Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

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Published online: 22 Aug 2006.

To cite this article: Joshi M. Ramanjulu, Madeleine M. Joullié & Wen-Ren Li (1997) Syntheses of Acyclic Analogs of Didemnin B, Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, 27:18, 3259-3272, DOI: 10.1080/00397919708004186

To link to this article: http://dx.doi.org/10.1080/00397919708004186

SYNTHETIC COMMUNICATIONS, 27(18), 3259-3272 (1997)

SYNTHESES OF ACYCLIC ANALOGS OF DIDEMNIN B

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Abstract: *The syntheses of three modified peptide fragments of the cyclodepsipeptide didemnin B are reported. The HIP and isostatine (Ist) units of the didemnin B macrocycle were simplified to a Z-alanine residue and the ester linkage (through threonine of the tetrapeptide) was replaced with amide linkages through the amines of glycine, D-alanine and an ethylenediamine linker. The latter permitted the attachment of a N-Me-D-Leu-Pro-Lac moiety to afford analogs* ***2****,* ***3*** *and* ***4*** *respectively.*

The didemnins are a new class of cyclodepsipeptides isolated and characterized in 1981 from a Caribbean tunicate of the family *Didemnidae*. Most didemnins contain a common macrocycle and differ only in the side chains attached to the backbone through the amino group of threonine. These natural products display a wide spectrum of biological activities including antiviral, antitumor, and immunomodulatory properties.[1] Didemnin B (**1b**) was tested extensively and until recently was thought to be the most active didemnin.[2-5] However, in preliminary studies *N*-pyruvyl-*N*-prolyl didemnin A (**1c**) has

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shown some exceptional activity in all areas.[6] This compound differs from didemnin B only in the lactyl portion which is present in its oxidized form (**Figure 1**).

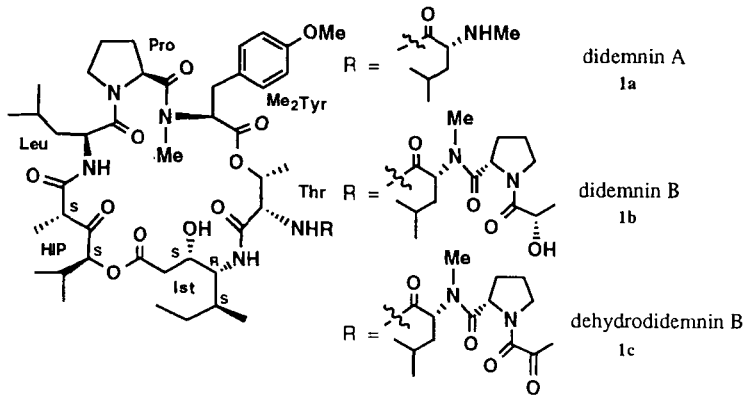


Figure 1. Some Naturally Occuring Didemnins (**1a-1c**).

Recently, a naturally occurring acyclic analog of didemnin A (**1a**) was isolated. This analog, in which the ester bond between tyrosine and threonine of the macrocycle had been cleaved, exhibited weaker cytotoxic activity (IC_{50} 0.2mg/mL).[7]

Cyclophilin (cyclosporin A binding protein) and FKBP (FK506 binding protein) catalyze the interconversion of *cis* and *trans* rotamers of peptidyl-prolyl amide bonds of peptide substrates.[8,9] However, the peptidyl prolyl *cis-trans* isomerase activities of cyclophilin and FKBP can be inhibited by their respective ligands with no cross-inhibition. Didemnin B is antiproliferative and more active than cyclosporin A but it does not bind to cyclosporin A receptor sites. Several reports show that the immunosuppressive agents FK506 and rapamycin act as leucine-(twisted amide)-proline peptidomimetics. Furthermore, these results suggest that selective inhibitors can be designed for cyclophilin,[10,11] FKBP, and other members of this type of enzyme. Based on studies of other immunosuppressors, we synthesized three analogs (**2-4**) of the Leu-Pro unit of didemnin B, which show noticeable resemblance to *cis-trans* isomerase inhibitors. All

analogs contain the altered tetrapeptide (Z-Ala-Leu-Pro-*N*,*O*-Me-Tyr) while the HIP and isostatine regions were simplified to Z-Ala. The first two analogs were obtained by replacing the ester linkage through threonine of the natural tetrapeptide with glycine amide (**2**) and D-alanine amide (**3**), whereas in the third analog (**4**) the ester linkage was replaced by an ethylenediamine linker. This unit allowed the attachment of the didemnin B side chain moiety (**Figure 2**).

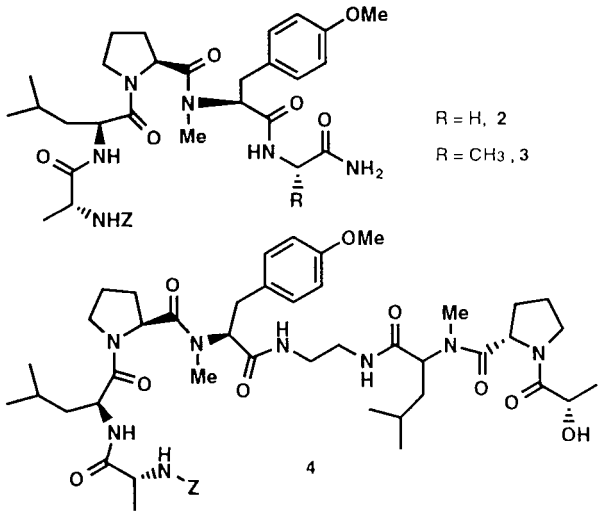
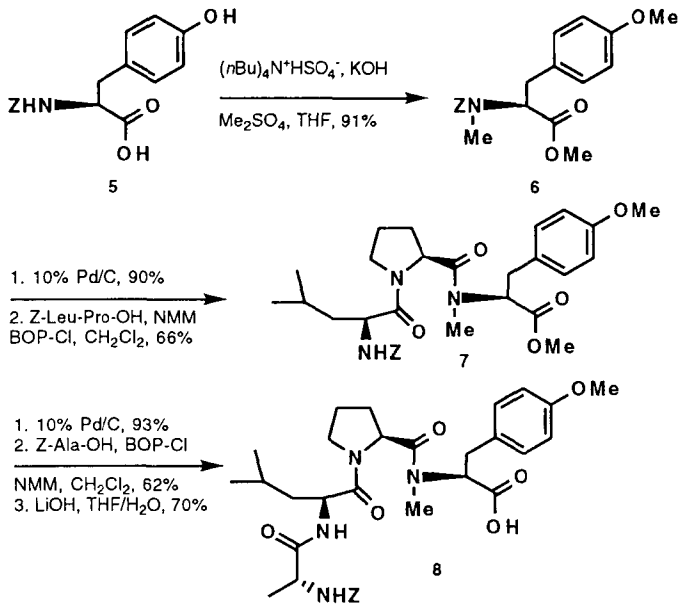


Figure 2. Acyclic Analogs of Didemnin B.

The syntheses of analogs **2** and **3** are shown in **Scheme 1**. Z-Tyrosine was permethylated under phase transfer conditions, using dimethyl sulfate, powdered potassium hydroxide and tetrabutylammonium hydrogen sulfate in THF to give compound **6**.[12] The Z protecting group was removed under catalytic hydrogenation conditions to give the secondary amine. Coupling of the amine with Z-leucylproline was accomplished using *N*,*N*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) activation[13] to afford tripeptide **7**. At this point the Z protecting group was removed and the resulting amine was

coupled with Z-alanine using BOP-Cl to obtain the desired tetrapeptide ester which after hydrolysis gave **8**, a common intermediate for all three analogs.

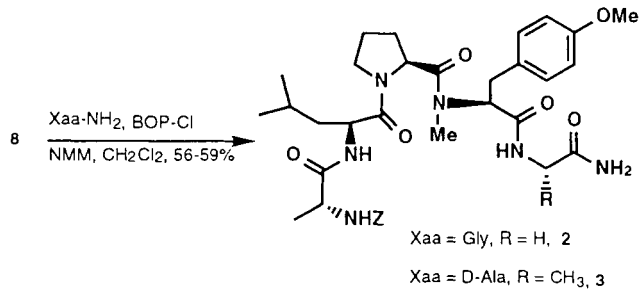
Scheme 1



Initial attempts to couple glycine amide or alanine amide[14] with **8** using 1H-1,2,3-benzotriazol-1-yloxytris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) reagent [15]gave only minor amounts of the desired products, however preactivated BOP-Cl was successful in producing analogs **2** and **3** in desirable yields (**Scheme 2**).

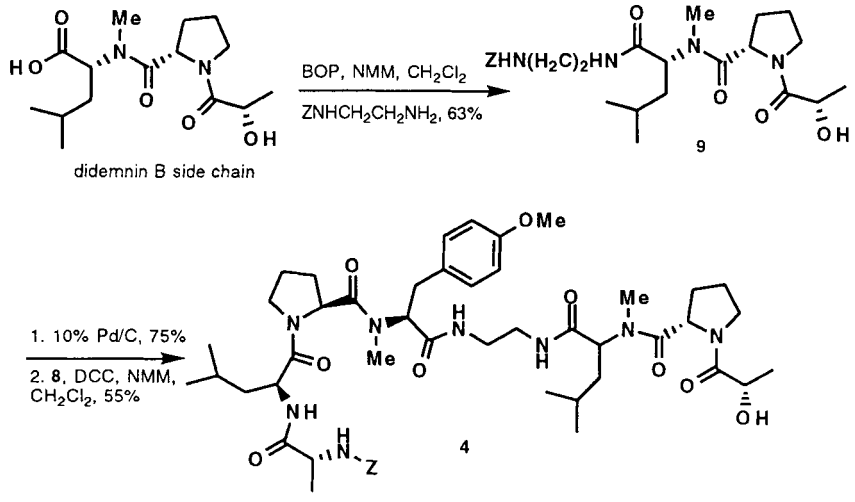
The synthesis of analog **4** began with the coupling of monoprotected ethylenediamine[16] and the didemnin B side chain[12] using BOP activation. The resulting compound **9** was hydrogenated to obtain the free amine which was to be coupled to acid **8**. The initial attempts to couple these two fragments using FDPP, BOP-Cl and BOP as coupling reagents were not successful. However the desired transformation was achieved using DCC in 56% yield to provide analog **4** (**Scheme 3**).

Scheme 2



In conclusion, we have synthesized three acyclic analogs of didemnin B with extensive modifications in the HIP and isostatine region of the macrocycle. The BOP-Cl coupling reagent was extremely useful in the syntheses of the advanced intermediates as well as of analogs **2** and **3**. However, DCC turned out to be the reagent of choice for the synthesis of analog **4**. Biological testing of these analogs will be reported in the future.

Scheme 3



Experimental

General: All manipulations were conducted under an inert atmosphere (argon or nitrogen). All solvents were reagent grade. Anhydrous tetrahydrofuran (THF) was distilled

from sodium/benzophenone. Dichloromethane (CH_2Cl_2) was distilled from calcium hydride (CaH_2). Organic acids and bases were reagent grade. All 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 with ultraviolet light, phosphomolybdic acid reagent (7% w/v) in absolute ethanol, and anisaldehyde reagent (5% v/v) in absolute ethanol containing 5% sulfuric acid and 1% acetic acid. Flash column chromatography was carried out on Merck silica gel 60 particle size (0.040-0.063 mm). Proton and carbon magnetic resonance spectra (^1H-, ^{13}C-NMR) were recorded on a Bruker AM-500 (500 MHz) Fourier transform spectrometer using $CDCl_3$ as the solvent. Chemical shifts were measured in parts per million (δ) relative to tetramethylsilane (TMS-0 ppm) or $CHCl_3$ as an internal reference (7.24 ppm for ^1H and 77.0 ppm for ^{13}C). Coupling constants (J values) are in Hertz (Hz). Multiplicities are designated as singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), doublet of doublet of doublets (ddd), doublet of triplets (dt), doublet of quartets (dq), triplet (t), triplet of doublets (td), quartet (q), and multiplet (m). Infrared spectra (IR) were obtained on a Perkin-Elmer Model 281-B or Perkin-Elmer Model 781 spectrometers. Oils were analyzed as neat films between sodium chloride plates. Absorptions are reported in wave numbers (cm^{-1}), and their intensities are designated as strong (s), medium (m), or weak (w). The spectra are calibrated against the 1601 cm^{-1} band of a polystyrene film, and only the most prominent or characteristic absorptions are noted. 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]. The mass spectrometer was interfaced to VG/DEC 11-73 data systems.

Z-*N,O*-Dimethyltyrosine Methyl Ester (6). To *N*-Z-L-tyrosine (1.00 g, 0.32 mmol) at ambient temperature, was added THF (16 mL). Finely powdered KOH (1.77 g, 0.032

mol) was then added in portions, followed by the addition of tetrabutylammonium hydrogen sulfate (0.10 g, 10% by weight). Rapid stirring was initiated, and dimethyl sulfate (1.8 mL, 0.019 mol) was added dropwise over a period of 15 min. After 1 h, the solid material was collected by filtration and washed with ethyl acetate. The filtrate was washed with 10% HCl, 5% $NaHCO_3$, and sat. NaCl solutions. The ethyl acetate layer was dried (Na_2SO_4), filtered, and concentrated. The crude oil was purified by column chromatography eluting with EtOAc/petroleum ether (15:85). The ester (**6**, 0.97 g, 85% yield) was obtained as an oil. R_f 0.55 (30:70 EtOAc:petroleum ether); $[\alpha]_D^{25}$ -51.00° (c=0.59, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 2.80 (d, J=12.90 Hz, 3H), 2.91-3.01 (m, 1H), 3.10-3.17 (m, 1H), 3.71, 3.64 and 3.75 (s, 3H, RI), 3.92 (s, 3H), 4.72-5.08 (m, 3H), 6.76-7.32 (m, 9H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 31.5, 32.0, 33.9 and 34.4 (RI), 52.1 and 55.1 (RI), 58.5, 60.7, 60.3, 67.3, 67.4, 113.9, 113.9, 136.4, 136.7, 127.4, 127.8, 127.9, 128.3, 128.9, 129.8, 136.4, 136.6, 155.9, 156.5 and 158.3 (RI), 171.5 and 171.2 (RI); IR (neat) 2400 (m), 1750 (s), 1710 (s), 1513 (s), 1247(m) cm^{-1}; HRMS *m/z* calcd for $C_{20}H_{24}NO_5$ (M+H) 358.1654, found 358.1640.

Z-Leucylprolyl-*N,O*-dimethyltyrosine Methyl Ester (7). To a CH_3OH/EtOAc solution (1:1, 15 mL) was added 10% Pd/C (0.34 g). To the resulting suspension was added Z-*N,O*-dimethyltyrosine methyl ester **6** (1.14 g, 3.16 mmol) in CH_3OH (2.00 mL). The solution was subjected to an atmosphere of hydrogen (40 psi) and shaken in a Parr apparatus for 3 h. The reaction mixture was filtered through Celite. The Celite was washed with CH_3OH, and the filtrate was concentrated. The resulting amine (1.04 g, 91%) was used directly in the next step. Z-Leucylproline (5.31 g, 0.015 mol) was dissolved in CH_2Cl_2 (50 mL) and the solution was cooled to -15 °C. BOP-Cl (4.48 g, 0.018 mol) was added followed by the dropwise addition of NMM (1.93 mL, 0.018 mol). The reaction mixture was stirred at -15 °C for 1 h. The solution was then concentrated to 20 mL, and the amine (2.53 g, 0.01 mol) and NMM (1.93 mL, 0.018 mol) were added. The solution was kept at 0 °C for 12 h and then diluted with ether (300 mL). The organic layer was washed with 10% HCl (50 mL), 5% $NaHCO_3$ (50 mL), and sat. NaCl (50 mL) solutions. The

organic layer was dried (Na_2SO_4), filtered, and concentrated. The crude oil was purified by column chromatography eluting with acetone/hexane (20:80). Compound **7** (4.59 g, 66%) was obtained as oil. R_f 0.42 acetone/hexane (40:60); $[\alpha]_D^{25}$ -64.63° (c=0.88, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.92 and 0.98 (dd, J=6.4 Hz, 6H), 1.44-1.60 (m, 2H), 1.76-2.45 (m, 6H), 3.03-3.09 and 3.30-3.36 (m, 2H), 2.96 (s, 3H), 3.64 (s, 3H), 3.81 (s, 3H), 3.66-3.79 (m, 2H), 4.51 (m, 1H), 4.78-4.80 (m, 1H), 5.05-5.13 (m, 3H), 5.41 (m, 1H), 6.78-6.83 (m, 2H), 7.05-7.15 (m, 2H), 7.44-7.60 (m, 5H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 22.0, 22.5, 23.5, 22.8, 28.1, 33.4, 33.5, 40.9, 46.9, 50.1, 52.1, 55.9, 56.9, 59.9, 66.7, 114.3, 128.9, 128.1, 129.4, 129.5, 129.7 (4 overlapping carbons), 130.1, 136.1, 159.9, 170.9, 171.1, 172.0; IR ($CHCl_3$) 3300 (w), 2900 (s), 1700 (s), 1620 (s), 1420 (s), 1210 (br), 1020 (w) cm^{-1}; HRMS *m/z* calcd for $C_{31}H_{42}N_3O_7$: 568.3022, found 568.3004.

Z-Alanineleucylprolyl-*N,O*-dimethyltyrosine (8). To a CH_3OH/EtOAc solution (1:1, 50 mL) was added 10% Pd/C (1.64 g). To the resulting suspension was added Z-leucylprolyl-*N,O*-dimethyltyrosine methyl ester **7** (5.47 g, 9.58 mmol) in CH_3OH (13.00 mL). The solution was subjected to an atmosphere of hydrogen (40 psi) and shaken in a Parr vessel for 3 h. The reaction mixture was filtered through Celite. The Celite was washed with CH_3OH, and the filtrate was concentrated. The resulting amine (5.09 g, 93%) was used directly in the next step. Z-Alanine (0.33 g, 1.46 mmol) was dissolved in CH_2Cl_2 (7.3 mL), and the solution was cooled to -15 °C. BOP-Cl (0.45 g, 1.75 mmol) was added, followed by the dropwise addition of NMM (0.18 mL, 1.46 mmol). The reaction mixture was stirred at -15 °C for 1 h. The solution was then concentrated to 3 mL, and leucylproline-*N,O*-dimethyltyrosine (0.53 g, 1.21 mmol) and NMM (0.18 mL, 1.46 mmol) were added. The solution was kept at 0 °C for 12 h and then diluted with ether (10 mL). The organic layer was washed with 10% HCl (3 mL), 5% $NaHCO_3$ (3 mL), and sat. NaCl (3 mL) solutions. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The crude oil was purified by column chromatography eluting with MeOH/CH_2Cl_2 (5:95). The pure product (0.48 g, 62%) was obtained as an oil. R_f 0.27 MeOH/$CHCl_3$ (5:95); $[\alpha]_D^{25}$ -82.35° (c=0.56, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.87 and 0.93 (dd, J=6.54 Hz, 6H),

1.37 (d, J=6.4 Hz, 3H), 1.50-1.70 (m, 2H), 1.82-2.20 (m, 5H), 2.91 (s, 3H), 2.94-2.99 and 3.24-3.29 (m, 2H), 3.71 (s, 3H), 3.81 (s, 3H), 3.77-3.82 (m, 2H), 4.24 (br s, 1H), 4.80-4.86 and 4.93-4.96 (m, 3H), 5.10 (s, 2H), 5.42 and 6.63 (br s, 2H), 6.82 and 7.11 ($[AX]_2$, J=8.54 Hz, 4H), 7.24-7.49 (m, 5H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 19.0, 22.1, 23.9, 24.5, 28.3, 36.6, 36.8, 40.3, 47.3, 49.7, 52.2, 50.2, 56.0, 57.1, 59.9, 66.7, 114.1, 128.1, 128.2, 128.3, 128.6, 128.9 (4 overlapping carbons), 130.1, 130.2, 136.1, 158.9, 170.2, 171.7, 171.8, 172.0; IR ($CHCl_3$) 3750 (w), 2900 (m), 1740 (s), 1650 (s), 1420 (br), 1240 (br), 1110 (w), 1030 (w), 900 (s) cm^{-1}; HRMS *m/z* calcd for $C_{34}H_{47}N_4O_8$ (M+H): 639.3394, found 639.3441. To Z-alanineleucylprolyl-*N,O*-dimethyltyrosine methyl ester (0.40 g, 0.62 mmol) was added a solution of THF, H_2O, and MeOH (1:1:1, 8 mL). The reaction was cooled to 0 °C, and lithium hydroxide monohydrate (0.052 g, 1.25 mmol) was added. The reaction was stirred at 0 °C for 6 h, concentrated to 3 mL, and washed with ether (2 x 3 mL). The combined ether layers were extracted with sat. $NaHCO_3$ solution (2 mL). The aqueous layers were combined and acidified to pH 1 with 1N potassium hydrogen sulfate solution. The acidified aqueous layer was extracted with ether (3 x 8 mL). The ether extracts were dried (Na_2SO_4), filtered and concentrated. Compound **8** (0.28 g, 70%) was obtained as a white hygroscopic foam. R_f 0.52 (10% MeOH/$CHCl_3$ (0.5% AcOH)); $[\alpha]_D^{25}$ -95.14° (c=0.35, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.90-1.00 (m, 6H), 1.30-1.51 (m, 5H), 1.70-1.82 and 2.10-2.20 (m, 4H), 2.34 (s, 3H), 2.78-2.80 and 2.91-3.00 (m, 4H), 3.21-3.26 and 3.52-3.60 (m, 2H), 3.77 and 3.80 (s, 3H, RI), 4.20-4.40 and 4.60-4.86 (m, 4H), 5.08-5.13 (m, 2H), 6.79-6.83 (m, 2H), 7.00-7.10 (m, 2H), 7.30-7.40 (m, 5H); IR ($CHCl_3$) 3450 (w), 3300 (w), 3000 (s), 1720 (s), 1670 (s), 1620 (s), 1470 (s), 1260 (br), 1050 (m) cm^{-1}; HRMS *m/z* calcd for $C_{33}H_{44}N_4O_8$ (M+H): 625.3237, found 625.3250.

Z-Alanineleucylprolyl-*N,O*-dimethyltyrosine Glycine Amide (2). Z-Alanine-leucylprolyl-*N,O*-dimethyltyrosine (0.30 g, 0.47 mmol) was dissolved in CH_2Cl_2 (5 mL), and the solution was cooled to -15 °C. BOP-Cl (0.14 g, 0.56 mmol) was added followed by the dropwise addition of NMM (0.062 mL, 0.56 mmol). The reaction mixture was stirred at

-15 °C for 1 h. The solution was then concentrated to 2 mL, and glycine amide (0.052 g, 0.047 mmol) and NMM (0.062 mL, 0.56 mmol) were added. The solution was kept at 0 °C for 12 h and then diluted with ether (6 mL). The organic layer was washed with 10% HCl (2 mL), 5% $NaHCO_3$ (2 mL), and sat. NaCl (2 mL) solutions. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The crude oil was purified by column chromatography eluting with MeOH/CH_2Cl_2 (7:93). Compound **2** (0.26 g, 80%) was obtained as an oil. R_f 0.33 MeOH/$CHCl_3$ (7:93); $[\alpha]_D^{25}$ =+40° (c=2.04, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.87-1.01 (m, 6H), 1.20-1.40 (m, 3H), 1.50-2.20 (m, 7H), 2.89-2.97 (m, 2H), 2.97 (s, 3H), 3.31 (s, 3H), 3.52-3.56 (m, 2H), 3.74 (s, 3H), 4.37-4.58 (m, 6H), 5.06 (s, 2H), 5.41-5.42 (m, 1H) and 8.00-8.02 (m, 1H), 6.79-6.89 (m, 2H), 7.14-7.18 (m, 2H), 7.29-7.36 (m, 5H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 18.9, 21.9, 23.6, 26.2, 28.1, 31.1, 33.5, 33.5, 40.8, 49.8, 51.0, 51.1, 55.6, 55.8, 56.4, 57.9, 66.7, 68.0, 114.1, 114.7, 125.8, 126.0, 128.0, 129.1, 129.5, 129.8, 130.0, 130.1, 136.1, 156.2, 158.3, 169.1, 170.0, 171.9, 173.6; IR (neat) 3013 (s), 2678 (w), 2541 (w), 1725 (s), 1664 (s), 1630 (s), 1610 (s), 1360 (w), 1228 (s), 1053 (s), 972 (w) cm^{-1}; HRMS *m/z* calcd for $C_{35}H_{49}N_6O_8$ (M+H): 681.3615, found 681.3651.

Z-Alanineleucylprolyl-*N,O*-dimethyltyrosine Alanine Amide (3). Z-Alanine-leucylprolyl-*N,O*-dimethyltyrosine (0.30 g, 0.47 mmol) was dissolved in CH_2Cl_2 (5 mL), and the solution was cooled to -15 °C. BOP-Cl (0.14 g, 0.56 mmol) was added, followed by the dropwise addition of NMM (0.062 mL, 0.56 mmol). The reaction mixture was stirred at -15 °C for 1 h. The solution was then concentrated to 2 mL, and D-alanine amide (0.038 g, 0.51 mmol) and NMM (0.062 mL, 0.56 mmol) were added. The solution was kept at 0 °C for 12 h and then diluted with ether (6 mL). The organic layer was washed with 10% HCl (2 mL), 5% $NaHCO_3$ (2 mL), and sat. NaCl (2 mL) solutions. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The crude oil was purified by column chromatography eluting with MeOH/CH_2Cl_2 (7:93). Compound **3** (0.26 g, 80%) was obtained as an oil. R_f 0.50 MeOH/CH_2Cl_2 (10:90); $[\alpha]_D^{25}$ -7.41° (c=0.18, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.91 and 0.97 (dd, J=6.51 Hz, 6H), 1.38 (d, J=6.91 Hz, 3H),

1.46 (d, J=6.93 Hz, 3H), 1.59-1.68 (m, 2H), 1.85-2.16 (m, 5H), 2.69-2.91 (m, 2H), 2.98 (s, 3H), 3.53-3.60 (m, 2H), 3.79 (s, 3H), 4.42-4.73 (m, 5H), 5.06-5.13 (m, 2H), 5.61-5.64 (m, 2H), 6.79 and 7.06 ($[AX]_2$, J=8.9 Hz, 4H), 7.40-7.55 (m, 5H), 7.61 (m, 3H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 21.2, 23.6, 25.4, 26.0, 28.1, 22.3, 31.1, 33.4, 33.6, 40.8, 47.1, 49.8, 51.0, 55.7, 56.7, 57.9, 66.8, 114.7, 114.1, 125.0, 126.0, 128.0, 129.1, 129.5, 129.5, 129.8, 129.9, 130.1, 130.1, 136.1, 156.1, 158.2, 169.1, 170.1, 171.8, 173.61; IR ($CHCl_3$) 3300 (br), 3000 (m), 1690 (s), 1630 (s), 1520 (m), 1460 (m), 1250 (s), 1050 (w) cm^{-1}; HRMS *m/z* calcd for $C_{36}H_{51}N_6O_8$ (M+H): 695.3768, found 695.3751.

[2-(2*S*-{[1*S*-(2*S*-Hydroxypropionyl)pyrrolidine-2-carbonyl]methyl-amino}4-methylpentanoylamine)ethyl]carbamic acid Benzyl Ester (9). To a solution of monoprotected ethylenediamine (0.19 g, 0.84 mol) and L-lactylprolyl-*N*-methyl-D-leucine (didemnin B side chain) (0.25 g, 0.80 mmol), in CH_2Cl_2 (5.5 mL) at 0 °C, were added BOP (0.39 g, 0.89 mmol) and NMM (0.27 mL, 2.45 mol). After 30 min, the solution was brought to room temperature and stirred for 6 h. After this time, the reaction mixture was treated with 3 mL of sat. NaCl solution and then extracted with 5 mL of EtOAc. The organic layers were combined and washed successively with 5% HCl, 5% $NaHCO_3$, and sat. NaCl solutions. The organic layer was dried ($NaSO_4$), filtered, and concentrated. The resulting crude oil was purified by column chromatography, eluting with acetone/CH_2Cl_2 (30:70) to afford 0.24 g (63%) of pure compound **9**. R_f 0.52 acetone/CH_2Cl_2 (30:70); $[\alpha]_D^{25}$ +46.62° (c=0.79, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.87 and 0.90 (dd, J=6.01 Hz, 6H), 1.41 d (6.71, 3H), 1.62-1.67 (m, 1H), 1.89-2.23 (m, 6H), 2.99 and 2.96 (s, 3H, RI), 3.10-3.20 and 3.45-3.55 (m, 5H), 3.55-3.70 (m, 2H), 4.30-4.50 (m, 1H), 4.70 (m, 1H), 5.10-5.20 (m, 2H), 5.30-5.32 (m, 1H), 6.70-6.80 (m, 2H), 7.30-7.50 (m, 5H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 14.0, 20.0, 22.2, 23.1, 23.91, 24.0, 31.1, 36.0, 41.1, 47.0, 55.8, 57.0, 66.9, 67.0, 128.11, 128.12, 128.15, 128.19, 128.2, 138.0, 137.0, 158.1, 158.9, 171.0, 173.1; IR ($CHCl_3$) 3400 (s), 2990 (s), 1720 (s), 1680 (s), 1630 (s), 1530 (s), 1460 (m), 1420 (m), 1250 (s), 1150 (s), 1120 (s), 850 (w) cm^{-1}; HRMS *m/z* calcd for $C_{25}H_{39}N_4O_6$ (M+H): 491.2869, found 491.2851.

{1*S*-[1*S*-(2*S*-{[1-[2-(2*S*-{[1*S*-(2*S*-Hydroxypropionyl)pyrrolidine-2-carbonyl]methylamino}4-methylpentanoylamine)ethylcarbamoyl]-2*S*-(4-methoxyphenyl)ethyl]methylcarbamoyl}pyrrolidine-1-carbonyl)-3-methyl-butylcarbamoyl]ethyl}carbamic Acid Benzyl Ester (4). To a CH_3OH/EtOAc solution (1:1, 3 mL) was added 10% Pd/C (0.036 g). To the resulting suspension was added compound **9** (0.12 g, 0.23 mmol) in CH_3OH (1 mL). The solution was subjected to an atmosphere of hydrogen (40 psi) and shaken in a Parr vessel for 2 h. The reaction mixture was filtered through Celite. The Celite was washed with CH_3OH, and the filtrate was concentrated. The resulting amine (0.061 g, 75%) was used directly in the next step. Acid **8** (0.32 g, 0.50 mmol) and the amine (0.17 g, 0.48 mmol) were dissolved in CH_2Cl_2 (6 mL). The reaction was cooled to 0 °C and NMM (0.12 mL, 1.05 mmol) was added followed by DCC (0.14 g, 0.65 mmol). The reaction was slowly warmed to RT and stirred for 24 h. After this time, the reaction mixture was filtered, and the collected solid was washed with CH_2Cl_2. The filtrate was concentrated and diluted with ether (25 mL). The ether layer was washed with 10% HCl (5 mL), 5% $NaHCO_3$ (5 mL), and sat. NaCl (40 mL) solutions. The organic layer was dried ($NaSO_4$), filtered, and concentrated. The resulting crude oil was purified by column chromatography eluting with MeOH/$CHCl_3$ (7:93) to afford compound **4** (0.292 g, 55%) as a white solid. Rf 0.40 MeOH/$CHCl_3$ (10:90); $[\alpha]_D^{25}$ +1.37° (c=0.95, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.83-0.97 (m, 12H), 1.29-1.71 (m, 10H), 1.87-2.23 (m, 10H), 2.43 (s, 3H), 2.81-2.99 (m, 4H), 2.86 (s, 3H), 3.20-3.70 (m, 7H), 3.78 (s, 3H), 4.30-4.80 (m, 6H), 5.20-5.30 (m, 1H), 5.04-5.15 (m, 2H), 6.15-6.84 and 7.15-7.22 (m, 4H), 7.04-7.13 (m, 4H), 7.24-7.35 (m, 5H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 20.1, 21.4, 21.8, 21.8, 23.2, 23.3, 24.5, 25.0, 25.1, 25.4, 25.8, 28.1, 29.2, 30.8, 30.9, 32.8 and 35.6 (RI), 35.7 and 38.5 (RI), 47.2, 47.4 55.0, 55.1, 55.2, 55.3, 56.8, 63.5, 65.8, 65.9, 66.5, 66.6, 113.5, 114.2, 125.2, 126.0, 127.8, 127.9, 128.0, 128.1, 128.4 (4 overlapping carbons), 130.2, 130.3, 137.8, 158.5, 169.4, 172.4, 172.5, 173.9; IR ($CHCl_3$) 3350 (m), 2995 (s), 1720 (w), 1680-1630 (br s), 1460 (s), 1250 (m), 1110 (w), 1050 (s) cm^{-1}; HRMS *m/z* calcd for $C_{50}H_{75}N_8O_{11}$ (M+H): 963.5555, found 963.5504.

Abbreviations. 1H-1,3-benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP); *N*-[(benzyloxy)carbonyl]-5-norbornene-2,3-dicarboximide (BCN); *N*,*N*-bis(2-oxo-3-oxazolidinyl)phosphonic chloride (BOP-Cl); N-methylmorpholine (NMM); tetrabutylammonium fluoride (TBAF); rotational isomers (RI).

Acknowledgments. Financial support by the National Institutes of Health (CA-40081) is gratefully acknowledged. We thank Dr. G. T. Furst and Mr. J. Dykins of the University of Pennsylvania Analytical Facility for their expert technical assistance.

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(Received in the USA 08 April 1997)