Total Synthesis of [(*2S*)-Hiv^2]Didemnin M

Bo Liang, Matthew D. Vera, and Madeleine M. Joullié*

Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6323

mjoullie@sas.upenn.edu

Received February 23, 2000

Introduction

The didemnins are cyclic depsipeptides isolated from the tunicate *Tridemnum solidum* by Rinehart and co-workers.[1] Several reviews discussing their syntheses and biological activities have been published.[2,3] Their structure−activity relationships have been reviewed.[4] Didemnin B (**2**) is the best-studied member of this group of natural products, and it exhibits antitumor, antiviral, immunosuppressive, and protein biosynthesis inhibition activities. Didemnin H,[5] also known as didemnin M[6](**3**), which contains glutamine and pyroglutamic acid residues in the side chain, displayed subpicomolar potency in an in vitro immunosuppressive assay,[4] roughly 1000-fold more potent than didemnin B.

Tamandarin A (**4**), a novel cyclodepsipeptide first isolated, identified and reported by Vervoort et al.,[7] was recently synthesized in our laboratory.[8] This natural product could be formally viewed as [(*2S*)-Hiv^2]didemnin B,[9] since it contains a hydroxyisovaleryl (Hiv) residue, rather than the more complex α-(α-hydroxyisovaleryl)-propionyl (HIP) unit at position 2 of the didemnin macrocycle. The structures of compounds **1**−**4** are shown in Figure 1. Tamandarin A has shown levels of cytotoxic and protein biosynthesis inhibition activities that are comparable to those of didemnin B (**2**),[7] indicating that the macrocylic HIP subunit may not be required for bioactivity. However, the immunosuppressive activity of tamandarin-type congeners has not been examined. Our current research effort is focused on the nature of the potency enhancement caused by the glutamine-containing side chain of **3**. We also wish to establish the consequences of the HIP-to-Hiv modification present in tamandarin-type congeners for the wide spectrum of biological effects exerted by this family of natural products. As part of this study, we now disclose the synthesis of [(*2S*)-Hiv^2]didemnin M (**1**). This analogue, which has not been reported as a natural product, contains both the simplified tamandarin-type macrocycle and the more complex side chain of didemnin M (**3**). Biological testing of this compound and comparison with **3** and **4** will establish the relevance of both the Hiv^2 residue and the glutamine-containing side chain for biological activities.

Results

Two semisynthetic approaches to **3** have been examined. Katauskas and co-workers[10,11] synthesized didemnin M (**3**) by derivatization of didemnin A. Wen and co-workers[12] reported methods for the synthesis of a depsipeptide containing residues 8−11. Deprotection operations and ester formation between Lac[9] and Gln[10] were cited as the primary difficulties. We report here an efficient total synthesis of the side chain containing residues 7−11 and its coupling to the tamandarin-type macrocycle to furnish analogue **1**. This method should also permit access to **3** by total synthesis. The retrosynthetic analysis of **1** showing the macrocycle (**6**) and the side chain (**5**) are shown in Figure 2.

The synthesis of the side chain started with the coupling of *O*-Bn-Lac-Proline[13] (**7**, Scheme 1) with the methyl ester of *N*-Me-D-Leu to afford compound **8**, followed by removal of the benzyl group, and then saponification of the ester to provide compound **10**, which is the didemnin B side chain.[14] Protection of the carboxyl functionality as its benzyl ester (**11**), followed by DCC coupling with BocGln(Xan)OH, afforded a high yield of compound **12**. Removal of the xanthyl group proved to be nontrivial. Although a 4.0 M HCl solution in dioxane could not accomplish deprotection, the cleavage of both the xanthyl and Boc groups was effected using HCl gas in EtOAc and anisole as a scavenger. The resulting free amine was coupled with the activated PFP ester of Z-pGlu to give compound **13**, followed by hydrogenolysis of both the benzyl and benzyloxycarbonyl protecting groups, to afford the free acid **5**. The coupling of the side chain **5** with the macrocycle **6**, synthesized according to our previous strategy,[8] using BOP and NMM in CH_2Cl_2 at 0 °C, afforded the desired product **1** in 32% yield.

The synthetic analogue (**1**) was tested by the National Cancer Institute against various cancer cell lines in vitro.[15] A representative sample of cytotoxic activity data,

(1) Rinehart, K. L., Jr.; Gloer, J. B.; Hughes, R. G., Jr.; Renis, H. E.; McGovren, J. P.; Swynenberg, E. B.; Stringfellow, D. A.; Kuentzel, S. L.; Li, L. H. *Science* **1981**, *212*, 933−935.

(2) Li, W.-R.; Joullié, M. M. In *Studies in Natural Products Chemistry, Vol. 10, Stereoselective Synthesis (Part F)*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 1992; pp 241−302.

(3) Wipf, P. *Chem. Rev.* **1995**, *95*, 2115−2134.

(4) Sakai, R.; Rinehart, K. L.; Kishore, V.; Kundu, B.; Faircloth, G.; Gloer, J. B.; Carney, J. R.; Namikoshi, M.; Sun, F.; Hughes, R. G., Jr.; Gravalos, G.; de Quesada, T. G.; Wilson, G. R.; Heid, R. M. *J. Med. Chem.* **1996**, *39*, 2819−2834.

(5) Boulanger, A.; Abou-Mansour, E.; Badre, A.; Banaigs, B.; Combaut, G.; Francisco, C. *Tetrahedron Lett.* **1994**, *35*, 4345−4348.

(6) Sakai, R.; Stroh, J. G.; Sullins, D. W.; Rinehart, K. L. *J. Am. Chem. Soc.* **1995**, *117*, 3734−3748.

(7) Vervoort, H.; Fenical, W.; de Epifanio, R. *J. Org. Chem.* **2000**, *65*, 782−792.

(8) Liang, B.; Portonovo, P.; Vera, M. D.; Xiao, D.; Joullié, M. M. *Org. Lett.* **1999**, *1*, 1319−1322.

(9) Because of the similarities among the didemnins, nordidemnins and tamandarins, we have adopted a systematic nomenclature for these natural products. To this end, we use the numbering convention as proposed by Sakai et al.[6] This system numbers isostatine as residue 1, HIP as residue 2, etc. Using this nomenclature, nordidemnin B (see Jouin et al. *J. Org. Chem.* **1989**, *54*, 617−627) would be named [Norsta^1]didemnin B and tamandarin A would be [(*2S*)-Hiv^2]didemnin B. This convention provides a convenient and unambiguous description of the natural products and their synthetic analogues and highlights the sites of the structural changes made.

(10) Katauskas, A. J.; Rinehart, K. L. 213th National Meeting of the American Chemical Society, San Francisco, CA, 1997; American Chemical Society: Washington, DC, 1997; pp ORG-408.

(11) Rinehart, K. L.; Katauskas, A. J. *PCT Int. Appl. WO 9817275*, April 30, 1998.

(12) Wen, J. J.; Crews, C. M. *Tetrahedron Lett.* **1998**, *39*, 779−782.

(13) Xiao, D. Ph.D. Thesis, University of Pennsylvania, Philadelphia, PA, 2000.

(14) Li, W.-R.; Ewing, W. R.; Harris, B. D.; Joullié, M. M. *J. Am. Chem. Soc.* **1990**, *112*, 7659−7672.

(15) Boyd, M. R.; Paul, K. D. *Drug Dev. Res.* **1995**, *34*, 91−109.

10.1021/jo000253a CCC: $19.00
Published on Web 06/23/2000

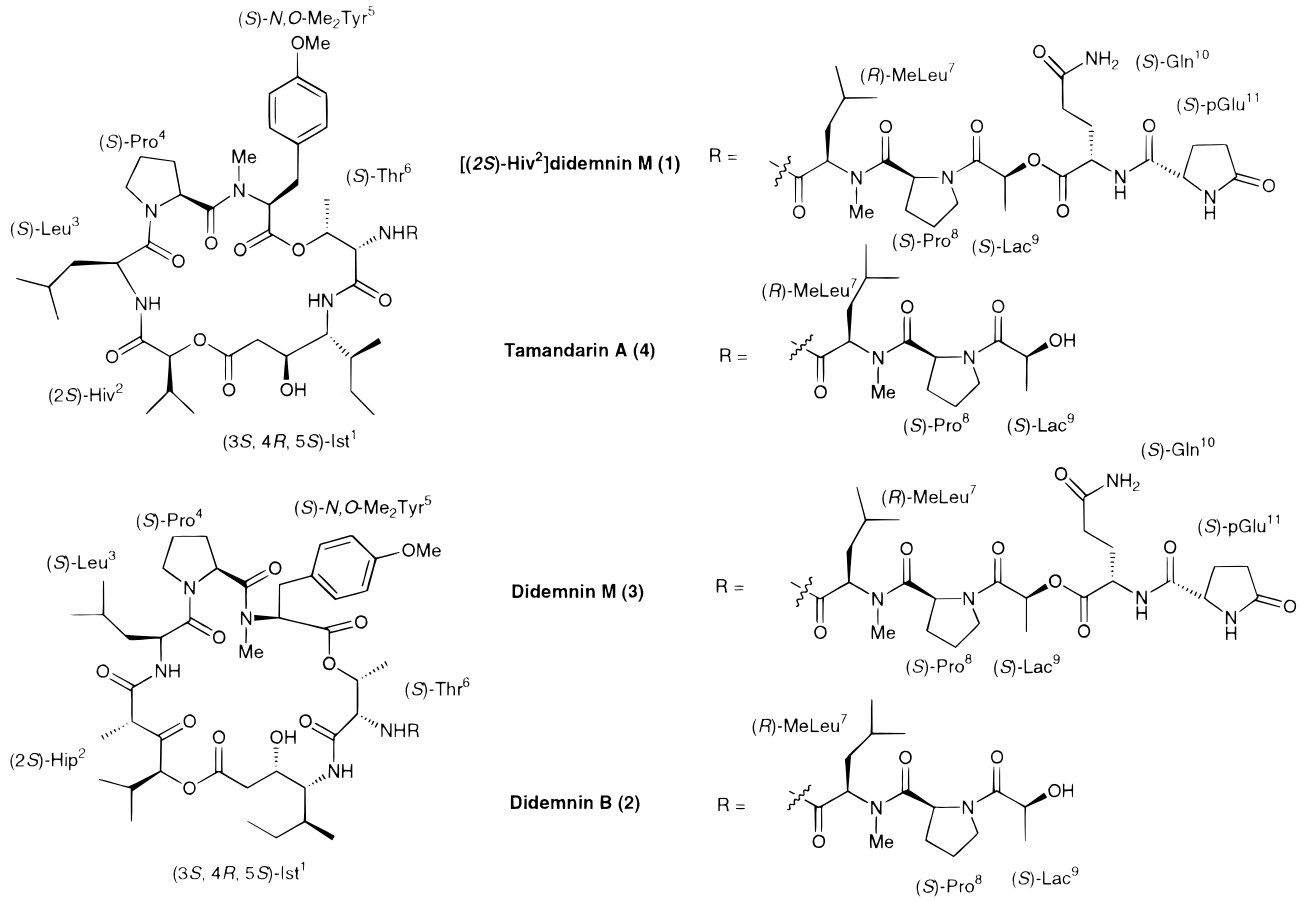


Figure 1. Structures of [(2*S*)-Hiv^2]didemnin M (**1**), didemnin B (**2**), didemnin M (**3**), and tamandarin A (**4**).

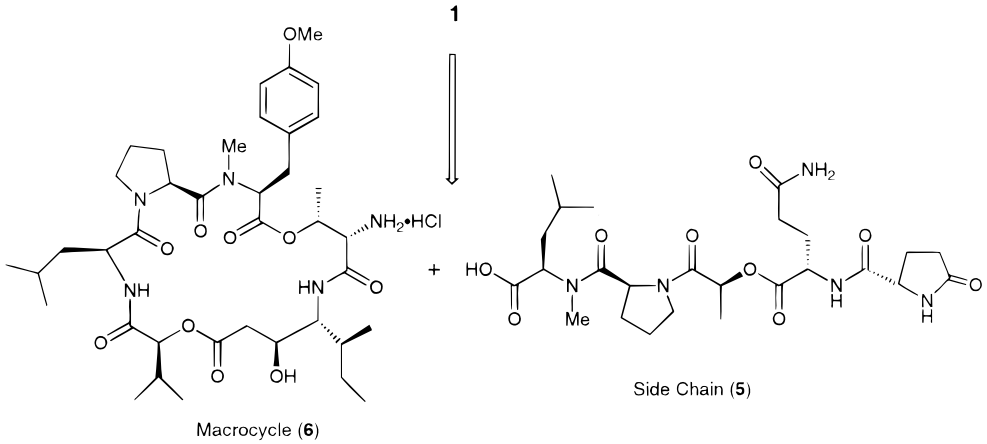


Figure 2. Retrosynthesis of [(2*S*)-Hiv^2]didemnin M (**1**).

derived from 48 h continuous drug exposure of at least five concentrations at 10-fold dilutions, is shown in Table 1. Growth inhibition concentrations (GI_{50}) were below 2.50 nM for 32 of 57 cell lines tested; the other 25 cell lines test data are all in the range of nanomolar concentrations. Interestingly, compound **1** is about 1000-fold more sensitive against colon cancer/HT29 cell line than didemnin B (**2**).

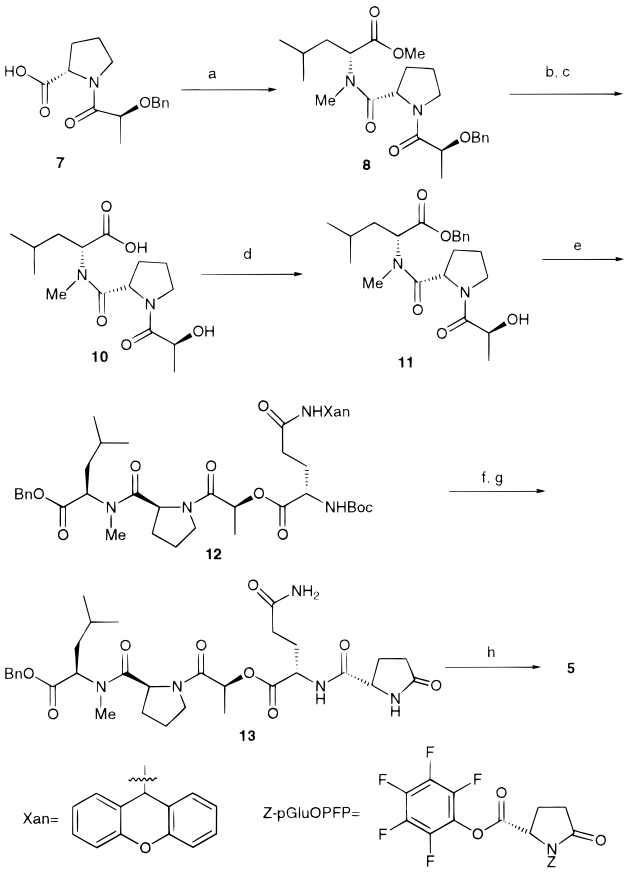
Compound **1** has shown comparable or even better antitumor activity than didemnin B (**2**). In conclusion, we have achieved the first total synthesis of **1**, which also provides a feasible synthetic route to **3**. The synthesis of **3** and the further biological characterization of **1** are underway. The results and the comparison with didemnin M (**3**) and tamandarin A (**4**) will be reported in due course.

Experimental Section

General Methods. All reactions were performed under nitrogen. THF and dichloromethane were distilled over sodium−benzophenone and calcium hydride, respectively. Proton and carbon magnetic resonance spectra were recorded (500 MHz for ^{1}H, 250 MHz for ^{13}C) on a Bruker AM-500 Fourier transform spectrometer. Flash column chromatography was carried out on E. Merck silica gel 60 (240−400 mesh) using the solvent systems listed under individual experiments.

***O*-Benzyl-L-lactyl-L-prolyl-*N*-methyl-D-leucine Methyl Ester (8).** To a solution of *O*-Bn-Lac-proline (**7**) (1.37 g, 4.95 mmol)

Scheme 1[a]



[a] Reagents and conditions: (a) *N*,*O*-dimethyl-D-Leu, BOP-Cl, NMM, CH_2Cl_2, −15 °C to room temperature (83%); (b) H_2, 10% Pd/C, MeOH/EtOAc, (98%); (c) 0.2 M LiOH solution, MeOH/THF/H_2O (1:1:1) (77%); (d) BnBr, K_2CO_3, DMF, Bu_4NI, (94%); (e) DCC, DMAP, BocGln(Xan)OH, CH_2Cl_2, rt (87%); (f) HCl (g), EtOAc/anisole (20:1); (g) Z-*p*-GluOPFP, DIEA, CH_2Cl_2, (67%, two steps overall); (h) H_2, 10% Pd/C, MeOH/EtOAc (1:1), (99%).

Table 1. Cytotoxic Activities of Didemnin B (2) and [(*2S*)-Hiv²]didemnin M (1)[a]

	didemnin B (**2**)	[(*2S*)-Hiv²]didemnin M (**1**)
GI_{50}	13 nM	4 nM
TGI	66 nM	126 nM
LC_{50}	3.8 μM	7.6 μM
colon cancer/HT29 (LC_{50})	38.2 μM	29.5 nM

[a] Preliminary results from the NCI-60 tumor cell screen. Concentrations for 50% growth inhibition (GI_{50}), total growth inhibition (TGI), and 50% cell kill (LC_{50}) are based upon the mean-graph midpoint across all cell lines.

in 15 mL of freshly distilled CH_2Cl_2 at 0 °C was added BOP-Cl (1.33 g, 5.2 mmol), followed by the dropwise addition of NMM (0.6 mL, 5.5 mmol), and the reaction was stirred at −15 °C for 30 min. To this mixture was added *N*,*O*-diMe-D-Leu (0.97 g, 4.95 mmol) and another portion of NMM (1.8 mL, 16.4 mmol) dropwise. The reaction was stirred at −15 °C for 10 min and then warmed to 0 °C for overnight stirring. The reaction mixture was diluted with EtOAc, washed with 10% HCl, 5% $NaHCO_3$, and saturated NaCl solution sequentially, and then dried (Na_2SO_4), filtered, and concentrated. The crude mixture was purified using flash column chromatography by eluting with MeOH/CH_2Cl_2 (5:95) to obtain the protected dipeptide **8** (1.73 g, 83% yield) as a yellow oil: R_f 0.64 (10:90 methanol/methylene chloride); ^{1}H NMR δ 0.86−1.04 (m, 6H), 1.40−1.44 (d, J = 6.6 Hz, 3H), 1.56−1.58 (m, 1H), 1.70−1.73 (m, 2H), 1.78−1.88 (m, 2H), 2.05−2.15 (m, 2H), 3.08 (s, 3H), 3.60−3.68 (m, 2H), 3.70 (s, 3H), 4.19−4.22 (m, 1H), 4.90−4.93 (m, 1H), 5.01−5.04 (t, 1H), 5.29 (s, 2H), 7.29−7.35 (m, 5H); HRMS (EI) *m*/*z* calcd for $C_{23}H_{34}N_2O_5$ (M + Na^+) 441.2365, found 441.2359.

L-Lactyl-L-prolyl-*N*-methyl-D-leucine Methyl Ester (9). The protected dipeptide **8** (0.51 g,1.2 mmol) was dissolved in MeOH/EtOAc (1:1)(10 mL), followed by the addition of 10% Pd/C (0.152 g), and the suspension was placed under an atmosphere of H_2 for 3 h. The catalyst was collected by filtration, the residue was washed with HPLC MeOH, and the filtrate was concentrated to give a white foam, which was recrystallized in ether/hexane to afford white crystals (**9**, 0.4246 g, 98% yield): R_f 0.68 (10:90 methanol/methylene chloride); mp 95.5−96.0 °C; ^{1}H NMR δ 0.84−1.01 (m, 6H), 1.34−1.36 (d, J = 6.60 Hz, 3H), 1.39−1.45 (m, 1H), 1.68−1.71 (m, 2H), 1.72−1.96 (m, 2H), 2.07−2.20 (m, 2H), 3.04 (s, 3H), 3.54−3.56 (t, d = 6.7 Hz, 2H), 3.67 (s, 3H), 4.28−4.32 (m, 1H), 4.90−4.93 (m, 1H), 5.06−5.09 (m, 1H); ^{13}C NMR δ 20.3, 21.1, 23.2, 25.0, 28.3, 31.9, 37.5, 46.6, 52.2, 55.5, 57.0, 65.4, 171.9, 172.1, 173.3; IR (KBr) 3409, 1736, 1642 cm^{-1}; HRMS (EI) *m*/*z* calcd for $C_{16}H_{29}N_2O_5$ (M + H^+) 329.2076, found 329.2081; Anal. Calcd for $C_{16}H_{28}N_2O_5$: C, 58.50; H, 8.60; N, 8.53. Found: C, 58.34; H, 8.67; N, 8.53.

L-Lactyl-L-prolyl-*N*-methyl-D-leucine (10). The white crystals (**9**, 0.31 g, 0.94 mmol) were dissolved in distilled THF/HPLC MeOH (1:1) (20 mL), 0.2 M LiOH solution (20 mL) was added, and the reaction mixture was stirred at room temperature overnight. The mixture was concentrated in vacuo, and the aqueous solution was cooled to 0 °C, then acidified to pH 3 with 1 N $KHSO_4$, and extracted with EtOAc. The organic layers were combined and dried (Na_2SO_4), filtered and concentrated to afford a white solid (0.23 g, 77% yield): R_f 0.08 (10:90-methanol: methylene chloride); ^{1}H NMR δ 0.87−0.88 (d, J = 6.6 Hz, 3H) and 0.91−0.92 (d, J = 6.7 Hz, 3H), 1.40−1.42 (d, J = 6.7 Hz, 3H), 1.65−1.68 (m, 1H), 1.80−1.85 (m, 2H), 1.98−2.02 (m, 2H), 2.07−2.18 (m, 2H), 2.99 (s, 3H), 3.61−3.63 (m, 2H), 4.42−4.43 (m, 1H), 4.86−4.87 (m, 1H), 5.35−5.38 (dd, J_1=11.3 Hz, J_2=4.4 Hz, 1H),; ^{13}C NMR δ 20.2, 21.3, 23.4, 25.1, 28.5, 31.2, 36.8, 47.0, 54.5, 57.2, 66.2, 171.8, 175.0, 175.1; IR (KBr) 3384, 1703, 1651, 1604 cm^{-1}; HRMS (EI) *m*/*z* calcd for $C_{15}H_{27}N_2O_5Na$ (M + H^+) 315.1922, found 315.1924; $[\alpha]^{20}_D$ − 24 (*c* 1.05, $CHCl_3$) [lit.[14] $[\alpha]^{25}_D$ −23.9 (*c* 0.85, EtOH)].

L-Lactyl-L-prolyl-*N*-methyl-D-leucine Benzyl Ester (11). A solution of compound **10** (0.163 g, 0.52 mmol) in redistilled DMF (5 mL) was added to anhydrous K_2CO_3 (0.075 g, 0.54 mmol), Bu_4NI (0.038 g, 0.10 mmol), and BnBr (0.25 mL, 2.08 mmol) sequentially. The reaction mixture was stirred at room temperature for 1 h, and the solvent was removed in vacuo. The residue was diluted with water and extracted with EtOAc. The organic layers were combined and washed with 10% HCl, 5% $NaHCO_3$, and brine and then dried (Na_2SO_4), filtered, and concentrated to afford a white solid (**11**, 0.197 g, 94% yield): R_f 0.31 (5:95 methanol/methylene chloride); ^{1}H NMR δ 0.87−1.02 (m, 6H), 1.36−1.37 (d, J = 6.64 Hz, 3H), 1.38−1.46 (m, 1H), 1.70−1.75 (m, 2H), 1.76−1.90 (m, 2H), 2.06−2.18 (m, 2H), 3.03 (s, 3H), 3.51−3.57 (m, 2H), 4.29−4.47 (m, 1H), 4.91−4.94 (m, 1H), 5.08−5.14 (m, 3H), 7.27−7.36 (m, 5H); ^{13}C NMR δ 20.4, 21.4, 23.2, 25.1, 28.4, 29.4, 37.4, 46.6, 55.7, 57.0, 65.6, 66.8, 128.1, 128.5, 128.7 and 135.8, 171.2, 172.2, 173.3; IR (KBr, $CHCl_3$) 3426, 1738 cm^{-1}; HRMS (EI) *m*/*z* calcd for $C_{22}H_{32}N_2O_5Na$ (M + Na^+) 427.2209, found 427.2213.

***N*²-Boc-*N*⁵-xanthyl-L-glutaminyl-*O*-L-lactyl-L-prolyl-*N*-methyl-D-leucine Benzyl Ester (12).** The alcohol **11** (0.1972 g, 0.49 mmol) was dissolved in CH_2Cl_2 (2 mL) under argon and cooled to 0 °C. To this solution were added BocGln(Xan)OH (0.3121 g, 0.73 mmol), DCC (0.151 g, 0.73 mmol), and DMAP (0.1192 g, 0.98 mmol) sequentially. The reaction mixture was stirred at 0 °C for 30 min and then at room temperature overnight, at which time it was quenched with AcOH/MeOH in EtOAc (2 mL). The solvent was evaporated in vacuo and the residue was dissolved in ether (10 mL) and the solid formed collected by filtration. The ether layer was washed with 10% citric acid, 5% $NaHCO_3$ and brine. The organic layer was then dried (Na_2SO_4), filtered, and concentrated. The resulting residue was purified by flash column chromatography eluting with acetone:hexane (10:90 to 30:70) to obtain **12** as a white solid (0.3461 g, 87% yield): R_f 0.25 (40:60 acetone/hexane); ^{1}H NMR δ 0.71−0.95 (m, 6H), 1.22−1.30 (m, 1H), 1.34−1.35 (d, J = 6.2 Hz, 3H), 1.43 (s, 9H), 1.62−1.67 (m, 2H), 1.77−2.05 (m, 4H), 2.15−2.39 (m, 4H), 2.70 (s, 3H), 3.43−3.68(m, 2H), 4.36−4.39

(m, 1H), 4.51 (m, 1H), 4.60−4.63 (m, 1H), 4.86−4.94 (m, 2H), 5.09−5.16 (m, 2H), 5.40−5.42 and 7.41−7.48 (m, 1H), 6.48−6.50 (m, 1H), 7.02−7.40 (m, 13H); ^{13}C NMR δ 15.9, 21.3, 21.7, 23.1, 24.9, 28.2 (overlap), 32.0, 37.3, 43.5, 46.6, 52.7, 55.7, 56.7, 66.5, 69.2, 79.8, 116.2, 121.3, 123.3 and 123.5, 127.9, 128.4, 128.8 and 135.7, 151.1, 168.7 (overlap), 171.0, 171.5, 172.0; IR (KBr) 3298, 1741, 1713, 1651 cm^{-1}; HRMS (EI) *m*/*z* calcd for $C_{45}H_{56}N_4O_{10}$-Na (M + Na$^+$) 835.3894, found 835.3921; $[\alpha]^{20}_D$ −23.3 (*c* 0.69, $CHCl_3$). Anal. Calcd for $C_{45}H_{56}N_4O_{10}$: C, 66.47; H, 6.95; N, 6.89. Found: C, 66.13; H, 7.06; N, 6.44.

L-pGlu-L-Glu-*O*-L-lactyl-L-prolyl-*N*-methyl-D-leucine Benzyl Ester (13). A solution of compound **12** (0.4065 g, 0.5 mmol) in EtOAc/MeOPh (20:1, 5 mL) was cooled to −20 °C, and gaseous HCl was introduced at such a rate that the temperature of the mixture was maintained between −10 and −20 °C at saturation. After being stirred for 30 min at this temperature, the reaction mixture was stirred at 0 °C for 1 h. The solution was then purged with N_2 for about 30 min, maintaining the temperature at 0 °C. After the solution was concentrated, the residue was triturated and washed by decantation with three 5.0 mL portions of *tert*-butyl methyl ether/hexane (1:4). The product was collected by filtration and dried in vacuo to provide the hydrochloride salt (0.285 g, quantitative yield) as a white solid, which was used directly in the next step. The crude hydrochloride salt was dissolved in fresh CH_2Cl_2 (2 mL) at 0 °C, followed by the addition of DIEA (0.35 mL, 2 mmol) and Z-pGluOPFP (0.215 g, 0.5 mmol). The reaction was stirred at 0 °C for 1 h, then at room temperature for another 1 h. The reaction mixture was quenched with saturated NaCl solution (3 mL), and diluted with CH_2Cl_2 (5 mL). The mixture was separated and the aqueous layer was extracted with CH_2Cl_2, the combined organic layers were washed with 10% HCl, 5% $NaHCO_3$ and brine, then dried (Na_2SO_4), filtered and concentrated. The crude residue was purified by flash column chromatography eluting with MeOH/CH_2Cl_2 (5:95) to provide a white solid (**13**, 0.2614 g, 67% yield, two steps overall): R_f 0.47 (10:90 MeOH/CH_2Cl_2); ^{1}H NMR δ 0.71−0.96 (m, 6H), 1.17−1.23 (m, 1H), 1.46−1.48 (d, J = 6.84 Hz, 3H), 1.68−1.75 (m, 2H), 1.92−2.25 (m, 10H), 2.35−2.41 (m, 2H), 2.60−2.70 (m, 1H) and 2.95 (s, 2H), 3.55−3.69 (m, 2H), 4.05−4.10 (dd, J_1=14.27 Hz, J_2=7.13 Hz, 1H), 4.41−4.50 (m, 1H), 4.54−4.59 (m, 1H), 4.82−4.92 (m, 1H), 5.04−5.13 (m, 1H), 5.15−5.24 (m, 4H), 5.54 (s, 1H) and 6.90 (s, 1H), 7.25−7.34 (m, 10H), 7.58−7.60 (d, J = 6.4 Hz, 1H); ^{13}C NMR δ 15.8, 21.1, 22.4, 23.1, 25.1, 27.2, 28.2, 31.1, 31.7, 37.4, 46.7, 52.0, 55.1, 57.0, 59.5, 66.7, 68.2, 69.2, 127.9, 128.0, 128.2, 128.4, 128.5, 128.6 and 135.1, 135.5, 151.3, 168.6, 170.7, 171.2, 171.5, 172.0, 173.5, 175.7; IR (KBr) 3333, 3211, 1791, 1741, 1661 cm^{-1}; HRMS (EI) *m*/*z* calcd for $C_{40}H_{51}N_5O_{11}Na$ (M + Na$^+$) 800.3483, found 800.3513.

L-pGlu-L-Glu-*O*-L-lactyl-L-prolyl-*N*-methyl-D-leucine (5). To the suspension of 10% Pd/C (0.023 g) in EtOAc/MeOH (1:1, 2 mL) was added a solution of compound **13** (0.0755 g, 0.097 mmol) in EtOAc/MeOH (1:1, 2 mL). The reaction mixture was shaken in a Parr hydrogenator under an atmosphere of H_2 for 4 h and then was filtered through a fine funnel to collect Pd/C. The residue was washed with MeOH, and the filtrate was concentrated in vacuo. The resulting product (**5**) was obtained (0.0534 g, 99%) as a white solid: R_f 0.05 (10:90 MeOH/CH_2Cl_2); ^{1}H NMR δ 0.82−0.98 (m, 6H), 1.26−1.41 (m, 1H), 1.44−1.45 (d, J = 6.68 Hz, 3H), 1.61−1.77 (m, 2H), 1.96−2.18 (m, 8H), 2.19−2.27 (m, 2H), 2.31−2.45 (m, 2H), 2.94 (s, 3H), 3.550−3.71 (m, 2H), 4.21−4.27 (m, 1H), 4.37−4.43 (m, 1H), 4.86−4.88 (m, 1H), 5.07−5.10 (m, 1H), 5.14−5.22 (m, 1H), 6.68(s, 1H) and 7.29 (s, 1H), 7.53 (s, 1H), 8.02 (s, 1H); ^{13}C NMR δ 15.7, 21.1, 23.2, 25.0, 25.1, 27.0, 28.4, 29.4, 31.0, 31.3, 37.1, 46.9, 52.1, 54.6, 56.8, 57.6, 69.3, 169.1, 171.3, 172.4, 173.0, 174.0, 176.9, 179.8; IR (KBr) 3300, 1734, 1654 cm^{-1}; HRMS (EI) *m*/*z* calcd for $C_{25}H_{39}N_5O_9Na$ (M + Na$^+$) 576.2645, found 576.2645.

[(*2S*)-Hiv^2]Didemnin M (1). To a mixture of the macrocycle amine salt (**6**, 84 mg, 0.11 mmol) and side chain (**5**) (53.4 mg, 0.096 mmol) in CH_2Cl_2 (0.30 mL) at 0 °C was added BOP (51 mg, 0.12 mmol) and NMM (0.042 mL, 0.39 mmol). After 30 min at 0 °C, the reaction mixture was allowed to warm to room temperature and stir overnight. The solution was then treated with saturated aqueous NaCl (2 mL) and extracted with EtOAc. The organic layer was washed with 10% aqueous HCl, 5% aqueous $NaHCO_3$, and saturated aqueous NaCl, dried (Na_2SO_4), filtered, and concentrated. The crude residue was purified by flash column chromatography (the silica gel was pretreated with ammonia washed hexane to neutral) eluting with MeOH/$CHCl_3$ (2:98 to 10:90) to obtain **1** (0.0331 g, 32% yield) as a pale yellow solid: R_f 0.27 (10:90 MeOH/$CHCl_3$); ^{1}H NMR δ 0.78−1.04 (m, 24H), 1.11−1.74 (m, 15H), 1.91−2.30 (m, 17H), 2.34−2.45 (m, 2H), 2.54 (s, 3H), 2.99 (s, 3H), 3.06−3.15 (m, 2H), 3.54−3.61 (m, 4H), 3.64−3.67 (m, 1H), 3.76 (s, 3H), 3.82−3.84 (m, 1H), 3.85−3.91 (m, 1H), 4.13−4.16 (m, 1H), 4.22−4.24 (m, 1H), 4.46−4.50 (m, 1H), 4.56−4.60 (m, 1H), 4.70−4.74 (m, 1H), 4.84−4.88 (m, 1H), 4.93−4.94 (d, J = 5.29 Hz, 1H), 4.98−5.03 (m, 1H), 5.11−5.17 (m, 1H), 5.24−5.27 (m, 1H), 6.81−6.82 (d, J = 8.57 Hz, 2H), 7.05−7.06 (d, J = 8.51 Hz, 2H), 7.29−7.31 (m, 1H), 7.42−7.45 (m, 1H), 7.50−7.55 (m, 1H), 7.62−7.66 (m, 1H), 7.79−7.81 (d, J = 9.83 Hz, 1H), 8.33−8.34 (d, J = 6.66 Hz, 1H); ^{13}C NMR δ 11.8, 14.1, 16.0, 17.9, 18.8, 20.8, 21.3, 23.5, 23.7, 24.6, 24.8 and 24.9 (overlap), 25.5, 25.9, 27.2 (overlap), 27.9 (overlap), 28.8, 29.3, 30.1 (overlap), 31.1, 33.6, 34.1, 36.0, 37.3, 38.6, 39.4, 46.8, 47.1, 48.3, 51.8, 54.5, 55.3, 56.5, 56.6, 56.7, 57.0, 66.0, 67.8, 68.7, 69.4, 71.2, 79.4, 114.1, 130.3, 132.1, 158.6, 168.5, 169.5, 170.4, 170.6, 170.7, 171.1, 171.3, 172.5 (overlap), 173.1, 174.0, 176.1, 178.6; IR (KBr, $CHCl_3$) 3335, 1742, 1641 cm^{-1}; HRMS (EI) *m*/*z* calcd for $C_{64}H_{98}N_{10}O_{18}Na$ (M + Na$^+$) 1317.6958, found 1317.6896; $[\alpha]^{20}_D$ −47.4 (*c* 3.96, $CHCl_3$).

Acknowledgment. We gratefully acknowledge the financial support of the National Institutes of Health (CA-40081), the National Science Foundation (CHE 99-01449), and the University of Pennsylvania.

Supporting Information Available: Copies of ^{1}H and ^{13}C NMR and IR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO000253A