Total Synthesis of Sarcophytonolide H and Isosarcophytonolide D: Structural Revision of Isosarcophytonolide D and Structure–Antifouling Activity Relationship of Sarcophytonolide H

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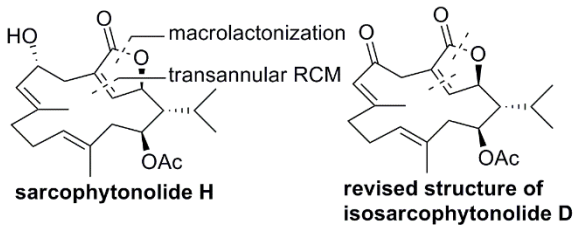
Supporting Information Placeholder



ABSTRACT: The first total syntheses of sarcophytonolide H and the originally proposed and correct structures of isosarcophytonolide D have been achieved via transannular ring-closing metathesis (RCM). These total syntheses culminated in the stereostructural confirmation of sarcophytonolide H and the reassignment of isosarcophytonolide D, respectively. The antifouling activity of the synthetic sarcophytonolide H and its analogues was also evaluated.

Corals have been recognized as a rich source of secondary metabolites with a variety of skeletons and biological activities.[1] Cembranolide diterpenes,[2] isolated from octocorals and soft corals, display a diverse range of biological activities such as antifouling,[3] antifungal,[4] antiviral,[5] cytotoxic,[6] and ichthyotoxic activities.[7] Ecologically, it has been suggested that these natural products play significant roles in the survival of corals as defensive, competitive, and reproductive substances.[1a,8] Sarcophytonolides are cembranolides isolated from the soft corals of genus *Sarcophyton* by Guo's research group since 2005.[9] As depicted in Figure 1, they have a 14-membered macrocycle and a butenolide unit as common structures. We previously established the absolute configuration of (+)-sarcophytonolide C (**1**) by the total synthesis of its two possible stereoisomers at the C8 position.[10,11] The absolute stereochemistry of sarcophytonolide H (**3**), which possesses a β-oriented acetoxy group at the C14 position, was determined by analysis of its 2D NMR spectra and the modified Mosher method.[9b] The relative configuration of sarcophytonolide D (**2**) bearing an α-positioned acetoxy moiety (C14) was elucidated by NOE observations.[9a] The relative stereochemistries at the C1, C2, and C14 positions of sarcophytonolides I (**4**), J (**5**), and isosarcophytonolide D (**6**) were determined by the similarity of their NMR data to those of sarcophytonolide D (**2**).[9c,9d] Qian and co-workers have reported that sarcophytonolides H (**3**) and J (**5**) show antifouling activity against the larval settlement of barnacle *Balanus* (*Amphibalanus*) *amphitrite* with EC_{50} values of 5.98 μg/mL and 7.50 μg/mL, respectively.[12] Herein, we describe the first total synthesis of sarcophytonolide H (**3**) and the originally proposed and correct structures of isosarcophytonolide D, which resulted in the stereostructural revision of isosarcophytonolide D. Furthermore, we also report the structure–antifouling activity relationship of **3**.

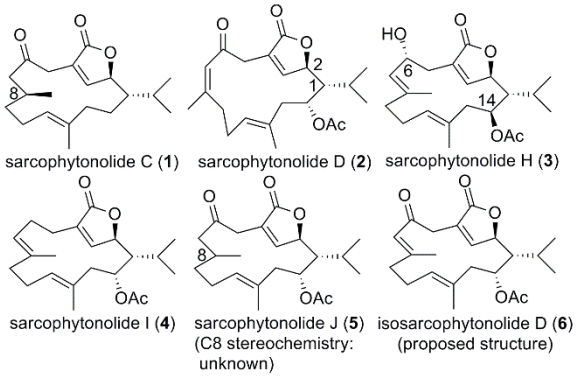
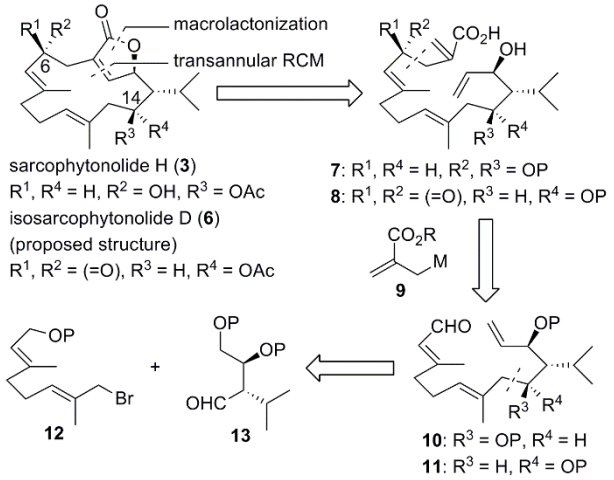


Figure 1. Structures of sarcophytonolides **1**–**6**.

Retrosynthetic analysis of sarcophytonolide H (**3**) and the proposed structure **6** of isosarcophytonolide D, wherein the structural differences are the oxidation degree at the C6 position and the stereochemistry at the C14 position, is depicted in Scheme 1. The common structures, namely, the 14-membered macrocycle and butenolide moiety, could potentially be constructed by macrolactonization[13] and subsequent transannular ring-closing metathesis (RCM)[14] of hydroxycarboxylic acids **7** and **8**, respectively. Macrolactonization precursors **7** and **8** could possibly be prepared by the addition reaction of 2-alkoxycarbonyl allylic

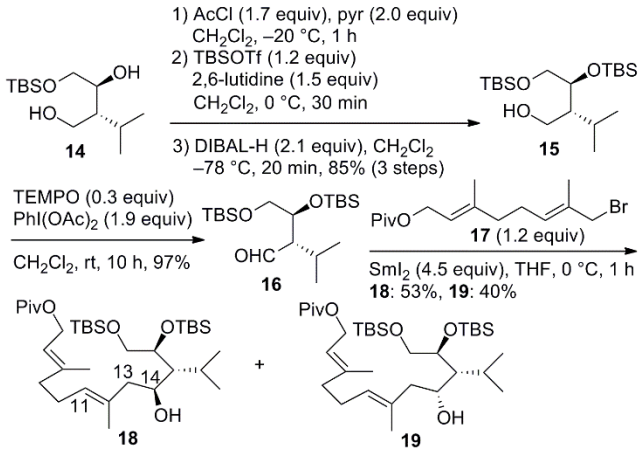
metal **9** to aldehydes **10** and **11**. The aldehydes **10** and **11** were broken down into allylic bromide **12** and aldehyde **13**.

Scheme 1. Retrosynthetic Analysis of Sarcophytonolide H (3) and the Proposed Structure 6 of Isosarcophytonolide D



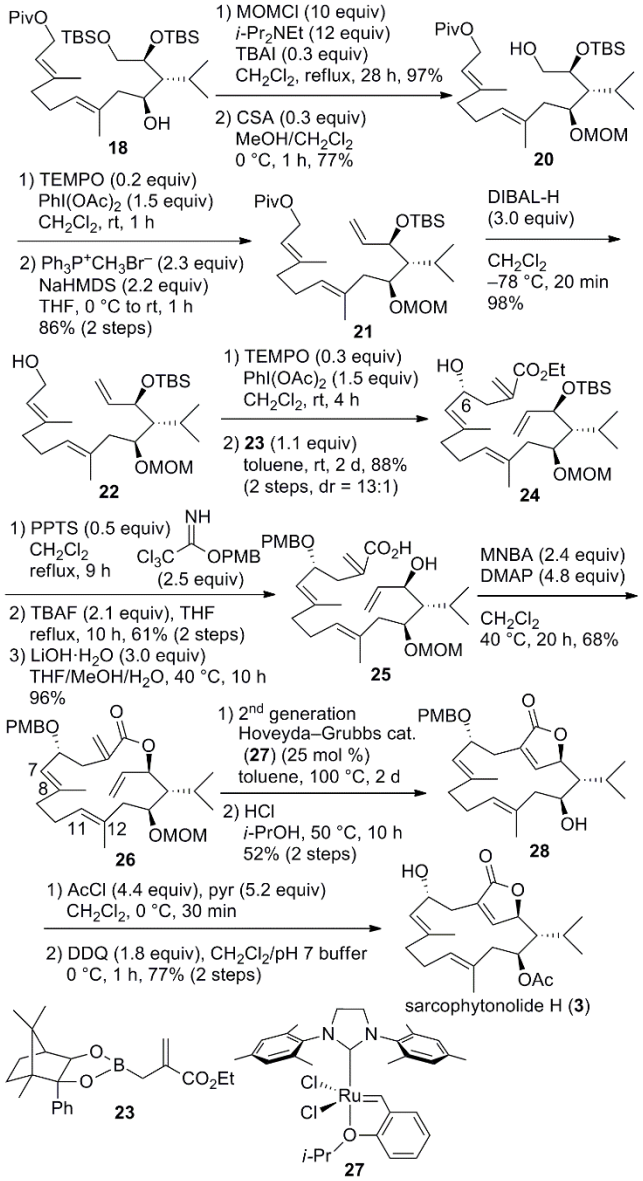
Our synthesis of the C14-stereoisomers **18** and **19** is described in Scheme 2. Selective acetylation of diol **14**[10] and subsequent protection of the remaining secondary hydroxy group with TBSOTf afforded the corresponding silyl ether. Reductive removal of the acetyl group with DIBAL-H followed by oxidation of alcohol **15** with TEMPO/PhI(OAc)$_2$[15] provided aldehyde **16**. Treatment of **16** with allylic bromide **17**[16] (1.2 equiv) in the presence of SmI_2[17,18] produced the desired α-adducts **18** and **19** in 53% and 40% yields, respectively. Formation of the corresponding γ-adduct was not observed at all in this reaction.[19] The C11/C12 alkene geometries of **18** and **19** were confirmed by the observed NOEs of H-11/H-13 (3% in **18** and 2% in **19**). The resulting stereochemistry at the C14 position of **18** was determined by the modified Mosher method.[20,21]

Scheme 2. Synthesis of Alcohols 18 and 19



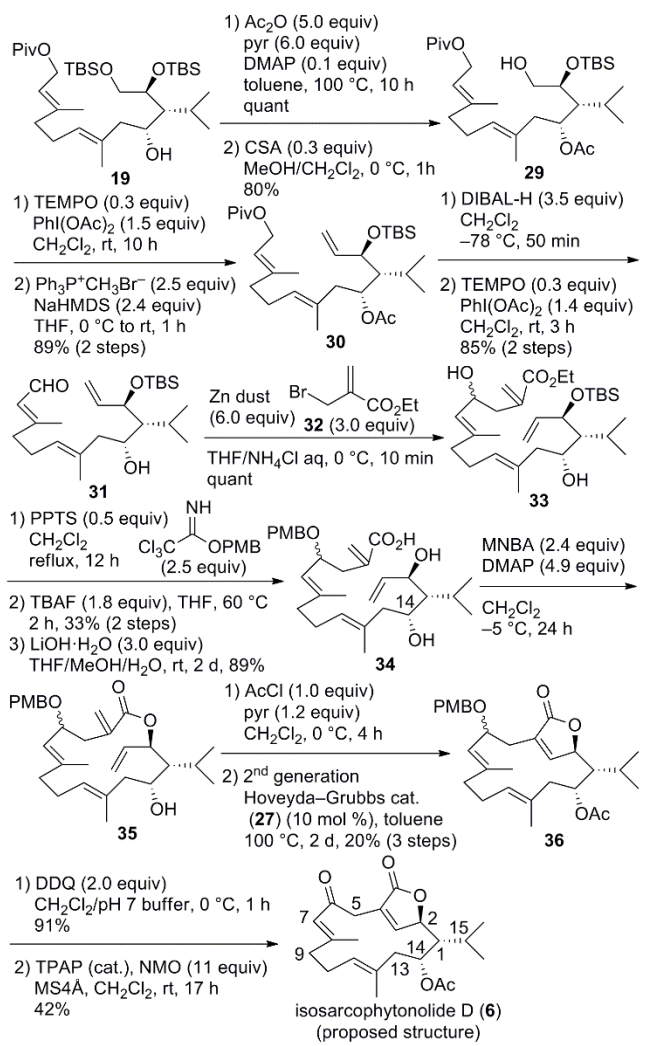
We next examined the transformation of **18** to sarcophytonolide H (**3**) possessing the 14*S* configuration. Thus, protection of the secondary alcohol **18** as the MOM ether followed by selective removal of the primary TBS group gave alcohol **20** (Scheme 3). TEMPO oxidation[15] of **20** and subsequent Wittig reaction afforded alkene **21**. The pivalate **21** was deprotected with DIBAL-H to yield alcohol **22**, which was oxidized to the corresponding aldehyde. The asymmetric alkoxycarbonylallylation of the resulting aldehyde was carried out by using the chiral allylic boronate **23** according to Chataingner's protocol[22] to provide the desired product **24** in 88% yield over two steps with a 13:1 diastereoselectivity[23]. Protection of the resulting hydroxy moiety in **24** as the PMB ether, removal of the TBS protective group, and hydrolysis of the ester portion produced hydroxycarboxylic acid **25**. Macrolactonization of **25** was performed with 2-methyl-6-nitrobenzoic anhydride (MNBA)/DMAP[24] to give the 15-membered lactone **26**. The tetraene **26** was treated with second-generation Hoveyda–Grubbs catalyst (**27**)[25] to afford the desired product, whose C7/C8 and C11/C12 alkene portions were inert to the reaction conditions.[26] The obtained butenolide underwent removal of the MOM group with HCl in *i*-PrOH[27] to provide alcohol **28** in 52% yield over two steps. The resulting hydroxy moiety in **28** was acetylated and the PMB ether was deprotected with DDQ to produce sarcophytonolide H (**3**). The synthetic sarcophytonolide H (**3**) displayed ^{1}H and ^{13}C NMR data[21] and specific rotation[28] which were in full agreement with those of the natural product.[9b,29] Thus, the absolute configuration of sarcophytonolide H was unambiguously confirmed.

Scheme 3. Synthesis of Sarcophytonolide H (3)



We next tried to synthesize the proposed structure **6** of isosarcophytonolide D with the 14*R* configuration. Thus, the alcohol **19** was transformed to **6** over 15 steps by the similar sequence to that used for sarcophytonolide H (**3**) (Scheme 4).[21]

Scheme 4. Synthesis of the Proposed Structure 6 of Isosarcophytonolide D



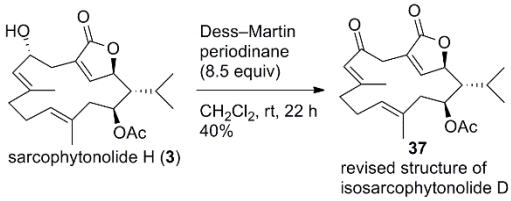
With the proposed structure **6** of isosarcophytonolide D in hand, we next carefully analyzed the ^{1}H–^{1}H COSY, HMQC, and HMBC spectra of **6** and compared the NMR data between the synthetic product **6** and the natural product. The ^{1}H and ^{13}C NMR data of the synthesized **6** were clearly different from the reported data of natural isosarcophytonolide D,[9d] respectively.[21] Especially, as shown in Table 1, the chemical shift deviations were found to be critical around the C14 position in both ^{1}H and ^{13}C NMR spectra. Therefore, we predicted the correct structure of isosarcophytonolide D to be that drawn as **37**, which is the C14-epimer of **6** (Scheme 5). The ketone **37** was synthesized by treatment of the synthetic sarcophytonolide H (**3**) with Dess–Martin periodinane.[30] As expected, the ^{1}H and ^{13}C NMR data of the synthetic product **37** matched with those of natural isosarcophytonolide D.[21] Furthermore, the measured specific rotation of the synthesized **37**, $[\alpha]_D^{24}$ = –50.9 (*c* = 0.07, $CHCl_3$), was agreement with the data reported for the natural product, $[\alpha]_D^{20}$ = –66 (*c* = 0.67, $CHCl_3$).[9d] Therefore, the absolute stereochemistry of isosarcophytonolide D was revised to be that described in **37**.[31]

Table 1. Chemical Shift Deviations ($\Delta\delta_{N–S}$) between Natural Isosarcophytonolide D and the Synthetic 6 in the ^{1}H and ^{13}C NMR[a]

position	^{1}H NMR	^{13}C NMR
1	–0.24	+3.3
2	–0.12	+0.9
13	–0.24	–1.6
	–0.02	
14	–0.37	+4.0
15	–0.10	–2.6

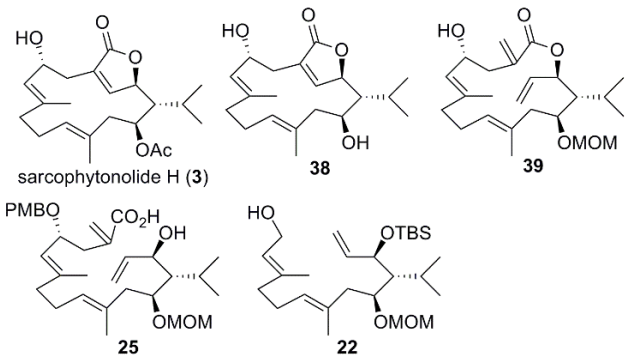
[a]δ_N and δ_S are chemical shifts of the natural product and the synthetic product. Chemical shifts are recorded in ppm with reference to the internal residual solvent signal ($CDCl_3$, 7.26 ppm in ^{1}H NMR and 77.0 ppm in ^{13}C NMR).

Scheme 5. Synthesis of the Predicted Structure 37 of Isosarcophytonolide D



We next evaluated the antifouling activity[32] and toxicity of the synthetic sarcophytonolide H (**3**) and its analogues **38**, **39**,[33] **25**, and **22** against the cypris larvae of barnacle *Balanus* (*Amphibalanus*) *amphitrite*. Our results described in Table 2 suggest that the tetraene **39**, which was the most antifouling active and non-toxic, is a good candidate for further pursuing the environmentally-benign antifouling compound.[21]

Table 2. Antifouling Activity (EC_{50}) and Toxicity (LC_{50}) of the Synthetic Sarcophytonolide H (3) and Its Analogues[a]



compound	EC_{50}	LC_{50}
3	3.36	>50
38	3.08	>50
39	1.61	>50
25	3.27	19.4
22	>50	>50

[a]Against the cypris larvae of barnacle *Balanus* (*Amphibalanus*) *amphitrite*. EC_{50} and LC_{50} values in μg/mL.

In conclusion, we have accomplished the first total synthesis of sarcophytonolide H (**3**) and the originally proposed and correct structures of isosarcophytonolide D, **6** and **37**, by using

transannular RCM as a key step. The structural revision of isosarcophytonolide D suggests that the C14 stereochemistries of sarcophytonolides D (**2**), I (**4**), and J (**5**) also need to be reconsidered. We also evaluated the antifouling activity and the toxicity of the synthetic sarcophytonolide H and its analogues, which denotes that the tetraene **39** is a good candidate for the creation of the antifouling agent without toxicity. Further synthetic and biological study of sarcophytonolides is currently underway.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Experimental procedures and characterization data of all new compounds, evaluation procedures of antifouling activity and toxicity, and NMR spectra of all new compounds (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) (a) Coll, J. C. *Chem. Rev.* **1992**, *92*, 613. (b) Berrue, F.; Kerr, R. G. *Nat. Prod. Rep.* **2009**, *26*, 681. (c) Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H. G.; Prinsep, M. R. *Nat. Prod. Rep.* **2013**, *30*, 237.

(2) (a) Roethle, P. A.; Trauner, D. *Nat. Prod. Rep.* **2008**, *25*, 298. (b) Li, Y.; Pattenden, G. *Nat. Prod. Rep.* **2011**, *28*, 429. (c) Li, Y.; Pattenden, G. *Nat. Prod. Rep.* **2011**, *28*, 1269. (d) Liang, L.-F.; Guo, Y.-W. *Chem. Biodiversity* **2013**, *10*, 2161.

(3) Sun, X.-P.; Wang, C.-Y.; Shao, C.-L.; Li, L.; Li, X.-B.; Chen, M.; Qian, P.-Y. *Nat. Prod. Commun.* **2010**, *5*, 1171.

(4) El Sayed, K. A.; Hamann, M. T. *J. Nat. Prod.* **1996**, *59*, 687.

(5) Cheng, S.-Y.; Chuang, C.-T.; Wen, Z.-H.; Wang, S.-K.; Chiou, S.-F.; Hsu, C.-H.; Dai, C.-F.; Duh, C.-Y. *Bioorg. Med. Chem.* **2010**, *18*, 3379.

(6) (a) Kusumi, T.; Ohtani, I.; Inouye, Y.; Kakisawa, H. *Tetrahedron Lett.* **1988**, *29*, 4731. (b) Duh, C.-Y.; Wang, S.-K.; Weng, Y.-L.; Chiang, M. Y.; Dai, C.-F. *J. Nat. Prod.* **1999**, *62*, 1518.

(7) Uchio, Y.; Eguchi, S.; Kuramoto, J.; Nakayama, M.; Hase, T. *Tetrahedron Lett.* **1985**, *26*, 4487.

(8) Coll, J. C.; Price, I. R.; König, G. M.; Bowden, B. F. *Mar. Biol.* **1987**, *96*, 129.

(9) (a) Jia, R.; Guo, Y.-W.; Mollo, E.; Cimino, G. *Helv. Chim. Acta* **2005**, *88*, 1028. (b) Jia, R.; Guo, Y.-W.; Mollo, E.; Gavagnin, M.; Cimino, G. *J. Nat. Prod.* **2006**, *69*, 819. (c) Yan, X.-H.; Li, Z.-Y.; Guo, Y.-W. *Helv. Chim. Acta* **2007**, *90*, 1574. (d) Yan, X.-H.; Gavagnin, M.; Cimino, G.; Guo, Y.-W. *Tetrahedron Lett.* **2007**, *48*, 5313. (e) Liang, L.-F.; Gao, L.-X.; Li, J.; Taglialatela-Scafati, O.; Guo, Y.-W. *Bioorg. Med. Chem.* **2013**, *21*, 5076.

(10) Takamura, H.; Iwamoto, K.; Nakao, E.; Kadota, I. *Org. Lett.* **2013**, *15*, 1108.

(11) For a report of the synthetic study of sarcophytonolides, see: Fernandes, R. A.; Ingle, A. B. *Tetrahedron Lett.* **2011**, *52*, 458.

(12) (a) Wang, C.-Y.; Chen, A.-N.; Shao, C.-L.; Li, L.; Xu, Y.; Qian, P.-Y. *Biochem. Syst. Ecol.* **2011**, *39*, 853. (b) Li, Y.-X.; Wu, H.-X.; Xu, Y.; Shao, C.-L.; Wang, C.-Y.; Qian, P.-Y. *Mar. Biotechnol.* **2013**, *15*, 552.

(13) For a review of macrolactonization, see: Parenty, A.; Moreau, X.; Campagne, J.-M. *Chem. Rev.* **2006**, *106*, 911.

(14) For reviews of RCM, see: (a) Fürstner, A. *Angew. Chem., Int. Ed.* **2000**, *39*, 3012. (b) Deiters, A.; Martin, S. F. *Chem. Rev.* **2004**, *104*, 2199. (c) Nicolaou, K. C.; Bulger, P. G.; Sarlah, D. *Angew. Chem., Int. Ed.* **2005**, *44*, 4490.

(15) De Mico, A.; Margarita, R.; Parlanti, L.; Vescovi; A.; Piancatelli, G. *J. Org. Chem.* **1997**, *62*, 6974.

(16) For the synthesis of **17**, see the Supporting Information.

(17) Souppe, J.; Namy, J. L.; Kagan, H. B. *Tetrahedron Lett.* **1982**, *23*, 3497.

(18) For reviews of SmI_2-mediated reaction in natural product synthesis, see: (a) Edmonds, D. J.; Johnston; D.; Procter, D. J. *Chem. Rev.* **2004**, *104*, 3371. (b) Nicolaou, K. C.; Ellery, S. P.; Chen, J. S. *Angew. Chem., Int. Ed.* **2009**, *48*, 7140.

(19) For related examples, see: (a) Tsushima, K.; Murai, A. *Tetrahedron Lett.* **1992**, *33*, 4345. (b) Burton, J. W.; Clark, J. S.; Derrer, S.; Stork, T. C.; Bendall, J. G.; Holmes, A. B. *J. Am. Chem. Soc.* **1997**, *119*, 7483.

(20) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.

(21) See the Supporting Information for details.

(22) Chataigner, I.; Zammattio, F.; Lebreton, J.; Villiéras, J. *Tetrahedron* **2008**, *64*, 2441.

(23) For the stereochemical determination, see the Supporting Information.

(24) Shiina, I.; Kubota, M.; Oshiumi, H.; Hashizume, M. *J. Org. Chem.* **2004**, *69*, 1822.

(25) Garber, S. B.; Kingsbury, J. S.; Gray, B. L.; Hoveyda, A. H. *J. Am. Chem. Soc.* **2000**, *122*, 8168.

(26) For the total synthesis of (–)-(*Z*)-deoxypkalide, which is a non-natural franocembranolide, by using transannular RCM, see: Donohoe, T. J.; Ironmonger, A.; Kershaw, N. M. *Angew. Chem., Int. Ed.* **2008**, *47*, 7314.

(27) Hall, D. G.; Deslongchamps, P. *J. Org. Chem.* **1995**, *60*, 7796.

(28) The synthetic **3**: $[\alpha]_D^{23}$ = +115 (*c* = 0.10, $CHCl_3$). The natural product: $[\alpha]_D^{20}$ = +74.7 (*c* = 0.20, $CHCl_3$).

(29) The ^1H NMR data of (*S*)- and (*R*)-MTPA esters (MTPA = α-methoxy-β-(trifluoromethyl)phenylacetyl) which were prepared from the synthetic **3** were identical to those of the (*S*)- and (*R*)-MTPA esters derived from the natural product, respectively. See the Supporting Information for details.

(30) (a) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155. (b) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277.

(31) For reviews of the structural elucidation of natural products by the chemical synthesis, see: (a) Nicolaou, K. C.; Snyder, S. A. *Angew. Chem., Int. Ed.* **2005**, *44*, 1012. (b) Maier, M. E. *Nat. Prod. Rep.* **2009**, *26*, 1105. (c) Suyama, T. L.; Gerwick, W. H.; McPhail, K. L. *Bioorg. Med. Chem.* **2011**, *19*, 6675.

(32) For reviews of antifouling active natural products, see: (a) Clare, A. S. *Biofouling* **1996**, *9*, 211. (b) Fusetani, N. *Nat. Prod. Rep.* **2004**, *21*, 94. (c) Qian, P.-Y.; Xu, Y.; Fusetani, N. *Biofouling* **2010**, *26*, 223. (d) Fusetani, N. *Nat. Prod. Rep.* **2011**, *28*, 400. (e) Qian, P.-Y.; Li, Z.; Xu, Y.; Li, Y.; Fusetani, N. *Biofouling* **2015**, *31*, 101.

(33) For the synthesis of **38** and **39**, see the Supporting Information.