Supporting Information for

Mycangimycin, a polyene peroxide from a mutualist *Streptomyces* sp.

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General experimental procedures

Optical rotation was measured on a Jasco P-2000 polarimeter with a 5 cm cell. IR spectra were recorded using a Perkin-Elmer 1600 FT-IR spectrometer. $^1$H and 2D NMR spectral data were collected in the inverse-detection probe on a Varian Inova 600 MHz spectrometer. $^{13}$C NMR spectrum was obtained in the broad-band probe on a Varian Inova 150 MHz spectrometer. UV spectra were acquired in an Amersham Biosciences Ultraspec 5300 Pro UV/Visible spectrometer with a path length of 1 cm. For HPLC and LC/MS analyses, 10 mL of the culture broth was collected and extracted with 15 mL of EtOAc. The EtOAc fraction was dried in vacuo and the dry material was resuspended in 500 $\mu$L of MeOH to make a HPLC and LC/MS sample. 10 $\mu$L of the prepared sample was injected to HPLC (Aglient 1100 Series, 4.6 × 150 mm, 5 $\mu$m, Zobax Eclipse XDB-C$_8$ reversed-phase column, 1 mL/min). For LC/MS analysis, Agilent 1200 Series HPLC (4.6 × 100 mm, 5 $\mu$m Phenomenex C$_{18}$(2) reversed-phase column / 4.6 × 50 mm, 5 $\mu$m, Zobax Eclipse XDB-C$_8$ reversed-phase column, 0.7 mL/min) and 6130 Series mass spectrometer were used. High resolution mass spectra were collected in a Waters Q-Tof Ultima mass spectrometer.

Cultivation of SPB74

The strain SPB74 was cultured in a 125 mL Erlenmeyer flask containing 25 mL of the medium YEME (4 g yeast extract, 10 g malt extract, and 4 g glucose per 1 L) at 30 °C with shaking at 250 rpm for 3 days. Then 20 mL of the YEME culture was inoculated to 200 mL of YPM (2 g yeast extract, 2 g peptone, and 4 g mannitol per 1 L) in 500 mL Erlenmeyer flask. The YPM culture was incubated at 30 °C with shaking at 250 rpm for 14 to 16 h before extraction. Multiple cultivations were carried out for necessary bioassays and NMR experiments.

Extraction and isolation of mycangimycin (1)

The whole culture (200 mL) was extracted with 250 mL of ethyl acetate (EtOAc) twice. The EtOAc layer was separated from the aqueous phase with a fractionating funnel. After partitioning, residual water in the EtOAc extract was removed by adding excess of sodium sulfate anhydrous. The dry EtOAc was evaporated by rotavap until the total volume reduced to approximately 100 mL. 0.2 g of celite was added to the EtOAc fraction and the mixture was dried by rotavap. The crude material-celite mixture was loaded on 1 g of a pre-packed C$_{18}$ Sepak resin and fractionated by eluting with a step gradient of water, methanol, and dichloromethane combinations. The methanol/dichloromethane 1:1 fraction contained almost pure polyene peroxide, which was subsequently purified by partitioning with hexane four times after evaporating dichloromethane and adding water to make 9:1 methanol/water solution. The overall yield of the pure polyene peroxide through this isolation scheme is approximately 5 mg / L.
$^1$H NMR spectrum of mycangimycin (1) in CD$_3$OD/CDCl$_3$ (5:2)
$^{13}$C NMR spectrum of mycangimycin (1) in CD$_3$OD/CDCl$_3$ (5:2)
gCOSY NMR spectrum of mycangimycin (1) in CD$_3$OD/CDCl$_3$ (5:2)
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gHMBC NMR spectrum of mycangimycin (1) in CD$_3$OD/CDCl$_3$ (5:2)
UV spectrum of mycangimycin (1) in tetrahydrofuran.

Wave length (nm)
1.36e-5 M in THF
Mycangimycin
Methyl ester (2) of mycangimycin (1)

A crude ethyl acetate extract of a 200 mL SPB74 culture was dried *in vacuo*. The dry material (~ 5 mg) was then dissolved in 2.5 mL of anhydrous MeOH. 200 µL of 2 M TMSdiazomethane was added to the solution and the reaction mixture was stirred. The completion of the reaction was confirmed with LC/MS analysis in an hour. The reaction mixture was dried with celite for purification through a 1g C18 Sepak column. A step-gradient of water-methanol combination (20%, 40%, 60%, 80%, and 100% aqueous MeOH) was applied for separation. The methyl ester (2) was recovered in the 100% MeOH fraction and purified by washing with hexane four times. The molecular formula of 2 was confirmed as C21H26O4 by ESI-HRMS analysis (obsd [M+Na]+ m/z 365.1724, calc m/z 365.1729).

Table S1. NMR spectral data for methyl ester (2) of 1 in CDCl3 (a600 MHz, b coupling constant in Hz, c150 MHz)

| C/H | δa | mult (Jb) | δc |
|-----|-----|-----------|-----|
| 1   | 173.8 | C         |     |
| 2a  | 2.78 | dd (16.0, 7.0) | 38.6 | CH3 |
| 2b  | 2.55 | dd (16.0, 6.5) |     |     |
| 3   | 4.68 | m         | 76.9 | CH  |
| 4a  | 2.88 | ddd (12.5, 7.5, 7.5) | 44.7 | CH2 |
| 4b  | 1.96 | ddd (12.5, 7.0, 6.0) |     |     |
| 5   | 4.31 | m         | 80.3 | CH  |
| 6a  | 2.59 | m         | 30.7 | CH2 |
| 6b  | 2.47 | m         |     |     |
| 7   | 5.50 | m         | 128.2 | CH |
| 8   | 6.61 | dd (11.0, 11.0) | 126.3 | CH |
| 9   | 6.23 | dd (11.0, 11.0) | 124.3 | CH |
| 10  | 6.12 | m         | 130.1 | CH |
| 11  | 6.73 | m         | 129.0 | CH |
| 12  | 6.71 | m         | 128.8 | CH |
| 13  | 6.12 | m         | 130.1 | CH |
| 14  | 6.49 | m         | 124.8 | CH |
| 15  | 6.49 | m         | 124.8 | CH |
| 16  | 6.08 | dd (12.0, 10.0) | 129.4 | CH |
| 17  | 6.70 | dd (15.0, 12.0) | 128.1 | CH |
| 18  | 6.28 | dd (15.0, 12.0) | 135.2 | CH |
| 19  | 6.38 | ddd (15.0, 12.0, 12.0) | 137.5 | CH |
| 20a | 5.26 | d (15.0) | 118.1 | CH3 |
| 20b | 5.13 | d (12.0) |     |     |
| O-Me | 3.68 | s         | 51.7 | CH3 |
$^1$H NMR of methyl ester (2) in CDCl$_3$
gCOSY NMR of methyl ester (2) in CDCl₃
gHMOC NMR of methyl ester (2) in CDCl$_3$
gHMBC NMR of methyl ester (2) in CDCl₃
Reduction product (3) of methyl ester (2)

A crude ethyl acetate extract of a 200 mL SPB74 culture was dried in vacuo. The crude material (~ 5 mg) was methylated by TMSdiazomethane as shown above. Without purification, the mixture was dissolved in 2 mL of anhydrous dichloromethane. 30 mg of metallic zinc and 50 µL of acetic acid were added sequentially. The reaction mixture was stirred at room temperature for 1 hour. The completion of the reaction was confirmed with LC/MS analysis in an hour. The reaction mixture was dried with celite for purification through a 1g C_{18} Sepak column. A step-gradient of water-methanol combination (20%, 40%, 60%, 80%, and 100% aqueous MeOH) was applied for separation. The reduction product (3) was purified in 80% MeOH fraction. The molecular formula of 3 was confirmed as C_{21}H_{28}O_{4} by ESI-HRMS analysis (obsd [M+Na]^+ m/z 367.1882, calc m/z 365.1885).

Table S2. NMR spectral data for reduction product (3) of 2 in CDCl₃ (a600 MHz, b coupling constant in Hz, c150 MHz).

| C/H | δ_Ha | mult (Jb) | δ_Cc |
|-----|------|-----------|------|
| 1   |      |           | 173.1 C |
| 2a  | 2.44 | m         | 35.8 CH₂ |
| 2b  | 2.36 | m         |       |
| 3   | 4.26 | m         | 69.0 CH |
| 4a  | 2.48 | m         | 41.5 CH₂ |
| 4b  | 1.62 | m         |       |
| 5   | 3.96 | m         | 71.8 CH |
| 6   | 2.33 | m         | 33.9 CH₂ |
| 7   | 5.59 | m         | 128.8 CH |
| 8   | 6.63 | dd (11.0, 11.0) | 126.4 CH |
| 9   | 6.27 | m         | 125.0 CH |
| 10  | 6.14 | m         | 130.5 CH |
| 11  | 6.74 | m         | 129.4 CH |
| 12  | 6.74 | m         | 129.4 CH |
| 13  | 6.14 | m         | 130.5 CH |
| 14  | 6.48 | m         | 125.0 CH |
| 15  | 6.48 | m         | 125.0 CH |
| 16  | 6.07 | m         | 130.5 CH |
| 17  | 6.70 | dd (14.5, 12.0) | 128.3 CH |
| 18  | 6.29 | m         | 135.3 CH |
| 19  | 6.42 | dddd(16.5, 11.0, 11.0) | 137.5 CH |
| 20a | 5.27 | d (16.5) | 118.6 CH₂ |
| 20b | 5.13 | d (11.0) |       |
| O-Me | 3.68 | s         | 51.9 CH₂ |
| OH  | 3.73 | br. s     |       |
| OH  | 3.29 | br. s     |       |
$^1$H NMR of reduction product (3) in CDCl$_3$
gCOSY NMR of reduction product (3) in CDCl₃
gHMQC NMR of reduction product (3) in CDCl₃
gHMBC NMR of reduction product (3) in CDCl₃
**Bis-MTPA esters (4a and 4b) of 3.**

In order to derivatize the 1,3-diol functional group of 3 to bis-<em>S</em>- and <em>R</em>-MTPA esters, duplicate dry samples (each 1 mg) were prepared. The samples were dissolved in 2 mL of freshly distilled dry pyridine and stirred for 20 min. A few crystals of dry DMAP were added and stirred again for 20 min. 30 µL of <em>R</em>- and <em>S</em>-MTPA chloride (5.36 µmol/µL) were added respectively to the separate reaction vials and the reaction mixtures were stirred at room temperature for 2 hours. The reactions were monitored by LC/MS. After 2 hours, MTPA-chloride was quenched by adding 100 µL of methanol. The acylation products (4a and 4b) were purified by reversed-phase HPLC (Agilent HPLC 1100 series, Alltech C<sub>18</sub> semi-preparative column, 10 mm × 250 mm, 2 mL/min, gradient 0-5 min: 20% aqueous CH<sub>3</sub>CN / 5-45 min 20 - 100% aqueous CH<sub>3</sub>CN / 45-60 min: 100% CH<sub>3</sub>CN). Bis-<em>S</em>-MTPA ester (4a) and bis-<em>R</em>-MTPA ester (4b) were eluted at 53.4 min. The molecular formulas for 4a and 4b were confirmed as C<sub>41</sub>H<sub>42</sub>F<sub>6</sub>O<sub>8</sub> by ESI-LC/MS analysis ([M+H]<sup>+</sup> m/z at 777). The <sup>1</sup>H chemical shifts around the stereogenic centers of 4a and 4b were assigned by <sup>1</sup>H, gCOSY, and TOCSY NMR spectral analysis.

**Bis-<em>S</em>-MTPA ester (4a):**<sup>a</sup> <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 1.92 (1H, m), 2.07 (1H, m), 2.44 (1H, m), 2.52 (1H, m), 2.65 (2H, m), 3.50 (6H, s), 3.62 (3H, s), 5.03 (1H, m), 5.14 (1H, d, <em>J</em> = 10.0 Hz), 5.24 (1H, m), 5.26 (1H, d, <em>J</em> = 17.5 Hz), 5.48 (1H, m), 6.05 – 6.16 (3H, m), 6.20 – 6.34 (3H, m), 6.42 (1H, m), 6.50 (2H, m), 6.60 – 6.78 (3H, m), 7.34 – 7.51 (10H, m).

**Bis-<em>R</em>-MTPA ester (4b):**<sup>a</sup> <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 1.96 (1H, m), 2.09 (1H, m), 2.51 (2H, m), 2.60 (1H, m), 2.68 (1H, m), 3.53 (3H, s), 3.64 (3H, s), 3.66 (3H, s), 5.14 (1H, d, <em>J</em> = 11.0), 5.15 (1H, m), 5.26 (1H, d, <em>J</em> = 17.0 Hz), 5.40 (1H, m), 5.42 (1H, m), 6.01 – 6.17 (3H, m), 6.18 – 6.40 (3H, m), 6.40 – 6.57 (3H, m), 6.61 (1H, dd, <em>J</em> = 11.5, 11.5 Hz), 6.66 – 6.78 (2H, m), 7.33 – 7.54 (10H, m).

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<sup>a</sup>Figure S1. <sup>1</sup>H NMR chemical shifts of (a) 4a and (b) 4b.
$^1$H NMR spectrum of bis-S-MTPA ester (4) of 3
$^1$H NMR spectrum of bis-$R$-MTPA ester (4) of 3