Review

Combinatorial Synthesis and Biological Evaluation of Destruxins

Masahito Yoshida

Faculty of Pure and Applied Sciences, University of Tsukuba;
1–1–1 Tennoudai, Tsukuba, Ibaraki 305–8571, Japan.
Received March 26, 2019

The combinatorial synthesis and biological evaluation of destruxins are described herein. First, the total synthesis of destruxin E was achieved, and its absolute configuration was successfully determined to be (*S*). In addition, the preparation of a combinatorial library based on the structure of destruxins was carried out by the split-and-pool method. Biological evaluation of the resulting analogs against osteoclast-like multinuclear cells (OCLs) revealed that the *N*-methyl-alanine residue was crucial to inducing morphological changes in OCLs. In particular, functionalization at the *β*-position of the proline (Pro) residue was found to be tolerant of the desired biological activity of destruxin E, suggesting that the *β*-position of the Pro residue should be a promising site for the introduction of a chemical tag toward the preparation of a molecular probe.

Key words　natural product; cyclic peptide; total synthesis; combinatorial library

1. Introduction

Natural products have long been a focus as promising chemical space for drug discovery.[1,2] In particular, biologically active peptides isolated from nature possess unique structural and biological properties; thus, peptidyl natural products are a potential source in the development of novel drug candidates.[3–5] On the other hand, an efficient method of synthesizing cyclic peptides should be established to achieve the rapid preparation of analogs for structure–activity relationship studies, and to provide a desired molecule on a gram scale for *in vivo* study. In this paper, we focus on the biologically active cyclodepsipeptide destruxin E, and describe its structure activity relationship study based on the total synthesis of destruxin E toward the elucidation of a mode of action.

2. Destruxin E

Destruxin E (**1**), isolated from *Metarhizium anisopliae* by Päis *et al.* in 1981, is a 19-membered cyclodepsipeptide, and consists of five amino acid residues (*β*-alanine (Ala), *N*-methyl-alanine (MeAla), *N*-methyl-valine (MeVal), isoleucine (Ile), and proline (Pro)) and *α*-hydroxy acid derivative containing a terminal epoxide in the side chain[6] (Fig. 1). Several

destruxin analogs have been isolated in recent decades,[7–13] and these analogs are known to show several biological activities, including the inhibition of V-ATPase which has been identified as a promising target in recent drug development for cancer therapeutics.[14–16] In particular, destruxin E (**1**) exhibits potent cytotoxic activity against cancer cells, for example, against KB-31 (IC$_{50}$ = 0.05 μM), HCT116 (IC$_{50}$ = 0.04 μM), and A549 (IC$_{50}$ = 0.22 μM).[17] In addition, Nakagawa *et al.* have reported that destruxin E (**1**) and B (**2**) reversibly inhibit the bone-resorbing activities of osteoclasts by inducing morphological changes without affecting cell viability.[18] In fact, most inhibitors of bone-resorbing activity are known to be irreversible, leading to the induction of apoptosis[19,20]; therefore, the mode of action of destruxin E (**1**) appears to be unique, and should be elucidated to develop a novel anti-resorptive agent for osteoporosis therapeutics. To date, synthetic studies on destruxins have been reported by several research groups.[21–29] Most of the recent synthetic studies reported have been carried out in solution-phase, and preparation of the 19-membered macrocyclic structure of destruxins was achieved by macro-
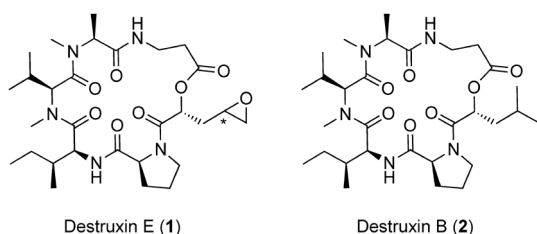

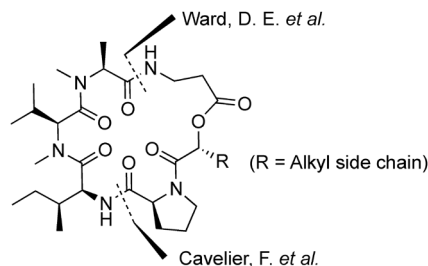Fig. 1.　Structures of Destruxin E (**1**) and B (**2**)

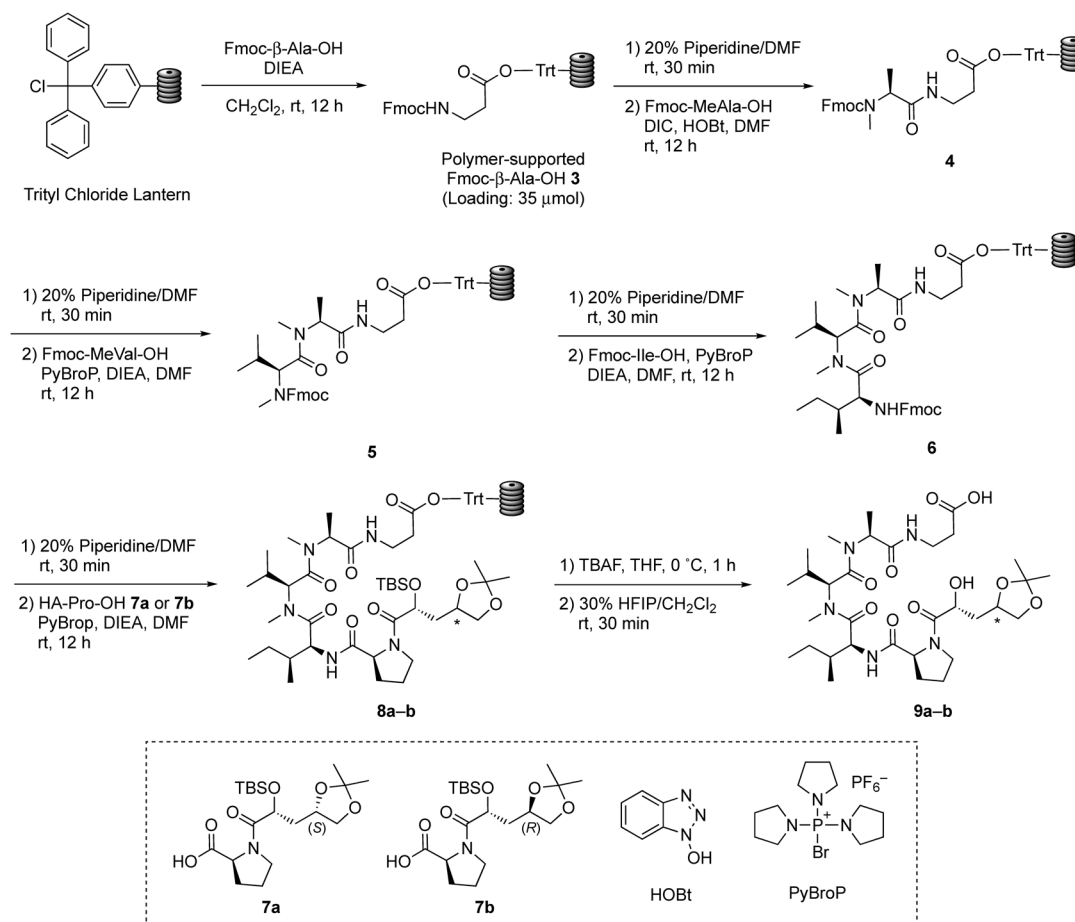Fig. 2.　Positions for the Formation of a Macrocycle

e-mail: masahito@chem.tsukuba.ac.jp

Chart 1. Preparation of Cyclization Precursors

lactamization (between the Pro and Ile residues, or between the MeAla and *β*-Ala residues) (Fig. 2). Recently, the biosynthesis of destruxins has been reported, and revealed that the macrocyclic structure of destruxins could be biosynthetically provided by macrolactamization after the nonribosomal peptide synthesis of a cyclization precursor.[30,31] Thus, the results of the above biosynthesis suggest that macrolactamization is an effective method for the formation of a macrocyclic structure in destruxin synthesis. On the other hand, solid-phase synthesis is a powerful method for preparing a combinatorial library toward the elucidation of structure–activity relationships. However, the formation of an ester linkage on polymer support is known to be problematic, compared to that of an amide bond, due to the low nucleophilicity of a corresponding hydroxyl group[32]; therefore, synthesis of a cyclization precursor for destruxins should be initiated from the *β*-Ala residue immobilized onto a polymer-support. Establishing reaction conditions for macrolactonization should be essential to achieving the total synthesis of destruxin E (**1**) and its analogs using solid-phase synthesis.

3. Total Synthesis and Structure Determination of Destruxin E (1)[33]

Toward understanding the structure–activity relationship

Biography

Masahito Yoshida received his Ph.D. in 2006 under the direction of Professors Takashi Takahashi and Takayuki Doi at the Tokyo Institute of Technology. After studying as a postdoctoral fellow at the Department of Chemical Biology at the Max-Planck Institute of Molecular Physiology (Dortmund, Germany) under Professor Herbert Waldmann, Dr. Yoshida worked with Prof. Doi at the Graduate School of Pharmaceutical Sciences in Tohoku University as COE fellow (2008–2011), and as Assistant Professor (2011–2018). He was appointed Associate Professor in 2018 at the Faculty of Pure and Applied Sciences in University of Tsukuba. He received The Japanese Peptide Society Award for Young Investigator 2016, and Pharmaceutical Society of Japan, Division of Organic Chemistry Prize 2018. Dr. Yoshida's research interest is the elucidation of the mode of action of biologically active natural products and their analogues, based on total synthesis.
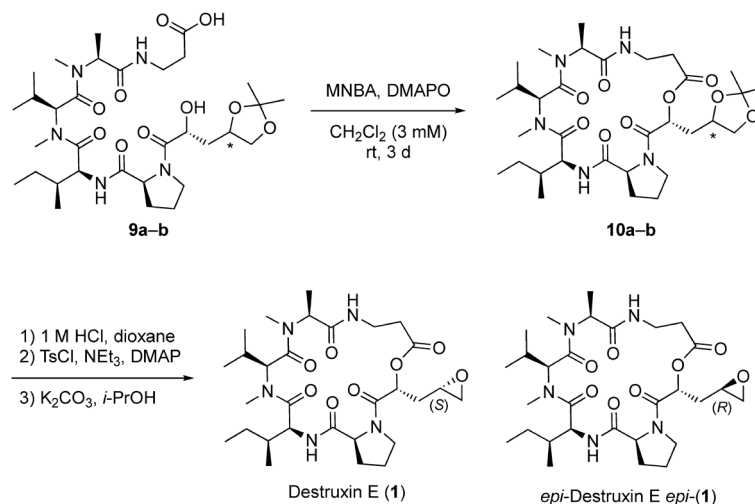
Masahito Yoshida

Chart 2.　Total Synthesis and Structure Determination of Destruxin E (**1**)

of destruxin E (**1**), we initially investigated the total synthesis of **1** using solid-phase synthesis that enables a combinatorial synthesis of destruxin analogs, and then attempted to determine the absolute configuration of the epoxide moiety. First, Fmoc-*β*Ala-OH was immobilized onto a trityl chloride-linked Lantern[34] under basic conditions, leading to polymer-supported **3**. Its loading amount was determined by gravimetric analysis to be 35 μmol/unit (quantitative) after cleavage from the polymer-support. After removal of the Fmoc group on the polymer-support, Fmoc-MeAla-OH was coupled using diisopropylcarbodiimide (DIC)/1-hydroxybenzotriazole (HOBt) to provide dipeptide **4**. In the next step, the acylation of polymer-supported *N*Me-amino acid is often problematic. In fact, the conventional method as mentioned above (DIC/HOBt) was not effective in coupling with Fmoc-MeVal-OH. After extensive investigations, the use of bromotri(pyrrolidino)phosphonium hexafluorophosphate (PyBroP)[35] was found to be best for the condensation of a polymer-supported *N*Me-amine leading to tripeptide **5**. Synthesis of the tetrapeptide **6** was also carried out using PyBroP/*N*,*N*-diisopropylethylamine (DIEA) for coupling with Fmoc-Ile-OH, and cyclization precursors **9** were successfully prepared through polymer-supported **8** by coupling with acylprolines **7a–b**, followed by removal of the *tert*-butyl dimethylsilyl (TBS) group and cleavage from polymer-support (Chart 1).

The macrolactonization of cyclization precursors **9a–b** was investigated, and we successfully found that macrolactonization proceeded smoothly by Shiina's method (2-methyl-6-nitrobenzoic anhydride (MNBA)/4-(dimethylamino)pyridine *N*-oxide (DMAPO))[36–40] under high dilution conditions (3 mM). The desired macrolactones **10a–b** were provided in moderate yields without the formation of a corresponding dimerized product. Finally, an epoxide in the side chain of an *α*-hydroxy acid derivative was successfully prepared, leading to destruxin E (**1**) and its epimer *epi*-**1** (Chart 2). Based on the above total synthesis, the stereochemistry of the epoxide in the natural product was determined to be (*S*). In addition, evaluation for V-ATPase inhibition of destruxin E (**1**) and *epi*-**1** revealed that the activity of the natural product is 10-fold stronger than that of its epimer, indicating that the epoxide moiety, including its stereochemistry, critically affected the biological potency of destruxin E (**1**).
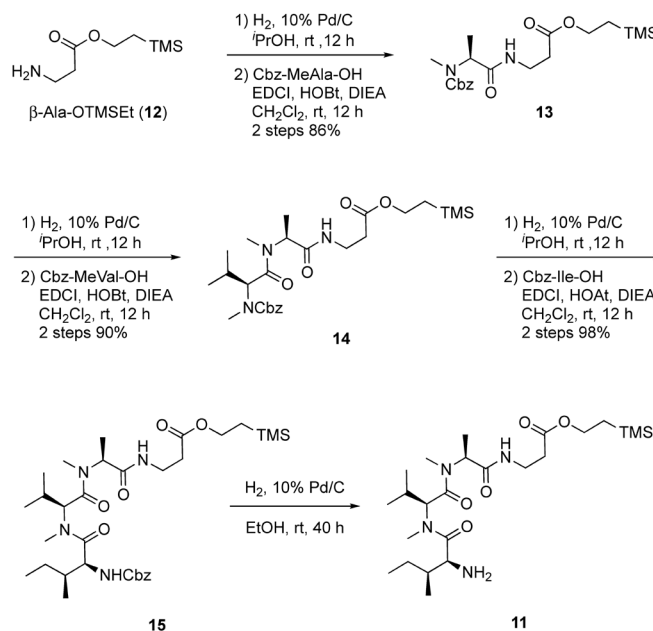
Chart 3.　Scalable Synthesis of Tetrapeptide **11**

4.　Scalable Synthesis of Destruxin E (1)[41]

The preparation of a natural product on a multi-gram scale is required for evaluating its biological activities *in vivo*. However, the production of destruxin E (**1**) from natural sources is known to be significantly low (approx. 7 mg/L)[6]; thus, establishment of a synthetic procedure for the multi-gram production of **1** would be necessary for our purposes. To achieve the scalable synthesis of destruxin E (**1**), we applied solution-phase synthesis for preparation of the cyclization precursor, which could then be synthesized by coupling with tetrapeptide **11** and acylproline **7a**. The solution-phase synthesis of tetrapeptide **11** was achieved using Cbz-protected amino acids rather than Boc-protected ones, due to the resulting *N*-methyl amide bonds that are unstable under acidic conditions for removal of the Boc group[42]; the corresponding **11** was readily provided from H-*β*-Ala-OTMSE (**12**) on a multi-gram scale (Chart 3).

On the other hand, acylproline **7a** should be prepared in

a stereoselective manner for scalable synthesis. Thus, we attempted Sharpless asymmetric dihydroxylation[43,44] of the terminal alkene in **14**. Although stereoselectivity is known to be moderate in the asymmetric dihydroxylation of a terminal alkene, we found that reaction conditions in the presence of (DHQD)$_2$PHAL favorably provided the desired diol **17** in a ratio of **17a** : **17b** = 86 : 14, whereas the reaction using an AD-mix β provided a diol in a ratio of **17a** : **17b** = 67 : 33. The diastereomers obtained were readily separated by silica gel column chromatography after protection of the resulting diol with an isopropylidene acetal. Finally, removal of the benzyl group by hydrogenolysis furnished the desired acylproline **7a** on a gram scale. The cyclization precursor **9a** was successfully prepared by coupling of the acylproline **7a** with tetrapeptide **11** using PyBroP/DIEA, followed by removal of the protecting groups at the N- and C-terminus (Chart 4).

Toward a scalable synthesis of **1**, the reaction conditions for macrolactonization should be re-investigated. In general, high

dilution conditions are employed for a macrolactonization to circumvent the formation of a corresponding dimerized product, resulting that the amount of solvent would also necessarily increase to achieve high dilution conditions in a scale-up synthesis. Therefore, the concentration of the substrate for macrolactonization was investigated to determine how best to reduce the amount of solvent; the reaction successfully proceeded at the concentration of 6 mM, leading to the macrolactone **10a** without the formation of the problematic dimerized product. Achieving the synthesis of macrolactone **10a** on a gram-scale, the scalable synthesis of destruxin E (**1**) was then accomplished as follows: removal of the isopropylidene acetal in **10a** under weakly acidic conditions, followed by the selective tosylation of the resulting primary alcohol, provided the tosylate **19**. Finally, formation of the epoxide under basic conditions furnished the desired destruxin E (**1**) on a gram scale with excellent purity (>95%) (Chart 5).

5. Combinatorial Synthesis of Destruxin Analogs Using the Split-and-Pool Method[45,46]

Based on synthetic methods for destruxin E (**1**), as mentioned above, a combinatorial synthesis of destruxin E analogs was attempted using the split-and-pool method.[47] We designed the analogs referring to the structure of destruxins and related derivatives previously isolated from natural sources, and planned to synthesize eighteen analogs containing amino acids A, B, and C, as shown in Chart 6.

Initially, the immobilization of Fmoc-βAla-OH onto lanterns was performed *via* a trityl linker that can be cleavable under weakly acidic conditions. Lanterns are pillar-shaped polymer-supports, and are readily distinguished by attachment with colored stems and cogs for the recognition of each analog on polymer-support. After dividing the lanterns into reaction vessels, the synthesis of tetrapeptide was accomplished in a combinatorial manner guided by stems and cogs. Acylation with the acylproline **7a**, followed by removal of the TBS group, afforded a polymer-supported hexapeptide which was cleaved from lanterns in parallel to provide the desired cyclization precursor. To this end, the synthesis of destruxin E analogs was successfully achieved by macrolactonization, followed by the formation of an epoxide in the same manner as used for the total synthesis of **1** (Chart 7). The resulting ana-
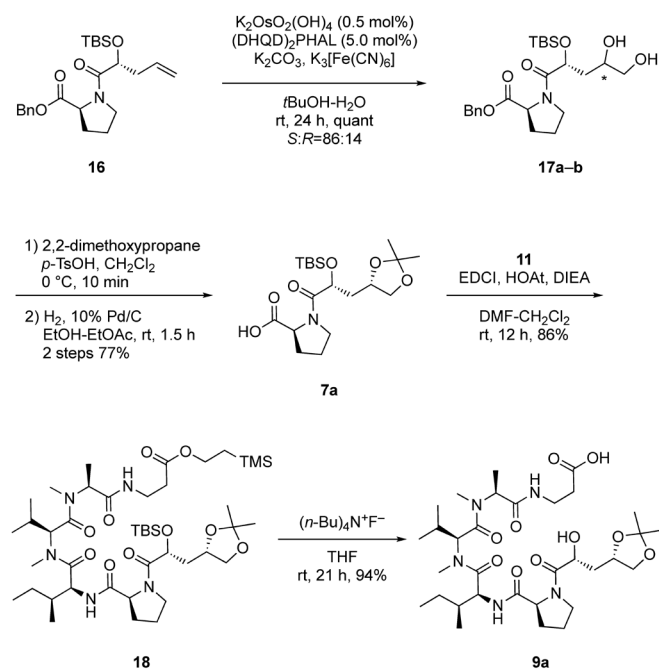

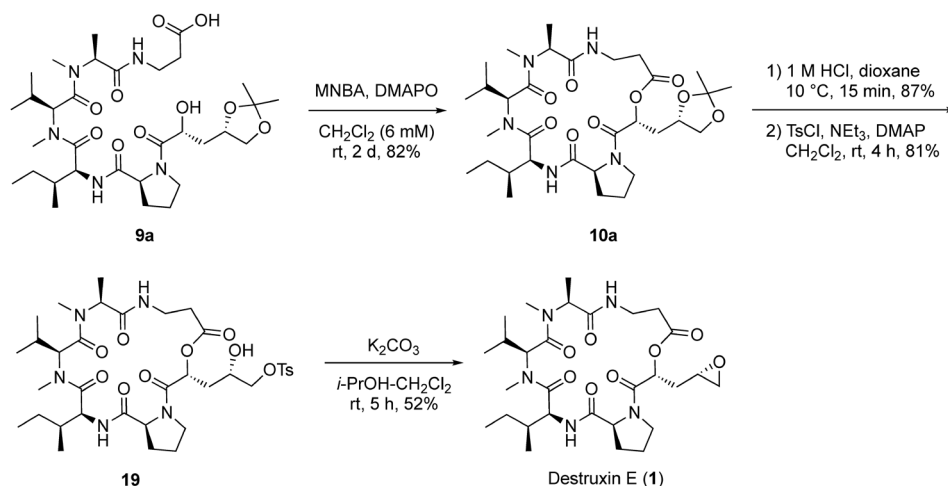Chart 4. Improved Synthesis of Cyclization Precursor **9a**

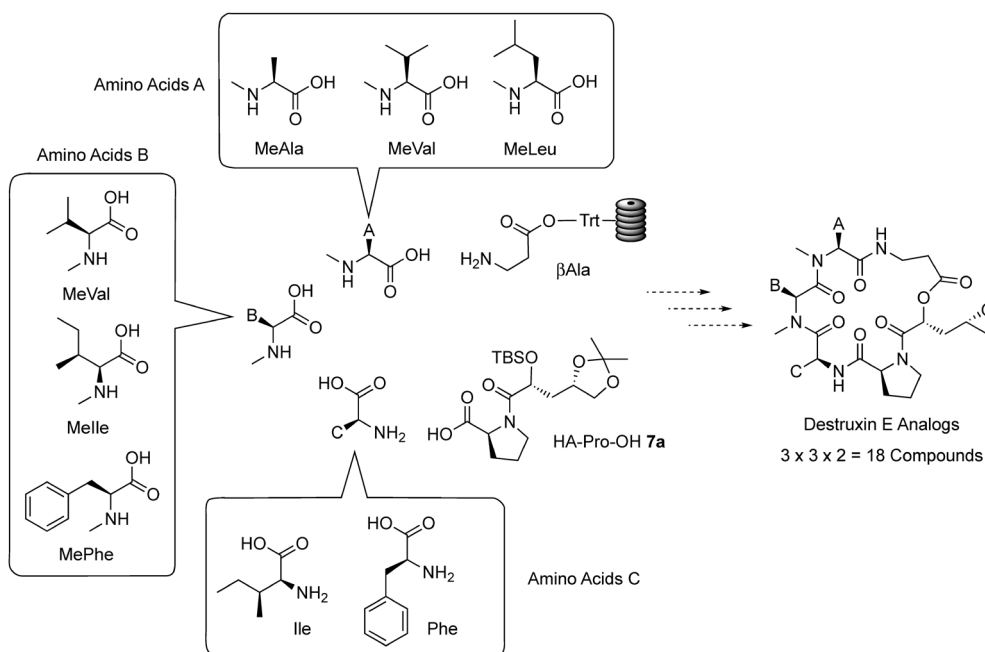Chart 5. Scalable Total Synthesis of Destruxin E (**1**)

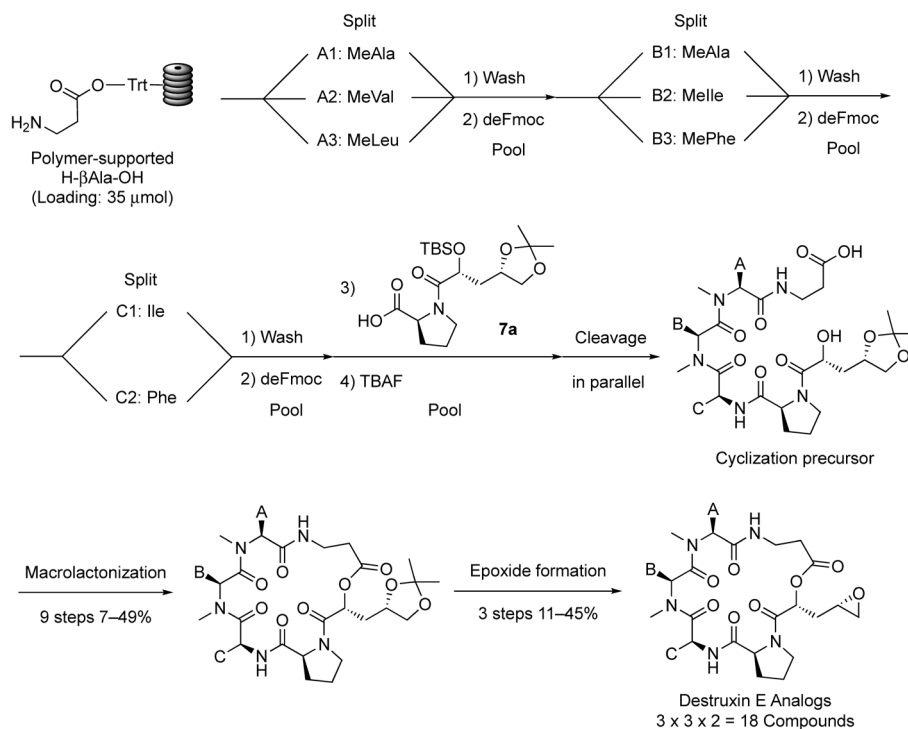Chart 6.   Synthetic Strategy of Destruxin E Library

Chart 7.   Combinatorial Synthesis of Destruxin E Analogs

logs were evaluated for morphological changes in the OCLs. It should be noted that MeAla residue was crucial to exhibiting the desired biological activity, and MeVal or Ile residues could be modified for the preparation of a chemical probe. However, substitution with amino acid derivatives possessing an alkyl azide significantly decreased the activity (Fig. 3), resulting in the need to perform another structure activity relationship study to determine a suitable position for the introduction of a chemical tag moiety toward the preparation of a molecular probe.

Nakagawa *et al.* have reported that the related analog, destruxin B, possessing an *iso*-butyl side chain, similarly exhibits a biological effect against osteoclasts,[18] and it can be readily prepared, in contrast to destruxin E possessing an epoxide moiety in the side chain on an α-hydroxy acid derivative. Therefore, toward further information on structure–activity relationships, we planned to prepare a combinatorial library based on the structures of destruxin A and B, which could be synthesized in an analogous manner, as used in the synthesis of destruxin E previously reported[33] (Fig. 4). In fact,
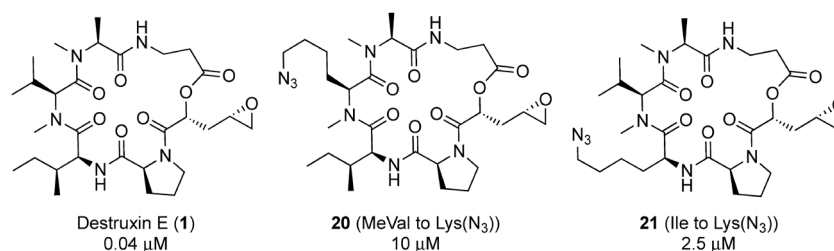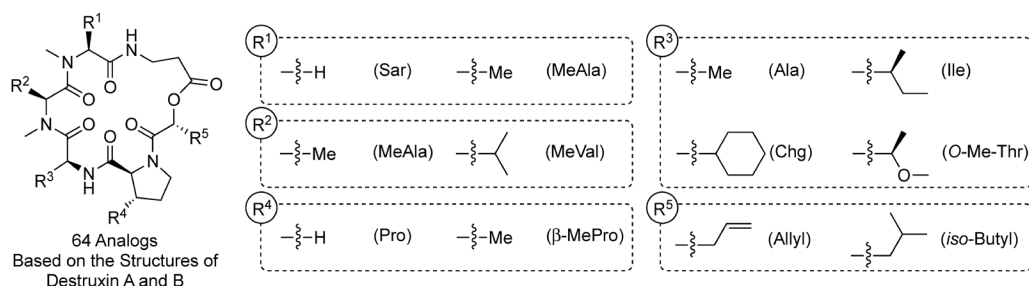
Fig. 3. Biological Evaluation of Alkyl Azide-Containing Analogs **20** and **21**

Fig. 4. Sixty-Four-Member Combinatorial Library Based on the Structures of Destruxins A and B

Table 1. Biological Evaluation of Destruxin Analogs (Selected)

Entry	Substituent					Activity [μM][e]
	R^1	R^2	R^3	R^4	R^5	
1[a]	Me	^{i}Pr	$CH(CH_3)C_2H_5$	H	^{i}Bu	0.78
2[b]	Me	^{i}Pr	$CH(CH_3)C_2H_5$	Me	^{i}Bu	0.78
3[c]	Me	^{i}Pr	$CH(CH_3)C_2H_5$	H	Allyl	1.56
4[d]	Me	^{i}Pr	$CH(CH_3)C_2H_5$	Me	Allyl	1.56
5	Me	^{i}Pr	C_6H_{11}	H	Allyl	3.13
6	Me	Me	Me	H	^{i}Bu	Not active
7	H	^{i}Pr	$CH(CH_3)C_2H_5$	H	^{i}Bu	50
8	Me	^{i}Pr	$CH(OMe)CH_3$	H	^{i}Bu	25

a) Destruxin B, *b*) Roseocardin, *c*) Destruxin A, *d*) Roseotoxin B, *e*) Minimum concentration for morphological changes in OCLs.

all of the analogs were successfully synthesized using a split-and-pool method, and the biological activities of the resulting analogs were evaluated against OCLs. Notably, the introduction of a methyl group to the β-position of the Pro residue did not decrease the potency against OCLs (Table 1, entry 1 *vs.* entry 2, entry 3 *vs.* entry 4). In addition, substitution of the Ile residue with the Chg residue did not influence the ability for morphological change (entry 5), whereas analogs possessing a smaller or polar substituent significantly diminished the desired activity (entries 6–8). Thus, the above results indicate that we succeeded in determination of a suitable position for introducing a chemical tag without affecting the ability to prepare a molecular probe for a target identification.

To this end, we have achieved the discovery of a suitable position for the attachment of a chemical tag toward the preparation of a molecular probe by structure–activity relationship study based on the total synthesis of destruxin E using solid- and solution-phase synthesis. The fruitful results described in this review should assist in determining a target molecule for destruxin E by a chemical biology approach. The development of a molecular probe and elucidation of the mode of action, including target identification, are now underway in our laboratory.

Conflict of Interest The author declares no conflict of interest.

References and Notes
1) Newman D. J., Cragg G. M., *J. Nat. Prod.*, **79**, 629–661 (2016).
2) Chen Y., de Lomana M. G., Friedrich N., Kirchmair J., *J. Chem. Inf. Model.*, **58**, 1518–1532 (2018).
3) Tsomaia N., *Eur. J. Med. Chem.*, **94**, 459–470 (2015).

4) Bhat A., Roberts L. R., Dwyer J. J., *Eur. J. Med. Chem.*, **94**, 471–479 (2015).

5) Walport L. J., Obexer R., Suga H., *Curr. Opin. Biotechnol.*, **48**, 242–250 (2017).

6) Païs M., Das B. C., Ferron P., *Phytochemistry*, **20**, 715–723 (1981).

7) Roberts D. W., *J. Invertebr. Pathol.*, **8**, 212–221 (1966).

8) Suzuki A., Taguchi H., Tamura S., *Agric. Biol. Chem.*, **34**, 813–816 (1970).

9) Kawazu K., Murakami T., Ono Y., Kanzaki H., Kobayashi A., Mikawa T., Yoshikawa N., *Biosci. Biotechnol. Biochem.*, **57**, 98–101 (1993).

10) Chen H. C., Yeh S. F., Ong G.-T., Wu S.-H., Sun C.-M., Chou C.-K., *J. Nat. Prod.*, **58**, 527–531 (1995).

11) Krasnoff S. B., Gibson D. M., Belofsky G. N., Gloer K. B., Gloer J. B., *J. Nat. Prod.*, **59**, 485–489 (1996).

12) Lira S. P., Vita-Marques A. M., Seleghim M. H. R., Bugni T. S., LaBarbera D. V., Sette L. D., Sponchiado S. R. P., Ireland C. M., Berlinck R. G. S., *J. Antibiot.*, **59**, 553–563 (2006).

13) Amagata T., Morinaka B. I., Amagata A., Tenney K., Valeriote F. A., Lobkovsky E., Clardy J., Crews P., *J. Nat. Prod.*, **69**, 1560–1565 (2006).

14) Muroi M., Shiragami N., Takatsuki A., *Biochem. Biophys. Res. Commun.*, **205**, 1358–1365 (1994).

15) Vázquez M. J., Albarrán M. I., Espada A., Rivera-Sagredo A., Díez E., Hueso-Rodríguez J. A., *Chem. Biodivers.*, **2**, 123–130 (2005).

16) Sasazawa Y., Futamura Y., Tashiro E., Imoto M., *Cancer Sci.*, **100**, 1460–1467 (2009).

17) Dornetshuber-Fleiss R., Heffeter P., Mohr T., Hazemi P., Kryeziu K., Seger C., Berger W., Lemmens-Gruber R., *Biochem. Pharmacol.*, **86**, 361–377 (2013).

18) Nakagawa H., Takami M., Udagawa N., Sawae Y., Suda K., Sasaki T., Takahashi N., Wachi M., Nagai K., Woo J. T., *Bone*, **33**, 443–455 (2003).

19) Hughes D. E., Wright K. R., Uy H. L., Sasaki A., Yoneda T., Roodman G. D., Mundy G. R., Boyce B. F., *J. Bone Miner. Res.*, **10**, 1478–1487 (1995).

20) Russell R. G., Rogers M. J., *Bone*, **25**, 97–106 (1999).

21) Kuyama S., Tamura S., *Agric. Biol. Chem.*, **29**, 168–169 (1965).

22) Lee S., Izumiya N., Suzuki A., Tamura S., *Tetrahedron Lett.*, **16**, 883–886 (1975).

23) Calmes M., Cavelier-Frontin F., Jacquier R., Mercadier J.-L., Sabil S., Verducci J., Qciot J.-M., Vey A., *Int. J. Pept. Protein Res.*, **41**, 528–535 (1993).

24) Cavelier F., Jacquier R., Mercadier J.-L., Verducci J., *Tetrahedron*, **52**, 6173–6186 (1996).

25) Ward D. E., Lazny R., Pedras M. S. C., *Tetrahedron Lett.*, **38**, 339–342 (1997).

26) Cavelier F., Jacquier R., Mercadier J.-L., Verducci J., Traris M., Vey A., *J. Pept. Res.*, **50**, 94–101 (1997).

27) Cavelier F., Verducci J., André F., Haraux F., Sigalat C., Traris M., Vey A., *Pestic. Sci.*, **52**, 81–89 (1998).

28) Ward D. E., Gai Y., Lazny R., Pedras M. S. C., *J. Org. Chem.*, **66**, 7832–7840 (2001).

29) Ast T., Barron E., Kinne L., Schmidt M., Germeroth L., Simmons K., Wenschuh H., *J. Pept. Res.*, **58**, 1–11 (2001).

30) Giuliano Garisto Donzelli B., Krasnoff S. B., Sun-Moon Y., Churchill A. C. L., Gibson D. M., *Curr. Genet.*, **58**, 105–116 (2012).

31) Romans-Fuertes P., Sondergaard T. E., Sandmann M. I. H., Wollenberg R. D., Nielsen K. F., Hansen F. T., Giese H., Brodersen D. E., Sørensen J. L., *Curr. Genet.*, **62**, 799–807 (2016).

32) Blankemeyer-Menge B., Nimtz M., Frank R., *Tetrahedron Lett.*, **31**, 1701–1704 (1990).

33) Yoshida M., Takeuchi H., Ishida Y., Yashiroda Y., Yoshida M., Takagi M., Shin-ya K., Doi T., *Org. Lett.*, **12**, 3792–3795 (2010).

34) Synphase lanterns were purchased from Mimotopes, 11 Duerdin Street, Clayton, Victoria, 3168, Australia.

35) Frérot E., Coste J., Pantaloni A., Dufour M.-N., Jouin P., *Tetrahedron*, **47**, 259–270 (1991).

36) Shiina I., Ibuka R., Kubota M., *Chem. Lett.*, **31**, 286–287 (2002).

37) Shiina I., Kubota M., Ibuka R., *Tetrahedron Lett.*, **43**, 7535–7539 (2002).

38) Shiina I., Kubota M., Oshiumi H., Hashizume M., *J. Org. Chem.*, **69**, 1822–1830 (2004).

39) Shiina I., Hashizume M., Yamai Y., Oshiumi H., Shimazaki T., Takasuna Y., Ibuka R., *Chem. Eur. J.*, **11**, 6601–6608 (2005).

40) A review: Shiina I., *Bull. Chem. Soc. Jpn.*, **87**, 196–233 (2014).

41) Yoshida M., Sato H., Ishida Y., Nakagawa H., Doi T., *J. Org. Chem.*, **79**, 296–306 (2014).

42) Anteunis M. J. O., Van Der Auwera C., *Int. J. Pept. Protein Res.*, **31**, 448–451 (1988).

43) Jacobsen E. N., Markó I., Mungall W. S., Schröder G., Sharpless K. B., *J. Am. Chem. Soc.*, **110**, 1968–1970 (1988).

44) Sharpless K. B., Amberg W., Bennari Y. L., Crispino G. A., Hartung J., Jeong K.-S., Kwong H.-L., Morikawa K., Wang Z.-M., Xu D., Zhang X.-L., *J. Org. Chem.*, **57**, 2768–2771 (1992).

45) Yoshida M., Ishida Y., Adachi K., Murase H., Nakagawa H., Doi T., *Chem. Eur. J.*, **21**, 18417–18430 (2015).

46) Sato H., Yoshida M., Murase H., Nakagawa H., Doi T., *ACS Combi. Sci.*, **18**, 590–595 (2016).

47) Geysen H. M., Meloen R. H., Barteling S. J., *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 3998–4002 (1984).