# Polyene Antibiotics from *Streptomyces mediocidicus*

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Three polyene antibiotics, mediomycins A (**1**), B (**2**), and clethramycin (**3**), were isolated from *Streptomyces mediocidicus* ATCC23936. Their structures were elucidated through extensive NMR study coupled with chemical reactions and MS/MS fragmentation analysis. All three compounds are linear polyenes consisting of a conjugated oxo-triene group and a hexaene moiety. Compounds **1** and **2** are new polyenes. All three compounds demonstrated a broad spectrum of antifungal activity *in vitro*.

Polyene antibiotics have received increasing interest in recent years because of their potent and broad spectrum of activity.[1] To date, about 200 polyenes produced by different *Streptomyces* species have been documented, including medicinally important compounds such as rapamycin (triene),[2] nystatin (tetraene),[3] filipins (pentaene),[4] and amphotericin B (heptaene).[5] However, outside of two oxo-hexaene macrolides, dermostatins A and B,[6] only one hexaene, clethramycin, has thus far been fully characterized.[7] The other hexaenes,[8,9] such as mediocidin, endomycin, hexaene-80, and hexaene-85, have been reported based on only UV absorption data; for these, neither isolation nor structural elucidation has been conducted. In our continuing search for new antimicrobial natural substances, three active components, **1**, **2**, and clethramycin (**3**), were isolated from the mycelia cake extract of *Streptomyces mediocidicus* ATCC23936 (Figure 1). All three compounds are linear polyenes consisting of a hexaene moiety and a conjugated oxo-triene group. Compounds **1** and **2** are new members of the polyene family, designated as mediomycins A (**1**) and B (**2**). Compound **3**, a known polyene, had been isolated from *Streptomyces hygroscopicus*. This paper describes the isolation, structure elucidation, and antifungal activity of these compounds.

## Results and Discussion

The mycelia cake from a 2 L fermentation broth of *S. mediocidicus* ATCC23936 was collected and extracted with MeOH. After the solvent was removed under vacuum, $Et_2O$ was added to the residue to produce the crude polyene precipitate. The precipitate (1.5 g) was then subjected to reversed-phase preparative HPLC isolation to yield pure **1** (125 mg), **2** (20 mg), and **3** (42 mg) as amorphous, yellow powders.

Mediomycin A (**1**), the major component, was soluble in MeOH, EtOH, and DMSO but insoluble in other organic solvents. It showed strong UV absorption maxima at 338, 357, and 379 nm and a broad UV absorption band in the range 295−306 nm with a shoulder at 320 nm, suggesting the presence of a conjugated hexaene moiety and an oxo-triene substructure. The molecular weight of **1** was determined to be 1175 by the negative EIMS, and the molecular formula was found to be $C_{62}H_{97}NO_{18}S$ by positive high-resolution FT-ICR mass spectroscopy.

The structure of **1** was elucidated by extensive NMR studies and confirmed by chemical derivatization and EI-TOF MS/MS fragmentation data. The complete assignments of all proton and carbon resonances, listed in Table 1, were achieved using various 2D NMR techniques including COSY, HSQC, gHMBC, and HSQC-TOCSY experiments.

The $^1$H NMR spectrum of **1** showed the complex nature of the molecule. The majority of the signals were not well resolved, particularly in the olefinic region of $\delta$ 6.2 to 6.3 and the aliphatic region $\delta$ 1.5 to 1.7, which had multiple overlapping resonances. The carbon signals were relatively well dispersed in the $^{13}$C NMR spectrum of **1**. When coupled with the HSQC experiment, four methyl, 15 methylene, and three methine carbon signals were observed in the aliphatic region of $\delta$ 9 to 51, and 12 oxygenated carbon (*C*H−OR) resonances were observed from $\delta$ 67 to 80. In the olefinic area, one quaternary carbon signal at $\delta$ 133.8 and 25 olefinic methine carbons between $\delta$ 127 and 139 were detected, accounting for 13 double bonds. The number of double bonds was further confirmed by hydrogenation. Treating **1** with $H_2$ and Pd/C yielded the derivative **1a** with a molecular mass 26 Da higher than the original compound (Figure 1), supporting the presence of 13 double bonds. The remaining two carbons required by the molecular formula could be assigned to the carboxylic carbonyl at $\delta$ 178.7 (C-1) and the ketone at $\delta$ 212.8 (C-31). This evidence revealed that compound **1** was a linear molecule.

Identification of the substructure from C-1 to C-17 was straightforward. A well-resolved methyl proton singlet at $\delta$ 1.93 (H-59) showed three cross-peaks to carboxylic carbon C-1 and double-bond carbons C-2/C-3 in the HMBC spectrum (Figure 2). The most downfield proton signal, olefinic proton H-3, showed a COSY correlation to an AB system for H-4 and H-5 and had a weak correlation to H-6, probably via zigzag path coupling. H-6 further correlated to H-7, which was resolved as a doublet of triplets with coupling constants of 15.1 and 7.5 Hz. This result demonstrated that H-7 was coupled to the *trans* proton H-6 (15.1 Hz) and equally coupled (7.5 Hz) to two aliphatic methylene protons H-8. The polarized chemical shifts of the triene unit C2 to C7 (Table 1) were in good agreement with the oxo-triene substructure, as indicated by the UV spectrum. The presence of the carboxylic acid group was further confirmed by methylation of **1** with diazomethane, which resulted in a mono-methyl ester **1b** (Figure 1). The presence of a methoxyl group in **1b** was indicated by the appearance of new signals in the $^1$H and $^{13}$C NMR spectra at $\delta$ 3.73 and 52.3, respectively, as well as the upfield shift of the carboxylic carbon from $\delta$ 178.7 in **1** to $\delta$ 172.5 in **1b**. Two methyl doublets, H-60 and H-61, showed all the expected two-bond and three-bond HMBC correlations to carbons C-11 through C-15 (Figure 2). The upfield chemical shift of the C14-methyl carbon (C-61: $\delta$ 8.3) was consistent with the presence of two $\gamma$-hydroxyls attached at C-13 and C-15, respectively.[10] COSY correlations were observed between H-8/H-9, H-9/H-10, H-10/H-11, H-15/H-16, and H-16/H-17. Since the chemical shift of H-8 was identical to those of H-38, H-46, and H-52, the connectivity of C-8/C-9 was verified by HMBC correlations from H-7 to C-8 and C-9, all resolved peaks.

A short spin system was easily identified from H-26 to the methyl doublet of H-62 in the COSY spectrum. The H-29, H-30, and H-62 all exhibited HMBC correlations to the most downfield resonance at $\delta$ 212.8 (C-31), appropriate for a ketone carbonyl carbon (Figure

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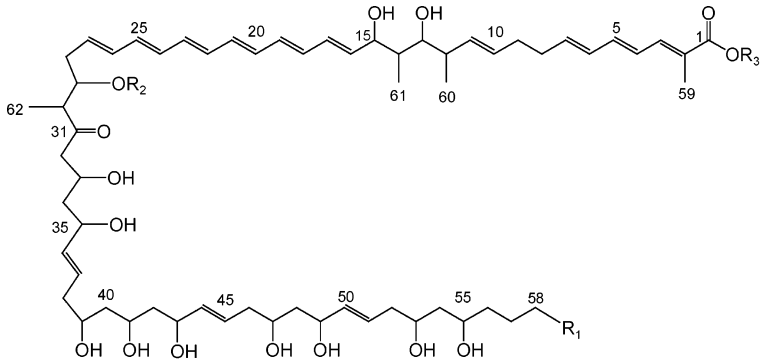
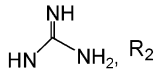



**1**. $R_1 = NH_2$, $R_2 = SO_3H$, $R_3 = H$

**2**. $R_1 = NH_2$, $R_2 = H$, $R_3 = H$

**3**. $R_1 =$  $R_2 = SO_3H$, $R_3 = H$

**1a**. $R_1 = NH_2$, $R_2 = SO_3H$, $R_3 = H$, all double bonds were reduced

**1b**. $R_1 = NH_2$, $R_2 = SO_3H$, $R_3 = CH_3$

**1c**. $R_1 = CH_3CONH$, $R_2 = SO_3H$, $R_3 = H$, all OH groups were converted to $CH_3COO$-

**Figure 1.** Structures of mediomycin A (**1**), mediomycin B (**2**), clethramycin (**3**), and the derivatives (**1a**, **1b**, and **1c**) of mediomycin A.

2). An additional HMBC correlation was observed between this ketone carbonyl C-31 and the methylene protons H-32. H-32 showed COSY correlations to H-33, which further coupled to the two methylene protons (H-34) at $\delta$ 1.56 and 1.69. This connectivity was supported by the HSQC-TOCSY correlation between H-33 and C-34, a resolved carbon resonance.

Theoretically, the remaining carbon skeleton C-34 to C-58 of **1** could be easily established by an interpretation of the COSY spectrum, since it possesses a continuous spin system up to the amino terminus. However, due to the multiple overlapping signals, combined analysis of COSY, HSQC-TOCSY with different mixing times, and HMBC data was absolutely essential. Detailed analysis of the 2D NMR data led to the identification of three identical six-carbon units: C-34 to C-39, C-42 to C-47, and C-48 to C-53. Each unit contained two hydroxylated carbons, two methylene carbons, and one *trans* double bond. For example, HSQC-TOCSY correlations observed from H-35 to C-34, C-35, C-36, and C-37; from H-38 (unresolved) to C-37 (resolved); and from H-39 to C-37 completed the assignment for the C-34 to C-39 unit. The olefinic carbon C-36, which resonated at the lower field, was attributed to the presence of a $\beta$-hydroxyl at C-35, while C-37 was shielded owing to the presence of $\gamma$-hydroxyls at C-36 and C-39. The units C-42 to C-47 and C-48 to C-53 were identified in a similar manner.

The connectivity between C-39/C-40 and C-47/C-48 was based on HMBC observations between H-41/C-39 and between H-49/C-47, respectively. Analysis of HSQC-COSY and HSQC-TOCSY data led to the NMR assignment of the tail segment C-54 to C-58, as shown in Figure 2. A comparison of the NMR data of **1** with those of linearmycin A provided the support for above assignments.[11] The $^1$H and $^{13}$C NMR chemical shifts assigned for the C-52 to C-58 segment of **1** were nearly identical to the chemical shifts of corresponding protons and carbons (C-54 to C-60) in linearmycin A, which indicated that these two compounds shared the same tail substructure.

The remaining unassigned eight olefinic carbons and eight protons comprised four double bonds, which, together with double bonds C-16/C-17 and C-26/C-27, formed the all-*trans* hexaene moiety revealed by the UV spectrum. The structure assigned to this point accounts for all atoms indicated by the molecular formula except of one sulfur and three oxygens. An intense IR band at 1240 cm$^{-1}$ suggested that the sulfur atom was present in a sulfate ester. Examination of the $^{13}$C NMR data readily identified the location of the sulfate group due to its deshielding effects.[12] The downfield chemical shifts for the oxygenated carbon at $\delta$ 80.0 (C-29) and its bonded proton at $\delta$ 4.67 indicated that the sulfate group was attached at this position. A comparison with the literature data for clethramycin ($\delta$ 79.9 and 4.67)[7] and linearmycin A ($\delta$ 72.5 and 3.95)[11] supported the above assignment. Acetylation of **1** with acetic anhydride in the presence of DMAP introduced 12 acetyl groups into the product **1c** (Figure 1). This was consistent with the fact that 11 of the 12 oxygenated carbons in **1** were present as free carbinols, plus one additional acetylation site on the amino group.

To confirm the proposed structure for **1**, both positive and negative EI-TOF MS/MS experiments were conducted. In the negative EIMS/MS spectrum, fragment ions at *m*/*z* 1076, 1058, 1040, and 1022, representing the loss of $H_2SO_4$ followed by the successive elimination of three water molecules, were detected. The strong fragmentation ions generated by allylic cleavage were observed in both the positive MS/MS spectrum (*m*/*z* 132, 246, and 404) and the negative MS/MS spectrum (*m*/*z* 151, 219, 277, 293, 896, 926, and 954). These fragmentations (Figure 3) corroborated the location of the double bonds and hydroxyl groups of **1** very well and supported the assigned structure. Observation of the fragmentation ions at *m*/*z* 657 and 559 in the negative MS/MS spectrum, resulting from the cleavage of the C-32/C-33 bond adjacent to the ketone group followed by $H_2SO_4$ loss, provided additional evidence for the assigned structure.

The relative configuration of **1** at C-13/C-14/C-15, C-33/C-35, C-39/C-41/C-43, C-47/C-49, and C-53/C-55 was assigned as *syn*/*syn*, *syn*, *syn*/*anti*, *anti*, and *syn* by application of the universal NMR database approach created by Dr. Kishi's group for acyclic 1,3-diol and 1,3,5-triol systems (Figure 4).[12,13] Comparing the C-41 chemical shift with the NMR database **A**, the relative configuration of the C-39/C-41/C-43 moiety could be either *syn*/*anti* or *anti*/*syn* (Figure 4). Since the C-43 chemical shift was very close to the value found for the *anti*-diol in the NMR database **B1**, the possibility of *anti*/*syn* was eliminated, thereby establishing the *syn*/*anti*-C-39/C-41/C-43 relative configuration (Figure 4). The chemical shifts of C-35 and C-49 were in good agreement with the values of *syn*-1,2 diol and *anti*-1,2-diol in the NMR database **B1**, respectively,

**Table 1.** $^1$H and $^{13}$C NMR Data for Compounds **1** and **2** in $CD_3OD$

| | **1** | | **2** | |
|---|---|---|---|---|
| C- | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 178.7 | | 179.1 | |
| 2 | 130.6 | | 130.9 | |
| 3 | 139.6 | 7.05, bd (9.5) | 139.1 | 6.99, bd, (9.5) |
| 4 | 128.0 | 6.44, dd (9.5, 15) | 128.0 | 6.35, dd (9.5, 15) |
| 5 | 138.6 | 6.42, dd (9.5, 15) | 138.2 | 6.32, dd (9.5, 15) |
| 6 | 132.2 | 6.22, dd (9.5, 15) | 132.3 | 6.19, dd (9.5, 15) |
| 7 | 137.9 | 5.84, dt (7.5, 15.1) | 137.4 | 5.79, dt (7.5, 15) |
| 8 | 34.0 | 2.23−2.21[a] | 34.0 | 2.22−2.21[a] |
| 9 | 33.4 | 2.14, dt (1.2, 7.0) | 33.4 | 2.07, dt (1.2, 7.0) |
| 10 | 131.3 | 5.48, dt (6.0, 15) | 131.9 | 5.40, dt (6.0, 15) |
| 11 | 134.7 | 5.44, dd (7.0, 15) | 134.5 | 5.42, dd (7.0, 15) |
| 12 | 41.7 | 2.33, m | 41.8 | 2.27, m |
| 13 | 77.9 | 3.40, dd (5.5, 7.5) | 77.8 | 3.35, dd (5.5, 7.5) |
| 14 | 42.5 | 1.63[b] | 42.4 | 1.58[b] |
| 15 | 75.4 | 4.25, m | 75.3 | 4.20, m |
| 16 | 137.1 | 5.72[c] | 137.1 | 5.66[c] |
| 17 | 132.0 | 6.25 | 132.0 | 6.21 |
| 18 | 134.1 | 6.26 | 133.8 | 6.22 |
| 19 | 134.3[d] | 6.24 | 134.0[d] | 6.21 |
| 20 | 133.8[d] | 6.25 | 134.1[d] | 6.21 |
| 21 | 134.2[d] | 6.25 | 134.1[d] | 6.21 |
| 22 | 134.4[d] | 6.25 | 134.3[d] | 6.21 |
| 23 | 134.5[d] | 6.25 | 134.4[d] | 6.21 |
| 24 | 134.6[d] | 6.25 | 134.5[d] | 6.21 |
| 25 | 133.3 | 6.24 | 133.1 | 6.20 |
| 26 | 135.0 | 6.17, dd (8.9, 15) | 134.6 | 6.15, dd (8.9, 15) |
| 27 | 130.8 | 5.74[c] | 130.9 | 5.68[c] |
| 28 | 37.0 | 2.55, m | 39.8 | 2.54, m |
| 29 | 80.0 | 4.67, q (6.0) | 72.5 | 3.82, q (6.0) |
| 30 | 51.0 | 2.88, dq (6.0, 7) | 53.1 | 3.08, dq (6.0, 7.0) |
| 31 | 212.8 | | 214.2 | |
| 32 | 50.8 | 2.73, dd (4.3, 17)<br>2.77, dd (8.0, 17) | 50.4 | 2.63, dd (4.3, 17)<br>2.67, dd (8.0, 17) |
| 33 | 66.7 | 4.14, m | 66.7 | 4.10, m |
| 34 | 45.0 | 1.56/1.69[b] | 45.0 | 1.57/1.67[b] |
| 35 | 71.8 | 4.21, q (6.9) | 71.8 | 4.12, q (6.9) |
| 36 | 136.5 | 5.56, dd (8.9, 15) | 136.5 | 5.50, dd (8.9, 15) |
| 37 | 129.1 | 5.70[c] | 129.1 | 5.60[c] |
| 38 | 41.8 | 2.23−2.21[a] | 41.7 | 2.22−2.20[a] |
| 39 | 71.2 | 3.83 | 71.1 | 3.74 |
| 40 | 44.7 | 1.58[b] | 44.9 | 1.56[b] |
| 41 | 68.4 | 3.99, m | 68.3 | 3.98, m |
| 42 | 44.7 | 1.58[b] | 45.7 | 1.59[b] |
| 43 | 70.2 | 4.26 | 70.1 | 4.24 |
| 44 | 137.4 | 5.58, dd (7.5, 15) | 137.4 | 5.50, dd (7.5, 15) |
| 45 | 127.7 | 5.69[c] | 127.6 | 5.61[c] |
| 46 | 41.6 | 2.23−2.21[a] | 41.6 | 2.22−2.20[a] |
| 47 | 68.9 | 3.86, m | 68.9 | 3.87, m |
| 48 | 45.7 | 1.52[b] | 44.8 | 1.54[b] |
| 49 | 70.2 | 4.29 | 70.2 | 4.26 |
| 50 | 137.1 | 5.54, dd (7.0, 15) | 137.2 | 5.52 |
| 51 | 127.6 | 5.68[c] | 127.5 | 5.64[c] |
| 52 | 41.6 | 2.23−2.21[a] | 41.4 | 2.22−2.20[a] |
| 53 | 71.1 | 3.80 | 71.1 | 3.74 |
| 54 | 44.2 | 1.55[b] | 44.2 | 1.52[b] |
| 55 | 70.7 | 3.79 | 70.7 | 3.74 |
| 56 | 35.0 | 1.43/1.52[b] | 35.1 | 1.35/1.48[b] |
| 57 | 24.9 | 1.68/1.73 | 24.9 | 1.62/1.71 |
| 58 | 40.9 | 2.95, m | 40.8 | 2.87, m |
| 59 | 13.5 | 1.93, s | 13.7 | 1.83, s |
| 60 | 18.4 | 0.95, d (7.2) | 18.4 | 0.89, d (7.2) |
| 61 | 8.3 | 0.91, d (7.0) | 8.3 | 0.84, d (7.0) |
| 62 | 11.8 | 1.12, d (7.0) | 11.1 | 1.02, d (7.0) |

[a] These signals overlapped. [b]These signals overlapped. [c]These signals overlapped. [d]These assignments are exchangeable in the column.

indicating the *syn*-C-33/C-35 and *anti*-C-47/C-49 relative configurations (Figure 4). The relative configuration of *syn*-C-53/C-55 was assigned on the basis of the chemical shift of C-55, which was close to the value of the *syn*-diol in the NMR database **B2** (Figure 4). The relative upfield chemical shifts of the C14-methyl carbon (C-61: $\delta$ 8.3 in $CD_3OD$ and $\delta$ 7.2 in DMSO-$d_6$) due to two $\gamma$-gauche effects suggested a *syn/syn*-C-13/C-14/C-15 relative configuration. This was supported by comparison with the methyl carbon chemical shifts in the NMR database **C** (Figure 4).

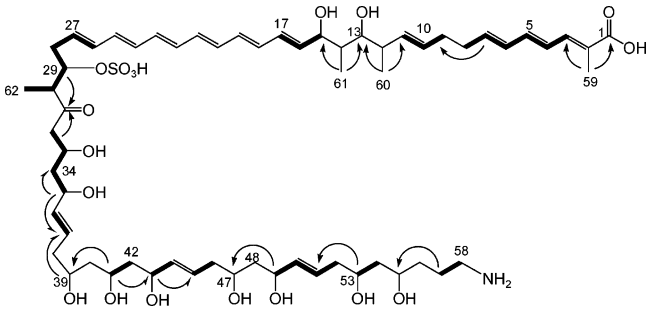



**Figure 2.** Key COSY (—), HMBC (→), and HSQC-TOCSY (⇀) correlations of **1**.

The UV spectrum of mediomycin B (**2**) was similar to that of **1**. The negative EIMS showed the molecular ion at *m*/*z* 1094, and the high-resolution FT-ICR mass spectrum revealed a molecular formula of $C_{62}H_{97}NO_{15}$ for **2**, suggesting one $SO_3$ group less than **1**. This suggestion was confirmed by its $^1$H and $^{13}$C NMR data (Table 1). Compared with **1**, C-29 of **2** showed a 7.5 ppm upfield shift, and C-28 and C-30 showed about 2 ppm downfield shifts due to the loss of the electron-withdrawing group ($SO_3$). In order to confirm that **2** was not simply an artificial product of **1**, a fermentation time-course study (1−3 days) by LC-MS was conducted. The detection of **2** in fresh mycelia extracts of day 1 to day 3 cultures suggested that **2** was not an isolation artifact.

Compound **3** showed the molecular ion at *m*/*z* 1216 in the negative EIMS, and the high-resolution FT-ICR mass spectrum revealed a molecular formula of $C_{63}H_{99}N_3O_{18}S$. The $^1$H and $^{13}$C NMR data of **3** closely resembled those of **1**, except for the terminal carbon C-58 and its attached protons. For **3**, the H-58 appeared at $\delta$ 3.18, instead of $\delta$ 2.95 in **1**, which showed strong HMBC correlation to a carbon at $\delta$ 158.8, highly diagnostic of a guanidine carbon. This indicated that a guanidine group was connected to C-58 in **3** instead of a free amino group in **1**. Therefore, **3** has the same structure as clethramycin. A direct comparison with the reported NMR data of clethramycin supported this conclusion.[7]

For compounds **2** and **3**, the relative configurations at C-13/C-14/C-15, C-33/C-35, C-39/C-41/C-43, C-47/C-49, and C-53/C-55 should be the same as those of **1** according to their carbon chemical shifts.

The antimicrobial activities of compounds **1**, **2**, **3**, and the methylation derivative **1b** were tested together with amphotericin B, a representative polyene antibiotic, against a variety of yeasts and fungi including pathogenic yeasts and filamentous fungi (Table 2). All three compounds exhibited a broad spectrum of activities against yeasts and filamentous fungi. Mediomycin B (**2**) demonstrated the most potent activity in most cases (MIC $\approx$ 1−2 $\mu$g/mL). However, methylation of the carboxylic acid group in **1b** led to the loss of antifungal bioactivity.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a Jasco P-1020 polarimeter. UV spectra were obtained on a Shimadzu UV-2501PC spectrophotometer. IR spectra were recorded on a Perkin-Elmer RX1 FT-IR spectrophotometer. $^1$H and $^{13}$C NMR spectra were acquired on a Bruker DRX 500 spectrometer in methanol-$d_4$ and DMSO-$d_6$. Electrospray mass spectra were recorded on an Agilent MSD 1100 instrument. High-resolution mass spectra were measured on a Bruker Apex-II 9.4 T electrospray FT-ICR mass spectrometer, and EI-TOF MS/MS experiments were carried out on a Micromass Q-TOF instrument. Analytical HPLC was conducted on a Waters Alliance HPLC system equipped with a Waters Model 996 photodiode array detector. The isolation and purification of **1**, **2**, **3**, and the derivatives were accomplished using a Waters Delta 4000 Prep-HPLC system.

**Fermentation.** *Streptomyces mediocidicus* ATCC23936 was cultivated in liquid culture, e.g., tryptic soy broth, at 30 °C and 200 rpm

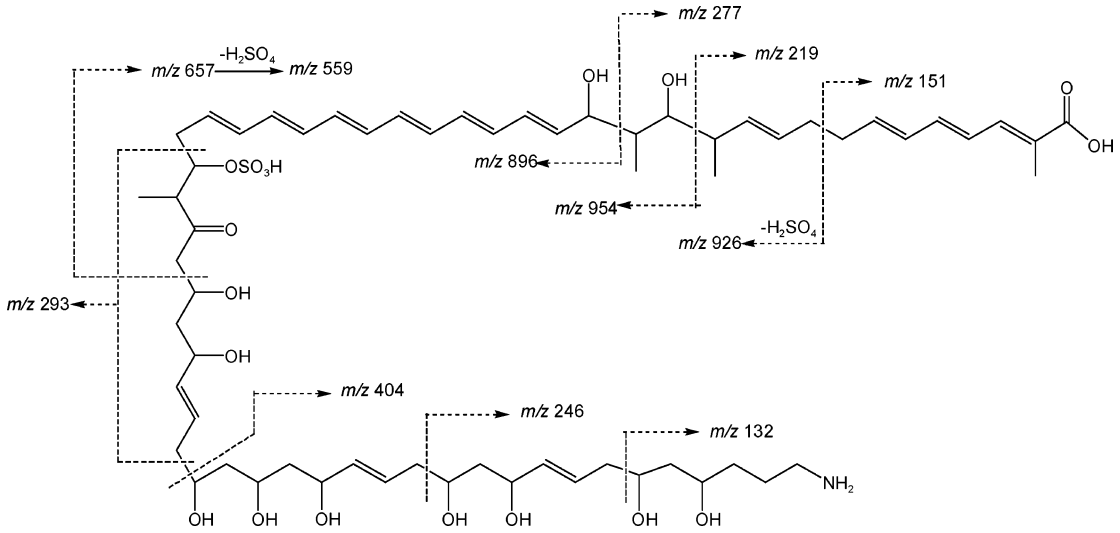



**Figure 3.** MS/MS fragmentation assignments of **1**.

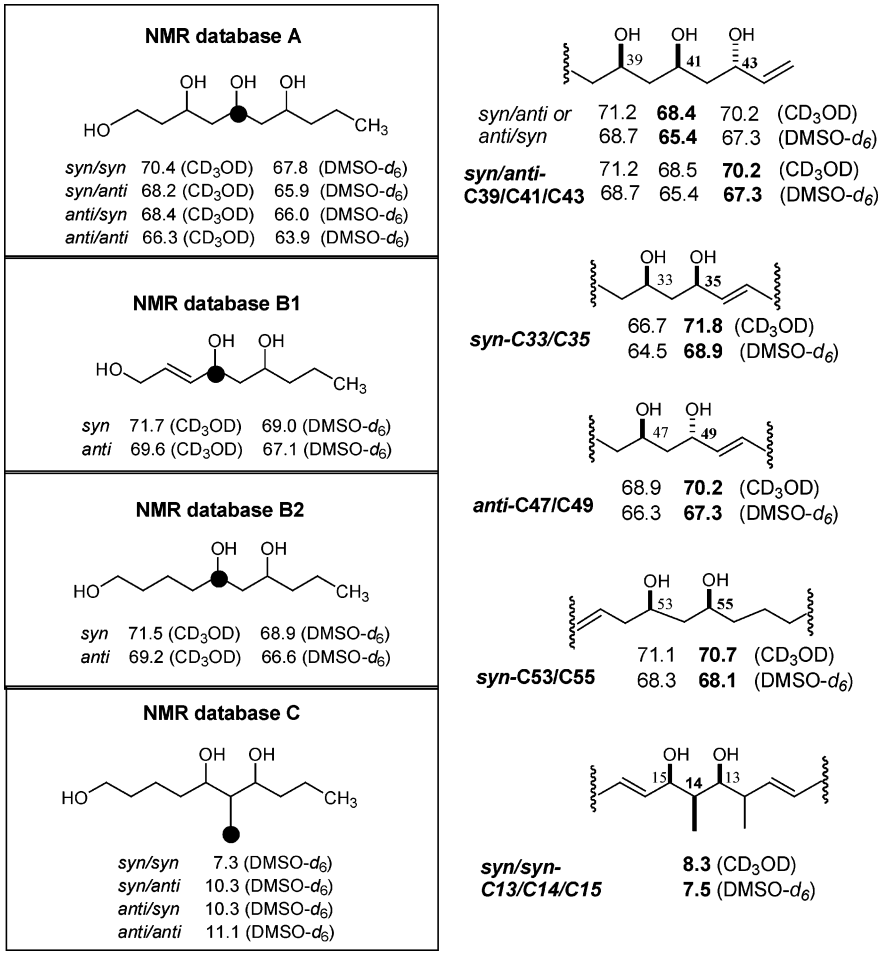



**Figure 4.** Chemical shift comparison of C-41, C-43, C-35, C-49, C-55, and C14-methyl (chemical shifts in bold) with Kishi's NMR databases (the carbon marked with a dot) suggesting the relative configuration at C-39/C-41/C-43, C-33/C-35, C-47/C-49, C-53/C-55, and C-13/C-14/C-15 to be *syn/anti*, *syn*, *anti*, *syn*, and *syn/syn*, respectively.

for 48 h. This seed culture was inoculated into the fermentation medium (25 mL) consisting of 2% Pharmamedia, 0.5% yeast extract, 2% glycerol, 0.1% $K_2HPO_4$, 0.1% $KH_2PO_4$, 0.5% NaCl, and 2% D-glucose in 250 mL Erlenmeyer flasks, which were incubated at 26 °C for 3 days with an agitation rate of 250 rpm.

**Extraction and Isolation.** The fermentation broth (2 L) was centrifuged, and the mycelia cake was extracted twice with MeOH (2 × 2 L). The MeOH extract after evaporation under vacuum was mixed with $Et_2O$ (500 mL) to produce a precipitate. The precipitate was collected and redissolved in MeOH for HPLC isolation. The HPLC column (HP/HPV C-18, 50 × 300 mm) was eluted with a gradient of MeOH/$H_2O$ containing 5 mM $NH_4OAc$ (65% to 90% MeOH in 70 min) at a flow rate of 30 mL/min. Compounds **1** ($t_R$ = 32.3 min), **2** ($t_R$ = 45.6 min), and **3** ($t_R$ = 31.4 min) were collected. After the MeOH was evaporated under vacuum, the resulting aqueous solutions were lyophilized to yield pure **1** (125 mg), **2** (20 mg), and **3** (42 mg) as amorphous, yellow powders.

**Mediomycin A (1):** $[\alpha]_D^{25}$ −10.4 (*c* 0.28, MeOH); IR (KBr) $\nu_{max}$ 3421, 3015, 2925, 2855, 1669, 1632, 1240 $cm^{-1}$; UV (MeOH/$H_2O$, 2:1) $\lambda_{max}$ 295, 306, 320 (sh), 338, 359, 379 nm; $^1H$ and $^{13}C$ NMR data,

**Table 2.** Antifungal Activity of **1**, **2**, **3**, and **1b** in Comparison to Amphotericin B

| | MIC ($\mu$g/mL) | | | | |
|---|---|---|---|---|---|
| organism | amphotericin B | **1** | **2** | **3** | **1b** |
| *Candida albicans* GC 3064 | 0.50 | 2 | 1 | 4 | 16 |
| *C. albicans* GC 3065 | 0.25 | 2 | 1 | 4 | 16 |
| *C. albicans* GC 3066 | 0.25 | 4 | 1 | 4 | 32 |
| *C. parapsilosis* GC 3074 | 0.25 | 16 | 2 | 16 | 128 |
| *C. parapsilosis* GC 3075 | 0.50 | 16 | 4 | 16 | >128 |
| *C. parapsilosis* GC 3076 | 0.50 | 16 | 2 | 16 | >128 |
| *C. pseudotropicalis* GC 3070 | 0.25 | 1 | 1 | 2 | 8 |
| *C. tropicalis* GC 3080 | 0.50 | 16 | 2 | 16 | 16 |
| *C. tropicalis* GC 3081 | 0.50 | 8 | 2 | 8 | 32 |
| *C. krussii* GC 3067 | 0.50 | 2 | 1 | 2 | 8 |
| *C.lusitaniae* GC 3068 | 0.25 | 2 | 2 | 4 | 16 |
| *C. rugosa* GC 3077 | 0.50 | 2 | 1 | 4 | 8 |
| *Aspergillus fumigatus* GC 3092 | 0.25 | 128 | 16 | >128 | >128 |
| *A. niger* GC 3091 | 0.25 | 1 | 1 | 4 | >128 |

see Table 1; EIMS *m*/*z* 1174.6 $[M]^-$ (100); HRMS *m*/*z* 1176.6478 $[M]^+$ (calcd for $C_{62}H_{98}NO_{18}S$ 1176.6499).

**Mediomycin B (2):** $[\alpha]_D^{25}$ −15.4 (*c* 0.21, MeOH); IR (KBr) $\nu_{max}$ 3421, 3015, 2925, 2855, 1669, 1630 $cm^{-1}$; UV (MeOH/$H_2O$, 2:1) $\lambda_{max}$ 295, 306, 320 (sh), 338, 359, 379 nm; $^1H$ and $^{13}C$ NMR data, see Table 1; EIMS *m*/*z* 1094.6 $[M]^-$ (100); HRMS *m*/*z* 1096.6921 $[M]^+$ (calcd for $C_{62}H_{98}NO_{15}$ 1096.6931).

**Compound 3:** $[\alpha]_D^{25}$ −13.5 (*c* 0.10, MeOH); IR (KBr) $\nu_{max}$ 3421, 3015, 2925, 2855, 1669, 1630, 1240, 972 $cm^{-1}$; UV (MeOH/$H_2O$, 4:1) $\lambda_{max}$ 306, 320 (sh), 339, 359, 379 nm; $^1H$ and $^{13}C$ NMR data, see Table 1; EIMS *m*/*z* 1216.6 $[M]^-$ (100); HRMS *m*/*z* 1216.6569 $[M]^-$ (calcd for $C_{62}H_{98}N_3O_{18}$ 1216.6571).

**Preparation of Hydrogenated Derivative 1a.** 10% Pd/C (5 mg) was added to a 2 mL MeOH solution of **1** (20 mg) at room temperature. After stirring under $H_2$ for 3 h, the mixture was filtered and the solvent was evaporated to yield 19 mg of **1a** as a white solid: EIMS *m*/*z* 1200.7 $[M]^-$ (100).

**Preparation of Methyl Ester 1b.** Freshly prepared diazomethane was added to a 2 mL MeOH solution of **1** (20 mg) at room temperature with stirring. After 40 min, the solvent was evaporated under vacuum to yield 20 mg of **1b**: $^1H$ NMR ($CD_3OD$) $\delta$ 6.93 (1H, bd, H-3), 6.42 (1H, H-4), 6.40 (1H, H-5), 6.25−6.21 (9H, H-17, H-18, H-19, H-20, H-21, H-22, H-23, H-24, H-25) 6.19 (1H, H-6), 6.17 (1H, H-26), 5.87 (1H, dt, H-7), 5.72−5.66 (5H, H-16, H-27, H-37, H-45, H-51), 5.59−5.46 (4H, H-10, H-36, H-44, H-50), 5.44 (1H, dd, H-11), 4.67 (1H, q, H-29), 4.29−4.22 (3H, , H-15, H-43, H-19), 4.21 (1H, dt, H-35), 4.14 (1H, m, H-33), 3.99 (1H, H-41), 3.86−3.79 (4H, H-39, H-47, H-53, H-55), 3.73 (3H, -$OCH_3$) 3.41 (1H, dd, H-13), 2.95 (2H, H-58), 2.88 (1H, q, H-30), 2.76 (1H, dd, H-32), 2.73 (1H, dd, H-32′), 2.55 (2H, m, H-28), 2.33 (1H, m, H-12), 2.23−2.21 (8H, H-8, H-38, H-46, H-52), 2.14 (2H, dt, H-9), 1.93 (3H, s, H-59), 1.78 (1H, H-57), 1.75−1.58 (13H, H-14, H-34, H-40, H-42, H-48, H− 54, H-57′), 1.43 (2H, H-56), 1.12 (3H, d, H-62), 0.95 (3H, d, H-60), 0.91 (3H, d, H-61); $^{13}C$ NMR ($CD_3OD$) $\delta$ 172.5 (C-1), 130.0 (C-2), 139.4 (C-3), 128.0 (C-4), 138.6 (C-5), 132.2 (C-6), 137.9 (C-7), 34.0 (C-8), 33.4 (C-9), 131.3 (C-10), 134.7 (C-11), 41.7 (C-12), 77.9 (C-13), 42.3 (C-14), 75.4 (C-15), 137.1 (C-16), 132.0 (C-17), 134.1 (C-18), 134.3 (C-19), 133.8 (C-20), 134.2 (C-21), 134.4 (C-22), 134.5 (C-23), 134.6 (C-24), 133.3 (C-25), 135.0 (C-26), 130.8 (C-27), 37.0 (C-28), 79.9 (C-29), 52.3 (−$OCH_3$), 51.0 (C-30), 212.8 (C-31), 50.8 (C-32), 66.7 (C-33), 45.0 (C-34), 71.8 (C-35), 136.5 (C-36), 129.1 (C-37), 41.8 (C-38), 68.9 (C-39), 44.7 (C-40), 68.4 (C-41), 44.4 (C-42), 70.2 (C-43), 137.4 (C-44), 127.7 (C-45), 41.6 (C-46), 68.4(C-47), 45.7 (C-48), 70.2 (C-49), 137.1 (C-50), 127.7 (C-51), 41.6 (C-52), 71.1 (C-53), 44.2 (C-54), 70.7 (C-55), 35.0 (C-56), 24.9 (C-57), 40.9 (C-58), 13.5 (C-59), 18.4 (C-60), 8.6 (C-61), 11.8 (C-62); EIMS *m*/*z* 1188.7 $[M]^-$ (100).

**Preparation of Acetate Derivative 1c.** Acetic anhydride (0.2 mL) and DMAP (1 mg) were added to a 1 mL pyridine solution of **1** (20 mg) at 0 °C under $N_2$ with stirring. The reaction mixture was gradually warmed to room temperature. After 8 h, the mixture was diluted with ice water (5 mL) and extracted twice with EtOAC. After the solvent was evaporated under vacuum, 25 mg of **1c** was obtained as a pale yellow powder: EIMS *m*/*z* 1678.7 $[M]^-$ (100); UV (MeOH) $\lambda_{max}$ 306, 323 (sh), 341, 359, 380 nm.

**Biological Activity Test.** The MIC of each antifungal agent was assessed using the National Committee for Clinical Laboratory Standard M-27A procedure. Briefly, antifungal agents were added to 96-well tissue culture plates at the final concentration of 0.03−128 $\mu$g/mL in RPMI-1640 medium; the cell inoculation was done as follows: yeasts 0.5−2.5 × $10^3$ cfu/mL; filamentous fungi (1−5) × $10^3$ cfu/mL. MIC values were measured following 30 °C incubation for 48 h for yeasts and 72 h for filmentous fungi.

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