Antineoplastic Agents. 571. Total Synthesis of Bacillistatin 2[†‡]

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The first total synthesis of bacillistain 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 the 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 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.

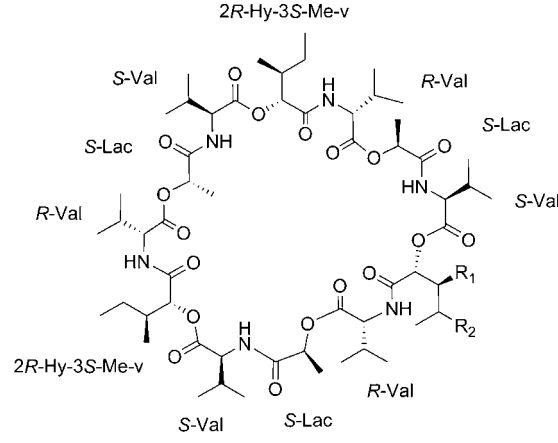
Another compelling need for a useful total synthesis of bacillistain 2 (**2**) is that the natural supply from the marine source, for practical purposes, is quite restricted. Thus, an efficient and flexible synthesis of bacillistain 2 was required also in order to provide enough material to evaluate 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**).

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



1, $R_1 = CH_3$, $R_2 = H$ (2*R*-Hy-3*S*-Me-v), bacillistatin 1

2, $R_1 = H$, $R_2 = CH_3$ (2*R*-Hy-4-Me-v), bacillistatin 2

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** 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 CH_2Cl_2 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 CH_2Cl_2 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 esterification[3] reaction using diethylazodicarboxylate (DEAD) and triphenylphosphine

† 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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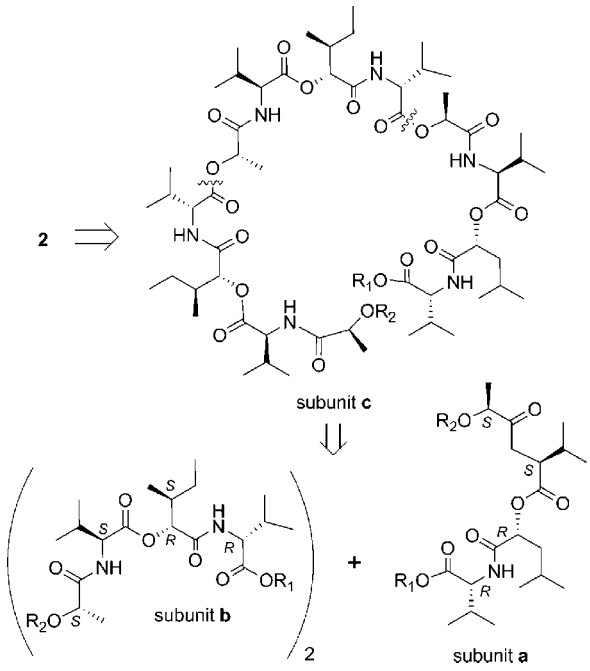


Figure 1. Retrosynthetic analysis of bacillistatin 2 (**2**).

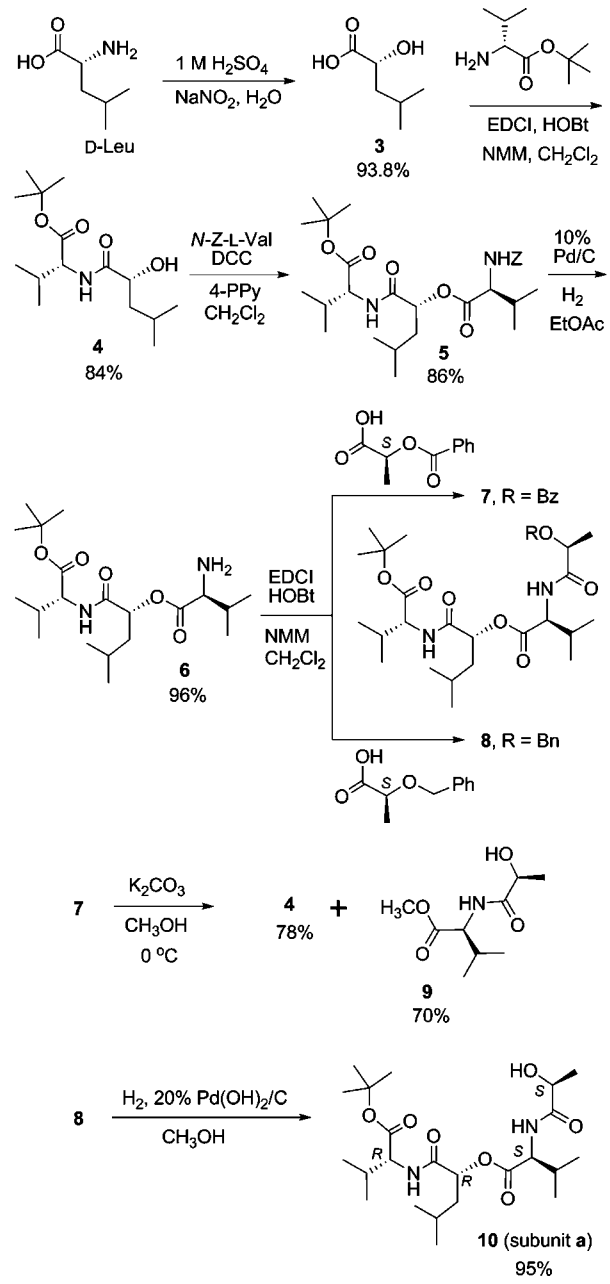
(Ph_3P) to condense alcohol **10** with acid **18**, the reaction proceeded smoothly and cleanly to furnish **19** (epi-subunit **a**−subunit **a**) in good yield with the expected hydroxyl configuration inversion (nearly 80%).

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 CH_2Cl_2 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 CH_2Cl_2, 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 Mitsunobu[3] 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 H_2 atmosphere in the presence of catalytic $Pd(OH)_2/C$ followed by treatment of the resulting **28** with TFA in CH_2Cl_2 afforded hydroxy acid **29**. Bacillistain 2 was now within reach via cyclization of **29**. Initially, the Yamaguchi reaction[4] (2,4,6-trichlorobenzoyl chloride, Et_3N, DMAP, toluene) was our choice for macrocyclization under

Scheme 1

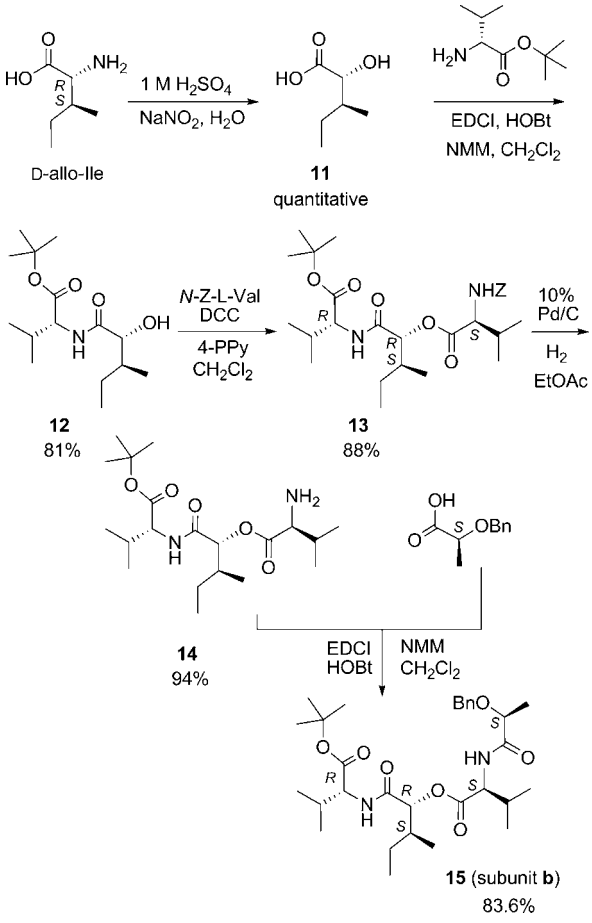


conditions of high dilution. However, this approach was unsuccessful, as monitoring by TLC showed formation of complex mixtures. After product separation, three unexpected byproducts were obtained, and **2** was not detected. Upon closer inspection of the ^1H NMR spectra of the products, it seemed apparent that depsipeptide **29** readily decomposed under the Yamaguchi reaction conditions.

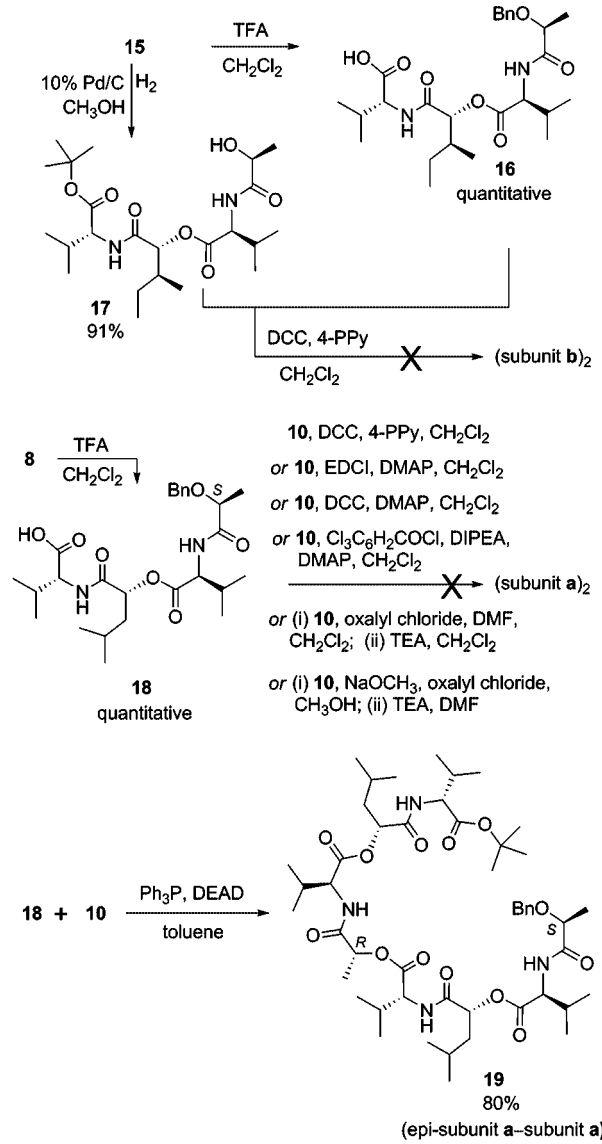
We next investigated application of the MNBA (2-methyl-6-nitrobenzoic anhydride) technique reported by Shiina et al.[5] That reagent and procedure has proved to be quite effective in the synthesis of carboxylic acid esters and lactones. Much to our satisfaction, treatment of hydroxy acid **29** with MNBA and DMAP in CH_2Cl_2 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, ^{13}C 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

Scheme 2



Scheme 3



subjected immediately to the Mitsunobu[3] reaction conditions under high dilution ($(5-8) \times 10^{-4}$M). Based on monitoring by TLC, ^{1}H 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. Cyclodepsipepide **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.

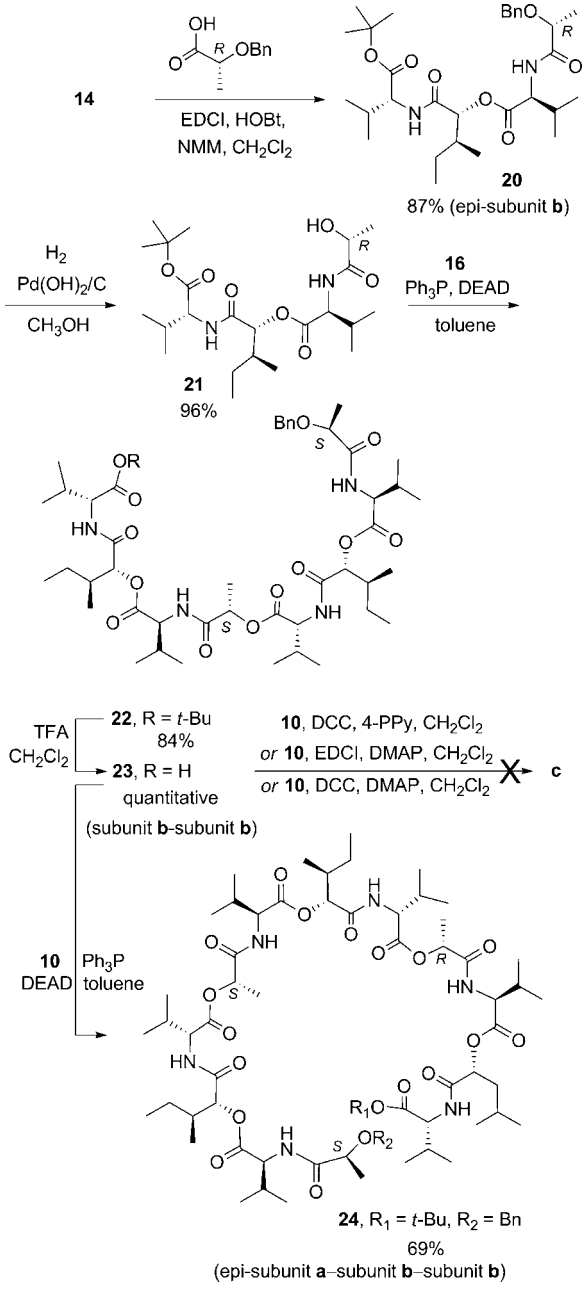
Experimental Section

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 (Ph_3P) 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 ^{1}H 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.

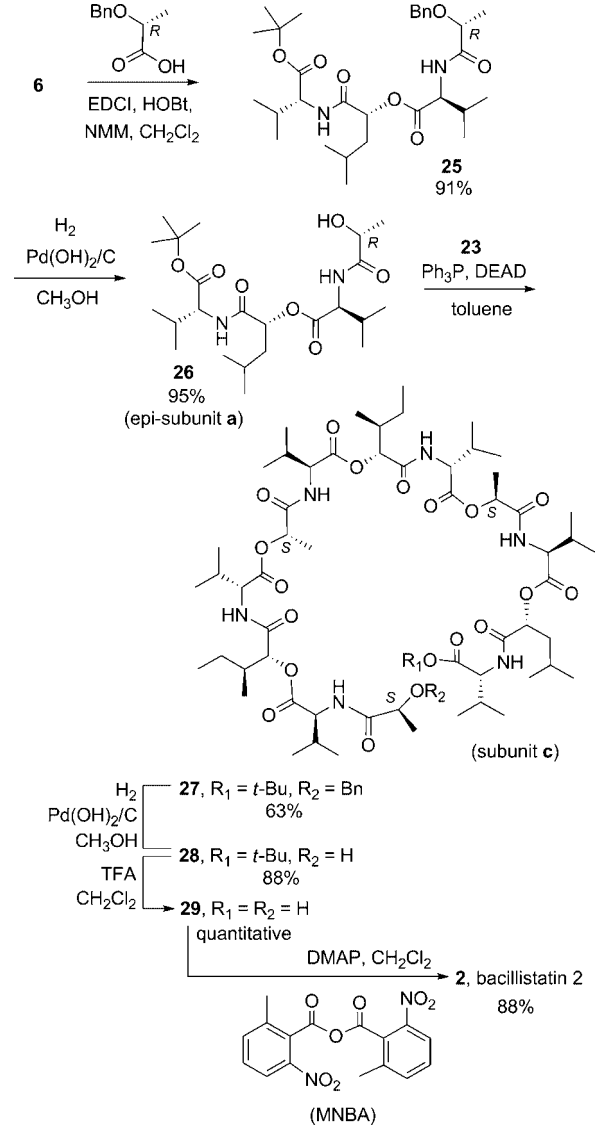
Carboxylic Acid 3.[2] To a cooled (ice bath) solution of D-leucine (16 g, 0.122 mol) in H_2SO_4 (1 M, 320 mL) was added dropwise a solution of $NaNO_2$ (64 g, 0.927 mol) in distilled water (300 mL). The reaction mixture temperature was kept below 5 °C for 10 h following the addition and was then allowed 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×) and brine (2×) 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; ^{1}H 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

Scheme 4



Scheme 5



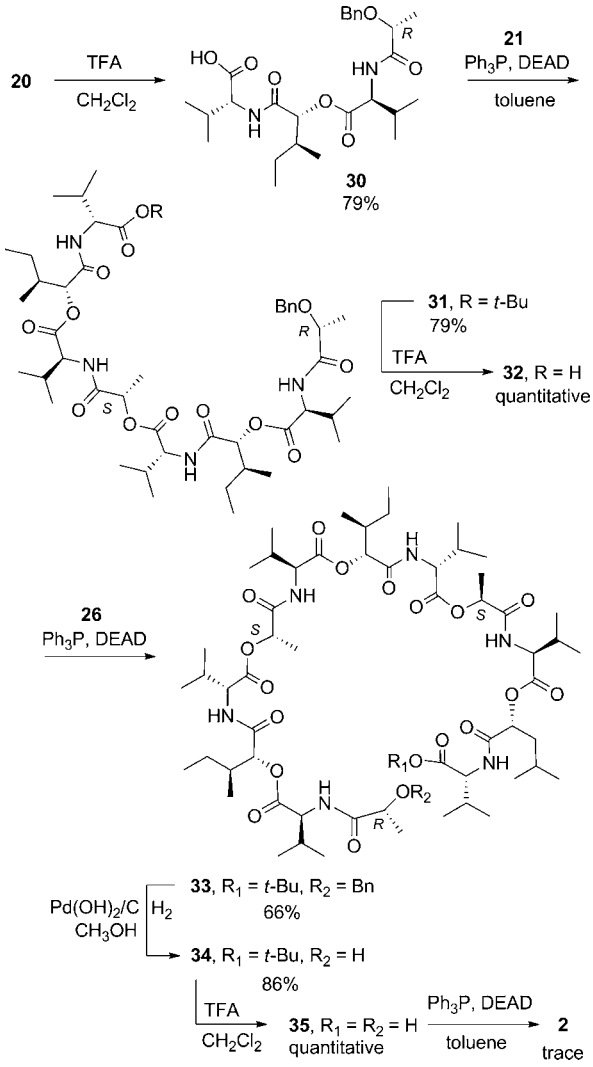
dried as stated previously. Removal of solvent followed by flash CC yielded compound **4** (0.97 g, 84%) as an oil: R_f 0.2 (4:1 hexane−EtOAc); $[\alpha]^{22}_D$ +21.9 (*c* 1.08, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3394 (br), 2962, 2360, 2339, 1734 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.95 (1H, d, J = 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 ($M^+ - CH_3$), 287 (M^+); HREIMS *m/z* 287.2110 $[M]^+$ (calcd for $C_{15}H_{29}NO_4$, 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×). 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); $[\alpha]^{22}_D$ +5.84 (*c* 1.25, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3337 (br), 2964, 2360, 1731, 1530 cm^{-1}; ^1H HMR ($CDCl_3$, 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 $[M + H]^+$ (calcd for $C_{28}H_{45}N_2O_7$, 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 the 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); $[\alpha]^{23}_D$ +0.36 (*c* 1.05, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3326 (br), 2963, 2360, 2340, 1737, 1675 cm^{-1}; ^1H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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 $C_{28}H_{45}N_2O_7$, 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 CH_2Cl_2 (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 NH_4Cl (10 mL), and extracted with CH_2Cl_2. 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);

Scheme 6



$[\alpha]^{23}_D$ +33.6 (*c* 1.7, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3322 (br), 1674, 1573, 1469 cm^{-1}; ^1H NMR ($CDCl_3$, 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 $C_{30}H_{47}N_2O_8$, 563.3332).

Alcohol 9. To a cooled (ice bath) solution of ester **7** (0.12 g, 0.21 mmol) in CH_3OH (10 mL) was added K_2CO_3 (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 Na_2SO_4. Flash CC of the residue led to alcohols **4** (46 mg, 78%) and **9** (29 mg, 70%). Alcohol **9**: ^1H NMR ($CDCl_3$, 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 CH_2Cl_2 (60 mL) that was stirring under argon were added EDCl (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 NH_4Cl (10 mL) and extracted with CH_2Cl_2. 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); $[\alpha]^{23}_D$ +4.15 (*c* 1.3, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1739, 1677, 1525, 1370, 1153 cm^{-1}; ^1H NMR ($CDCl_3$, 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 $C_{30}H_{49}N_2O_7$, 549.3540).

Alcohol 10. To a solution of benzyl ester **8** (0.46 g) in CH_3OH (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, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3312 (br), 2966, 2936, 2360, 1739, 1662, 1528, 1149 (s) cm^{-1}; ^1H NMR ($CDCl_3$, 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 $C_{23}H_{43}N_2O_7$, 459.3070).

Hydroxycarboxylic Acid 11. A solution of $NaNO_2$ (42 g, 611 mmol) in distilled H_2O (200 mL) was added (dropwise) over 4 h to a cooled (ice bath) solution of D-*allo*-isoleucine (10 g, 76.3 mmol) in H_2SO_4 (1 M, 200 mL) with stirring. Evolution of NO_2 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 ($CDCl_3$, 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 CH_2CL_2 (40 mL) that was stirring at 0 °C were added EDCl (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 NH_4Cl was added, followed by extraction with CH_2Cl_2 (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, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3393 (br), 2970, 1734, 1656, 1525 cm^{-1}; ^1H NMR ($CDCl_3$, 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 (APCl) *m/z* 288 (MH^+), 232; HRMS (APCl) *m/z* 288.2176 $[M + H]^+$ (calcd for $C_{15}H_{30}NO_4$, 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 CH_2Cl_2 (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 NH_4Cl, and extracted with CH_2Cl_2 (2×). The combined CH_2Cl_2 phase was washed first with $NaHCO_3$(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); $[\alpha]^{23}_D$ −0.61 (*c* 1.65, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3336 (br), 1696, 1531 cm^{-1}; ^1H NMR ($CDCl_3$, 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 (APCl) *m/z* 521.3213 $[M + H]^+$ (calcd for $C_{28}H_{45}N_2O_7$, 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

Table 1. Inhibition of the Murine P388 Lymphocytic Leukemia (ED_{50} μg/mL) and Human Cancer Cell Lines (GI_{50} μg/mL)[a]

compound	P388 leukemia	BXPC-3 pancreas	MCF-7 breast	SF268 CNS	NCI-460 lung	KM20L2 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

[a] DMSO was used as vehicle in the testing.

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); $[\alpha]^{23}_D$ +14 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3317 (br), 2967, 1737, 1708 cm^{-1}; ^1H NMR ($CDCl_3$, 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 $C_{20}H_{39}N_2O_5$, 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 CH_2Cl_2 (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 NH_4Cl (10 mL). The mixture was extracted with CH_2Cl_2 (30 mL × 3), and the combined organic phase was first washed successively with saturated $NaHCO_3$ 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); $[\alpha]^{23}_D$ −1.41 (*c* 0.85, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3325, 1735, 1673, 1519, 1148 cm^{-1}; ^1H NMR ($CDCl_3$, 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 $C_{30}H_{49}N_2O_7$, 549.3540).

Carboxylic Acid 16. To a cold (ice bath) solution of *tert*-butyl ester **15** (68 mg) in anhydrous CH_2Cl_2 (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 CH_2Cl_2 (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 CH_2Cl_2−CH_3OH); $[\alpha]^{23}_D$ +24 (*c* 0.7, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3298 (br), 2361, 2331, 1744, 1669, 1528 cm^{-1}; ^1H NMR ($CDCl_3$, 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 $C_{26}H_{41}N_2O_7$, 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 CH_3OH (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); $[\alpha]^{23}_D$ −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 $[M + H]^+$ (calcd for $C_{23}H_{43}N_2O_7$, 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 $[M + H]^+$ (calcd for $C_{49}H_{81}N_4O_{13}$, 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), EDCl (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); $[\alpha]^{26}_D$ +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 $[M + H]^+$ (calcd for $C_{30}H_{49}N_2O_7$, 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% $Pd(OH)_2$/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); $[\alpha]^{26}_D$ −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, quintet, 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 $[M + H]^+$ (calcd for $C_{23}H_{43}N_2O_7$, 459.3070); *anal.* C 60.75%, H 9.58%, N 5.90%, calcd for $C_{23}H_{42}N_2O_7$, 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), Ph_3P (97 mg, 0.372 mmol), and DEAD (55 μL, 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. Workup 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); $[\alpha]^{28}_D$ +12 (*c* 0.51, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3320, 2971, 1752, 1671, 1532, 1624 cm^{-1}; ^1H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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 ($CDCl_3$, 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 $C_{49}H_{81}N_4O_{13}$, 933.5800).

Depsipeptide 24. Selective deprotection of *tert*-butyl ester **22** (50 mg, 0.054 mmol) in anhydrous CH_2Cl_2 (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), Ph_3P (42 mg, 0.162 mmol), and DEAD (25.6 μL, 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 ($CDCl_3$, 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 $C_{68}H_{113}N_6O_{19}$, 1317.8061).

Depsipeptide 25. To a cooled (ice bath) solution of amine **6** (0.18 g, 0.47 mmol) in anhydrous CH_2Cl_2 (8 mL) that was stirring under argon were added *O*-benzyl-D-lactic acid (0.10 g, 0.56 mmol), EDCl (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); $[\alpha]^{26}_D$ +22.5 (*c* 0.59, $CHCl_3$); ^1H NMR ($CDCl_3$, 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 $C_{30}H_{49}N_2O_7$, 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 CH_3OH (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 ($CHCl_3$) ν_{max} 3310 (br), 2967, 1739, 1661, 1530, 370, 1150 cm^{-1}; ^1H NMR ($CDCl_3$, 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 Ph_3P (45 mg, 0.171 mmol) and DEAD (27 μL, 0.171 mmol). The mixture was stirred at 0 °C for 5 h and at room temperature overnight, by which time the 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 ($CHCl_3$) ν_{max} 3307, 2963, 2927, 2359, 1750, 1655, 1534, 1370, 1145 cm^{-1}; ^1H NMR ($CDCl_3$, 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, dd, 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 ($CDCl_3$, 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 $C_{68}H_{113}N_6O_{19}$, 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 CH_3OH (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 ($CHCl_3$) ν_{max} 3306, 2966, 1750, 1656, 1534, 1181,1146 cm^{-1}; ^1H NMR ($CDCl_3$, 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 $C_{61}H_{107}N_6O_{19}$, 1227.759).

Bacillistatin 2. To a cooled (ice bath) solution of *tert*-butyl ester **28** (38 mg, 0.031 mmol) in anhydrous CH_2Cl_2 (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 × 4) and CH_2Cl_2 (3 mL × 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 CH_2Cl_2 (13 mL) under argon was added dropwise (over 3 h from a syringe) a solution of **29** (18 mg, 0.0154 mmol) in anhydrous CH_2Cl_2 (2 mL). The mixture was stirred overnight and then cooled to 0 °C before termination of the reaction with water (2 mL). The mixture was extracted with CH_2Cl_2 (30 mL × 2), and the combined extract was washed and dried as stated previously. The solvent was removed, and flash CC of the residue afforded bacillistatin 2 (**2**, 15.6 mg, 88%) as a colorless solid: R_f 0.55 (4:1 hexane−EtOAc); mp 143−146 °C; $[\alpha]^{20}_D$ +33.9 (*c* 0.44, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3004, 2965, 1757, 1659, 1539, 1184 cm^{-1}; ^1H NMR ($CDCl_3$, 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 ($CDCl_3$, 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 $C_{61}H_{107}N_6O_{19}$, 1153.686). The synthetic specimen of bacillistatin 2 was found to be identical (TLC, HPLC, ^1H NMR, ^{13}C NMR, HRMS) with the natural product.[1]

Compound 31. The *tert*-butyl group was removed from **20** (94 mg, 0.172 mmol) in anhydrous CH_2Cl_2 (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), Ph_3P (135 mg, 0.516 mmol), and DEAD (82 μL, 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 ($CHCl_3$) ν_{max} 3307, 2966, 2935, 1751, 1658, 1532, 1465, 1180, 1147 cm^{-1}; ^1H NMR ($CDCl_3$, 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); APT ($CDCl_3$, 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 $C_{49}H_{81}N_4O_{13}$, 933.5800).

Compound 33. Cleavage of the *tert*-butyl group from **31** (102 mg, 0.109 mmol) in anhydrous CH_2Cl_2 (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 $[M + H]^+$ (calcd for $C_{68}H_{113}N_6O_{19}$, 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 $[M + H]^+$ (calcd for $C_{61}H_{107}N_6O_{19}$, 1227.759).

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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