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Macrocyclic inhibitors for the serine protease plasmin

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Abstract
Macrocyclic inhibitors for the serine protease plasmin were synthesized and evaluated. The inhibitors were constructed starting from a cyclohexanone core. This core was linked to either the *C*- or *N*-terminus of a peptide so that the inhibitors were designed to interact with the non-primed or primed binding sites of the protease. Macrocycles were prepared by connecting the side chain of Tyr or Trp, via a short linker, to one end of the peptide. The activities of the macrocyclic inhibitors, while modest, were up to 10-fold more potent than a related non-cyclic analog.

Keywords: *Serine protease, plasmin, macrocyclic, conformational constraint, inhibition*

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Introduction

Plasmin is a serine protease that plays important roles in the proteolytic modification of the extracellular matrix (ECM). These remodeling events are key steps involved in the cancer-related processes of angiogenesis and metastases [1–2]. Plasmin degrades a variety of ECM components, and also activates other important proteases such as the matrix metalloproteases (MMPs) 1, 3 and 9 [3]. The pivotal regulatory role of plasmin in the ECM remodeling process makes it a potential therapeutic target for the treatment of cancer.

Plasmin is also a key player in the dissolution of fibrin clots because it is the major enzyme responsible for cleaving fibrin. Plasminogen, the inactive precursor to plasmin, initially binds to fibrin via its lysine binding site. Plasminogen is then converted to active plasmin by several proteases including tissue plasminogen activator, urokinase, factor XIIa and kallikrein. The activated plasmin subsequently cleaves the fibrin mesh into smaller fragments. Several fibrinolysis inhibitors have been used clinically to reduce bleeding during surgery [4]. Aprotinin is a protein-based inhibitor that targets both plasmin and kallikrein, and is produced by Bayer under the name Trasylol. It is also known as bovine pancreatic trypsin inhibitor (BPTI). Aprotinin was recently withdrawn from the market because of concerns over side effects and increased risk of mortality. By contrast, small molecule antifibrinolytic drugs such as ϵ-aminocaproic acid and *trans*-4-(aminomethyl)cyclohexanecarboxylic acid (tranexamic acid) continue to be safe alternatives to aprotinin. However, these two small molecules target the lysine binding site of plasminogen, and do not influence the catalytic activity of activated plasmin. These observations highlight the need for development of new fibrinolysis inhibitors with a mechanism of action that targets the active site of plasmin and modifies its catalytic activity.

Over the last several years we have designed and synthesized a series of plasmin inhibitors **1** (Figure 1) [5]. These inhibitors were constructed around a cyclic ketone core, and were designed to react with the active site serine residue to give a reversibly formed hemiacetal linkage [5b]. The inhibitors also incorporated two peptide side chains, the identity of which were derived from the substrate specificity of plasmin. Several analogs of compound **1** showed potency in the low micromolar range and selectivities of greater than 100-fold for plasmin over other related serine proteases [5f]. However, peptidic inhibitors are often

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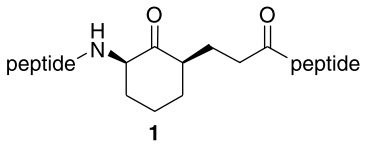

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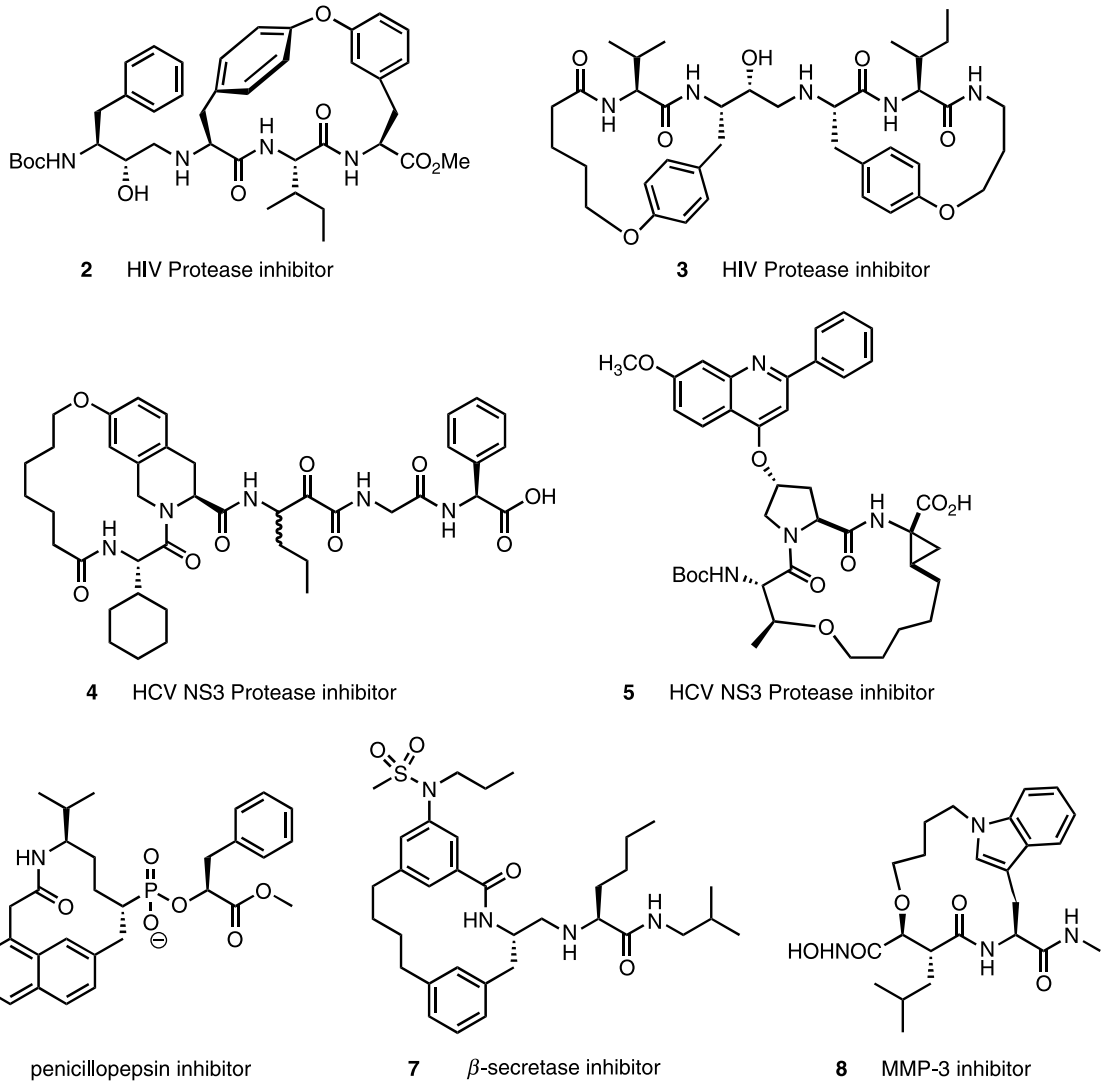
Figure 1. Structure of inhibitor **1**.

associated with undesirable pharmacokinetic properties including poor oral bioavailability and cell membrane permeability.

Macrocyclization of peptides has been a widely adopted strategy for designing peptidomimetic protease inhibitors with improved pharmacokinetic properties [6]. For example, compounds **2** [7] and **3** [8] are potent and selective inhibitors of the human immunodeficiency virus type 1 (HIV-1) protease (Figure 2). Compounds **4** [9] and **5** [10] are novel inhibitors of the hepatitis C virus (HCV) NS3 protease. Other macrocyclic peptidomimetics, such as compounds **6** [11], **7** [12] and **8** [13] inhibit the aspartic proteases β-secretase and penicillopepsin, and the metalloprotease MMP-3.

In addition to their desirable pharmacokinetic characters, macrocyclic compounds provide several other advantages over peptides. First, the macrocycle often preorganizes the molecule into an extended conformation, which can be an ideal conformation for binding to the target enzyme [6]. Second, the macrocycle decreases the conformational entropic penalty for binding to the enzyme when compared to more flexible non-cyclic analogs [14]. Consequently, macrocyclic inhibitors often display enhanced activities.

In previous studies we found that plasmin prefers to bind compounds with aromatic amino acids (e.g. Phe and Trp) at the P2 position, and the large aromatic amino acid Trp at the P2′ position (Figure 3) [5f]. We also found that plasmin prefers hydrophobic amino acids at both the P3 and P3′ positions. Based on these



2 HIV Protease inhibitor

3 HIV Protease inhibitor

4 HCV NS3 Protease inhibitor

5 HCV NS3 Protease inhibitor

6 penicillopepsin inhibitor

7 *β*-secretase inhibitor

8 MMP-3 inhibitor

Figure 2. Macrocyclic protease inhibitors.

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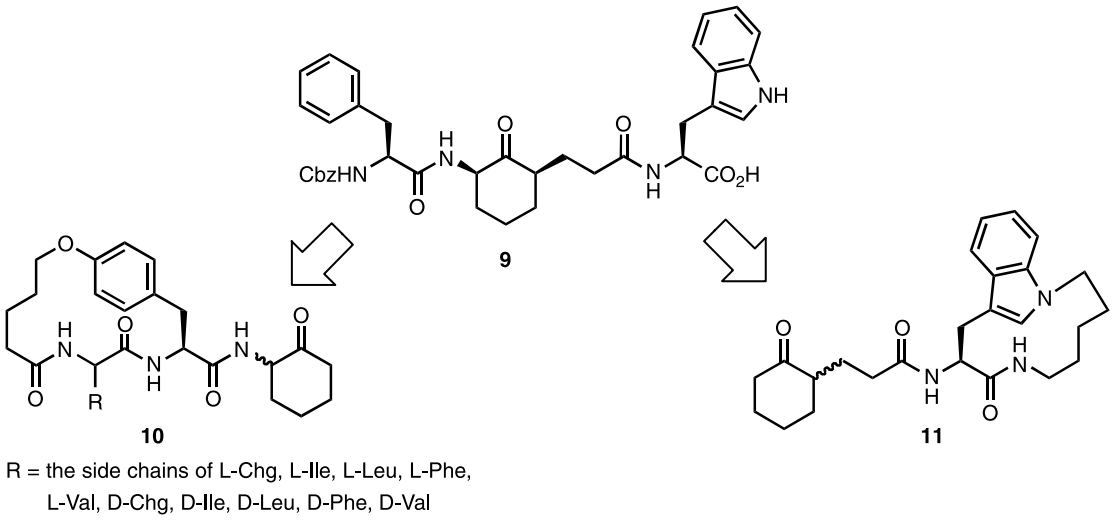

Figure 3. Development of the macrocyclic inhibitors **10** and **11**.

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results, we hypothesized that an intramolecular linkage between the P2 aromatic group and the *N*-terminus of P3, or between the P2′ Trp side chain and its *C*-terminus could provide favorable conformational constraints. These approaches, as shown in Figure 3, lead to the design of the macrocyclic inhibitors **10** and **11**.

Materials and 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, sodium carbonate 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 δ scale relative to δ = 0.00 ppm for TMS), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant (*J*/Hz), which were 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 δ scale, with an internal reference of δ 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.

Inhibition studies

Inhibitors **10a-j** and **11** were assayed against plasmin using H-D-Val-Ile-Lys-*p*NA (*p*NA = *p*-nitroanilide) as the substrate [5f]. Initial rates were measured using UV spectroscopy to monitor formation of *p*-nitroaniline (405 nm). The assay mixtures contained 50 mM sodium phosphate buffer at pH 7.4, and 10% DMSO to ensure solubility of the inhibitors. Under these conditions, the K_m value for the substrate was measured to be 170 μM.

Chemistry

*(*S*)-Benzyl 2-((*S*)-2-(*tert*-butoxycarbonylamino)-2-cyclohexylethanamido)-3-(4-hydroxyphenyl)propanoate (Boc-Chg-Tyr-OBn) (**12a**)*. Boc-Chg-OH (1.5 mmol) was dissolved in DMF (10 mL). To this solution was added H-Tyr-OBn (1.5 mmol), HBTU (758 mg, 2.0 mmol), and DIEA (530 μL, 390 mg, 3.0 mmol). The reaction was stirred at room temperature for 2 h, and then 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) to yield dipeptide **12a** (710 mg, 1.40 mmol, 93%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80-1.02 (m, 2H), 1.08-1.22 (m, 3H), 1.47 (s, 9H), 1.55-1.82 (m, 6H), 2.95-3.10 (d, *J* = 4.8 Hz, 2H), 3.80-4.00 (t, *J* = 8.0 Hz, 1H), 4.85-5.00 (dt, *J* = 3.6, 8.0 Hz, 1H), 5.05-5.25 (d, *J* = 12.0 Hz, 1H), 5.15-5.25 (d, *J* = 12.0 Hz, 1H), 5.25-5.35 (d, *J* = 9.2 Hz, 1H), 6.60-6.70 (d, *J* = 8.0 Hz, 2H), 6.80-6.90 (m, 3H), 5.30-5.45 (m, 5H), 7.66 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 25.8, 26.1, 28.4, 29.5, 37.1, 42.2, 53.4, 59.5, 67.3, 80.2, 115.6, 126.5, 128.6, 128.7, 130.4, 135.0, 155.6,

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156.2, 171.2, 171.6; HRMS-FAB (M + Na^+) calcd for $C_{29}H_{38}NaN_2O_6$ 533.2628, found 533.2638.

*(S)-Benzyl 2-((R)-2-(*tert*-butoxycarbonylamino)-2-cyclohexylethanamido)-3-(4-hydroxyphenyl)propanoate (Boc-D-Chg-Tyr-OBn)* ***(12b)***. Compound **12b** was synthesized using a procedure analogous to compound **12a** (665 mg, 1.30 mmol, 86%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05-1.12 (m, 1H), 1.13-1.25 (m, 2H), 1.46 (s, 9H), 1.55-1.65 (m, 3H), 1.65-1.75 (m, 3H), 1.80-1.90 (m, 2H), 2.90-3.10 (d, J = 6.8 Hz, 2H), 3.90-4.10 (t, J = 8.0 Hz, 1H), 4.85-4.95 (dd, J = 3.6, 8.0 Hz, 1H), 5.05-5.25 (m, 3H), 6.25-6.45 (br s, 1H), 6.55-6.61 (d, J = 7.8 Hz, 1H), 6.65-6.71 (d, J = 8.4 Hz, 2H), 6.85-6.95 (d, J = 8.0 Hz, 2H), 7.30-7.50 (m, 5H), 7.66 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 25.9, 26.0, 27.6, 28.3, 29.7, 37.2, 40.4, 53.2, 59.3, 67.4, 80.1, 115.6, 128.6, 128.7, 130.3, 135.0, 155.9, 156.2, 171.4, 171.5; HRMS-FAB (M + Na^+) calcd for $C_{29}H_{38}NaN_2O_6$ 533.2628, found 533.2643.

*(S)-Benzyl 2-((2S,3S)-2-(*tert*-butoxycarbonylamino)-3-methylpentanamido)-3-(4-hydroxyphenyl)propanoate (Boc-Ile-Tyr-OBn)* ***(12c)***. Compound **12c** was synthesized using a procedure analogous to compound **12a** (670 mg, 1.30 mmol, 86%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80-0.90 (m, 6H), 1.00-1.15 (m, 1H), 1.48 (s, 9H), 1.70-1.85 (m, 1H), 2.97-3.12 (d, J = 4.8 Hz, 2H), 3.85-4.00 (t, J = 8.0 Hz, 1H), 4.85-5.00 (td, J = 5.4, 8.0 Hz, 1H), 5.05-5.25 (m, 3H), 6.50-6.70 (d, J = 8.0 Hz, 2H), 6.75-6.90 (d, J = 8.0 Hz, 2H), 7.00-7.20 (br s, 1H), 7.30-7.50 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.2, 15.4, 24.7, 28.4, 53.3, 59.2, 76.7, 80.3, 115.5, 126.6, 128.6, 128.7, 130.4, 135.0, 155.4, 156.0, 171.1, 171.5; HRMS-FAB (M + Na^+) calcd for $C_{27}H_{36}NaN_2O_6$ 507.2471, found 507.2485.

*(S)-Benzyl 2-((2R,3R)-2-(*tert*-butoxycarbonylamino)-3-methylpentanamido)-3-(4-hydroxyphenyl)propanoate (Boc-D-Ile-Tyr-OBn)* ***(12d)***. Compound **12d** was synthesized using a procedure analogous to compound **12a** (700 mg, 1.37 mmol, 91%): ^{1}H NMR (400 MHz, CD_3OD) δ 0.70-0.90 (m, 6H), 0.93-1.10 (m, 1H), 1.30-1.50 (m, 11H), 1.60-1.75 (m, 1H), 2.85-2.95 (dd, J = 10.0, 12.0 Hz, 1H), 3.05-3.15 (dd, J = 8.0, 12.0 Hz, 1H), 3.90-4.00 (d, J = 7.6 Hz, 1H), 4.60-4.75 (m, 1H), 5.10-5.20 (dd, J = 10.0, 12.0 Hz, 2H), 6.65-6.75 (d, J = 8.0 Hz, 2H), 6.95-7.05 (d, J = 8.0 Hz, 2H), 7.25-7.45 (m, 5H); ^{13}C NMR (100 MHz, CD_3OD) δ 10.3, 14.4, 24.0, 27.3, 36.1, 37.2, 54.1, 59.1, 66.6, 79.2, 115.0, 127.1, 127.9, 128.2, 129.8, 135.7, 156.1, 156.4, 171.4, 172.9; HRMS-FAB (M + Na^+) calcd for $C_{27}H_{36}NaN_2O_6$ 507.2471, found 507.2468.

*(S)-Benzyl 2-((S)-2-(*tert*-butoxycarbonylamino)-4-methylpentanamido)-3-(4-hydroxyphenyl)propanoate (Boc-Leu-Tyr-OBn)* ***(12e)***. Compound **12e** was synthesized using a procedure analogous to compound **12a** (665 mg, 1.3 mmol, 86%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80-1.00 (d, J = 2.0 Hz, 6H), 1.47 (s, 9H), 1.50-1.70 (m, 2H), 2.97-3.12 (d, J = 4.8 Hz, 2H), 4.10-4.30 (m, 1H), 4.85-5.00 (td, J = 5.4, 8.0 Hz, 1H), 5.05-5.25 (m, 3H), 6.50-6.70 (d, J = 8.0 Hz, 2H), 6.75-6.90 (d, J = 8.0 Hz, 2H), 6.90-7.00 (d, J = 7.6 Hz, 1H), 7.30-7.50 (m, 5H), 7.50-7.90 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.0, 22.8, 24.7, 28.4, 37.0, 41.2, 53.0, 53.5, 67.3, 80.4, 115.5, 126.4, 128.6, 128.8, 130.4, 135.0, 155.5, 156.0, 171.2, 172.5; HRMS-FAB (M + Na^+) calcd for $C_{27}H_{36}NaN_2O_6$ 507.2471, found 507.2485.

*(S)-Benzyl 2-((R)-2-(*tert*-butoxycarbonylamino)-4-methylpentanamido)-3-(4-hydroxyphenyl)propanoate (Boc-D-Leu-Tyr-OBn)* ***(12f)***. Compound **12f** was synthesized using a procedure analogous to compound **12a** (690 mg, 1.37 mmol, 91%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80-0.90 (d, J = 6.4 Hz, 6H), 1.40-1.50 (m, 10H), 1.55-1.70 (m, 2H), 2.97-3.12 (m, 2H), 4.10-4.30 (m, 1H), 4.85-5.00 (td, J = 5.4, 8.0 Hz, 1H), 4.95-5.05 (d, J = 7.6 Hz, 1H), 5.05-5.15 (d, J = 12.4 Hz, 1H), 5.15-5.30 (d, J = 12.0 Hz, 1H), 6.60-6.70 (d, J = 7.6 Hz, 2H), 6.80-6.95 (m, 3H), 7.30-7.50 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.8, 22.9, 24.7, 28.3, 37.0, 41.1, 53.0, 53.3, 67.4, 80.4, 115.7, 126.7, 128.6, 128.7, 130.3, 135.0, 155.6, 155.8, 171.6, 172.7; HRMS-FAB (M + Na^+) calcd for $C_{27}H_{36}NaN_2O_6$ 507.2471, found 507.2490.

*(S)-Benzyl 2-((S)-2-(*tert*-butoxycarbonylamino)-3-phenylpropanamido)-3-(4-hydroxyphenyl)propanoate (Boc-Phe-Tyr-OBn)* ***(12g)***. Compound **12g** was synthesized using a procedure analogous to compound **12a** (770 mg, 1.50 mmol, 99%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (s, 9H), 2.90-3.12 (m, 4H), 4.40-4.50 (m, 1H), 4.70-4.90 (dd, J = 6.0, 12.8 Hz, 1H), 5.00-5.20 (d, J = 1.6 Hz, 1H), 6.50-6.60 (d, J = 7.6 Hz, 1H), 6.60-6.70 (d, J = 8.0 Hz, 2H), 6.70-6.80 (d, J = 8.4 Hz, 2H), 7.01 (s, 1H), 7.10-7.20 (m, 2H), 7.20-7.30 (m, 3H), 7.30-7.50 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.3, 37.1, 38.3, 53.4, 55.7, 67.3, 80.5, 95.7, 115.6, 126.6, 127.0, 128.6, 128.7, 129.3, 130.4, 135.0, 136.4, 155.5, 171.0, 171.3; HRMS-FAB (M + Na^+) calcd for $C_{30}H_{34}NaN_2O_6$ 541.2315, found 541.2325.

*(S)-Benzyl 2-((R)-2-(*tert*-butoxycarbonylamino)-3-phenylpropanamido)-3-(4-hydroxyphenyl)propanoate (Boc-D-Phe-Tyr-OBn)* ***(12h)***. Compound **12h** was synthesized using a procedure analogous to compound **12a** (725 mg, 1.40 mmol, 93%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (s, 9H), 2.80-2.90 (m, 1H), 2.90-3.05

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(m, 2H), 3.05-3.20 (m, 1H), 4.30-4.50 (m, 1H), 4.75-4.90 (dd, $J = 6.0$, 12.8 Hz, 1H), 5.00-5.20 (m, 3H), 6.40-6.50 (s, 1H), 6.50-6.60 (d, $J = 7.6$ Hz, 2H), 6.60-6.75 (m, 4H), 7.10-7.20 (d, $J = 7.2$ Hz, 2H), 7.20-7.30 (m, 5H), 7.30-7.50 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.3, 37.0, 53.2, 67.4, 115.6, 126.6, 127.0, 128.5, 128.6, 128.7, 129.3, 130.3, 135.0, 136.5, 155.3, 171.2, 171.3; HRMS-FAB ($M + Na^+$) calcd for $C_{30}H_{34}NaN_2O_6$ 541.2315, found 541.2298.

*(S)-Benzyl 2-((S)-2-(*tert*-butoxycarbonylamino)-3-methylbutanamido)-3-(4-hydroxyphenyl)propanoate (Boc-Val-Tyr-OBn) (**12i**)*. Compound **12i** was synthesized using a procedure analogous to compound **12a** (635 mg, 1.35 mmol, 90%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80-0.90 (d, $J = 6.4$ Hz, 3H), 0.90-1.00 (d, $J = 6.8$ Hz, 3H), 1.47 (s, 9H), 1.95-2.15 (m, 1H), 3.00-3.10 (d, $J = 4.8$ Hz, 2H), 3.80-3.95 (dd, $J = 8.0$, 8.4 Hz, 1H), 4.85-4.95 (dd, $J = 5.6$, 13.2 Hz, 1H), 5.05-5.25 (m, 3H), 6.45-6.55 (d, $J = 7.6$ Hz, 1H), 6.65-6.75 (d, $J = 12.0$ Hz, 2H), 6.75 (s, 1H), 6.75-6.90 (d, $J = 8.0$ Hz, 2H), 7.30-7.50 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.9, 19.2, 28.4, 31.0, 37.0, 53.3, 60.0, 67.3, 80.3, 115.6, 126.7, 128.6, 128.7, 130.4, 135.0, 155.3, 156.1, 171.2, 171.4; HRMS-FAB ($M + Na^+$) calcd for $C_{26}H_{34}NaN_2O_6$ 493.2315, found 493.2326.

*(S)-Benzyl 2-((R)-2-(*tert*-butoxycarbonylamino)-3-methylbutanamido)-3-(4-hydroxyphenyl)propanoate (Boc-D-Val-Tyr-OBn) (**12j**)*. Compound **12j** was synthesized using a procedure analogous to compound **12a** (635 mg, 1.35 mmol, 90%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80-0.90 (d, $J = 6.4$ Hz, 3H), 0.90-1.00 (d, $J = 6.8$ Hz, 3H), 1.47 (s, 9H), 1.95-2.15 (m, 1H), 3.00-3.10 (d, $J = 4.8$ Hz, 2H), 3.80-3.95 (dd, $J = 8.0$, 8.4 Hz, 1H), 4.85-4.95 (dd, $J = 5.6$, 13.2 Hz, 1H), 5.05-5.25 (m, 3H), 6.45-6.55 (d, $J = 7.6$ Hz, 1H), 6.65-6.75 (d, $J = 12.0$ Hz, 2H), 6.75 (s, 1H), 6.75-6.90 (d, $J = 8.0$ Hz, 2H), 7.30-7.50 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.9, 19.2, 28.4, 31.0, 37.0, 53.3, 60.0, 67.3, 80.3, 115.6, 126.7, 128.6, 128.7, 130.4, 135.0, 155.3, 156.1, 171.2, 171.4; HRMS-FAB ($M + Na^+$) calcd for $C_{26}H_{34}NaN_2O_6$ 493.2315, found 493.2330.

*(S)-Benzyl 2-((S)-2-cyclohexyl-2-(5-iodopentanamido)ethanamido)-3-(4-hydroxyphenyl)propanoate (**13a**)*. To a solution of compound **12a** (510 mg, 1.0 mmol) in CH_2Cl_2 (10 mL) was added TFA (5 mL). The reaction was stirred at room temperature for 30 min, and the solvent was removed by rotary evaporation to yield the free amine. To a solution of the resulting amine 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 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 the corresponding primary bromide (535 mg, 0.95 mmol, 95%). This compound (280 mg, 0.5 mmol) was dissolved in acetone (20 mL) and NaI (150 mg, 1.0 mmol) was added. The reaction 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 passing it through a short silica plug (EtOAc:hexanes 1:1-2:1) to give the corresponding primary iodide **13a** (310 mg, 0.5 mmol, 100%): ^{1}H NMR (400 MHz, CD_3OD) δ 0.85-1.10 (m, 3H), 1.10-1.30 (m, 3H), 1.50-1.90 (m, 11H), 2.10-2.30 (br s, 2H), 2.80-2.95 (m, 1H), 2.96-3.10 (m, 1H), 3.20-3.30 (m, 1H), 3.31-3.40 (s, 2H), 4.10-4.22 (m, 1H), 4.60-4.70 (m, 1H), 5.00-5.20 (s, 2H), 6.60-6.75 (d, $J = 7.6$, 2H), 6.90-7.05 (d, $J = 7.6$, 2H), 7.25-7.50 (m, 5H), 7.80-8.00 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 4.7, 25.6, 25.9, 26.4, 28.5, 29.3, 32.8, 34.1, 36.3, 39.8, 54.1, 58.0, 66.6, 114.8, 127.1, 127.9, 128.1, 128.2, 130.0, 135.6, 156.0, 171.3, 172.1, 174.0; HRMS-FAB ($M + Na^+$) calcd for $C_{29}H_{37}INaN_2O_5$ 643.1645, found 643.1660.

*(S)-Benzyl 2-((R)-2-cyclohexyl-2-(5-iodopentanamido)ethanamido)-3-(4-hydroxyphenyl)propanoate (**13b**)*. The synthesis of compound **13b** was analogous to the procedure used for compound **13a** (280 mg, 0.45 mmol, 90% for two steps): ^{1}H NMR (400 MHz, CD_3OD) δ 0.70-1.00 (m, 2H), 1.00-1.25 (m, 3H), 1.26-1.40 (m, 1H), 1.45-1.75 (m, 7H), 1.76-2.00 (m, 2H), 2.15-2.30 (m, 2H), 2.75-2.95 (m, 1H), 3.10-3.40 (m, 3H), 4.10-4.30 (m, 1H), 4.60-4.75 (m, 1H), 5.00-5.20 (m, 2H), 6.50-6.75 (d, $J = 7.6$, 2H), 6.90-7.10 (d, $J = 7.6$, 2H), 7.25-7.50 (m, 5H); ^{13}C NMR (75 MHz, CD_3OD) δ 5.0, 26.0, 26.2, 26.8, 28.5, 29.6, 33.1, 34.5, 36.4, 40.2, 40.4, 54.3, 58.1, 66.9, 115.2, 115.3, 127.6, 128.2, 128.4, 128.5, 128.6, 130.1, 130.3, 136.2, 156.5, 171.7, 172.4, 174.3; HRMS-FAB ($M + Na^+$) calcd for $C_{29}H_{37}INaN_2O_5$ 643.1645, found 643.1652.

*(S)-Benzyl 3-(4-hydroxyphenyl)-2-((S)-2-(5-iodopentanamido)-4-methylpentanamido)propanoate (**13e**)*. The synthesis of compound **13e** was analogous to the procedure used for compound **13a** (280 mg, 0.47 mmol, 95% for two steps): ^{1}H NMR (300 MHz, CD_3OD) δ 0.80-0.95 (m, 6H), 1.30-1.50 (m, 3H), 1.55-1.90 (m, 4H), 2.10-2.30 (m, 2H), 2.80-3.00 (m, 1H), 3.05-3.20 (m, 1H), 4.35-4.50 (dd, $J = 4.8$, 7.5 Hz; 1H), 4.60-4.75 (dd, $J = 5.7$, 7.2 Hz; 1H), 5.00-5.20 (d, $J = 4.2$ Hz, 2H), 6.60-6.70 (d, $J = 8.1$ Hz, 2H), 6.90-7.00

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(d, $\mathcal{J}$ = 8.1 Hz, 2H), 7.25-7.40 (m, 5H), 7.95-8.15 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 5.3, 21.2, 22.5, 24.8, 26.8, 33.1, 34.6, 36.6, 41.0, 51.9, 54.4, 67.0, 115.4, 127.3, 128.3, 128.4, 128.6, 130.4, 136.0, 156.5, 171.7, 173.7, 174.4; HRMS-FAB (M + Na^+) calcd for $C_{27}H_{35}INaN_2O_5$ 617.1488, found 617.1492.

*(S)-Benzyl 3-(4-hydroxyphenyl)-2-((S)-2-(5-iodopentanamido)-3-phenylpropanamido)propanoate (**13g**)*. The synthesis of compound **13g** was analogous to the procedure used for compound **13a** (280 mg, 0.45 mmol, 90% for two steps): ^{1}H NMR (400 MHz, CD_3OD) δ 1.45-1.70 (m, 4H), 2.10-2.20 (m, 2H), 2.70-2.80 (dd, $\mathcal{J}$ = 4.0, 14.0 Hz, 1H), 2.85-2.95 (m, 1H), 3.00-3.20 (m, 4H), 4.60-4.75 (m, 2H), 5.05-5.15 (s, 2H), 6.60-6.70 (dd, $\mathcal{J}$ = 2.0, 6.8 Hz, 2H), 6.90-7.00 (dd, $\mathcal{J}$ = 2.0, 6.8 Hz, 2H), 7.15-7.27 (m, 5H), 7.27-7.45 (m, 5H); ^{13}C NMR (100 MHz, CD_3OD) δ 4.8, 26.2, 32.3, 34.1, 36.2, 37.4, 54.1, 54.2, 66.6, 114.9, 126.3, 127.0, 127.9, 128.0, 128.1, 128.2, 128.9, 135.6, 137.1, 156.1, 171.2, 172.2, 173.9; HRMS-ESI (M + Na^+) calcd for $C_{30}H_{33}INaN_2O_5$ 651.1332, found 651.1347.

*(S)-Benzyl 3-(4-hydroxyphenyl)-2-((R)-2-(5-iodopentanamido)-3-phenylpropanamido)propanoate (**13h**)*. The synthesis of compound **13h** was analogous to the procedure used for compound **13a** (270 mg, 0.44 mmol, 88% for two steps): ^{1}H NMR (400 MHz, CD_3OD) δ 1.45-1.60 (m, 4H), 2.05-2.20 (m, 2H), 2.65-2.75 (dd, $\mathcal{J}$ = 9.2, 13.6 Hz, 1H), 2.80-2.95 (dd, $\mathcal{J}$ = 9.2, 13.6 Hz, 1H), 2.92-3.05 (m, 2H), 3.06-3.20 (m, 2H), 4.50-4.80 (m, 2H), 5.00-5.20 (m, 2H), 6.60-6.70 (d, $\mathcal{J}$ = 8.4 Hz, 2H), 6.80-7.00 (d, $\mathcal{J}$ = 8.4 Hz, 2H), 7.05-7.15 (d, $\mathcal{J}$ = 7.2 Hz, 2H), 7.15-7.90 (m, 8H); ^{13}C NMR (100 MHz, CD_3OD) δ 4.9, 26.2, 32.3, 34.1, 36.2, 37.6, 54.0, 66.6, 115.0, 126.4, 126.9, 128.0, 128.1, 128.2, 128.9, 130.0, 135.7, 137.0, 156.1, 171.2, 172.0, 173.9; HRMS-ESI (M + Na^+) calcd for $C_{30}H_{33}INaN_2O_5$ 651.1332, found 651.1346.

*(S)-Benzyl 3-(4-hydroxyphenyl)-2-((S)-2-(5-iodopentanamido)-3-methylbutanamido)propanoate (**13i**)*. The synthesis of compound **13i** was analogous to the procedure used for compound **13a** (265 mg, 0.45 mmol, 91% for two steps): ^{1}H NMR (400 MHz, CD_3OD) δ 0.85-0.95 (d, $\mathcal{J}$ = 6.8 Hz, 6H), 1.65-1.75 (m, 1H), 1.76-1.90 (m, 1H), 1.95-2.05 (m, 1H), 2.20-2.30 (dt, $\mathcal{J}$ = 0.9, 6.8 Hz, 2H), 2.85-2.95 (dd, $\mathcal{J}$ = 8.0, 11.2 Hz, 1H), 2.95-3.05 (dd, $\mathcal{J}$ = 6.4, 14.0 Hz, 1H), 3.20-3.30 (m, 2H), 4.10-4.20 (d, $\mathcal{J}$ = 7.6 Hz, 1H), 4.60-4.70 (dd, $\mathcal{J}$ = 6.0, 7.6 Hz, 1H), 5.05-5.15 (d, $\mathcal{J}$ = 0.9 Hz, 2H), 6.60-6.70 (dd, $\mathcal{J}$ = 2.0, 6.4 Hz, 2H), 6.90-7.00 (dd, $\mathcal{J}$ = 2.0, 6.4 Hz, 2H), 7.25-7.30 (m, 2H), 7.30-7.40 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 4.7, 17.4, 18.3, 26.4, 30.5, 32.7, 34.1, 36.3, 54.2, 58.6, 66.6, 114.9, 127.1, 128.0, 128.1, 128.2, 130.0, 135.6, 156.0, 171.3, 172.2, 174.1; HRMS-FAB (M + Na^+) calcd for $C_{26}H_{33}INaN_2O_5$ 603.1332, found 603.1351.

*(S)-Benzyl 3-(4-hydroxyphenyl)-2-((R)-2-(5-iodopentanamido)-3-methylbutanamido)propanoate (**13j**)*. The synthesis of compound **13j** was analogous to the procedure used for compound **13a** (260 mg, 0.45 mmol, 90% for two steps): ^{1}H NMR (400 MHz, CD_3OD) δ 0.70-0.85 (d, $\mathcal{J}$ = 6.8 Hz, 6H), 1.65-1.75 (m, 1H), 1.76-1.85 (m, 1H), 1.86-2.00 (m, 1H), 2.15-2.35 (m, 2H), 2.80-2.90 (dd, $\mathcal{J}$ = 4.8, 14.0 Hz, 1H), 3.00-3.10 (dd, $\mathcal{J}$ = 4.2, 13.6 Hz, 1H), 3.15-3.25 (t, $\mathcal{J}$ = 6.8 Hz, 2H), 3.30-3.50 (m, 1H), 4.20-4.30 (dd, $\mathcal{J}$ = 7.2, 8.4 Hz, 1H), 4.60-4.70 (m, 1H), 5.05-5.15 (d, $\mathcal{J}$ = 5.6 Hz, 2H), 6.60-6.70 (d, $\mathcal{J}$ = 8.4 Hz, 2H), 6.90-7.00 (d, $\mathcal{J}$ = 8.4 Hz, 2H), 7.25-7.40 (m, 5H), 7.85-7.95 (d, $\mathcal{J}$ = 8.8 Hz, 1H), 8.15-8.25 (d, $\mathcal{J}$ = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 4.9, 17.0, 18.4, 26.5, 30.6, 32.7, 34.2, 36.2, 54.0, 54.1, 58.4, 58.5, 66.6, 115.0, 127.1, 127.9, 128.0, 128.2, 129.9, 135.7, 156.1, 171.31, 171.33, 172.3, 174.2; HRMS-FAB (M + Na^+) calcd for $C_{26}H_{33}INaN_2O_5$ 603.1332, found 603.1355.

*Benzyl ester **14a***. The iodide **13a** (310 mg, 0.5 mmol) was dissolved in DMF (30 mL). To this solution was added K_2CO_3 (342 mg, 2.5 mmol). The reaction 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 **14a** (172 mg, 0.35 mmol, 70%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85-1.00 (m, 2H), 1.00-1.20 (m, 3H), 1.30-1.50 (m, 3H), 1.51-1.80 (m, 7H), 2.00-2.10 (m, 1H), 2.15-2.20 (m, 1H), 2.50-2.60 (t, $\mathcal{J}$ = 13.2 Hz, 1H), 3.40-3.50 (dd, $\mathcal{J}$ = 4.8, 13.2 Hz, 1H), 3.96-4.06 (t, $\mathcal{J}$ = 8.0 Hz, 1H), 4.10-4.30 (m, 2H), 5.00-5.10 (m, 1H), 5.15-5.20 (d, $\mathcal{J}$ = 12.4 Hz, 1H), 5.21-5.30 (d, $\mathcal{J}$ = 12.4 Hz, 1H), 5.70-5.80 (d, $\mathcal{J}$ = 8.8 Hz, 1H), 6.10-6.20 (d, $\mathcal{J}$ = 10.0 Hz, 1H), 6.70-6.80 (dd, $\mathcal{J}$ = 2.4, 8.0 Hz, 1H), 6.80-6.90 (ddd, $\mathcal{J}$ = 2.0, 8.4, 13.6 Hz, 2H), 7.15-7.25 (dd, $\mathcal{J}$ = 2.0, 8.4 Hz, 1H), 7.30-7.50 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.7, 25.8, 25.9, 26.0, 28.8, 29.2, 36.1, 38.4, 41.2, 52.4, 57.6, 67.4, 67.8, 116.5, 118.9, 128.4, 128.5, 128.7, 128.8, 130.0, 131.2, 135.1, 155.6, 170.2, 171.3, 172.0; HRMS-FAB (M + Na^+) calcd for $C_{29}H_{36}NaN_2O_5$ 515.2522, found 515.2531.

*Benzyl ester **14b***. Compound **14b** was synthesized using a procedure analogous to benzyl ester **14a** (184 mg, 0.38 mmol, 75%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.70-0.95 (m, 2H), 1.00-1.15 (m, 1H), 1.16-1.30 (m, 2H),

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1.35-1.75 (m, 11H), 1.80-2.00 (m, 3H), 2.05-2.20 (m, 1H), 3.15-3.25 (d, $J = 5.6$ Hz, 2H), 4.10-4.25 (m, 2H), 4.30-4.45 (m, 1H), 4.55-4.65 (dd, $J = 6.8$, 13.6 Hz, 1H), 5.05-5.15 (d, $J = 8.8$ Hz, 1H), 5.15-5.25 (d, $J = 12.0$ Hz, 1H), 5.26-5.35 (d, $J = 12.4$ Hz, 1H), 5.80-5.90 (d, $J = 6.8$ Hz, 1H), 6.88 (s, 2H), 6.95-7.00 (d, $J = 6.8$ Hz, 1H), 7.01-7.10 (d, $J = 8.0$ Hz, 1H), 7.30-7.50 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.8, 25.9, 26.1, 27.6, 27.9, 29.9, 34.0, 35.5, 38.8, 52.5, 58.4, 67.4, 68.5, 118.1, 118.4, 128.3, 128.6, 128.7, 130.2, 130.7, 158.0, 170.9, 171.5, 172.3; HRMS-FAB ($M + Na^+$) calcd for $C_{29}H_{36}NaN_2O_5$ 515.2522, found 515.2536.

*Benzyl ester **14c***. Compound **14c** was synthesized using a procedure analogous to benzyl ester **14a** (140 mg, 0.30 mmol, 60%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.70-0.90 (m, 6H), 0.95-1.10 (m, 1H), 1.30-1.55 (m, 3H), 1.56-1.85 (m, 4H), 1.95-2.10 (m, 1H), 2.12-2.30 (m, 1H), 2.45-2.60 (m, 1H), 3.35-3.50 (dd, $J = 5.6$, 9.9 Hz, 1H), 3.95-4.05 (t, $J = 8.4$ Hz, 1H), 4.10-4.35 (m, 2H), 5.00-5.15 (m, 1H), 5.15-5.25 (d, $J = 12.0$ Hz, 1H), 5.26-5.35 (d, $J = 12.4$ Hz, 1H), 5.60-5.75 (d, $J = 6.8$ Hz, 1H), 5.80-5.90 (d, $J = 8.4$ Hz, 1H), 6.25-6.35 (m, 1H), 6.80-7.00 (m, 2H), 7.15-7.25 (m, 1H), 7.30-7.50 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.3, 15.0, 21.6, 25.1, 26.0, 36.1, 38.1, 38.4, 52.4, 57.3, 67.5, 67.8, 115.4, 116.5, 118.9, 128.4, 128.5, 128.6, 128.7, 128.8, 130.0, 130.9, 131.2, 135.1, 155.7, 170.1, 171.3, 171.9; HRMS-FAB ($M + Na^+$) calcd for $C_{27}H_{34}NaN_2O_5$ 489.2365, found 489.2380.

*Benzyl ester **14d***. Compound **14d** was synthesized using a procedure analogous to benzyl ester **14a** (155 mg, 0.33 mmol, 65%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80-0.90 (m, 6H), 0.90-1.00 (m, 1H), 1.30-1.55 (m, 3H), 1.56-1.75 (m, 1H), 1.80-2.00 (m, 3H), 2.05-2.20 (m, 1H), 3.15-3.25 (d, $J = 8.8$ Hz, 2H), 4.15-4.25 (m, 2H), 4.30-4.45 (m, 1H), 4.50-4.70 (dd, $J = 8.0$, 12.4 Hz, 1H), 5.10-5.25 (m, 2H), 5.26-5.35 (d, $J = 12.4$ Hz, 1H), 5.85-5.95 (d, $J = 8.0$ Hz, 1H), 6.80-6.90 (m, 2H), 6.95-7.00 (m, 1H), 7.00-7.10 (m, 1H), 7.30-7.50 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.4, 15.7, 20.7, 24.5, 27.6, 34.0, 35.5, 35.7, 52.5, 58.4, 67.4, 68.5, 118.2, 118.4, 128.3, 128.6, 128.7, 130.2, 130.7, 135.3, 158.0, 170.9, 171.5, 172.4; HRMS-FAB ($M + Na^+$) calcd for $C_{27}H_{34}NaN_2O_5$ 489.2365, found 489.2375.

*Benzyl ester **14e***. Compound **14e** was synthesized using a procedure analogous to benzyl ester **14a** (155 mg, 0.33 mmol, 65%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80-0.90 (d, $J = 6.3$ Hz, 6H), 1.30-1.55 (m, 5H), 1.60-1.85 (m, 2H), 1.90-2.10 (m, 2H), 2.10-2.25 (m, 1H), 2.50-2.65 (t, $J = 9.3$ Hz, 1H), 3.35-3.50 (dd, $J = 4.8$, 13.5 Hz, 1H), 4.10-4.35 (m, 3H), 4.90-5.10 (m, 1H), 5.15-5.22 (d, $J = 12.3$ Hz, 1H), 5.23-5.35 (d, $J = 12.3$ Hz, 1H), 5.55-5.65 (d, $J = 8.7$ Hz, 1H), 6.00-6.10 (d, $J = 9.6$ Hz, 1H), 6.70-7.00 (m, 3H), 7.15-7.25 (d, $J = 8.4$ Hz, 1H), 7.30-7.50 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.9, 22.8, 23.0, 25.0, 26.5, 36.3, 38.5, 42.8, 51.5, 52.9, 67.8, 68.3, 117.4, 119.1, 128.8, 129.0, 129.1, 130.3, 131.6, 135.5, 156.2, 171.6, 171.8, 172.1; HRMS-FAB ($M + Na^+$) calcd for $C_{27}H_{34}NaN_2O_5$ 489.2365, found 489.2381.

*Benzyl ester **14f***. Compound **14f** was synthesized using a procedure analogous to benzyl ester **14a** (128 mg, 0.28 mmol, 55%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80-0.95 (m, 6H), 1.30-1.55 (m, 4H), 1.65-1.75 (m, 2H), 1.80-1.95 (m, 2H), 2.00-2.20 (m, 1H), 3.15-3.25 (d, $J = 6.4$ Hz, 2H), 4.15-4.25 (m, 1H), 4.30-4.40 (m, 2H), 4.50-4.60 (dd, $J = 4.4$, 5.2 Hz, 1H), 5.10-5.20 (m, 2H), 5.21-5.30 (d, $J = 12.0$ Hz, 1H), 5.95-6.05 (d, $J = 7.2$ Hz, 1H), 6.80-6.9 (m, 2H), 6.95-7.05 (dd, $J = 2.0$, 8.4 Hz, 1H), 7.05-7.10 (dd, $J = 1.6$, 8.4 Hz, 1H), 7.30-7.50 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 21.7, 22.9, 24.8, 27.6, 33.9, 35.5, 40.0, 51.8, 52.8, 67.4, 68.5, 118.3, 118.6, 128.4, 128.6, 128.7, 130.2, 130.7, 135.4, 157.9, 171.4, 171.8, 172.4; HRMS-FAB ($M + Na^+$) calcd for $C_{27}H_{34}NaN_2O_5$ 489.2365, found 489.2375.

*Benzyl ester **14g***. Compound **14g** was synthesized using a procedure analogous to benzyl ester **14a** (150 mg, 0.30 mmol, 60%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30-1.55 (m, 1H), 1.60-1.80 (m, 2H), 1.81-2.00 (m, 1H), 2.00-2.25 (m, 2H), 2.40-2.60 (m, 1H), 2.74-3.00 (m, 2H), 3.00-3.15 (m, 1H), 3.25-3.40 (dd, $J = 5.2$, 14.0 Hz, 1H), 4.10-4.30 (m, 1H), 4.30-4.40 (m, 1H), 5.20-5.35 (m, 1H), 6.55-6.75 (m, 1H), 6.76-6.90 (m, 2H), 7.05-7.25 (m, 6H), 7.30-7.50 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.7, 26.0, 36.0, 38.3, 39.2, 52.5, 54.3, 67.3, 67.6, 115.6, 116.2, 118.6, 127.0, 128.2, 128.4, 128.5, 128.6, 128.7, 128.8, 129.3, 129.4, 130.4, 131.1, 135.2, 136.2, 155.8, 169.9, 170.9, 172.0; HRMS-FAB ($M + Na^+$) calcd for $C_{30}H_{32}NaN_2O_5$ 523.2209, found 523.2220.

*Benzyl ester **14h***. Compound **14h** was synthesized using a procedure analogous to benzyl ester **14a** (125 mg, 0.25 mmol, 50%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20-1.50 (m, 3H), 1.50-1.65 (m, 1H), 1.70-1.90 (m, 3H), 1.90-2.00 (m, 1H), 2.90-3.00 (m, 1H), 3.00-3.30 (m, 3H), 4.10-4.20 (m, 1H), 4.20-4.30 (m, 1H), 5.00-5.30 (m, 3H), 5.80-5.90 (d, $J = 8.8$ Hz, 1H), 6.70-6.80 (m, 1H), 6.80-6.85 (m, 1H), 6.95-7.00 (m, 1H), 7.00-7.05 (m, 1H), 7.10-7.15 (m, 2H), 7.20-7.25 (m, 4H), 7.30-7.50 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 27.6, 34.1, 35.5, 36.4, 52.6, 54.0, 67.4, 68.4, 118.6, 118.7, 126.9, 128.1, 128.6, 128.7, 129.3, 129.9, 131.0, 135.3, 136.6, 157.9, 170.9, 171.4, 172.5; HRMS-FAB

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($M + Na^+$) calcd for $C_{30}H_{32}NaN_2O_5$ 523.2209, found 523.2222.

*Benzyl ester **14i***. Compound **14i** was synthesized using a procedure analogous to benzyl ester **14a** (158 mg, 0.35 mmol, 70%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80-0.90 (d, J = 6.8 Hz, 6H), 1.80-2.00 (m, 2H), 1.70-1.82 (m, 2H), 1.83-1.90 (m, 1H), 2.00-2.10 (m, 1H), 2.15-2.25 (m, 1H), 2.50-2.60 (t, J = 12.8 Hz, 1H), 3.40-3.50 (dd, J = 4.8, 13.6 Hz, 1H), 3.95-4.05 (dd J = 7.6, 8.4 Hz, 1H), 4.10-4.30 (m, 2H), 5.00-5.10 (m, 1H), 5.15-5.25 (d, J = 12.0 Hz, 1H), 5.25-5.35 (d, J = 12.0 Hz, 1H), 5.70-5.80 (d, J = 8.8 Hz, 1H), 6.10-6.15 (d, J = 10.0 Hz, 1H), 6.75-6.82 (dd, J = 2.4, 8.4 Hz, 1H), 6.85-6.95 (m, 2H), 7.17-7.25 (dd, J = 2.4, 8.8 Hz, 1H), 7.30-7.50 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.5, 18.8, 21.7, 26.4, 31.8, 36.2, 52.5, 58.1, 67.5, 116.6, 118.9, 128.6, 128.7, 128.8, 130.0, 131.2, 155.7, 170.2, 171.4, 172.0; HRMS-FAB ($M + Na^+$) calcd for $C_{26}H_{32}NaN_2O_5$ 475.2209, found 475.2221.

*Benzyl ester **14j***. Compound **14j** was synthesized using a procedure analogous to benzyl ester **14a** (158 mg, 0.35 mmol, 70%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.75-0.85 (d, J = 6.8 Hz, 3H), 0.85-0.95 (d, J = 6.8 Hz, 3H), 1.40-1.60 (m, 2H), 1.60-1.70 (m, 1H), 1.80-2.12 (m, 3H), 2.10-2.20 (m, 1H), 2.22-2.32 (m, 1H), 3.15-3.30 (dd, J = 1.6, 7.6 Hz, 1H), 4.10-4.15 (dd, J = 6.4, 8.4 Hz, 1H), 4.15-4.25 (m, 1H), 4.30-4.40 (m, 1H), 4.50-4.60 (dd, J = 6.8, 13.6 Hz, 1H), 5.10-5.20 (d J = 12.0 Hz, 1H), 5.20-5.30 (m, 2H), 5.95-6.05 (d, J = 7.2 Hz, 1H), 6.80-6.90 (m, 2H), 6.92-7.00 (dd, J = 3.2, 11.6 Hz, 1H), 7.00-7.05 (dd, J = 3.2, 11.6 Hz, 1H), 7.30-7.50 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.6, 19.4, 20.7, 27.6, 29.2, 33.9, 35.5, 52.5, 59.1, 67.4, 68.5, 118.2, 118.4, 128.3, 128.6, 128.7, 130.3, 130.7, 135.3, 158.0, 171.1, 171.5, 172.5; HRMS-FAB ($M + Na^+$) calcd for $C_{26}H_{32}NaN_2O_5$ 475.2209, found 475.2225.

*Amide **17a***. A solution benzyl ester **14a** (150 mg, 0.30 mmol) in MeOH (20 mL) was hydrogenated using 1 atm of H_2 gas over 10% $Pd(OH)_2$/C (20 mg) at room temperature for 4 h. The catalyst was removed by filtration and the solvent was removed by rotary evaporation to give the corresponding carboxylic acid **15a**. The carboxylic acid was dissolved in CH_2Cl_2 (10 mL). To this solution were added HBTU (340 mg, 0.90 mmol), DIEA (210 μL, 156 mg, 1.20 mmol) and primary amine **16** (46 mg, 0.45 mmol) as a solution in DMF (100 μL). The reaction 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 (gradient of 100% EtOAc to 10% MeOH/CH_2Cl_2) to yield the corresponding amide **17a** (118 mg, 0.21 mmol, 70%) as a mixture of two diastereomers: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85-1.05 (m, 3H), 1.06-1.20 (m, 4H), 1.30-1.55 (m, 9H), 1.56-1.75 (m, 9H), 1.76-1.85 (m, 3H), 1.86-2.00 (m, 2H), 2.00-2.15 (m, 2H), 2.15-2.25 (m, 1H), 2.65-2.80 (m, 2H), 3.25-3.40 (m, 1H), 3.75-3.90 (m, 3H), 3.91-4.10 (m, 5H), 4.11-4.30 (m, 3H), 4.80-5.00 (m, 1H), 5.70-5.90 (m, 1H), 6.40-6.60 (m, 2H), 6.75-6.85 (m, 1H), 6.85-6.90 (m, 1H), 6.90-7.00 (m, 1H), 7.20-7.26 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.7, 25.5, 25.7, 25.8, 25.9, 26.1, 27.7, 28.8, 28.9, 29.0, 29.2, 29.3, 35.9, 36.0, 38.6, 38.8, 41.2, 41.3, 53.7, 53.9, 57.5, 57.7, 59.2, 59.3, 59.4, 67.8, 67.9, 97.5, 97.6, 116.4, 116.5, 116.6, 118.6, 118.8, 128.5, 129.3, 129.4, 130.0, 131.2, 131.3, 131.4, 155.5, 155.6, 170.2, 170.3, 170.4, 170.5, 170.6, 171.8, 171.9, 172.2; HRMS-FAB ($M + Na^+$) calcd for $C_{31}H_{45}NaN_3O_6$ 578.3206, found 578.3218.

*Amide **17b***. Compound **17b** was synthesized using a procedure analogous to amide **17a** (100 mg, 0.18 mmol, 60%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80-1.00 (m, 2H), 1.05-1.30 (m, 5H), 1.35-1.55 (m, 7H), 1.56-1.75 (m, 8H), 1.85-2.05 (m, 5H), 2.10-2.20 (m, 1H), 2.60-2.80 (m, 1H), 3.20-3.40 (m, 2H), 3.80-4.13 (m, 6H), 4.15-4.32 (m, 2H), 4.35-4.65 (m, 2H), 5.15-5.40 (m, 1H), 6.00-6.30 (m, 1H), 6.85-7.00 (m, 3H), 7.00-7.10 (m, 1H), 7.11-7.25 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.2, 21.3, 21.4, 22.1; 24.1, 25.5, 25.6, 26.1, 27.0, 27.4, 28.1, 28.3, 28.8, 29.0, 29.7, 29.8, 33.7, 33.8, 34.1, 35.1, 35.6, 35.8, 36.1, 38.8, 38.9, 39.3, 41.1, 53.5, 54.3, 55.3, 58.2, 58.6, 58.8, 59.3, 59.5, 62.0, 68.3, 68.7, 97.4, 97.7, 117.7, 117.8, 118.2, 118.3, 128.8, 130.2, 130.7, 130.8, 131.3, 157.4, 157.7, 157.9, 171.0, 171.2, 172.3, 172.5; HRMS-FAB ($M + Na^+$) calcd for $C_{31}H_{45}NaN_3O_6$ 578.3206, found 578.3225.

*Amide **17c***. Compound **17c** was synthesized using a procedure analogous to amide **17a** (121 mg, 0.23 mmol, 75%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.70-0.90 (m, 6H), 0.91-1.10 (m, 1H), 1.15-1.55 (m, 9H), 1.55-1.70 (m, 3H), 1.71-1.83 (m, 3H), 1.84-2.00 (m, 2H), 2.05-2.15 (m, 1H), 2.16-2.30 (m, 1H), 2.65-2.80 (m, 1H), 3.20-3.40 (m, 1H), 3.70-4.13 (m, 5H), 4.14-4.30 (m, 3H), 4.80-5.00 (m, 1H), 5.90-6.10 (m, 1H), 6.50-6.70 (m, 1H), 6.75-6.90 (m, 2H), 6.91-7.05 (m, 1H), 7.15-7.35 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.3, 15.0, 15.1, 20.9, 21.6, 21.7, 23.7, 25.0, 25.1, 25.4, 25.7, 26.2, 27.3, 29.0, 29.1, 31.5, 36.0, 36.5, 38.2, 38.3, 38.4, 38.6, 38.8, 52.4, 52.6, 53.8, 54.0, 57.1, 57.2, 59.1, 59.2, 59.3, 68.0, 68.1, 97.5, 97.6, 116.7, 116.8, 118.6, 118.8, 120.1, 125.6, 129.5, 129.6, 129.7, 129.8, 129.9, 131.4, 131.5, 155.5, 155.6, 170.4, 170.5, 170.7, 170.8, 171.8, 171.9, 172.3, 174.0; HRMS-ESI ($M + Na^+$) calcd for $C_{29}H_{43}NaN_3O_6$ 552.3050, found 552.3060.

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*Amide **17d***. Compound **17d** was synthesized using a procedure analogous to amide **17a** (121 mg, 0.23 mmol, 75%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.75-0.90 (m, 6H), 0.91-1.10 (m, 1H), 1.15-1.30 (m, 1H), 1.31-1.58 (m, 7H), 1.59-1.71 (m, 2H), 1.72-1.85 (m, 2H), 1.86-2.10 (m, 4H), 2.10-2.30 (m, 1H), 2.60-2.80 (m, 1H), 3.20-3.30 (m, 1H), 3.80-4.30 (m, 6H), 4.35-4.70 (m, 2H), 5.10-5.40 (m, 1H), 6.10-6.40 (m, 1H), 6.70-7.05 (m, 3H), 7.06-7.25 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.2, 11.4, 15.4, 15.7, 21.0, 21.7, 23.9, 24.0, 24.7, 25.5, 25.6, 26.0, 27.3, 28.9, 29.0, 34.0, 34.1, 35.9, 36.3, 38.6, 53.4, 55.1, 58.6, 58.7, 59.2, 59.3, 59.4, 68.3, 68.7, 97.5, 97.7, 117.5, 117.6, 117.8, 118.1, 128.6, 128.7, 129.0, 129.3, 130.1, 130.7, 130.9, 131.3, 157.4, 157.6, 157.9, 171.0, 171.1, 171.2, 171.3, 172.1, 172.3; HRMS-FAB ($M + Na^+$) calcd for $C_{29}H_{43}NaN_3O_6$ 552.3050, found 552.3060.

*Amide **17e***. Compound **17e** was synthesized using a procedure analogous to amide **17a** (120 mg, 0.23 mmol, 75%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 075-0.95 (m, 6H), 1.20-1.60 (m, 10H), 1.61-1.85 (m, 4H), 1.86-2.15 (m, 3H), 2.16-2.30 (m, 1H), 2.65-2.85 (m, 2H), 3.20-3.40 (m, 1H), 3.70-4.10 (m, 4H), 4.11-4.30 (m, 2H), 4.31-4.50 (m, 1H), 4.60-4.90 (m, 1H), 5.60-5.90 (m, 1H), 6.40-6.60 (m, 1H), 6.75-6.90 (m, 2H), 6.95-7.05 (m, 1H), 7.15-7.26 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.4, 22.8, 22.9, 24.6, 24.7, 25.5, 25.7, 26.1, 29.0, 29.1, 36.0, 36.1, 38.6, 39.1, 42.9, 43.0, 51.1, 51.2, 53.7, 53.9, 59.1, 59.2, 59.3, 59.4, 67.9, 68.0, 97.5, 97.7, 116.8, 118.5, 118.6, 129.3, 130.0, 130.1, 131.5, 131.6, 155.7, 155.8, 170.4, 170.6, 171.4, 171.5, 171.6, 171.7; HRMS-FAB ($M + Na^+$) calcd for $C_{29}H_{43}NaN_3O_6$ 552.3050, found 552.3061.

*Amide **17f***. Compound **17f** was synthesized using a procedure analogous to amide **17a** (105 mg, 0.20 mmol, 65%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.75-0.95 (m, 6H), 1.15-1.60 (m, 9H), 1.61-1.83 (m, 5H), 1.84-2.05 (m, 3H), 2.05-2.30 (m, 2H), 2.60-2.80 (m, 1H), 3.20-3.30 (m, 1H), 3.75-4.10 (m, 4H), 4.15-4.30 (m, 1H), 4.31-4.70 (m, 3H), 5.20-5.70 (m, 1H), 6.20-6.40 (m, 1H), 6.85-7.05 (m, 2H), 7.06-7.26 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.7, 21.8, 21.9, 22.9, 23.0, 24.1, 24.8, 24.9, 25.4, 25.7, 27.1, 27.5, 28.1, 28.8, 29.0, 34.1, 34.2, 36.1, 36.7, 40.6, 40.7, 52.2, 52.3, 53.5, 54.6, 59.3, 59.4, 68.3, 68.6, 97.4, 97.7, 117.6, 117.8, 118.1, 118.2, 128.9, 129.2, 130.4, 130.9, 131.2, 157.5, 157.7, 171.1, 171.7, 171.8, 172.1, 172.2; HRMS-ESI ($M + Na^+$) calcd for $C_{29}H_{43}NaN_3O_6$ 552.3050, found 552.3048.

*Amide **17g***. Compound **17g** was synthesized using a procedure analogous to amide **17a** (95 mg, 0.17 mmol, 65%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30-1.50 (m, 7H), 1.60-1.80 (m, 3H), 1.80-1.95 (m, 3H), 2.00-2.20 (m, 3H), 2.70-2.85 (m, 2H), 2.86-2.95 (m, 2H), 3.10-3.30 (m, 1H), 3.80-4.00 (m, 4H), 4.01-4.10 (m, 1H), 4.11-4.20 (m, 1H), 4.21-4.30 (m, 1H), 4.40-4.50 (m, 1H), 4.70-4.80 (m, 1H), 5.10-5.30 (m, 1H), 6.00-6.20 (m, 1H), 6.30-6.40 (m, 1H), 6.70-6.90 (m, 2H), 6.91-7.00 (m, 1H), 7.00-7.18 (m, 3H), 7.19-7.26 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.6, 21.7, 25.5, 25.7, 26.2, 26.3, 29.1, 35.9, 38.6, 39.0, 39.1, 53.6, 53.7, 54.0, 54.2, 59.2, 59.3, 59.4, 67.6, 67.7, 97.5, 97.6, 116.4, 118.3, 118.4, 126.9, 127.0, 128.5, 129.0, 129.3, 130.5, 136.1, 136.3, 156.0, 170.0, 170.1, 171.8, 171.9; HRMS-FAB ($M + Na^+$) calcd for $C_{32}H_{41}NaN_3O_6$ 586.2893, found 586.2875.

*Amide **17h***. Compound **17h** was synthesized using a procedure analogous to amide **17a** (100 mg, 0.18 mmol, 60%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20-1.40 (m, 4H), 1.41-1.55 (m, 3H), 1.56-1.70 (m, 3H), 1.75-1.90 (m, 3H), 1.90-2.10 (m, 4H), 2.60-2.80 (m, 1H), 2.90-3.00 (m, 1H), 3.05-3.25 (m, 2H), 3.26-3.40 (m, 1H), 3.70-4.10 (m, 5H), 4.12-4.25 (m, 1H), 4.28-4.40 (m, 1H), 4.40-4.55 (m, 1H), 4.55-4.65 (m, 1H), 5.20-5.60 (m, 1H), 6.00-6.20 (m, 1H), 6.50-7.00 (m, 3H), 7.00-7.30 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.7, 21.7, 25.5, 25.7, 27.3, 27.7, 34.1, 35.8, 36.5, 36.7, 53.2, 54.9, 55.0, 59.3, 59.4, 68.6, 97.6, 97.8, 117.8, 118.2, 118.7, 126.8, 126.9, 128.6, 128.7, 129.1, 129.2, 130.6, 130.7, 131.1, 136.8, 137.0, 157.9, 170.8, 170.9, 171.0, 172.4, 172.5; HRMS-FAB ($M + Na^+$) calcd for $C_{32}H_{41}NaN_3O_6$ 586.2893, found 586.2882.

*Amide **17i***. Compound **17i** was synthesized using a procedure analogous to amide **17a** (123 mg, 0.24 mmol, 80%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.75-0.90 (m, 6H), 1.15-1.55 (m, 8H), 1.56-2.02 (m, 8H), 2.03-2.35 (m, 3H), 2.60-2.80 (m, 2H), 3.20-3.40 (m, 1H), 3.70-4.10 (m, 5H), 4.11-4.30 (m, 3H), 4.30-4.50 (m, 1H), 5.80-6.00 (m, 1H), 6.50-6.90 (m, 4H), 6.91-7.01 (m, 1H), 7.15-7.30 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.9, 19.0, 19.2, 19.3, 22.0, 22.1, 24.1, 25.8, 26.0, 26.5, 28.0, 29.4, 29.5, 32.3, 36.5, 38.9, 39.4, 54.2, 54.4, 58.3, 58.4, 59.5, 59.6, 59.7, 68.3, 68.5, 97.9, 98.0, 117.0, 117.1, 119.1, 129.8, 129.9, 130.4, 130.5, 131.8, 131.9, 156.0, 170.8, 170.9, 171.1, 172.3, 172.4; HRMS-FAB ($M + Na^+$) calcd for $C_{28}H_{41}NaN_3O_6$ 538.2893, found 538.2881.

*Amide **17j***. Compound **17j** was synthesized using a procedure analogous to amide **17a** (92 mg, 0.18 mmol, 60%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.70-1.00 (m, 6H), 1.15-1.60 (m, 7H), 1.61-1.83 (m, 3H), 1.84-2.50 (m, 6H), 2.60-2.80 (m, 1H), 3.20-3.40 (m, 2H), 3.70-4.15 (m, 5H), 4.17-4.30 (m, 1H), 4.31-4.40 (m, 1H), 4.41-4.80 (m, 1H), 5.30-5.70 (m, 1H), 6.40-6.60 (m, 1H), 6.90-7.00 (m, 2H), 7.00-7.21 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.1, 18.3, 19.2, 19.5, 21.0, 21.7, 25.4, 25.6, 27.1, 27.3, 29.0, 29.9, 30.0, 33.9, 34.1, 35.8,

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36.6, 54.9, 59.3, 59.4, 59.6, 59.9, 68.3, 68.6, 97.4, 97.7, 117.6, 117.7, 117.8, 117.9, 129.0, 129.3, 129.9, 130.5, 131.2, 131.5, 157.5, 157.7, 171.2, 171.3, 171.4, 171.5, 172.2, 172.3; HRMS-FAB ($M + Na^+$) calcd for $C_{28}H_{41}NaN_3O_6$ 538.2893, found 538.2899.

*Inhibitor **10a***. To compound **17a** (110 mg, 0.2 mmol), an aqueous TFA solution (10 mL of a 33% solution) was added at 0°C. The reaction was warmed to room temperature, stirred for an additional 12h, and then concentrated by rotary evaporation. The resulting residue was diluted with EtOAc (50 mL) and washed with saturated aqueous Na_2CO_3 (50 mL) and brine (50 mL). It was then dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The crude oil was purified by flash chromatography (EtOAc:hexanes 2:1) to yield inhibitor **10a** as a mixture of two diastereomers (50 mg, 0.1 mmol, 50%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80-1.00 (m, 2H), 1.10-1.25 (m, 3H), 1.30-1.55 (m, 3H), 1.56-1.71 (m, 7H), 1.72-1.85 (m, 7H), 2.00-2.30 (m, 3H), 2.35-2.55 (m, 1H), 2.56-2.75 (m, 3H), 3.25-3.40 (m, 1H), 4.00-4.10 (m, 1H), 4.11-4.30 (m, 2H), 4.40-4.60 (m, 1H), 4.80-5.00 (m, 1H), 5.60-5.85 (m, 1H), 6.20-6.40 (m, 1H), 6.75-6.90 (m, 2H), 6.90-7.00 (m, 1H), 7.01-7.11 (m, 1H), 7.15-7.25 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.1, 22.2, 24.1, 26.8, 26.9, 28.8, 29.5, 29.6, 29.7, 35.5, 35.6, 36.1, 37.6, 38.8, 38.9, 39.1, 41.3, 41.4, 41.5, 53.2, 54.4, 54.5, 58.2, 58.5, 64.4, 68.2, 117.8, 117.9, 119.0, 129.3, 129.4, 130.0, 130.1, 131.8, 155.9, 171.1, 171.2, 171.4, 171.5, 174.0, 207.4, 207.6; HRMS-ESI ($M + H^+$) calcd for $C_{28}H_{40}N_3O_5$ 498.2968, found 498.2970.

*Inhibitor **10b***. Compound **10b** was synthesized using a procedure analogous to inhibitor **10a** (50 mg, 0.10 mmol, 50%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80-1.00 (m, 2H), 1.01-1.30 (m, 3H), 1.45-1.55 (m, 2H), 1.56-1.82 (m, 8H), 1.83-2.00 (m, 3H), 2.10-2.25 (m, 2H), 2.35-2.50 (m, 1H), 2.51-2.75 (m, 2H), 3.10-3.40 (m, 2H), 4.00-4.15 (m, 1H), 4.16-4.32 (m, 1H), 4.31-4.45 (m, 1H), 4.46-4.60 (m, 2H), 5.40-5.65 (m, 1H), 6.20-6.60 (m, 2H), 6.85-7.00 (m, 2H), 7.01-7.11 (m, 2H), 7.30-7.50 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.2, 24.7, 24.8, 26.0, 26.4, 27.6, 28.4, 28.9, 30.2, 34.0, 35.3, 36.0, 39.2, 39.3, 41.4, 41.5, 55.0, 55.2, 58.7, 58.8, 59.4, 59.5, 64.4, 68.6, 118.2, 118.3, 118.5, 118.6, 128.9, 130.3, 130.4, 131.5, 131.7, 158.2, 158.3, 172.0, 173.6, 207.4, 207.5; HRMS-ESI ($M + H^+$) calcd for $C_{28}H_{40}N_3O_5$ 498.2968, found 498.2977.

*Inhibitor **10c***. Compound **10c** was synthesized using a procedure analogous to inhibitor **10a** (56 mg, 0.12 mmol, 60%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.70-0.90 (m, 6H), 0.91-1.10 (m, 1H), 1.25-1.75 (m, 7H), 1.76-1.95 (m, 4H), 2.05-2.25 (m, 2H), 2.25-2.40 (m, 1H), 2.41-2.65 (m, 3H), 2.66-2.81 (m, 1H), 3.20-3.40 (m, 1H), 4.00-4.30 (m, 3H), 4.40-4.70 (m, 1H), 4.85-5.10 (m, 1H), 6.70-6.90 (m, 2H), 6.90-7.00 (m, 1H), 7.10-7.46 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.6, 11.7, 15.5, 21.9, 24.4, 25.4, 26.5, 28.3, 31.3, 35.3, 35.6, 36.1, 38.3, 38.5, 38.7, 39.1, 41.5, 41.6, 54.3, 57.7, 58.3, 58.4, 68.4, 118.0, 118.2, 119.1, 120.5, 120.6, 129.6, 130.0, 130.1, 130.2, 130.3, 132.0, 155.8, 171.3, 171.5, 173.7, 207.4, 207.5; HRMS-ESI ($M + H^+$) calcd for $C_{26}H_{38}N_3O_5$ 472.2811, found 472.2825.

*Inhibitor **10d***. Compound **10d** was synthesized using a procedure analogous to inhibitor **10a** (52 mg, 0.11 mmol, 55%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.70-0.90 (m, 6H), 0.91-1.11 (m, 1H), 1.30-1.60 (m, 4H), 1.61-1.75 (m, 2H), 1.76-2.00 (m, 4H), 2.01-2.10 (m, 1H), 2.11-2.31 (m, 2H), 2.35-2.75 (m, 2H), 3.20-3.40 (m, 2H), 4.10-4.30 (m, 2H), 4.30-4.45 (m, 1H), 4.45-4.65 (m, 2H), 5.50-5.80 (m, 1H), 6.40-6.70 (m, 1H), 6.90-7.00 (m, 2H), 7.00-7.15 (m, 2H), 7.40-7.60 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.2, 11.3, 11.9, 21.0, 21.1, 24.4, 25.2, 27.5, 27.6, 28.3, 33.9, 34.0, 35.2, 35.9, 36.2, 36.3, 41.4, 41.5, 55.1, 55.2, 58.7, 58.8, 59.2, 59.3, 64.4, 68.5, 68.6, 118.0, 118.1, 118.5, 118.6, 128.9, 129.0, 129.1, 130.2, 130.3, 131.6, 131.7, 158.2, 158.3, 172.1, 172.2, 172.4, 172.5, 173.6, 207.4, 207.5; HRMS-ESI ($M + H^+$) calcd for $C_{26}H_{38}N_3O_5$ 472.2811, found 472.2820.

*Inhibitor **10e***. Compound **10e** was synthesized using a procedure analogous to inhibitor **10a** (45 mg, 0.10 mmol, 50%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.70-0.90 (m, 6H), 1.20-1.30 (m, 1H), 1.30-1.60 (m, 7H), 1.75-2.00 (m, 3H), 2.00-2.15 (m, 1H), 2.16-2.25 (m, 1H), 2.30-2.40 (m, 1H), 2.50-2.65 (m, 2H), 2.66-2.80 (m, 1H), 3.20-3.40 (m, 1H), 4.10-4.30 (m, 2H), 4.40-4.70 (m, 3H), 4.90-5.10 (m, 1H), 6.65-6.81 (m, 2H), 6.86-6.90 (m, 1H), 6.90-7.00 (m, 1H), 7.10-7.20 (m, 1H), 7.27-7.35 (m, 1H), 7.40-7.60 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.7, 22.4, 22.9, 24.2, 24.3, 25.0, 25.2, 26.2, 27.5, 28.3, 28.4, 35.5, 35.9, 39.0, 39.1, 41.3, 41.4, 42.7, 42.8, 51.8, 51.9, 54.8, 58.6, 58.7, 64.4, 65.4, 68.2, 113.4, 118.5, 118.6, 118.9, 129.0, 129.1, 129.9, 130.0, 131.9, 155.9, 159.7, 160.3, 171.2, 171.4, 172.8, 172.9, 174.6, 174.7, 207.5, 207.8; HRMS-ESI ($M + H^+$) calcd for $C_{26}H_{38}N_3O_5$ 472.2811, found 472.2816.

*Inhibitor **10f***. Compound **10f** was synthesized using a procedure analogous to inhibitor **10a** (61 mg, 0.13 mmol, 65%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.70-0.90 (m, 6H), 1.30-1.75 (m, 9H), 1.75-2.00 (m, 3H), 2.00-2.25 (m, 3H), 2.30-2.70 (m, 3H), 3.00-3.20 (m, 1H), 3.20-3.40 (m, 1H), 4.10-4.40 (m, 3H), 4.45-4.75 (m, 3H), 5.70-5.90 (m, 1H), 6.50-6.80 (m, 1H), 6.90-7.25 (m, 4H), 7.60-7.70 (m, 1H); ^{13}C NMR

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(100 MHz, $CDCl_3$) δ 21.8, 21.9, 23.3, 23.4, 24.4, 25.7, 25.8, 27.7, 27.8, 28.2, 34.0, 35.0, 35.1, 36.3, 36.8, 40.2, 40.3, 41.3, 41.4, 52.8, 52.9, 54.8, 54.9, 58.7, 64.4, 68.4, 118.0, 118.1, 118.7, 118.8, 128.6, 128.7, 130.4, 131.7, 131.8, 158.4, 158.5, 159.4, 160.0, 172.5, 173.0, 173.1, 174.0, 174.1, 207.4, 207.5; HRMS-ESI ($M + H^+$) calcd for $C_{26}H_{38}N_3O_5$ 472.2811, found 472.2814.

*Inhibitor **10g***. Compound **10g** was synthesized using a procedure analogous to inhibitor **10a** (60 mg, 0.12 mmol, 60%): ^1H NMR (400 MHz, $CDCl_3$) δ 1.30-1.58 (m, 3H), 1.60-1.80 (m, 3H), 1.81-2.00 (m, 2H), 2.10-2.35 (m, 3H), 2.40-2.70 (m, 4H), 2.80-3.00 (m, 2H), 3.10-3.30 (m, 1H), 4.10-4.30 (m, 2H), 4.40-4.60 (m, 3H), 4.70-4.90 (m, 1H), 6.40-6.60 (m, 1H), 6.70-6.85 (m, 2H), 6.86-7.00 (m, 2H), 7.00-7.26 (m, 7H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.9, 24.4, 26.1, 26.3, 28.2, 28.3, 35.2, 35.5, 36.0, 38.8, 38.9, 39.6, 39.7, 41.4, 54.5, 54.8, 54.9, 58.5, 58.6, 67.8, 68.0, 117.2, 117.5, 119.0, 127.5, 128.7, 128.8, 128.9, 129.5, 129.6, 130.3, 131.8, 135.9, 136.0, 156.0, 156.1, 170.8, 170.9, 171.1, 174.2, 207.3, 207.4; HRMS-ESI ($M + H^+$) calcd for $C_{29}H_{36}N_3O_5$ 506.2655, found 506.2624.

*Inhibitor **10h***. Compound **10h** was synthesized using a procedure analogous to inhibitor **10a** (51 mg, 0.10 mmol, 50%): ^1H NMR (400 MHz, $CDCl_3$) δ 1.30-1.50 (m, 2H), 1.50-1.70 (m, 4H), 1.75-1.90 (m, 2H), 1.90-2.00 (m, 3H), 2.10-2.25 (m, 1H), 2.40-2.50 (m, 1H), 2.50-2.65 (m, 2H), 3.00-3.10 (m, 2H), 3.15-3.25 (m, 1H), 3.35-3.45 (m, 1H), 4.20-4.30 (m, 1H), 4.30-4.40 (m, 1H), 4.40-4.55 (m, 3H), 5.25-5.35 (m, 1H), 5.95-6.05 (m, 1H), 6.90-7.00 (m, 2H), 7.00-7.10 (m, 2H), 7.11-7.20 (m, 2H), 7.20-3.28 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.1, 21.3, 24.2, 27.8, 27.9, 28.2, 28.3, 34.1, 34.2, 35.0, 35.2, 35.8, 36.1, 36.5, 36.6, 41.4, 54.8, 55.3, 55.6, 58.6, 58.7, 68.5, 68.6, 118.6, 118.7, 118.9, 127.3, 128.6, 128.7, 129.0, 129.2, 129.5, 129.6, 129.9, 130.7, 131.4, 131.5, 136.9, 137.0, 158.3, 158.5, 171.6, 171.7, 171.9, 173.8, 173.9, 207.5, 207.6; HRMS-ESI ($M + H^+$) calcd for $C_{29}H_{36}N_3O_5$ 506.2655, found 506.2670.

*Inhibitor **10i***. Compound **10i** was synthesized using a procedure analogous to inhibitor **10a** (50 mg, 0.11 mmol, 55%): ^1H NMR (400 MHz, $CDCl_3$) δ 0.70-0.90 (m, 6H), 1.25-1.40 (m, 1H), 1.45-1.65 (m, 3H), 1.70-2.00 (m, 5H), 2.10-2.25 (m, 2H), 2.35-2.80 (m, 5H), 3.20-3.40 (m, 1H), 4.00-4.25 (m, 2H), 4.26-4.35 (m, 1H), 4.40-4.55 (m, 1H), 4.55-4.70 (m, 1H), 4.90-5.11 (m, 1H), 6.70-7.00 (m, 4H), 7.15-7.25 (m, 1H), 7.30-7.70 (m, 1H), 7.70-7.90 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.1, 19.2, 19.3, 21.9, 24.3, 26.3, 27.5, 28.3, 32.0, 32.1, 35.3, 35.5, 35.9, 39.0, 39.1, 41.4, 41.5, 54.8, 58.6, 58.7, 58.9, 64.4, 68.3, 118.4, 118.5, 119.0, 129.2, 129.3, 130.0, 130.1, 131.9, 155.8, 171.4, 171.5, 171.7, 171.8, 175.0, 175.1, 207.5, 207.6; HRMS-ESI ($M + H^+$) calcd for $C_{35}H_{36}N_3O_5$ 458.2655, found 458.2664.

*Inhibitor **10j***. Compound **10j** was synthesized using a procedure analogous to inhibitor **10a** (55 mg, 0.12 mmol, 60%): ^1H NMR (400 MHz, $CDCl_3$) δ 0.60-1.00 (m, 6H), 1.40-1.60 (m, 3H), 1.60-1.75 (m, 2H), 1.75-2.05 (m, 4H), 2.10-2.30 (m, 3H), 2.40-2.70 (m, 3H), 3.10-3.20 (m, 1H), 3.20-3.40 (m, 1H), 4.00-4.10 (m, 1H), 4.15-4.40 (m, 2H), 4.50-4.70 (m, 2H), 5.80-6.00 (m, 1H), 6.70-7.00 (m, 3H), 7.00-7.25 (m, 2H), 7.60-7.80 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.4, 18.5, 19.5, 21.2, 24.4, 27.5, 27.6, 28.2, 30.1, 33.9, 34.0, 35.0, 35.1 36.1, 36.4, 41.3, 41.4, 55.1, 58.8, 60.6, 64.5, 68.4, 117.2, 117.8. 117.9, 118.5, 128.8, 130.0, 131.8, 131.9, 158.3, 158.4, 159.5, 160.1, 172.5, 172.6, 172.7, 174.2, 207.2, 207.3; HRMS-ESI ($M + H^+$) calcd for $C_{35}H_{36}N_3O_5$ 458.2655, found 458.2660.

*7-(But-3-enyl)-1,5-dioxaspiro[5.5]undecane (**21**)*. To a solution of diisopropylamine (8.42 mL, 6.06 g, 60.0 mmol) in THF (60 mL), *n*-butyllithium (23.5 mL, 58.8 mmol, 2.5 M in hexanes) was added at −78°C under an atmosphere of nitrogen. The temperature of the solution was slowly increased to 0°C and maintained at that temperature for an additional 10 min. To this solution ketoester **18** (5.0 g, 29.4 mmol) was slowly added. After 15 min, 4-bromo-but-1-ene (3.80 mL, 5.34 g, 44.0 mmol) was added dropwise. The reaction was stirred at room temperature for 30 h, and then quenched with water. The THF was removed by rotary evaporation, and the mixture was partitioned between EtOAc (500 mL) and 1 N HCl (250 mL). The organic layer was washed with 1 N HCl (250 mL), saturated $NaHCO_3$ (250 mL), and brine (250 mL). It was then dried over $MgSO_4$, and the resulting solution was concentrated by rotary evaporation. The crude oil was purified by flash chromatography (EtOAc:hexanes 1:18) to yield **19** as a mixture of ketone and enol tautomers (5.16 g, 22.9 mmol, 78%). To a solution of compound **19** (6.0 g, 19.7 mmol) in MeOH (50 mL), 2 N aqueous NaOH (50 mL) was added. The reaction was heated at reflux for 24 h, and then cooled to room temperature. The MeOH was removed by rotary evaporation. The resulting aqueous solution was extracted with EtOAc (3 × 50 mL), the organic layers were combined and the solvent was removed by rotary evaporation to yield the corresponding alkene **20** (2.75 g, 90%). A solution of compound **20** (3.0 g, 20 mmol) in THF (10 mL) was cooled in an ice bath. To this solution, 1,3-propanediol (30 mL, 31.7 g, 417 mmol) and TMSCl (5.0 mL, 4.3 g, 40 mmol) were added. The reaction was stirred at room temperature for 48 h, and then

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partitioned between EtOAc (500 mL) and saturated $NaHCO_3$ (400 mL). The organic layer was washed with saturated $NaHCO_3$ (400 mL) and brine (400 mL). It was then dried over $MgSO_4$, and the resulting solution was concentrated by rotary evaporation. The crude oil was purified by flash chromatography (EtOAc:hexanes 1:18) to yield **21** (4.0 g, 19.0 mmol, 95%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.15-1.30 (m, 4H), 1.30-1.40 (m, 1H), 1.40-1.50 (m, 1H), 1.50-1.65 (m, 3H), 1.66-1.75 (m, 1H), 1.80-2.00 (m, 3H), 2.05-2.20 (m, 1H), 2.35-2.55 (m, 1H), 3.70-3.83 (m, 2H), 3.85-3.95 (td, J = 3.2, 11.2 Hz, 1H), 3.96-4.05 (td, J = 3.2, 11.2 Hz, 1H), 4.87-4.93 (dd, J = 1.2, 10.4 Hz, 1H), 4.95-5.05 (dd, J = 1.6, 17.2 Hz, 1H), 5.70-5.90 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.3, 25.7, 26.7, 26.9, 28.2, 32.1, 58.8, 58.9, 99.1, 114.0, 139.5; HRMS-EI ($M + H^+$) calcd for $C_{13}H_{23}O_2$ 210.1620, found 210.1615.

*3-(1,5-Dioxaspiro[5.5]undecan-7-yl)propanoic acid (**22**).* Compound **21** (250 mg, 1.0 mmol) was dissolved in a 2:1 mixture of acetone and water (60 mL). To this solution $NaIO_4$ (1.1 mg, 5.4 mmol), $KMnO_4$ (120 mg, 750 μmol), and $NaHCO_3$ (100 mg, 1.0 mmol) were added. The reaction was stirred at room temperature for 4 h, and then the acetone was removed by rotary evaporation. The remaining material was partitioned between EtOAc (100 mL) and 1 N HCl (75 mL). The organic layer was washed with 1 N HCl (3 × 75 mL), brine (75 mL), and dried over $MgSO_4$. The solvent was removed by rotary evaporation to give the carboxylic acid **22** (205 mg, 0.9 mmol, 90%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.15-1.45 (m, 5H), 1.46-1.75 (m, 5H), 1.85-2.05 (m, 1H), 2.10-2.30 (m, 1H), 2.30-2.70 (m, 3H), 3.70-3.85 (m, 2H), 3.85-3.98 (m, 1H), 4.00-4.10 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.1, 23.4, 25.6, 27.5, 28.0, 32.8, 58.8, 58.9, 99.1, 180.3; HRMS-FAB ($M + Na^+$) calcd for $C_{12}H_{20}NaO_4$ 251.1259, found 251.1269.

*(S)-tert-Butyl 1-(5-hydroxypentylamino)-3-(1H-indol-3-yl)-1-oxopropan-2-ylcarbamate (**24**).* Boc-Trp-OH (456 mg, 1.5 mmol) was dissolved in DMF (10 mL). To this solution were added 5-aminohexan-1-ol (206 mg, 2.0 mmol), HBTU (758 mg, 2.0 mmol), and DIEA (530 μL, 390 mg, 3.0 mmol). The reaction was stirred at room temperature for 2 h, and then 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) to yield alcohol **24** (560 mg, 1.43 mmol, 95%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90-1.10 (m, 2H), 1.15-1.31 (m, 4H), 1.35-1.51 (m, 11H), 2.50-2.60 (s, 1H), 2.90-3.20 (m, 3H), 3.21-3.40 (m, 1H), 3.50-3.60 (m, 2H), 4.30-4.50 (br s, 1H), 5.20-5.45 (br s, 1H), 6.95-7.05 (s, 1H), 7.05-7.25 (m, 2H), 7.30-7.40 (d, J = 11.2 Hz, 1H), 7.60-7.70 (d, J = 11.2 Hz, 1H), 8.80-9.00 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.6, 26.6, 28.7, 29.1, 29.4, 32.6, 39.6, 62.8, 111.8, 119.1, 119.9, 122.4, 123.8, 127.7, 136.7, 156.0, 172.3; HRMS-FAB ($M + Na^+$) calcd for $C_{21}H_{31}NaN_3O_4$ 412.2212, found 412.2220.

*(S)-5-(2-(tert-butoxycarbonylamino)-3-(1H-indol-3-yl)propanamido)pentyl 4-methylbenzenesulfonate (**25**).* Alcohol **24** (400 mg, 1.0 mmol) was dissolved in CH_2Cl_2 (10 mL). To this solution were added TsCl (285 mg, 1.5 mmol) and pyridine (530 μL, 390 mg, 3.0 mmol). The reaction 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) to yield compound **25** (560 mg, 1.43 mmol, 97%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90-1.15 (m, 4H), 1.40-1.60 (m, 11H), 2.40-2.50 (m, 3H), 2.90-3.25 (m, 3H), 3.25-3.40 (m, 1H), 3.30-3.50 (m, 2H), 4.30-4.50 (br s, 1H), 5.20-5.40 (br s, 1H), 5.70-5.80 (br s, 1H), 6.90-7.23 (m, 3H), 7.30-7.45 (m, 3H), 7.55-7.70 (d, J = 9.6 Hz, 1H), 7.75-7.85 (d, J = 10.4 Hz, 2H), 8.80-9.00 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.6, 22.6, 28.3, 28.4, 39.1, 70.6, 110.3, 111.5, 118.8, 119.5, 122.0, 123.4, 127.4, 127.8, 130.0, 132.8, 136.4, 145.0, 155.5, 171.7; HRMS-FAB ($M + Na^+$) calcd for $C_{29}H_{39}NaN_3O_6S$ 580.2457, found 580.2437.

*Macrocycle **26**.* Compound **25** (290 mg, 0.5 mmol) was dissolved in THF (10 mL). To this solution was added NaH (150 mg, 2.5 mmol). The reaction was stirred at room temperature for 48 hr, 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) to yield macrocyclic compound **26** (56 mg, 0.15 mmol, 30%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.05-0.30 (br s, 1H), 0.40-0.60 (br s, 1H), 1.35-1.60 (m, 12H), 1.70-1.85 (br s, 1H), 1.86-2.00 (br s, 1H), 2.70-2.90 (br s, 1H), 2.95-3.10 (m, 1H), 3.15-3.50 (m, 2H), 4.10-4.30 (m, 3H), 5.10-5.40 (m, 1H), 6.90-7.00 (s, 1H), 7.15-7.30 (m, 2H), 7.31-7.40 (m, 1H), 7.60-7.80 (d, J = 12.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.9, 25.8, 25.9, 28.7, 29.0, 38.4, 44.2, 56.7, 80.3, 108.4, 110.9, 119.0, 119.8, 121.9, 128.3, 131.0, 136.9, 155.4, 172.1; HRMS-FAB ($M + Na^+$) calcd for $C_{21}H_{29}NaN_3O_3$ 394.2107, found 394.2115.

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*Amide **28***. To a solution of compound **26** (190 mg, 0.5 mmol) in CH_2Cl_2 (10 mL) was added TFA (5 mL). The reaction was allowed to stir at room temperature for 30 min. The solvent was removed to yield the crude amine **27**. The resulting compound **27** was dissolved in DMF (10 mL). To this solution were added HBTU (379 mg, 1.0 mmol) and DIEA (265 μL, 195 mg, 1.5 mmol). The reaction was stirred at room temperature for 2 h, and then partitioned between EtOAc (150 mL) and 1 N HCl (100 mL). The organic layer was washed with 1 N HCl (100 mL), saturated $NaHCO_3$ (100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$ and concentrated by rotary evaporation. The crude material was purified by flash chromatography (EtOAc: hexanes) to yield amide **28** as a mixture of two diastereomers (170 mg, 0.35 mmol, 70%): ^{1}H NMR (400 MHz, $CDCl_3$) δ -0.10-0.25 (m, 1H), 0.40-0.80 (m, 1H), 1.00-1.20 (m, 1H), 1.30-1.90 (m, 10H), 2.15-2.50 (m, 4H), 2.51-2.75 (m, 2H), 2.76-2.85 (m, 2H), 2.90-3.10 (m, 1H), 3.25-3.55 (m, 3H), 3.65-4.00 (m, 3H), 4.20-4.40 (m, 1H), 4.40-4.70 (m, 1H), 5.00-5.60 (m, 1H), 5.80-6.80 (m, 1H), 6.80-7.00 (m, 1H), 7.05-7.25 (m, 2H), 7.30-7.40 (m, 1H), 7.60-7.70 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.4, 20.0, 20.2, 23.0, 25.3, 25.6, 25.8, 27.9, 28.3, 28.6, 29.6, 34.0, 34.1, 34.2, 35.4, 38.1, 38.4, 42.0, 43.1, 43.6, 44.1, 49.8, 50.0, 55.0, 55.2, 58.6, 58.9, 62.0, 99.4, 107.8, 108.1, 110.6, 110.9, 111.2, 118.3, 118.8, 119.7, 119.9, 121,4, 121.7, 127.9, 128.5, 130.6, 131.2, 136.4, 136.7, 170.0, 171.7, 171.8, 172.7, 173.5, 213.4; HRMS-FAB ($M + Na^+$) calcd for $C_{28}H_{39}NaN_3O_4$ 504.2838, found 504.2852.

*Inhibitor **11***. To compound **28** (250 mg, 0.5 mmol), an aqueous TFA solution (10 mL of a 33% solution) was added at 0°C. The reaction was warmed to room temperature, stirred for an additional 12h, and then concentrated by rotary evaporation. The resulting residue was diluted with EtOAc (50 mL) and washed with saturated aqueous Na_2CO_3 (50 mL) and brine (50 mL). It was then dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The crude oil was purified by flash chromatography (EtOAc:hexanes 2:1) to yield inhibitor **11** as a mixture of two diastereomers (97 mg, 224 μmol, 55%): ^{1}H NMR (300 MHz, $CDCl_3$) δ -0.10-0.10 (br s, 1H), 0.60-0.90 (br s, 1H), 1.20-1.80 (m, 9H), 1.81-2.00 (m, 1H), 2.00-2.20 (m, 5H), 2.21-2.50 (m, 5H), 2.55-2.80 (m, 1H), 2.95-3.10 (m, 1H), 3.25-3.40 (m, 1H), 3.41-3.60 (m, 1H), 4.00-4.20 (m, 1H), 4.21-4.40 (m, 1H), 4.40-4.60 (m, 1H), 5.50-5.70 (m, 1H), 6.40-6.60 (m, 1H), 6.89 (s, 1H), 7.10-7.30 (m, 2H), 7.31-7.45 (m, 1H), 7.60-7.80 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.0, 26.0, 26.2, 28.4, 29.0, 34.3, 34.4, 34.6, 34.7, 38.5, 42.4, 42.5, 44.4, 50.2, 50.4, 55.3, 108.5, 111.0, 119.1, 120.0, 122.0, 128.3, 131.0, 137.0, 172.1, 172.9, 213.6, 213.7; HRMS-FAB ($M + Na^+$) calcd for $C_{25}H_{33}N_3NaO_3$ 446.2420, found 446.2410.

Results and discussion

Chemistry

The synthesis of inhibitors **10a-j** is detailed in Scheme 1. Boc-protected amino acids were coupled with H-Tyr-OBn using HBTU to give dipeptides **12a-j**. The Boc protecting groups were removed using 1:2 TFA/CH_2Cl_2 to generate the corresponding primary amines. Acylation of these amines with 5-bromopentanoyl chloride under the Schotten-Baumann conditions provided the primary bromides in excellent yield. These were treated with NaI in acetone to generate compounds **13a-j**. Ring closure of compounds **13a-j** in the presence of K_2CO_3 gave macrocycles **14a-j**. The benzyl esters of compounds **14a-j** were removed by catalytic hydrogenation, and the resulting carboxylic acids **15a-j** were coupled with racemic primary amine **16** [5e] in the presence of HBTU to yield amides **17a-j**. Finally, the acetal protecting group was removed using aqueous TFA to give inhibitors **10a-j** as mixtures of two diastereomers.

The synthesis inhibitor **11** required carboxylic acid **22**, which was prepared starting from ketoester **18** (Scheme 2). Compound **18** was treated with two equivalents of LDA, and the resulting dianion was reacted with 4-bromo-1-butene to generate alkene **19**. Hydrolysis of the ester using NaOH/MeOH, followed by spontaneous decarboxylation gave compound **20**. The ketone was converted to acetal **21** using 1,3-propanediol in the presence of TMSCl. Finally, oxidative cleavage of the alkene in **21** using $NaIO_4$ and $KMnO_4$ provided acid **22**.

Inhibitor **11** was prepared as shown in Scheme 3. The coupling reaction between Boc-Trp-OH **23** and 5-amino-1-hexanol using HBTU gave alcohol **24**. The hydroxy group was converted to the corresponding tosylate, and the resulting compound **25** was subjected to a series of bases to determine optimal cyclization conditions. We found that NaH gave a modest but acceptable yield of the desired macrocycle **26**. The Boc protecting group in **26** was removed with TFA to generate amine **27**, which was then coupled with acid **22** to give compound **28**. Finally, the acetal was hydrolyzed using aqueous TFA to yield inhibitor **11**.

Inhibition of plasmin

The assay results for inhibitors **10a-j**, evaluated as mixtures of the two diastereomers, against plasmin are shown in Figure 4. None of these compounds showed $>20\%$ inhibition at a concentration of 250 μM. We measured the IC_{50} values of three inhibitors with the highest activities, compounds **10c**, **10d** and **10h** (Table I), and compared these values with the activity of a closely related non-cyclic inhibitor, compound **29**.

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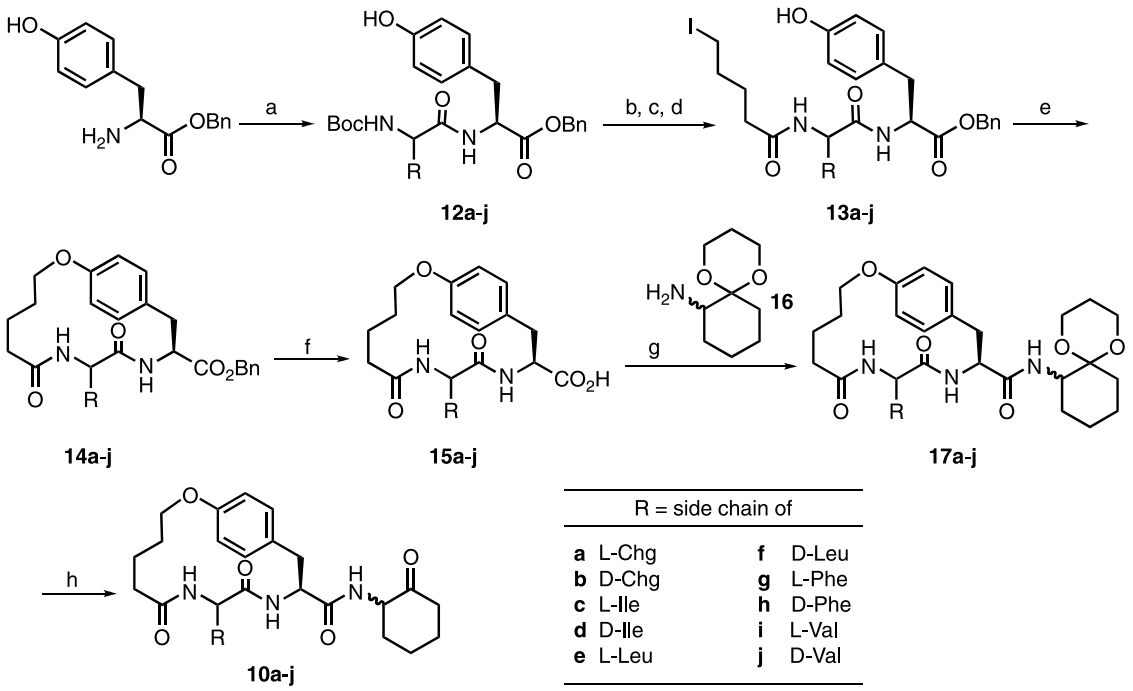
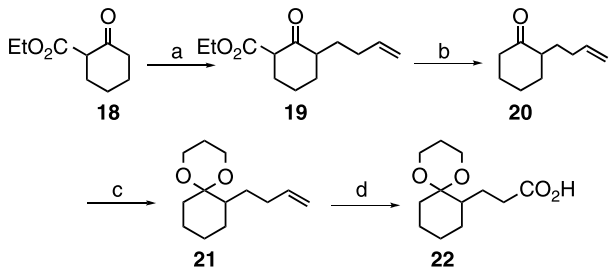


Scheme 1. Reagents and conditions: (a) Boc-aa-OH, HBTU, DIEA, rt, 2 h (86-99%); (b) TFA/CH_2Cl_2 (1:2), rt, 30 min; (c) 20% K_2CO_3, 5-bromopentanoyl chloride, rt, 8 min; (d) NaI, acetone, reflux, 2 h (88-100%); (e) K_2CO_3, rt, 10 h (50-75%); (f) H_2, $Pd(OH)_2$/C, rt, 4 h; (g) **16**, HBTU, DIEA, rt, 24 h (60-80%); (h) TFA/H_2O (1:2), rt, 12 h (50-65%). Compounds **13c**, **13d** and **13f** were not isolated in pure form, but instead the crude materials were used directly in the next reaction. Compounds **17a-j** and **10a-j** are 1:1 mixtures of two diastereomers where the stereochemistry of the R substituent is defined, but the stereocenter on the cyclohexane ring is not.

Inhibitor **10d**, with R = the side chain of D-Ile, is the best inhibitor with an IC_{50} value of 450 μM. This compound is closest in structure with its linear analog, compound **29**. Inhibitor **10d** is greater than 10-fold more potent than the linear analog **29**, suggesting that the combination of macrocyclization and acylation of the *N*-terminus significantly improves its interactions with the active site of plasmin. The enhancement in activity caused by macrocyclization is further accentuated by the fact that **29** contains a sulfur atom, while **10d** contains a methylene group at the analogous position. A sulfur atom at this position has been shown to enhance the electrophilicity of the ketone, and improve inhibition activity by approximately three-fold [5a].

Inhibitor **10c**, with R = the side chain of L-Ile, is the second most potent compound with an IC_{50} value of 550 μM. The observed preference of the enzyme for a D-Ile residue at the P3 position is consistent with results reported by Okada and coworkers [15]. Additionally, inhibitor **10h**, where R = the side chain of D-Phe, has an IC_{50} value of 930 μM, which is still >5 fold more potent than **29**. The other seven inhibitors, which showed $<10\%$ inhibition at a concentration of 250 μM, were poor inhibitors of plasmin. This result suggests that, within the context of the macrocyclic scaffold **10**, plasmin prefers Ile at P3 over other similar amino acids including Leu, Val, and cyclohexylglycine.

We also examined the activity of compound **11** against plasmin, but it showed no detectable inhibition at concentrations up to 500 μM. Two factors may contribute to the low activity of this compound. First, the linker between the indole nitrogen atom and the *C*-terminus of the inhibitor may be too long so that it does not significantly limit the conformational freedom of the molecule. Second, the inhibitor incorporates only a single amino acid unit, Trp, at the P2′ position. The corresponding S2′ subsite on plasmin is known to have a relatively minor influence on binding interactions with peptide substrates. Thus, there



Scheme 2. (a) LDA (2 equiv), 0°C, then 1-bromo-4-butene, rt, 30 h (78%); (b) 2 N NaOH:MeOH (1:1), reflux, 24 h (90%); (c) 1,3-propanediol, TMSCl, 0°C to rt, 48 h (95%); (d) $NaIO_4$, $KMnO_4$, $NaHCO_3$, acetone/water (2:1), rt, 4 h (90%).

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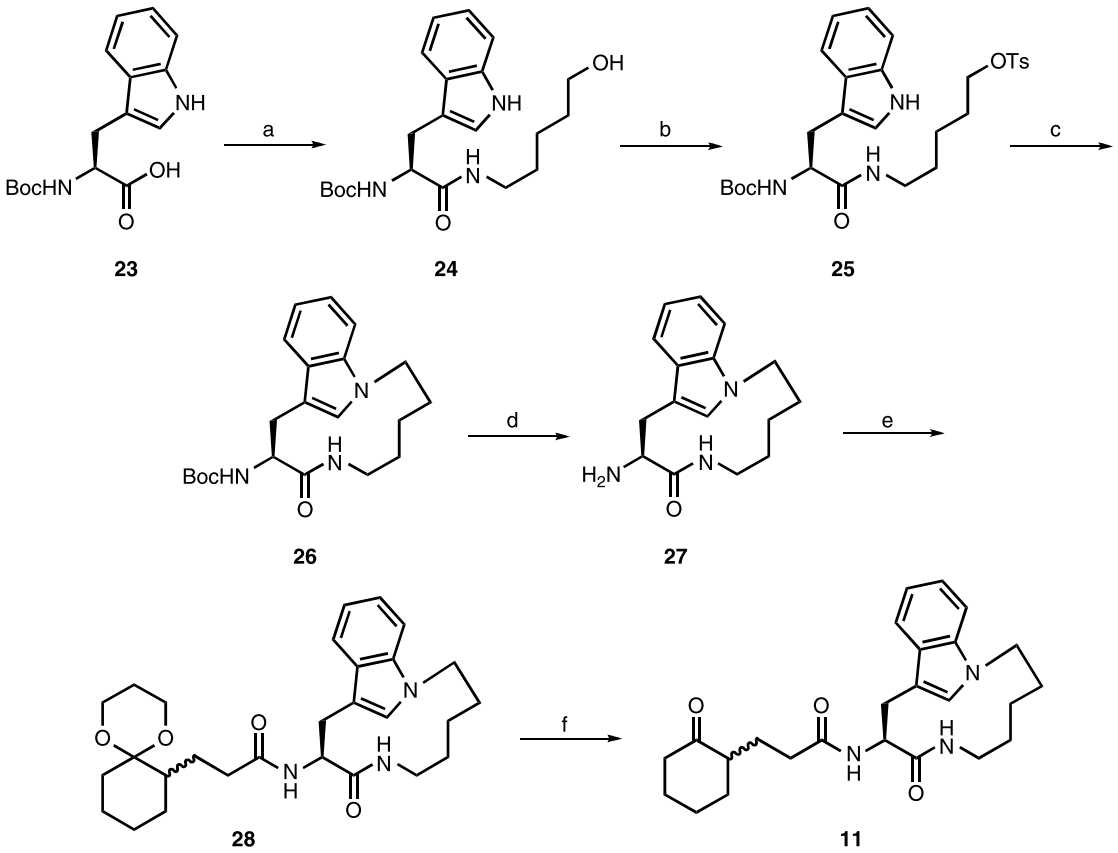

Scheme 3. (a) 5-amino-1-hexanol, HBTU, DIEA, rt, 2 h (95%); (b) TsCl, pyridine, rt, 2 h (97%); (c) NaH, rt, 2 d (30%); (d) TFA/CH_2Cl_2 (1:2), rt, 30 min; (e) **22**, HBTU, DIEA, rt, 2 h (70% for two steps); (f) TFA/H_2O (1:2), rt, 12 h (55%).

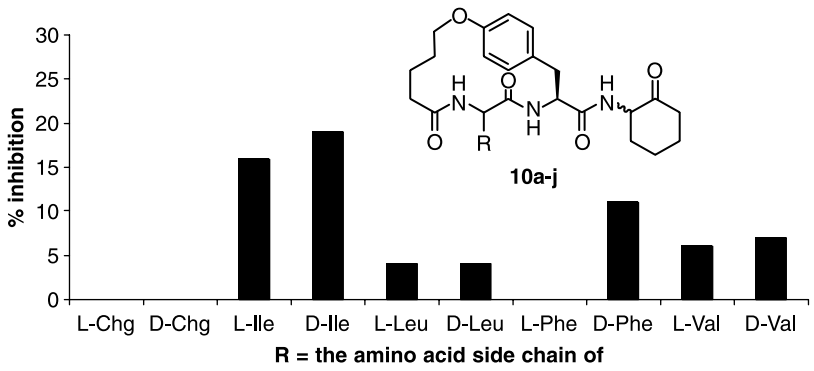

Figure 4. Assay results for inhibitors **10a-j** (250 μM) against plasmin. The R group in each inhibitor is defined by the side chain of the amino acid shown on the *x*-axis of the plot. The data are an average of three independent measurements.

Table I. IC_{50} values of inhibitors **10c**, **10d** and **10h** against plasmin.

Inhibitor	R = the side chain of	IC_{50} (μM)
10c	L-Ile	550 ± 40
10d	D-Ile	450 ± 50
10h	D-Phe	930 ± 120

is likely limited affinity between the S2′ subsite and the peptide portion of inhibitor **11**.

In summary, we have designed and synthesized several macrocyclic inhibitors of the serine protease plasmin. While the inhibitors showed only modest activities, we did observe greater than 10-fold improvement in activity for several of the compounds

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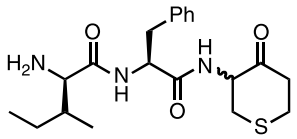
(**10c** and **d**) when compared to the related linear analog compound **29**.



29

The two diastereomers of 29 have IC_{50} values of 6 and 9mM.

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