Supporting Information

Structure Determination of Mycolactone C via Total Synthesis

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General Procedures and Methods

Except as otherwise indicated, reactions were carried out under an nitrogen atmosphere in flame- or oven-dried glassware. Reagents and solvents are commercial grade and were used as supplied, with the following exceptions: THF was distilled from sodium/benzophenone; toluene, diethyl ether and dichloromethane were purified by passing through two alumina (A-2) columns using the J. C. Myer (Glass Contour) system; benzene was distilled from calcium hydride and stored over 3Å MS; and triethylamine was distilled from calcium hydride and stored over potassium hydroxide. Reactions were monitored by thin layer chromatography (TLC) with 0.25-mm E. Merck pre-coated silica gel plates. Silica gel 60 for flash chromatography (particle size 0.040-0.063 mm) was supplied by E. Merck. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise noted. ^1H and ^{13}C NMR spectra were recorded on a Varian Inova 600 MHz, Varian Inova 500 MHz or Varian Mercury 400 MHz spectrometer. Chemical shifts are reported as δ values (ppm) relative to internal chloroform (δ 7.26), benzene (δ 7.15), methanol (δ 3.34), or acetone (δ 2.04) for ^1H spectra and either chloroform (δ 77.0), benzene (δ 128.0), methanol (δ 49.0), or acetone (δ 29.8) for ^{13}C spectra. Optical rotations were measured on a Perkin-Elmer model 241 polarimeter. Low- and high-resolution mass spectra were measured at the Harvard University Chemistry and Chemical Biology Mass Spectrometry Facility.

To a solution of (+)-Ipc₂BOMe (516 mg, 1.63 mmol) in Et₂O (24 mL) at -78 °C was added allyl magnesium bromide (1.63 mL, 1.63 mmol, 1M in Et₂O) by dropwise addition. After stirring 10 min, the resulting solution was allowed to come to ambient temperature for 1h and recooled to -78 °C. The known aldehyde **4** (300 mg, 1.48 mmol; Paterson, I.; Craw, P. A. *Tetrahedron Lett.* **1989**, 30, 5799) in Et₂O was added in dropwise fashion and the reaction was allowed to stir for 1h at -78 °C. After warming to 0 °C, the reaction was quenched by careful addition of aqueous 3N NaOH (1.1 mL) and

30% H₂O₂ (0.44 mL) and stirred 12 hrs at ambient temperature and diluted with H₂O. The layers were separated, and the aqueous layer was extracted 3 x Et₂O. The combined organic layers were washed 1 x H₂O, 1 x brine, and dried over MgSO₄. Concentration *in vacuo* and purification by flash chromatography (9:1 Hex/EtOAc) afforded the corresponding allylic alcohol (243 mg, 67% yield) as a single diastereomer. (Note- in the case of the *anti*-1,3 diols used to prepare **11c** and **11d**, the diastereoselectivity was ca. 8:1 in favor of the desired product. The minor diastereomer was separated following DiBAL-H reduction at a later stage *vide supra*).

A solution of the allylic alcohol described above, TBSCl, and imidazole in DMF (800 μ L) was stirred for 2.5h at ambient temperature. The reaction was quenched by dilution with Et₂O and H₂O. The layers were separated, and the aqueous layer was extracted 2 X Et₂O. The combined organic layers were washed 2 x H₂O, 1 x brine, and dried over MgSO₄. Concentration *in vacuo* and purification by flash chromatography (20:1 Hex/EtOAc) afforded the corresponding bis TBS-protected diol as a colorless oil (346 mg, 97% yield). $[\alpha]^{23}_D = -2.4$ (c 1.4, MeOH) ¹H NMR (400 MHz, CDCl₃) δ 5.85 (m, 1H), 5.05 (m, 1H), 5.01 (m, 1H), 3.90 (m, 1H), 3.79 (m, 1H), 2.26 (m, 1H), 2.16 (m, 1H), 1.67 (ddd, $J = 13.6, 6.4, 6.4$ Hz, 1H), 1.51 (ddd, $J = 13.6, 6.4, 6.4$ Hz, 1H), 1.13 (d, $J = 6.4$ Hz, 3H), 0.88 (s, 18H), 0.05 (s, 6H), 0.04 (s, 6H); ¹³C NMR (CDCl₃, 100 MHz) δ 135.1, 116.8, 69.3, 65.8, 47.1, 41.8, 25.9 (x3), 25.8 (x3), 23.9, -4.3, -4.3, -4.6, -4.8; HRMS (ES+) 359.2804, calcd 359.2801.

Anti-1,3 bis TBS-protected diol for the preparation of **11c** and **11d**:

¹H NMR (400 MHz, CDCl₃) δ 5.85 (m, 1H), 5.05 (d, $J = 3.6$ Hz, 1H), 5.02 (s, 1H), 3.89 (m, 1H), 3.82 (m, 1H), 2.22 (m, 2H), 1.64 (ddd, $J = 7.2, 14.0, 5.2$ Hz, 1H), 1.47 (ddd, $J = 6.8, 14.0, 5.2$ Hz, 1H), 1.15 (d, $J = 6.0$ Hz, 3H), 0.88 (s, 18H), 0.06 (s, 12H).

Ozone was passed through a solution of bis TBS-protected diol (346 mg, 0.965 mmol) described above in CH₂Cl₂ (50 mL) at -78 °C until a blue solution was observed. Oxygen followed by nitrogen gas was bubbled through the solution followed by addition of triphenylphosphine (313 mg, 1.29 mmol). After stirring at room temperature for 3h, the solution was concentrated *in vacuo* and purified by flash chromatography (20:1 Hex/EtOAc) to furnish the corresponding aldehyde (337 mg, 97% yield). ¹H NMR (400 MHz, CDCl₃) δ 9.79 (dd, $J = 2.0, 3.2$ Hz, 1H), 4.32 (m, 1H), 3.86 (m, 1H), 2.56 (ddd, $J =$

4.4, 15.2, 1.6 Hz, 1H), 2.49 (ddd, J = 7.2, 16.0, 3.6 Hz, 1H), 1.78 (ddd, J = 13.6, 8.0, 5.6 Hz, 1H), 1.56 (ddd, J = 13.6, 8.0, 4.8 Hz, 1H), 1.15 (d, J = 6.0, 3H), 0.87 (s, 9H), 0.86 (s, 9H), 0.07 (s, 3H), 0.04 (s, 6H), 0.03 (s, 3H).

To a solution of the above aldehyde (337 mg, 0.934 mmol) in toluene (10 mL) was added carbethoxyethylidene triphenylphosphorane (792 mg, 2.19 mmol). The reaction was heated to 110 °C for 12h, cooled to room temperature, and then concentrated *in vacuo*. Flash chromatography (20:1 Hex/ Et_2O) gave ester **5** (357 mg, 86% yield) as a colorless oil. $[\alpha]_D^{23} = +9.4$ (c 1.0, MeOH) ^1H NMR (400 MHz, CDCl_3) δ 6.83 (ddd, J = 1.2, 7.6, 76 Hz 1 H), 4.20 (q, J = 7.2 Hz, 2H), 3.89 (m, 2H), 2.35 (m, 1H), 2.30 (m, 1H), 1.82 (s, 3H), 1.70 (m, 1H), 1.49 (m, 1H), 1.27 (t, J = 7.2 Hz, 3H), 1.13 (d, J = 5.6 Hz, 3H), 0.87 (s, 18 H), 0.04 (s, 6H), 0.03 (s, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ 168.0, 138.7, 128.9, 68.8, 65.7, 60.3, 47.7, 36.5, 25.8 (x 3), 25.8 (x 3), 23.9, 18.0, 17.9, 14.2, 12.6, -4.3, -4.6, -4.6, -4.8; HRMS (ES+) 445.3163, calcd 445.3169.

To a solution of ester **5** (357 mg, 0.803 mmol) in CH_2Cl_2 (10 mL) at -78 °C was slowly added DiBAL-H (2.0 ml, 2.0 mmol, 1M in hexanes). The resulting solution was stirred at -78 °C for 1.5h and quenched with saturated aqueous potassium sodium tartrate (25 mL). The mixture was stirred vigorously for 6h while warming to ambient temperature and diluted with EtOAc. The layers were separated, and the aqueous layer was extracted 2 X EtOAc. The combined organic layers were washed 1 x H_2O , 1 x brine, and dried over MgSO_4 . Concentration *in vacuo* and purification by flash chromatography (10:1 Hex/EtOAc) afforded the corresponding alcohol. $[\alpha]_D^{23} = -8.6$ (c 1.2, MeOH) ^1H NMR (400 MHz, CDCl_3) δ 5.43 (ddd, J = 1.2, 7.2, 7.2 Hz 1 H), 3.99 (s, 2H), 3.90 (m, 1H), 3.77 (m, 1H), 2.19 (m, 2H), 1.65 (s, 3H), 1.63 (m, 1H), 1.49 (m, 1H), 1.12 (d, J = 6.4 Hz, 3H), 0.86 (s, 18 H), 0.03 (s, 3H), 0.03 (s, 3 H), 0.02 (s, 6 H); ^{13}C NMR (CDCl_3 , 100

MHz,) δ 136.2, 122.4, 69.5, 69.0, 65.9, 47.2, 35.5, 25.9 (x 3), 25.8 (x3), 23.8, 18.1, 18.0, 13.9, -4.3 (x 2), -4.6, -4.8. HRMS (ES+) 403.3062 (M+H), calcd. 403.3064.

To a stirred solution of the above alcohol (45 mg, 112 μ mol) in CH_2Cl_2 (1.2 ml) was added Dess-Martin periodane (66 mg, 156 μ mol) in one portion. The resulting solution was stirred for 1h and quenched by addition of saturated aqueous NaHCO_3 and $\text{Na}_2\text{S}_2\text{O}_4$. Following dilution with Et_2O , the mixture was stirred vigorously for 20 min. The layers were separated, and the aqueous layer was extracted 2 x Et_2O . The combined organic layers were washed 1 x 50% saturated aqueous NaHCO_3 , 1 x H_2O , 1 x brine, and dried over MgSO_4 . Concentration *in vacuo* and purification by flash chromatography (20:1 Hex/ Et_2O) afforded aldehyde **6** (43 mg, 96% yield) as a colorless oil. ^1H NMR (400 MHz, CDCl_3) δ 9.41 (s, 1H), 6.59 (t, J = 7.6 Hz, 1H), 3.99 (m, 1H), 3.88 (m, 1H), 2.52 (m, 2H), 1.74 (s, 3H), 1.72 (m, 1H), 1.50 (ddd, J = 12.8, 7.2, 5.2 Hz, 1H), 1.15 (d, J = 6.4 Hz, 3H), 0.88 (s, 9H), 0.87 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H), 0.04 (s, 6H).

Each diastereomer of the polyunsaturated carboxylic acid was prepared analogous to our previously reported conditions, see: Song, F.; Fidanze, S.; Benowitz; Kishi, Y. *Org. Lett.* **2002**, 4, 647.

To a stirred solution of aldehyde **6** (32 mg, 79.0 μ mol) and phosphonate **7** (50 mg, 158 μ mol; Gurjar, M. K.; Cherian, J; *Heterocycles* **2001**, 55, 1095) in THF (0.6 mL) at -78°C was added dropwise freshly prepared LDA (0.3 mL, 150 μ mol, 0.5M in THF). The resulting dark red solution was stirred at -78°C for 10 min, then allowed to warm to 0°C over 1h. After stirring for an additional 1h at ambient temperature, the reaction was quenched by addition of saturated aqueous NH_4Cl followed by dilution with EtOAc . The layers were separated, and the aqueous layer was extracted 3 x EtOAc . The combined organic layers were washed 1 x H_2O , 1 x brine, and dried over Na_2SO_4 . Concentration *in*

vacuo and purification by flash chromatography (20:1 Hex/EtOAc) furnished the corresponding ester (50 mg, 96%) as a yellow oil. This ester exists as a 1:1 mixture of 4'Z:4'E isomers.

To a stirred solution of the above ester (25 mg, 44.4 μ mol) in 4:1:1 THF/MeOH/H₂O (1.4 mL) was added LiOH (430 μ l, 0.43 mmol, 1.0M in H₂O). The resulting solution was protected from light and stirred for 18h at ambient temperature. The reaction was quenched by addition of saturated aqueous NH₄Cl followed by dilution with EtOAc. The layers were separated, and the aqueous layer was extracted 3 x EtOAc. The combined organic layers were washed 1 x H₂O, 1 x brine, and dried over Na₂SO₄. Concentration *in vacuo* and purification by flash chromatography (4:1 Hex/EtOAc) furnished the acid **8** (23 mg, 96%) as a yellow oil. This acid exists as a ca. 1:1 mixture of 4'Z:4'E isomers. $[\alpha]_D^{23} = +12.3$ (c 0.76, MeOH); ¹H NMR (400 MHz, CD₃COCD₃, 4'Z isomer) δ 7.95 (d, *J* = 16.0 Hz, 1H), 6.54 (dd, *J* = 14.8, 10.8 Hz, 1H), 6.44 (s, 1H), 6.37 (d, *J* = 10.4 Hz, 1H), 6.17 (d, *J* = 11.2 Hz, 1H), 5.89 (d, *J* = 15.6, 1H), 5.71 (dd, *J* = 8.0, 16.0 Hz, 1H), 3.93 (m, 2H), 2.37 (m, 2H), 2.01 (s, 3H), 1.96 (s, 3H), 1.84 (s, 3H), 1.71 (m, 1H), 1.51 (m, 1H), 1.14 (d, *J* = 6.0 Hz, 3H), 0.89 (s, 9H), 0.08 (s, 6H). ¹H NMR (400 MHz, CD₃COCD₃, 4'E isomer) δ 7.36 (d, *J* = 15.6 Hz, 1H), 6.56 (dd, *J* = 14.8, 10.8 Hz, 1H), 6.48 (d, *J* = 14.8, 1H), 6.44 (s, 1H), 6.33 (d, *J* = 8.0 Hz, 1H), 5.87 (d, *J* = 16.0 Hz, 1H), 5.71 (dd, *J* = 8.0, 16.0 Hz, 1H), 3.98 (m, 2H), 2.07 (s, 3H), 2.05 (s, 3H), 1.84 (s, 3H), 1.68 (m, 1H), 1.54 (m, 1H), 1.15 (d, *J* = 5.6 Hz, 3H), 0.89 (s, 9H), 0.08 (s, 6H). ¹³C NMR (100 MHz, CD₃COCD₃, 4'Z and 4'E isomers); MS (ES+) 549.3800 (M+H), calcd. 549.3795.

Anti-1,3 bis TBS-protected diol carboxylic acid for the preparation of **11c** and **11d**:) ¹H NMR (400 MHz, CD₃COCD₃, 4'Z isomer) δ 7.95 (d, *J* = 15.6 Hz, 1H), 6.54 (dd, *J* = 14.8, 10.8 Hz, 1H), 6.44 (d, *J* = 15.2 Hz, 1H), 6.39 (d, *J* = 11.6 Hz, 1H), 6.19 (d, *J* = 10.4 Hz, 1H), 5.94 (d, *J* = 16.0, 1H), 5.70 (dd, *J* = 8.0, 16.0 Hz, 1H), 3.99 (m, 2H), 2.39 (m, 2H), 2.01 (s, 3H), 1.96 (s, 3H), 1.84 (s, 3H), 1.62 (m, 1H), 1.52 (m, 1H), 1.14 (d, *J* = 6.0 Hz, 3H), 0.89 (s, 9H), 0.08 (s, 6H). ¹H NMR (400 MHz, CD₃COCD₃, 4'E isomer) δ 7.36 (d, *J* = 15.6 Hz, 1H), 6.56 (dd, *J* = 14.8, 10.8 Hz, 1H), 6.48 (d, *J* = 14.4 Hz, 1H), 6.46 (s, 1H), 6.34 (s, 1H), 5.88 (d, *J* = 15.6 Hz, 1H), 5.70 (dd, *J* = 8.0, 16.0 Hz, 1H), 3.98

(m, 2H), 2.40 (m, 2H), 2.07 (s, 3H), 2.05 (s, 3H), 1.84 (s, 3H), 1.62 (m, 1H), 1.52 (m, 1H), 1.15 (d, $J = 6.0$ Hz, 3H), 0.89 (s, 9H), 0.08 (s, 6H).

To a stirred solution of acid **8** (19 mg, 34.6 μmol) in benzene (1.4 mL) was added $i\text{-Pr}_2\text{NEt}$ (16 μL , 92.4 μmol) $\text{Cl}_3\text{C}_6\text{H}_2\text{COCl}$ (6.8 μL , 43.8 μmol) and DMAP (11.3 mg, 92.4 μmol). After 20 min, alcohol **9** (15 mg, 23.1 μmol) was added as a solution in benzene (1.6 mL). The reaction was protected from light and stirred 24 h. The reaction was quenched by addition of saturated aqueous NaHCO_3 and diluted with EtOAc. The layers were separated, and the aqueous layer was extracted 3 x EtOAc. The combined organic layers were washed 1 x H_2O , 1 x brine, and dried over Na_2SO_4 . Concentration *in vacuo* and purification by flash chromatography (20:1 Hex/EtOAc) gave the corresponding ester (25 mg, 91% yield) as a yellow oil. This ester exists as a ca. 1:1 mixture of 4'Z:4'E isomers.

To a stirred solution of the above ester **10** (25 mg, 21.1 μmol) in THF (2.3 mL) was added TBAF (190 μL , 190 μmol , 1M in THF). The reaction was protected from light and stirred 18 h. The reaction was quenched by addition of saturated aqueous NH_4Cl and diluted with EtOAc. The layers were separated, and the aqueous layer was extracted 3 x EtOAc. The combined organic layers were washed 1 x H_2O , 1 x brine, and dried over

Na₂SO₄. Concentration *in vacuo* and purification by flash chromatography (100:1 EtOAc/MeOH) gave the corresponding synthetic mycolactone **11** as a yellow oil (12.8 mg, 84% yield). The synthetic mycolactones exist as a ca. 1:1 mixture of 4'Z:4'E isomers. $[\alpha]_D^{23} = -15.5$ (c 0.48, MeOH) ¹H NMR (500 MHz, CD₃COCD₃, 4'Z isomer) δ 7.93 (d, *J*= 16.0 Hz, 1H), 6.51 (dd, *J*= 11.0, 15.5 Hz, 1H), 6.45 (d, *J*= 15.5 Hz, 1H), 6.32 (s, 1H), 6.15 (d, *J*=11.0 Hz, 1H), 5.93 (d, *J*= 16.0 Hz, 1H), 5.72 (dd, *J*= 7.5, 15.5 Hz, 1H), 5.12 (m, 1H), 5.04 (d, *J*= 10.0 Hz, 1H), 4.88 (m, 1H), 4.70 (m, 1H), 4.23 (dd, *J*= 3.5, 3.5 Hz, 1H), 4.19 (d, *J*= 3.5 Hz, 1H), 3.50 (m, 1H), 2.49 (m, 1H), 2.38 (m, 2H), 2.34 (m, 2H), 2.07-1.85 (m, 8H), 2.01 (s, 3H), 1.99 (s, 3H), 1.84 (s, 3H), 1.69 (s, 3H), 1.64 (s, 3H), 1.83 (m, 1H), 1.70 (m, 1H), 1.66 (m, 1H), 1.55-1.60 (m, 4H), 1.48 (m, 1H), 1.38 (m, 1H), 1.13 (d, *J*= 5.5 Hz, 3H), 1.11 (d, *J*= 6.0 Hz, 3H), 0.97 (d, *J*= 7.0 Hz, 3H), 0.90 (d, *J*= 6.5 Hz, 1H), 0.88 (d, *J*= 7.0 Hz, 3H).

¹H NMR (500 MHz, CD₃COCD₃, 4'E isomer) δ 7.37 (d, *J*= 15.5 Hz, 1H), 6.52 (dd, *J*= 10.5, 15.0 Hz), 6.45 (s, 1H), 6.42 (d, *J*= 15.5 Hz, 1H), 6.34 (d, *J*= 12.0 Hz, 1H), 5.88 (d, *J*= 16.0 Hz, 1H), 5.72 (dd, *J*= 7.5, 15.5 Hz, 1H), 5.12 (m, 1H), 5.04 (d, *J*= 10.0 Hz, 1H), 4.88 (m, 1H), 4.70 (m, 1H), 4.22 (d, *J*= 3.5 Hz, 1H), 4.09 (dd, *J*= 3.0, 5.5 Hz, 1H), 3.50 (m, 1H), 2.49 (m, 1H), 2.38 (m, 2H), 2.34 (m, 2H), 2.07-1.85 (m, 8H), 2.07 (s, 3H), 1.96 (s, 3H), 1.83 (s, 3H), 1.71 (s, 3H), 1.64 (s, 3H), 1.83 (m, 1H), 1.70 (m, 1H), 1.66 (m, 1H), 1.55-1.60 (m, 4H), 1.48 (m, 1H), 1.38 (m, 1H), 1.13 (d, *J*= 5.5 Hz, 3H), 1.11 (d, *J*= 6.0 Hz, 3H), 0.97 (d, *J*= 7.0 Hz, 3H), 0.90 (d, *J*= 6.5 Hz, 1H), 0.87 (d, *J*= 6.5 Hz, 3H). ¹³C NMR (100 MHz, CD₃COCD₃, 4'E and 4'Z isomer) δ 173.2 (x 2), 166.9 (x 2), 151.2, 144.5, 143.2, 141.9, 141.0, 140.6, 137.3 (x 2), 136.6, 136.4 (x 2), 135.3, 134.4, 133.9, 133.4 (x 2), 132.1, 131.9, 131.8, 131.2 (x 3), 123.8 (x 4), 123.4 (x 2), 119.4, 117.1, 79.2 (x 3), 76.9 (x 2), 76.2, 76.2, 72.2 (x 3), 69.0 (x 2), 68.2 (x 2), 46.4 (x 2), 45.8 (x 2), 44.3 (x 2), 43.8, 40.5 (x 2), 38.0, 36.0 (x 2), 35.9, 35.3 (x 2), 32.8, 32.7, 31.5, 31.3, 24.6 (x 2), 24.4 (x 2), 20.9, 20.7, 20.6 (x 2), 20.5, 17.5, 17.1 (x 2), 17.0, 16.2, 15.9, 14.9 (x 2), 14.3 (x 2), 12.7 (x 2). HRMS (ES⁺) 727.5143 (M+H), calcd 727.5149. UV (MeOH) λ_{max} 365 nm (log ϵ 4.69).

¹H NMR spectrum for each of the four diastereomers 11a, 11b, 11c, and 11d, are included.

11a (¹H NMR, 500 MHz, acetone-*d*₆)

11a (¹H NMR, 500 MHz, acetone-*d*₆)

11a (¹H NMR, 500 MHz, acetone-*d*₆)

11a (¹H NMR, 500 MHz, acetone-*d*₆)

11b (^1H NMR, 500 MHz, acetone- d_6)

11b (¹H NMR, 500 MHz, acetone-*d*₆)

11b (¹H NMR, 500 MHz, acetone-*d*₆)

11b (¹H NMR, 500 MHz, acetone-*d*₆)

11c (¹H NMR, 500 MHz, acetone-*d*₆)

11c (¹H NMR, 500 MHz, acetone-*d*₆)

11c (¹H NMR, 500 MHz, acetone-*d*₆)

11c (¹H NMR, 500 MHz, acetone-*d*₆)

11d (¹H NMR, 500 MHz, acetone-*d*₆)

11d (¹H NMR, 500 MHz, acetone-*d*₆)

11d (¹H NMR, 500 MHz, acetone-*d*₆)

11d (¹H NMR, 500 MHz, acetone-*d*₆)

11b (¹H NMR, 500 MHz, (*R*)-DMBA-*d*₁₃)

11b (¹H NMR, 500 MHz, (*R*)-DMBA-*d*₃)

11a (¹H NMR, 500 MHz, (*R*)-DMBA-*d*₁₃)

11a (H NMR, 500 MHz, (*R*)-DMBA-*d*₁₃)

Partially Purified Natural Mycolactone C
(¹H NMR, 600 MHz, acetone-*d*₆)

11a. Synthetic Mycolactone C
(¹H NMR, 600 MHz, acetone-*d*₆)