Supplementary Information for

A MACROCYCLE DIRECTED TOTAL SYNTHESIS OF DI-O-METHYLENDIANDRIN A

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General experimental conditions

All reactions were run in flame or oven-dried (120 °C) glassware and cooled under a positive pressure of ultra high pure nitrogen or argon gas. All chemicals were used as received from commercial sources, unless otherwise stated. Anhydrous reaction solvents were purified and dried by passing HPLC grade solvents through activated columns of alumina (Glass Contour SDS). All solvents used for chromatographic separations were HPLC grade (hexanes, ethyl acetate, dichloromethane, chloroform, methanol, and acetone). Chromatographic separations were performed using flash chromatography, as originally reported by Still and co-workers, on silica gel 60 (particle size 43-60 µm), and all chromatography conditions have been reported as height × diameter in centimeters. Reaction progress was monitored by thin layer chromatography (TLC), on glass-backed silica gel plates (pH = 7.0). TLC plates were visualized using a handheld UV lamp (254 nm) and stained using either an aqueous ceric ammonium molybdate (CAM) stain, an aqueous vanillin stain, or an iodine chamber. Plates stained with CAM or vanillin were dipped, wiped clean, and heated from the back of the plate. ¹H and ¹³C nuclear magnetic resonance (NMR) spectra were recorded at 400, 500, or 600 MHz, calibrated using residual undeuterated solvent as an internal reference (CHCl₃, δ 7.27 and 77.2 ppm), reported in parts per million relative to trimethylsilane (TMS, δ 0.00 ppm), and presented as follows: chemical shift (δ , ppm), multiplicity (s = singlet, br s = broad singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublets, t = triplet, m = multiplet), coupling constants (J, Hz). High-resolution mass spectrometric (HRMS) data were obtained using a quadrupole time-of-flight (Q-TOF) spectrometer and electrospray ionization (ESI).



Figure SI-1: Compounds reported in experimental procedures, but not appearing in the manuscript

New compounds: procedures and characterization data (listed in chronological order)



1,4-diketone 10: 1,6-Dibromohexane (1.1 mL, 7.2 mmol) was added to a stirred solution of isovanillin (2.06 g, 13.6 mmol), K_2CO_3 (4.16 g, 30.1 mmol), and TBAI (0.076 g, 0.21 mmol) in DMF (27 mL) at room temperature. The reaction was heated at 80 °C for 20 h, cooled to room temperature, followed by the addition of water (30 mL) and 1 M HCI (30 mL). The resulting mixture was extracted with ethyl acetate (3 × 30 mL). The organic extracts were combined and washed with 1 M HCI (2 × 30 mL) and brine (30 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The yellow oil was

dissolved in dichloromethane (100 mL) and vinylmagnesium bromide (0.7 M in THF, 42 mL, 29 mmol) was added. After 1 h, the reaction was poured into water (100 mL) and further diluted with 1 M HCl (100 mL). The resulting mixture was extracted with dichloromethane (3 × 100 mL). The combined organic extracts were washed with a saturated solution NaHCO₃ (100 mL) and brine (100 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (18 × 5.0 cm, 50% to 70% EtOAc/hexanes) to afford allylic diol **18** as a colorless oil. Hoveyda-Grubbs second-generation catalyst (0.0520 g, 0.0830 mmol)

was added, to a stirred solution of 18 (1.50 g, 3.40 mmol) in dichloromethane (345 mL) and heated at 40 °C. 2 h, the reaction mixture was concentrated under reduced pressure. The dark brown residue was After dissolved in 1:7 methanol/dichloromethane (36.5 mL), and sodium borohydride (0.525 g, 13.9 mmol) was added. After 3 h, the reaction poured into ice cold water (35 mL) and further diluted with 1 M HCl (35 mL). The layers were separated and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The combined organic extracts were washed with water (40 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (15 × 3.5 cm, 80% EtOAc/hexanes) to afford macrocyclic 1,4diol 19 as a pale yellow oil, which was dissolved in dichloromethane (15 mL), followed by the sequential addition of NaHCO₃ (0.556 g, 6.61 mmol) and Dess-Martin periodinane (1.70 g, 4.00 mmol). After 3 h, the reaction was poured into water (20 mL). The layers were separated and the aqueous phase was extracted with dichloromethane (3 × 15 mL). The combined organic extracts were washed with water (20 mL), dried over MgSO₄, filtered and concentrated under reduced pressure to afford 1,4-diketone **10** as a colorless solid (0.405 g, 15% from isovanillin): $R_f = 0.41$ (60% EtOAc/hexanes); ¹H NMR (600 MHz, CDCl₃) δ 7.57 (dd, J = 8.5, 2.3 Hz, 2H), 7.44 (d, J = 2.5 Hz, 2H), 6.89 (d, J = 8.5 Hz, 2H), 4.20 (t, J = 7.0 Hz, 4H), 3.91 (s, 6H), 3.32 (s, 4H), 1.72 (q, J = 6.8 Hz, 4H), 1.49 (d, J = 6.6 Hz, 4H); ¹³C NMR (151 MHz, CDCl₃) δ 198.83, 154.33, 147.38, 129.57, 123.00, 114.36, 111.29, 68.37, 56.21, 34.57, 28.07, 24.45; HRMS (ESI) calculated for $C_{24}H_{38}O_6$ ([M+Na]⁺) m/z =calculated 435.1784, found 435.1790.



(*rac*)-2,3-Dimethyl-1,4-Dione 14: Diketone 10 (0.100 g, 0.242 mmol) and NaH (0.034 g, 1.5 mmol) were dissolved in THF (6 mL). After stirring for 1 h at 0 °C, Mel (16 μ L, 1.4 mmol) was added and the reaction stirred for an additional 6 h at 0 °C. A saturated solution of NH₄Cl (10 mL) was slowly added, and the reaction mixture was poured into water. The resulting mixture was extracted with ethyl acetate (4 × 15 mL). The organic

extracts were combined and washed with water (2 \times 10 mL) and brine (2 \times 10 mL), dried over MgSO₄, filtered,

and concentrated under reduced pressure. The residue was purified via flash chromatography (20% EtOAc/hexanes) to afford (*rac*)-2,3-dimethyl-1,4-dione **14** as a white solid. (0.083 g, 78%): $R_f = 0.25$ (20% EtOAc/hexanes); ¹H NMR (600 MHz, CDCl₃) δ 7.50 (dd, J = 8.4, 1.8 Hz, 2H), 7.27 (d, J = 1.8 Hz, 2H), 6.85 (d, J = 8.4 Hz, 2H), 4.15 (t, J = 6.2 Hz, 4H), 3.90 (s, 6H), 3.82–3.74 (m, 2H), 1.68–1.56 (m, 4H), 1.52–1.44 (m, 2H), 1.44–1.37 (m, 2H), 1.27 (d, J = 6.0 Hz, 6H); ¹³C NMR (151 MHz, CDCl₃) δ 203.54, 153.92, 146.68, 129.85, 122.90, 113.10, 110.35, 67.97, 55.88, 43.88, 26.90, 23.61, 15.00; HRMS (ESI) calculated for C₂₆H₃₄O₆ ([M+H⁺) m/z = 441.2277, found 441.2284.



Cyclobutene 9: Titanium(IV) chloride (0.22 mL, 2.0 mmol) was added to a slurry of zinc powder (0.272 g, 4.14 mmol) in THF (15 mL) at 0 °C. After dissipation of the resulting yellow gas, the solution was heated to 68 °C. After 50 min., pyridine (0.41 mL, 5.0 mmol) was added, and 10 min. later (*rac*)-2,3-dimethyl-1,4-dione **14** (0.159 g, 0.361 mmol) as a solution in THF (25 mL) was added. The reaction was heated at 68 °C for 3 h, and then poured into chloroform (25 mL), filtered through Celite, and concentrated under reduced

pressure. The residue was purified via flash chromatography (30% EtOAc/hexanes) to afford **10** as a white solid (0.135 g, 92%): $R_f = 0.30$ (25% EtOAc/hexanes); ¹H NMR (600 MHz, CDCl₃) δ 7.36 (d, J = 1.7 Hz, 2H), 6.88 (d, J = 8.3 Hz, 2H), 6.80 (dd, J = 8.2, 1.7 Hz, 2H), 4.20 (dt, J = 11.8, 6.1 Hz, 2H), 4.01 (dt, J = 12.0, 6.1 Hz, 2H), 3.88 (s, 6H), 2.60 (q, J = 6.6 Hz, 2H), 1.78–1.68 (m, 4H), 1.60–1.49 (m, 8H), 1.21 (d, J = 6.7 Hz, 6H); ¹³C NMR (101 MHz, CDCl₃) δ 150.02, 148.01, 139.52, 128.45, 120.40, 114.24, 111.99, 69.00, 56.10, 42.61, 28.84, 23.75, 17.12; HRMS (ESI) calculated for C₂₆H₃₄O₆ ([M+H]⁺) *m/z* = calculated 409.2379, found 409.2371.



Macrocyclic cyclobutane 20: 10% Pd/C (0.0138 g, 0.0130 mmol) was added to a stirred solution of **9** (0.0447 g, 0.109 mmol) in a 10:1 mixture of methanol/EtOAc (11 mL) under a atmosphere (balloon) of hydrogen. After 2 h, the reaction was filtered through a pad of Celite, washed with methanol (10 mL) and chloroform (10 mL), and concentrated under reduced pressure. This material was used on without further purification (0.034 g, 75%): $R_f = 0.36$ (dichloromethane); ¹H NMR (600 MHz, CDCl₃) δ 6.65 (dd, J = 8.3, 5.5 Hz, 2H),

6.59 (s, 1H), 6.56–6.52 (m, 1H), 6.51–6.49 (m, 1H), 6.43 (d, J = 8.3 Hz, 1H), 4.19–4.06 (m, 2H), 3.88 (t, J = 9.0 Hz, 1H), 3.82 (t, J = 7.3 Hz, 2H), 3.78 (d, J = 4.1 Hz, 6H), 3.58 (t, J = 9.8 Hz, 1H), 2.58–2.51 (m, 1H), 2.38 (q, J = 8.6 Hz, 1H), 1.79–1.49 (m, 8H), 1.32 (d, J = 6.5 Hz, 3H), 0.73 (d, J = 7.0 Hz, 3H); ¹³C NMR (151 MHz, CDCl₃) δ 147.29, 147.26, 146.49, 146.43, 134.75, 131.97, 123.25, 119.92, 116.13, 113.01, 111.70, 110.51, 68.64, 68.16, 55.95, 55.70, 47.50, 47.42, 42.43, 39.39, 27.64, 27.51, 23.58, 23.29, 20.83, 14.90; HRMS (ESI) calculated for $C_{26}H_{34}O_4$ ([M+H]⁺) m/z = calculated 411.2535, found 411.2526.



*syn/anti/anti/syn-*cyclobutane 17: A solution of boron tribromide (1 M in dichloromethane, 0.70 mL, 0.70 mmol) was added dropwise over 5 min. to a stirred solution of **20** (0.062 g, 0.15 mmol) in dichloromethane (2.5 mL) at 0 °C. After 25 min., the reaction was added carefully to ice water (10 mL). The organic layers were separated and the aqueous phase extracted with ethyl acetate (6 × 10 mL). The organic extracts were combined and washed with brine (10 mL), dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was

passed through a short pad of silica gel (50% EtOAc/hexanes), and the filtrate was concentrated under reduced pressure. The residue (4.0 mg, 0.013 mmol) was dissolved in acetonitrile (1.0 mL) followed by the addition of methyl iodide (6.5 µL, 0.10 mmol) and cesium carbonate (0.0338 g, 0.104 mmol). The reaction was heated to 82 °C for 30 min., cooled and concentrated under reduced pressure. The residue was dissolved in ethyl acetate (5 mL), washed with water (2 × 3 mL) and brine (1 × 3 mL), dried over MgSO₄, and concentrated under reduced pressure. The residue was purified via flash chromatography (30% EtOAc/hexanes) to afford **17** as a white solid (4.3 mg, 80% over 2 steps): R_f = 0.40 (40% EtOAc/hexanes); ¹H NMR (600 MHz, CDCl₃) δ 6.71 (d, *J* = 8.2 Hz, 1H), 6.62 (d, *J* = 8.2 Hz, 1H), 6.59 (d, *J* = 8.1 Hz, 1H), 6.51 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.44 (d, *J* = 1.7 Hz, 1H), 6.42 (s, 1H), 3.89 (dd, *J* = 11.0, 6.9 Hz, 1H), 3.84 (dd, *J* = 11.1, 6.7 Hz, 1H), 3.80 (s, 3H), 3.78 (s, 3H), 3.66 (s, 3H), 3.58 (s, 3H), 2.63 – 2.51 (m, 1H), 2.43 – 2.29 (m, 1H), 1.32 (d, *J* = 6.4 Hz, 3H), 0.71 (d, *J* = 7.0 Hz, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 148.76, 147.82, 146.85, 135.03, 132.13, 122.77, 118.70, 113.88, 111.01, 110.92, 110.36, 56.05, 55.88, 55.79, 55.74, 47.76, 47.47, 42.16, 39.52, 20.59, 14.89; HRMS (ESI) calculated for C₂₆H₃₄O₆ ([M+H]⁺) *m/z* =357.1467, found 357.1453.



Di-O-methylendiandrin A (3): Potassium *tert*-butoxide (0.0038 g, 0.031 mmol) was added to a stirred solution of **17** (1.0 mg, 0.0030 mmol) in dimethyl sulfoxide (0.3 mL) and heated to 100°C under argon. After 2 h, the reaction mixture was poured into water (2 mL) extracted with ethyl acetate (4 × 5 mL) and dichloromethane (4 × 5 mL). The combined organic extracts were washed with water (5 × 5 mL), dried over MgSO₄, and concentrated under reduced pressure to afford **3** as a colorless oil (1.0 mg, 95%): R_f = 0.30 (40% EtOAc/hexanes); ¹H NMR

(500 MHz, CDCl₃) δ 6.81 (d, *J* = 8.2 Hz, 2H), 6.77 (dd, *J* = 8.2, 1.8 Hz, 2H), 6.71 (d, *J* = 1.7 Hz, 2H), 3.85 (s, 6H), 3.84 (s, 6H), 2.79 (dd, *J* = 5.7, 3.3 Hz, 2H), 1.90 – 1.81 (m, *J* = 9.0, 3.7 Hz, 2H), 1.20 (d, *J* = 5.9 Hz, 6H); ¹³C NMR (126 MHz, CDCl₃) δ 149.00, 147.60, 136.65, 118.74, 111.36, 110.40, 56.12, 56.01, 53.20, 43.12, 19.10; HRMS (ESI) calculated for $C_{22}H_{28}O_4$ ([M+Na]⁺) *m/z* = 379.1885, found 379.1090.

¹H NMR (600 MHz, DMSO) δ 6.84 (d, *J* = 8.2 Hz, 2H), 6.78 (d, *J* = 1.8 Hz, 2H), 6.73 (dd, *J* = 8.2, 1.9 Hz, 2H), 3.72 (s, 6H), 3.69 (s, 6H), 2.77 (dd, *J* = 5.8, 3.2 Hz, 2H), 1.79 - 1.70 (m, Hz, 2H), 1.13 (d, *J* = 5.8 Hz, 6H).

¹³C NMR (126 MHz, DMSO) δ 148.67, 147.28, 135.84, 118.68, 111.95, 110.69, 55.57, 55.45, 52.38, 42.71, 18.52.

TABLE SI-1: ¹H and ¹³C NMR data for di-O-methylendiandrin A (**3**) reported in this work and in the original isolation paper



POSITION	¹ H (MULT., <i>J</i> , INT.) REPORTED	¹³ C REPORTED	¹ H (MULT., <i>J</i> , INT.) ISOLATED ²	¹³ C ISOLATED ²
1		135.84		135.8
2	6.78 (d, <i>J</i> = 1.8 Hz, 1H)	110.69	6.77 (d, <i>J</i> = 1.8 Hz, 1H)	110.7
3		148.67		148.7
3-OME	3.72 (s, 3H)	54.45	3.71 (s, 3H)	55.4
4		147.28		147.3
4-OME	3.69 (s, 3H)	55.57	3.69 (s, 3H)	55.5
5	6.84 (d, <i>J</i> = 8.2 Hz, 1H),	111.95	6.84 (d, <i>J</i> = 8.4 Hz, 1H)	112.0
6	6.73 (dd, <i>J</i> = 8.2, 1.9 Hz, 1H)	118.68	6,73 (dd, <i>J</i> = 8.4, 1.8 Hz, 1H)	118.6
7	2.77 (dd, <i>J</i> = 5.8, 3.2 Hz, 1H)	52.38	2.77 (d, <i>J</i> = 9.0 Hz, 1H)	52.3
8	1.79 – 1.70 (m, 1H)	42.71	1.75 (m, 1H)	42.6
9	1.13 (d, <i>J</i> = 5.8 Hz, 3H)	18.52	1.13 (d, <i>J</i> = 6.0 Hz, 3H)	18.4
1'		135.84		135.8
2'	6.78 (d, <i>J</i> = 1.8 Hz, 1H)	110.69	6.77 (d, <i>J</i> = 1.8 Hz, 1 H)	110.7
3'		148.67		148.7
3'-OME	3.72 (s, 3H)	54.45	3.71 (s, 3H)	55.4
4'		147.28		147.3
4'-OME	3.69 (s, 3H)	55.57	3.69 (s, 3H)	55.5
5'	6.84 (d, <i>J</i> = 8.2 Hz, 1H),	111.95	6.84 (d, <i>J</i> = 8.4 Hz, 1H)	112.0
6'	6.73 (dd, <i>J</i> = 8.2, 1.9 Hz, 1H)	118.68	6,73 (dd, <i>J</i> = 8.4, 1.8 Hz, 1H)	118.6
7'	2.77 (dd, <i>J</i> = 5.8, 3.2 Hz, 1H)	52.38	2.77 (d, <i>J</i> = 9.0 Hz, 1H)	52.3
8'	1.79 – 1.70 (m, 1H)	42.71	1.75 (m, 1H)	42.6
9'	1.13 (d, <i>J</i> = 5.8 Hz, 3H)	18.52	1.13 (d, <i>J</i> = 6.0 Hz, 3H)	18.4















