Potent 7-Hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid-Based Macrocyclic Inhibitors of Hepatitis C Virus NS3 Protease

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The NS3 protease of hepatitis C virus (HCV) has emerged as one of the best characterized targets for next-generation HCV therapy. The tetrapeptide **1** and pentapeptide **2** are α-ketoamide-type HCV serine protease inhibitors with modest potency. We envisioned that the 1,2,3,4-tetrahydroisoquinoline-3-carboxylamide (Tic) moiety could be cyclized to the P3 capping group. The resulting macrocycle could enhance the binding through its extra contact with the Ala156 methyl group. Macrocyclization could also provide a less peptidic HCV inhibitor. Synthesis started from dipeptide **5**, which was obtained via a coupling of two amino acid derivatives. The N-terminal was capped as hept-6-enoylamide to give **6**. Hydroboration of the double bond afforded alcohol **7**, the precursor to the macrocycle **8**. The macrocyclization was achieved under Mitsunobu conditions (PPh_3, ADDP). The macrocyclic acid **9** was then combined with appropriate right-hand fragments **12**, **14**, or **16**, which was prepared from common intermediate **11**. Finally, oxidation of α-hydroxyamide provided target molecule α-ketoamides **17**, **18**, and **21**. The C-terminal esters were then elaborated to carboxylic acids **19** and **20**, and amides **20** and **23**. The inhibitors **17**−**23** were tested in HCV NS3 protease continuous assay. Tripeptide **17** was more potent than the larger acyclic tetrapeptide **1**. The tetrapeptides **18**−**20** were as active as **17**. Most significantly, the pentapeptides (**21**−**23**) were much better inhibitors (K_i* = 0.015−0.26 μM). The carboxylic acid (**22**) and amide (**23**) were 57−80 times more potent than the acyclic analogue **2**. The X-ray crystal structure of compound **23** bound to the protease revealed that the macrocycle adopted a donutlike conformation and had close contact with the Ala156 methyl group. The ketone carbonyl formed a reversible covalent bond with Ser139. The *n*-propyl of P1 novaline and the aromatic ring of P2′ phenylglycine formed a C-shaped clamp around the Lys136 side chain.

Introduction

The hepatitis C virus (HCV) is the principal etiologic agent of chronic hepatitis C infection, which could lead to cirrhosis, hepatocellular carcinoma, and, ultimately, liver failure. It has infected more than 170 million people worldwide and is emerging as a global health problem.[1] Current standard therapy involves pegylated α-interferon administered subcutaneously, either alone or in combination with the broad-spectrum antiviral agent ribavirin. These therapies have limited efficacy and frequently are accompanied by serious side effects.[2] Given the prevalence of HCV infections, a more effective, convenient, and well-tolerated small molecule drug is highly desirable.

Responsible for processing the nonstructural (NS) portion of the polyprotein in hepatitis C virus, the virally encoded NS3 protease is located in the N-terminal portion of the NS3 protein. Studies have confirmed that NS3 protease belongs to the trypsin or chymotrypsin super family of serine protease.[3] It is unique in that it requires a cofactor protein NS4A for efficient processing. The NS3 protease is essential for viral replication,[4] and it has been a prime target for drug discovery.[5] However, the fact that the binding pockets of the NS3 protease are shallow, solvent-exposed, and relatively featureless has made it a challenging endeavor. Although a number of potent inhibitors have been reported by various research laboratories around the world,[6] none has gone beyond phase II clinical trial.

Since the discovery of competitive inhibitors of the HCV NS3/NS4 protease from the hexapeptide cleavage products of the substrates,[7] intensive research has been focused on various derivatives of these peptide inhibitors. Our early efforts led to moderately potent tetrapeptide inhibitor **1** and pentapeptide inhibitor **2**, with binding affinity (K_i*) of 15 and 1.2 μM, respectively[8] (Figure 1). Both compounds have tetrahydroisoquinoline-3-carboxylamide (Tic) as the P2 residue. The challenge was to enhance the potency while the peptidic nature of these inhibitors was decreased. Peptides are known to be easily cleaved by peptidases and thus suffer from low bioavailability and poor pharmacological profiles.[9] One promising approach in depeptization is macrocyclization.[10] Macrocycles are conformationally preorganized for enzyme binding and are not

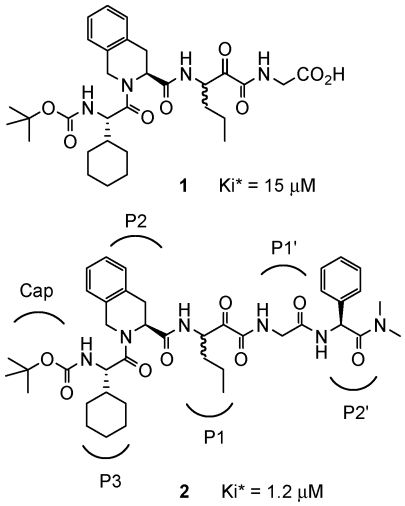

Figure 1. Tetra- and pentapeptide HCV protease inhibitors.

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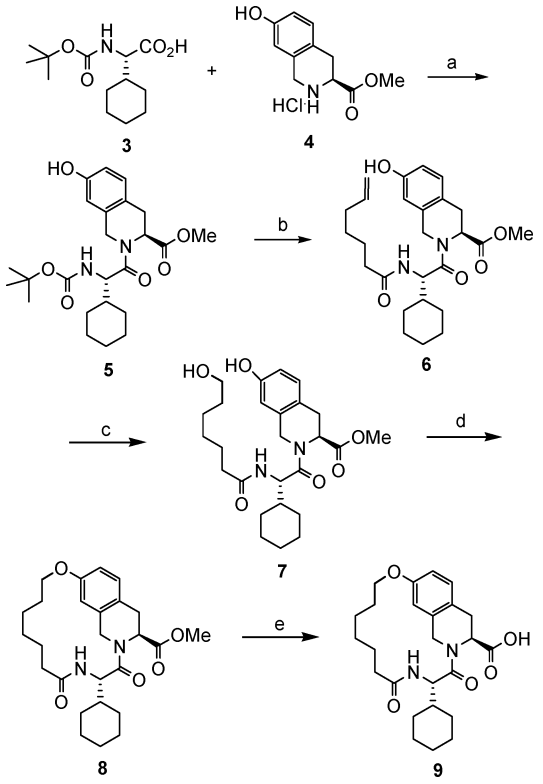
Scheme 1[a]



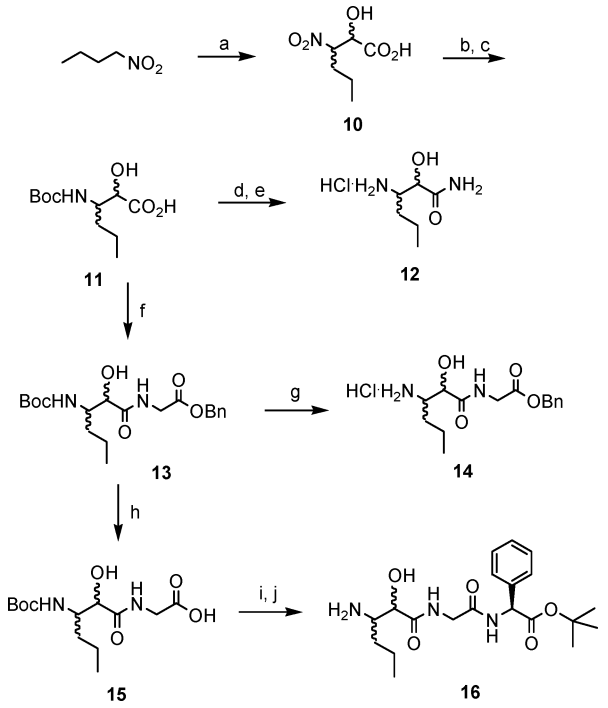
[a] Reaction conditions: (a) HOOBt, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 97%; (b) 4 M HCl, 1,4-dioxane; then 1-hept-6-enoic acid, HOOBt, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 31%; (c) BH_3·THF, THF, 0 °C, 93%; (d) PPh_3, ADDP, CH_2Cl_2, rt, 66%; (e) LiOH, MeOH/THF/H_2O, rt, 98%.

easily recognized by peptidases. As a result, they could provide better potency and stability.[11] Using X-ray crystal structure data of the enzyme, computer modeling of compounds **1** and **2** at the enzyme active site indicated that the P2 moiety was close to the P3 Boc capping group. We envisioned that macrocyclization from the P2 benzene ring to the P3 capping group would not only provide additional binding but also effectively depeptize the P2−P3 moiety. On the basis of molecular modeling results, the optimal ring size was estimated to be 17. The macrocycle formation could be accomplished through an aryl−alkyl ether linkage created by a Mitsunobu reaction.[12] Herein, we report the discovery of highly potent Tic-based macrocyclic HCV NS3 protease inhibitors.

Synthesis of Inhibitors

Synthesis of the macrocyclic left-hand portion of the inhibitors is outlined in Scheme 1. Amino acids Boc-cyclohexylglycine (**3**) and 7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic) methyl ester hydrochloride (**4**) were coupled to give dipeptide **5** under standard conditions (HOOBt, EDC, NMM). After removal of the Boc protecting group, the resulting amine hydrochloride was coupled to 1-hept-6-enoic acid to afford compound **6**. Hydroboration of the terminal alkene provided primary alcohol **7**. The key step, macrocyclization of this phenol alcohol, was accomplished under Mitsunobu reaction[12] conditions using triphenylphosphine and 1,1′-(azodicarbonyl) dipiperidine (ADDP) in dichloromethane at room temperature. Macrocyclic ester **8** was obtained in good yield (66%). To complete the synthesis of the left-hand section of the target molecule, the methyl ester was hydrolyzed to carboxylic acid **9**.

Scheme 2[a]

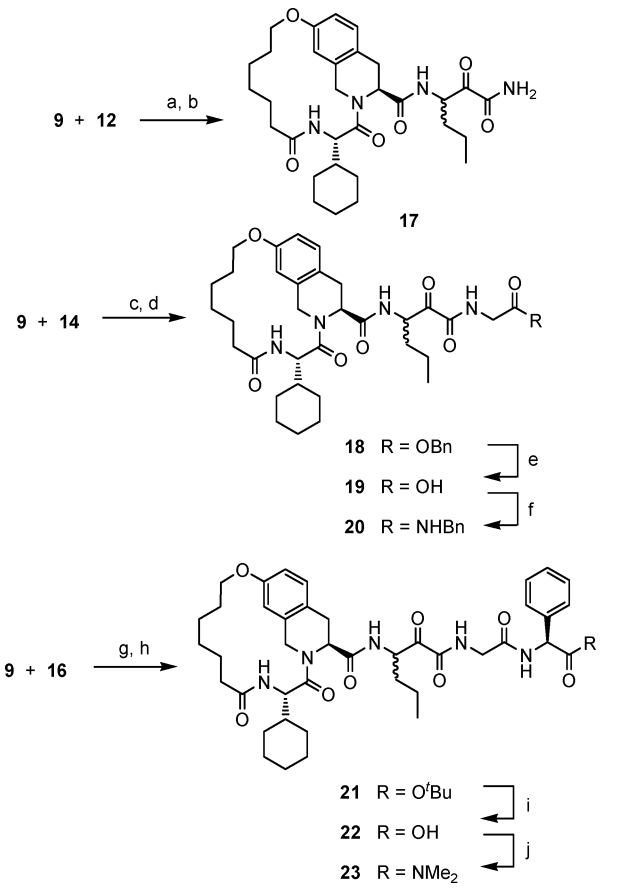


[a] Reaction conditions: (a) glyoxylic acid monohydrate, Et_3N, MeOH, 0 °C, then rt, 99%; (b) H_2, Pd−C, AcOH, rt, 66%; (c) $(Boc)_2O$, dioxane/H_2O, rt, 89%; (d) NH_4Cl, HOOBt, EDC, NMM, DMF −20 °C, 31%; (e) 4 M HCl, dioxane, rt, quantitative; (f) glycine benzyl ester hydrochloride, HOOBt, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 95%; (g) 4 M HCl, dioxane, rt, quantitative; (h) H_2, Pd−C, EtOH, rt, 98%; (i) phenylglycine *tert*-butyl ester hydrochloride, HOOBt, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 80%; (j) 2 M HCl in EtOAc/dioxane (1:1), rt, quantitative.

Preparation of the right-hand fragments of the inhibitors started with synthesis of α-hydroxy-*β*-amino acid derivative **11** (Scheme 2). Thus, in the presence of triethylamine, 1-nitrobutane reacted with glyoxylic acid to afford α-hydroxy-*β*-nitrohexanoic acid **10**. Low-pressure hydrogenation reduced the nitro group to an amino group, which was protected as a Boc derivative. The acid **11** was converted to primary amide, which, after removal of Boc-protecting group, gave desired intermediate **12**. Similarly, coupling of **11** with glycine benzyl ester hydrochloride provided dipeptide **13**, which was then converted to intermediate **14**. On the other hand, hydrogenation of benzyl ester **13** provided acid **15**. Standard coupling of **15** with phenylglycine *tert*-butyl ester furnished a tripeptide, whereupon Boc-deprotection, gave the desired intermediate **16**.

With synthesis of both left-hand and right-hand subunits accomplished, time came for the assembly of the final targets. Thus, macrocyclic acid **9** was coupled to amine **12** under standard conditions (Scheme 3). The resulting α-hydroxyamide was oxidized to tripeptide α-ketoamide **17** via a modified Moffatt oxidation[13] (DMSO, EDC, Cl_2CHCO_2H). The coupling between **9** and dipeptide amine **14** provided an α-hydroxyamide intermediate, which, upon Dess−Martin periodinane oxidation,[14] afforded tetrapeptide α-ketoamide **18**. The benzyl ester **18** was converted to its corresponding carboxylic acid **19** through catalytic hydrogenation. Amide bond formation from **19** furnished the benzyl amide derivative **20**. Similarly, coupling of compounds **9** and **16** and subsequent oxidation gave pentapeptide α-ketoamide **21**. When treated with trifluoroacetic acid, *tert*-butyl ester **21** was converted to carboxylic acid **22**. Finally, dimethyl amide derivative **23** was obtained from acid **22** through amide formation.

Scheme 3[a]



[a] Reaction conditions: (a) HOOBt, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 92%; (b) EDC, Cl_2CHCO_2H, DMSO/toluene, rt, 33%; (c) same as step a, 66%; (d) Dess−Martin periodinane, CH_2Cl_2, rt, 87%; (e) H_2, Pd−C, EtOH/MeOH, rt, 98%; (f) benzylamine, HOOBt, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 53%; (g) same as step a, 80%; (h) same as step d, 88%; (i) CF_3CO_2H, CH_2Cl_2, rt, quantitative; (j) Me_2NH·HCl, HOOBt, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 56%.

Table 1. Inhibitory Activity against HCV NS3 Protease

compd	K_i* (μM)	compd	K_i* (μM)
1	15	**20**	2.0
2	1.2	**21**	0.26
17	2.1	**22**	0.015
18	1.7	**23**	0.021
19	3.0		

Results and Discussions

The macrocyclic α-ketoamides (**17**−**23**) were examined in a continuous spectrophotometric assay[15] for inhibitory activity against the NS4A-tethered single chain NS3 serine protease.[16] The assay was based on the proteolysis of chromogenic ester substrates and was suitable for continuous monitoring of HCV NS3 protease activity. The inhibition constants (K_i*)[17] were determined and are listed in Table 1. Due to the acidic nature of the P1 α-proton, all the compounds were tested as a mixture of two diastereomers. When pure P1 isomers were used, extensive racemization was observed under assay conditions. The small molecule tripeptide **17** was modestly potent (K_i* = 2.1 μM). The tetrapeptide inhibitors (**18**−**20**) had similar binding affinity (K_i* = 1.7−3.0 μM). Additional amino acid (P1′ glycine) did not provide much enhancement to potency. However, when a second amino acid residue (P2′ phenylglycine) was incorporated, the resulting pentapeptide inhibitors were significantly more active against HCV protease. The K_i* of the *tert*-butyl ester derivative (**21**) was in the sub-micromolar range (0.26 μM). More encouraging was that the K_i* values of carboxylic acid **22** and dimethyl carboxamide **23** were 15 and 21 nM, respectively.

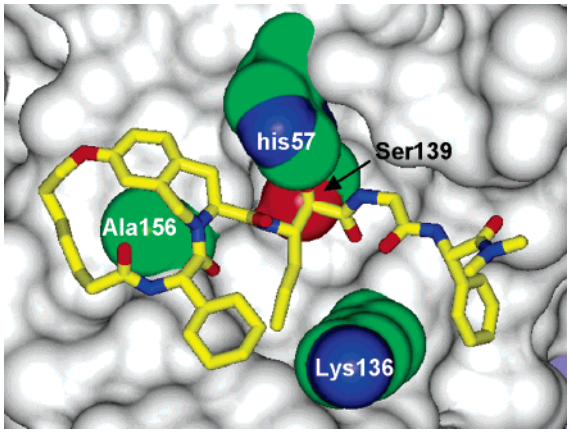


Figure 2. X-ray crystal structure of compound **23** bound to HCV NS3 protease.

Comparing the results of new macrocyclic inhibitors to that of acyclic analogues, it was clear that macrocyclization significantly enhanced potency. Tripeptide **17** was a smaller molecule than acyclic tetrapeptide **1**, yet it was a more effective inhibitor. Compounds **18**−**20** were similar in size to **1**, but the potency was improved 5−9-fold. More dramatic enhancement was observed from cyclization of acyclic pentapeptide **2** to macrocyclic compounds **22** and **23**. The improvement in K_i* was 50−80-fold.

Compound **23** was soaked with crystalline HCV NS3/4A protease protein, and an X-ray crystal structure of the inhibitor bound to the enzyme was obtained. The reversible covalent bond between the hydroxyl of the enzyme active site serine (Ser139) and the ketone carbonyl of the inhibitor was confirmed. The most interesting feature, however, was that the macrocycle encircled the methyl group of Ala156. This donutlike conformation provided additional interaction between the inhibitor and the protease. The *n*-propyl side chain of P1 norvaline extended deep inside the S1 pocket. On the prime side, the phenyl ring of the P2′ residue extended over the far side of residue Lys136 in such a way that the P1 propyl group and P2′ phenyl group formed a C-shaped clamp around the lysine side chain. There were also several hydrogen-bonding interactions between the inhibitor amide chain and the protease backbone. To examine the pharmacokinetic properties of these macrocyclic inhibitors, compound **23** was also selected for rat PK studies. It has good AUC in rats (5.5 μM h) when the compounds were administered subcutaneously. However, oral dosing gave a low AUC in rats (0.27 μM·h), which led a low bioavailability (<10%). The results were not surprising, considering the fact that the backbone of the molecule is still mostly peptidic in nature, even though macrocyclization reduced some peptide characteristics at the P2−P3 moiety. Further depeptization and designing of smaller potent inhibitors are in progress.

Conclusion

A short synthesis of a 17-membered macrocycle **8** has been accomplished. The macrocyclization of the phenol alcohols was achieved through a Mitsunobu protocol (PPh_3, ADDP). The macrocyclic acid **9** was coupled to mono-, di-, and tripeptide right-hand intermediates **12**, **14**, and **16**. The resulting α-hydroxyamides were oxidized to the final target α-ketoamides. The inhibition constants (K_i*) from HCV protease continuous assay demonstrated that these macrocyclic inhibitors were much

more potent (5−80-fold) than acyclic analogues of comparable molecular weight. The pentapeptide acid **22** and dimethyl amide **23** were the most potent inhibitors, with K_i* values (15 and 21 nM, respectively) approaching single digit nanomolar range. The X-ray structure of compound **23** bound to the enzyme revealed that the macrocycle formed a donutlike shape around the methyl group of Ala156. It also confirmed the formation of a covalent bond between the ketone carbonyl and Ser139 hydroxyl. A C-shaped clamp around the Lys136 side chain formed by P1 norvaline and P2′ phenylglycine was also observed.

Experimental Section

General Methods. Chemical reagents and solvents, including anhydrous THF, dichloromethane, and DMF, were purchased from Aldrich or other commercial sources and were used without further purification. Reactions that were moisture sensitive or using anhydrous solvents were performed under either a nitrogen or an argon atmosphere. Analytical thin-layer chromatography (TLC) was performed on precoated silica gel plates obtained from Analtech. Visualization was accomplished with UV light or by staining with basic $KMnO_4$ solution, ethanolic H_2SO_4, or Vaughn's reagent. Compounds were purified by flash chromatography either on a glass column using Merck silica gel 60 (230−400 mesh) or on an ISCO RediSep disposable silica gel column. NMR spectra were recorded at 300, 400, or 500 MHz for ^{1}H NMR and at 75, 100, or 125 MHz for ^{13}C NMR, on a Bruker or Varian spectrometer with $CDCl_3$ or d_6-DMSO as solvent. The chemical shifts are given in ppm, referenced to the internal TMS or deuterated solvent signal.

2-(2-*tert*-Butoxycarbonylamino-2-cyclohexylacetyl)-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid Methyl Ester (5). To a solution of *N*-Boc-cyclohexylglycine (**3**) (3.50 g, 12.7 mmol), 7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic) methyl ester hydrochloride (**4**) (3.03 g, 12.4 mmol), 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (HOOBt) (2.15 g, 13.2 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) hydrochloride (2.85 g, 14.9 mmol) in anhydrous DMF (100 mL), and CH_2Cl_2 (100 mL) at −20 °C was added 4-methylmorpholine (NMM) (4.10 mL, 37.3 mmol). After being stirred at −20 °C for 30 min, the reaction mixture was kept in a freezer overnight (18 h). It was then warmed to room temperature. EtOAc (300 mL), brine (100 mL), and 5% H_3PO_4 (100 mL) were added and the layers were separated. The organic solution was washed consecutively with 5% H_3PO_4 (200 mL), saturated aqueous sodium bicarbonate solution (2 × 200 mL), water (200 mL), and brine (200 mL); dried with magnesium sulfate; filtered; and concentrated under reduced pressure to afford dipeptide **5** (5.35 g, 97% yield) as a pale yellow solid. An analytical sample was obtained through flash chromatography (2−5% MeOH/CH_2Cl_2): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.87 (d, J = 7.9 Hz, 1 H), 6.59 (d, J = 2.2 Hz, 1 H), 6.56 (dd, J = 8.0, 2.5 Hz, 1 H), 5.49 (d, J = 9.2 Hz, 1 H), 5.42 (dd, J = 6.0, 4.0 Hz, 1 H), 4.76 (d, J = 5.5 Hz, 1 H), 4.67 (dd, J = 9.2, 5.4 Hz, 1 H), 4.55 (d, J = 5.5 Hz, 1 H), 3.60 (s, 3 H), 3.10 (dd, J = 15.7, 4.0 Hz, 1 H), 2.89 (dd, J = 15.7, 5.8 Hz, 1 H), 1.90−1.67 (m, 4 H), 1.44 (s, 9 H), 1.38−0.76 (m, 7 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 171.2, 156.3, 155.3, 132.7, 129.1, 123.0, 114.1, 112.9, 80.3, 55.1, 52.3, 51.9, 45.4, 41.0, 29.7, 29.6, 28.4, 28.3, 27.3, 26.2, 26.1, 26.0; HRMS calcd for $C_{24}H_{35}N_2O_6$ $(M + H)^+$ 447.2492, found 447.2495.

2-(2-Cyclohexyl-2-hept-6-enoylaminoacetyl)-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid Methyl Ester (6). The dipeptide **5** (7.20 g, 16.1 mmol) was dissolved in 4 M HCl (100 mL, 400 mmol), and the resulting solution was stirred at room temperature. The progress of the reaction was monitored by TLC. After 4 h, the solution was concentrated and the residue was left under vacuum overnight to give product amine hydrochloride as an off-white solid: HRMS calcd for $C_{19}H_{27}N_2O_4$ $(M + H)^+$ 347.1971, found 347.1965. The desired product **6** was prepared from the amine hydrochloride and 1-hept-6-enoic acid (2.90 g, 22.6 mmol) under similar coupling conditions as described above for the preparation of compound **5**. Flash chromatography (5−30% EtOAc−CH_2Cl_2) of the crude product afforded **6** (2.30 g, 31%, two steps) as a white solid. Two rotamers were observed (ca., 4:1 ratio) according to ^{1}H NMR spectra. The two sets of peaks only partially collapsed when the NMR sample was heated to 90 °C. They were, however, proved to be exchangeable protons in a 2D NOESY experiment: ^{1}H (500 MHz, DMSO-d_6) δ 9.37 & 9.31 (s, 1 H), 8.12 & 8.09 (d, J = 8.8 & 9.4 Hz, 1 H), 6.99 & 6.97 (d, J = 8.2 & 8.2 Hz, 1 H), 6.64−6.54 (m, 2 H), 5.79−5.39 (m, 1 H), 5.05−4.89 (m, 4 H), 4.71 & 4.62 (t, J = 8.8 & 9.2 Hz, 1 H), 4.51 & 4.23 (d, J = 15.6 & 17.7 Hz, 1 H), 3.54 & 3.46 (s, 3 H), 3.12−2.90 (m, 2 H), 2.16−1.93 (m, 4 H), 1.75−1.59 (m, 5 H), 1.50−0.97 (m, 10 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.9, 171.6, 171.5, 171.3, 171.1, 170.7, 156.0, 155.9, 138.5, 134.0, 132.8, 129.1, 128.6, 122.5, 121.3, 114.6, 114.5, 114.1, 114.0, 112.4, 54.2, 52.9, 52.5, 52.2, 52.1, 51.9, 45.0, 43.1, 34.48, 34.46, 32.8, 32.7, 30.6, 29.4, 29.0, 28.6, 28.0, 27.9, 27.7, 27.6, 25.84, 25.78, 25.52, 25.46, 25.39, 25.35, 24.7; HRMS calcd for $C_{26}H_{37}N_2O_5$ $(M + H)^+$ 457.2702, found 457.2712.

2-[2-Cyclohexyl-2-(7-hydroxyheptanoylamino)acetyl]-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid Methyl Ester (7). To the solution of **6** (2.20 g, 4.82 mmol) in anhydrous THF (100 mL) under nitrogen at 0 °C was added borane−THF solution (20 mL, 1.0 M, 20 mmol) cautiously. The mixture was stirred at 0 °C under nitrogen for 1 h 40 min. Then, ethanol (10 mL) and pH 7 buffer (15 mL) were added, followed by 30% aqueous hydrogen peroxide solution (15 mL). After 20 min, the mixture was warmed to room temperature and stirred for 2 h. EtOAc (400 mL) and brine (200 mL) were added and the two layers were separated. Aqueous solution was extracted with EtOAc (2 × 150 mL). The combined organic solution was dried with magnesium sulfate, filtrated, and concentrated in vacuo. Flash chromatography (3−5% MeOH−CH_2Cl_2) afforded **7** (2.18 g, 4.47 mmol, 93%) as a white solid. Similar to the NMR spectra of compound **6**, two rotamers were observed (ca., 4:1 ratio) in both ^{1}H and ^{13}C NMR spectra at room temperature. The two sets of peaks only partially collapsed in ^{1}H NMR at 90 °C. They were, however, proved to be exchangeable protons in a 2D NOESY experiment. One set of peaks were observed in ^{13}C NMR at 90 °C: ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.37 & 9.31 (s, 1 H), 8.09 & 8.07 (d, J = 8.9 & 10.4 Hz, 1 H), 6.99 & 6.97 (d, J = 8.2 & 8.2 Hz, 1 H), 6.63−6.54 (m, 2 H), 5.04−5.02 (d, J = 5.4 & 5.4 Hz, 1 H), 4.70 & 4.61 (t, J = 8.5 & 9.2 Hz, 1 H), 4.51 & 4.23 (d, J = 15.4 & 17.9 Hz, 1 H), 4.33 (t, J = 5.2 Hz, 1 H), 3.54 & 3.46 (s, 3 H), 3.36 (s, 1 H), 3.35 & 3.32 (t, J = 2.6 & 5.9 Hz, 2 H), 3.15−2.90 (m, 2 H), 2.14−1.99 (m, 2 H), 1.74−1.57 (m, 5 H), 1.49−1.31 (m, 4 H), 1.27−0.84 (m, 10 H); ^{13}C NMR (125 MHz, DMSO-d_6, 90 °C) δ 172.1, 171.4, 171.0, 156.0, 134.0, 128.5, 122.6, 114.3, 112.6, 60.8, 53.0, 52.3, 51.6, 45.1, 35.0, 32.3, 29.5, 28.9, 28.4, 28.0, 25.8, 25.5, 25.4, 25.2, 25.1; HRMS calcd for $C_{26}H_{39}N_2O_6$ $(M + H)^+$ 475.2812, found 475.2808.

3-Cyclohexyl-2,5-dioxo-12-oxa-1,4-diazatricyclo[11.5.3.0^{16,20}]-henicosa-13(21),14,16(20)-triene-18-carboxylic Acid Methyl Ester (8). A solution of phenol alcohol **7** (2.08 g, 4.38 mmol) and 1,1′-(azodicarbonyl)dipiperidine (ADDP) (3.00 g, 11.9 mmol) in anhydrous CH_2Cl_2 was bubbled with argon through a frit-glass bubbler for 20 min. To this solution at 0 °C was added triphenylphosphine (3.45 g, 13.2 mmol). After stirring at 0 °C for 20 min, the solution was warmed to room temperature and stirred overnight (18 h) under argon. TLC indicated the presence of starting material. A second batch of ADDP (3.00 g, 11.9 mmol) and triphenylphosphine (3.45 g, 13.2 mmol) was added, and the mixture was stirred under argon for 2 days and 16 h. The reaction was concentrated under reduced pressure. The residue was purified by flash chromatography (1−2% MeOH in CH_2Cl_2) to afford the macrocycle **8** (1.31 g, 66% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.08 (d, J = 8.2 Hz, 1 H), 6.93 (d, J = 2.5 Hz, 1 H), 6.79 (dd, J = 8.2, 2.5 Hz, 1 H), 5.92 (d, J = 10.0 Hz, 1 H), 5.22 (d, J = 14.5 Hz, 1 H), 5.02 (t, J = 10.3 Hz, 1 H), 4.52 (dd, J = 11.9, 6.3 Hz, 1 H), 4.36−4.31 (m, 1 H), 4.31 (d, J = 14.5 Hz, 1 H), 4.14−4.07 (m, 1 H), 3.77 (s, 3 H), 3.20 (dd, J = 14.9, 6.3 Hz, 1 H), 2.88 (dd, J = 14.4, 11.7 Hz, 1 H), 2.28 (ddd, J = 14.9, 7.4, 3.1 Hz, 1 H), 2.01−1.95 (m, 1

H), 1.87−1.62 (m, 10 H), 1.42−0.98 (m, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.3, 171.8, 171.5, 158.0, 136.8, 128.0, 126.1, 116.5, 112.7, 66.9, 54.9, 52.8, 52.3, 46.2, 40.2, 35.1, 30.2, 29.5, 28.6, 27.5, 27.4, 26.4, 25.61, 25.57, 24.4, 24.0; HRMS calcd for $C_{26}H_{37}N_2O_5$ $(M + H)^+$ 457.2707, found 457.2702.

3-Cyclohexyl-2,5-dioxo-12-oxa-1,4-diazatricyclo[11.5.3.0^{16,20}]-henicosa-13(21),14,16(20)-triene-18-carboxylic Acid (9). A solution of lithium hydroxide (0.21 g, 8.75 mmol) in water (30 mL) was added to a solution of methyl ester **8** (1.30 g, 2.85 mmol) in THF (30 mL) and methanol (30 mL) at 0 °C. The mixture was allowed to warm to room temperature along with the ice bath in 4 h. The progress of the reaction was monitored by TLC. The mixture was concentrated under reduced pressure to one-third of its original volume. EtOAc (50 mL) and water (30 mL) were added and the two layers were separated. The aqueous solution was washed with CH_2Cl_2 (50 mL), after which it was acidified to pH 1 using 2 M aqueous hydrochloric acid. EtOAc was then added (100 mL) and the aqueous solution was saturated with solid sodium chloride. After separation of the layers, the aqueous layer was extracted with EtOAc (2 × 60 mL). Organic solutions were combined, dried with magnesium sulfate, filtered, and concentrated in vacuo to afford **9** (1.23 g, 98% yield) as an off-white solid, which was used without further purification: ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.64 (bs, 1 H), 7.99 (d, J = 9.9 Hz, 1 H), 7.16 (d, J = 8.2 Hz, 1 H), 6.80 (dd, J = 8.2, 2.6 Hz, 1 H), 6.75 (d, J = 2.2 Hz, 1 H), 5.10 (d, J = 14.8 Hz, 1 H), 4.74 (t, J = 10.0 Hz, 1 H), 4.32 (dd, J = 11.3, 6.4 Hz, 1 H), 4.25 (d, J = 14.4 Hz, 1 H), 4.22−4.18 (m, 2 H), 4.07−4.03 (m, 1 H), 3.16 (dd, J = 14.8, 6.3 Hz, 1 H), 2.84 (dd, J = 14.4, 11.3 Hz, 1 H), 2.0.05−2.04 (m, 1H), 1.83−1.77 (m, 1 H), 1.69−1.47 (m, 8 H), 1.35−0.82 (m, 11 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.5, 171.7, 169.9, 157.0, 137.0, 128.2, 126.6, 115.7, 112.5, 66.4, 54.2, 52.2, 44.8, 33.6, 30.3, 29.4, 29.1, 27.9, 27.3, 27.0, 26.0, 25.3, 25.2, 24.3, 23.8; HRMS calcd for $C_{25}H_{35}N_2O_5$ $(M + H)^+$ 443.2546, found 443.2558.

3-*tert*-Butoxycarbonylamino-2-hydroxyhexanoic Acid (11). To a stirred solution of 1-nitrobutane (16.5 g, 0.160 mol) and glyoxylic acid monohydrate (28.1 g, 0.305 mol) in MeOH (120 mL) at 0 °C was added triethylamine (93.0 mL, 0.667 mol) dropwise over 2 h. The solution was warmed to room temperature, stirred overnight, and concentrated to give an oil. The oil was dissolved in water (100 mL), and the solution was acidified to pH 1 with 10% aqueous HCl solution and extracted with EtOAc (3 × 200 mL). The combined organic solution was washed with brine, dried over Na_2SO_4, filtered, and concentrated to dryness to give the 3-nitro-2-hydroxyhexanoic acid (28.1 g, 99% yield). To a stirred solution of the nitro acid (240 g, 1.35 mol) in acetic acid (1.25 L) was added 10% Pd/C (37 g). The resulting solution was hydrogenated at 59 psi for 20 h. The acetic acid was then evaporated and the residue was azeotroped three times with toluene. It was then triturated with MeOH and ether. The solid that formed was separated by filtration and azeotroped twice with toluene to give the amino acid product as an off-white solid (131 g, 66% yield). To a stirred solution of this amino acid (2.0 g, 13.6 mmol) in dioxane (10 mL) and H_2O (5 mL) at 0 °C was added 1 N aqueous NaOH solution (14.0 mL, 14.0 mmol). After stirring for 10 min, di-*tert*-butyl dicarbonate (3.10 g, 14.0 mmol) was added and the mixture was stirred for 15 min at 0 °C. It was then warmed to room temperature and stirred for an additional 45 min before it was placed in a refrigerator overnight. The reaction was then concentrated to dryness. The residue was dissolved in EtOAc (100 mL). Ice (∼50 g), $KHSO_4$ (3.36 g), and H_2O (32 mL) were added, and the mixture was stirred for 5 min. The layers were then separated, the aqueous layer was extracted twice with EtOAc (2 × 50 mL), and the combined organic solution was washed with water and brine, dried (Na_2SO_4), filtered, and concentrated to dryness to yield the product **11** (3.0 g, 89% yield) as a mixture of four diastereomers: ^{1}H NMR (500 MHz, CD_3OD,) δ, 4.19 & 4.15 (d, J = 4, 2.5 Hz, 1 H), 3.98−3.95 & 3.91−3.90 (m, 1 H), 1.59−1.35 (m, 10 H), 1.46 & 1.43 (s, 9 H), 0.98 & 0.94 (t, 3 H, J = 7.0 Hz).^{13}C NMR (125 MHz, CD_3OD) δ, 175.2, 174.8, 157.1, 156.9, 79.1, 73.3, 72.1, 53.2, 42.9, 34.1, 31.1, 27.8, 27.7, 19.4, 19.3, 13.2, 13.1; HRMS calcd for $C_{11}H_{22}NO_5$ 248.1498 $(M + H)^+$, found 248.1510.

3-Amino-2-hydroxyhexanoic Acid Amide Hydrochloride (12). Carboxylic acid **11** (1.50 g, 6.07 mmol) was converted to the primary amide (0.462 g, 31% yield) under standard coupling conditions as described above for the preparation of **5**, except that excess ammonium chloride (5 equivalent) was used as amine and DMF was the only solvent used in the reaction: HRMS calcd for $C_{11}H_{23}N_2O_4$ $(M + H)^+$ 247.1658, found 247.1652. This product was treated with 4 M hydrochloric acid in dioxane at room temperature for 3 h. It was concentrated under reduced pressure to give an pale yellow solid (quantitative yield) as a mixture of four diastereomers, which was used in subsequent reactions without further purification: HRMS calcd for $C_6H_{15}N_2O_2$ $(M + H)^+$ 147.1134, found 147.1139.

(3-*tert*-Butoxycarbonylamino-2-hydroxyhexanoylamino)acetic Acid Benzyl Ester (13). The coupling of carboxylic acid **11** (3.00 g, 12.0 mmol) and glycine benzyl ester hydrochloride (2.56 g, 13.0 mmol) was carried out in a manner similar to that described above for the preparation of **5**. The crude product was purified by flash chromatography (25−50% acetone/hexanes) to afford the dipeptide product (4.50 g, 95%) as a mixture of four diastereomers: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.21 & 8.18 (t, 1 H, J = 6.5 Hz), 7.40−7.33 (m, 5 H), 6.39 & 5.94 (d, J = 9.0, 9.5 Hz, 1 H), 5.89 &5.76 (d, J = 6, 5.5 Hz, 1 H), 5.14 (bs, 2 H), 3.99−3.70 (m, 4 H), 1.39 &1.36 (s, 9 H), 1.48−1.07 (m, 4 H), 0.85 & 0.77 (t, J = 7.0, 6.5 Hz, 3 H); ^{13}C NMR (125 MHz, DMSO-d_6), δ, 173.9, 173.2, 170.5, 170.4, 156.1, 136.8, 129.3, 128.9, 128.8, 128.7, 78.5, 78.4, 74.5, 73.1, 66.6, 53.5, 41.4, 30.5, 29.1, 29.0, 19.8, 19.7, 14.7, 14.6; LRMS m/z MH^+ = 395; HRMS calcd for $C_{20}H_{31}N_2O_6$ 395.2182 $(M + H)^+$, found 395.2199.

(3-Amino-2-hydroxyhexanoylamino)acetic Acid Benzyl Ester Hydrochloride (14). Compound **12** was treated with 4 M hydrochloric acid in dioxane at room temperature for 3 h. It was concentrated under reduced pressure to give an off-white solid as a mixture of four diastereomers (quantitative yield), which was used in subsequent reactions without further purification: HRMS calcd for $C_{15}H_{23}N_2O_4$ 295.1658 $(M + H)^+$, found 295.1654.

(3-*tert*-Butoxycarbonylamino-2-hydroxyhexanoylamino)acetic Acid (15). To a solution of the benzyl ester **13** (4.50 g, 11.4 mmol) in ethanol (50 mL) was added 5% Pd−C (1.0 g). The mixture was stirred vigorously under a hydrogen atmosphere at room temperature for 3 h before it was filtered through a Celite pad. The filter cake was washed with EtOAc (2 × 30 mL). The solution was concentrated under reduced pressure to afford the product **15** (3.40 g, 98% yield) as a mixture of four diastereomers: HRMS calcd for $C_{13}H_{25}N_2O_6$ $(M + H)^+$ 305.1713, found 305.1699.

[2-(3-Amino-2-hydroxyhexanoylamino)acetylamino]phenylacetic Acid *tert*-butyl Ester Hydrochloride (16). The coupling of carboxylic acid **15** (2.00 g, 6.57 mmol) and phenyl glycine *tert*-butyl ester hydrochloride (1.76 g, 6.57 mmol) was carried out in a manner similar to that described above for the preparation of **5**. The crude product was purified by flash chromatography (1:1 EtOAc/hexanes) to afford a tripeptide product (2.60 g, 80%) as a mixture of four diastereomers: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.64 & 8.58 (d, J = 7.0 Hz, 1 H), 7.91 &7.87 (m, 1 H), 7.42−7.32 (m, 5 H), 6.40 & 5.99 (dd, J = 4.5 & 5.00 Hz, 1 H), 5.77−5.73 (m, 1 H), 5.30−5.28 (m, 1 H), 3.97−3.70 (m, 4 H), 1.51−1.08 (m, 22 H), 0.86 & 0.79 (t, J = 6.0 Hz, 3 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.4, 172.8, 170.3, 169.4, 169.3, 155.7, 137.5, 129.5, 129.0, 128.3, 128.2, 82.2, 82.1, 78.4, 74.6, 73.2, 57.7, 53.4, 42.3, 33.7, 29.1, 29.0, 28.3, 19.7, 14.7, 14.6; HRMS calcd for $C_{25}H_{40}N_3O_7$ 494.2866 $(M + H)^+$, found 494.2863. A solution of this *tert*-butyl ester (2.6 g, 5.30 mmol) in 2 M HCl in EtOAc/dioxane (1:1) was stirred at room temperature for 15 min. The reaction mixture was concentrated in vacuo to yield **16** (quantitative yield) as a pale yellow solid which was used in subsequent reactions without further purification: HRMS calcd for $C_{20}H_{32}N_3O_5$ 394.2342 $(M + H)^+$, found 394.2336.

3-Cyclohexyl-2,5-dioxo-12-oxa-1,4-diazatricyclo [11.5.3.0^{16,20}]-henicosa-13(21),14,16(20)-triene-18-carboxylic Acid (1-Ami-

nooxalylbutyl)amide (17). Coupling of acid **9** (59 mg, 0.133 mmol) and amine hydrochloride **12** (34 mg, 0.187 mmol) was carried out in a manner similar to that described above for the preparation of **5**. The intermediate product was obtained as a mixture of diastereomers (70 mg, 92% yield). To the solution of this product and EDC (235 mg, 1.23 mmol) in DMSO/toluene (1:1, 10 mL) at 0 °C was added dichloroacetic acid (0.055 mL, 0.667 mmol) dropwise. The mixture was stirred at 0 °C for 15 min and at room temperature for 2 h. EtOAc (100 mL) was added and the mixture was washed with 5% H_3PO_4 (2 × 60 mL), saturated aqueous sodium bicarbonate solution (2 × 60 mL), and brine (60 mL). It was then dried with magnesium sulfate, filtered, and concentrated in vacuo. Flash chromatography (3−6% MeOH/CH_2Cl_2) afforded **16** (25 mg, 33% yield) as a mixture of diastereomers. The ratio of the two diastereomers was variable and dependent on the reaction conditions: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.21 & 8.08 (d, J = 7.3 & 7.3 Hz, 1 H), 8.00 (s, 1 H), 7.91 (t, J = 9.6 Hz, 1 H), 7.87 (d, J = 10.4 Hz, 1 H), 7.13 (t, J = 8.2 Hz, 1 H), 6.82−6.79 (m, 1 H), 6.74 (dd, J = 11.0, 2.4 Hz, 1 H), 5.09−4.98 (m, 1 H), 4.74 (ABq, J = 9.8 Hz, 1 H), 4.38 & 4.36 (dd, J = 8.9, 6.5 & 9.5, 6.5 Hz, 1 H), 4.30 & 4.21 (d, J = 14.7 & 14.7 Hz, 1 H), 4.21−4.18 (m, 1 H), 4.06−4.03 (m, 1 H), 3.14−3.09 (m, 1 H), 2.75 (ddd, J = 15.5, 11.2, 5.7 Hz, 1 H), 2.06 (t, J = 5.7 Hz, 2 H), 1.80−1.45 (m, 12 H), 1.43−0.82 (m, 14 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 197.6, 197.5, 171.72, 171.69, 171.1, 171.0, 170.2, 169.8, 163.1, 163.0, 157.04, 156.96, 137.4, 137.2, 128.0, 127.8, 127.0, 126.8, 115.7, 115.6, 112.6, 112.5, 66.7, 66.6, 54.9, 54.7, 53.2, 53.1, 52.5, 52.4, 45.4, 45.3, 33.5, 33.4, 31.74, 31.65, 30.0, 29.9, 29.6, 29.4, 28.1, 28.0, 27.2, 27.1, 27.0, 26.9, 26.0, 25.34, 25.28, 24.1, 23.9, 23.8, 23.7, 18.63, 18.55, 13.5, 13.4; HRMS calcd for $C_{31}H_{45}N_4O_6$ $(M + H)^+$ 569.3339, found 569.3336.

{3-[(3-Cyclohexyl-2,5-dioxo-12-oxa-1,4-diazatricyclo[11.5.3.0^{16,20}]-henicosa-13(21),14,16(20)-triene-18-carbonyl)amino]-2-oxohexanoylamino}acetic Acid Benzyl Ester (18). Coupling of carboxylic acid **9** (150 mg, 0.339 mmol) and amine hydrochloride **14** (155 mg, 0.468 mmol) was carried out in a manner similar to that described above for the preparation of compound **5**. After purification by flash chromatography (2−5% MeOH/CH_2Cl_2), the intermediate product (160 mg, 66% yield) was obtained as a mixture of diastereomers. To the solution of this product (150 mg, 0.209 mmol) in anhydrous CH_2Cl_2 (80 mL) at room temperature was added Dess−Martin periodinane (0.220 g, 0.519 mmol). The mixture was stirred for 4 h. Saturated aqueous sodium bicarbonate and sodium thiosulfate solutions (40 mL each) were added. After stirring for 10 min, the layers were separated. The aqueous solution was extracted with CH_2Cl_2 (2 × 60 mL). The combined organic solution was dried with magnesium sulfate, filtered, and concentrated in vacuo. Flash chromatography (1−5% MeOH/CH_2Cl_2) afforded **18** (130 mg, 87% yield) as a mixture of diastereomers. The ratio of the two diastereomers was variable and dependent on the reaction conditions: ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.08 (t, J = 6.0 Hz, 1 H), 8.27 & 8.14 (d, J = 6.8 & 7.1 Hz, 1 H), 7.92 (t, J = 9.5 Hz, 1 H), 7.39−7.32 (m, 5 H), 7.13 (dd, J = 10.6, 8.4 Hz, 1 H), 6.80 (ddd, J = 8.4, 6.0, 2.4 Hz, 1 H), 6.74 (dd, J = 10.2, 2.4 Hz, 1 H), 5.17−5.10 (m, 2 H), 5.09−5.00 (m, 2 H), 4.74 (dd, J = 18.7, 10.0 Hz, 1 H), 4.40−4.35 (m, 1 H), 4.33−4.18 (m, 2 H), 4.06−4.00 (m, 1 H), 3.99−3.93 (m, 1H), 3.11 (dd, J = 14.8, 6.3 Hz, 1 H), 2.78−2.71 (m, 1 H), 2.07−2.05 (m, 2 H), 1.77−1.46 (m, 10 H), 1.42−0.84 (m, 16 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 196.5, 196.4, 171.74, 171.69, 171.2, 171.1, 170.2, 169.8, 168.7, 161.2, 161.1, 157.1, 157.0, 137.4, 137.3, 135.7, 128.3, 128.03, 127.99, 127.92, 127.86, 127.83, 127.0, 126.8, 115.7, 115.6, 112.54, 112.50, 66.7, 66.6, 66.0, 55.0, 54.7, 53.4, 53.3, 52.6, 51.8, 45.3, 40.6, 33.5, 33.4, 31.6, 31.46, 31.45, 31.2, 30.0, 29.9, 29.6, 29.5, 28.1, 28.0, 27.2, 27.1, 27.0, 26.9, 26.01, 26.02, 25.34, 25.29, 24.1, 23.9, 23.8, 23.7, 18.6, 18.5, 13.5, 13.4; HRMS calcd for $C_{40}H_{52}N_4O_8$ $(M + H)^+$ 717.3863, found 717.3859.

{3-[(3-Cyclohexyl-2,5-dioxo-12-oxa-1,4-diazatricyclo [11.5.3.0^{16,20}]henicosa-13(21),14,16(20)-triene-18-carbonyl)amino]-2-oxohexanoylamino}acetic Acid (19). To a solution of the benzyl ester **18** (120 mg, 0.167 mmol) in ethanol/methanol (2:1, 45 mL) was added 5% Pd−C (0.50 g). The mixture was stirred vigorously under hydrogen at room temperature for 3 h before it was filtered through a 1 in. Celite pad. The filter cake was washed with EtOAc (2 × 30 mL). The solution was concentrated under reduced pressure to afford the product **19** (103 mg, 98% yield) as a mixture of diastereomers: ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.7 (bs, 1 H), 8.88 (t, J = 6.0 Hz, 1 H), 8.26 & 8.13 (d, J = 7.0 & 7.5 Hz, 1 H), 7.92 (t, J = 9.4 Hz, 1 H), 7.14 (dd, J = 12.0, 8.2 Hz, 1 H), 6.80 (td, J = 7.2, 2.4 Hz, 1 H), 6.74 (dd, J = 9.5, 2.4 Hz, 1 H), 5.09−5.01 (m, 2 H), 4.74 (ABq, J = 9.7 Hz, 1 H), 4.39−4.34 (m, 1 H), 4.33−4.18 (m, 2 H), 3.76 (d, J = 6.0 Hz, 2 H), 3.11 (dd, J = 15.0, 6.3 Hz, 1 H), 2.75 (ddd, J = 15.0, 11.2, 7.0 Hz, 1 H), 2.07−2.05 (m, 2 H), 1.79−1.01 (m, 21 H), 0.94−0.79 (m, 5 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 196.8, 196.7, 171.74, 171.69, 171.2, 171.1, 170.2, 169.8, 161.0, 160.9, 157.1, 157.0, 137.4, 137.3, 128.0, 127.9, 126.8, 126.5, 115.6, 112.5, 66.7, 66.6, 56.2, 55.0, 54.7, 53.4, 53.3, 52.6, 45.3, 40.5, 33.5, 33.4, 31.6, 31.5, 30.0, 29.9, 29.6, 29.4, 28.1, 28.0, 27.2, 27.1, 27.0, 26.9, 26.3, 26.0, 25.34, 25.29, 24.1, 23.8, 23.7, 18.7, 18.6, 13.5, 13.4; LRMS m/z $(M + H)^+$ = 627.3.

3-Cyclohexyl-2,5-dioxo-12-oxa-1,4-diazatricyclo[11.5.3.0^{16,20}]-henicosa-13(21),14,16(20)-triene-18-carboxylic Acid [1-(Benzylcarbamoylmethyl)aminooxalyl]butyl)amide (20). The coupling of carboxylic acid **19** (90 mg, 0.144 mmol) and benzylamine (0.020 mL, 0.183 mmol) was carried out in a manner similar to that described above for the preparation of compound **5**. The product was purified by flash chromatography (2−5% MeOH/CH_2Cl_2) to afford **20** (55 mg, 53% yield) as a mixture of diastereomers: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.83−8.79 (m, 1 H), 8.44 (dd, J = 10.3, 5.5 Hz, 1 H), 8.27 & 8.13 (d, J = 7.6 & 6.6 Hz, 1 H), 7.93 (t, J = 10.2 Hz, 1 H), 7.34−7.23 (m, 5 H), 7.14 (t, J = 8.7 Hz, 1 H), 6.83−6.80 (m, 1 H), 6.76−6.74 (m, 1 H), 5.11−5.03 (m, 2 H), 4.75 (ABq, J = 10.5 Hz, 1 H), 4.41−4.36 (m, 1 H), 4.33−4.19 (m, 4 H), 4.07−4.04 (m, 1 H), 3.82 (d, J = 6.1 Hz, 1 H), 3.12 (td, J = 14.4, 6.2 Hz, 1 H), 2.75 (t, J = 13.1 Hz, 1 H), 2.08 (s, 2 H), 1.77−1.25 (m, 18 H), 1.14−0.87 (8 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 196.4, 196.3, 171.73, 171.69, 171.2, 171.0, 170.2, 170.0, 167.7, 160.8, 160.7, 157.1, 157.0, 139.1, 137.4, 137.3, 128.1, 128.0, 127.8, 127.1, 127.0, 126.8, 126.7, 115.7, 115.5, 112.54, 112.52, 66.7, 66.6, 55.0, 54.7, 53.3, 53.2, 45.4, 45.3, 42.0, 41.9, 41.8, 33.5, 33.4, 31.88, 31.85, 29.97, 29.93, 29.6, 29.4, 28.1, 28.0, 27.2, 27.1, 27.0, 26.9, 26.0, 25.33, 25.29, 24.1, 23.91, 23.90, 23.8, 23.7, 18.6, 18.5, 13.5, 13.4; HRMS calcd for $C_{40}H_{54}N_5O_7$ $(M + H)^+$ 716.4023, found 716.4021.

(2-{3-[(3-Cyclohexyl-2,5-dioxo-12-oxa-1,4-diazatricyclo[11.5.3.0^{16,20}]henicosa-13(21),14,16(20)-triene-18-carbonyl)amino]-2-oxohexanoylamino}acetylamino)phenylacetic Acid *tert*-Butyl Ester (21). The coupling of carboxylic acid **9** (390 mg, 0.881 mmol) and amine hydrochloride **16** (390 mg, 0.907 mmol) was carried out in a manner similar to that described above for the preparation of **5**. The intermediate product (580 mg, 80% yield) was obtained as a mixture of diastereomers. To the solution of this product (0.360 g, 0.440 mmol) in anhydrous CH_2Cl_2 (80 mL) at room temperature was added Dess−Martin reagent (0.467 g, 1.10 mmol). The mixture was stirred for 4 h. Saturated aqueous sodium bicarbonate and sodium thiosulfate solutions (50 mL each) were added. After stirring for 10 min, the layers were separated. The aqueous solution was extracted with CH_2Cl_2 (2 × 80 mL). The organic solutions were combined, dried with magnesium sulfate, filtered, and concentrated in vacuo. Flash chromatography (2−5% MeOH/CH_2Cl_2) afforded **21** (0.316 g, 88% yield) as a mixture of diastereomers. The ratio of the two diastereomers was variable and dependent on the reaction conditions: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.74 (ABq, J = 5.9 Hz, 1 H), 8.70 (t, J = 8.0 Hz, 1 H), 8.25 & 8.11 (d, J = 6.9 & 8.0 Hz, 1 H), 7.92 (t, J = 10.0 Hz, 1 H), 7.40−7.33 (m, 5 H), 7.13 (dd, J = 10.4, 8.1 Hz, 1 H), 6.81 (ddd, J = 7.8, 5.2, 2.6 Hz, 1 H), 6.74 (dd, J = 9.0, 2.3 Hz, 1 H), 5.26 (t, J = 7.4 Hz, 1 H), 5.09−5.01 (m, 2 H), 4.74 (ABq, J = 10.0 Hz, 1 H), 4.36 (td, J = 11.3, 5.6 Hz, 1 H), 4.31 & 4.22 (d, J = 14.5 & 14.9 Hz, 1 H), 4.21−4.19 (m, 1 H), 4.06−4.02 (m, 1 H), 3.92−3.80 (m, 2 H), 3.11 (ddd, J = 14.8, 6.3, 2.74 (ddd, J = 14.8, 11.2, 8.0 Hz, 1 H), 2.07−2.05 (m, 2 H), 1.77−1.42 (m, 12 H), 1.34 & 1.33 (s, 9 H), 1.41−1.23

(m, 4 H), 1.21−0.91 (m, 5 H), 0.89−0.82 (m, 5 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 197.6, 197.5, 172.7, 172.6, 172.1, 172.0, 171.3, 171.1, 170.8, 170.3, 168.49, 168.47, 161.8, 161.7, 158.0, 157.9, 138.4, 138.3, 137.4, 132.91, 132.89, 132.4, 132.3, 129.7, 129.6, 129.5, 129.0, 128.8, 128.3, 128.0, 127.8, 116.6, 116.5, 113.49, 113.47, 82.2, 82.1, 67.6, 67.5, 57.8, 57.7, 56.0, 55.7, 54.3, 54.2, 46.3, 42.31, 42.27, 34.48, 34.46, 34.3, 32.65, 32.60, 32.1, 30.954, 30.947, 30.9, 30.52, 30.51, 30.4, 29.04, 28.99, 28.3, 28.2, 28.1, 28.0, 27.9, 27.0, 26.3, 26.2, 25.1, 24.9, 24.7, 24.6, 19.6, 19.5, 14.43, 14.35; HRMS calcd for $C_{45}H_{62}N_5O_9$ $(M + H)^+$ 816.4548, found 816.4554.

(2-{3-[(3-Cyclohexyl-2,5-dioxo-12-oxa-1,4-diazatricyclo[11.5.3.0^{16,20}]henicosa-13(21),14,16(20)-triene-18-carbonyl)amino]-2-oxohexanoylamino}acetylamino)phenylacetic Acid (22). A solution of *tert*-butyl ester **21** (210 mg, 0.257 mmol) in trifluoroacetic acid (15 mL) and CH_2Cl_2 (15 mL) was stirred at room temperature for 4 h. The mixture was concentrated in vacuo. The residue was redissolved in 1:1 MeOH/CH_2Cl_2 (10 mL) and concentrated to dryness to afford product **22** (198 mg, quantitative) as a mixture of diastereomers: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.74 (t, J = 6.1 Hz, 1 H), 8.63 (d, J = 6.9 Hz, 1 H), 8.25 & 8.11 (d, J = 7.0 & 7.6 Hz, 1 H), 7.91 (t, J = 9.4 Hz, 1 H), 7.38−7.33 (m, 5 H), 7.31−7.28 (m, 1 H), 7.13 (t, J = 9.1 Hz, 1 H), 6.80 (ddd, J = 7.5, 5.0, 2.5 Hz, 1 H), 6.74 (dd, J = 8.2, 2.2 Hz, 1 H), 5.25 (t, J = 7.5 Hz, 1 H), 5.08−5.01 (m, 2 H), 4.74 (ABq, J = 10.2 Hz, 1 H), 4.36 (td, J = 11.4, 6.3 Hz, 1 H), 4.31 & 4.22 (d, J = 14.8 & 14.5 Hz, 1 H), 4.20−4.18 (m, 1 H), 4.06−4.03 (m, 1 H), 3.86 (ddd, J = 20.3, 16.5, 6.3 Hz, 2 H), 3.11 (ddd, J = 14.8, 6.2, 4.1 Hz, 1 H), 2.74 (ddd, J = 14.8, 11.2, 8.3 Hz, 1 H), 2.06 (t, J = 6.0 Hz, 2 H), 1.77−1.45 (m, 12 H), 1.44−1.21 (m, 4 H), 1.19−1.03 (m, 5 H), 0.91−0.80 (m, 5 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 196.7, 196.5, 171.73, 171.68, 171.5, 171.2, 171.1, 170.2, 169.8, 167.1, 160.8, 160.7, 157.1, 157.0, 137.4, 137.3, 128.3, 128.24, 128.18, 128.0, 127.8, 127.6, 127.5, 127.30, 127.27, 127.1, 126.8, 115.7, 115.6, 112.53, 112.52, 66.7, 66.6, 56.5, 55.0, 54.7, 53.4, 53.2, 52.4, 48.5, 45.4, 45.3, 41.5, 33.5, 33.4, 31.7, 31.6, 30.0, 29.9, 29.6, 29.4, 28.1, 28.0, 27.2, 27.1, 27.0, 26.9, 26.0, 25.34, 25.27, 24.1, 23.9, 23.8, 23,7, 18.6, 18.5, 13.5, 13.4; HRMS calcd for $C_{41}H_{54}N_5O_9$ $(M + H)^+$ 760.3922, found 760.3943.

3-Cyclohexyl-2,5-dioxo-12-oxa-1,4-diazatricyclo[11.5.3.0^{16,20}]-henicosa-13(21),14,16(20)-triene-18-carboxylic Acid [1-(Dimethylcarbamoylphenylmethyl)carbamoyl]methyl)aminooxalyl)butyl)-amide (23). The coupling of carboxylic acid **22** (50 mg, 0.0658 mmol) and dimethylamine hydrochloride (10 mg, 0.123 mmol) was carried out in a manner similar to that described above for the preparation of **5**. The product was purified by flash chromatography (2−5% MeOH/CH_2Cl_2) to afford **23** (29 mg, 56% yield) as a mixture of diastereomers. The ratio of the two diastereomers was variable and dependent on the reaction conditions: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.73 (ABq, J = 6.0 Hz, 1 H), 8.57−8.54 (m, 1 H), 8.24 & 8.09 (d, J = 7.3 & 7.5 Hz, 1 H), 7.91 (t, J = 9.4 Hz, 1 H), 7.39−7.28 (m, 6 H), 7.13 (t, J = 8.8 Hz, 1 H), 6.82−6.73 (m, 2 H), 5.81 & 5.78 (d, J = 7.8 & 7.8 Hz, 1 H), 5.09−5.01 (m, 2 H), 4.77 (dd, J = 21.1, 10.0 Hz, 1 H), 4.36 (td, J = 11.3, 6.0 Hz, 1 H), 4.28−4.18 (m, 2 H), 4.10−4.02 (m, 1 H), 3.87−3.76 (m, 2 H), 3.17−3.09 (m, 1 H), 2.91 & 2.89 (s, 3 H), 2.84 & 2.83 (s, 3 H), 2.80−2.71 (m, 1 H), 2.07−2.05 (m, 2 H), 1.77−1.24 (m, 16 H), 1.19−1.05 (m, 5 H), 0.89−0.77 (m, 5 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 196.7, 196.5, 171.72, 171.67, 171.14, 170.07, 170.2, 169.8, 169.15, 169.12, 166.9, 166.8, 160.9, 160.7, 157.1, 156.9, 137.41, 137.38, 137.3, 128.5, 127.83, 127.77, 127.70, 127.65, 127.1, 126.8, 115.6, 115.5, 112.54, 112.51, 66.7, 66.6, 55.0, 54.8, 54.70, 54.68, 53.3, 53.2, 52.94, 52.92, 45.4, 45.3, 41.51, 41.47, 36.48, 36.46, 35.26, 35.24, 33.5, 33.3, 31.7, 31.6, 30.0, 29.9, 29.6, 29.4, 28.1, 28.0, 27.2, 27.1, 27.0, 26.9, 26.02, 25.98, 25.33, 25.28, 24.1, 23.9, 23.8, 23.7, 18.6, 18.5, 13.5, 13.4; HRMS calcd for $C_{43}H_{59}N_6O_8$ $(M + H)^+$ 787.4394, found 787.4404.

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