Design, Synthesis, and Biological Activity of *m*-Tyrosine-Based 16- and 17-Membered Macrocyclic Inhibitors of Hepatitis C Virus NS3 Serine Protease

Kevin X. Chen,* F. George Njoroge, John Pichardo, Andrew Prongay, Nancy Butkiewicz, Nanhua Yao, Vincent Madison, and Viyyoor Girijavallabhan

Schering-Plough Research Institute, 2015 Galloping Hill Road, K-15-3-3545, Kenilworth, New Jersey 07033

Received April 8, 2005

The limited efficacy and considerable side effects of currently available therapies for the treatment of hepatitis C virus (HCV) infection have prompted significant efforts toward the development of safe and effective new therapeutics. The pentapeptide α-ketoamides of type **1** were weak HCV inhibitors with a binding constant, Ki*, above 5 μM. We envisioned that cyclization of a P2 phenyl side chain to a P3 capping group could enhance binding through an interaction of the resulting macrocycle with the methyl group of Ala156 on the enzyme backbone. The macrocyclic dipeptide moiety would also decrease the peptidic nature of the inhibitors. The synthesis of macrocyclic HCV inhibitors started from *m*-tyrosine methyl ester. Two consecutive couplings, first, with Boc-cyclohexylglycine and, then, with hept-6-enoic acid, provided compound **6**. The alkene was converted to an alcohol via hydroboration. The key macrocyclization of phenol alcohol **7** was achieved through a Mitsunobu reaction. Both 16- and 17-membered macrocycles (**8** and **21**) were prepared. After hydrolysis, the macrocyclic acids (**15** and **22)** were coupled to the right-hand tripeptide (**14**) to afford α-hydroxyamides, which upon Dess−Martin periodinane oxidation furnished the desired α-ketoamides. Esters, acids, and amides were incorporated at the C-terminal of these peptides. These inhibitors were tested in an HCV protease continuous assay. The binding constants (Ki*) indicated that the 16-membered macrocyclic inhibitors (**23** and **24**) were less potent than the 17-membered analogues (**16**−**19**). It was also evident that C-terminal acids (i.e., **17**) and amides **(18** and **19)** (Ki* range: 0.16−0.31 μM) were much better inhibitors than *tert*-butyl esters (**16** and **23**). The X-ray crystal structure of compound **17** bound to the enzyme revealed that the macrocycle formed a "donut"-shaped ring around the methyl group of Ala156. P2′ phenyl and P1 propyl groups wrapped around the Lys136 side chain, forming a "C"-shaped clamp. The 17-membered macrocyclic inhibitors **17**−**19** were significantly more potent than the acyclic pentapeptide **1**.

Introduction

The hepatitis C virus (HCV) has infected more than 170 million people worldwide. It is the major cause of chronic liver disease leading to cirrhosis, hepatocellular carcinoma, and, ultimately, liver failure. HCV is emerging as a global health crisis.[1] The only currently available therapies are subcutaneous α-interferon or pegylated α-interferon monotherapy or α-interferon and oral ribavirin combination therapy. These therapeutics are only effective in up to 50% of the patients and have considerable side effects in certain patients.[2] Given the prevalence of HCV infections, a small molecule drug is highly desirable.

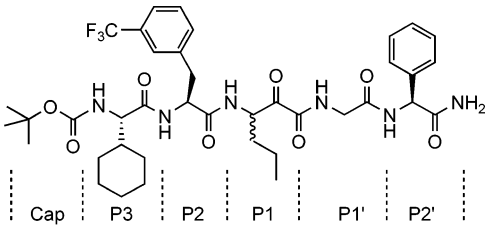
In the hepatitis C virus, the virally encoded protease responsible for processing the nonstructural (NS) portion of the polyprotein is located in the N-terminal portion of the NS3 protein. Studies have confirmed that the NS3 protease belongs to the trypsin or chymotrypsin superfamily of serine proteases and that it is essential for viral replication.[3] However, the NS3 protease is unique, in that it requires a small polypeptide cofactor (NS4A) for efficient processing. The fact that the binding pockets of the NS3 protease are very shallow, solvent-exposed, and relatively featureless makes it a difficult target in drug design. Nonetheless, considerable efforts have been targeting the NS3 serine protease and a number of potent inhibitors have been identified by various research laboratories around the world.[4]

Many protease inhibitors developed in recent years are peptides because their design is based on the cleavage products of the substrates. Peptides are easily cleaved by peptidases and, thus, suffer from low bioavailability and poor pharmacological profiles.[5] Major efforts have been undertaken to design peptidomimetics with less peptide character. One such approach that is showing considerable promise is through macrocyclization.[6] Many biologically active macrocyclic molecules exist in nature. The vancomycin family of antibiotics and chloropeptins are well-known examples.[7] Macrocycles offer certain advantages over peptides as drug candidates. They are conformationally preorganized for stronger binding. They are also potentially more stable toward degradation by peptidases.

It has been shown that N-terminal cleavage products (e.g., DDIVPC-OH) of the peptide substrates are competitive inhibitors of HCV NS3 protease.[8] We and others have been using the hexapeptide as the starting point in developing more potent and smaller HCV inhibitors. Among the different classes of inhibitors discovered, α-ketoacids and ketoamides, with their electrophilic

* To whom correspondence should be addressed. Tel: (908) 740-7556. Fax: (908) 740-7152. E-mail: kevin.chen@spcorp.com.

10.1021/jm050323b CCC: $30.25
Published on Web 08/31/2005



1 Ki* = 8 μM

Figure 1. Pentapeptide HCV inhibitor.

"war-heads", have demonstrated their potential in VX-950, a clinical candidate in development from Vertex Pharmaceuticals.[9] In the course of our search for potent HCV inhibitors, we found that compound **1** (Figure 1), a pentapeptide α-ketoamide, had modest potency against NS3 protease (Ki* = 8 μM).[10] We envisioned that cyclization between the P2 phenyl group and the N-terminal P3 capping group would provide a macrocycle which could have enhanced binding with the protease. Macrocyclization would also reduce the peptidic nature of the inhibitor. Computer modeling using an enzyme surface from the X-ray crystal structure of the protein confirmed that the strategy was feasible and that the appropriate ring size would be 16 or 17.

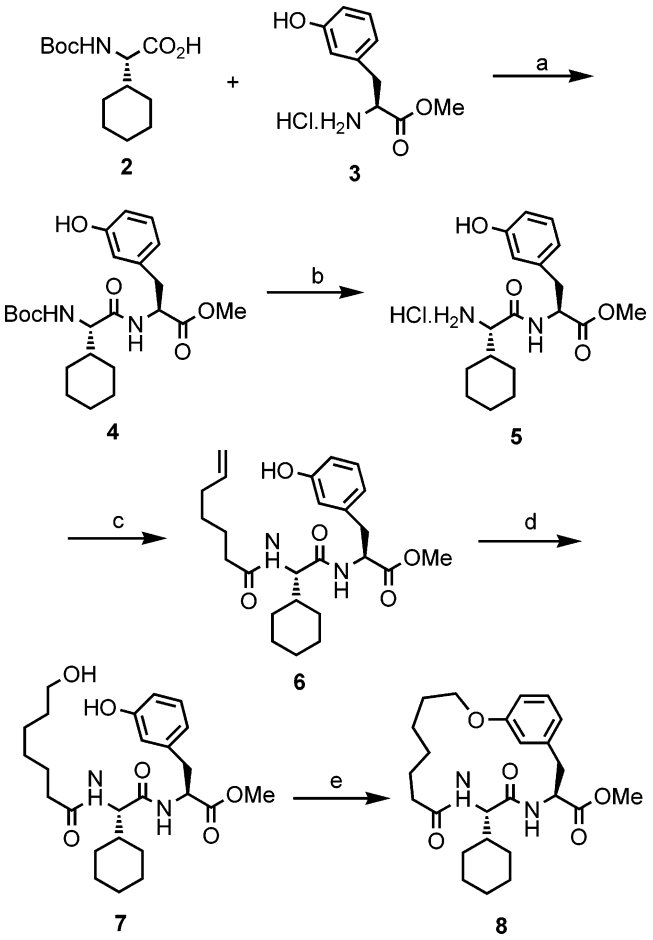
Synthesis

The key step in the synthesis of a compound such as **8** is macrocyclization. Among various synthetic methodologies available, we envisioned that ring closure through an aryl alkyl ether formation from a phenol alcohol precursor could be accomplished under Mitsunobu reaction conditions.[11]

Our synthesis started with commercially available *N*-Boc-cyclohexylglycine **2** and *m*-tyrosine methyl ester hydrochloride **3** (Scheme 1). Standard coupling between **2** and **3** using 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (DhBtOH), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) hydrochloride, and 4-methylmorpholine (NMM) afforded dipeptide **4**. The Boc protecting group was removed, and the resulting amine hydrochloride **5** was coupled with hept-6-enoic acid under the same coupling conditions to provide compound **6**. The terminal alkene was then converted to primary alcohol **7** through hydroboration using borane−THF. With the macrocycle precursor in hand, macrocyclization of **7** was attempted under Mitsunobu reaction conditions.[11] The reaction was first conducted in benzene using tri-*n*-butylphosphine and 1,1′-(azodicarbonyl)dipiperidine (ADDP). Under these conditions, a very low yield of **8** was obtained, possibly due to low solubility of the substrate in benzene. When the solvent was changed to dichloromethane, the desired macrocycle (**8**) was obtained in good yield.

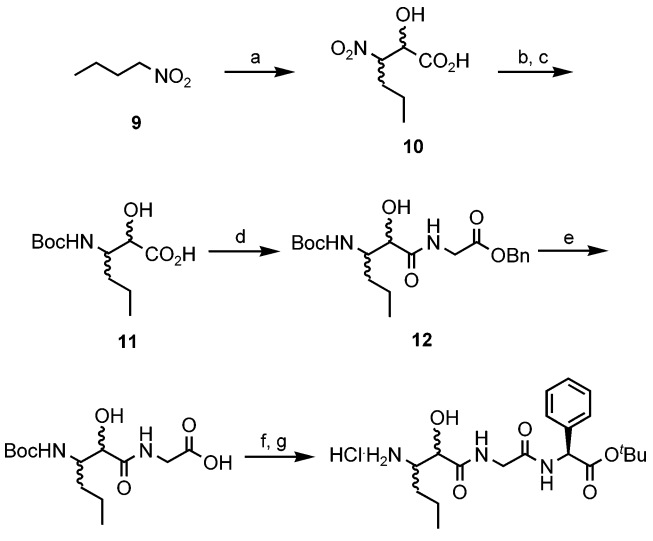
The right-hand tripeptide segment of the inhibitors was prepared starting from 1-nitrobutane **9** (Scheme 2). Thus, when treated with triethylamine, the reaction between **9** and glyoxylic acid afforded compound **10** as a diastereomeric mixture. The nitro group was reduced to an amine via low-pressure catalytic hydrogenation. The resulting amino acid was converted to a Boc protected norvaline α-hydroxyacid (**11**). Coupling of **11** with glycine benzyl ester under standard conditions gave dipeptide **12**, which, upon hydrogenation, provided

Scheme 1[a]



[a] Reaction conditions: (a) DhBtOH, EDC, NMM, CH_2Cl_2/DMF, −20 °C, 88%; (b) 4 N HCl, *p*-dioxane, rt, 96%; (c) hept-6-enoic acid, DhBtOH, EDC, NMM, CH_2Cl_2/DMF, −20 °C, 94%; (d) BH_3·HF, THF, 0 °C, 68%; (e) (*n*-Bu)$_3$P, ADDP, CH_2Cl_2, rt, 68%.

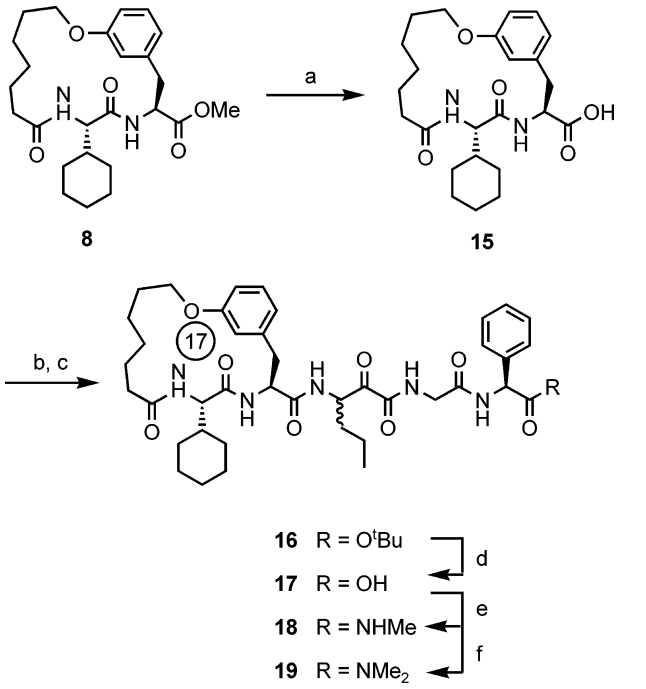
Scheme 2[a]



[a] Reaction conditions: (a) glyoxylic acid monohydrate, Et_3N, MeOH, 0 °C, then rt, 99%; (b) H_2, 59 psi, Pd/C, AcOH, rt, 66%; (c) NaOH, $(Boc)_2O$, dioxane/H_2O, rt, 89%; (d) glycine benzyl ester hydrochloride, DhBtOH, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 95%; (e) H_2, Pd/C, EtOH, rt, 98%; (f) phenylglycine *tert*-butyl ester hydrochloride, DhBtOH, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 80%; (g) 2 M HCl in EtOAc/dioxane (1:1), rt, quantitative.

carboxylic acid **13**. Compound **13** was coupled to phenylglycine *tert*-butyl ester to afford a tripeptide. Treat-

Scheme 3[a]



[a] Reaction conditions: (a) LiOH, MeOH/THF/H_2O, rt, 81%; (b) **14**, DhBtOH, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 74%; (c) Dess−Martin periodinane, CH_2Cl_2, rt, 35%; (d) TFA, CH_2Cl_2, rt, quantitative; (e) methylamine hydrochloride, DhBtOH, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 58%; (f) dimethylamine, DhBtOH, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 46%.

ment of this product with 2 M hydrochloric acid selectively removed the Boc protecting group in the presence of *tert*-butyl ester to give the desired amine (**14**).

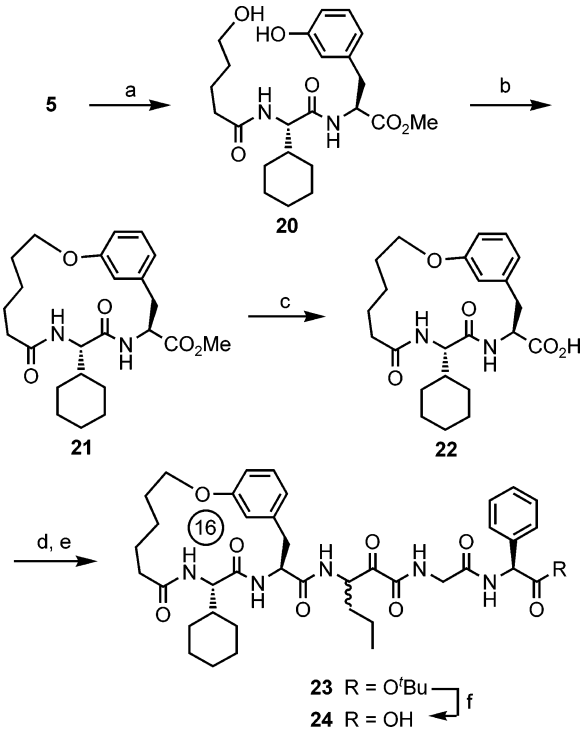
With both left-hand macrocycle and right-hand tripeptide fragments in hand, the stage was set to assemble the final targets. Thus, methyl ester **8** was hydrolyzed to acid **15** using lithium hydroxide (Scheme 3). Compound **15** was coupled with amine **14** to afford an α-hydroxyamide, which was oxidized to α-ketoamide **16** using Dess−Martin periodinane.[12] Under low-temperature coupling conditions, no racemization occurred at the phenylalanine center. Treatment of **16** with trifluoroacetic acid provided carboxylic acid **17**. Coupling of **17** with methylamine or dimethylamine under standard conditions gave methyl amide **18** and dimethyl amide **19**, respectively. The compounds were isolated as an inseparable mixture of two diastereomers. The ratio of the two diastereomers was in the range of 4:6 to 6:4 but was compound specific.

To determine the effect of ring size on the potency of the inhibitors, 16-membered macrocycle **21** was also prepared through a reaction sequence similar to that described in the synthesis of 17-membered macrocycle **8** in Scheme 1. Starting from amine **5**, the ultimate product α-ketoamide **23** was obtained after a number of operations. Treatment of **23** with trifluoroacetic acid converted it to carboxylic acid **24** (Scheme 4).

Results and Discussions

Macrocyclic compounds **16**−**19**, **23**, and **24** were tested in an HCV continuous assay[13] using the NS4A-tethered single chain NS3 serine protease.[14] The Ki* value is a collective reflection of the equilibrium constant, determined by the reversible covalent bond

Scheme 4[a]



[a] Reaction conditions: (a) 6-hydroxyhexanoic acid, DhBtOH, EDC, NMM, LiOH, DMF/CH_2Cl_2, −20 °C, 39%; (b) *n*-Bu_3P, ADDP, CH_2Cl_2, rt, 74%; (c) LiOH, MeOH/THF/H_2O, rt, 88%; (d) **14**, DhBtOH, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 48%; (e) Dess−Martin periodinane, CH_2Cl_2, rt, 70%; (f) TFA, CH_2Cl_2, rt, quantitative.

Table 1. Inhibitors of HCV NS3 Protease

compound	R	macrocycle ring size	Ki* (μM)
1	NH_2		8.0 ± 1.5
16	O^{t}Bu	17	5.5 ± 1.3
17	OH	17	0.16 ± 0.02
18	NHMe	17	0.17 ± 0.02
19	NMe_2	17	0.31 ± 0.11
23	O^{t}Bu	16	>8.9
24	OH	16	1.2 ± 0.10

formed between the ketone and serine hydroxyl, and other interactions between the inhibitor and enzyme.[15] The Ki* values along with standard errors for these compounds are listed in Table 1. It was evident that 17-membered macrocyclic inhibitors were more potent than 16-membered analogues. *tert*-Butyl ester **16** (17-membered) was a modest inhibitor with a Ki* of 5.5 μM, while **23** (16-membered) was inactive. Presumably, the bulky *tert*-butyl group not only prevented hydrogen bonding of the P2′ carbonyl with the enzyme backbone but also had some detrimental steric interactions with the protein surface. Seventeen-membered acid **17** (Ki* = 0.16 μM) was a potent inhibitor that was eight times more active than 16-membered derivative **24** (Ki* = 1.2 μM). Both monomethyl and dimethyl amides (**18** and **19**) were also potent inhibitors (Ki* = 0.17 and 0.31 μM, respectively). This study clearly demonstrated that, with the right ring size, macrocyclization increases the potency of these inhibitors over that of acyclic analogue **1** 26−50 times (**17**−**19** vs **1**).

The selectivity of compound **17** was measured against human neutrophil elastase (HNE), which is the closest serine protease to human hepatitis C virus NS3 pro-

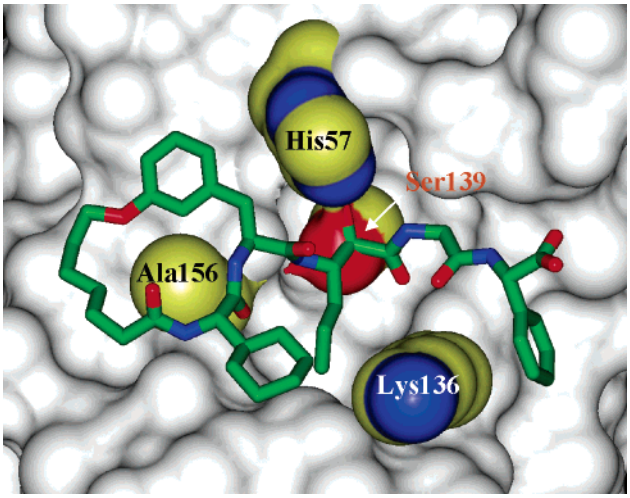


Figure 2. X-ray structure of compound **17** bound to HCV NS3 protease.

tease. The ratio of Ki* values (HNE/HCV) was 5. This indicated that compound **17** has a moderate selectivity against another closely related protease.

The X-ray crystal structure of inhibitor **17** bound to HCV NS3/NS4A protease was obtained (Figure 2). As expected, the P1 (*S*)-diastereomer was more potent and was observed in the crystal structure. A reversible covalent bond was formed between the enzyme active site serine (Ser139) hydroxyl and the ketone carbonyl of the inhibitor. The resulting oxygen anion was stabilized by hydrogen bonding with His57. The macrocycle encircled the methyl group of Ala156. This established a donut-shaped conformation that provided extra interaction between the inhibitor and the protease. The inhibitor−protease complex indicated that the smaller 16-membered ring was not large enough to properly interact with the Ala156 residue. The *n*-propyl side chain of the P1 norvaline fit very well within the S1 pocket. In addition, the aromatic ring of the P2′ phenylglycine reaches to the other side of the Lys136 side chain such that it formed a C-shaped clamp around the lysine residue along with a P1 propyl group. Although this phenomenon is not unique for macrocyclic inhibitors, the clamping is part of the overall binding that contributes to the potency of the compound. The inhibitor also made multiple hydrogen bonds with the protease through its amide chain.

Conclusion

A concise synthesis of both 16- and 17-membered macrocycles **8** and **21** has been accomplished. The macrocyclization of phenol alcohols was achieved through a Mitsunobu protocol using tri-*n*-butylphosphine and 1,1′-(azodicarbonyl)dipiperidine. The macrocycles have been incorporated into HCV protease inhibitors as peptidomimetics for the P2−P3 dipeptide moiety. When tested in an HCV continuous assay, 17-membered macrocyclic inhibitors were more potent than their 16-membered analogues. Carboxylic acid **17** and amides **18** and **19** were potent inhibitors with Ki* values in the range of 0.16−0.31 μM. They were 26−50 times more active than acyclic analogue **1**. *tert*-Butyl esters (**16** and **23**) were much less potent. The X-ray structure of compound **17** bound to enzyme confirmed the formation of a covalent bond between the ketone carbonyl and the Ser139 hydroxyl of the protease. It also revealed that the macrocycle adopted a donut-shaped conformation on top of Ala156. A C-shaped clamping around the Lys136 side chain by the P1 norvaline and the P2′ phenylglycine was also observed.

Experimental Section

General Methods. Reagents and solvents, including anhydrous tetrahydrofuran (THF), dichloromethane, and DMF, were purchased from Aldrich or other commercial sources and were used without further purification. Reactions that were moisture sensitive or used 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 via 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 and at 75, 100, or 125 MHz for ^{13}C on a Bruker or Varian spectrometer with $CDCl_3$ or DMSO-d_6 as a solvent. The chemical shifts are given in ppm and referenced to the internal TMS or deuterated solvent signal.

***N*-*tert*-Butoxycarbonyl-(*S*)-cyclohexylglycine-(*S*)-*m*-tyrosine Methyl Ester (4).** To a solution of (*S*)-*m*-tyrosine methyl ester hydrochloride **3** (2.00 g, 8.63 mmol), *N*-*tert*-butoxycarbonyl-(*S*)-cyclohexylglycine (2.30 g, 8.94 mmol), 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (DhBtOH) (1.50 g, 9.20 mmol), 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC) hydrochloride (1.99 g, 10.38 mmol) in anhydrous DMF (100 mL) and CH_2Cl_2 (100 mL) at −20 °C was added 4-methylmorpholine (NMM) (3.00 mL, 27.3 mmol). After stirring at this temperature for 30 min, the reaction mixture was kept in a freezer for 18 h. It was then allowed to warm to room temperature. EtOAc (300 mL), brine (100 mL), and 5% aqueous H_3PO_4 solution (100 mL) were added. After separation of the layers, the organic solution was washed consecutively with 5% aqueous H_3PO_4 solution (150 mL), aqueous saturated sodium bicarbonate solution (2 × 150 mL), water (150 mL), and brine (150 mL). It was then dried over magnesium sulfate, filtered, and concentrated in vacuo. Flash chromatography (2−5% MeOH/CH_2Cl_2) afforded **4** (3.43 g, 88% yield) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (s, 1H), 7.12 (t, J = 7.6 Hz, 1H), 6.76 (dd, J = 8.1, 1.7 Hz, 1H), 6.58−6.54 (m, 2H), 6.47 (d, J = 7.5 Hz, 1H), 5.28 (d, J = 9.1 Hz, 1H), 4.89−4.85 (m, 1H), 3.96 (dd, J = 9.0, 7.0 Hz, 1H), 3.73 (s, 3H), 3.13 (dd, J = 13.7, 5.7 Hz, 1H), 3.04 (dd, J = 13.7, 4.2 Hz, 1H), 1.75−1.58 (m, 6H), 1.46 (s, 9H), 1.28−0.93 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.5, 171.4, 156.5, 156.3, 136.3, 129.7, 120.4, 117.0, 114.5, 80.6, 59.8, 53.0, 52.4, 40.3, 36.8, 29.5, 28.4, 28.3, 25.9, 25.8. HRMS calcd for $C_{23}H_{35}N_2O_6$ $(M + H)^+$: 435.2495. Found: 435.2491.

***H*-(*S*)-Cyclohexylglycine-(*S*)-*m*-tyrosine Methyl Ester Hydrochloride (5).** A solution of **4** (10.0 g, 23.0 mmol) was dissolved in a 4 M HCl solution in dioxane (100 mL) and stirred at room temperature for 4 h. The reaction mixture was concentrated in vacuo to give a solid residue, which was suspended in ether (200 mL). The suspension was filtered, and the solid was washed with ether and dried under vacuum to give a white solid **5** (8.2 g, 96% yield). It was used in subsequent reactions without further purification. ^{1}H NMR (400 MHz, CD_3OD) δ 7.09 (t, J = 8.0 Hz, 1H), 6.71−6.36 (m, 3H), 4.69 (dd, J = 6.0, 3.2 Hz, 1H), 3.69 (s, 3H), 3.66 (d, J = 5.2 Hz, 1H), 3.15−3.10 (dd, J = 5.6, 4.0 Hz, 1H), 1.87−1.69 (m, 6H), 1.32−1.10 (m, 5H).

***N*-6-Heptenoyl-(*S*)-cyclohexylglycine-(*S*)-*m*-tyrosine Methyl Ester (6).** The coupling of amine hydrochloride **5** (1.20 g, 3.23 mmol) and hept-6-enoic acid (0.610 g, 4.68 mmol) was carried out in a manner similar to that described for the preparation of **4**. The crude product was purified by flash chromatography (2−5% MeOH/CH_2Cl_2), which gave compound

6 (1.36 g, 94% yield) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 (s, 1H), 7.11 (t, J = 7.7 Hz, 1H), 7.78 (dd, J = 8.2, 2.6 Hz, 1H), 6.51 (d, J = 7.2 Hz, 1H), 6.45 (d, J = 1.5 Hz, 1H), 6.36 (d, J = 7.9 Hz, 1H), 6.26 (d, J = 9.2 Hz, 1H), 5.81−5.73 (m, 1H), 5.01−4.93 (m, 2H), 4.84−4.81 (m, 1H), 4.22 (t, J = 8.7 Hz, 1H), 3.75 (s, 3H), 3.13 (dd, J = 14.0, 5.4 Hz, 1H), 3.01 (dd, J = 13.8, 4.3 Hz, 1H), 2.30 (t, J = 7.6 Hz, 2H), 2.06 (q, J = 7.0 Hz, 2H), 1.81−1.64 (m, 8H), 1.46−1.39 (m, 2H), 1.26−0.96 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.4, 171.2, 170.8, 171.1, 156.7, 138.2, 135.9, 129.7, 120.3, 117.8, 114.9, 114.8, 58.7, 53.1, 52.5, 39.7, 36.5, 33.3, 29.6, 29.1, 28.4, 26.0, 25.7, 25.0. HRMS calcd for $C_{25}H_{37}N_2O_5$ $(M + H)^+$: 445.2702. Found: 445.2683.

***N*-7-Hydroxyheptanoyl-(*S*)-cyclohexylglycine-(*S*)-*m*-tyrosine Methyl Ester (7).** To the solution of **6** (1.46 g, 3.28 mmol) in anhydrous THF (60 mL) under nitrogen at 0 °C was added a borane−THF solution (12 mL, 1.0 M, 12 mmol) cautiously. The resulting solution was stirred at 0 °C for 1 h and 40 min. Ethanol (4 mL) and a pH 7 buffer (8 mL) were added, followed by aqueous 30% H_2O_2 solution (7.5 mL). After stirring at 0 °C for 20 min, the mixture was warmed to room temperature and stirred for 2 h. EtOAc (200 mL) and brine (100 mL) were added, and the layers were separated. An aqueous solution was extracted with EtOAc (2 × 150 mL). The combined organic solution was dried with magnesium sulfate, filtered, and concentrated under reduced pressure. Flash chromatography (2−5% MeOH/CH_2Cl_2) afforded **7** (1.05 g, 2.18 mmol, 68% yield) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.28 (s, 1H), 8.35 (d, J = 7.3 Hz, 1H), 7.73 (d, J = 9.2 Hz, 1H), 7.04−7.00 (m, 1H), 6.60−6.58 (m, 3H), 4.40−4.33 (m, 2H), 4.20 (dd, J = 8.8, 7.3 Hz, 1H), 3.54 (s, 3H), 3.36−3.33 (m, 2H), 2.89 (dd, J = 13.9, 6.3 Hz, 2H), 2.81 (dd, J = 13.9, 8.7 Hz, 2H), 2.16−2.02 (m, 2H), 1.65−1.34 (m, 10H), 1.27−0.88 (m, 9H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.9, 172.7, 172.1, 158.1, 139.3, 130.0, 120.4, 116.7, 114.4, 61.5, 57.3, 54.4, 52.6, 40.9, 37.4, 35.9, 33.3, 29.8, 29.4, 28.8, 26.7, 26.4, 26.3, 26.1. HRMS calcd for $C_{25}H_{39}N_2O_6$ $(M + H)^+$: 463.2813. Found: 463.2808.

Methyl 11(*S*)-Cyclohexyl-9,12-dioxo-2-oxa-10,13-diazabicyclo[14.3.1]eicosa-1(20),16,18-triene-14(*S*)-carboxylate (8). To a solution of phenol alcohol **7** (1.00 g, 2.16 mmol) and tri-*n*-butylphosphine (1.10 mL, 4.28 mmol) in anhydrous CH_2Cl_2 (100 mL) and THF (40 mL) at 0 °C under an argon atmosphere was added 1,1′-(azodicarbonyl)dipiperidine (ADDP) (1.08 g, 4.28 mmol). After stirring at 0 °C for 1 h, the solution was warmed to room temperature and stirred for another 3 h. TLC indicated complete consumption of the starting material. After removal of solvent in vacuo, the residue was purified by flash chromatography (0−3% MeOH/CH_2Cl_2) to afford compound **8** (650 mg, 1.46 mmol, 68% yield). An analytical sample was obtained through a second flash column chromatography eluted with a gradient of 0−80% acetone/hexanes. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.61 (d, J = 8.3 Hz, 1H), 7.81 (d, J = 9.4 Hz, 1H), 7.15 (t, J = 7.9 Hz, 1H), 6.74 (d, J = 7.6 Hz, 1H), 6.69 (dd, J = 8.2, 2.2 Hz, 1H), 6.63 (s, 1H), 4.75−4.71 (m, 1H), 4.29 (t, J = 8.6 Hz, 1H), 3.96−3.92 (m, 1H), 3.79−3.74 (m, 1H), 3.66 (s, 3H), 3.15 (d, J = 14.2 Hz, 1H), 2.94 (dd, J = 16.0, 12.0 Hz, 2H), 2.30−2.25 (m, 1H), 2.00−1.96 (m, 1H), 1.70−0.83 (m, 19H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.1, 171.7, 171.5, 158.9, 139.0, 129.1, 121.1, 113.0, 111.9, 66.4, 56.1, 52.1, 50.7, 40.7, 34.9, 34.2, 28.8, 28.4, 26.8, 26.3, 26.0, 25.6, 25.5, 25.3, 24.2. HRMS calcd for $C_{25}H_{37}N_2O_5$ $(M + H)^+$: 445.2685. Found: 445.2702.

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 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 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 a 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, and the aqueous layer was extracted twice with EtOAc (2 × 50 mL); 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. HRMS calcd for $C_{11}H_{22}NO_5$ $(M + H)^+$: 248.1498. Found: 248.1510.

(3-*tert*-Butoxycarbonylamino-2-hydroxyhexanoylamino)acetic Acid Benzyl Ester (12). 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 for the preparation of **4**. The crude product was purified by flash chromatography (25−50% acetone/hexanes) to afford dipeptide **12** (4.50 g, 95% yield) as a mixture of four diastereomers. HRMS calcd for $C_{20}H_{31}N_2O_6$ $(M + H)^+$: 395.2182. Found: 395.2199.

(3-*tert*-Butoxycarbonylamino-2-hydroxyhexanoylamino)acetic Acid (13). To a solution of the benzyl ester **12** (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, **13** (3.40 g, 98% yield), as a mixture of four diastereomers. LRMS *m/z* MH^+: 305. 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 (14). The coupling of carboxylic acid **13** (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 for the preparation of **4**. The crude product was purified by flash chromatography (1:1 EtOAc/hexanes) to afford a tripeptide product (2.60 g, 80% yield) as a mixture of four diastereomers. HRMS calcd for $C_{25}H_{40}N_3O_7$: 494.2866. 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 **14** (2.11 g, quantitative yield) as a pale yellow solid, which was used in subsequent reactions without further purification. HRMS calcd for $C_{20}H_{32}N_3O_5$ $(M + H)^+$: 394.2342. Found: 394.2336.

11(*S*)-Cyclohexyl-9,12-dioxo-2-oxa-10,13- diazabicyclo-[14.3.1]eicosa-1(20),16,18-triene-14(*S*)-carboxylic Acid (15). To a solution of methyl ester **8** (330 mg, 0.742 mmol) in THF (20 mL) and ethanol (10 mL) at room temperature was added an aqueous solution of lithium hydroxide (70 mg, 15 mL of H_2O, 2.92 mmol). The mixture was stirred at room temperature for 3 h. The progress of the reaction was monitored by TLC. After the solution was concentrated in vacuo to one-third of its original volume, EtOAc (60 mL), 1 N HCl solution (10 mL), and water (20 mL) were added and the layers were separated. The aqueous solution was extracted with EtOAc (2 × 50 mL). Organic solutions were combined, dried with magnesium sulfate, filtered, and concentrated to afford **15** (260 mg, 81% yield) as a white solid. ^{1}H NMR (500 MHz, DMSO-

d_6) δ 12.7 (bs, 1H), 8.46 (d, J = 8.2 Hz, 1H), 7.78 (d, J = 9.4 Hz, 1H), 7.16−7.13 (m, 1H), 6.75 (d, J = 7.5 Hz, 1H), 6.69 (dd, J = 8.2, 1.9 Hz, 1H), 6.63 (s, 1H), 4.64−4.60 (m, 1H), 4.28 (t, J = 8.5 Hz, 1H), 3.96−3.92 (m, 1H), 3.78−3.74 (m, 1H), 3.14 (d, J = 14.3 Hz, 1H), 3.92 (dd, J = 16.2, 11.7 Hz, 1H), 2.30−2.25 (m, 1H), 2.00−1.96 (m, 1H), 1.69−1.39 (m, 10H), 1.34−0.85 (m, 9H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.3, 171.6, 171.3, 158.8, 139.4, 129.1, 121.2, 113.1, 111.8, 66.4, 56.2, 50.8, 40.8, 35.1, 34.2, 28.8, 28.3, 26.9, 26.3, 26.0, 25.6, 25.5, 25.3, 24.2. HRMS calcd for $C_{24}H_{35}N_2O_5$ (M + H)$^+$: 431.2564. Found: 431.2546.

1,1-Dimethylethyl-α-(*S*)-[[[[3-[[(11(*S*)-cyclohexyl-2-oxa-10,13-diazabicyclo[14.3.1]eicosa-1(20),16,18-trien-14(*S*)-yl)carbonyl]amino]-1,2-dioxohexyl]amino]acetyl]amino]-benzeneacetate (16). The coupling of macrocyclic acid **15** (0.215 g, 0.499 mmol) and tripeptide amine hydrochloride **14** (0.220 g, 0.513 mmol) was carried out in a manner similar to that described for the preparation of **4**. The intermediate product, α-hydroxyamide, was obtained as a mixture of diastereomers (0.270 g, 0.335 mmol, 74% yield), which was used in subsequent reactions without further purification. To the solution of this product (0.290 g, 0.36 mmol) in anhydrous CH_2Cl_2 (60 mL) at room temperature was added 1,1,1-tris(acetyloxy)-1,1-dihydro-1,2-benziodoxol-3-(1*H*)-one (Dess−Martin reagent) (0.450 g, 1.06 mmol). The mixture was stirred for 3 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 × 50 mL). The organic solutions were combined, dried with magnesium sulfate, filtered, and concentrated in vacuo. Flash chromatography (1−5% MeOH/CH_2Cl_2) afforded **16** (100 mg, 0.124 mmol, 35% yield) as a mixture of diastereomers. The two diastereomers were inseparable under a variety of solvent combinations. The ratio of the two isomers was in the range of 4:6 to 6:4 but was compound specific and depended on the reaction conditions. HRMS calcd for $C_{44}H_{61}N_5O_9$ (M + H)$^+$: 804.4542. Found: 804.4548.

1,1-Dimethylethyl-α-(*S*)-[[[[3-[[(11(*S*)-cyclohexyl-2-oxa-10,13-diazabicyclo[14.3.1]eicosa-1(20),16,18-trien-14(*S*)-yl)carbonyl]amino]-1,2-dioxohexyl]amino]acetyl]amino]-benzeneacetic Acid (17). A solution of *tert*-butyl ester **16** (56.8 mg, 0.0706 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 (3 mL) and concentrated to dryness to afford product **17** (53 mg, 0.0707 mmol, quantitative) as a mixture of diastereomers. HRMS calcd for $C_{40}H_{54}N_5O_9$ (M + H)$^+$: 748.3947. Found: 748.3922.

11(*S*)-Cyclohexyl-*N*-[1-[2-[[2-[[2-(methylamino)-2-oxo-1(*S*)-phenylethyl]amino]-2-oxoethyl]amino]-1,2-dioxoethyl]butyl]-9,12-dioxo-2-oxa-10,13-diazabicyclo[14.3.1]-eicosa-1(20),16,18-triene-14(*S*)-carboxamide (18). Coupling of acid **17** (60 mg, 0.080 mmol) and methylamine hydrochloride (7.0 mg, 0.104 mmol) was carried out in a manner similar to that described for the preparation of **4**. The product was purified by flash chromatography (0−5% MeOH/CH_2Cl_2) to afford compound **18** as a mixture of diastereomers (35 mg, 58% yield). HRMS calcd for $C_{41}H_{57}N_6O_8$ (M + H)$^+$: 761.4238. Found: 761.4230.

11(*S*)-Cyclohexyl-*N*-[1-[2-[[2-[[2-(dimethylamino)-2-oxo-1(*S*)-phenylethyl]amino]-2-oxoethyl]amino]-1,2-dioxoethyl]butyl]-9,12-dioxo-2-oxa-10,13-diazabicyclo[14.3.1]-eicosa-1(20),16,18-triene-14(*S*)-carboxamide (19). Coupling of acid **17** (65 mg, 0.087 mmol) and dimethylamine (6.0 μL, 0.113 mmol) was carried out in a manner similar to that described for the preparation of **4**. The product was purified by flash chromatography (0−5% MeOH/CH_2Cl_2) to afford compound **19** as a mixture of diastereomers (31 mg, 46% yield). HRMS calcd for $C_{42}H_{59}N_6O_8$ (M + H)$^+$: 775.4394. Found: 775.4406.

2-[2-Cyclohexyl-2-(5-hydroxypentanoylamino)acetylamino]-3-(3-hydroxyphenyl)propionic Acid Methyl Ester (20). Coupling of 6-hydroxyhexanoic acid (0.620 g, 75% pure, 3.52 mmol) and amine hydrochloride **5** (0.922 g, 2.37 mmol) was carried out in a manner similar to that described for the preparation of **4**. The product was purified by flash chromatography (10−50% EtOAc/CH_2Cl_2) to afford compound **20** (0.430 g, 39% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.11 (t, J = 8.0 Hz, 1H), 6.78−6.76 (m, 1H), 6.52−6.49 (m, 2H), 6.43−6.35 (m, 2H), 4.88−4.83 (m, 1H), 4.18 (t, J = 8.6 Hz, 1H), 4.09−4.05 (m, 1H), 3.75 (s, 3H), 3.68−3.66 (m, 1H), 3.11 (dd, J = 13.5, 5.3 Hz, 1H), 3.04 (dd, J = 13.5, 4.4 Hz, 1H), 2.68 (bs, 1H), 2.36−2.28 (m, 1H), 1.81−1.56 (m, 12H), 1.46−1.37 (m, 2H), 1.25−0.94 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.5, 171.2, 171.0, 156.8, 136.0, 129.8, 120.2, 117.5, 114.9, 62.1, 58.9, 53.0, 52.5, 39.6, 36.7, 36.0, 31.5, 29.6, 29.1, 26.0, 25.8, 25.7, 24.8, 24.3. HRMS calcd for $C_{24}H_{36}N_2O_6$: 449.2652. Found: 449.2641.

10-Cyclohexyl-8,11-dioxo-2-oxa-9,12-diazabicyclo[13.3.1]-nonadeca-1(19),15,17-triene-13-carboxylic Acid Methyl Ester (21). Macrocyclization of **20** (0.420 g, 0.936 mmol) was carried out in a manner similar to that described for the preparation of **8**. The product was purified by flash chromatography (1−5% MeOH/CH_2Cl_2) to afford compound **21** (0.300 g, 74% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18 (t, J = 7.8 Hz, 1H), 6.72 (dd, J = 7.5, 2.2 Hz, 1H), 6.69 (d, J = 7.5 Hz, 1H), 6.58 (d, J = 2.0 Hz, 1H), 6.37 (d, J = 7.8 Hz, 1H), 5.99 (d, J = 9.2 Hz, 1H), 4.86 (td, J = 8.1, 4.0 Hz, 1H), 4.25 (t, J = 8.5 Hz, 1H), 4.06−4.02 (m, 2H), 3.82 (s, 3H), 4.32 (dd, J = 14.3, 3.8 Hz, 1H), 2.83 (dd, J = 14.3, 8.5 Hz, 1H), 2.31 (ddd, J = 14.2, 7.3, 5.0 Hz, 1H), 2.12 (ddd, J = 14.2, 9.2, 4.6 Hz, 1H), 1.82−1.39 (m, 14H), 1.29−0.96 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 172.2, 171.2, 159.0, 137.9, 130.1, 121.6, 117.7, 113.0, 68.2, 58.3, 53.4, 53.2, 41.5, 38.2, 36.5, 29.8, 29.3, 28.3, 26.6, 26.3, 26.2, 25.8, 25.2. HRMS calcd for $C_{24}H_{34}N_2O_5$ (M + H)$^+$: 431.2546. Found: 431.2545.

10-Cyclohexyl-8,11-dioxo-2-oxa-9,12-diazabicyclo[13.3.1]-nonadeca-1(19),15,17-triene-13-carboxylic Acid (22). Hydrolysis of methyl ester **21** (0.280 g, 0.650 mmol) was carried out in a manner similar to that described for the preparation of compound **15** to afford carboxylic acid **22** (0.238 g, 88% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.74 (bs, 1H), 8.47 (d, J = 8.8 Hz, 1H), 7.72 (d, J = 9.4 Hz, 1H), 7.14 (t, J = 7.9 Hz, 1H), 6.78−6.76 (m, 1H), 6.68 (s, 1H), 4.50−4.45 (m, 1H), 4.24 (t, J = 9.1 Hz, 1H), 4.08−4.03 (m, 1H), 4.01−3.96 (m, 1H), 3.07 (dd, J = 14.3, 2.0 Hz, 1H), 2.77 (dd, J = 14.3, 11.8 Hz, 1H), 2.26−2.20 (m, 1H), 1.93−1.88 (m, 1H), 1.63−1.43 (m, 10H), 1.38−1.22 (m, 4H), 1.20−0.84 (m, 5H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.1, 171.7, 171.0, 158.2, 139.7, 129.2, 121.3, 116.8, 112.0, 66.9, 56.5, 52.1, 40.5, 35.9, 34.6, 28.9, 28.6, 27.7, 26.0, 25.5, 25.4, 24.9, 24.4. HRMS calcd for $C_{23}H_{32}N_2O_5$ (M + H)$^+$: 417.2389. Found: 417.2379.

1,1-Dimethylethyl-α-(*S*)-[[[[3-[[(10(*S*)-cyclohexyl-8,11-dioxo-2-oxa-9,12-diazabicyclo[13.3.1]nonadeca-1(19),15,-17-trien-13(*S*)-yl)carbonyl]amino]-1,2-dioxohexyl]amino] acetyl]amino]benzene Acetate (23). Coupling of carboxylic acid **22** (0.230 g, 0.552 mmol) and tripeptide amine hydrochloride **14** (0.262 g, 0.609 mmol) was carried out in a manner similar to that described for the preparation of **4** to give an α-hydroxy amide pentapeptide intermediate (0.210 g, 48% yield). This crude product (0.200 g, 0.253 mmol) was oxidized with Dess−Martin periodinane in a manner similar to that described for the preparation of compound **16**. The product was purified by flash chromatography (1−5% MeOH/CH_2Cl_2) to afford compound **23** (0.140 g, 70% yield) as a mixture of diastereomers. HRMS calcd for $C_{43}H_{60}N_5O_9$ (M + H)$^+$: 790.4391. Found: 790.4426.

α-(*S*)-[[[[3-[[(10(*S*)-Cyclohexyl-8,11-dioxo-2-oxa-9,12-diazabicyclo[13.3.1]nonadeca-1(19),15,17-trien-13(*S*)-yl)-carbonyl]amino]-1,2-dioxohexyl]amino]acetyl]amino]-benzeneacetic Acid (24). Carboxylic acid **24** was obtained, as a mixture of diastereomers, in a manner similar to that described for the preparation of compound **17** in quantitative yield. HRMS calcd for $C_{39}H_{52}N_5O_9$ (M + H)$^+$: 734.3765. Found: 734.3747.

References

(1) (a) Cohen, J. The Scientific Challenge of Hepatitis C. *Science* **1999**, *285*, 26−30. (b) Alter, M. J.; Kruszon-Moran, D.; Nainan, O. V.; McQuillan, G. M.; Gao, F.; Moyer, L. A.; Kaslow, R. A.; Margolis, H. S. The Prevalence of Hepatitis C Virus Infection in The United States, 1988 Through 1994. *N. Engl. J. Med.* **1999**, *341*, 556−562. (c) Cuthbert, J. A. Hepatitis C: Progress and Problems. *Clin. Microbiol. Rev.* **1994**, *7*, 505−532.

(2) (a) Neumann, A. U.; Lam, N. P.; Dahari, H.; Gretch, D. R.; Wiley, T. E.; Layden, T. J.; Perelson, A. S. Hepatitis C Virus Dynamics in vivo and the Antiviral Efficacy of Interferon-α Therapy. *Science* **1998**, *282*, 103−107. (b) Rosen, H. R.; Gretch, D. R. Hepatitis C Virus: Current Understanding and Prospects for Future Therapies. *Mol. Med. Today* **1999**, *5*, 393−399. (c) Di Bisceglie, A. M.; McHutchison, J.; Rice, C. M. New therapeutic strategies for hepatitis C. *Hepatology* **2002**, *35*, 224−231.

(3) (a) Kwong, A. D.; Kim, J. L.; Rao, G.; Lipovsek, D.; Raybuck, S. A. Hepatitis C Virus NS3/4A Protease. *Antiviral Res.* **1998**, *40*, 1−18. (b) Yan, Y.; Li, Y.; Munshi, S.; Sardana, V.; Cole, J. L.; Sardana, M.; Steinkuehler, C.; Tomei, L.; De Francesco, R.; Kuo, L. C.; Chen, Z. Complex of NS3 protease and NS4A Peptide of BK strain Hepatitis C Virus: a 2.2 A resolution structure in a hexagonal crystal form. *Protein Sci.* **1998**, *7*, 837−847.

(4) (a) Tan, S.-L.; He, Y.; Huang, Y.; Gale, M., Jr. Strategies for Hepatitis C Therapeutic Intervention: Now and Next. *Curr. Opin. Pharmacol.* **2004**, *4*, 465−470. (b) Malancona, S.; Colarrusso, S.; Ontoria, J. M.; Marchetti, A.; Poma, M.; Stansfield, I.; Laufer, R.; Marco, A. D.; Taliani, M.; Verdirame, M.; Gonzalezpaz, O.; Matassa, V. G.; Narjes, F. SAR and Pharmacokinetics Studies on Phenethylamide Inhibitors of the Hepatitis C Virus. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4575−4579. (c) Llinas-Brunet, M.; Bailey, M. D.; Ghiro, E.; Gorys, V.; Halmos, T.; Poirier, M.; Rancourt, J.; Goudreau, N. A Systematic Approach to the Optimization of Substrate-Based Inhibitors of the Hepatitis C Virus NS3 Protease: Discovery of Potent and Specific Tripeptide Inhibitors. *J. Med. Chem.* **2004**, *47*, 6584−6594. (d) Zhang, X. Inhibitors of Hepatitis C − A Review of the Current Patent Literature. *IDrugs* **2002**, *5*, 154−158. (e) Dymock, B. W. Emerging Therapies for Hepatitis C Virus Infection. *Expert Opin. Emerging Drugs* **2001**, *6*, 13−42. (f) Dymock, B. W.; Jones, P. S.; Wilson, F. X. Novel Approaches to the Treatment of Hepatitis C Virus. *Antiviral. Chem. Chemother.* **2000**, *11*, 79−96. (g) Zhang, R.; Durkin, J. P.; Windsor, W. T. Azapeptides as Inhibitors of the Hepatitis C Virus NS3 Serine Protease. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1005−1008. (h) Ingallinella, P.; Fattori, D.; Altamura, S.; Steinkuhler, C.; Koch, U.; Cicero, D.; Bazzo, R.; Cortese, R.; Bianchi, E.; Pessi, A. Prime Site Binding Inhibitors of a Serine Protease: NS3/4A of Hepatitis C Virus. *Biochemistry* **2002**, *41*, 5483−5492. (i) Beevers, R.; Carr, M. G.; Jones, P. S.; Jordan, S.; Kay, P. B.; Lazell, R. C.; Raynham, T. M. Solution and Solid-Phase Synthesis of Potent Inhibitors of Hepatitis C Virus NS3 Protease. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 641−643.

(5) West, M. L.; Fairlie, D. P. Targeting HIV-1 protease: A Test of Drug-Design Methodologies. *Trends Pharm. Sci.* **1995**, *16*, 67−75.

(6) Fairlie, D. P.; West, M. L.; Wong, A. K. Towards Protein Surface Mimetics. *Curr. Med. Chem*. **1998**, *5*, 29−62.

(7) (a) Boger, D. L.; Kim, S. H.; Miyazaki, S.; Strittmatter, H.; Weng, J.-H.; Mori, Y.; Rogel, O.; Castle, S. L.; McAtee, J. J. Total Synthesis of the Teicoplanin Aglycon. *J. Am. Chem. Soc*. **2000**, *122*, 7416−7417. (b) Evans, D. A.; Wood, M. R.; Trotter, B. W.; Richardson, T. I.; Barrow, J. C.; Katz, J. L. Total Syntheses of Vancomycin and Eremomycin Aglycons. *Angew. Chem., Int. Ed.* **1998**, *37*, 2700−2704. (c) Evans, D. A.; Dinsmore, C. J.; Watson, P. S.; Wood, M. R.; Richardson, T. I.; Trotter, B. W.; Katz, J. L. Nonconventional Stereochemical Issues in the Design of the Synthesis of the Vancomycin Antibiotics: Challenges Imposed by Axial and Nonplanar Chiral Elements in the Heptapeptide Aglycons. *Angew. Chem., Int. Ed.* **1998**, *37*, 2704−2708. (d) Rama Rao, A.; Garjar, M.; Reddy, K.; Rao, A. Studies directed toward the synthesis of vancomycin and related cyclic peptides. *Chem. Rev.* **1995**, *95*, 2135−2167. (e) Mtsuzaki, K.; Ikeda, H.; Ogino, T.; Matsumoto, A.; Woodruff, H. B.; Tanaka, H.; Omura, S. Chloropeptins I and II, Novel Inhibitors Against gp120-CD4 Binding from *Streptomyces* sp. *J. Antibiot.* **1994**, *47*, 1173−1174. (f) Matsuzaki, K.; Ogino, T.; Sunazuka, T.; Tanaka, H.; Omura, S. Chloropeptins, New Anti-HIV Antibiotics Inhibiting gp120-CD4 Binding from *Streptomyces* sp. II. Structure Elucidation of Chloropeptin I. *J. Antibiot.* **1997**, *50*, 66−69.

(8) (a) Llinas-Brunet, M.; Bailey, M. D.; Fazal, G.; Goulet, S.; Halmos, T.; LePlante, S.; Maurice, R.; Poirier, M.; Poupart, M.-A.; Thibeault, D.; Wernic, D.; Lamarre, D. Peptide-Based Inhibitors of the Hepatitis C Virus Serine Protease. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 1713−1718. (b) Steinkuhler, C.; Biasiol, G.; Brunetti, M.; Urbani, A.; Koch, U.; Cortese, R.; Pessi, A.; De Francesco, R. Product Inhibition of the Hepatitis C Virus NS3 Protease. *Biochemistry* **1998**, *37*, 8899−8905.

(9) (a) Lin, C.; Lin, K.; Luong, Y.-P.; Rao, B. G.; Wei, Y.; Brennan, D. L.; Fulghum, J. R.; Hsiao, H.-M.; Ma, S.; Maxwell, J. P.; Cottrell, K. M.; Perni, R. B.; Gates, C. A.; Kwong, A. D. In Vitro Resistance Studies of Hepatitis C Virus Serine Protease Inhibitors, VX-950 and BILN 2061. *J. Biol. Chem.* **2004**, *279*, 17508−17514. (b) Sun, D. X.; Liu, L.; Heinz, B.; Kolykhalov, A.; Lamar, J.; Johnson, R. B.; Wang, Q. M.; Yip, Y.; Chen, S.-H. P4 Cap Modified Tetrapeptidyl α-Ketoamides as Potent HCV NS3 Protease Inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4333−4338. (c) Yip, Y.; Victor, F.; Lamar, J.; Johnson, R.; Wang, Q. M.; Glass, J. I.; Yumibe, N.; Wakulchik, M.; Munroe, J.; Chen, S.-H. P4 and P1′ Optimization of Bicycloproline P2 Bearing Tetrapeptidyl α-Ketoamides as HCV Protease Inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5007−5011. (d) Perni, R. B.; Farmer, L. J.; Cottrell, K. M.; Court, J. J.; Courtney, L. F.; Deininger, D. D.; Gates, C. A.; Harbeson, S. L.; Kim, J. L.; Lin, C.; Lin, K.; Luong, Y.-P.; Maxwell, J. P.; Murcko, M. A.; Pitlik, J.; Rao, B. G.; Schairer, W. C.; Tung, R. D.; Van Drie, J. H.; Wilson, K.; Thomson, J. A. Inhibitors of Hepatitis C Virus NS3/4A Protease. Part 3: P2 Proline Variants. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1939−1942.

(10) Schering-Plough Research Institute: unpublished results.

(11) (a) Hughes, D. L. The Mitsunobu Reaction. *Org. React.* **1992**, *42*, 335. (b) Mitsunobu, O. The Use of Diethyl Azodicarboxylate and Triphenylphosphine in Synthesis and Transformation of Natural Products. *Synthesis* **1981**, 1−28.

(12) Dess, D. B.; Martin, J. C. A Useful 12-I-5 Triacetoxyperiodinane (the Dess−Martin Periodinane) for the Selective Oxidation of Primary or Secondary Alcohols and a Variety of Related 12-I-5 Species. *J. Am. Chem. Soc.* **1991.** *113*, 7277−7287.

(13) Zhang, R.; Beyer, B. M.; Durkin, J.; Ingram, R.; Njoroge, F. G.; Windsor, W. T.; Malcolm, B. A. A Continuous Spectrophotometric Assay for the Hepatitis C Virus Serine Protease. *Anal. Biochem.* **1999**, *270*, 268−275; The substrate Ac-DTEDVVP(Nva)-O−PAP was used in the present study.

(14) Taremi, S. S.; Beyer, B.; Maher, M.; Yao, N.; Prosise, W.; Weber, P. C.; Malcolm, B. A. Construction, Expression, and Characterization of a Novel Fully Activated Recombinant Single-Chain Hepatitis C Virus Protease. *Protein Sci.* **1998**, *7*, 2143−1249.

(15) For a definition of Ki* and discussions, see: Morrison, J. F.; Walsh, C. T. The Behavior and Significance of Slow-binding Enzyme Inhibitors. *Adv. Enzymol.* **1998**, *61*, 201−301.

JM050323B