Published in final edited form as:

J Nat Prod. 2009 March 27; 72(3): 372–379. doi:10.1021/np800607x.

Antineoplastic Agents 571. Total Synthesis of Bacillistatin 2^{†,‡}

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Abstract

The first total synthesis of bacillistatin 2 (**2**) has been achieved in 24 steps and 22.9% overall yield, providing a quite efficient route with maximal convergence. Notable features of this approach include two successful applications of the Mitsunobu reaction during respective assemblies of key intermediates **22** and **27**, successful employment of 2-methyl-6-nitrobenzoic anhydride (MNBA) in formation by lactonization of a macrocyclic (36-membered) ring, and very flexible access to structural modifications of the bacillistatin-type cyclodepsipeptides.

In 1998 we began an investigation of microorganisms associated with a marine crab that we obtained during a field expedition along the Pacific Ocean coast of southern Chile. While evaluating these marine microorganisms for potential antineoplastic constituents, we isolated a bacillus subsequently identified as *Bacillus silvestris*. After an extensive research endeavor, we were able to isolate from the fermentation broth two new and remarkably potent cancer cell growth inhibitors designated bacillistatins 1 (**1**) and 2 (**2**) in 34-mg and 20.1-mg yields (total), respectively. The structure determination proved to be challenging and, as reported in the preceding contribution,¹ even the X-ray crystal structure analysis of **2** gave results that were in conflict with degradation and spectroscopic results. Eventually the chemical degradation experiment proved to be decisive and revealed bacillistatin 2 to be structure **2**. Now we are pleased to report the first total synthesis of bacillistatin 2, which in turn provides unequivocal confirmation for structure **2** as summarized below.

Another compelling need for a useful total synthesis of bacillistatin 2 (**2**) is that the natural supply from the marine source, for practical purposes, is quite restricted. Thus, an efficient and flexible synthesis of bacillistatin 2 was required also in order to provide enough material to evaluate further its anticancer potential and to open up a synthetic pathway to bacillistatin 1 and novel structural analogues.

Results and Discussion

Structurally, bacillistatins 1 (**1**) and 2 (**2**) are cyclodepsipeptides containing six amino acid and six α -hydroxy carboxylic acid units coupled to form a 36-membered ring. Retrosynthetic analysis of **2** revealed three principal fragments: one **a** subunit and two **b** subunits. Our synthetic strategy (Figure 1) called for sequential connection of these subunits, utilizing an esterification protocol to achieve the key intermediate (**c**), macrocyclization of which would provide the target 36-membered ring of bacillistatin 2 (**2**).

[†]Dedicated to Dr. David G. I. Kingston of Virginia Polytechnic Institute and State University for his pioneering work on bioactive natural products.

[‡]Dedicated also to Diane Middlebrook Djerassi (1939–2007), a great humanities scholar.

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First, subunit **a** was constructed as shown in Scheme 1. *D*-Leucine was diazotized to yield alcohol **3** with configuration retention as previously reported,² and the product was coupled with *D*-valine *tert*-butyl ester via activation with *N*-ethyl-*N'*-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) in the presence of 1-hydroxybenzotriazole (HOBt) and *N*-methylmorpholine (NMM) to afford amide **4**.

Condensation of **4** with *N*-*Z*-*L*-valine using 1,3-dicyclohexylcarbodiimide (DCC) and 4-pyrrolidinopyridine (4-PPy) gave ester **5**, which was subjected to hydrogenolysis to remove the *Z*-protecting group and provide amine **6**. Next, *O*-benzoyl-*L*-lactic acid was coupled with **6** to give **7**. However, subsequent removal of the benzoyl group by mild saponification resulted in alcohols **4** (above 78%) and **9** rather than the desired product. When we substituted *O*-benzyl-*L*-lactic acid for the *O*-benzoyl derivative and coupled it with **6** to give **8**, subsequent removal of the benzyl protecting group by hydrogenolysis smoothly generated alcohol **10**.

In a parallel synthesis (Scheme 2) of subunit **b**, *D*-alloisoleucine was converted to hydroxy acid **11** with retention of configuration, and the synthesis was advanced via amide **12**, ester **13**, amine **14** and amide **15**. Next, the *tert*-butyl group was selectively removed from **15** by treatment with TFA in anhydrous CH₂Cl₂ to furnish carboxylic acid **16** (Scheme 3); compound **15** also provided alcohol **17** via selective removal of the benzyl group under a hydrogen atmosphere.

Once **16** and **17** were in hand, each an intermediate related to subunit **b**, we attempted to couple them utilizing esterification protocols, a step that we expected to be routine but that proved to be difficult to accomplish. For example, DCC and 4-PPy in CH₂Cl₂ led to a very complex mixture. Because subunit **a** was synthesized from more economical commercial reagents than was subunit **b**, we used the subunit **a** intermediates in order to explore esterification methods, namely the condensation of alcohol **10** with carboxylic acid **18**, which was derived from **8**. The first attempts, with reaction variations (Scheme 3) such as use of EDCI or DCC with dimethylaminopyridine (DMAP), failed to provide a workable yield of the desired product and instead resulted in complex mixtures. However, when we employed the Mitsunobu esterification³ reaction using diethylazodicarboxylate (DEAD) and triphenylphosphine (Ph₃P) to condense alcohol **10** with acid **18**, the reaction proceeded smoothly and cleanly to furnish **19** (epi-subunit **a**–subunit **a**) in good yield with the expected hydroxyl configuration inversion (nearly 80%).

With a useful esterification method in hand, we were now able to approach again the synthesis of the desired intermediate (subunit **b**–subunit **b**). In order to obtain the correct stereochemistry, we replaced *O*-benzyl-*L*-lactic acid (used in the synthesis of alcohol **17**) with *O*-benzyl-*D*-lactic acid, and this modification in the synthesis led via **20** and **21** to ester **22** with the required hydroxyl configuration (Scheme 4). Removal of the *tert*-butyl group by treatment with TFA in anhydrous CH₂Cl₂ provided carboxylic acid **23** in quantitative yield.

We had now succeeded in building the subunit **b**–subunit **b** dimer and needed to add subunit **a** (**10**) in order to have the key linear precursor of the target molecule. As shown in Scheme 4, we made several attempts to perform this step using a variety of reactants in CH₂Cl₂, including (1) DCC and 4-PPy, (2) EDCI and DMAP, and (3) DCC and DMAP. The results were analogous to those from the initial attempts at condensation of the subunits shown in Scheme 3, that is, we obtained only very complex mixtures (judged from monitoring by TLC), and none of the desired subunit **c** was detected. Again it became necessary to adopt the Mitsunobu approach. In a trial reaction of this type, the esterification of alcohol **10** with carboxylic acid **23** produced ester **24** (69% yield) with an inverted hydroxyl group, and therefore it was expected that use of *O*-benzyl-*D*-lactic acid instead of the *L*-isomer to synthesize the **a**-subunit epimer would lead to a subunit **c** with the desired configuration. The coupling of amine **6** and

O-benzyl-D-lactic acid was performed smoothly as before (see Scheme 1) to generate amide **25** in high yield (91%, Scheme 5), and hydrogenolysis of the benzyl ether protecting group in the presence of Pd(OH)₂/C (cat.) led to **26**. When carboxylic acid **23** and alcohol **26** were subjected to the Mitsunobu³ esterification reaction, the result was the key precursor (**27**, 63% yield) to the target cyclic depsipeptide.

Cleavage of the benzyl group from **27** under a H₂ atmosphere in the presence of catalytic Pd (OH)₂/C followed by treatment of the resulting **28** with TFA in CH₂Cl₂ afforded hydroxy acid **29**. Bacillistatin 2 was now within reach via cyclization of **29**. Initially, the Yamaguchi reaction⁴ (2,4,6-trichlorobenzoyl chloride, Et₃N, DMAP, toluene) was our choice for macrocyclization under conditions of high dilution. However, this approach was unsuccessful, as monitoring by TLC showed formation of complex mixtures. After product separation, three unexpected byproducts were obtained, and **2** was not detected. Upon closer inspection of the ¹H NMR spectra of the products, it seemed apparent that depsipeptide **29** readily decomposed under the Yamaguchi reaction conditions.

We next investigated application of the MNBA (2-methyl-6-nitrobenzoic anhydride) technique reported by Shiina et al.⁵ That reagent and procedure has proved to be quite effective in the synthesis of carboxylic acid esters and lactones. Much to our satisfaction, treatment of hydroxy acid **29** with MNBA and DMAP in CH₂Cl₂ under high dilution conditions (1.0 × 10⁻³ M) turned out to be very successful and smoothly provided bacillistatin 2 (**2**) in excellent yield (88%). The product exhibited physical properties (TLC, HPLC, ¹H NMR, ¹³C NMR, HRMS) identical with those of natural bacillistatin 2.¹

We also explored application of the Mitsunobu esterification reaction to the final ring-closure step. The required hydroxy acid precursor **35** was prepared as outlined in Scheme 6 and was subjected immediately to the Mitsunobu³ reaction conditions under high dilution (5~8 × 10⁻⁴M). Based on monitoring by TLC, ¹H NMR spectroscopy and flash column chromatography, this reaction completely consumed precursor **35** but resulted in a very complex mixture that contained only trace amounts of **2**, as identified by high-resolution mass spectroscopy.

Bioactivity

The synthetic product **2** and the precursors listed in Table 1 were evaluated against a minipanel of human cancer cell lines and the murine P388 lymphocytic leukemia cell line. Cyclodepsipeptide **2** itself has the most significant activity, in line with that of the natural product.¹ Of the precursors, the subunit-*c* epimers **27** and **33** are the most active.

Experimental Section

General Experimental Procedures

Solvents used for the chromatographic procedures were redistilled. 2-Methyl-6-nitrobenzoic anhydride (MNBA) was obtained from TCI America; other starting materials including *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI), 1-hydroxybenzotriazole (HOBt), *N*-methylmorpholine (NMM), 1,3-dicyclohexylcarbodiimide (DCC), 4-pyrrolidinopyridine (4-PPy), dimethylaminopyridine (DMAP), diethylazodicarboxylate (DEAD), and triphenylphosphine (Ph₃P) were acquired from Acros Organics and Sigma-Aldrich. Reagents were used as received. Thin-layer chromatography (TLC) was carried out with Analtech 250-μm silica gel GHLF plates supplied by Analtech, Inc., Newark, DE. The TLC plates were viewed under UV light and developed with ceric sulfate–sulfuric acid (heating for 3 min). The crude products were separated by flash column chromatography (CC) on flash (230–400 mesh ASTM) silica from E. Merck. Analytical HPLC

was conducted with a Hewlett-Packard model 1100 HPLC coupled with a diode-array detector and an evaporative light scattering detector. Melting points are uncorrected and were determined on an Electrothermal Mel-Temp apparatus. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. IR spectra were obtained with a Thermo Nicolet Avatar 360 FT-IR instrument equipped with a single reflection horizontal ATR sampling device from PIKE Technologies. The ^1H and ^{13}C NMR spectra were recorded on Varian Gemini 300, Varian Unity 400, or Varian Unity 500 instruments in CDCl_3 . High-resolution mass spectra were obtained on a JEOL LCmate magnetic sector instrument by APCI+ with a poly(ethylene glycol) reference. Elemental analyses were determined by Galbraith Laboratories, Inc., Knoxville, TN.

Carboxylic Acid **3**.²

To a cooled (ice bath) solution of *D*-leucine (16 g, 0.122 mol) in H_2SO_4 (1 M, 320 mL) was added dropwise a solution of NaNO_2 (64 g, 0.927 mol) in distilled water (300 mL). The reaction mixture temperature was kept below 5 °C for 10 h following the addition and was then allowed to warm to room temperature and stirred for four days. The mixture was then saturated with NaCl and extracted with ethyl acetate (3×500 mL). The combined organic phase was washed with water ($2 \times$) and brine ($2 \times$) and dried over Na_2SO_4 . Removal of solvent under reduced pressure afforded chiral hydroxy acid **3** (15.2 g, 93.8%) as a colorless solid: mp 76–80 °C; ^1H NMR (CDCl_3 , 300 MHz) δ 4.30 (1H, dd, $J_1 = 6.0$ Hz, $J_2 = 9.0$ Hz), 1.98–1.83 (1H, m), 1.66–1.60 (2H, m), 0.99 (3H, s), 0.96 (3H, s).

Amide **4**

To a solution of *D*-valine *tert*-butyl ester (0.90 g, 5.2 mmol) in anhydrous CH_2Cl_2 (35 mL) were added hydroxy acid **3** (0.53 g, 4 mmol), EDCI (1.2 g, 6.4 mmol), HOBt (0.864 g, 6.4 mmol), and NMM (1.7 mL, 16 mmol) under an argon atmosphere at 0 °C. After 5 min, the reaction mixture was allowed to warm to room temperature and stirred for 2 h, by which time TLC analysis showed that the starting material was completely consumed. The mixture was cooled to 0 °C and the reaction terminated by addition of saturated aqueous NH_4Cl (5 mL). After extraction with CH_2Cl_2 , the organic layer was washed and dried as stated previously. Removal of solvent followed by flash CC yielded compound **4** (0.97 g, 84%) as an oil: R_f 0.2 (4:1 hexane–EtOAc); $[\alpha]_D^{22} +21.9$ (*c* 1.08, CHCl_3); IR (CHCl_3) ν_{max} 3394 (br), 2962, 2360, 2339, 1734 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 6.95 (1H, d, $J = 8.4$ Hz); 4.45 (1H, dd, $J_1 = 4.8$ Hz, $J_2 = 9.0$ Hz), 4.48–4.15 (1H, m), 2.80 (1H, d, $J = 5.4$ Hz), 2.24–2.14 (1H, m), 1.92–1.82 (1H, m), 1.70–1.52 (2H, m), 1.48 (9H, s), 0.98–0.90 (12H, m); EIMS m/z 175, 186, 214, 231, 272 ($\text{M}^+ - \text{CH}_3$), 287 (M^+); HREIMS m/z 287.2110 [$\text{M}]^+$ (calcd for $\text{C}_{15}\text{H}_{29}\text{NO}_4$, 287.2097).

Ester **5**

To a solution of alcohol **4** (72 mg, 0.25 mmol) and *N*-*Z*-*L*-valine (82 mg, 0.326 mmol) in anhydrous CH_2Cl_2 (7 mL) that was stirring in an ice bath were added DCC (82 mg, 0.326 mmol) and 4-PPy (59 mg, 0.40 mmol). The reaction mixture was stirred for 48 h at room temperature (reaction complete by TLC). The mixture was cooled to 0 °C, diluted with H_2O (2 mL), and extracted with CH_2Cl_2 ($2 \times$). The combined CH_2Cl_2 phase was first washed with NaHCO_3 (aq) and then washed and dried as above. Removal of solvent followed by flash CC gave compound **5** (0.112 g, 86%) as an oil: R_f 0.3 (3:1 hexane–EtOAc); $[\alpha]_D^{22} +5.84$ (*c* 1.25, CHCl_3); IR (CHCl_3) ν_{max} 3337 (br), 2964, 2360, 1731, 1530 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 7.36–7.33 (5H, m), 6.63 (1H, d, $J = 8.7$ Hz), 5.41 (1H, d, $J = 8.7$ Hz); 5.22 (1H, dd, $J_1 = 3.3$ Hz, $J_2 = 9.0$ Hz), 5.14 (1H, d, $J = 12.0$ Hz), 5.08 (1H, d, $J = 12.0$ Hz), 4.37 (1H, dd, $J_1 = 4.8$ Hz, $J_2 = 8.7$ Hz), 4.33 (1H, dd, $J_1 = 5.4$ Hz, $J_2 = 8.7$ Hz), 2.25–2.12 (2H, m), 1.82–1.69 (3H, m), 1.44 (9H, s), 1.02–0.89 (18H, m); MS (FAB) m/z 185(100), 421 (9.4), 465 (30.8), 521 (MH^+ , 17.4); HRMS (FAB) m/z 521.3239 [$\text{M} + \text{H}]^+$ (calcd for $\text{C}_{28}\text{H}_{45}\text{N}_2\text{O}_7$, 521.3227).

Amine 6

To a solution of ester **5** (90 mg, 0.173 mmol) in EtOAc (3 mL) was added 10% Pd/C (20 mg), and the reaction mixture was stirred under hydrogen for 21 h, by which time reaction was complete. The catalyst was collected by filtration and the solvent was removed from the filtrate. The resulting residue was separated by flash CC to give the oily amine **6** (64 mg, 96%): R_f 0.3 (1:1 hexane–EtOAc); $[\alpha]_D^{23} +0.36$ (c 1.05, CHCl₃); IR (CHCl₃) $\nu_{\max}$ 3326 (br), 2963, 2360, 2340, 1737, 1675 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 6.49 (1H, d, J = 9.0 Hz), 5.12 (1H, dd, J_1 = 3.6 Hz, J_2 = 10.2 Hz), 4.34 (1H, dd, J_1 = 4.5 Hz, J_2 = 8.7 Hz), 3.32 (1H, d, J = 5.4 Hz), 2.15–1.97 (2H, m), 1.75–1.56 (3H, m), 1.39 (9H, s), 0.96–0.82 (18H, m); ¹³C NMR (CDCl₃, 300 MHz) δ 17.2, 17.6, 18.8, 19.4, 21.4, 23.2, 24.5, 28.0, 31.5, 31.7, 40.9, 56.9, 60.0, 73.2, 82.3, 170.1, 170.9, 175.0; HRMS (FAB) m/z 387.2856 [M + H]⁺ (calcd for C₂₈H₄₅N₂O₇, 387.2859).

Benzoyl Ester 7

To a cooled (ice bath) solution of amine **6** (0.49 g, 1.27 mmol) and *O*-benzoyl-L-lactic acid (0.32 g, 1.65 mmol) in anhydrous CH₂Cl₂ (40 mL) that was stirring under argon were added EDCI (0.39 g, 2.03 mmol), HOBt (0.27 g, 2.03 mmol) and NMM (0.6 mL, 5.08 mmol). The reaction mixture was stirred for 43 h at room temperature, cooled to 0 °C, quenched with aqueous NH₄Cl (10 mL) and extracted with CH₂Cl₂. The organic layer was washed and dried as stated previously. After removal of solvent, flash CC gave product **7** (0.62 g, 87%) as a colorless solid: R_f 0.3 (4:1 hexane–EtOAc); $[\alpha]_D^{23} +33.6$ (c 1.7, CHCl₃); IR (CHCl₃) $\nu_{\max}$ 3322 (br), 1674, 1573, 1469 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 8.07–8.05 (2H, m), 7.61–7.58 (2H, m), 7.51–7.45 (2H, m), 6.80 (1H, d, J = 8.4 Hz), 6.58 (1H, d, J = 8.7 Hz), 5.50 (1H, q, J = 6.9 Hz), 5.18 (1H, dd, J_1 = 3.9 Hz, J_2 = 8.7 Hz), 4.60 (1H, dd, J_1 = 5.4 Hz, J_2 = 8.1 Hz), 4.35 (1H, dd, J_1 = 4.8 Hz, J_2 = 8.7 Hz), 2.30–2.20 (1H, m), 2.20–2.10 (1H, m), 1.80–1.65 (3H, m), 1.62 (3H, d, J = 6.6 Hz), 1.41 (9H, s), 1.03–0.89 (18H, m); HRMS (FAB) m/z 563.3314 [M + H]⁺ (calcd for C₃₀H₄₇N₂O₈, 563.3332).

Alcohol 9

To a cooled (ice bath) solution of ester **7** (0.12 g, 0.21 mmol) in CH₃OH (10 mL) was added K₂CO₃ (43 mg, 0.3 mmol) and the mixture was stirred for 1.5 h before being neutralized with cold 1 N HCl. The solvent was removed and the residue was extracted with ethyl acetate (30 mL × 2). The combined organic phase was washed with water and brine and dried over Na₂SO₄. Flash CC of the residue led to alcohols **4** (46 mg, 78%) and **9** (29 mg, 70%). Alcohol **9**: ¹H NMR (CDCl₃, 300 MHz) δ 7.55 (1H, dd, J_1 = 5.4 Hz, J_2 = 9.0 Hz), 7.01 (1H, d, J = 9.6 Hz), 4.28 (1H, dq, J_1 = 6.6 Hz, J_2 = 5.1 Hz), 3.75 (3H, s), 2.98 (1H, d, J = 4.5 Hz), 2.25–2.15 (1H, m), 1.45 (3H, d, J = 6.9 Hz), 0.95 (3H, d, J = 7.2 Hz), 0.92 (3H, d, J = 7.2 Hz).

Benzyl Ether 8

To a cooled (ice bath) solution of amine **6** (2.2 g, 5.7 mmol) and *O*-benzyl-L-lactic acid (1.3 g, 7.4 mmol) in anhydrous CH₂Cl₂ (60 mL) that was stirring under argon were added EDCI (1.7 g, 9.12 mmol), HOBt (1.23 g, 9.12 mmol) and NMM (2.5 mL, 22.8 mmol). The mixture was stirred for 18 h at room temperature and was then cooled to 0 °C before being quenched with aqueous NH₄Cl (10 mL) and extracted with CH₂Cl₂. The organic layer was washed and dried as stated above. After removal of solvent, flash CC provided benzyl ether **8** (2.78 g, 89%) as a colorless solid: mp 110–112 °C; R_f 0.25 (4:1 hexane–EtOAc); $[\alpha]_D^{23} +4.15$ (c 1.3, CHCl₃); IR (CHCl₃) $\nu_{\max}$ 1739, 1677, 1525, 1370, 1153 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 7.38–7.31 (5H, m), 7.11 (1H, d, J = 8.7 Hz), 6.65 (1H, d, J = 8.4 Hz), 5.23 (1H, dd, J_1 = 4.2 Hz, J_2 = 9.0 Hz), 4.65 (1H, d, J = 11.7 Hz), 4.53 (1H, d, J = 11.7 Hz), 4.55 (1H, m), 4.37 (1H, dd, J_1 = 5.4 Hz, J_2 = 8.7 Hz), 4.01 (1H, q, J = 6.6 Hz), 2.24–2.17 (2H, m), 1.82–1.64 (3H, m),

1.43 (9H, s), 1.42 (3H, d, $J = 6.6$ Hz), 1.01–0.92 (18H, m); HRMS (FAB) m/z 549.3561 [$M + H$]⁺ (calcd for C₃₀H₄₉N₂O₇, 549.3540).

Alcohol 10

To a solution of benzyl ester **8** (0.46 g) in CH₃OH (5 mL) was added 10% Pd (0.10 g) and the mixture was stirred under hydrogen. After 24 h, TLC showed that almost half the starting material had been consumed. The suspension was collected by filtration using a pad of celite. To the filtrate was added 20% Pd(OH)₂/C (0.10 g), and the mixture was stirred under hydrogen for 10 h, by which time the starting material had been completely consumed. Removal of solvent followed by flash CC of the residue provided alcohol **10** (0.37 g, 95%) as an oil: R_f 0.45 (1:1 hexane–EtOAc); $[\alpha]^{24}_D -59.3$ (c 0.45, CHCl₃); IR (CHCl₃) ν_{max} 3312 (br), 2966, 2936, 2360, 1739, 1662, 1528, 1149 (s) cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 6.98 (1H, d, $J = 8.7$ Hz), 6.52 (1H, d, $J = 8.7$ Hz), 5.24 (1H, dd, $J_1 = 4.5$ Hz, $J_2 = 9.0$ Hz), 4.49 (1H, dd, $J_1 = 9.3$ Hz, $J_2 = 17.1$ Hz), 4.47 (1H, dd, $J_1 = 9.3$ Hz, $J_2 = 14.1$ Hz), 4.25 (1H, qd, $J_1 = 4.2$ Hz, $J_2 = 7.2$ Hz), 3.63 (1H, d, $J = 3.9$ Hz), 2.34–2.12 (2H, m), 1.83–1.70 (3H, m), 1.45 (9H, s), 1.43 (3H, d, $J = 7.2$ Hz), 1.00–0.89 (18H, m); HRMS (FAB) m/z 459.3077 [$M + H$]⁺ (calcd for C₂₃H₄₃N₂O₇, 459.3070).

Hydroxycarboxylic acid 11

A solution of NaNO₂ (42 g, 611 mmol) in distilled H₂O (200 mL) was added (dropwise) over 4 h to a cooled (ice bath) solution of *D*-*allo*-isoleucine (10 g, 76.3 mmol) in H₂SO₄ (1 M, 200 mL) with stirring. Evolution of NO₂ took place. The reaction temperature was kept below 5 °C, and after completion of addition the mixture was stirred for another 8–10 h at less than 5 °C followed by four days at room temperature. The mixture was then saturated with NaCl and extracted with ethyl acetate (3 × 500 mL). The combined organic phase was washed and dried as stated previously. Removal of solvent afforded **11** (10 g, quantitative): ¹H NMR (CDCl₃, 300 MHz) δ 4.29 (1H, d, $J = 2.7$ Hz), 1.89 (1H, ddd, $J_1 = 2.7$ Hz, $J_2 = 7.2$ Hz, $J_3 = 14.4$ Hz), 1.62–1.51 (1H, m), 1.41–1.28 (1H, M), 0.97 (3H, t, $J = 7.3$ Hz), 0.88 (3H, d, $J = 7.2$ Hz).

Alcohol 12

To a solution of *D*-valine *tert*-butyl ester (0.90 g, 5.2 mmol) and acid **11** (0.53 g, 4 mmol) in anhydrous CH₂Cl₂ (40 mL) that was stirring at 0 °C were added EDCI (1.2 g, 6.4 mmol), HOBt (864 mg, 6.4 mmol), and NMM (1.7 mL, 16 mmol). After 15 min, the mixture was allowed to warm to room temperature and was stirred for 45 h (reaction complete by TLC). The mixture was cooled to 0 °C and saturated aqueous NH₄Cl was added, followed by extraction with CH₂Cl₂ (50 mL × 3). The combined organic layer was washed first with cold 1 *N* HCl and then washed and dried as stated above. Removal of solvent followed by flash CC of the residue gave alcohol **12** (0.93 g, 81%) as an oil: R_f 0.8 (1:1 hexane–EtOAc); $[\alpha]^{23}_D +15$ (c 1.2, CHCl₃); IR (CHCl₃) ν_{max} 3393 (br), 2970, 1734, 1656, 1525 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 7.04 (1H, br), 4.47 (1H, dd, $J_1 = 4.7$ Hz, $J_2 = 8.8$ Hz), 4.17 (1H, dd, $J_1 = 2.7$ Hz, $J_2 = 5.4$ Hz), 3.10–2.85 (1H, br, OH), 2.19 (1H, dt, $J_1 = 2.1$ Hz, $J_2 = 6.9$ Hz), 1.93 (1H, dt, $J_1 = 2.4$ Hz, $J_2 = 7.2$ Hz), 1.48 (9H, s), 1.55–1.25 (2H, m), 0.98–0.92 (9H, m), 0.85 (3H, d, $J = 6.9$ Hz); MS (APCI) m/z 288 (MH⁺), 232; HRMS (APCI) m/z 288.2176 [$M + H$]⁺ (calcd for C₁₅H₃₀NO₄, 288.2175).

N-Z-Amide 13

To a cooled (ice bath) solution of alcohol **12** (0.58 g, 2 mmol) and *N*-Z-*L*-valine (0.653 g, 2.6 mmol) in anhydrous CH₂Cl₂ (7 mL) were added DCCI (0.66 g, 3.2 mmol) and 4-PPy (0.47 g, 3.2 mmol). The mixture was allowed to warm to room temperature and was stirred for 48 h (reaction complete by TLC). The mixture was cooled to 0 °C, treated with saturated aqueous NH₄Cl, and extracted with CH₂Cl₂ (2 ×). The combined CH₂Cl₂ phase was washed first with

NaHCO₃ (aq) and then washed and dried as stated above. Removal of solvent and flash CC provided oily *N*-Z-amide **13** (0.92 g, 88%): *R_f* 0.3 (5:1 hexane–EtOAc); [α]_D²³ –0.61 (*c* 1.65, CHCl₃); IR (CHCl₃) $\nu_{\max}$ 3336 (br), 1696, 1531 cm^{–1}; ¹H NMR (CDCl₃, 300 MHz) δ 7.36–7.32 (5H, m), 6.71 (1H, d, *J* = 5.4 Hz), 5.40 (1H, d, *J* = 5.4 Hz), 5.25 (1H, d, *J* = 3.0 Hz), 5.15 (1H, d, *J* = 12.3 Hz), 5.07 (1H, d, *J* = 12.3 Hz), 4.37 (1H, dd, *J*₁ = 5.1 Hz, *J*₂ = 8.4 Hz), 4.33 (1H, dd, *J*₁ = 5.4 Hz, *J*₂ = 8.7 Hz), 2.26–2.14 (2H, m), 1.43 (9H, s), 1.30–1.20 (3H, m), 1.04–0.90 (18H, m); HRMS (APCI) *m/z* 521.3213 [M + H]⁺ (calcd for C₂₈H₄₅N₂O₇, 521.3227).

Amine 14

A suspension of 10% Pd/C (80 mg) in a solution of **13** (0.33 g, 0.60 mmol) in EtOAc (10 mL) was stirred under hydrogen for 4 h. The catalyst was collected and the solvent was removed (in vacuo). Separation of the residue by flash CC gave amine **14** (0.24 g, 94%) as an oil: *R_f* 0.28 (1:1 hexane–EtOAc); [α]_D²³ +14 (*c* 1.0, CHCl₃); IR (CHCl₃) $\nu_{\max}$ 3317 (br), 2967, 1737, 1708 cm^{–1}; ¹H NMR (CDCl₃, 300 MHz) δ 6.68 (1H, d, *J* = 8.4 Hz), 5.24 (1H, d, *J* = 3.9 Hz), 4.40 (1H, dd, *J*₁ = 4.4 Hz, *J*₂ = 8.3 Hz), 3.54 (1H, d, *J* = 4.8 Hz), 2.25–2.02 (3H, m), 1.45 (9H, s), 1.40–1.20 (2H, m), 1.08–0.94 (18H, m); HRMS (FAB) *m/z* 387.2863 [M + H]⁺ (calcd for C₂₀H₃₉N₂O₅, 387.2859).

Depsipeptide 15

To a cooled (ice bath) solution of amine **14** (0.19 g, 0.5 mmol) and *O*-benzyl-L-lactic acid (0.13 g, 0.65 mmol) in anhydrous CH₂Cl₂ (15 mL) that was stirring under argon were added EDCI (0.15 g, 0.8 mmol), HOBT (0.11 g, 0.8 mmol) and NMM (0.22 mL, 2 mmol). The mixture was stirred under argon for 48 h at room temperature and then cooled to 0 °C before the reaction was quenched with aqueous NH₄Cl (10 mL). The mixture was extracted with CH₂Cl₂ (30 mL × 3), and the combined organic phase was first washed successively with saturated NaHCO₃ and cold 1*N* HCl and then washed and dried as above. After removal of solvent, flash CC of the residue gave depsipeptide **15** (0.23 g, 84%) as a liquid: *R_f* 0.25 (4:1 hexane–EtOAc); [α]_D²³ –1.41 (*c* 0.85, CHCl₃); IR (CHCl₃) $\nu_{\max}$ 3325, 1735, 1673, 1519, 1148 cm^{–1}; ¹H NMR (CDCl₃, 300 MHz) δ 7.38–7.31 (5H, m), 7.12 (1H, d, *J* = 8.1 Hz), 6.78 (1H, d, *J* = 9.0 Hz), 5.27 (1H, d, *J* = 3.3 Hz), 4.65 (1H, d, *J* = 11.7 Hz), 4.55 (1H, d, *J* = 11.1 Hz), 4.53 (1H, dd, *J*₁ = 6.3 Hz, *J*₂ = 9.0 Hz), 4.38 (1H, dd, *J*₁ = 5.4 Hz, *J*₂ = 8.7 Hz), 4.02 (1H, q, *J* = 6.9 Hz), 2.28–2.08 (3H, m), 1.43 (1H, d, *J* = 6.6 Hz), 1.30–1.20 (2H, m), 1.42 (9H, s), 1.02–0.94 (18H, m); HRMS (FAB) *m/z* 549.3542 [M + H]⁺ (calcd for C₃₀H₄₉N₂O₇, 549.3540).

Carboxylic Acid 16

To a cold (ice bath) solution of *tert*-butyl ester **15** (68 mg) in anhydrous CH₂Cl₂ (2 mL) was added TFA (2 mL dropwise) under argon. The solution was stirred overnight at room temperature (deprotection complete by TLC), and the solvent was removed (in vacuo). Toluene (3 mL × 3) and CH₂Cl₂ (2 mL × 2) were added successively to the residue, the solvent being removed after each addition. Acid **16** was obtained as a solid (almost quantitative yield): *R_f* 0.15 (10:1 CH₂Cl₂–CH₃OH); [α]_D²³ +24 (*c* 0.7, CHCl₃); IR (CHCl₃) $\nu_{\max}$ 3298 (br), 2361, 2331, 1744, 1669, 1528 cm^{–1}; ¹H NMR (CDCl₃, 300 MHz) δ 7.35–7.28 (6H, m), 7.18 (1H, d, *J* = 7.2 Hz), 5.30 (1H, d, *J* = 2.7 Hz), 4.56 (2H, s), 4.36 (1H, dd, *J*₁ = 6.6 Hz, *J*₂ = 8.1 Hz), 4.27 (1H, t, *J* = 6.9 Hz), 3.98 (1H, q, *J* = 6.9 Hz), 2.34–2.24 (1H, m), 2.18–2.08 (2H, m), 1.39 (3H, d, *J* = 6.6 Hz), 1.36–1.26 (2H, m), 1.01–0.93 (18H, m); HRMS (FAB) *m/z* 493.2913 [M + H]⁺ (calcd for C₂₆H₄₁N₂O₇, 493.2914).

Depsipeptide 17

Hydrogenolysis was conducted by suspending 20% Pd/C (80 mg) in a solution of benzyl ester **15** (0.12 g) in CH₃OH (3 mL) under hydrogen for 3 h (complete by TLC). The catalyst was collected on a pad of celite and the solvent was removed in vacuo. The residue was separated

by flash CC to give the desired alcohol **17** (90 mg, 91%) as an oil: R_f 0.5 (1:1, hexane–EtOAc); $[\alpha]_D^{23} -61$ (c 1.0, CHCl_3); IR (CHCl_3) ν_{max} 3307 (br), 1739, 1660, 1529, 1149 (s) cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 7.06 (1H, d, $J = 9.3$ Hz), 6.65 (1H, d, $J = 9.0$ Hz), 5.30 (1H, d, $J = 2.7$ Hz), 4.47 (1H, dd, $J_1 = 9.0$ Hz, $J_2 = 13.8$ Hz), 4.45 (1H, dd, $J_1 = 9.0$ Hz, $J_2 = 10.5$ Hz), 4.23 (1H, dq, $J_1 = 6.9$ Hz, $J_2 = 4.5$ Hz), 3.75 (1H, d, $J = 4.5$ Hz), 2.26–2.08 (3H, m), 1.45 (9H, s), 1.43 (3H, d, $J = 7.2$ Hz), 1.00–0.92 (18H, m); HRMS (FAB) m/z 459.3090 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{23}\text{H}_{43}\text{N}_2\text{O}_7$, 459.3070).

Depsipeptide 19

After deprotection (overnight) of *tert*-butyl ester **8** (93 mg) as described above (see **16**) in anhydrous CH_2Cl_2 (2 mL) with TFA (2 mL), C-terminal carboxylic acid **18** was isolated as a solid in almost quantitative yield. Next, to a solution of alcohol **10** (71 mg, 0.154 mmol) and acid **18** (76 mg, 0.154 mmol) in anhydrous toluene (2 mL) at 0 °C under an argon atmosphere was added Ph_3P (121 mg, 0.46 mmol), followed by dropwise addition of DEAD (73 μL , 0.462 mmol). The mixture was stirred at 0 °C for 10 h and at room temperature for another 10 h (reaction complete by TLC). The mixture was cooled to 0 °C before termination of reaction with H_2O (2 mL). Following extraction with EtOAc (20 mL), the organic phase was washed and dried as described previously. Removal of solvent and flash CC of the residue gave a crude sample of depsipeptide **19** in about 80% yield: ^1H NMR (CDCl_3 , 300 MHz) δ 7.36–7.34 (5H, m), 7.12 (1H, d, $J = 7.8$ Hz), 7.08 (1H, d, $J = 7.2$ Hz), 6.96 (1H, d, $J = 8.4$ Hz), 6.76 (1H, d, $J = 7.5$ Hz), 5.29–5.21 (3H, m), 4.61 (1H, d, $J = 11.4$ Hz), 4.56 (1H, d, $J = 12.0$ Hz), 4.47–4.45 (1H, m), 4.42–4.37 (1H, m), 4.34–4.18 (2H, m), 4.01 (1H, q, $J = 6.6$ Hz), 2.39–2.10 (4H, m), 1.83–1.68 (6H, m), 1.44 (9H, s), 1.41 (6H, d, $J = 7.2$ Hz), 1.02–0.93 (36H, m); HRMS (EI) m/z 933.5812 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{49}\text{H}_{81}\text{N}_4\text{O}_{13}$, 933.5800).

Depsipeptide 20

The peptide-bond-forming reaction described above (see **15**) was employed here using amine **14** (0.18 g, 0.45 mmol), *O*-benzyl- D -lactic acid (97 mg, 0.54 mmol), anhydrous CH_2Cl_2 (18 mL) at 0 °C under argon, EDCI (0.14 g, 0.72 mmol), HOBt (97 mg, 0.72 mmol), and NMM (0.2 mL, 1.8 mmol). Reaction went to completion in 24 h. Flash CC provided depsipeptide **20** (0.22 g, 87%) as an oil: R_f 0.25 (4:1 hexane–EtOAc); $[\alpha]_D^{26} +18$ (c 0.56, CHCl_3); IR (CHCl_3) ν_{max} 3418(br), 3322, 2967, 2934, 2876, 1739 (s), 1673 (s), 1518, 1148 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 7.37–7.31 (5H, m), 7.67 (1H, d, $J = 7.8$ Hz), 7.14 (1H, d, $J = 8.4$ Hz), 5.24 (1H, d, $J = 3.3$ Hz), 4.62 (1H, d, $J = 12.0$ Hz), 4.57 (1H, d, $J = 12.3$ Hz), 4.56 (1H, dd, $J_1 = 5.4$ Hz, $J_2 = 8.4$ Hz), 4.34 (1H, dd, $J_1 = 5.4$ Hz, $J_2 = 8.4$ Hz), 4.01 (1H, q, $J = 6.6$ Hz), 2.05–2.03 (3H, m), 1.43 (3H, d, $J = 6.6$ Hz), 1.43 (9H, s), 1.31–1.18 (2H, m), 1.01–0.93 (18H, m); HRMS(EI) m/z 549.3532 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{30}\text{H}_{49}\text{N}_2\text{O}_7$, 549.3540).

Depsipeptide 21

Selective deprotection of depsipeptide **20** (0.21 g, 0.38 mmol) in CH_3OH (6 mL) by use of a suspension of 20% $\text{Pd}(\text{OH})_2/\text{C}$ (46 mg) under a hydrogen atmosphere (overnight) was achieved as described above (see **17**) to afford alcohol **21** (0.17 g, 96%) as a colorless solid: mp 104–106 °C; R_f 0.5 (1:1 hexane–EtOAc); $[\alpha]_D^{26} -18$ (c 1.11, CHCl_3); IR (CHCl_3) ν_{max} 3309 (br), 2968, 2934, 1739, 1658, 1533, 1148 cm^{-1} ; ^1H NMR (CDCl_3 , 300 MHz) δ 6.78 (1H, d, $J = 8.7$ Hz), 6.56 (1H, d, $J = 8.4$ Hz), 5.15 (1H, d, $J = 3.3$ Hz), 4.46 (1H, d, $J_1 = 6.6$ Hz, $J_2 = 8.7$ Hz), 4.30 (1H, dd, $J_1 = 5.4$ Hz, $J_2 = 8.7$ Hz), 4.19 (1H, p, $J = 6.6$ Hz), 2.94 (1H, d, $J = 6.0$ Hz), 2.18–1.96 (3H, m), 1.37 (3H, d, $J = 6.6$ Hz), 1.35 (9H, s), 1.30–1.15 (2H, m), 0.92–0.81 (18H, m); HRMS (FAB) m/z 459.3075 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{23}\text{H}_{43}\text{N}_2\text{O}_7$, 459.3070); *anal.* C 60.75%, H 9.58%, N 5.90%, calcd for $\text{C}_{23}\text{H}_{42}\text{N}_2\text{O}_7$, C 60.24%, H 9.23%, N 6.11%.

Depsipeptide 22

The Mitsunobu reaction (see **19** above) was carried out with alcohol **21** (57 mg, 0.214 mmol), carboxylic acid **16** (67 mg, 0.136 mmol), anhydrous toluene (8 mL), Ph₃P (97 mg, 0.372 mmol) and DEAD (55 μ L, 0.372 mmol); reaction was complete after the mixture was stirred at 0 °C for several hours and then at room temperature for several hours. Work-up as before and flash CC of the residue provided depsipeptide **22** (97 mg, 84%) as an oil: *R_f* 0.5 (2:1 hexane–EtOAc); [α]_D²⁸ +12 (*c* 0.51, CHCl₃); IR (CHCl₃) ν_{max} 3320, 2971, 1752, 1671, 1532, 1624 cm^{−1}; ¹H NMR (CDCl₃, 500 MHz) δ 7.51 (1H, d, *J* = 7.0 Hz), 7.44 (1H, d, *J* = 6.5 Hz), 7.37–7.31 (5H, m), 7.12 (1H, d, *J* = 8.5 Hz), 7.09 (1H, d, *J* = 6.5 Hz), 5.33 (1H, q, *J* = 7.0 Hz), 5.28 (1H, d, *J* = 2.5 Hz), 5.24 (1H, d, *J* = 2.5 Hz), 4.61 (1H, d, *J* = 11.0 Hz), 4.58 (1H, d, *J* = 11.0 Hz), 4.31 (1H, dd, *J*₁ = 6.0 Hz, *J*₂ = 8.0 Hz), 4.25 (1H, t, *J* = 7.3 Hz), 4.19 (1H, t, *J* = 7.3 Hz), 4.13 (1H, t, *J* = 7.5 Hz), 4.05 (1H, q, *J* = 6.8 Hz), 2.36–2.01 (6H, m), 1.45 (3H, d, *J* = 6.5 Hz), 1.41 (3H, d, *J* = 7.5 Hz), 1.40 (9H, s), 1.35–1.26 (4H, m), 1.05–0.91 (36H, m); ¹³C NMR (CDCl₃, 500 MHz) δ 174.7, 171.5, 170.9, 170.8, 170.4, 170.3, 169.5, 137.3, 128.6, 128.1, 127.9, 81.4, 76.9, 76.0, 72.2, 70.4, 59.3, 59.2, 58.6, 58.1, 37.0, 36.8, 30.5, 29.9, 29.8, 29.7, 29.4, 28.0, 26.1, 19.3, 19.2, 19.1, 19.0, 18.9, 18.6, 18.5, 18.4, 17.2, 14.1, 14.0, 11.8; APT (CDCl₃, 500 MHz) δ −174.7, −171.5, −170.9, −170.8, −170.4, −170.3, −169.5, −137.3, 128.6, 128.1, 127.9, −81.4, −76.8, 76.0, −72.2, 70.4, 59.3, 59.2, 58.6, 58.1, 37.0, 36.8, 30.5, 29.9, 29.8, 29.7, 29.4, 28.0, −26.1, 19.3, 19.2, 19.1, 19.0, 18.8, 18.6, 18.5, 18.4, 17.2, 14.1, 14.0, 11.8; HRMS (APCI) *m/z* 933.5759 [M + H]⁺ (calcd for C₄₉H₈₁N₄O₁₃, 933.5800).

Depsipeptide 24

Selective deprotection of *tert*-butyl ester **22** (50 mg, 0.054 mmol) in anhydrous CH₂Cl₂ (3 mL) and TFA (3.5 mL) under argon with cooling (ice bath) was performed as described above (see **16**) to yield carboxylic acid **23**. The product was obtained as a solid in almost quantitative yield and was used without further purification in a Mitsunobu reaction as described above (see **19**) with alcohol **10** (29.6 mg, 0.064 mmol), anhydrous toluene (4.5 mL), Ph₃P (42 mg, 0.162 mmol) and DEAD (25.6 μ L, 0.162 mmol). The mixture was stirred at 0 °C for 5 h and at room temperature overnight (reaction complete by TLC). Flash CC led to separation of the crude product (**24**, 48 mg, 69%) as a liquid: *R_f* 0.5 (2:1 hexane–EtOAc); ¹H NMR (CDCl₃, 300 MHz) δ 7.71 (1H, d, *J* = 5.7 Hz), 7.67 (1H, d, *J* = 7.8 Hz), 7.64 (1H, d, *J* = 6.3 Hz), 7.37–7.31 (5H, m), 7.13 (1H, d, *J* = 8.4 Hz), 7.09 (1H, d, *J* = 6.6 Hz), 6.82 (1H, d, *J* = 8.7 Hz), 5.32–5.17 (6H, m), 4.60 (2H, s), 4.54–4.40 (2H, m), 4.33–3.96 (4H, m), 2.40–2.06 (9H, m), 1.45–1.40 (18H, m), 1.32–1.25 (6H, m), 1.07–0.90 (54H, m); HRMS (APCI) *m/z* 1317.8137 [M + H]⁺ (calcd for C₆₈H₁₁₃N₆O₁₉, 1317.8061).

Depsipeptide 25

To a cooled (ice bath) solution of amine **6** (0.18 g, 0.47 mmol) in anhydrous CH₂Cl₂ (8 mL) that was stirring under argon were added *O*-benzyl-D-lactic acid (0.10 g, 0.56 mmol), EDCI (144 mg, 0.75 mmol), HOBT (101 mg, 0.75 mmol) and NMM (0.21 mL, 1.88 mmol). The mixture was stirred overnight and then reaction was terminated and the mixture treated as described above (see **15**). Flash CC gave product **25** (0.23 g, 91%) as a liquid: *R_f* 0.3 (4:1 hexane–EtOAc); [α]_D²⁶ +22.5 (*c* 0.59, CHCl₃); ¹H NMR (CDCl₃, 300 MHz) δ 7.36–7.31 (5H, m), 7.10 (1H, d, *J* = 8.4 Hz), 6.58 (1H, d, *J* = 7.5 Hz), 5.21 (1H, dd, *J*₁ = 3.9 Hz, *J*₂ = 9.3 Hz), 4.62 (1H, d, *J* = 12.3 Hz), 4.57 (1H, d, *J* = 12.3 Hz), 4.54 (1H, dd, *J*₁ = 5.1 Hz, *J*₂ = 8.4 Hz), 4.34 (1H, dd, *J*₁ = 5.1 Hz, *J*₂ = 8.4 Hz), 4.01 (1H, q, *J* = 6.9 Hz), 2.28–2.14 (2H, m), 1.82–1.66 (3H, m), 1.44 (9H, s), 1.42 (3H, d, *J* = 7.2 Hz), 0.99–0.91 (18H, m); HRMS (APCI) *m/z* 549.3549 [M+H]⁺ (calcd for C₃₀H₄₉N₂O₇, 549.3540).

Depsipeptide 26

Selective benzyl group cleavage from **25** (230 mg) was carried out as described above (see **21**) using 20% Pd(OH)₂/C (60 mg) in CH₃OH (15 mL) under hydrogen (overnight) to provide, following flash CC, alcohol **26** (0.18 g, 95%) as a syrup/solid: *R*_f 0.4 (1:1 hexane–EtOAc); IR (CHCl₃) ν_{max} 3310 (br), 2967, 1739, 1661, 1530, 370, 1150 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 6.90 (1H, d, *J* = 8.4 Hz), 6.58 (1H, d, *J* = 8.7 Hz), 5.21 (1H, m), 4.56 (1H, dd, *J*₁ = 6.3 Hz, *J*₂ = 9.0 Hz), 4.41 (1H, dd, *J*₁ = 5.0 Hz, *J*₂ = 8.8 Hz), 4.27 (1H, dq, *J*₁ = 6.6 Hz, *J*₂ = 6.6 Hz), 3.08 (1H, d, *J* = 6.6 Hz), 2.30–2.14 (2H, m), 1.84–1.68 (3H, m), 1.46 (3H, d, *J* = 7.2 Hz), 1.45 (9H, s), 1.01–0.91 (18H, m).

Depsipeptide 27

Alcohol **26** (31 mg, 0.068 mmol) was condensed with carboxylic acid **23** (0.057 mmol) in anhydrous toluene (4.5 mL at 0 °C under argon) under Mitsunobu reaction conditions (see **19**) with Ph₃P (45 mg, 0.171 mmol) and DEAD (27 μ L, 0.171 mmol). The mixture was stirred at 0 °C for 5 h and at room temperature overnight, by which time reaction was complete, and the product mixture was extracted (see **19**). Separation by flash CC yielded depsipeptide **27** (35 mg, 63%) as a liquid: *R*_f 0.4 (2:1 hexane–EtOAc); IR (CHCl₃) ν_{max} 3307, 2963, 2927, 2359, 1750, 1655, 1534, 1370, 1145 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 7.87 (1H, d, *J* = 6.8 Hz), 7.70 (1H, d, *J* = 5.6 Hz), 7.62 (1H, d, *J* = 6.4 Hz), 7.56 (1H, d, *J* = 7.6 Hz), 7.34–7.28 (5H, m), 7.08 (1H, d, *J* = 6.0 Hz), 7.07 (1H, d, *J* = 9.2 Hz), 5.29 (1H, q, *J* = 6.8 Hz), 5.25–5.22 (4H, m), 4.58 (1H, d, *J* = 11.2 Hz), 4.55 (1H, d, *J* = 11.6 Hz), 4.26 (1H, dd, *J*₁ = 5.6 Hz, *J*₂ = 8.4 Hz), 4.27–4.24 (1H, m), 4.10 (1H, dd, *J*₁ = 7.6 Hz, *J*₂ = 6.8 Hz), 4.02–3.98 (2H, m), 3.94 (1H, *J*₁ = 5.6 Hz, *J*₂ = 8.0 Hz), 3.90 (1H, dd, *J*₁ = 6.6 Hz, *J*₂ = 8.2 Hz), 2.34–2.22 (4H, m), 2.16–2.03 (4H, m), 1.79–1.58 (3H, m), 1.42 (3H, d, *J* = 6.8 Hz), 1.41 (3H, d, *J* = 6.8 Hz), 1.39 (9H, s), 1.38 (3H, d, *J* = 6.8 Hz), 1.35–1.19 (4H, m), 1.02 (3H, d, *J* = 6.2 Hz), 0.99–0.83 (51H, m); APT (CDCl₃, 400 MHz) δ 174.9, 171.6, 171.0, 170.9, 170.86, 170.8, 170.46, 170.4, 170.3, 170.0, 137.2, –128.6, –128.1, –127.9, 81.4, –76.6, –76.5, –76.0, –73.1, 72.2, –70.5, –70.0, –60.2, –59.7, –59.6, –58.7, –57.9, 41.0, –36.8, –36.7, –30.9, –30.1, 29.7, –29.3, –29.2, –29.0, –27.9, 26.3, 26.1, –24.4, –23.1, –21.4, –19.7, –19.4, –19.36, –19.3, –19.2, –19.1, –19.0, –18.9, –18.8, –18.4, –18.3, –17.4, –17.1, –14.1, –13.9, –11.8, –11.7; HRMS (APCI) *m/z* 1317.809 [M+H]⁺, (calcd for C₆₈H₁₁₃N₆O₁₉, 1317.806).

Depsipeptide 28

Cleavage of the benzyl group from **27** (55 mg) was carried out as described above (see **17**) using 20% Pd(OH)₂/C (40 mg) in CH₃OH (5 mL) under hydrogen (overnight). Purification by flash CC gave alcohol **28** (45 mg, 88%) as an oily solid: *R*_f 0.6 (1:1 hexane–EtOAc); IR (CHCl₃) ν_{max} 3306, 2966, 1750, 1656, 1534, 1181, 1146 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 7.80 (1H, d, *J* = 6.0 Hz), 7.65 (1H, d, *J* = 8.8 Hz), 7.63 (1H, d, *J* = 7.2 Hz), 7.57 (1H, d, *J* = 6.8 Hz), 7.55 (1H, d, *J* = 6.4 Hz), 7.14 (1H, d, *J* = 8.4 Hz), 5.33 (1H, q, *J* = 7.2 Hz), 5.25 (1H, d, *J* = 2.4 Hz), 5.20–5.17 (2H, m), 5.09 (1H, d, *J* = 2.8 Hz), 4.50 (1H, dd, *J*₁ = 7.2 Hz, *J*₂ = 8.4 Hz), 4.21 (1H, dd, *J*₁ = 5.6 Hz, *J*₂ = 8.4 Hz), 4.10 (1H, dd, *J*₁ = 7.0 Hz, *J*₂ = 6.9 Hz), 4.00–3.90 (4H, m), 2.32–2.03 (8H, m), 1.74–1.64 (3H, m), 1.35 (9H, s), 1.37–1.35 (6H, m), 1.25 (3H, d, *J* = 6.8 Hz), 1.32–1.14 (4H, m), 1.03–0.84 (54H, m); HRMS (APCI) *m/z* 1227.757 [M+H]⁺ (calcd for C₆₁H₁₀₇N₆O₁₉, 1227.759).

Bacillistatin 2

To a cooled (ice bath) solution of *tert*-butyl ester **28** (38 mg, 0.031 mmol) in anhydrous CH₂Cl₂ (3 mL) under argon was added TFA (3 mL) dropwise. The solution was stirred overnight at room temperature to complete the reaction, and the solvent was removed (in vacuo). Toluene (3 mL $\times$ 4) and CH₂Cl₂ (3 mL $\times$ 4) were added successively to the residue, the solvent being removed after each addition, to yield hydroxy acid **29** as a syrup/solid (almost

quantitative yield) that was used without further purification. Next, to a stirred solution of MNBA (6.8 mg, 0.02 mmol) and DMAP (6.5 mg, 0.054 mmol) in anhydrous CH₂Cl₂ (13 mL) under argon was added dropwise (over 3 h from a syringe) a solution of **29** (18 mg, 0.0154 mmol) in anhydrous CH₂Cl₂ (2 mL). The mixture was stirred overnight and then cooled to 0 °C before termination of reaction with water (2 mL). The mixture was extracted with CH₂Cl₂ (30 mL × 2), and the combined extract was washed and dried as stated previously. The solvent was removed, and flash CC of the residue afford bacillistatin 2 (**2**, 15.6 mg, 88%) as a colorless solid: *R_f* 0.55 (4:1 hexane–EtOAc); mp 143–146 °C; [α]_D²⁰ +33.9 (*c* 0.44, CHCl₃); IR (CHCl₃) ν_{max} 3004, 2965, 1757, 1659, 1539, 1184 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 7.92 (1H, d, *J* = 8.0 Hz), 7.83 (1H, d, *J* = 8.0 Hz), 7.80 (1H, d, *J* = 5.5 Hz), 7.74 (1H, d, *J* = 7.0 Hz), 7.70 (1H, d, *J* = 6.5 Hz), 7.61 (1H, d, *J* = 5.5 Hz), 5.25 (1H, dd, *J*₁ = 7.0 Hz, *J*₂ = 10.0 Hz), 5.18 (1H, d, *J* = 2.5 Hz), 5.28–5.17 (3H, m), 5.11 (1H, d, *J* = 2.5 Hz), 4.22 (1H, dd, *J*₁ = 9.5 Hz, *J*₂ = 8.5 Hz), 4.12 (1H, dd, *J*₁ = 8.0 Hz, *J*₂ = 9.5 Hz), 2.33–2.09 (8H, m), 1.77–1.72 (3H, m), 1.45 (3H, d, *J* = 7.0 Hz), 1.44 (3H, d, *J* = 7.0 Hz), 1.43 (3H, d, *J* = 6.5 Hz), 1.38–1.27 (4H, m), 1.08–0.87 (54H, m); APT (CDCl₃, 500 MHz) δ 172.6, 172.2, 172.17, 172.13, 171.8, 171.6, 171.5, 170.8, 170.23, 170.17, 169.8, -76.6, -73.4, -70.6, -70.5, -70.1, -60.6, -60.5, -59.6, -59.15, -59.07, -58.4, 40.7, -36.9, -36.7, -28.7, -28.5, -28.42, -28.39, -28.35, -28.3, 26.22, 26.19, -24.5, -23.1, -21.5, -19.9, -19.8, -19.6, -19.45, -19.41, -19.38, -19.3, -19.23, -19.17, -19.14, -19.12, -19.0, -17.3, -17.1, -16.9, -14.2, -11.8; HRMS (APCI) *m/z* 1153.691 [M+H]⁺ (calcd for C₆₁H₁₀₇N₆O₁₉, 1153.686). The synthetic specimen of bacillistatin 2 was found to be identical (TLC, HPLC, ¹H NMR, ¹³C NMR, HRMS) with the natural product.¹

Compound 31

The *tert*-butyl group was removed from **20** (94 mg, 0.172 mmol) in anhydrous CH₂Cl₂ (2 mL) and TFA (3 mL) (ice bath under argon) as described earlier (see **16**) to yield carboxylic acid **30**. The product was obtained as a solid in quantitative yield and was used without further purification in a Mitsunobu reaction as described above (see **19**) with alcohol **21** (92 mg, 0.20 mmol), anhydrous toluene (8 mL), Ph₃P (135 mg, 0.516 mmol) and DEAD (82 μ L, 0.516 mmol). The resulting mixture was stirred at 0 °C for 5 h and at room temperature overnight (reaction complete by TLC). Flash CC led to separation of the crude product (**31**, 125 mg, 79%) as a liquid: IR (CHCl₃) ν_{max} 3307, 2966, 2935, 1751, 1658, 1532, 1465, 1180, 1147 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 7.47 (1H, d, *J* = 6.5 Hz), 7.45 (1H, d, *J* = 6.5 Hz), 7.39–7.33 (5H, m), 7.10 (1H, d, *J* = 9.0 Hz), 7.08 (1H, d, *J* = 7.0 Hz), 5.35 (1H, q, *J* = 7.0 Hz), 5.27 (1H, d, *J* = 2.5 Hz), 5.25 (1H, d, *J* = 3.5 Hz), 4.59 (1H, d, *J* = 11.5 Hz), 4.56 (1H, d, *J* = 12.0 Hz), 4.31 (1H, dd, *J*₁ = 6.0 Hz, *J*₂ = 8.5 Hz), 4.25 (1H, t, *J* = 7.2 Hz), 4.18 (1H, t, *J* = 7.2 Hz), 4.08 (1H, dd, *J*₁ = 7.0 Hz, *J*₂ = 8.5 Hz), 4.00 (1H, q, *J* = 6.8 Hz), 2.34–2.09 (6H, m), 1.45 (3H, d, *J* = 6.5 Hz), 1.42 (3H, d, *J* = 6.0 Hz), 1.41 (9H, s), 1.36–1.25 (4H, m), 1.04 (3H, d, *J* = 7.0 Hz), 1.02–0.90 (33H, m); ¹³C NMR (CDCl₃, 500 MHz) δ 174.4, 171.2, 170.9, 170.7, 170.44, 170.4, 170.3, 169.6, 137.2, 128.6, 128.1, 127.6, 81.4, 76.9, 76.6, 75.7, 71.8, 70.3, 59.5, 59.2, 58.7, 58.1, 37.0, 36.9, 30.5, 29.8, 29.7, 29.3, 27.9, 26.1, 26.08, 19.3, 19.24, 19.22, 19.17, 19.1, 18.8, 18.7, 18.4, 18.1, 17.3, 14.1, 14.0, 11.78, 11.76; APT (CDCl₃, 500 MHz) δ 174.4, 171.2, 170.9, 170.7, 170.44, 170.4, 170.3, 169.5, 137.2, -128.6, -128.1, -127.6, 81.4, -76.9, -76.6, -75.7, 71.8, -70.3, -59.5, -59.2, -58.7, -58.1, -37.0, -36.9, -30.5, -29.8, -29.7, -29.3, -27.9, 26.1, 26.08, -19.3, -19.24, -19.22, -19.17, -19.1, -18.8, -18.7, -18.4, -18.1, -17.3, -14.1, -14.0, -11.78, -11.77; HRMS (FAB) *m/z* 933.5795 [M + H]⁺ (calcd for C₄₉H₈₁N₄O₁₃, 933.5800).

Compound 33

Cleavage of the *tert*-butyl group from **31** (102 mg, 0.109 mmol) in anhydrous CH₂Cl₂ (3 mL) and TFA (5 mL) (ice bath under argon) was carried out as described earlier (see **16**) to yield carboxylic acid **32**. The product was obtained as a solid in quantitative yield and was used without further purification in a Mitsunobu reaction as described above (see **19**) with alcohol

26 (80 mg, 0.174 mmol), anhydrous toluene (10 mL), Ph_3P (87 mg, 0.327 mmol) and DEAD (52 μL , 0.327 mmol). The resulting mixture was stirred at 0 °C for 5 h and at room temperature overnight, by which time reaction was complete (by TLC). Flash column chromatography led to separation of the crude product (**33**, 95 mg, 66%) as a liquid: R_f 0.30 (3:2 hexane–EtOAc); IR (CHCl_3) ν_{max} 3309, 2965, 2935, 1750, 1655, 1533, 1180, 1146 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz) δ 7.91 (1H, d, J = 6.5 Hz), 7.77 (1H, d, J = 5.5 Hz), 7.64 (1H, d, J = 6.0 Hz), 7.58 (1H, d, J = 8.0 Hz), 7.39–7.33 (5H, m), 7.10 (1H, d, J = 9.5 Hz), 7.09 (1H, d, J = 6.5 Hz), 5.34 (1H, q, J = 7.0 Hz), 5.31–5.26 (4H, m), 4.62 (1H, d, J = 12.0 Hz), 4.56 (1H, d, J = 11.5 Hz), 4.31–4.27 (2H, m), 4.11 (1H, dd, J_1 = 7.0 Hz, J_2 = 8.0 Hz), 3.99 (1H, q, J = 7.0 Hz), 3.94 (1H, dd, J_1 = 6.5 Hz, J_2 = 8.0 Hz), 3.98–3.93 (2H, m), 2.38–2.24 (4H, m), 2.22–2.08 (4H, m), 1.84–1.68 (3H, m), 1.47 (3H, d, J = 6.5 Hz), 1.45 (3H, d, J = 6.0 Hz), 1.44 (3H, d, J = 6.0 Hz), 1.42 (9H, s), 1.39–1.23 (4H, m), 1.06 (3H, d, J = 6.0 Hz), 1.02–0.91 (51H, m); APT (CDCl_3 , 500 MHz) δ 174.7, 171.7, 171.4, 170.98, 170.92, 170.90, 170.8, 170.6, 170.44, 170.37, 170.3, 169.9, 137.2, –128.6, –128.1, –127.6, 81.4, –76.6, –76.4, –75.7, –73.1, 71.8, –70.6, –69.9, –60.3, –60.0, –59.7, –58.9, –58.8, –57.9, 41.3, –36.9, –36.7, –30.9, –30.1, –29.7, –29.3, –29.2, –28.9, –28.0, –27.9, 26.3, 26.1, –24.4, –23.1, –21.4, –19.7, –19.5, –19.4, –19.21, –19.18, –18.96, –18.94, –18.9, –18.3, –18.1, –17.5, –17.1, –14.1, –13.8, –11.84, –11.79; HRMS (APCI) m/z 1317.804 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{68}\text{H}_{113}\text{N}_6\text{O}_{19}$, 1317.806).

Compound 34

Cleavage of the benzyl group from **33** (75 mg) was carried out as described above (see **17**) using 20% Pd (OH) $_2$ /C (15 mg) in CH_3OH (5 mL) under hydrogen (overnight) to provide (following flash chromatography) alcohol **34** (60 mg, 86%) as an oily solid: R_f 0.50 (1:1 hexane–EtOAc); IR (CHCl_3) ν_{max} 3306 (br), 2966, 1750, 1656, 1536, 1182, 1147 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 7.77 (1H, d, J = 5.2 Hz), 7.75 (1H, d, J = 5.2 Hz), 7.66 (1H, d, J = 5.6 Hz), 7.64 (1H, d, J = 6.8 Hz), 7.52 (1H, d, J = 5.2 Hz), 7.33 (1H, d, J = 6.8 Hz), 5.40 (1H, q, J = 5.2 Hz), 5.33–5.27 (2H, m), 5.24 (1H, d, J = 2.0 Hz), 5.20 (1H, d, J = 2.0 Hz), 5.15–5.10 (1H, m), 4.29 (1H, dd, J_1 = 6.8 Hz, J_2 = 8.4 Hz), 4.27 (1H, dd, J_1 = 4.8 Hz, J_2 = 6.4 Hz), 4.16 (1H, dd, J_1 = 5.2 Hz, J_2 = 6.4 Hz), 4.08 (1H, dd, J_1 = 5.6 Hz, J_2 = 8.0 Hz), 4.03 (1H, dd, J_1 = 2.8 Hz, J_2 = 5.6 Hz), 4.01 (1H, dd, J_1 = 2.4 Hz, J_2 = 5.2 Hz), 3.91–3.87 (1H, m), 2.42–2.31 (2H, m), 2.30–2.01 (3H, m), 2.16–2.04 (3H, m), 1.84–1.70 (3H, m), 1.45 (3H, d, J = 5.6 Hz), 1.43 (9H, s), 1.41 (3H, d, J = 5.2 Hz), 1.37–1.22 (4H, m), 1.33 (3H, d, J = 5.6 Hz), 1.11–0.91 (54H, m); ^{13}C NMR (CDCl_3 , 500 MHz) δ 176.5, 172.8, 172.2, 171.3, 171.2, 171.1, 170.93, 170.89, 170.86, 170.5, 170.1, 81.6, 77.1, 76.4, 72.9, 70.9, 70.2, 67.8, 59.98, 59.7, 59.6, 58.4, 58.2, 40.9, 37.2, 36.7, 30.5, 29.6, 29.4, 29.3, 28.5, 28.4, 27.98, 26.2, 26.1, 24.4, 23.3, 21.3, 20.4, 19.5, 19.4, 19.3, 19.2, 19.1, 19.05, 18.8, 18.4, 17.8, 16.8, 14.2, 14.1, 11.8, 11.75; HRMS (APCI) m/z 1227.746 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{61}\text{H}_{107}\text{N}_6\text{O}_{19}$, 1227.759).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are pleased to acknowledge financial support provided by Outstanding Investigator Grant CA44344–10–12, grant R01 CA90441–01–05, grant 2R56 CA090441–06A1, and grant 5R01 CA090441–07 from the Division of Cancer Treatment, Diagnosis and Centers, National Cancer Institute, DHHS; the Fannie E. Rippel Foundation; Dr. Alec D. Keith; the Arizona Disease Control Research Commission; the Robert B. Dalton Endowment Fund; Dr. William Crisp and Mrs. Anita Crisp; and Dr. John C. Budzinski. We also thank Drs. Ernest Hamel, Fiona Hogan, and Ron Nieman; and Felicia Craciunescu, Natalie Fuller, Christine Weber, and Lee Williams for other helpful assistance.

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1, R₁ = CH₃, R₂ = H (2*R*-Hy-3*S*-Me-v), bacillistatin 1

2, R₁ = H, R₂ = CH₃ (2*R*-Hy-4-Me-v), bacillistatin 2

Figure 1.
Retrosynthetic analysis of bacillistatin 2 (2).

Scheme 1.

Scheme 2.

Scheme 3.

Scheme 4.

Scheme 5.

Scheme 6.

Table 1

Inhibition of the Murine P388 Lymphocytic Leukemia (ED₅₀ µg/mL) and Human Cancer Cell Lines (GI₅₀ µg/mL)^a

compound	P388 Leukemi	BXPC-3 pancreas	MCF-7 breast	SF268 CNS	NCI-460 lung	KM20 Colon	DU145 Prostate
2	0.016	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
5	0.42	5.4	2.8	5.1	3.6	4.7	5.8
14	1.2	>10	>10	>10	>10	>10	>10
18	>10	>10	>10	>10	>10	>10	>10
20	2.3	5.7	3.0	8.5	3.7	3.3	>10
21	3.8	>10	>10	>10	>10	>10	>10
22	>10	>10	>10	8.2	>10	5.4	>10
25	0.076	7.7	3.3	5.4	5.2	4.6	6.5
26	>10	>10	>10	>10	>10	>10	>10
27	2.1	0.89	0.67	0.60	4.9	0.49	2.1
28	2.0	2.0	2.2	1.5	3.4	0.48	2.3
31	0.69	>10	7.5	8.8	>10	7.1	10.5
33	0.62	0.55	0.39	0.27	0.47	0.43	1.3
34	1.1	1.9	2.0	1.5	2.2	1.3	3.1

^aDMSO was used as vehicle in the testing.