# Abyssomicin I, a Modified Polycyclic Polyketide from *Streptomyces* sp. CHI39

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Abyssomicin I (**1**), a new modified polycyclic polyketide, was isolated from the culture extract of a soil-derived *Streptomyces* sp. The structure of **1** was elucidated by interpretation of NMR and other spectroscopic data. The stereochemistry of the new compound was assigned by NOE analysis, chemical derivatization, and application of the modified Mosher method. While **1** was inactive against bacteria and yeasts, the oxidized derivative **7** showed weak activities against Gram-positive bacteria. Compounds **1** and **7** exhibited inhibitory effects on tumor cell invasion with IC$_{50}$ values of 11 and 0.21 $\mu$M, respectively.

Actinomycetes are known to be a rich source of structurally unique and complex polyketides. Intact polyketide backbones are frequently modified with structural units from other biosynthetic pathways such as amino acids and isoprenes, leading to formation of molecules with higher structural variation and complexity.[1] Tetronic acid is one of such key structural motifs often found in bacterial PKS products.[2] Feeding studies with $^{13}$C-labeled precursors and biochemical analysis using recombinant enzymes have revealed that during the biosynthesis of tetronate compounds in actinomycetes the tetronic acid portion is formed by the addition of a glyceryl unit to the polyketide chain, followed by a Dieckmann-type intramolecular condensation.[3–6] In our HPLC/UV-based chemical screening for structurally unique metabolites from actinomycetes, a soil-derived *Streptomyces* strain was found to produce abyssomicin I (**1**), a new member of the unique spirotetronate polyketides. Herein, we report the isolation, structure elucidation, and absolute configuration of **1**.

The producing strain CHI39 was isolated from a rock soil sample collected in Campeche, Mexico. On the basis of the result of a 16S rRNA gene sequence similarity, the isolate was designated *Streptomyces* sp. CHI39. This strain was cultured in A-3 M medium, and the whole culture broth was subsequently extracted with 1-butanol. The crude extract was subjected to silica gel column chromatography, followed by C-18 column chromatography, to yield abyssomicin I (**1**).
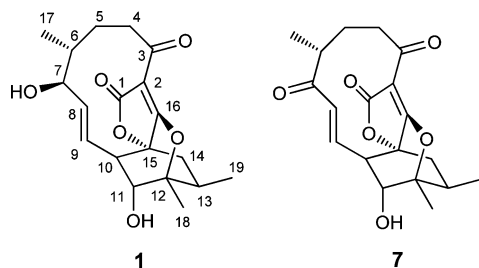


Abyssomicin I (**1**) was obtained as an optically active, colorless, amorphous solid that gave an [M − H]$^-$ peak at *m/z* 347.1508 in the negative ion HR-ESITOFMS appropriate for a molecular
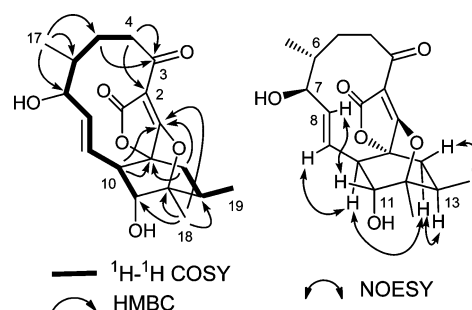
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**Figure 1.** COSY, key HMBC, and key NOESY correlations for **1**.

formula of C$_{19}$H$_{24}$O$_6$ (calcd for C$_{19}$H$_{23}$O$_6$ 347.1500), which was consistent with both the $^1$H and $^{13}$C NMR data. The IR spectrum contained absorption bands for hydroxyl, ester, and ketone functional groups (3389, 1743, and 1670 cm$^{-1}$, respectively). $^{13}$C NMR and HSQC spectral analysis confirmed the presence of 19 carbons, which were assigned to six quaternary, seven methine, three methylene, and three methyl carbons.

Analysis of the COSY spectrum led to two proton-bearing fragments, H$_2$-4 to H-11 and H$_2$-14/H-13/H$_3$-19 (Figure 1). A secondary methyl group (H$_3$-17) was attached to C-6 in the first fragment on the basis of a series of HMBC correlations from H$_3$-17 to C-5, C-6, and C-7. This fragment was expanded to include two quaternary sp$^2$ carbons by HMBC correlations from H$_2$-5 and H$_2$-4 to C-3 and from H$_2$-4 to C-2. The magnitude of the $^3J_{H,H}$ vicinal coupling constant (16.5 Hz) between H-8 and H-9 indicated an *E* geometry for the C-8–C-9 olefinic bond. The chemical shift of C-7 ($\delta_C$ 75.4) indicated an oxygen substitution at this carbon. The first and the second fragments were connected through the oxygenated quaternary sp$^3$ carbons C-12 ($\delta_C$ 90.1) and C-15 ($\delta_C$ 82.0) on the basis of HMBC correlations from the angular methyl proton H$_3$-18 to C-11, C-12, and C-13 and from H-10, H-13, and H$_2$-14 to C-15, establishing a cyclohexane ring with the linear chain connected at C-10. HMBC correlations were observed from H-10 and H$_2$-14 to a deshielded carbon at $\delta_C$ 186.6 (C-16). This carbon also had a four-bond HMBC correlation from H$_3$-18. These data established the connectivity between C-15 and C-16 and the oxygen bridge between C-12 and C-16. Therefore, C-16 was assigned as an enol ether carbon connected to C-2 through a double bond. The remaining carbonyl carbon C-1 could be placed adjacent to C-2 and connected to C-15 through an ester linkage to form an α,β-unsaturated δ-lactone with a carbonyl functionality at the α-position. This was supported by the $^{13}$C chemical shifts of the carbons C-1,
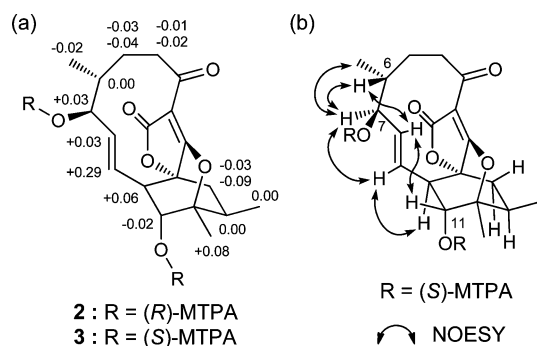
**Figure 2.** $\Delta\delta_{S-R}$ values for MTPA esters of **1** (a) and key NOESY correlations for (S)-MTPA ester **3** (b).
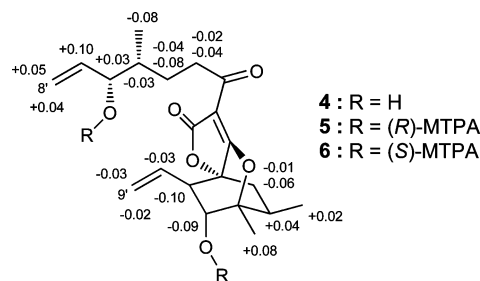


**Figure 3.** $\Delta\delta_{S-R}$ values for MTPA esters of **4**.

C-2, C-3, and C-16 that closely matched those published for this structure in abyssomicins.[7–9] Lack of signals for the two hydroxyl protons at C-7 and C-11 suggested that these were exchangeable in CD$_3$OD solution as expected.

NOESY correlations for H-10/H-14b, H-13/H-14b, and H-8/H-11 indicated that H-10, H-13, H-14b, and the hydroxyl group at C-11 were located on the same face of the cyclohexane ring. NOESY correlations were also observed for H-9/H-10, H-8/H-11, and H-7/H-8, but the conformation analysis of the C-3 to C-7 chain part was difficult because of the presence of several possible rotamers. The configuration at C-6 could not be determined by NOESY analysis due to the signal overlapping of H-6 and H$_3$-17. The relative and absolute configurations of C-6 and C-7 were first proposed by application of the modified Mosher method[10] and NOESY analysis of the MTPA esters of **1**. Treatment of **1** with (S)- and (R)-MTPA chlorides gave the bis-(R)- and (S)-MTPA esters (**2** and **3**), respectively. The signs of $\Delta\delta_{S-R}$ ($\delta_S - \delta_R$) values for the protons from H-8 to H-10 were positive, while those for H$_2$-4, H$_2$-5, and H$_3$-17 were negative (Figure 2a). These data suggested the absolute configuration at C-7 as S, although the conformation of the C-3 to C-8 chain was unclear from only the observed NMR data. An α-orientation of the methyl group (H$_3$-17) at C-6 was suggested by the NOESY spectra of **2** and **3**, which clearly showed a correlation between H-6 and H-8, along with correlations for H-9/H-10 and H-8/H-11 (Figure 2b). Assignment of the absolute configuration at C-11 was not possible because the distribution of signs of $\Delta\delta_{S-R}$ values was not consistent. To confirm the proposed configurations at C-6 and C-7, the C-8–C-9 double bond was cleaved by olefin metathesis using the Grubbs second-generation catalyst[11] under an ethylene atmosphere to afford the ring-opened derivative **4**,[12] which was further transformed to bis-(R)- and (S)-MTPA esters (**5** and **6**) by treatment with (S)- and (R)-MTPA chlorides. In the $^1$H NMR spectra of **5** and **6**, positive $\Delta\delta_{S-R}$ values were observed for H-8 and H$_2$-8′, while negative $\Delta\delta_{S-R}$ values were observed for H$_3$-17 and the protons from H$_2$-4 to H-6 (Figure 3). These data also indicated that the absolute configuration of C-7 was S. The 6R configuration was established on the basis of the small vicinal coupling constants between H-6 and H-7 ($J = 5.3-5.9$ Hz) in **4–6** that indicated the *syn* relationship of the methyl group at C-6 and the oxygen functionality at C-7. The sign distribution
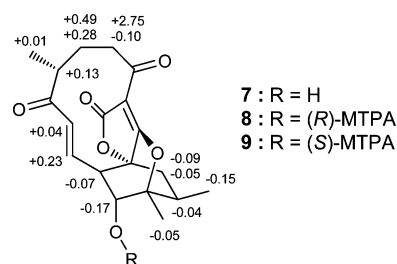


**Figure 4.** $\Delta\delta_{S-R}$ values for MTPA esters of **7**.

of $\Delta\delta_{S-R}$ values around the axial MTPA group at C-11 was opposite of that expected from the absolute configuration of the known abyssomicins presumably due to the steric hindrance. As it was reported that the modified Mosher method was applicable to the C-11 axial hydroxyl group of abyssomicins,[7] the allylic secondary hydroxyl group at C-7 was transformed to the ketone by selective oxidation with manganese dioxide, yielding **7**, which was derivatized to the mono-(R)- and (S)-MTPA esters (**8** and **9**) by DCC coupling with (R)- and (S)-MTPA acids. Analysis of $^1$H NMR data for these MTPA esters allowed the assignment of the $\Delta\delta_{S-R}$ values, which were positive for the protons from H-9 to H$_2$-5, while those of H-12, H-13, H$_2$-14, H$_3$-18, and H$_3$-19 were negative (Figure 4). This is sufficiently consistent to assign the absolute configuration of C-11 as R. The absolute configurations of the polycyclic stereocenters of **1** were identical with those of the known abyssomicins.

Biological activities of **1** and **7** were evaluated in our standard assays. The oxidized derivative **7** showed antimicrobial activity against Gram-positive bacteria including *Micrococcus luteus*, *Bacillus subtilis*, and *Staphylococcus aureus* with MIC values of 50 $\mu$M (29 $\mu$g/mL), while **1** was inactive even at 100 $\mu$M. This result is consistent with the previously reported structure−activity relationship of abyssomicins in which the enone system is essential to exert antibacterial properties.[13] Both compounds were inactive against Gram-negative bacteria (*Escherichia coli*) and yeast (*Candida albicans*). In addition, **1** and **7** inhibited the tumor cell invasion into the reconstituted extracellular matrix protein Matrigel with IC$_{50}$ values of 11 and 0.21 $\mu$M, respectively, at noncytotoxic concentrations.[14,15]

To date seven abyssomicin congeners have been reported.[7–9] Abyssomicin I (**1**) has the same tetracyclic skeleton as abyssomicins C and G but a different methylation pattern previously not described. In addition, **1** possesses a hydroxyl group at C-7 instead of a ketone or its equivalent functionality common to other abyssomicins. It is postulated that the polycyclic framework of abyssomicins is constructed through a polyketide biosynthetic pathway, followed by an intramolecular Diels−Alder reaction between the diene part in the polyketide chain and the *exo*-methylene dienophile in the tetronic acid moiety in a similar fashion to the biosynthesis of kijanimicin-type spirotetronate compounds.[5,6,16] Hence, the position of methyl branching is determined by the acyltransferase domain in PKS, which recruits methylmalonyl-CoA for chain elongation.[1] **1** is a new abyssomicin variant derived from a different incorporation pattern of extender units. Production of the known abyssomicins was not detected in the culture extract of strain CHI39.

## Experimental Section

**Isolation and Characterization of the Microorganism.** Strain CHI39 was isolated from a rock soil sample collected in Campeche,

Mexico. The rock sample was ground using a sterile mortar and pestle, and the ground sample (1 g) was resuspended in 9 mL of Ringer's solution (NaCl 0.9%, KCl 0.01%, CaCl$_2$ 0.01%, NaHCO$_3$ 0.01%). This suspension was heated at 85 °C for 15 min to eliminate Gram-negative bacteria, and serial dilutions from $10^{-1}$ to $10^{-5}$ were prepared in Ringer's solution. One milliliter of $10^{-3}$ to $10^{-5}$ dilutions was inoculated on humic acid agar[17] and incubated at 28 °C. Strain CHI39 was recovered after three weeks and identified as a member of the genus *Streptomyces* on the basis of 16S rRNA gene sequence (1460 nucleotides; EMBL accession number FM875937). Strain CHI39 shared a sequence similarity of 99.8% with *S. fragilis* NRRL 2424$^T$ (16S rRNA gene accession number AY999917).

**Fermentation.** Strain CHI39 cultured on a slant agar medium consisting of soluble starch 0.5%, glucose 0.5%, meat extract (Kyokuto Pharmaceutical Industrial Co., Ltd.) 0.1%, yeast extract (Difco Laboratories) 0.1%, NZ-case (Wako Chemicals USA, Inc.) 0.2%, NaCl 0.2%, CaCO$_3$ 0.1%, and agar 1.5% was inoculated into 500 mL K-1 flasks each containing 100 mL of the V-22 seed medium consisting of soluble starch 1%, glucose 0.5%, NZ-case 0.3%, yeast extract 0.2%, tryptone (Difco Laboratories) 0.5%, K$_2$HPO$_4$ 0.1%, MgSO$_4$·7H$_2$O 0.05%, and CaCO$_3$ 0.3% (pH 7.0). The flasks were cultivated on a rotary shaker (200 rpm) at 30 °C for 4 days. The seed culture (3 mL) was transferred into 500 mL K-1 flasks each containing 100 mL of the A-3 M production medium consisting of glucose 0.5%, glycerol 2%, soluble starch 2%, Pharmamedia (Traders Protein) 1.5%, yeast extract 0.3%, and Diaion HP-20 (Mitsubishi Chemical Co.) 1%. The pH of the medium was adjusted to 7.0 before sterilization. The inoculated flasks were cultured on a rotary shaker (200 rpm) at 30 °C for 6 days.

**Extraction and Isolation.** At the end of the fermentation period, 50 mL of 1-butanol was added to each flask, and they were allowed to shake for 1 h. The mixture was centrifuged at 6000 rpm for 10 min, and the organic layer was separated from the aqueous layer containing the mycelium. Evaporation of the solvent gave approximately 1.4 g of extract from 2 L of culture. The crude extract (1.4 g) was subjected to silica gel column chromatography with a step gradient of CHCl$_3$/MeOH (1:0, 20:1, 10:1, 4:1, 2:1, 1:1, and 0:1 v/v). Fraction 5 was concentrated to provide 127 mg of brown oil, which was further purified by reversed-phase ODS column chromatography with a gradient of MeCN/0.1% HCO$_2$H solution (2:8, 3:7, 4:6, 5:5, 6:4, 7:3, and 8:2 v/v). Fraction 3 was evaporated, and the remaining aqueous solution was extracted with EtOAc. After drying with anhydrous Na$_2$SO$_4$, the organic layer was concentrated to give abyssomicin I (**1**, 31 mg).

**Abyssomicin I (1):** colorless, amorphous solid; [α]$^{24}$$_D$ +27 (*c* 0.40, MeOH); IR (ATR) ν$_{max}$ 3389, 1743, 1670, 1604 cm$^{-1}$; UV (MeOH) λ$_{max}$ (log ε) 215 (3.98), 255 (4.09); $^1$H and $^{13}$C NMR data, see Table 1; HR-ESITOFMS [M − H]$^-$ 347.1508 (calcd for C$_{19}$H$_{23}$O$_6$, 347.1500).

**Bis-(R)-MTPA Ester of 1 (2).** To a solution of **1** (2.0 mg, 5.7 μmol) in dry CH$_2$Cl$_2$ (100 μL) was added (S)-MTPA chloride (7 μL, 37 μmol) at room temperature. After standing for 1 h, the reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 8:1−1:1) to give bis-(R)-MTPA ester **2** (2.1 mg, 47% yield): $^1$H NMR (500 MHz, CDCl$_3$) δ 1.00 (3H, d, J = 7.2 Hz, H-19), 1.02 (3H, d, J = 7.0 Hz, H-17), 1.14 (1H, m, H-5), 1.36 (1H, dq, J = 7.0, 7.0 Hz, H-6), 1.43 (3H, s, H-18), 1.45 (1H, dd, J = 13.0, 2.0 Hz, H-14), 1.83 (1H, dd, J = 14.6, 14.6 Hz, H-5), 2.19 (1H, ddd, J = 14.6, 13.3, 2.8 Hz, H-4), 2.23 (1H, m, H-13), 2.38 (1H, dd, J = 13.0, 10.7 Hz, H-14), 2.62 (1H, d, J = 6.2 Hz, H-10), 3.22 (1H, ddd, J = 13.3, 5.4, 2.7 Hz, H-4), 5.03 (1H, ddd, J = 16.6, 6.2, 1.9 Hz, H-9), 5.04 (1H, d, J = 1.3 Hz, H-11), 5.21 (1H, dd, J = 16.6, 2.9 Hz, H-8), 5.59 (1H, br s, H-7); HR-ESITOFMS *m/z* 779.2292 [M − H]$^-$ (calcd for C$_{39}$H$_{37}$F$_6$O$_{10}$ 779.2296).

**Bis-(S)-MTPA Ester of 1 (3).** In the same manner as described for **2**, **3** was prepared from **1** and (R)-MTPA chloride: $^1$H NMR (500 MHz, CDCl$_3$) δ 1.00 (3H, d, J = 7.0 Hz, H-17), 1.00 (3H, d, J = 6.9 Hz, H-19), 1.10 (1H, m, H-5), 1.36 (1H, dq, J = 7.0, 7.0 Hz, H-6), 1.42 (1H, dd, J = 12.5, 1.1 Hz, H-14), 1.51 (3H, s, H-18), 1.80 (1H, dd, J = 14.5, 14.5 Hz, H-5), 2.18 (1H, ddd, J = 14.5, 13.3, 3.0 Hz, H-4), 2.23 (1H, m, H-13), 2.29 (1H, dd, J = 12.5, 10.7 Hz, H-14), 2.68 (1H, d, J = 6.0 Hz, H-10), 3.20 (1H, ddd, J = 13.3, 5.5, 2.8 Hz, H-4), 5.02 (1H, d, J = 1.3 Hz, H-11), 5.24 (1H, dd, J = 16.7, 2.7 Hz, H-8), 5.32 (1H, ddd, J = 16.7, 6.0, 1.6 Hz, H-9), 5.62 (1H, br s, H-7); HR-ESITOFMS *m/z* 779.2292 [M − H]$^-$ (calcd for C$_{39}$H$_{37}$F$_6$O$_{10}$ 779.2296).

**Olefin Metathesis of 1 to Yield 4.** A mixture of **1** (2 mg, 5.7 μmol) and Grubbs second-generation catalyst ([RuCl$_2$(IMes)(PCy$_3$)=CHPh],

**Table 1.** $^1$H and $^{13}$C NMR Data for Abyssomicin I (**1**) in CD$_3$OD

| no. | δ$_H$ mult (J in Hz)$^a$ | δ$_C$$^b$ | HMBC$^{a,c}$ |
|---|---|---|---|
| 1 | | 172.1 | |
| 2 | | 103.9 | |
| 3 | | 198.1 | |
| 4a | 2.14, ddd (13.6, 13.0, 5.2) | 43.3 | 3, 5, 6 |
| 4b | 3.18, dd (13.0, 5.2) | | 2, 3, 5, 6 |
| 5a | 1.09, m | 26.7 | 3, 4, 6, 7, 17 |
| 5b | 1.90, dd (15.6, 13.6, 1.0) | | |
| 6 | 1.09, m | 37.1 | |
| 7 | 4.14, br s | 75.4 | 5, 6, 8, 9, 17 |
| 8 | 5.20, dd (16.5, 2.5) | 139.9 | 6, 7, 9, 10, 15 |
| 9 | 5.76, ddd (16.5, 6.4, 1.8) | 122.6 | 7, 10 |
| 10 | 2.96, dd (6.4, 0.9) | 52.2 | 8, 9, 11, 14, 15, 16 |
| 11 | 3.99, d (0.9) | 75.9 | 9, 10, 12, 13, 15, 18 |
| 12 | | 90.1 | |
| 13 | 2.59, ddq (10.6, 1.9, 7.1) | 29.9 | 14, 15, 19 |
| 14a | 1.44, dd (12.3, 1.9) | 35.5 | 10, 12, 13, 15, 16, 19 |
| 14b | 2.70, dd (12.3, 10.6) | | 10, 12, 13, 15, 16, 19 |
| 15 | | 82.0 | |
| 16 | | 186.6 | |
| 17 | 1.09, d$^d$ | 20.3 | 5, 6, 7 |
| 18 | 1.58, s | 19.9 | 11, 12, 13, 16 |
| 19 | 1.05, d (7.1) | 16.4 | 12, 13, 14 |

$^a$ Recorded at 500 MHz. $^b$ Recorded at 100 MHz. $^c$ HMBC correlations are from proton(s) stated to the indicated carbon. $^d$ Coupling constant could not be determined due to signal overlapping.

trace amount) in dry CH$_2$Cl$_2$ (1 mL) was stirred under ethylene gas (99.5% purity) at room temperature. After stirring for 4 h, a saturated solution of Na$_2$S$_2$O$_3$ was added to the reaction mixture, which was then extracted with EtOAc. The organic layer was washed with 0.5 N HCl, saturated NaHCO$_3$ solution, and brine and dried over anhydrous Na$_2$SO$_4$. The organic layer was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 8:1−1:1) to give **4** (1.6 mg, 75% yield): colorless oil; $^1$H NMR (500 MHz, CDCl$_3$) δ 0.91 (3H, d, J = 6.9 Hz, H-17), 1.07 (3H, d, J = 7.1 Hz, H-19), 1.25 (1H, dd, J = 12.4, 4.8 Hz, H-14), 1.52 (1H, m, H-5), 1.59 (3H, s, H-18), 1.66 (1H, m, H-6), 1.86 (1H, m, H-5), 2.56 (1H, m, H-13), 2.70 (1H, dd, J = 12.4, 11.3 Hz, H-14), 2.75 (1H, dd, J = 8.7, 4.3 Hz, H-10), 2.82 (1H, ddd, J = 17.0, 8.6, 6.6 Hz, H-4), 2.96 (1H, ddd, J = 17.0, 8.8, 5.7 Hz, H-4), 3.94 (1H, dd, J = 6.6, 5.9 Hz, H-7), 3.73 (1H, d, J = 4.3 Hz, H-11), 5.16 (1H, d, J = 10.4 Hz, H-8′a), 5.25 (1H, d, J = 17.1 Hz, H-8′b), 5.26 (1H, d, J = 9.8 Hz, H-9′a), 5.28 (1H, d, J = 17.1 Hz, H-9′b), 5.41 (1H, ddd, J = 17.1, 9.8, 8.7 Hz, H-9), 5.87 (1H, ddd, J = 17.1, 10.4, 6.6 Hz, H-8); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 15.3 (C-17), 16.5 (C-19), 19.2 (C-18), 25.9 (C-5), 28.2 (C-13), 37.9 (C-14), 38.3 (C-6), 39.0 (C-4), 52.6 (C-10), 76.5$^a$ (C-15), 77.0$^a$ (C-7), 77.0$^a$ (C-11), 88.2 (C-12), 99.6 (C-2), 115.9 (C-8′), 120.9 (C-9′), 132.0 (C-9), 139.2 (C-8), 169.3 (C-1), 186.1 (C-16), 194.5 (C-3); $^a$overlapped with the solvent peak; HR-ESITOFMS *m/z* 375.1808 [M − H]$^-$ (calcd for C$_{21}$H$_{27}$O$_6$ 375.1813).

**Bis-(R)-MTPA Ester of 4 (5).** To a solution of **4** (0.8 mg, 2.1 μmol) in dry CH$_2$Cl$_2$ (100 μL) was added (S)-MTPA chloride (7 μL, 37 μmol) at room temperature. After standing for 1 h, the reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc, 8:1−1:1) to give bis-(R)-MTPA ester **5** (0.5 mg, 29% yield): $^1$H NMR (500 MHz, CDCl$_3$) δ 0.95 (3H, d, J = 6.8 Hz, H-17), 1.00 (3H, d, J = 7.1 Hz, H-19), 1.29 (1H, dd, J = 12.9, 4.4 Hz, H-14), 1.43 (3H, s, H-18), 1.44 (1H, m, H-5), 1.84 (H, m, H-5), 1.91 (1H, m, H-6), 2.28 (1H, m, H-13), 2.60 (1H, dd, J = 12.9, 11.0 Hz, H-14), 2.78 (1H, dd, J = 8.5, 4.2 Hz, H-10), 2.80 (1H, ddd, J = 17.4, 9.2, 6.1 Hz, H-4), 2.94 (1H, ddd, J = 17.4, 9.2, 5.7 Hz, H-4), 5.21 (1H, d, J = 4.2 Hz, H-11), 5.26 (1H, d, J = 10.5 Hz, H-8′), 5.27 (1H, d, J = 17.4 Hz, H-8′), 5.28 (1H, d, J = 16.9 Hz, H-9′), 5.34 (1H, d, J = 10.3 Hz, H-9′), 5.37 (1H, d, J = 7.0, 5.4 Hz, H-7), 5.48 (1H, ddd, J = 16.9, 10.3, 8.5 Hz, H-9), 5.73 (1H, ddd, J = 17.4, 10.5, 7.0 Hz, H-8); HR-ESITOFMS *m/z* 807.2595 [M − H]$^-$ (calcd for C$_{41}$H$_{41}$F$_6$O$_{10}$ 807.2609).

**Bis-(S)-MTPA Ester of 4 (6).** In the same manner as described for **5**, **6** was prepared from **4** and (R)-MTPA chloride: $^1$H NMR (500 MHz, CDCl$_3$) δ 0.87 (3H, d, J = 6.9 Hz, H-17), 1.02 (3H, d, J = 7.1 Hz, H-19), 1.28 (1H, dd, J = 12.8, 4.0 Hz, H-14), 1.40 (1H, m,, H-5), 1.51 (3H, s, H-18), 1.76 (1H, m, H-5), 1.88 (1H, m, H-6), 2.32 (1H, m,

H-13), 2.54 (1H, dd, $J$ = 12.8, 11.0 Hz, H-14), 2.68 (1H, dd, $J$ = 8.5, 3.7 Hz, H-10), 2.78 (1H, ddd, $J$ = 17.4, 9.2, 6.1 Hz, H-4), 2.90 (1H, ddd, $J$ = 17.4, 9.4, 5.6 Hz, H-4), 5.12 (1H, d, $J$ = 3.7 Hz, H-11), 5.25 (1H, d, $J$ = 16.9 Hz, H-9′), 5.32 (1H, d, $J$ = 10.4 Hz, H-9′), 5.32 (1H, d, $J$ = 10.4 Hz, H-8′), 5.36 (1H, dd, $J$ = 17.4 Hz, H-8′), 5.40 (1H, dd, $J$ = 7.3, 5.3 Hz, H-7), 5.45 (1H, ddd, $J$ = 16.9, 10.4, 8.5 Hz, H-9), 5.83 (1H, ddd, $J$ = 17.4, 10.4, 7.3 Hz, H-8); HR-ESITOFMS $m/z$ 807.2604 [M − H]$^-$ (calcd for $C_{41}H_{41}F_6O_{10}$ 807.2609).

**Oxidation of 1 to Yield 7.** To a solution of **1** (15 mg, 0.040 mmol) in CHCl$_3$ was added activated MnO$_2$ (0.10 g, 98 mmol), and the mixture was vigorously stirred for 24 h at room temperature. The reaction mixture was filtered through Celite, and the filtrate was concentrated *in vacuo*. The residue was chromatographed on a silica gel column ($n$-hexane/EtOAc, 10:1−1:2) to give **7** (7.6 mg) in 55% yield: colorless needles; mp 229.0−229.5 °C; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 1.09 (3H, d, $J$ = 6.8 Hz, H-17), 1.11 (3H, d, $J$ = 7.6 Hz, H-19), 1.53 (1H, dd, $J$ = 12.6, 2.6 Hz, H-14), 1.57 (1H, dddd, $J$ = 16.2, 4.5, 4.5, 4.4 Hz, H-5), 1.63 (3H, s, H-18), 1.90 (1H, dddd, $J$ = 16.2, 13.0, 3.8, 3.7 Hz, H-5), 2.38 (1H, m, H-6), 2.43 (1H, ddd, $J$ = 13.5, 13.0, 4.4 Hz, H-4), 2.66 (1H, ddq, $J$ = 11.0, 7.6, 2.6 Hz, H-13), 2.75 (1H, dd, $J$ = 12.6, 11.0 Hz, H-14), 3.15 (1H, dd, $J$ = 6.9, 2.2 Hz, H-10), 3.21 (1H, ddd, $J$ = 13.5, 4.5, 3.8 Hz, H-4), 3.44 (1H, d, $J$ = 4.2 Hz, OH-11), 3.81 (1H, br s, H-11), 5.80 (1H, d, $J$ = 16.7 Hz, H-8), 6.35 (1H, dd, $J$ = 16.7, 6.9 Hz, H-9); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 16.0 (C-17), 16.3 (C-19), 19.7 (C-18), 28.3 (C-13), 30.2 (C-5), 34.4 (C-14), 42.2 (C-6), 42.4 (C-4), 51.1 (C-10), 75.4 (C-11), 79.9 (C-15), 88.1 (C-12), 104.2 (C-2), 135.2 (C-8), 136.2 (C-9), 169.4 (C-1), 183.6 (C-16), 194.5 (C-3), 204.6 (C-7); HR-ESITOFMS $m/z$ 345.1349 [M − H]$^-$ (calcd for $C_{19}H_{21}O_6$ 345.1344).

**(R)-MTPA Ester of 7 (8).** (R)-MTPA acid (1.5 mg, 6.4 μmol), DCC (2.0 mg, 9.7 μmol), and DMAP (1.0 mg, 8.2 μmol) were added to a solution of **7** (2.0 mg, 5.8 μmol) in dry CH$_2$Cl$_2$ (100 μL). After stirring for 18 h at room temperature, the reaction mixture was separated by silica gel column chromatography ($n$-hexane/EtOAc, 10:1−1:1), which gave **8** (1.0 mg, 31%): $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 1.08 (3H, d, $J$ = 7.2 Hz, H-19), 1.13 (3H, d, $J$ = 6.8 Hz, H-17), 1.48 (3H, s, H-18), 1.57 (1H, m, H-14), 1.62 (1H, m, H-5), 1.89 (1H, m, H-5), 2.32 (1H, m, H-13), 2.43 (1H, m, H-6), 2.48 (1H, ddd, $J$ = 13.4, 13.4, 4.4 Hz, H-4), 2.53 (1H, dd, $J$ = 13.8, 10.0 Hz, H-14), 3.03 (1H, dd, $J$ = 6.8, 2.2 Hz, H-10), 3.18 (1H, ddd, $J$ = 13.4, 4.9, 3.8 Hz, H-4), 5.04 (1H, d, $J$ = 2.2 Hz, H-11), 5.99 (1H, d, $J$ = 16.5 Hz, H-8), 6.34 (1H, dd, $J$ = 16.5, 6.8 Hz, H-9); HR-ESITOFMS $m/z$ 585.1717 [M + Na]$^+$ (calcd for $C_{29}H_{29}F_3O_8Na$ 585.1707).

**(S)-MTPA Ester of 7 (9).** In the same manner as described for **8**, **9** was prepared from **7**: $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 0.93 (3H, d, $J$ = 7.2 Hz, H-19), 1.14 (3H, d, $J$ = 6.9 Hz, H-17), 1.43 (3H, s, H-18), 1.52 (1H, m, H-14), 1.90 (1H, m, H-5), 2.28 (1H, m, H-13), 2.38 (2H, m, H-4 and H-5), 2.44 (1H, dd, $J$ = 12.9, 11.0 Hz, H-14), 2.56 (1H, m, H-6), 2.96 (1H, dd, $J$ = 7.4, 2.4 Hz, H-10), 4.87 (1H, d, $J$ = 2.4 Hz, H-11), 5.93 (1H, dd, $J$ = 11.7, 7.3 Hz, H-4), 6.03 (1H, d, $J$ = 16.7 Hz, H-8), 6.57 (1H, dd, $J$ = 16.7, 7.4 Hz, H-9); HR-ESITOFMS $m/z$ 585.1704 [M + Na]$^+$ (calcd for $C_{29}H_{29}F_3O_8Na$ 585.1707).

**Biological Assays.** Antimicrobial assays were carried out using *Eschcerichia coli* NIH-JC2, *Micrococcus luteus* ATCC9343, *Bacillus subtilis* PCI219, *Staphylococcus aureus* IFO12732, and *Candida albicans* IFO1594. Mueller Hinton broth (DIFCO) was used for bacteria, and yeast nitrogen base (DIFCO) supplemented with 2% glucose was used for *C. albicans*. Test microorganisms were inoculated into a 32 mL test tube containing 8 mL of the liquid medium. After incubation

on a reciprocal shaker for 20 h at 30 °C, the cells were collected by centrifugation (3000 rpm, 5 min) and the cell suspension (1 × 10$^5$ cells/mL) was prepared in saline. Then, the liquid medium (135 μL), the cell suspension (15 μL), and the sample solution in DMSO (0.5 to 1 μL) were added into the wells of a 96-well culture plate, and the plate was agitated gently to mix the solution. After incubation for 20 h at 37 °C (*E. coli*) or 30 °C (other bacteria and *C. albicans*), the absorbance at 650 nm was measured using a microplate reader. Cytotoxic assay and invasion assay were carried out according to the procedures previously described.[14,15]

**Supporting Information Available:** 1D and 2D NMR spectra of abyssomicin I (**1**) and its derivatives (**4** and **7**) and $^1$H NMR spectra of the MTPA esters **2**, **3**, **5**, **6**, **8**, and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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