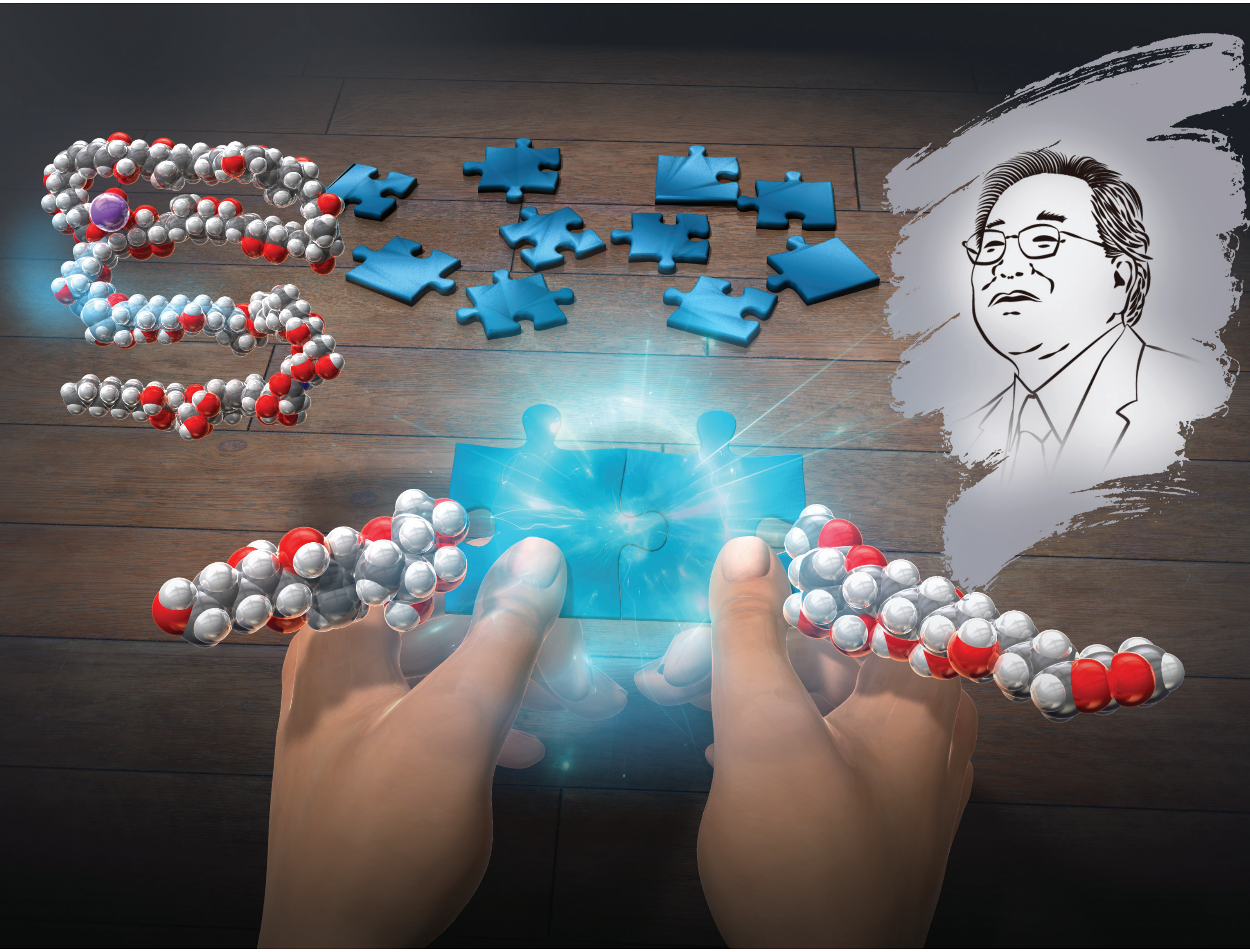
Volume 21
Number 44
28 November 2023
Pages 8785-8946

Organic & Biomolecular Chemistry

rsc.li/obc



ISSN 1477-0520

ROYAL SOCIETY OF CHEMISTRY

PAPER

Hiroyoshi Takamura *et al.*
Relative stereochemical determination of the C61–C83 fragment of symbiodinolide using a stereodivergent synthetic approach

Organic &
Biomolecular Chemistry

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Check for updates

Cite this: *Org. Biomol. Chem.*, 2023, **21**, 8837

Received 5th September 2023,
Accepted 27th September 2023

DOI: 10.1039/d3ob01420g

rsc.li/obc

Relative stereochemical determination of the C61–C83 fragment of symbiodinolide using a stereodivergent synthetic approach†‡

Hiroyoshi Takamura, * Kosuke Hattori, Takumi Ohashi, Taichi Otsu and Isao Kadota

Structural determination is required in the use of marine natural products to create novel drugs and drug leads in medicinal chemistry. Symbiodinolide, which is a polyol marine natural product with a molecular weight of 2860, increases the intracellular Ca^{2+} concentration and exhibits inhibitory activity against cyclooxygenase-1. Seventy percent of the structure of symbiodinolide has been stereochemically clarified. Herein, we report the elucidation of the relative configuration of the C61–C83 fragment, which is among the remaining thirty percent, using a stereodivergent synthetic strategy. We first assigned the relative configuration of the C61–C74 fragment. Two candidate diastereomers of the C61–C74 fragment were synthesized, and their NMR data were compared with those of the natural product, revealing the relative stereochemistry of this component. We then narrowed down the candidate compounds for the C69–C83 fragment from 16 possible diastereomers by analyzing the NMR data of the natural product, and we thus selected eight candidate diastereomers. Stereodivergent synthesis of the candidates for this fragment and comparison of the NMR data of the natural product and the eight synthetic products resulted in the relative stereostructural clarification of the C69–C83 fragment. These individually determined relative stereochemistries of the C61–C74 and C69–C83 fragments were connected *via* the common C69–C73 tetrahydropyran moiety of the fragments. Finally, the relative configuration of the C61–C83 fragment of symbiodinolide was determined. The stereodivergent synthetic approach used in this study can be extended to the stereochemical determination of other fragments of symbiodinolide.

Introduction

Marine natural products (MNPs) are critical in drug discovery and development.[1] Structural determination of MNPs, including their planar structures and stereochemistries, is a fundamental research topic and a requirement in using MNPs to create novel drugs and drug leads in medicinal chemistry. Remarkable recent advancements in computational chemistry enhanced our ability to determine the configurations of structurally complex natural products.[2] Thus, over the decades, computational modeling of the ^{1}H and ^{13}C nuclear magnetic resonance (NMR) chemical shifts of organic compounds resulted in marked increases in availability, accuracy, and application. Nevertheless, if the target natural product contains conformationally flexible acyclic motifs and/or remote stereogenic centers, a synergistic combination of spectroscopic analysis and chemical synthesis is effective in unambiguous structural elucidation.[3]

Symbiodinolide (**1**, Fig. 1) is a secondary metabolite that was isolated from the cultured dinoflagellate *Symbiodinium* sp. by Uemura *et al.*[4] Natural product **1** increased the intracellular free Ca^{2+} concentration at 7 nM against IMR-32 human neuroblastoma cells and exhibited cyclooxygenase-1 inhibitory activity (65% inhibition) at 2 μM. Furthermore, **1** ruptured the tissue surface of the flatworm *Amphiscolops* sp., which is a host organism containing the symbiotic dinoflagellate *Symbiodinium* sp., at 2.5 μM. In an isolation study, detailed 2D NMR analysis and chemical degradation of **1** revealed its planar structure and partial stereochemistry. The structure of **1** displays a molecular weight of 2860, 61 chiral centers, and an extended carbon skeleton that is highly functionalized with oxygen atoms. The large, complicated chemical structure of **1** hampers its configurational assignment. Therefore, our group investigated the chemical synthesis of each fragment of **1**,

---

Department of Chemistry, Graduate School of Natural Science and Technology, Okayama University, 3-1-1 Tsushimanaka, Kita-ku, Okayama 700-8530, Japan. E-mail: takamura@cc.okayama-u.ac.jp

† We dedicate this work to the memory of Prof. Daisuke Uemura, who sadly passed away on 13th April 2021.

‡ Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d3ob01420g

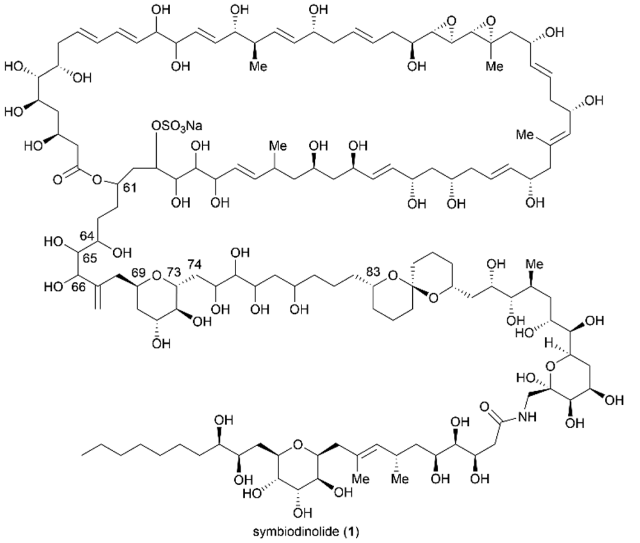


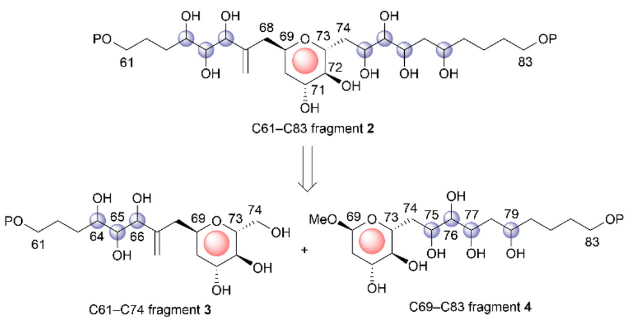
Fig. 1 Structure of **1**.

including the stereoisomers, leading to the stereochemical clarification of the moieties corresponding to 70% of the structure of **1**.[5] Herein, we report the stereodivergent synthesis[6] of the C61–C74 and C69–C83 fragments (**3** and **4**), resulting in the determination of the relative stereochemistry of the C61–C83 fragment **2** that is among the remaining 30%.

Results and discussion

Strategy for the stereochemical determination of fragment 2

The relative configuration of the tetrahydropyran domain at positions C69–C73 of **1** was verified *via* the $^3J_{H,H}$ coupling constants and nuclear Overhauser effect (NOE) observations.[4] The large coupling constants ($^3J_{71,72}$ and $^3J_{72,73}$ = 8.3 Hz) indicate that H-71, H-72, and H-73 are oriented axially. The stereochemistry at the C69 position was elucidated using the NOEs of Ha-68/H-71 and Ha-68/H-73. To determine the stereostructure of fragment **2**, we divided it into two fragments, *i.e.*, fragments **3** and **4** (Scheme 1). The C69–C73 tetrahydropyran ring, with a known relative stereochemistry, is a common structure in fragments **3** and **4**. Therefore, if the relative configurations of **3** and **4** are established individually, these two stereostructures may be connected by this common tetrahydropyran moiety to finally reveal the relative stereochemistry of fragment **2**.



Scheme 1 Strategy for the configurational determination of fragment **2**.

Fragment 3

The relative configurations of the three contiguous oxymethine stereogenic centers at the C64, C65, and C66 positions of **1** were determined *via* ^{1}H NMR data.[4] The coupling constants $^3J_{64,65}$ (5.5 Hz) and $^3J_{65,66}$ (7.4 Hz) indicate that the respective relative relationships of C64/C65 and C65/C66 are *syn* and *anti*. The relative stereochemistries at the C66 and C69 positions are not correlated, and thus, we propose two candidate diastereomers, **3a** and **3b**, for fragment **3**, as shown in Fig. 2.

To stereochemically elucidate fragment **3** of **1**, we planned to synthesize **3a** and **3b** and compare the NMR data of these synthetic products with those of natural product **1**. In our retrosynthetic analysis, target molecules **3a** and **3b** may be synthesized *via* regio- and diastereoselective dihydroxylation at the C64 and C65 positions of dienes **5** and **6**, respectively (Scheme 2). Alcohols **5** and **6**, which are diastereomers at the C66 position, would be formed by coupling alkenyl iodide **7** and aldehyde **8**. Aldehyde **8** would be synthesized by introducing a C4 side-chain into tri-*O*-acetyl-D-glucal (**9**) as a starting material.

We first examined the stereoselective synthesis of aldehyde **14**, which is a branching synthetic intermediate of **3a** and **3b** (Scheme 3). The reaction of the known tetraacetate **10**, which was prepared using **9** in one step,[7] with 2-[(acetoxymethyl)allyl] trimethylsilane (**11**)[8] proceeded in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a Lewis acid to yield allylated product **12** as a single diastereomer.[9] The stereo-

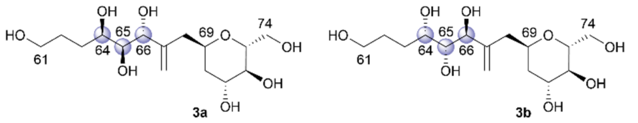
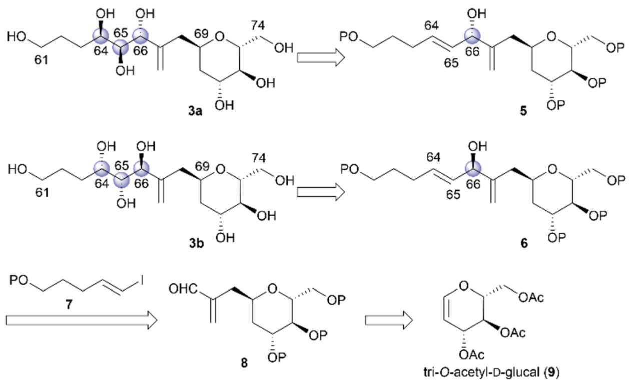
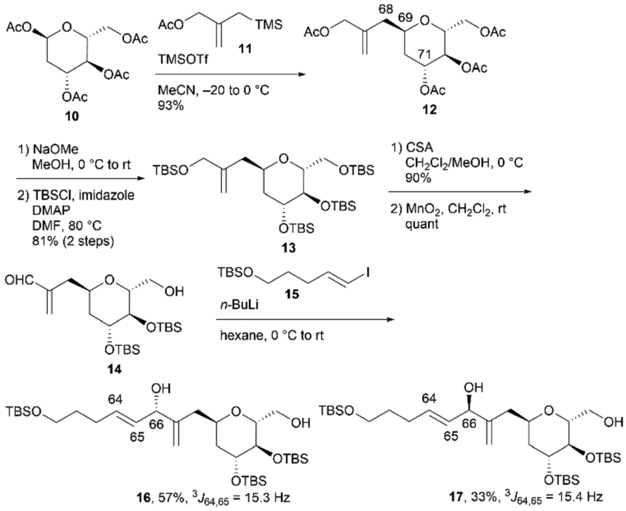


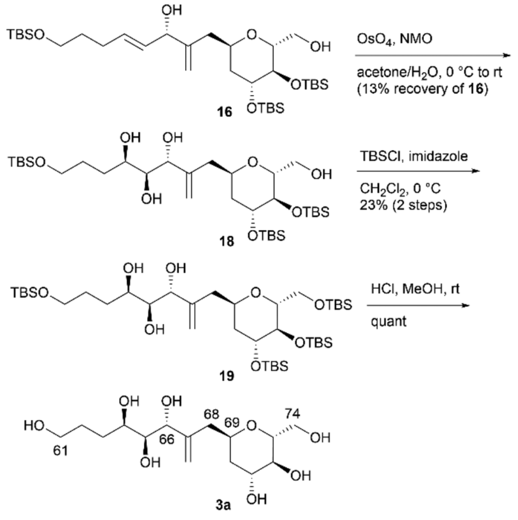
Fig. 2 Candidate diastereomers **3a** and **3b** for fragment **3**.



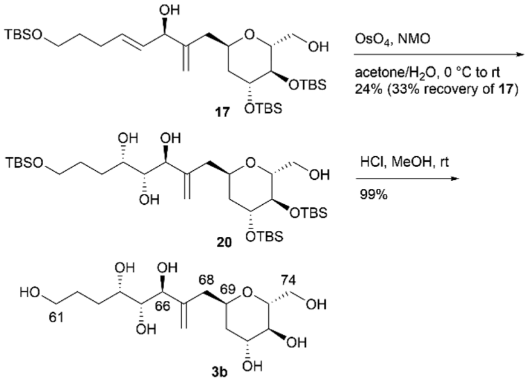
Scheme 2 Retrosynthetic analysis of candidate diastereomers **3a** and **3b**.



Scheme 3 Synthesis of alcohols **16** and **17**.



Scheme 4 Synthesis of candidate compound **3a**.

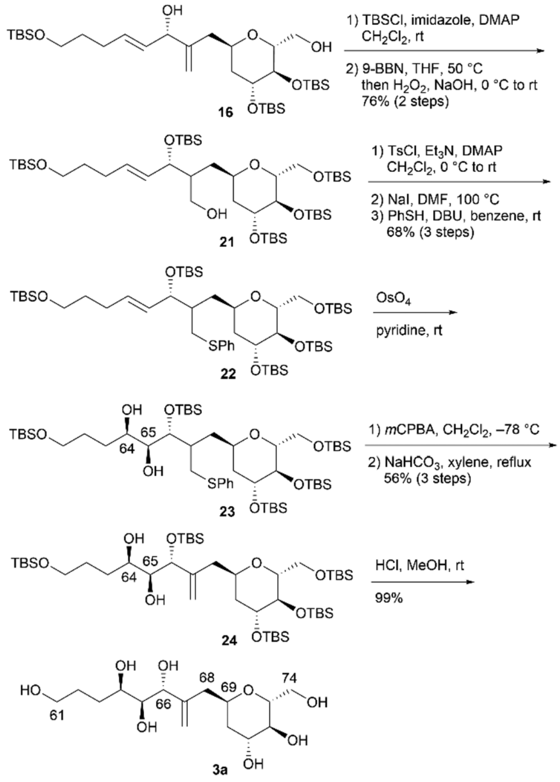


Scheme 5 Synthesis of candidate compound **3b**.

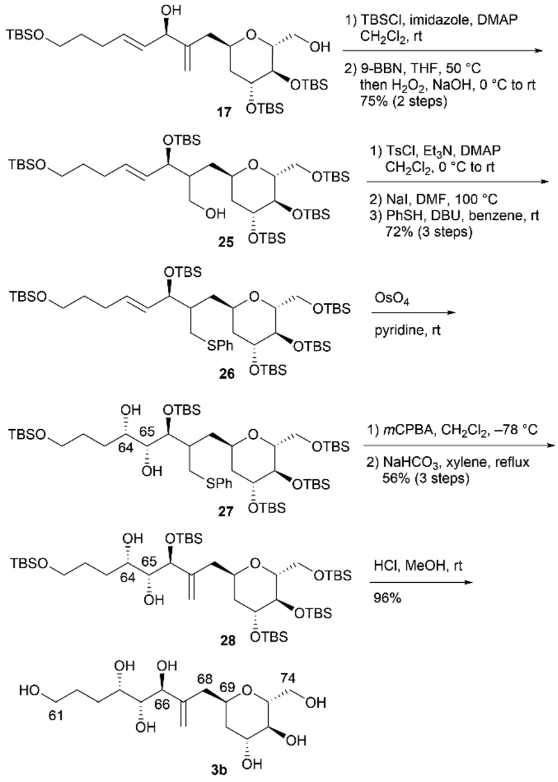
chemistry at the C69 position was confirmed by the NOE correlations between H_2-68 and H-71, which indicate that the introduced side-chain moiety is positioned axially. Global deprotection of **12** *via* methanolysis and subsequent protection of the resulting tetraol using *tert*-butyldimethylsilyl chloride (TBSCl) afforded silyl ether **13** in 81% yield over two steps. The removal of the two primary *tert*-butyldimethylsilyl (TBS) groups of **13** with camphorsulfonic acid (CSA) in CH_2Cl_2/methanol (MeOH), followed by selective oxidation of the allylic alcohol component with MnO_2, produced aldehyde **14**. The coupling reaction of an alkenyl lithium species, which was generated *via* lithium–iodine exchange between alkenyl iodide **15**[10] and *n*-butyllithium (*n*-BuLi) in hexane,[11] with aldehyde **14** generated diols **16** and **17** in respective yields of 57% and 33%.[12] The C64/C65 geometries in **16** and **17** were confirmed as *trans* based on the coupling constants ($^3J_{64,65}$ = 15.3 Hz for **16** and 15.4 Hz for **17**).

We then attempted to convert **16** to the first target molecule **3a** (Scheme 4). Treatment of allylic alcohol **16** with OsO_4/*N*-methylmorpholine *N*-oxide (NMO) in acetone/H_2O[13] produced the desired tetraol **18**, along with its structural isomer, wherein the *exo*-olefin moiety was dihydroxylated.[14] This obtained mixture could not be easily separated, and thus, the primary hydroxy group of **18** was protected with TBSCl/imidazole to furnish tetrakis-TBS ether **19**, which was purified using silica gel column chromatography. The use of AD-mix-α or AD-mix-β[15] in dihydroxylation did not improve the regioselectivity or chemical yield. The four TBS protective groups of **19** were removed using HCl in MeOH to quantitatively obtain the first target molecule, **3a**. The second target molecule, **3b**, was synthesized using **17** *via* a transformation similar to that employed in synthesizing **3a** using **16** (Scheme 5). Thus, dihydroxylation of the disubstituted alkene moiety of **17** was conducted using OsO_4/NMO[13] to afford the desired tetraol **20**[14] in 24% yield, along with recovered **17** in 33% yield. When the reaction time was extended, dihydroxylation of the *exo*-olefin component of **20** was observed. Finally, tris-TBS ether **20** was deprotected using HCl in MeOH to furnish the second target molecule, **3b**. Although we obtained candidate compounds **3a** and **3b**, the regioselectivities in the dihydroxylation of **16** and **17** were uncontrolled, and the chemical yields of **18** and **20** were low. Therefore, alternative synthetic routes to **3a** and **3b** were investigated.

In an alternative synthetic route, we temporarily and regioselectively protected the *exo*-olefin moieties of dienes **16** and **17** prior to dihydroxylation. The second synthesis of **3a** is shown in Scheme 6. After protecting diol **16** using TBSCl, regioselective hydroboration of the obtained diene was examined by changing the borane reagent. The use of $BH_3{\cdot}SMe_2$ (SMe_2 = dimethylsulfide) or thexylborane was ineffective in the regioselective hydroboration of the *exo*-olefin moiety. When we treated the obtained diene with 9-borabicyclo[3.3.1]nonane (9-BBN) and performed an oxidative work-up, the desired alcohol **21** was obtained in 76% yield over two steps. Tosylation of **21**, iodination of the tosylate with NaI, and treatment of the iodoalkane with thiophenol (PhSH)/1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) afforded sulfide **22** in 68% yield



Scheme 6 Improved synthesis of candidate compound **3a**.



Scheme 7 Improved synthesis of candidate compound **3b**.

over three steps. Diastereoselective dihydroxylation of alkene **22** with a stoichiometric amount of OsO_4 in pyridine[13,16] afforded the desired diol **23** and its C64,C65-epimer (dr = 6.2 : 1). The alkene component of **24** was regenerated *via* the following sequence: (1) *meta*-chloroperoxybenzoic acid (*m*CPBA) oxidation of sulfide **23** to the corresponding sulfoxide and (2) elimination of the sulfoxide with $NaHCO_3$ in xylene at reflux. Diol **24** and its C64,C65-epimer were separated, and **24** was obtained in 56% yield in three steps from **22**. Finally, pentakis-TBS ether **24** was deprotected to afford **3a**. The respective total number of steps and overall yield of **3a** from **16** in the second synthesis, as shown in Scheme 6, were 9 and 29%. Although the number of steps was increased compared to that in the first synthesis (3 steps from **16**, as shown in Scheme 4), the overall yield was improved compared to that in the first synthesis (23% from **16**, as shown in Scheme 4). We then investigated the second synthesis of **3b** using a conversion similar to that shown in Scheme 6 (Scheme 7). TBS protection of diol **17** and subsequent regioselective hydroboration with 9-BBN afforded alcohol **25** in 75% yield over two steps. Tosylation, nucleophilic iodination, and thioetherification afforded sulfide **26**. Alkene **26** reacted with OsO_4 in pyridine[13,16] to produce diol **27** (dr = 4.0 : 1), which was then converted to alkene **28** *via* oxidation with *m*CPBA and elimination of the resulting sulfoxide. Global removal of the TBS protecting groups from **28** provided **3b**. As in the case of **3a**, although the transformation from **17** to **3b** in the second synthesis (9 steps, Scheme 7) was lengthy, the overall yield of the second synthesis (29%) was higher than that of the first synthesis (24%, Scheme 5).

After obtaining candidate compounds **3a** and **3b**, we analyzed their 2D NMR spectra. The differences in the ^1H NMR chemical shifts representing the C66, C68, and C69 positions of **1** and the synthetic products **3a** and **3b** are graphically illustrated in Fig. 3.[17] The chemical shift deviations of **3b** were smaller than those of **3a**, as the sums of the magnitudes of the differences in the ^1H NMR chemical shifts representing the C66, C68, and C69 positions were 0.23 (**3a**) and 0.15 (**3b**). Therefore, the relative configuration of fragment **3** of **1** is that of **3b**.

Fragment 4

We then focused on the stereochemical clarification of fragment **4**. This fragment contains four unidentified chiral

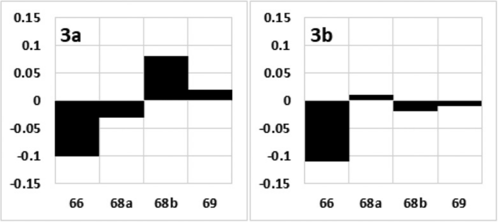
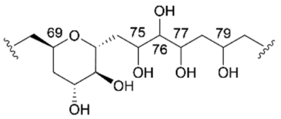


Fig. 3 Deviations in the ^1H NMR chemical shifts of the synthetic products **3a** and **3b** ($\Delta\delta = \delta_1 - \delta_3$ in ppm) relative to those of **1**. The *x* and *y* axes show the carbon number and $\Delta\delta$, respectively.

centers at positions C75, C76, C77, and C79, and thus, 16 diastereomers are possible. Prior to commencing the synthetic approach for structural determination, we analyzed the NMR data of natural product **1** to identify candidate compounds. The NMR chemical shifts of positions C75–C77 of **1**, which are reported in an isolation study,[4] are shown in Table 1. A detailed comparison of these chemical shifts with data reported in the Kishi universal NMR database for 1,2,3-triols[18] clarified the relative relationships of C75–C76/C76–C77 as *syn*/*syn* or *anti*/*anti*. Therefore, eight hexaols, **4a–4h**, as shown in Fig. 4, were used as candidate compounds for fragment **4**.

The stereodivergent synthetic strategy[6] for candidate compounds **4a–4h** is outlined in Scheme 8. The reaction of aldehyde **29** and alkyne **30** would give two propargylic alcohols, **31** and **32**, which are stereoisomeric at the C75 position (Scheme 8a). This coupling reaction is the first step in stereodivergence. Respective *trans*- and *cis*-alkenes **33** and **34** could be synthesized *via* the stereoselective reduction of alkyne **31**, which is the second stereodivergence step (Scheme 8b). The dihydroxylation of *trans*-alkene **33** would afford hexaol **4a**, and hexaol **4b**, which is the C79-epimer of **4a**, could be synthesized *via* stereoinversion at the C79 position, which is the third stereodivergence step, after the dihydroxylation of **33**. Similarly, hexaols **4c** and **4d**, which are the respective C76-stereoisomers of **4a** and **4b**, could be produced using *cis*-alkene **34**. Furthermore, the other four candidates, **4e–4h**, could be prepared using propargylic alcohol **32**, with the respective *trans*- and *cis*-alkenes **35** and **36** as the key synthetic intermediates, *via* a similar transformation. We then commenced the stereodivergent synthesis of candidate compounds **4a–4h**.

Table 1 NMR chemical shifts of **1** in CD_3OD^a



C69–C79 moiety of symbiodinolide (**1**)

Position	^1H NMR	^{13}C NMR
75	3.89	69.6
76	3.18	78.5
77	3.95	70.5

[a] Data reported in ref. 4. Chemical shifts are reported in ppm with reference to the internal residual solvent (^1H NMR: 3.30 ppm, ^{13}C NMR: 49.0 ppm).

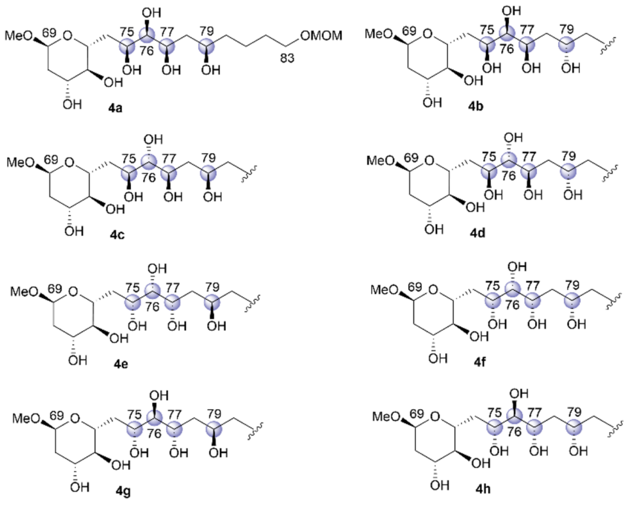
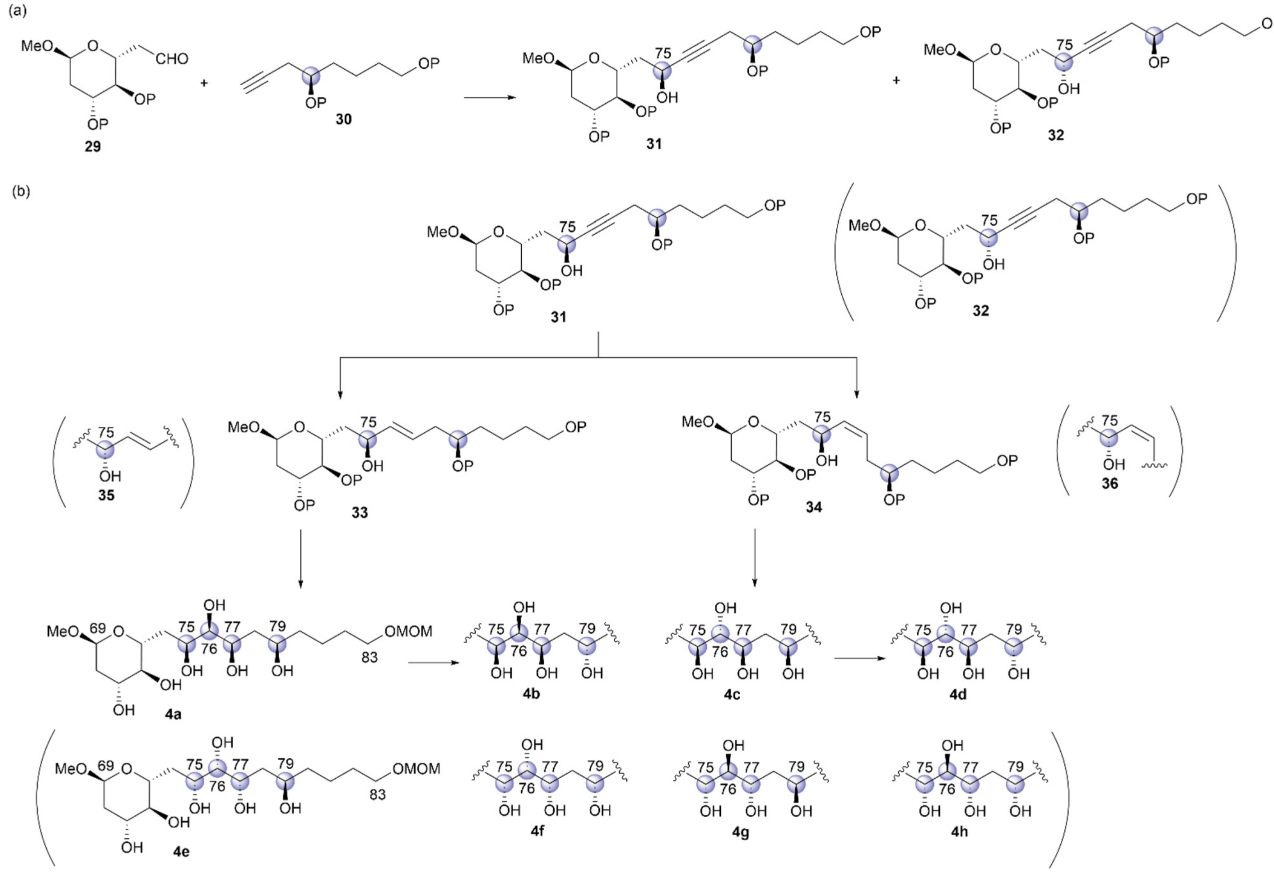


Fig. 4 Candidate diastereomers **4a–4h** for fragment **4**.

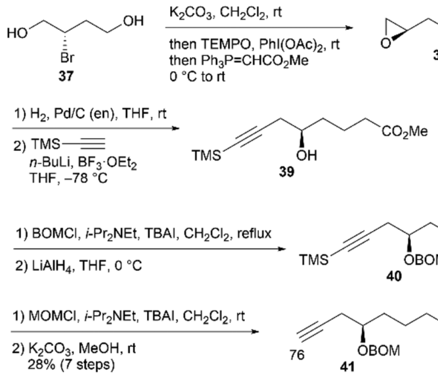
First, we examined the synthesis of alkyne **41**, which corresponds to the C76–C83 fragment (Scheme 9). The synthesis commenced with (*S*)-2-bromobutane-1,4-diol (**37**), which was prepared in two steps using L-aspartic acid.[19] Compound **37** was treated with K_2CO_3 in CH_2Cl_2 to yield a known epoxy alcohol,[19] which underwent oxidation with 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO)/PhI(OAc)$_2$ [20] and subsequent Wittig olefination with $Ph_3P{=}CHCO_2Me$ in one-pot to afford unsaturated ester **38**. The alkene moiety of **38** was chemoselectively hydrogenated with a Pd/C-ethylenediamine complex catalyst in tetrahydrofuran (THF)[21] to provide the saturated ester, with the epoxide structure retained. The resulting epoxide was reacted with trimethylsilylacetylene/*n*-BuLi/$BF_3{\cdot}OEt_2$ [22] (OEt_2 = diethyl ether) to produce alcohol **39**. The hydroxy group of **39** was protected as the benzyloxymethyl (BOM) ether, and the ester group was reduced using $LiAlH_4$ to yield alcohol **40**. Protection of **40** with methoxymethyl chloride/*N*,*N*-diisopropylethylamine/tetra-*n*-butylammonium iodide (MOMCl/*i*-Pr_2NEt/TBAI), followed by removal of the trimethylsilyl (TMS) moiety with K_2CO_3 in MeOH, afforded alkyne **41** in 28% yield in seven steps from **37**.

With coupling precursor **41** in hand, we then synthesized aldehyde **44**, which is the coupling partner of **41** (Scheme 10). Deacetylation of the known methyl glucoside **42**,[23] TBS protection of the resulting triol, and selective deprotection of the primary TBS ether provided alcohol **43** in 76% yield over three steps. One-carbon elongation from **43** was conducted *via* a combination of tosylation and cyanation to yield a nitrile, which was reduced with diisobutylaluminum hydride (DIBAL-H) to afford aldehyde **44**. Deprotonation of alkyne **41** using *n*-BuLi and subsequent addition of the resulting anion to aldehyde **44** afforded the desired alcohols **45** and **46** in respective yields of 28% and 57%.[24]

We then converted **45** to **4a** (Scheme 11). Propargylic alcohol **45** was reduced using Red-Al[25] to yield *trans*-allylic alcohol **47**. The *trans*-geometry of **47** was determined based on the observed coupling constant ($^3J_{76,77}$ = 15.4 Hz). After protection of **47** with *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf)/2,6-lutidine, *trans*-alkene **48**, with an allylic siloxy group, was dihydroxylated with OsO_4 in pyridine[13,16] to afford the desired diol **49** in 28% yield and its C76,C77-epimer in 55% yield (dr = 1 : 2.0). These products could be separated *via* silica gel column chromatography.[14,26] The hydrogenation of BOM ether **49** with $Pd(OH)_2$/C in MeOH generated triol **50**. The three TBS groups of **50** were removed using tetra-*n*-buty-

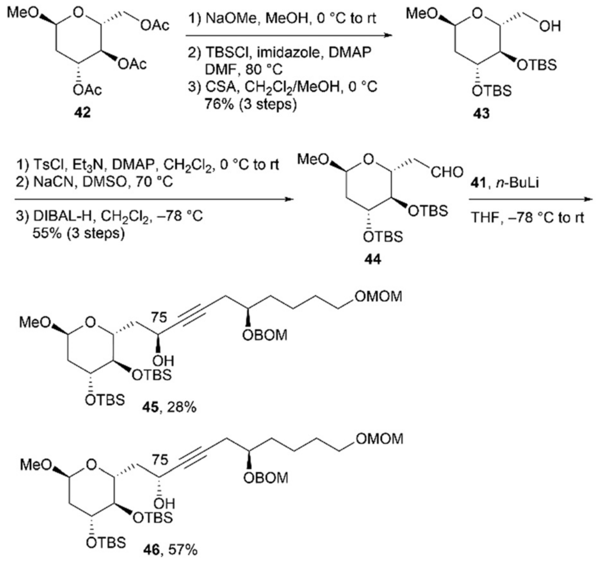


Scheme 8 Stereodivergent synthetic strategy of candidate compounds **4a–4h**.



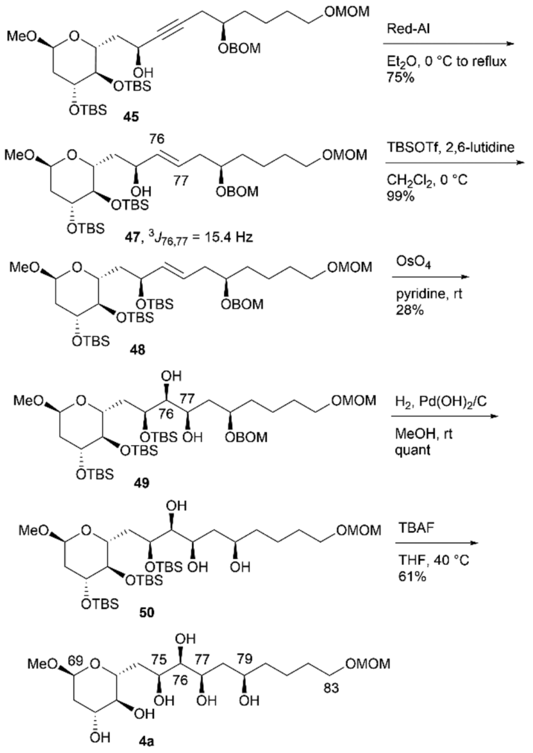
Scheme 9 Synthesis of alkyne **41**.

lammonium fluoride (TBAF) to produce candidate compound **4a**. We then focused on the synthesis of candidate compound **4b**, which is the C79-stereoisomer of **4a** (Scheme 12). Diol **49**, which is the dihydroxylation product in the synthesis of **4a**, was acetylated to afford the diacetate, and the obtained BOM ether was deprotected to yield the corresponding C79-alcohol. The stereochemistry at the C79 position was inverted under Mitsunobu conditions[27] using *p*-nitrobenzoic acid/diethyl azodicarboxylate (DEAD)/triphenylphosphine (Ph$_3$P)[28] to produce *p*-nitrobenzoate **51**. The simultaneous removal of the acetyl

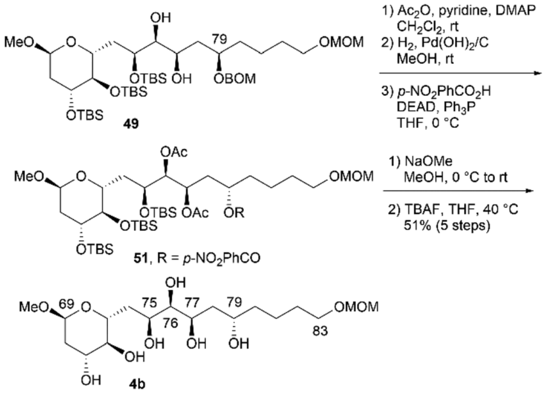


Scheme 10 Synthesis of alcohols **45** and **46**.

and benzoyl groups of triester **51** *via* methanolysis afforded the triol, which was reacted with TBAF to afford candidate compound **4b** in 51% yield in five steps. The ^{1}H and ^{13}C NMR data of **4b** differed from those of **4a**, and thus, the C79-stereo-



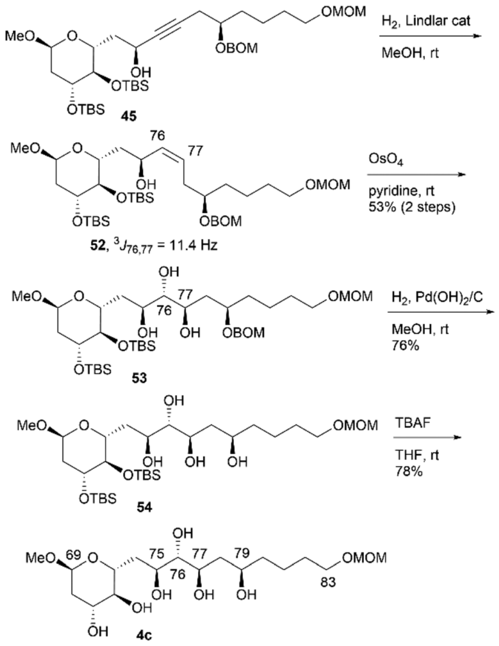
Scheme 11 Synthesis of candidate compound **4a**.



Scheme 12 Synthesis of candidate compound **4b**.

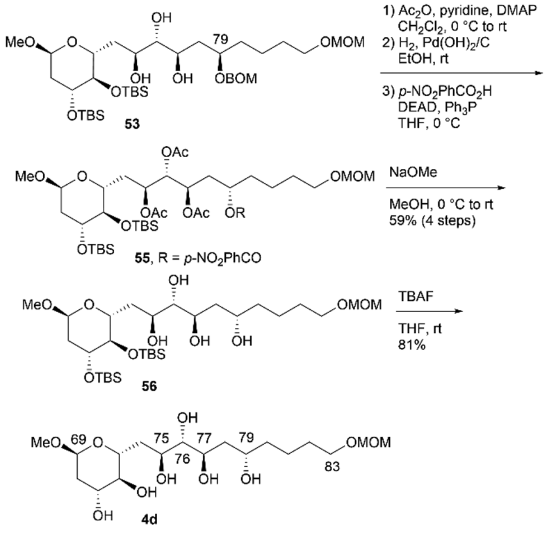
chemistry was inverted in the Mitsunobu reaction, leading to the formation of **51**.

We then investigated the synthesis of candidate compound **4c**, which is the C76-epimer of **4a** (Scheme 13). The hydrogenation of alkyne **45** with a Lindlar catalyst produced *cis*-alkene **52**, with the geometry confirmed by the coupling constant $^3J_{76,77}$ = 11.4 Hz. The osmylation of *cis*-allylic alcohol **52** with OsO_4 in pyridine[13,16] generated the desired triol **53** in 53% yield in two steps,[14] with the C76,C77-epimer of **53** obtained in 44% yield (dr = 1.2 : 1). The two diastereomers were separated using silica gel column chromatography. The deprotection of BOM ether **53** *via* hydrogenation provided tetraol **54**, which was desilylated using TBAF to afford candidate compound **4c**.



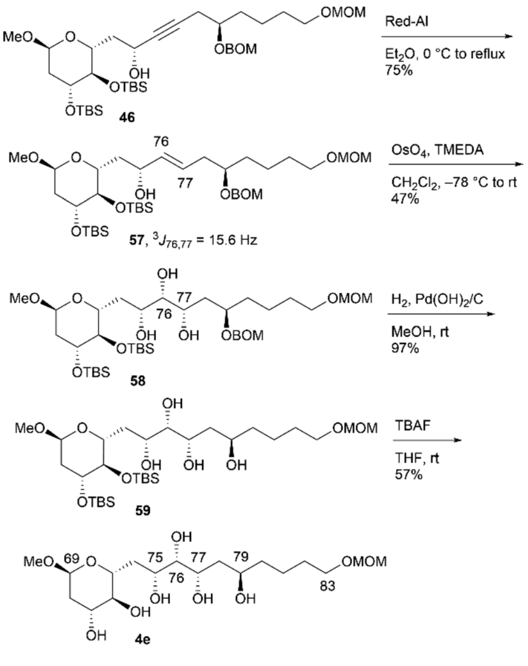
Scheme 13 Synthesis of candidate compound **4c**.

Candidate compound **4d**, which is the C79-epimer of **4c**, was synthesized *via* a conversion similar to that used in synthesizing **4b** (Scheme 14). Thus, acetyl protection of triol **53**, which is a synthetic intermediate of **4c**, removal of the BOM moiety, and Mitsunobu esterification[27,28] with configurational inversion provided the desired *p*-nitrobenzoate **55**. The methanolysis of tetraester **55** afforded tetraol **56** in 59% yield in four steps from **53**. The stereochemical inversion from **53** to **55** *via* Mitsunobu esterification was confirmed by the difference in the NMR data of **56** and **54**. Finally, bis-TBS ether **56** was deprotected to produce candidate compound **4d**.

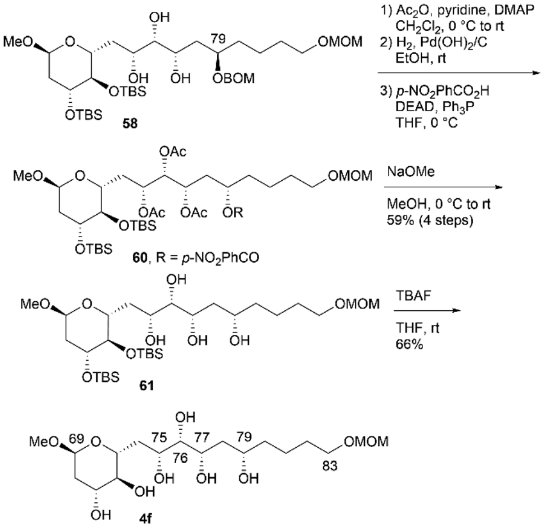


Scheme 14 Synthesis of candidate compound **4d**.

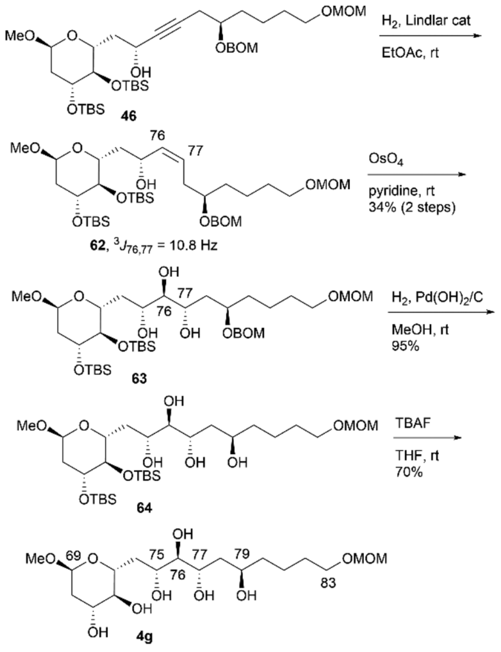
Having synthesized candidate compounds **4a–4d** by branching from the common synthetic intermediate **45** with the 75*S* absolute configuration, we then attempted to synthesize the other four candidate compounds, **4e–4h**, using propargylic alcohol **46**, with 75*R* stereochemistry, as the common intermediate. The transformation of **46** into candidate compound **4e** is shown in Scheme 15. Propargylic alcohol **46** was treated with Red-Al[25] to produce *trans*-allylic alcohol **57** in 75% yield, and the *trans*-structure of alkene **57** was elucidated using the coupling constant $^3J_{76,77}$ = 15.6 Hz. Allylic alcohol **57** was subjected to the directed dihydroxylation in accordance with the protocol reported by Donohoe *et al.*[29] Thus, the reaction of **57** with OsO_4/tetramethylethylenediamine (TMEDA) in CH_2Cl_2 afforded the desired triol **58** in 47% yield[14] and its C76,C77-stereoisomer in 39% yield (dr = 1.2 : 1). The two triols were separated using silica gel column chromatography. The removal of the BOM protecting group *via* hydrogenation and the two TBS groups using TBAF provided candidate compound **4e**. Triol **58** was then converted to candidate compound **4f** *via* stereoinversion at the C79 position (Scheme 16). The acetylation of **58**, removal of the BOM group, and Mitsunobu esterification[27,28] produced inverted *p*-nitrobenzoate **60**, which underwent methanolysis to afford tetraol **61** in 59% yield over four steps. The configurational inversion under Mitsunobu conditions was verified by comparing the NMR data of **61** and its C79-epimer **59**. The deprotection of bis-TBS ether **61** afforded candidate compound **4f** in 66% yield.



Scheme 15 Synthesis of candidate compound **4e**.

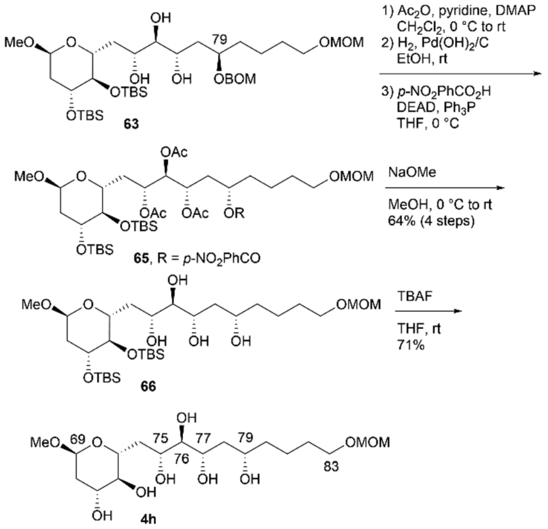


Scheme 16 Synthesis of candidate compound **4f**.



Scheme 17 Synthesis of candidate compound **4g**.

We then attempted to convert **46** to candidate compound **4g**, which is the C76-epimer of **4e** (Scheme 17). Alkyne **46** was hydrogenated using a Lindlar catalyst to yield *cis*-alkene **62**, with the geometry elucidated using the coupling constant $^3J_{76,77}$ = 10.8 Hz. Treatment of *cis*-allylic alcohol **62** with OsO_4 in pyridine[13,16] afforded the desired triol **63** and its C76,C77-stereoisomer in respective yields of 34% and 26% in two steps (dr = 1.3 : 1).[14] Synthetic **63** and its diastereomer were separated *via* silica gel column chromatography. The removal of the BOM group of **63**, followed by desilylation, yielded candidate compound **4g**. Finally, we investigated the synthesis of candidate compound **4h**, which is the C79-stereoisomer of **4g** (Scheme 18). Triol **63** was converted to *p*-nitrobenzoate **65** *via* the following sequence: (1) acetylation, (2) deprotection of the BOM ether, and (3) Mitsunobu esterification.[27,28] Methanolysis of tetraester **65** and subsequent deprotection of bis-TBS ether **66** produced candidate compound **4h**. The difference in the



Scheme 18 Synthesis of candidate compound **4h**.

NMR data of **66** and **64** confirmed the stereoinversion in the Mitsunobu reaction during the synthesis of **65** using **63**.

With candidate compounds **4a–4h** in hand, we compared the NMR data of these eight synthetic products with those of natural **1**. Deviations in the ^{1}H NMR chemical shifts of natural **1** and the synthesized samples **4a–4h** at the C75, C76, C77, and C79 positions were calculated, as shown in Fig. 5.[17] The ^{1}H NMR characteristics of **4e** and **4f** were more similar to those of the natural product than those of the other candidate compounds. The total magnitudes of the differences in the ^{1}H NMR chemical shifts at the C75, C76, C77, and C79 positions are shown in Table 2, and those of **4e** and **4f** were 0.21. We then compared the ^{13}C NMR chemical shifts of the eight synthetic candidates with those of the natural product. As shown

Table 2 Sums of the magnitudes of the differences in the NMR chemical shifts (ppm) of natural **1** and the synthetic products **4a–4h** at the C75, C76, C77, and C79 positions

Compound	^{1}H NMR	^{13}C NMR
4a	0.33	8.0
4b	0.26	5.4
4c	0.35	7.5
4d	0.25	4.9
4e	0.21	2.1
4f	0.21	3.1
4g	0.37	5.2
4h	0.30	3.2

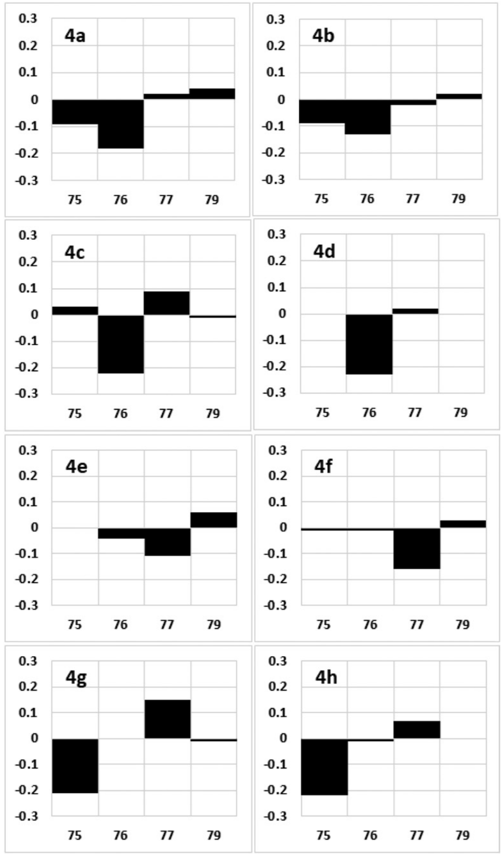


Fig. 5 Deviations in the ^{1}H NMR chemical shifts of the synthetic products **4a–4h** ($\Delta\delta = \delta_1 - \delta_4$ in ppm) relative to those of **1**. The *x* and *y* axes show the carbon number and $\Delta\delta$, respectively.

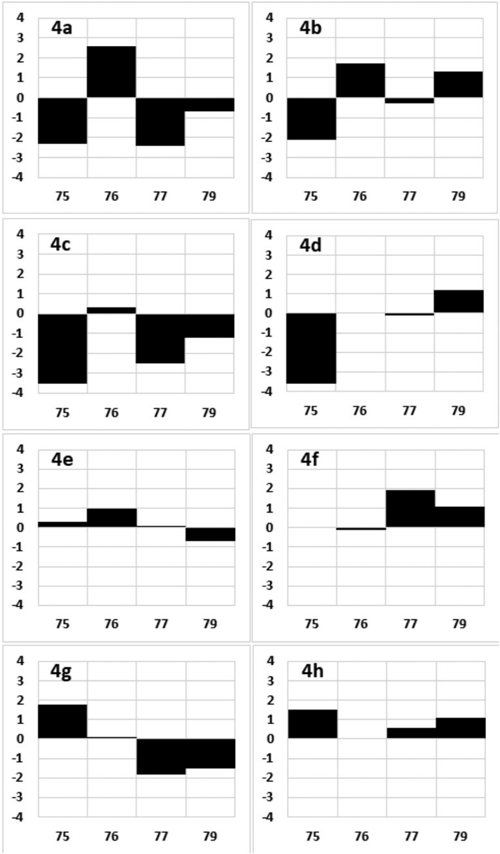
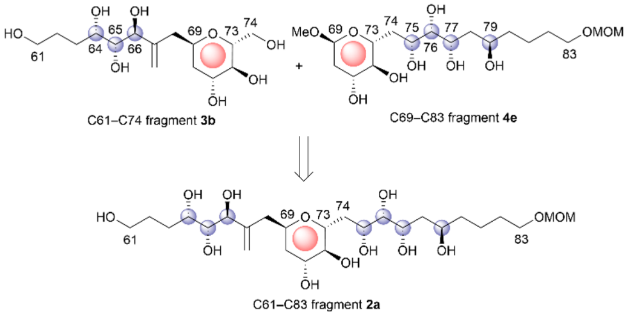


Fig. 6 Deviations in the ^{13}C NMR chemical shifts of the synthetic products **4a–4h** ($\Delta\delta = \delta_1 - \delta_4$ in ppm) relative to those of **1**. The *x* and *y* axes show the carbon number and $\Delta\delta$, respectively.



Scheme 19 Relative configuration of fragment **2**.

in Fig. 6, candidate compound **4e** exhibited the most similar characteristics of candidate compounds **4a–4h** to those of the natural product.[17] The sum of the magnitudes of the deviations in the ^{13}C NMR chemical shifts of **4e** was 2.1, as shown in Table 2, which was the smallest of those calculated for **4a–4h**. Therefore, the relative stereochemistry of fragment **4** is shown in **4e**.

Relative stereochemistry of fragment 2

We determined the relative configurations of fragments **3** and **4** in **1** as those described by **3b** and **4e**, respectively, by synthesizing all candidate compounds for each fragment and comparing their NMR chemical shifts with those of the natural product (Scheme 19). The C69–C73 tetrahydropyran moiety is a common structure in both fragments. Therefore, by connecting the relative stereostructures **3b** and **4e** *via* the common C69–C73 component, we elucidated the relative stereochemistry of fragment **2** in **1** as that of **2a**.

Conclusions

Fragment **2** is among the 30% of the structure of **1** that has not been previously clarified. To determine the stereochemistry of fragment **2**, we divided it into fragments **3** and **4** and elucidated the relative configurations of these two fragments using a stereodivergent synthetic approach. For fragment **3**, the first synthesis of candidate compounds **3a** and **3b** was performed by coupling aldehyde **14** and alkenyl iodide **15**, with the dihydroxylation of dienes **16** and **17**, wherein the regioselectivities were uncontrolled. To overcome this problem, in the second synthesis of **3a** and **3b**, the *exo*-olefin components of dienes **16** and **17** were temporally and regioselectively protected prior to dihydroxylation. The overall yields of **3a** and **3b** in the second synthesis were improved compared to those in the first synthesis. A comparison of the NMR chemical shifts of these two synthetic products and the natural product revealed that natural product **1** displayed the relative stereochemistry of **3b** in fragment **3**. For fragment **4**, we first analyzed the NMR data of positions C75–C77 of the natural product and selected candidate compounds **4a–4h** from 16 possible diastereomers. The stereodivergent, unified synthesis of target molecules **4a–4h** was performed *via* the coupling of alkyne **41** and aldehyde **44**, stereoselective reduction of alkynes **45** and **46**, and Mitsunobu inversion as the stereodivergence steps. The NMR chemical shifts of the synthetic products **4a–4h** were compared with those of natural product **1**, which clarified that the relative configuration in this domain of **1** was that of **4e**. The tetrahydropyran component at positions C69–C73 is a common structure in fragments **3** and **4**. Therefore, by overlapping the common tetrahydropyran moieties in **3b** and **4e**, the relative stereochemistry of fragment **2** of **1** is shown as **2a**. The stereodivergent synthetic strategy used in this study can be employed for the configurational determination of other fragments of **1**. Further study for the complete structural clarification of **1** is currently underway in our group.

Author contributions

H. T. conceived and directed the study. K. H., T. Ohashi, and T. Otsu conducted the syntheses and collected the data. All authors analyzed the collected data. H. T. prepared the manuscript.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We are grateful to Mr Soichiro Inoue (Okayama University, Okayama, Japan) for experimental support. We appreciate the assistance of the Division of Instrumental Analysis at Okayama University with NMR spectroscopy and high-resolution mass spectrometry. This study was supported by a Japan Society for the Promotion of Science KAKENHI grant (grant number: JP21H01938).

References

1 For selected reviews, see: (*a*) D. J. Newman and G. M. Cragg, Drugs and drug candidates from marine sources: An assessment of the current "state of play", *Planta Med.*, 2016, **82**, 775–789; (*b*) K.-H. Altmann, Drugs from the oceans: Marine natural products as leads for drug discovery, *Chimia*, 2017, **71**, 646–652; (*c*) C. Alves, J. Silva, S. Pinteus, H. Gaspar, M. C. Alpoim, L. M. Botana and R. Pedrosa, From marine origin to therapeutics: The antitumor potential of marine algae-derived compounds, *Front. Pharmacol.*, 2018, **9**, 777; (*d*) A. R. Carroll, B. R. Copp, R. A. Davis, R. A. Keyzers and M. R. Prinsep, Marine natural products, *Nat. Prod. Rep.*, 2023, **40**, 275–325.

2 For selected reviews, see: (*a*) M. Elyashberg, A. J. Williams and K. Blinov, Structural revisions of natural products by

Computer-Assisted Structure Elucidation (CASE) systems, *Nat. Prod. Rep.*, 2010, **27**, 1296–1328; (*b*) M. W. Lodewyk, M. R. Siebert and D. J. Tantillo, Computational prediction of ^{1}H and ^{13}C chemical shifts: A useful tool for natural product, mechanistic, and synthetic organic chemistry, *Chem. Rev.*, 2012, **112**, 1839–1862; (*c*) N. Grimblat and A. M. Sarotti, Computational chemistry to the rescue: Modern toolboxes for the assignment of complex molecules by GIAO NMR calculations, *Chem. – Eur. J.*, 2016, **22**, 12246–12261; (*d*) R. B. Nazarski, Summary of DFT calculations coupled with current statistical and/or artificial neural network (ANN) methods to assist experimental NMR data in identifying diastereomeric structures, *Tetrahedron Lett.*, 2021, **71**, 152548.

3 For selected reviews, see: (*a*) K. C. Nicolaou and S. A. Snyder, Chasing molecules that were never there: Misassigned natural products and the role of chemical synthesis in modern structure elucidation, *Angew. Chem., Int. Ed.*, 2005, **44**, 1012–1044; (*b*) M. E. Maier, Structural revisions of natural products by total synthesis, *Nat. Prod. Rep.*, 2009, **26**, 1105–1124; (*c*) T. L. Suyama, W. H. Gerwick and K. L. McPhail, Survey of marine natural product structure revisions: A synergy of spectroscopy and chemical synthesis, *Bioorg. Med. Chem.*, 2011, **19**, 6675–6701; (*d*) T. F. Molinski and B. I. Morinaka, Integrated approaches to the configurational assignment of marine natural products, *Tetrahedron*, 2012, **68**, 9307–9343; (*e*) H. Fuwa, Structure determination, correction, and disproof of marine macrolide natural products by chemical synthesis, *Org. Chem. Front.*, 2021, **8**, 3990–4023; (*f*) M. W. Ha, J. Kim and S.-M. Paek, Recent achievements in total synthesis for integral structural revisions of marine natural products, *Mar. Drugs*, 2022, **20**, 171.

4 M. Kita, N. Ohishi, K. Konishi, M. Kondo, T. Koyama, M. Kitamura, K. Yamada and D. Uemura, Symbiodinolide, a novel polyol macrolide that activates N-type Ca^{2+} channel, from the symbiotic marine dinoflagellate *Symbiodinium* sp., *Tetrahedron*, 2007, **63**, 6241–6251.

5 (*a*) H. Takamura, J. Ando, T. Abe, T. Murata, I. Kadota and D. Uemura, Stereocontrolled synthesis of the C79–C96 fragment of symbiodinolide, *Tetrahedron Lett.*, 2008, **49**, 4626–4629; (*b*) T. Murata, M. Sano, H. Takamura, I. Kadota and D. Uemura, Synthesis and structural revision of symbiodinolide C23–C34 fragment, *J. Org. Chem.*, 2009, **74**, 4797–4803; (*c*) H. Takamura, T. Murata, T. Asai, I. Kadota and D. Uemura, Stereoselective synthesis and absolute configuration of the C1′–C25′ fragment of symbiodinolide, *J. Org. Chem.*, 2009, **74**, 6658–6666; (*d*) H. Takamura, Y. Kadonaga, Y. Yamano, C. Han, Y. Aoyama, I. Kadota and D. Uemura, Synthesis and structural determination of the C33–C42 fragment of symbiodinolide, *Tetrahedron Lett.*, 2009, **50**, 863–866; (*e*) H. Takamura, Y. Kadonaga, Y. Yamano, C. Han, I. Kadota and D. Uemura, Stereoselective synthesis and absolute configuration of the C33–C42 fragment of symbiodinolide, *Tetrahedron*, 2009, **65**, 7449–7456; (*f*) H. Takamura, Y. Kadonaga, I. Kadota and D. Uemura, Stereoselective synthesis of the C14–C24 degraded fragment of symbiodinolide, *Tetrahedron Lett.*, 2010, **51**, 2603–2605; (*g*) H. Takamura, Y. Kadonaga, I. Kadota and D. Uemura, Stereocontrolled synthesis and structural confirmation of the C14–C24 degraded fragment of symbiodinolide, *Tetrahedron*, 2010, **66**, 7569–7576; (*h*) H. Takamura, K. Tsuda, Y. Kawakubo, I. Kadota and D. Uemura, Stereoselective synthesis of the C94–C104 fragment of symbiodinolide, *Tetrahedron Lett.*, 2012, **53**, 4317–4319; (*i*) H. Takamura, T. Fujiwara, I. Kadota and D. Uemura, Stereoselective synthesis of the C79–C97 fragment of symbiodinolide, *Beilstein J. Org. Chem.*, 2013, **9**, 1931–1935; (*j*) H. Takamura, H. Wada, M. Ogino, T. Kikuchi, I. Kadota and D. Uemura, Stereodivergent synthesis and relative stereostructure of the C1–C13 fragment of symbiodinolide, *J. Org. Chem.*, 2015, **80**, 3111–3123; (*k*) H. Takamura, T. Fujiwara, Y. Kawakubo, I. Kadota and D. Uemura, Stereoselective synthesis of the proposed C79–C104 fragment of symbiodinolide, *Chem. – Eur. J.*, 2016, **22**, 1979–1983; (*l*) H. Takamura, T. Fujiwara, Y. Kawakubo, I. Kadota and D. Uemura, Stereodivergent synthesis and stereochemical reassignment of the C79–C104 fragment of symbiodinolide, *Chem. – Eur. J.*, 2016, **22**, 1984–1996.

6 For selected reviews, see: (*a*) H. Takamura, Recent topics of the stereodivergent synthesis of natural products, *Tetrahedron Lett.*, 2018, **59**, 955–966; (*b*) C. Nájera, F. Foubelo, J. M. Sansano and M. Yus, Stereodivergent routes in organic synthesis: Carbohydrates, amino acids, alkaloids and terpenes, *Org. Biomol. Chem.*, 2020, **18**, 1232–1278; (*c*) C. Nájera, F. Foubelo, J. M. Sansano and M. Yus, Stereodivergent routes in organic synthesis: Marine natural products, lactones, other natural products, heterocycles and unnatural compounds, *Org. Biomol. Chem.*, 2020, **18**, 1279–1336.

7 S. N. Lam and J. Gervay-Hague, Efficient route to 2-deoxy β-*O*-aryl-D-glycosides *via* direct displacement of glycosyl iodides, *Org. Lett.*, 2003, **5**, 4219–4222.

8 (*a*) B. M. Trost and D. M. T. Chan, New conjunctive reagents. 2-Acetoxymethyl-3-allyltrimethylsilane for methylenecyclopentane annulations catalyzed by palladium(0), *J. Am. Chem. Soc.*, 1979, **101**, 6429–6432; (*b*) B. M. Trost, M. Buch and M. L. Miller, Convenient alternative approach to 2-(acetoxymethyl)-3-(trimethylsilyl)propene, *J. Org. Chem.*, 1988, **53**, 4887–4888.

9 (*a*) M. Morimoto, H. Matsukura and T. Nakata, Total synthesis of hemibrevetoxin B, *Tetrahedron Lett.*, 1996, **37**, 6365–6368; (*b*) I. Kadota, H. Takamura, H. Nishii and Y. Yamamoto, Total synthesis of brevetoxin B, *J. Am. Chem. Soc.*, 2005, **127**, 9246–9250.

10 D. P. Curran, Q. Zhang, C. Richard, H. Lu, V. Gudipati and C. S. Wilcox, Total synthesis of a 28-member stereoisomer library of murisolins, *J. Am. Chem. Soc.*, 2006, **128**, 9561–9573.

11 T. Yokoo, H. Shinokubo, K. Oshima and K. Utimoto, A room temperature preparation of alkenyllithiums by lithium-halogen exchange between alkenyl iodides and *n*-BuLi in hydrocarbon solvents, *Synlett*, 1994, 645–646.

12 For the stereochemical determination at the C66 position of **17**, see ESI.‡

13 (*a*) J. K. Cha, W. J. Christ and Y. Kishi, On stereochemistry of osmium tetraoxide oxidation of allylic alcohol systems. Empirical rule, *Tetrahedron*, 1984, **40**, 2247–2255; (*b*) J. K. Cha and N.-S. Kim, Acyclic stereocontrol induced by allylic alkoxy groups. Synthetic applications of stereoselective dihydroxylation in natural product synthesis, *Chem. Rev.*, 1995, **95**, 1761–1795.

14 For the stereostructural determination, see ESI.‡

15 For a review, see: H. C. Kolb, M. S. VanNieuwenhze and K. B. Sharpless, Catalytic asymmetric dihydroxylation, *Chem. Rev.*, 1994, **94**, 2483–2547.

16 J. S. Baran, Notes – Method for the cleavage of osmate esters, *J. Org. Chem.*, 1960, **25**, 257.

17 The chemical shifts are described in ESI.‡

18 S. Higashibayashi, W. Czechtizky, Y. Kobayashi and Y. Kishi, Universal NMR databases for contiguous polyols, *J. Am. Chem. Soc.*, 2003, **125**, 14379–14393.

19 C. D. Donner, The divergent asymmetric synthesis of kalafungin, 5-*epi*-frenolicin B and related pyranonaphthoquinone antibiotics, *Tetrahedron*, 2013, **69**, 377–386.

20 A. De Mico, R. Margarita, L. Parlanti, A. Vescovi and G. Piancatelli, A versatile and highly selective hypervalent iodine (III)/2,2,6,6-tetramethyl-1-piperidinyloxyl-mediated oxidation of alcohols to carbonyl compounds, *J. Org. Chem.*, 1997, **62**, 6974–6977.

21 H. Sajiki, K. Hattori and K. Hirota, Highly chemoselective hydrogenation with retention of the epoxide function using a heterogeneous Pd/C-ethylenediamine catalyst and THF, *Chem. – Eur. J.*, 2000, **6**, 2200–2204.

22 M. Yamaguchi and I. Hirao, An efficient method for the alkynylation of oxiranes using alkynyl boranes, *Tetrahedron Lett.*, 1983, **24**, 391–394.

23 (*a*) C. J. France, I. M. McFarlane, C. G. Newton, P. Pitchen and M. Webster, Straightforward homochiral synthesis of the lactone moiety of mevinic acids, *Tetrahedron Lett.*, 1993, **34**, 1635–1638; (*b*) H. Fuwa, Y. Okamura and H. Natsugari, Synthetic studies on antascomicin A: Construction of the C18–C34 fragment, *Tetrahedron*, 2004, **60**, 5341–5352.

24 For the stereochemical determination at the C75 position of **46**, see ESI.‡

25 S. E. Denmark and T. K. Jones, (*E*)-3-(Trimethylsilyl)-2-propen-1-ol. An improved preparation, *J. Org. Chem.*, 1982, **47**, 4595–4597.

26 When we treated allylic alcohol **47** with OsO_4/TMEDA in CH_2Cl_2,[29] the desired triol and its C76,C77-epimer were obtained as an inseparable mixture in respective yields of 38%. Our attempts to derivatize and separate this mixture failed.

27 For selected reviews, see: (*a*) O. Mitsunobu, The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformation of natural products, *Synthesis*, 1981, 1–28; (*b*) K. C. K. Swamy, N. N. B. Kumar, E. Balaraman and K. V. P. P. Kumar, Mitsunobu and related reactions: Advances and applications, *Chem. Rev.*, 2009, **109**, 2551–2651.

28 S. F. Martin and J. A. Dodge, Efficacious modification of the mitsunobu reaction for inversions of sterically hindered secondary alcohols, *Tetrahedron Lett.*, 1991, **32**, 3017–3020.

29 (*a*) T. J. Donohoe, N. J. Newcombe and M. J. Waring, *syn* Stereocontrol in the directed dihydroxylation of acyclic allylic alcohols, *Tetrahedron Lett.*, 1999, **40**, 6881–6885; (*b*) T. J. Donohoe, K. Blades, P. R. Moore, M. J. Waring, J. J. G. Winter, M. Helliwell, N. J. Newcombe and G. Stemp, Directed dihydroxylation of cyclic allylic alcohols and trichloroacetamides using OsO_4/TMEDA, *J. Org. Chem.*, 2002, **67**, 7946–7956.