The total synthesis of destruxin E (1) has been achieved for the first time, and the stereochemistry of its chiral center at the epoxide has been determined to be (S). The cyclization precursor 3a was synthesized by solid-phase peptide synthesis. Macrolactonization of 3a utilizing MNBA-DMAPO, followed by formation of the epoxide, then furnished destruxin E. Its diastereomer, epi-destruxin E (2), was also synthesized in the same manner. Furthermore, the biological evaluation indicated that destruxin E exhibits V-ATPase inhibitory activity 10-fold greater than that of epi-destruxin E.

Destruxin E (1) is a 19-membered cyclic depsipeptide, consisting of five amino acids (proline, isoleucine, NMe-valine, NMe-alanine, and \( \beta \)-alanine) and epoxide-containing \( \alpha \)-hydroxy acid. It was first isolated from a culture medium of the strain of \textit{Metarhizium anisopliae} by Païs et al. in 1981.\(^1\) To date, more than 35 destruxin derivatives have been found from cultures such as \textit{Oospora destructor},\(^2\) \textit{Alternaria brassica},\(^3\) \textit{Nigrosabulum globosum},\(^4\) \textit{Beauveria felina},\(^5\) and \textit{Metarhizium anisopliae}.

Among the destruxins, destruxin E (1) has been shown to strongly inhibit V-ATPase (IC\(_{50}\) 0.4 \( \mu \)M)\(^7\) that is localized on the membranes enclosing the organelles such as the lysosomes and function to acidify a wide array of intracellular compartments.\(^8\) V-ATPases have also an important role for the control of tumor microenvironment by proton extrusion, and therefore V-ATPase inhibitors can be a drug candidate for the cancer therapy.\(^9\) Moreover, destruxin E (1) can reversibly induce morphologi-
cal changes in osteoclast-like multinucleated cells (OCLs) in a dose-dependent manner (0.01–0.05 µM). It has recently been reported that 1 can also reduce β-amyloid generation without affecting β-amyloid-cleaving enzyme (BACE) or PS/γ-secretase activity similar to how bafilomycin does. Several groups have previously reported the (BACE) or PS/β-secretase activity similar to how bafilomycin does. Several groups have previously reported the generation without affecting absolute stereochemistry of the epoxide and evaluate the two possible diastereomers of destruxin E in order to determine the absolute stereochemistry of the epoxide and evaluate the biological activity of destruxin E.

Our synthetic strategy is illustrated in Scheme 1. Destruxin E (1) and epi-destruxin E (2) can be synthesized from cyclization precursor 3 through macrolactonization, followed by formation of the epoxide in the side chain. The cyclization precursor 3 would be obtained by sequential benzoyloxyacetyl chloride, followed by removal of the benzyl group and protection of the resulting alcohol with a TBS group provided 6 in 98% yield. The Evans asymmetric alkylation of 5 proceeded smoothly at −30 °C (LiHMDS/allyl bromide) to afford allylated product 8 with high diastereoselectivity (81%, dr >98:2). Dihydroxylation of 8 with OsO4/NMO induced concomitant formation of γ-lactones, which were separated by silica gel column chromatography to afford the two desired diastereomers 5a (44%) and 5b (42%). Acid 9a was formed by hydrolysis of the

**Scheme 1. Retrosynthetic Analysis of Destruxin E (1)**

Our synthetic strategy is illustrated in Scheme 1. Destruxin E (1) and epi-destruxin E (2) can be synthesized from cyclization precursor 3 or 4 through macrolactonization, followed by formation of the epoxide in the side chain. The cyclization precursor 3 would be obtained by sequential benzoyloxyacetyl chloride, followed by removal of the benzyl group and protection of the resulting alcohol with a TBS group provided 6 in 98% yield. The Evans asymmetric alkylation of 5 proceeded smoothly at −30 °C (LiHMDS/allyl bromide) to afford allylated product 8 with high diastereoselectivity (81%, dr >98:2). Dihydroxylation of 8 with OsO4/NMO induced concomitant formation of γ-lactones, which were separated by silica gel column chromatography to afford the two desired diastereomers 5a (44%) and 5b (42%). Acid 9a was formed by hydrolysis of the
lactone 5a with LiOH·H₂O, followed by protection of the resulting diols as an acetone. Owing to the instability of 9 under acidic and basic conditions, the acid 9a was immediately utilized in the next step without further purification. The amidation of 9a with L-proline benzyl ester was performed using PyBroP²¹/DIEA to afford 10a in 70% yield. Finally, removal of the benzyl ester at the C-terminus of 10a provided the desired acid 4a in quantitative yield. According to the synthesis of 4a, 4b was also prepared from lactone 5b.

With the desired 4a and 4b in hand, the cyclization precursors 3a and 3b were prepared by solid-phase peptide synthesis on SynPhase lanterns¹⁹ (Scheme 3). Attachment of Fmoc-βAla-OH onto trityl alcohol lantern 11²⁰ provided polymer-supported 12. The quantitative immobilization of the Fmoc-βAla-OH was observed by gravimetric analysis after acidic cleavage from the polymer support (30% hexafluoroisopropyl alcohol (HFIP)/CH₂Cl₂, rt, 1 h).²¹ After removal of the Fmoc group with 20% piperidine/DMF, acylation of the resulting amine with Fmoc-MeAla-OH was performed utilizing DIC/HOBt to afford dipeptide 13 with high purity, which was monitored by reversed-phase HPLC-MS analysis (UV 214 nm) after cleavage from the polymer support. Removal of the Fmoc group in 13 (20% piperidine/DMF, rt, 30 min), followed by amidation with Fmoc-MeVal-OH utilizing PyBroP/DIEA provided tripeptide 14 (purity 99%, UV 214 nm). We next attempted the synthesis of tetrapeptide 15. The coupling reaction of 14 with Fmoc-Ile-OH was, however, not complete due to the difficulty of the coupling between sterically hindered N-Me valine and isoleucine. Therefore, we repeated the reaction under the same conditions to obtain the tetrapeptide 15 with high conversion (purity 92%, UV 214 nm). After removal of the Fmoc group of 15, coupling with acids 4a and 4b using PyBroP/DIEA in parallel, followed by removal of the TBS group utilizing TBAF, and cleavage from the polymer support under weakly acidic conditions yielded the desired cyclization precursors 3a and 3b, respectively.²² Macrolactonization of 3a was performed using 2-methyl-6-nitrobenzoic anhydride (MNBA)/4-(dimethylamino)pyridine N-oxide (DMAPO)²³ under high dilution conditions (3 mM) for 3 d to afford the desired macrocycle 16a in 65% yield; 16b was also synthesized in the same manner.

After synthesizing macrocycles 16a and 16b, the epoxide was formed, leading to the synthesis of destruxin E (1). Removal of the acetonide in 16a was performed with 3 M HCl aq/1,4-dioxane at 10 °C to yield the diol 17a (73%). Regioselective tosylation of the primary alcohol was carefully achieved with TsCl/NET₃/DMAP at room temperature to provide desired tosylate 18a (87%). Initial attempt for the formation of epoxide using K₂CO₃ in MeOH resulted in the ring opening of the macrocycle. Therefore, we used less nucleophilic isopropyl alcohol instead of MeOH. Finally, formation of the epoxide with K₂CO₃ in isopropyl alcohol/CH₂Cl₂ afforded destruxin E (1). Spectral data, including optical rotation, of synthetic destruxin E (1) are in good agreement with those of the natural product, destruxin E.¹,²⁴ On the other hand, the ¹H NMR spectrum of epi-destruxin E (2) synthesized from macrocycle 16b was found to be distinguishable from that of natural and synthetic 1. Specifically, the chemical shift of the α-proton of the α-hydroxy acid in ¹H NMR of 2 was found to be δ 5.07 ppm, which is obviously different from those of natural destruxin E (δ 4.91 ppm) and synthetic 1 (δ 4.99 ppm). Further drastic differences between natural destruxin E and epi-2 were observed in C1α (+0.5 ppm), C1β (+0.6 ppm), C1γ (+0.7 ppm), and C1δ (+0.8 ppm) positions, though the corresponding chemical shifts of synthetic 1 were identical to those of the natural product (see Supporting Information).


(20) Trityl alcohol lantern was purchased from Mimotopes Pty Ltd., Clayton, Vic 3168, Australia.


Scheme 3. Synthesis of Macrocycles 3a and 3b

of Fmoc-βAla-OH onto trityl alcohol lantern 11²⁰ provided polymer-supported 12. The quantitative immobilization of the Fmoc-βAla-OH was observed by gravimetric analysis after acidic cleavage from the polymer support (30% hexafluoroisopropyl alcohol (HFIP)/CH₂Cl₂, rt, 1 h).²¹ After removal of the Fmoc group with 20% piperidine/DMF,
Synthetic destruxin E (1) and epi-destruxin E (2) were evaluated for their V-ATPase inhibitory activity by acridine orange staining of V-ATPase-dependent acidic organelles.\(^{(25,26)}\) Accumulation of the dye was not detected when V-ATPase was inhibited. Both natural and synthetic destruxin E were found to be active at 0.63 \(\mu\)M, although epi-destruxin E was not active until 10 \(\mu\)M. Therefore, 1 exhibited V-ATPase inhibitory activity 10-fold greater than that of epi-destruxin E (2) (Table 1).

In summary, we have accomplished the first total synthesis of destruxin E (1) and its diastereomer epi-destruxin E (2) by solid-phase synthesis. On the basis of the synthesis, the stereochemistry of the chiral center at the epoxide was determined to be (S). It was found that natural destruxin E (1) exhibits V-ATPase inhibitory activity 10-fold greater than that of 2, which indicates that the stereochemistry of the epoxide is crucial for exhibiting the potent V-ATPase inhibitory activity. The library synthesis of destruxin E based on the solid-phase synthesis and the biological evaluations are underway in our laboratory.

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**Supporting Information Available:** Experimental details and NMR spectra of 1–6, 8, 10, and 16–18. This material is available free of charge via the Internet at http://pubs.acs.org.
