Supporting Information

Stereospecificity of Ketoreductase Domains of the 6-Deoxyerythronolide B Synthase

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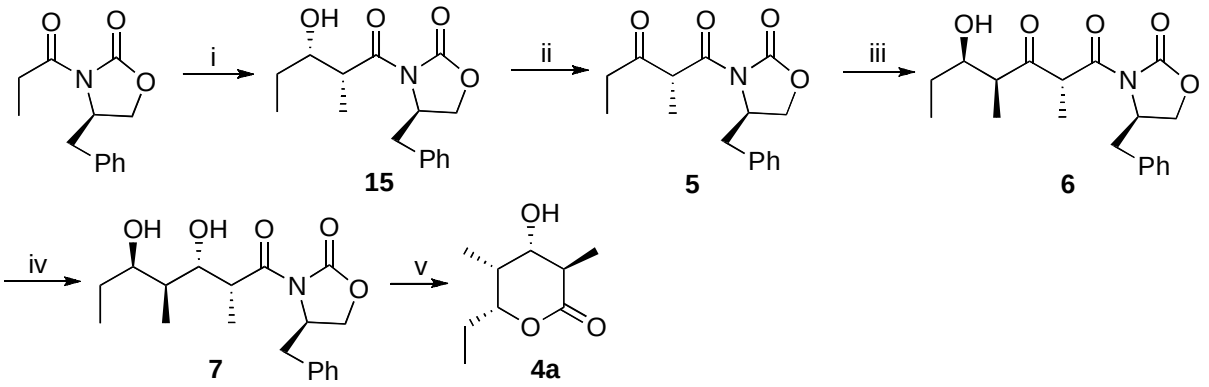
Materials and Methods

HPLC was carried out on a Rainin HPLC equipped with a dual HPXL solvent delivery system. ^{1}H and ^{13}C NMR (300 and 400 MHz) utilized Bruker Avance AM 300 and AM 400 spectrometers. All non-enzymatic reactions were carried out with dry solvents under anhydrous conditions. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials. Reagents were of the highest commercial quality and were used without further purification. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as a visualizing agent and *p*-anisaldehyde stain and heat as developing agent. Sorbent silica gel (60, particle size 0.040-0.063 mm) was used for flash column chromatography.

Experimental Procedures



i) Bu_2BOTf, i-Pr_2NEt, propionaldehyde, CH_2Cl_2, -78 °C, 60%; ii) SO_3•Pyr, Et_3N, DMSO, 97%; iii) $Sn(OTf)_2$, Et_3N, propionaldehyde, CH_2Cl_2, -78 °C, 61%; iv)$Na(OAc)_3BH$, AcOH, 25 °C, 89%; v) LiOOH, 0 °C, 68%

Scheme S1. Synthesis of triketide lactone **4a**

(4*R*,2′*R*,3′*S*)-3-(2′-methyl-3′-hydroxypentanoyl)-4-benzyl-2-oxazolidinone (15):[1] To a dried flask was added (*R*)-(-)-4-Benzyl-3-Propionyl-2-oxazolidinone (910 mg, 3.89 mmol) and CH_2Cl_2 (10 mL) under nitrogen. The solution was cooled to 0 °C and n-Bu_2BOTf (4.3 mL, 4.28 mmol, 1.1 equiv) was added. Then i-Pr_2NEt (0.88 mL, 5.06 mmol, 1.3 equiv) was added dropwise. The reaction was stirred at 0 °C for 1 h before being cooled down to - 78 °C. Propionaldehyde (3.37 mL, 46.68 mmol, 12 equiv) was added dropwise. The reaction was stirred at - 78 °C for 5 h and then quenched by addition of sodium phosphate buffer (10 mL, 100 mM, pH 7.0). The mixture was extracted with $CHCl_3$ (3 × 10 mL). The combined organic layers were dried and concentrated. The residue was dissolved in methanol (7 mL), sodium phosphate buffer (7 mL, 100 mM, pH 7.0), and H_2O_2 (7 mL, 30 %, aq). The solution was stirred at 0 °C for 30 minutes and then extracted with ethyl acetate (3 × 15 mL). The organic phase was washed with $Na_2S_2O_3$ (1 M, aq) and saturated NaCl (aq), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography (5-50% ethyl acetate in hexanes) to obtain **15** (680 mg, 60 %). TLC R_f = 0.20 (3:1 hexanes/ethyl acetate, anisaldehyde stain); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.38-7.20 (m, 5H, aromatic C-**H**), 4.72 (m, 1H, **H**-3), 4.22 (m, *2*H, **H**-4 and **H**-5), 3.88 (m, 1H, C3-**H**), 3.80 (dq, *J* = 2.7, 7.0 Hz, 1H, C2-**H**), 3.27 (dd, 1H, *J* = 3.3, 13.4 Hz, **H**-1), 2.85 (d, *J* = 3.3 Hz, 1H, O**H**),

2.80 (dd, *J* = 9.6, 13.5 Hz, 1H, **H**-2), 1.54 (m, 2H, C4-**H**), 1.26 (d, *J* = 7.0 Hz, 3H, C6-**H**), 0.99 (t, *J* = 7.4 Hz, 3H, C5-**H**).

(4*R*,2′*R*)-3-(2′-methyl-3′-oxopentanoyl)-4-benzyl-2-oxazolidinone (5):[1] The aldol adduct (**14**, 680 mg, 2.441 mmol) in CH_2Cl_2/DMSO (12 mL each) was cooled to ca. - 15 °C and Et_3N (0.984 mL, 7.062 mmol, 3.03 equiv) was added. A solution of SO_3•Pyr (1.124 g, 7.062 mmol, 3.03 equiv) in DMSO (10 mL) was transferred at a rate slow enough to maintain the temperature under 0 °C in the reaction vessel. After 4 h, the reaction mixture was diluted with ether (50 mL) and extracted with successive portions of 1 M $NaHSO_4$ (aq.), 1 M $NaHCO_3$ (aq.), and satd. NaCl (aq., 50 mL each). The organic phase was dried over Na_2SO_4. After the removal of solvent in vacuo, the residue was purified by flash chromatography (10-25% ethyl acetate in hexanes) to afford **5** (653 mg, 97%). TLC R_f = 0.30 (3:1 Hexanes/EtOAc, anisaldehyde stain); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.31-7.16 (m, 5H, aromatic C-**H**), 4.71 (m, 1H, **H**-3), 4.57 (q, 1H, *J* = 7.3 Hz, C2-**H**), 4.15 (m, *2*H, **H**-4 and **H**-5), 3.25 (dd, 1H, *J* = 3.2, 13.4 Hz, **H**-1), 2.77 (dd, *J* = 9.4, 13.4 Hz, 1H, **H**-2), 2.62 (m, 2H, C4-**H**), 1.40 (d, *J* = 7.3 Hz, 3H, C6-**H**), 1.03 (t, *J* = 7.2 Hz, 3H, C5-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 208.0, 170.1, 153.8, 135.2, 129.4, 128.9, 127.3, 66.5, 55.1, 52.7, 37.8, 33.9, 12.9, 7.5.

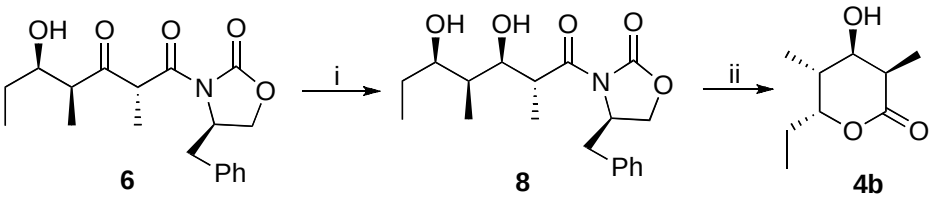
(4*R*,2′*R*,4′*S*,5′*R*)-3-(2′,4′-dimethyl-3′-oxo-5′-hydroxyheptanoyl)-4-benzyl-2-oxazolidinone (6):[1] A magnetically stirred suspension of anhydrous, acid-free $Sn(OTf)_2$ (988 mg, 2.371 mmol, 1.05 equiv, ca. 0.25 M in CH_2Cl_2) was treated with Et_3N (330 L, 2.371 mmol, 1.05 equiv) and then immediately cooled to -20 °C. After 5 min, a solution of the β–keto imide (**5**, 653 mg, 2.258 mmol, ca. 0.40 M in CH_2Cl_2) was added dropwise over a 5-min period. The resultant suspension was stirred for 1 h and then cooled to -78 °C prior to treatment with propionaldehyde (523 μL, 7.113 mmol, 3 equiv). The reaction mixture was stirred at -78 °C for 90 min and then transferred rapidly to a cool and vigorously stirred 1:1 mixture of CH_2Cl_2/1 M $NaHSO_4$ (250 mL each) at 0 °C. After 10 min of vigorous stirring at 0 °C, the mixture was diluted with additional 1:1 CH_2Cl_2/1 M $NaHSO_4$ (125 mL each). The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were washed with 1 M $NaHCO_3$ (aq.), dried

over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography (10-40% EtOAc in hexanes) to afford **6** (478 mg, 61%). TLC R_f = 0.13 (3:1 Hexanes/EtOAc, anisaldehyde stain); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36-7.20 (m, 5H, aromatic C-**H**), 4.88 (q, 1H, *J* = 7.2 Hz, C2-**H**), 4.76 (m, 1H, **H**-3), 4.24 (m, *2*H, **H**-4 and **H**-5), 3.83 (m, 1H, C5-**H**), 3.31 (dd, 1H, *J* = 3.2, 13.4 Hz, **H**-1), 2.81 (m, *2*H, **H**-2 and C4-**H**), 2.53 (br s, 1H, O**H**), 1.60-1.36 (m, 2H, C6-**H**), 1.49 (d, *J* = 7.3 Hz, 3H, C9-**H**), 1.24 (d, *J* = 7.2 Hz, 3H, C8-**H**), 0.97 (t, *J* = 7.4 Hz, 3H, C7-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 212.4, 170.5, 153.8, 135.2, 129.6, 129.2, 127.6, 66.7, 55.5, 52.1, 48.2, 38.1, 27.0, 13.1, 10.8, 10.2.

(4*R*,2′*R*,3′*S*,4′*S*,5′*R*)-3-(2′,4′-dimethyl-3′,5′-dihydroxyheptanoyl)-4-benzyl-2-oxazolidinone (7):[1] To 0.972 mL of acetic acid at 0 °C was added portionwise $NaBH_4$ (23.5 mg, 0.622 mmol). Upon completion of gas evolution, the reaction was allowed to warm to ambient temperature where it was stirred for 1 h. To this solution was added a solution of **6** (21.6 mg, 0.0622 mmol) in 0.194 mL of acetic acid. After 20 min, the reaction was concentrated in vacuo before it was partitioned between 1 mL of satd. $NaHCO_3$ (aq.) and 1 mL of CH_2Cl_2. The aqueous layer was extracted by CH_2Cl_2 (2 × 1 mL). The combined organic layers were then dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was azeotroped with methanol (5 × 1 mL) with the addition of 12.5 μL of acetic acid during the first round, and with heptane (1 × 1 mL) to obtain **7** (17.4 mg, 89%). TLC R_f = 0.23 (1:1 Hexanes/EtOAc, anisaldehyde stain); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36-7.20 (m, 5H, aromatic C-**H**), 4.71 (m, 1H, **H**-3), 4.22 (m, *2*H, **H**-4 and **H**-5), 4.00 (dd, 1H, *J* = 2.6, 9.0 Hz, C3-**H**), 3.91 (dq, 1H, *J* = 2,6, 7.0 Hz, C2-**H**), 3.78 (ddd, 1H, *J* = 2.3, 4.1, 9.0 Hz, C5-**H**), 3.26 (dd, 1H, *J* = 3.3, 13.4 Hz, **H**-1), 2.80 (dd, 1H, J = 9.4, 13.4 Hz, **H**-2), 1.86 (m, 1H, C4-**H**), 1.52 (m, 2H, C6-**H**), 1.28 (d, *J* = 7.1 Hz, 3H, C9-**H**), 1.00 (t, *J* = 7.4 Hz, 3H, C7-**H**), 0.87 (d, 3H, *J* = 7.1 Hz, C8-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.1, 153.1, 135.2, 129.6, 129.2, 127.7, 75.5, 73.9, 66.4, 55.3, 39.9, 39.1, 38.0, 26.1, 11.6, 11.2, 10.3.

Synthesis of (2*R*,3*S*,4*R*,5*R*)-3,5-dihydroxy-2,4-dimethyl-*n*-heptanoic acid-δ-lactone (**4a**):[2] A 30 % aq. solution of H_2O_2 (19 μL, 0.189 mmol, 3.8 equiv) was added to a stirring solution of **7** (17.4 mg, 0.0498 mmol) in THF/H_2O (4:1, v/v, 1.25 mL) at 0 °C followed by addition of 1 M LiOH (aq., 75 μL,

0.0747 mmol, 1.5 equiv). The reaction was stirred 2 h at 0 °C then quenched by the dropwise addition of 2 M Na_2SO_3 (aq., 125 μL, 0.249 mmol, 5 equiv.). After stirring at 0 °C for 1 h, the reaction was concentrated *in vacuo*. The residue was extracted with CH_2Cl_2 (1 × 1 mL) to remove the oxazolidinone auxiliary. The aqueous phase was acidified with 1 M HCl (aq.) to pH 3 and stirred 5 h at room temperature. The aqueous phase was extracted with ethyl acetate (3 × 1 mL). The organic extracts were washed with satd. $NaHCO_3$ (aq.), satd. NaCl (aq.), dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford **4a** (5.8 mg, 68 %). TLC R_f = 0.33 (1:1 Hexanes/EtOAc, anisaldehyde stain); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.13 (ddd, 1H, *J* = 2.4, 6.4, 7.6 Hz, C5-**H**), 3.83 (dd, 1H, *J* = 4.3, 10.3 Hz, C3-**H**), 2.47 (qd, 1H, *J* = 7.1, 10.3 Hz, C2-**H**), 2.17 (ddq, 1H, *J* = 2.4, 4.3, 6.9 Hz, C4-**H**), 1.94 (br s, 1H, O**H**), 1.82 (m, 1H, C6-**H**), 1.58 (m, 1H, C6-**H**), 1.41 (d, 3H, *J* = 7.1 Hz, C8-**H**), 1.01 (t, 3H, *J* = 7.5 Hz, C7-**H**), 0.97 (d, 3H, *J* = 7.1 Hz, C9-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.8, 81.6, 74.1, 40.0, 37.0, 25.5, 14.5, 10.1, 4.6; HRMS (FAB+) *m*/*z* 195.0990 ($C_9H_{16}O_3$ + Na^+ requires 195.0997).



i) DIBAL-H, THF, -78 °C, 86%; ii) LiOOH, 0 °C, 51%

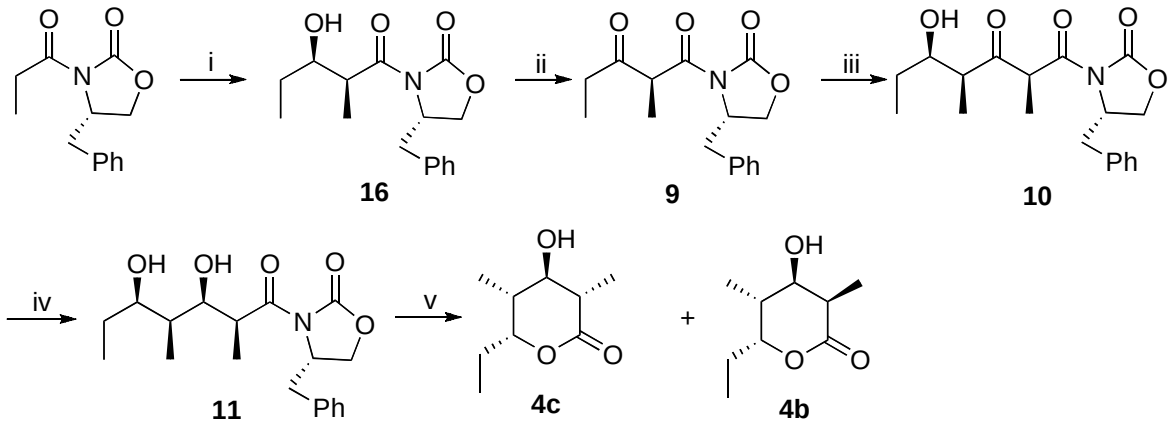
Scheme S2. Synthesis of triketide lactone **4b**.

(4*R*,2′*R*,3′*R*,4′*S*,5′*R*)-3-(2′,4′-dimethyl-3′,5′-dihydroxyheptanoyl)-4-benzyl-2-oxazolidinone (8):[1]

A solution of **6** (59.5 mg, 0.1713 mmol) in THF (5 mL) was cooled to -78 °C and diisobutylaluminum hydride (257 μL, 1.0 M in hexanes, 0.2569 mmol, 1.5 equiv.) was added dropwise. After 90 min, the excess of hydride was consumed by addition of acetone (1 mL). After 5 min, the solution was poured into a vigorously stirred mixture of 10 mL of tartaric acid (0.5 M, aq.) and 10 mL of CH_2Cl_2. The mixture was stirred 30 min at room temperature and then the phases were separated. The organic layer was washed with satd. NaCl (aq., 10 mL) and H_2O (10 mL). The combined aqueous phases were extracted with CH_2Cl_2. The organic layers were combined, dried over Na_2SO_4, filtered and concentrated

in vacuo to afford **8** (51.5 mg, 86%). TLC R_f = 0.38 (1:1 Hexanes/EtOAc, anisaldehyde stain); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36-7.21 (m, 5H, aromatic C-**H**), 4.75 (m, 1H, **H**-3), 4.22 (m, *2*H, **H**-4 and **H**-5), 4.12 (dd, 1H, *J* = 1.4, 9.4 Hz, C3-**H**), 4.00 (m, 1H, C2-**H**), 3.80 (m, 1H, C5-**H**), 3.27 (dd, 1H, *J* = 3.2, 13.5 Hz, **H**-1), 2.81 (dd, 1H, J = 9.5, 13.4 Hz, **H**-2), 1.73 (m, 1H, C4-**H**), 1.53 (m, 2H, C6-**H**), 1.18 (d, *J* = 6.83 Hz, 3H, C9-**H**), 0.97 (t, *J* = 7.5 Hz, 3H, C7-**H**), 0.95 (d, 3H, *J* = 7.1 Hz, C8-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.8, 153.6, 135.4, 129.6, 129.2, 127.6, 79.2, 68.1, 66.4, 55.5, 41.4, 38.2, 37.0, 25.8, 14.8, 10.6, 4.6.

(2*R*,3*R*,4*R*,5*R*)-3,5-dihydroxy-2,4-dimethyl-heptanoic acid-δ-lactone (4b):[2] A 30 % aq. solution of H_2O_2 (43 μL, 0.4374 mmol, 3.8 equiv) was added to a stirring solution of **8** (40.2 mg, 0.1151 mmol) in THF/H_2O (4:1, v/v, 1.25 mL) at 0 °C followed by addition of 1 M LiOH (aq., 173 μL, 0.1727 mmol, 1.5 equiv). The reaction was stirred 1 h at 0 °C then quenched by the dropwise addition of 2 M Na_2SO_3 (aq., 288 μL, 0.5755 mmol, 5 equiv.). After stirring at 0 °C 30 min, the reaction was concentrated in vacuo. The residue was extracted with CH_2Cl_2 (1 × 2 mL) to remove the oxazolidinone auxiliary. The aqueous phase was acidified with 1 M HCl (aq.) to pH 3 and stirred 3 h at room temperature. The aqueous phase was extracted with ethyl acetate (3 × 3 mL). The organic extracts were washed with satd. $NaHCO_3$ (aq.), satd. NaCl (aq.), dried over Na_2SO_4, filtered and concentrated in vacuo to afford **4b** (10.1 mg, 51 %). TLC R_f = 0.46 (1:1 Hexanes/EtOAc, anisaldehyde stain); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.70 (ddd, 1H, *J* = 2.9, 6.2, 8.3 Hz, C5-**H**), 3.95 (dd, 1H, *J* = 3.7, 3.7 Hz, C3-**H**), 2.66 (dq, 1H, *J* = 3.7, 7.2 Hz, C2-**H**), 2.10 (m, 1H, C4-**H**), 1.80 (m, 1H, C6-**H**), 1.54 (m, 1H, C6-**H**), 1.35 (d, 3H, *J* = 7.2 Hz, C8-**H**), 1.02 (t, 3H, *J* = 7.4 Hz, C7-**H**), 0.99 (d, 3H, *J* = 7.2 Hz, C9-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.1, 79.8, 73.2, 36.8, 36.7, 25.2, 12.4, 10.2, 10.1; HRMS (FAB+) *m*/*z* 195.0995 ($C_9H_{16}O_3$ + Na^+ requires 195.0997).



i) Bu_2BOTf, Et_3N, propionaldehyde, CH_2Cl_2, -78 °C, 54%; ii) SO_3•Pyr, Et_3N, DMSO, 93%; iii) $TiCl_4$, i-Pr_2NEt, propionaldehyde, CH_2Cl_2, -78 °C, 80%; iv) $Zn(BH_4)_2$, CH_2Cl_2, -78 °C, 99%; v) LiOOH, 0°C, 59%

Scheme S3. Synthesis of triketide lactone **4c**.

(4*S*,2′*S*,3′*R*)-3-(2′-methyl-3′-hydroxypentanoyl)-4-benzyl-2-oxazolidinone (16): To a dried flask was added (*S*)-(+)-4-benzyl-3-propionyl-2-oxazolidinone (1.012 g, 4.34 mmol) and CH_2Cl_2 (10 mL) under nitrogen. The solution was cooled to 0 °C and n-Bu_2BOTf (4.77 mL, 4.77 mmol, 1.1 equiv) was added. Then Et_3N (0.82 mL, 5.64 mmol, 1.3 equiv) was added dropwise. The reaction was stirred at 0 °C for 1 h before being cooled down to -78 °C. Propionaldehyde (3.76 mL, 52.1 mmol, 12 equiv) was added dropwise. The reaction was stirred at - 78 °C for 5 h and then quenched by addition of sodium phosphate buffer (10 mL, 100 mM, pH 7.0). The mixture was extracted with $CHCl_3$ (3 × 10 mL). The combined organic layers were dried and concentrated. The residue was dissolved in methanol (7 mL), sodium phosphate buffer (7 mL, 100 mM, pH 7.0), and H_2O_2 (7 mL, 30 %, aq). The solution was stirred at 0 °C for 30 min and then extracted with ethyl acetate (3 × 15 mL). The organic phase was washed with $Na_2S_2O_3$ (1 M, aq) and saturated NaCl (aq), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography (5-50% ethyl acetate in hexanes) to obtain **16** (680 mg, 54 %). TLC R_f = 0.20 (3:1 hexanes/ethyl acetate, anisaldehyde stain); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.38-7.20 (m, 5H, aromatic C-**H**), 4.72 (m, 1H, **H**-3), 4.20 (m, *2*H, **H**-4 and **H**-5), 3.88 (m, 1H, C3-**H**), 3.80 (dq, *J* = 2.7, 7.0 Hz, 1H, C2-**H**), 3.27 (dd, 1H, *J* = 3.3, 13.4 Hz, **H**-1), 2.86 (d, *J* = 3.0 Hz, 1H, O**H**),

2.80 (dd, *J* = 9.4, 13.4 Hz, 1H, **H**-2), 1.54 (m, 2H, C4-**H**), 1.26 (d, *J* = 7.0 Hz, 3H, C6-**H**), 0.99 (t, *J* = 7.4 Hz, 3H, C5-**H**).

(4*S*,2′*S*)-3-(2′-methyl-3′-oxo-pentanoyl)-4-benzyl-2-oxazolidinone (9): The aldol adduct (**16**, 489 mg, 1.678 mmol) in CH_2Cl_2/DMSO (10 mL each) was cooled to ca. -15 °C and Et_3N (0.708 mL, 5.084 mmol, 3.03 equiv) was added. A solution of SO_3•Pyr (809 mg, 5.084 mmol, 3.03 equiv) in DMSO (10mL) was transferred at a rate slow enough to maintain the temperature under 0 °C in the reaction vessel. After 4 h, the reaction mixture was diluted with ether (50 mL) and extracted with successive portions of 1 M $NaHSO_4$ (aq.), 1 M $NaHCO_3$ (aq.), and satd. NaCl (aq., 50 mL each). The organic phase was dried over Na_2SO_4. After the removal of solvent *in vacuo*, the residue was purified by flash chromatography (10-25% EtOAc in hexanes) to afford **9** (454 mg, 93 %). TLC R_f = 0.30 (3:1 hexanes/ethyl acetate, anisaldehyde stain); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.32-7.16 (m, 5H, aromatic C-**H**), 4.72 (m, 1H, **H**-3), 4.58 (q, 1H, *J* = 7.3 Hz, C2-**H**), 4.17 (m, *2*H, **H**-4 and **H**-5), 3.26 (dd, 1H, *J* = 3.2, 13.4 Hz, **H**-1), 2.77 (dd, *J* = 9.4, 13.4 Hz, 1H, **H**-2), 2.64 (m, 2H, C4-**H**), 1.41 (d, *J* = 7.3 Hz, 3H, C6-**H**), 1.04 (t, *J* = 7.3 Hz, 3H, C5-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 208.1, 170.2, 153.8, 135.2, 129.4, 128.9, 127.3, 66.5, 55.2, 52.7, 37.8, 34.0, 12.9, 7.5.

(4*S*,2′*S*,4′*S*,5′*R*)-3-(2′,4′-dimethyl-3′-oxo-5′-hydroxyheptanoyl)-4-benzyl-2-oxazolidinone (10): The β-keto imide (**9**, 372.2 mg, 1.286 mmol) was dissolved in dry CH_2Cl_2 (5 mL, ca. 0.25 M) under nitrogen. The solution was cooled to -10 °C. $TiCl_4$ (155 μL, 1.415 mmol, 1.1 equiv.) was added (as a neat liquid) dropwise, followed by i-Pr_2NEt (246 μL, 1.415 mmol, 1.1 equiv.), and the reaction mixture was stirred at -10 °C for 1 h. The enolate solution was then cooled to -78 °C, and propionaldehyde was added dropwise. The reaction mixture was stirred at -78 °C for 30 min and then allowed to come to -40 °C over a 1-h period. Upon warming to 0 °C, the reaction was quenched by the addition of pH 7 buffer (4 mL, 100 mM sodium phosphate). After stirring for an additional 5 min at 0 °C, the solution was transferred into CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2 (3 × 3 mL). The combined organic layers were washed with 1 M $NaHCO_3$ (aq.), dried over Na_2SO_4, and concentrated *in vacuo*.

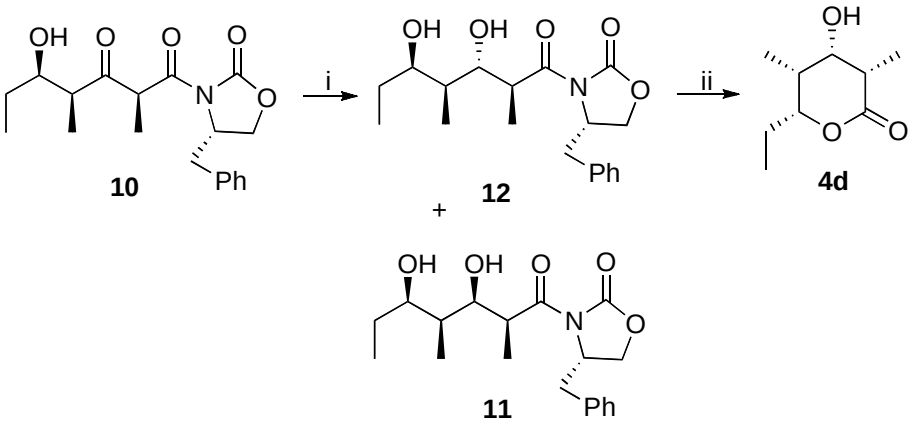
The residue was purified by flash chromatography (10-35% ethyl acetate in hexanes) to afford **10** (358 mg, 80 %). TLC R_f = 0.17 (3:1 hexanes/ethyl acetate, anisaldehyde stain); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36-7.20 (m, 5H, aromatic C-**H**), 4.87 (q, 1H, *J* = 7.3 Hz, C2-**H**), 4.78 (m, 1H, **H**-3), 4.24 (m, *2*H, **H**-4 and **H**-5), 4.04 (m, 1H, C5-**H**), 3.30 (dd, 1H, *J* = 3.1, 13.4 Hz, **H**-1), 2.90 (dq, 1H, *J* = 2.1, 7.0 Hz, C4-**H**), 2.81 (m, *2*H, **H**-2 and O**H**), 1.64-1.40 (m, 2H, C6-**H**), 1.49 (d, *J* = 7.3 Hz, 3H, C9-**H**), 1.13 (d, *J* = 7.0 Hz, 3H, C8-**H**), 0.99 (t, 3H, *J* = 7.4 Hz, C7-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 211.8, 170.4, 154.2, 135.1, 129.5, 129.2, 127.6, 66.8, 55.5, 52.2, 48.7, 38.1, 27.0, 13.3, 10.9, 9.4.

(4*S*,2′*S*,3′*R*,4′*S*,5′*R*)-3-(2′,4′-dimethyl-3′,5′-dihydroxyheptanoyl)-4-benzyl-2-oxazolidinone (11): To a clear solution of **10** (31.5 mg, 0.09067 mmol) in 5 mL of CH_2Cl_2 at -78 °C was added a solution of $Zn(BH_4)_2$ in ether (0.944 mL, 0.1378 mmol, 1.52 equiv., ca. 0.146 M). The resultant clear solution was stirred for 15 min at -78 °C before the reaction was quenched by the addition of 5 mL of satd. NH_4Cl (aq.) at -78 °C. The mixture was stirred vigorously as it was warmed to ambient temperature. After 10 min at ambient temperature, the mixture was diluted with 5 mL of CH_2Cl_2, the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 5 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford **11** (31.3, 99%). TLC R_f = 0.35 (1:1 Hexanes/EtOAc, anisaldehyde stain); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.37-7.21 (m, 5H, aromatic C-**H**), 4.71 (m, 1H, **H**-3), 4.23 (m, *2*H, **H**-4 and **H**-5), 4.08 (dd, 1H, *J* = 5.6, 10.1 Hz, C3-**H**), 4.02 (m, 1H, C2-**H**), 3.78 (ddd, 1H, *J* = 1.9, 5.6, 7.5 Hz, C5-**H**), 3.27 (dd, 1H, *J* = 3.0, 13.4 Hz, **H**-1), 2.80 (dd, 1H, J = 9.5, 13.3 Hz, **H**-2), 1.65 (m, 1H, C4-**H**), 1.57 (m, 1H, C6-$\mathbf{H_a}$), 1.47 (m, 1H, C6-$\mathbf{H_b}$), 1.33 (d, *J* = 6.7 Hz, 3H, C9-**H**), 0.97 (d, *J* = 7.0 Hz, 3H, C8-**H**), 0.96 (t, 3H, *J* = 7.6 Hz, C7-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.6, 153.3, 135.2, 129.6, 129.2, 127.6, 76.6, 76.0, 66.4, 55.4, 41.0, 39.4, 37.9, 28.1, 13.0, 10.7, 6.5.

Preparation of $Zn(BH_4)_2$:[3] A mixture of zinc chloride (2.0 g, 14.67 mmol, 98%, Aldrich) with 25 mL of dry ether was boiled until most of the solid had dissolved. The mixture was allowed to stand, and the supernatant liquid was carefully decanted from insoluble material (ca. 0.2 g). The ethereal zinc chloride solution was added dropwise at room temperature to a stirred suspension of sodium borohydride (1.31 g,

34.26 mmol, 2.33 equiv., 99%, Aldrich) in 75 mL of absolute ether. Stirring was continued overnight. The solids were allowed to settle, and the liquid was removed by decantation and clarified by centrifugation. The ethereal solution of zinc borohydride was stored in a stoppered bottle at 4 °C.

(2*S*,3*R*,4*R*,5*R*)-3,5-dihydroxy-2,4-dimethyl-heptanoic acid-δ-lactone (4c): A 30 % aq. solution of H_2O_2 (31.8 μL, 0.3230 mmol, 3.8 equiv) was added to a stirring solution of **11** (29.7 mg, 0.0850 mmol) in THF/H_2O (4:1, v/v, 1.25 mL) at 0 °C followed by addition of 1 M LiOH (aq., 127.5 μL, 0.1275 mmol, 1.5 equiv). The reaction was stirred 2 h at 0 °C then quenched by the dropwise addition of 2 M Na_2SO_3 (aq., 212.5 μL, 0.425 mmol, 5 equiv.). After stirring at 0 °C for 30 min, the reaction was concentrated in vacuo. The residue was extracted with CH_2Cl_2 (1 × 1 mL) to remove the oxazolidinone auxiliary. The aqueous phase was acidified with 1 M HCl (aq.) to pH 3 and stirred 3 hours at room temperature. The aqueous phase was extracted with ethyl acetate (3 × 1 mL). The organic extracts were washed with satd. $NaHCO_3$ (aq.), satd. NaCl (aq.), dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford a mixture of **4c** and **4b** (ca. 1:1, 10.9 mg, 75%). Attempts to separate them by flash chromatography failed. **4c**: TLC R_f = 0.40 (1:1 hexanes/ethyl acetate, anisaldehyde stain); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.41 (ddd, 1H, *J* = 2.9, 5.2, 8.6 Hz, C5-**H**), 3.40 (dd, 1H, *J* = 2.5, 7.5 Hz, C3-**H**), 2.55 (m, 1H, C2-**H**), 1.99 (m, 1H, C4-**H**), 1.77 (m, 1H, C6-**H**), 1.54 (m, 1H, C6-**H**), 1.34 (d, 3H, *J* = 6.7 Hz, C8-**H**), 1.02 (t, 3H, *J* = 7.3 Hz, C7-**H**), 0.95 (d, 3H, *J* = 7.4 Hz, C9-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.5, 79.5, 76.9, 42.6, 41.1, 24.7, 14.1, 12.2, 10.4; HRMS (FAB+) *m*/*z* 195.0995 ($C_9H_{16}O_3$ + Na^+ requires 195.0997).



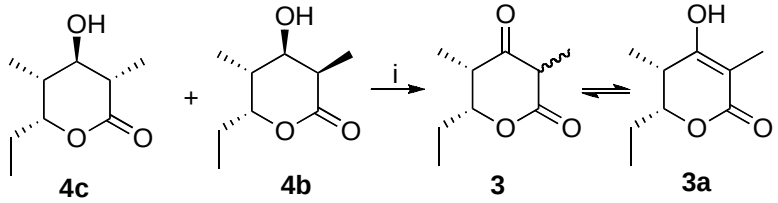
i) $Na(OAc)_3BH$, 25 °C, AcOH, 63 %; ii) LiOOH, 0 °C, 64%

Scheme S4. Synthesis of triketide lactone **4d**.

(4*S*,2′*S*,3′*S*,4′*S*,5′*R*)-3-(2′,4′-dimethyl-3′,5′-dihydroxyheptanoyl)-4-benzyl-2-oxazolidinone (12): To 5.48 mL of acetic acid at 0 °C was added portionwise $NaBH_4$ (132.7 mg, 3.509 mmol). Upon completion of gas evolution, the reaction was allowed to warm to ambient temperature where it was stirred for 1 h. To this solution was added a solution of **10** (121.9 mg, 0.3509 mmol) in 1.097 mL of acetic acid. After 1 h, the reaction was concentrated *in vacuo* before it was partitioned between 100 mL of satd. $NaHCO_3$ (aq.) and 100 mL of CH_2Cl_2. The aqueous layer was extracted by CH_2Cl_2 (2 × 100 mL). The combined organic layers were then dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was azeotroped with methanol (5 × 5 mL) with the addition of 62.5 μL of acetic acid during the first round, and with heptane (1 × 5 mL) to obtain a mixture of **11** and **12** (117.0 mg, 95%).

The two diastereomers were separated and purified by reverse phase HPLC. The separation conditions were scouted on an analytical RP-HPLC column (Thermo Hypersil-Keystone, BDS Hypersil, C18, 250 mm × 4.6 mm), using a linear gradient [35-70 % acetonitrile in water, 0.1 % (v/v) TFA] over 20 min at the flow rate of 1 mL/min. Purification was carried out on a reverse phase semi-preparative column (Phenomenex, Jupiter 5μ, C4 300A, 250 mm × 10 mm). A linear gradient [40-50 % acetonitrile in water, 0.1 % (v/v) TFA] was run over 21 min at a flow rate of 4 mL/min. The HPLC was monitored by UV detection at 230 nm. The fractions corresponding to the two diastereomers were combined respectively. Acetonitrile was removed by rotovap and then water was removed by lyophilizer to afford **11** (58.3 mg, 48 %) and **12** (35.5 mg, 29 %) at a molar ratio of 1.6:1. **12**: TLC R_f = 0.39 (1:1 hexanes/ethyl acetate, anisaldehyde stain); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38-7.20 (m, 5H, aromatic C-**H**), 4.77 (m, 1H, **H**-3), 4.24 (m, *3*H, **H**-4, **H**-5 and C2-**H**), 4.01 (ddd, 1H, *J* = 1.9, 5.5, 7.8 Hz, C5-**H**), 3.85 (dd, 1H, *J* = 4.5, 8.1 Hz, C3-**H**), 3.26 (dd, 1H, *J* = 3.3, 13.4 Hz, **H**-1), 2.82 (dd, 1H, J = 9.4, 13.3 Hz, **H**-2), 1.83 (m, 1H, C4-**H**), 1.52 (m, 2H, C6-$\mathbf{H_a}$ and C6-$\mathbf{H_b}$), 1.24 (d, *J* = 6.9 Hz, 3H, C9-**H**), 1.05 (d, *J* = 7.1 Hz, 3H, C8-**H**), 0.96 (t, 3H, *J* = 7.4 Hz, C7-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.0, 153.7, 135.2, 129.6, 129.2, 127.7, 79.4, 74.1, 66.5, 55.4, 40.6, 38.2, 37.0, 27.3, 11.5, 11.2, 10.7.

(2*S*,3*S*,4*R*,5*R*)-3,5-dihydroxy-2,4-dimethyl-heptanoic acid-δ-lactone (4d): A 30 % aq. solution of H_2O_2 (28.8 μL, 0.2925 mmol, 3.8 equiv) was added to a stirring solution of **12** (26.9 mg, 0.07698 mmol) in THF/H_2O (4:1, v/v, 1.25 mL) at 0 °C followed by addition of 1 M LiOH (aq., 115.5 μL, 0.1155 mmol, 1.5 equiv). The reaction was stirred 2 h at 0 °C then quenched by the dropwise addition of 2 M Na_2SO_3 (aq., 192.5 μL, 0.3849 mmol, 5 equiv.). After stirring at 0 °C 30 min, the reaction was concentrated *in vacuo*. The residue was extracted with CH_2Cl_2 (1 × 1 mL) to remove the oxazolidinone auxiliary. The aqueous phase was acidified with 1 M HCl (aq.) to pH 3 and stirred 3 h at room temperature. The aqueous phase was extracted with ethyl acetate (3 × 1 mL). The organic extracts were washed with satd. $NaHCO_3$ (aq.), satd. NaCl (aq.), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (1:1 ethyl acetate/hexanes) to yield lactone **12** (2.6 mg, 20 %). TLC R_f = 0.44 (1:1 hexanes/ethyl acetate, anisaldehyde stain); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.25 (td, 1H, *J* = 4.7, 9.4 Hz, C5-**H**), 4.19 (m, 1H, C3-**H**), 2.80 (dq, 1H, *J* = 4.5, 7.0 Hz, C2-**H**), 2.38 (m, 1H, C4-**H**), 1.85 (m, 1H, C6-$\mathbf{H_a}$), 1.61 (m, 1H, C6-$\mathbf{H_b}$), 1.34 (d, 3H, *J* = 7.0 Hz, C8-**H**), 1.05 (t, 3H, *J* = 7.4 Hz, C7-**H**), 1.05 (d, 3H, *J* = 7.3 Hz, C9-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.0, 81.7, 71.2, 40.7, 36.5, 29.9, 25.0, 9.4, 8.8.



i) Dess-Martin Periodinane, CH_2Cl_2, rt, 38 %

Scheme S5. Synthesis of triketide ketolactone **3**.

(4*R*,5*R*)-3-oxo-2,4-dimethyl-5-hydroxy-heptanoic acid-δ-lactone (3): To a solution of triketide hydroxylactones **4b** and **4c** (9.3 mg, 0.054 mmol) in CH_2Cl_2 was added Dess-Martin periodinane (24.2 mg, 0.059 mmol, 1.1 equiv.). The reaction was stirred at room temperature overnight (16 h). The mixture was then filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (1:2 ethyl acetate/hexanes) to afford a ~2:1 mixture of lactone **3** and the enol isomer **3a**. (3.5 mg, 38 %).

TLC R_f = 0.55 (1:1 hexanes/ethyl acetate, anisaldehyde stain). **3**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.68 (ddd, 1H, *J* = 3.0, 5.4, 8.3 Hz, C5-**H**), 3.63 (q, 1H, *J* = 6.7 Hz, C2-**H**), 2.65 (dq, 1H, *J* = 2.9, 7.5 Hz, C4-**H**), 1.90 (m, 1H, C6-$\mathbf{H_a}$), 1.69 (m, 1H, C6-$\mathbf{H_b}$), 1.39 (d, 3H, *J* = 6.7 Hz, C8-**H**), 1.15 (d, 3H, *J* = 7.5 Hz, C9-**H**), 1.10 (t, 3H, *J* = 7.4 Hz, C7-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 205.6, 170.3, 78.8, 50.6, 44.6, 24.3, 9.9, 9.5, 8.5. **3a**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.56 (m, 1H, C5-**H**), 2.95 (dq, 1H, *J* = 3.5, 7.3 Hz, C4-**H**), 1.77 (m, 1H, C6-$\mathbf{H_a}$), 1.69 (m, 1H, C6-$\mathbf{H_b}$), 1.61 (s, 3H, C8-**H**), 1.21 (d, 3H, *J* = 7.3 Hz, C9-**H**), 1.09 (t, 3H, *J* = 7.5 Hz, C7-**H**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 204.1, 170.4, 87.4, 79.2, 44.7, 24.7, 20.8, 10.0, 9.5.

Table 6-1. ^{1}H and ^{13}C NMR data for the triketide lactones **4a** – **4d**.

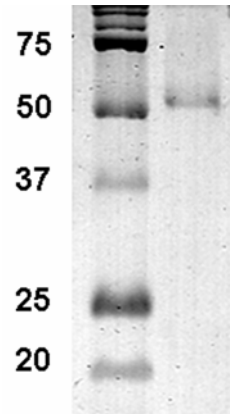
Lactone	*^{1}H NMR (ppm)*				*^{13}C NMR (ppm)*			
	H-3	H-5	H-8	H-9	C-8	C-3	C-9	C-5
4a	3.83	4.13	1.41	0.97	14.5	74.1	10.1	81.6
4b	3.95	4.70	1.35	0.99	12.4	73.2	10.2	79.8
4c	3.40	4.41	1.34	0.95	14.1	76.9	12.2	79.5
4d	4.19	4.25	1.34	1.05	25.0	71.2	9.4	81.7

Table 6-2. Selected ^{1}H NMR coupling constants for triketide lactones **4a** – **4d**.

Lactone	*Coupling constant (Hz)*						
	$J_{2,3}$	$J_{2,8}$	$J_{3,4}$	$J_{4,5}$	$J_{4,9}$	$J_{5,6a}$	$J_{5,6b}$
4a	10.3	7.1	4.3	2.4	7.1	6.4	7.6
4b	3.7	7.2	3.7	2.9	7.2	6.2	8.3
4c	7.5	6.7	2.5	2.9	7.4	5.2	8.6
4d	4.5	7.0	N.D.*	9.4	7.3	4.7	4.7

*N.D. = Not Determined

Figure S1. SDS-PAGE (10 % running gel) of [$KR3^0$] from DEBS. Lane 1, molecular marker (in kDa). Lane 2, [$KR3^0$].



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