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Synthetic Studies on Amphirionin-5: Stereochemical Assignment/Reassignment of the C1−C9 Portion through Stereodivergent Synthesis

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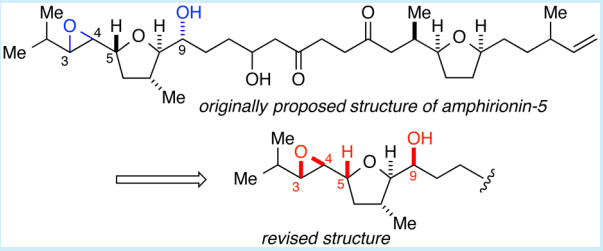
Supporting Information

ABSTRACT: Synthesis of four diastereomers of the C1−C12 fragment of amphirionin-5 has been achieved in a convergent and stereodivergent manner. Detailed comparison of the ^{1}H and ^{13}C NMR data of each compound with those reported for the natural product led to not only the stereochemical assignment of the relative configuration of the C4/C5 stereogenic centers but also reassignment of the proposed relative configuration at C9 of amphirionin-5.



Dinoflagellates of the genus *Amphidinium* are an enormously rich source of structurally diverse secondary metabolites of complex molecular architecture with potent biological activities. In particular, a number of potent cytotoxic macrolides, amphidinolides and iriomoteolides, have been isolated from *Amphidinium* sp. to date.[1] Recently, novel complex tetrahydrofuran-containing linear polyketide natural products with intriguing biological activities have been isolated from *Amphidinium* sp. by Tsuda and co-workers.[2−4] Of these, amphirionin-5 (**1**, Figure 1) was isolated in 2014 by Tsuda and co-workers from cultivated algal cells of the benthic dinoflagellate *Amphidinium* sp. (KCA09053 strain) collected off the coast of Iriomote Island, Okinawa Prefecture, Japan.[2] The gross structure and partial stereochemical assignment of amphirionin-5 was elucidated on the basis of 2D-NMR data and *J*-based configurational analysis[5] and found to consist of a linear polyketide skeleton containing two tetrahydrofuran rings, a *trans*-epoxide, and 11 stereogenic centers. However, despite extensive NMR studies, the relative configurations of the C4/C5 stereogenic centers and the stereochemistry of the two isolated stereogenic centers at C12 and C26 could not be resolved, and the absolute configuration has also remained undefined. These stereochemical problems can only be addressed efficiently using a synthetic approach.

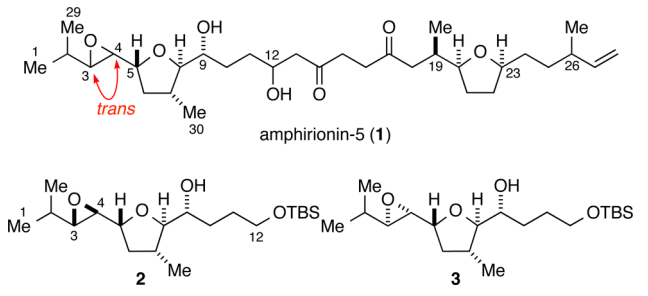


Figure 1. Structures of amphirionin-5 (**1**) and two possible diastereomeric C1−C12 fragments **2** and **3**.

Interestingly, this linear polyketide natural product was found to exhibit potent proliferation-promoting activity on murine bone marrow stromal ST-2 cells (282%) and murine osteoblastic MC3T3-E1 cells (320%) at a dose of 10 ng/mL, whereas it did not induce cellular differentiation or cellular morphological changes at a dose range of 0.001−1000 ng/mL and also exhibited no cytotoxicity at high doses (1−10 μg/mL).[2]

As part of our program toward the total synthesis and complete stereochemical assignment of amphirionin-5, we herein report the stereodivergent synthesis of four diastereomeric C1−C12 fragments and comparison of their NMR data with those reported for the natural product. This has led both to an assignment of the relative configuration of the C4/C5 stereogenic centers and a reassignment of the proposed relative configuration at C9 of amphirionin-5.

Our stereochemical-determination strategy for establishing the relative configuration of the C4/C5 stereogenic centers of amphirionin-5 relied on the synthesis of two possible diastereomers of the epoxide-containing C1−C12 fragments **2** and **3** (Figure 1). Comparison of their NMR spectroscopic data with those of the natural product would assign the relative configuration of C4/C5.[6−8] Our retrosynthetic plan for the C1−C12 fragments **2** and **3** is depicted in Scheme 1. We envisioned that the 2,5-*trans*-substituted tetrahydrofuran ring of **2** and **3** would be constructed through a domino Sharpless asymmetric dihydroxylation (SAD)[9]/stereospecific cyclization of mesylates **4** and **5**, respectively.[10] The two requisite diastereomeric epoxides **4** and **5** would be derived by branching from allylic alcohol **6** by Katsuki−Sharpless asymmetric epoxidation[11] using (+)- or

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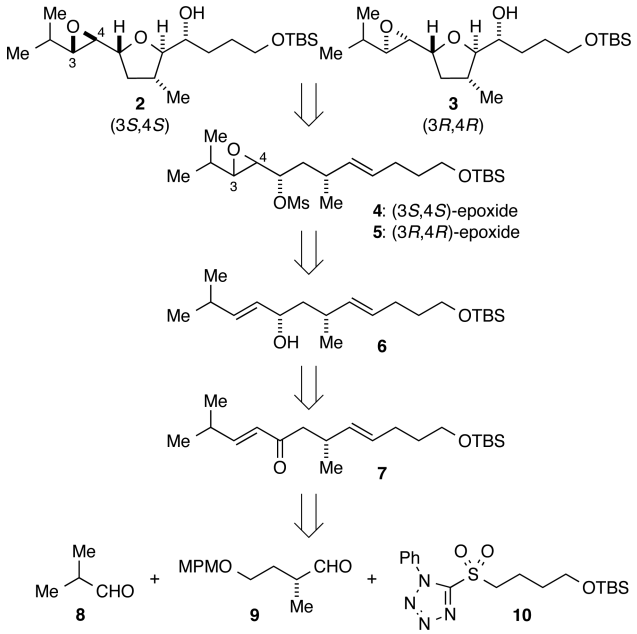
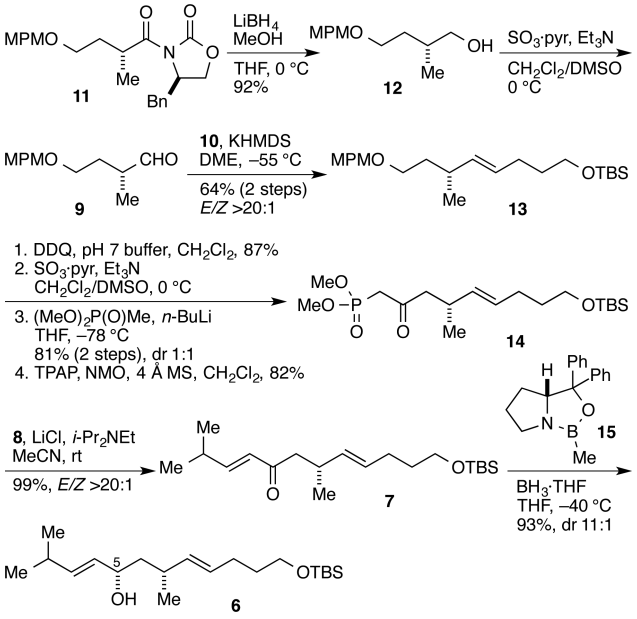
Scheme 1. Retrosynthesis of Two Diastereomeric C1−C12 Fragments 2 and 3



(−)-tartrate ester. Compound **6** would be accessed by means of Corey−Bakshi−Shibata (CBS) reduction[12] of enone **7**, which in turn would be assembled from three fragments, isobutyraldehyde (**8**), aldehyde **9**, and sulfone **10**, through Julia−Kocienski olefination[13] and Horner−Wadsworth−Emmons (HWE) reaction, in a convergent manner.

The synthesis of allylic alcohol **6** started with the known imide **11**.[14] Reductive removal of the chiral auxiliary in **11** with $LiBH_4$ (MeOH, THF, 0 °C)[15] afforded alcohol **12** in 92% yield (Scheme 2). Parikh−Doering oxidation[16] of **12** provided aldehyde **9**, which was then coupled with the known sulfone **10**[17] through Julia−Kocienski olefination (KHMDS, DME, −55 °C)[13] to give (*E*)-alkene **13** in 64% yield from alcohol **12** (*E*/*Z* > 20:1). The *p*-methoxyphenylmethyl (MPM) group of **13** was oxidatively removed with DDQ (87%), and the resultant primary alcohol was oxidized to the corresponding aldehyde. Treatment of the aldehyde with lithiated dimethyl methylphosphonate, followed by oxidation with tetra-*n*-propylammonium perruthenate (TPAP)/*N*-methylmorpholine *N*-oxide (NMO),[18] provided *β*-keto phosphonate **14** (66% yield for the three steps). HWE reaction of **14** with isobutyraldehyde (**8**) under Masamune−Roush conditions (*i*-Pr_2NEt, LiCl, MeCN)[19] led to (*E*)-enone **7** in 99% yield as a single stereoisomer (*E*/*Z* > 20:1). Finally, CBS reduction[12] of enone **7** using (*R*)-2-methyl-CBS-oxazaborolidine **15** provided the desired allylic alcohol **6** in 93% yield with an 11:1 diastereomer ratio.[20] The absolute configuration of the C5[21] stereogenic center was unambiguously established by a modified Mosher analysis.[22]

Scheme 2. Synthesis of Allylic Alcohol 6



Katsuki−Sharpless asymmetric epoxidation[11] of allylic alcohol **6** using (+)-diisopropyl tartrate (DIPT) as a chiral ligand delivered epoxy alcohol **16**[23] in 90% yield with high diastereoselectivity (dr ca. 23:1) (Scheme 3). Alcohol **16** was converted to the corresponding mesylate **4** (MsCl, Et_3N), which was then subjected to SAD using AD-mix-*β*.[9] Diastereoselective dihydroxylation and concomitant stereospecific cyclization proceeded smoothly to form a tetrahydrofuran ring, and the desired C1−C12 fragment **2** was obtained in 90% yield for the two steps. The 2,5-*trans* configuration of the tetrahydrofuran ring in **2** was confirmed by means of HMBC spectra and NOE data, and the absolute configuration of the C9 stereogenic center was unambiguously established by a modified Mosher analysis.[22,23]

The diastereomeric fragment **3** was prepared in a similar fashion from allylic alcohol **6** via epoxy alcohol **17** (Scheme 3). In this case, Katsuki−Sharpless asymmetric epoxidation of **6**[24] with (−)-DIPT is a typical "mismatched" pair[11b,c] and thus resulted in a 1.4:1 mixture of epoxides **17** and **16**, which were readily separated by flash column chromatography on silica gel. Epoxide **17** was advanced by employing the sequence described in the conversion of **16** to **2** to furnish **3** (86% yield, two steps).[23]

The ^1H and ^{13}C NMR chemical shifts in the C1−C9 region of the two diastereomeric C1−C12 fragments **2** and **3** were compared in detail with those of the corresponding moiety of the natural product.[25] As shown in Figure 2, the ^{13}C NMR chemical shifts in the C1−C6 region of fragment **2** matched with those reported for the natural product within ±0.7 ppm, while the diastereomer **3** displayed obviously different chemical shifts. In particular, the observed ^{13}C NMR chemical shifts for C5 and C6 of **3** distinctively deviated from those of the natural product ($\Delta\delta$ > 1.0 ppm). These results strongly suggested that natural amphirionin-5 has the (3*S**,4*S**,5*R**)-configuration shown for structure **2**. In contrast, there were large and similar discrepancies in the ^{13}C NMR chemical shifts for C7, C9, and C30 in the right-hand region of both compounds **2** and **3**. From these significant deviations in the NMR data between the synthetic fragments **2**/**3** and the natural product, we inferred that the C9 stereogenic center of amphirionin-5 might be misassigned and that the most likely configuration of the C1−C9 portion of amphirionin-5 is represented by the revised structure **18** (see Scheme 4).

Thus, inversion of the C9 hydroxy group of **2** and **3** was performed using modified Mitsunobu conditions (*p*-$NO_2C_6H_4CO_2H$, Ph_3P, diethyl azodicarboxylate (DEAD), THF)[26] followed by methanolysis (K_2CO_3, MeOH) to afford alcohols **18** and **19**, respectively, as shown in Scheme 4. Their NMR data were again compared with those of the natural product (Figure 3). Clearly, the ^{13}C NMR chemical shifts in the C1−C9 region of diastereomer **18** were virtually identical to

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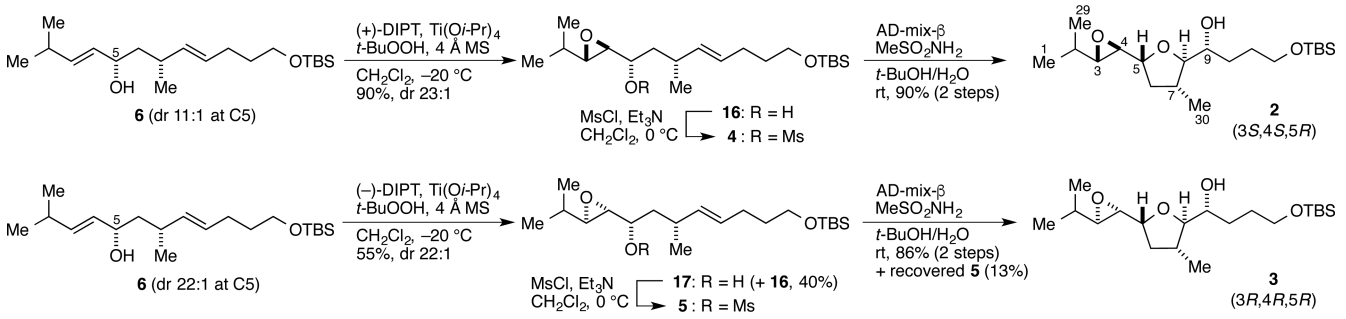
Scheme 3. Synthesis of the Diastereomeric C1−C12 Fragments 2 and 3



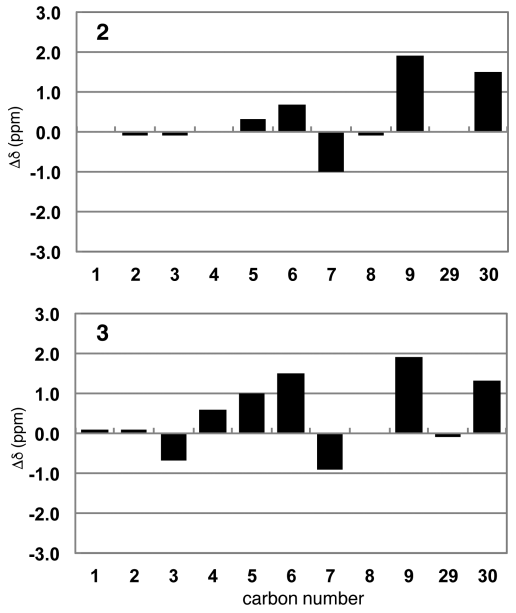


Figure 2. Differences in ^{13}C NMR chemical shifts between amphirionin-5 (125 MHz) and synthetic fragments **2** and **3** (150 MHz). $\Delta\delta = \delta$ (natural product) − δ (synthetic fragment) in ppm ($CDCl_3$).

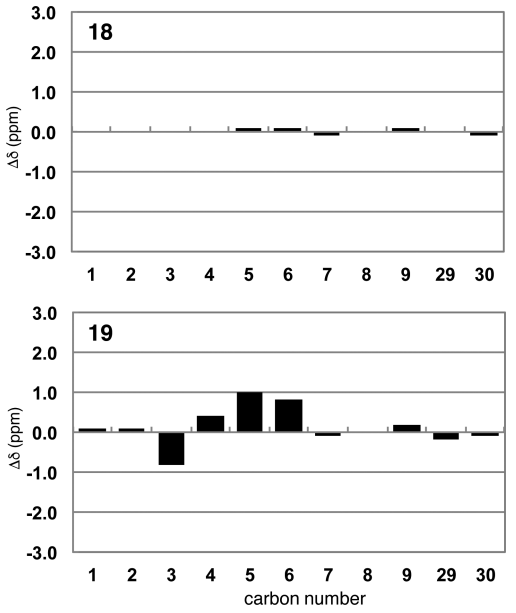
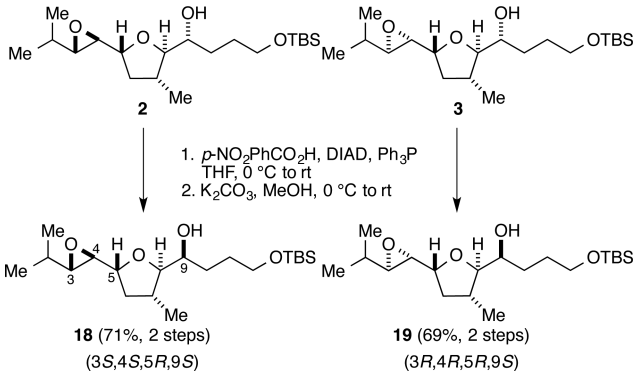


Figure 3. Differences in ^{13}C NMR chemical shifts between amphirionin-5 (125 MHz) and synthetic fragments **18** and **19** (150 MHz). $\Delta\delta = \delta$ (natural product) − δ (synthetic fragment) in ppm ($CDCl_3$).

Scheme 4. Synthesis of Diastereomers 18 and 19



those reported for the natural product.[25] In contrast, as with the case of compound **3**, distinct differences were observed in the ^{13}C NMR chemical shifts of the diastereomer **19** and the natural product in the C3−C6 region. Furthermore, $^{3}J_{H,H}$ data of the C1−C9 portion of **18** correspond well to the data of amphirionin-5.[25] These results convincingly defined the relative configuration of the C1−C9 portion of amphirionin-5 as that represented by structure **18** with the (3*S**,4*S**,5*R**,9*S**)-stereochemistry.

In conclusion, four diastereomers C1−C12 fragments of amphirionin-5 have been synthesized in a stereodivergent manner. The key features of the synthesis route include (1) convergent synthesis of the common intermediary allylic alcohol by employing Julia−Kocienski olefination, Horner−Wadsworth−Emmons reaction, and Corey−Bakshi−Shibata reduction and (2) efficient construction of the 2,5-*trans*-substituted tetrahydrofuran ring by a domino Sharpless asymmetric dihydroxylation/stereospecific cyclization. Comparison of the NMR data of the four diastereomers with those of the natural product allowed not only assignment of the relative configuration of the C4/C5 stereogenic centers but also reassignment of the proposed configuration at C9 of amphirionin-5. Further studies aimed at the complete stereochemical assignment and total synthesis of amphirionin-5 are underway and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b03346.

Experimental procedures, characterization data for all new compounds, stereochemical assignments for selected compounds, comparison of the NMR data of compounds

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2, **3**, **18**, and **19** with the data of amphirionin-5, and ^{1}H and ^{13}C NMR spectra for all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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(24) Asymmetric epoxidation using (−)-DIPT employed allylic alcohol **6** in a diastereomerically enriched form (dr 22:1) obtained by kinetic resolution. See the Supporting Information for details.

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