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Synthesis, Structure Determination, and Biological Evaluation of Destruxin E

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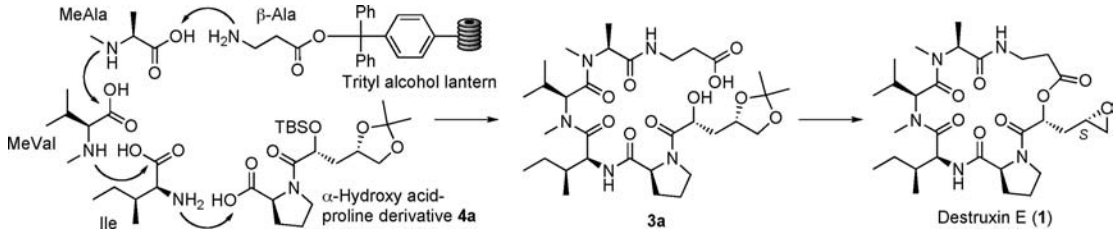
ABSTRACT



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 β-alanine) and epoxide-containing α-hydroxy acid. It was first isolated from a culture medium of the strain of *Metarhizium anisopliae* by Païs et al. in 1981.[1] To date, more than 35 destruxin derivatives have been found from cultures such as *Oospora destructor*,[2] *Alternaria brassica*,[3] *Nigrosabulum globosum*,[4] *Beauveria felina*,[5] and *Metarhizium anisopliae*.[6] Among the destruxins, destruxin E (**1**) has been shown to strongly inhibit V-ATPase (IC_{50} 0.4 μM)[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-

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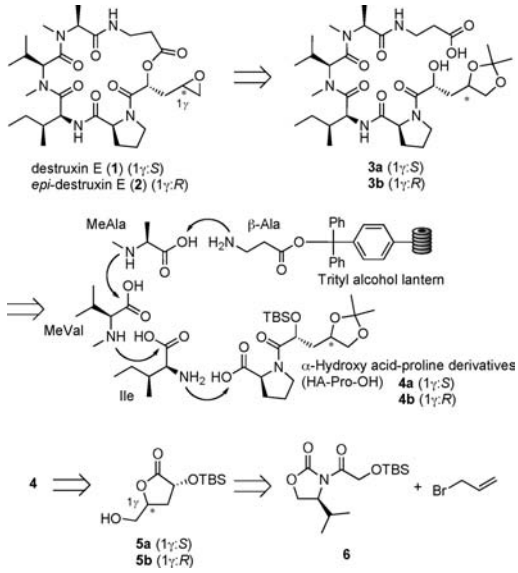
cal changes in osteoclast-like multinucleated cells (OCLs) in a dose-dependent manner (0.01−0.05 μM).[10] It has recently been reported that **1** can also reduce β-amyloid generation without affecting β-amyloid-cleaving enzyme (BACE) or PS/γ-secretase activity similar to how bafilomycin does.[11,12] Several groups have previously reported the synthesis of destruxin analogues and evaluated their biological activities.[13] These reports demonstrated that the epoxide in the side chain could be crucial for exhibiting the biological activity. The absolute stereochemistry of the epoxide, however, has not been established. To elucidate the structure−activity relationships involving destruxin E (**1**), we are interested in the total synthesis and the library synthesis of **1** via solid-phase synthesis. We thus report herein a solid-phase-assisted total synthesis of 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.

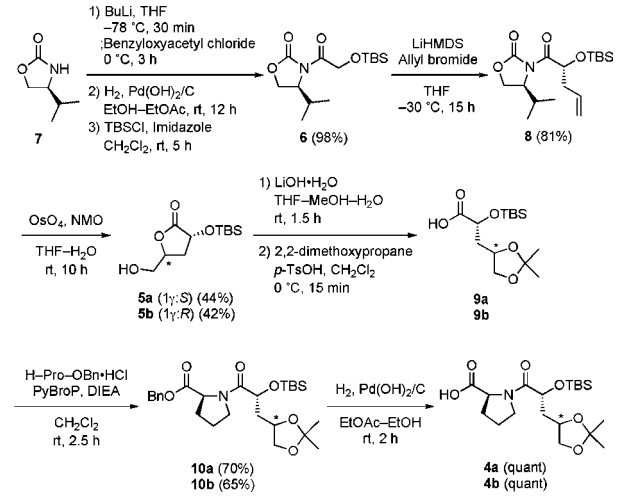
Scheme 1. Retrosynthetic Analysis of Destruxin E (**1**)



Our synthetic strategy is illustrated in Scheme 1. Destruxin E (**1**) and *epi*-destruxin E (**2**) can be synthesized from cyclization precursor **3a** or **3b** through macrolactonization,[14] followed by formation of the epoxide in the side chain. The cyclization precursor **3** would be obtained by sequential coupling of five fragments, β-alanine, NMe-alanine, NMe-valine, isoleucine, and α-hydroxy acid-proline derivative (HA-Pro-OH) **4**, by a solid-phase peptide synthesis using a trityl linker. Both diastereomers **4** will be prepared from chiral lactones **5a** and **5b** by hydrolysis of the lactones, followed by amidation with proline. The optically active lactones **5** would be provided utilizing Evans asymmetric allylation of **6**, followed by dihydroxylation and concomitant cyclization.

Initially, HA-Pro-OH **4a** and **4b** were prepared according to Scheme 2. Acylation of Evans chiral auxiliary **7** with benzyloxyacetyl 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[15] of **6** proceeded smoothly at −30 °C (LiHMDS/allyl bromide) to afford allylated product **8** with high diastereoselectivity (81%, dr >98:2). Dihydroxylation of **8** with OsO_4/NMO induced concomitant formation of γ-lactones, which were separated by silica gel column chromatography to afford the two desired diastereomers **5a**[16] (44%) and **5b**[17] (42%). Acid **9a** was formed by hydrolysis of the

Scheme 2. Synthesis of **4a** and **4b**



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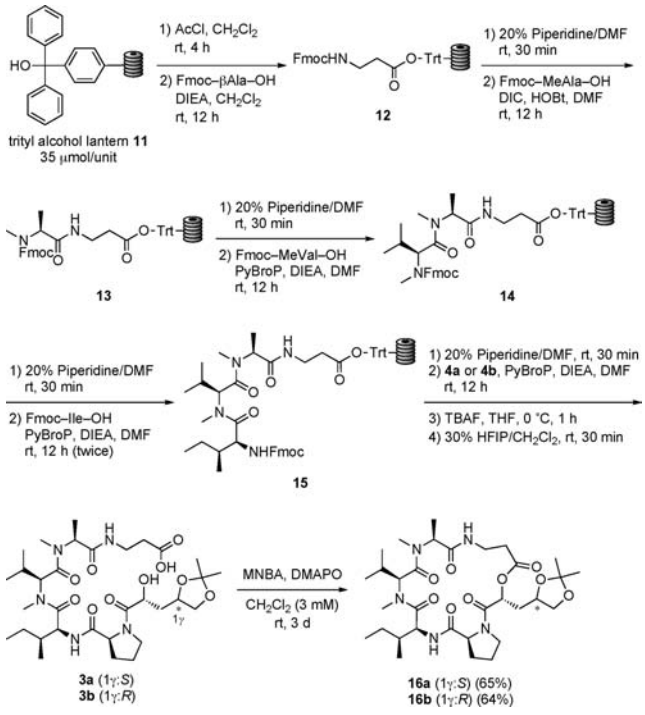
(16) **5a** was converted into reported (2*R*,4*S*)-2-hydroxy-4-hydroxymethyl-4-butanolide, whose spectral data were in good agreement with those previously reported: Uchikawa, O.; Okukado, N.; Sakata, T.; Arase, T.; Terada, K. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2025–2029.

lactone **5a** with $LiOH{\cdot}H_2O$, followed by protection of the resulting diols as an acetonide. 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 PyBroP[18]/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 lanterns[19] (Scheme 3). Attachment

Scheme 3. Synthesis of Macrocycles **3a** and **3b**



of Fmoc-βAla-OH onto trityl alcohol lantern **11**[20] 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)/CH_2Cl_2, 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 $TsCl/NEt_3$/DMAP at room temperature to provide desired tosylate **18a** (87%). Initial attempt for the formation of epoxide using K_2CO_3 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 K_2CO_3 in isopropyl alcohol/CH_2Cl_2 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).

(17) Spectral data of synthetic chiral lactone **5b** were in good agreement with those previously reported . (a) Enantiomer: Zhen, Z.-B.; Gao, J.; Wu, Y. *J. Org. Chem.* **2008**, *73*, 7310–7316. (b) Liu, S. Y.; Huang, D. F.; Huang, H. H.; Huang, L. *Chin. Chem. Lett.* **2000**, *11*, 957–960.

(18) PyBroP: bromo-tris-pyrrolidino-phosphonium hexafluorophosphate. Frérot, E.; Coste, J.; Pantaloni, A.; Dufour, M.-N.; Jouin, P. *Tetrahedron* **1991**, *47*, 259–270.

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

(21) Wade, J. D.; Bedford, J.; Sheppard, R. C.; Tregear, G. W. *Pep. Res.* **1991**, *4*, 194–199.

(22) The linear precursors **3a** and **3b** have several conformational isomers shown in ^1H NMR spectra (see Supporting Information). Therefore, we identified the compounds after macrolactonization.

(23) MNBA: 2-methyl-6-nitrobenzoic anhydride. DMAPO: 4-(dimethylamino)pyridine *N*-oxide. (a) Shiina, I.; Ibuka, R.; Kubota, M. *Chem. Lett.* **2002**, 286–287. (b) Shiina, I.; Kubota, M.; Ibuka, R. *Tetrahedron Lett.* **2002**, *43*, 7535–7539. (c) Shiina, I.; Kubota, M.; Oshiumi, H.; Hashizume, M. *J. Org. Chem.* **2004**, *69*, 1822–1830.

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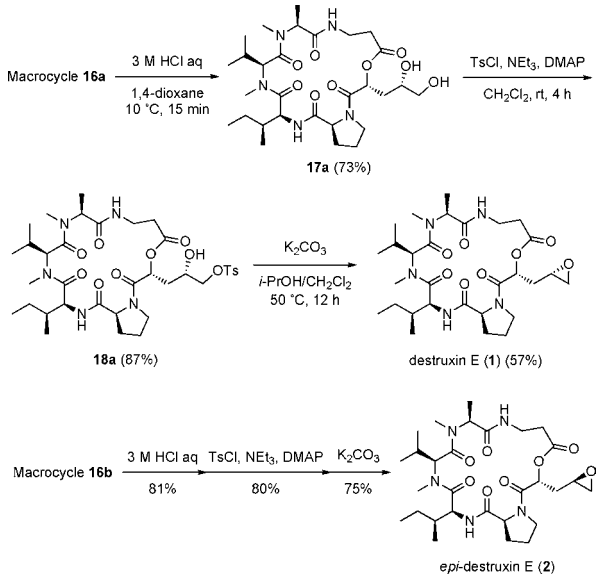
Scheme 4. Total Synthesis of Destruxin E (**1**) and *epi*-Destruxin E (**2**)



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 μM, although *epi*-destruxin E was not active until 10 μM. 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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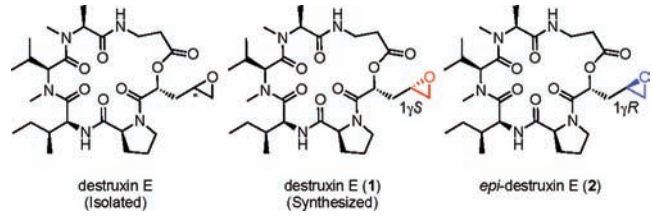
Table 1. V-ATPase Inhibitory Activity of Destruxin E (**1**) and *epi*-Destruxin E (**2**)[a]



	concentration [μM]						
compound	40	10	2.5	0.63	0.16	0.04	0
destruxin E (isolated)	N/D	−	−	−	+	+	+
destruxin E (synthesized)	−	−	−	−	+	+	+
epi-destruxin E	−	−	+	+	+	+	+

[a] : Positive (+) = V-ATPase activity detected. Negative (−) = inhibition of V-ATPase activity.

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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.

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