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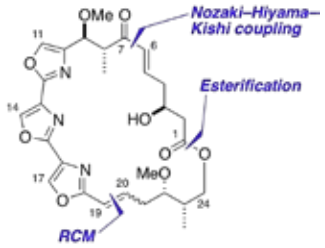

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# Synthesis and biological activities of the tris-oxazole macrolactone analogs of mycalolides

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ARTICLE INFO

*Article history:*
Received
Received in revised form
Accepted
Available online

*Keywords:*
Natural products
Structure-activity relationships
Ring-closing metathesis
Actin depolymerization
Antimycotic activity

ABSTRACT

Mycalolides are trisoxazole macrolides isolated from the marine sponge *Mycale* sp., which shows cytotoxic, antifungal, and actin-depolymerizing activities. To develop an efficient synthetic route of mycalolides and to evaluate its functional mechanism of biological activities, tris-oxazole macrolactone analogs of mycalolides were synthesized through the use of ring-closing metathesis (RCM). The presence/absence of protecting groups at C3, solvent polarity, and reaction temperature significantly affected the stereoselectivity of RCM (*E*:*Z* = 2.5/1.0 to 1.0/2.5). The 19*E*- and 19*Z*-stereoisomers both exhibited moderate cytotoxicity against tumor cells, but neither showed significant actin-depolymerizing properties or antimycotic activity against pathogenic fungi. Thus, both the side-chain (actin-binding) moiety and the macrolactone moiety were suggested to be essential for the potent biological activities of the parent molecules.



## 1. Introduction

Various actin-depolymerizing agents have been found in marine invertebrates, and some show extremely potent cytotoxicity. They are considered to be useful for the development of novel pharmacological tools for analyzing actin-mediated cell functions, such as muscle contraction, cell motility, and cytokinesis. Among them, mycalolide B (**1**) is a unique tris-oxazole macrolide that was isolated from the marine sponge *Mycale* sp. (Fig. 1),[1] which exhibits various biological activities, such as cytotoxicity against B16 melanoma cells, inhibition of actomyosin $Mg^{2+}$–ATPase,[2] and antimycotic activity against several pathogenic fungi.[3] It has been suggested that several antifungal compounds might serve as a chemical defense against microbial attack for marine invertebrates that lack cellular immune systems.[4]

Many tris-oxazole macrolides that are structurally related to mycalolides have been isolated, such as ulapualides,[5] halichondramides,[6] jaspisamides,[7] and kabiramides;[8] and all of these exhibit potent actin-depolymerizing properties. X-ray analyses of the actin–kabiramide C,[9] actin–jaspisamide A,[9] and actin–ulapualide A complexes [10] revealed that their side-chain parts (C25–C35) intercalate into the hydrophobic cleft between subdomains 1 and 3 of actin. Furthermore, aplyronine A (**4**), which shows potent antitumor activities against several cancers, also possesses an actin-binding side-chain part similar to that of mycalolides.[11–13] Photoaffinity labeling experiments [14] and X-ray crystallographic analysis [15] have also established that the interactions of actin–aplyronines are closely related to those of mycalolides. Meanwhile, the macrocyclic functional groups of aplyronines, i.e., the C7 trimethylserine and C9 hydroxyl groups, have been shown to be essential for its potent cytotoxicity.[16] Thus, we expected that the structurally unique macrolactone moieties in mycalolides and related tris-oxazole macrolides could serve as a pharmacophore that enable them to specifically interact with biomacromolecules to exhibit various biological activities.

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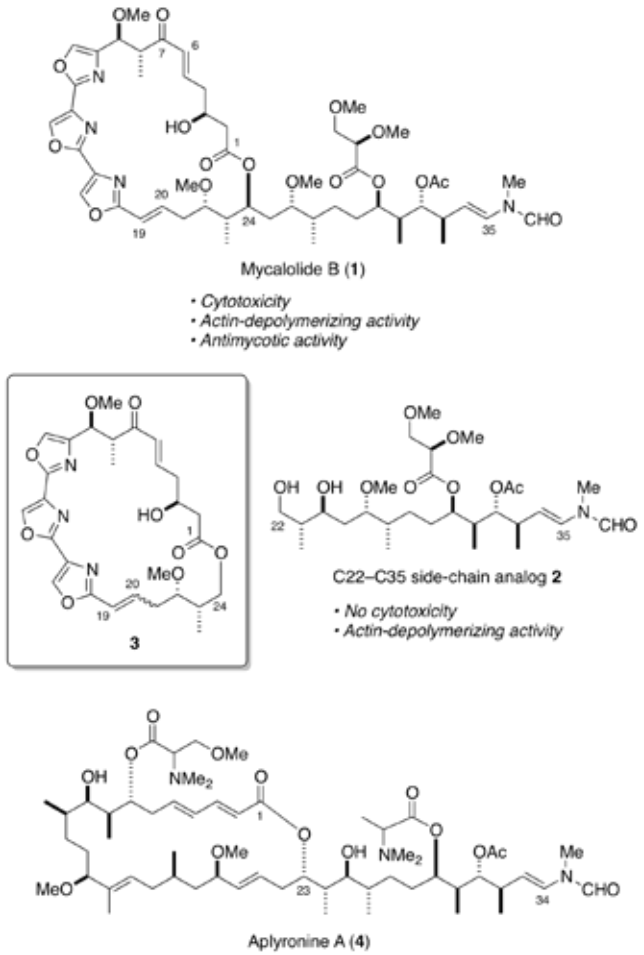



**Fig. 1.**

Due to their complexity and unique biological activities, there have been several synthetic studies on tris-oxazole macrolides.[17] Recently, total syntheses of mycalolide A [18] and ulapualide A [19] have been described. We previously synthesized the C22–C35 part **2** of mycalolide B, which showed significant actin-depolymerizing activity corresponding to that of **1**, but no cytotoxicity.[20] However, mycalolide derivatives that consist of the tris-oxazole macrolactone part alone have not yet been synthesized and characterized. It was expected that ulapualides and halichondramide could incorporate several metal ions (i.e. Fe, Co, Cu) to form coordination complexes, in which the oxazole N atoms and the O atoms of C3 and C7 oxygenated functional groups may coordinate to metals.[21] Thus, it is possible that the lactone moiety of mycalolides and related tris-oxazole macrolides might serve as an ionophore, which could contribute to their unique bioactivities. Here we describe the synthesis of tris-oxazole macrolactone analogs **3** through the use of ring-closing metathesis (RCM) along with their biological activities.

## 2. Results and discussion

### 2.1 Molecular modeling studies

First, we estimated the three-dimensional structure of the macrolactone part of **1** by using a molecular modeling method. A conformational analysis and density functional theory (DFT) calculation of tris-oxazole macrolactone analogs **3** revealed that the 19*E* isomer is lower in energy than the 19*Z* isomer by 5.4 kJ/mol at the B3LYP/6-31G(d) level of theory. (Fig. 2) [22] This difference was almost identical to that for (*E*)- and (*Z*)-2-(1-butenyl)oxazoles (6.2 kJ/mol). Conjugated tris-oxazoles, the C7 conjugated ketones, and the C19 olefin moieties in both (*E*)- and (*Z*)-**3** have an almost planar orientation, which suggested that their conformations are considerably fixed. The distances between the C3 hydroxyl H atom and two oxazole N atoms of (*E*)-**3** were less than 3.1 Å, which strongly indicated the presence of intramolecular hydrogen bonding. In contrast, the C3 hydroxyl group in (*Z*)-**3** did not interact with the tris-oxazole moiety. These significant differences in the stereostructures of *E*- and *Z*-isomers led us to synthesize both isomers and compare their biological activities.

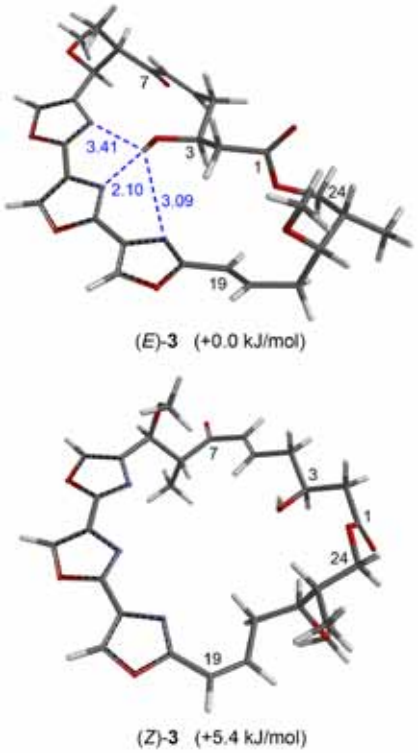
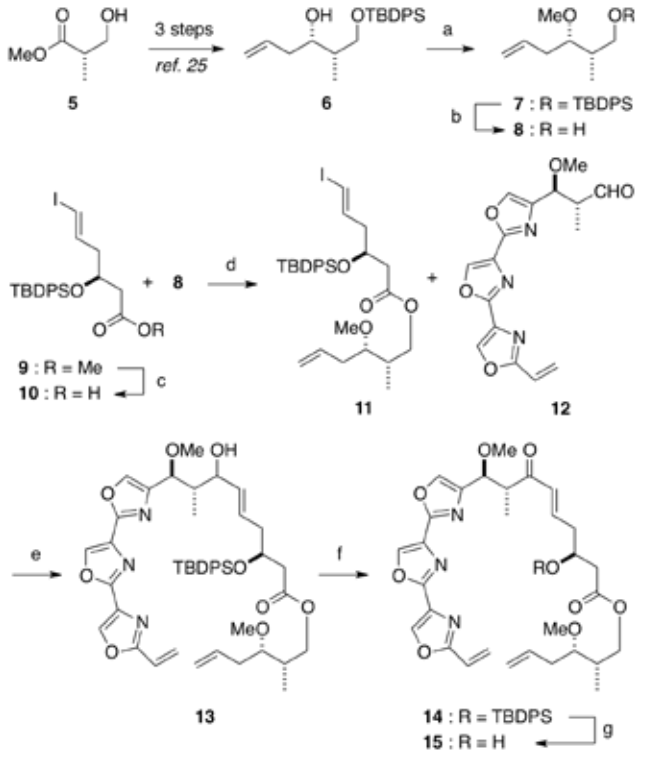



**Fig. 2.** Optimized structures and relative energies of **3** at the B3LYP/6-31G(d) level of theory. Values in (*E*)-**3** represent the calculated distances between the C3 hydroxyl H atom and the three oxazole N atoms (Å).

### 2.2 Synthesis of the tris-oxazole macrolactone analogs of mycalolides

Based on the finding that olefin metathesis is a useful method for connecting the C19–C20 double bonds in mycalolide analogs,[23] we considered that the assembly of two fragments via Ni/Cr-mediated Nozaki–Hiyama–Kishi coupling [24] at C6–C7 and RCM at C19 could efficiently afford **3**. The synthesis started with *syn*-homoallylic alcohol **6**, which was prepared from methyl (*S*)-(+)-3-hydroxy-2-methylpropionate (**5**) [25] (Scheme 1). Methylation of compound **6** by methyl trifluoromethanesulfonate (MeOTf) (90%) and removal of the *tert*-butyldiphenylsilyl (TBDPS) group by tetra-*n*-butylammonium fluoride (TBAF) gave primary alcohol **8** (96%). Hydrolysis of methyl ester in **9** [23b] with LiOH afforded carboxylic acid **10** (80%), which was condensed with **8** by the Yamaguchi procedure to afford ester **11** in 92% yield. Fragment coupling between **11** and aldehyde **12** [23b] by a Ni/Cr-mediated reaction (73%) was followed by oxidation of the C7 allylic alcohol with Dess–Martin periodinane (DMP) to afford the RCM precursor **14** (94%). Alternatively, removal of the TBDPS group of **14** by TBAF with acetic acid (AcOH) gave another RCM precursor **15** in 72% yield.

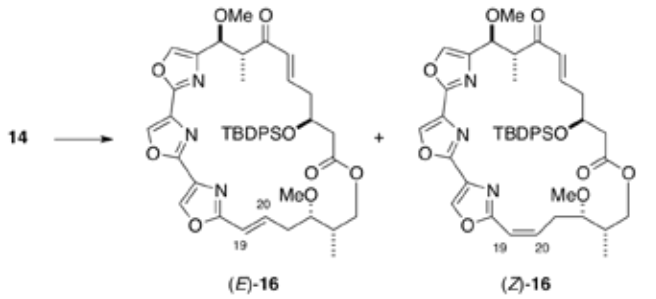



**Scheme 1.** Synthesis of the RCM precursors **14** and **15**. Reagents and conditions: (a) MeOTf, 2,6-di-*tert*-butylpyridine, $CH_2Cl_2$, rt, 90%; (b) TBAF, THF, rt, 96%; (c) LiOH, THF–$H_2O$, 40 °C, 80%; (d) 2,4,6-$Cl_3C_6H_2COCl$, $Et_3N$, THF, rt, then DMAP, benzene, rt, 92%; (e) $CrCl_2$, $NiCl_2$, THF–DMF (3:1), rt, 73%; (f) Dess–Martin periodinane, pyridine, $CH_2Cl_2$, 0 °C, 94%; (g) TBAF, AcOH, THF, rt, 72%.

With the key intermediates **14** and **15** in hand, RCM reactions were examined (Table 1). Despite the calculation results that 19*E*-macrolactone **3** was lower in energy than its 19*Z*-isomer, treatment of **14** with 30 mol% of 2nd-generation Hoveyda–Grubbs catalyst [26] in refluxing toluene (1 mM) gave tris-oxazole lactone **16** as a separable 1:1.9 mixture of *E*- and *Z*-isomers in 61% yield (entry 1). Similarly, the reaction at 40 °C in hexane preferred the *Z*-isomer (*E*/*Z* = 1.0/2.5) (entry 2).[27] With the use of benzotrifluoride as a solvent,[28] the RCM reaction was completed in 1 h, and the yield of the *E*-isomer was improved (entry 3). Interestingly, the *E*/*Z* product ratio was reversed at lower temperature in the same solvent (entry 4) or in ethyl acetate (entry 5). Furthermore, refluxing conditions in dichloromethane gave the best results (82%, *E*/*Z* = 1.8/1.0) (entry 6).[29] These results suggested that both the solvent polarity and reaction temperature significantly affected the stereoselectivity of the RCM reaction of **14**, and that the formation of C=C bonds in **16** would take place under kinetic control.[23b]

**Table 1**

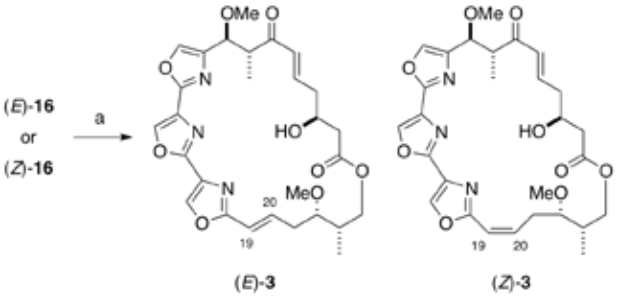
Ring-closing metathesis of **14**




| Entry | Reaction conditions [a] | Yield of **16** (%) | *E*/*Z* ratio | Yield of dimer (%) |
|---|---|---|---|---|
| 1 | toluene, reflux, 1.5 h | 61 | 1.0 / 1.9 | 5 |
| 2 | Hexane, 40 °C, 24 h | 73 | 1.0 / 2.5 | 9 |
| 3 | $C_6H_5CF_3$, reflux, 1 h | 69 | 1.0 / 1.3 | 7 |
| 4 | $C_6H_5CF_3$, 40 °C, 24 h | 77 | 1.3 / 1.0 | 6 |
| 5 | EtOAc, 40 °C, 9 h | 78 | 1.2 / 1.0 | 9 |
| 6 | $CH_2Cl_2$, reflux, 9 h | 82 | 1.8 / 1.0 | 5 |

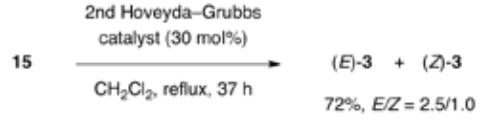
[a] Concentration = 1 mM. 2nd Hoveyda–Grubbs catalyst (30 mol%) was used.

Finally, removal of the TBDPS groups of the stereoisomers (*E*)- and (*Z*)-**16** by TBAF with AcOH afforded trisoxazole macrolactones (*E*)- and (*Z*)-**3** in respective yields of 87% and 91% (Scheme 2).




**Scheme 2.** Synthesis of tris-oxazole macrolactones **3**. Reagents and conditions: (a) TBAF, AcOH, THF, rt, 87% for (*E*)-**3** and 91% for (*Z*)-**3**.

For comparison, we also examined the RCM reaction of C3 hydroxyl analog **15** (Scheme 3). While the reaction was slower than that of silyl ether **14** (see Table 1, entry 6), refluxing conditions in dichloromethane (1 mM) gave macrolactone **3** in 72% yield, and the *E*-selectivity was slightly improved (*E*/*Z* = 2.5/1.0). Thus, it is expected that the RCM precursor **15** would have a specific conformation to prefer the formation of the *E*-isomer, due to intramolecular hydrogen bonding between the hydroxyl H atom and oxazole N atom(s), which might enable terminal olefins to move toward each other.




**Scheme 3.** Ring-closing metathesis of **15**.

### 2.3 Biological activities

While the *E*- and *Z*-stereoisomers of tris-oxazole macrolactone analogs **3** both exhibited moderate cytotoxicity against human epithelial carcinoma HeLa S3 cells, they were approximately 100 times less cytotoxic than mycalolide B (**1**) (Table 2). Furthermore, they exhibited no significant actin-depolymerizing effects. In addition, neither (*E*)- nor (*Z*)-**3** exhibited *in vitro* antimycotic activity against several pathogenic fungi (*Aspergillus fumigatus*, *Candida albicans*, and *Trichophyton mentagrophytes*) or normal fungi (*Mucor hiemalis* and *Rhizopus nigricans*) at 30 μg/mL. Thus, both the side-chain and the macrolactone moieties were suggested to be essential for the potent cytotoxicity or antimycotic activity of the parent molecules.[30]

**Table 2**

Biological activities of mycalolide analogs

| Compound | Cytotoxicity (HeLa S3) $IC_{50}$ (μg/mL) | Actin-depolymerizing activity $EC_{50}$ (μM) [a] |
|---|---|---|
| Mycalolide B (**1**) | 0.020 | 1.4 [b] |
| **2** | >10 [b] | 2.7 [b] |
| (*E*)-**3** | 2.4 | >30 |
| (*Z*)-**3** | 1.9 | >30 |

[a] Activity was monitored by measuring the fluorescence intensity of pyrenyl actin. Values indicate the concentrations required to depolymerize *F*-actin (3 μM) to 50% of its control amplitude.

[b] See ref. 20.

## 3. Conclusions

In conclusion, we concisely synthesized tris-oxazole macrolactone analogs of mycalolides by using Nozaki–Hiyama–Kishi coupling at C6–C7, esterification, and RCM at C19 as key steps. We established that both the side-chain (actin-binding) moiety and the macrolactone moiety were essential for the potent biological activities of the parent molecules. Further studies on the structure-activity relationships for mycalolides and related actin-targeting natural products as well as their mechanisms of action are currently underway.

## 4. Experimental Section

### 4.1 General

All chemicals were obtained commercially unless otherwise noted. Organic solvents and reagents for moisture-sensitive reactions were distilled by the standard procedure. Anhydrous $CH_2Cl_2$, tetrahydrofuran (THF), and *N*,*N*-dimethylformamide (DMF) were obtained commercially. Column chromatography was performed using silica gel BW-820MH or FL60D (75–200 or 45–75 μm, Fuji Silysia Co., Aichi, Japan) or a Yamazen preparative silica gel (40 μm). All moisture-sensitive reactions were performed under an atmosphere of argon or nitrogen, and the starting materials were azeotropically dried with benzene before use. Merck precoated silica gel 60 F254 plates were used for thin layer chromatography (TLC).

### 4.2 Spectroscopic analysis

$^1$H and $^{13}$C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Biospin AVANCE 600 spectrometer (600 MHz for $^1$H and 150 MHz for $^{13}$C), or a JEOL EX-270 spectrometer (270 MHz for $^1$H). Chemical shifts were reported in parts per million (ppm) with coupling constants (*J*) in hertz relative to the solvent peaks, $\delta_H$ 7.26 (residual C<u>H</u>$Cl_3$) and $\delta_C$ 77.0 for <u>C</u>$DCl_3$, respectively. Optical rotations were measured with a JASCO DIP-1000 polarimeter using the sodium D line. Infrared (IR) spectra were recorded on a JASCO FT/IR-230 spectrometer. High-resolution electrospray ionization mass spectra (HR-ESIMS) were measured on a QSTAR Pulsar *i* spectrometer (Applied Biosystems) or an AccuTOF CS spectrometer (JEOL).

### 4.3 Synthesis and spectroscopic data of mycalolide derivatives

*4.3.1. Methyl ether **7**.* To a stirred solution of (–)-secondary alcohol **6** (787 mg, 2.14 mmol, >99%ee, *syn*/*anti* = 93/7) in dry $CH_2Cl_2$ (4.8 mL) were added methyl trifluoromethanesulfonate (0.72 mL, 6.4 mmol) and 2,6-di-*tert*-butylpyridine (1.5 mL, 6.4 mmol) at 0 °C under a nitrogen atmosphere. After being stirred for 20 h at room temperature, the reaction mixture was quenched with sat. sodium bicarbonate aq. (15 mL) at 0 °C, stirred for 45 min at room temperature, and extracted with $CH_2Cl_2$ (5 mL × 4). The combined extracts were washed with water and brine, dried with $Na_2SO_4$, and concentrated. The crude material was purified with a Yamazen preparative $SiO_2$ column (30 g, hexane / EtOAc = 100/0 to 95/5) to give methyl ether **7** (737 mg, 90%, *syn*/*anti* = 93/7) as a colorless oil. **7**: $R_f$ 0.41 (hexane / EtOAc = 9:1); $[\alpha]_D^{25}$ +9.0 (*c* 1.09, $CHCl_3$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.72–7.65 (m, 4H), 7.46–7.36 (m, 6H), 5.81 (ddt, *J* = 17.2, 10.2, 7.0 Hz, 1H), 5.08 (d, *J* = 17.2 Hz, 1H), 5.05 (d, *J* = 10.2 Hz, 1H), 3.69 (dd, *J* = 10.0, 7.2 Hz, 1H), 3.54 (dd, *J* = 10.0, 6.1 Hz, 1H), 3.45 (dt, *J* = 3.9, 6.8 Hz, 1H), 3.36 (s, 3H), 2.36 (dddt, *J* = 13.9, 7.0, 6.8, 1.2 Hz, 1H), 2.23 (dddt, *J* = 13.9, 7.0, 6.8, 1.0 Hz, 1H), 1.87 (dddq, *J* = 7.8, 6.1, 3.9, 7.0 Hz, 1H), 1.08 (s, 9H), 0.89 (d, *J* = 7.0 Hz, 3H); $^{13}$C NMR (150 MHz, $CDCl_3$) δ 135.6 (d, 4C), 135.5 (d), 134.0 (s), 133.9 (s), 129.5 (d, 2C), 127.6 (d, 4C), 116.5 (t), 80.6 (d), 65.9 (t), 57.9 (q), 38.7 (d), 35.6 (t), 26.9 (q, 3C), 19.3 (s), 10.9 (q); IR ($CHCl_3$) 3073, 3009, 2962, 2932, 2859, 1640, 1589, 1472, 1428, 1389, 1362, 1217, 1112, 1088, 998, 918, 824, 705 $cm^{-1}$; HRMS (ESI) *m/z* 405.2207 (calcd for $C_{24}H_{34}NaO_2Si$ $[M+Na]^+$, Δ –1.9 mmu).

*4.3.2. Primary alcohol **8**.* To a stirred solution of **7** (1.01 g, 2.64 mmol, *syn*/*anti* = 93/7) in dry THF (18 mL) was added a 1 M solution of tetra-*n*-butylammonium fluoride in THF (3.2 mL, 3.2 mmol) at 0 °C under a nitrogen atmosphere. After being stirred for 4 h at room temperature, the reaction mixture was quenched with sat. $NH_4Cl$ aq. (10 mL), and extracted with $Et_2O$ (10 mL × 3). The combined extracts were washed with brine, dried with $Na_2SO_4$, and concentrated. The crude material was purified with a $SiO_2$ column (40 g, hexane / $Et_2O$ = 9/1 to 1/1) to give primary alcohol **8** (366 mg, 96%, *syn*/*anti* = 93/7) as a colorless oil. **8**: $R_f$ 0.15 (hexane / $Et_2O$ = 7:3); $[\alpha]_D^{25}$ +17.2 (*c* 1.31, $CHCl_3$); $^1$H NMR (600 MHz, $CDCl_3$) δ 5.81 (ddt, *J* = 17.0, 10.7, 7.2 Hz, 1H), 5.10 (d, *J* = 17.0 Hz, 1H), 5.04 (d, *J* = 10.7 Hz, 1H), 3.65 (dd, *J* = 10.7, 7.1 Hz, 1H), 3.58 (dd, *J* = 10.7, 4.6 Hz, 1H), 3.38 (s, 3H), 3.36 (dt, *J* = 3.4, 6.6 Hz, 1H), 2.56 (br s, 1H), 2.38 (dddt, *J* = 14.0, 7.2, 6.6, 1.3 Hz, 1H), 2.20 (dddt, *J* = 14.0, 7.2, 6.6, 1.1 Hz, 1H), 1.95 (m, 1H), 0.89 (d, *J* = 7.1 Hz, 3H); $^{13}$C NMR (150 MHz, $CDCl_3$) δ 135.2 (d), 116.8 (t), 83.8 (d), 66.3 (t), 57.6 (q), 37.0 (d), 34.6 (t), 11.1 (q); IR ($CHCl_3$) 3632, 3484, 3081, 3009, 2979, 2935, 2832, 1641, 1460, 1428, 1380, 1361, 1236, 1192, 1083, 1032, 918, 780, 773, 726 $cm^{-1}$; HRMS (ESI) *m/z* 167.1050 (calcd for $C_8H_{16}NaO_2$ $[M+Na]^+$, Δ +0.2 mmu).

*4.3.3. Carboxylic acid **10**.* To a stirred solution of (+)-methyl ester **9** (302 mg, 0.593 mmol, 99%ee, *E*/*Z* = 6.7/1) in THF (3 mL) was added 1 M lithium hydroxide aq. (3.6 mL, 3.6 mmol) at 0 °C. After being stirred for 23 h at 40 °C, the reaction mixture was acidified with 1 M HCl aq. (5 mL) and extracted with $Et_2O$ (5 mL × 4). The combined extracts were washed with brine, dried with $Na_2SO_4$, and concentrated. The crude material was purified with a $SiO_2$ column (10 g, hexane / EtOAc = 9/1 to 5/1) to give carboxylic acid **10** (234 mg, 80%, *E*/*Z* = 6.7/1) as a colorless oil. **10**: $R_f$ 0.42 (hexane / EtOAc = 2:1); $[\alpha]_D^{25}$ +33.7 (*c* 1.22, $CHCl_3$); $^1$H NMR (270 MHz, $CDCl_3$) δ 7.75–7.64 (m, 4H), 7.50–7.35 (m, 6H), 6.38 (dt, *J* = 14.4, 7.6 Hz, 1H), 5.98 (d, *J* = 14.4 Hz, 1H), 4.18 (tt, *J* = 5.9, 5.9 Hz, 1H), 2.49 (dd, *J* = 6.7, 6.5 Hz, 2H), 2.30–2.16 (m, 2H), 1.06 (s, 9H). COO<u>*H*</u> signal was not observed; $^{13}$C NMR (150 MHz, $CDCl_3$) δ 177.1 (s), 141.5 (d), 135.9 (d, 4C), 133.4 (s, 2C), 129.9 (d), 129.8 (d), 127.7 (d, 4C), 78.2 (d), 68.8 (d), 43.1 (t), 41.2 (t), 26.9 (q, 3C), 19.3 (s); IR ($CHCl_3$) 3073, 3054, 3010, 2961, 2933, 2897, 2860, 1712, 1606, 1589, 1472, 1428, 1363, 1221, 1111, 951, 822, 786, 704 $cm^{-1}$; HRMS (ESI) *m/z* 517.0701 (calcd for $C_{22}H_{27}INaO_3Si$ $[M+Na]^+$, Δ +2.9 mmu).

*4.3.4. Ester **11**.* To a stirred solution of carboxylic acid **10** (234 mg, 0.473 mmol) in dry THF (8.6 mL) under a nitrogen atmosphere were added triethylamine (72 µL, 0.52 mmol) and 2,4,6-trichlorobenzoyl chloride (81 µL, 0.52 mmol). After the mixture was stirred for 5 h at room temperature, the precipitate was removed by centrifugation. After the precipitate was washed with dry THF (4 mL), the combined filtrates and washings were concentrated *in vacuo* and dissolved in dry benzene (11.8 mL). To the activated ester solution was added a solution of primary alcohol **8** (75.3 mg, 0.522 mmol, *syn*/*anti* = 93/7) and *N*,*N*-dimethyl-4-aminopyridine (124 mg, 1.02 mmol) in dry benzene (4 mL) under a nitrogen atmosphere. After being stirred for 2 h at room temperature, the reaction mixture was quenched with sat. sodium bicarbonate aq. (10 mL), and extracted with EtOAc (3 mL × 5). The combined extracts were washed with brine, dried with $Na_2SO_4$, and concentrated. The crude oil was purified with a $SiO_2$ column (FL60D, 9 g, hexane / EtOAc = 49/1 to 4/1) to give ester **11** (269 mg, 92%, *syn*/*anti* = 99/1, *E*/*Z* = 6.1/1) as a colorless oil. **11**: $R_f$ 0.60 (hexane / EtOAc = 5:1); $[\alpha]_D^{25}$ +26.8 (*c* 1.31, $CHCl_3$); $^1$H NMR (600 MHz, $CDCl_3$) δ 7.68–7.64 (m, 4H), 7.46–7.37 (m, 6H), 6.38 (dt, *J* = 14.5, 7.4 Hz, 1H), 5.95 (d, *J* = 14.5 Hz, 1H), 5.76 (ddt, *J* = 17.2, 10.1, 7.1 Hz, 1H), 5.10 (d, *J* = 17.2 Hz, 1H), 5.06 (d, *J* = 10.1 Hz, 1H), 4.20 (tt, *J* = 5.9, 5.9 Hz, 1H), 3.99 (dd, *J* = 10.7, 6.8 Hz, 1H), 3.87 (dd, *J* = 10.7, 6.8 Hz, 1H), 3.31 (s, 3H), 3.16 (dt, *J* = 3.7, 6.5 Hz, 1H), 2.45 (dd, *J* = 6.5, 5.9 Hz, 2H), 2.34 (m, 1H), 2.27–2.15 (m, 3H), 1.90 (m, 1H), 1.04 (s, 9H), 0.87 (d, *J* = 6.9 Hz, 3H); $^{13}$C NMR (150 MHz, $CDCl_3$) δ 170.9 (s), 141.8 (d), 135.9 (d, 2C), 135.8 (d, 2C), 134.9 (d), 133.6 (s), 133.5 (s), 129.8 (d, 2C), 127.7 (d, 2C), 127.6 (d, 2C), 117.1 (t), 80.9 (d), 77.8 (d), 69.0 (d), 66.8 (t), 58.0 (q), 43.1 (t), 41.5 (t), 35.4 (d), 35.3 (t), 26.9 (q, 3C), 19.2 (s), 11.0 (q); IR ($CHCl_3$) 3074, 3054, 3010, 2962, 2933, 2897, 2860, 2829, 1729, 1641, 1472, 1428, 1363, 1233, 1190, 1105, 998, 952, 920, 822, 735, 704 cm$^{-1}$; HRMS (ESI) *m/z* 643.1729 (calcd for $C_{30}H_{41}INaO_4Si$ $[M+Na]^+$, Δ +1.3 mmu).

*4.3.5. Allylic alcohol **13**.* To a stirred solution of (–)-aldehyde **12** (23.3 mg, 70.8 µmol) and ester **11** (65.9 mg, 106 µmol, *E*/*Z* = 6.1/1) in degassed dry THF / DMF (3:1, 1.3 mL) under an argon atmosphere was added a 99:1 (w/w) mixture of chromium chloride (II) – nickel chloride (II) (56.5 mg, $CrCl_2$ 455 µmol and $NiCl_2$ 4.36 µmol). After being stirred for 14 h, the reaction mixture was diluted with EtOAc (4 mL), sat. ammonium chloride aq. (4 mL), and water (1 mL), and extracted with EtOAc (4 mL × 3). The combined extracts were washed with brine, dried with $Na_2SO_4$, and concentrated. The crude oil was purified with a $SiO_2$ column (3.1 g, hexane / $CHCl_3$ / EtOAc = 1/1/0, 3/7/0, 0/1/0, 0/4/1 and 0/3/1) to give allylic alcohol **13** (42.7 mg, 73%, 5*E*-isomer only, ca. 3:2 diastereomer mixture at C7) as a colorless oil. **13**: $R_f$ 0.50 (hexane / acetone = 7:3); $[\alpha]_D^{25}$ +5.7 (*c* 0.93, $CHCl_3$); $^1$H NMR (600 MHz, $CDCl_3$) δ 8.32 (s, 0.4H), 8.32 (s, 0.6H), 8.31 (s, 0.6H), 8.30 (s, 0.4H), 7.72–7.61 (m, 5H), 7.44–7.32 (m, 6H), 6.67 (dd, *J* = 17.7, 11.2 Hz, 1H), 6.34 (d, *J* = 17.7 Hz, 1H), 5.77 (d, *J* = 11.2 Hz, 1H), 5.74 (m, 1H), 5.55 (m, 1H), 5.39 (m, 1H), 5.08 (d, *J* = 17.2 Hz, 1H), 5.03 (d, *J* = 10.2 Hz, 1H), 4.30–4.26 (m, 0.4H), 4.26 (d, *J* = 7.9 Hz, 0.6H), 4.25–4.19 (m, 0.6H), 4.17 (d, *J* = 7.8 Hz, 0.4H), 4.01 (m, 1H), 3.97 (m, 1H), 3.84 (m, 1H), 3.30 (s, 1.2H), 3.30 (s, 1.8H), 3.29 (s, 1.8H), 3.28 (s, 1.2H), 3.16 (m, 1H), 2.51–2.43 (m, 2H), 2.36–2.12 (m, 6H), 1.89 (m, 1H), 1.02 (s, 9H), 0.87 (d, *J* = 6.8 Hz, 1.2H), 0.86 (d, *J* = 6.8 Hz, 1.8H), 0.75 (d, *J* = 7.1 Hz, 1.2H), 0.65 (d, *J* = 6.9 Hz, 1.8H); $^{13}$C NMR (150 MHz, $CDCl_3$) δ 171.34 [171.27], 161.90 [161.89], 156.0, 155.3 [155.0], 141.0 [140.6], 138.96 [138.95], 138.43 [138.39], 136.7 [136.5], 135.8 (4C), 134.9 [134.8], 134.3, 134.0 [133.9], 133.7 (2C), 131.54 [131.50], 130.7, 129.7 [129.6], 127.8, 127.5 (4C), 126.4 [124.2], 122.5, 117.0, 80.9, 80.1 [79.9], 75.7 [73.0], 70.2 [70.1], 66.6, 58.0 [57.5], 57.2 [56.9], 42.5, 42.1, 41.5 [41.4], 40.0, 39.8, 35.3, 26.9, 19.2, 12.0, 11.4, 11.02 [11.01] (sprit signals derived from the C7 diastereomer were shown in parenthesis); IR ($CHCl_3$) 3673, 3462, 3167, 3072, 3029, 3009, 2966, 2933, 2899, 2859, 2828, 1729, 1651, 1542, 1462, 1428, 1380, 1308, 1238, 1218, 1191, 1111, 980, 942, 918, 822 cm$^{-1}$; HRMS (ESI) *m/z* 846.3741 (calcd for $C_{46}H_{57}N_3NaO_9Si$ $[M+Na]^+$, Δ –2.1 mmu).

*4.3.6. RCM precursor **14**.* To a stirred solution of allylic alcohol **13** (26.1 mg, 28.3 µmol) in dry $CH_2Cl_2$ (0.63 mL) under a nitrogen atmosphere were added pyridine (26 µL) and Dess–Martin periodinane (20.2 mg, 47.6 µmol). After being stirred for 2.5 h at 0 °C, the reaction mixture was quenched with a 1:1:1 mixture of sat. sodium thiosulfate aq. – sat. sodium bicarbonate aq. – water (5 mL), and extracted with $CH_2Cl_2$ (2 mL × 4). The combined extracts were washed with brine, dried with $Na_2SO_4$, and concentrated. The crude oil was purified with a $SiO_2$ column (FL60D 2 g, hexane / $CHCl_3$ = 1/1 to 0/1) to give ketone **14** (24.6 mg, 94%) as a colorless oil. **14**: $R_f$ 0.42 ($CHCl_3$ / EtOAc = 4:1); $[\alpha]_D^{25}$ –5.5 (*c* 0.54, $CHCl_3$); $^1$H NMR (600 MHz, $CDCl_3$) δ 8.332 (s, 1H), 8.327 (s, 1H), 7.70 (s, 1H), 7.70–7.65 (m, 4H), 7.44–7.35 (m, 6H), 6.83 (dt, *J* = 15.8, 7.6 Hz, 1H), 6.67 (dd, *J* = 17.7, 11.3 Hz, 1H), 6.34 (d, *J* = 17.7 Hz, 1H), 6.12 (d, *J* = 15.8 Hz, 1H), 5.78 (d, *J* = 11.3 Hz, 1H), 5.75 (ddt, *J* = 17.2, 10.2, 7.1 Hz, 1H), 5.07 (d, *J* = 17.2 Hz, 1H), 5.04 (d, *J* = 10.2 Hz, 1H), 4.40 (d, *J* = 9.7 Hz, 1H), 4.32 (tt, *J* = 6.1, 5.9 Hz, 1H), 4.01 (dd, *J* = 10.8, 6.8 Hz, 1H), 3.86 (dd, *J* = 10.8, 6.9 Hz, 1H), 3.44 (dq, *J* = 9.7, 7.1 Hz, 1H), 3.31 (s, 3H), 3.21–3.13 (m, 1H), 3.17 (s, 3H), 2.51 (dd, *J* = 15.1, 6.1 Hz, 1H), 2.46 (dd, *J* = 15.1, 6.1 Hz, 1H), 2.47–2.35 (m, 2H), 2.32 (m, 1H), 2.19 (m, 1H), 1.90 (m, 1H), 1.04 (s, 9H), 0.87 (d, *J* = 6.9 Hz, 3H), 0.84 (d, *J* = 7.1 Hz, 3H); $^{13}$C NMR (150 MHz, $CDCl_3$) δ 201.7 (s), 170.9 (s), 161.9 (s), 156.0 (s), 155.5 (s), 142.8 (d), 140.0 (s), 139.0 (d), 138.5 (d), 137.2 (d), 135.9 (d, 2C), 135.8 (d, 2C), 134.8 (d), 133.5 (s), 133.4 (s), 132.9 (d), 131.6 (s), 130.7 (s), 129.9 (d), 129.8 (d), 127.7 (d, 4C), 124.2 (t), 122.6 (d), 117.1 (t), 80.9 (d), 77.6 (d), 69.2 (d), 66.8 (t), 58.0 (q), 57.0 (q), 47.0 (d), 41.6 (t), 40.0 (t), 35.3 (d), 35.3 (t), 26.9 (q, 3C), 19.2 (s), 14.1 (q), 11.0 (q); IR ($CHCl_3$) 3163, 3027, 3010, 2933, 2859, 1729, 1694, 1665, 1637, 1542, 1462, 1428, 1376, 1308, 1111, 978, 943, 919, 823, 703 cm$^{-1}$; HRMS (ESI) *m/z* 844.3618 (calcd for $C_{46}H_{55}N_3NaO_9Si$ $[M+Na]^+$, Δ +1.3 mmu).

*4.3.7. Secondary alcohol **15**.* To a stirred solution of ketone **14** (22.7 mg, 27.6 µmol) in THF (10.2 mL) under a nitrogen atmosphere at 0 °C were added a 1 M solution of tetra-*n*-butylammonium fluoride in THF (0.86 mL, 0.86 mmol) and acetic acid (49 µL). After being stirred for 18 h at room temperature, the reaction mixture was diluted with sat. sodium bicarbonate aq. (10 mL) and extracted with EtOAc (5 mL × 4). The combined extracts were washed with brine, dried with $Na_2SO_4$, and concentrated. The crude oil was purified with $SiO_2$ columns (FL60D 1.2 g, $CHCl_3$ / EtOAc = 4/1 to 1/1; FL60D 0.4 g, EtOAc) to give secondary alcohol **15** (11.6 mg, 72%) as a colorless oil. **15**: $R_f$ 0.66 ($CHCl_3$ / MeOH = 10:1); $[\alpha]_D^{25}$ –39.7 (*c* 0.46, $CHCl_3$); $^1$H NMR (600 MHz, $CDCl_3$) δ 8.34 (s, 1H), 8.33 (s, 1H), 7.71 (s, 1H), 6.97 (dt, *J* = 15.8, 7.3 Hz, 1H), 6.67 (dd, *J* = 17.7, 11.5 Hz, 1H), 6.34 (d, *J* = 17.7 Hz, 1H), 6.29 (d, *J* = 15.8 Hz, 1H), 5.78 (d, *J* = 11.5 Hz, 1H), 5.77 (ddt, *J* = 17.8, 10.0, 7.3 Hz, 1H), 5.11 (d, *J* = 17.8 Hz, 1H), 5.06 (d, *J* = 10.0 Hz, 1H), 4.42 (d, *J* = 9.6 Hz, 1H), 4.21 (m, 1H), 4.12 (dd, *J* = 10.9, 7.0 Hz, 1H), 4.05 (dd, *J* = 10.9, 6.5 Hz, 1H), 3.56 (dq, *J* = 9.6, 7.1 Hz, 1H), 3.35 (s, 3H), 3.34 (m, 1H), 3.23 (dt, *J* = 3.5, 6.5 Hz, 1H), 3.19 (s, 3H), 2.55 (dd, *J* = 16.6, 3.1 Hz, 1H), 2.48 (dd, *J* = 16.6, 9.2 Hz, 1H), 2.52–2.40 (m, 2H), 2.36 (m, 1H), 2.21 (m, 1H), 2.04 (m, 1H), 0.93 (d, *J* = 7.0 Hz, 3H), 0.90 (d, *J* = 7.1 Hz, 3H); $^{13}$C NMR (150 MHz, $CDCl_3$) δ 202.0 (s), 172.4 (s), 161.9 (s), 156.0

(s), 155.6 (s), 142.8 (d), 139.8 (s), 139.0 (d), 138.6 (d), 137.2 (d), 134.8 (d), 132.9 (d), 131.6 (s), 130.7 (s), 124.2 (t), 122.6 (d), 117.6 (t), 81.3 (d), 77.7 (d), 67.1 (d), 66.8 (t), 58.0 (q), 57.0 (q), 46.8 (d), 41.0 (t), 39.4 (t), 35.2 (d), 35.1 (t), 14.2 (q), 11.1 (q); IR ($CHCl_3$) 3674, 3481, 3168, 3079, 3010, 2934, 2826, 1720, 1665, 1628, 1542, 1459, 1377, 1233, 1222, 1210, 1187, 1092, 980, 943, 919, 787, 771, 756, 741, 731 $cm^{-1}$; HRMS (ESI) *m/z* 606.2411 (calcd for $C_{30}H_{37}N_3NaO_9$ $[M+Na]^+$, Δ –1.6 mmu).

*4.3.8. Macrolactone **16**.* To a stirred solution of ketone **14** (7.8 mg, 9.5 μmol) in dry $CH_2Cl_2$ (8.8 mL) under a nitrogen atmosphere was added a 2 mM solution of 2nd generation Hoveyda–Grubbs catalyst in dry $CH_2Cl_2$ (1.4 mL, 2.8 μmol). After being stirred for 9 h at reflux, the reaction mixture was concentrated. The crude material was purified with a $SiO_2$ column (FL60D 0.5 g, hexane / EtOAc = 2/1 to 1/1) to give macrolactone (*E*)-**16** (4.0 mg, 53%), its stereoisomer (*Z*)-**16** (2.2 mg, 29%), and its dimer (0.8 mg, 5%) as colorless oils. (*E*)-**16**: $R_f$ 0.16 ($CHCl_3$ / EtOAc = 4:1); $[\alpha]_D^{25}$ –38.1 (*c* 0.63, $CHCl_3$); $^1H$ NMR (600 MHz, $CDCl_3$) δ 8.13 (s, 1H), 8.08 (s, 1H), 7.71–7.66 (m, 4H), 7.66 (s, 1H), 7.44–7.35 (m, 6H), 7.07 (td, *J* = 7.6, 15.8 Hz, 1H), 6.94 (td, *J* = 7.2, 16.2 Hz, 1H), 6.35 (d, *J* = 15.8 Hz, 1H), 5.97 (d, *J* = 16.2 Hz, 1H), 4.45 (m, 1H), 4.36 (d, *J* = 9.6 Hz, 1H), 4.13 (qd, *J* = 7.0, 9.6 Hz, 1H), 4.00 (dd, *J* = 6.8, 10.4 Hz, 1H), 3.92 (dd, *J* = 6.8, 10.4 Hz, 1H), 3.39 (m, 1H), 3.35 (s, 3H), 3.09 (s, 3H), 2.77 (m, 1H), 2.72 (dd, *J* = 5.6, 15.6 Hz, 1H), 2.66 (dd, *J* = 6.8, 15.6 Hz, 1H), 2.45 (m, 2H), 2.42 (m, 1H), 2.06 (ddtq, *J* = 2.0, 6.8, 6.8, 6.8 Hz, 1H), 1.02 (s, 9H), 0.88 (d, *J* = 6.8 Hz, 3H), 0.81 (d, *J* = 7.0 Hz, 3H); $^{13}C$ NMR (150 MHz, $CDCl_3$) δ 203.2 (s), 171.1 (s), 162.7 (s), 156.5 (s), 155.6 (s), 143.3 (d), 139.6 (d), 139.0 (s), 137.4 (d), 137.3 (d), 137.2 (d), 135.9 (d, 2C), 135.83 (d, 2C), 135.0 (d), 133.7 (s), 133.6 (s), 131.5 (s), 130.3 (s), 129.9 (d), 129.7 (d), 127.7 (d, 2C), 127.6 (d, 2C), 116.3 (d), 79.7 (d), 76.7 (d), 68.7 (d), 67.5 (t), 57.7 (q), 56.2 (q), 42.6 (d), 41.7 (t), 40.3 (t), 35.0 (d), 32.4 (t), 26.9 (t, 3C), 19.2 (s), 14.9 (q), 9.3 (q); IR ($CHCl_3$) 3160, 3005, 2932, 2856, 1731, 1662, 1560, 1485, 1424, 1220, 1209, 1181, 1104, 997, 979, 909, 821, 790, 721, 703 $cm^{-1}$; HRMS (ESI) *m/z* 816.3320 (calcd for $C_{44}H_{51}N_3O_9SiNa$ $[M+Na]^+$, Δ +2.8 mmu). (*Z*)-**16**: $R_f$ 0.21 ($CHCl_3$ / EtOAc = 4:1); $[\alpha]_D^{25}$ –67.8 (*c* 0.57, $CHCl_3$); $^1H$ NMR (600 MHz, $CDCl_3$) δ 8.13 (s, 1H), 8.10 (s, 1H), 7.69–7.62 (m, 4H), 7.61 (s, 1H), 7.44–7.35 (m, 6H), 6.84 (td, *J* = 6.8, 16.0 Hz, 1H), 6.43 (td, *J* = 7.6, 11.8 Hz, 1H), 6.33 (d, *J* = 11.8 Hz, 1H), 6.01 (d, *J* = 16.0 Hz, 1H), 4.38–4.29 (m, 1H), 4.35 (d, *J* = 8.4 Hz, 1H), 4.16 (dd, *J* = 5.2, 10.8 Hz, 1H), 3.95 (m, 1H), 3.94 (dd, *J* = 7.6, 10.8 Hz, 1H), 3.50–3.41 (m, 2H), 3.37 (s, 3H), 3.16 (s, 3H), 2.88 (m, 1H), 2.60 (dd, *J* = 6.4, 15.8 Hz, 1H), 2.53 (dd, *J* = 6.4, 15.8 Hz, 1H), 2.40 (m, 2H), 2.19 (m, 1H), 1.07 (d, *J* = 6.8 Hz, 3H), 1.02 (s, 9H), 0.93 (d, *J* = 6.8 Hz, 3H); $^{13}C$ NMR (150 MHz, $CDCl_3$) δ 202.5 (s), 170.9 (s), 161.7 (s), 156.6 (s), 155.7 (s), 142.7 (d), 141.1 (d), 139.3 (s), 137.6 (d), 137.4 (d), 137.0 (d), 135.80 (d, 2C), 135.79 (d, 2C), 134.7 (d), 133.7 (s), 133.5 (s), 131.6 (s), 130.7 (s), 129.83 (d), 129.78 (d), 127.71 (d, 2C), 127.67 (d, 2C), 114.3 (d), 81.7 (d), 77.6 (d), 69.0 (d), 66.9 (t), 58.3 (q), 56.9 (q), 44.0 (d), 41.0 (t), 39.4 (t), 36.8 (d), 31.9 (t), 26.9 (q, 3C), 19.2 (s), 14.0 (q), 12.6 (q); IR ($CHCl_3$) 3162, 3005, 2932, 2856, 1730, 1663, 1558, 1458, 1424, 1179, 1103, 980, 917, 820, 790, 758, 739, 702 $cm^{-1}$; HRMS (ESI) *m/z* 816.3318 (calcd for $C_{44}H_{51}N_3O_9SiNa$ $[M+Na]^+$, Δ +2.4 mmu).

*4.3.9. Mycalolide macrolactone analog (E)-**3**.* To a stirred solution of silyl ether (*E*)-**16** (7.7 mg, 9.7 μmol) in THF (3.7 mL) under a nitrogen atmosphere at 0 °C were added a 1 M solution of tetra-*n*-butylammonium fluoride in THF (0.29 mL, 0.29 mmol) and acetic acid (16.5 μL, 0.29 mmol). After being stirred for 60 h at room temperature, the reaction mixture was diluted with sat. sodium bicarbonate aq. (5 mL), and extracted with EtOAc (5 mL × 3). The combined extracts were washed with brine, dried with $Na_2SO_4$, and concentrated. The crude oil was purified with a $SiO_2$ column (FL60D 0.5 g, hexane / EtOAc = 3/7 to 1/9) to give (*E*)-lactone **3** (4.7 mg, 87%) as a colorless oil. (*E*)-**3**: $R_f$ 0.30 (hexane/EtOAc = 1/9); $[\alpha]_D^{25}$ –54 (*c* 0.38, $CHCl_3$); $^1H$ NMR (600 MHz, $CDCl_3$) δ 8.12 (s, 1H), 8.08 (s, 1H), 7.66 (s, 1H), 7.05 (dt, *J* = 15.8, 7.7 Hz, 1H), 7.04 (dt, *J* = 15.9, 7.5 Hz, 1H), 6.35 (dt, *J* = 15.8, 1.6 Hz, 1H), 6.23 (dt, *J* = 15.9, 1.3 Hz, 1H), 4.40 (d, *J* = 8.9 Hz, 1H), 4.34 (m, 1H), 4.16 (dd, *J* = 10.7, 8.2 Hz, 1H), 4.10 (m, 1H), 4.04 (dd, *J* = 10.7, 5.9 Hz, 1H), 4.00 (dt, *J* = 8.9, 7.0 Hz, 1H), 3.53 (ddd, *J* = 10.2, 4.7, 2.6 Hz, 1H), 3.39 (s, 3H), 3.15 (s, 3H), 2.78 (dddd, *J* = 14.8, 7.7, 4.7, 1.6 Hz, 1H), 2.71 (dd, *J* = 15.8, 2.9 Hz, 1H), 2.61 (dd, *J* = 15.8, 10.0 Hz, 1H), 2.57–2.54 (m, 2H), 2.41 (dddd, *J* = 14.8, 10.2, 7.7, 1.6 Hz, 1H), 2.05 (m, 1H), 0.93 (d, *J* = 6.9 Hz, 3H), 0.88 (d, *J* = 7.0 Hz, 3H); $^{13}C$ NMR (150 MHz, $CDCl_3$) δ 202.6, 172.3, 162.7, 156.5, 155.5, 143.6, 139.8, 139.2, 137.4, 137.3, 137.2, 134.2, 131.3, 130.1, 116.3, 79.2, 77.1, 67.2, 66.9, 57.9, 56.6, 43.6, 41.5, 40.4, 35.7, 33.2, 13.8, 9.5; IR ($CHCl_3$) 3690, 3167, 3026, 3003, 2929, 2846, 1719, 1661, 1609, 1563, 1458, 1386, 1261, 1215, 1091, 1100, 1023, 977, 918 $cm^{-1}$; HRMS (ESI) *m/z* 578.2087 (calcd for $C_{28}H_{33}N_3NaO_9$ $[M+Na]^+$, Δ –2.7 mmu).

*4.3.10. Mycalolide macrolactone analog (Z)-**3**.* To a stirred solution of silyl ether (*Z*)-**16** (3.3 mg, 4.2 μmol) in THF (1.6 mL) under a nitrogen atmosphere at 0 °C were added a 1 M solution of tetra-*n*-butylammonium fluoride in THF (0.125 mL, 125 μmol) and acetic acid (7.1 μL, 125 μmol). After being stirred for 48 h at room temperature, the reaction mixture was diluted with sat. sodium bicarbonate aq. (5 mL), and extracted with EtOAc (5 mL × 4). The combined extracts were washed with brine, dried with $Na_2SO_4$, and concentrated. The crude oil was purified with a $SiO_2$ column (FL60D 0.5 g, hexane / EtOAc = 1/9) to give (*Z*)-lactone **3** (2.1 mg, 91%) as a colorless oil. (*Z*)-**3**: $R_f$ 0.39 (hexane/EtOAc = 1/9); $[\alpha]_D^{25}$ –71 (*c* 0.36, $CHCl_3$); $^1H$ NMR (600 MHz, $CDCl_3$) δ 8.17 (s, 1H), 8.14 (s, 1H), 7.63 (s, 1H), 6.91 (dt, *J* = 16.1, 6.7 Hz, 1H), 6.42 (dt, *J* = 11.7, 7.5 Hz, 1H), 6.35 (d, *J* = 11.7 Hz, 1H), 6.18 (d, *J* = 16.1 Hz, 1H), 4.37 (d, *J* = 7.5 Hz, 1H), 4.18 (m, 1H), 4.16 (dd, *J* = 10.9, 5.3 Hz, 1H), 4.09 (dd, *J* = 10.9, 5.6 Hz, 1H), 3.89 (qd, *J* = 7.1, 7.5 Hz, 1H), 3.75 (br s, 1H), 3.51 (dt, *J* = 4.7, 6.6 Hz, 1H), 3.40 (s, 3H), 3.36 (dddd, *J* = 16.2, 7.5, 4.7, 1.4 Hz, 1H), 3.24 (s, 3H), 3.08 (dddd, *J* = 16.2, 7.5, 6.6, 1.4 Hz, 1H), 2.60 (dd, *J* = 16.0, 3.2 Hz, 1H), 2.51 (dd, *J* = 16.0, 9.5 Hz, 1H), 2.46–2.41 (m, 2H), 2.15 (m, 1H), 1.08 (d, *J* = 7.0 Hz, 3H), 1.01 (d, *J* = 7.1 Hz, 3H); $^{13}C$ NMR (150 MHz, $CDCl_3$) δ 202.2, 172.2, 161.8, 156.6, 155.6, 143.4, 140.4, 139.7, 137.9, 137.7, 136.8, 133.9, 131.4, 130.6, 114.6, 81.0, 78.2, 67.0, 66.8, 58.1, 57.3, 44.3, 41.2, 39.6, 37.0, 31.4, 13.5, 12.7; IR ($CHCl_3$) 3675, 3167, 3026, 3007, 2931, 2846, 1724, 1663, 1557, 1459, 1377, 1279, 1261, 1220, 1182, 1100, 1013, 975, 918 $cm^{-1}$; HRMS (ESI) *m/z* 578.2091 (calcd for $C_{28}H_{33}N_3NaO_9$ $[M+Na]^+$, Δ –2.3 mmu).

*4.3.11. Ring-closing metathesis of **15**.* To a stirred solution of secondary alcohol **15** (5.8 mg, 9.9 μmol) in dry $CH_2Cl_2$ (9.2 mL) under a nitrogen atmosphere was added a 2 mM solution of 2nd generation Hoveyda–Grubbs catalyst in dry $CH_2Cl_2$ (1.5 mL, 3.0 μmol). After being stirred for 37 h at reflux, the reaction mixture was concentrated. The crude material was purified with $SiO_2$ columns (FL60D 0.5 g, $CHCl_3$ / MeOH = 100/1 to 10/1; FL60D 0.5 g, hexane / EtOAc = 3/7 to 1/9) to give macrolactone (*E*)-**3** (2.9 mg, 52%), its stereoisomer (*Z*)-**3** (1.1 mg, 20%), and its dimer (0.8 mg, 7%) as colorless oils.

### 4.4 Bioassay of mycalolide B and its synthetic analogs

The cytotoxicities of the probes and model compounds were measured by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) method.[19a] The actin-depolymerizing activities of the compounds were measured based on their ability to attenuate the fluorescence of pyrene-conjugated (pyrenyl) actin, as previously described.[12a] Antimycotic assays against several fungi were conducted at Ricerca Biosciences Inc. (Taipei, Taiwan).

## Acknowledgments

This work was supported by Grants-in-Aid for Scientific Research from JSPS (21681028 and 21651091 for M.K.; 23102014 and 23310148 for H.K.). Support was also provided by the Naito Foundation, the Uehara Memorial Foundation, and the Takeda Science Foundation. We thank the Kaneka Corporation for their gift of methyl (*S*)-(+)-3-hydroxy-2-methylpropionate.

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