ChemPubSoc Europe

DOI: 10.1002/chem.201504674

CHEMISTRY A European Journal Communication

Natural Products

Total Synthesis of the 7,10-Epimer of the Proposed Structure of Amphidinolide N, Part I: Synthesis of the C1–C13 Subunit

Koji Ochiai,[a] Sankar Kuppusamy,[a] Yusuke Yasui,[a] Tsubasa Okano,[a] Yasunobu Matsumoto,[a] Nishant R. Gupta,[a] Yohei Takahashi,[b] Takaaki Kubota,[b, c] Jun'ichi Kobayashi,[b] and Yujiro Hayashi*[a, d]

Abstract: Amphidinolide N, the structure of which has been recently revised, is a 26-membered macrolide featuring allyl epoxide and tetrahydropyran moieties with 13 chiral centers. Due to its challenging structure and extraordinary potent cytotoxicity, amphidinolide N is a highly attractive target of total synthesis. During our total synthesis studies of the 7,10-epimer of the proposed structure of amphidinolide N, we have synthesized the C1–C13 subunit enantio- and diastereoselectively. Key reactions include an L-proline catalyzed enantioselective intramolecular aldol reaction, Evans aldol reaction, Sharpless asymmetric epoxidation and Tamao–Fleming oxidation. To aid late-stage manipulations, we also developed the 4-(*N*-benzyloxycarbonyl-*N*-methylamino)butyryl group as a novel ester protective group for the C9 alcohol.

The amphidinolides are a series of cytotoxic macrolides possessing unique structural features originating from laboratory cultured, marine dinoflagellates *Amphidinium* sp. Specifically, Kobayashi and co-workers showed amphidinolide N to possess the most potent cytotoxic activity against murine lymphoma L1210 and KB cells (IC_{50} = 0.00005 and 0.00006 μg mL^{-1}, respectively) among all amphidinolides isolated so far.[1] The structure of amphidinolide N was elucidated by NMR technique by Kobayashi as shown **1** in 1994 (Figure 1). In 2012, Trost presented the structure **2** in their paper during synthesis of the C13–C29 subunit.[2] In 2013, Kobayashi revised the structure to be **3** via extensive NMR studies.[3] Amphidinolide N (**3**) is thus a unique 26-membered macrolide possessing allyl epoxide and tetrahydropyran moieties with 13 chiral centers.

To date, no total synthesis of amphidinolide N has been reported.[4] Due to the unique structure and the high potent cytotoxicity, amphidinolide N is an important target of the total synthesis, as well as other members of the amphidinolide family.[5] Nicolaou synthesized the iso-epoxy-amphidinolide N stereoisomer in 2006.[4a,b] Recently, Trost[2] and Sasaki[4c] reported their independent stereoselective syntheses of the C13–C29 subunit.

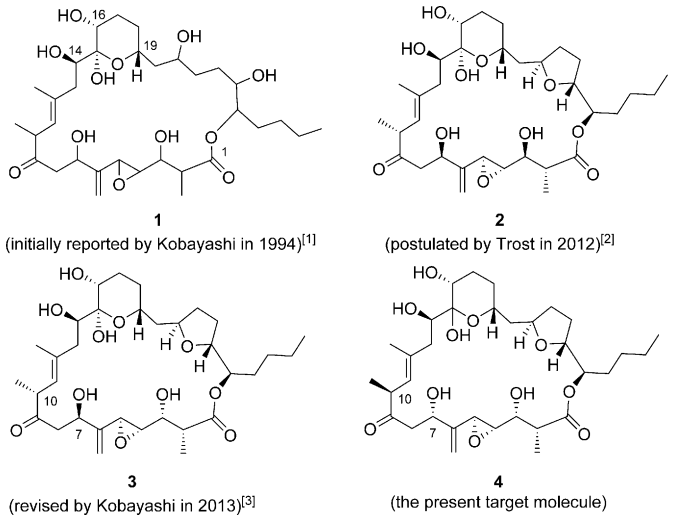


Figure 1. Structure of amphidinolide N and 7,10-*epi*-amphidinolide N (**4**).

When we initiated the total synthesis of amphidinolide N, the relative stereochemistry was not completely elucidated. We selected the structure **4** as our target molecule.[6] According to the latest revised structure of amphidinolide N (**3**) in 2013,[3] **4** is epimeric at the C7 and C10 positions. Herein, and in the following paper, we describe our efforts that have culminated in the synthesis of 7,10-epimer of the proposed structure of amphidinolide N (**4**). Clearly, a synthetic strategy toward **4** would be beneficial for the total synthesis of amphidinolide N.

Our retrosynthetic analysis of **4** is shown in Scheme 1. We considered the allyl epoxy moiety to be relatively labile and thus should be constructed under mild and neutral conditions

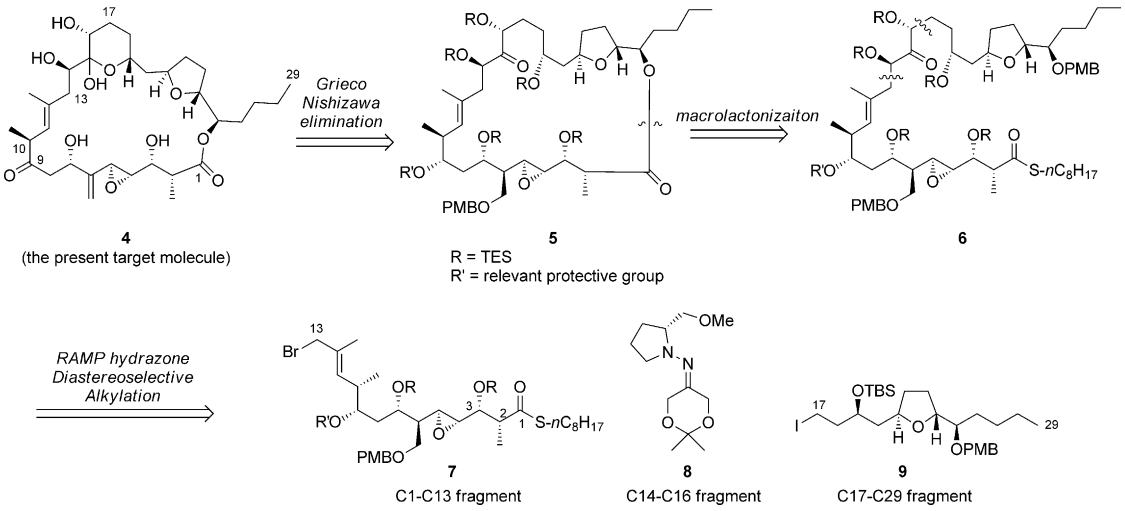
[a] Dr. K. Ochiai, Dr. S. Kuppusamy, Y. Yasui, T. Okano, Y. Matsumoto, Dr. N. R. Gupta, Prof. Dr. Y. Hayashi
Before July, 2012: Department of Industrial Chemistry
Faculty of Engineering, Tokyo University of Science
Kagurazaka, Shinjuku-ku, Tokyo 162-8601 (Japan)

[b] Dr. Y. Takahashi, Prof. Dr. T. Kubota, Prof. Dr. J. Kobayashi
Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812 (Japan)

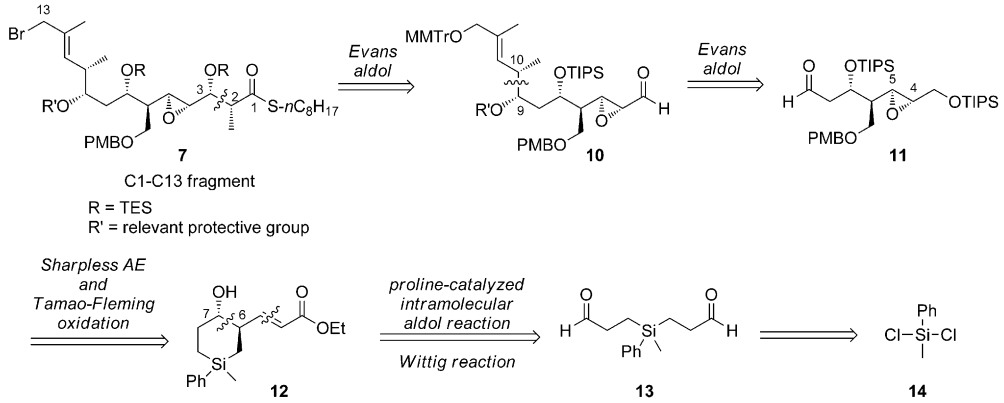
[c] Prof. Dr. T. Kubota
Present address: Showa Pharmaceutical University, 3-3165 Higashi-Tamagawagakuen, Machida, Tokyo 194-8543 (Japan)

[d] Prof. Dr. Y. Hayashi
Present address: Department of Chemistry, Graduate School of Science, Tohoku University, Aoba-ku, Sendai 980-8578 (Japan)
Fax: (+81) 22-795-6566
E-mail: yhayashi@m.tohoku.ac.jp

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201504674.



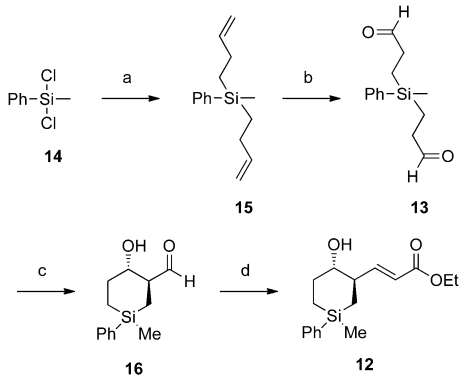
Scheme 1. Retrosynthesis of C7,C10-*epi*-amphidinolide N (**4**), PMB = *p*-methoxybenzyl, TES = triethylsilyl, TIPS = triisopropylsilyl, TBS = *tert*-butyldimethylsilyl.



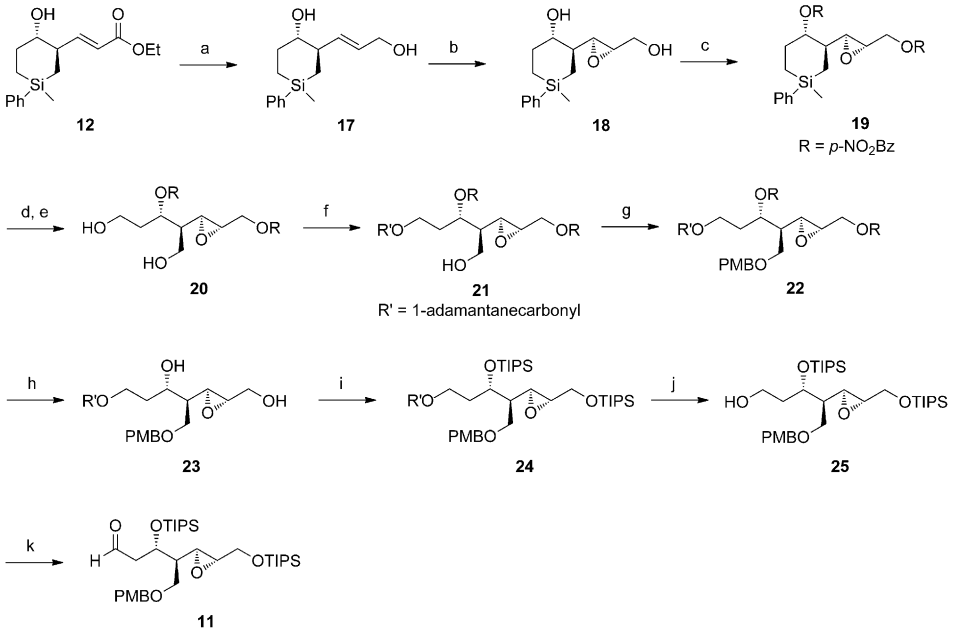
Scheme 2. Retrosynthesis of the C1–C13 Subunit **7**. PMB = *p*-methoxybenzyl, MMTr = (*p*-methoxyphenyl)diphenylmethyl, TES = triethylsilyl, TIPS = triisopropylsilyl, Tr = trityl.

at a late stage of the synthesis. Moreover, the ketone at C9 would be constructed at a late stage to suppress possible dehydration and epimerization at C10, in which the selection of the protecting group at the C9 hydroxy group would be critical. Therefore, **4** would be synthesized from **5** via Grieco–Nishizawa elimination[7] and oxidation of alcohol at C9. Compound **5** would be constructed by macrolactonization;[8] **6** would be constructed with three subunits, C1–C13 (**7**), C14–C16 (**8**),[4a,b] and C17–C29 (**9**). All carbon frameworks would be coupled by a double Enders RAMP hydrazone diastereoselective alkylation,[4a,b,9] which has been employed by Nicolaou and co-workers.[4a,b] Herein, we report the synthesis of the C1–C13 subunit **7** of 7,10-epimer of the proposed structure of amphidinolide N (**4**) with 8 chiral centers, among which six are contiguous.

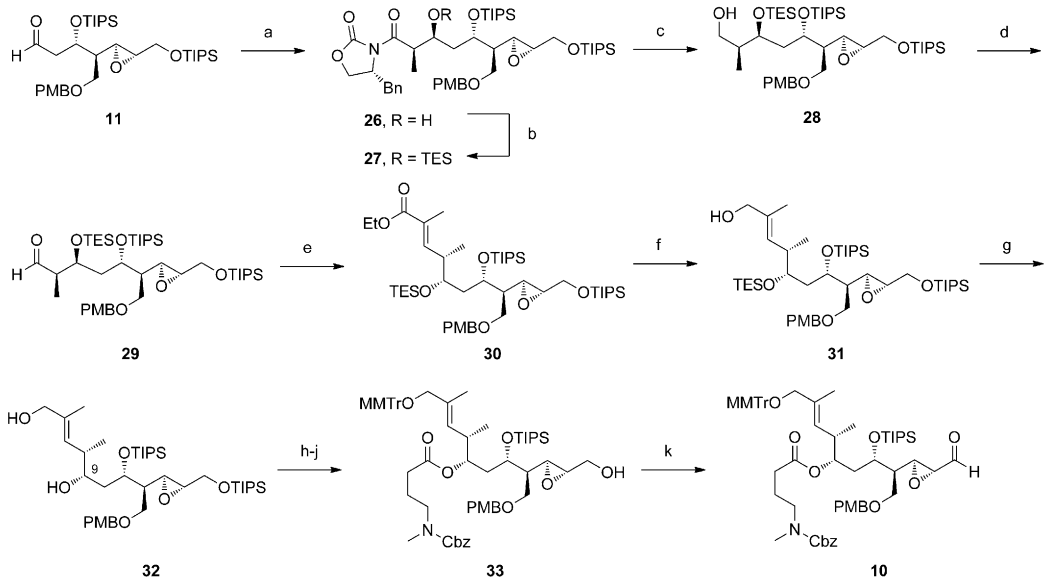
Retrosynthesis of **7** is illustrated in Scheme 2. The stereogenic centers at C2 and C3 of **7** could be constructed by Evans *syn*-aldol reaction.[10] The stereogenic centers at C9 and C10 of **10** would also be controlled by Evans *syn*-aldol reaction.[10] Sharpless asymmetric epoxidation,[11] differentiation of two primary hydroxyl groups, followed by Tamao–Fleming oxidation[12] will lead to chiral epoxide **11**. The stereocenters at C6 and C7 of **12** would be constructed by proline-catalyzed intramolecular aldol reaction of **13**.[13] Dialdehyde **13** could be derived from commercially available **14**.



Scheme 3. Synthesis of **12** via proline catalyzed intramolecular aldol reaction. a) Homoallylmagnesium bromide/THF, RT, 98%; b) O_3/DCM, −78 °C then Ph_3P/MeOH, −78 °C to RT, quant.; c) L-proline (10 mol%)/DCM, RT; d) Ph_3P=CHCOOEt/toluene, RT, 54% (2 steps, *anti*/*syn* = 10: 1, 99% *ee* for *anti*).



Scheme 4. Synthesis of **11**. a) DIBAL-H/DCM, −30 °C, 82%; b) TBHP, (+)-DET, Ti(O*i*Pr)$_4$, MS4 Å/DCM, −50 °C to −30 °C, 98%; c) *p*-NO$_2$-BzCl, Et$_3$N, DMAP/DCM, RT, quant.; d) Hg(CF$_3$COO)$_2$, Et$_3$N/DCM, −30 °C then NaBH$_4$/MeOH, EtOAc, −50 °C; e) *m*CPBA, KHF$_2$/MeOH, EtOAc, RT, 57% (2 steps); f) 1-adamantanecarbonyl chloride, pyridine/DCM, −78 °C, 63%; g) PMBOC(=NH)CCl$_3$, TfOH/Et$_2$O, RT; h) K$_2$CO$_3$/MeOH, THF, 0 °C, 67% (2 steps); i) TIPSOTf, 2,6-lutidine/DCM, 0 °C; j) DIBAL-H/DCM, −78 °C; k) SO$_3$-pyridine, Et$_3$N, DMSO/DCM, RT, 47% (3 steps). DIBAL-H = diisobutylaluminium hydride, TBHP = *tert*-butyl hydroperoxide, DET = diethyl tartrate, Bz = benzoyl, MS = molecular sieves, DMAP = 4-dimethylaminopyridine, *m*CPBA = *m*-chloroperbenzoic acid, PMB = *p*-methoxybenzyl, DMSO = dimethylsulfoxide.

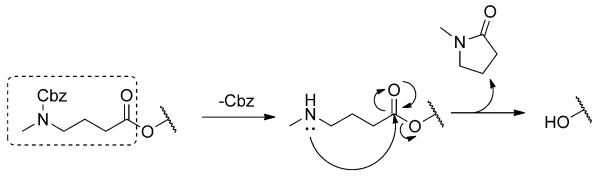


Scheme 5. Synthesis of intermediate **10**. a) (*R*)-4-benzyl-3-propionyl-2-oxazolidinone, *n*Bu$_2$BOTf, Et$_3$N/DCM, −78 °C then 0 °C; b) TESOTf, 2,6-lutidine/DCM, −78 °C, 80% (2 steps); c) LiBH$_4$/THF, MeOH, 0 °C, 95%; d) SO$_3$-pyridine, Et$_3$N, DMSO/DCM, RT, 86%; e) Ph$_3$P=C(Me)COOEt/toluene, reflux, 90%; f) DIBAL-H/Et$_2$O, −78 °C, 93%; g) PPTS/DCM, MeOH, 0 °C, 95%; h) MMTrCl, DMAP/pyridine, RT; i) 4-(*N*-benzyloxycarbonyl-*N*-methylamino)butyric acid, 2,4,6-trichlorobenzoyl chloride, Et$_3$N/toluene, RT then DMAP, RT; j) NH$_4$F/MeOH, reflux, 82% (3 steps); k) SO$_3$-pyridine, Et$_3$N, DMSO/DCM, RT, 98%. TIPS = triisopropylsilyl, PMB = *p*-methoxybenzyl, Bn = benzyl, TES = triethylsilyl, DMSO = dimethylsulfoxide, DIBAL-H = diisobutylaluminium hydride, PPTS = pyridinium *p*-toluenesulfonate, Cbz = benzyloxycarbonyl, DMAP = 4-dimethylaminopyridine, MMTr = (*p*-methoxyphenyl)diphenylmethyl.

Our synthesis starts from **14** (Scheme 3), which was treated with homoallyl Grignard reagent to give bis-homoallyl silane **15**. Ozonolysis of **15** afforded bis-aldehyde **13** in quantitative yield. Organocatalysis is a powerful tool for asymmetric synthesis. In particular, proline is inexpensive, stable to air and water, and an environmentally friendly catalyst.[14] In this case, we utilized the L-proline-catalyzed intramolecular aldol reaction as developed by List[13] for the construction of the C6 and C7 ste-

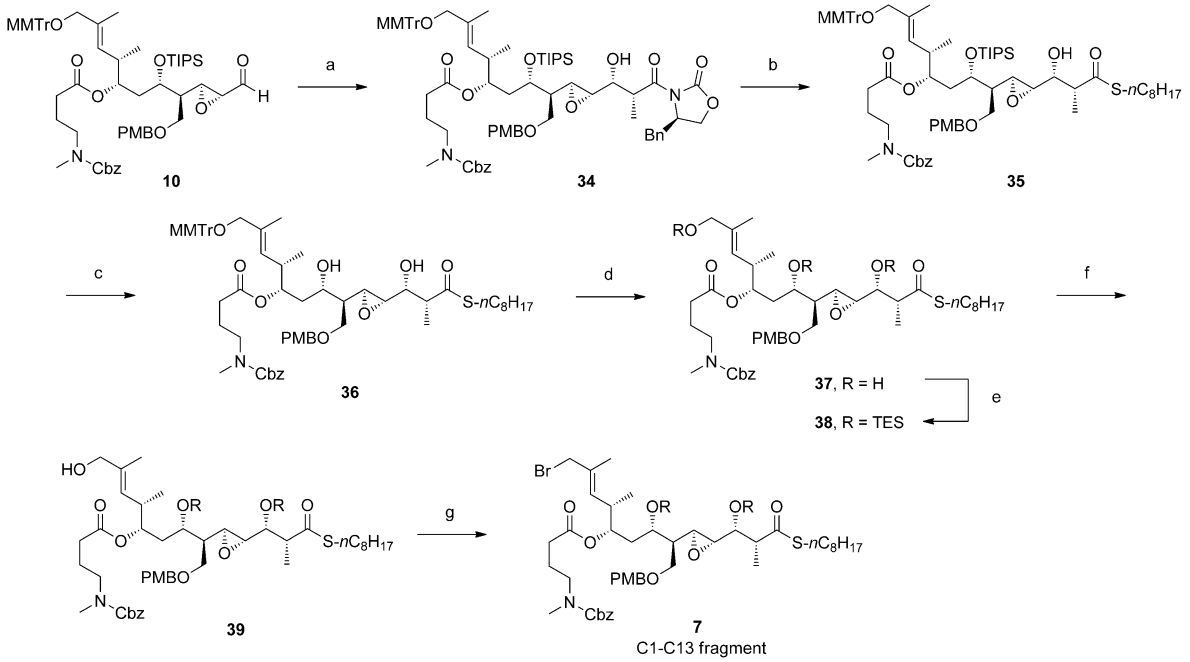
reocenters. The reaction afforded **12** in good yield with excellent diastereo- and enatio-selectivity (*anti*/*syn* 10:1, 99% *ee* for *anti* isomer, diastereomer ratio on Si = 1:1) after Wittig reaction, and this reaction is appropriate for the large scale preparation.[15]

Synthesis of the epoxy aldehyde intermediate **11** is shown in Scheme 4. DIBAL-H reduction of **16** gave allyl alcohol **17**. Sharpless asymmetric epoxidation[11] of **17** using (+)-diethyl tartrate as a ligand afforded the epoxide **18** in excellent yield and diastereoselectivity. Two hydroxyl groups of **18** were protected with *p*-nitrobenzoyl chloride to afford bis-*p*-nitrobenzoate **19**. This protecting group was selected because it has to be discriminated from other acyl protecting groups afterwards (**22**→**23**). Tamao-Fleming oxidation[12] of **19** gave diol **20** in moderate yield. Selective protection of the two primary hydroxyl groups of **20** was troublesome, but treatment of **20** with bulky 1-adamantanecarbonyl chloride at low temperature afforded **21** in 63% yield, importantly with recovery of the starting material (30%). **21** was converted into aldehyde **11** in a five-step sequence consisting of PMB protection,[16] removal of the two *p*-nitrobenzoyl groups, TIPS protection, DIBAL-H reduction, and Parikh–Doering oxidation.[17]



Scheme 6. Concept for novel protective group, Cbz = benzyloxycarbonyl.

Synthesis of intermediate **10** is described in Scheme 5. Evans aldol reaction[10] of **11** proceeded in a highly diastereoselective manner, followed by TES protection to afford **27** in good yield. Reductive removal of the chiral auxiliary was accomplished by treatment of $LiBH_4$ to furnish **28**, which was converted into **32** in a four-step sequence consisting of Parikh–Doering oxidation,[17] Wittig reaction, reduction of ester, followed by removal of TES group with PPTS. We protected the hydroxyl group at the C9 position after protection of the allyl alcohol. We first selected the acetyl group as a protective group at C9 but we could not remove it under various conditions without affecting other functionality before and after macrolactonization (see Supporting Information). Presumably, steric hindrance at the C9 position suppressed its deprotection. The protecting group at C9 had to be deprotected at the late stage of the synthesis under neutral conditions among other protecting groups. Thus, the novel alcohol protective group, that is, the 4-(*N*-benzyloxycarbonyl-*N*-methylamino)butyryl group, was developed. The ester formed would thus be readily removed under neutral hydrogenation conditions, followed by intramolecular nucleophilic substitution (Scheme 6).[18] The new protective group was installed by Yamaguchi esterification.[8] TIPS protecting group of the primary alchol was selectively removed with NH_4F in MeOH[19] to afford alchol **33**, which was converted into aldehyde **10** via Parikh-Doering oxidation.[17]



Scheme 7. Synthesis of the C1–C13 subunit **7**. a) (*R*)-4-benzyl-3-propionyl-2-oxazolidinone, nBu_2BOTf, Et_3N/DCM, −78 °C then 0 °C, 84%; b) lithium *n*-octanethiolate/THF, −40 °C, 91%; c) TAS-F, H_2O/THF, DMF, RT, 84%; d) PPTS/MeOH, DCM, 0 °C then RT, 96%; e) TESOTf, 2,6-lutidine/DCM, −78 °C, 98%; f) NH_4F/MeOH, DCM, 0 °C to RT, 85%; g) MsCl, Et_3N/THF, 0 °C then LiBr, RT, 98%. Cbz = benzyloxycarbonyl, TIPS = triisopropylsilyl, PMB = *p*-methoxybenzyl, Bn = benzyl, MMTr = (*p*-methoxyphenyl)diphenylmethyl, TES = triethylsilyl, DMSO = dimethylsulfoxide, TAS-F = tris(dimethylamino)sulfonium difluorotrimethylsilicate, PPTS = pyridinium *p*-toluenesulfonate.

Synthesis of the C1–C13 subunit **7** from **10** is shown in Scheme 7. Evans aldol reaction[10] afforded **34** with highly diastereoselectivity. Removal of the chiral auxiliary was accomplished by the treatment of **34** with lithium *n*-octanethiolate[20] to give thioester **35**. As the TIPS protecting group was difficult to be deprotected at a later stage of the synthesis, without affecting labile moieties such as allyl epoxide, it was replaced by TES. Removal of the TIPS group was troublesome. When TBAF was employed, the yield was low largely due to dehydration to afford α,β-unsaturated thioester. On the other hand, treatment with TAS-F in the presence of water afforded **36** in good yield.[21] Addition of water and TAS-F were essential for suppressing the dehydration.[22] Deprotection of MMTr group with PPTS gave the triol **37**. After TES protection of all free hydroxyl groups of **37**, the treatment of **38** with NH_4F in MeOH[19] resulted in the selective deprotection of the allyl silyl ether to give **39**. Finally, bromination of allyl alcohol **39** afforded the C1–C13 subunit **7**.

In summary, the synthesis of the C1–C13 subunit **7** of 7,10-epimer of the proposed structure of amphidinolide N (**4**) was accomplished. The stereogenic centers at C6 and C7 were controlled by a large-scale, L-proline mediated intramolecular aldol reaction in a highly practical and diastereo/enantioselective manner. The C2, C3, C9 and C10 stereogenic centers were constructed by Evans *syn*-aldol reaction, while those at C4 and C5 were controlled by Sharpless epoxidation. We also developed the novel protective group 4-(*N*-benzyloxycarbonyl-*N*-methylamino)butyryl group for ester protection of the C9 alcohol. Stereoselective synthesis of the C1–C13 subunit culminated in the total synthesis of 7,10-epimer of the proposed structure of amphidinolide N (**4**). These efforts are disclosed in the following paper.[23]

Keywords: aldol reaction · organocatalyst · Sharpless epoxidation · Tamao-Fleming oxidation · total synthesis

[1] M. Ishibashi, N. Yamaguchi, T. Sasaki, J. Kobayashi, *J. Chem. Soc. Chem. Commun.* **1994**, 1455.
[2] B. M. Trost, J. Rey, *Org. Lett.* **2012**, *14*, 5632.
[3] Y. Takahashi, T. Kubota, M. Imachi, M. R. Wälchli, J. Kobayashi, *J. Antibiot.* **2013**, *66*, 277 – 279.
[4] For partial synthesis, see: a) K. C. Nicolaou, W. E. Brenzovich, P. G. Bulger, T. M. Francis, *Org. Biomol. Chem.* **2006**, *4*, 2119; b) K. C. Nicolaou, P. G. Bulger, W. E. Brenzovich, *Org. Biomol. Chem.* **2006**, *4*, 2158; c) M. Sasaki, Y. Kawashima, H. Fuwa, *Heterocycles* **2015**, *90*, 579.
[5] For review, see: a) J. Kobayashi, *J. Antibiot.* **2008**, *61*, 271; b) J. Kobayashi, T. Kubota, *J. Nat. Prod.* **2007**, *70*, 451; c) J. Kobayashi, M. Tsuda, *Nat. Prod. Rep.* **2004**, *21*, 77; d) J. Kobayashi, K. Shimbo, T. Kubota, M. Tsuda, *Pure Appl. Chem.* **2003**, *75*, 337; e) M. Ishibashi, J. Kobayashi, *Heterocycles* **1997**, *44*, 543.
[6] J. Kobayashi, Personal communication.
[7] P. A. Grieco, S. Gilman, M. Nishizawa, *J. Org. Chem.* **1976**, *41*, 1485.
[8] J. Inanaga, K. Hirata, H. Saeki, T. Katsuki, M. Yamaguchi, *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989.
[9] a) D. Enders, M. Voith, A. Lenzen, *Angew. Chem. Int. Ed.* **2005**, *44*, 1304; *Angew. Chem.* **2005**, *117*, 1330; b) D. Enders, M. Voith, S. J. Ince, *Synthesis* **2002**, 1775; c) For review, see: A. Job, C. F. Janeck, W. Bettray, R. Peters, D. Enders, *Tetrahedron* **2002**, *58*, 2253.
[10] D. A. Evans, J. Bartroli, T. L. Shih, *J. Am. Chem. Soc.* **1979**, *101*, 6120.
[11] a) R. M. Hanson, K. B. Sharpless, *J. Org. Chem.* **1986**, *51*, 1922; b) T. Katsuki, K. B. Sharpless, *J. Am. Chem. Soc.* **1980**, *102*, 5974.
[12] a) K. Tamao, M. Akita, M. Kumada, *J. Organomet. Chem.* **1983**, *254*, 13; b) I. Fleming, R. Henning, H. Plaut, *J. Chem. Soc. Chem. Commun.* **1984**, 29.
[13] C. Pidathala, L. Hoang, N. Vignola, B. List, *Angew. Chem. Int. Ed.* **2003**, *42*, 2785; *Angew. Chem.* **2003**, *115*, 2891.
[14] a) Z. G. Hajos, D. R. Parrish, *J. Org. Chem.* **1974**, *39*, 1615; b) U. Eder, G. Sauer, R. Wiechert, *Angew. Chem. Int. Ed. Engl.* **1971**, *10*, 496; *Angew. Chem.* **1971**, *83*, 492; c) B. List, R. A. Lerner, C. F. Barbas, III, *J. Am. Chem. Soc.* **2000**, *122*, 2395; d) K. Sakthivel, W. Notz, T. Bui, C. F. Barbas, III, *J. Am. Chem. Soc.* **2001**, *123*, 5260; e) For review, see: B. List, *Tetrahedron* **2002**, *58*, 5573.
[15] The reaction proceeded successfully using 60 g of bis-aldehyde **13**.
[16] N. Nakajima, K. Hirota, R. Abe, O. Yonemitsu, *Tetrahedron Lett.* **1988**, *29*, 4139.
[17] J. P. Parikh, W. E. Doering, *J. Am. Chem. Soc.* **1967**, *89*, 5505.
[18] For assisted cleavage, see: a) S. Kusumoto, K. Sakai, T. Shiba, *Bull. Chem. Soc. Jpn.* **1986**, *59*, 1296; b) S. Velarde, J. Urbina, M. R. Pena, *J. Org. Chem.* **1996**, *61*, 9541.
[19] a) Y. Hayashi, M. Shoji, J. Yamaguchi, K. Sato, S. Yamaguchi, T. Mukaiyama, K. Sakai, Y. Asami, H. Kakeya, H. Osada, *J. Am. Chem. Soc.* **2002**, *124*, 12078 – 12079; b) J. D. White, J. C. Amedio, Jr., S. Gut, L. Jayasinghe, *J. Org. Chem.* **1989**, *54*, 4268; c) X.-F. Zhu, H. J. Williams, A. I. Scott, *Synth. Commun.* **2003**, *33*, 2011; d) D. Crich, F. Hermann, *Tetrahedron Lett.* **1993**, *34*, 3385.
[20] a) K. Narasaka, M. Saitou, N. Iwasawa, *Tetrahedron: Asymmetry* **1991**, *2*, 1305; b) R. E. Damon, G. M. Coppola, *Tetrahedron Lett.* **1990**, *31*, 2849.
[21] a) K. A. Scheidt, H. Chen, B. C. Follows, S. R. Chemler, D. S. Coffey, W. R. Roush, *J. Org. Chem.* **1998**, *63*, 6436; b) R. Noyori, I. Nishida, J. Sakata, *J. Am. Chem. Soc.* **1983**, *105*, 1598; c) C. J. Douglas, S. Hiebert, L. E. Overman, *Org. Lett.* **2005**, *7*, 933.
[22] A. Fürstner, L. C. Bouchez, J.-A. Funel, V. Liepins, F.-H. Poreé, R. Gilmour, F. Beaufils, D. Laurich, M. Tamiya, *Angew. Chem. Int. Ed.* **2007**, *46*, 9265; *Angew. Chem.* **2007**, *119*, 9425.
[23] K. Ochiai, S. Kuppusamy, Y. Yasui, K. Harada, N. R. Gupta, J. Kobayashi, Y. Hayashi, *Chem. Eur. J.* **2016**, *22*, DOI: 10.1002/chem.20154675.

Received: November 20, 2015
Published online on February 4, 2016