Synthesis of New Didemnin B Analogs for Investigations of Structure/Biological Activity Relationships

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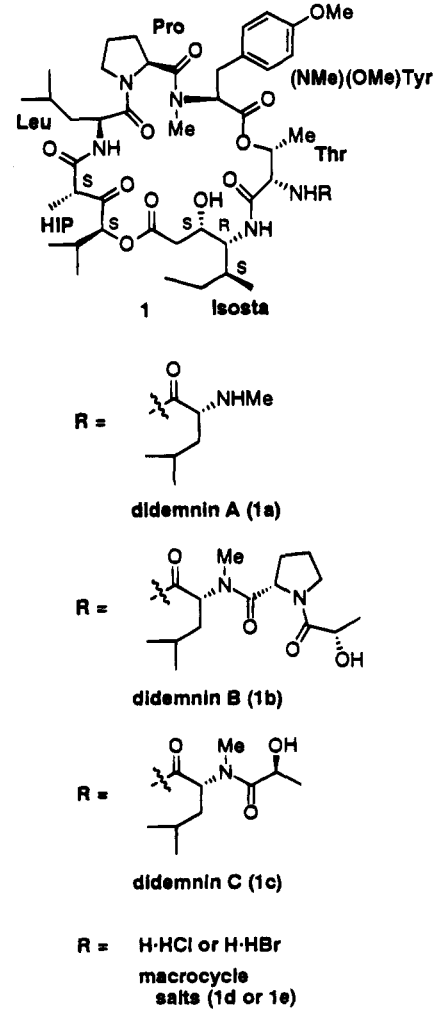
Modifications were introduced in the side chain of didemnin B to afford several analogs (**1f-1j**) for biological testing in order to identify the features responsible for the bioactivity of the natural products (**1a-1c**). To achieve our goal, two changes were made in the proline ring of the β-turn side chain. Initially, a hydroxyl group was incorporated at the C-4 position of the ring to increase the polar nature of the molecule. Secondly, unsaturation was introduced at C-3 and C-4 to increase the rigidity of the ring and to provide a site for tritiation to follow the drug pathway in biological systems. Improvements were also introduced in the macrocycle construction to produce gram quantities of this unit (**1d**) for the preparation of the planned analogs. The linear precursor to the macrocycle was oxidized more effectively with 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one (Dess–Martin periodinane reagent), and cyclization yields were increased substantially by using a new coupling reagent, pentafluorophenyl diphenylphosphinate (FDPP). (1*H*-1,2,3-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) and pentafluorophenyl trifluoroacetate were also used to improve other coupling reactions.

Introduction

The didemnins (**1**) are a class of novel cyclodepsipeptides isolated in the Caribbean from a marine tunicate of the family *Didemnidae* (*Tridemnum genus*) by Rinehart and co-workers in 1981 using extraction and column chromatography.[1] Didemnins A–C (**1a-1c**) contain a common macrocycle and differ only in the side chains attached to the backbone through the amino group of threonine (Chart 1). They have been the subject of much study because of their potent antitumor, antiviral, and immunosuppressive activities. The syntheses of didemnins A–C[2–5] and nordidemnins[6,7] have been reported, and the synthetic chemistry and biological profile of the didemnins were reviewed in 1992.[8] Didemnin A (**1a**) has *N*-methyl-D-leucine attached to the amino group of threonine. *N*-Methyl-D-leucine and the amino acids attached to it will be referred to as the "side chains". Most didemnins are derivatives of didemnin A, in which different amino acid residues are attached to the *N*-methylamino group of D-leucine. Didemnin B (**1b**) contains a L-lactyl-L-prolyl residue, whereas didemnin C (**1c**) contains only the L-lactyl portion.

Didemnin B is the most potent of the natural didemnins shown in Chart 1, and it is undergoing phase II clinical trials for antitumor activity.[9–12] Both didemnins A and B show antiviral activity against DNA and RNA viruses, with didemnin B being more active.[13,14] Didemnin B has significantly greater immunosuppressive

Chart 1. Some Naturally Occurring Didemnins



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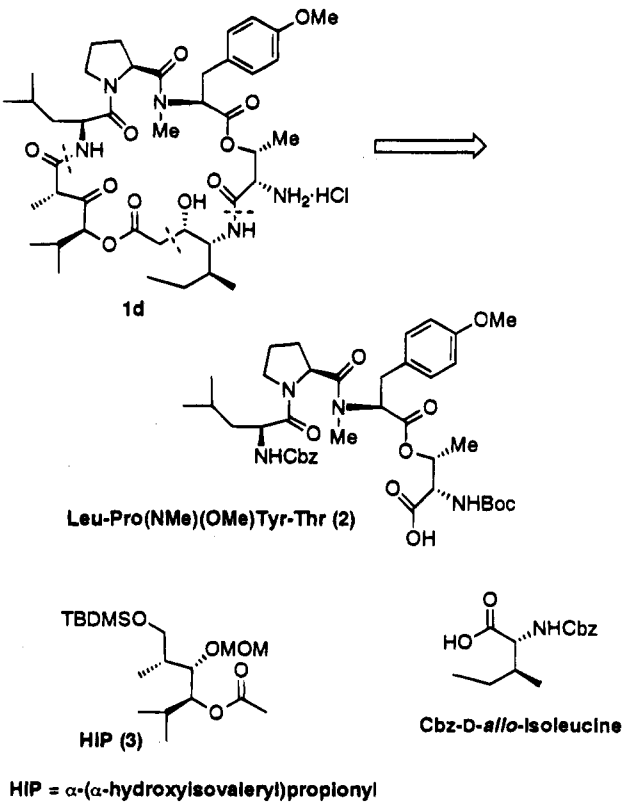
0022-3263/94/1959-5192$04.50/0

activity than cyclosporin A, but it does not bind to the same receptor site.[15] The pharmacophore of the didemnins is not well understood. An X-ray crystal structure of didemnin B by van der Helm and co-workers[16] shows that the β-turn side chain, the isostatine hydroxyl group, and the tyrosine residue extend conspicuously outward from the rest of the molecule, leading to speculation about their importance for biological activity. Experimental work has indeed shown that biological activity is sensitive to changes in those areas.[13,17-25]

The mechanism of action of the didemnins has not yet been established. However, recent studies of possible binding sites have yielded important results. Shen and co-workers[19] have documented that didemnin B binds specifically to a site on Nb2 node lymphoma cells and that binding to this site mediates immunosuppressive activity. Very recently, Schreiber and co-workers[26] have reported the GTP-dependent binding of didemnin A derivatives to elongation factor 1α(EF-1α), which may be linked to the ability of the didemnins to inhibit protein synthesis. Toogood and co-workers[27] have shown that didemnin B inhibits the ability of eEF-2 to catalyze polypeptide elongation, which demonstrates that it is an inhibitor of ribosomal translocation.

In this paper, we present the synthesis and biological testing of several analogs of didemnin B in which modifications of the L-lactyl-L-prolyl portion of the side chain have been made to examine the structure–activity relationships in this region of the molecule. We also report some significant improvements in the synthesis of the didemnin macrocycle.

Scheme 1



HIP = α-(α-hydroxyisovaleryl)propionyl

Results and Discussion

Previously our efforts were directed to the stereocontrolled macrocycle construction with subsequent attachment of optically active side chains to the amino group of threonine in order to produce the natural products in sufficient quantities for testing.[5] The next goal was to determine the structural features responsible for the biological activity. Since the side chain length was believed to be a key structural feature for bioactivity, investigations of new side chain analogs were undertaken to produce compounds for testing. The construction of the macrocycle for these new didemnin analogs incorporated methodology developed in our laboratory,[5] but several steps needed to be improved before gram quantities of the targets could be prepared. The original retrosynthetic analysis of the macrocycle established three disconnection points which afforded three subunits: the tetrapeptide (**2**) comprised of leucine, proline, *N*-Me,*O*-Me-tyrosine, and threonine; the isostatine portion derived from D-*allo*-isoleucine; and the carbon unit α-(α-hydroxyisovaleryl)propionyl (HIP, **3**, Scheme 1).

The condensation reaction responsible for the synthesis of the HIP–isostatine portion, which provides the lower half of macrocycle **1d**, was accomplished in better yield by using pentafluorophenyl trifluoroacetate[28] as the activating agent instead of 1,1-carbonyldiimidazole (CDI). The starting material units (Cbz-D-*allo*-isoleucine and **3**) were reported in an earlier publication.[5] Potassium borohydride reduction[29] of the β-keto ester (**4**) afforded a diastereomeric mixture of alcohols which, after protection with triisopropylsilyl triflate, provided a chromatographically separable mixture of silyl ethers (12.6:1 ratio). The benzoxycarbonyl (Cbz) protecting group of the primary

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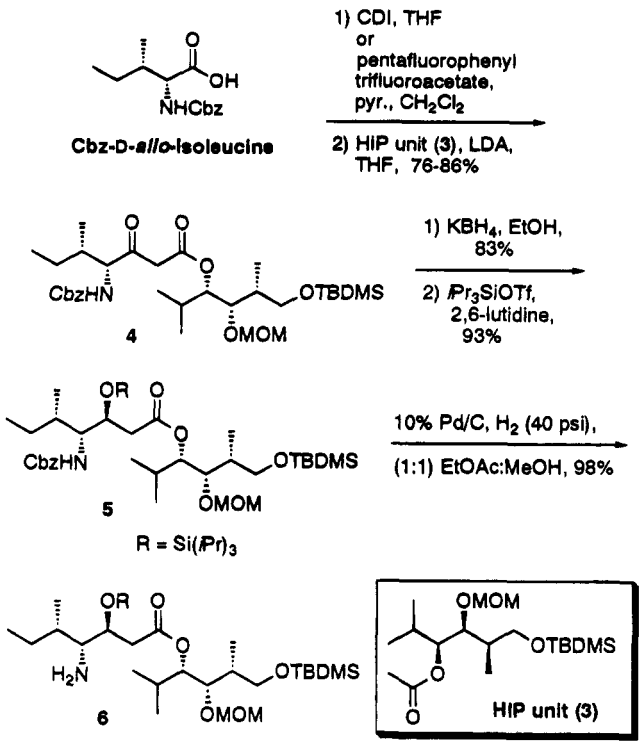
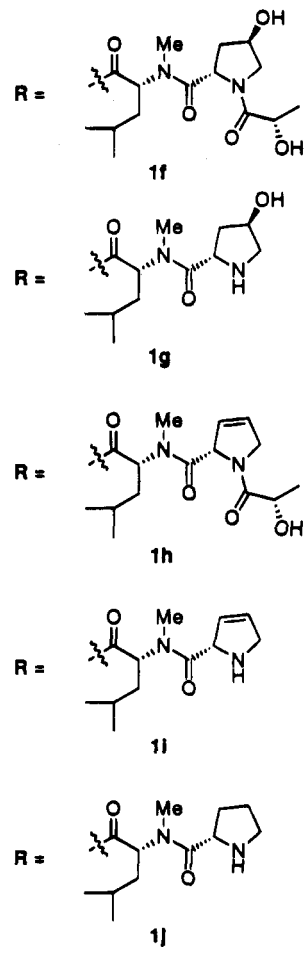
Scheme 2



amine of the major isomer (**5**) was removed by catalytic hydrogenation to afford the HIP–isostatine subunit (**6**, Scheme 2).

Originally, the coupling of the tetrapeptide acid portion (**2**) with amine **6** was accomplished using isopropenyl chloroformate activation at −15 °C.[30] This reaction afforded a modest 60% yield of **7**; a major side reaction was carbamate formation between the amino portion of the HIP-isostatine unit and the reagent. By using BOP[31] with a catalytic amount of DMAP at room temperature, we increased the yield of **7** to 72% (Scheme 3). The primary alcohol (**8**), resulting from removal of the TBDMS group under acidic conditions, was subjected to a two-stage oxidation protocol to afford the carboxylic acid (**9**) needed for macrolactamization. Previously, Swern oxidation conditions were used to convert the primary alcohol to the intermediate aldehyde. However, the reproducibility of this step was erratic, especially on a microscale, due to traces of moisture and other contaminants. The impure aldehyde could not be purified by flash column chromatography due to extensive decomposition. By using the Dess–Martin periodinane reagent[32] this transformation could be accomplished in quantitative yield at room temperature in 30 min. The new procedure afforded the intermediate aldehyde, which could be oxidized further without purification using the Masamune protocol.[33] This reaction incorporates a buffered potassium permanganate solution to generate the acid functionality needed for cyclization. Neither procedure interferes with any other functionality in the linear chain. The Cbz group was then removed with catalytic hydrogenation to give the deprotected linear precursor (**10**) which could be cyclized.

Chart 2. Synthetic Side Chains



A last modification was achieved in the cyclization step (Scheme 3). Originally, diphenyl phosphorazidate (DPPA)[5] was used for this transformation; however, the reaction took 72 h at 0 °C, and at best the yields were only 42%. A major contaminant was the dimerization product even under very dilute conditions. By examining the literature[34] and testing different coupling reagents,[35] a new phosphinate (FDPP) was found, which achieved cyclization in 68% yield. The reaction was performed at room temperature and was completed in 3–4 h. The new reagent was a significant improvement in the cyclization step as it afforded a better yield under mild conditions.

After cyclization, the MOM protecting group was removed with dimethylboron bromide[36,37] and the resulting secondary alcohol (**12**) oxidized using the periodinane reagent.[32] The protected target **13** was then treated with anhydrous HCl or HBr to provide the corresponding macrocycle salts (**1d** or **1e**). These salts were used for the synthesis of new analogs and also for an X-ray analysis.[38]

With the macrocycle in hand, the preparation of new side chains was examined. Since didemnin B with a

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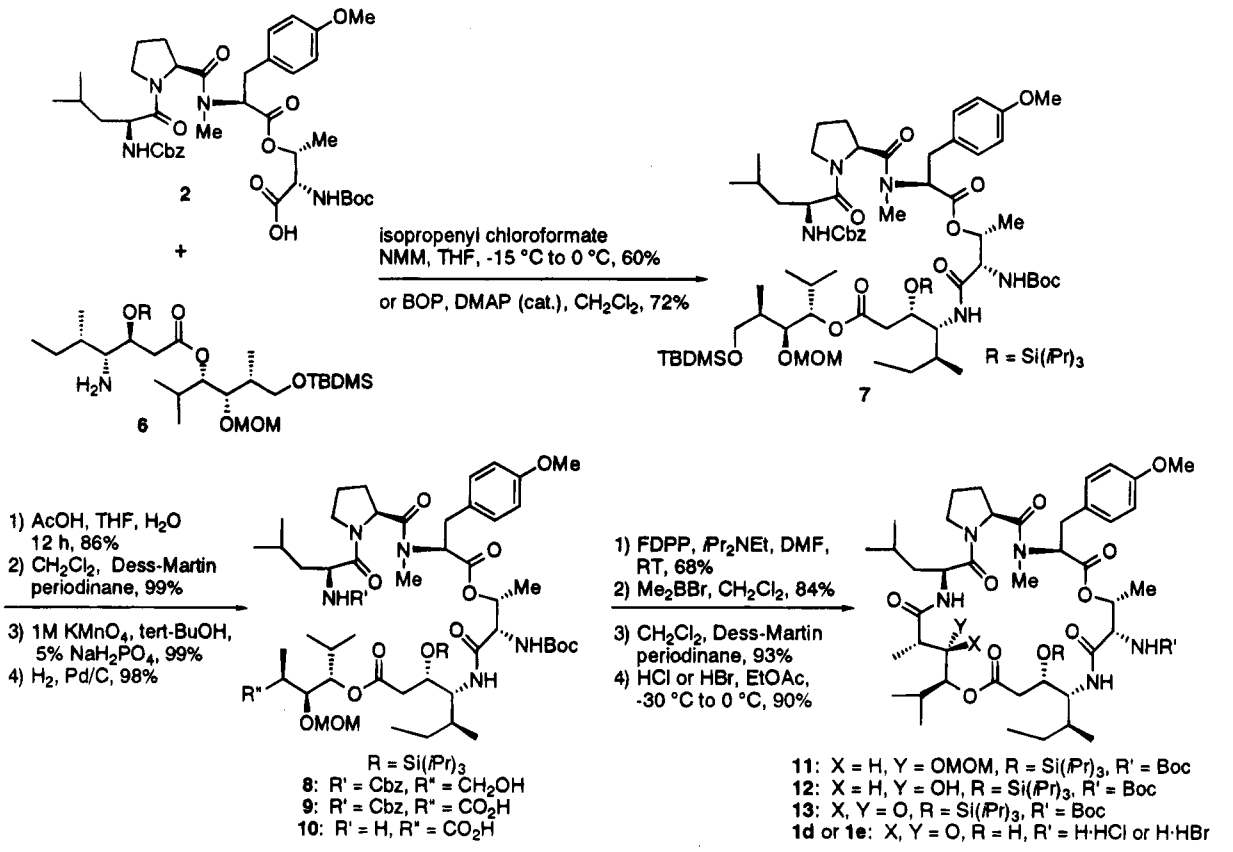
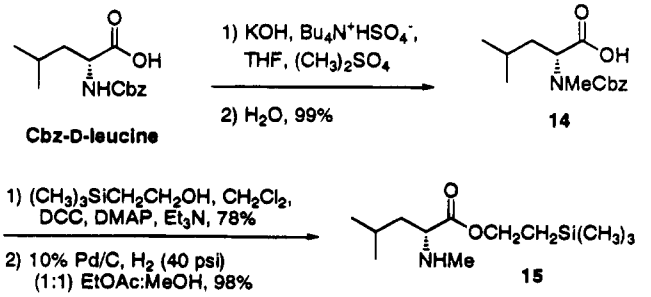
Scheme 3



β-turn side chain had shown the most promising bioactivity, variations in its side chain length, rigidity, and polar character were investigated. Two types of functionality were introduced into the proline ring to achieve these modifications. The hydroxy proline derivatives (**1f-1g**, Chart 2) could elucidate whether polarity plays an important role in the biological activity and whether the hydroxyl functionality could offer an additional binding site. The 3,4-didehydroproline ring (**1h-1i**)[39] was introduced for two reasons: first, to examine the effect of a more rigid ring on the bioactivity and, second, to investigate the possibility of tritiating the molecule at the C-3, C-4 positions in order to follow the drug pathway in biological systems. This information could help in confirming a protein binding receptor for the didemnin class of natural products which was recently isolated by Dr. Schreiber's group at Harvard University.[26] Finally, new side chains were prepared with and without the lactyl portion to ascertain the importance of side chain length. Previously, didemnin B was diacetylated with a tritium species; however, this methodology incorporated an additional tritiated unit into didemnin B.[40]

As *N*-methyl-D-leucine is a common intermediate to all new analogs, it was prepared first (Scheme 4). Cbz-D-leucine was methylated with dimethyl sulfate to afford **14**. To use *N*-methyl-D-leucine for the synthesis of the other side chains, its carboxylic acid (**14**) was then protected as a (trimethylsilyl)ethyl ester and the resulting compound hydrogenated to the secondary amine **15** to be used for coupling in subsequent steps. Compound **14** was initially coupled to the amino group of threonine using the BOP reagent[31] to produce didemnin A (**1a**,

Scheme 4



Scheme 5). Didemnin A was tested by Dr. Stuart Schreiber at Harvard University.[41] He confirmed earlier reports[13] that the acetylated form of didemnin A showed comparable cytotoxic activity to didemnin B. This observation restated the importance of side chain length for activity. Didemnin A could be labeled with a tritiated acetyl group to study its biological course of action.

The lactylhydroxyproline didemnin B side chain was prepared by coupling the *tert*-butyldimethylsilyl ether of L-lactic acid with *trans*-4-hydroxyproline methyl ester using the BOP reagent for activation (84% yield, Scheme 6). The secondary alcohol **16** was protected with another TBDMS group, and then the ester was hydrolyzed to acid **17**. The secondary amine **15** (Scheme 4) was introduced into the side chain by using *N,N*-bis(2-oxo-3-oxazolidinyl)-phosphonic chloride (BOP-Cl).[42] This reaction consistently afforded a 60–65% yield of one diastereomer after column chromatography, and to date BOP-Cl has been the reagent of choice in coupling secondary amines. This

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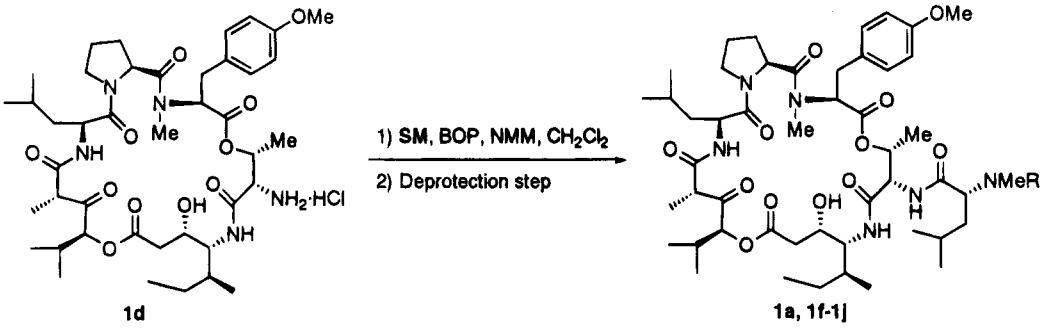
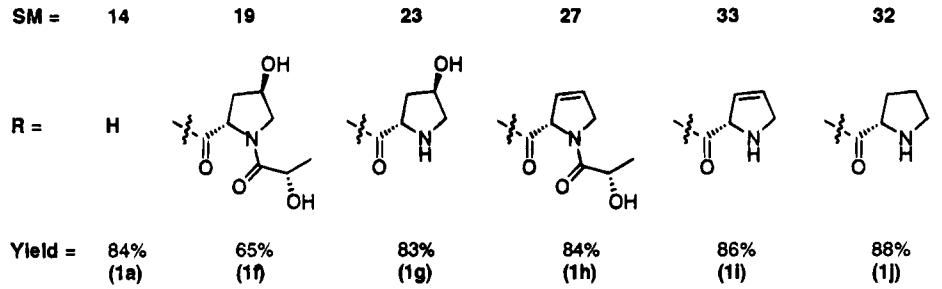
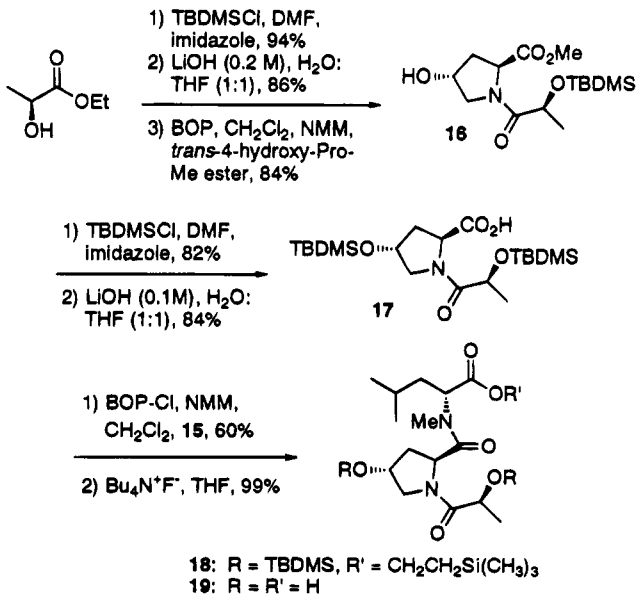
Scheme 5



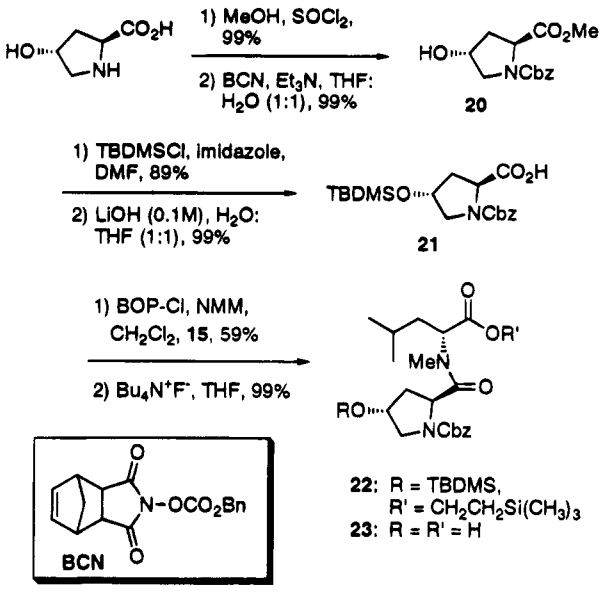
Deprotection: Pd/C, H_2 (40 psi), EtOAc:MeOH (1:1) for **1a**, **1g**, and **1j**, TFA, CH_2Cl_2 for **1i**.



Scheme 6



Scheme 7



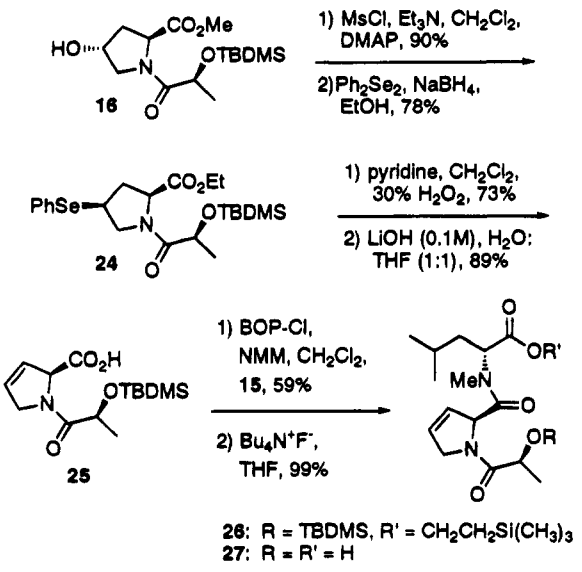
reagent suppresses racemization substantially. The reason for using several similar silyl protecting groups is evident in the last step in which tetrabutylammonium fluoride (TBAF) removes all three groups simultaneously to afford the first modified side chain **19**.

The remaining side chains were synthesized and attached to the macrocycle using previous methodology. To generate the Cbz-protected hydroxyproline side chain, Cbz-*trans*-4-hydroxyproline methyl ester (**20**) (Scheme 7) was protected with TBDMSCl and the ester group hydrolyzed with aqueous lithium hydroxide to the acid **21**, which was subsequently coupled to compound **15** using BOP-Cl. Again TBAF was used to deprotect the silyl groups and generate a second side chain (**23**).

To obtain the lactyl-3,4-didehydroproline didemnin B side chain,[39] the secondary alcohol (**16**) from Scheme 6 was first mesylated and then displaced by phenyl selenide (Scheme 8). During this step, the ester was transesterified by refluxing with ethanol. The use of methanol was found to afford much lower yields. By oxidizing the phenyl selenide **24** with 30% aqueous hydrogen peroxide and pyridine, the elimination product was obtained as a single structural isomer. After hydrolysis, acid **25** could be coupled with secondary amine **15** using BOP-Cl. The final deprotection utilized previous methodology to afford compound **27**.

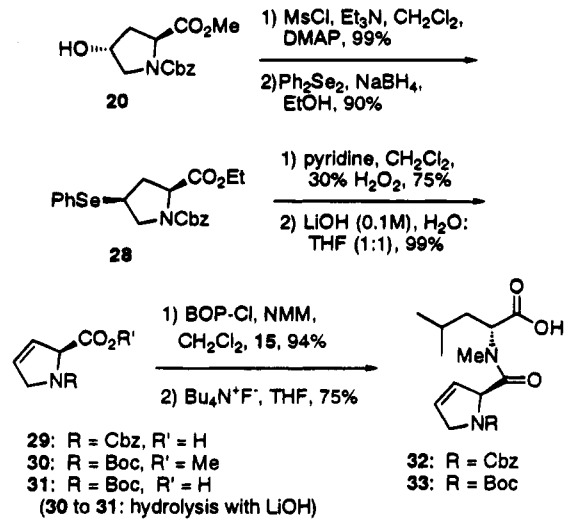
The last side chain incorporating dehydroproline without the lactyl portion was synthesized as previously described (Scheme 9).[39] The only difference was that both the Cbz-protected and the Boc-protected *trans*-4-hydroxyproline methyl esters were used as starting materials for two separate synthetic investigations. The side chain protected with the Cbz-group (**32**) could be hydrogenated to effect, in one step, the removal of the protecting group and the reduction of the dehydroproline double bond. The Boc-protected side chain (**33**) could be removed without

Scheme 8



26: R = TBDMS, R' = $CH_2CH_2Si(CH_3)_3$
27: R = R' = H

Scheme 9



29: R = Cbz, R' = H
30: R = Boc, R' = Me
31: R = Boc, R' = H
(**30** to **31**: hydrolysis with LiOH)

32: R = Cbz
33: R = Boc

affecting the double bond. The significance of synthesizing two differently protected dehydroproline side chains will be seen after coupling these new chains to the macrocycle.

The attachment of these new side chains to the macrocycle salt utilized the BOP reagent to activate the acid functionality (Scheme 5). All coupling reactions were successful, and two new didemnin B analogs with the lactyl portion (**1f** and **1h**), along with two new protected didemnin B analogs, were generated. The Cbz-protected hydroxyproline analog was hydrogenated under standard conditions to produce a third didemnin B analog (**1g**). The removal of the Cbz group and the saturation of the double bond of the final 3,4-didehydroproline analog could be achieved in one step under hydrogenation conditions without affecting the rest of the macrocycle (**1j**). This observation will play a key role in further testing because it will permit tritiation of this species as the last step.

The three new analogs (**1f-1h**) and the *N*-prolyldidemnin A (**1j**) are currently being screened by different groups. Preliminary test results performed at the National Cancer Institute (Table 1) confirm that the cytotoxicity of **1j** is comparable to or exceeds that of didemnin B.[25] The cytotoxic activity displayed by analog **1j** suggests that the lactyl portion may not be significant for this activity and that at least three amino acids are needed to retain the side chain turn as previously seen with acetylated didemnin A.[13,41] A similar trend was also noted in the antiproliferation protency of these new analogs determined using an LAF assay[43] at Wyeth–Ayerst Research (Table 2). Although replacement of the lactyl unit with either a mandelyl or a 3-(*p*-hydroxyphenyl)propionyl unit was shown not to affect cytotoxic activity,[7] replacement with a pyruvyl residue improved this activity significantly.[22] However, elimination of the lactyl unit had not been extensively tested previously. A molecular modeling study[44,45] revealed that the macrocycle core of this new analog and acetylated didemnin A overlaid well with the didemnin B backbone. The more restricted chains are held by a hydrogen bond between the threonine amide NH and the prolyl or acetyl carbonyl in **1j** and acetylated didemnin A, respectively. In the potent natural product, **1b**, the lactyl carbonyl is the acceptor for the same donor. The side chain may not be directly involved in the bioactivity, but rather it might force the macrocycle into a bioactive conformation.[19] To support this hypothesis, the macrocycle salt was tested for bioactivity and shown to have minimal cytotoxic activity compared to dideminin B. Therefore, the presence of the side chain is important to the bioactivity.

The added hydroxyl group on the proline ring analogs **1f** and **1g** decreased the cytotoxic activity as well as antiproliferation potency. Molecular modeling studies[44,45] are being conducted to see if this added functionality destroys the bioactive β-turn, thereby changing the conformation drastically. The polar hydroxyl group could also be hydrogen bonded to a hydrophilic region of the receptor reducing the activity. Preliminary results,

Table 1. Cytotoxic Activity of New Didemnin B Analogs

	Panel/Cell Line LC_{50}[a] (μM)			
Compound	melanoma/ SK-MEL-5	CNS cancer/ SNB-75	renal cancer/ RXF-393	breast cancer/ HS 578T
didemnin B (**1b**)	<0.0100	0.432	0.494	N/A
1f	0.495	3.21	1.59	0.658
1g	0.162	0.935	0.737	1.90
1h	<0.0100	0.0542	<0.0100	0.140
1j	0.0368	0.344	0.0256	0.0372

[a] Testing performed at the National Cancer Institute.

Table 2. Antiproliferation Potency of New Didemnin B Analogs[a]

compd	IC_{50} (nM)	compound	IC_{50} (nM)
didemnin B (**1b**)	4.4	**1h**	2.6
1f	10 < IC_{50} < 100	**1j**	1.9
1g	10 < IC_{50} < 100		

[a] Testing performed at Wyeth–Ayerst Research using LAF assay. Checked for cell viability using MTT reduction over dose range (1 μm to 0.1 nM).

(43) Staruch, M. J.; Wood, D. D. *J. Leuk. Biol.* **1985**, *37*, 193–207.
(44) All molecular modeling calculations were performed using MacroModel v3.1X on a Silicon Graphics Iris 4D/320S computer. Minimizations were generated from X-ray coordinates using PRCG followed by FMNR with the MM2 force field to obtain overlays with RMS values less than 0.050.
(45) Still, W. C.; Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Lipton, M.; Liskamp, R.; Chang, G.; Hendrickson, T.; DeGunst, F.; Hasel, W. Department of Chemistry, Columbia University, New York, NY 10027. Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440–467.

obtained by a torsional angle minimization study, favor the latter hypothesis because no altered hydrogen bonding is evident in the β-turn by the addition of this hydroxyl group. Further synthetic studies such as methylation of this hydroxyl group could clarify the observed decrease in biological activity. Finally, the Boc-protected acid (**33**) has been prepared and is currently being attached to the macrocycle to generate the unsaturated side chain analog (**1i**) needed for the tritiation study.

Conclusions

Previous studies of the didemnins proposed several structural features which were judged to be essential for the biological activity: the side chain attached to the amino group of threonine, isostatine hydroxyl group, and tyrosine side chain.[13,16-25] Since didemnin B was shown to be one of the most active member of the family, we elected to introduce modifications in its β-turn side chain to ascertain features essential to bioactivity: side chain length, rigidity, or hydrophilicity. Initial results reveal that only three residues, threonine, *N*-methyl-D-leucine, and proline, are needed to enforce the hydrogen bond of the side chain turn. Similarly, only three residues are present in the acetylated didemnin A side chain, which has also been found to have bioactivity comparable to that of didemnin B.[13,41] In addition, all new analogs had cytotoxic and immunosuppressive activities comparable to those of didemnin B (**1b**).

In preparing the macrocycle core for attachment of these new side chains, several transformations were modified to improve the yield of the following steps: HIP–isostatine condensation, HIP–isostatine and tetrapeptide coupling, the oxidation steps leading to the macrocycle, and the cyclization incorporating FDPP activation.

Experimental Section

Provided in the supplementary material are the analytical data for the intermediates (**1d**, **1e**, **4**, **7–9**, and **11–14**) prepared previously by our group[5] using improved methodology. All spectroscopic data compare favorably with the reported values in the earlier publication.

General Procedures. All manipulations were conducted under an inert atmosphere (argon or nitrogen). All solvents were reagent grade. Anhydrous ether, tetrahydrofuran (THF), benzene, and toluene were distilled from sodium and/or benzophenone ketyl. Dichloromethane (CH_2Cl_2) and dichloroethane (DCE) were distilled from calcium hydride (CaH_2). *N,N*-Dimethylformamide (DMF) and acetonitrile were distilled from phosphorus pentoxide and calcium hydride. Methanol was distilled from magnesium and iodine. Organic acids and bases were reagent grade. Triethylamine, diisopropylethylamine, and *N*-methylmorpholine were distilled from calcium hydride. All the other reagents were commercial compounds of the highest purity available. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel (60 F-254) plates (0.25 mm) precoated with a fluorescent indicator (0.50 mm plates were used for preparatory thin-layer chromatography). Visualization was effected using standard procedures unless otherwise stated. Flash column chromatography was carried out on Merck silica gel 60 particle size (0.040–0.063 mm). Melting points (mp) were determined with a Thomas-Hoover capillary melting point apparatus. Proton and carbon magnetic resonance spectra (^{1}H-, ^{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 Perkin-Elmer Model 281-B or Perkin-Elmer Model 781 spectrometers. 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 crosslinked methyl silicone gum capillary column. Elemental analyses were performed by either Desert Analytics, Tucson, AZ, or University of Pennsylvania Chemistry Department Elemental Analysis facility.

Didemnin A (1a).[5,13] To a mixture of the macrocycle amine salt (25.0 mg, 29.3 μmol) and Cbz-*N*-methyl-D-leucine (12.3 mg, 43.9 μmol) in CH_2Cl_2 (0.250 mL) at 0 °C was added BOP (19.4 mg, 43.9 μmol) and *N*-methylmorpholine (NMM, 8.10 μL, 73.7 μmol).[31] After 30 min at 0 °C, the reaction mixture was allowed to warm to rt and stir for an additional 4 h. The solution was then treated with saturated aqueous NaCl (2 mL) and extracted with EtOAc (10 mL). The organic layer was washed with 5% aqueous HCl (1 mL), 5% aqueous $NaHCO_3$, and saturated aqueous NaCl (1 mL), dried (Na_2SO_4), filtered, and concentrated. The crude residue was purified by flash column chromatography eluting with methanol:chloroform (3:97) to obtain Cbz didemnin A (27.2 mg, 86% yield) as a white foam: R_f 0.68 (10:90 methanol:chloroform); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.70–1.05 (m, 24H), 1.12–1.25 (d, J = 6 Hz, 3H), 1.31–1.50 (d, J = 7 Hz, 3H), 1.50–1.71 (m, 7H), 1.72–1.79 (m, 1H), 1.80–1.89 (m, 1H), 2.01–2.10 (m, 2H), 2.12–2.19 (m, 2H), 2.31–2.38 (m, 1H), 2.45–2.52 (m, 1H), 2.54 (s, 3H), 2.80–2.86 (s, 3H), 2.85–2.94 (m, 1H), 3.15–3.22 (m, 2H), 3.34–3.42 (m, 1H), 3.52–3.61 (m, 2H), 3.65–3.71 (m, 1H), 3.8 (s, 3H), 3.96–4.01 (m, 1H), 4.06–4.12 (m, 1H), 4.14–4.19 (m, 1H), 4.58–4.62 (m, 1H), 4.75–4.85 (m, 2H), 4.96–5.05 (m, 1H), 5.15–5.25 (m, 3H), 6.82 (m, 1H), 6.85–6.90 (d, J = 8.9 Hz, 1H), 7.06–7.11 (d, J = 9.5 Hz, 2H), 7.35 (m, 1H), 7.36–7.47 (m, 5H), 7.9 (d, J = 6.5 Hz, 1H); IR ($CHCl_3$) 3360 (m), 3000 (s), 1745 (s), 1680 (s), 1655 (s), 1525 (m), 1470 (m), 1330 (m), 1180 (w) cm^{-1}; HRMS *m/z* calcd for $C_{57}H_{85}N_6O_{14}$ (M + H) 1077.612, found 1077.618; $[\alpha]^{20}_D$ −104° (c = 0.550, $CHCl_3$). To a suspension of 10% Pd/C (5.50 mg, 256 μmol) in CH_3OH:EtOAc (1:1, 0.38 mL) was added the Cbz didemnin A (27.2 mg, 25.2 μmol) in CH_3OH (0.13 mL). The mixture was placed under an atmosphere of hydrogen (40 psi) and shaken in a Parr apparatus for 4.5 h. The reaction mixture was filtered through Celite and the Celite washed with an excess of CH_3OH:EtOAc (1:1). The filtrate was concentrated, and the residue was purified by recrystallization from $CHCl_3$:hexane to obtain the product (**1a**, 20.0 mg, 84% yield) as colorless crystals: mp 141–143 °C; R_f 0.39 (10:90 methanol:chloroform); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.84 (d, J = 6.8 Hz, 3H), 0.88–0.95 (m, 21H), 1.19 (dd, J = 7.4, 14.4 Hz, 1H), 1.25 (d, J = 6.6 Hz, 1H), 1.31 (d, J = 6.4 Hz, 3H), 1.33 (d, J = 7.0 Hz, 3H), 1.37–1.45 (m, 2H), 1.58–1.65 (m, 2H), 1.67–1.72 (m, 1H), 1.73–1.79 (m, 1H), 1.81–1.85 (m, 1H), 2.02–2.07 (m, 2H), 2.11–2.16 (m, 2H), 2.33–2.37 (m, 1H), 2.39 (s, 3H), 2.47–2.54 (m, 2H), 2.57 (s, 3H), 2.95 (t, J = 6.3 Hz, 1H), 3.10 (d, J = 17.0 Hz, 1H), 3.19 (dd, J = 10.9, 14.2 Hz, 1H), 3.38 (dd, J = 4.0, 14.0 Hz, 1H), 3.36–3.45 (m, 1H), 3.58 (dd, J = 4.0, 10.2 Hz, 1H), 3.58–3.63 (m, 1H), 3.69–3.74 (m, 1H), 3.80 (s, 3H), 4.00 (t, J = 8.1 Hz, 1H), 4.08 (dd, J = 4.1, 9.4 Hz, 1H), 4.16 (d, J = 6.9 Hz, 1H), 4.61 (dd, J = 6.0, 7.9 Hz, 1H), 4.80 (t, J = 9.5 Hz, 1H), 4.88 (dd, J = 3.2, 9.3 Hz, 1H), 5.02 (dd, J = 3.3, 6.3 Hz, 1H), 5.20 (d, J = 3.3 Hz, 1H), 6.85 (d, J = 8.4 Hz, 2H), 7.10 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 9.9 Hz, 1H), 7.74 (d, J = 9.2 Hz, 1H), 8.23 (d, J = 9.1 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.6, 14.87, 14.95, 15.4, 16.8, 18.6, 20.9, 22.3, 22.8, 23.7, 24.8, 25.0, 25.1, 26.9, 27.9, 31.2, 34.0, 34.1, 35.4, 38.5, 38.6, 41.3, 42.4, 47.1, 49.4, 49.8, 54.6, 55.2, 55.3, 57.3, 63.1, 66.2, 67.6, 71.1, 81.5, 114.1, 128.2, 129.0, 129.8, 130.3, 158.6, 168.6, 169.6, 169.9, 170.4, 171.3, 172.3, 175.3, 205.3; IR ($CHCl_3$) 3325 (m), 3000 (s), 1730 (s), 1650 (s), 1630 (s), 1540 (m), 1510 (m), 1450 (m), 1380 (w), 1320 (w), 1300 (w), 1270 (m), 1170 (m), 1080 (w), 830 (w) cm^{-1}; HRMS *m/z* calcd for

$C_{49}H_{79}N_6O_{12}$ (M + H) 943.5756, found 943.5744; $[\alpha]^{20}_D$ −141° (c = 0.950, $CHCl_3$).

2-(Trimethylsilyl)ethyl *N*-Methyl-D-leucinate (15). To a solution of acid **14** (0.250 g, 0.895 mmol) in CH_2Cl_2 (5 mL) cooled to 0 °C, triethylamine (Et_3N, 0.156 mL, 1.12 mmol) was added dropwise, followed by the addition of DCC (0.430 g, 2.08 mmol). DMAP (0.600 g, 4.91 mmol) was added to the reaction mixture, followed by dropwise addition of 2-(trimethylsilyl)-ethanol (0.193 mL, 1.34 mmol), also at 0 °C for 30 min.[5] The solution was brought to ambient temperature, and stirring was continued for an additional 22 h. The solid formed was removed by filtration and washed with Et_2O. The filtrate was diluted with 70 mL of Et_2O. The organic layer was washed with 10% aqueous HCl (8 mL), 5% aqueous $NaHCO_3$ (8 mL), and saturated aqueous NaCl (8 mL), dried ($MgSO_4$), filtered, and concentrated. The crude residue was purified by flash column chromatography eluting with EtOAc:petroleum ether (5:95) to afford the product as a clear oil (0.265 g, 78% yield): R_f 0.44 (5:95 EtOAc: petroleum ether); ^1H NMR (500 MHz, $CDCl_3$) δ 0.02−0.05 (m, 9H), 0.89 (dd, J = 6.4, 32.5 Hz, 3H), 0.93−1.00 (m, 5H), 1.51−1.57 (m, 1H), 1.64−1.68 (m, 1H), 1.71 (t, J = 7.8 Hz, 1H), 2.86 and 2.88 (s, 3H), 4.13−4.21 (m, 2H), 4.70 and 4.90 (dd and t, J = 4.9, 10.7 and 8.1 Hz, 1H), 5.13−5.19 (m, 2H), 7.29−7.36 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −1.6, 17.4, 21.2, 21.3, 23.2, 24.6, 24.9, 30.1, 30.6, 37.4, 37.8, 56.76, 56.84, 63.37, 63.43, 67.3, 67.4, 127.7, 127.9, 128.0, 128.4, 136.6, 136.8, 156.5, 157.0, 172.0, 172.3 (rotamers present in NMR); IR ($CHCl_3$) 3520 (w), 2980 (s), 2920 (m), 2900 (m), 2470 (w), 2110 (w), 1960 (w), 1685−1750 (s), 1595 (w), 1460−1475 (s), 1410 (s), 1375 (m), 1325 (m), 1210−1260 (s), 1160 (s), 1115−1130 (m), 1065 (m), 1030−1045 (m), 985 (m), 940 (m), 840−860 (s), 665−700 (w) cm^{-1}; HRMS m/z calcd for $C_{20}H_{34}NSiO_4$ (M + H) 380.2257, found 380.2251; $[\alpha]^{20}_D$ +30.8° (c = 0.825, $CHCl_3$). Under an atmosphere of hydrogen (40 psi), a mixture of 2-(trimethylsilyl)ethyl *N*-Cbz-*N*-methyl-D-leucinate (0.417 g, 1.10 mmol), MeOH:EtOAc (1:1, 6.00 mL), and 10% Pd/C (83.7 mg, 3.90 mmol) was shaken for 5 h in a Parr apparatus. The slurry was filtered through Celite and the filter cake washed with excess solvent mixture. The filtrate was dried (Na_2SO_4), filtered, and concentrated to produce **15** as a clear oil (0.264 g, 98% yield), which needed no further purification: R_f 0.33 (30:70 EtOAc:petroleum ether); ^1H NMR (500 MHz, $CDCl_3$) δ 0.06 (s, 9H), 0.91 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H), 1.00−1.03 (m, 2H), 1.45 (ddd, J = 6.6, 12.4, 19.8 Hz, 2H), 1.69−1.73 (m, 1H), 2.36 (s, 3H), 3.15 (t, J = 7.3 Hz, 1H), 4.20−4.24 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −1.5, 17.5, 22.4, 22.7, 24.9, 34.7, 42.7, 61.8, 62.7, 176.0; IR (neat) 3340 (w), 2970 (s), 2910 (m), 2880 (m), 2805 (w), 1730 (s), 1450−1480 (m), 1385 (w), 1370 (w), 1330 (w), 1310 (w), 1260 (s), 1220 (m), 1170 (s), 1140 (m), 1090 (w), 1060 (m), 1040 (m), 980 (m), 860 (s), 840 (s), cm^{-1}; HRMS m/z calcd for $C_{12}H_{28}NSiO_2$ (M + H) 246.1889, found 246.1873; $[\alpha]^{20}_D$ +2.9° (c = 0.715, $CHCl_3$).

Methyl (2*S*)-2-[[(*tert*-Butyldimethylsilyl)oxy]lactyl]-*trans*-4-hydroxy-L-prolinate (16). To a solution of (*S*)-ethyl lactate (2.00 g, 16.9 mmol) in DMF (57.3 mL) was added with stirring *tert*-butyldimethylsilyl chloride (3.83 g, 25.4 mmol), followed by imidazole (4.04 g, 59.3 mmol). After the mixture was stirred at rt for 4 h, it was diluted with saturated aqueous NaCl (80 mL) and then extracted with Et_2O (3 × 40 mL). The organic layers were washed with ice cold 5% aqueous HCl (10 mL) and saturated aqueous NaCl (10 mL). The resulting organic layer was dried (Na_2SO_4), filtered, and concentrated. The crude oil was purified by flash column chromatography eluting with EtOAc:petroleum ether (3:97) to give the intermediate as a clear oil (3.69 g, 94% yield): R_f 0.77 (5:95 EtOAc: petroleum ether); ^1H NMR (500 MHz, $CDCl_3$) δ 0.07 (s, 3H), 0.10 (s, 3H), 0.91 (s, 9H), 1.27 (t, J = 7.1 Hz, 3H), 1.40 (d, J = 6.8 Hz, 3H), 4.17 (dd, J = 7.1, 10.3 Hz, 2H), 4.31 (q, J = 6.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.3, −5.0, 14.2, 18.3, 21.3, 25.7, 60.7, 68.5, 174.1; IR (neat) 2990 (m), 2970 (s), 2940 (s), 2900 (m), 2870 (s), 1755 (s), 1740 (m), 1465 (m), 1445 (w), 1390 (w), 1370 (m), 1300 (w), 1255 (m), 1190 (m), 1150 (s), 1115 (m), 1060 (m), 1020 (m), 975 (m), 940 (w), 890 (w), 830 (s), 810 (m) cm^{-1}; HRMS m/z calcd for $C_{11}H_{25}SiO_3$ (M + H) 233.1573, found 233.1548; $[\alpha]^{20}_D$ −28.2° (c = 0.615, $CHCl_3$). To a mixture of protected ethyl lactate (1.00 g, 4.30 mmol) and THF (43 mL) at 0 °C was added a cooled 0.20 M aqueous solution of LiOH (43.0 mL) dropwise over a 10 min period. After being stirred at rt for 4 h, the solution was concentrated to one-half volume. The resulting aqueous mixture was washed with Et_2O (2 × 5 mL). The Et_2O layers were extracted with saturated aqueous $NaHCO_3$ (5 mL). The aqueous layers were combined, acidified to pH 4 with 1 N aqueous $KHSO_4$, and then extracted with Et_2O (3 × 40 mL). The resulting organic layers were combined, dried ($MgSO_4$), filtered, and concentrated to afford the product as a clear oil (0.756 g, 86% yield): R_f 0.50 (10:90 EtOAc:petroleum ether); ^1H NMR (500 MHz, $CDCl_3$) δ 0.15 (s, 6H), 0.93 (s, 9H), 1.46 (d, J = 6.8 Hz, 3H), 4.36 (q, J = 6.8 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.3, −5.0, 18.0, 21.1, 25.6, 68.6, 175.3; IR (neat) 2380−3700 (s), 2960 (s), 2940 (s), 2900 (s), 2870 (s), 1730 (s), 1465 (m), 1410 (w), 1390 (w), 1365 (m), 1340 (w), 1260 (s), 1190 (w), 1150 (s), 1060 (m), 1005 (w), 970 (m), 935 (w), 840 (s), 810 (m) cm^{-1}; HRMS m/z calcd for $C_9H_{21}SiO_3$ (M + H) 205.1260, found 205.1247; $[\alpha]^{20}_D$ +7.45° (c = 0.725, $CHCl_3$). A mixture of (2*S*)-2-[(*tert*-butyldimethylsilyl)oxy]lactic acid (100 mg, 0.489 mmol), methyl *trans*-4-hydroxy-L-prolinate (80.8 mg, 0.445 mmol), and CH_2Cl_2 (6 mL) was cooled to 0 °C. The coupling reagent BOP (215 mg, 0.487 mmol) was added followed by NMM (0.122 mL, 1.11 mmol), and the solution was stirred at 0 °C for 30 min. It was stirred at rt for 5 h before it was quenched with saturated aqueous NaCl (6 mL). This mixture was extracted with EtOAc (40 mL), and the resulting organic layer was washed with 5% aqueous HCl (4 mL), 5% aqueous $NaHCO_3$ (4 mL), and saturated aqueous NaCl (4 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by flash column chromatography eluting with EtOAc: petroleum ether (50:50) to produce a viscous oil (**16**, 123 mg, 84% yield): R_f 0.32 (50:50 EtOAc:petroleum ether); ^1H NMR (500 MHz, $CDCl_3$) δ 0.09 (s, 3H), 0.11 (s, 3H), 0.91 (s, 9H), 1.39 (d, J = 6.8 Hz, 3H), 1.97−2.02 (m, 1H), 2.24−2.29 (m, 1H), 3.72 (s, 3H), 3.74−3.78 (m, 1H), 3.87 (d, J = 11.9 Hz, 1H), 4.46 (q, J = 6.8 Hz, 1H), 4.54 (t, J = 1.9 Hz, 1H), 4.64 (t, J = 8.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.3, −5.0, 18.2, 20.9, 21.0, 25.8, 36.8, 52.1, 55.4, 58.2, 70.6, 172.6, 173.1; IR (neat) 3100−3680 (s), 2930−2980 (s), 2900 (s), 2860 (s), 1740 (s), 1630 (s), 1425−1485 (s), 1365 (s), 1175−1320 (m), 1140 (s), 1090 (s), 1050 (m), 1030 (m), 1010 (m), 925−975 (m), 810−885 (s), cm^{-1}; HRMS m/z calcd for $C_{15}H_{30}NSiO_5$ (M + H) 332.1893, found 332.1884; $[\alpha]^{20}_D$ −57.8° (c = 0.710, EtOH).

(2*S*)-2-[[(*tert*-Butyldimethylsilyl)oxy]lactyl]-*trans*-4-[(*tert*-butyldimethylsilyl)oxy]-L-proline (17). To a solution of **16** (0.484 g, 1.46 mmol) and DMF (14.5 mL) at rt was added *tert*-butyldimethylsilyl chloride (0.334 g, 2.22 mmol), followed by imidazole (0.348 g, 5.12 mmol). The reaction was stirred at this temperature for 18 h and then quenched with saturated aqueous NaCl (12 mL). The mixture was extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with ice cold 5% aqueous HCl (15 mL) and saturated aqueous NaCl (15 mL), dried (Na_2SO_4), filtered, and concentrated. The crude residue was purified by flash column chromatography eluting with EtOAc:petroleum ether (20:80) to give the intermediate as a low melting solid (0.534 g, 82% yield): mp 51−53 °C; R_f 0.53 (20:80 EtOAc:petroleum ether); ^1H NMR (500 MHz, $CDCl_3$) δ 0.07 (s, 3H), 0.08 (s, 3H), 0.09 (s, 3H), 0.11 (s, 3H), 0.86 (s, 9H), 0.91 (s, 9H), 1.38 (d, J = 6.8 Hz, 3H), 1.89−1.94 (m, 1H), 2.16 (ddd, J = 2.5, 7.9, 12.9 Hz, 1H), 3.72 (s, 3H), 3.72−3.76 (m, 2H), 4.43−4.47 (m, 2H), 4.60 (t, J = 8.3 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.3, −5.0, −4.9, −4.8, 17.8, 18.2, 20.8, 25.6, 25.8, 37.3, 52.1, 55.8, 58.3, 70.7, 71.1, 172.8, 173.0; IR (neat) 2960 (s), 2940 (s), 2900 (m), 2870 (s), 1755 (s), 1650 (s), 1465 (m), 1430 (m), 1390 (w), 1365 (m), 1290 (w), 1260 (s), 1230 (w), 1200 (m), 1180 (m), 1135 (s), 1095 (s), 1055 (w), 1025 (m), 1010 (w), 970 (m), 930 (m), 900 (w), 830 (s), 810 (m) cm^{-1}; HRMS m/z calcd for $C_{21}H_{44}NSi_2O_5$ (M + H) 446.2758, found 446.2740; $[\alpha]^{20}_D$ −43.6° (c = 1.64, $CHCl_3$). A mixture of the fully protected lactylproline unit (0.412 g, 0.924 mmol) and THF (25.0 mL) was prepared and cooled to 0 °C. A cold 0.10 M aqueous solution of LiOH (25.0 mL) was added dropwise over 5 min. The reaction was stirred at rt for 18 h and then concentrated to one-half volume. The resulting aqueous layer

was washed with Et_2O (2 × 5 mL). The Et_2O layers were extracted with saturated aqueous $NaHCO_3$ (10 mL). The aqueous layers were combined and acidified with 1 N aqueous $KHSO_4$ to pH 4. The aqueous layer was extracted with Et_2O (3 × 50 mL). The organic layers were dried ($MgSO_4$), filtered, and concentrated to afford the product (**17**) as a white solid (0.335 g, 84% yield): mp 106.5–107.5 °C; R_f 0.29 (10:90 methanol:chloroform); ^1H NMR (500 MHz, $CDCl_3$) δ 0.08–0.09 (m, 12H), 0.87 (s, 9H), 0.90 (s, 9H), 1.40 (d, J = 6.8 Hz, 3H), 2.10–2.14 (m, 1H), 2.27–2.32 (m, 1H), 3.61 (dd, J = 3.7, 11.7 Hz, 1H), 3.83 (d, J = 11.8 Hz, 1H), 4.45 (bs, 1H), 4.51 (dd, J = 6.9, 13.2 Hz, 1H), 4.70 (t, J = 8.1 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.3, −5.0, −4.9, −4.8, 17.9, 18.2, 20.8, 25.6, 25.8, 36.1, 56.0, 59.0, 70.5, 70.8, 173.5, 175.6; IR ($CHCl_3$) 2600–3550 (w), 2980 (s), 2950 (s), 2910 (w), 2880 (m), 1740 (w), 1615–1665 (s), 1470 (m), 1405–1450 (m), 1370 (w), 1305 (w), 1260 (s), 1135 (s), 1100 (s), 1055 (w), 1025 (w), 1010 (w), 930–945 (w), 905 (w), 840 (s) cm^{-1}; HRMS *m/z* calcd for $C_{20}H_{42}NSi_2O_5$ (M + H) 432.2601, found 432.2587; $[\alpha]^{20}_D$ −83.5° (*c* = 0.825, $CHCl_3$). Anal. Calcd for $C_{20}H_{41}NSi_2O_5$: C, 55.64; H, 9.57; N, 3.24. Found: C, 55.91; H, 9.95; N, 3.38.

2-(Trimethylsilyl)ethyl (2*S*)-2-[[(*tert*-Butyldimethylsilyl)oxy]lactyl]-*trans*-4-[(*tert*-butyldimethylsilyl)oxy]-L-prolyl-*N*-methyl-D-leucinate (18). To a cooled solution of acid **17** (0.253 g, 0.586 mmol) in CH_2Cl_2 (5 mL) at −15 °C (ethylene glycol–dry ice) was added BOP-Cl (0.163 g, 0.640 mmol) and NMM (70.0 μL, 0.640 mmol).[42] The reaction was stirred at −15 °C for 30 min, and a cold solution of **15** (0.131 g, 0.533 mmol) in CH_2Cl_2 (2 mL) was added dropwise. The solution was concentrated to one-half its volume keeping the temperature below 0 °C. To the resulting mixture was added another equivalent of NMM (70.0 μL, 0.640 mmol). The reaction was stirred at 0 °C for 7 h and then diluted with Et_2O (100 mL). The ether layer was washed with 10% aqueous HCl (10 mL), 5% aqueous $NaHCO_3$ (10 mL), and saturated aqueous NaCl (10 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash column chromatography eluting with EtOAc:petroleum ether (20:80) to afford the product (**18**, 0.211 g, 60% yield) as a clear oil: R_f 0.77 (20:80 EtOAc:petroleum ether); ^1H NMR (500 MHz, $CDCl_3$) δ 0.01–0.14 (m, 21H), 0.87 (s, 9H), 0.90 (s, 9H), 0.93 (d, J = 6.7 Hz, 3H), 0.85–1.04 (m, 5H), 1.37 and 1.28 (d, J = 6.7 Hz, 3H), 1.43–1.50 (m, 1H), 1.68–1.85 (m, 2H), 1.91–2.05 (m, 2H), 3.12 and 2.85 (s, 3H), 3.79 (td, J = 4.4, 11.2 Hz, 1H), 3.71–3.75 (m, 1H), 4.11–4.22 (m, 2H), 4.44–4.63 (m, 2H), 4.96 (dt, J = 5.8, 9.8 Hz, 1H), 5.02 (t, J = 7.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.3, −5.0, −4.9, −4.8, −1.5, 17.4, 18.2, 20.9, 21.5, 22.3, 22.8, 23.3, 25.1, 25.7, 25.9, 32.5, 37.4, 37.6, 55.6, 55.9, 63.3, 70.3, 71.4, 171.7, 172.5, 172.8 (rotamers present in NMR); IR ($CHCl_3$) 2970 (s), 2940 (s), 2900 (m), 2870 (m), 1730 (m), 1660 (s), 1635 (s), 1535 (w), 1465 (m), 1440 (m), 1415 (w), 1390 (w), 1370 (w), 1310 (w), 1255 (s), 1180 (m), 1130 (m), 1095 (s), 1050 (w), 1020 (w), 1005 (w), 940 (w), 905 (w), 860 (m), 840 (s) cm^{-1}; HRMS *m/z* calcd for $C_{32}H_{67}N_2Si_3O_6$ (M + H) 659.4307, found 659.4335; $[\alpha]^{20}_D$ +9.20° (*c* = 0.550, $CHCl_3$).

Cyclo[*N*-(*N*-L-lactyl-*trans*-4-hydroxy-L-prolyl-*N*-methyl-D-leucyl)-*O*-[[*N*-[(2*S*,3*S*,4*S*)-4-[[(3*S*,4*R*,5*S*)-4-amino-3-hydroxy-5-methylheptanoyl]oxy]-3-oxo-2,5-dimethylhexanoyl]-L-leucyl]-L-prolyl-*N*,*O*-dimethyl-L-tyrosyl]-L-threonyl] (1f). A mixture of fully protected tripeptide (**18**, 94.0 mg, 143 μmol) and THF (5 mL) was cooled to 0 °C in an ice bath. A 1.0 M solution of TBAF in THF (0.700 mL) was added dropwise, and the solution was stirred at 0 °C for 1 h and at rt for 4 h. The solution was quenched with saturated aqueous NaCl (0.5 mL) and then concentrated *in vacuo*. The resulting aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with 5% aqueous HCl (3 mL) and saturated aqueous NaCl (3 mL), dried (Na_2SO_4), filtered, and concentrated. The crude acid **19** (~50 mg) was contaminated with a tetrabutylammonium byproduct which could not be removed by recrystallization, trituration, or HPLC purification; however, it did not interfere in the subsequent coupling step. To a solution of the macrocycle HCl salt (**1d**, 25.0 mg, 29.3 μmol) and crude acid **19** (14.5 mg, 44.0 μmol) in CH_2Cl_2 (1.0 mL) at 0 °C was added the BOP reagent (19.5 mg, 44.0 μmol), followed by NMM (8.10 μL, 73.3 μmol) dropwise. After 30 min at 0 °C and 2 h at rt, the mixture was diluted with saturated aqueous NaCl (1 mL). This solution was extracted with EtOAc (20 mL), and the resulting organic layer was washed with 5% aqueous HCl (2 mL), 5% aqueous $NaHCO_3$ (2 mL), and saturated aqueous NaCl (2 mL). The organic phase was then dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by flash column chromatography eluting first with methanol:chloroform (4:96), a second column with EtOAc:petroleum (90:10) to EtOAc (100%), and finally one more column with methanol:chloroform (4:96) followed by recrystallization from $CHCl_3$:hexane to give **1f** as a yellow solid (21.5 mg, 65% yield): mp 178–180 °C; R_f 0.44 (10:90 methanol:chloroform); ^1H NMR (500 MHz, acetone-d_6) δ 0.84 (d, J = 6.9 Hz, 3H), 0.87 (d, J = 6.5 Hz, 3H), 0.90 (d, J = 6.8 Hz, 3H), 0.92 (d, J = 1.0 Hz, 3H), 0.93 (d, J = 1.0 Hz, 3H), 0.94 (d, J = 2.4 Hz, 3H), 0.95 (d, J = 2.2 Hz, 3H), 0.96 (d, J = 6.8 Hz, 3H), 1.18–1.26 (m, 2H), 1.28 (d, J = 6.8 Hz, 3H), 1.30 (d, J = 6.6 Hz, 3H), 1.33 (d, J = 6.3 Hz, 3H), 1.36–1.43 (m, 2H), 1.45–1.53 (m, 1H), 1.53–1.62 (m, 1H), 1.63–1.86 (m, 5H), 2.00–2.15 (m, 3H), 2.22–2.29 (m, 2H), 2.30–2.36 (m, 1H), 2.43 (dd, J = 10.0, 17.7 Hz, 1H), 2.69 (s, 3H), 2.79 (s, 1H), 3.15 (dd, J = 10.6, 13.9 Hz, 1H), 3.20 (s, 3H), 3.30 (dd, J = 4.4, 14.0 Hz, 1H), 3.58–3.83 (m, 4H), 3.78 (s, 3H), 3.92 (d, J = 6.2 Hz, 1H), 3.98 (dd, J = 4.4, 10.6 Hz, 1H), 3.98–4.09 (m, 2H), 4.17 (q, J = 6.8 Hz, 1H), 4.37 (d, J = 3.8 Hz, 1H), 4.40–4.45 (m, 1H), 4.56 (dd, J = 2.2, 5.4 Hz, 1H), 4.64 (bs, 1H), 4.77–4.83 (m, 2H), 5.03 (dd, J = 7.1, 9.3 Hz, 1H), 5.08 (d, J = 3.6 Hz, 1H), 5.33 (dt, J = 2.2, 6.3 Hz, 1H), 5.40 (dd, J = 3.9, 11.6 Hz, 1H), 6.90 (d, J = 8.6 Hz, 2H), 7.11 (d, J = 9.7 Hz, 1H), 7.20 (d, J = 8.6 Hz, 2H), 7.75 (d, J = 5.4 Hz, 1H), 7.89 (d, J = 9.4 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d_6) δ 12.3, 14.9, 15.6, 16.9, 17.1, 19.3, 20.6, 21.2, 21.6, 23.7, 23.8, 24.2, 25.4, 25.5, 28.2, 28.5, 30.1, 30.9, 31.5, 34.7, 36.9, 37.8, 39.0, 40.4, 42.2, 47.7, 49.8, 49.9, 55.4, 55.5, 55.8, 56.2, 56.3, 58.2, 58.6, 66.2, 66.7, 67.8, 70.9, 71.4, 80.8, 114.7, 131.1, 131.4, 159.6, 169.5, 169.7, 171.3, 171.6, 172.4, 172.5, 172.7, 173.7, 174.8, 205.1; IR ($CHCl_3$) 3210–3590 (w), 3320 (m), 2960 (s), 2930 (m), 2870 (m), 1730 (s), 1650 (s), 1640 (s), 1560 (w), 1545 (m), 1530 (m), 1510 (m), 1425–1470 (s), 1410 (m), 1370 (s), 1300 (m), 1265 (s), 1245 (s), 1170 (m), 1115 (m), 1070–1085 (m), 1040 (m), 1000 (w), 965 (w), 935 (w), 915 (w), 820 (w) cm^{-1}; HRMS *m/z* calcd for $C_{57}H_{90}N_7O_{16}$ (M + H) 1128.6443, found 1128.6413; $[\alpha]^{20}_D$ −67.0° (*c* = 0.750, $CHCl_3$).

Methyl *N*-Cbz-4-*trans*-hydroxy-L-prolinate (20). To a solution of *trans*-4-hydroxy-L-proline (0.500 g, 3.81 mmol) in MeOH (4 mL), at 0 °C was added thionyl chloride (0.306 mL, 4.19 mmol) dropwise. The reaction was stirred at rt for 1 h before it was heated at reflux for 22 h. The resulting solution was concentrated, and the residue was azeotroped with MeOH (3 × 25 mL). The white product formed (0.689 g, 99% yield) was kept under reduced pressure at 10 μmHg for 2 h: mp 168–170 °C; R_f 0.44 (6:4:1 chloroform:methanol:water); ^1H NMR (500 MHz, MeOH-d_4) δ 2.21 (ddd, J = 4.2, 10.7, 14.9 Hz, 1H), 2.40–2.44 (m, 1H), 3.30–3.33 (m, 1H), 3.46 (dd, J = 3.8, 12.2 Hz, 1H), 3.86 (s, 3H), 4.59–4.63 (m, 2H); ^{13}C NMR (125 MHz, MeOH-d_4) δ 38.6, 54.0, 55.0, 59.4, 70.6, 170.6; IR (KBr Pellet) 3380 (s), 2650–3200 (br), 2950 (s), 2600 (w), 2560 (w), 2500 (w), 2420 (w), 1745 (s), 1585 (m), 1450 (m), 1420 (w), 1360 (s), 1325 (m), 1310 (m), 1280–1290 (s), 1245 (s), 1215 (m), 1185 (m), 1080 (m), 1060 (w), 1040 (m), 1025 (m), 980 (w), 955 (m), 920 (w), 900 (m), 855–865 (w), 780 (w), 740 (w), 700 (w), 625 (w) cm^{-1}; HRMS *m/z* calcd for $C_6H_{12}NO_3$ (M − Cl) 146.0817, found 146.0810; $[\alpha]^{20}_D$ − 19.5° (*c* = 0.910, EtOH). Anal. Calcd for $C_6H_{12}ClNO_3$: C, 39.68; H, 6.66; N, 7.71. Found: C, 39.92; H, 6.88; N, 7.60. A mixture of methyl *trans*-4-hydroxy-L-prolinate (0.400 g, 2.20 mmol) and THF:H_2O (1:1, 22.0 mL) was cooled to 0 °C. With efficient stirring, BCN (0.765 g, 2.44 mmol) was added, followed by Et_3N (0.774 mL, 5.55 mmol) dropwise. The reaction was stirred at rt for 12 h and then concentrated to one-half volume. This residue was extracted with Et_2O (100 mL); the organic layer was washed with 5% aqueous HCl (10 mL), 5% aqueous $NaHCO_3$ (10 mL), and saturated aqueous NaCl (10 mL), dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by flash column chromatography eluting with EtOAc:petroleum ether (60:40) to provide **20** as a viscous oil (0.583 g, 99% yield): R_f 0.28 (50:

50 EtOAc:petroleum ether); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.01–2.11 (m, 2H), 2.28–2.32 (m, 1H), 3.55 and 3.75 (s, 3H), 3.63–3.71 (m, 2H), 4.48–4.54 (m, 2H), 5.01 and 5.10–5.21 (d and m, J = 12.4 Hz, 2H), 7.29–7.36 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 38.4, 39.2, 52.1, 52.4, 54.6, 55.2, 57.7, 57.9, 67.3, 69.4, 70.2, 127.8, 127.9, 128.0, 128.4, 128.5, 136.3, 173.2, 173.3 (rotamers present in NMR); IR ($CHCl_3$) 3610 (m), 3360–3570 (br), 3050 (m), 3020 (m), 2970 (m), 2410–2460 (w), 1750 (s), 1705 (s), 1610 (w), 1500 (w), 1420 (s), 1360 (s), 1320 (m), 1280 (m), 1230–1250 (m), 1175 (s), 1125 (s), 1085 (m), 990–1045 (m), 955 (m), 915 (w), cm^{-1}; HRMS m/z calcd for $C_{14}H_{18}NO_5$ (M + H) 280.1185, found 280.1165; $[\alpha]^{20}_D$ −58.8° (c = 1.04, $CHCl_3$).

***N*-Cbz-4-*trans*-[(*tert*-Butyldimethylsilyl)oxy]-L-proline (21).** Following the procedure described for compound **17**, a solution of alcohol **20** (0.581 g, 2.17 mmol) in DMF (7.4 mL) was prepared and treated with *tert*-butyldimethylsilyl (0.491 g, 3.26 mmol) and imidazole (0.518 g, 7.61 mmol). After the reaction was stirred at rt for 7 h, it was worked up as previously described and then purified by flash chromatography eluting with EtOAc:petroleum ether (5:95) to provide TBDMS-protected **20** (0.739 g, 89% yield) as an oil: R_f 0.76 (5:95-acetone:chloroform); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.04–0.06 (m, 6H), 0.86 (d, J = 5.3 Hz, 9H), 2.02–2.07 (m, 1H), 2.18–2.22 (m, 1H), 3.46 (ddd, J = 2.0, 11.0, 42.4 Hz, 1H), 3.54 and 3.75 (s, 3H), 3.66 (ddd, J = 4.6, 8.0, 12.7 Hz, 1H), 4.42–4.51 (m, 2H), 5.07 (dd, J = 12.4, 32.7 Hz, 1H), 5.20 (d, J = 12.4 Hz, 1H), 7.28–7.35 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −4.9, −4.8, 17.8, 17.9, 25.6, 25.7, 38.9, 39.9, 52.0, 52.2, 54.7, 55.2, 57.8, 58.0, 67.1, 69.7, 70.4, 127.8, 127.88, 127.91, 128.3, 128.4, 136.4, 136.6, 154.3, 155.0, 173.1, 173.2 (rotamers present in NMR); IR ($CHCl_3$) 3500 (w), 3010 (m), 2960 (s), 2940 (s), 2900 (m), 2860 (s), 2440 (w), 1745 (s), 1700 (s), 1605 (w), 1585 (w), 1495 (m), 1415–1465 (s), 1355 (s), 1315 (m), 1290 (m), 1170–1260 (s), 1095–1140 (s), 1065 (m), 1020 (s), 935 (m), 905 (s), 835 (s) cm^{-1}; HRMS m/z calcd for $C_{20}H_{32}NSiO_5$ (M + H) 394.2050, found 394.2045; $[\alpha]^{20}_D$ −39.0° (c = 0.775, $CHCl_3$). To a solution of TBDMS-protected **20** (0.450 g, 1.14 mmol) in THF (31.5 mL) at 0 °C was added dropwise a cold 0.10 M solution of LiOH (aq, 31.5 mL). After stirring for 20 h at rt, the mixture was worked up using the previous conditions (compound **17**) to afford **21** (0.430 g, 99% yield) as a clear viscous oil: R_f 0.36 (10:90 methanol:chloroform); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.02–0.07 (m, 6H), 0.85 (s, 9H), 2.13–2.32 (m, 2H), 3.45 (dd, J = 2.8, 11.0 Hz, 1H), 3.59 (dd, J = 4.7, 11.1 Hz, 1H), 4.43–4.56 (m, 2H), 5.11–5.22 (m, 2H), 7.27–7.39 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −4.9, −4.8, 17.9, 25.6, 25.7, 38.2, 39.8, 54.6, 54.8, 59.4, 67.2, 67.8, 69.7, 70.1, 127.5, 127.9, 128.1, 128.4, 128.5, 136.1, 136.3, 154.5, 156.3, 175.8; IR (neat) 2350–3760 (br), 2940–2960 (s), 2900 (m), 2860 (m), 1650–1760 (s), 1500 (w), 1400–1470 (s), 1360 (s), 1315 (m), 1255 (m), 1185–1215 (m), 1170 (m), 1120 (s), 1090 (s), 1065 (m), 1020 (m), 1005 (m), 985 (w), 965 (w), 920 (w) 895 (w), 835 (m) cm^{-1}; HRMS m/z calcd for $C_{19}H_{30}NSiO_5$ (M + H) 380.1893, found 380.1899; $[\alpha]^{20}_D$ −47.8° (c = 1.48, $CHCl_3$).

2-(Trimethylsilyl)ethyl *N*-Cbz-4-*trans*-[(*tert*-butyldimethylsilyl)oxy]-L-prolyl-*N*-methyl-D-leucinate (22). The BOP-Cl activation protocol as described in the procedure for compound **18** was used to couple acid **21** (34.0 mg, 92.5 μmol) with **15** (34.1 mg, 139 μmol) using CH_2Cl_2 (1 mL), BOP-Cl (28.3 mg, 111 μmol), and NMM (24.4 μL, 224 μmol). The reaction was stirred for 6 h at 0 °C and then worked up as previously described. The crude residue was purified by flash column chromatgraphy eluting with EtOAc:petroleum ether (20:80) to afford **22** as a white solid (32 mg, 55% yield): mp 77–78 °C; R_f 0.69 (5:95 acetone:chloroform); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.02–0.10 (m, 15H), 0.69 (t, J = 6.1 Hz, 1H), 0.86 and 0.87 (s, 9H), 0.88–0.96 (m, 6H), 0.96–1.05 (m, 1H), 1.37–1.50 (m, 1H), 1.70–1.76 (m, 2H), 1.97–2.17 (m, 2H), 3.07 and 2.97 (s, 3H), 3.44 (ddd, J = 3.6, 10.8, 30.6 Hz, 1H), 3.75 (td, J = 5.1, 11.4 Hz, 1H), 4.10 (td, J = 3.6, 7.3 Hz, 1H), 4.17–4.28 (m, 1H), 4.47–4.57 (m, 1H), 4.85 (ddd, J = 5.5, 8.1, 24.6 Hz, 1H), 5.03 (dd, J = 12.5, 19.6 Hz, 1H), 5.07–5.26 (m, 2H), 7.29–7.35 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −4.9, −4.8, −1.58, −1.56, 17.37, 17.43, 17.6, 17.9, 21.3, 21.4, 23.3, 25.1, 25.2, 25.68, 25.73, 37.5, 37.7, 38.7, 39.6, 54.6, 55.0, 55.3, 55.6, 55.8, 56.0, 63.3, 63.4, 66.7, 66.9, 69.8, 70.6, 127.4, 127.6, 127.7, 127.8, 127.9, 128.25, 128.32, 128.4, 136.8, 137.0, 154.3, 154.9, 171.5, 171.6, 172.9, 173.0 (rotamers present in NMR); IR (neat) 3070 (w), 3040 (w), 2860–2980 (s), 1710–1725 (s), 1660 (s), 1400–1475 (s), 1360 (s), 1300–1325 (m), 1250 (s), 1175–1220 (s), 1115 (s), 1060 (s), 1020 (m), 1005 (m), 985 (w), 920–940 (m), 900 (m), 840–870 (s) cm^{-1}; HRMS m/z calcd for $C_{31}H_{55}N_2Si_2O_6$ (M + H) 607.3598, found 607.3604; $[\alpha]^{20}_D$ +19.5° (c = 0.810, $CHCl_3$). Anal. Calcd for $C_{31}H_{54}N_2Si_2O_6$: C, 61.35; H, 8.97; N, 4.62. Found: C, 61.09; H, 9.19; N, 4.16.

Cyclo[*N*-(*trans*-4-hydroxy-L-prolyl-*N*-methyl-D-leucyl)-*O*-[[*N*-[(2*S*,3*S*,4*S*)-4-[[(3*S*,4*R*,5*S*)-4-amino-3-hydroxy-5-methylheptanoyl]oxy]-3-oxo-2,5-dimethylhexanoyl]-L-leucyl]-L-prolyl-*N*,*O*-dimethyl-L-tyrosyl]-L-threonyl] (1g). To a solution of 4-*trans*-[(*tert*-butyldimethylsilyl)oxy]-L-prolyl-*N*-methyl-D-leucine (**22**, 0.287 g, 473 μL) in THF (14 mL) cooled to 0 °C was added a 1.0 M solution of TBAF in THF (1.55 mL) dropwise. After being stirred at 0 °C for 1 h and then at rt for 1 h, the reaction was quenched with saturated aqueous NaCl (10 mL). The solution was concentrated *in vacuo* before extracting with EtOAc (3 × 20 mL). The organic layers were combined, washed with 5% aqueous HCl (3 mL) and saturated aqueous NaCl (3 mL), dried (Na_2SO_4), filtered, and concentrated. The crude acid **23** (~250 mg) was contaminated with a tetrabutylammonium byproduct which could not be removed by recrystallization, trituration, or HPLC purification. However, its presence did not interfere with the subsequent coupling step. The crude acid (**23**, 17.3 mg, 44.0 μmol) was then combined with the macrocycle HCl salt (**1d**, 25.0 mg, 29.3 μmol), CH_2Cl_2 (1 mL), BOP reagent (19.5 mg, 44.0 μmol), and NMM (8.10 μL, 73.3 μmol) as previously described (procedure for compound **1f**). The crude residue was purified by flash column chromatography eluting first with methanol:chloroform (4:96), a second column with EtOAc:petroleum (90:10), and finally two more columns with methanol:chloroform (4:96) followed by recrystallization with $CHCl_3$:hexane to afford Cbz protected **1g** as a white solid (25.6 mg, 73% yield): mp 165–167 °C; R_f 0.44 (10:90 methanol:chloroform); ^{1}H NMR (500 MHz, acetone-d_6) δ 0.84 (d, J = 6.7 Hz, 3H), 0.88 (d, J = 6.5 Hz, 3H), 0.90 (d, J = 7.0 Hz, 3H), 0.92 (d, J = 1.2 Hz, 3H), 0.93 (d, J = 4.1 Hz, 3H), 0.94 (d, J = 4.4 Hz, 3H), 0.95 (d, J = 3.8 Hz, 3H), 0.97 (d, J = 7.0 Hz, 3H), 1.20–1.27 (m, 2H), 1.29 (d, J = 6.9 Hz, 3H), 1.32 (d, J = 6.3 Hz, 3H), 1.34–1.46 (m, 1H), 1.50–1.57 (m, 1H), 1.58–1.63 (m, 1H), 1.64–1.87 (m, 5H), 2.00–2.04 (m, 1H), 2.06–2.14 (m, 2H), 2.21–2.30 (m, 2H), 2.31–2.36 (m, 1H), 2.44 (dd, J = 10.0, 17.7 Hz, 1H), 2.73 (s, 3H), 2.80 (s, 1H), 3.13 (dd, J = 6.4, 10.1 Hz, 1H), 3.22 (s, 3H), 3.31 (dd, J = 9.1, 14.0 Hz, 1H), 3.57–3.69 (m, 4H), 3.77 (s, 3H), 3.93 (d, J = 6.2 Hz, 1H), 3.97 (dd, J = 4.9, 10.1 Hz, 1H), 3.99–4.09 (m, 2H), 4.16 (q, J = 6.8 Hz, 1H), 4.30 (d, J = 7.1 Hz, 1H), 4.57 (ddd, J = 1.8, 3.7, 7.8 Hz, 2H), 4.78–4.83 (m, 2H), 4.99 (dd, J = 6.9, 9.0 Hz, 1H), 5.08 (d, J = 3.7 Hz, 1H), 5.23 (AB, J = 12.8 Hz, 1H), 5.31 (dd, J = 2.2, 6.3 Hz, 1H), 5.40 (AB, J = 12.8 Hz, 1H), 5.44 (dd, J = 3.8, 11.6 Hz, 1H), 6.88 (d, J = 8.7 Hz, 2H), 7.10 (d, J = 9.8 Hz, 1H), 7.20 (d, J = 8.6 Hz, 2H), 7.31–7.41 (m, 3H), 7.50 (d, J = 7.5 Hz, 2H), 7.69 (d, J = 5.4 Hz, 1H), 7.91 (d, J = 9.4 Hz, 1H); ^{13}C NMR (125 MHz, acetone-d_6) δ 12.3, 14.8, 15.6, 17.0, 17.1, 19.4, 21.2, 21.4, 21.6, 23.8, 24.3, 25.3, 25.4, 25.6, 28.2, 28.5, 30.9, 31.8, 34.6, 36.9, 38.9, 39.0, 40.5, 42.3, 47.7, 49.8, 50.0, 55.4, 55.5, 55.6, 55.8, 56.2, 58.2, 58.8, 66.2, 67.8, 68.0, 70.9, 71.0, 80.8, 114.6, 128.5, 128.6, 129.0, 129.2, 129.7, 131.1, 131.5, 137.8, 156.1, 159.5, 169.5, 169.7, 169.8, 171.3, 171.5, 172.4, 172.7, 174.0, 205.1; IR ($CHCl_3$) 3670 (w), 3250–3550 (w), 3330 (m), 3000 (m), 2960 (s), 2930 (m), 2870 (m), 1730 (s), 1680 (s), 1640–1660 (s), 1545 (m), 1530 (m), 1515 (m), 1415–1470 (s), 1375 (s), 1360 (m), 1300 (m), 1265 (s), 1250 (s), 1170 (m), 1145 (w), 1070 (m), 1045 (s), 995 (w), 960 (w), 935 (w), 915 (w), 815–850 (w) cm^{-1}; HRMS m/z calcd for $C_{62}H_{91}N_7O_{16}Na$ (M + Na) 1212.6420, found 1212.6478; $[\alpha]^{20}_D$ −77.5° (c = 1.25, $CHCl_3$). Under a hydrogen atmosphere (40 psi) the Cbz-protected **1g** (23.6 mg, 19.8 μmol) was combined with MeOH:EtOAc (1:1, 2.0 mL) and 10% Pd/C (4.2 mg, 196 μmol) and shaken in a Parr apparatus for 8 h. The slurry was filtered through Celite, followed by thorough washing with MeOH:EtOAc. The filtrate was concentrated *in vacuo*, and the residue was azeotroped

with toluene several times followed by recrystallization from $CHCl_3$:hexane to give the product (**1g**) as a beige solid (20.7 mg, 99% yield): mp 156–158 °C; R_f 0.27 (10:90 methanol:chloroform); ^1H NMR (500 MHz, $CDCl_3$) δ 0.85–0.95 (m, 24H), 1.25 (dd, J = 5.6, 12.9 Hz, 3H), 1.15–1.28 (m, 2H), 1.36 (d, J = 6.8 Hz, 3H), 1.37–1.68 (m, 6H), 1.69–1.76 (m, 1H), 1.77–1.87 (m, 2H), 1.93–2.01 (m, 1H), 2.03–2.10 (m, 1H), 2.10–2.26 (m, 4H), 2.32–2.39 (m, 1H), 2.48–2.78 (m, 2H), 2.55 (s, 3H), 2.97 (s, 3H), 2.91–3.11 (m, 3H), 3.19 (t, J = 11.0 Hz, 1H), 3.37 (d, J = 13.9 Hz, 1H), 3.56–3.65 (m, 2H), 3.68–3.75 (m, 1H), 3.80 (s, 3H), 3.98–4.05 (m, 2H), 4.16 (dd, J = 6.7, 13.7 Hz, 1H), 4.34–4.42 (m, 1H), 4.52 (bs, 1H), 4.56–4.62 (m, 1H), 4.81 (dd, J = 9.8, 27.4 Hz, 2H), 5.03–5.09 (m, 1H), 5.14–5.22 (m, 2H), 6.85 (d, J = 8.5 Hz, 2H), 7.09 (d, J = 8.3 Hz, 2H), 7.35 (d, J = 9.1 Hz, 1H), 7.51 (d, J = 7.0 Hz, 1H), 7.96 (d, J = 9.2 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.45, 11.52, 15.0, 15.2, 15.9, 16.9, 18.5, 18.6, 20.9, 22.1, 22.2, 22.9, 23.1, 23.7, 24.9, 25.1, 26.8, 27.9, 30.3, 31.3, 33.9, 34.1, 35.9, 38.4, 38.6, 41.8, 47.1, 49.6, 54.9, 55.3, 55.8, 57.3, 66.2, 67.6, 70.2, 70.5, 71.7, 73.2, 81.6, 114.1, 128.2, 129.0, 129.8, 130.3, 158.7, 168.4, 169.6, 169.7, 169.9, 170.3, 171.5, 171.9, 172.1, 204.8; IR ($CHCl_3$) 3140–3460 (w), 3340 (m), 3040 (w), 3000 (w), 2970 (m), 2940 (m), 2880 (w), 1735 (s), 1665 (s), 1645 (s), 1570 (w), 1550 (m), 1535 (m), 1520 (m), 1445–1470 (m), 1410 (w), 1390 (w), 1370 (w), 1350 (w), 1320 (w), 1305 (m), 1275 (m), 1250 (m), 1170 (m), 1120 (w), 1110 (w), 1080 (w), 1000 (w), 965 (w), 790–870 (w) cm^{-1}; HRMS m/z calcd for $C_{54}H_{85}N_7O_{14}Na$ (M + Na) 1078.6052, found 1078.6006; $[\alpha]^{20}_D$ −73.5° (c = 1.02, $CHCl_3$). Anal. Calcd for $C_{54}H_{85}N_7O_{14}\cdot H_2O$: C, 60.37; H, 8.16; N, 9.13. Found: C, 60.19; H, 8.14; N, 8.68.

Ethyl (2*S*)-2-[[(*tert*-Butyldimethylsilyl)oxy]lactyl]-*cis*-4-(phenylselenenyl)-L-prolinate (24).[39] To a solution of alcohol **16** (0.496 g, 1.50 mmol) in CH_2Cl_2 (15 mL) at 0 °C was added Et_3N (0.230 mL, 1.65 mmol) dropwise, followed by mesyl chloride (0.151 mL, 1.95 mmol). A catalytic amount of DMAP (55.0 mg, 0.450 mmol) was added, and the reaction was stirred at 0 °C for 1 h, warmed to rt and stirred an additional 1.5 h. The mixture was poured into 27 g of ice/H_2O with stirring. The product was extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with 10% aqueous HCl (20 mL), 5% aqueous $NaHCO_3$ (20 mL), and H_2O (20 mL), dried (Na_2SO_4), filtered, and concentrated. The crude oil was purified by flash column chromatography eluting with EtOAc:petroleum ether (40:60) to give the intermediate (0.553 g, 90% yield) as a colorless oil: R_f 0.58 (30:70 EtOAc:petroleum ether); ^1H NMR (500 MHz, $CDCl_3$) δ 0.10 (s, 3H), 0.11 (s, 3H), 0.90 (s, 9H), 1.40 (d, J = 6.9 Hz, 3H), 2.13–2.18 (m, 1H), 2.59 (qt, J = 1.9, 8.1, 14.4 Hz, 1H), 3.05 (s, 3H), 3.74 (s, 3H), 3.85 (dd, J = 3.7, 13.3 Hz, 1H), 4.30 (d, J = 13.4 Hz, 1H), 4.47 (q, J = 6.9 Hz, 1H), 4.67 (t, J = 8.5 Hz, 1H), 5.33 (bs, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.2, −5.0, 18.2, 20.9, 25.8, 34.7, 38.8, 52.4, 53.4, 57.7, 71.1, 78.8, 171.7, 172.9; IR (neat) 2960 (s), 2940 (s), 2900 (m), 2870 (m), 1750–1760 (s), 1660 (s), 1645 (s), 1465 (m), 1430 (s), 1365 (s), 1260 (m), 1200 (m), 1180 (s), 1140 (s), 1100 (m), 1050 (m), 1025 (w), 1005 (w), 960 (m), 900 (s), 815 (w) cm^{-1}; HRMS m/z calcd for $C_{16}H_{32}NO_7SSi$ (M + H) 410.1669, found 410.1654; $[\alpha]^{20}_D$ −34.5° (c = 0.905, $CHCl_3$). Sodium borohydride (6.00 mg, 159 μmol) was added in small portions, at rt, to a solution of diphenyl selenide (23.8 mg, 76.3 μmol) in EtOH (2.5 mL). The mixture was stirred for ~5 min until the bright yellow color disappeared. The previously prepared mesylate (50.0 mg, 122 μmol) was added, the solution was refluxed for 2 h, and the solvent was distilled *in vacuo*. The residue was diluted with Et_2O (100 mL), and the organic layer was washed with H_2O (10 mL) and saturated aqueous NaCl (10 mL). The resulting organic phase was dried ($MgSO_4$), filtered, and concentrated. The crude oil was purified by flash column chromatography eluting with EtOAc:petroleum ether (15:85) to afford **24** (45.6 mg, 78% yield): R_f 0.53 (20:80 EtOAc:petroleum ether); ^1H NMR (500 MHz, $CDCl_3$) δ 0.07 (s, 3H), 0.08 (s, 3H), 0.89 (s, 9H), 1.23 (t, J = 7.1 Hz, 3H), 1.32 (d, J = 6.8 Hz, 3H), 1.87–1.94 (m, 1H), 2.64 (td, J = 7.8, 19.4 Hz, 1H), 3.52–3.60 (m, 2H), 4.12–4.19 (m, 2H), 4.20–4.26 (m, 1H), 4.43 (dt, J = 8.4, 16.7 Hz, 2H), 7.29–7.36 (m, 3H), 7.54–7.58 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.2, −5.0, 14.1, 18.2, 20.9, 25.8, 35.6, 37.5, 53.8, 59.6, 61.1, 70.6, 127.1, 128.1, 128.4, 129.3, 134.8, 135.3, 171.1, 172.0; IR (neat) 3200–3640 (m), 3060–3080 (w), 2980 (s), 2960 (s), 2940 (s), 2900 (m), 2860 (s), 1750 (s), 1665 (s), 1645 (s), 1580 (w), 1410–1480 (s), 1395 (m), 1370 (m), 1350 (m), 1300 (m), 1260 (s), 1190 (s), 1135 (s), 1100 (m), 1025–1040 (m), 950 (m), 885 (w), 835 (s), 815 (m), cm^{-1}; HRMS m/z calcd for $C_{22}H_{36}NO_4SeSi$ (M + H) 486.1579, found 486.1563; $[\alpha]^{20}_D$ −29.5° (c = 0.800, $CHCl_3$).

(2*S*)-2-[(*tert*-Butyldimethylsilyl)oxy]lactyl]-3,4-didehydro-L-proline (25).[39] A mixture of selenide **24** (50.0 mg, 0.103 mmol) and CH_2Cl_2 (1.3 mL) was initially cooled to 0 °C in an ice bath. Pyridine (12.4 μL, 0.153 mmol) was added dropwise to this solution. A solution of 30% aqueous H_2O_2 (26.0 μL, 0.253 mmol) was then gradually added over a 5 min period. The reaction was stirred at rt for 1 h and then diluted with EtOAc (25 mL). The organic phase was washed with 5% HCl aqueous (2 × 2 mL), saturated aqueous Na_2CO_3 (2 mL), and H_2O (3 × 2 mL). The resulting solution was dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash column chromatography eluting with acetone:chloroform (2:98) to give the product (24.6 mg, 73% yield) as an oil: R_f 0.61 (5:95 acetone:chloroform); ^1H NMR (500 MHz, $CDCl_3$) δ 0.10 (s, 3H), 0.13 (s, 3H), 0.91 (s, 9H), 1.26 (t, J = 7.2 Hz, 3H), 1.40 (d, J = 6.7 Hz, 3H), 4.18 (q, J = 7.1 Hz, 2H), 4.40–4.59 (m, 3H), 5.23 (td, J = 2.2, 4.4 Hz, 1H), 5.81–5.84 (m, 1H), 6.00 (dd, J = 2.1, 4.2 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.2, −5.0, 14.1, 18.2, 20.6, 25.8, 53.4, 61.2, 67.2, 70.1, 124.4, 128.8, 169.5, 172.0; IR ($CHCl_3$) 3620 (m), 3350–3550 (w), 2960–2990 (s), 2940 (s), 2900 (m), 2860 (m), 1745 (s), 1645 (s), 1620 (m), 1460 (m), 1445 (m), 1425 (s), 1390 (m), 1370 (m), 1340 (m), 1300 (w), 1185–1255 (s), 1135 (s), 1095 (m), 1080 (m), 1045 (s), 1030 (s), 950 (m), 915 (w), 875 (m), 835 (s) cm^{-1}; HRMS m/z calcd for $C_{16}H_{30}NSiO_4$ (M + H) 328.1944, found 328.1958; $[\alpha]^{20}_D$ −162° (c = 1.39, $CHCl_3$). To a solution of the above ester (70.3 mg, 0.215 mmol) in THF (5 mL), cooled to 0 °C, was added a cold 0.10 M aqueous solution of LiOH (5.00 mL) dropwise over a 5 min period. The reaction was stirred at rt for 18 h. The resulting solution was concentrated to one-half volume, and the aqueous phase was washed with Et_2O (2 × 5 mL). The Et_2O layers were extracted with saturated aqueous $NaHCO_3$ (15 mL). The aqueous layers were combined and acidified to pH 4 with 1 M aqueous $KHSO_4$. This solution was extracted with EtOAc (3 × 50 mL), and the organic layers were dried ($MgSO_4$), filtered, and concentrated to provide **25** (57.2 mg, 89% yield) as an oil: R_f 0.30 (10:90 methanol:chloroform); ^1H NMR (500 MHz, $CDCl_3$) δ 0.09 (s, 3H), 0.10 (s, 3H), 0.90 (s, 9H), 1.40 (d, J = 6.7 Hz, 3H), 4.46–4.57 (m, 3H), 5.29 (dd, J = 2.2, 4.9 Hz, 1H), 5.89–5.91 (m, 1H), 6.01–6.03 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.0, −4.9, 18.2, 20.4, 25.8, 53.6, 67.2, 70.2, 124.2, 128.7, 172.9, 173.3; IR ($CHCl_3$) 2380–3460 (w), 3060 (m), 3020 (m), 2980 (s), 2960 (s), 2880 (m), 1740 (s), 1650 (s), 1630 (m), 1470 (s), 1435 (s), 1360–1380 (m), 1310 (w), 1265 (s), 1195 (m), 1145 (s), 1105 (m), 1010 (m), 945 (m), 915 (m), 840 (s), 665–690 (m) cm^{-1}; HRMS m/z calcd for $C_{14}H_{26}NSiO_4$ (M + H) 300.1631, found 300.1647; $[\alpha]^{20}_D$ −112° (c = 1.80, $CHCl_3$).

2-(Trimethylsilyl)ethyl (2*S*)-2-[[(*tert*-Butyldimethylsilyl)oxy]lactyl]-3,4-didehydro-L-prolyl-*N*-methyl-D-leucinate (26). To a cooled solution of **25** (127 mg, 0.424 mmol) and CH_2Cl_2 (6 mL) at − 15 °C (ethylene glycol–dry ice) was added BOP-Cl (128 mg, 0.502 mmol), followed by NMM (56.0 μL, 0.510 mmol). After the solution was stirred for 30 min at this temperature, a cold solution of **15** (94.6 mg, 0.386 mmol) in CH_2Cl_2 (1 mL) was added dropwise. The mixture was concentrated to one-half volume keeping the temperature below 0 °C. Another equivalent of NMM (56.0 μL, 0.510 mmol) was added dropwise and the reaction stirred at 0 °C for 5 h. The mixture was diluted with Et_2O (80 mL) and the organic layer washed with 10% aqueous HCl (8 mL), 5% aqueous $NaHCO_3$ (8 mL), and saturated aqueous NaCl (8 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated. The product was purified by flash column chromatography eluting with acetone:chloroform (12:88) to obtain a clear oil (**26**, 120 mg, 59% yield): R_f 0.41 (50:1 methylene chloride:acetone); ^1H NMR (500 MHz, $CDCl_3$) δ 0.03–0.15 (m, 15H), 0.90 (s, 9H), 0.88–1.05 (m, 8H), 1.40 (d, J = 6.6 Hz, 3H), 1.44–1.52 (m, 1H), 1.67–1.87 (m, 2H), 3.14 and 2.85 (s, 3H), 4.10–

4.25 (m, 2H), 4.41–4.60 (m, 3H), 5.05 and 4.69 (t, J = 7.9 Hz, 1H), 5.63 (dd, J = 2.0, 5.0 Hz, 1H), 5.78 (ddd, J = 2.0, 6.2, 32.9 Hz, 1H), 5.99–6.12 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −4.9, −4.6, −1.6, 17.3, 20.5, 21.5, 23.1, 24.9, 25.8, 29.3, 31.8, 37.5, 53.5, 55.7, 63.3, 65.2, 69.6, 124.1, 129.0, 169.9, 171.1, 171.2 (rotamers present in NMR); IR ($CHCl_3$) 3620 (m), 3370–3520 (w), 2990 (s), 2900 (m), 2390–2440 (w), 1725 (m), 1660 (m), 1390–1445 (m), 1200–1255 (s), 1130 (m), 1045 (s), 950 (w), 920 (w), 870 (m), 850 (m), 830 (m) cm^{-1}; HRMS m/z calcd for $C_{26}H_{51}N_2Si_2O_5$ (M + H) 527.3336, found 527.3349; $[\alpha]^{20}_D$ −57.2° (c = 0.945, $CHCl_3$).

Cyclo[*N*-(*N*-L-lactyl-3,4-didehydro-L-prolyl-*N*-methyl-D-leucyl)-*O*-[[*N*-[(2*S*,3*S*,4*S*)-4-[[(3*S*,4*R*,5*S*)-4-amino-3-hydroxy-5-methylheptanoyl]oxy]-3-oxo-2,5-dimethylhexanoyl]-L-leucyl]-L-prolyl-*N*,*O*-dimethyl-L-tyrosyl]-L-threonyl] (1h). A solution of **26** (92.0 mg, 175 μmol) in THF (2 mL) was cooled to 0 °C, and a 1.0 M solution of TBAF in THF (0.573 mL) was added dropwise. After the reaction stirred for 1 h at 0 °C and 1.5 h at rt, it was diluted with saturated aqueous NaCl (1.0 mL). The mixture was concentrated *in vacuo*, and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with 5% aqueous HCl (2 mL) and saturated aqueous NaCl (2 mL), dried (Na_2SO_4), filtered, and concentrated. The crude acid **27** (~95 mg) was contaminated with a tetrabutylammonium byproduct which could not be removed by recrystallization, trituration, or HPLC purification. However, this impurity did not interfere with the subsequent coupling step. A solution of the macrocycle HCl salt (**1d**, 25.0 mg, 29.3 μmol), crude acid **27** (13.7 mg, 44.0 μmol), CH_2Cl_2 (1.00 mL), BOP reagent (19.5 mg, 44.0 μmol), and NMM (8.10 μL, 73.3 μmol) was prepared according to the procedure described for compound **1f**. The reaction was stirred at 0 °C for 0.5 h and then at rt for 3 h before the usual workup procedure. The crude residue was purified by flash column chromatography eluting with methanol:chloroform (4:96), followed by a second column with EtOAc:petroleum ether (90:10) followed by recrystallization from $CHCl_3$:hexane to obtain the pure product (**1h**) as a white solid (27.4 mg, 84% yield): mp 167–168 °C; R_f 0.41 (10:90 methanol:chloroform); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85–0.97 (m, 24H), 1.17–1.27 (m, 3H), 1.32 (d, J = 6.9 Hz, 3H), 1.40 (d, J = 6.3 Hz, 3H), 1.43 (d, J = 6.7 Hz, 3H), 1.45 (d, J = 6.5 Hz, 1H), 1.46–1.50 (m, 1H), 1.61 (t, J = 11.6 Hz, 1H), 1.68–1.73 (m, 1H), 1.74–1.79 (m, 1H), 1.82–1.88 (m, 2H), 2.02–2.08 (m, 1H), 2.11–2.17 (m, 2H), 2.34–2.37 (m, 1H), 2.57 (s, 3H), 2.63 (dd, J = 10.6, 17.0 Hz, 1H), 2.95 (d, J = 4.8 Hz, 1H), 3.18 (dd, J = 10.9, 14.2 Hz, 1H), 3.23 (s, 3H), 3.28 (d, J = 15.8 Hz, 1H), 3.33 (d, J = 9.9 Hz, 1H), 3.39 (dd, J = 4.2, 14.3 Hz, 1H), 3.57–3.61 (m, 2H), 3.69–3.71 (m, 1H), 3.80 (s, 3H), 4.05–4.14 (m, 2H), 4.24 (q, J = 6.9 Hz, 1H), 4.36–4.48 (m, 3H), 4.56 (dd, J = 2.1, 5.1 Hz, 1H), 4.65 (dd, J = 5.0, 8.0 Hz, 1H), 4.81 (t, J = 7.0 Hz, 1H), 5.19 (d, J = 3.5 Hz, 1H), 5.35 (dd, J = 4.1, 11.2 Hz, 1H), 5.42 (dd, J = 2.3, 6.4 Hz, 1H), 5.61 (dd, J = 2.1, 4.7 Hz, 1H), 5.73–5.75 (m, 1H), 6.11 (dd, J = 2.0, 6.4 Hz, 1H), 6.85 (d, J = 8.6 Hz, 2H), 7.08 (d, J = 8.6 Hz, 2H), 7.20 (d, J = 9.9 Hz, 1H), 7.54 (d, J = 5.2 Hz, 1H), 7.78 (d, J = 9.3 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.7, 14.7, 15.2, 16.3, 16.9, 18.6, 20.1, 20.9, 21.4, 23.3, 23.7, 24.8, 24.9, 25.0, 25.1, 27.2, 27.9, 31.2, 31.3, 33.9, 34.0, 36.2, 38.7, 38.8, 41.3, 46.9, 49.46, 49.52, 53.0, 55.3, 55.4, 57.2, 57.6, 63.9, 65.9, 66.5, 67.9, 70.4, 81.5, 114.1, 124.0, 129.1, 130.0, 130.3, 158.6, 168.5, 169.3, 169.6, 170.5, 170.6, 171.3, 171.5, 172.4, 173.9, 204.9; IR ($CHCl_3$) 3780 (w), 3420–3550 (w), 3340 (m), 3040 (w), 2980 (s), 2940 (s), 2880 (m), 2400–2460 (w), 1740 (s), 1665 (s), 1650 (s), 1550 (m), 1535 (m), 1520 (m), 1465 (s), 1410 (m), 1380 (m), 1350 (w), 1305 (m), 1270 (m), 1250 (s), 1175 (m), 1125 (m), 1110 (w), 1095 (w), 1080 (m), 1040 (m), 1010 (w), 965 (w), 925 (w), 890 (w), 845 (w) cm^{-1}; HRMS m/z calcd for $C_{57}H_{88}N_7O_{15}$ (M + H) 1110.6338, found 1110.6371; $[\alpha]^{20}_D$ −124° (c = 0.875, $CHCl_3$). Anal. Calcd for $C_{57}H_{87}N_7O_{15}\cdot H_2O$: C, 60.67; H, 7.95; N, 8.69. Found: C, 60.97; H, 7.97; N, 8.19.

Ethyl *N*-Cbz-4-*cis*-(phenylselenenyl)-L-prolinate (28).[39] Following the procedure described for mesylation (compound **24**), alcohol **20** (0.586 g, 2.19 mmol), CH_2Cl_2 (22 mL), Et_3N (0.336 mL, 2.41 mmol), mesyl chloride (MsCl, 0.220 mL, 2.85 mmol), and DMAP (80.3 mg, 0.657 mmol) were combined. After being stirred for 1 h at 0 °C, the solution was warmed to ambient temperature and stirred for another 2 h. After workup, the product was purified by flash column chromatography eluting first with acetone:hexane (5:95) and then with acetone:chloroform (2:98) to provide a clear oil (0.749 g, 99% yield): R_f 0.41 (50:50 EtOAc:petroleum ether); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.26–2.32 (m, 1H), 2.60–2.70 (m, 1H), 3.02 (d, J = 10.9 Hz, 3H), 3.56 and 3.77 (s, 3H), 3.79–3.84 (m, 1H), 3.92 (dd, J = 12.8, 37.3 Hz, 1H), 4.52 (dt, J = 7.8, 20.5 Hz, 1H), 5.03–5.28 (m, 3H), 7.30–7.36 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 36.3, 37.4, 38.6, 38.7, 52.3, 52.6, 52.7, 57.2, 57.4, 67.5, 77.5, 77.8, 127.9, 128.0, 128.1, 128.2, 128.4, 128.5, 135.95, 136.05, 153.9, 154.4, 172.1, 172.2 (rotamers present in NMR); IR ($CHCl_3$) 3770 (w), 3500–3570 (w), 3020 (m), 2960 (m), 2890 (m), 2850 (w), 2440 (w), 2250 (w), 1950 (w), 1695–1765 (s), 1605 (w), 1585 (w), 1495 (m), 1415 (s), 1350 (s), 1285 (m), 1265 (m), 1235 (m), 1170 (s), 1125 (s), 1050 (m), 1010 (m), 950–970 (s), 900 (s), 835 (m) cm^{-1}; HRMS m/z calcd for $C_{15}H_{19}NO_7S$ (M^+) 357.0855, found 357.0882; $[\alpha]^{20}_D$ −43.2° (c = 0.795, $CHCl_3$). A mixture of diphenyl selenide (56.3 mg, 0.180 mmol), absolute EtOH (4.5 mL), $NaBH_4$ (14.1 mg, 3.73 mmol), and the intermediate mesylate (100 mg, 0.290 mmol) was then reacted as previously described. The crude product was purified by flash column chromatography eluting with EtOAc:petroleum ether (20:80) to afford **28** as a yellow oil (106 mg, 90% yield): R_f 0.44 (20:80 EtOAc:petroleum ether); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.11 and 1.25 (t, J = 7.1 Hz, 3H), 2.08 (dt, J = 8.7, 13.0 Hz, 1H), 2.69 (dd, J = 7.0, 13.4 Hz, 1H), 3.50 and 3.59 (dd, J = 8.5, 19.3 Hz, 2H), 3.97–4.08 (m, 2H), 4.20 (q, J = 7.1 Hz, 1H), 4.33 (dt, J = 7.7, 20.5 Hz, 1H), 5.02–5.18 (m, 2H), 7.25–7.35 (m, 8H), 7.53–7.55 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.9, 14.0, 36.4, 37.1, 37.2, 37.9, 52.9, 53.5, 58.9, 59.1, 61.2, 61.3, 67.1, 127.76, 127.83, 127.91, 127.95, 128.0, 128.1, 128.2, 128.3, 128.4, 129.2, 134.8, 134.9, 135.1, 135.2, 136.2, 136.4, 153.8, 154.2, 171.5, 171.8 (rotamers present in NMR); IR (neat) 3040–3070 (m), 2990 (m), 2880 (m), 1745 (s), 1710 (s), 1580 (m), 1530 (w), 1500 (w), 1470–1480 (m), 1440 (m), 1415 (s), 1355 (s), 1295 (m), 1255 (m), 1195 (s), 1165 (m), 1110 (m), 1075 (w), 1030 (m), 1000 (w), 965 (w), 915 (w), 860 (w) cm^{-1}; HRMS m/z calcd for $C_{21}H_{24}NO_4Se$ (M + H) 434.0870, found 434.0855; $[\alpha]^{20}_D$ −18.0° (c = 1.87, $CHCl_3$).

***N*-Cbz-3,4-didehydro-L-proline (29).**[39] The elimination reaction used to obtain compound **25** was employed to provide **29**, using selenide **28** (93.4 mg, 0.222 mmol), CH_2Cl_2 (3 mL), pyridine (26.8 μL, 0.331 mmol), and 30% aqueous H_2O_2 (56.8 μL). The product was purified by flash column chromatography eluting with EtOAc:petroleum ether (15:85) to give the product as a clear oil (44.0 mg, 75% yield): R_f 0.59 (30:70 EtOAc:petroleum ether); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.14 and 1.27 (t, J = 7.1 Hz, 3H), 4.06 and 4.21 (t and dd, J = 7.3 and 7.1, 14.3 Hz, 2H), 4.25–4.38 (m, 2H), 5.03–5.23 (m, 3H), 5.76 (dddd, J = 2.2, 4.5, 8.5, 24.0 Hz, 1H), 5.97 (dddd, J = 2.0, 4.1, 8.3, 26.2 Hz, 1H), 7.28–7.39 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.0, 14.1, 53.4, 53.9, 61.3, 61.4, 66.4, 66.7, 67.1, 124.77, 124.80, 127.77, 127.86, 127.9, 128.0, 128.4, 128.9, 129.0, 129.2, 136.4, 136.6, 153.9, 154.3, 169.8, 170.1 (rotamers present in NMR); IR (neat) 3070–3100 (w), 3040 (w), 2920–2990 (m), 2880 (m), 1760 (s), 1715 (s), 1625 (w), 1590 (w), 1525–1550 (w), 1500 (m), 1470 (m), 1450 (m), 1415 (s), 1370–1330 (m), 1310 (m), 1260 (m), 1190 (s), 1095–1130 (s), 1080 (m), 1030 (m), 1010 (m), 975 (m), 930–955 (m), 820–840 (w) cm^{-1}; HRMS m/z calcd for $C_{15}H_{18}NO_4$ (M + H) 276.1236, found 276.1199; $[\alpha]^{20}_D$ −104° (c = 1.72, $CHCl_3$). A mixture of the previously prepared dehydroproline ester (34.4 mg, 0.131 mmol) and THF (3.6 mL) was cooled to 0 °C. A cooled 0.10 M aqueous solution of LiOH (3.6 mL) was added over a 5 min period, and the resulting mixture was stirred for 18 h until completion. The mixture was then concentrated to one-half its volume and extracted with Et_2O (2 × 3 mL). The organic layers were combined and extracted with saturated aqueous $NaHCO_3$ (7 mL). The aqueous layers were acidified with 1 M aqueous $KHSO_4$ to pH 2 and extracted with EtOAc (3 × 20 mL). The combined EtOAc layers were dried ($MgSO_4$), filtered, and concentrated to give **29** (30.6 mg, 99% yield) as a clear oil: R_f 0.30 (10:90 methanol:chloroform); ^{1}H NMR (500 MHz,

$CDCl_3$) δ 4.31 (dd, J = 1.7, 16.3 Hz, 2H), 5.08–5.25 (m, 3H), 5.82 (ddd, J = 2.1, 6.1, 45.9 Hz, 1H), 6.01 (ddd, J = 1.8, 6.2, 28.3 Hz, 1H), 7.26–7.38 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 53.5, 53.9, 66.0, 66.5, 67.3, 67.7, 124.3, 124.5, 127.7, 128.0, 128.2, 128.4, 128.5, 128.9, 129.6, 136.1, 154.0, 155.3, 173.6, 175.0; IR (neat) 2400–3600 (m), 2930 (s), 2880 (s), 1665–1760 (s), 1620 (m), 1590 (w), 1500 (m), 1425 (s), 1360 (s), 1345 (m), 1320 (m), 1250 (m), 1235–1170 (s), 1130 (s), 1080 (w), 1030 (w), 1005 (m), 975 (m), 910–955 (w), 885 (w), 815 (w) cm^{-1}; HRMS *m/z* calcd for $C_{13}H_{17}N_2O_4$ (M + NH_4) 265.1189, found 265.1194; $[\alpha]^{20}_D$ −116.9° (*c* = 1.61, $CHCl_3$).

***N*-Cbz-3,4-didehydro-L-prolyl-*N*-methyl-D-leucine (32).** The BOP-Cl activation protocol as described to prepare compound **26** was used to couple acid **29** (30.6, 0.124 mmol) with **15** (45.6 mg, 0.186 mmol) using CH_2Cl_2 (2 mL), BOP-Cl (37.9 mg, 0.149 mmol), and NMM (32.8 μL, 0.298 μmol). After the solution was stirred for 6 h at 0 °C, the reaction was worked up using the previously reported conditions. The crude residue was purified by flash column chromatography eluting with acetone:chloroform (5:95) to provide a beige solid (55.4 mg, 94% yield): mp 81–82 °C; R_f 0.60 (5:95 acetone:chloroform); ^1H NMR (500 MHz, $CDCl_3$) δ 0.02–0.07 (m, 9H), 0.71 (t, J = 6.6 Hz, 1H), 0.81–1.05 (m, 7H), 1.42–1.53 (m, 1H), 1.64–1.80 (m, 2H), 3.01 and 3.10 (s, 3H), 4.10–4.43 (m, 4H), 4.90–5.36 (m, 3H), 5.43–5.49 (m, 1H), 5.71 and 5.74–5.81 (tdd and m, J = 2.1, 4.3, 8.5 Hz, 1H), 5.95–6.04 (m, 1H), 7.27–7.38 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −1.58, −1.56, 17.39, 17.44, 21.2, 21.5, 22.8, 23.2, 25.0, 25.1, 37.5, 37.6, 53.5, 54.1, 55.5, 55.7, 63.3, 63.5, 65.17, 65.21, 65.4, 66.9, 67.0, 67.3, 124.1, 124.5, 127.6, 127.7, 127.8, 127.9, 128.28, 128.30, 128.36, 128.40, 129.2, 129.4, 136.8, 154.0, 154.2, 170.2, 170.3, 171.5, 171.6 (rotamers present in NMR); IR (neat) 3070 (w), 3040 (w), 2970 (s), 2940 (m), 1720 (s), 1670 (s), 1620 (m), 1590 (w), 1440–1485 (m), 1415 (s), 1360 (s), 1315 (s), 1250 (s), 1215 (s), 1180 (s), 1100–1140 (s), 1060 (m), 1045 (m), 980 (m), 935–955 (m), 860 (s), 840 (s) cm^{-1}; HRMS *m/z* calcd for $C_{25}H_{39}N_2SiO_5$ (M + H) 475.2628, found 475.2613; $[\alpha]^{20}_D$ −59.1° (*c* = 1.15, $CHCl_3$). Anal. Calcd for $C_{25}H_{38}N_2SiO_5$: C, 63.26; H, 8.07; N, 5.90. Found: C, 62.99; H, 8.35; N, 5.93. To a mixture of the fully protected dehydroproline dipeptide (96.2 mg, 0.203 mmol) in THF (4 mL) cooled to 0 °C was added a 1.0 M solution of TBAF in THF (0.332 mL). The reaction mixture was stirred for 1 h at 0 °C and then quenched with saturated NaCl solution (2 mL). The solvent was distilled *in vacuo*, and the resulting slurry was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with 5% aqueous HCl (3 mL) and saturated aqueous NaCl (3 mL). The resulting organic phase was dried (Na_2SO_4), filtered, and concentrated. The crude solid was triturated with 20% CH_3CN:H_2O to afford **32** as a white solid (57.0 mg, 75% yield): mp 166–168 °C; R_f 0.49 (10:90 methanol:chloroform); ^1H NMR (500 MHz, $CDCl_3$) δ 0.70–1.05 (m, 6H), 1.42–1.52 (m, 1H), 1.63–1.85 (m, 2H), 3.03 and 3.10 (s, 3H), 3.98 (bs, 1H), 4.29 (dt, J = 2.5, 13.3 Hz, 1H), 4.35–4.41 (m, 1H), 4.96–5.28 (m, 3H), 5.38 and 5.46–5.49 (d and m, J = 2.9 Hz, 1H), 5.69–5.85 (m, 1H), 5.97–6.06 (m, 1H), 7.28–7.37 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.0, 21.4, 22.7, 22.9, 24.0, 24.7, 29.3, 31.2, 37.2, 37.9, 53.3, 53.7, 64.9, 65.1, 67.1, 67.4, 123.3, 123.6, 124.1, 124.2, 127.5, 127.6, 127.7, 128.1, 128.2, 128.9, 129.2, 129.3, 136.1, 136.2, 154.1, 154.5, 170.2, 170.3, 172.2, 173.1 (rotamers present in NMR); IR ($CHCl_3$) 2600–3650 (s), 3010 (m), 2970 (m), 2880 (m), 1710 (s), 1665 (m), 1470 (m), 1420 (m), 1365 (m), 1345 (w), 1315 (w), 1235–1265 (m), 1190 (w), 1130 (m), 1110 (m), 1040 (w), 1005 (w), 970 (w), 920 (w), 880 (w) cm^{-1}; HRMS *m/z* calcd for $C_{20}H_{27}N_2O_5$ (M + H) 375.1920, found 375.1907; $[\alpha]^{20}_D$ −112° (*c* = 0.825, $CHCl_3$).

Cyclo[(*N*-L-prolyl-*N*-methyl-D-leucyl)-*O*-[[*N*-[(2*S*,3*S*,4*S*)-4-[[(3*S*,4*R*,5*S*)-4-amino-3-hydroxy-5-methylheptanoyl]oxy]-3-oxo-2,5-dimethylhexanoyl]-L-leucyl]-L-prolyl-*N*,*O*-dimethyl-L-tyrosyl]-L-threonyl] (1j). The crude acid (**32**, 19.1 mg, 51.0 μmol) was combined with the macrocycle HCl salt (**1d**, 29.0 mg, 34.0 μmol), CH_2Cl_2 (1 mL), BOP reagent (22.6 mg, 51.0 μmol), and NMM (9.30 μL, 85.0 μmol) as previously described (procedure for compound **1f**). The crude residue was purified by flash column chromatography eluting with methanol:chloroform (3:97) to give Cbz protected dehydroproline analog **1j** as a waxy solid (36.8 mg, 92% yield): R_f 0.63 (10:90 methanol:chloroform); ^1H NMR (500 MHz, $CDCl_3$) δ 0.83–1.00 (m, 24H), 1.12–1.26 (m, 2H), 1.34 (d, J = 6.9 Hz, 3H), 1.37 (d, J = 6.2 Hz, 3H), 1.40–1.45 (m, 3H), 1.51–1.54 (m, 1H), 1.58–1.63 (m, 1H), 1.67–1.80 (m, 2H), 1.81–1.85 (m, 1H), 2.02–2.07 (m, 1H), 2.10–2.20 (m, 2H), 2.34–2.39 (m, 1H), 2.60 (s, 3H), 2.60–2.65 (m, 1H), 2.98 (d, J = 4.8 Hz, 1H), 3.15 (dd, J = 10.4, 14.1 Hz, 1H), 3.22 (s, 3H), 3.27 (d, J = 16.0 Hz, 1H), 3.37 (dd, J = 4.5, 14.4 Hz, 1H), 3.56 (dd, J = 4.4, 10.4 Hz, 1H), 3.58–3.62 (m, 1H), 3.69 (dd, J = 9.5, 15.5 Hz, 1H), 3.79 (s, 3H), 4.06–4.12 (m, 2H), 4.21–4.27 (m, 2H), 4.48 (dd, J = 5.6, 15.3 Hz, 1H), 4.56 (dd, J = 2.1, 5.5 Hz, 1H), 4.63 (dd, J = 5.1, 7.8 Hz, 1H), 4.81 (t, J = 9.9 Hz, 1H), 5.16–5.20 (m, 2H), 5.34 (dd, J = 2.2, 6.3 Hz, 1H), 5.40–5.44 (m, 2H), 5.49 (d, J = 2.9 Hz, 1H), 5.65–5.66 (m, 1H), 6.08 (dd, J = 1.9, 6.4 Hz, 1H), 6.83 (d, J = 8.5 Hz, 2H), 7.09 (d, J = 8.5 Hz, 2H), 7.17 (d, J = 9.9 Hz, 1H), 7.28–7.36 (m, 3H), 7.43 (d, J = 7.2 Hz, 2H), 7.56 (d, J = 5.6 Hz, 1H), 7.86 (d, J = 9.2 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.3, 14.7, 15.2, 16.4, 16.9, 18.6, 20.9, 21.4, 23.4, 23.9, 24.7, 24.9, 25.0, 25.1, 27.1, 27.9, 31.3, 31.4, 33.9, 34.0, 36.3, 38.6, 38.8, 41.2, 47.0, 49.5, 53.5, 55.1, 55.2, 55.4, 57.1, 57.9, 63.1, 66.4, 67.6, 68.0, 70.6, 81.4, 114.0, 124.1, 127.6, 127.8, 128.3, 130.00, 130.03, 130.3, 136.6, 154.8, 158.6, 168.1, 169.5, 169.6, 170.4, 170.5, 171.1, 171.7, 172.4, 204.9; IR ($CHCl_3$) 3350–3620 (w), 3330 (m), 3040 (m), 2970 (m), 2880 (m), 1730 (s), 1660 (s), 1645 (s), 1510 (m), 1415–1455 (m), 1360 (w), 1345 (m) 1300–1320 (w), 1200–1255 (m), 1170 (m), 1135 (w), 1070 (w), 1030 (w), 920 (w), 820 (w) cm^{-1}; HRMS *m/z* calcd for $C_{62}H_{90}N_7O_{15}$ (M + H) 1172.6495, found 1172.6571; $[\alpha]^{20}_D$ −120° (*c* = 1.12, $CHCl_3$). Under a hydrogen atmosphere (40 psi) a solution of the Cbz-protected dehydroproline analog **1j** (33.0 mg, 28.1 μmol), 10% Pd/C (6.00 mg, 281 μmol), and MeOH:EtOAc (1:1, 2.0 mL) was shaken in a Parr hydrogenator for 12 h. The slurry was filtered through Celite, followed by thorough washing with MeOH:EtOAc, and the filtrate was concentrated *in vacuo*. The residue was azeotroped with toluene several times followed by recrystallization from $CHCl_3$:hexane to afford the product (**1j**) as a white solid (28.1 mg, 96% yield): mp 164–165 °C; R_f 0.21 (10:90 methanol:chloroform); ^1H NMR (500 MHz, $CDCl_3$) δ 0.83–0.98 (m, 24H), 1.18–1.26 (m, 2H), 1.24 (d, J = 6.4 Hz, 3H), 1.29 (d, J = 6.2 Hz, 3H), 1.35–1.38 (m, 4H), 1.39–1.52 (m, 1H), 1.56–1.72 (m, 2H), 1.64 (t, J = 12.8 Hz, 2H), 1.75–1.82 (m, 2H), 1.83–1.90 (m, 1H), 2.02–2.07 (m, 1H), 2.08–2.18 (m, 3H), 2.29–2.35 (m, 1H), 2.55 (d, J = 6.0 Hz, 3H), 2.56–2.70 (m, 2H), 2.97 (d, J = 10.1 Hz, 3H), 2.96–3.31 (m, 5H), 3.34–3.37 (m, 1H), 3.53–3.63 (m, 2H), 3.71–3.77 (m, 1H), 3.80 (s, 3H), 3.96–4.12 (m, 2H), 4.17–4.22 (m, 1H), 4.58 (dd, J = 5.9, 12.6 Hz, 1H), 4.75–4.89 (m, 2H), 5.08 (ddd, J = 2.4, 6.0, 31.1 Hz, 1H), 5.18 (dd, J = 3.0, 6.3 Hz, 1H), 5.21 (t, J = 6.4 Hz, 1H), 6.85 (dd, J = 5.9, 8.2 Hz, 2H), 7.08 (t, J = 7.7 Hz, 2H), 7.44 (d, J = 10.2 Hz, 1H), 7.93 (d, J = 8.9 Hz, 1H), 7.98 (d, J = 9.2 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.5, 15.00, 15.03, 15.2, 15.6, 15.9, 16.9, 18.5, 20.9, 21.0, 22.1, 22.6, 23.0, 23.1, 23.7, 24.5, 24.8, 25.1, 26.8, 27.9, 31.3, 33.9, 34.2, 38.6, 41.5, 47.0, 49.6, 49.7, 55.2, 55.5, 57.3, 57.4, 58.2, 66.2, 68.0, 70.7, 71.1, 81.5, 125.3, 128.2, 129.0, 130.1, 158.6, 168.3, 169.5, 169.7, 170.0, 170.5, 171.2, 171.7, 172.4, 204.7; IR ($CHCl_3$) 3680 (w), 3200–3620 (w), 3340 (m), 3030 (m), 2970 (s), 2880 (m), 2400 (w) 1735 (s), 1660 (s), 1640 (s), 1550 (m), 1530 (m), 1515 (m), 1465 (m), 1450 (m), 1415 (w), 1390 (w), 1370 (w), 1345 (w), 1335 (w), 1320 (w), 1305 (w), 1270 (m), 1240 (m), 1170 (m), 1120 (w), 1110 (w) 1075 (w), 1035 (w), 965 (w), 925 (w), 910 (w) cm^{-1}; HRMS *m/z* calcd for $C_{54}H_{85}N_7O_{13}Na$ (M + Na) 1062.6102, found 1062.6163; $[\alpha]^{20}_D$ −84.0° (*c* = 1.40, $CHCl_3$). Anal. Calcd for $C_{54}H_{85}N_7O_{13}{\cdot}2H_2O$: C, 60.26; H, 8.33; N, 9.11. Found: C, 60.57; H, 8.47; N, 8.77.

Methyl *N*-Boc-3,4-didehydro-L-prolinate (30):[46] R_f 0.31 (20:80 EtOAc:petroleum ether); ^1H NMR (250 MHz, $CDCl_3$) δ 1.46 (d, J = 12.0 Hz, 9H), 3.73 (s, 3H), 4.12–4.35 (m, 2H), 4.88–5.09 (m, 1H), 5.65–5.80 (m, 1H), 5.88–6.03 (m, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 28.6, 52.3, 53.7, 66.9, 80.8, 125.2, 129.7, 153.8, 171.3 (rotamers present in NMR); IR ($CHCl_3$) 3790 (w), 3330–3620 (w), 3040 (m), 3010 (m), 2990 (m), 2960

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(m), 2400 (w), 1740 (s), 1700 (s), 1625 (w), 1480 (w), 1440 (m), 1390 (s), 1370 (s), 1330 (m), 1230–1255 (m), 1160 (s), 1240 (s), 1065 (m), 1020 (m), 970 (w), 925 (w), 905 (w), 850 (w) cm^{-1}; HRMS *m/z* calcd for $C_{11}H_{18}NO_4$ (M + H) 228.1236, found 228.1247; $[\alpha]^{20}_D$ −105° (*c* = 1.62, $CHCl_3$).

***N*-Boc-3,4-didehydro-L-proline (31).** Methyl ester **30** (0.600 g, 2.64 mmol) was dissolved in THF (25 mL) and the solution cooled to 0 °C. To this solution was added a cooled 0.10 M aqueous solution of LiOH (25.0 mL) dropwise over a 10 min period. After being stirred at rt for 18 h, the reaction was concentrated to one-half volume. The residue was washed with Et_2O (2 × 10 mL), and the organic layers were extracted with saturated aqueous $NaHCO_3$ (15 mL). The aqueous layers were combined and acidified to pH 4 with 1 M aqueous $KHSO_4$ and then extracted with EtOAc (3 × 75 mL). The resulting organic phase was dried ($MgSO_4$), filtered, and concentrated to yield the product (**31**) as a solid (0.552 g, 98% yield): mp 93–94 °C; R_f 0.49 (10:90 methanol:chloroform); ^1H NMR (500 MHz, $CDCl_3$) δ 1.47 (d, *J* = 27.4 Hz, 9H), 4.20–4.31 (m, 2H), 5.03 (d, *J* = 54.0 Hz, 1H), 5.80 (dd, *J* = 4.2, 51.7 Hz, 1H), 5.99 (dd, *J* = 4.5, 29.4 Hz, 1H), 9.36 (bs, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.2, 28.4, 53.3, 53.7, 66.2, 66.3, 80.7, 81.3, 124.3, 124.6, 128.8, 129.7, 153.5, 155.2, 173.9, 175.8 (rotamers present in NMR); IR ($CHCl_3$) 2420–3440 (m), 3030 (m), 2990 (m), 2940 (m), 2880 (m), 1730 (s), 1700 (s), 1620 (m), 1480 (m), 1410 (s), 1370 (s), 1350 (m), 1310–1335 (m), 1260 (m), 1230 (m), 1175 (s), 1135 (s), 1110 (m), 1035 (w), 1005 (w), 960 (m), 930 (w), 890 (m), 860 (w), 820 (w) cm^{-1}; HRMS *m/z* calcd for $C_{10}H_{16}NO_4$ (M + H): 214.1079, found 214.1075; $[\alpha]^{20}_D$ −116° (*c* = 1.32, $CHCl_3$).

***N*-Boc-3,4-didehydro-L-prolyl-*N*-methyl-D-leucine (33).** BOP-Cl (0.503 g, 1.97 mmol) was added to a solution of *N*-Boc-3,4-didehydro-L-proline (**31**, 0.360 g, 1.69 mmol) in CH_2Cl_2 (25 mL) cooled to −15 °C (ethylene glycol–dry ice bath). NMM (0.205 mL, 1.86 mmol) was added dropwise, and the mixture was stirred at this temperature for 0.5 h. A cold solution of *N*-methylsilyl-protected D-leucine (0.346 g, 1.41 mmol) in CH_2Cl_2 (5 mL) was added dropwise, and the resulting slurry was concentrated to one-half volume keeping the temperature below 0 °C. Another equivalent of NMM (0.205 mL, 1.86 mmol) was added *via* a syringe and the mixture stirred at 0 °C for 6 h. The reaction was worked up as described for compound **26**. The crude product was purified twice by flash column chromatography eluting with acetone:chloroform (1:99) to give the product as a pale solid (0.391 g, 63% yield): mp 90–91 °C; R_f 0.43 (20:80 EtOAc:petroleum ether); ^1H NMR (500 MHz, $CDCl_3$) δ 0.01–0.07 (m, 9H), 0.90 (d, *J* = 6.5 Hz, 3H), 0.95 (d, *J* = 6.7 Hz, 3H), 0.89–1.04 (m, 2H), 1.44 and 1.47 (s, 9H), 1.42–1.48 (m, 1H), 1.68–1.73 (m, 2H), 3.03 and 3.07 (s, 3H), 4.10–4.40 (m, 4H), 5.12 and 5.22 (t and dd, *J* = 8.0 and 5.7, 10.2 Hz, 1H), 5.34–5.42 (m, 1H), 5.65–5.78 (m, 1H), 5.98 (ddt, *J* = 2.0, 6.3, 15.9 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −1.6, 17.5, 21.5, 23.2, 25.1, 28.2, 30.9, 37.7, 53.5, 55.1, 63.3, 65.5, 80.0, 124.1, 129.4, 153.5, 170.6, 172.1 (rotamers present in NMR); IR ($CHCl_3$) 3460 (w), 3010 (m), 2970 (s), 2880 (m), 1735 (s), 1700 (s), 1670 (s), 1625 (m), 1455–1485 (m), 1410 (s), 1370 (m), 1320 (m), 1300 (m), 1255 (s), 1180 (s), 1135 (s), 1100 (m), 1045 (m), 1010 (w), 990 (w), 965 (m), 935 (m), 885 (w), 865 (m), 840 (m) cm^{-1}; HRMS *m/z* calcd for $C_{22}H_{41}N_2SiO_5$ (M + H) 441.2774, found 441.2791; $[\alpha]^{20}_D$ −43.2° (*c* = 2.43, $CHCl_3$). To a solution of 2-(trimethylsilyl)ethyl *N*-Boc-3,4-didehydro-L-prolyl-*N*-methyl-D-leucinate (100 mg, 0.227 mmol) in THF (4 mL) cooled to 0 °C was added a 1.0 M solution of TBAF in THF (0.370 mL) dropwise. The reaction was stirred for 1 h at 0 °C and then 3 h at rt. The resulting mixture was quenched with saturated aqueous NaCl (2 mL), and the THF was distilled *in vacuo*. The aqueous layer was extracted with EtOAc (3 × 20 mL). The organic layers were combined, dried (Na_2SO_4), filtered, and concentrated. The crude residue was triturated with 20% acetonitrile:H_2O to afford **33** as a granular white solid (71.4 mg, 92% yield). Presently this compound is being coupled to the macrocycle using BOP activation, and then the Boc group will be removed with TFA to provide the fifth analog: mp 182–183 °C; R_f 0.52 (10:90 methanol:chloroform); ^1H NMR (500 MHz, $CDCl_3$) δ 0.90 (dd, *J* = 1.9, 6.5 Hz, 3H), 0.95 (dd, *J* = 6.8, 8.7 Hz, 3H), 1.45 (d, *J* = 16.7 Hz, 9H), 1.70–1.83 (m, 3H), 3.06 (d, *J* = 23.8 Hz, 3H), 4.15–4.31 (m, 2H), 5.20 (dd, *J* = 5.2, 10.4 Hz, 1H), 5.39 (ddd, *J* = 2.2, 5.5, 25.5 Hz, 1H), 5.68 (ddd, *J* = 2.1, 4.2, 16.8 Hz, 1H), 6.00–6.03 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.4, 23.15, 23.18, 24.9, 25.0, 28.2, 28.4, 31.2, 31.7, 37.0, 37.2, 53.5, 53.7, 55.4, 56.1, 64.5, 65.5, 80.4, 80.8, 123.9, 129.6, 129.8, 153.6, 154.2, 171.2, 171.4, 173.6, 175.2 (rotamers present in NMR); IR ($CHCl_3$) 2360–3470 (w), 2960 (m), 2940 (m), 2870 (m), 1695 (s), 1665 (s), 1620 (m), 1420–1430 (m), 1410 (s), 1370 (m), 1315 (w), 1300 (w), 1260 (m), 1175 (m), 1135 (m), 1095 (w), 960 (w), 880–910 (w), 860 (w), 830 (w) cm^{-1}; HRMS *m/z* calcd for $C_{17}H_{29}N_2O_5$ (M + H) 341.2076, found 341.2065; $[\alpha]^{20}_D$ −125° (*c* = 0.940, $CHCl_3$). Anal. Calcd for $C_{17}H_{28}N_2O_5$: C, 59.98; H, 8.29; N, 8.23. Found: C, 59.52; H, 8.40; N, 7.80.

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Abbreviations: (1*H*-1,3-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP); *N*-[(benzyloxy)carbonyl]-5-norbornene-2,3-dicarboximide (BCN); *N*,*N*-bis(2-oxo-3-oxazolidinyl)phosphonic chloride (BOP-Cl); 1,1-carbonyldiimidazole (CDI); diethyl ether (Et_2O); diisopropylethylamine (iPr_2NEt); diphenyl phosphorazidate (DPPA); ethyl acetate (EtOAc); α-(α-hydroxyisovaleryl)propionyl (HIP); *N*-methylmorpholine (NMM); pentafluorophenyl diphenylphosphinate (FDPP); tetrabutylammonium fluoride (TBAF); 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one (Des–Martin periodinane reagent); triethylamine (Et_3N).

Supplementary Material Available: Analytical data for the intermediates and copies of NMR spectra (60 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.