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Total Synthesis of (−)-Zampanolide and Questionable Existence of (−)-Dactylolide as the Elusive Biosynthetic Precursor of (−)-Zampanolide in an Okinawan Sponge

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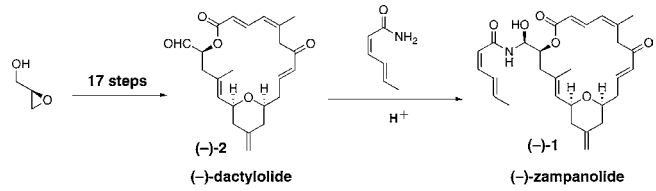
ABSTRACT



A new and concise total synthesis of (−)-zampanolide, (−)-1, and (−)-dactylolide, (−)-2, is described. Synthetic highlights include (i) a mild Horner-Wadsworth-Emmons reaction providing the seco acid, (ii) an unusual stepwise cross-coupling reaction of a 1,1-dibromodiene with inversion of olefin geometry, and (iii) specific *O*-Michael reaction conditions using catalytic LHMDS with TMEDA for the synthesis of functionalized 2,6-*cis*-tetrahydropyran. A marine sponge extract was analyzed for the presence of (−)-2 as the biosynthetic precursor of (−)-zampanolide.

In 1996, Tanaka and Higa reported isolation of (−)-zampanolide, (−)-**1**, the first representative of a novel class of 20-membered cytotoxic macrolides endogenous to the marine sponge *Fasciospongia rimosa* found at Cape Zampa in Okinawa.[1] Five years later, Riccio et al. isolated (+)-dactylolide, (+)-**2**, the structurally related metabolite from a different marine sponge belonging to the genus *Dactylospongia* collected off the coast of Vanuatu island.[2] Through total synthesis of both structures, Smith and co-workers were first to observe the apparent enantiomeric relationship vis-à-vis the common macrocyclic domains of (−)-**1** and (+)-**2**, a rare occurrence in marine natural products (Figure 1).[3,4c] This observation aptly suggests that if dactylolides were either a direct biosynthetic precursor to zampanolide or perhaps a degradation product thereof, one should expect to

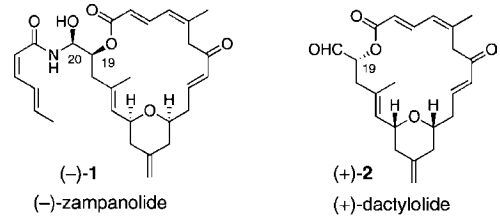


Figure 1. Structures of (−)-zampanolide and (+)-dactylolides.

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(1) Tanaka, J.; Higa, T. *Tetrahedron Lett.* **1996**, *37*, 5535–5538.
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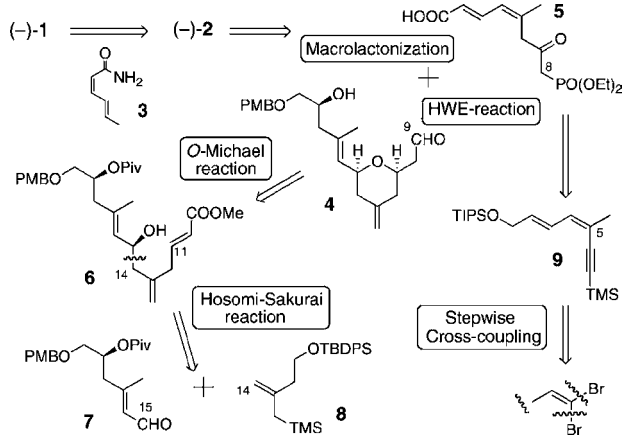
either find both (−)-**1** and (−)-**2** in *F. rimosa* or (+)-**1** together with (+)-**2** in *Dactylospongia* sp.; however, neither was the case.[1,2] Indeed, to our knowledge no isolation reports of (+)-**1** and (−)-**2** from any marine organisms have been disclosed in the literature.

Figure 2. Marine sponge *Fasciospongia rimosa* residing at a 20 m depth at Cape Zampa in Okinawa.

A potent cytotoxic agent (IC_{50} = 1−5 ng/mL against several cancer cell lines), zampanolide possesses a unique molecular architecture comprising a highly unsaturated macrolactone ring, a bridged 2,6-*cis*-tetrahydropyran unit, and an exocyclic *N*-acylhemiaminal group with a pendant *N*-(2*Z*,4*E*)-hexadienoylamide (**3**).[1] The latter structural feature has recently been implicated in the much enhanced biological activity of (−)-**1** relative to that of (−)-**2**.[5c] Not surprisingly, zampanolide and dactylolide have drawn much attention from synthetic chemists over the past few years, culminating in several total syntheses of these natural products.[4,5]

Herein, we disclose a new concise total synthesis of (−)-**1** and (−)-**2**, the former arising from an acid-catalyzed union of (−)-**2** with **3**, and discuss the questionable existence of (−)-dactylolide as an elusive biosynthetic precursor to (−)-zampanolide in *F. rimosa*.

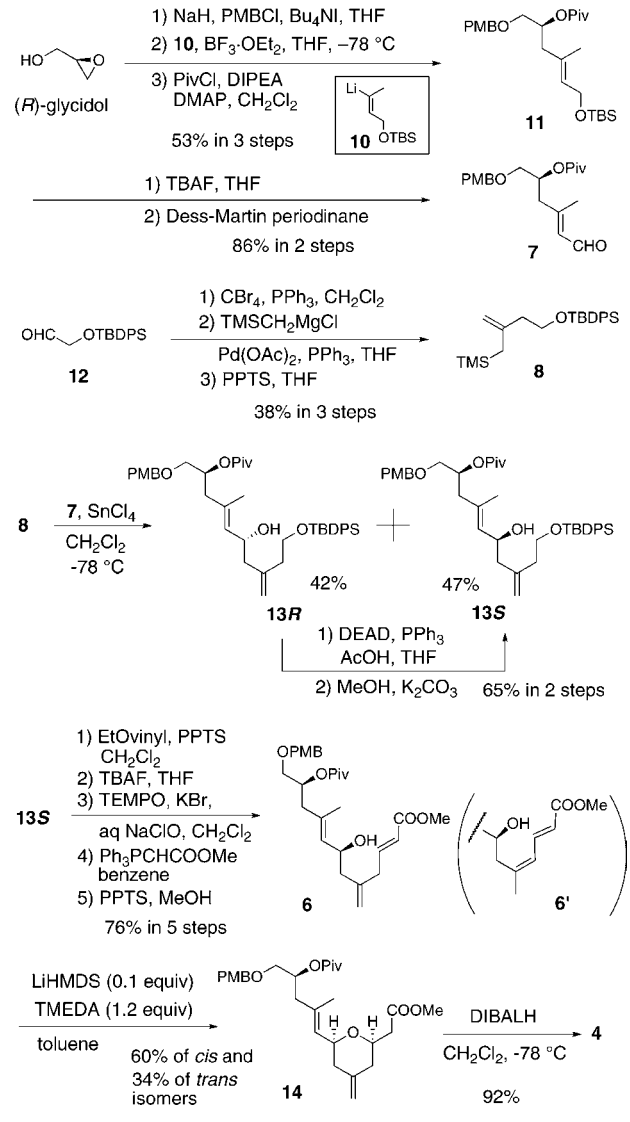
Scheme 1. Retrosynthetic Analysis of (−)-Zampanolide, (−)-**1**



Our overall synthetic strategy is outlined in Scheme 1. We envisaged the entire carbon framework of the macrolactone ring to be rapidly assembled via the Horner−Wadsworth−Emmons (HWE) reaction of fragments **4** and **5** immediately followed by macrolactonization.[6] Acid-catalyzed *N*-hemiacetalization of **2** with **3** would afford **1** in the final step. Stereoselective intramolecular *O*-Michael reaction of **6** would result in the formation of the desired *cis* tetrahydropyran **4**. The C14−C15 bond in **6** would be constructed by a Hosomi−Sakurai reaction of aldehyde **7** with allylsilane **8**. The remaining fragment, *β*-keto-phosphonate **5**, would be derived from the conjugated dienyne **9** by exploiting the potential reversal of the double bond geometry via the order in which the Sonogashira and Kumada−Tamao−Corriu couplings are performed on the 1,1-dibromoalkene precursor, a strategy previously reported by us.[7]

The synthesis of **4**, shown in Scheme 2, commenced from PMB protection of commercial (*R*)-glycidol. Ring opening

Scheme 2. Synthesis of C9−C20 Fragment **4**



of epoxide with (*Z*)-1-(*tert*-butyldimethylsilyl)-oxy-2-buten-3-yllithium **10**[8] in the presence of BF_3•OEt_2 followed by protection of the derived hydroxy group with PivCl afforded

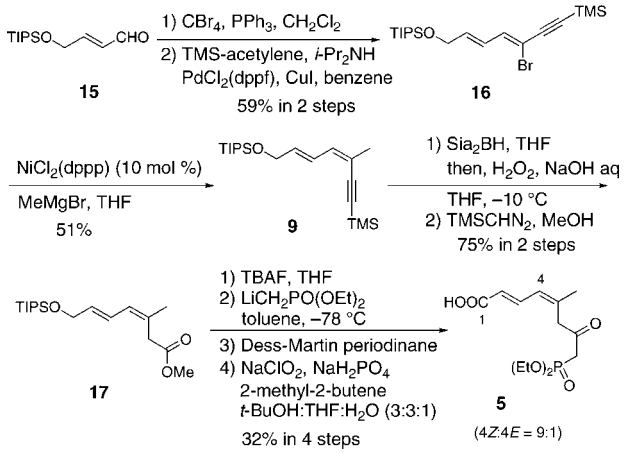
11. Deprotection of silyl ether and Dess−Martin oxidation gave aldehyde **7** in 46% overall yield for the five-step sequence. Allylsilane **8**, the coupling partner of **7** in the proposed Hosomi−Sakurai reaction, was prepared from aldehyde **12** in three steps: (i) dibromomethylenation,[9] (ii) Kumada−Tamao−Corriu coupling with $TMSCH_2MgCl$, and (iii) protodesilylation of the resulting bisallylsilane.[10] The $SnCl_4$-promoted Hosomi−Sakurai reaction of **7** with **8** gave the desired **13*S*** in 47% yield and its isomer **13*R*** in 42% yield, which was converted to **13*S*** in 65% yield by Mitsunobu reaction and methanolysis of the resulting acetate. Conversion of **13*S*** to **6** entailed the efficient five-step sequence of the following transformations proceeding in 76% overall yield: 1-ethoxyethyl ether protection of the secondary alcohol, removal of the TBDPS group and TEMPO oxidation of the derived primary alcohol to the corresponding aldehyde, two-carbon Wittig homologation to the (*E*)-α,*β*-unsaturated ester, and hydrolysis of the ethoxyethyl ether.

The THP ring of **14** was constructed by the intramolecular *O*-Michael reaction. Originally, standard cyclization conditions such as *t*-BuOK in THF[11] gave poor yields of cyclized products. Switching to LHMDS as a base and running the reaction below −40 °C predominantly gave the undesired *trans* isomer over the *cis* isomer in a 2: 1 ratio in 60−79% yield.[12] However, raising the reaction temperatures above −10 °C resulted in the formation of thermodynamic *cis* isomer (20%) rather than the *trans* isomer (8%), and acyclic conjugated ester **6′** (66%) was produced by the isomerization of the *exo* double bond. In the end, the optimized reaction conditions (LHMDS 0.1 equiv, TMEDA 1.2 equiv; toluene, rt, 30 min) provided a 1.8:1 *cis/trans* mixture of the two tetrahydropyrans, readily separable by chromatography, in 94% combined yield.[13] Gratifyingly, exposure of **14** to DIBALH (3 equiv) resulted in reduction of the ester moiety to the aldehyde with concomitant removal of the pivaloate group to give hydroxy aldehyde **4** in 92% yield.

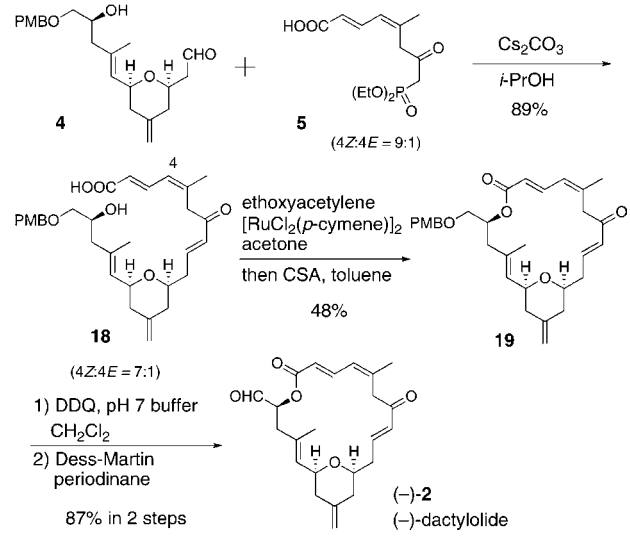
Preparation of *β*-ketophosphonate **5** is summarized in Scheme 3. Dibromomethylenation[9] of aldehyde **15** followed by the stereoselective Sonogashira coupling with TMS-acetylene gave (3*Z*,5*E*)-3-bromo-3,5-dienyne **16**. Introduction of the methyl group in **16** via the Kumada−Tamao−Corriu coupling proceeded with the anticipated inversion[7] of olefin geometry, providing the desired trisubstituted dienyne **9** as the major component of a 4:1 isomeric mixture. The terminal TMS-acetylene unit was then converted to the corresponding methyl ester by way of a hydroboration−oxidation−esterification sequence to give **17** in 75% overall yield. Deprotection of the TIPS ether, introduction of diethyl methylphosphonate, and a two-step oxidation of the primary alcohol completed the synthesis of **5** in 32% overall yield from **17**.[14]

The synthesis of (−)-dactylolide was completed as shown in Scheme 4. To our delight, the HWE reaction of aldehyde **4** and *β*-ketophosphonate **5** successfully forged the C8−C9 bond in the presence of unprotected hydroxy group and carboxylic acid moieties; seco acid **18** was thus obtained in

Scheme 3. Synthesis of C1−C8 Fragment **5**



Scheme 4. Synthesis of (−)-Dactylolide



(3) Another example of antipodal natural products of marine orgin was reported in the case of (+)-wistarin and (−)-wistarin, see: (a) Gregson, R. P.; Ouvrier, D. J. *J. Nat. Prod.* **1982**, *45*, 412–414. (b) Fontana, A.; Fakhr, I.; Mollo, E.; Cimino, G. *Tetrahedron: Asymmetry* **1999**, *10*, 3869–3872.

(4) Synthesis of (−)-**1**: (a) Hoye, T. R.; Hu, M. *J. Am. Chem. Soc.* **2003**, *125*, 9576–9577. Synthesis of (+)-**1**: (b) Smith, A. B., III; Safonov, I. G.; Corbett, R. M. *J. Am. Chem. Soc.* **2001**, *123*, 12426–12427. (c) Smith, A. B., III; Safonov, I. G.; Corbett, R. M. *J. Am. Chem. Soc.* **2002**, *124*, 11102–11113.

(5) Synthesis of (−)-**2**: (a) Ding, F.; Jennings, M. P. *Org. Lett.* **2005**, *7*, 2321–2324. (b) Louis, I.; Hungerford, N. L.; Humphries, E. J.; McLeod, M. D. *Org. Lett.* **2006**, *8*, 1117–1120. (c) Ding, F.; Jennings, M. P. *J. Org. Chem.* **2008**, *73*, 5965–5976. Synthesis of (+)-**2**: (d) Smith, A. B., III; Safonov, I. G. *Org. Lett.* **2002**, *4*, 635–637. (e) Aubele, D. L.; Wan, S.; Floreancig, P. E. *Angew. Chem., Int. Ed.* **2005**, *44*, 3485–3488. (f) Sanchez, C. C.; Keck, G. E. *Org. Lett.* **2005**, *7*, 3053–3056.

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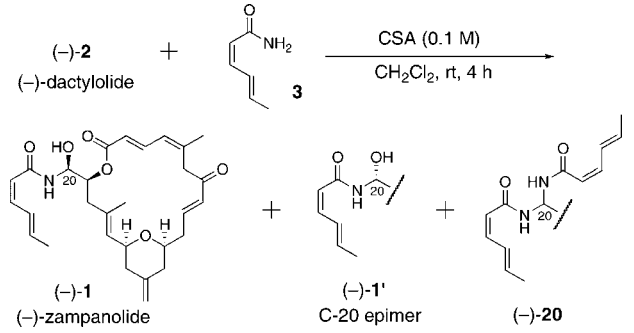
(7) Uenishi, J.; Matsui, K.; Ohmi, M. *Tetrahedron Lett.* **2005**, *46*, 225–228.

(8) Vinyllithium **10** was generated from the corresponding tributylvinylstanne with BuLi. The stanne, see: Uenishi, J.; Kawahama, R.; Yonemitsu, O.; Wada, A.; Ito, M. *Angew. Chem., Int. Ed.* **1998**, *37*, 320–323.

89% yield.[14,15] Cyclization of **18** using the Trost−Kita method[16] gave **19** in 48% yield.[17] Finally, deprotection of the PMB group and oxidation of the resulting alcohol furnished (−)-**2**[18] in 87% yield over two steps.

Next, we turned our attention to the conversion of (−)-dactylolide to (−)-zampanolide. Although Hoye et al. used the aluminum reagent of **3** with (−)-**2** in the *N*-hemiaminalization step,[4a] we anticipated a simple acid-promoted union of (−)-**2** with hexadienoylamide **3**. Indeed, as shown in Scheme 5, a reaction of (−)-**2** and **3** in the presence of

Scheme 5. Synthesis of (−)-Zampanolide



CSA afforded a mixture of (−)-zampanolide (−)-**1**[18,19] (12%), its C20-epimer (−)-**1′** (12%),[18] and bis(*N*-acyl)product (−)-**20** (16%) along with the recovery of (−)-**2** (35%).[20]

In our hand, (−)-**1** exhibited potent cytotoxic activity against SKM-1 and U937 cell lines with IC_{50} values of 1.1−2.9 nM. Interestingly, (−)-**1′** and (−)-**20** are approximately 10- and 500-fold less active, respectively.[21]

At this stage, we had access to four spectroscopically characterized 20-membered macrocylic compounds. An extract obtained from freshly isolated *F. rimosa* was probed by LC-MS for the presence of these macrolides in the sponge tissue. Surprisingly, this analysis turned up no evidence of (−)-dactylolide as a distinct entity in the sponge extract; compounds (−)-**1′** and (−)-**20** were not observed either. Since thermolysis of (+)-zampanolide in benzene provided (+)-dactylolide,[4c] one may consider the possibility that the latter is an artifact produced from decomposition of (+)-**1** during isolation. However, upon individual exposure of (−)-zampanolide and the sponge to the extraction conditions reported by Riccio et al., no trace of (−)-dactylolide was detected by us in either case. Therefore, we conclude that (+)-dactylolide is indeed a naturally occurring entity, not a degradation artifact of yet to be isolated (−)-zampanolide. Currently, we hypothesized that the macrolactone ring may form after formation of *N*-acylhemiaminal in the sponge.[22]

In summary, the total synthesis of (−)-dactylolide was accomplished in 17 linear steps in 6.5% overall yield from commercial (*R*)-glycidol via a short access to seco acid **18** by the HWE reaction of hydroxyaldehyde **4** with β-ketophosphonate **5**. (−)-Zampanolide was derived synthetically from (−)-dactylolide in one step, though natural (−)-zampanolide would not be presumed to be produced directly from (−)-dactylolide because the latter was not present in the sponge extract.

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Supporting Information Available: Experimental procedures and product characterization data including copies of NMR spectra, This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Uenishi, J.; Iwamoto, T.; Ohmi, M. *Tetrahedron Lett.* **2007**, *48*, 1237–1240.
(11) For examples of THP ring formation by the intramolecular *O*-Michael reactions, see: (a) Fettes, A.; Carreira, E. M. *J. Org. Chem.* **2003**, *68*, 9274–9283. (b) Bhattacharjee, A.; Soltani, O.; De Brabander, J. K. *Org. Lett.* **2002**, *4*, 481–484.
(12) Discussion of stereochemistry for intramolecular *O*-Michael reaction: (a) Betancort, J. M.; Martin, V. S.; Padron, J. M.; Palazon, J. M.; Ramirez, M. A.; Soler, M. A. *J. Org. Chem.* **1997**, *62*, 4570–4583. (b) Ramirez, M. A.; Padron, J. M.; Palazon, J. M.; Martin, V. S. *J. Org. Chem.* **1997**, *62*, 4583–4590.
(13) The undesired *trans* isomer was isomerized in the presence of NHMDS in toluene to a 3:2 mixture of *cis* and *trans* isomers in 65% yield.
(14) A δ-methyl-ζ-keto-$\alpha\beta$, γ,δ-unsaturated acid unit was isomerized slowly under the acidic and basic conditions to give geometric mixtures.
(15) The analogous common approach was unsuccessful in the case listed in ref 6.
(16) (a) Trost, B. M.; Chisholm, J. D. *Org. Lett.* **2002**, *4*, 3743–3745. (b) Ohba, Y.; Takatsuji, M.; Nakahara, K.; Fujioka, H.; Kita, Y. *Chem.−Eur. J.* **2009**, *15*, 3526–3537.
(17) The corresponding Yamaguchi and Shiina macrolactonization methods gave the desired product in only 33% and 20% yields, respectively. Dienoic substrates occasionally gave unsatisfactory yields in macrolactonizations; for example, see: Amans, D.; Bellosta, V.; Cossy, J. *Org. Lett.* **2007**, *9*, 4761–4764.
(18) All spectroscopic data, including the optical rotation, were in complete agreement with those reported previously: (−)-**2** in refs 2 and 5, (−)-**1** in refs 1 and 4, and (−)-**1′** in ref 4.
(19) Use of chiral organic acids failed to improve upon diastereoselectivity of this transformation.
(20) We have found that the reaction of (−)-**2** and **3** to (−)-**1** and (−)-**1′** to (−)-**20** was irreversible, though that of (−)-**2** and **3** to (−)-**1** and (−)-**1′** was reversible. When the reaction continued for additional 20 h, (−)-**20** was obtained exclusively.
(21) Measured IC_{50} values against SKM-1 and U937 cell lines: (−)-**1**, 1.1 and 2.9 nM, respectively; (−)-**1′**, 10 and 27 nM; and (−)-**20**, 490 and 950 nM.
(22) Still, the possibility of existence of (−)-**2** in *F. rimosa* can not be ruled out completely. Further studies are in progress.