JOC The Journal of Organic Chemistry

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Scalable Solution-Phase Synthesis of the Biologically Active Cyclodepsipeptide Destruxin E, a Potent Negative Regulator of Osteoclast Morphology

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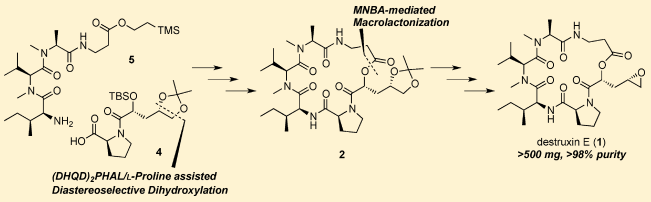
Supporting Information

ABSTRACT: The scalable solution-phase synthesis of the cyclodepsipeptide destruxin E (**1**) has been achieved. Diastereoselective dihydroxylation of the terminal alkene in a 2-alkoxy-4-pentenoic amide, **7**, was successfully accomplished utilizing $(DHQD)_2PHAL$ as the chiral ligand, and it was found that the use of the L-proline moiety in the substrate as a chiral auxiliary was essential for the induction of high diastereoselectivity to afford the key compound **4** on a gram scale. MNBA-mediated macrolactonization of **3** was also performed without formation of the dimerized product even under higher-dilution conditions, and it is noteworthy that the internal hydrogen bonds and *s-cis* configuration of the amide bond between *N*-methylalanine and *N*-methylvaline in the cyclization precursor **3** would assist in the macrolactonization to provide the macrolactone **2** without forming a dimerized product. Finally, epoxide formation in the side chain afforded destruxin E (**1**) on a gram scale in high purity (>98%).



INTRODUCTION

Osteoporosis, characterized by the loss of bone mass and bone mineral density, is a worldwide health problem, particularly among postmenopausal women and elderly people.[1] Osteoporosis is caused by disorders of bone homeostasis including excessive bone resorption by osteoclasts, and thus, several antiresorptive agents such as bisphosphonate and denosumab have been developed and are widely used for osteoporosis treatment.[2] The antiresorptive agents currently in use efficiently inhibit osteoclastic bone resorption by reducing the number of osteoclasts. However, it is difficult for patients to completely recover their bone health because bone remodeling is regulated by a balance between osteoblasts and osteoclasts and is occasionally hampered during treatment with antiresorptive agents. Thus, a therapy for osteoporosis that inhibits bone resorption without affecting osteoclast viability is desired. Recently, a vacuolar-type H^+-ATPase (V-ATPase) in osteoclasts was postulated as an attractive and a potential drug target for osteoporosis therapeutics.[3] The pumping of protons by the osteoclastic V-ATPase is a prerequisite for promoting bone resorption; therefore, specific inhibition of the osteoclastic V-ATPase without affecting the viability of osteoclasts would be a desirable mode of action in the treatment of osteolytic diseases.

Destruxin E (**1**), isolated from *Metarhidium anisopliae* by Païs et al. in 1981, is a 19-membered cyclodepsipeptide consisting of five amino acids (L-proline, L-isoleucine, *N*-methyl-L-valine, *N*-methyl-L-alanine, and *β*-alanine) and an epoxide-containing hydroxy acid derivative.[4] It has recently been reported that destruxin derivatives exhibit potent V-ATPase inhibitory activity, and **1** in particular strongly inhibits the activity of V-ATPase.[5] To date, the synthesis of destruxin and its derivatives has been reported by several research groups.[6] We have recently achieved the first total synthesis and structural determination of **1** and also reported that the stereochemistry of the epoxide is crucial for potent V-ATPase inhibitory activity.[7] In addition, destruxin E (**1**) intriguingly induces morphological changes in osteoclast-like multinuclear cells (OCLs) at low concentration without affecting the V-ATPase activity of the OCLs.[8] Therefore, **1** is consequently considered as a new type of antiresorptive agent. We are thus interested in the elucidation of the mode of action of destruxin E (**1**) in OCLs and its effect on bone metabolism in vivo. However, the limited production of **1** from fungi such as *M. anisopliae* has prevented a detailed study of its in vivo mechanism. Hence, the development of an efficient synthetic route to **1** is essential for obtaining sufficient quantities of **1** for in vivo studies. We herein report the scalable solution-phase synthesis of the naturally occurring cyclodepsipeptide destruxin E (**1**) via a 2-methyl-6-nitrobenzoic anhydride (MNBA)-mediated macrolactonization[9] and a diastereoselective dihydroxylation[10] of the terminal alkene in a 2-alkoxy-4-pentenoic amide using $(DHQD)_2PHAL$ as the chiral ligand and the L-proline moiety in the substrate as a chiral auxiliary.

Received: November 1, 2013
Published: November 19, 2013

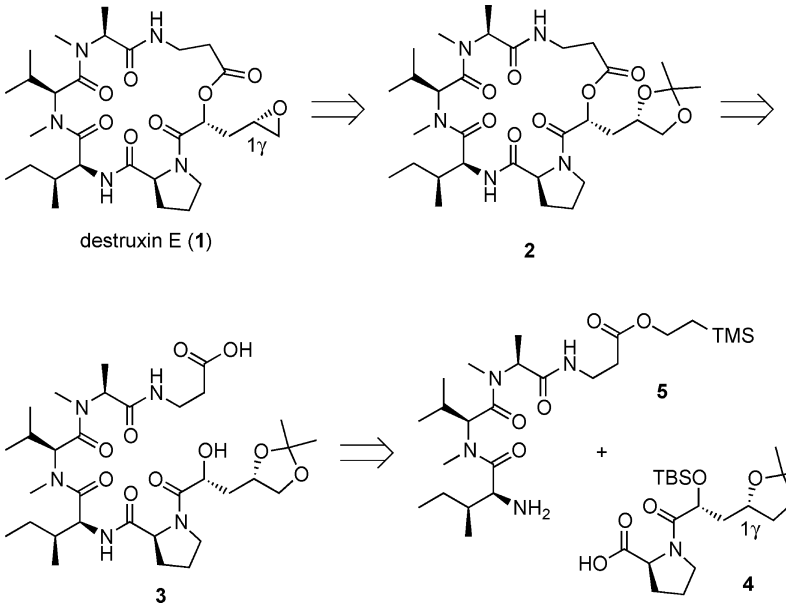


Figure 1. Retrosynthesis of destruxin E (**1**).

RESULTS AND DISCUSSION

To achieve the preparation of destruxin E (**1**) on a gram scale, we planned a solution-phase synthesis of **1**, and the synthetic outline is illustrated in Figure 1. The unstable epoxide side chain would be synthesized from macrolactone **2** in the final step. The selection of the cyclization site was important for obtaining the desired macrolactone **2**, and thus, the macrolactonization at the β-Ala–α-hydroxy acid site was chosen in accordance with our previous sysnthesis.[6a,b,7] The cyclization precursor **3** would be obtained via coupling of acid **4** and tetrapeptide **5** followed by simultaneous deprotection of the N- and C-termini. A TBS group was chosen to protect the hydroxyl group at the N-terminus of **3** and a 2-(trimethylsilyl)-ethyl (TMSEt) group was selected as the protecting group at the C-terminus of **5** because both the TBS and TMSEt groups can be readily removed by treatment with TBAF under ambient conditions without decomposition of the peptide sequence.[11]

The efficient preparation of **4** is a key issue for achieving the gram-scale synthesis of destruxin E (**1**). The secondary alcohol at the 1γ position should be diastereoselectively prepared because the stereochemistry at the 1γ position is important for the inhibition of V-ATPase activity.[7] We previously reported the synthesis of compound **4** from **8** through lactone **6**; however, the total yield of **4** was not reproducible on a gram scale because of the high polarity of the resulting acid after hydrolysis of lactone **6a** (Figure 2). Moreover, the overall yield of the desired lactone **6a** was less than 50% because of the non-stereoselective dihydroxylation of alkene **8**, which was utilized to prepare both lactones **6a** and **6b** for structural determination of destruxin E (**1**). Therefore, preparation of the key compound **4** utilizing lactone **6a** is problematic on a gram scale. Thus, an alternative route to **4** involving diastereoselective dihydroxylation of alkene **7** followed by acetal formation and removal of the benzyl group was anticipated to provide a higher yield. In general, it is known that dihydroxylation of terminal alkenes proceeds with lower enantioselectivity than that of internal alkenes.[12] Therefore, we expected that the proline moiety in **7** might act as a chiral auxiliary in the diastereoselective dihydroxylation of the terminal alkene moiety. Compound **7**

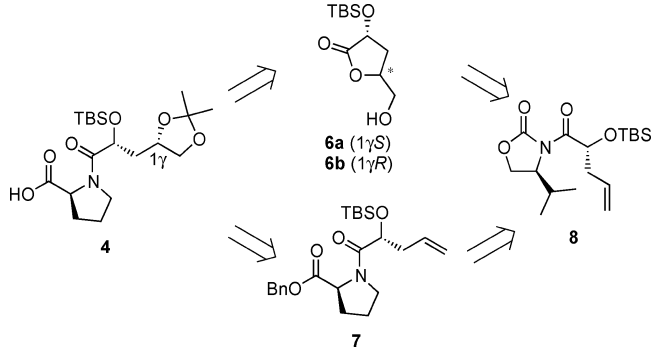


Figure 2. Retrosynthesis of key compound **4**.

can be readily synthesized via the coupling of L-proline benzyl ester and the acid obtained by hydrolysis of the previously synthesized compound **8**.

Compound **7** was initially synthesized from the previously reported[7] TBS ether **9** (Scheme 1). In our previous report, the asymmetric allylation of **9** was performed using allyl bromide/lithium hexamethyldisilazide (LiHMDS) at −30 °C for 16 h to achieve reproducible yield and diastereoselectivity. To improve on these results, highly reactive allyl iodide was used as the electrophile.[13] Expectedly, the allylation with NaHMDS at −45 °C was smoothly completed within 1.5 h, affording the desired product **8** in 94% yield with >95% diastereoselectivity. However, acid **11** was not formed by direct hydrolysis of **8** under basic conditions because the cyclic carbamate was readily hydrolyzed under the reaction conditions. The stepwise modification to give **11** through methyl ester **10** was then attempted via methanolysis of **8** utilizing samarium trifluoromethanesulfonate.[14] However, this reaction took over 48 h to go to completion, and the TBS group in **11** was easily removed under the acidic workup. Thus, the nucleophilic cleavage of **8** with MeOMgBr was investigated;[15] this reaction proceeded successfully at room temperature to provide **10** in 94% yield without removal of the TBS group. Hydrolysis of the methyl ester followed by amidation of the resulting acid **11** with L-Pro-OBn using bromotripyrrolidinophosphonium hexafluorophos-

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Scheme 1. Synthesis of 7

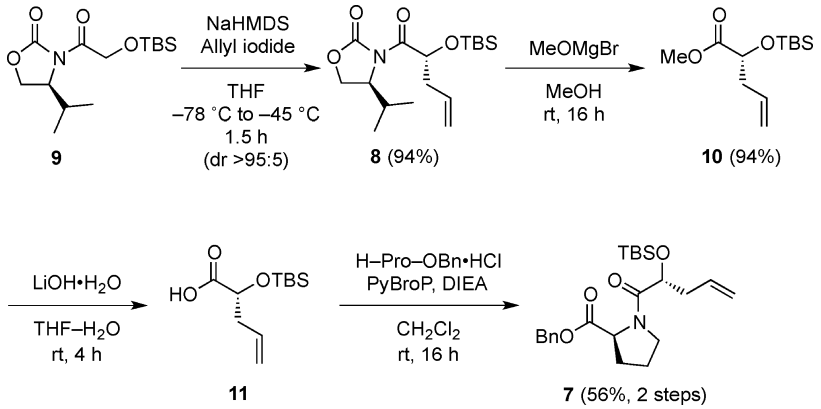
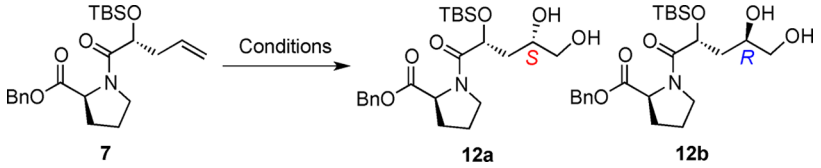


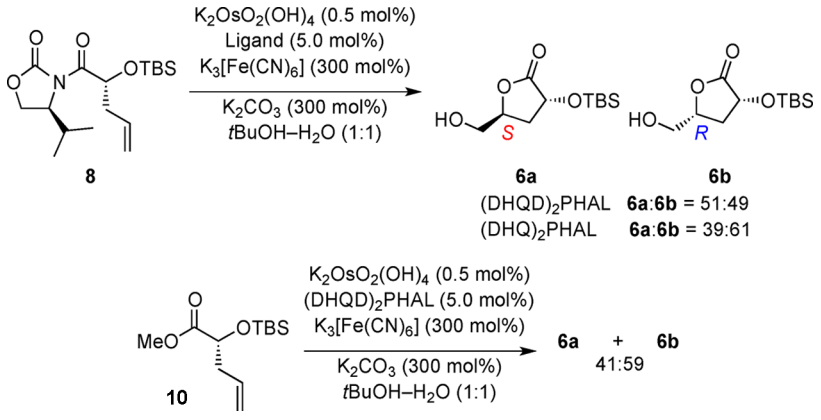
Table 1. Investigation of the Reaction Conditions for Diastereoselective Dihydroxylation of 7



entry	conditions[a]	*S*:*R* ratio[b]	yield (%)[c]
1	AD-mix-β (1.4 g/mmol)	67:33	51
2	AD-mix α (1.4 g/mmol)	50:50	43
3	OsO_4 (1.0 mol %), $(DHQD)_2PHAL$ (5.0 mol %)	77:23	71
4	$K_2OsO_2(OH)_4$ (1.0 mol %), $(DHQD)_2PHAL$ (5.0 mol %)	86:14	97
5	$K_2OsO_2(OH)_4$ (0.5 mol %), $(DHQD)_2PHAL$ (5.0 mol %)	86:14	quant
6	$K_2OsO_2(OH)_4$ (1.0 mol %), $(DHQD)_2AQN$ (5.0 mol %)	84:16	90
7	$K_2OsO_2(OH)_4$ (1.0 mol %), $(DHQD)_2PYR$ (5.0 mol %)	31:69	92

[a] $K_3[Fe(CN)_6]$ (300 mol %), K_2CO_3 (300 mol %), *t*BuOH–H_2O (1:1), rt, 18–24 h. [b] Determined by ^1H NMR analysis of the crude mixtures. [c] Combined yields.

Scheme 2. Attempts at Diastereoselective Dihydroxylation of 8



phate (PyBroP)[16] then afforded the desired product **7** in 56% yield.

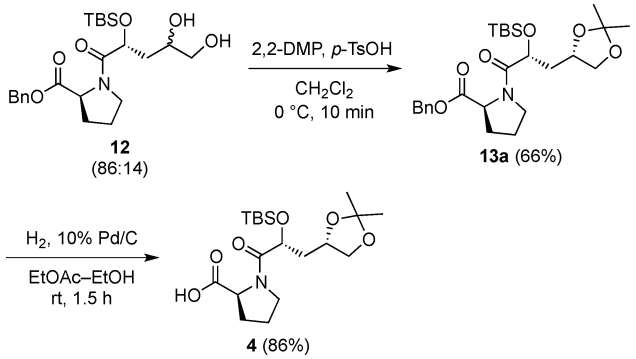
Once the gram-scale synthesis of the desired **7** was achieved, we next investigated the diastereoselective dihydroxylation of the terminal alkene in **7**. It is known that the asymmetric dihydroxylation of terminal alkenes generally proceeds with poor enantioselectivity. Thus, extensive studies of the asymmetric dihydroxylation of terminal alkenes have been conducted, and bis(cinchona alkaloid) ligands with 1,4-phthalazine (PHAL) or pyrimidine (PYR) spacers have been shown to be effective for obtaining the corresponding diols with high enantioselectivity.[12] As an initial attempt at the synthesis of the desired diol **12a**, the dihydroxylation of **7** utilizing AD-mix-β was examined (Table 1). This reaction provided the desired diol **12a** and its diastereomer **12b** in a ratio of 67:33 (entry 1).[17] By contrast, dihydroxylation utilizing AD-mix-α provided a 50:50 mixture of diastereomers (entry 2). Thus, the dihydroxylation was further investigated using cinchonidine-based chiral ligands. When the reaction was performed using $(DHQD)_2PHAL$ (5 mol %) in the presence of OsO_4 (1 mol %), the diols **12** were formed in a 77:23 ratio (entry 3). After extensive investigations, it was found that the dihydroxylation

dx.doi.org/10.1021/jo402437z | J. Org. Chem. 2014, 79, 296–306

of **7** utilizing $K_2OsO_2(OH)_4$–$(DHQD)_2PHAL$ quantitatively afforded **12a** with 86% diastereoselectivity and that reduction of the osmium catalyst loading to 0.5 mol % did not affect the diastereoselectivity (entry 5). On the other hand, it was found that with other chiral ligands, such as $(DHQD)_2AQN$[18] or $(DHQD)_2PYR$,[12a] the yield or diastereoselectivity was decreased (entries 6 and 7). Therefore, $(DHQD)_2PHAL$ was selected as the chiral ligand for the diastereoselective dihydroxylation of the terminal alkene in **7**. In contrast to the dihydroxylation of **7**, the dihydroxylation of **8** under the optimized conditions utilizing $(DHQD)_2PHAL$ and $(DHQ)_2PHAL$ resulted in poor stereoselectivity (**6a**:**6b** = 51:49 and 39:61, respectively; Scheme 2). In addition, the dihydroxylation of methyl ester **10** in the presence of $K_2OsO_2(OH)_4$–$(DHQD)_2PHAL$ afforded a mixture of lactones **6a** and **6b** in a 41:59 ratio. Thus, it seemed that the chirality at the α-position in the hydroxyl acid derivative was mismatched in the dihydroxylation utilizing $(DHQD)_2PHAL$ for the induction of the desired stereochemistry at the 1γ position. Therefore, the chirality of the proline moiety in **7** also would assist in the induction of the high diastereoselectivity obtained for **12a**.

Because it was difficult to separate the resulting diols **12** using conventional column chromatography, acetal formation with 2,2-dimethoxypropane was performed on the mixture of diastereomers **12**, and this was followed by isolation of the resulting acetal **13a** via flash column chromatography in 66% overall yield. Finally, removal of the benzyl group by hydrogenolysis furnished the desired compound **4** in 86% yield (Scheme 3).

Scheme 3. Synthesis of Compound 4



With the desired **4** in hand, the gram-scale synthesis of the cyclization precursor **3** was then investigated. Although we have reported the solid-phase synthesis of cyclization precursor **3** using an Fmoc method, we considered performing the gram-scale synthesis of **3** in solution because of facile scale-up and yield. As it is well-known that peptide sequences including *N*-methylamide are readily hydrolyzed at the *N*-methylamide bond under acidic conditions,[19] peptide elongation utilizing the Boc strategy had to be avoided for the synthesis of **5**. Instead, the benzyloxycarbonyl (Cbz) group was selected as the protecting group.[6a,c−h] The Cbz group can be removed by hydrogenolysis under ambient conditions without cleavage of *N*-methylamide bonds, and Cbz-protected *N*-methylamino acids can be readily prepared in one step from the corresponding amino acids on a large scale.[20] The starting material Cbz-β-Ala-OH (**14**) was thus converted to the corresponding TMSEt ester **15**[11b] via condensation with 2-(trimethylsilyl)ethanol. Hydrogenolysis of **15** in isopropanol provided amine **16** without transesterification at the C-terminus. The resulting amine **16** was then coupled with Cbz-NMeAla-OH using EDCI–HOBt, and dipeptide **17** was obtained in 86% yield. However, when the preparation of **18** from **17** and Cbz-NMeVal-OH was carried out in the same manner as for the preparation of **17**, the yield of **18** was moderate (56%). After several attempts at the tripeptide synthesis, it was found that the use of HOAt instead of HOBt enabled efficient amidation to provide the desired product **18** in 90% yield without formation of the diketopiperazine through displacement of the C-terminal β-Ala residue.[6e,21] Next, tetrapeptide **5** was prepared through **19** in the same manner as used for **18**, and then hexapeptide **20** was obtained via coupling of **5** with **4** using EDCI–HOAt. Concomitant removal of the TBS and TMSEt groups with TBAF furnished the desired cyclization precursor **3** on a multigram scale (Scheme 4).

Next, we investigated the MNBA-mediated macrolactonization of **3** (Scheme 5).[8] Previously, we performed the macrolactonization of **3** at 3 mM. However, in the present study it was found that the macrocyclization of **3** could be achieved at 6 mM on a gram scale, providing the corresponding product **2** in 81% yield without formation of the dimerized byproduct (Scheme 5). On the other hand, we also attempted the macrolactamization of L-Pro and L-Ile residues of **21** based on the previous reports.[6c,f,g] Although the macrolactamization of **21**[22] at 1 mM utilizing EDCI–HOAt proceeded smoothly at room temperature, a mixture of **2** and its dimer **22** in a 53:47 ratio was obtained (Scheme 6). On the basis of previously reported conformational studies of destruxin A and roseotoxin B using NMR and X-ray crystallographic analyses, it has been suggested that the amido bond between *N*-MeAla and *N*-MeVal adopts the *s-cis* configuration, leading to a β-turn-shaped structure stabilized by two internal hydrogen bonds.[23] Therefore, we believe that the cyclization precursor **3** forms a similar conformation as **A**, which is proper for the macrolactonization (Figure 3). Wang et al.[24] recently reported that the destruxins are produced via nonribosomal peptide synthesis of a linear peptide, which undergoes macrolactonization in the biosysnthesis. Thus, the macrolactonization between β-Ala and the hydroxy acid would be structurally suitable for the ring construction of destruxins, rather than the macrolactamization between the proline and isoleucine residues.

With the desired macrolactone **2** on a gram scale, removal of the acetonide in **2** was performed [3 M aqueous HCl/dioxane (1:2), 10 °C, 10 min] in accordance with our previous report. However, the yield of **26** was moderate because the *N*-methylamide bond was partially cleaved under the acidic conditions. After further investigation, it was found that the ratio of water and dioxane was crucial for removal of the acetonide without cleavage of the peptide bond. The reaction was complete under milder conditions [1.5 M aqueous HCl/dioxane (2:1), 0 °C, 2 h], providing diol **26** in 87% yield. For completion of the scalable synthesis of **1**, selective tosylation of the primary alcohol in **26** was carefully performed via treatment with 2 equiv of TsCl at room temperature to afford the desired tosylate **27** in 81% yield. Finally, formation of the epoxide under basic conditions furnished destruxin E (**1**) in several hundred milligram yield with high purity (>98%) after silica gel column chromatography (Scheme 7).

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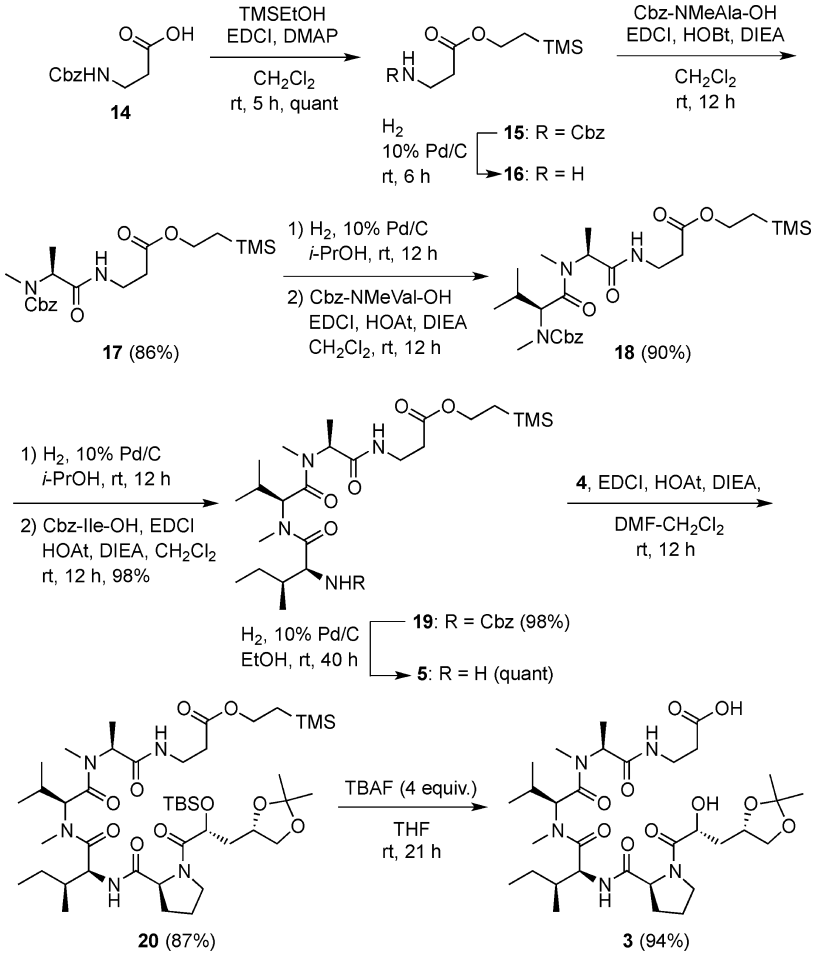
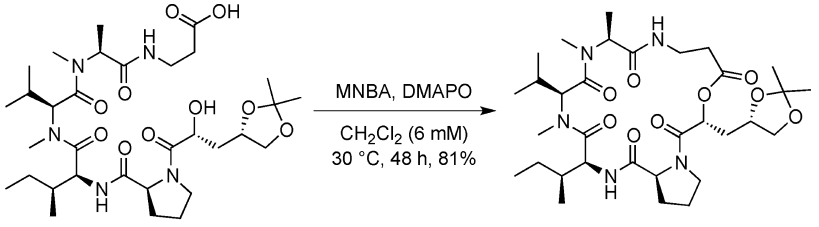
Scheme 4. Synthesis of Cyclization Precursor 3



Scheme 5. Synthesis of Macrolactone 2 via Macrolactonization



CONCLUSION

In conclusion, we have accomplished the improved solution-phase synthesis of destruxin E (**1**) and established an efficient synthetic process for the production of a sufficient quantity of **1** in high purity for use in vivo experiments. Diastereoselective dihydroxylation of the terminal alkene in **7** was successfully performed utilizing $(DHQD)_2PHAL$, and the L-proline moiety in the substrate was essential for inducing the high diastereoselectivity in this reaction. It is also noteworthy that the β-turn-shaped structure stabilized by internal hydrogen bonds and the *s-cis* configuration of the NMe amido bonds assisted in the efficient macrolactonization of **3** without high-dilution conditions, leading to macrolactone **2** without formation of the dimerized byproduct. These improved synthetic processes made it possible to access a sufficient quantity of destruxin E (**1**) in high purity (>98%), and in vivo experiments on **1** to study its effect on bone metabolism are currently underway in our laboratories.

EXPERIMENTAL SECTION

General Techniques. Chemicals and solvents were all purchased from commercial suppliers and used without further purification. All reactions in the solution phase were monitored by thin-layer chromatography carried out on glass-packed silica gel plates (60F-254) with UV light and visualized by *p*-anisaldehyde H_2SO_4–ethanol solution or phosphomolybdic acid ethanol solution. Flash column chromatography was carried out with silica gel (40–100 μm) with the indicated solvent system. ^{1}H NMR spectra (400 MHz, 600 MHz) and ^{13}C NMR spectra (100 MHz) were recorded in the indicated solvents. Chemical shifts (δ) are reported in units of parts per million (ppm) relative to the signal for internal tetramethylsilane (0.00 ppm for ^{1}H) for solutions in chloroform-*d*. NMR spectral data are reported as follows: chloroform-*d* (77.0 ppm for ^{13}C), methanol-d_3 (3.30 ppm for

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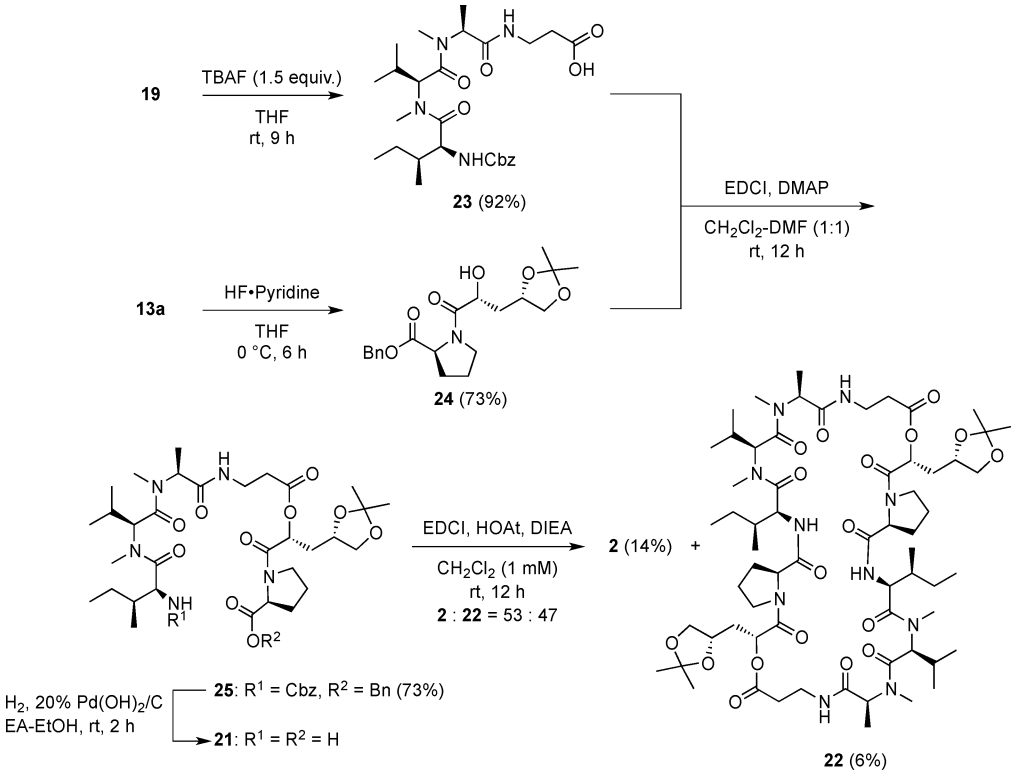
Scheme 6. Alternative Synthesis of Macrolactone 2 via Macrolactamization



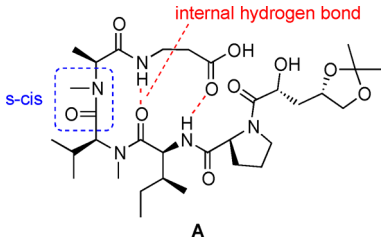
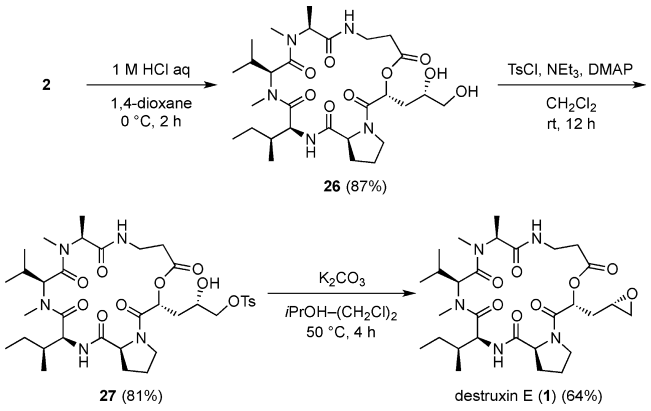


Figure 3. Plausible conformation of cyclization precursor **3**.

Scheme 7. Achievement of the Scalable Synthesis of 1



^{1}H), dimethyl sulfoxide-d_4 (2.49 ppm for ^{1}H and 39.5 ppm for ^{13}C) when an internal standard is not indicated. Multiplicities are reported using the following abbreviations: s (singlet), d (doublet), t (triplet), m (multiplet), dd (doublet of doublets), dt (doublet of triplets), dq (doublet of quartets), ddd (doublet of doublets of doublets), ddt (doublet of doublets of triplets). Coupling constants (*J*) are reported in hertz (Hz). High-resolution mass spectra were measured on TOF-MS with ESI or FAB probe. Infrared spectra are reported in reciprocal centimeters (cm^{-1}). Melting points were measured on a melting point apparatus and are not corrected. Optical rotations were measured with a polarimeter at 589 nm. HPLC analysis was performed on an HPLC system with a photodiode array detector and an analytical C_{18} column (3.5 mm, 4.6 × 100 mm i.d.) at a flow rate of 1.1 mL/min with a gradient solvent (0 min, 10% MeOH/H_2O; 4 min, 95% MeOH/H_2O; 11 min, 95% MeOH/H_2O; 11.1 min, 10% MeOH/H_2O; 15 min, 10% MeOH/H_2O). HPLC solvents (MeOH and H_2O) were buffered with 0.1% LC–MS-grade formic acid. Purity was measured with the peak area at UV (214 nm).

Syntheses. *(R)-2-(tert-Butyldimethylsilyloxy)pentenoyl Oxazolidinone (**8**).* To a solution of TBS ether **9**[7] (17.5 g, 58.1 mmol, 1 equiv) in dry THF (200 mL, 3.4 mL/mmol) was added a solution of NaHMDS in THF (1.00 M, 116 mL, 116 mmol, 2 equiv) dropwise at −78 °C under an argon atmosphere. After the reaction mixture was stirred at −78 °C for 30 min, a solution of allyl iodide (15.9 mL, 174 mmol, 3 equiv) in dry THF (50 mL, 0.29 mL/mmol) was added dropwise at −78 °C. After being stirred at −45 °C for 1 h, the reaction mixture was poured into saturated aqueous NH_4Cl, and then the aqueous layer was extracted with ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by silica gel column chromatography (eluted with hexane/AcOEt = 20:1) to afford the allylated product **8** (18.7 g, 54.8 mmol, 94%, >95% ds) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.84–5.95 (1H, ddt, *J* = 6.4, 8.4, 16.0 Hz), 5.41 (1H, dd, *J* = 4.0, 7.2 Hz), 5.13 (1H, d, *J* = 16.0 Hz), 5.10 (1H, d, *J* = 8.4 Hz), 4.51 (1H, dt, *J* = 3.6, 8.4 Hz), 4.34 (1H, t, *J* = 8.8 Hz), 4.23 (1H, dd, *J* = 3.6, 8.8 Hz), 2.56–2.63 (1H, m), 2.38–2.46 (1H, m), 2.31 (1H, dq, *J* = 3.6, 7.2 Hz), 0.90 (9H, s), 0.90 (3H, d, *J* = 6.8 Hz), 0.87 (3H, d, *J* = 6.8 Hz), 0.07 (3H, s), 0.05 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 153.6, 133.4, 118.1, 71.0, 63.9, 58.2, 40.0, 28.2, 25.7, 18.3, 17.8, 14.7, −4.9, −5.2; IR ($CHCl_3$) 2958, 2931, 1781, 1717, 1389, 1249, 1209, 1116, 837 cm^{-1}; $[\alpha]_D^{26}$ +63.5 (*c* 1.03, $CHCl_3$); HRFABMS calcd for $C_{17}H_{31}NO_4Si$ $[M + H]^+$ 342.2101, found 342.2086.

*(R)-2-(tert-Butyldimethylsilyloxy)pent-4-enoic Acid Methyl Ester (**10**).* Dry MeOH (30 mL, 0.83 mL/mmol) was added to a solution of

dx.doi.org/10.1021/jo402437z | J. Org. Chem. 2014, 79, 296–306

methylmagnesium bromide in THF (0.97 M, 74 mL, 72 mmol, 2 equiv) dropwise at 0 °C under an argon atmosphere. After the reaction mixture was stirred at 0 °C for 15 min, a solution of **8** (12.3 g, 36.0 mmol, 1 equiv) in dry MeOH (90 mL, 2.5 mL/mmol) was added dropwise at 0 °C. After being stirred at room temperature for 16 h, the reaction mixture was poured into saturated aqueous NH_4Cl, and then the aqueous layer was extracted with ether. The organic layer was washed with brine, and its organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by silica gel column chromatography (eluted with hexane/AcOEt = 20:1) to afford methyl ester **10** (8.26 g, 33.8 mmol, 94%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82 (1H, ddt, J = 7.2, 10.4, 17.0 Hz), 5.06−5.15 (2H, m), 4.26 (1H, dd, J = 4.8, 7.2 Hz), 3.72 (3H, s), 2.40−2.53 (2H, m), 0.90 (9H, s), 0.08 (3H, s), 0.06 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 133.4, 117.9, 72.2, 51.8, 39.8, 25.8, 18.4, −4.9, −5.2; IR ($CHCl_3$) 2953, 2935, 1760, 1473, 1257, 1143, 837, 779 cm^{-1}; $[\alpha]_D^{26}$ +11.6 (c 0.900, $CHCl_3$); HRFABMS calcd for $C_{12}H_{24}O_3Si$ $[M + H]^+$ 245.1573, found 245.1587.

*N-[(2R)-tert-Butyldimethylsiloxypent-4-enoyl]-L-proline Benzyl Ester (**7**).* To a solution of methyl ester **10** (8.02 g, 32.8 mmol, 1 equiv) in THF (126 mL, 3.8 mL/mmol) and H_2O (126 mL, 3.8 mL/mmol) was added lithium hydroxide monohydrate (2.75 g, 65.6 mmol, 2 equiv) at 0 °C. After the reaction mixture was stirred at room temperature for 4 h, 2 M sodium dihydrogen phosphate (pH 3, 70 mL, 140 mmol, 4.3 equiv) was added at 0 °C, and the aqueous layer was extracted with ether. The organic layer was washed with brine, and its organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was used for the next reaction without further purification.

To a solution of the crude carboxylic acid **11**, L-proline benzyl ester hydrochloride (8.73 g, 36.1 mmol, 1.1 equiv), and *N*,*N*-diisopropylethylamine (17.1 mL, 98.4 mmol, 3 equiv) in dry CH_2Cl_2 (164 mL, 5.00 mL/mmol) was added PyBroP (23.0 g, 49.2 mmol, 1.5 equiv) at 0 °C under an argon atmosphere. After the reaction mixture was stirred at room temperature for 16 h, H_2O was added, and the aqueous layer was extracted with CH_2Cl_2. The organic layer was washed with brine, and its organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by silica gel column chromatography (eluted with hexane/AcOEt = 7:1) to afford amide **7** (7.67 g, 18.4 mmol, 56%) as a pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26−7.40 (5H, m), 5.81 (1H, ddt, J = 7.2, 10.0, 17.2 Hz), 5.16 (2H, s), 5.10 (1H, d, J = 17.2 Hz), 5.06 (1H, d, J = 10.0 Hz), 4.52 (1H, dd, J = 3.6, 8.8 Hz), 4.32, (1H, t, J = 6.8 Hz), 3.87 (1H, dt, J = 6.8, 10.4 Hz), 3.68−3.74 (1H, m), 2.39−2.48 (2H, m), 2.10−2.17 (1H, m), 1.99−2.05 (1H, m), 1.88−1.94 (2H, m), 0.89 (9H, s), 0.07 (3H, s), 0.05 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 171.3, 135.8, 133.7, 128.4, 128.1, 128.0, 117.8, 74.9, 66.6, 59.6, 46.7, 39.2, 28.4, 25.9, 25.3, 18.3, −4.7, −5.1; IR ($CHCl_3$) 2954, 2928, 2856, 1746, 1641, 1430, 1258, 1169, 837, 779 cm^{-1}; $[\alpha]_D^{25}$ −34.4 (c 0.450, $CHCl_3$); HRFABMS calcd for $C_{23}H_{35}NO_4Si$ $[M + H]^+$ 418.2414, found 418.2412.

*Benzyl Ester **13a**.* To a solution of alkene 7 (7.67 g, 18.4 mmol, 1 equiv) in *t*-BuOH (90 mL, 4.89 mL/mmol) and H_2O (90 mL, 4.89 mL/mmol) were added $(DHQD)_2PHAL$ (717 mg, 0.92 mmol, 5.0 mol %), K_2CO_3 (7.63 g, 55.2 mmol, 3 equiv), $K_3[Fe(CN)_6]$ (18.2 g, 55.2 mmol, 3 equiv), and a solution of $K_2OsO_2(OH)_4$ in H_2O (0.05 M, 1.84 mL, 0.092 mmol, 0.5 mol %) at 0 °C. After the reaction mixture was stirred at room temperature for 8 h, saturated aqueous $Na_2S_2O_3$ was added, and the aqueous layer was extracted with ethyl acetate. The organic layer was washed with brine, and its organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was filtered through a short pad of silica gel (eluted with AcOEt/MeOH = 10:1) to afford diol **12** (86:14 diastereomeric mixture, 8.26 g, 18.4 mmol, quant) as a colorless oil.

To a solution of diol **12** (8.26 g, 18.4 mmol, 1 equiv) and 2,2-dimethoxypropane (12.1 mL, 92.0 mmol, 5.00 equiv) in dry CH_2Cl_2 (123 mL, 6.7 mL/mmol) was added *p*-TsOH (317 mg, 1.78 mmol, 0.10 equiv) at 0 °C under an argon atmosphere. After being stirred at the same temperature for 10 min, the reaction mixture was quenched with *N*,*N*-diisopropylethylamine. The reaction mixture was concentrated in vacuo, and the resulting residue was purified by silica gel column chromatography (eluted with hexane/AcOEt = 6:1) to afford **13a** (5.82 g, 11.8 mmol, 66% over two steps) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25−7.37 (5H, m), 5.18 (1H, d, J = 12.0 Hz), 5.13 (1H, d, J = 12.0 Hz), 4.52 (1H, dd, J = 3.6, 8.4 Hz), 4.45 (1H, t, J = 6.4 Hz), 4.11 (1H, q, J = 6.4 Hz), 4.04 (1H, dd, app. dt, J = 6.8 Hz), 3.81−3.88 (1H, m), 3.63−3.70 (1H, m), 3.57 (1H, dd, app. dt, J = 7.2 Hz), 1.88−2.20 (6H, m), 1.40 (3H, s), 1.32 (3H, s) 0.89 (9H, s), 0.08 (3H, s), 0.07 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 170.9, 135.8, 128.5, 128.1, 128.0, 108.5, 72.8, 71.4, 69.5, 66.6, 59.4, 46.6, 38.7, 28.4, 27.0, 25.7, 25.6, 25.1, 18.1, −4.8, −5.3; IR ($CHCl_3$) 2956, 2934, 1755, 1666, 1643, 1442, 1256, 1157, 835 cm^{-1}; $[\alpha]_D^{28}$ −32.9 (c 1.00, $CHCl_3$); HRFABMS calcd for $C_{26}H_{41}NO_6Si$ $[M + H]^+$ 492.2781, found 492.2787.

*Acid **4**.* To a solution of benzyl ester **13a** (5.82 g, 11.8 mmol, 1 equiv) in AcOEt (23.6 mL, 2.00 mL/mmol) and EtOH (23.6 mL, 2.00 mL/mmol) was added 10% Pd/C (580 mg, 10 wt %) at room temperature under an argon atmosphere, and the flask was purged with hydrogen three times. After being stirred at room temperature for 1 h, the reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated in vacuo. The resulting residue was recrystallized from hexane to afford carboxylic acid **4** (4.06 g, 10.1 mmol, 86%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.62 (1H, dd, J = 2.4, 8.4 Hz), 4.55 (1H, dd, app. dt, J = 6.0 Hz), 4.10−4.18 (1H, m), 4.07 (1H, dd, app. dt, J = 6.8 Hz), 3.80−3.90 (1H, m), 3.60−3.68 (1H, m), 3.58 (1H, dd, app. dt, J = 7.6 Hz), 2.43−2.53 (1H, m), 1.85−2.10, (5H, m), 1.40 (3H, s), 1.32 (3H, s), 0.90 (9H, s), 0.09 (3H, s), 0.08 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 173.1, 109.0, 72.3, 71.2, 69.4, 60.4, 47.2, 38.8, 27.2, 26.9, 25.7, 25.6, 25.1, 18.1, −4.8, −5.2; IR ($CHCl_3$) 2928, 2857, 1741, 1616, 1462, 1369, 1255, 1159, 1063 cm^{-1}; mp 84−87 °C; $[\alpha]_D^{26}$ −75.9 (c 1.03, $CHCl_3$); HRFABMS calcd for $C_{19}H_{35}NO_6Si$ $[M + H]^+$ 402.2312, found 402.2325.

*Cbz-β-Ala-OTMSEt (**15**).* To a solution of Cbz-β-Ala-OH (**14**) (3.00 g, 13.4 mmol, 1 equiv), 2-(trimethylsilyl)ethanol (2.12 mL, 14.8 mmol, 1.1 equiv), and DMAP (164 mg, 1.34 mmol, 0.1 equiv) in dry CH_2Cl_2 (54 mL, 4 mL/mmol) was added EDCI (2.84 g, 14.8 mmol, 1.1 equiv) at 0 °C under an argon atmosphere. After being stirred at room temperature for 5 h, the reaction mixture was quenched with 3 M HCl at 0 °C. The aqueous layer was extracted with ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to afford TMSEt ester **15** (4.34 g, 13.4 mmol, quant) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26−7.36 (5H, m), 5.29 (1H, br s), 5.09 (2H, s), 4.18 (2H, t, J = 8.6 Hz), 3.46 (2H, dt, J = 5.6, 6.0 Hz), 2.52 (2H, t, J = 6.0 Hz), 0.98 (2H, t, J = 8.6 Hz), 0.04 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 156.2, 136.5, 128.5, 128.08, 128.05, 66.7, 63.0, 36.6, 34.6, 17.3, −1.52; IR ($CHCl_3$) 3353, 2954, 1729, 1523, 1250, 1175, 860, 837, 697 cm^{-1}; HRFABMS calcd for $C_{16}H_{26}NO_4Si$ $[M + H]^+$ 324.1631, found 324.1641; HPLC retention time 8.73 min, purity >97%.

*Cbz-MeAla-β-Ala-OTMSEt (**17**).* To a solution of **15** (1.36 g, 4.21 mmol, 1 equiv) in *i*-PrOH (14 mL, 3.3 mL/mmol) was added 10% Pd/C (140 mg, 10 wt %) at room temperature under an argon atmosphere, and the flask was purged with hydrogen three times. After being stirred at room temperature for 6 h, the reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated in vacuo. The crude residue was used for the next reaction without further purification.

To a solution of the crude amine **16** (0.781 g, 4.13 mmol, 1 equiv), Cbz-MeAla-OH (1.00 g, 4.21 mmol, 1.0 equiv), HOBt (0.670 g, 4.96 mmol, 1.2 equiv), and DIEA (1.08 mL, 6.20 mmol, 1.5 equiv) in dry CH_2Cl_2 (16.5 mL, 4 mL/mmol) was added EDCI (0.951 g, 4.96 mmol, 1.2 equiv) at 0 °C under an argon atmosphere. After being stirred at room temperature for 12 h, the reaction mixture was quenched with saturated aqueous NH_4Cl at 0 °C. The aqueous layer was extracted with $CHCl_3$. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by silica gel column chromatography

dx.doi.org/10.1021/jo402437z | J. Org. Chem. 2014, 79, 296−306

(eluted with hexane/AcOEt = 1:1) to afford dipeptide **17** (1.46 g, 3.57 mmol, 86%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33−7.23 (5H, m), 6.50 (1H, br s), 5.13 (2H, s), 4.72 (1H, br s), 4.13 (2H, t, J = 8.6 Hz), 3.46 (2H, dt, J = 5.2, 6.4 Hz), 2.81 (3H, s), 2.44 (2H, t, J = 5.2 Hz), 1.32 (3H, d, J = 7.2 Hz), 0.94 (2H, t, J = 8.6 Hz), 0.03 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 171.0, 136.4, 128.5, 128.1, 127.9, 67.6, 63.0, 35.0, 34.1, 17.3, −1.54; IR ($CHCl_3$) 3340, 2953, 2898, 1732, 1703, 1683, 1527, 1455, 1400, 1313, 1250, 1218, 1172, 1061, 860, 838 cm^{-1}; $[\alpha]_D^{26}$ −44 (c 0.66, $CHCl_3$); HRFABMS calcd for $C_{20}H_{33}N_2O_5Si$ $[M + H]^+$ 409.2159, found 409.2158; HPLC retention time 8.75 min, purity 94%.

*Cbz-MeVal-MeAla-β-Ala-OTMSEt (**18**).* To a solution of **17** (1.45 g, 3.55 mmol, 1 equiv) in *i*-PrOH (14 mL, 3.9 mL/mmol) was added 10% Pd/C (145 mg, 10 wt %) at room temperature under an argon atmosphere, and the flask was purged with hydrogen three times. After being stirred at room temperature for 12 h, the reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated in vacuo. The crude residue was used for the next reaction without further purification.

To a solution of the crude amine (0.931 g, 3.39 mmol, 1 equiv), Cbz-MeVal-OH (1.08 g, 4.07 mmol, 1.2 equiv), HOAt (0.693 g, 5.09 mmol, 1.5 equiv), and DIEA (1.78 mL, 10.2 mmol, 3 equiv) in dry CH_2Cl_2 (14 mL, 4 mL/mmol) was added EDCI (0.975 g, 5.09 mmol, 1.5 equiv) at 0 °C under an argon atmosphere. After being stirred at room temperature for 12 h, the reaction mixture was quenched with saturated aqueous NH_4Cl at 0 °C. The aqueous layer was extracted with ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by silica gel column chromatography (eluted with hexane/AcOEt = 1:1) to afford tripeptide **18** (1.67 g, 3.21 mmol, 90%) as a colorless oil. ^{1}H NMR (600 MHz, DMSO-d_6, 80 °C) δ 7.15−7.50 (6H, m), 5.00−5.14 (2H, m), 4.86 (1H, d, J = 6.6 Hz), 4.48−4.58 (1H, m), 4.09 (2H, t, J = 7.8 Hz), 3.20−3.30 (2H, m), 2.66−2.83 (6H, m), 2.38 (2H, t, J = 7.2 Hz), 2.15−2.25 (1H, m), 1.13−1.30 (3H, m), 0.76−0.91 (8H, m), 0.00 (9H, s); ^{13}C NMR (100 MHz, DMSO-d_6, mixture of rotamers) δ 171.23, 171.19, 170.5, 169.7, 169.6, 169.5, 156.2, 156.0, 155.2, 136.9, 136.7, 136.5, 128.37, 128.34, 128.0, 127.8, 127.6, 127.25, 127.23, 79.1, 66.8, 66.7, 66.5, 61.9, 60.0, 59.3, 54.6, 52.0, 51.9, 35.0, 34.84, 34.81, 33.8, 33.7, 30.5, 30.3, 29.3, 29.0, 28.9, 28.8, 27.1, 26.96, 26.92, 19.42, 19.35, 19.32, 18.3, 18.09, 18.05, 16.75, 16.73, 15.7, 14.4, −1.55, −1.58; IR ($CHCl_3$) 3334, 2958, 2898, 1731, 1692, 1646, 1522, 1498, 1472, 1456, 1396, 1370, 1303, 1250, 1224, 1174, 1133, 1111, 860, 838 cm^{-1}; $[\alpha]_D^{27}$ −117 (c 1.00, $CHCl_3$); HRFABMS calcd for $C_{26}H_{44}N_3O_6Si$ $[M + H]^+$ 522.2999, found 522.3011; HPLC retention time 9.18 min, purity >98%

*Cbz-Ile-MeVal-MeAla-β-Ala-OTMSEt (**19**).* To a solution of **18** (1.67 g, 3.21 mmol, 1 equiv) in *i*-PrOH (17 mL, 5.3 mL/mmol) was added 10% Pd/C (167 mg, 10 wt %) at room temperature under an argon atmosphere, and the flask was purged with hydrogen three times. After being stirred at room temperature for 12 h, the reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated in vacuo. The crude residue was used for the next reaction without further purification.

To a solution of the crude amine (1.24 g, 3.21 mmol, 1 equiv), Cbz-Ile-OH (1.30 g, 4.91 mmol, 1.5 equiv), HOAt (0.668 g, 4.91 mmol, 1.5 equiv), and DIEA (1.71 mL, 9.84 mmol, 3 equiv) in dry CH_2Cl_2 (13 mL, 4 mL/mmol) was added EDCI (0.941 g, 4.91 mmol, 1.5 equiv) at 0 °C under an argon atmosphere. After being stirred at room temperature for 12 h, the reaction mixture was quenched with saturated aqueous NH_4Cl at 0 °C. The aqueous layer was extracted with ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by silica gel column chromatography (eluted with hexane/AcOEt = 1:1) to afford tetrapeptide **19** (1.99 g, 3.13 mmol, 98%) as a colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6, 80 °C) δ 7.35−7.45 (1H, br), 7.23−7.33 (5H, m), 6.95−7.15 (1H, br), 4.93−5.10 (3H, m), 4.89 (1H, q, J = 7.2 Hz), 4.30 (1H, dd, app. dt, J = 8.4 Hz), 4.09 (2H, t, J = 8.2 Hz), 3.23−3.35 (1H, m), 2.93 (3H, s), 2.85 (3H, s), 2.39 (2H, t), 2.10−2.26 (1H, m), 1.68−1.75 (1H, m), 1.40−1.52 (1H, m), 1.05−1.25 (1H, m), 1.13 (3H, d, J = 7.2 Hz), 0.92 (2H, t, J = 8.4 Hz), 0.83 (3H, d, J = 6.8 Hz), 0.79 (3H, t, J = 7.6 Hz), 0.78 (3H, d, J = 6.8 Hz), 0.68 (3H, d, J = 6.8 Hz), 0.00 (9H, s); ^{13}C NMR (100 MHz, DMSO-d_6, mixture of rotamers) δ 172.4, 171.3, 170.3, 169.6, 156.2, 137.2, 128.3, 127.7, 127.5, 79.2, 65.3, 61.9, 57.4, 54.9, 51.8, 35.6, 34.9, 33.8, 30.4, 30.0, 26.6, 24.2, 19.5, 17.9, 16.8, 15.4, 14.7, 14.3, 10.6, −1.51; IR ($CHCl_3$) 3314, 2961, 2876, 1720, 1685, 1629, 1527, 1467, 1455, 1408, 1388, 1293, 1250, 1226, 1173, 1038, 860, 838 cm^{-1}; $[\alpha]_D^{28}$ −100 (c 1.01, $CHCl_3$); HRFABMS calcd for $C_{32}H_{55}N_4O_7Si$ $[M + H]^+$ 635.3840, found 635.3810; HPLC retention time 9.25 min, purity >97%.

*TBS-HA-Pro-Ile-MeVal-MeAla-β-Ala-OTMSEt (**20**).* To a solution of **19** (5.00 g, 7.88 mmol, 1 equiv) in EtOH (50 mL, 6.3 mL/mmol) was added 10% Pd/C (500 mg, 10 wt %) at room temperature under an argon atmosphere, and the flask was purged with hydrogen three times. After being stirred at room temperature for 40 h, the reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated in vacuo. The crude residue was used for the next reaction without further purification.

To a solution of the crude amine **5** (3.73 g, 7.45 mmol, 1 equiv), acid **4** (2.99 g, 7.45 mmol, 1 equiv), HOAt (1.52 g, 11.2 mmol, 1.5 equiv), and DIEA (3.89 mL, 22.4 mmol, 3 equiv) in dry DMF−CH_2Cl_2 (1:1, 30 mL, 4 mL/mmol) was added EDCI (2.15 g, 11.2 mmol, 1.5 equiv) at 0 °C under an argon atmosphere. After being stirred at room temperature for 12 h, the reaction mixture was quenched with saturated aqueous NH_4Cl at 0 °C. The aqueous layer was extracted with ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by silica gel column chromatography (eluted with $CHCl_3$/MeOH = 50:1) to afford hexapeptide **20** (5.71 g, 6.46 mmol, 87%) as a colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6, 80 °C) δ 7.75−7.85 (1H, br), 7.43 (1H, br t, J = 5.2 Hz), 5.09 (1H, d, J = 10.4 Hz), 4.91 (1H, q, J = 7.2 Hz), 4.52−4.62 (1H, m), 4.30−4.50 (2H, m), 4.12 (2H, t, J = 8.4 Hz), 4.00−4.15 (1H, m), 3.97 (1H, dd, app. dt, J = 7.0 Hz), 3.40−3.75 (3H, m), 3.25−3.35 (2H, m), 2.94 (3H, s), 2.88 (3H, s), 2.42 (2H, t, J = 6.6 Hz), 2.15−2.30 (1H, m), 1.65−2.00 (7H, m), 1.50−1.58 (1H, m), 1.31 (3H, s), 1.24 (3H, s), 1.16 (3H, d, J = 7.2 Hz), 1.05−1.15 (1H, m), 0.95 (2H, t, J = 8.4 Hz), 0.86 (9H, s), 0.75−0.90 (9H, m), 0.72 (3H, d, J = 6.4 Hz), 0.04 (3H, s), 0.038 (3H, s), 0.03 (9H, s); ^{13}C NMR (100 MHz, DMSO-d_6, mixture of rotamers) δ 172.0, 171.4, 171.3, 170.4, 169.6, 169.5, 107.6, 79.2, 72.5, 72.4, 70.1, 68.9, 68.7, 61.9, 59.2, 57.5, 55.0, 52.5, 51.9, 46.3, 38.0, 35.9, 34.9, 33.8, 30.4, 29.9, 28.6, 26.83, 26.80, 26.6, 25.7, 25.57, 25.55, 24.4, 23.8, 19.6, 17.9, 17.7, 16.8, 14.9, 14.3, 10.7, −1.52, −4.78, −5.49; IR ($CHCl_3$) 3320, 2958, 2931, 2877, 2858, 1733, 1683, 1634, 1538, 1471, 1463, 1446, 1410, 1379, 1369, 1314, 1251, 1174, 1118, 1098, 860, 838 cm^{-1}; $[\alpha]_D^{27}$ −101 (c 1.00, $CHCl_3$); HRFABMS calcd for $C_{43}H_{82}O_{10}N_5Si_2$ $[M + H]^+$ 884.5595, found 884.5569; HPLC retention time 10.23 min, purity >98%.

*Cyclization Precursor **3**.* To a solution of **20** (5.71 g, 6.46 mmol, 1 equiv) in dry THF (32 mL, 5 mL/mmol) was slowly added TBAF (1.0 M solution in THF, 19.4 mL, 19.4 mmol, 3 equiv) at 0 °C under an argon atmosphere. After the reaction mixture was stirred at room temperature for 9 h, TBAF (1.0 M solution in THF, 6.5 mL, 6.5 mmol, 1 equiv) was added at 0 °C. After the resulting mixture was stirred at room temperature for 12 h, DOWEX 50WX8-400 (7.0 g) was added at 0 °C, and the reaction mixture was filtered through a pad of Celite. The filtrate was concentrated in vacuo, and the resulting residue was purified by silica gel column chromatography (eluted with $CHCl_3$/MeOH = 30:1) to afford cyclization precursor **3** (4.2 g, 6.40 mmol, 94%) as a colorless oil. ^{1}H NMR (600 MHz, DMSO-d_6, 80 °C) δ 7.75−7.90 (1H, br), 7.35−7.45 (1H, br), 5.10 (1H, d, J = 9.6 Hz), 4.93 (1H, q, J = 6.6 Hz), 4.48−4.58 (1H, m), 4.35−4.45 (1H, m), 4.18−4.30 (1H, m), 4.02−4.13 (1H, m), 3.88−4.00 (1H, m), 3.35−3.70 (3H, m), 3.20−3.35 (2H, m), 2.95 (3H, s), 2.88 (3H, s), 2.33−2.41 (2H, m), 2.18−2.40 (1H, m), 1.60−2.00 (7H, m), 1.50−1.60 (1H, m), 1.31 (3H, s), 1.24 (3H, s), 1.16 (3H, d, J = 7.2 Hz), 1.05−1.15 (1H, m), 0.75−0.90 (9H, m), 0.72 (3H, d, J = 6.0 Hz); ^{13}C NMR (100

dx.doi.org/10.1021/jo402437z | J. Org. Chem. 2014, 79, 296−306

MHz, DMSO-d_6, mixture of rotamers) δ 172.9, 172.4, 172.2, 171.9, 171.4, 171.0, 170.8, 170.3, 169.58, 169.55, 169.1, 107.6, 107.4, 79.1, 72.7, 72.6, 68.7, 68.5, 66.8, 66.7, 59.0, 58.5, 57.4, 57.4, 56.8, 54.9, 53.1, 52.9, 51.8, 51.8, 46.6, 46.3, 37.6, 37.3, 35.9, 35.7, 35.2, 34.9, 33.7, 33.6, 31.7, 30.4, 30.1, 29.94, 29.90, 28.96, 28.93, 28.8, 27.0, 26.79, 26.77, 26.60, 26.56, 25.7, 25.6, 24.3, 24.2, 24.12, 24.07, 24.04, 21.4, 19.5, 19.4, 17.7, 15.4, 15.0, 14.86, 14.68, 14.3, 10.9, 10.7; IR (neat) 3311, 2966, 2938, 2877, 1725, 1624, 1540, 1457, 1409, 1380, 1241, 1221, 1159, 1098, 1065, 754 cm^{-1}; $[\alpha]_D^{27}$ −158 (c 0.80, $CHCl_3$); HRFABMS calcd for $C_{32}H_{55}N_5O_{10}Na$ $[M + Na]^+$ 692.3841, found 692.3808; HPLC retention time 7.18 min, purity 94%.

Macrolactone ***2***. To a solution of cyclization precursor **3** (4.20 g, 6.40 mmol, 1 equiv) and DMAPO (1.77 g, 12.8 mmol, 2 equiv) in dry CH_2Cl_2 (1060 mL, 166 mL/mmol) was added MNBA (6.61 g, 19.2 mmol, 3 equiv) at room temperature under an argon atmosphere. After the reaction mixture was stirred at 30 °C for 48 h, saturated aqueous $NaHCO_3$ was added at 0 °C, and the aqueous layer was extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by silica gel column chromatography (eluted with $CHCl_3$/MeOH = 30:1) to afford macrolactone **2** (3.32 g, 5.21 mmol, 81%) as a colorless amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (1H, d, J = 8.0 Hz), 7.15 (1H, d, J = 9.2 Hz), 5.15 (1H, q, J = 6.8 Hz), 5.06 (1H, dd, J = 5.8, 7.6 Hz), 4.97 (1H, d, J = 11.2 Hz), 4.90 (1H, dd, J = 6.8, 9.0 Hz), 4.69 (1H, d, J = 6.8 Hz), 4.00−4.15 (3H, m), 3.86−3.94 (1H, m), 3.73−3.80 (1H, m), 3.62 (1H, dd, app. dt, J = 6.8 Hz), 3.23 (3H, s), 3.08 (1H, dd, J = 11.6, 13.4 Hz), 2.72 (3H, s), 2.45−2.75 (3H, m), 2.26−2.39 (1H, m), 1.83−2.20 (6H, m), 1.24−1.46 (2H, m), 1.49 (3H, s), 1.33 (3H, s), 1.29 (3H, d, J = 6.8 Hz), 0.93 (3H, d, J = 6.8 Hz), 0.89 (3H, d, J = 6.8 Hz), 0.87 (3H, d, J = 7.2 Hz), 0.86 (3H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 173.4, 171.0, 170.8, 169.6, 168.8, 109.2, 71.5, 70.8, 69.1, 60.8, 58.0, 55.5, 53.5, 46.4, 37.4, 34.5, 34.4, 33.2, 30.8, 29.2, 28.1, 27.2, 26.8, 25.5, 24.3, 23.9, 20.0, 19.6, 15.4, 15.1, 11.3; IR ($CHCl_3$) 3853, 3744, 3385, 3308, 2965, 2928, 2879, 2360, 2341, 1733, 1683, 1669, 1653, 1635, 1628, 1539, 1521, 1441, 1381, 1183, 1101 cm^{-1}; $[\alpha]_D^{28}$ −199 (c 1.00, $CHCl_3$); HRESIMS calcd for $C_{32}H_{53}N_5O_9Na$ $[M + Na]^+$ 674.3735, found 674.3714.

*Cbz-Ile-MeVal-MeAla-β-Ala-OH (****23****)*. To a solution of ester **19** (0.100 g, 0.158 mmol, 1 equiv) in dry THF (1.2 mL, 7.6 mL/mmol) was slowly added TBAF (0.237 mL, 0.237 mmol, 1.5 equiv) at 0 °C under an argon atmosphere. After the reaction mixture was stirred at room temperature for 9 h, DOWEX 50WX8-400 (0.200 g) was added at 0 °C, and the resulting mixture was filtered through a pad of Celite. The filtrate was concentrated in vacuo, and the resulting residue was purified by silica gel column chromatography (eluted with $CHCl_3$/MeOH = 30:1) to afford carboxylic acid **23** (0.0779 g, 0.146 mmol, 92%) as a colorless oil. ^{1}H NMR (600 MHz, DMSO-d_6, 80 °C) δ 7.35−7.45 (1H, br), 7.20−7.35 (5H, m), 7.00−7.25 (1H, br), 5.10 (1H, d, J = 10.2 Hz), 4.95−5.18 (2H, m), 4.92 (1H, q, J = 6.6 Hz), 4.33 (1H, dd, app. dt, J = 8.7 Hz), 3.20−3.35 (2H, m), 2.96 (3H, s), 2.88 (3H, s), 2.37 (2H, t, J = 6.6 Hz), 2.15−2.26 (1H, m), 1.74−1.82 (1H, m), 1.45−1.54 (1H, m), 1.15 (3H, d, J = 6.6 Hz), 1.10−1.20 (1H, m), 0.86 (3H, d, J = 6.6 Hz), 0.82 (3H, d, J = 7.8 Hz), 0.80 (3H, t, J = 7.8 Hz), 0.71 (3H, d, J = 6.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9, 172.4, 170.3, 169.7, 156.2, 137.1, 128.3, 127.7, 127.5, 65.3, 57.4, 54.9, 51.8, 35.6, 34.9, 33.7, 30.4, 30.0, 26.6, 24.2, 19.4, 17.9, 14.7, 14.3, 10.6; IR ($CHCl_3$) 3310, 2965, 2938, 2876, 1716, 1681, 1628, 1530, 1456, 1408, 1344, 1293, 1251, 1123, 1099, 1027, 755, 698 cm^{-1}; $[\alpha]_D^{25}$ −152 (c 1.04, $CHCl_3$); HRESIMS calcd for $C_{27}H_{42}N_4O_7Na$ $[M + Na]^+$ 557.2946, found 557.2927; HPLC retention time 8.05 min, purity >98%.

Alcohol ***24***. To a solution of TBS ether **13a** (0.500 g, 1.02 mmol, 1 equiv) in THF (4.0 mL, 3.9 mL/mmol) was added HF−pyridine (~70% HF, 1.0 mL) at 0 °C under an argon atmosphere. After being stirred at same temperature for 6 h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ at 0 °C. The aqueous layer was extracted with ethyl acetate, and the organic layer was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by silica gel column chromatography (eluted with hexane/AcOEt = 1:1) to afford alcohol **24** (0.281 g, 0.745 mmol, 73%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30−7.45 (5H, m), 5.18 (2H, d, J = 11.2 Hz), 4.53 (1H, dd, J = 2.8, 8.8 Hz), 4.41 (1H, dd, J = 6.4, 10.8 Hz), 4.18−4.25 (1H, m), 4.10 (1H, dd, J = 5.8, 8.2 Hz), 3.70 (1H, dd, J = 7.2, 8.2 Hz), 3.60−3.70 (1H, m), 3.58 (1H, d, J = 6.4 Hz), 1.80−2.25 (6H, m), 1.39 (3H, s), 1.34 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 171.6, 135.6, 128.5, 128.3, 128.1, 108.6, 72.7, 69.2, 67.2, 66.9, 59.6, 46.5, 37.5, 28.8, 26.8, 25.6, 24.6; IR (neat) 3422, 2983, 2956, 2935, 2880, 1744, 1645, 1456, 1380, 1212, 1170, 1051 cm^{-1}; mp 105−106 °C; $[\alpha]_D^{27}$ −54.5 (c 1.03, $CHCl_3$); HRESIMS calcd for $C_{20}H_{27}NO_6Na$ $[M + Na]^+$ 400.1731, found 400.1717.

*Cbz-Ile-MeVal-MeAla-β-Ala-HA-Pro-OBn (****25****)*. To a solution of acid **23** (20.7 mg, 0.0346 mmol, 1 equiv) in CH_2Cl_2/DMF (1:1, 1 mL, 23 mL/mmol) were added alcohol **24** (17.0 mg, 0.0450 mmol, 1.3 equiv), DMAP (1.3 mg, 0.0104 mmol, 0.3 equiv), and EDCI (9.9 mg, 0.0519 mmol, 1.5 equiv) at 0 °C under an argon atmosphere. After being stirred at room temperature for 12 h, the reaction mixture was quenched with saturated aqueous NH_4Cl, and the aqueous layer was extracted with ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by silica gel column chromatography (eluted with hexane/AcOEt = 1:3) to afford amide **25** (28.8 mg, 0.0322 mmol, 73%) as an amorphous solid. ^{1}H NMR (600 MHz, DMSO-d_6, mixture of rotamers) δ 7.60−7.85 (2H, m), 7.31−7.50 (10H, m), 4.80−5.42 (7H, m), 3.80−4.45 (4H, m), 3.60−3.75 (2H, m), 3.30−3.65 (2H, m), 2.80−3.14 (6H, m), 2.50−2.65 (2H, m), 1.70−2.30 (8H, m), 1.10−1.60 (11H, m), 0.60−1.00 (12H, m); ^{13}C NMR (100 MHz, DMSO-d_6, mixture of rotamers) δ 172.4, 171.2, 170.4, 170.2, 169.7, 166.99, 166.95, 156.2, 137.2, 136.0, 128.5, 128.4, 128.3, 127.9, 127.7, 127.6, 127.5, 108.1, 108.1, 79.2, 72.0, 69.0, 68.5, 68.1, 66.7, 65.7, 65.3, 59.7, 59.1, 58.9, 58.7, 57.4, 54.9, 51.8, 46.3, 35.6, 34.7, 34.2, 33.5, 30.5, 30.0, 28.4, 26.71, 26.66, 26.62, 25.5, 24.4, 24.1, 20.7, 19.4, 17.9, 14.7, 14.31, 14.27, 14.1, 10.6, 10.5; IR (neat) 3309, 2963, 2936, 2877, 1740, 1721, 1639, 1527, 1455, 1407, 1380, 1341, 1249, 1226, 1170, 1095, 1040, 751 cm^{-1}; $[\alpha]_D^{27}$ −120 (c 1.00, $CHCl_3$); HRESIMS calcd for $C_{47}H_{67}N_5O_{12}Na$ $[M + Na]^+$ 916.4678, found 916.4650; HPLC retention time 8.95 min, purity 97%.

Dimer ***22***. To a solution of benzyl ester **25** (20.0 mg, 0.0224 mmol, 1 equiv) in AcOEt/EtOH (1:1, 1.0 mL, 45 mL/mmol) was added 20% $Pd(OH)_2$/C (3.1 mg, 4.48 μmol, 0.2 equiv) under an argon atmosphere, and the flask was purged with hydrogen three times. After being stirred at room temperature for 2 h, the reaction mixture was filtered through a pad of Celite. The filtrate was concentrared in vacuo, and the resulting residue was used without further purification.

To a solution of amino acid **21** (10.5 mg, 0.0157 mmol, 1 equiv) in CH_2Cl_2 (15.7 mL, 1 mM) were added DIEA (13.7 μL, 0.0785 mmol, 5.0 equiv), HOAt (8.5 mg, 0.0628 mmol, 4.0 equiv), and EDCI (9.0 mg, 0.0471 mmol, 3 equiv) at 0 °C under an argon atmosphere. After being stirred at room temperature for 12 h, the reaction mixture was quenched with saturated aqueous NH_4Cl, and the aqueous layer was extracted with $CHCl_3$. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by reversed-phase HPLC (eluted with CH_3CN/H_2O) to afford macrolactone **2** (1.4 mg, 0.00215 mmol, 14%) and dimer **22** (1.3 mg, 0.997 μmol, 6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00−7.80 (4H, m), 1.80−5.50 (54H, m), 0.70−1.55 (46H, m); ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers) δ 173.4, 170.85, 170.83, 170.2, 168.7, 109.3, 71.5, 70.7, 69.2, 60.4, 57.3, 56.0, 53.6, 46.5, 36.4, 36.0, 34.5, 33.7, 31.9, 31.0, 29.7, 29.5, 29.4, 28.9, 27.8, 26.9, 25.6, 25.2, 23.9, 22.7, 19.7, 18.7, 15.1, 14.9, 14.1, 10.5; HRESIMS calcd for $C_{64}H_{106}N_{10}O_{18}Na$ $[M + Na]^+$ 1325.7579, found 1325.7548; HPLC retention time 8.65 min, purity >98%.

Diol ***26***. To a solution of macrolactone **2** (1.33 g, 2.04 mmol, 1 equiv) in 1,4-dioxane (6.7 mL, 3.3 mL/mmol) was slowly added 1.5 M HCl(aq) (13.3 mL, 6.5 mmol) at 0 °C. After the reaction mixture was stirred at 0 °C for 2 h, saturated aqueous $NaHCO_3$ was added, and the aqueous layer was extracted with ethyl acetate. The organic layer was

dx.doi.org/10.1021/jo402437z | J. Org. Chem. 2014, 79, 296−306

washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by silica gel column chromatography (eluted with $CHCl_3$/MeOH = 30:1) to afford diol **26** (1.08 g, 1.77 mmol, 87%) as a colorless amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (1H, d, J = 10.4 Hz), 7.13 (1H, d, J = 9.2 Hz), 5.19 (1H, q, J = 6.8 Hz), 5.12 (1H, dd, J = 5.2, 6.4 Hz), 4.98 (1H, d, J = 11.2 Hz), 4.89 (1H, dd, J = 6.4, 9.2 Hz), 4.68 (1H, d, J = 6.0 Hz), 3.96−4.10 (2H, m), 3.85−3.95 (1H, m), 3.63−3.73 (2H, m), 3.45−3.55 (1H, m), 3.23 (3H, s), 3.05−3.15 (1H, m), 2.73 (3H, s), 2.45−2.70 (3H, m), 2.28−2.38 (1H, m), 1.85−2.10 (5H, m), 1.25−1.45 (2H, m), 1.31 (3H, d, J = 6.8 Hz), 0.93 (3H, d, J = 6.8 Hz), 0.89 (3H, d, J = 6.8 Hz), 0.86 (3H, d, J = 6.8 Hz), 0.86 (3H, t, J = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 173.5, 171.1, 171.0, 169.9, 70.8, 67.7, 66.4, 60.9, 58.1, 55.5, 53.7, 46.7, 37.3, 34.5, 33.9, 33.3, 30.8, 29.3, 28.1, 27.2, 24.4, 23.9, 19.9, 19.5, 15.4, 15.1, 11.3; IR ($CHCl_3$) 3345, 3301, 2961, 2886, 2360, 1734, 1720, 1652, 1634, 1630, 1622, 1538, 1455, 1414, 1262, 1179, 1025 cm^{-1}; $[\alpha]_D^{28}$ −230.4 (c 1.00, $CHCl_3$); HRESIMS calcd for $C_{29}H_{49}N_5O_9Na$ $[M + Na]^+$ 634.3422, found 634.3397.

Tosylate 27. To a solution of diol **26** (1.08 g, 1.77 mmol, 1 equiv), triethylamine (0.371 mL, 2.12 mmol, 1.5 equiv), and DMAP (21.6 mg, 0.177 mmol, 0.1 equiv) in dry CH_2Cl_2 (18 mL, 10 mL/mmol) was added *p*-toluenesulfonyl chloride (0.405 g, 2.12 mmol, 1.2 equiv) at 0 °C under an argon atmosphere. After the reaction mixture was stirred at room temperature for 6 h, additional triethylamine (0.246 mL, 1.77 mmol, 1.0 equiv) and *p*-toluenesulfonyl chloride (0.270 g, 1.416 mmol, 0.8 equiv) were added at 0 °C. After the reaction mixture was stirred at room temperature for 6 h, saturated aqueous NH_4Cl was added, and the aqueous layer was extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by silica gel flash column chromatography (eluted with $CHCl_3$/MeOH = 30:1) to afford tosylate **27** (1.10 g, 1.44 mmol, 81%) as a colorless amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (1H, d, J = 8.4 Hz), 7.79 (2H, d, J = 8.2 Hz), 7.37 (2H, d, J = 8.2 Hz), 7.09 (1H, d, J = 9.2 Hz), 5.16 (1H, q, J = 6.8 Hz), 5.10 (1H, dd, app. dt, J = 5.8 Hz), 4.96 (1H, d, J = 11.2 Hz), 4.89 (1H, dd, J = 6.2, 9.4 Hz), 4.65 (1H, d, J = 6.8 Hz), 4.10−4.16 (1H, m), 3.95−4.09 (3H, m), 3.87−3.94 (1H, m), 3.57−3.67 (1H, m), 3.22 (3H, s), 3.00−3.10 (1H, m), 2.72 (3H, s), 2.48−2.68 (2H, m), 2.47 (3H, s), 2.28−2.38 (1H, m), 1.85−2.15 (5H, m), 1.23−1.48 (2H, m), 1.30 (3H, d, J = 6.8 Hz), 1.28−1.33 (1H, m), 0.92 (3H, d, J = 6.8 Hz), 0.88 (3H, d, J = 6.8 Hz), 0.86 (3H, d, J = 7.2 Hz), 0.85 (3H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 173.3, 171.0, 170.7, 169.7, 169.5, 145.3, 132.3, 130.0, 127.9, 73.2, 70.0, 65.2, 60.9, 58.0, 55.4, 53.6, 46.7, 37.3, 34.4, 33.6, 33.1, 30.8, 29.1, 28.1, 27.2, 24.3, 23.9, 21.6, 19.9, 19.5, 15.4, 15.1, 11.3; IR ($CHCl_3$) 3387, 3301, 2962, 2922, 2852, 1733, 1673, 1632, 1630, 1627, 1622, 1520, 1464, 1362, 1265, 1177, 1098 cm^{-1}; $[\alpha]_D^{28}$ −202 (c 1.00, $CHCl_3$); HRESIMS calcd for $C_{36}H_{55}N_5O_{11}SNa$ $[M + Na]^+$ 788.3511, found 788.3485.

Destruxin E (1). To a solution of tosylate **27** (1.10 g, 1.44 mmol, 1 equiv) in *i*-PrOH/$(CH_2Cl)_2$ (10:1, 15 mL, 10 mL/mmol) was added K_2CO_3 (0.793 g, 5.74 mmol, 4 equiv) at 0 °C under an argon atmosphere. After the reaction mixture was stirred at 50 °C for 4 h, saturated aqueous NH_4Cl was added at 0 °C, and the aqueous layer was extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the resulting residue was purified by silica gel flash column chromatography twice (eluted with $CHCl_3$/MeOH = 50:1, ethyl acetate/MeOH = 20:1) to afford destruxin E (**1**) (0.547 g, 0.921 mmol, 64%) as a colorless amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (1H, d, J = 10.0 Hz), 7.16 (1H, d, J = 9.2 Hz), 5.16 (1H, q, J = 6.8 Hz), 4.99 (1H, dd, app. dt, J = 7.2 Hz), 4.97 (1H, d, J = 10.8 Hz), 4.89 (1H, dd, J = 6.8, 9.2 Hz), 4.70 (1H, d, J = 7.2 Hz), 4.00−4.10 (1H, m), 3.92−3.99 (1H, m), 3.23 (3H, s), 3.08 (1H, br t, J = 12.0 Hz), 2.95−3.00 (1H, m), 2.83 (1H, dd, app. dt, J = 4.4 Hz), 2.72 (3H, s), 2.62−2.72 (1H, m), 2.45−2.59 (3H, m), 2.24−2.40 (2H, m), 2.02−2.12 (1H, m), 1.85−2.01 (4H, m), 1.38−1.46 (1H, m), 1.31 (3H, d, J = 6.8 Hz), 1.28−1.33 (1H, m), 0.93 (3H, d, J = 6.8 Hz), 0.89 (3H, d, J = 6.8 Hz), 0.87 (3H, d, J = 7.2 Hz), 0.86 (3H, t, J = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5 173.4, 171.0, 170.8, 169.7, 168.6, 70.6, 60.9, 58.0, 55.5, 53.6, 47.8, 47.1, 46.6, 37.5, 34.5, 33.6, 33.2, 30.8, 29.2, 28.1, 27.2, 24.4, 24.0, 20.0, 19.6, 15.4, 15.2, 11.4; IR ($CHCl_3$) 3385, 3298, 2960, 2923, 2851, 1731, 1673, 1670, 1636, 1631, 1622, 1519, 1445, 1413, 1378, 1278, 1178, 1100 cm^{-1}; $[\alpha]_D^{28}$ −247 (c 1.00, $CHCl_3$) {lit[4b] $[\alpha]_D^{25}$ −253 (c 1.00, $CHCl_3$)}; HRESIMS calcd for $C_{29}H_{47}N_5O_8Na$ $[M + Na]^+$ 616.3317, found 616.3297.

■ ASSOCIATED CONTENT

Supporting Information

Copies of ^{1}H and ^{13}C NMR spectra for **1**−**4**, **7**, **8**, **13a**, **15**, **17**−**20**, **22**−**27**; copy of the ^{1}H spectrum for **21**; and HPLC chromatograms for **1**, **3**, **15**, **17**−**23**, and **25**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by a Grant-in-Aid for Young Scientists (B) (M.Y.) from JSPS and the Japan Science and Technology Agency (A-STEP, AS242Z00980Q). We thank Mr. Hisayuki Takeuchi for initial investigations on the synthesis of destruxin E.

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