Supporting Information

Synthesis of Proposed Oxidation-Cyclization-Methylation Intermediates of the Coumarin Antibiotic Biosynthetic Pathway

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Materials and methods. The enzymes for the preparation of screening kits were obtained from various enzyme suppliers including Amano (Nagoya, Japan), Roche (Basel, Switzerland), Novo Nordisk (Bagsvaerd, Denmark), Altus Biologics Inc. (Cambridge, MA), Biocatalytics (Pasadena, CA), Toyobo (Osaka, Japan), Sigma-aldrich (St. Louis, MO, USA). The diastereoselectivity was analyzed by HPLC using a C18 column (3 , 4.6 mm x 100 mm). The enantioselectivity was analyzed by chiral HPLC on Chiracel OJ-RH or AS-RH column (3 , 4.6 mm x 150 mm); mobile phase, ACN/H₂O (containing 0.1% TFA); detection at 220 nm. LC-MS conditions: ESI (positive and negative modes), LC chromatography runs on tuna C18 column (3 , 30 x 4.6 mm) using ACN/H₂O (containing 0.1% formic acid) as mobile phases: gradient 10% to 90% ACN in 3.5 min, then isocratic for 1 min, flow rate 0.8 mL/min, detection at 220 nm and 254 nm.

Screening of enzyme plates. A screening kit was thawed for 5 minutes. 80 L of potassium phosphate buffer (0.1 M, pH 7.2) were then dispensed into the wells via a multi-channel pipette, and the plate was agitated for 5 minutes in an incubator/shaker. 10 L of the substrate stock solution (20 mg in 1-3 mL ACN) were then added to each well, and the 96 reactions were incubated at 30 C and 800 rpm. The reactions were quenched after 6 h with 100 L of acetonitrile. The 96-well plate was then centrifuged, and the supernatant was transferred from each well into another 96-well plate. Sampled reactions were then sealed using a penetrable mat cover and transferred to a HPLC system for reactivity analysis.

Preparation of 14. To a 250 mL of flask was added diethyl 2-*t*-Boc-aminomalonate (10g, 36.4 mmol) and PLE (3.3 g, from Sigma) in 70 mL of phosphate buffer (pH 7.0, 0.5 M). The mixture was stirred at 23 °C and the conversion was monitored by HPLC. After 2 h, the reaction mixture was washed with EtOAc (x 2) to remove the remaining diester. For the aqueous layer the pH was adjusted to 3.5 and further extracted with EtOAc (x 3), which was dried and concentrated to yield the monoacid **14** (7.6 g, 84%). The spectral data was identical to what was reported in the literature.¹

Preparation of 16. To an ice cold solution of **14** (0.51 g, 2.1 mmol) in THF (10 mL) was added Et₃N (0.43 g, 4.3 mmol) and MgCl₂ (0.23 g, 2.3 mmol). The resulting slurry was stirred vigorously at the same temperature for 2.5 h and then treated with acid chloride **15** (0.22 g, 1.0 mmol) in THF (5 mL) (**15** was prepared in a standard protocol²). The mixture was then stirred at rt overnight. The reaction is complete after 12 h by HPLC and added EtOAc (50 mL), quenched with sat. NH₄Cl (50 mL). The organic layer was separated, washed with brine (50 mL) and dried over Na₂SO₄ to give **16** (0.315 g, 86%) upon rotary evaporation. The crude product is carried over without further purification. ESI m/z [M+Na]⁺ 388.1. HRMS for C₁₈H₂₄NO₇Na [M+H]⁺ calcd 366.1553, found 366.1537. IR 3324, 2981, 1753, 1678, 1530, 1190, 912, 858 cm⁻¹. ¹H NMR (300 MHz, CDCl₃): 8.08 (d, *J* = 8.7, 2H), 7.16 (d, *J* = 8.7, 2H), 5.83 (s, 1H), 4.11 (dd, *J* = 3.0, 7.2 Hz, 2H), 2.26 (s, 3H), 1.38 (s, 9H), 1.10 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (75 ppm, CDCl₃) •191.13, 168.89, 167.33, 155.66, 155.36, 132.18, 131.55, 122.28, 81.03, 63.03, 59.80, 53.76, 28.61, 21.48, 14.23.

Preparation of 17. To a 20 mL vial was added ester **16** (100 mg, 0.274 mmol) in 0.5 mL of ACN and *Candida rugosa* lipase (200 mg, Amano AY 30G) in 4.5 mL of phosphate buffer (pH 7.0, 0.5 M). The mixture was stirred at 23 °C and the conversion was monitored by HPLC. After 16 h, the mixture was extracted with EtOAc (x 3) and the organic layer was dried over MgSO₄. After evaporation of solvent *in vacuo*, the crude product was purified by flash chromatography using CH₂Cl₂/EtOAc (10:1 and 4:1) to give 90 mg of **17** (91%). ESI m/z [M+Na]⁺ 348.2.

Preparation of 21. To **20** (550 mg, 1.57 mmol, prepared according to a literature protocol³) in MeOH (15 mL) was added Boc₂O (0.5 mL) and Pd/C (300 mg). The reaction mixture was hydrogenated under 1 atm at 23 °C. After 3 h, the reaction mixture was centrifuged and the supernatant was extracted with EtOAc, washed by saturated NaHCO₃ (x 2) and dried over Na₂SO₄. The organic layer was evaporated *in vacuo* to yield ester **21** (540 mg, 98% yield, *syn/anti* >100:1, 97% ee). The diastereoselectivity was analyzed by HPLC using a C18 column (3 , 4.6 mm x 100 mm). The enantioselectivity was analyzed by chiral HPLC on Chiracel OJ-RH column (3 , 4.6 mm x 150 mm; mobile phase, ACN/H₂O: 20%:80%, 0.6 mL/min; detection at 220 nm). ESI m/z [M+Na]⁺ 348.2. HRMS for C₁₆H₂₄NO₆ [M+H]⁺ calcd 326.1604, found 326.1608. ¹H NMR (300 MHz, CDCl₃): 7.10 (d, *J* = 8.4 Hz, 2H), 6.67 (d, *J* = 8.4 Hz, 2H), 5.57 (d, *J* = 9.0 Hz, 1H), 5.13 (d, *J* = 9.0 Hz, 1H), 4.39 (br s, 1H), 4.26 (t, *J* = 7.2 Hz, 2H), 1.40 (s, 9H), 1.29 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (75 ppm, CDCl₃) •173.17, 158.96, 158.61, 134.53, 129.82, 117.27, 81.74, 75.13, 62.21, 56.10, 28.27.

Preparation of (2*S*,3*R*)-22. To a 20 mL flask was added ester **21** (150 mg, 0.462 mmol) and MML (150 mg, Biocatalytics, CA) in 15 mL of phosphate buffer (pH 7.0, 0.5 M). The mixture was stirred at 23 °C and

the conversion was monitored by HPLC. After 36 h, the mixture was extracted with EtOAc (x 3) and the starting ester was recovered (45 mg, 30%). The aqueous layer was adjusted to pH 3.0 and extracted by EtOAc (x 3). The organic layer was dried and concentrated *in vacuo* to afford pure acid **22** (94 mg, 69%; 95% *ee*). The *ee* value was determined by converting **22** to the corresponding ester **21** via DCC/HOBT coupling with EtOH. ESI *m/z* [M-H]⁻ 296.1. HRMS for C₁₄H₁₉NO₆Na [M+Na]⁺ calcd 320.1110, found 320.1105. ¹H NMR (300 MHz, CDCl₃): 7.22 (d, *J* = 8.4 Hz, 2H), 6.75 (d, *J* = 8.4 Hz, 2H), 5.17 (br s, 1H), 4.35 (br s, 1H), 1.40 (s, 9H). ¹³C NMR (75 ppm, CDCl₃) 175.33, 158.61, 134.53, 130.12, 129.82, 117.28, 81.74, 75.14, 62.21, 19.41.

Preparation of 23. To acid **22** (50 mg, 0.168 mmol) in ACN (5 mL) was added N-acetyl cysteamine (300 mg, 2.52 mmol), HOBT (27.0 mg, 0.201 mmol) and DCC (41 mg, 0.201 mmol). The mixture was stirred at 23 °C for 1 h. After the removal of the precipitate by centrifugation, and the evaporation of the solvent, the residue was dissolved in CH₂Cl₂ and washed with phosphate buffer (pH 3.0, 0.5 M) twice, dried over MgSO₄ to afford the desired product **23** (>90% purity based on HPLC and LC-MS, 95% *ee*), which was directly used for the next step without further purification. HRMS for C₁₈H₂₇N₂O₆S [M+H]⁺ calcd 399.1590, found 399.1607. IR 3284, 2934, 1738, 1644, 1546, 1366, 1290 cm⁻¹. The *ee* was determined by converting **23** to the corresponding ethyl ester **21** via transesterification with EtOH at rt for 2 h.

Preparation of 10. To crude product **23** (130 mg) was added 4M HCl in dioxane (1 mL) at 0 °C and the mixture was stirred for 1 h. After the removal of excess HCl and dioxane, the crude residue was washed with EtOAc and ACN (x 3) to afford the desired product in 70% overall yield for two steps from **22**. ESI [M+H]⁺ 299.3. HRMS for C₁₃H₁₉N₂O₄S [M+H]⁺ calcd 299.1066, found 299.1078. ¹H NMR (300 MHz, D₂O): 7.27 (d, *J* = 8.4, 2H), 6.88 (d, *J* = 8.4, 2H), 5.10 (d, *J* = 6.6 Hz, 1H), 4.47 (d, *J* = 6.3 Hz, 1H), 3.43 (t, *J* = 6.3 Hz, 1H), 2.90-3.27 (m, 2H), 2.78 (t, *J* = 6.6 Hz, 1H), 1.95 (s, 3H). ¹³C NMR (75 ppm, D₂O) •200.54, 174.73, 156.81, 129.96, 128.82, 116.53, 72.16, 65.04, 38.93, 29.08, 22.22. For storage the salt product was dissolved in phosphate buffer (pH 3.5, 0.1 M) and lyophilized as a powder and kept at -80 °C.

Preparation of 24. To a stirred solution of **16** (0.25 g, 0.68 mmol) in EtOH (5 mL) at -20 °C was added NaBH₄ (0.26 g, 0.68 mmol). The reaction is complete after 15 min at the same temperature by monitoring the disappearance of starting material following TLC (hexanes/ethyl acetate = 1:1) and was quenched by the addition of sat. NH₄Cl (10 mL). EtOH was then removed by rotary evaporation and the aqueous layer was extracted with CH₂Cl₂ (10 mL x 3). The combined organic layers were dried over Na₂SO₄ to provide **25** (0.32 g, 85%, dr 12:1, *anti/syn*) upon rotary evaporation. The crude was used for enzymatic resolution without further purification. ESI [M+Na]⁺ 390.1. HRMS for C₁₈H₂₆NO₇ [M+H]⁺ calcd 368.1709, found 368.1696. IR 3424, 2980, 1737, 1692, 1505, 1368, 1192, 1016, 752 cm⁻¹. ¹H NMR (300 MHz, CDCl₃): 7.29 (br s, 2H), 7.05 (br s, 2H), 5.36 (br s, 1H), 5.18 (br s, 1H), 4.66 (br s, 1H), 4.15 (d, *J* = 6.0, 13.5 Hz, 1H), 2.28 (s, 3H), 1.43 (s, 9H), 1.18(t, *J* = 4.0 Hz, 3H). ¹³C NMR (75 ppm, CDCl₃) •169.83, 150.76, 137.34, 127.57, 121.68, 115.57, 81.09, 74.92, 62.12, 60.16, 28.73, 21.44, 14.31.

Preparation of (2S, 3S)-25 and (2R,3R)-26. To a 20 mL vial was added racemic ester **24** (100 mg, 0.308 mmol) and *Aspergillus melleus* protease (200 mg, Amano protease P) in 10 mL phosphate buffer (pH 7.0,

0.5 M). The mixture was stirred at 23 °C and the conversion was monitored by HPLC. After 3 h, the mixture was adjusted to pH 8.0 and extracted with EtOAc (x 3). The organic layer was dried and evaporated *in vacuo* to give enantioenriched ester **26** (32 mg, 35%; >94% ee) based on LC-MS (ESI m/z [M+Na]⁺ 348.1). The ee was determined by chiral HPLC using Chiracel AS-RH column (3 , 4.6 mm x 150 mm); mobile phase, ACN/H₂O: 20%:80%, 0.6 ml/min; detection at 220 nm. The aqueous layer was adjusted to pH 3.0 and extracted by EtOAc (x 3). The organic layer was dried and concentrated *in vacuo* to afford pure acid **25** (45 mg, 49%; 96%ee). The ee was determined by converting **25** to its corresponding ethyl ester via DCC/HOBT coupling with EtOH. **25**: ESI m/z [M-H]⁻ 296.1. ¹H NMR (300 MHz, CDCl₃): 7.21 (d, *J* = 8.1 Hz, 2H), 6.75 (d, *J* = 8.4 Hz, 2H), 4.85(br s, 1H), 4.38 (d, *J* = 7.2 Hz, 1H), 1.36 (s, 9H). ¹³C NMR (75 ppm, CDCl₃) 172.61, 156.18, 131.27, 127.44, 127.44, 114.62, 79.84, 73.13, 59.10, 26.89. The absolute configurations of **25** and **26** were determined by comparing to the *anti*-ester produced from *syn*-ester **21** via epimerization under basic conditions. After the hydrolysis of **26** with 10% K₂CO₃ in H₂O/MeOH (1:1) at 23 °C for 10 h, the crude acid was afforded after usual work-up, which was used for the coupling without further purification.

Preparation of 11 and 12. The coupling and deprotection procedures were the same as that for **10**. After the usual work-up, both enantiomers **11** and **12** were produced in 65% and 70% overall yields for two and three steps, respectively. ESI m/z [M+H]⁺ 299.1. ¹H NMR (300 MHz, D₂O): 7.23 (d, *J* = 8.3, 2H), 6.86 (d, *J* = 8.4, 2H), 5.18 (d, *J* = 4.8 Hz, 1H), 4.6 (d, *J* = 5.4 Hz, 1H), 3.42 (t, *J* = 5.3 Hz, 1H), 2.90-3.27 (m, 2H), 2.78 (t, *J* = 5.7 Hz, 1H), 1.92 (s, 3H). ¹³C NMR (75 ppm, D₂O) • 194.46, 173.46, 155.40, 127.77, 127.49, 114.98, 70.45, 62.99, 37.34, 30.61, 22.26. The ee's were determined for **27** (95% ee by chiral HPLC) and **28** (94% ee by chiral HPLC) by converting both of them to the corresponding ethyl esters via transesterification with EtOH.

Preparation of 29. To **16** (100 mg, 0.274 mmol) was added 4M HCl in dioxane (1 mL) at 0 °C and the mixture was stirred for 1 h. After the removal of excess HCl and dioxane, the crude residue was washed with EtOAc to provide **30** (80 mg, 94%). ESI m/z [M+H]⁺ 266.2. ¹H NMR (300 MHz, D₂O): 8.96 (br s, 2H), 8.10 (br s, 2H), 8.12 (br s, 2H), 4.15 (br s, 1H), 4.05 (m, 2H), 2.24 (s, 3H), 0.96 (br s, 3H). ¹³C NMR (75 ppm, D₂O) • 187.94, 168.79, 163.74, 156.05, 132.52, 131.37, 122.22, 64.36, 59.28, 21.54, 13.96.

Preparation of 30. To a 20 mL flask was added **29** (30 mg, 0.113 mmol) and MML (100 mg, Biocatalytics, CA) in phosphate buffer (pH 7.0, 0.5 M, 3 mL). The mixture was stirred at 23 °C and the conversion was monitored by HPLC. After 6 h, the mixture was ultrafiltered and lyophilized to yield **30** as a salt, which was readily decarboxylated at acidic solution to give the corresponding amino-keto product (85%). ESI m/z [M-H]⁻ 150.1. ¹H NMR (300 MHz, D₂O): 7.85 (d, *J* = 8.3, 2H), 6.80 (d, *J* = 8.4, 2H), 4.40 (s, 2H). ¹³C NMR (75 ppm, D₂O) • 192.50, 162.72, 131.63, 126.16, 116.31, 45.09.

Preparation of 31 and 32. To a 20 mL flask was added **16** (100 mg, 0.274 mmol) in ACN (0.5 mL) and MML (200 mg, Biocatalytics, CA) in phosphate buffer (pH 7.0, 0.5 M, 5 mL). The mixture was stirred at 23 °C and the conversion was monitored by HPLC. After 16 h, the mixture was ultrafiltered and lyophilized to yield **31** as a salt, which was spontaneously decarboxylated upon acidic work-up to give N-Boc

aminoketone **32**. ESI m/z $[M+Na]^+$ 274.1. HRMS for $C_{13}H_{17}NO_4Na$ $[M+Na]^+$ calcd 274.1055, found 274.1067. IR 3381, 3227, 1691, 1668, 1605, 1293, 1165, 934, 830 cm^{-1} . 1H NMR (300 MHz, MeOD- d_4): 7.90 (d, $J = 8.4$, 2H), 6.86 (d, $J = 8.4$, 2H), 4.46 (s, 2H), 1.40 (s, 9H). ^{13}C NMR (75 ppm, D_2O) •195.86, 164.52, 158.94, 131.89, 128.58, 116.84, 80.99, 55.17, 29.12.

Preparation of 34. Compound **34** was prepared by the same procedure as that for **16** (80%). ESI m/z $[M+Na]^+$ 446.1. 1H NMR (300 MHz, $CDCl_3$): 7.98 (d, $J = 8.7$, 2H), 7.14 (dd, $J = 2.1$, 8.4, 2H), 7.04 (s, 1H), 5.87 (s, 1H), 4.21 (m, 2H), 2.33 (s, 3H), 2.31 (s, 3H), 1.45 (s, 9H), 1.10 (t, $J = 3.6$ Hz, 1H). ^{13}C NMR (75 ppm, $CDCl_3$) •191.19, 168.98, 168.46, 166.89, 155.36, 155.04, 150.99, 132.23, 126.03, 119.34, 117.76, 81.01, 62.77, 61.89, 28.68, 21.35, 21.19, 14.09.

Preparation of 8. To a stirred solution of **34** (150 mg, 3.54 mmol) in MeOH (1.2 mL) was added NaOH (6%, 1.5 mL). The reaction was in completion at rt for 3 h by HPLC. The pH was adjusted to 4.0 using 1N HCl and the aqueous layer was extracted with EtOAc (2 mL x 4). The combined organic layers were dried over Na_2SO_4 to provide **35** (89 mg, 86%) upon rotary evaporation. Without further purification, the crude (77 mg, 0.26 mmol) was dissolved into MeOH (1 mL) and added 1N HCl (in ether, 5 mL). The reaction is finished after 5 h at rt and the precipitate was collected to give **8** (56 mg, 93%) as a yellow solid after washing with ether (1 mL x 2). ESI m/z $[M+H]^+$ 194.1. HRMS for $C_9H_8NO_4$ $[M+H]^+$ calcd 194.0453, found 194.0463. IR 3268, 2889, 2176, 1687, 1601, 1420, 1200, 1107, 951, 801, 730 cm^{-1} . 1H NMR (300 MHz, D_2O): 7.80 (dd, $J = 0.6$, 9.0 Hz, 1H), 6.80 (ddd, $J = 0.9$, 2.4, 9.0 Hz, 1H), 6.68 (dd, $J = 0.6$, 2.1 Hz, 1H). ^{13}C NMR (75 ppm, D_2O) •164.82, 162.87, 162.04, 156.14, 126.56, 115.33, 108.81, 104.38, 96.79.

References

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