Synthesis of a Reduced Ring Analog of Didemnin B

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As part of investigations directed toward the determination of the essential/nonessential structural features for the bioactivities of didemnin B, we designed a reduced ring analog in which three moieties, namely the tyrosine side chain, the isostatine hydroxyl, and the side chain (L-lactyl-L-prolyl-*N*-Me-D-leucine), were in their presumed bioactive conformation. In designing the reduced ring analog, we eliminated the leucine-proline portion of the macrocycle core and replaced it with an *n*-butyl linker in order to elucidate its role. According to MM2 calculations (MacroModel molecular modeling), this analog was of lower energy than the natural product didemnin B, and both structures were superimposable. The synthetic strategy involved four disconnections. Macrocyclization was accomplished at the activated carboxylic acid of the α-(α-hydroxyisovaleryl)-propionyl unit (HIP) and the protected amine of the *n*-butyl linker using a modification of Schmidt's protocol. After selective deprotection of the hydroxyl and amino groups of the macrocycle, the peptide side chain was introduced using (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) as the activating reagent.

Introduction

The didemnins are an interesting class of cyclodepsipeptides isolated from a marine tunicate of the family *Didemnidae*.[1] Didemnins possess a varied degree of bioactivities: antiviral, antitumor, and immunosuppressive.[2−7] Didemnin B (**1b,** Figure 1) was tested extensively. Until now, **1b** was thought to be the most active didemnin.[4−9] Recently isolated dehydrodidemnin B (**1c**), an oxidized form of didemnin B, glutaminyldidemnin B, and didemnin M have shown comparable or superior cytotoxic activity.[10,11] Most didemnins contain a common macrocycle and differ only in the side chains attached to the backbone through the amino group of threonine.

Didemnin B has undergone phase II clinical trials for antitumor activity.[8,12−16] Both didemnins A (**1a**) and B (**1b**) show antiviral activity against DNA and RNA viruses,[6,17] with didemnin B being more active. Didemnin

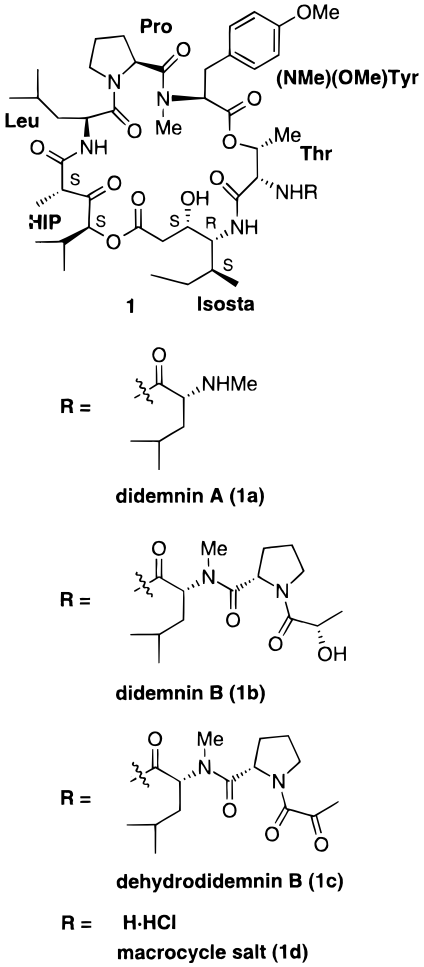

Figure 1. Some naturally occurring didemnins.

[⊗] Abstract published in *Advance ACS Abstracts,* July 1, 1997.

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B has significantly greater immunosuppressive activity than cyclosporin A, but it does not bind to the same receptor site.[18] Three sites were originally proposed as being essential to biological activity on the basis of their spatial relationship as determined from X-ray analysis[19] and solution conformation of didemnin B:[20] the tyrosine side chain of the tetrapeptide region, the hydroxyl group of isostatine, and the side chain attached to the amino group of threonine. These three moieties lie on the periphery of the highly irregular "bent figure eight" macrocycle.

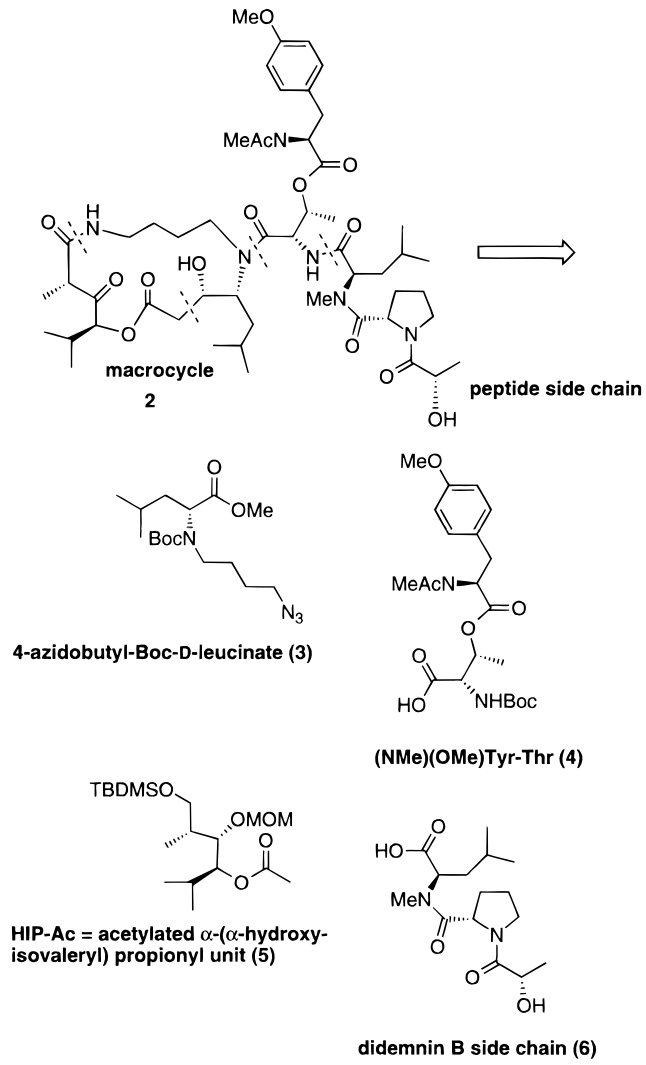
Previous experimental work has indeed shown that biological activity is sensitive to changes in these proposed areas. Removal of the *O*-methyl of the tyrosine side chain reduced potency in all areas of activity[21] and the macrocycle salt (**1d**) without the side chain was essentially inactive.[22] Acetylation of the isostatine hydroxyl group and of side-chain functionalities caused several changes in the activity of didemnin B.[10,17] Finally, natural didemnin B and nordidemnin B, which differ in the isostatine region of the macrocycle where isobutyl is replaced by isopropyl, were shown to have comparable activity.[23]

The mechanism of action of the didemnins has not yet been established. However, recent studies of possible binding sites have yielded important results. In 1992, Shen and co-workers reported that the immunosuppressive activity of didemnin B is mediated through binding to a site on Nb2 node lymphoma cells.[21] In 1994, Schreiber and co-workers found that a didemnin A derivative binds to elongation factor 1α (eEF-1α) in a GTP-dependent manner, which in turn inhibits protein synthesis.[24] Recently, Toogood and co-workers reported that didemnin B inhibits the ability of eEF-2 to catalyze polypeptide elongation, which demonstrates that it is an inhibitor of ribosomal translocation.[25]

Results and Discussion

In recent years, introduction of covalent linkers replacing amino acids that do not participate in receptor occupation or activation has led to the syntheses of comparable or more active analogs. This method was used to develop analogs of smaller molecules such as jaspamide and large peptides such as atrial natriuretic factor.[26] Upon examining the X-ray structure[19] and NMR spectra of didemnin B,[20] we opted to replace the leucine and proline residues with a covalent linker to introduce conformational constraints and eliminate amino acids that are not essential to biological activity. Elimination of the proline-leucine portion might also reduce the toxicity of didemnin B and increase its oral activity. Various bridging units, needed to replace the only transannular hydrogen bond present in the macrocycle, were examined as options for connecting the free ends of the isostatine-HIP unit. After examination of several bridging units, a *n*-butyl linker was found to be a suitable replacement. The resulting structure is a 15-membered ring (**2**, Scheme 1). This modification left all three potentially active sites in a presumed "bioactive conformation". Visualization of this process was aided by using a structure superposition program (MacroModel molecular modeling). Calculations were performed using MacroModel v3.1X on a Silicon Graphics Iris 4D/320S computer. Minimizations were generated using PRCG, followed by FNMR with MM2 force field. The proposed structure was then compared to the presumed bioactive didemnin B conformation. The two structures proved to be remarkably similar by visual overlap comparison. In an effort to compare this overlap by other means, we also used MacroModel's superposition subroutine, which calculates the best fit and overlap standard deviation of the selected atoms in the molecule. On a 3-D computer screen, the overlay of the above-designed structure and

Scheme 1



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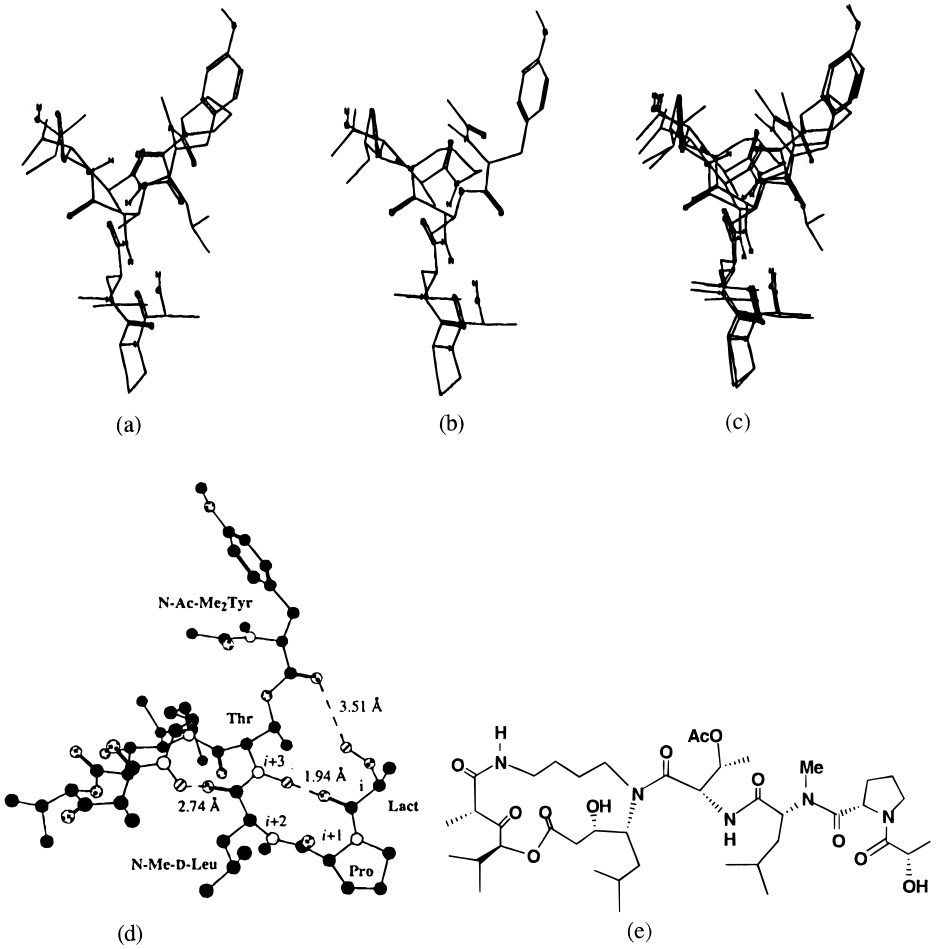

Figure 2. (a) View of the minimized structure of didemnin B showing the fold of the cyclic peptide ring and the disposition of possible interacting groups: the tyrosine side chain, the lactylproline moiety, and the hydroxyl group in isostatine. (b) Minimized structure of reduced ring analog **2**. (c) Overlay of didemnin B with reduced ring analog **2**. (d) Another view (Chem 3D) of the reduced ring analog **2** showing the intramolecular H-bond pattern. (e) Structure of reduced ring analog **2a**.

didemnin B shows a rather remarkable correspondence of the important functionalities, particularly the tyrosine side chain, the lactyl proline moiety, and the hydroxyl group of isostatine, as shown in Figure 2c. We retained three hydrogen bonds (d, Figure 2) present in the natural product. The side chain is held onto the main macrocycle by means of a strong hydrogen bond between the carbonyl of the side chain D-leucine and the amide of the n-butyl linker. The conformation of the β(II) turn is dictated by a hydrogen bond between the lactyl carbonyl and the amide group of the threonine, and the turn is further stabilized by the third hydrogen bond between the lactyl hydroxyl and the tyrosine carbonyl.

At this time, we would like to emphasize two points:

1. The conformation of the threonine residue plays a critical role in shaping the overall molecular conformation. Rotation of the tyrosine-threonine portion dramatically interferes with two hydrogen bonds in the lactylprolyl D-(NMe)-leucyl side chain and increases the molecular mechanics energy (conformer energies vary from 23 to 42 kcal/mol). Local conformation searching has been carried out and has proven that b in Figure 2 is the local minimum conformer (E = 23.03 kcal/mol, rms (root mean square) = 0.014 kJ/Å mol), even lower than didemnin B (E = 36.57 kcal/mol, rms = 0.014 kJ/Å-mol).

2. After the role of the tetrapeptide is elucidated, and if it is established that the *N*,*O*-dimethyltyrosine is not essential to the specific biological activity, analog **2a** (e, Figure 2), which does not contain the *N*,*O*-dimethyltyrosine portion, will be prepared in order to confirm that the active region is the β(II)-turn region, as was observed in jaspamide analogs.[26] Preliminary biological testing of a didemnin B analog in which the *N*,*O*-dimethyltyrosine portion was replaced with N-MeLeu retained both cytotoxic and protein synthesis inhibition activities.[27]

Synthetic Strategy. Our synthetic strategy involved four disconnections that afforded four subunits: the methyl 4-(azidobutyl)-Boc-D-leucinate (**3**); the dipeptide *N*,*O*-diMeTyr-Thr (**4**); acetylated α-(α-hydroxyisovaleryl)-propionyl) unit (acetylated HIP, **5**); and didemnin B side chain (**6**, Scheme 1). We introduced the optically pure peptide side chain on the macrocycle as a separate step at the end of the synthesis so that it could be easily functionalized. The linear precursor leading to the macrocycle was prepared in a stereoselective manner, specifically preserving the stereochemistry of the 2-methyl group of the HIP unit. We introduced the 3-keto group of the HIP unit masked as a methoxymethyl (MOM) ether to preserve the stereochemistry of the HIP unit, so that deprotection and oxidation to the corresponding ketone would occur only after macrocyclization. In the macrocycle, the 3-keto group and the amide cannot lie in the same plane; therefore, racemization at the 2-position cannot occur readily.

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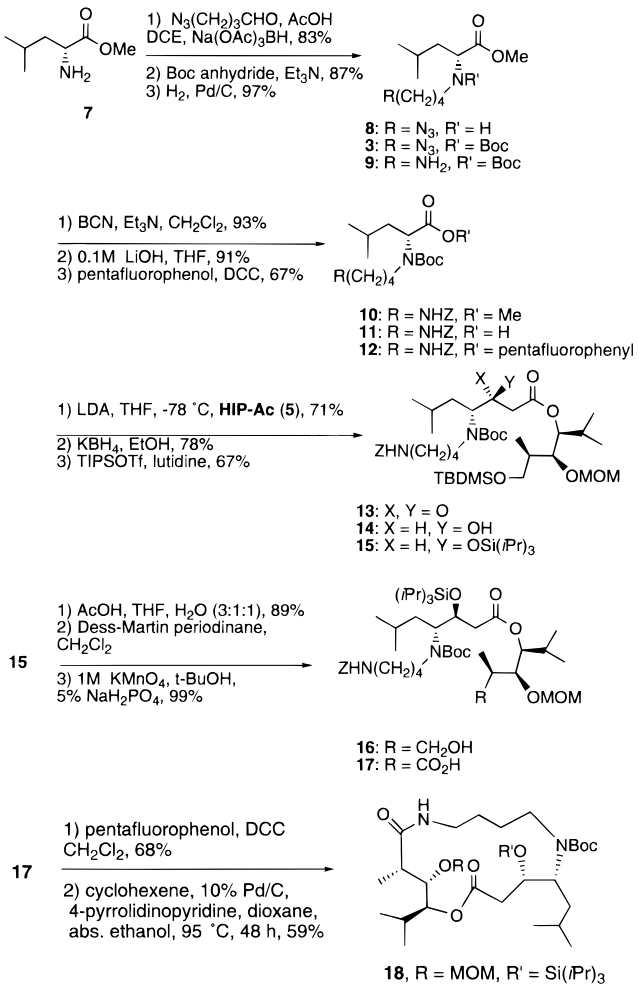
Scheme 2



Synthesis of the Macrocycle. Preparation of the macrocycle began with the reductive amination[28,29] of D-leucine methyl ester (**7**) with 4-azidobutanal (prepared by a two-step sequence: nucleophilic substitution of 4-chloro-1-butanol, followed by Swern oxidation of the corresponding alcohol) to afford a secondary amine (**8**, Scheme 2). The amine functionality was immediately protected as its Boc derivative, and the azide was reduced to a primary amine (**9**), which was converted to its *N*-(benzyloxycarbonyl) (Z) derivative (**10**). The methyl ester was saponified to afford the corresponding acid (**11**). The acid was activated using pentafluorophenol and DCC, and the resulting pentafluorophenyl ester (**12**) was directly condensed with the lithium enolate of the HIP-acetate to produce the β-keto ester **13** in 71% yield.

HIP-acetate was synthesized using previous methodology.[30] The reduction of the β-keto ester was accomplished with selectivity for the *anti* diastereomer, using potassium borohydride in ethanol[31] to afford a diastereomeric mixture of alcohols (**14**, Scheme 2), which was not separated but treated with triisopropylsilyl triflate, to give a chromatographically separable mixture of two silyl ethers. The ratio of these two diastereomers was found to be 6:1 (**15**, Scheme 2). To effect cyclization, the protected primary hydroxyl group in the HIP-unit had to be oxidized to a carboxylic acid. This operation was accomplished by first removing the *tert*-butyldimethylsilyl group with HOAc/THF/H_2O (3:1:1) to give the corresponding alcohol **16**, in 89% yield (Scheme 2). The primary alcohol was then oxidized to its corresponding carboxylic acid by a two-step oxidation method. Conversion of the alcohol to the aldehyde was accomplished using Dess−Martin periodinane reagent.[32] The resulting aldehyde was further oxidized without purification using Masamune's procedure.[33] Treatment of the aldehyde with potassium permanganate in *tert*-butyl alcohol, buffered with 5% sodium hydrogen phosphate, gave the carboxylic acid **17**, in excellent yield.

To accomplish the cyclization, various phosphorus coupling reagents as well as substituted carbodiimides were used. However, macrocyclization proved far from trivial. BOP, DPPA, 1,3-dicyclohexylcarbodiimide (DCC), and BOP-Cl were unsuccessful. *O*-Benzotriazolyl-*N*,*N*,-*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) only gave minute amounts of product. Although pentafluorophenyl diphenylphosphinate (FDPP) had been used successfully in the macrocyclization of didemnin B,[34] in this case, only a 20% yield could be obtained. As a last resort, we utilized a modification of Schmidt's method.[35] The Z-protected acid was treated with pentafluorophenol to form the pentafluorophenyl ester. The cyclization of the active ester to the desired macrocyle was effected by in *situ* removal of the Z-protecting group using cyclohexene or cyclohexadiene as the hydrogen source to produce a slow evolution of hydrogen in the presence of the catalyst (10% Pd/C), under high dilution conditions, in dioxane at 95 °C, and with 4-pyrrolidinopyridine as the base. The desired macrocycle (**18**) was obtained in 59% yield (Scheme 2).

The next task was the deprotection of the various protecting groups in a selective manner (Scheme 3). The MOM group was removed with dimethylboron bromide to give the corresponding secondary alcohol (**19**). Dess−Martin oxidation[32] of **19** afforded the ketone **20** in 99% yield. Both the triisopropylsilyl group and the Boc group were removed with hydrogen chloride gas in EtOAc, at −30 to 0 °C, to yield the corresponding hydrochloride salt of the macrocycle **21**.

Synthesis of the Peptide Side Chain. Our approach to the peptide side chain began with Z-L-tyrosine (Scheme 4). Treatment of Z-L-tyrosine in THF with dimethyl sulfate and powdered KOH in the presence of a phase-transfer catalyst, tetrabutylammonium hydrogen sulfate, gave the ester **22** in good yield (Scheme 4).[30] The Z-protecting group was removed to yield **23**,[36] and the secondary amine functionality was acetylated to give **24**, using acetic anhydride. Saponification of the methyl ester of **24** gave the corresponding acid (**25**). Reaction of the acid with Boc-Thr-OBn in presence of isopropenyl chloroformate gave ester **26**, which upon hydrogenation provided the corresponding acid **4**.

With the macrocycle salt **21** and the peptide side chain **4** in hand, we used the BOP reagent to provide **27** in 67% yield (Scheme 3). The Boc group was removed under

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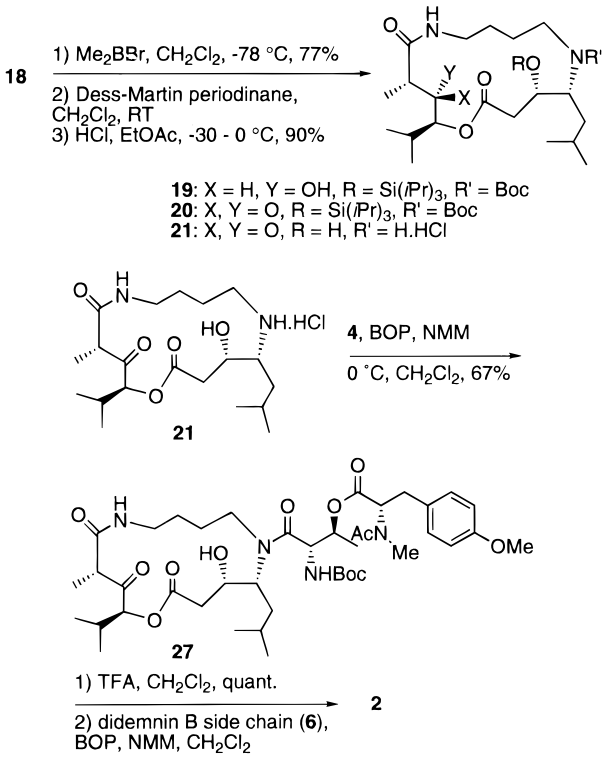
Scheme 3



standard conditions, and the resulting amine was coupled to the didemnin B side chain (**6**), thereby completing the synthesis of the reduced ring analog **2**.

Conclusions

The X-ray analysis of the didemnins suggested that three structural features are essential for their biological activity: the side chain attached to the amino group of threonine, the isostatine hydroxyl group, and the tyrosine side chain. Since no modifications in the leucine-proline dipeptide unit of the macrocycle had been reported, we decided to elucidate its role in determining bioactivity and overall conformation. A molecular-modeling study revealed that the reduced ring analog (**2**) and didemnin B overlaid well and that three of the four hydrogen bonds were retained.

The synthesis of the macrocycle was accomplished mainly by three key reactions: reductive amination using sodium triacetoxyborohydride, a stereoselective reduction of the β-keto ester, and finally *in situ* deprotection of the amino group and cyclization in one pot via an improved version of Schmidt's protocol. Selective removal of the protecting groups and the attachment of the side chain were accomplished using previously reported methodologies.[30,34] The diverse biological activities of this reduced ring analog will be tested and compared to those of didemnin B in the near future.

Experimental Section

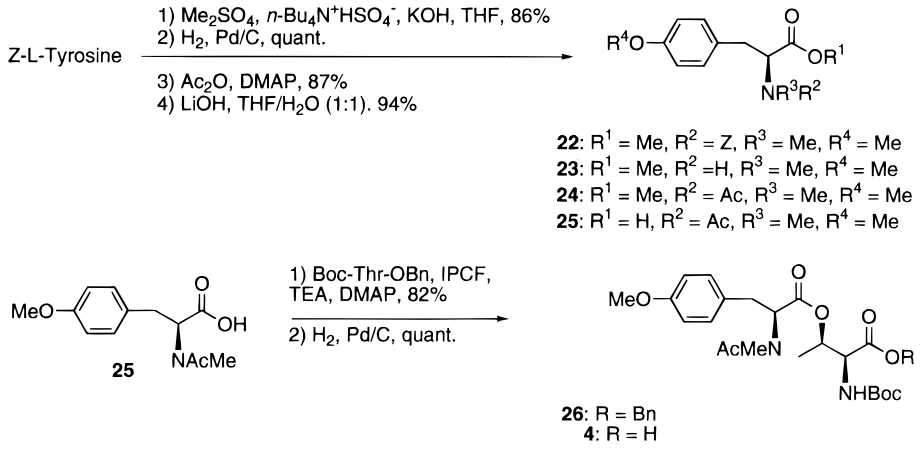
General Procedures. Materials were described previously.[34] Proton and carbon magnetic resonance spectra (^{1}H- and ^{13}C-NMR) were recorded on a Bruker AM-500 (500 MHz) Fourier transform spectrometer, and chemical shifts were expressed in parts per million (δ) relative to tetramethylsilane (TMS 0 ppm) or $CHCl_3$ as an internal reference (7.24 ppm for ^{1}H and 77.0 ppm for ^{13}C). Infrared spectra (IR) were obtained on a Perkin-Elmer Model 281-B or a Perkin-Elmer Model 781 spectrometer. Absorptions are reported in wavenumbers (cm^{-1}), and the spectra are calibrated against the 1601 cm^{-1} band of a polystyrene film. Optical rotations (in degrees) were recorded on a Perkin-Elmer Model 241 polarimeter at the sodium D line. High-resolution mass spectra (HRMS) were obtained on either a VG 70-70HS [a high-resolution double-focusing mass spectrometer using ammonia chemical ionization (CI) or electron impact (EI)] or a ZAB-E [using fast atom bombardment (FAB), CI, or EI]. Gas chromatograms were obtained on a Hewlett-Packard 5890 GC incorporating a HP-1 cross-linked methyl silicone gum capillary column. Elemental analyses were performed by either Desert Analytics, Tucson, AZ, or University of Pennsylvania Chemistry Department Elemental Analysis Facility.

All molecular modeling calculations were performed using Macro Model v3.1X on a Silicon Graphics Iris 4D/320S computer. Minimizations were generated from X-ray coordinates using PRCG followed by FNMR with the MM2 force field to obtain overlays with rms values less than 0.050.

Methyl *N*-(4-Azidobutyl)-D-leucinate (8). 4-Azido-1-butanal was prepared from commercially available 4-chloro-1-butanol. Displacement of chlorine with sodium azide, followed by oxidation under Swern's conditions, gave the aldehyde in 72% overall yield: R_f 0.58 (20% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 1.80−2.00 (m, 2H), 2.40−2.60 (m, 2H), 3.20−3.45 (m, 2H), 9.80 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.47, 40.76, 50.58, 200.80; IR (neat) 2922 (w), 2360 (w), 2099 (s), 1715 (s), 1257 (w) cm^{-1}.

To a solution of **7** (0.83 g, 5.71 mmol) in 20 mL of 1,2 dichloroethane (DCE) was added 4-azido-1-butanal (0.65 g, 5.75 mmol) in 3 mL of DCE. To this mixture were added acetic acid (0.33 mL, 5.70 mmol) and sodium triacetoxyborohydride (1.70 g, 8.02 mmol). After 12 h, the reaction was diluted with CH_2Cl_2 (30 mL), and the excess reagent was quenched by dropwise addition of ammonium chloride. The reaction mixture was washed with 5% HCl, 5% $NaHCO_3$, and saturated

Scheme 4



NaCl solutions. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The resulting crude oil was purified by column chromatography, eluting with MeOH/$CHCl_3$ (5:95). Pure compound **8** (1.22 g, 83%) was obtained as an oil: R_f 0.55 (10:90 MeOH:$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.93 (d, J = 6.6 Hz, 3H) and 0.91 (d, J = 6.6 Hz, 3H), 1.42−1.49 (m, 2H), 1.51−1.58 (m, 2H), 1.62−1.68 (m, 3H), 1.71−1.73 (m, 1H), 2.45 and 2.63 (ABX, J = 6.90, 4.30 Hz, 2H), 3.25−3.29 (m, 3H), 3.72 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 22.2, 22.7, 24.7, 24.8, 26.5, 27.2, 42.7, 47.4, 51.2, 59.8, 176.4; IR (neat) 2956 (s), 2097 (s), 1744 (s) cm^{-1}; HRMS m/z calcd for $C_{11}H_{23}N_4O_2$ (M + H) 243.1821, found 243.1825; $[\alpha]^{25}_D$ +8.89° (c = 1.35, $CHCl_3$).

Methyl *N*-Boc-*N*-(4-azidobutyl)-D-leucinate (3). Amine **8** (4.00 g, 0.0155 mol) was dissolved in CH_2Cl_2 (70 mL) and treated with triethylamine (6.59 mL, 0.047 mol) and Boc anhydride (6.90 g, 0.032 mol). The reaction mixture was refluxed overnight. After this time, the reaction was cooled and diluted with ether. The organic solution was washed with 5% HCl, 5% $NaHCO_3$, and saturated NaCl solutions. The ether layer was dried (Na_2SO_4), filtered, and concentrated. The resulting crude oil was purified by column chromatography, eluting with EtOAc/petroleum ether (10:90). Pure compound **3** (4.63 g, 87%) was obtained as an oil: R_f 0.37 (10:90 EtOAc: petroleum ether); ^{1}H NMR ($CDCl_3$) δ 0.87−0.90 (m, 6H), 1.53 (s, 9H), 1.58−1.70 (m, 5H), 2.95 (m, 1H), 3.20 (m, 2H), 3.30 (m, 2H), 3.60 (s, 3H), 4.10 and 4.50 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 21.8, 22.9, 24.7, 26.4, 26.7, 28.3, 45.3, 46.4, 50.7, 51.1, 51.9, 56.9, 58.1, 80.2, 155.0, 172.5; IR (neat) 2956 (s), 2870 (s), 2096 (s), 1744 (s), 1697 (s) cm^{-1}; HRMS m/z calcd for $C_{16}H_{30}N_4O_4$ (M + H) 343.2345, found 343.2348; $[\alpha]^{25}_D$ +36.89° (c = 5.45, $CHCl_3$).

Methyl *N*-Boc-*N*-(4-aminobutyl)-D-leucinate (9). To a CH_3OH/EtOAc solution (1:1, 20 mL) was added 10% Pd/C (1.0 g). To the resulting suspension was added compound **3** (3.50 g, 9.78 mmol) in CH_3OH (5 mL). The solution was subjected to an atmosphere of hydrogen (40 psi) and shaken for 3 h in a Parr hydrogenation apparatus. The reaction mixture was filtered through Celite. The Celite bed was washed with CH_3OH, and the filtrate was concentrated. The resulting amine **9** (3.0 g, 97%) was used directly in the next step: ^{1}H NMR ($CDCl_3$) δ 0.84 (d, J = 6.56 Hz, 6H), 1.32 (s, 9H), 1.41−1.66 (m, 7H), 2.87−2.92 (m, 1H) and 3.15−3.22 (m, 3H), 3.31−3.32 (m, 2H), 3.58 (s, 3H), 4.01−4.07 and 4.78−4.81 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 21.6, 23.0, 24.7, 25.6, 27.1, 28.0, 44.7, 51.0, 51.9, 57.3, 58.1, 80.1, 155.1, 171.2; IR (neat) 3364 (br), 2955 (s), 1743 (m), 1695 (s), cm^{-1}; HRMS m/z calcd for $C_{16}H_{33}N_2O_4$ (M + H) 317.2440, found 317.2447. $[\alpha]^{25}_D$ +39.90° (c = 4.52, $CHCl_3$).

Methyl *N*-Boc-*N*-[4-[*N*-(benzyloxycarbonyl)amino]-butyl]-D-leucinate (10). The primary amine **9** (1.07 g, 3.37 mmol) was dissolved in CH_2Cl_2 (20 mL) and cooled to 0 °C. To this solution were added *N*-[(benzyloxycarbonyl)oxy]succinimide (1.0 g, 4.04 mmol) and triethylamine (0.56 mL, 4.04 mmol). The reaction was stirred overnight and diluted with ether (100 mL). The organic solution was washed with 5% HCl, 5% $NaHCO_3$, and saturated NaCl solutions. The ether layer was dried (Na_2SO_4), filtered, and concentrated. The resulting crude oil was purified by column chromatography, eluting with EtOAc/petroleum ether (30:70). Pure compound **10** (1.41 g, 93%) was obtained as an oil: R_f 0.53 (30:70 EtOAc:petroleum ether); ^{1}H NMR ($CDCl_3$) δ 0.86 (d, J = 3.2 Hz, 3H) and 0.87 (d, J = 3.0 Hz, 3H), 1.35 (s, 9H), 1.42−1.67 (m, 6H), 1.68−1.70 (m, 1H), 2.90−2.92 (m, 1H), 3.12−3.20 (m, 3H), 3.59 (s, 3H), 4.01-4.07 and 4.49−4.54 (m, 1H), 5.00 (s, 2H), 6.07 (s, 1H), 7.20−7.29 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 21.7, 22.9, 24.6, 25.7, 26.6, 27.1, 28.2 (3 overlapping carbons), 44.7, 46.4, 51.9, 56.9, 66.4, 80.2, 128.4, 129.1, 133.3, 133.5, 134.6, 136.6, 155.1, 156.6, 169.4; IR (neat) 3353 (m), 2955 (s), 2869 (m), 1741 (s), 1697 (s) cm^{-1}; HRMS m/z calcd for $C_{24}H_{39}N_2O_6$ (M + H) 451.2808, found 451.2803; $[\alpha]^{25}_D$ +32.29° (c = 1.25, $CHCl_3$). Anal. Calcd for $C_{24}H_{38}N_2O_6$: C, 63.98; H, 8.50; N, 6.22. Found: C, 63.93; H, 8.23; N, 5.89.

***N*-Boc-*N*-[4-[*N*-(benzyloxycarbonyl)amino]butyl]-D-leucine (11).** Compound **10** (8.01 g, 0.018 mol) was dissolved in THF/H_2O (1:1, 100 mL). The reaction was cooled to 0 °C, and lithium hydroxide monohydrate (4.45 g, 0.106 mol) was added. The reaction was stirred at 0 °C for 4 h, concentrated to 50 mL, and washed with ether (2 × 20 mL). The combined ether layers were extracted with saturated $NaHCO_3$ (10 mL) solution. The aqueous layers were combined and acidified to pH 1 with 1 N potassium hydrogen sulfate solution. The acidified aqueous layer was extracted with ether (3 × 100 mL). The ether extracts were dried (Na_2SO_4), filtered, and concentrated. Compound **11** (6.34 g, 91%) was used directly in the next step: R_f 0.60 (10:90 MeOH:$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.94 (d, J = 5.2 Hz, 6H), 1.44 (s, 9H), 1.62−1.79 (m, 7H), 3.00−3.41 (m, 4H), 4.19−4.22 and 4.34−4.36 (m, 1H), 5.09 (m, 3H), 7.31−7.34 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 23.1, 24.9, 25.9, 26.2, 27.3, 28.3 (3 overlapping carbons), 40.6, 46.1, 58.3, 58.5, 66.6, 80.5, 128.5, 128.0, 136.9 (3 overlapped carbons), 156.5 (overlapping carbon), 177.0; IR (neat) 3339 (br), 2957 (s), 1695 (br and s) cm^{-1}; HRMS m/z calcd for $C_{23}H_{37}N_2O_6$ (M + H) 437.2651, found 437.2643; $[\alpha]^{25}_D$ +21.99° (c = 4.30, $CHCl_3$); Anal. Calcd for $C_{23}H_{36}N_2O_6$: C, 63.28; H, 8.31; N, 6.42. Found: C, 63.45; H, 8.03; N, 6.09.

Pentafluorophenyl *N*-Boc-*N*-[4-[*N*-(benzyloxycarbonyl)amino]butyl]-D-leucinate (12). To the solution of **11** (0.45 g, 1.28 mmol) in CH_2Cl_2 (3 mL) were added DCC (0.32 g, 1.53 mmol) and pentafluorophenol (0.24 g, 1.53 mmol). The reaction was stirred at room temperature for 12 h. After this time, the reaction mixture was diluted and the solid material collected by filtration. The solid was washed with ether, and the organic layer was washed with 5% HCl, 5% $NaHCO_3$, and saturated NaCl solutions. The ether layer was dried (Na_2SO_4), filtered, and concentrated. The resulting crude oil was purified by column chromatography, eluting with EtOAc/petroleum ether (20:80). Pure compound **12** (0.67 g, 67%) was obtained as an oil: R_f 0.60 (20:80 EtOAc:petroleum ether); ^{1}H NMR ($CDCl_3$) δ 0.92−0.99 (m, 6H), 1.46 (s, 9H), 1.50−2.00 (m, 7H), 3.15−3.25 (m, 2H), 3.71−3.78 (m, 2H), 4.50−4.80 (m, 1H), 4.90 (m, 1H), 5.10 (s, 2H), 7.25−7.40 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 22.0, 23.1, 24.9, 25.9, 26.17, 27.3, 28.3, 40.6, 46.1, 66.6, 80.5, 125.5, 127.9, 128.0, 128.3, 134.9, 136.8, 136.8, 138.3, 140.0, 142.2, 154.5 (overlapping carbon), 154.7, 156.5, 169.1; IR (neat) 3336 (m), 2960 (m), 1789 (s), 1697 (s) cm^{-1}; HRMS m/z calcd for $C_{29}H_{39}N_3O_6F_5$ (M+NH_4) 620.2759, found 620.2752; $[\alpha]^{25}_D$ +21.20° (c = 1.37, $CHCl_3$).

(4*R*)-4-[[4-[(Benzyloxycarbonyl)amino]butyl](*tert*-butoxycarbonyl)amino]-6-methyl-3-oxoheptanoic Acid (1*S*,2*S*,3*R*)-4-[(*tert*-Butyldimethylsilyl)oxy]-1-isopropyl-2-(methoxymethoxy)-3-methylbutyl Ester (13). To the solution of HIP-Ac (**5**, 6.50 g, 0.018 mol) in dry THF (50 mL) at −78 °C was added LDA (90 mL, 0.018 mol). The solution was stirred at −78 °C for 1 h. The solution containing the enolate of HIP-Ac was then added dropwise, with vigorous stirring, to the solution containing the pentafluorophenyl ester **12** (2.32 g, 4.48 mmol). The reaction mixture was stirred for 4 h at −78 °C. After this time, saturated ammonium chloride (100 mL) and ether (500 mL) were added. The aqueous layer was separated and extracted with ether (2 × 100 mL), and the combined ether layers were washed with 5% HCl, 5% $NaHCO_3$, and saturated NaCl solutions. The ether layer was dried (Na_2SO_4), filtered, and concentrated. The resulting crude oil was purified by column chromatography, eluting with EtOAc/petroleum ether (20:80). Pure compound **13** (2.21 g, 71%) was obtained as an oil: R_f 0.58 (20:80 EtOAc:petroleum ether); ^{1}H NMR ($CDCl_3$) δ 0.02 (s, 6H), 0.74−0.98 (m, 21H), 1.21−1.62 (m, 18H), 1.74−1.88 (m, 3H), 2.92−3.26 (m, 4H), 3.29 (s, 3H), 3.63−3.67 and 3.30−3.48 (m, 4H), 3.78 (d, J = 5.9 Hz, 1H), 4.58−4.61 (m, 3H), 4.89−5.21 (m, 4H), 7.26−7.34 (m, 5H); ^{13}C NMR ($CDCl_3$) δ −5.49 (3 overlapping carbons), 10.5, 15.8, 18.1, 19.8, 24.8, 28.3 (3 overlapping carbons), 28.8 (3 overlapping carbons), 37.4, 37.5, 37.7, 40.4, 45.05, 46.1, 55.9, 62.4, 64.5 66.6, 78.9, 79.7, 81.3, 98.9, 128.0, 128.5, 128.6, 136.6 (3 overlapping carbons), 156.4, 157.0, 167.3, 200.8; IR (neat) 3343 (br), 2958 (s), 2933 (s), 1710 (s), 1696 (s), 1643 (w) cm^{-1}; HRMS m/z calcd for $C_{41}H_{72}N_2O_{10}Si$ (M + Na) 803.4854, found 803.4869; $[\alpha]^{25}_D$ +19.97° (c = 1.89, $CHCl_3$).

(4*R*)-4-[[4-[(Benzyloxycarbonyl)amino]butyl](*tert*-butoxycarbonyl)amino]-3-hydroxy-6-methylheptanoic Acid (1*S*,2*S*,3*R*)-4-[(*tert*-Butyldimethylsilyl)oxy]-1-isopropyl-2-(methoxymethoxy)-3-methylbutyl Ester (14). Potas-

sium borohydride (0.58 g, 0.011 mol) was added in portions to β-keto ester **13** (1.66 g, 2.39 mmol) in absolute ethanol (20 mL) and at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and then at ambient temperature for 24 h. A 1 N HOAc solution was added dropwise until the aqueous layer was neutral to litmus. The resulting solution was concentrated, dissolved in ether, and washed with 5% HCl, 5% $NaHCO_3$, and saturated NaCl solutions. The ether layer was dried (Na_2SO_4), filtered, and concentrated. The resulting crude oil was purified by column chromatography eluting with EtOAc/petroleum ether (20:80). Pure compound **14** (1.46 g, 78%) was obtained as an oil: R_f 0.49 (20:80 EtOAc:petroleum ether); ^{1}H NMR ($CDCl_3$) δ 0.05 (s, 6H), 0.89−0.92 (m, 24H), 1.45 (s, 9H), 1.16−1.76 (m, 9H), 2.50−2.55 (m, 2H), 3.08−3.17 (m, 5H), 3.34 (s, 3H), 3.39−3.82 (m, 3H), 3.96−4.00 (brs, 1H), 4.20−4.31 (m, 1H), 4.59−4.64 (m, 2H), 5.04−5.08 (m, 4H), 7.26−7.34 (m, 5H); ^{13}C NMR ($CDCl_3$) δ −5.5 (3 overlapping carbons), 10.4, 15.8, 18.1, 19.9, 20.1, 20.4, 20.6, 20.9, 22.7, 25.8, 28.4 (3 overlapping carbons), 28.7 (3 overlapping carbons), 37.5, 37.5, 39.1, 39.5, 40.4, 42.3, 42.5, 46.1, 48.1, 56.0, 64.9, 66.5, 78.6, 78.8, 78.9, 79.3, 99.0, 127.9, 128.4, 128.4, 136.7 (2 overlapping carbons) 155.1, 156.4, 170.8; IR ($CHCl_3$) 3344 (br), 2958 (s), 2932 (s), 1693 (br and s), 1534 (m), 1516 (s) cm^{-1}; HRMS *m/z* calcd for $C_{41}H_{74}N_2O_{10}Si$ (M + Na) 805.5011, found 805.5003. $[\alpha]^{25}_D$ +17.68° (*c* = 6.55, $CHCl_3$).

(3*S*,4*R*)-4-[[4-[(Benzyloxycarbonyl)amino]butyl](*tert*-butoxycarbonyl)amino]-6-methyl-3-[(triisopropylsilyl)-oxy]heptanoic Acid (1*S*,2*S*,3*R*)-4-[(*tert*-Butyldimethylsilyl)oxy]-1-isopropyl-2-(methoxymethoxy)-3-methylbutyl Ester (15). To alcohol **14** (0.69 g, 0.88 mmol), in CH_2Cl_2 (5 mL) and at 0 °C, was added 2,6-lutidine (0.31 mL, 2.66 mmol). To the resulting solution was added dropwise triisopropylsilyl triflate (0.36 mL, 1.34 mmol). The reaction mixture was stirred at 0 °C for 6 h. After the reaction was complete, it was diluted with 20 mL of ether. The organic layer was washed with 5% HCl, 5% $NaHCO_3$, and saturated NaCl solutions. The ether layer was dried (Na_2SO_4), filtered, and concentrated. The resulting crude oil was purified by column chromatography eluting with EtOAc/petroleum ether (5:95). Pure compound **15** (1.46 g, 78%) was obtained as an oil: R_f 0.25 (20:80 EtOAc:petroleum ether); ^{1}H NMR ($CDCl_3$) δ 0.04 (s, 6H), 0.89−1.08 (m, 47H), 1.41 (s, 9H), 1.41−1.95 (m, 7H), 2.42−2.50 and 2.70−2.85 (m, 2H), 3.05−3.50 (m, 7H), 3.34 (s, 3H), 3.79 (m, 1H), 4.27−4.35 (m, 1H), 4.57−4.64 (m, 3H), 4.91−4.97 (m, 1H), 5.09 (s, 2H), 7.26−7.35 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 12.7 (9 overlapping carbons), 16.3, 17.7, 18.2, 18.3, 19.7, 20.1, 20.4, 20.6, 20.9, 22.1, 28.4 (3 overlapping carbons), 28.7 (3 overlapping carbons), 37.6, 39.1, 40.9, 41.0, 43.0, 55.9, 56.0, 65.2, 66.6, 72.6, 78.7, 78.8, 79.9, 98.4, 127.9, 128.0, 128.4, 136.8 (2 overlapping carbon) 156.3 (overlapping carbon), 170.8; IR (neat) 3356 (w), 2955 (s), 2867 (m), 1731 (m), 1691 (s), 1591 (s) cm^{-1}; HRMS *m/z* calcd for $C_{50}H_{94}N_2O_{10}Si_2$ (M + Na) 961.6345, found 961.6301; $[\alpha]^{25}_D$ −8.19° (*c* = 2.02, $CHCl_3$).

(3*S*,4*R*)-4-[[4-[(Benzyloxycarbonyl)amino]butyl](*tert*-butoxycarbonyl)amino]-6-methyl-3-[(triisopropylsilyl)-oxy]heptanoic Acid (1*S*,2*S*,3*R*)-4-Hydroxy-1-isopropyl-2-(methoxymethoxy)-3-methylbutyl Ester (16). To compound **15** (0.15 g, 0.16 mmol) in THF (2 mL) was added HOAc/H_2O (3:1, 8 mL). After 16 h, the reaction was diluted with toluene (40 mL) and concentrated until no HOAc remained. The crude oil was then purified by column chromatography eluting with EtOAc/petroleum ether (20:80). Pure compound **16** (0.12 g, 89%) was obtained as an oil: R_f 0.22 (20:80 EtOAc:petroleum ether); ^{1}H NMR ($CDCl_3$) δ 0.83−0.99 (m, 15H), 1.09 (s, 21H), 1.42 (s, 9H), 1.43−1.89 (m, 9H), 2.41−2.53 and 2.83−2.88 (m, 2H), 3.06−3.56 (m, 8H), 3.40 (s, 3H), 3.37−3.84 (m, 1H), 4.21−4.40 (m, 2H), 4.55 (d, *J* = 6.0 Hz, 1H) and 4.68−4.72 (m, 2H), 5.07−5.09 (m, 2H), 7.31-7.35 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 10.3, 12.5 (9 overlapping carbons), 12.7, 15.9, 18.3, 19.8, 20.2, 20.3, 20.9, 22.3, 28.6 (3 overlapping carbons), 35.4, 39.2, 39.4, 40.8, 40.9, 42.8, 54.0, 56.2, 64.5, 66.6, 72.6, 78.6, 79.1, 98.6, 127.9, 128.1, 128.1, 128.3, 128.5, 136.0, 156.2, 156.4, 170.4; IR ($CHCl_3$) 3354 (br), 2946 (s), 2868 (s), 1729 (s), 1691 (s) cm^{-1}; HRMS *m/z* calcd for $C_{44}H_{80}N_2O_{10}Si$ (M + Na) 847.5480, found 847.5461; $[\alpha]^{25}_D$ −16.51° (*c* = 1.89, $CHCl_3$).

(3*S*,4*R*)-4-[[4-[(Benzyloxycarbonyl)amino]butyl](*tert*-butoxycarbonyl)amino]-6-methyl-3-[(triisopropylsilyl)-oxy]heptanoic Acid (1*S*,2*S*,3*R*)-3-Carboxy-1-isopropyl-2-(methoxymethoxy)butyl Ester (17). To a solution of the alcohol **16** (27 mg, 0.033 mmol) in CH_2Cl_2 (1.0 mL) was added 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one (Dess−Martin periodinane reagent, 19 mg, 0.05 mmol). The reaction was stirred for 0.5 h and diluted with ether (5.5 mL). This slurry was poured into saturated aqueous $NaHCO_3$ (2.1 mL) containing $Na_2S_2O_3{\cdot}5H_2O$ (86.38 mg). After the slurry was stirred for 5 min, an additional amount of Et_2O (5 mL) was added. The combined organic layers were washed with saturated aqueous $NaHCO_3$ (2.1 mL) and H_2O (2.1 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was dissolved in *tert*-BuOH (1.91 mL), keeping the temperature at 25 °C. To this solution was added 5% aqueous NaH_2PO_4 (1.26 mL) followed by 1 M aqueous $KMnO_4$ (1.91 mL) dropwise. The reaction was stirred at 25 °C for 1 h at which time Et_2O (66 mL) was added. After the solution was cooled to 0 °C, saturated aqueous Na_2SO_3 (30 drops) was added with efficient stirring. A 10% aqueous HCl solution was added until the pH of the aqueous layer reached exactly 3 (use caution not to go below). The aqueous layer was extracted with EtOAc (30 mL), and the combined organic layers were dried (Na_2SO_4), filtered, and concentrated to obtain the product **17** (27 mg) in 99% yield: R_f 0.71 (12:4:1 $CHCl_3$:MeOH:NH_3); ^{1}H NMR ($CDCl_3$) δ 0.83−1.12 (m, 34H), 1.23−1.82 (m, 16H), 1.98−2.05 (m, 1H), 2.53−2.74 (m, 2H), 2.81−2.88 (m, 1H), 3.03−3.40 (m, 8H), 3.39 (s, 3H), 3.95−3.97 (m, 1H), 4.25−4.41 (m, 2H), 4.65−4.78 (m, 3H), 5.09 (s, 2H), 7.33−7.36 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 12.6 (9 overlapping carbons), 17.7, 18.3, 19.1, 22.1, 28.4 (3 overlapping carbons), 28.8, 28.7, 29.7, 39.6, 40.4, 40.8, 41.1, 43.1 56.1, 59.7, 66.6, 72.2, 72.6, 73.1, 78.5, 79.6, 98.3, 128.1, 128.3, 128.4, 128.5, 129.9, 130.1, 156.3, 156.8, 169.6, 176.5; IR (neat) 3400 (br), 2946 (s), 1733 (s), 1690 (s) cm^{-1}; HRMS *m/z* calcd for $C_{44}H_{78}N_2O_{11}$ (M + Na) 861.1967, found 861.1957; $[\alpha]^{25}_D$ +3.31° (*c* = 0.83, $CHCl_3$).

5(*R*)-Isobutyl-15(*S*)-isopropyl-14(*S*)-(methoxymethoxy)-13(*S*)-methyl-2,12-dioxo-4(*S*)-[(triisopropylsilyl)oxy]-1-oxa-6,11-diazacyclopentadecane-6-carboxylic Acid *tert*-Butyl Ester (18). Acid **17** (30 mg, 0.036 mmol) was dissolved in CH_2Cl_2 (2 mL). To this solution were added DCC (9 mg, 0.043 mmol), pentafluorophenol (8 mg, 0.043 mmol), and DMAP (2 mg, 0.016 mmol) sequentially. The reaction was stirred at room temperature for 12 h. The reaction mixture was diluted with ether (5 mL) and filtered. The solid was washed with an additional 5 mL of ether, and the organic layer was washed with 5% HCl, 5% $NaHCO_3$, and saturated NaCl solutions. The ether layer was dried (Na_2SO_4), filtered, and concentrated. The resulting crude oil was purified by column chromatography eluting with EtOAc/petroleum ether (20:80). The corresponding pure pentafluorophenyl ester (0.025 g, 68%) was used in the next step. To a mixture of freshly distilled dioxane (22 mL) containing 10% palladium on carbon (8.4 mg), absolute ethanol (0.52 mL), and 4-pyrrolidinopyridine (9 mg, 0.065 mmol) at 95 °C was added over a period of 1.5 h a solution of the activated ester (22 mg, 0.022 mmol) and cyclohexene (1.97 mL) in dioxane (4 mL). After the addition, the reaction was allowed to stir for 48 h, at which time it was cooled to 25 °C and filtered through a bed of Celite. The filtrate was concentrated under reduced pressure to afford a crude oil. This oil was purified by column chromatography eluting with EtOAc/petroleum ether (50:50) to provide pure macrocycle **18** (8.6 mg, 58%): R_f 0.57 (50% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 0.91−1.23 (m, 36H), 1.37−1.38 (m, 2H), 1.46 (s, 9H), 1.57−1.94 (m, 5H), 2.06−2.10 (m, 1H), 2.31−2.33 and 2.45−2.48 (m, 2H), 2.63−2.64 and 2.82−2.84 (m, 2H), 2.84−2.86 (m, 1H), 3.16−3.20 and 3.40−3.43 (m, 2H), 3.45 (s, 3H), 3.78−3.80 (m, 1H), 3.90 (d, *J* = 6.5 Hz, 1H), 4.23 (dd, *J* = 8.8, 10.8 Hz, 1H), 4.76−4.78 (m, 3H) and 6.03 (brs, 1H); ^{13}C NMR ($CDCl_3$) δ 13.1 (9 overlapping carbons), 14.2, 17.6, 18.3, 18.3, 19.9, 19.2, 21.8, 23.3, 23.9, 24.7, 27.1, 28.3 (3 overlapping carbons), 28.8, 33.5, 36.7, 40.8, 41.4, 42.3, 56.4, 59.1, 73.9, 75.7, 80.3, 98.1, 155.8, 170.9, 173.3; IR ($CHCl_3$) 3282 (br), 2943 (s), 2868 (s), 2359 (s), 1739 (s), 1693 (s), 1633 (s), 1157 (s), 1027 (s) cm^{-1}; HRMS *m/z* calcd for $C_{36}H_{70}N_2O_8Si$ (M + Na) 709.4803, found 709.4861; $[\alpha]^{25}_D$ −16.48° (*c* = 1.07, $CHCl_3$). Anal. Calcd

for $C_{36}H_{70}N_2O_8Si$: C, 62.94; H, 10.27; N, 4.08. Found: C, 62.97; H, 9.95; N, 4.03.

14(*S*)-Hydroxy-5(*R*)-isobutyl-15(*S*)-isopropyl-14-(methoxymethoxy)-13(*S*)-methyl-2,12-dioxo-4(*S*)-[(triisopropylsilyl)oxy]-1-oxa-6,11-diazacyclopentadecane-6-carboxylic Acid *tert*-Butyl Ester (19). To a cold (n-78 °C), stirred solution of the MOM ether **18** (35 mg, 0.05 mmol) in dry CH_2Cl_2 (1 mL) was added dropwise a solution of dimethylboron bromide (1.40 M, 0.11 mL) in CH_2Cl_2. After 1 h at −78 °C, a mixture of THF/$NaHCO_3$ (2:1) was added dropwise into a vigorously stirring reaction mixture. After 5 min, the mixture was diluted with ether (5 mL), and the organic layer was separated and washed successively with water, 10% aqueous sodium bisulfate, and saturated NaCl solutions. The aqueous layers were reextracted with ether (5 mL), and the organic layers were combined, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography eluting with EtOAc/petroleum ether (50:50). Pure compound **19** (0.023 g, 77%) was obtained as an oil: R_f 0.55 (50% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 0.84−1.21 (m, 34H), 1.31−1.39 (m, 2H), 1.39 (s, 9H), 1.44−1.97 (m, 5H), 2.07−2.09 (m, 1H), 2.37−2.44 (m, 2H), 2.74−2.91 (m, 3H), 3.10−3.16 (m, 2H), 3.78−3.80 (m, 1H), 3.89 (d, J = 5.6 Hz, 1H), 4.04−4.15 (m, 1H), 4.66 (d, J = 6.9 Hz, 1H), 5.76 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 13.1 (9 overlapping carbons), 13.3, 13.6, 17.7, 18.3, 18.4, 18.7, 19.8, 21.7, 23.9, 24.3, 24.8, 26.7, 28.4, 29.4, 33.7, 36.8, 40.3 (overlapping carbon), 42.2, 43.9, 59.3, 61.0, 72.4, 73.2, 80.3, 155.8, 171.2 (overlapping carbon); IR (neat) 3318 (br), 2960 (m), 2868 (m), 1739 (s), 1692 (s), 1643 (s) cm^{-1}; HRMS m/z calcd for $C_{34}H_{66}N_2O_7Si$ (M + H) 643.4717, found 643.4738; $[\alpha]^{25}_D$ −0.13° (c = 4.64, $CHCl_3$). Anal. Calcd for $C_{34}H_{65}N_2O_7Si$: C, 63.61; H, 10.21; N, 4.36. Found: C, 63.45; H, 10.25; N, 4.39.

5(*R*)-Isobutyl-15(*S*)-isopropyl-14-(methoxymethoxy)-13(*S*)-methyl-2,12,14-trioxo-4(*S*)-[(triisopropylsilyl)oxy]-1-oxa-6,11-diazacyclopentadecane-6-carboxylic Acid *tert*-Butyl Ester (20). To a mixture of the 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one (Dess−Martin periodinane reagent, 0.021 g, 0.048 mmol) and CH_2Cl_2 (1 mL) was added the alcohol **19** (0.023 g, 0.036 mmol) in CH_2Cl_2 (1 mL). The reaction was stirred for 1 h and diluted with ether (5 mL). This slurry was poured into saturated aqueous $NaHCO_3$ (2.5 mL) containing $Na_2S_2O_3{\cdot}5H_2O$ (90 mg). After the mixture was stirred for 5 min, an additional amount of Et_2O (5 mL) was added. The combined organic layers were washed with saturated aqueous $NaHCO_3$ (2.5 mL) and H_2O (2.5 mL), dried (Na_2SO_4), filtered, and concentrated. The crude oil was purified by column chromatography eluting with EtOAc/petroleum ether (40:60). Pure compound **20** (0.023 g, 99%) was obtained as an oil. R_f 0.36 (40% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$) δ 0.85−1.30 (m, 35H), 1.30−1.33 (m, 2H), 1.46 (s, 9H), 1.60−1.82 (m, 5H), 2.05−2.11 (m, 1H), 2.50−2.92 (m, 5H), 3.12−3.20 and 3.41−3.55 (m, 2H), 3.63−3.75 (m, 1H), 4.20−4.21 (m, 1H), 5.08−5.10 (m, 1H), 5.48 (brs, 1H); ^{13}C NMR ($CDCl_3$) δ 12.2, 12.8 (3 overlapping carbons), 18.0, 18.2, 18.3, 21.8, 23.9, 24.7, 27.15, 25.1 28.4 (3 overlapping carbons), 35.0, 37.9, 38.4, 40.0, 42.0, 51.8, 59.5, 72.7, 81.1, 156.4, 168.7 and 170.1, 204.1; IR (neat) 3302 (br), 2944 (s), 2868 (s), 2360 (m), 1751 (s), 1693 (s), 1638 (s) cm^{-1}; HRMS m/z calcd for $C_{34}H_{64}N_2O_7Si$ (M + H) 641.4561, found 641.4550; $[\alpha]^{25}_D$ −23.58° (c = 1.65, $CHCl_3$).

4(*S*)-Hydroxy-5(*R*)-isobutyl-15(*S*)-isopropyl-13(*S*)-methyl-1-oxa-6,11-diazacyclopentadecane-2,12,14-trione (21). A solution of ketone **20** (0.023 g, 0.036 mmol) in 3.0 mL of EtOAc was cooled to −30 °C. Gaseous HCl was then introduced at such a rate that the temperature of the reaction mixture was maintained between −10 and −20 °C at saturation. The solution was kept for 2 h at this temperature and then kept at 0 °C for 4 h. The solution was then purged with nitrogen (g) for about 30 min, maintaining the temperature at 0 °C. After the solution was concentrated, the residue was triturated and washed by decantation with three 3-mL portions of *tert*-butyl methyl ether/hexane (1:4). The product was collected by filtration and dried. Compound **20** (0.015 g, 90%) was obtained as a white solid: R_f 0.30 (15:85 MeOH:chloroform); ^{1}H NMR ($CDCl_3$) δ 0.84−1.19 (m, 12H), 1.27 (s, 3H), 1.32−1.92 (m, 8H), 2.19−2.29 (m, 1H), 2.78−3.28 (m, 8H), 3.78 (m, 1H), 4.15 (m, 1H), 5.18 (m, 1H), 6.69 (s, 1H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 14.2, 16.6, 17.6, 17.6, 19.5, 21.9, 22.9, 24.8, 25.8, 28.3, 38.8, 39.4, 39.5, 46.3, 51.6 and 58.9 (RI), 66.1, 84.3 and 84.4 (RI), 168.2, 172.8, 205.5; IR (neat) 3251 (br), 2962 (s), 2359 (m), 1723 (s), 1652 (s) cm^{-1}; HRMS m/z calcd for $C_{20}H_{36}N_2O_5$ (M + H) 385.2702, found 385.2715.

Z-*N*,*O*-dimethyltyrosine Methyl Ester (22).[30] To *N*-Z-L-tyrosine (1.00 g, 0.32 mmol) at ambient temperature was added THF (16 mL). Finely powdered KOH (1.77 g, 0.032 mol) was then added in portions, followed by the addition of tetrabutylammonium hydrogen sulfate (0.10 g, 10% by weight). Rapid stirring was initiated, and dimethyl sulfate (1.8 mL, 0.019 mol) was added dropwise over a period of 15 min. After 1 h, the solid material was collected by filtration and washed with ethyl acetate. The filtrate was washed with 10% HCl, 5% $NaHCO_3$, and saturated NaCl solutions. The ethyl acetate layer was dried (Na_2SO_4), filtered, and concentrated. The crude oil was purified by column chromatography eluting with EtOAc/petroleum ether (15:85). The ester (**22**, 0.97 g, 85% yield) was obtained as an oil: R_f 0.55 (30:70 EtOAc:petroleum ether); ^{1}H NMR ($CDCl_3$) δ 2.80 (d, J = 12.90 Hz, 3H), 2.91−3.01 (m, 1H), 3.10−3.17 (m, 1H), 3.71, 3.64 and 3.75 (s, 3H, RI), 3.92 (s, 3H), 4.72−5.08 (m, 3H), 6.76−7.32 (m, 9H); ^{13}C NMR ($CDCl_3$) δ 31.5, 32.0, 33.9 and 34.4 (RI), 52.1 and 55.1 (RI), 58.5, 60.7, 60.3, 67.3, 67.4, 113.9, 113.9, 136.4, 136.7, 127.4, 127.8, 127.9, 128.3, 128.9, 129.8, 136.4, 136.6, 155.9, 156.5 and 158.3 (RI), 171.5 and 171.2 (RI); IR (neat) 2400 (m), 1750 (s), 1710 (s), 1513 (s), 1247(m) cm^{-1}; HRMS m/z calcd for $C_{20}H_{24}NO_5$ (M + H) 358.1654, found 358.1640; $[\alpha]^{25}_D$ −51.00° (c = 0.59, $CHCl_3$).

***N*-Acetyl-*N*,*O*-dimethyl-L-tyrosine Methyl Ester (24).** To a CH_3OH/EtOAc solution (1:1, 15 mL) was added 10% Pd/C (0.34 g). To the resulting suspension was added Z-*N*,*O*-dimethyltyrosine methyl ester (**22**, 1.14 g, 3.16 mmol) in CH_3OH (2.00 mL). The solution was subjected to an atmosphere of hydrogen (40 psi) in a Parr apparatus and shaken for 3 h. The reaction mixture was filtered through Celite. The Celite was washed with CH_3OH, and the filtrate was concentrated. The resulting amine (1.00 g, 91% yield) was used directly in the next step.[36] The crude secondary amine was dissolved in methylene chloride and cooled to 0 °C. To this solution was added triethylamine (0.62 mL, 4.70 mmol) followed by acetic anhydride (0.47 mL, 4.93 mmol) and the reaction stirred for 12 h. After the reaction was complete, it was diluted with ether and washed with 10% HCl, 5% $NaHCO_3$, and saturated NaCl solutions. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The resulting crude oil was purified by column chromatography eluting with acetone/hexane (30:70). Compound **24** (1.04 g, 87% yield) was obtained as an oil: R_f 0.30 (30:70 acetone:hexane); ^{1}H NMR ($CDCl_3$) δ 1.95 (s, 3H), 2.78 and 2.84 (s, 3H, RI), 2.92−2.95 and 3.22−3.26 (m, 2H), 3.67 (s, 3H), 3.73 (s, 3H), 4.49−4.52 and 5.10−5.20 (m, 1H), 6.70 and 7.05 ([AX]$_2$, J = 8.2 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 21.0 and 21.6 (RI), 34.3 (33.8 and 33.5, (RI), 52.1 and 52.4 (RI), 55.1, 58.0, 62.7, 113.8, 114.2, 128.9, 129.6, 129.8, 158.3, 171.2, 171.4; IR (neat) 3000 (w), 2359 (s), 1739 (s), 1651 (s), 1513 (s) cm^{-1}; HRMS m/z calcd for $C_{14}H_{20}NO_4$ (M + H) 266.1392, found 266.1403; $[\alpha]^{25}_D$ −63.53° (c = 0.40, $CHCl_3$).

***N*-Acetyl-*N*,*O*-dimethyl-L-tyrosine (25).** *N*-Acetyl-*N*,*O*-dimethyl-L-tyrosine methyl ester (**24**, 0.24 g, 0.902 mmol) was dissolved in 20 mL of THF. The reaction mixture was cooled to 0 °C, and 20 mL of 0.2 M LiOH was added. The reaction was stirred for 6 h, concentrated to 20 mL, and washed with ether (2 × 20 mL). The combined organic layers were extracted with saturated $NaHCO_3$ solution. The aqueous layers were combined and acidified to pH 1 with 2 N $KHSO_4$ solution. The acidified aqueous layer was extracted with ethyl acetate (3 × 25 mL). The organic layers were dried (Na_2SO_4), filtered, and concentrated. The acid (**25**, 0.213 g, 94% yield) was obtained as a white foam: R_f 0.11 (40:60 acetone:hexane); ^{1}H NMR ($CDCl_3$) δ 1.71 and 1.95 (s, 3H, RI), 2.75 and 2.85 (s, 3H, RI), 2.96−3.01 and 3.23−3.26 (m, 2H), 3.71 (s, 3H), 4.40−4.49 and 5.04−5.07 (m, 1H), 6.74−6.77 and 7.00−7.04 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 21.6, 33.6 and 34.2 and 34.5 (RI), 55.2, 59.6, 62.1, 114.3, 114.0, 128.6, 129.7, 129.8, 158.4, 172.6, 174.0;

IR (neat) 2957 (br), 1731 (s), 1595 (s), 1513 s), 1249 (s) cm^{-1}; HRMS *m/z* calcd for $C_{13}H_{18}NO_4$ (M + H) 252.1236, found 252.1229; $[\alpha]^{25}_D$ −56.64 (*c* = 0.52, $CHCl_3$). Anal. Calcd for $C_{13}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.57. Found: C, 62.02; H, 6.71; N, 5.91.

***N*-Acetyl-*N*,*O*-dimethyl-L-tyrosine-*O*-Boc-L-threonine Benzyl Ester (26).** To *N*-acetyl-*N*,*O*-dimethyltyrosine (**25**, 0.41 g, 0.16 mmol), in CH_2Cl_2 (5 mL) and at 0 °C, was added Boc-threonine benzyl ester (0.50 g, 1.62 mmol). To the resulting solution were added triethylamine (0.48 mL, 0.36 mmol), DMAP (0.04 g, 0.32 mmol), and isopropenyl chloroformate (0.19 mL, 0.18 mmol). The reaction was stirred at 0 °C for 1 h and diluted with ether (50 mL), and the organic layer was washed with 5% HCl, 5% $NaHCO_3$, and saturated NaCl solutions. The ether layer was dried (Na_2SO_4), filtered, and concentrated. The resulting crude oil was purified by column chromatography eluting with EtOAc/petroleum ether (40:60). Compound **26** (0.72 g, 82%) was obtained as an oil: R_f 0.71 (40:60 acetone:hexane); ^{1}H NMR ($CDCl_3$) δ 1.28 (d, *J* = 6.35 Hz, 3H), 1.45 (s, 9H), 1.99 (s, 3H), 2.64 (s, 3H), 2.92 (t, *J* = 10.4 Hz, 1H) and 3.15 and 3.18 (dd, *J* = 5.6, 5.7 Hz, 1H), 3.78 (s, 3H), 4.40 (d, *J* = 9.3 Hz, 1H), 4.86−4.87 (m, 1H), 5.12−5.16 (m, 3H), 5.40−5.41 (m, 1H), 6.81 and 7.60 ($[AX]_2$, *J* = 8.6 Hz, 4H), 7.33−7.46 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 16.4, 20.9, 30.1 (3 overlapping carbons), 33.8, 34.3, 55.7, 57.8, 59.5, 67.0, 71.2, 80.1, 113.9, 114.3, 127.2, 127.8, 128.9, 128.9, 129.1, 129.1, 129.9, 131.1, 137.9, 156.1, 158.1, 169.9, 170.3, 171.6; IR ($CHCl_3$) 3400 (br), 2950 (s), 1745 (s), 1650 (br and s), 1500 (s) cm^{-1}; HRMS *m/z* calcd for $C_{29}H_{38}N_2O_8$ (M + Na) 565.2526, found 565.2541; $[\alpha]^{25}_D$ −29.60° (*c* = 0.38, $CHCl_3$).

***N*-Acetyl-*N*,*O*-dimethyl-L-tyrosine-*O*-Boc-L-threonine (4).** To a CH_3OH/EtOAc solution (1:1, 2 mL) was added 10% Pd/C (0.014 g). To the resulting suspension was added *N*-acetyl-*N*,*O*-dimethyl-L-tyrosine-*O*-Boc-L-threonine-OBn (**26**, 0.048 g, 0.065 mmol) in CH_3OH (1.00 mL). The solution was subjected to an atmosphere of hydrogen (40 psi) and stirred for 3 h in a Parr apparatus. The reaction mixture was filtered through Celite. The Celite was washed with CH_3OH, and the filtrate was concentrated. The resulting acid **4** (0.038 g, 91% yield) was used directly in the next step: R_f 0.13 (40:60 acetone:hexane); ^{1}H NMR ($CDCl_3$) δ 1.29 (d, *J* = 5.9 Hz, 3H), 1.49 (s, 9H), 2.01 (s, 3H), 2.77 (s, 3H), 2.96−3.01 and 3.20−3.22 (m, 2H), 3.75 (s, 3H), 4.44 (m, 1H), 4.84 (m, 1H), 5.22 (m, 1H), 5.41 (s, 1H), 6.92 and 7.06 ($[AX]_2$, 8.2, 4H); ^{13}C NMR ($CDCl_3$) δ 16.4, 21.2, 28.2 (3 overlapping carbons), 33.3, 35.2, 55.1, 60.3, 62.9, 72.44, 80.1, 113.9, 114.3, 128.9, 129.8, 155.9 (overlapping carbon), 158.4, 169.2, 172.9; IR ($CHCl_3$) 3350 (br), 2979 (m), 1740 (s), 1715 (s), 1610 (m), 1513 (s), 1165 (s) cm^{-1}; HRMS *m/z* calcd for $C_{22}H_{32}N_2O_8$ (M + Na) 475.2057, found 475.2035; $[\alpha]^{25}_D$ −10.57° (*c* = 1.94, $CHCl_3$).

2-(Acetylmethylamino)-3-(4-methoxyphenyl)propionic Acid 2-[(*tert*-butoxycarbonyl)amino]-3-(4-hydroxy-5-isobutyl-15-isopropyl-13-methyl-2,12,14-trioxo-1-oxa-6,-11-diazacyclopentadec-6-yl)-1-methyl-3-oxopropyl Ester (27). To a solution of acid **4** (0.01 g, 0.02 mol) and amine **21** (0.009 g, 0.02 mmol), in CH_2Cl_2 (1.0 mL) and at 0 °C, were added BOP (0.0122 g, 0.027 mmol) and NMM (0.011 mL, 0.102 mmol). After 30 min, the solution was brought to room temperature and stirred for 6 h. After this time, the reaction mixture was treated with 3 mL of saturated NaCl solution and then extracted with 5 mL of Et_2O. The organic layers were combined and washed successively with 5% HCl, saturated NaCl, 5% $NaHCO_3$, and saturated NaCl solutions. The organic layer was dried ($NaSO_4$), filtered, and concentrated. The resulting crude oil was purified by column chromatography eluting with MeOH/chloroform (10:90) to afford 0.011 g (67%) of pure compound **27**: R_f 0.5 (90:10 chloroform:MeOH); ^{1}H NMR ($CDCl_3$) 0.87−1.34 (m, 18H), 1.46 (s, 9H), 1.61−1.91 (m, 7H), 1.99 and 2.00 (s, 3H, RI), 2.19−2.24 (m, 1H), 2.77 (s, 3H), 2.94−3.23 (m, 6H), 3.15−3.32(m, 4H), 3.77 (s, 3H), 4.61−4.90 (m, 4H), 5.10−5.53 (m, 4H), 6.81−6.83 and 7.25−7.26 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 16.2, 16.7, 17.2, 17.5, 17.6, 19.3, 20.3, 21.8, 22.0, 24.8, 25.1, 26.2, 28.2 (3 overlapping carbons), 29.7, 33.8, 34.5, 37.3, 38.9, 39.9, 46.2, 52.2, 57.7, 59.0, 66.4, 71.5, 80.3, 83.0, 113.9, 128.2, 129.0, 129.8, 137.8, 155.9, 158.4, 169.3, 169.4, 169.6, 171.1, 171.8, 205.5; IR (neat) 3310 (br), 2935 (s), 1735 (s), 1717 (s), 1652 (s), 1513 (s), 1173 (s) cm^{-1}; HRMS calcd for $C_{42}H_{66}O_{12}N_4$ (M + Na) *m/z* 841.4779, found 841.4795; $[\alpha]^{25}_D$ −1.62° (*c* = 1.79, $CHCl_3$).

3(*S*)-[[2(*S*)-(Acetylmethylamino)-3-(4-methoxyphenyl)-propionyl]oxy]-2(*S*)-(2-[[[1-(2(*S*)-hydroxypropionyl)pyrrolidine-2(*S*)-carbonyl]methyl]amino]-4(*S*)-methylpentanoylamino)butyric Acid 4(*S*)-Hydroxy-5(*R*)-isobutyl-15(*S*)-isopropyl-13(*S*)-methyl-1-oxa-6,11-diazacyclopentadecane-2,12,14-trione (2). Compound **27** (0.013 g, 15 μM) was dissolved in 1 mL of dry methylene chloride and cooled to 0 °C. To this solution was added trifluoroacetic acid (0.024 mL, 0.03 mM), and the reaction was stirred for 6 h at ambient temperature. Upon completion of the reaction, the solution was concentrated under reduced pressure, the resulting residue was azeotroped (3 × 2 mL) with toluene, and the resulting TFA salt was used in the next step without purification. To a solution of TFA salt (0.013 g, 15 μM) and didemnin B side chain (**6**, 0.005 g, 15 μM) in CH_2Cl_2 (1.0 mL) and at 0 °C were added BOP (8.5 mg, 19 μM) and NMM (8.0 μL, 7.16 μM). After 30 min, the solution was brought to room temperature and stirred for 6 h. After this time, the reaction mixture was diluted with 5 mL of Et_2O. The organic layer was washed successively with 5% HCl, 5% $NaHCO_3$, and saturated NaCl solutions. The organic layer was dried ($NaSO_4$), filtered, and concentrated. The resulting crude oil was purified by column chromatography eluting with MeOH/$CHCl_3$ (10:90) to afford 9.0 mg (53%) of pure analog **2**: R_f 0.61 (15:85 MeOH:$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.87−1.34 (m, 33H), 1.61−1.91 (m, 11H), 1.99 and 2.00 (s, 3H, RI), 2.19−2.24 (m, 1H), 2.95 and 2.82 (s, 6H), 2.94−3.23 (m, 6H), 3.15−3.32(m, 6H), 3.77 (s, 3H), 4.61−4.90 (m, 5H), 5.10−5.53 (m, 5H), 6.81−6.83 and 7.25−7.26 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 15.8, 19.5, 17.2, 21.1, 21.4, 21.6, 21.9, 22.3, 23.1, 23.3, 23.5, 23.7, 24.4, 24.8, 24.9, 25.1, 26.0, 28.3, 28.4, 28.4, 28.9, 29.7, 30.9, 36.0, 39.0, 39.3, 45.3, 46.9, 49.5, 55.3, 55.1, 56.9, 57.7, 61.5, 66.0, 70.5, 80.7, 83.9, 113.9, 114.1, 128.5, 129.5, 129.7, 158.3, 168.3, 168.9, 170.0, 171.1, 172.3, 172.8, 173.3, 205.1; IR (neat) 3323 (br), 2929 (s), 1738 (s), 1731 (s), 1659 (s), 1643 (s), 1632 (s), 1514 (s) cm^{-1}; HRMS calcd for $C_{52}H_{82}O_{14}N_6$ (M + Na) *m/z* 1037.5787, found 1037.5738; $[\alpha]^{25}_D$ +9.71° (*c* = 0.57, $CHCl_3$).

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Abbreviations: (1*H*-1,3-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP); *N*-(benzyloxycarbonyl)-5-norbornene-2,3-dicarboximide (BCN); *N*,*N*-bis(2-oxo-3-oxazolidinyl)phosphonic chloride (BOP-Cl); diethyl ether (Et_2O); diisopropylethylamine (DIEA); diphenyl phosphorazidate (DPPA); ethyl acetate (EtOAc); α-(α-hydroxyisovaleryl)propionyl (HIP); isopropenyl chloroformate (IPCF); *N*-methylmorpholine (NMM); pentafluorophenyl diphenylphosphinate (FDPP); tetrabutylammonium fluoride (TBAF); triisopropylsilyl triflate (TIPSOTf); 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one (Dess−Martin periodinane reagent); triethylamine (Et_3N); rotational isomers (RI).

Supporting Information Available: NMR data for obtained compounds (44 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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