd, $J$ = 4.0, 10.0 Hz, 1H), 3.62–3.68 (dd, $J$ = 3.6, 10.0 Hz, 1H), 4.00–4.10 (m, 2H), 4.10–4.15 (m, 1H); $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 58.9, 72.5, 73.3, 77.1; HRMS-ESI ($M+H^+$) calcd for $C_4H_{10}NO_2$ 104.0712, found 104.0714.

**4.3.10.12. Aminoalcohol 11b.** Compound **11b** was synthesized starting from **10b** using general method D (310 mg, 3.0 mmol, 65%): $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 3.30–3.40 (d, $J$ = 12 Hz, 2H), 3.45–3.55 (dd, $J$ = 3.0, 12.0 Hz, 2H), 3.90–4.00 (d, $J$ = 2.0 Hz, 2H); $^{13}$C NMR (75 MHz, acetone-$d_6$) $\delta$ 53.6, 56.4, 60.5, 70.8; HRMS-FAB ($M+H^+$) calcd for $C_4H_9NaNO_3S$ 174.0201, found 174.0206.

**4.3.10.13. Aminoalcohol 11c.** Compound **11c** was synthesized starting from **10c** using general method D (310 mg, 3.0 mmol, 100%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.44 (s, 9H), 2.50–2.90 (br s, 3H), 3.10–3.50 (m, 3H), 3.70–3.95 (m, 4H), 3.95–4.20 (m, 1H), 5.60–5.70 (br s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 28.4, 42.9, 51.2, 51.4, 51.5, 51.8, 56.0, 57.7, 74.6, 76.0, 79.9, 156.1, 167.9, 168.0; HRMS-FAB ($M+Na^+$) calcd for $C_{11}H_{21}NaN_3O_4$ 282.1430, found 282.1442.

**4.3.10.14. Aminoalcohol 11d.** Compound **11d** was synthesized starting from **10d** using general method D (300 mg, 100%): $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 1.30–1.40 (m, 1H), 1.45–1.60 (m, 1H), 1.65–1.80 (m, 2H), 1.90–2.10 (m, 2H), 2.90–3.10 (ddd, $J$ = 1.5, 3, 9 Hz, 1H), 3.70–3.80 (dd, $J$ = 3, 9 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 20.0, 32.2, 32.3, 60.0, 79.7; HRMS-ESI ($M+H^+$) calcd for $C_5H_9O$ 85.0653, found 85.0650.

**4.3.10.15. Aminoalcohol 11e.** Compound **11e** was synthesized starting from **10e** using general method D (345 mg, 3.0 mmol, 90%): $^1$H NMR (400 MHz, $CDCl_3$)

$\delta$ 0.90–1.10 (m, 1H), 1.10–1.30 (m, 3H), 1.50–1.70 (m, 2H), 1.71–1.82 (m, 1H), 1.83–1.95 (m, 1H), 2.30–2.40 (m, 1H), 2.45–2.85 (br s, 2H), 2.90–3.10 (m, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 24.8, 25.0, 33.9, 34.3, 56.9, 75.6; HRMS-ESI ($M+H^+$) calcd for $C_6H_{14}NO$ 116.1075, found 116.1078.

**4.3.10.16. Ketone 12a.** Compound **12a** was synthesized starting from **8a** using general method E (81 mg, 0.10 mmol, 62% for two steps): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.55–1.75 (m, 18H), 2.75–2.98 (m, 1H), 3.00–3.30 (m, 3H), 3.45–3.95 (m, 2H), 3.97–4.50 (m, 4H), 4.70–4.81 (m, 1H), 4.82–5.05 (m, 2H), 5.20–5.70 (m, 1H), 6.50–6.80 (m, 2H), 6.95–7.19 (m, 2H), 7.20–7.40 (m, 8H), 7.41–7.62 (m, 3H), 8.00–8.20 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 27.0, 27.7, 27.8, 28.4, 53.3, 53.7, 54.6, 54.7, 54.9, 55.0, 55.5, 56.0, 67.5, 67.6, 67.7, 69.4, 69.7, 69.9, 70.2, 77.5, 84.0, 84.2, 84.3, 114.4, 114.6, 115.0, 115.2, 115.3, 115.7, 118.5, 118.6, 119.1, 119.3, 122.80, 122.82, 123.0, 123.1, 123.2, 124.7, 124.8, 124.9, 125.0, 125.1, 125.2, 128.10, 128.17, 128.20, 128.4, 128.5, 128.6, 128.7, 128.8, 128.9, 130.2, 130.3, 130.5, 135.5, 135.6, 135.7, 135.9, 136.0, 137.0, 170.9, 171.0, 171.1, 171.3, 171.4, 171.6, 171.7, 210.3, 210.4, 210.8, 210.9; HRMS-FAB ($M+Na^+$) calcd for $C_{44}H_{49}NaN_5O_{10}$ 830.3377, found 830.3362.

**4.3.10.17. Ketone 12b.** Compound **12b** was synthesized starting from **8b** using general method E (62 mg, 93 μmol, 55% for two steps): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.60–1.70 (m, 9H), 2.50–2.90 (m, 1H), 2.90–3.30 (m, 3H), 3.50–4.55 (m, 6H), 4.60–4.78 (m, 1H), 4.90–5.10 (m, 2H), 5.30–5.70 (m, 1H), 6.20–6.60 (m, 1H), 6.65–6.90 (m, 1H), 6.90–7.10 (m, 2H), 7.10–7.27 (m, 4H), 7.29–7.41 (m, 4H), 7.41–7.60 (m, 2H), 8.00–8.20 (m, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 28.0, 28.6, 37.6, 54.0, 54.9, 55.1, 55.2, 55.7, 56.4, 67.7, 67.9, 69.5, 70.0, 70.2, 70.5, 84.5, 115.1, 115.2, 115.3, 115.8, 115.9, 119.3, 119.4, 123.3, 124.9, 125.3, 127.5, 128.3, 128.4, 128.5, 128.6, 128.7, 128.8, 129.0, 129.1, 129.6, 129.7, 130.3, 135.8, 136.0, 136.1, 136.2, 149.9, 156.8, 171.0, 171.2, 171.3, 171.4, 171.6, 171.7, 210.8, 211.4; HRMS-FAB ($M+Na^+$) calcd for $C_{37}H_{40}NaN_4O_8$ 691.2744, found 691.2765.

**4.3.10.18. Ketone 12c.** Compound **12c** was synthesized starting from **8c** using general method E (64 mg, 0.10 mmol, 60% for two steps): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.20–1.45 (m, 9H), 1.60–1.75 (m, 9H), 2.40–2.70 (m, 1H), 2.90–3.30 (m, 3H), 3.60–4.00 (m, 2H), 4.01–4.20 (m, 2H), 4.21–4.50 (m, 2H), 4.55–4.80 (m, 1H), 4.90–5.28 (m, 1H), 6.00–6.50 (m, 1H), 6.70–6.97 (m, 3H), 7.10–7.30 (m, 5H), 7.31–7.45 (m, 1H), 7.46–7.65 (m, 2H), 8.00–8.20 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 27.6, 28.4, 28.5, 36.9, 53.5, 54.6, 54.8, 55.0, 55.3, 55.4, 56.1, 69.1, 69.6, 69.8, 70.3, 70.4, 80.0, 80.2, 80.4, 84.2, 84.4, 84.5, 115.1, 115.2, 115.5, 115.6, 115.7, 115.8, 119.1, 119.2, 119.4, 119.5, 123.09, 123.13, 123.17, 124.56, 124.59, 125.09, 125.17, 125.24, 127.4, 129.0, 129.5, 129.6, 130.4, 135.6, 135.7, 135.9, 149.9, 170.9, 171.0, 171.2, 171.4, 210.3, 210.5, 210.7, 210.9; HRMS-FAB ($M+Na^+$) calcd for $C_{34}H_{42}NaN_4O_8$ 657.2900, found 657.2915.

**4.3.10.19. Ketone 13a.** Compound **13a** was synthesized starting from **8b** and **11d** using general method E (73 mg, 0.11 mmol, 61% for two steps):$^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.50–1.85 (m, 11H), 1.85–2.15 (m, 2H), 2.25–2.50 (m, 2H), 2.65–3.20 (m, 4H), 3.70–4.25 (m, 1H), 4.32–4.60 (m, 1H), 4.65–4.80 (m, 1H), 4.85–5.12 (m, 2H), 5.40–5.80 (m, 1H), 6.50–6.70 (m, 1H), 6.70–6.90 (m, 1H), 6.90–7.10 (m, 2H), 7.11–7.27 (m, 5H), 7.29–7.42 (m, 4H), 7.42–7.50 (m, 1H), 7.51–7.60 (m, 1H), 7.65–8.25 (m, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 18.5, 18.7, 20.4, 28.2, 28.6, 29.6, 29.9, 35.5, 35.6, 38.3, 54.3, 55.6, 56.0, 57.8, 58.1, 67.6, 67.7, 84.2, 115.4, 115.6, 115.7, 115.8, 119.4, 119.5, 123.2, 124.9, 125.1, 125.2, 127.3, 128.3, 128.4, 128.58, 128.62, 128.7, 128.9, 129.0, 129.7, 130.5, 131.7, 132.1, 133.5, 133.6, 135.8, 136.3, 136.4, 136.5, 136.7, 136.9, 149.9, 171.0, 171.1, 171.4, 171.6, 214.7, 215.0; HRMS-ESI ($M+Na^+$) calcd for $C_{38}H_{42}NaN_4O_7$ 689.2951, found 689.2932.

**4.3.10.20. Ketone 13b.** Compound **13b** was synthesized starting from **8b** and **11e** using general method E (68 mg, 0.10 mmol, 59% for two steps): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.45–1.90 (m, 12H), 2.00–2.20 (m, 1H), 2.20–2.60 (m, 3H), 2.80–3.05 (m, 2H), 3.05–3.25 (m, 2H), 4.20–4.75 (m, 3H), 4.95–5.15 (m, 2H), 5.40–5.60 (m, 1H), 6.50–6.80 (m, 2H), 6.90–7.10 (m, 2H), 7.10–7.25 (m, 4H), 7.21–7.41 (m, 5H), 7.42–7.50 (m, 1H), 7.50–7.65 (m, 1H), 8.00–8.20 (m, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 23.9, 24.0, 28.3, 28.5, 28.6, 35.1, 35.4, 38.8, 38.9, 41.4, 54.5, 54.6, 54.8, 55.5, 58.3, 58.5, 67.5, 67.6, 77.7, 84.1, 115.5, 115.7, 115.8, 119.5, 123.2, 124.9, 125.0, 125.1, 127.3, 127.4, 128.4, 128.56, 128.58, 128.63, 129.0, 129.6, 129.7, 130.6, 135.8, 136.3, 136.4, 136.5, 136.7, 149.9, 170.0, 170.2, 171.1, 207.1, 207.3; HRMS-FAB ($M+Na^+$) calcd for $C_{39}H_{44}NaN_4O_7$ 703.3108, found 703.3125.

**4.3.10.21. Ketone 13c.** Compound **13c** was synthesized starting from **8b** and **11b** using general method E (58 mg, 83 μmol, 47% for two steps): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.55–1.72 (m, 9H), 2.40–2.87 (m, 1H), 2.90–3.25 (m, 3H), 3.30–3.65 (m, 1H), 4.00–4.30 (m, 1H), 4.31–4.75 (m, 2H), 4.85–5.15 (m, 2H), 5.30–5.70 (m, 1H), 6.05–6.55 (m, 1H), 6.60–6.82 (m, 1H), 6.83–7.00 (m, 1H), 7.05–7.26 (m, 4H), 7.30–7.60 (m, 7H), 8.00–8.20 (m 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 27.8, 28.6, 36.4, 36.9, 37.2, 53.3, 53.6, 53.9, 54.4, 55.7, 55.9, 56.5, 56.6, 57.1, 57.3, 58.3, 67.8, 67.9, 68.1, 84.7, 114.9, 115.1, 115.9, 116.0, 119.1, 119.4, 123.3, 124.9, 125.4, 127.7, 128.0, 128.1, 128.4, 128.5, 128.8, 128.9, 129.0, 129.1, 129.2, 129.4, 129.5, 129.6, 130.2, 135.4, 135.7, 135.8, 135.9, 136.1, 149.9, 157.0, 171.4, 171.5, 171.6, 172.0, 196.6, 196.7; HRMS-ESI ($M+Na^+$) calcd for $C_{37}H_{40}NaN_4O_9S$ 739.2414, found 739.2430.

**4.3.10.22. Ketone 13d.** Compound **13d** was synthesized starting from **8b** and **11c** using general method E (82 mg, 0.10 mmol, 61% for two steps): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.40–1.50 (m, 9H), 1.50–1.60 (m, 9H), 2.60–3.40 (m, 5H), 3.55–4.20 (m, 5H), 4.21–4.60 (m, 2H), 4.61–4.82 (m, 1H), 4.85–5.10 (m, 2H), 5.35–5.60 (m, 1H), 5.61–6.20 (m, 1H), 6.65–7.05 (m, 3H), 7.06–7.40 (m, 11H), 7.41–7.65 (m, 2H), 8.00–8.20 (m,

1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 28.1, 28.3, 29.0, 37.4, 37.7, 37.8, 42.7, 43.5, 46.9, 47.1, 47.2, 47.5, 51.1, 51.4, 53.6, 53.8, 54.0, 54.2, 55.1, 55.2, 55.6, 56.1, 67.6, 67.8, 84.4, 115.4, 115.5, 115.8, 115.9, 119.3, 119.5, 124.8, 124.9, 128.0, 128.2, 128.3, 128.6, 130.5, 135.7, 135.8, 136.1, 136.2, 136.3, 150.0, 156.2, 156.9, 168.2, 168.3, 168.4, 171.3, 171.5, 171.6, 171.8, 172.1, 205.4, 205.5, 205.6, 205.7; HRMS-ESI ($M+Na^+$) calcd for $C_{44}H_{52}NaN_6O_{10}$ 824.3645, found 824.3665.

**4.3.10.23. Inhibitor 3a.** Compound **3a** was synthesized starting from **12a** using general method I (52 mg, 85 μmol, 88%): $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 2.55–2.82 (m, 1H), 2.83–3.20 (m, 3H), 3.55–3.82 (m, 1H), 3.83–4.04 (m, 2H), 4.05–4.45 (m, 4H), 4.50–4.75 (m, 1H), 4.76–5.05 (m, 2H), 6.90–7.12 (m, 1H), 7.13–7.42 (m, 9H), 7.45–7.65 (m, 3H), 7.66–7.82 (m, 2H), 8.00–8.20 (m, 2H), 8.25–8.80 (m, 2H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 28.1, 28.4, 28.6, 28.7, 53.1, 54.3, 55.4, 55.9, 56.4, 66.0, 66.1, 69.2, 69.4, 69.6, 70.5, 110.4, 110.9, 111.0, 112.1, 115.6, 116.4, 116.5, 116.8, 117.1, 117.2, 117.3, 119.1, 119.2, 119.3, 120.1, 121.7, 123.1, 124.6, 125.0, 125.3, 128.1, 128.2, 128.3, 128.5, 129.1, 129.2, 131.0, 131.1, 135.8, 136.8, 136.9, 137.8, 152.2, 152.3, 156.6, 156.7, 156.8, 171.7, 171.9, 172.1, 172.2, 172.3, 172.5, 172.6, 212.4, 212.6; HRMS-FAB ($M+Na^+$) calcd for $C_{34}H_{33}NaN_5O_6$ 630.2329, found 630.2302.

**4.3.10.24. Inhibitor 3b.** Compound **3b** was synthesized starting from **12b** using general method I (46 mg, 81 μmol, 90%): $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 2.80–3.25 (m, 4H), 3.26–3.40 (m, 1H), 3.45–3.75 (m, 1H), 3.76–4.13 (m, 2H), 4.15–4.55 (m, 3H), 4.56–4.75 (m, 1H), 4.95–5.15 (m, 2H), 6.90–7.11 (m, 1H), 7.12–7.40 (m, 10H), 7.41–7.70 (m, 2H), 8.05–8.20 (m, 1H); $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 29.1, 29.3, 39.2, 39.4, 56.0, 56.1, 56.3, 56.4, 56.7, 56.8, 57.0, 57.1, 57.3, 57.9, 68.4, 70.8, 71.0, 71.7, 74.0, 74.3, 74.9, 75.4, 76.3, 76.6, 105.9, 106.1, 107.0, 117.8, 117.9, 118.0, 118.1, 120.6, 120.7, 124.4, 126.1, 126.2, 128.5, 129.2, 129.8, 130.5, 130.9, 131.3, 132.3, 132.4, 132.5, 134.2, 137.7, 138.8, 143.0, 153.9, 159.0, 159.1, 173.6, 173.7, 173.9, 174.4, 174.6, 174.8, 212.8, 212.9; HRMS-ESI ($M+H^+$) calcd for $C_{32}H_{33}N_4O_6$ 569.2400, found 569.2428.

**4.3.10.25. Inhibitor 3c.** Compound **3c** was synthesized starting from **12c** using general method I (45 mg, 104 μmol, 89%): $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 2.50–3.25 (m, 4H), 3.30–3.48 (m, 1H), 3.50–4.00 (m, 3H), 4.02–4.40 (m, 3H), 4.50–4.80 (m, 1H), 7.00–7.40 (m, 8H), 7.41–7.75 (m, 2H), 8.10–8.25 (m, 1H); $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 28.5, 28.7, 29.1, 29.3, 39.2, 39.4, 39.5, 54.2, 55.0, 55.1, 55.4, 55.5, 55.7, 55.8, 56.4, 56.5, 56.6, 56.7, 56.8, 57.7, 70.4, 70.5, 70.7, 76.3, 105.5, 105.7, 105.8, 108.1, 108.2, 114.6, 114.7, 116.8, 119.5, 120.2, 120.7, 123.3, 124.3, 125.9, 126.2, 126.3, 126.7, 126.9, 127.3, 128.3, 128.4, 128.7, 128.8, 130.7, 131.5, 131.6, 137.6, 138.1, 138.2, 138.3, 138.4, 138.5, 138.6, 138.7, 153.6, 170.0, 170.3, 170.4, 173.1, 173.3, 212.7, 212.8; HRMS-FAB ($M+Na^+$) calcd for $C_{24}H_{26}NaN_4O_4$ 457.1852, found 457.1861.

**4.3.10.26. Inhibitor 4a.** Compound **4a** was synthesized starting from **13a** using general method I (32 mg, 55 μmol, 92%) as a mixture of two diastereomers: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.20–1.40 (m, 1H), 1.60–2.25 (m, 3H), 2.26–2.55 (m, 2H), 2.56–3.03 (m, 2H), 3.04–3.30 (m, 2H), 3.70–4.30 (m, 1H), 4.50–4.90 (m, 2H), 4.90–5.25 (m, 2H), 5.85–6.20 (m, 1H), 6.80–7.27 (m, 9H), 7.29–7.44 (m, 3H), 7.45–7.65 (m, 2H), 7.90–8.10 (m, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 18.1, 18.3, 28.1, 28.9, 29.1, 34.9, 35.2, 37.8, 38.2, 54.5, 55.3, 57.8, 57.9, 67.4, 94.8, 113.4, 115.6, 116.3, 119.0, 123.3, 124.5, 125.0, 127.2, 127.9, 128.0, 128.1, 128.3, 128.5, 128.6, 129.2, 129.3, 130.5, 132.0, 133.3, 133.5, 135.3, 135.8, 136.2, 141.9, 153.4, 156.4, 159.3, 159.7, 169.9, 171.9, 172.0, 172.3, 172.7, 214.5, 214.6; HRMS-ESI ($M+H^+$) calcd for $C_{33}H_{35}N_4O_5$ 576.2607, found 576.2625.

**4.3.10.27. Inhibitor 4b.** Compound **4b** was synthesized starting from **13b** using general method I (52 mg, 90 μmol, 91%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.80–1.35 (m, 1H), 1.48–1.65 (m, 1H), 1.66–1.95 (m, 2H), 2.00–2.20 (m, 1H), 2.21–2.45 (m, 2H), 2.46–2.80 (m, 2H), 2.81–3.00 (m, 1H), 3.00–3.30 (m, 2H), 3.90–4.40 (m, 1H), 4.50–4.90 (m, 2H), 4.91–5.25 (m, 2H), 5.90–6.30 (m, 1H), 6.80–7.11 (m, 3H), 7.11–7.27 (m, 6H), 7.28–7.40 (m, 4H), 7.45–7.60 (m, 1H), 8.00–8.20 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 23.8, 27.8, 28.0, 28.2, 34.2, 34.5, 40.8, 54.6, 54.9, 55.0, 55.4, 58.1, 58.2, 58.3, 67.5, 68.4, 110.6, 111.3, 111.4, 113.4, 115.2, 115.7, 116.2, 116.4, 118.5, 119.1, 119.9, 122.3, 122.4, 123.3, 124.4, 124.6, 124.9, 125.1, 125.5, 127.3, 127.8, 127.9, 128.0, 128.3, 128.4, 128.6, 128.7, 129.2, 129.3, 130.6, 132.0, 135.2, 135.8, 136.2, 141.9, 153.5, 156.3, 159.2, 159.6, 160.1, 170.8, 170.9, 171.1, 171.3, 172.4, 172.7, 206.5, 206.8; HRMS-ESI ($M+H^+$) calcd for $C_{34}H_{37}N_4O_5$ 581.2764, found 581.2755.

**4.3.10.28. Inhibitor 4c.** Compound **4c** was synthesized starting from **13c** using general method I (45 mg, 72 μmol, 80%): $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 2.65–3.20 (m, 4H), 3.21–3.30 (m, 3H), 3.40–3.80 (m, 2H), 4.20–4.50 (m, 1H), 4.51–4.75 (m, 1H), 4.76–4.85 (m, 2H), 4.95–5.20 (m, 2H), 6.80–7.40 (m, 12H), 7.41–7.70 (m, 2H), 8.0–8.20 (m, 1H); $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 28.9, 29.0, 29.1, 37.7, 38.7, 39.0, 39.2, 39.5, 56.1, 56.2, 56.3, 56.7, 57.1, 57.3, 57.4, 57.8, 58.0, 58.4, 58.8, 68.5, 68.6, 102.3, 102.4, 111.2, 111.4, 113.1, 116.9, 117.9, 118.1, 120.0, 120.7, 123.2, 124.4, 124.6, 125.3, 126.1, 126.2, 128.5, 128.6, 128.8, 129.2, 129.3, 129.4, 129.6, 129.7, 130.2, 130.9, 131.0, 131.1, 131.2, 131.7, 132.3, 132.4, 132.5, 134.2, 137.7, 138.7, 143.2, 153.9, 159.0, 174.0, 174.1, 174.2, 174.4, 174.5; HRMS-ESI ($M+H^+$) calcd for $C_{32}H_{33}N_4O_7S$ 616.2070, found 616.2098.

**4.3.10.29. Inhibitor 4d.** Compound **4d** was synthesized starting from **13d** using general method I (60 mg, 92 μmol, 92%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.65–3.25 (m, 5H), 3.40–3.90 (m, 5H), 3.97–4.17 (m, 1H), 4.18–4.58 (m, 3H), 4.59–4.76 (m, 1H), 4.77–4.94 (m, 1H), 5.00–5.20 (m, 2H), 6.85–7.20 (m, 6H), 7.22–7.36 (m, 6H), 7.40–7.60 (m, 2H), 8.00–8.20 (m, 1H); $^{13}$C

NMR (100 MHz, $CDCl_3$) $\delta$ 29.1, 29.6, 39.0, 39.1, 39.4, 41.5, 41.8, 42.0, 52.4, 52.7, 53.5, 53.8, 54.2, 54.4, 54.6, 54.9, 55.2, 55.4, 55.8, 56.1, 56.5, 57.0, 57.1, 58.2, 68.3, 68.4, 102.8, 103.0, 103.2, 104.2, 104.5, 111.3, 113.0, 115.2, 116.8, 116.9, 117.8, 117.9, 118.0, 119.6, 119.9, 120.5, 120.6, 123.1, 124.3, 125.3, 126.1, 126.2, 126.9, 128.5, 129.2, 129.4, 130.9, 131.1, 132.4, 137.5, 138.6, 153.8, 153.9, 158.9, 159.1, 162.3, 162.7, 166.5, 166.6, 166.7, 167.0, 174.0, 174.1, 174.5, 174.6, 175.0, 175.2, 207.1 ; HRMS-FAB ($M+Na^+$) calcd for $C_{34}H_{36}NaN_6O_6S$ 647.2594, found 647.2580.

**4.3.10.30. Carboxylic acid 15.** To a solution of *trans*-4-(aminomethyl)cyclohexane carboxylic acid **14** (500 mg, 3.20 mmol) in THF/$H_2O$ (2:1, 15 mL) were added CbzCl (473 mg, 3.30 mmol) and 2 N NaOH (2 mL). The reaction mixture was stirred at room temperature for 2 h and then partitioned between EtOAc (300 mL) and 1 N HCl (250 mL). The aqueous layer was extracted once with EtOAc (150 mL). The combined organic layers were washed with brine (200 mL) and dried over $MgSO_4$. The solvent was removed to give carboxylic acid **15** (900 mg, 3.10 mmol, 97%) without further purification: $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 0.80–1.10 (m, 2H), 1.30–1.60 (m, 3H), 1.70–1.90 (d, $J = 8.4$ Hz, 2H), 1.95–2.15 (d, $J = 8.4$ Hz, 2H), 2.15–2.40 (m, 1H), 2.90–3.20 (m, 2H), 4.80–5.25 (m, 3H), 6.05–6.20 (br s, 1H), 7.28–7.50 (m, 5H), 10.00–11.80 (br s, 1H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 28.2, 29.5, 37.6, 43.0, 47.0, 66.8, 95.7, 128.2, 128.5, 136.5, 156.7, 181.6; HRMS-FAB ($M+Na^+$) calcd for $C_{15}H_{19}NaNO_4$ 314.1368, found 314.1350.

**4.3.10.31. Carbamate 16.** Compound **15** (700 mg, 2.5 mmol) was dissolved in toluene (25 mL). To this solution were added DIEA (660 μL, 488 mg, 3.75 mmol) and $(PhO)_2PON_3$ (810 μL, 1.0 g, 3.75 mmol). The reaction mixture was heated at 85 °C under nitrogen for 20 h and then cooled to room temperature. To a separated flask containing a solution of potassium *tert*-butoxide (560 mg, 5.0 mmol) in THF (30 mL) at 0 °C the isocyanate solution was added slowly. The reaction was allowed to warm to room temperature over 30 min and quenched with water (30 mL). The THF was removed by rotary evaporation, and the resulting material was partitioned between EtOAc (250 mL) and 1 N HCl (250 mL). The organic layer was washed with 1 N HCl (150 mL), saturated $NaHCO_3$ (200 mL), and brine (200 mL). It was dried over $MgSO_4$ and the solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (EtOAc/hexanes 1:2–1:1) to give compound **16** (825 mg, 2.27 mmol, 91%): $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 0.90–1.20 (dd, $J = 9.3$, 18.3 Hz, 4H), 1.45 (s, 9H), 1.70–1.90 (d, $J = 8.1$ Hz, 2H), 1.90–2.10 (d, $J = 7.5$ Hz, 2H), 2.90–3.10 (t, $J = 5.2$ Hz, 2H), 3.38 (br s, 1H), 4.42 (br s, 1H), 4.92 (br s, 1H), 5.10 (s, 2H), 7.27–7.48 (m, 5H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 28.5, 29.3, 32.9, 37.4, 46.8, 49.7, 66.7, 79.1, 128.2, 128.6, 136.6, 155.2, 156.5; HRMS-FAB ($M+Na^+$) calcd for $C_{20}H_{30}NaN_2O_4$ 385.2103, found 385.2111.

**4.3.10.32. Aminoalcohol 18a.** Compound **18a** was synthesized starting from **10a** and **17a** using general method F (356 mg, 1.3 mmol, 87%): $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 1.41 (s, 9H), 1.42–1.57 (m, 4H), 2.55–2.75 (m, 2H), 2.95–3.15 (m, 4H), 3.16–3.25 (m, 1H), 3.55–3.61 (dd, $J = 3.0$, 12.0 Hz, 1H), 3.62–3.72 (dd, $J = 3.0$, 9.0 Hz, 1H), 3.90–4.10 (m, 2H), 4.15–4.25 (m, 1H), 4.90–5.10 (br s, 1H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 27.3, 28.1, 28.8, 40.5, 48.0, 50.7, 66.9, 72.3, 74.4, 76.4, 79.6, 156.5; HRMS-FAB ($M+H^+$) calcd for $C_{13}H_{26}N_2O_4$ 275.1971, found 275.1976.

**4.3.10.33. Aminoalcohol 18b.** Compound **18b** was synthesized starting from **10a** and **17b** using general method F (366 mg, 1.27 mmol, 85%): $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 1.25–1.60 (m, 15H), 2.55–2.75 (t, $J = 7.5$ Hz, 2H), 2.95–3.15 (dd, $J = 6.0$, 15.0 Hz, 2H), 3.15–3.30 (m, 3H), 3.50–3.65 (dd, $J = 3.0$, 9.0 Hz, 1H), 3.66–3.75 (dd, $J = 3.0$, 9.0 Hz, 1H), 3.90–4.10 (m, 2H), 4.12–4.28 (m, 1H), 4.65–4.90 (br s, 1H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 24.7, 28.2, 29.6, 30.2, 40.7, 48.3, 67.0, 72.3, 74.4, 76.5, 79.6, 156.5.; HRMS-FAB ($M+H^+$) calcd for $C_{14}H_{28}N_2O_4$ 288.2049, found 288.2047.

**4.3.10.34. Aminoalcohol 18c.** Compound **18c** was synthesized starting from **10a** and **17c** using general method F (407 mg, 1.35 mmol, 90%): $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 1.25–1.40 (m, 4H), 141–1.60 (m, 13H), 2.10–2.25 (t, $J = 7.5$ Hz, 2H), 3.00–3.15 (dd, $J = 6.0$, 15.0 Hz, 2H), 3.15–3.35 (m, 3H), 3.55–3.65 (dd, $J = 3.0$, 9.0 Hz, 1H), 3.66–3.75 (dd, $J = 3.0$, 9.0 Hz, 1H), 3.95–4.15 (m, 2H), 4.20–4.32 (m, 1H), 4.55–4.72 (br s, 1H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 26.8, 27.1, 28.8, 29.7, 30.2, 40.8, 48.3, 50.8, 66.9, 72.0, 74.4, 76.3, 79.6, 156.6; HRMS-FAB ($M+Na^+$) calcd for $C_{15}H_{30}NaN_2O_4$ 325.2103, found 325.2109.

**4.3.10.35. Aminoalcohol 18d.** Compound **18d** was synthesized starting from **10a** and **17d** using general method F (425 mg, 1.35 mmol, 90%): $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 1.25–1.40 (m, 7H), 1.41–1.60 (m, 14H), 2.55–2.70 (t, $J = 7.0$ Hz, 2H), 3.05–3.13 (dd, $J = 6.0$, 15.0 Hz, 2H), 3.14–3.22 (m, 1H), 3.50–3.60 (dd, $J = 4.0$, 8.0 Hz, 1H), 3.65–3.75 (dd, $J = 4.0$, 8.0 Hz, 1H), 3.95–4.02 (dd, $J = 6.0$, 10.0 Hz, 1H), 4.05–4.12 (dd, $J = 6.0$, 10.0 Hz, 1H), 4.15–4.22 (m, 1H), 4.45–4.65 (br s, 1H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 26.6, 27.1, 28.4, 29.1, 30.0, 30.1, 40.5, 48.3, 66.9, 72.5, 74.1, 77.3, 79.1, 156.0; HRMS-FAB ($M+Na^+$) calcd for $C_{16}H_{32}NaN_2O_4$ 339.2260, found 339.2263.

**4.3.10.36. Aminoalcohol 18e.** Compound **18e** was synthesized starting from **10a** and **17e** using general method F (390 mg, 1.27 mmol, 85%): $^1H$ NMR (300 MHz, $CD_3OD$) $\delta$ 1.53 (s, 9H), 3.10–3.20 (m, 1H), 3.50–3.70 (m, 2H), 3.70–3.80 (d, $J = 3.0$ Hz, 2H) 3.95–4.05 (m, 2H), 4.15–4.25 (m, 1H), 7.20–7.30 (d, $J = 9.0$ Hz, 2H), 7.30–7.45 (d, $J = 9.0$ Hz, 2H); $^{13}C$ NMR (75 MHz, $CD_3OD$) $\delta$ 27.7, 51.3, 65.7, 71.9, 74.1, 76.0, 79.8, 118.8, 129.1, 133.7, 138.7, 154.3; HRMS-FAB ($M+H^+$) calcd for $C_{16}H_{24}N_2O_4$ 331.1638, found 331.1631.

**4.3.10.37. Aminoalcohol 18f.** Compound **18f** was synthesized starting from **10a** and **17f** using general method F (375 mg, 1.20 mmol, 80%): $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 0.95–1.30 (m, 4H), 1.45 (s, 9H), 2.30–2.50 (m, 4H), 2.40–2.60 (m, 2H), 3.10–3.20 (m, 1H), 3.20–3.30 (m, 1H), 3.50–3.70 (m, 2H), 3.90–4.10 (m, 2H), 4.10–4.20 (m, 1H); $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 27.8, 30.1, 32.7, 36.9, 50.0, 54.2, 66.9, 71.8, 74.1, 75.7, 78.8, 156.8; HRMS-FAB ($M+Na^+$) calcd for $C_{16}H_{30}N_2O_4$ 337.2103, found 337.2125.

**4.3.10.38. Aminoalcohol 18g.** Compound **18g** was synthesized starting from **10b** and **17c** using general method F (480 mg, 1.38 mmol, 92%) as a mixture of isomers: $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 1.20–1.40 (m, 4H), 1.42–1.60 (s, 14H), 2.55–2.70 (m, 2H), 2.90–3.20 (m, 4H), 3.21–3.41 (m, 2H), 3.42–3.60 (m, 2H), 4.30–4.50 (m, 1H), 4.55–4.75 (br s, 1H); $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 26.7, 26.8, 27.0, 28.8, 29.9, 30.22, 30.24, 30.3, 40.7, 40.8, 47.6, 48.0, 53.9, 56.5, 57.9, 59.0, 60.1, 62.4, 68.3, 72.3, 77.7, 79.7, 156.6; HRMS-FAB ($M+Na^+$) calcd for $C_{15}H_{30}N_2O_5S$ 373.1773, found 373.1782.

**4.3.10.39. TMS ether 19a.** Compound **18a** (300 mg, 1.1 mmol) was dissolved in $CH_2Cl_2$ (20 mL). To this solution TMSCl (900 μL, 545 mg, 5.0 mmol) and DIEA (880 μL, 650 mg, 5.0 mmol) were added. The reaction mixture was stirred at room temperature for 15 min and then partitioned between $CH_2Cl_2$ (200 mL) and $H_2O$ (200 mL). The organic layer was washed twice with $H_2O$ (200 mL) and brine (200 mL), and the solvent was removed by rotary evaporation to yield the *N*,*O*-bis-TMS-protected aminoalcohol (460 mg, 1.1 mmol, 100%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.14 (s, 9H), 0.23 (s, 9H), 1.35–1.55 (m, 13H), 2.55–2.70 (m, 2H), 3.00–3.20 (m, 3H), 3.50–3.60 (m, 2H), 3.90–4.00 (dd, $J$ = 4.0, 8.0 Hz, 1H), 4.00–4.08 (dd, $J$ = 4.0, 8.0 Hz, 1H), 4.10–4.15 (m, 1H). The *N*,*O*-bis-TMS–protected aminoalcohol was dissolved in dry MeOH (20 mL) and the reaction mixture was stirred at room temperature overnight. The solvent was removed by rotary evaporation, and the crude material was purified by flash chromatography (5% MeOH/$CH_2Cl_2$) to yield TMS ether **19a** (385 mg, 1.1 mmol, 100%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.14 (s, 9H), 1.43 (s, 9H), 1.50–1.60 (m, 4H), 2.55–2.75 (m, 2H), 3.00–3.20 (m, 3H), 3.50–3.65 (m, 2H), 3.90–4.00 (dd, $J$ = 4.0, 8.0 Hz, 1H), 4.00–4.08 (dd, $J$ = 4.0, 8.0 Hz, 1H), 4.10–4.15 (m, 1H), 4.70–4.90 (br s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 0.1, 0.2, 27.6, 27.9, 28.4, 40.4, 47.9, 66.7, 72.4, 74.4, 79.1, 156.0; HRMS-FAB ($M+Na^+$) calcd for $C_{16}H_{34}NaN_2O_4Si$ 369.2186, found 369.2175.

**4.3.10.40. TMS ether 19b.** Compound **19b** was synthesized starting from **18b** using general method G (395 mg, 1.1 mmol, 100%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.14 (s, 9H), 1.25–1.65 (m, 15H), 2.50–2.80 (td, $J$ = 3.0, 6.6 Hz, 2H), 3.00–3.25 (m, 3H), 3.50–3.70 (td, $J$ = 3.0, 9.0 Hz, 2H), 3.90–4.10 (m, 2H), 4.10–4.15 (m, 1H), 4.45–4.60 (br s, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 0.1, 0.2, 24.5, 28.5, 29.9, 50.0, 40.5, 48.2, 66.7, 72.3, 74.4, 76.8, 79.1, 156.0; HRMS-ESI ($M+H^+$) calcd for $C_{17}H_{37}N_2O_4Si$ 353.2523, found 353.2540.

**4.3.10.41. TMS ether 19c.** Compound **19c** was synthesized starting from **18c** using general method G (410 mg, 1.1 mmol, 100%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.12 (s, 9H), 1.20–1.40 (m, 5H), 1.41–1.60 (m, 12H), 2.50–2.70 (td, $J$ = 3.0, 6.3 Hz, 2H), 3.00–3.20 (m, 3H), 3.50–3.70 (td, $J$ = 3.0, 9.0 Hz, 2H), 3.90–4.10 (m, 2H), 4.10–4.15 (m, 1H), 4.50–4.65 (br s, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 0.5, 27.0, 27.3, 28.8, 30.4, 30.5, 40.9, 48.6, 67.1, 72.7, 74.7, 79.4, 156.4; HRMS-ESI ($M+H^+$) calcd for $C_{18}H_{39}N_2O_4Si$ 375.2679, found 375.2666.

**4.3.10.42. TMS ether 19d.** Compound **19d** was synthesized starting from **18d** using general method G (425 mg, 1.1 mmol, 100%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.14 (s, 9H), 1.20–1.40 (m, 7H), 1.41–1.60 (m, 12H), 2.50–2.70 (td, $J$ = 3.0, 9.0 Hz, 2H), 3.00–3.20 (m, 3H), 3.50–3.70 (td, $J$ = 3.0, 9.0 Hz, 2H), 3.90–4.08 (m, 2H), 4.10–4.15 (m, 1H), 4.45–4.65 (br s, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 0.5, 27.1, 27.6, 28.8, 29.6, 30.4, 30.5, 41.0, 48.7, 67.1, 72.7, 74.8, 77.2, 79.4, 156.4; HRMS-FAB ($M+H^+$) calcd for $C_{19}H_{40}NaN_2O_4Si$ 411.2655, found 411.2671.

**4.3.10.43. TMS ether 19e.** Compound **19e** was synthesized starting from **18e** using general method G (415 mg, 1.1 mmol, 100%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.13 (s, 9H), 1.53 (s, 9H), 3.15–3.25 (dd, $J$ = 4.0, 4.4 Hz, 1H), 3.50–3.70 (m, 2H), 3.77 (s, 2H), 3.90–4.08 (m, 2H), 4.15–4.25 (m, 1H), 6.58 (s, 1H), 7.20–7.30 (d, $J$ = 8.0 Hz, 2H), 7.30–7.40 (d, $J$ = 8.0 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 0.1, 0.2, 28.4, 51.7, 66.0, 72.4, 74.4, 77.0, 80.5, 118.6, 128.7, 134.6, 137.4, 152.8; HRMS-FAB ($M+H^+$) calcd for $C_{19}H_{33}N_2O_4Si$ 381.2210, found 381.2219.

**4.3.10.44. TMS ether 19f.** Compound **19f** was synthesized starting from **18f** using general method G (415 mg, 1.1 mmol, 98%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.13 (s, 9H), 0.90–1.20 (m, 5H), 1.25–1.40 (m, 1H), 1.44 (s, 9H), 1.70–1.90 (m, 2H), 1.90–2.10 (m, 2H), 2.40–2.50 (d, $J$ = 6.0 Hz, 2H), 3.00–3.15 (m, 1H), 3.30–3.45 (br s, 1H), 3.50–3.70 (ddd, $J$ = 3.0, 9.0, 18.0 Hz, 2H), 3.90–3.98 (dd, $J$ = 3.0, 9.0, 1H), 3.99–4.08 (dd, $J$ = 3.0, 9.0, 1H), 4.05–4.15 (m, 1H), 4.30–4.50 (br s, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 0.5, 28.8, 30.4, 33.5, 37.9, 50.3, 54.8, 67.1, 72.9, 74.7, 77.2, 79.5, 155.6; HRMS-ESI ($M+H^+$) calcd for $C_{19}H_{39}N_2O_4Si$ 387.2679, found 387.2690.

**4.3.10.45. TMS ether 19g.** Compound **18g** (140 mg, 0.29 mmol) was dissolved in $CH_2Cl_2$ (15 mL). To this solution TMSCl (180 μL, 153 mg, 1.4 mmol) and DIEA (250 μL, 182 mg, 1.40 mmol) were added. The reaction mixture was stirred at room temperature for 45 min, then the solution was partitioned between $CH_2Cl_2$ (75 mL) and $H_2O$ (75 mL). The organic layer was washed twice with $H_2O$ (75 mL) and brine (75 mL), the solvent was removed by rotary evaporation, and the crude product was purified by flash chromatography (5% MeOH/$CH_2Cl_2$) to yield TMS ether **19g** as a mixture of isomers (118 mg, 0.28 mmol, 97%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.10–0.20 (m, 9H), 1.25–1.40 (m,

4H), 1.41–1.55 (m, 13H), 2.50–2.65 (dd, $J$ = 4.0, 8.0 Hz, 2H), 2.85–3.35 (m, 5H), 3.36–3.55 (m, 2H), 4.25–4.70 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ −0.1, 0.0, 26.5, 26.6, 26.8, 26.9, 28.4, 30.0, 30.1, 40.5, 47.2, 48.0, 54.1, 55.6, 58.0, 59.9, 60.1, 62.7, 70.0, 72.6, 79.1, 156.0; HRMS-FAB (M+Na$^+$) calcd for $C_{18}H_{38}NaN_2O_5SSi$ 445.2168, found 445.2145.

**4.3.10.46. Ketone 20a.** Compound **20a** was synthesized starting from **19a** using general method H (41 mg, 49 μmol, 81%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.10–1.37 (m, 4H), 1.38–1.52 (m, 10H), 1.53–1.72 (m, 9H), 2.50–3.30 (m, 8H), 3.35–3.60 (m, 1H), 3.85–4.25 (m, 4H), 4.30–4.60 (m, 1H), 4.75–5.04 (m, 2H), 5.05–5.20 (m, 2H), 5.20–5.70 (m, 1H), 6.25–6.75 (m, 1H), 6.95–7.17 (m, 2H), 7.18–7.26 (m, 3H), 7.28–7.40 (m, 6H), 7.41–7.51 (m, 1H), 7.55–7.70 (m, 1H), 8.00–8.20 (m, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 26.5, 26.7, 26.8, 27.0, 28.2, 28.5, 28.6, 28.8, 39.5, 39.7, 39.9, 49.9, 50.1, 50.4, 55.4, 55.7, 62.1, 62.3, 67.3, 67.4, 67.6, 68.0, 68.2, 70.0, 79.7, 84.1, 84.3, 84.4, 115.4, 115.8, 119.4, 123.2, 124.7, 124.8, 124.9, 125.0, 125.2, 127.6, 127.7, 128.5, 128.6, 128.7, 128.8, 128.9, 129.0, 129.8, 129.9, 130.2, 130.5, 135.6, 135.7, 135.8, 135.9, 136.1, 136.4, 149.9, 150.0, 156.2, 156.5, 170.0, 170.1, 170.3, 170.4, 170.6, 208.1, 208.2; HRMS-ESI (M+Na$^+$) calcd for $C_{46}H_{57}NaN_5O_{10}$ 862.4003, found 862.4025.

**4.3.10.47. Ketone 20b.** Compound **20b** was synthesized starting from **19b** using general method H (47 mg, 55 μmol, 62%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.00–1.30 (m, 3H), 1.31–1.51 (m, 11H), 1.52–1.70 (m, 9H), 2.50–3.30 (m, 8H), 3.33–3.50 (m, 1H), 3.85–4.06 (m, 2H), 4.07–4.30 (m, 2H), 4.35–4.60 (m, 1H), 4.62–5.04 (m, 2H), 5.05–5.15 (m, 2H), 5.20–5.70 (m, 1H), 6.25–6.80 (m, 1H), 6.95–7.17 (m, 2H), 7.19–7.27 (m, 3H), 7.29–7.33 (m, 6H), 7.34–7.51 (m, 1H), 7.50–7.70 (m, 1H), 8.00–8.20 (m, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 23.7, 28.5, 28.6, 28.8, 29.1, 29.2, 29.3, 29.9, 39.6, 40.5, 50.3, 50.4, 50.5, 62.1, 62.2, 67.4, 67.5, 67.7, 68.1, 70.0, 77.7, 84.1, 84.3, 115.8, 119.4, 119.5, 123.1, 123.2, 124.7, 124.8, 124.9, 125.1, 125.2, 127.5, 127.6, 127.7, 128.5, 128.6, 128.7, 129.0, 129.8, 129.9, 130.2, 135.7, 135.9, 136.5, 170.3, 170.6, 208.1, 208.2; HRMS-ESI (M+Na$^+$) calcd for $C_{47}H_{59}NaN_5O_{10}$ 876.4160, found 876.4176.

**4.3.10.48. Ketone 20c.** Compound **20c** was synthesized starting from **19c** using general method H (51 mg, 59 μmol, 66%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.00–1.35 (m, 7H), 1.36–1.52 (m, 9H), 1.53–1.70 (m, 9H), 2.50–3.30 (m, 8H), 3.30–3.50 (m, 1H), 3.85–4.25 (m, 4H), 4.35–4.65 (m, 1H), 4.66–5.02 (m, 2H), 5.03–5.15 (m, 2H), 5.30–5.70 (m, 1H), 6.40–6.80 (m, 1H), 6.97–7.17 (m, 2H), 7.18–7.27 (m, 4H), 7.29–7.40 (m, 6H), 7.41–7.49 (m, 1H), 7.50–7.65 (m, 1H), 8.00–8.20 (m, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 26.1, 26.5, 28.3, 28.6, 28.9, 29.4, 29.6, 30.1, 39.6, 39.9, 40.7, 50.3, 50.4, 50.5, 55.3, 62.2, 62.3, 67.4, 67.5, 68.1, 68.2, 70.0, 77.7, 79.5, 84.2, 84.3, 115.5, 115.6, 115.7, 119.4, 123.1, 123.2, 124.7, 124.8, 125.0, 125.1, 127.5, 127.6, 127.7, 128.5, 128.6, 128.9, 129.8, 129.9, 130.2, 130.5, 130.6, 135.7, 135.8, 136.0, 136.2, 136.5, 149.9, 156.2, 156.5, 170.0, 170.1, 170.3, 170.4, 170.5, 170.6, 208.0, 208.1, 208.2, 208.3; HRMS-FAB (M+Na$^+$) calcd for $C_{48}H_{61}NaN_5O_{10}$ 890.4316, found 890.4334.

**4.3.10.49. Ketone 20d.** Compound **20d** was synthesized starting from **19d** using general method H (50 mg, 57 mmol, 65%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.00–1.35 (m, 10H), 1.40–1.55 (m, 12H), 1.56–1.70 (m, 9H), 2.50–2.85 (m, 2H), 2.86–3.30 (m, 7H), 3.40–3.55 (m, 1H), 3.85–4.05 (m, 2H), 4.06–4.30 (m, 2H), 4.35–4.60 (m, 1H), 4.62–4.80 (m, 1H), 4.81–5.05 (m, 1H), 5.05–5.20 (m, 2H), 5.25–5.65 (m, 1H), 6.30–6.80 (m, 1H), 6.96–7.27 (m, 7H), 7.30–7.42 (m, 6H), 7.43–7.54 (m, 1H), 7.55–7.70 (m, 1H), 8.05–8.20 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 26.0, 26.5, 28.1, 28.4, 28.6, 29.1, 29.8, 39.2, 39.5, 40.4, 50.0, 50.1, 54.9, 61.7, 61.8, 61.9, 66.9, 67.1, 67.7, 67.8, 69.5, 79.0, 83.6, 83.7, 115.3, 115.5, 119.0, 122.7, 122.8, 124.2, 124.3, 124.6, 124.7, 127.0, 127.1, 127.2, 128.1, 128.2, 128.5, 128.6, 129.3, 129.4, 129.7, 135.2, 135.3, 135.5, 135.7, 136.0, 149.4, 155.8, 156.0, 169.5, 169.8, 169.9, 170.0, 207.6, 207.7; HRMS-FAB (M+Na$^+$) calcd for $C_{49}H_{63}NaN_5O_{10}$ 904.4473, found 904.4450.

**4.3.10.50. Ketone 20e.** Compound **20e** was synthesized starting from **19e** using general method H (40 mg, 46 μmol, 52%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.40–1.55 (m, 9H), 1.56–1.70 (m, 9H), 2.55–3.10 (m, 2H), 3.11–3.27 (m, 2H), 3.30–3.50 (m, 1H), 3.65–3.5 (m, 3H), 4.05–4.20 (m, 1H), 4.25–4.65 (m, 2H), 4.90–5.25 (m, 3H), 5.25–5.65 (m, 1H), 6.40–6.95 (m, 3H), 7.00–7.08 (m, 1H), 7.09–7.20 (m, 2H), 7.21–7.30 (m, 3H), 7.31–7.42 (m, 7H), 7.43–7.70 (m, 2H), 8.00–8.15 (m, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 14.6, 28.2, 28.6, 28.7, 29.4, 50.4, 50.7, 53.1, 53.2, 61.1, 61.3, 61.6, 67.5, 67.6, 67.8, 68.0, 70.0, 77.7, 81.2, 84.1, 84.2, 84.3, 115.6, 115.7, 115.8, 119.2, 119.3, 119.5, 123.1, 123.2, 124.7, 124.8, 124.9, 125.1, 127.6, 127.7, 128.5, 128.6, 128.7, 128.9, 129.0, 129.1, 129.2, 129.6, 129.8, 130.0, 130.1, 130.2, 131.7, 135.4, 135.8, 136.1, 136.5, 138.8, 139.0, 149.9, 153.0, 156.3, 170.3, 170.5, 170.6, 170.7, 170.8, 208.3, 208.4; HRMS-ESI (M+Na$^+$) calcd for $C_{49}H_{55}NaN_5O_{10}$ 896.3847, found 896.3862.

**4.3.10.51. Ketone 20f.** Compound **20f** was synthesized starting from **19f** using general method H (43 mg, 49 μmol, 56%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.70–1.10 (m, 5H), 1.11–1.31 (m, 1H), 1.35–1.55 (m, 10H), 1.56–1.70 (m, 9H), 1.80–2.00 (m, 2H), 2.45–3.25 (m, 6H), 3.26–3.55 (m, 2H), 3.90–4.28 (m, 4H), 4.29–4.40 (m, 1H), 4.41–4.55 (m, 1H), 4.75–5.02 (m, 1H), 5.03–5.13 (m, 2H), 5.20–5.60 (m, 1H), 6.20–6.70 (m, 1H), 6.95–7.16 (m, 2H), 7.17–7.28 (m, 4H), 7.30–7.42 (m, 6H), 7.43–7.53 (m, 1H), 7.55–7.74 (m, 1H), 8.00–8.20 (m, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 28.5, 28.6, 28.8, 29.1, 29.2, 29.4, 33.0, 33.1, 37.8, 39.5, 39.9, 49.8, 50.5, 52.2, 56.1, 63.1, 67.1, 67.3, 67.6, 67.8, 67.9, 70.0, 70.1, 77.7, 79.6, 84.1, 84.3, 115.5, 115.7, 115.8, 119.5, 123.2, 123.3, 124.7, 125.0, 125.2, 127.5, 127.6, 127.7, 128.5, 128.7, 129.0, 129.8, 129.9, 130.2, 130.5, 135.7, 135.9, 136.2, 136.4, 149.9, 155.5, 156.2, 170.3, 170.4, 170.6, 170.7, 170.8, 207.9, 208.0, 208.1, 208.3; HRMS-ESI (M+Na$^+$) calcd for $C_{49}H_{61}NaN_5O_{10}$ 902.4316, found 902.4301.

**4.3.10.52. Ketone 20g.** Compound **20g** was synthesized starting from **19g** using general method H (41 mg, 45 μmol, 50%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.00–1.34 (m, 6H), 1.35–1.58 (m, 9H), 1.59–1.85 (m, 9H), 2.55–3.03 (m, 3H), 3.04–3.40 (m, 4H), 3.41–3.95 (m, 3H), 4.10–4.30 (m, 1H), 4.35–4.62 (m, 1H), 4.63–4.90 (m, 1H), 4.95–5.15 (m, 2H), 5.20–5.89 (m, 1H), 6.30–6.80 (m, 1H), 6.90–7.11 (m, 2H), 7.12–7.28 (m, 4H), 7.30–7.45 (m, 5H), 7.46–7.51 (m, 1H), 7.52–7.77 (m, 1H), 8.00–8.20 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.6, 21.5, 26.2, 26.5, 28.5, 28.6, 28.8, 29.1, 29.3, 30.1, 39.1, 39.8, 40.7, 50.4, 52.2, 52.3, 52.8, 55.3, 57.6, 60.8, 64.2, 64.3, 67.6, 67.8, 79.6, 84.3, 84.4, 115.3, 115.8, 119.5, 123.2, 124.7, 124.9, 125.1, 125.2, 127.7, 128.0, 128.4, 128.5, 128.7, 128.8, 129.0, 129.1, 129.7, 129.8, 129.9, 130.6, 136.4, 149.9, 156.4, 170.9, 171.3, 194.3, 194.4; HRMS-FAB (M+Na$^+$) calcd for $C_{48}H_{61}NaN_5O_{11}S$ 938.3986, found 938.3966.

**4.3.10.53. Inhibitor 5a.** Compound **5a** was synthesized starting from **20a** using general method I (31 mg, 48 μmol, 80%): $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 1.30–1.70 (m, 4H), 2.70–2.95 (m, 3H), 2.96–3.25 (m, 3H), 3.70–4.05 (m, 3H), 4.10–4.55 (m, 3H), 5.00–5.20 (m, 2H), 6.90–7.50 (m, 14H), 8.00–8.50 (m, 1H); $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 24.1, 24.2, 26.0, 26.2, 27.9, 28.1, 28.3, 37.8, 38.1, 38.6, 39.2, 39.3, 50.3, 50.5, 50.8, 56.4, 56.5, 61.7, 61.9, 62.0, 66.6, 66.9, 67.2, 67.9, 69.5, 69.6, 109.6, 111.4, 115.3, 118.3, 118.8, 119.0, 121.4, 122.7, 123.7, 123.8, 124.4, 127.0, 127.2, 127.7, 127.8, 127.9, 128.0, 128.5, 128.6, 129.5, 129.6, 129.7, 129.8, 136.6, 136.7, 136.8, 136.9, 137.1, 170.6, 170.7, 172.8, 173.1, 209.3, 209.8; HRMS-ESI (M+H$^+$) calcd for $C_{36}H_{42}N_5O_6$ 640.3135, found 640.3146.

**4.3.10.54. Inhibitor 5b.** Compound **5b** was synthesized starting from **20b** using general method I (33 mg, 50 mmol, 77%): $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 1.00–1.40 (m, 4H), 1.41–1.75 (m, 2H), 2.35–2.64 (m, 1H), 2.65–3.25 (m, 7H), 3.65–4.07 (m, 3H), 4.08–4.32 (m, 2H), 4.33–4.54 (m, 1H), 4.65–5.00 (m, 1H), 5.00–5.15 (m, 2H), 6.90–7.15 (m, 3H), 7.16–7.45 (m, 10H), 7.46–7.72 (m, 2H); $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 22.8, 22.9, 26.9, 27.0, 27.9, 28.5, 28.6, 37.9, 38.3, 38.6, 39.3, 39.4, 50.3, 50.4, 50.6, 50.8, 56.4, 56.6, 61.9, 66.7, 66.8, 67.4, 67.9, 69.6, 109.5, 109.6, 111.3, 115.3, 118.3, 118.8, 119.0, 121.4, 122.7, 123.7, 123.9, 124.5, 126.9, 127.1, 127.7, 127.8, 127.9, 128.0, 128.1, 128.5, 128.6, 129.5, 129.6, 129.7, 129.8, 130.8, 136.7, 136.8, 136.9, 137.1, 157.1, 170.5, 172.8, 172.9, 173.0, 209.4, 209.7; HRMS-FAB (M+Na$^+$) calcd for $C_{37}H_{43}NaN_5O_6$ 676.3111, found 676.3131.

**4.3.10.55. Inhibitor 5c.** Compound **5c** was synthesized starting from **20c** using general method I (36 mg, 54 mmol, 80%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.90–1.35 (m, 6H), 1.40–1.65 (m, 2H), 2.35–3.28 (m, 8H), 3.30–3.60 (m, 1H), 3.70–4.02 (m, 2H), 4.03–4.29 (m, 2H), 4.32–4.65 (m, 1H), 4.70–5.20 (m, 3H), 5.60–6.20 (m, 1H), 6.85–7.22 (m, 10H), 7.30–7.40 (m, 2H), 7.41–7.80 (m, 6H), 7.90–9.00 (m, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 25.2, 25.4, 26.7, 26.9, 28.0, 28.4, 28.7, 30.1, 39.0, 40.1, 49.7, 50.1, 50.6, 51.1, 56.2, 62.1, 62.3, 67.2, 67.6, 68.3, 69.9, 70.1, 109.7, 109.8, 110.5, 111.9, 114.3, 118.2, 118.6, 120.1, 122.0, 122.6, 124.0, 124.2, 125.2, 127.7, 127.8, 127.9, 128.3, 128.4, 128.7, 129.0, 129.1, 129.7, 130.0, 135.5, 136.3, 136.4, 136.5, 136.6, 153.1, 156.6, 156.7, 156.8, 160.9, 161.4, 161.9, 162.4, 170.5, 170.8, 171.2, 172.1, 208.1, 208.2; HRMS-FAB (M+Na$^+$) calcd for $C_{38}H_{45}NaN_5O_6$ 690.3268, found 690.3287.

**4.3.10.56. Inhibitor 5d.** Compound **5d** was synthesized starting from **20d** using general method I (36 mg, 54 mmol, 81%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.00–1.35 (m, 8H), 1.36–2.24 (m, 2H), 2.50–3.30 (m, 8H), 3.35–3.55 (m, 1H), 3.80–4.02 (m, 2H), 4.03–4.25 (m, 2H), 4.30–4.70 (m, 1H), 4.70–4.95 (m, 1H), 4.96–5.20 (m, 2H), 5.70–6.20 (m, 1H), 6.85–7.25 (m, 9H), 7.30–7.90 (m, 7H), 8.00–8.80 (m, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 25.3, 25.5, 25.7, 26.8, 26.9, 27.2, 27.9, 28.3, 28.6, 28.8, 30.1, 38.8, 39.5, 40.3, 50.1, 50.4, 50.5, 50.6, 56.2, 56.6, 62.2, 62.5, 67.2, 67.6, 68.3, 69.9, 70.2, 109.1, 109.7, 111.9, 114.3, 115.9, 118.1, 118.6, 118.8, 120.1, 121.3, 122.6, 123.9, 124.1, 124.4, 125.2, 127.6, 127.8, 127.9, 128.3, 128.4, 128.7, 129.0, 129.1, 129.7, 130.0, 135.5, 136.3, 136.4, 136.6, 151.5, 156.7, 156.8, 161.4, 161.9, 170.2, 170.6, 170.9, 171.3, 172.3, 208.0, 208.2; HRMS-FAB (M+Na$^+$) calcd for $C_{39}H_{47}NaN_5O_6$ 704.3424, found 704.3435.

**4.3.10.57. Inhibitor 5e.** Compound **5e** was synthesized starting from **20e** using general method I (37 mg, 55 mmol, 75%): $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 2.45–3.25 (m, 4H), 3.55–4.05 (m, 4H), 4.06–4.55 (m, 4H), 4.56–4.90 (m, 1H), 5.00–5.15 (m, 2H), 6.75–7.43 (m, 18H), 7.44–7.65 (m, 2H), 8.00–8.20 (m, 1H); $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 27.7, 27.9, 28.4, 37.6, 37.9, 38.2, 47.2, 47.4, 47.7, 50.7, 51.0, 52.4, 55.3, 56.1, 61.6, 61.8, 66.7, 67.4, 67.7, 69.6, 109.6, 111.4, 115.3, 116.2, 118.4, 118.8, 119.1, 121.4, 122.6, 123.1, 123.8, 124.4, 124.7, 126.9, 127.1, 127.2, 127.7, 127.8, 127.9, 128.5, 128.6, 128.7, 128.8, 128.9, 129.0, 129.2, 129.3, 129.4, 129.6, 129.7, 129.8, 130.8, 135.9, 136.5, 136.6, 136.7, 136.9, 137.1, 152.2, 157.1, 170.8, 172.8, 208.8, 208.9; HRMS-FAB (M+Na$^+$) calcd for $C_{39}H_{39}NaN_5O_6$ 696.2798, found 696.2788.

**4.3.10.58. Inhibitor 5f.** Compound **5f** was synthesized starting from **20f** using general method I (42 mg, 62 mmol, 81%): $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 0.70–1.40 (m, 5H), 1.42–2.15 (m, 4H), 2.80–3.20 (m, 5H), 3.40–3.86 (m, 2H), 3.87–4.05 (m, 2H), 4.10–4.30 (m, 2H), 4.31–4.80 (2H), 5.00–5.15 (m, 2H), 6.90–7.18 (m, 3H), 7.19–7.42 (m, 9H), 7.45–7.70 (m, 2H), 8.00–8.20 (m, 1H); $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 27.5, 27.8, 28.0, 28.3, 30.2, 36.5, 36.7, 36.8, 37.9, 38.2, 38.8, 50.3, 50.5, 50.7, 55.1, 55.3, 55.4, 56.4, 62.3, 62.5, 66.6, 67.3, 67.5, 67.6, 69.6, 109.7, 111.3, 115.3, 118.3, 118.8, 119.1, 121.4, 122.6, 123.7, 124.4, 124.6, 127.0, 127.1, 127.7, 127.8, 127.9, 128.0, 128.5, 128.6, 129.7, 129.8, 129.9, 130.8, 135.9, 136.6, 136.7, 136.9, 137.1, 170.6, 170.8, 172.1, 172.7, 172.9, 209.1, 209.2; HRMS-FAB (M+Na$^+$) calcd for $C_{39}H_{45}NaN_5O_6$ 702.3268, found 702.3285.

**4.3.10.59. Inhibitor 5g.** Compound **5g** was synthesized starting from **20g** using general method I (30 mg, 42 mmol, 59%): $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 1.10–1.45 (m, 6H), 1.46–1.78 (m, 2H), 2.68–3.22 (m, 7H), 3.45–3.82 (m, 2H), 4.15–4.40 (m, 1H), 4.41–4.65 (m, 1H), 4.90–5.02 (m, 1H), 5.02–5.15 (m, 2H), 6.90–7.45 (m, 13H), 7.46–7.70 (m, 2H), 8.05–8.30 (m, 1H); $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 25.3, 25.6, 25.7, 25.8, 26.9, 27.1, 27.8, 28.4, 28.5, 28.9, 29.6, 37.4, 37.8, 39.4, 49.4, 52.4, 52.7, 56.1, 56.2, 64.0, 64.2, 66.5, 66.6, 109.4, 111.2, 118.2, 118.7, 121.3, 121.4, 123.6, 126.7, 127.1, 127.5, 127.6, 127.7, 127.8, 128.1, 128.3, 128.5, 129.2, 129.4, 129.5, 136.5, 136.6, 136.7, 136.8, 136.9, 170.9, 172.6, 172.7, 172.9, 195.9, 196.0; HRMS-FAB ($M+Na^+$) calcd for $C_{38}H_{45}NaN_5O_7S$ 738.2937, found 738.2962.

**4.3.10.60. Fmoc-Trp(Boc)-Tyr-OBn (22).** Compound **22** was synthesized starting from **21** using general method A (1.21 g, 1.56 mmol, 97%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.64 (s, 9H), 2.06 (s, 1H), 2.75–3.00 (m, 2H), 3.00–3.30 (m, 2H), 4.00–4.50 (m, 3H), 4.50–4.70 (d, $J$ = 4.8 Hz, 1H), 4.72–4.85 (br s, 1H), 4.90–5.15 (dd, $J$ = 12.0, 20.0 Hz, 2H), 5.60–5.70 (d, $J$ = 6.0 Hz, 1H), 6.40–6.80 (m, 6H), 7.10–7.30 (m, 5H), 7.30–7.45 (m, 5H), 7.46–7.65 (m, 4H), 7.65–7.85 (m, 2H), 8.00–8.20 (br s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.3, 21.1, 28.2, 37.0, 47.0, 53.6, 54.9, 60.6, 67.3, 67.4, 83.8, 115.2, 115.4, 115.5, 119.0, 120.0, 122.8, 124.6, 124.7, 125.2, 126.7, 127.2, 127.8, 130.2, 130.4, 135.0, 135.5, 141.3, 143.7, 149.6, 155.2, 156.2, 170.8, 170.9; HRMS-FAB ($M+Na^+$) calcd for $C_{47}H_{45}NaN_3O_8$ 802.3104, found 802.3128.

**4.3.10.61. $H_2N$-Trp(Boc)-Tyr-OBn (23).** To a solution of dipeptide **22** (5 g, 6.4 mmol) in $CH_2Cl_2$ (150 mL) was added dry piperidine (50 mL). The reaction mixture was stirred at room temperature for 35 min, then partitioned between $CH_2Cl_2$ (1 L) and saturated $NH_4Cl$ (1 L). The organic layer was washed with saturated $NH_4Cl$ (750 mL), brine (750 mL), and dried over $Na_2CO_3$. The solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (EtOAc/hexanes 1:1–9:1) to yield the free amine **23** as a white solid (3.15 g, 5.4 mmol, 84%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.65 (s, 9H), 2.30–2.40 (dd, $J$ = 10.0, 14.4 Hz, 1H), 2.85–2.95 (dd, $J$ = 8.0, 14.0 Hz, 1H), 2.95–3.05 (dd, $J$ = 3.2, 14.4 Hz, 1H), 3.10–3.20 (dd, $J$ = 5.6, 14.0 Hz, 1H), 3.55–3.65 (dd, $J$ = 3.2, 9.6 Hz, 1H), 4.90–5.05 (td, $J$ = 5.6, 14.0 Hz, 1H), 5.10–5.25 (dd, $J$ = 12.0, 21.2 Hz, 2H), 6.70–6.80 (d, $J$ = 8.4 Hz, 2H), 6.85–6.95 (d, $J$ = 8.4 Hz, 2H), 7.10–7.25 (m, 2H), 7.25–7.30 (m, 2H), 7.31–7.45 (m, 5H), 7.55–7.60 (d, $J$ = 8.0 Hz, 1H), 7.80–7.90 (d, $J$ = 8.8 Hz, 1H), 7.90–8.10 (br s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.2, 28.2, 30.0, 37.6, 52.7, 54.4, 60.5, 67.3, 77.3, 83.7, 115.1, 115.5, 116.1, 119.2, 122.6, 124.0, 124.6, 126.6, 128.5, 128.6, 128.7, 129.8, 130.4, 135.5, 155.8, 171.6, 174.8; HRMS-FAB ($M+Na^+$) calcd for $C_{32}H_{35}NaN_3O_6$ 580.2424, found 580.2415.

**4.3.10.62. 5-Bromopentanoyl-L-Trp(Boc)-L-Tyr-OBn (24a).** To a solution of compound **23** (560 mg, 1.0 mmol) in THF (4 mL) were added $H_2O$ (4 mL), aqueous $K_2CO_3$ (20%, 1.25 mL), and 5-bromovaleryl chloride (146 μL, 220 mg, 1.1 mmol) as a solution in THF (1 mL). The reaction mixture was stirred vigorously at room temperature for 8 min and then partitioned between EtOAc (300 mL) and 1 N HCl (250 mL). The organic layer was washed with saturated $NaHCO_3$, brine (200 mL), and dried over $MgSO_4$. The solvent was removed by rotary evaporation. The crude material was purified by flash chromatography (EtOAc/hexanes 1:2–1:1) to yield **24a** (710 mg, 0.99 mmol, 95%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.65 (s, 9H), 2.00–2.20 (m, 3H), 2.80–2.95 (dd, $J$ = 6.9, 14.0 Hz, 1H), 2.95-3.05 (dd, $J$ = 6.0, 13.6 Hz, 1H), 3.10–3.20 (d, $J$ = 6.9 Hz, 2H), 3.25–3.35 (t, $J$ = 6.0 Hz, 2H), 4.15–4.35 (m, 2H), 4.95–5.15 (d, $J$ = 6.9 Hz, 2H), 6.45–6.65 (m, 3H), 6.65–6.75 (m, 3H), 7.10–7.22 (m, 1H), 7.25–7.45 (m, 7H), 7.48 (s, 1H), 7.50–7.60 (d, $J$ = 7.5 Hz, 1H), 8.00–8.20 (br d, $J$ = 6.6 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 24.3, 28.4, 28.6, 32.2, 33.6, 35.6, 37.3, 53.5, 54.0, 67.7, 84.2, 115.5, 115.7, 115.9, 119.4, 123.1, 125.0, 126.9, 129.0, 129.1, 130.6, 130.7, 135.4, 135.7, 150.0, 155.8, 171.1, 171.3, 173.3; HRMS-FAB ($M+Na^+$) calcd for $BrC_{31}H_{39}NaN_3O_7$ 742.2104, found 742.2129.

**4.3.10.63. 6-Bromohexanoyl-L-Trp(Boc)-L-Tyr-OBn (24b).** Compound **24b** was synthesized starting with 6-bromohexanoyl chloride using a procedure described for the preparation of compound **24a** (725 mg, 0.99 mmol, 99%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.20–1.40 (m, 2H), 1.50–1.60 (m, 2H), 1.65 (s, 9H), 2.20–2.35 (m, 2H), 2.05–2.20 (t, $J$ = 6.0 Hz, 2H), 2.80–2.95 (dd, $J$ = 6.0, 14.6 Hz, 1H), 2.96–3.05 (dd, $J$ = 6.0, 14.6 Hz, 1H), 3.10–3.20 (t, $J$ = 6.0 Hz, 2H), 3.25–3.40 (t, $J$ = 9.0 Hz, 2H), 4.15–4.40 (m, 2H), 5.00–5.20 (dd, $J$ = 12.0, 15.0 Hz, 2H), 6.35–6.65 (m, 4H), 6.66–6.75 (d, $J$ = 9.0 Hz, 2H), 7.10–7.20 (m, 2H), 7.27–7.46 (m, 5H), 7.48 (s, 1H), 7.50–7.60 (d, $J$ = 6.0 Hz, 1H), 8.00–8.15 (d, $J$ = 9.0 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 25.0, 28.0, 28.4, 28.6, 32.8, 33.9, 36.6, 37.3, 53.0, 54.0, 67.7, 77.7, 84.2, 115.6, 115.7, 116.0, 119.4, 123.1, 125.0, 126.9, 129.0, 120.1, 130.6, 130.7, 135.4, 135.8, 150.0, 155.8, 171.1, 171.2, 173.6; HRMS-FAB ($M+Na^+$) calcd for $BrC_{38}H_{44}NaN_3O_7$ 756.2260, found 756.2275.

**4.3.10.64. 8-Bromooctanoyl-L-Trp(Boc)-L-Tyr-OBn (24c).** 8-Bromooctanoic acid (224 mg, 1.0 mmol) was dissolved in $CH_2Cl_2$ (20 mL). To this solution was added oxalyl chloride (170 μL, 254 mg, 2.0 mmol). The reaction mixture was stirred at room temperature for 6 h. The solvent was removed by rotary evaporation to yield the corresponding acid chloride (240 mg, 1.0 mmol, 100%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.22–1.58 (m, 6H), 1.70–1.80 (m, 2H), 1.80–2.00 (m, 2H), 2.82–2.95 (t, $J$ = 7.2 Hz, 2H), 3.40–3.50 (t, $J$ = 7.6 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 25.0, 27.9, 28.3, 32.6, 33.8, 33.9, 47.0, 173.8. Using the resulting acid chloride as the starting material, compound **24c** was synthesized with the procedure described for the preparation of compound **24a** (750 mg, 0.99 mmol, 100%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.10–1.40 (m, 7H), 1.45–1.56 (m, 2H), 1.65 (s, 9H), 1.70–1.90 (m, 2H), 2.10–2.20 (m, 2H), 2.80–2.95 (dd, $J$ = 6.0, 12.0 Hz, 1H), 2.96–3.05 (dd, $J$ = 6.0, 12.0 Hz, 1H), 3.06–3.21 (m, 2H), 3.30–

3.40 (t, $J$ = 6.0 Hz, 2H), 4.15–4.40 (m, 2H), 5.00–5.20 (dd, $J$ = 6.0, 21.0 Hz, 2H), 6.40–6.50 (d, $J$ = 9.0 Hz, 1H), 6.55–6.65 (m, 3H), 6.66–6.75 (d, $J$ = 6.0 Hz, 2H), 7.10–7.20 (m, 1H), 7.20–7.40 (m, 6H), 7.48 (s, 1H), 7.50–7.60 (d, $J$ = 9.0 Hz, 1H), 8.00–8.15 (d, $J$ = 9.0 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 25.8, 28.3, 28.4, 28.6, 28.8, 29.3, 33.1, 34.3, 36.8, 37.3, 53.5, 54.0, 67.7, 84.1, 115.6, 115.7, 116.0, 119.4, 123.1, 125.0, 126.8, 129.0, 129.1, 130.6, 130.7, 135.4, 135.8, 150.0, 155.9, 171.1, 171.2, 174.0; HRMS-FAB (M+Na$^+$) calcd for $BrC_{40}H_{48}NaN_3O_7$ 784.2573, found 784.2596.

**4.3.10.65. Compound 25a.** To a solution of **24a** (360 mg, 0.5 mmol) in acetone (20 mL) was added NaI (150 mg, 1.0 mmol). The reaction mixture was stirred under reflux for 2 h and cooled to room temperature. The white precipitate was removed by filtration, and the filtrate was concentrated by rotary evaporation. The crude material was purified by a short silica pack (EtOAc/hexanes, 1:1–2:1) to give the corresponding iodide (381 mg, 0.5 mmol, 100%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.50–1.80 (m, 13H), 2.00–2.20 (m, 2H), 2.85–2.95 (dd, $J$ = 4.8, 10.5 Hz, 1H), 2.95–3.30 (m, 5H), 4.75–4.87 (dd, $J$ = 4.5, 9.9 Hz, 1H), 4.88–4.95 (dd, $J$ = 5.7, 10.8 Hz, 1H), 5.00–5.20 (dd, $J$ = 9.0, 17.4 Hz, 1H), 6.55–6.65 (d, $J$ = 9.0, 6.3 Hz, 2H), 6.65–6.80 (m, 3H), 6.80–7.00 (br s, 1H), 7.10–7.20 (m, 1H), 7.25–7.30 (m, 1H), 7.31–7.40 (m, 5H), 7.45–7.55 (m, 2H), 7.81 (s, 1H), 8.00–8.20 (d, $J$ = 4.5 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 6.2, 14.3, 21.2, 24.0, 26.2, 28.2, 31.8, 32.5, 33.2, 35.0, 36.9, 53.1, 53.7, 60.6, 67.3, 77.4, 83.8, 115.3, 115.6, 119.0, 122.7, 124.6, 126.5, 128.6, 128.7, 130.3, 130.4, 135.1, 149.7, 155.6, 170.8, 171.2, 173.1; HRMS-FAB (M+Na$^+$) calcd for $C_{37}H_{42}INaN_3O_7$ 790.1965, found 790.1951. The iodide was redissolved in DMF (30 mL). To this solution was added $K_2CO_3$ (342 mg, 2.5 mmol). The reaction mixture was stirred at room temperature for 10 h and then diluted with EtOAc (200 mL). The organic layer was washed with 1 N HCl (3× 250 mL), saturated $NaHCO_3$ (200 mL), and brine (200 mL). The organic layer was dried over $MgSO_4$ and concentrated by rotary evaporation. The crude material was purified by flash chromatography (EtOAc/hexanes 1:1–2:1) to yield **25a** (224 mg, 0.35 mmol, 70%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.60–1.70 (m, 11H), 2.10–2.20 (m, 2H), 2.35–2.45 (dd, $J$ = 11.6, 13.4 Hz, 1H), 2.80–2.92 (dd, $J$ = 9.2, 14.0 Hz, 1H), 3.05–3.15 (dd, $J$ = 4.8, 14.0 Hz, 1H), 3.20–3.30 (dd, $J$ = 5.2, 13.6 Hz, 1H), 4.10–4.20 (td, $J$ = 4.0, 14.4 Hz, 1H), 4.25–4.32 (td, $J$ = 4.0, 14.0 Hz, 1H), 4.33–4.45 (td, $J$ = 4.0, 14.4 Hz, 1H), 4.85–4.95 (td, $J$ = 3.6, 14.4 Hz, 1H), 5.00–5.20 (dd, $J$ = 12.4, 24.0 Hz, 2H), 5.50–5.55 (d, $J$ = 9.6 Hz, 1H), 5.80–5.90 (d, $J$ = 9.6 Hz, 1H), 6.70–6.80 (m, 2H), 6.80–6.90 (dd, $J$ = 2.4, 12.4 Hz, 1H), 7.05–7.15 (dd, $J$ = 1.6, 8.4 Hz, 1H), 7.20–7.25 (m, 1H), 7.30–7.45 (m, 9H), 7.60–7.70 (d, $J$ = 8.0 Hz, 1H), 8.05–8.20 (br s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 21.8, 25.9, 28.2, 28.9, 36.3, 38.3, 52.3, 53.4, 67.2, 67.4, 77.3, 83.6, 115.1, 115.3, 115.7, 118.6, 119.1, 122.7, 124.5, 124.6, 128.1, 128.4, 128.5, 128.6, 128.7, 130.2, 130.3, 130.5, 131.1, 135.1, 149.6, 155.7, 169.8, 170.6, 172.0; HRMS-FAB (M+Na$^+$) calcd for $C_{37}H_{41}NaN_3O_7$ 662.2842, found 662.2858.

**4.3.10.66. Compound 25b.** Compound **25b** was synthesized starting with **24b** using a procedure that was similar to that used to prepare **25a** (245 mg, 0.38 mmol, 75%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.15–1.55 (m, 4H), 1.56–1.80 (m, 12H), 1.90–2.10 (m, 1H), 2.20–2.30 (m, 1H), 2.38–2.55 (dd, $J$ = 12.0, 14.6 Hz, 1H), 2.85–3.00 (dd, $J$ = 9.0, 15.0 Hz, 1H), 3.05–3.20 (dd, $J$ = 6.0, 15.0 Hz, 1H), 3.21–3.31 (dd, $J$ = 12.0, 14.6 Hz, 1H), 4.05–4.30 (m, 2H), 4.40–4.55 (m, 1H), 4.85–5.03 (m, 1H), 5.05–5.25 (m, 2H), 5.80–5.90 (d, $J$ = 9.0, 1H), 6.10–6.20 (d, $J$ = 6.0, 1H), 6.70–6.80 (d, $J$ = 9.0, 2H), 6.82–7.00 (m, 2H), 7.15–7.25 (m, 1H), 7.28–7.48 (m, 7H), 7.60–7.70 (d, $J$ = 6.0, 1H), 8.05–8.25 (d, $J$ = 9.0, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 24.7, 25.0, 28.2, 28.8, 28.9, 35.9, 38.0, 52.4, 52.7, 67.2, 67.7, 77.4, 83.5, 115.0, 115.3, 116.2, 119.2, 122.7, 124.5, 124.8, 127.7, 128.4, 128.6, 128.7, 130.3, 130.6, 135.2, 135.5, 149.6, 157.4, 169.9, 170.9, 171.9; HRMS-FAB (M+Na$^+$) calcd for $C_{38}H_{43}NaN_3O_7$ 676.2999, found 676.2977.

**4.3.10.67. Compound 25c.** Compound **25c** was synthesized starting with **24c** using a procedure that was similar to that used to prepare **25a** (259 mg, 0.38 mmol, 76%): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.10–1.50 (m, 9H), 1.50–1.80 (m, 12H), 1.90–2.02 (m, 1H), 2.04–2.20 (m, 1H), 2.60–2.70 (dd, $J$ = 8.0, 12.0 Hz, 1H), 2.95–3.07 (dd, $J$ = 8.0, 16.0 Hz, 1H), 3.07–3.15 (dd, $J$ = 4.0, 9.0 Hz, 1H), 3.25–3.35 (dd, $J$ = 4.0, 12.0 Hz, 1H), 3.90–4.10 (m, 2H), 4.60–4.70 (m, 1H), 4.85–4.95 (m, 1H), 5.17 (s, 2H), 5.80–5.90 (d, $J$ = 8.0 Hz, 1H), 6.00–6.10 (d, $J$ = 7.6 Hz, 1H), 6.55–6.65 (d, $J$ = 8.0 Hz, 2H), 6.66–6.75 (d, $J$ = 8.4 Hz, 2H), 7.20–7.30 (m, 2H), 7.30–7.45 (m, 4H), 8.48 (s, 1H), 7.60–7.70 (d, $J$ = 7.6 Hz, 1H), 8.10–8.25 (d, $J$ = 8.0 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 23.5, 24.4, 26.9, 27.4, 27.5, 27.8, 28.2, 36.4, 37.2, 52.7, 52.8, 60.4, 66.8, 67.3, 77.4, 83.7, 115.1, 115.2, 115.3, 119.2, 122.8, 124.7, 124.8, 127.3, 128.5, 128.6, 128.7, 128.8, 130.2, 130.3, 130.5, 135.2, 135.5, 149.6, 157.3, 170.1, 171.0, 172.7; HRMS-FAB (M+Na$^+$) calcd for $C_{40}H_{47}NaN_3O_7$ 704.3312, found 704.3329.

**4.3.10.68. Ketone 27a.** A solution of benzyl ester **25a** (192 mg, 0.30 mmol) in MeOH (20 mL) was hydrogenated over 10% $Pd(OH)_2$/C (20 mg) at room temperature for 4 h. The catalyst was filtered off and the solvent was removed by rotary evaporation to get the corresponding carboxylic acid. The carboxylic acid was dissolved in $CH_2Cl_2$ (10 mL). To this solution were added HOBt (122 mg, 0.90 mmol), EDC (173 mg, 0.90 mmol), DIEA (210 μL, 156 mg, 1.20 mmol) and **11a** (46 mg, 0.45 mmol) as a solution in DMF (100 μL). The reaction mixture was stirred at room temperature for 24 h then partitioned between $CH_2Cl_2$ (100 mL) and 1 N HCl (100 mL). The organic layer was washed with saturated $NaHCO_3$ (75 mL) and brine (75 mL), dried over $MgSO_4$, and concentrated. The crude material was purified by flash chromatography (EtOAc to 10% MeOH/$CH_2Cl_2$) to yield the corresponding alcohol **26a** (140 mg, 0.21 mmol, 70%) as a mixture of two diastereomers. To a solution of the resulting alcohol in $CH_2Cl_2$ (10 mL) was added Dess–Martin periodinane (135 mg, 0.32 mmol). The reaction mixture was stirred at room temperature for 4 h and then partitioned between $CH_2Cl_2$ (50 mL) and 10 % $Na_2S_2O_3$

(50 mL). The organic layer was washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$, and concentrated. The crude material was purified by flash chromatography (EtOAc/hexanes, 4:1) to yield ketone **27a** (105 mg, 0.17 mmol, 81%) as a mixture of two diastereomers: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.35–1.60 (m, 3H), 1.60–1.70 (m, 9H), 1.80–2.25 (m, 3H), 2.70–3.10 (m, 2H), 3.10–3.60 (m, 1H), 3.61–3.85 (m, 1H), 3.85–4.05 (m, 1H), 4.10–4.25 (m, 2H), 4.26–4.55 (m, 4H), 4.60–4.80 (m, 1H), 5.45–5.75 (m, 1H), 5.76–6.10 (m, 1H), 6.20–6.60 (m, 1H), 6.65–6.80 (m, 1H), 6.80–7.10 (m, 3H), 7.10–7.26 (m, 1H), 7.28–7.40 (m, 2H), 7.48–7.52 (m, 1H), 7.53–7.65 (m, 1H), 8.00–8.20 (m, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 21.9, 27.4, 27.9, 28.1, 28.4, 28.6, 35.6, 35.7, 36.7, 53.1, 54.5, 54.7, 55.3, 55.5, 68.1, 68.4, 68.9, 69.8, 70.4, 70.5, 84.8, 84.9, 115.4, 115.5, 115.9, 116.6, 117.5, 118.1, 118.3, 118.7, 118.8, 119.5, 119.6, 119.7, 123.1, 123.4, 124.5, 124.6, 124.7, 125.1, 125.4, 128.6, 128.7, 130.3, 130.4, 130.5, 131.5, 131.6, 135.6, 150.0, 157.4, 157.8, 170.5, 170.9, 171.1, 171.3, 171.6, 171.9, 173.3, 173.6, 210.8, 211.1; HRMS-FAB (M+Na$^+$) calcd for $C_{34}H_{40}NaN_4O_8$ 655.2744, found 655.2736.

**4.3.10.69. Ketone 27b.** Ketone **27b** was synthesized starting with **25b** using a procedure that was similar to that used to prepare **27a** (116 mg, 0.18 mmol, 48% for two steps): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.10–1.32 (m, 4H), 1.33–1.50 (m, 1H), 1.55–1.78 (m, 11H), 1.80–2.25 (m, 3H) 2.62–3.12 (m, 2H), 3.13–3.42 (m, 2H), 3.78–4.02 (m, 2H), 4.03–4.33 (m, 4H), 4.35–4.65 (m, 3H), 4.66–4.82 (m, 1H), 5.75–6.15 (m, 1H), 6.16–6.38 (m, 1H), 6.60–6.95 (m, 4H), 6.98–7.12 (m, 1H), 7.15–7.26 (m, 1H), 7.30–7.65 (m, 3H), 8.00–8.20 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 23.0, 24.0, 24.2, 25.0, 25.1, 25.3, 25.5, 27.6, 27.9, 28.2, 28.4, 28.6, 35.2, 35.4, 35.7, 35.8, 36.4, 52.8, 52.9, 53.1, 53.2, 53.8, 53.9, 54.4, 54.5, 54.9, 68.0, 68.2, 68.8, 68.9, 69.4, 69.6, 69.8, 70.1, 77.3, 84.0, 84.4, 115.0, 115.1, 115.4, 115.5, 116.1, 116.8, 116.9, 117.9, 118.1, 118.9, 119.0, 119.1, 122.8, 123.0, 124.2, 124.3, 124.7, 125.0, 125.1, 127.4, 127.6, 127.8, 130.2, 130.3, 130.4, 130.6, 130.8, 135.3, 149.6, 157.9, 158.0, 158.4, 159.5, 170.5, 170.6, 170.7, 170.8, 170.9, 171.2, 171.4, 171.6, 173.4, 173.6, 173.8, 173.9, 210.5, 210.9, 211.1; HRMS-FAB (M+Na$^+$) calcd for $C_{35}H_{42}NaN_4O_8$ 669.2900, found 669.2918.

**4.3.10.70. Ketone 27c.** Ketone **27c** was synthesized starting with **25c** using a procedure that was similar to that used to prepare **27a** (121 mg, 0.18 mmol, 52% for two steps): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.10–1.50 (m, 10H), 1.55–1.85 (m, 13H), 1.80–2.00 (m, 2H), 2.30–2.50 (m, 1H), 3.10–3.40 (m, 3H), 3.70–4.07 (m, 3H), 4.08–4.25 (m, 3H), 4.35–4.55 (m, 2H), 4.15–4.30 (m, 1H), 5.65–5.90 (m, 2H), 6.55–6.65 (m, 2H), 6.66–6.75 (m, 2H), 6.80–6.95 (m, 1H), 6.96–7.10 (m, 1H), 7.30–7.40 (m, 1H), 7.35–7.48 (m, 1H), 7.50–7.58 (m, 1H), 7.59–7.65 (m, 1H), 8.00–8.20 (m, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 23.5, 26.0, 26.2, 26.7, 26.8, 27.4, 27.5, 28.2, 35.2, 36.0, 52.1, 52.2, 54.1, 54.4, 54.5, 54.8, 66.4, 66.6, 68.8, 69.6, 70.1, 70.2, 84.5, 114.7, 114.8, 115.0, 115.2, 115.5, 115.6, 116.1, 118.9, 119.0, 119.1, 122.8, 123.2, 124.3, 124.8, 125.3, 125.4, 126.6, 126.7, 130.1, 130.4, 130.5, 130.7, 130.8, 135.4, 149.6, 157.7, 170.2, 170.3, 170.8, 171.1, 174.7, 174.8, 211.3, 211.8; HRMS-FAB (M+Na$^+$) calcd for $C_{37}H_{46}NaN_4O_8$ 697.3213, found 697.3223.

**4.3.10.71. Inhibitor 6a.** Compound **6a** was synthesized starting with **27a** using general method I (50 mg, 94 μmol, 78%) as a mixture of two diastereomers: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.10–1.65 (m, 4H), 1.66–1.95 (m, 1H), 1.96–2.15 (m, 1H), 2.35–3.50 (m, 1H), 2.65–2.95 (m, 2H), 2.98–3.10 (m, 1H), 3.70–3.90 (m, 2H), 3.91–4.20 (m, 4H), 4.22–4.40 (m, 2H), 4.45–4.75 (m, 1H), 6.55–6.82 (m, 1H), 6.83–7.10 (m, 2H), 7.11–7.35 (m, 3H), 7.36–7.50 (m, 1H), 7.51–7.70 (m, 1H), 7.95–8.50 (m, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 22.0, 26.2, 29.5, 35.4, 37.7, 37.8, 52.7, 53.7, 53.8, 54.1, 54.2, 54.3, 68.4, 69.0, 69.1, 70.1, 70.2, 110.5, 110.6, 110.7, 110.8, 111.7, 111.9, 118.0, 118.1, 118.56, 118.61, 118.8, 119.0, 119.1, 121.2, 121.3, 121.4, 123.8, 124.7, 128.0, 129.8, 129.9, 130.5, 131.8, 136.5, 136.6, 152.0, 155.5, 157.9, 171.5, 171.6, 171.7, 171.8, 171.9, 212.0, 212.1; HRMS-FAB (M+Na$^+$) calcd for $C_{29}H_{32}NaN_4O_6$ 555.2220, found 555.2232.

**4.3.10.72. Inhibitor 6b.** Compound **6b** was synthesized starting with **27b** using general method I (42 mg, 77 μmol, 64%): $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$; $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 23.7, 25.3, 26.0, 28.8, 29.3, 29.6, 34.6, 35.0, 36.9, 52.1, 52.9, 53.8, 53.9, 54.4, 54.6, 55.3, 67.7, 68.5, 69.4, 70.6, 110.6, 111.1, 112.0, 112.2, 115.4, 116.6, 116.9, 117.7, 118.7, 119.1, 121.7, 122.9, 124.2, 124.8, 128.0, 128.4, 129.1, 130.0, 130.8, 131.1, 131.4, 135.6, 136.8, 136.9, 152.3, 157.4, 157.8, 171.3, 171.5, 171.6, 171.7, 172.0, 172.1, 172.2, 172.3, 172.4, 212.5, 212.8; HRMS-FAB (M+Na$^+$) calcd for $C_{30}H_{34}NaN_4O_6$ 569.2376, found 569.2391.

**4.3.10.73. Inhibitor 6c.** Compound **6c** was synthesized starting with **27c** using general method I (46 mg, 81 μmol, 68%): $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.90–1.10 (m, 1H), 1.11–1.45 (m, 5H), 1.46–1.65 (m, 2H), 1.70–1.90 (m, 1H), 1.95–2.10 (m, 1H), 2.55–3.05 (m, 3H), 3.70–3.85 (m, 1H), 3.86–4.20 (m, 4H), 4.20–4.65 (m, 3H), 6.70–6.80 (m, 2H), 6.90–7.15 (m, 2H), 7.16–7.35 (m, 1H), 7.36–7.70 (m, 2H), 7.90–8.15 (m, 1H), 8.16–8.50 (m, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 24.2, 25.0, 27.1, 27.5, 28.0, 28.3, 28.6, 35.5, 37.1, 37.2, 52.2, 52.8, 53.0, 53.5, 53.6, 54.1, 54.2, 54.3, 66.8, 69.0, 69.2, 70.2, 70.3, 110.4, 110.5, 111.7, 115.2, 115.5, 115.8, 115.9, 116.6, 116.7, 118.6, 118.7, 118.8, 119.1, 121.3, 124.0, 124.5, 124.6, 124.7, 128.1, 129.6, 129.7, 130.6, 130.8, 131.1, 135.4, 136.5, 136.6, 152.0, 156.8, 171.1, 171.2, 171.6, 171.8, 171.9, 172.0, 212.1, 212.2; HRMS-FAB (M+Na$^+$) calcd for $C_{32}H_{38}NaN_4O_6$ 597.2689, found 597.2672.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.08.040.

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