Natural Products Synthesis

Total Synthesis and Structural Assignment of Spongidepsin through a Stereodivergent Ring-Closing-Metathesis Strategy**

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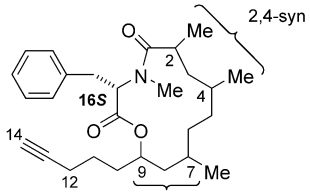
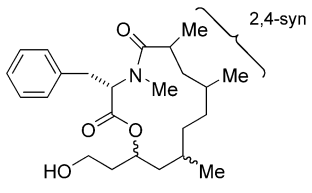
Spongidepsin (**1**) is a remarkable natural product isolated recently from a *Spongia* sp. sponge collected off the Vanuatu Islands, Australia, by Riccio and co-workers.[1] Its cytotoxic and antiproliferative activities against J774.A1, WEHI-164, and HEK-293 cancer cell lines are accompanied by an unprecedented structure.[1] The genus *Spongia* is a well-known source of diterpenoid and polyketide natural products, such as epispongiadiol[2] and spongistatin,[3] respectively. However, **1** reflects a distinct biogenetic origin that combines



7,9-unassigned
1: Spongidepsin



[(2*S*,4*S*) or (2*R*,4*R*) + 7*R*/*S*,9*R*/*S*)]-**2**

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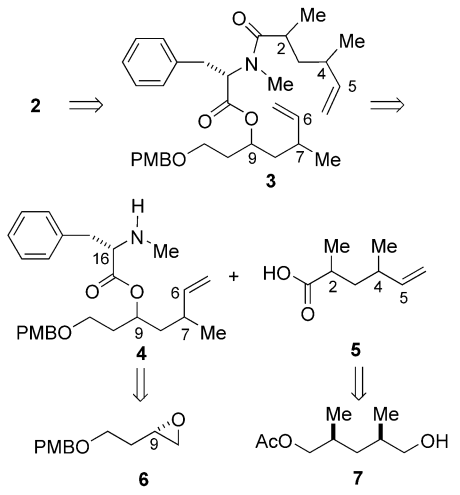
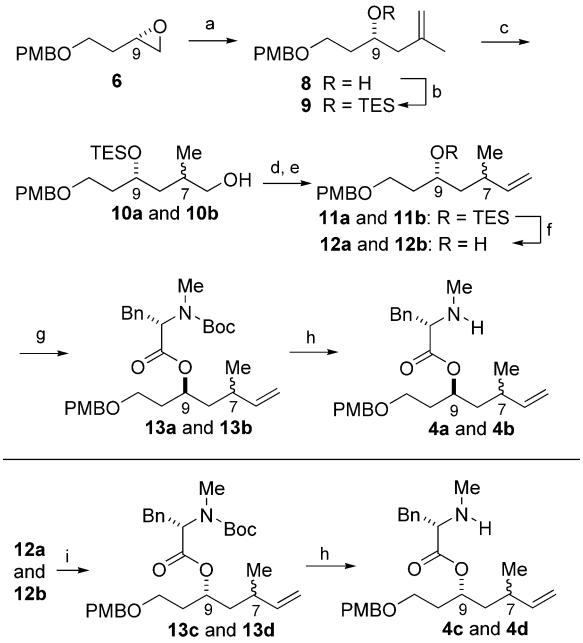
amino acid and unprecedented ketide motifs within a 13-membered macrocycle. The ketide domain is comprised of a 9-hydroxy-2,4,7-trimethyltetradeca-14-ynoic acid, while the amino acid was established as (*S*)-*N*-methylphenylalanine by Marfey analysis of the acidic hydrosylate of **1**.[1,4] The dimethyl substitution at C2 and C4 of **1** was determined to be *syn* by application of Murata's NMR spectroscopic-based method, but the absolute configuration was not established.[1,5] Neither the relative, nor the absolute stereochemistry of the two remaining stereogenic centers at C7 and C9 were originally assigned, partly owing to unfavorable ^{1}H NMR spectral overlap. Hence, the actual structure of **1** could have been one of eight possible stereoisomers (2*S*,4*S* or 2*R*,4*R* + 7*R*/*S*,9*R*/*S*). The complete structural definition of **1** should extend our understanding of the complex biosynthetic diversity of *Spongia* isolates, while the development of a total synthesis should facilitate the complete biological evaluation of **1**. For this, a stereodivergent total synthesis strategy that features macrocycle formation through ring-closing metathesis (RCM) as the key step was employed. The successful implementation of this plan culminated in the full structural elucidation and total synthesis of spongidepsin, as summarized herein.

The stereochemical-determination strategy relied on the preparation of all eight possible diastereoisomers of the macrolide-containing portion **2** of spongidepsin incorporating (*S*)-*N*-methylphenylalanine. These include both the *2S*,4*S* and *2R*,4*R* enantiomers of the *syn*-2,4-dimethyl moiety conjoined with the four diastereomeric combinations of *R*,*S* isomers at C7 and C9. Comparison of the spectral data of each of the diastereomeric probes **2** with those of natural spongidepsin would, ideally, indicate which isomer to advance selectively in the total synthesis of **1**. As shown in Scheme 1, the 13-membered macrolides **2** would be prepared by RCM of dienes **3** and subsequent alkene hydrogenation. The RCM substrates **3**, in turn, be derived from the C1–C5 and C6–C11 fragments **5** and **4**, respectively. The two enantiomers of *syn*-2,4-dimethyl carboxylic acid **5** are derivable from acetate alcohol **7** by alternative manipulations of the terminal functional groups. Each of the four stereoisomers of **4** would originate from the known L-malate-derived epoxide **6**, which bears a C9 stereogenic center.[6]



Scheme 1. Retrosynthesis of macrolides **2**.

The synthesis began with the CuI-mediated opening of epoxide **6** with a 2-bromopropene-derived Grignard reagent to give secondary alcohol **8** (Scheme 2). The hydroxy group of

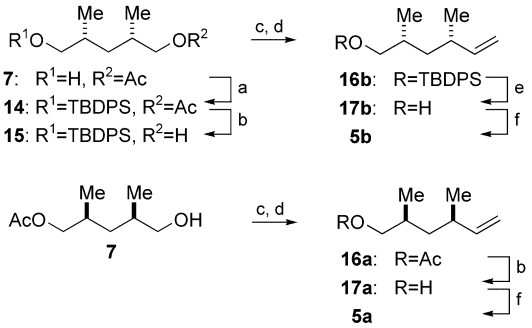


Scheme 2. Synthesis of esters **4a**–**d**. Reagents and conditions: a) 2-bromopropene (3 equiv), Mg, CuI (0.3 equiv), THF, −60°C, 30 min, 86%; b) TESCl (1.5 equiv), imidazole (3 equiv), DMAP (0.1 equiv), CH_2Cl_2, 1 h, 97%; c) BH_3·THF (2.2 equiv), THF 0°C, 2 h; NaOH, H_2O_2, 2 h, 95%; d) TPAP (0.08 equiv), NMO (1.5 equiv), molecular sieves (4 Å; 500 mg mmol^{-1}), CH_2Cl_2, 30 min; e) CH_2Br_2, Zn, $TiCl_4$, CH_2Cl_2, 10 min, 75% over two steps; f) TBAF (1.5 equiv), THF, 1 h, 99%; g) DIAD (3 equiv), Ph_3P (3 equiv), THF, *N*-Me-*N*-Boc-Phe (1.5 equiv), 10 min, 91%; h) TBSOTf (1.5 equiv), 2,6-lutidine (2 equiv), CH_2Cl_2, 1.5 h; TBAF (1.1 equiv), THF, 1 h, 82% over two steps for **4a** and **4b**, 85% over two steps for **4c** and **4d**; i) $Cl_3C_6H_2COCl$ (1.2 equiv), DIPEA (3 equiv), *N*-Me-*N*-Boc-Phe (1.1 equiv), THF; DMAP, toluene, 89%. *N*-Me-*N*-Boc-Phe = (*S*)-*N*-methyl-*N*-Boc-phenylalanine, PMB = 4-methoxybenzyl, TES = triethylsilyl, Boc = *tert*-butyl carbamate, Bn = benzyl, DMAP = 4-dimethylaminopyridine, TPAP = tetrapropylammonium perruthenate, NMO = 4-methylmorpholine *N*-oxide, TBAF = tetra-*n*-butylammonium fluoride, DIAD = diisopropyl azodicarboxylate, TBS = *tert*-butyldimethylsilyl, Tf = trifluoromethanesulfonyl.

8 was converted into TES ether **9**, and the alkene was subjected to a hydroboration–oxidation sequence to install the C7 stereogenic center intentionally as an approximately equal molar ratio of primary alcohols (7*R*,9*R*)-**10a** and (7*S*,9*R*)-**10b**. Attempts to separate **10a** and **10b** or various simple derivatives thereof from one another were unsuccessful. It was anticipated, however, that the C7 epimers would be separated at the stage of the conformationally constrained 13-membered macrolides resulting from RCM. Thus, the diastereomeric mixture of **10a** and **10b** was converted into the corresponding alkenes **11a** and **11b** through an oxidation[7]–olefination[8] sequence. Liberation of the secondary hydroxy group of **11a** and **11b** followed by Mitsunobu esterification[9] with (*S*)-*N*-methyl-*N*-Boc-phenylalanine

yielded C9-inverted esters (7*R*,9*S*)-**13a** and (7*S*,9*S*)-**13b**. Stepwise cleavage of the *N*-Boc carbamates[10] from **13a** and **13b** generated secondary amines **4a** and **4b**. The other two C7–C9 stereoisomers (7*R*,9*R*)-**4c** and (7*S*,9*R*)-**4d** were prepared from **12a** and **12b** and (*S*)-*N*-methyl-*N*-Boc-phenylalanine with retention of (9*R*)-configuration via Yamaguchi esterification.[11]

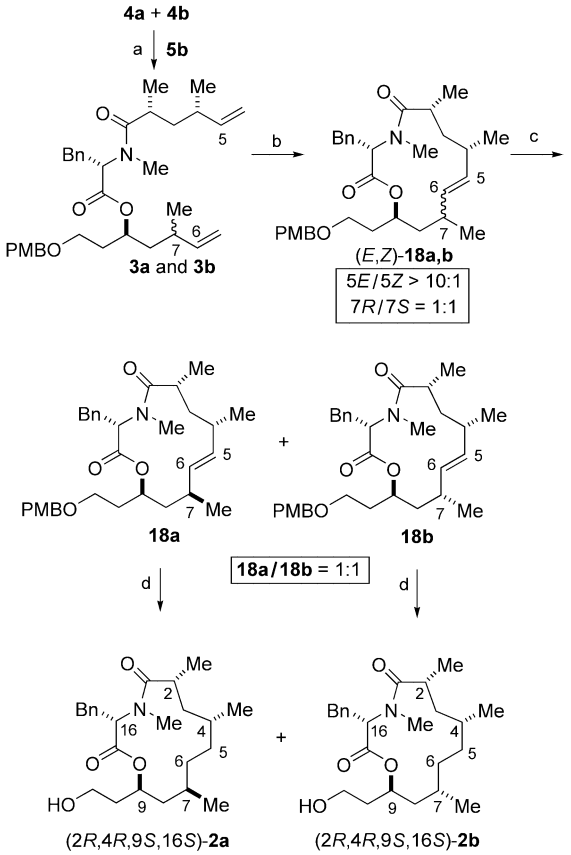
The enantiomeric *syn*-2,4-dimethyl-substituted carboxylic acids (2*S*,4*R*)-**5a** and (2*R*,4*S*)-**5b**, one of which corresponds to the C1–C5 moiety of **1**, were prepared from monoacetate (2*S*,4*R*)-**7** (Scheme 3). Acetate **7**, in turn, was obtained by



Scheme 3. Synthesis of carboxylic acids **5a** and **5b**. Reagents and conditions: a) TBDPSCl (1.5 equiv), imidazole (2.5 equiv), DMAP (0.1 equiv), CH_2Cl_2, 1.5 h, 93%; b) K_2CO_3 (1.5 equiv), MeOH, 4 h, ≈87%; c) TPAP (0.08 equiv), NMO (1.5 equiv), molecular sieves (4 Å; 500 mg mmol^{-1}), CH_2Cl_2, 20 min; d) CH_2Br_2, Zn, $TiCl_4$, CH_2Cl_2, 10 min, ≈67% over two steps; e) TBAF (1.5 equiv), THF, 3 h, ≈86%; f) Jones reagent (excess), acetone, 30 min, 65%. TBDPS = *tert*-butyldiphenylsilyl.

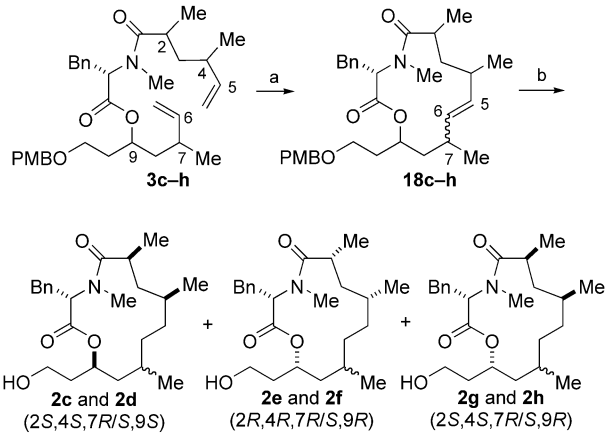
enzymatic resolution of the corresponding *meso* diol.[12] For the synthesis of **5b**, alcohol **7** was silylated to yield **14**, then the acetate terminus was converted into an alkene (**16b**) in a stepwise fashion culminating in a Lombardo olefination.[8] Desilylation of **16b** followed by Jones oxidation of the resultant alcohol **17b** furnished carboxylate **5b**. Its enantiomer **5a** was similarly obtained from monoacetate **7** through the complementary set of terminal functionalizations indicated in Scheme 3.

With each of the four amine diastereomers **4a–d** and the two enantiomeric carboxylic acids **5a** and **5b** available, the synthesis of the eight diastereomeric macrolides **2** was addressed. PyAOP-mediated amide formation[13] of the diastereomeric mixture of amines **4a** and **4b** with carboxylic acid **5b** proceeded smoothly to afford the corresponding amides **3a** and **3b** (Scheme 4). Exposure of **3a** and **3b** to the second-generation Grubbs catalyst[14] in refluxing toluene yielded the four possible macrocycle 5*E*/*Z*,7*R*/*S* diastereomers in 80% combined yield. The two *E* alkenes (**18a** and **18b**) were obtained in a 1:1 ratio and predominated over the *Z* isomers by >10:1. As anticipated, the two C7 epimers **18a** and **18b** were separated from one another easily by flash column chromatography. The absolute stereochemical assignment of C7 in compounds **18a** and **18b** was not made at this stage, although each isomer could be obtained in diastereomerically pure form. The two diastereomeric alkenes **18a** and **18b** were separately subjected to palladium-catalyzed hydrogenation to provide the corresponding saturated macrolides **2a** and **2b**.

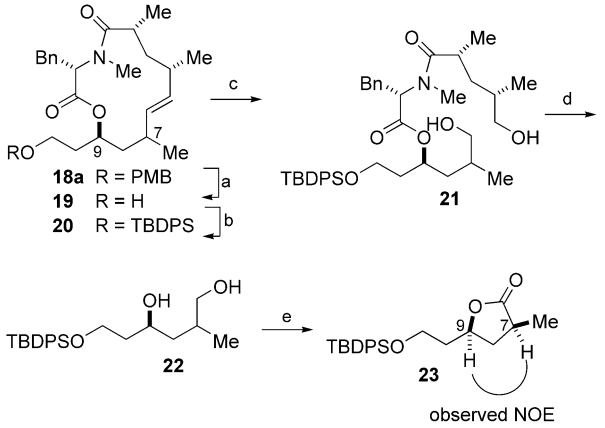


Scheme 4. Synthesis of macrolides **2a–b**. Reagents and conditions: a) PyAOP (1.2 equiv), DIPEA (2 equiv), DMF, 24 h, 87%; b) second-generation Grubbs catalyst[14] (0.1 equiv), toluene, 110°C, 20 min, 80% combined yield; c) silica gel chromatographic separation; d) H_2, Pd/C (0.1 equiv), EtOAc, 8 h, ≈88%. PyAOP = (7-azabenzotriazole-1-yloxy)-tripyrrodinophosphonium hexafluorophosphate, DIPEA = diisopropylethylamine, DMF = *N*,*N*-dimethylformamide.

The remaining six diastereoisomeric macrolides **2c–h** were prepared in a similar fashion from the corresponding acids and amines through amide formation, RCM, and hydrogenation (Scheme 5). Among the eight diastereoisomers of **2** prepared, the ^{1}H and ^{13}C NMR spectral data of (2*R*,4*R*,9*S*,16*S*)-**2a** best matched those of natural spongidepsin.[15] To determine the configuration at C7, the RCM adduct **18a** (the direct precursor to macrolide **2a**) was chosen for degradative analysis. First, the PMB ether **18a** was converted into TBDPS ether **20** as a prelude to ozonolytic cleavage of the alkene moiety (Scheme 6). Ozonolysis of **20** followed by reductive workup afforded diol **21**. Hydrolysis of the ester moiety of **21** with LiOH in aqueous *t*BuOH yielded 1,4-diol **22**, which was oxidatively cyclized into five-membered lactone **23** with TEMPO/BAIB.[16] Extensive NOE studies and detailed ^{1}H–^{1}H coupling-constant analysis with reference to analogous known *cis* and *trans* lactones,[17,18] indicated that the methyl and (silyloxy)ethyl substituents were *cis* to each other in lactone (*S*,*S*)-**23**. Hence, macrolide **2a** was assigned the corresponding 7*R*,9*S* stereochemistry. Given that the configuration at C9 is established from L-malic acid via



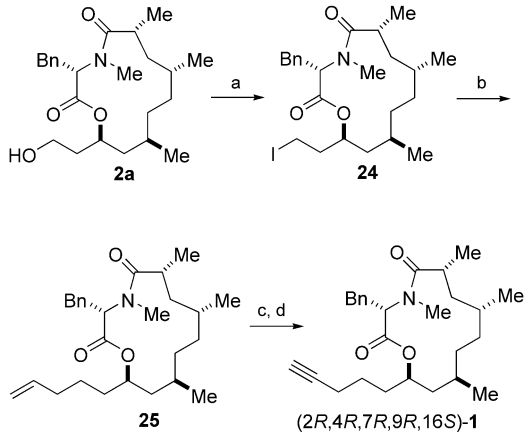
Scheme 5. Synthesis of macrolides **2c–h**. Reagents and conditions: a) second-generation Grubbs catalyst[14] (0.1 equiv), toluene, 110 °C, 20 min; b) H_2, Pd/C (0.1 equiv), EtOAc, 8 h.



Scheme 6. Elucidation of the configuration of **18a** at C7. Reagents and conditions: a) DDQ (5 equiv), *t*BuOH, H_2O, CH_2Cl_2, 10 min sonication, 89%; b) TBDPSCl (1.5 equiv), imidazole (2 equiv), DMAP (0.1 equiv), CH_2Cl_2, 3 h, 85%; c) O_3, MeOH, 10 min; $NaBH_4$ (2 equiv), 3 h, 77%; d) LiOH (6 equiv), *t*BuOH/H_2O (4:1), 2 h, 74%; e) TEMPO (0.3 equiv), BAIB (3 equiv), CH_2Cl_2, 2 h, 65%. DDQ = 2,3-dichloro-5,6-dicyanoquinone, TEMPO = 2,2,6,6-tetramethyl-1-piperidinyloxy, BAIB = iodobenzene diacetate.

epoxide **6** with inversion of configuration during the formation of **13** and that (*S*)-*N*-methylphenylalanine[1] was employed throughout, **2a** was assigned the 2*R*,4*R*,7*R*,9*S*,16*S* configuration.[19]

To extend the stereochemical assignment of **2a** unambiguously to **1**, the former was further functionalized to complete a total synthesis. This involved conversion of the C11 alcohol of **2a** into the alkyne-terminated side chain of (2*R*,4*R*,7*R*,9*R*,16*S*)-**1**. First, the primary alcohol was transformed into iodide **24**, which was then treated with allyl tri-*n*-butyltin and catalytic AIBN to generate the allylation product **25** (Scheme 7). The resultant alkene was cleaved with K_2OsO_4 and $NaIO_4$ to give the corresponding aldehyde. Finally, the Bestmann reagent[20] was employed to convert the aldehyde into the corresponding terminal alkyne (2*R*,4*R*,7*R*,9*R*,16*S*)-**1**, which matched natural spongidepsin by ^1H and ^{13}C NMR spectroscopy, HRMS, and specific rotation [synthetic



Scheme 7. Total synthesis of (2*R*,4*R*,7*R*,9*R*,16*S*)-spongidepsin (**1**). Reagents and conditions: a) Ph_3P (2 equiv), imidazole (3 equiv), I_2 (1.5 equiv), THF, 20 min, 82%; b) allyl tri-*n*-butyltin (3 equiv), AIBN (0.5 equiv), benzene, 80 °C, 4 h, 85%; c) K_2OsO_4 (0.2 equiv), $NaIO_4$ (6 equiv), THF-H_2O (2:1), 1.5 h, 87%; d) K_2CO_3 (1.5 equiv), Bestmann reagent (1.5 equiv), MeOH, 3 h, 75%. AIBN = 2,2′-azobisisobutyronitrile, Bestmann reagent = dimethyl-1-diazo-2-oxopropylphosphonate.

(2*R*,4*R*,7*R*,9*R*,16*S*)-**1**: $[\alpha]_D = -67.3$ ($c = 1.00$, MeOH); *Spongia* isolate **1**:[1] $[\alpha]_D = -61.8$ ($c = 1.4$, MeOH)].

In summary, this work highlights the convergence of synthetic design, methodology, and spectroscopic analyses to fully define the structure and provide an alternative source of the recently described antiproliferative natural product spongidepsin. The complete stereochemical assignment and the total synthesis of **1** have been achieved through a stereochemically divergent strategy that employed macrolide-closure by ring-closing metathesis as a key step. Finally, the stereochemical assignments of the unprecedented 9-hydroxy-2,4,7-trimethyltetradeca-14-ynoic acid moiety may be relevant to biosynthetic congeners of **1**.

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Keywords: cyclization · metathesis · natural products · structure elucidation · total synthesis

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