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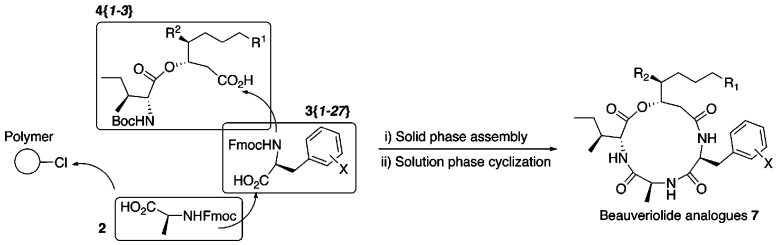

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Synthesis and Biological Evaluation of a Beauveriolide Analogue Library

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Synthesis of beauveriolide III (**1b**), which is an inhibitor of lipid droplet accumulation in macrophages, was achieved by solid-phase assembly of linear depsipeptide using a 2-chlorotrityl linker followed by solution-phase cyclization. On the basis of this strategy, a combinatorial library of beauveriolide analogues was carried out by radio frequency-encoded combinatorial chemistry. After automated purification using preparative reversed-phase HPLC, the library was tested for inhibitory activity of CE synthesis in macrophages to determine structure−activity relationships of beauveriolides. Among them, we found that diphenyl derivative **7**{*9,1*} is 10 times more potent than **1b**.

Introduction

Lipid droplet accumulation in macrophages is a critical stage in foam cell formation which ultimately leads to the development of atherosclerosis in the arterial wall. The inhibition of lipid droplet accumulation in macrophages may be useful in treating atherosclerogenesis.[1] Beauveriolides I (**1a)** and III (**1b)**, isolated from a culture broth of *Beauveria* sp. FO-6979, were found to reduce the number and size of lipid droplets in macrophages without exhibiting cytotoxic effects and to inhibit cholesteryl ester (CE) synthesis with IC_{50} values of 0.78 and 0.41 μM, respectively.[2a−c] Analysis using microsomes prepared from mouse macrophages revealed that beauveriolides inhibit acyl-CoA/cholesterol acyltransferase (ACAT) activity, resulting in blockage of CE synthesis in macrophages and suppression of foam cell formation. Furthermore, the in vivo efficacy of beauveriolides was demonstrated in ApoE and LDL receptor knockout mice, in which these compounds reduced atherogenic lesions in the aorta and heart.[2d] A number of synthetic ACAT inhibitors, including amides, ureas, and imidazoles, have been reported, but their success has been limited.[3] Beauveriolides are structurally different from such synthetic inhibitors and are expected to be highly promising lead compounds for the treatment of atherosclerosis. Therefore, a method for rapid synthesis of beauveriolide analogues is required to elucidate structure−activity relationships (SAR). Combinatorial libraries based on natural products have recently become a powerful tool for discovering lead compounds and developing drugs to improve activities and pharmacokinetic profiles.[4]

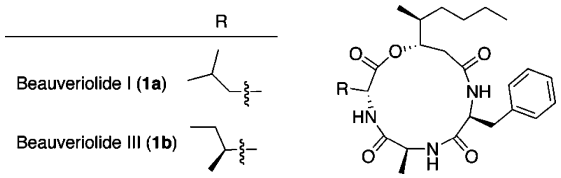

Figure 1. Structures of beauveriolides I and III.

Beauveriolide derivatives could be produced by means of solid-phase peptide synthesis, because they are cyclic depsipeptides containing L-Ala, L-Phe, D-Leu (or D-*allo*-Ile), and (*3S*,*4S*)-3-hydroxy-4-methyloctanoic acid. Herein, we report an efficient route for synthesis of a beauveriolide analogue library using a solid support and an evaluation of CE synthesis inhibition in macrophages.

Results and Discussion

Our synthetic approach toward beauveriolide analogues **7** is shown in Scheme 1. Linear depsipeptide **5** composed of blocks **2**, **3**, and **4** can be assembled by solid-phase synthesis using a trityl linker that is tolerant to reaction conditions in solid-phase synthesis and stable under cleavage conditions. Block **4** was prepared in solution to avoid the risk of esterification on the solid support. The Boc group was selected for protection of block **4** because deprotection and cleavage of **5** from the solid support can be simultaneously carried out under acidic conditions. Subsequent cyclization of **6** in the solution phase could yield **7**.

Synthesis of building blocks **4**{*1*−*3*} is summarized in Scheme 2. Condensation of (*S*)-oxazolidinone **8** and hexanoyl chrolide **9**, followed by Evans asymmetric alkylation of the imide with NaHMDS and methyl iodide gave methylated imide **10**.[5] After removal of the chiral auxiliary, the carboxylic acid was treated with CDI and then subjected to addition of ethylmagnesium malonate to afford β-keto ester

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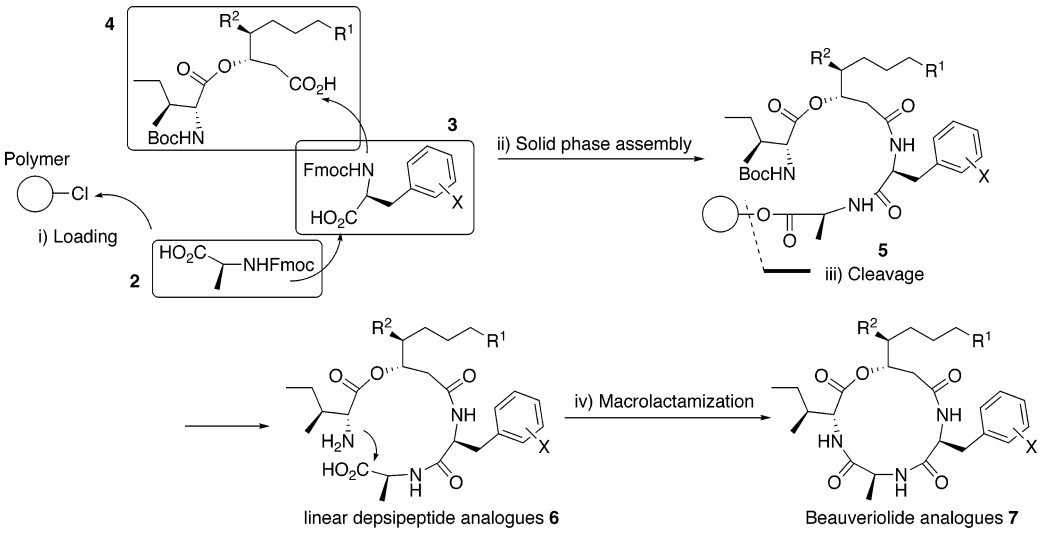
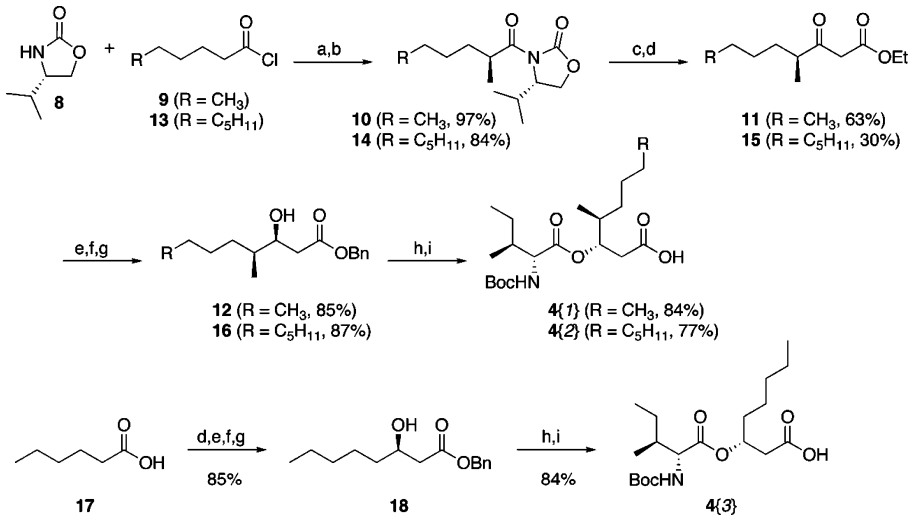
Scheme 1. Strategy for Synthesis of a Beauveriolide Analogue Library



Scheme 2. Synthesis of Building Blocks **4**{*1*−*3*}[a]



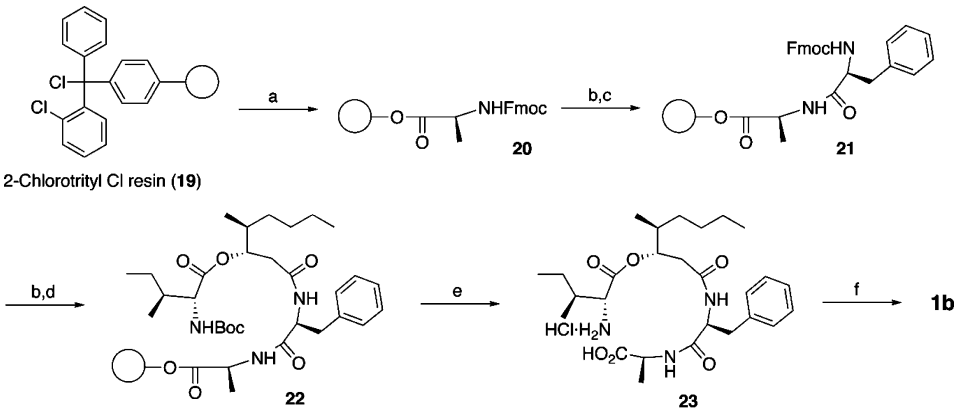
[a] (a) *n*-BuLi, THF, −78 °C, 30 min; (b) NaHMDS, MeI, THF, −78 °C, 1 h; (c) 1 M LiOH, THF, rt, 2.5 h; (d) CDI, $(EtO_2CCH_2CO_2)_2Mg$, THF, rt, 12 h; (e) $RuCl_2$[(*R*)-binap], ethanol, H_2, 90 atm, 45 °C, 40 h; (f) 1 M LiOH, THF, rt, 2.5 h; (g) Cs_2CO_3, BnBr, DMF, rt, 12 h; (h) Boc-D-*allo*-Ile-OH, DCC, DMAP, CH_2Cl_2, 0 °C to room temperature, 22 h; (i) 10% Pd/C, H_2, ethanol, rt, 12 h.

11. Asymmetric hydrogenation of **11** with $RuCl_2$[(*R*)-binap][6] under 90 atm of hydrogen at 45 °C provided the desired stereochemistry of the hydroxyl group (>95% selectivity by ^{1}H NMR analysis),[7] and the ethyl ester was converted to benzyl ester **12** through alkali hydrolysis and benzylation. Coupling of the secondary alcohol with Boc-D-*allo*-Ile-OH using DCC in the presence of DMAP, followed by hydrogenolysis of the benzyl group, produced ester unit **4**{*1*}. Long side chain compound **4**{*2*} and desmethyl compound **4**{*3*} were prepared using the same method.

We investigated a synthesis of beauveriolide III (**1b**) using 2-chlorotrityl chloride resin **19** (Scheme 3). The carboxylic acid in Fmoc-L-Ala-OH **2** was loaded onto **19** in the presence of *i*-Pr_2NEt in CH_2Cl_2. Loading yield was quantitative (1.25 mmol/g), calculated from the weight of **2** after TFA cleavage. The Fmoc group was removed with 20% piperidine in DMF, and subsequent condensation with Fmoc-L-Phe-OH **3**{*1*} afforded dipeptide **21**. After subsequent deprotection, coupling of the resulting amine and ester unit **4**{*1*} using PyBrop resulted in depsipeptide **22**.[8] When other coupling reagents were used, β-elimination of **4**{*1*} was observed. Deprotection of the Boc group and cleavage from the polymer support with 4 M HCl in 1,4-dioxane released the desired depsipeptide **23** in 93% purity and 98% crude yield. Finally, the cyclization of **23** with EDCI and *i*-Pr_2NEt under high dilution conditions proceeded to provide beauveriolide III (**1b)** in 51% yield. The use of EDCI was crucial for reducing the cyclic dimer of **23**. Spectral data of the synthetic beauveriolide III were found to be identical to those of the natural product.[2b]

To design a beauveriolide library, we next focused on the phenyl group in L-Phe and the side chain in the fatty acid. The phenyl group in these cyclic depsipeptides was found to be essential for the inhibition of CE synthesis. For example, beauveriolide V, in which L-Phe is replaced with L-Val, exhibited markedly decreased activity (>20 μM).[2e] Synthetic ACAT inhibitors are known to contain a variety of long alkyl chains and aromatic rings.[3] We hypothesized that replacement of the phenyl group and the fatty acid in cyclic depsipeptides may enhance potency. We designed an 81-member library based on natural products from building blocks **2**, **3**{*1*−*27*}, and **4** {*1*−*3*}, as summarized in Figure

Scheme 3. Total Synthesis of Beauveriolide III (**1b**) Using Solid Support **19**[a]



[a] (a) Fmoc-L-Ala-OH **2**, *i*-Pr_2NEt, CH_2Cl_2, rt, 2 h; (b) 20% piperidine/DMF, rt, 1 h; (c) Fmoc-L-Phe-OH **3**{*1*}, DIPCI, HOBt, CH_2Cl_2/DMF (4/1), rt, 2 h; (d) **4**{*1*}, PyBrop, *i*-Pr_2NEt, CH_2Cl_2/DMF (4/1), rt, 1.5 h; (e) 4 M HCl/1,4-dioxane, rt, 2h, crude yield = 93%, purity = 98%; (f) EDCI·HCl, *i*-Pr_2NEt, CH_2Cl_2, rt, 2 h, 51%.

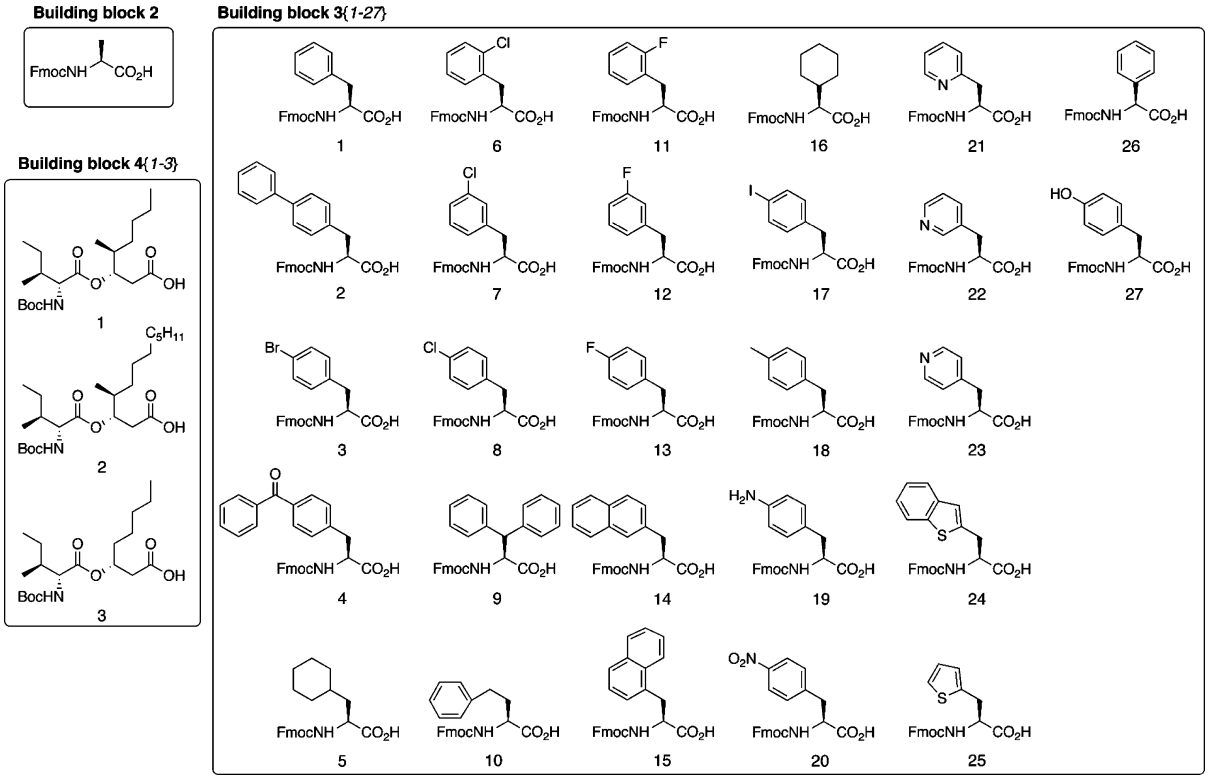


Figure 2. Building blocks **2**, **3**, and **4** for a combinatorial library of beauveriolides.

2. According to the above strategy, synthesis of a combinatorial library of beauveriolide analogues was carried out using radio frequency-encoded combinatorial (REC) chemistry.[9] Linear depsipeptides **6** were stocked without purification (~1 mg) and used in the assay systems. The identification of each product was accomplished by LC/MS analysis. Cyclic compounds were purified by preparative reversed-phase HPLC to give 77 beauveriolide analogues **7** from 81 trials. Aniline derivatives **7**{*19,1*−*3*} were not provided. Low isolation yields were attributed to loss during the preparative HPLC process due to a lack of product solubility.

The inhibitory activity of **7** against CE synthesis in mouse peritoneal macrophages[2d] was evaluated (Table 1). Linear depsipeptides **6** were found to be inactive (data not shown). The ring construction plays a crucial role in inhibiton of CE synthesis. With regard to building block **4**{*1*−*3*}, the natural fatty acid **4**{*1*} displayed the most potent activity. The long side chain derivatives **7**{*2*−*27,2*} and demethylated side chain derivatives **7**{*2*−*27,3*} showed a lack of inhibitory activity relative to **1b**. However, it is interesting to note that **7**{*1,2*} and **7**{*1,3*}, which possessed L-Phe, retained the activity. Building block **3**{*1*−*27*} gave different activity profiles. *p*-Bromo and *p*-iodophenyl derivatives (**7**{*3,1*} and **7**{*17,1*}) showed an inhibitory profile similar to **1b**. *m*- and *p*-Chlorophenyl derivatives (**7**{*7,1*} and **7**{*8,1*}) exhibited more potent activity (3−4-fold) than **1b**, whereas *o*-chlorophenyl derivative **7**{*6,1*} gave moderate activity. Fluorophenyl derivatives (**7**{*11,1*}, **7**{*12,1*}, and 7{*13,1*}) showed slightly less activity. Thus, the type and position of halogen substituents on the phenyl ring are important to enhance activity. Substitution of phenyl and benzoyl groups at the 4-position resulted in a loss of activity (**7**{*2,1*} and **7**{*4,1*}),

Table 1. Combinatorial Library of Beauveriolide Analogues **7**

compd	yield (%)[a]	IC_{50} (μM)[b]	R_t (min)[c]	MS (*m*/*z*)[d]	compd	yield (%)[a]	IC_{50} (μM)[b]	R_t (min)[c]	MS (*m*/*z*)[d]
7{*1,1*}[e]	38	0.41	8.20	488	**7**{*15,2*}	27	>20	10.41	594
7{*2,1*}	12	11	9.12	564	**7**{*16,2*}	16	>20	10.43	536
7{*3,1*}	17	0.37	8.69	566	**7**{*17,2*}	11	>20	10.29	670
7{*4,1*}	19	18.6	8.64	592	**7**{*18,2*}	21	>20	10.50	558
7{*5,1*}	18	1.8	8.97	494	**7**{*19,2*}				
7{*6,1*}	17	1.34	8.56	522	**7**{*20,2*}	14	>20	10.25	589
7{*7,1*}	9	0.14	8.58	522	**7**{*21,2*}	21	>20	9.90	545
7{*8,1*}	19	0.095	8.61	522	**7**{*22,2*}	25	>20	8.29	545
7{*9,1*}	27	0.040	8.79	564	**7**{*23,2*}	18	>20	7.24	548
7{*10,1*}	23	1.2	8.52	502	**7**{*24,2*}	19	>20	10.38	600
7{*11,1*}	20	1.98	8.30	506	**7**{*25,2*}	19	>20	10.40	550
7{*12,1*}	19	1.19	8.30	506	**7**{*26,2*}	19	>20	9.96	530
7{*13,1*}	17	1.82	8.27	506	**7**{*27,2*}	12	>20	10.02	560
7{*14,1*}	15	0.09	8.77	538	**7**{*1,3*}	27	0.60	7.93	474
7{*15,1*}	19	0.74	8.79	538	**7**{*2,3*}	27	>20	7.80	550
7{*16,1*}	18	>20	8.50	480	**7**{*3,3*}	4	>20	8.89	552
7{*17,1*}	13	0.41	8.89	614	**7**{*4,3*}	21	>20	8.46	578
7{*18,1*}	25	0.40	8.54	502	**7**{*5,3*}	23	>20	8.42	480
7{*19,1*}					**7**{*6,3*}	7	>20	8.69	508
7{*20,1*}	15	1.6	8.16	533	**7**{*7,3*}	22	>20	8.33	508
7{*21,1*}	15	1.54	6.08	489	**7**{*8,3*}	13	9.6	8.35	508
7{*22,1*}					**7**{*9,3*}	31	16.4	8.56	550
7{*23,1*}	20	>20	8.51	492	**7**{*10,3*}	26	>20	8.24	488
7{*24,1*}	18	0.59	8.74	544	**7**{*11,3*}	14	>20	8.02	492
7{*25,1*}	16	2.84	8.12	494	**7**{*12,3*}	27	>20	8.04	492
7{*26,1*}	7	>20	8.13	474	**7**{*13,3*}	27	>20	8.00	492
7{*27,1*}	12	2.40	7.40	504	**7**{*14,3*}	25	>20	8.54	524
7{*1,2*}	22	0.60	9.96	544	**7**{*15,3*}	31	>20	8.55	524
7{*2,2*}	13	>20	10.63	620	**7**{*16,3*}	11	>20	8.18	466
7{*3,2*}	25	>20	10.34	622	**7**{*17,3*}	7	>20	8.65	600
7{*4,2*}	20	>20	10.23	648	**7**{*18,3*}	8	>20	8.26	488
7{*5,2*}	15	>20	10.67	550	**7**{*19,3*}				
7{*6,2*}	26	>20	10.28	578	**7**{*20,3*}	19	>20	7.94	519
7{*7,2*}	24	>20	10.27	578	**7**{*21,3*}	23	>20	5.15	475
7{*8,2*}	21	0.35	10.27	578	**7**{*22,3*}	30	>20	5.77	475
7{*9,2*}	21	1.0	10.29	620	**7**{*23,3*}	17	>20	8.23	478
7{*10,2*}	21	>20	10.37	558	**7**{*24,3*}	17	>20	8.50	530
7{*11,2*}	20	>20	10.25	562	**7**{*25,3*}	21	>20	7.83	480
7{*12,2*}	16	>20	10.07	562	**7**{*26,3*}	23	>20	9.30	460
7{*13,2*}	19	>20	10.04	562	**7**{*27,3*}	13	>20	7.13	490
7{*14,2*}	23	>20	10.01	594					

[a] Isolated yield by automated preparative HPLC (UV at 215 nm, Waters Symmetry C18, 5-μm, 19 × 50 mm with a linear gradient of 10% acetonitrile containing 0.1% formic acid/aqueous 0.1% formic acid to 100% acetonitrile containing 0.1% formic acid over 20 min at a flow rate of 10 mL/min). [b] Inhibitory activity of CE synthesis. [c] Retention time (UV at 215 nm, Waters Symmetry C18 5 μm, 4.6 × 50 mm with a linear gradient of 10% acetonitrile containing 0.1% formic acid/aqueous 0.1% formic acid to 100% acetonitrile containing 0.1% formic acid over 12 min at a flow rate of 1.0 mL/min). [d] Positive ion electrospray MS data, $[M + H]^+$ were recorded. [e] Beauveriolide III (**1b**).

whereas introduction of a methyl group retained activity (**7**{*18,1*}). The length of the methylene in L-Phe is also important for activity. Phenylglycine **7**{*26,1*} was inactive, but homophenylalanine **7**{*10,1*} retained activity. Cyclohexyl derivative **7**{*5,1*} was slightly less active than **1b**. Diphenyl derivative **7**{*9,1*} is the most potent, with an IC_{50} value of 40 nM , which is 10 times higher potency than **1b**. Differences in inhibition were also observed at the position of the naphthyl group (**7**{*14,1*} and **7**{*15,1*}). Replacement of the phenyl group with 2-pyridyl, 3-benzothienyl and 2-thienyl groups resulted in slightly less activity (**7**{*21,1*}, **7**{*24,1*} and **7**{*25,1*}), whereas exchange of the phenyl group with 3-pyridyl moiety gave **7**{*23,1*} which showed markedly reduced potency.

Conclusion

In conclusion, we demonstrated an efficient route for synthesis of a beauveriolide analogues library using the solid support. Seventy-seven beauveriolide analogues, including beauveriolide III (**1b**), have been obtained using REC chemistry. The library allowed the SAR of beauveriolides to be determined. Among them, we found that diphenyl derivative **7**{*9,1*} is 10 times more potent than **1b**. It should be noted that combinatorial synthesis based on natural products is very effective in determining SAR and in discovering more active leads for drug discovery. Further development of atherosclerotic agents is underway in our laboratory.

Experimental Section

Reagents and solvents were purchased from commercial sources and used without further purification. 2-Chlorotrity chloride resin **19** (100−200 mesh, 1% cross-linked, 1.25 mmol/g; batch, U1125032) was purchased from IRORI.

All radio frequency encoding/sorting equipment utilized herein is available from IRORI Quantum Microchemistry.

A combinatorial synthesis of a beauveriolide library was carried out in a MicroKan microreactor initially filled with ~30 mg of **19** and a radio frequency chip. The resin in the MicroKan microreactor was swelled in solvent for 1 h before use.

NMR spectra were recorded on a Varian Mercury-300 instrument with $CDCl_3$, CD_3OD, or DMSO-d_6. ^1H NMR spectral data are reported as follows: chemical shifts relative to chloroform (7.26 ppm), methanol (3.30 ppm), or DMSO (2.49 ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling, and integration. ^{13}C NMR spectral data are reported in parts per million relative to $CDCl_3$ (77. 0 ppm), CD_3OD (49.0 ppm), or DMSO (39.7 ppm). Melting points were measured with a Yanaco micromelting point apparatus. FT-IR spectra were recorded on a Horiba FT-210 spectrometer. Optical rotation was obtained with a JASCO DIP-370 polarimeter. HR-FABMS were measured with a JEOL JMS-AX505 HA mass spectrometer.

LC/MS spectra were obtained on an AppliedBioSystems Mariner TK3500 Biospectrometry Workstation (ESI-TOF) mass spectrometer and Hewlett-Packerd Series 1100 (Waters Symmetry C18 5 μm, 4.6 × 50 mm) with a linear gradient of 10% acetonitrile containing 0.1% formic acid/water containing 0.1% formic acid to 100% acetonitrile containing 0.1% formic acid over 9 min at 1.0 mL/min flow rate. The peak areas were detected by UV at 215 nm. Preparative HPLC was performed on a Gilson 506 system using a Waters Symmetry C18 5-μm, 19 × 50 mm with a linear gradient of 10% acetonitrile containing 0.1% formic acid/water containing 0.1% formic acid to 100% acetonitrile containing 0.1% formic acid over 20 min at 10 mL/min flow rate.

General Procedure for Loading of Building Block 2 (Fmoc-Ala-OH) onto 2-Chlorotrityl Chloride Resin 19. The 96 MicroKan microreactors were placed into a 300-mL flask and treated with a cocktail of *N*-diisopropylethylamine (4.60 mL, 25.0 mmol) and **2** (3.10 g, 10.0 mmol) in CH_2Cl_2 (100 mL). The mixture was vigorously agitated at room temperature. After being agitated for 2 h, the reaction was quenched with methanol (1 mL) to cap remaining reactive sites and agitated for additional 10 min. The MicroKans were filtered; washed with DMF (2 min × 4), methanol (2 min × 1), and CH_2Cl_2 (2 min × 4,); and dried in vacuo. The loading of the resin was calculated by the weight of the first amino acid after acid cleavage.

General Procedure for Removal of the Fmoc Group. The 96 MicroKan microreactors were placed into a 300-mL flask and treated with a solution of 20% piperidine in DMF (100 mL). The mixture was vigorously agitated at room temperature for 1 h. The MicroKans were filtered; washed with DMF (2 min × 4), methanol (2 min × 1), and CH_2Cl_2 (2 min × 4); and dried in vacuo.

General Procedure for Coupling of Building Block 3{*1*−*27*} with Polymer-Supported Alanine. The 81 MicroKan microreactors were split into 27 vessels (3 microreactors in each **3**{*1*−*27*}) and treated with a cocktail of **3**{*1*−*27*} (500 μmol) and HOBt (80.0 mg, 600 μmol) in CH_2Cl_2/DMF (4/1, 5 mL), and DIC (100 μL, 600 μmol) was added to this mixture. After being agitated at room temperature for 2 h, the MicroKans were filtered and washed with CH_2Cl_2 (2 min × 1). The MicroKans were pooled into a 300-mL flask; washed with DMF (2 min × 4), methanol (2 min × 1), and CH_2Cl_2 (2 min × 4); and dried in vacuo.

General Procedure for Coupling of Building Block 4{*1*−*3*} with Polymer-Supported N-Free Dipeptide. The 81 MicroKan microreactors were split into three 100-mL flasks (27 microreactors in each **4**{*1*−*3*}) and treated with a cocktail of **4**{*1*−*3*} (2.50 mmol) and *N*-diisopropylethylamine (1.1 mL, 6.00 mmol) in CH_2Cl_2/DMF (4/1, 30 mL), and PyBrop (1.30 g, 3.00 mmol) was added to this mixture. After being agitated at room temperature for 2 h, the MicroKans were filtered and washed with CH_2Cl_2 (2 min × 1). The MicroKans were pooled into a 300-mL flask; washed with DMF (2 min × 4), methanol (2 min × 1), and CH_2Cl_2 (2 min × 4); and dried in vacuo.

General Procedure for Deprotection of the Boc Group and Cleavage of Linear Depscipeptide 5 from the Polymer Support. The 81 MicroKan microreactors were split into 81 vessels and treated with 4 M HCl in dioxane (4 mL), and the mixture was agitated at room temperature for 1 h. The MicroKan was filtered and washed with CH_2Cl_2 (2 min × 3). The collected filtrate was dried in a stream of N_2 and pumped on at 40 °C for 3 h to give linear depsipeptide **6**.

General Procedure for the Cyclization. Linear depsipeptide **6** (~37.5 μmol) was treated with a cocktail of *N*-diisopropylethylamine (20 μL, 112 μmol) and EDCI (10.0 mg, 56.3 μmol) in CH_2Cl_2 (25 mL), and the mixture was stirred at room temperature for 2 h. The reaction was quenched with methanol and evaporated under reduced pressure. The crude was purified by preparative HPLC to provide beauveriolide analogue **7**.

Selected Spectral Data of Beauveriolide Analogues 7. 7{*1,1*} (Synthetic Beauveriolide III). mp: 246−248 °C; $[\alpha]_D^{28}$ = −17 (c 0.13, DMF); HRFABMS: calcd for $C_{27}H_{42}N_3O_5$ $[M + H]^+$ 488.3124, found 488.3122; IR (KBr): 3305, 1726, 1686, 1639, 1539 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3/CD_3OD$ = 4:1) δ 7.28−7.11 (m, 5H), 4.94 (dt, J = 10.0, 4.5 Hz, 1H), 4.26 (d, J = 10.0 Hz, 1H), 4.17 (dd, J = 8.5, 7.5 Hz, 1H), 3.82 (q, J = 7.0 Hz, 1H), 3.02 (dd, J = 13.5, 8.5 Hz, 1H), 2.94 (dd, J = 13.5, 7.5 Hz, 1H), 2.46 (dd, J = 14.0, 5.0 Hz, 1H), 2.38 (dd, J = 14.0, 10.0 Hz, 1H), 2.03 (m, 1H), 1.67 (m, 1H), 1.44−1.26 (m, 3H), 1.22 (d, J = 7.0 Hz, 3H), 1.21−1.13 (m, 5H), 1.00 (m, 1H), 0.88 (d, J = 7.0 Hz, 3H), 0.86 (d, J = 7.0 Hz, 3H), 0.85 (t, J = 6.0 Hz, 3H), 0.84 (t, J = 7.0 Hz, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3/CD_3OD$ = 4:1) δ 172.1, 171.5, 171.4, 169.4, 136.5, 129.0 (×2), 128.5 (×2), 126.9, 76.6, 59.4, 57.0, 49.3, 37.2, 35.9, 35.7 (×2), 30.6, 29.7, 26.0, 23.1, 15.7, 15.0, 14.8, 14.1, 11.1.

Beauveriolide III (Natural Product). mp: 245−247 °C; $[\alpha]_D^{28}$ = −18 (c 0.12, DMF); HRFABMS: calcd for $C_{27}H_{42}N_3O_5$ $[M + H]^+$ 488.3124, found 488.3127; IR (KBr): 3300, 1724, 1684, 1641, 1540 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3/CD_3OD$ = 4:1) δ 7.23 (ddd, J = 8.0, 7.0, 1.0 Hz, 2H), 7.18 (ddd, J = 7.0, 1.5 Hz, 1H), 7.14 (ddd, J = 8.0, 1.5, 1.0 Hz, 2H), 4.93 (ddd, J = 10.0, 4.5, 4.0 Hz, 1H), 4.27 (d, J = 10.0 Hz, 1H), 4.19 (dd, J = 8.5, 7.5 Hz, 1H), 3.80 (q, J = 7.0 Hz, 1H), 3.04 (dd, J = 13.5, 7.5 Hz, 1H), 2.95

(dd, $J = 13.5$, 8.5 Hz, 1H), 2.46 (dd, $J = 14.0$, 4.5 Hz, 1H), 2.38 (dd, $J = 14.0$, 10.0 Hz, 1H), 2.04 (m, 1H), 1.67 (m, 1H), 1.36 (m, 1H), 1.32 (m, 1H), 1.32 (m, 1H), 1.22 (d, $J = 7.0$ Hz, 3H), 1.21 (m, 2H), 1.20 (m, 2H), 1.11 (m, 1H), 0.99 (m, 1H), 0.88 (d, $J = 7.0$ Hz, 3H), 0.86 (t, $J = 7.0$ Hz, 3H), 0.85 (d, $J = 6.0$ Hz, 3H), 0.84 (t, $J = 7.0$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD = 4:1$) δ 172.4, 171.8 (×2), 169.7, 136.7, 129.3 (×2), 128.8 (×2), 127.2, 76.8, 59.6, 57.2, 49.7, 37.4, 36.0, 35.9, 35.8, 30.7, 29.8, 26.1, 23.2, 15.7, 15.1, 14.8, 14.1, 11.1.

7{*9,1*}. HRFABMS: calcd for $C_{33}H_{46}N_3O_5$ $[M + H]^+$ 564.3437, found 564.3418; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.45 (d, $J = 7.0$ Hz, 1H), 8.32 (d, $J = 7.5$ Hz, 1H), 7.34−6.99 (m, 10H), 6.58 (d, $J = 9.5$ Hz, 1H), 4.89 (d, $J = 12.0$, 7.5 Hz, 1H), 4.85 (ddd, $J = 10.0$, 4.5, 4.0 Hz, 1H), 4.46 (d, $J = 12.0$ Hz, 1H), 4.11 (t, $J = 9.5$ Hz, 1H), 3.66 (dq, $J = 7.5$, 7.0 Hz, 1H), 2.31 (dd, $J = 14.0$, 4.5 Hz, 1H), 2.20 (dd, $J = 14.0$, 10.0 Hz, 1H), 1.94 (m, 1H), 1.60 (m, 1H), 1.44−0.94 (m, 11H), 0.87 (t, $J = 7.0$ Hz, 3H), 0.85−0.84 (m, 9H); ^{13}C NMR (75.4 MHz, $CDCl_3/CD_3OD = 4:1$) δ 170.5, 170.0, 169.3, 168.5, 141.2, 140.9, 128.1 (×2), 128.0 (×2), 127.8 (×2), 127.7 (×2), 126.2, 161.1, 75.7, 67.3, 58.3, 50.5, 48.2, 36.6, 35.5, 35.0, 29.9, 28.8, 25.0, 23.1, 22.1 15.0, 14.3, 14.1, 13.5, 10.4.

7{*10,1*}. HRFABMS: calcd for $C_{28}H_{44}N_3O_5$ $[M + H]^+$ 502.3281, found 502.3293; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD = 4:1$) δ 7.27−7.21 (m, 2H), 7.17−7.10 (m, 3H), 4.92 (dt, $J = 9.5$, 5.0 Hz, 1H), 4.27 (d, $J = 9.5$ Hz, 1H), 3.97 (t, $J = 7.0$ Hz, 1H), 3.94 (q, $J = 7.0$ Hz, 1H), 2.63−2.56 (m, 2H), 2.59 (dd, $J = 14.0$, 5.0 Hz, 1H), 2.42 (dd, $J = 14.0$, 10.0 Hz, 1H), 2.10−1.90 (m, 3H), 1.60 (m, 1H), 1.40−0.96 (m, 11H), 0.87 (t, $J = 7.0$ Hz, 3H), 0.86 (d, $J = 7.0$ Hz, 3H), 0.85 (d, $J = 7.0$ Hz, 3H), 0.84 (t, $J = 7.0$ Hz, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3/CD_3OD = 4:1$) δ 172.2, 172.0, 171.7, 169.7, 140.5, 128.6 (×2), 128.5 (×2), 126.4, 76.7, 59.3, 55.0, 49.6, 37.3, 35.9, 36.6, 32.1, 31.2, 30.7, 29.6, 26.0, 23.1, 15.6, 15.2, 14.7, 14.0, 11.1.

7{*13,1*}. HRFABMS: calcd for $C_{27}H_{41}FN_3O_5$ $[M + H]^+$ 506.3030, found 506.3011; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD = 4:1$) δ 7.10 (m, 2H), 6.92 (m, 2H), 4.93 (dt, $J = 10.0$, 5.0 Hz, 1H), 4.27 (d, $J = 10.0$ Hz, 1H), 4.13 (dd, $J = 8.0$, 7.5 Hz, 1H), 3.82 (q, $J = 7.0$ Hz, 1H), 3.02 (dd, $J = 13.5$, 8.0 Hz, 1H), 2.90 (dd, $J = 13.5$, 7.5 Hz, 1H), 2.45 (dd, $J = 14.0$, 5.0 Hz, 1H), 2.37 (dd, $J = 14.0$, 10.0 Hz, 1H), 2.02 (m, 1H), 1.66 (m, 1H), 1.43−0.93 (m, 11H), 0.87 (d, $J = 7.0$ Hz, 3H), 0.86 (t, $J = 7.0$ Hz, 3H), 0.85 (t, $J = 6.0$ Hz, 3H), 0.84 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3/CD_3OD = 4:1$) δ 172.3, 171.6, 171.5, 169.6, 132.3, 138.0 (×2), 127.4, 111.5 (×2), 76.6, 59.4, 56.9, 49.6, 37.2, 35.9, 35.7, 34.8, 30.6, 29.8, 26.0, 23.1, 15.6, 15.0, 14.7, 14.0, 11.0.

7{*24,1*}. HRFABMS: calcd for $C_{29}H_{42}N_3O_5S$ $[M + H]^+$ 544.2845, found 544.2852; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD = 4:1$) δ 7.81 (m, 1H), 7.73 (m, 1H), 7.38−7.28 (m, 3H), 6.75 (d, $J = 10.0$ Hz, 1H), 4.94 (ddd, $J = 10.0$, 5.5, 5.0 Hz, 1H), 4.37 (dd, $J = 8.5$, 7.5 Hz, 1H), 4.28 (d, $J = 10.0$ Hz, 1H), 3.82 (q, $J = 7.5$ Hz, 1H), 3.32 (dd, $J = 14.5$, 8.0 Hz, 1H), 3.21 (dd, $J = 14.5$, 7.5 Hz, 1H), 2.48 (dd, $J = 14.0$, 5.0 Hz, 1H), 2.39 (dd, $J = 14.0$, 10.0 Hz, 1H), 2.04 (m, 1H), 1.66 (m, 1H), 1.44−0.94 (m, 11H), 0.87 (d, $J = 7.5$ Hz, 3H), 0.86 (t, $J = 7.0$ Hz, 3H), 0.84 (t, $J = 7.0$ Hz, 3H), 0.83 (t, $J = 7.0$ Hz, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3/CD_3OD = 4:1$) δ 172.3, 171.6, 171.5, 169.6, 132.3, 138.0 (×2), 127.4, 111.5 (×2), 76.6, 59.4, 56.9, 49.6, 37.2, 35.9, 35.7, 34.8, 30.6, 29.8, 26.0, 23.1, 15.6, 15.0, 14.7, 14.0, 11.0.

7{*1,2*}. HRFABMS: calcd for $C_{31}H_{50}N_3O_5$ $[M + H]^+$ 544.3750, found 544.3763; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD = 4:1$) δ 7.25−7.18 (m, 2H), 7.17−7.11 (m, 3H), 4.93 (dt, $J = 10.5$, 4.5 Hz, 1H), 4.26 (d, $J = 10.0$ Hz, 1H), 4.16 (dd, $J = 8.5$, 8.0 Hz, 1H), 3.80 (q, $J = 7.0$ Hz, 1H), 3.04 (dd, $J = 13.5$, 8.5 Hz, 1H), 2.94 (dd, $J = 13.5$, 8.0 Hz, 1H), 2.45 (dd, $J = 14.0$, 4.5 Hz, 1H), 2.36 (dd, $J = 14.0$, 10.5 Hz, 1H), 2.03 (m, 1H), 1.66 (m, 1H), 1.39−0.90 (m, 19H), 0.86 (t, $J = 7.5$ Hz, 3H), 0.85 (d, $J = 7.0$ Hz, 3H), 0.84 (d, $J = 6.0$ Hz, 3H), 0.81 (t, $J = 6.5$ Hz, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3/CD_3OD = 4:1$) δ 172.2, 171.5, 171.4, 169.5, 136.5, 129.1 (×2), 128.6 (×2), 127.0, 76.6, 59.3, 57.0, 49.5, 37.2, 35.9, 35.6 (×2), 31.9, 30.9, 30.0, 29.6, 29.3, 27.4, 25.9, 22.7, 15.6, 14.9, 14.7, 14.0, 11.0.

7{*20,2*}. HRFABMS: calcd for $C_{31}H_{49}N_4O_7$ $[M + H]^+$ 589.3601, found 589.3595; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD = 4:1$) δ 8.11 (m, 2H), 7.35 (m, 2H), 4.95 (dt, $J = 10.0$, 5.0 Hz, 1H), 4.26 (dd, $J = 8.5$, 8.0 Hz, 1H), 4.16 (dd, $J = 10.0$ Hz, 1H), 3.84 (q, $J = 7.0$ Hz, 1H), 3.16 (dd, $J = 13.5$, 8.0 Hz, 1H), 3.07 (dd, $J = 13.5$, 8.5 Hz, 1H), 2.45 (dd, $J = 14.0$, 5.0 Hz, 1H), 2.37 (dd, $J = 14.0$, 10.0 Hz, 1H), 2.02 (m, 1H), 1.67 (m, 1H), 1.41−0.93 (m, 19H), 0.87 (t, $J = 7.0$ Hz, 3H), 0.85 (d, $J = 7.0$ Hz, 3H), 0.83 (t, $J = 6.5$ Hz, 3H), 0.81 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3/CD_3OD = 4:1$) δ 172.1, 171.4, 170.9, 169.7, 147.0, 130.2 (×2), 123.8 (×2), 123.7, 76.5, 59.3, 56.7, 49.7, 37.3, 35.9, 35.6, 35.4, 32.0, 30.9, 30.1, 29.7, 29.4, 27.5, 26.0, 22.7, 15.5, 15.0, 14.7, 14.0, 11.0.

7{*1,3*}. HRFABMS: calcd for $C_{26}H_{40}N_3O_5$ $[M + H]^+$ 474.2968, found 474.2962; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD = 4:1$) δ 7.27−7.19 (m, 2H), 7.18−7.11 (m, 3H), 5.01 (ddt, $J = 7.0$, 5.5, 5.0 Hz, 1H), 4.38 (d, $J = 7.5$ Hz, 1H), 4.34 (t, $J = 7.0$ Hz, 1H), 3.99 (q, $J = 7.0$ Hz, 1H), 3.05 (dd, $J = 13.5$, 7.5 Hz, 1H), 2.90 (dd, $J = 13.5$, 8.0 Hz, 1H), 2.52 (dd, $J = 14.5$, 5.0 Hz, 1H), 2.34 (dd, $J = 14.5$, 6.5 Hz, 1H), 1.76 (m, 3H), 1.38−1.03 (m, 11H), 0.87 (t, $J = 7.0$ Hz, 3H), 0.83 (d, $J = 7.0$ Hz, 3H), 0.82 (t, $J = 7.0$ Hz, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3/CD_3OD = 4:1$) δ 171.8, 171.7, 171.5, 170.0, 136.4, 129.1 (×2), 128.7 (×2), 127.1, 72.9, 58.2, 56.3, 49.3, 38.9, 37.7, 36.1, 33.6, 31.5, 26.0, 24.9, 22.5, 15.0, 14.7, 13.9, 11.1.

7{*7,3*}. HRFABMS: calcd for $C_{26}H_{39}ClN_3O_5$ $[M + H]^+$ 508.2578, found 508.2578; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD = 4:1$) δ 7.18−7.14 (m, 3H), 7.02 (m, 1H), 5.00 (ddt, $J = 6.5$, 5.5, 5.0 Hz, 1H), 4.37 (d, $J = 7.5$ Hz, 1H), 4.32 (t, $J = 8.0$ Hz, 1H), 3.98 (q, $J = 7.0$ Hz, 1H), 3.02 (dd, $J = 13.5$, 8.0 Hz, 1H), 2.86 (dd, $J = 13.5$, 8.0 Hz, 1H), 2.53 (dd, $J = 14.5$, 5.0 Hz, 1H), 2.34 (dd, $J = 14.5$, 7.0 Hz, 1H), 1.75 (m, 3H), 1.38−1.03 (m, 11H), 0.87 (t, $J = 7.5$ Hz, 3H), 0.83 (d, $J = 7.0$ Hz, 3H), 0.82 (t, $J = 6.0$ Hz, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3/CD_3OD = 4:1$) δ 171.7, 171.6, 171.4, 169.9, 138.6, 134.4, 130.0, 129.3, 127.3 (×2), 72.9, 58.3, 56.1, 49.5, 39.0, 37.7, 35.8, 33.6, 31.6, 26.0, 24.9, 22.6, 15.1, 14.7, 13.9, 11.1.

7{***27,3***}**.** HRFABMS: calcd for $C_{26}H_{40}N_3O_6$ $[M + H]^+$ 490.2917, found 490.2910; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ = 4:1) δ 8.47 (brs,1H), 6.98 (m, 2H), 6.70 (m, 2H), 5.01 (ddt, J = 7.0, 5.5, 5.0 Hz, 1H), 4.38 (d, J = 8.0 Hz, 1H), 4.30 (t, J = 8.0 Hz, 1H), 4.02 (q, J = 7.0 Hz, 1H), 2.90 (dd, J = 14.0, 8.0 Hz, 1H), 2.80 (dd, J = 14.0, 8.0 Hz, 1H), 2.55 (dd, J = 14.0, 5.0 Hz, 1H), 2.34 (dd, J = 14.0, 7.0 Hz, 1H), 1.78 (m, 3H), 1.39−1.07 (m, 11H), 0.89 (t, J = 7.0 Hz, 3H), 0.85 (t, J = 7.0 Hz, 3H), 0.84 (d, J = 7.0 Hz, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3/CD_3OD$ = 4:1) δ 172.3, 172.0, 171.7, 170.1, 130.3 (×2), 127.5, 125.2, 124.7, 115.7 (×2), 73.2, 58.6, 57.0, 49.5, 39.1, 38.0, 35.6, 33.9, 31.7, 26.2, 25.1, 22.7, 15.2, 14.8, 13.9, 11.2.

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Supporting Information Available. Synthesis details, LC/MS charts of beauveriolide analogues **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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