A Journal of the Gesellschaft Deutscher Chemiker

Angewandte International Edition

GDCh

Chemie

www.angewandte.org

Accepted Article

Title: Synthesis and Stereochemical Revision of the C31-C67 Section of Amphidinol 3

Authors: Yuma Wakamiya, Makoto Ebine, Mariko Murayama, Hiroyuki Omizu, Nobuaki Matsumori, Michio Murata, and Tohru Oishi

To be cited as: *Angew. Chem. Int. Ed.* 10.1002/anie.201712167
Angew. Chem. 10.1002/ange.201712167

Link to VoR: http://dx.doi.org/10.1002/anie.201712167
http://dx.doi.org/10.1002/ange.201712167

WILEY-VCH

10.1002/anie.201712167

WILEY-VCH

Synthesis and Stereochemical Revision of the C31–C67 Section of Amphidinol 3

*Yuma Wakamiya, Makoto Ebine, Mariko Murayama, Hiroyuki Omizu, Nobuaki Matsumori, Michio Murata, and Tohru Oishi**

Abstract: *Amphidinol 3 (AM3) is a marine natural product produced by the dinoflagellate Amphidinium klebsii. Although the absolute configuration of AM3 was determined in 1999 by extensive NMR analysis and degradation of the natural product, it was a daunting task due to the presence of numerous stereogenic centers on the acyclic carbon chain and the limited availability from natural sources. Thereafter, revisions of the absolute configurations at C2 and C51 were reported in 2008 and 2013, respectively. Herein, we revised the absolute configuration of AM3 to be 32S, 33R, 34S, 35S, 36S, and 38S based on the chemical synthesis of partial structures corresponding to the C31–C67 section of AM3 in combination with degradation of the natural product. The revised structure is unique in that both antipodal tetrahydropyran counterparts exist on a single carbon chain. The structural revision of AM3 may affect proposed structures of congeners related to the amphidinols.*

Amphidinol 3 (AM3) is a marine natural product produced by the dinoflagellate *Amphidinium klebsii*.[1] AM3 elicits potent antifungal and hemolytic activities, while its mode of action is not fully understood.[2] The planar structure of AM3 was reported in 1991,[1a] however, determination of the absolute configuration was a daunting task due to the limited availability of the natural product and the presence of a number of stereogenic centers located on the acyclic carbon chain. It was finally achieved in 1999 with the aid of extensive NMR analysis including the *J*-based configuration analysis (JBCA) method[3] (Figure 1a).[1b] To date, a number of congeners of amphidinols and related compounds,[4] have been identified. Among these congeners, the structure of karlotoxin-2 (KmTx2), reported in 2010[4f] and revised in 2015,[4g] is interesting in that the THP rings are reported to be antipodal to those of AM3 (Figure 1c).

Because the absolute configuration of the B-ring of KmTx2 is antipodal to that of AM3, we have confirmed the absolute configuration of the B-ring of AM3 to be correct by degradation of the natural product and chemical correlation.[5c] On the other hand, the absolute configurations at C2 and C51 were revised to be *R* and *S*, respectively, by chemical syntheses of partial structures and chemical correlation (Figure 1b).[5a,d] The reason for the misassignment at C51 was the difficulty in applying the JBCA method because the key *J* value was in the medium range (i.e., $^{3}J_{H50,H51}$ = 3.4 Hz). A similar situation was also observed for the assignment of the absolute configuration at C38, that is, although the absolute configuration at C39 was determined by the modified Mosher method[6] and the relative configuration at C38–C39 was determined by the JBCA method, the observed key *J* value for C38–C39 was in the medium range (i.e., $^{3}J_{H38,H39}$ = 5.1 Hz). If the assignment of the absolute configuration at C38 of *R* determined by the JBCA method is wrong, the absolute configurations at C32, C33, C34, C35, and C36 should be also incorrect. Therefore, it is important to confirm the absolute configuration at C38. The similar situation was observed for C36–C37 of KmTx2, and the proposed structure was supported by DP4 chemical shift analysis.[4g] Herein, we report the chemical synthesis of the partial structures of AM3, degradation of the natural product and synthetic intermediates, and chemical correlation of these compounds to confirm the absolute configuration of AM3.

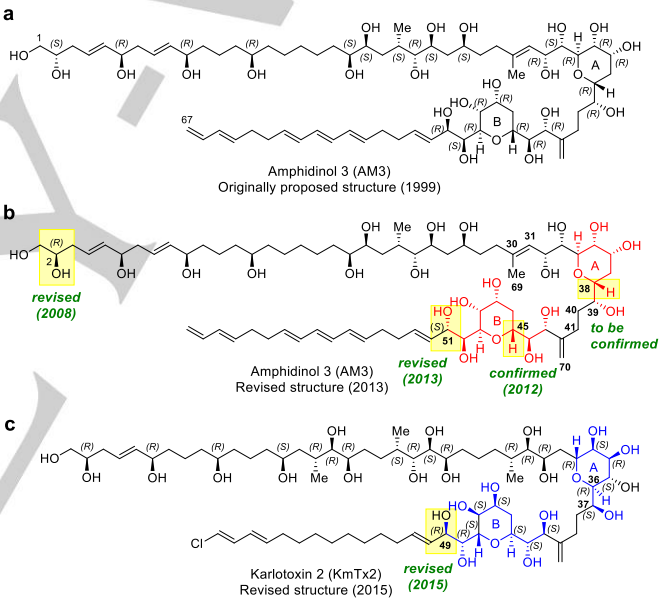


Figure 1. (a) Originally proposed structure of amphidinol 3 (AM3) in 1999. (b) Structure of AM3 revised in 2013. (c) Structure of karlotoxin 2 (KmTx2) revised in 2015.

The unique structural features of AM3, represented by the presence of a long hydrophilic polyol chain, highly substituted tetrahydropyran ring systems, and a hydrophobic polyene unit, have attracted considerable attention in the synthetic community.[7,8] Although pioneering synthetic studies of AM3 including the tetrahydropyran ring and the polyene unit, the C31–C67 section by Rychnovsky[8f] and the C43–C67 section by Roush[8d,e] and Paquette[8i] have been reported, all of them were based on the originally proposed structure reported in 1999. Therefore, there was no argument regarding the stereochemistry of AM3 except for the synthetic study of the C1–C31 polyol part by Evans[8l] based on the revised structure in 2013.

We planned to confirm the absolute configuration at C38 of AM3 by comparing the NMR data between natural product and the synthetic model compounds **1a** or **1b** (Figure 2). The C31–C67 section **1a** corresponds to the revised structure of AM3 in 2013, whereas **1b** has an opposite stereochemistry at C32–C38. Both **1a** and **1b** would be synthesized via alkenyllithium–aldehyde coupling[8f,j] between the B-

[*] Y. Wakamiya, Dr. M. Ebine, M. Murayama, H. Omizu, Prof. Dr. N. Matsumori, Prof. Dr. T. Oishi,
Department of Chemistry, Faculty and Graduate School of Science, Kyushu University
744 Motooka, Nishi-ku, Fukuoka 819-0395 (Japan)
E-mail: oishi@chem.kyushu-univ.jp
Prof. Dr. M. Murata
Department of Chemistry, Graduate School of Science, Osaka University
1-1 Machikeneyama, Toyonaka, Osaka 560-0043 (Japan)

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under:

10.1002/anie.201712167

ring and the A-ring or its enantiomer with inversion of C39, followed by Julia–Kocienski olefination,[9] respectively.

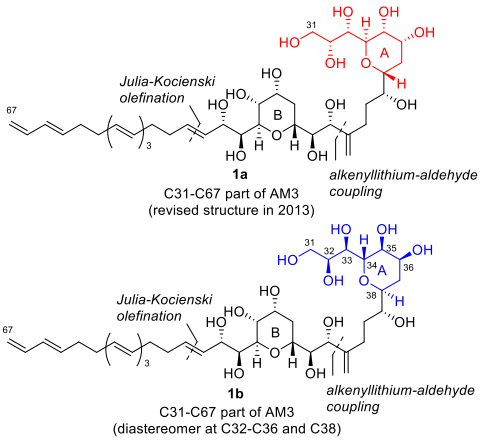
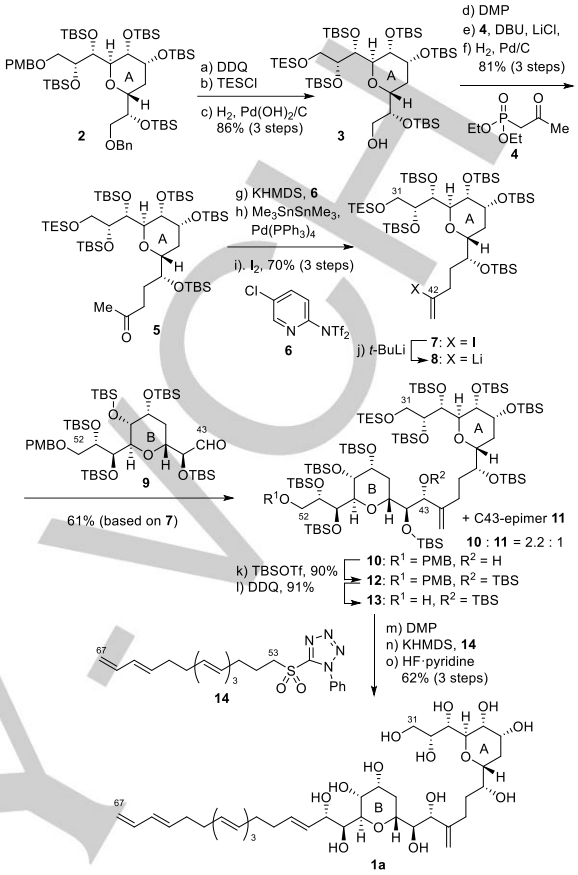


Figure 2. Structures and synthetic plan of the model compounds **1a** and **1b**.

The synthesis of the C31–C67 part (**1a**) was achieved as shown in Scheme 1. Protecting group manipulation of the known compound **2**[5d] (86%, 3 steps) afforded primary alcohol **3.** Dess–Martin oxidation followed by Horner–Wadsworth–Emmons olefination under the Masamune–Roush protocol[10] using phosphonate **4** afforded an α,β-unsaturated ketone, which was hydrogenated to furnish saturated methyl ketone **5** (81%, 3 steps). The ketone **5** was converted to alkenyliodide **7** (70%, 3 steps) via enol triflation[8f] with Comins reagent **6**,[11] Stille coupling of the triflate with $Me_3SnSnMe_3$, and subsequent tin-iodine exchange reaction. Iodide **7** was treated with *t*-BuLi to form alkenyllithium **8**, which was then coupled with aldehyde **9**[12] to afford secondary alcohol **10** with concomitant formation of the C43-epimer **11**[13] in a 2.2:1 ratio (61%, based on **7**). After separation and protecting group manipulation, the resulting primary alcohol **13** was oxidized to an aldehyde and coupled with sulfone **14**[5f] by Julia–Kocienski olefination.[8f,i] Global deprotection with HF·pyridine afforded the C31–C67 part **1a** as a single *E*-isomer (62%, 3 steps).

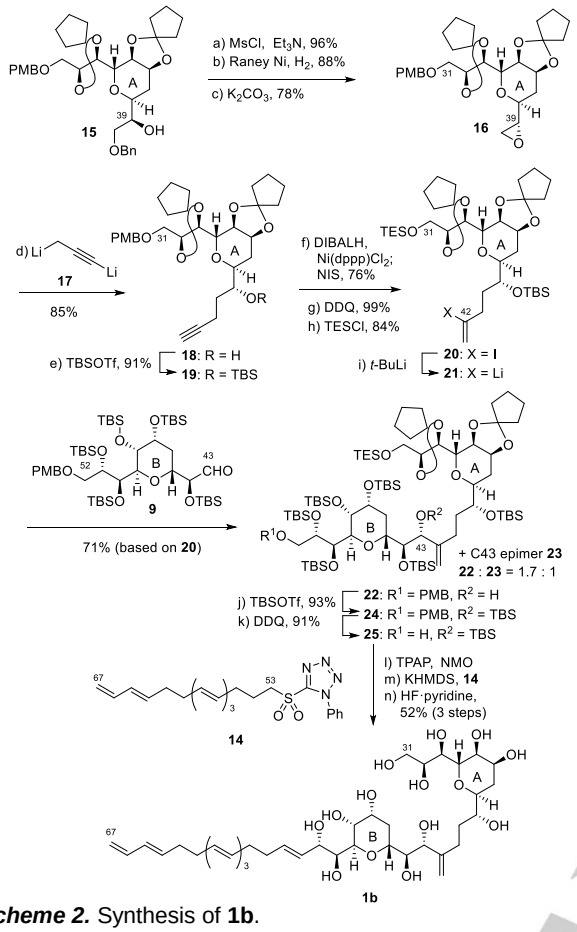
Next, we turned our attention to the synthesis of the diastereomer **1b** as shown in Scheme 2. The secondary alcohol **15**[12] was converted to mesylate (96%). Removal of the Bn group (88%) followed by intramolecular S_N2 reaction by treatment with K_2CO_3 afforded epoxide **16** (78%) with inversion of stereochemistry at C39. Nucleophilic ring-opening of **16** by dilithium reagent **17**[14] furnished alcohol **18** (85%).[8e,j] After protection of **18** as a TBS ether **19** (91%), the resulting terminal alkyne was converted to iodoolefin via Ni-catalyzed regioselective hydroalumination/iodination (76%).[15] Removal of the PMB group (99%) followed by protection as TES ether furnished iodoolefin **20** (84%). In an analogous sequence as shown in Scheme 1, coupling of iodide **20** and aldehyde **9** was carried out to afford secondary alcohol **22** and C43-epimer **23**[13] (71% based on **20, 22:23** = 1.7:1). Further transformation similar to Scheme 1 afforded the C31–C67 part **1b** as a mixture of the geometrical isomers at C52–C53 (*E*:*Z* = 3:1). The isomers were separated by HPLC (33%).



Scheme 1. Synthesis of **1a**.

Having synthesized the model compounds **1a** and **1b**, their NMR data were compared with those of the natural product. The differences in the chemical shifts at C31–C51 portion of AM3 and **1a** or **1b** are shown in Figure 3. For both diastereomers, chemical shifts at C52–C67 corresponding to the polyene moiety are identical to those of AM3,[12] but those at the C31–C33 terminus deviate because the structures are different from AM3. Large deviations for **1a** were observed in both ^1H and ^{13}C chemical shifts, which clearly indicates that the proposed structure of AM3 is incorrect. As for **1b**, deviations are almost within error range, however, non-negligible deviations were observed for C38 and H40a. Considering the observed NOE between CH_3 (C69) and CH_2 (C70) groups, AM3 is likely to take tightly bending conformation with C30–C31 olefin close to C38–C41.[2a] Conformational differences between the model compounds and natural product may cause the difference in NMR chemical shifts, but analysis of **1b** revealed the compound to have a conformation similar to that of AM3.[12] Therefore, the deviation of the chemical shifts at C38–C41 may be due to the magnetic anisotropic effect caused by C30–C31 olefin which is lacking in the structure of **1b**. It is noteworthy that the estimated $^3J_{H38,H39}$ values of **1a** and **1b** (4.6 Hz and 5.4 Hz, respectively) support the difficulty in applying the JBCA method to this system.

10.1002/anie.201712167



Scheme 2. Synthesis of **1b**.

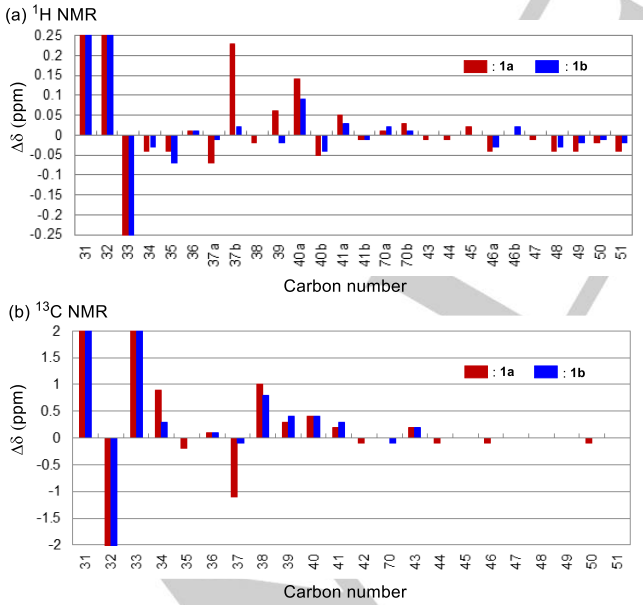
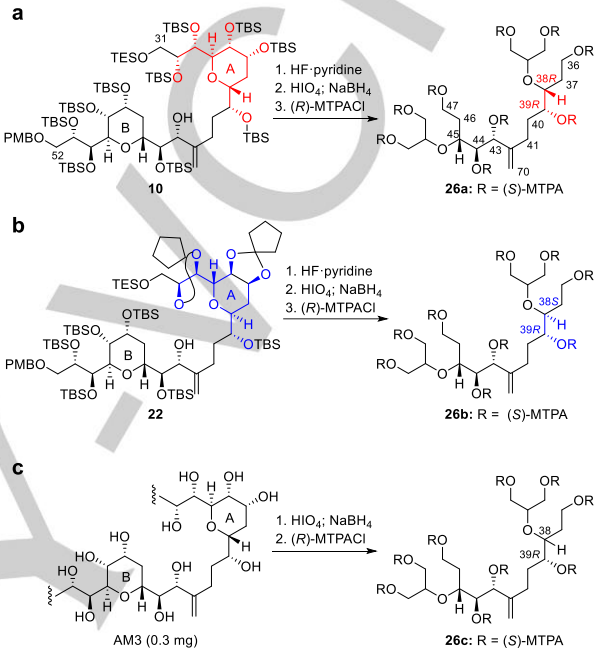


Figure 3. Differences in chemical shifts between AM3 and the synthetic fragments **1a** and **1b**. (a) ^{1}H NMR (600 MHz, 1:2 C_5D_5N/CD_3OD), (b) ^{13}C NMR (150 MHz, 1:2 C_5D_5N/CD_3OD). The x- and y-axes represent carbon number and Δδ in ppm, respectively. Red and blue bars represent Δδ = δAM3 – δsynthetic **1a** or **1b**, respectively.

Therefore, as shown in Scheme 3, we decided to convert our samples to MTPA esters **26a**–**c** which lack the C30–C31 double bond but retain the C38–C39 portion.[1b] The sample corresponding to 38*R* diastereomer **26a** was prepared from **10**, the precursor of **1a**, and the 38*S* diastereomer **26b** from **22**, the precursor of **1b**. Although preparation of **26c** from natural product was not an easy task due to the limited availability of AM3, we obtained an adequate amount of **26c** for ^{1}H NMR analysis from no more than ca. 0.3 mg of AM3.



Scheme 3. Degradation of **10**, **22**, and AM3.

Then, ^{1}H NMR data (600 MHz, $CDCl_3$) of (*S*)-MTPA esters **26a** (38*R*, 39*R*) and **26b** (38*S*, 39*R*) were compared with those of (*S*)-MTPA ester **26c** derived from the natural product. The differences in the chemical shifts at C36–C47 between **26c** and **26a** or **26b** are shown in Figure 4. It is obvious that deviations between **26c** and **26a** are large (red bars), but chemical shifts of **26c** are identical to those of **26b** (blue bars). Therefore, the correct absolute configurations at C32–C36 and C38 are opposite to those in the originally proposed structure, namely, they should be revised to 32*S*, 33*R*, 34*S*, 35*S*, 36*S*, and 38*S* (Figure 5a). Based on these results, it was revealed that AM3 is a unique natural product having both antipodal tetrahydropyran counterparts on a single carbon chain (C33–C38 and C45–C50 sections).

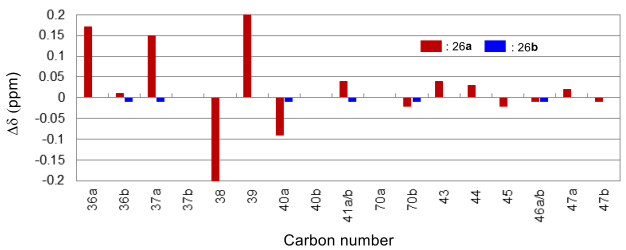


Figure 4. Differences in ^{1}H NMR (600 MHz, $CDCl_3$) chemical shifts between degradation products **26c** derived from AM3 and the synthetic fragments **26a** and **26b**. Red and blue bars represent Δδ = δ**26c** – δ**26a** or **26b**, respectively.

Although biosynthetic pathways of the amphidinols are not fully elucidated, a ^{13}C metabolite labeling pattern was reported by the feeding experiment of ^{13}C-enriched acetate (Figure 5b).[16] The labeling patterns are not symmetrical between the A- and B-ring moieties, and the arrangement of the A- and B-rings is anti-parallel, head-to-tail to tail-to-head (Figure 5c). Therefore, it is plausible that the two antipodal tetrahydropyran moieties were constructed coincidentally in the biosynthetic pathway, and it is not considered to be unnatural phenomenon. It has been reported that enantiomeric natural products can arise from a single or different species, and that both diastereomers possessing enantiomeric partial structures can arise from a single species.[17,18] It is interesting to note that both enantiomers of the partial structures exist in a single molecule in the nonactines[19] and oxasqualenoids.[20] However, to the best of our knowledge, this is the first example of two antipodal tetrahydropyran rings existing on a single carbon chain in the family of amphidinols and related compounds. It is also noteworthy that stereochemical revision of AM3 would afford significant contribution to elucidate the 3D structure and mode of action of AM3.

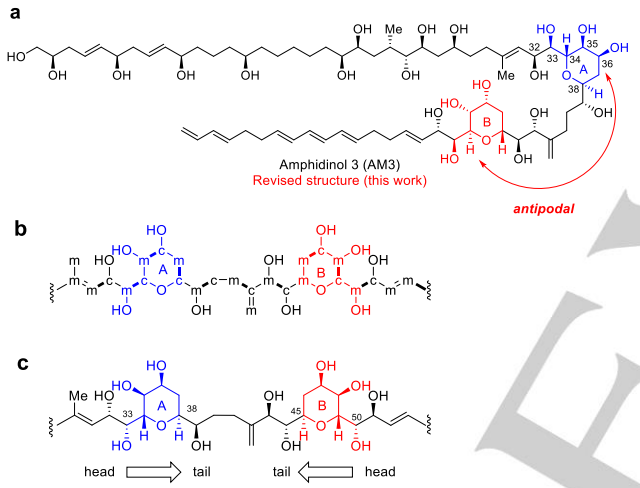


Figure 5. (a) Revised structure of AM3. (b) ^{13}C metabolite labeling pattern of AM3. (c) Arrangement of the A- and B-rings of AM3.

In conclusion, syntheses of the C31–C67 part (**1a**) of AM3 and the diastereomer at C32–C36 and C38 (**1b**) were achieved to confirm the absolute configuration of the natural product. By comparison of the NMR data of **1a** and **1b** with those of AM3 in combination with the degradation of the natural product, the absolute configuration of AM3 was revised to be 32*S*, 33*R*, 34*S*, 35*S*, 36*S*, and 38*S*. The present results suggest that structures of AM3 congeners should also be corrected, and investigations toward this end are currently in progress.

Acknowledgements

This work was supported in part by JST ERATO Lipid Active Structure, and JSPS KAKENHI Grant Numbers JP24750092, JP15K17857, JP15K13645, and JP16H01159 in Middle Molecular Strategy.

Conflict of interest

The authors declare no conflict of interest.

Keywords: Amphidinol 3 • Natural Product• Structure Elucidation • Organic Synthesis • Stereochemistry

[1] a) M. Satake, M. Murata, T. Yasumoto, T. Fujita, H. Naoki, *J. Am. Chem. Soc.* **1991**, *113*, 9859. b) M. Murata, S. Matsuoka, N. Matsumori, G. K. Paul, K. Tachibana, *J. Am. Chem. Soc.* **1999**, *121*, 870.

[2] a) T. Houdai, S. Matsuoka, N. Morsy, N. Matsumori, M. Satake, M. Murata, *Tetrahedron* **2005**, *61*, 2795. b) R. T. Swasono, R. Mouri, N. Morsy, N. Matsumori, T. Oishi, M. Murata, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2215. c) R. A. Espiritu, N. Matsumori, M. Tsuda, M. Murata, *Biochemistry* **2014**, *53*, 3287.

[3] N. Matsumori, D. Kaneno, M. Murata, H. Nakamura, Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866.

[4] a) Y. Meng, R. M. Van Wagoner, I. Misner, C. Tomas, J. L. C. Wright, *J. Nat. Prod.* **2010**, *73*, 409. b) J. Kobayashi, T. Kubota, *J. Nat. Prod.* **2007**, *70*, 451. c) K. Washida, T. Koyama, K. Yamada, M. Kita, D. Uemura, *Tetrahedron Lett.* **2006**, *47*, 2521. d) S.-J. Huang, C.-M. Kuo, Y.-C. Lin, Y.-M. Chen, C.-K. Lu, *Tetrahedron Lett.* **2009**, *50*, 2512. e) T. Inuzuka, Y. Yamamoto, K. Yamada, D. Uemura, *Tetrahedron Lett.* **2012**, *53*, 239. f) J. Peng, A. R. Place, W. Yoshida, C. Anklin, M. T. Hamann, *J. Am. Chem. Soc.* **2010**, *132*, 3277. g) Waters, A. L.; Oh, J.; Place, A. R.; Hamann, M. T. *Angew. Chem., Int. Ed.* **2015**, *54*, 15705.

[5] a) T. Oishi, M. Kanemoto, R. Swasono, N. Matsumori, M. Murata, *Org. Lett.* **2008**, *10*, 5203. b) M. Kanemoto, M. Murata, T. Oishi, *J. Org. Chem.* **2009**, *74*, 8810. c) Y. Manabe, M. Ebine, N. Matsumori, M. Murata, T. Oishi, *J. Nat. Prod.* **2012**, *75*, 2003. d) M. Ebine, M. Kanemoto, Y. Manabe, Y. Konno, K. Sakai, N. Matsumori, M. Murata, T. Oishi, *Org. Lett.* **2013**, *15*, 2846. e) T. Tsuruda, M. Ebine, A. Umeda, T. Oishi *J. Org. Chem.* **2015**, *80*, 859. f) M. Ebine, Y. Takada, N. Yanai, T. Oishi, *Chem. Lett.* **2017**, *46*, 662.

[6] I. Ohtani, T. Kusumi, Y. Kashman, H. Kakisawa, *J. Am. Chem. Soc.* **1991**, *113*, 4092.

[7] For a review, see: C. Bensoussan, N. Rival, G. Hanquet, F. Colobert, S. Reymond, J. Cossy, *Nat. Prod. Rep.* **2014**, *31*, 468.

[8] a) C. Bensoussan, N. Rival, G. Hanquet, F. Colobert, S. Reymond, J. Cossy, *Tetrahedron* **2013**, *69*, 7759. b) N. Rival, G. Hanquet, C. Bensoussan, S. Reymond, J. Cossy, F. Colobert, *Org. Biomol. Chem.* **2013**, *11*, 6829. c) E. M. Flamme, W. R. Roush, *Org. Lett.* **2005**, *7*, 1411. d) J. D. Hicks, E. M. Flamme, W. R. Roush, *Org. Lett.* **2005**, *7*, 5509. e) J. D. Hicks, W. R. Roush, *Org. Lett.* **2008**, *10*, 681. f) J. de Vicente, J. R. Huckins, S. D. Rychnovsky, *Angew. Chem. Int. Ed.* **2006**, *45*, 7258. g) J. R. Huckins, J. de Vicente, S. D. Rychnovsky, *Org. Lett.* **2007**, *9*, 4757. h) L. A. Paquette, S.-K. Chang, *Org. Lett.* **2005**, *7*, 3111. i) S.-K. Chang, L. A. Paquette, *Synlett* **2005**, 2915. j) M. W. Bedore, S.-K. Chang, L. A. Paquette, *Org. Lett.* **2007**, *9*, 513. k) M. T. Crimmins, T. J. Martin, T. A. Martinot, *Org. Lett.* **2010**, *12*, 3890. l) A. Grisin, P. A. Evans, *Chem. Sci.* **2015**, *6*, 6407. m) J. S. Yadav, Y. Gopalarao, D. Chandrakanth, B. V. S. Reddy, *Helv. Chim. Acta* **2016**, *99*, 436.

[9] P. R. Blakemore, W. J. Cole, P. J. Kocienski, A. Morley, *Synlett* **1998**, 26.

[10] M. A. Blanchette, W. Choy, J. T. Davis, A. P. Essenfeld, S. Masamune, W. R. Roush, T. Sakai, *Tetrahedron Lett.* **1984**, *25*, 2183.

[11] D. L. Comins, A. Dehghani, *Tetrahedron Lett.* **1992**, *33*, 6299.

[12] See Supporting Information.

[13] The absolute configuration was determined by the modified Mosher method.

[14] A. R. Pereira, J. A. Cabezas, *J. Org. Chem.* **2005**, *70*, 2594.

[15] F. Gao, A. H. Hoveyda, *J. Am. Chem. Soc.* **2010**, *132*, 10961.

[16] T. Houdai, S. Matsuoka, M. Murata, M. Satake, S. Ota, Y. Oshima, L. L. Rhodes, *Tetrahedron* **2001**, *57*, 5551.

[17] J. M. Finefield, D. H. Sherman, M. Kreitman, R. M. Williams, *Angew. Chem., Int. Ed.* **2012**, *51*, 4802.

[18] A. Hoshino, H. Nakai, M. Morino, K. Nishikawa, T. Kodama, K. Nishikibe, Y. Morimoto, *Angew. Chem. Int. Ed.* **2017**, *56*, 3064.

[19] W. C. Smith, L. Xiang, B. Shen, *Antimicrob. Agents Chemother.* **2000**, *44*, 1809.

[20] J. J. Fernández, M. L. Souto, M. Norte, *Nat. Prod. Rep.* **2000**, *17*, 235.

10.1002/anie.201712167

WILEY-VCH

Entry for the Table of Contents (Please choose one layout)

Layout 1:

COMMUNICATION

Text for Table of Contents

((Insert TOC Graphic here))

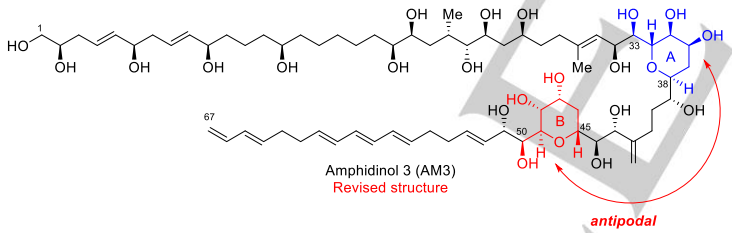
*Author(s), Corresponding Author(s)**

Page No. – Page No.

Title

Layout 2:

COMMUNICATION



*Yuma Wakamiya, Makoto Ebine, Mariko Murayama, Hiroyuki Omizu, Nobuaki Matsumori, Michio Murata, and Tohru Oishi**

Page No. – Page No.

Synthesis and Stereochemical Revision of the C31–C67 Section of Amphidinol 3