Peptide-catalyzed synthesis of a series of erythromycin analogs

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General Procedures. Proton NMR spectra were recorded on either a Bruker 400 or 500 MHz spectrometer or a Varian 500 or 600 MHz spectrometer. Proton chemical shifts are reported in ppm (δ) relative to internal tetramethylsilane (δ 0.0) or solvent peak ($CDCl_3$ or CD_3OD, 7.26 or 3.30 ppm respectively). Data are reported as follows: chemical shift (multiplicity [singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m)], coupling constants [Hz], integration). NMR data were collected at 25 °C. Infrared spectra were obtained on a ThermoNicolet Avatar 210 spectrometer. Analytical thin-layer chromatography (TLC) was performed using Silica Gel 60 Å F-254 precoated plates (0.25 mm thickness). Visualization was accomplished after staining with cerium ammonium molybdenate (CAM) solution. Flash column chromatography was performed using Silica Gel 60 Å (32-63 μm). Optical rotations were recorded on a Rudolf Research Analytical Autopol IV Automatic polarimeter at the sodium D line (path length 50 mm, corrected to 20.0 °C). High-resolution mass spectra were obtained at the Mass Spectrometry Facility at the University of Illinois Urbana-Champaign. The method of ionization is given in parentheses.

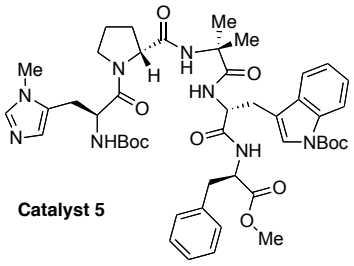
Analytical and preparative reverse phase HPLC were run on a Rainin SD-200 chromatograph equipped with a single wavelength UV detector (214 nm). Analytical reverse phase HPLC was performed on a Hewlett-Packard 1100 Series chromatograph equipped with a diode array detector. All reactions were carried out under a nitrogen

atmosphere employing oven or flame-dried glassware. All solvents were either distilled or obtained from passing through activated alumina.

General Procedure for the Acetylation of Erythromycin A (2).

Erythromycin A (**2**, 100 mg, 0.136 mmol) was dissolved in $CHCl_3$ (100 mM soln., 1.36 mL) in a flame-dried vial. Triethylamine (5 equiv., 93.0 μL, 0.681 mmol) and the peptide catalyst (5 mol%, 20.0 mM soln., in CH_2Cl_2, 0.340 mL, 6.81 μmol) were then introduced sequentially. Acetic anhydride (10 equiv., 128 μL, 1.36 mmol) was introduced and the reaction was allowed to stir at 25 °C. Reaction progress was monitored by ^{1}H NMR (400 MHz) by removing 100 μL aliquots followed by a methanol quench. The resulting solutions were passed through a silica gel plug eluting with a $CHCl_3$/MeOH (95/5 v/v) solvent system and concentrated under high vacuum. After an appropriate time interval, the full reaction mixture was quenched by addition of methanol and passed through a silica gel plug and concentrated to dryness. If complete cleavage of the labile 2'-acetyl was desired, then the unpurified reaction mixture was redissolved in MeOH, and allowed to stir for 72 h. Following concentration of the reaction mixture, reaction selectivity was analyzed by ^{1}H NMR (400 MHz). Individual products were isolated by silica gel column chromatography ($CHCl_3$/MeOH/NH_4OH, 14 M; 95/5/1 v/v/v) and/or semipreparative HPLC techniques.

Preparation of Boc-Pmh-D-Pro-Aib-D-Trp(Boc)-D-Phe-OMe (5)



Catalyst 5

Fmoc-D-Phe Wang resin (0.50 g, 0.70 mmol/g) was swelled for 30 min in THF. Typical solid phase peptide couplings were performed with 5 equiv of amino acid, 5 equiv of HBTU, and 10 equiv of Hünig's base in DMF for 3 h. The resin was then washed three times with DMF, MeOH, and CH_2Cl_2, respectively. Deprotections were performed with 20% piperidine in DMF for 30 min. (except the second coupling utilized 50% piperidine in DMF for 5 min). The peptide was cleaved from the resin with 9:1:1 MeOH:DMF:NEt_3 for 5 days. The crude peptide was purified by reverse phase HPLC using a Waters XTerra Prep RP_{18} (10 μm, 7.8 x 150 mm) eluting at 4 mL/min. with 60% MeOH/water for 10 min, then 65% MeOH for 5 min, and then 70% MeOH for 5-10 min.

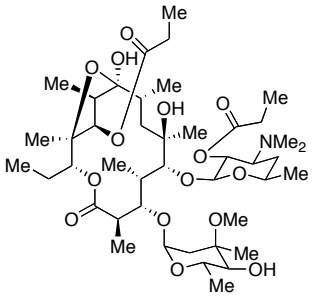
$[\alpha]^{20.0}$: -9.6 (*c* 1.0, $CHCl_3$); **^{1}H NMR** (CD_3OD, 400 MHz) δ 7.99 (d, J = 8.0 Hz, 1H), 7.88 (s, 1H), 7.53 (d, J = 7.2 Hz, 1H), 7.41 (s, 1H), 7.23-7.13 (m, 7H), 6.80 (s, 1H), 4.61 (dd, J_1 = 8.8 Hz, J_2 = 6.4 Hz, 1H), 4.51 (dd, J_1 = 9.2 Hz, J_2 = 6.0 Hz, 1H), 4.37 (dd, J_1 = 8.8 Hz, J_2 = 5.6 Hz, 1H), 4.11 (t, J = 7.6 Hz, 1H), 3.69-3.62 (m, 4H), 3.56 (s, 3H), 3.22-3.18 (m, 1H), 3.14-3.09 (m, 3H), 3.03 (d, J = 8.8 Hz, 1H), 2.99 (d, J = 8.8 Hz, 1H), 2.90 (dd, J_1 = 14.8 Hz, J_2 = 5.2 Hz, 1H), 2.68 (dd, J_1 = 15.2 Hz, J_2 = 9.2 Hz, 1H), 2.21-2.15 (m, 1H), 2.04-1.98 (m, 1H), 1.88-1.78 (m, 2H), 1.62 (s, 9H), 1.44 (s, 3H), 1.41 (s, 3H), 1.26 (s, 9H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 173.7, 171.9, 171.6, 155.7, 150.3, 137.9, 137.7, 137.2, 135.0, 130.6, 129.3, 128.9, 128.1, 126.9, 126.5, 125.2, 124.6, 124.2, 122.6, 119.3, 117.2, 115.1, 84.0, 80.3, 77.4, 62.2, 57.5, 54.7, 53.8, 52.9, 52.2, 48.0, 37.8, 31.5, 29.3, 28.4, 28.4, 27.1, 26.6, 25.9, 25.2, 25.1, 21.6; **IR** (film, cm^{-1}) 3314, 2986, 2942,

1740, 1671, 1545, 1457, 1369, 1256, 1161, 752; **HRMS** (ESI^+) *m/z* Calc'd for $C_{47}H_{63}N_8O_{10}$ 899.4667, found 899.4685 ($M + H^+$). ***Ent*-5** $[\alpha]^{20.0}$: +9.3 (*c* 1.0, $CHCl_3$);

Preparation of Erythromycin Analogs

Preparation of 2',11-Dipropionylerythromycin (6)

11-Propionylerythromycin (**16**, 10.0 mg, 0.0127 mmol) was dissolved in chloroform (1.0 mL), and triethylamine (5.3 μL, 0.0380 mmol) was added. Propionic anhydride (4.9 μL, 0.0380 mmol) was then added and the reaction was allowed to stir for 22 hours. The complete reaction mixture was then loaded directly onto a silica gel column ($CHCl_3$/MeOH 95/5 v/v) to provide a colorless solid (9.7 mg, 91%).

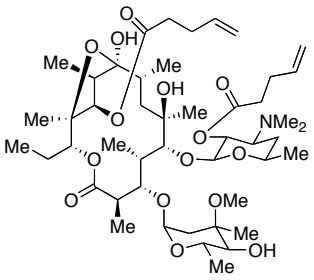


$[\alpha]^{20.0}$: -38.7 (*c* 0.5, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 5.03 (dd, J_1 = 10.4 Hz, J_2 = 2.6 Hz, 1H), 4.85 (s, 1H), 4.83 (d, J = 4.5 Hz, 1H), 4.80 (dd, J_1 = 10.6 Hz, J_2 = 7.6 Hz, 1H), 4.51 (d, J = 7.5 Hz, 1H), 4.35 (d, J = 7.3 Hz, 1H), 3.99 (dd, J_1 = 9.4 Hz, J_2 = 6.2 Hz, 1H), 3.57 (d, J = 8.6 Hz, 1H), 3.57 (d, J = 8.6 Hz, 1H), 3.51-3.48 (m, 1H), 3.37-3.35 (m, 1H), 3.33 (s, 3H), 3.03 (d, J = 9.3 Hz, 1H), 2.70-2.61 (m, 2H), 2.39-2.26 (m, 7H), 2.26 (s, 6H), 2.19-2.10 (m, 3H), 1.80 (dd, J_1 = 14.7 Hz, J_2 = 2.3 Hz, 1H), 1.75-1.71 (m, 1H), 1.65-1.61 (m, 1H), 1.55 (dd, J_1 = 15.2 Hz, J_2 = 4.8 Hz, 1H), 1.49 (s, 3H), 1.46-1.37 (m, 1H), 1.35-1.22 (m, 15H), 1.19-1.12 (m, 12H), 0.94 (d, J = 7.3 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 176.8, 173.7, 173.2, 109.2, 101.8, 96.5, 85.5, 85.3, 83.6, 78.4, 78.2, 77.7, 75.3, 72.9, 71.7, 69.0, 65.6, 63.7, 49.5, 47.2, 45.6, 40.7, 38.9, 36.3, 35.1, 30.5, 28.2, 28.0, 27.4, 25.8, 25.4, 23.9, 21.8, 21.2, 18.7, 18.4, 15.9, 14.2, 11.3, 10.1, 9.3, 9.2; **IR** (film, cm^{-1}) 3465, 2974, 2937, 2880, 2831, 1736,

1462, 1372, 1168, 1082, 1054, 1017; **TLC** R_f = 0.40 (9/1 $CHCl_3$/MeOH v/v); **HRMS** (ESI^+) *m/z* Calc'd for $C_{43}H_{76}NO_{15}$ 846.5215, found 846.5189 ($M + H^+$).

Preparation of 2', 11-Di-pent-4-enoate erythromycin (7)

11-pent-4-enoate erythromycin (**17**, 20.0 mg, 0.0245 mmol) was dissolved in chloroform (1.0 mL) and triethylamine (10.2 μL, 0.0735 mmol) was added. The symmetrical anhydride (13.4 μL, 0.0735 mmol) was then added and the mixture was stirred for 20 hours. The reaction was deemed complete by TLC and the crude reaction loaded directly onto the top of the silica gel column ($CHCl_3$/MeOH 95/5 v/v). The colorless solid product was isolated (21.4 mg, 88%).

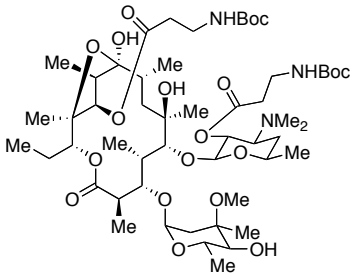


$[\alpha]^{20.0}$: -32.0 (*c* 1.0, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 5.89-5.79 (m, 2H), 5.09-4.99 (m, 6H), 4.87-4.82 (m, 2H), 4.54 (d, J = 9.3 Hz, 1H), 4.32 (d, J = 8.9 Hz, 1H), 3.98 (dd, J_1 = 7.6 Hz, J_2 = 3.4 Hz, 1H), 3.59-3.51 (m, 2H), 3.33 (s, 3H), 3.04 (d, J = 11.7 Hz, 1H), 2.87-2.81 (m, 1H), 2.63 (t, J = 8.9 Hz, 1H), 2.48-2.33 (m, 14H), 2.31 (s, 6H), 2.19-2.12 (m, 3H), 1.82-1.78 (m, 2H), 1.65-1.54 (m, 3H), 1.49 (s, 3H), 1.46-1.22 (m, 15H), 1.17 (d, J = 8.8 Hz, 3H), 1.11 (d, J = 8.8 Hz, 3H), 0.94 (d, J = 9.1 Hz, 3H), 0.84 (t, J = 9.1 Hz, 3H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 177.1, 176.5, 172.3, 171.9, 137.0, 137.0, 136.6, 115.9, 115.5, 115.4, 109.1, 101.6, 96.5, 85.6, 85.3, 83.5, 78.3, 78.2, 75.4, 72.9, 71.6, 68.9, 65.6, 63.4, 49.5, 47.2, 45.6, 40.8, 40.4, 38.9, 36.3, 35.1, 33.9, 33.9, 33.7, 30.4, 29.0, 28.9, 28.8, 25.8, 25.4, 23.9, 21.8, 21.2, 18.7, 18.4, 15.9, 14.2, 11.3, 10.2; **IR** (film, cm^{-1}) 3461, 2970, 2942, 1732, 1638, 1462, 1381,

1168, 1111, 1053, 1009, 911; **TLC** R_f = 0.33 (9/1 $CHCl_3$/MeOH v/v); **HRMS** (ESI^+) *m/z* Calc'd for $C_{47}H_{80}NO_{15}$ 898.5528, found 898.5534 ($M + H^+$).

Preparation of 2', 11-Di-3-(Boc)-propanoylerythromycin (8)

11-3-(Boc)-propanoylerythromycin (**18**, 15.4 mg, 0.0135 mmol) was dissolved in chloroform (1.0 mL), and triethylamine (5.7 μL, 0.0406 mmol) was added. The mixed anhydride (11.1 mg, 0.0406 mmol) was added and the reaction was allowed to stir for 23 hours. The reaction was deemed complete by TLC and loaded directly onto a silica gel column ($CHCl_3$/MeOH 95/5 v/v). The product was isolated and the ^1H NMR examined whereupon rotamers were observed. The major rotamer was characterized (16.4 mg, 90%).

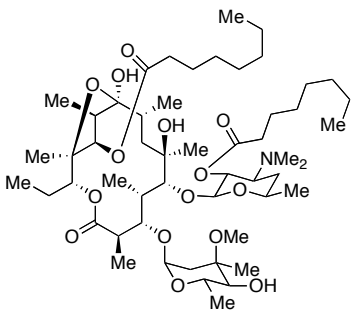


$[\alpha]^{20.0}$: -40.5 (*c* 0.5, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 6.83 (s, 1H), 5.28 (s, 1H), 5.04 (dd, J_1 = 10.4 Hz, J_2 = 2.3 Hz, 1H), 4.89 (s, 1H), 4.81 (dd, J_1 = 12.1 Hz, J_2 = 7.7 Hz, 1H), 4.53 (d, J = 7.5 Hz, 1H), 4.34 (d, J = 7.2 Hz, 1H), 3.98 (dd, J_1 = 9.4 Hz, J_2 = 6.4 Hz, 1H), 3.57-3.49 (m, 4H), 3.37-3.33 (m, 1H), 3.31 (s, 3H), 3.04 (t, J = 9.8 Hz, 1H), 2.70-2.36 (m, 5H), 2.25 (s, 6H), 2.21-2.02 (m, 4H), 1.78-1.76 (m, 2H), 1.66-1.61 (m, 3H), 1.55 (dd, J_1 = 15.3 Hz, J_2 = 4.9 Hz, 1H), 1.49 (s, 3H), 1.43-1.42 (m, 21H), 1.31-1.23 (m, 18H), 1.16-1.13 (m, 6H), 0.91-0.84 (m, 6H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 176.8, 171.6, 170.8, 156.4, 109.2, 101.8, 96.6, 85.7, 85.2, 84.2, 79.5, 79.0, 78.5, 78.2, 77.8, 76.3, 75.7, 75.2, 73.4, 73.0, 71.6, 69.1, 65.6, 64.0, 49.5, 47.3, 45.3, 44.4, 40.8, 40.0, 39.8, 38.9, 37.0, 36.4, 36.0, 35.4, 35.1, 28.7, 28.6, 25.7, 25.4, 23.9, 22.4, 21.8, 21.2, 18.7, 18.3, 18.0, 17.9, 15.9, 14.2, 11.4, 10.1; **IR** (film, cm^{-1}) 3395,

2978, 2937, 2884, 1732, 1716, 1699, 1507, 1458, 1373, 1258, 1164, 1062, 1001; **TLC** R_f = 0.48 (9/1 $CHCl_3$/MeOH v/v); **HRMS** (ESI^+) *m/z* Calc'd for $C_{53}H_{94}N_3O_{19}$ 1076.6482, found 1076.6449 ($M + H^+$).

Preparation of 2', 11-Dioctanoylerythromycin (9)

11-Octanoylerythromycin (**19**, 20.0 mg, 0.0234 mmol) was dissolved in chloroform (1.0 mL) and triethylamine (9.7 μL, 0.0698 mmol) was added. The symmetrical anhydride (18.9 mg, 0.0698 mmol) was added last and the reaction was allowed to stir for 44 hours. The crude mixture was loaded directly onto a silica gel column ($CHCl_3$/MeOH 95/5 v/v) and purified to afford a colorless solid (18.3 mg, 80%).

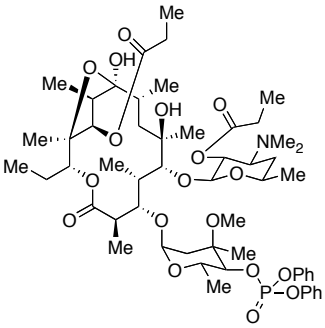


$[\alpha]^{20.0}$: -29.4 (*c* 1.0, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 5.02 (dd, J_1 = 10.4 Hz, J_2 = 2.5 Hz, 1H), 4.84-4.82 (m, 2H), 4.80 (dd, J_1 = 10.5 Hz, J_2 = 7.6 Hz, 1H), 4.53 (d, *J* = 7.5 Hz, 1H), 4.34 (d, *J* = 6.8 Hz, 1H), 3.99 (dd, J_1 = 9.3 Hz, J_2 = 6.3 Hz, 1H), 3.57 (d, *J* = 8.4 Hz, 1H), 3.52-3.46 (m, 1H), 3.34 (s, 3H), 3.04 (d, *J* = 9.4 Hz, 1H), 2.71-2.60 (m, 2H), 2.38 (d, *J* = 15.2 Hz, 1H), 2.34-2.10 (m, 14H), 1.80 (d, *J* = 12.9 Hz, 1H), 1.74 (d, *J* = 12.4 Hz, 1H), 1.66-1.54 (m, 6H), 1.48 (s, 3H), 1.45-1.40 (m, 1H), 1.35-1.21 (m, 35H), 1.17 (d, *J* = 7.1 Hz, 3H), 1.12 (d, *J* = 7.1 Hz, 3H), 0.94 (d, *J* = 7.3 Hz, 3H), 0.89 (m, 9H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 176.8, 173.1, 172.6, 109.2, 101.7, 96.4, 85.4, 85.2, 83.4, 78.2, 77.8, 75.3, 73.0, 71.5, 69.0,. 65.6, 63.6, 49.4, 47.1, 45.5, 41.0, 40.6, 38.9, 36.6, 35.1, 34.9, 34.8, 34.3, 31.9, 31.8, 31.8, 30.4, 29.4, 29.3, 29.2, 29.1, 25.8, 25.4, 25.1, 25.0, 23.8, 22.8, 22.7, 21.8, 18.6, 18.4, 15.9, 14.2, 14.2, 14.0, 11.3, 10.2; **IR** (film, cm^{-1}) 3461, 2962, 2934, 2860, 1732, 1454,

1381, 1250, 1168, 1107, 1058, 1009, 956; **TLC** R_f = 0.42 (9/1 $CHCl_3$/MeOH v/v); **HRMS** (ESI^+) *m/z* Calc'd for $C_{53}H_{96}NO_{15}$ 986.6780, found 986.6778 ($M + H^+$).

Preparation of 2',11-Dipropionyl-4"-diphenylphosphate erythromycin (10)

2',11-Dipropionate (**6**, 10.0 mg, 0.0118 mmol) was dissolved in chloroform (1.0 mL) and triethylamine (11.5 μL, 0.0827 mmol) were added. 4-Dimethylaminopyridine (0.4 mg, 3.55 μmol) and diphenyl chlorophosphate (14.7 μL, 0.0709 mmol) was added. The reaction was complete after 6 hours and was purified using two silica gel columns ($CHCl_3$/MeOH 95/5 v/v and EtOAc/hexanes 4/1 v/v) to afford the product (10.4 mg, 82%)

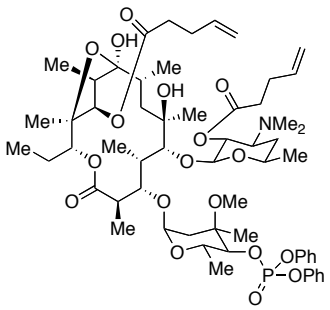


$[\alpha]^{20.0}$: -36.1 (*c* 0.5, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.29-7.26 (m, 4H), 7.21-7.15 (m, 6H), 5.03 (dd, J_1 = 10.3 Hz, J_2 = 2.4 Hz, 1H), 4.88 (d, J = 4.7 Hz, 1H), 4.84 (s, 1H), 4.79 (dd, J_1 = 10.5 Hz, J_2 = 7.5 Hz, 1H), 4.61 (d, J = 7.5 Hz, 1H), 4.48-4.43 (m, 1H), 4.34 (d, J = 7.0 Hz, 1H), 4.28 (t, J =10.1 Hz, 1H), 3.58-3.50 (m, 2H), 3.39-3.33 (m, 4H), 2.71-2.64 (m, 2H), 2.45 (d, J = 15.2 Hz, 1H), 2.40-2.28 (m, 4H), 2.25 (s, 6H), 2.21-2.10 (m, 3H), 2.05-2.02 (m, 1H), 1.80 (dd, J_1 = 14.5 Hz, J_2 = 1.7 Hz, 1H), 1.73-1.54 (m, 5H), 1.49 (s, 3H), 1.46-1.41 (m, 1H), 1.35 (d, J = 6.3 Hz, 3H), 1.27-1.21 (m, 6H), 1.19-1.12 (m, 18H), 0.98 (d, J = 7.3 Hz, 3H), 0.85 (t, J = 7.3 Hz, 3H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 176.8, 173.7, 173.3, 150.9, 150.9, 150.8, 150.8, 129.9, 129.8, 125.5, 125.4, 120.4, 120.4, 120.0, 119.9, 109.2, 101.3, 96.2, 86.5, 86.5, 85.5, 85.3, 83.1, 78.3, 77.7, 75.3, 73.3, 73.3, 71.8, 68.6, 63.7, 63.6, 63.5, 49.6, 47.2, 45.3, 40.9, 39.0, 36.3, 35.8, 30.8, 28.2, 28.0, 25.6, 25.4, 23.9, 21.9, 21.3, 18.7,

18.3, 15.9, 14.1, 11.3, 9.9, 9.2; **^{31}P NMR** ($CDCl_3$, 162 MHz) δ -11.7; **IR** (film, cm^{-1}) 3436, 2970, 2937, 2880, 1732, 1491, 1454, 1376, 1291, 1189, 1168, 1050, 1013, 956; **TLC** R_f = 0.27 (9/1 $CHCl_3$/MeOH v/v); **HRMS** (ES^+) *m/z* Calc'd for $C_{55}H_{85}NO_{18}P$ 1078.5504, found 1078.5468 ($M + H^+$).

Preparation of 2', 11-Di-pent-4-enoate-4"-diphenylphosphate erythromycin (11)

2',11-Di-4-pentenoate (**7**, 15.0 mg, 0.0167 mmol) was dissolved in chloroform (1.5 mL) and triethylamine (16.3 μL, 0.117 mmol) was added. 4-Dimethylaminopyridine catalyst (0.6 mg, 4.91 μmol) and diphenyl chlorophosphate (20.8 μL, 0.100 mmol) was added last. The reaction was allowed to stir for 6 hours at which time, was deemed complete by TLC. The crude mixture was loaded onto a silica gel column ($CHCl_3$/MeOH 95/5 v/v) and purified. Residual diphenylmethylphosphate coeluted with the product and was removed with a second silica gel purification (EtOAc/hexanes 4/1 v/v) to afford the colorless solid (9.8 mg, 52%).

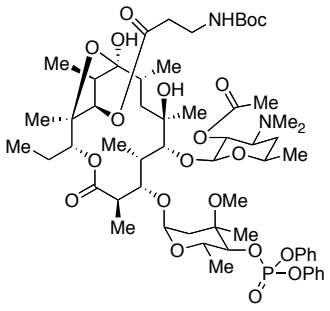


$[\alpha]^{20.0}$: -35.0 (*c* 0.5, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.33-7.29 (m, 4H), 7.21-7.15 (m, 6H), 5.89-5.79 (m, 2H), 5.09 (s, 1H), 5.05-4.99 (m, 4H), 4.88 (d, *J* = 4.7 Hz, 1H), 4.86 (s, 1H), 4.77 (dd, J_1 = 10.5 Hz, J_2 = 7.6 Hz, 1H), 4.63 (d, *J* = 7.5 Hz, 1H), 4.47-4.44 (m, 1H), 4.34 (d, *J* = 6.8 Hz, 1H), 4.28 (t, *J* =10.1 Hz, 1H), 3.61-3.53 (m, 2H), 3.38 (s, 1H), 3.36 (s, 3H), 2.68-2.63 (m, 2H), 2.43-2.35 (m, 9H), 2.24 (s, 6H), 2.20-2.12 (m, 3H), 1.95 (s, 1H), 1.81 (d, *J* = 14.3 Hz, 1H), 1.65-1.59 (m, 4H), 1.49 (s, 3H), 1.46-1.41 (m, 1H), 1.35 (d, *J* = 6.3 Hz, 3H), 1.28 (d, *J* = 7.6 Hz, 3H), 1.27-1.22 (m, 5H), 1.19-1.12 (m, 11H), 0.98 (d, *J* = 7.3 Hz, 3H), 0.85 (t,

J = 7.3 Hz, 3H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 207.1, 176.7, 172.3, 171.9, 150.9, 150.8, 150.8, 150.7, 137.1, 136.5, 129.9, 129.8, 125.5, 125.4, 120.4, 120.4, 120.0, 119.9, 115.9, 115.3, 109.1, 101.2, 96.1, 86.5, 86.4, 85.5, 85.3, 82.9, 78.0 77.6, 75.2, 73.3, 73.3, 71.9, 68.6, 63.8, 63.5, 63.5, 49.6, 47.2, 45.3, 40.8, 39.0, 36.3, 35.8, 34.0, 33.9, 31.1, 30.3, 29.1, 28.8, 25.6, 25.4, 23.9, 21.9, 21.3, 18.7, 18.3, 15.9, 14.1, 11.3, 10.0; **^{31}P NMR** ($CDCl_3$, 162 MHz) δ -11.7; **IR** (film, cm^{-1}) 3436, 2970, 2937, 2884, 1736, 1495, 1454, 1381, 1291, 1193, 1168, 1050, 1009, 960; **TLC** R_f = 0.52 (9/1 $CHCl_3$/MeOH v/v); **HRMS** (ES^+) *m/z* Calc'd for $C_{59}H_{89}NO_{18}P$ 1130.5817, found 1130.5825 ($M + H^+$).

Preparation of 2'-Acetyl-11-3-(Boc)-propanoyl-4"-diphenylphosphate erythromycin (12)

2'-acetyl-11-3-(Boc)-propanoyl erythromycin (**14**, 12.3 mg, 0.0130 mmol) was dissolved in chloroform (1.0 mL), and triethylamine (12.7 μL, 0.0909 mmol) was added. 4-Dimethylaminopyridine (0.5 mg, 3.90 μmol) and diphenyl chlorophosphate (16.1 μL, 0.0779 mmol) was added. The reaction was complete after 2 hours and was purified by loading directly onto a silica gel column ($CHCl_3$/MeOH 95/5 v/v). A minor impurity was detected so the product was repurified by silica gel chromatography (EtOAc) to afford the pure product (10.6 mg, 69%).

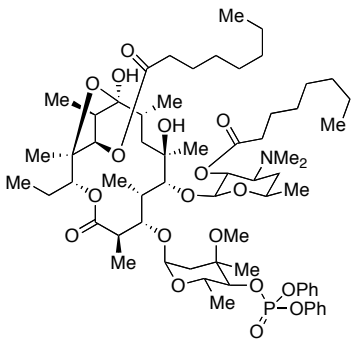


$[\alpha]^{20.0}$: -45.7 (*c* 0.5, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.32-7.30 (m, 4H), 7.21-7.15 (m, 6H), 5.27 (br s, 1H), 5.04 (dd, J_1 = 10.3 Hz, J_2 = 2.4 Hz, 1H), 4.90 (s, 1H), 4.86 (d, J = 4.8 Hz, 1H), 4.77 (dd, J_1 = 10.5 Hz, J_2 = 7.6 Hz, 1H), 4.60 (d, J = 7.5 Hz, 1H), 4.47-4.40 (m, 1H), 4.35 (d, J = 6.8 Hz, 1H),

4.32-4.24 (m, 1H), 3.57 (d, J = 8.1 Hz, 1H), 3.55-3.44 (m, 2H), 3.40-3.36 (m, 1H), 3.35 (s, 3H), 3.25-2.80 (m, 2H), 2.73-2.56 (m, 3H), 2.51-2.39 (m, 2H), 2.26 (s, 6H), 2.20-2.08 (m, 3H), 2.07 (s, 3H), 1.98 (s, 1H), 1.90 (qd, J_1 = 7.3 Hz, J_2 = 3.1 Hz, 1H), 1.78 (d, J = 12.8 Hz, 1H), 1.67-1.56 (m, 4H), 1.50 (s, 3H), 1.43 (s, 9H), 1.34 (d, J = 6.3 Hz, 3H), 1.30-1.12 (m, 18H), 0.98 (d, J = 7.2 Hz, 3H), 0.88 (t, J = 7.3 Hz, 3H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 176.8, 175.8, 171.7, 170.0, 155.9, 150.9, 150.9, 150.8, 150.8, 129.9, 129.8, 125.5, 125.4, 120.4, 120.4, 120.0, 120.0, 109.2, 101.3, 99.2, 96.3, 94.3, 86.5, 86.5, 85.9, 84.6, 83.2, 79.6, 78.3, 77.8, 76.4, 76.2, 75.3, 73.3, 73.3, 72.0, 68.6, 63.6, 63.2, 49.5, 49.0, 47.2, 45.1, 44.4, 40.9, 39.6, 39.1, 38.5, 36.7, 35.8, 35.4, 33.8, 31.5, 31.0, 30.9, 28.6, 25.6, 25.5, 23.9, 21.9, 21.6, 21.2, 18.6, 18.3, 15.9, 14.0, 11.4, 10.1, 8.6; **^{31}P NMR** ($CDCl_3$, 162 MHz) δ -11.8; **IR** (film, cm^{-1}) 3415, 2974, 2933, 2886, 1736, 1716, 1491, 1454, 1364, 1246, 1164, 1042, 1009, 960; **TLC** R_f = 0.46 (9/1 $CHCl_3$/MeOH v/v); **HRMS** (ES^+) *m/z* Calc'd for $C_{59}H_{92}N_2O_{20}P$ 1179.5981, found 1179.5956 ($M + H^+$).

Preparation of 2', 11-Dioctanoyl-4"-diphenylphosphate erythromycin (13)

2',11-Di-octonoate (**9**, 15.0 mg, 0.0152 mmol) was dissolved in chloroform (1.5 mL) and triethylamine (9.0 μL, 0.0638 mmol) was added. 4-Dimethylaminopyridine (0.2 mg, 1.52 μmol) and diphenyl chlorophosphate (10.8 μL, 0.0517 mmol) was added. The reaction was incomplete after 4 hours so another portion of 4-dimethylaminopyridine was added. After 7 hours, the reaction was deemed complete and was loaded directly onto a silica gel column ($CHCl_3$/MeOH 95/5 v/v) to afford material that had minor impurities. These impurities were easily separated by a second silica gel purification (hexanes/EtOAc 1/1 v/v) to afford the product (10.8 mg, 58%).

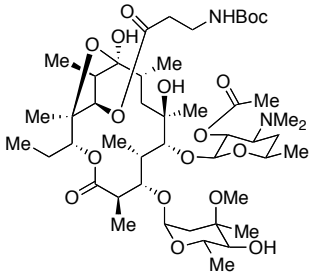


$[\alpha]^{20.0}$: -29.3 (*c* 0.5, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.32-7.29 (m, 4H), 7.21-7.15 (m, 6H), 5.03 (dd, J_1 = 10.2 Hz, J_2 = 2.2 Hz, 1H), 4.88 (d, J = 4.6 Hz, 1H), 4.83 (s, 1H), 4.78 (dd, J_1 = 10.4 Hz, J_2 = 7.6 Hz, 1H), 4.64 (d, J = 7.5 Hz, 1H), 4.48-4.42 (m, 1H), 4.34 (d, J = 6.3 Hz, 1H), 4.28 (t, J = 10.0 Hz, 1H), 3.59-3.51 (m, 2H), 3.40 (s, 1H), 3.37 (s, 3H), 2.69-2.64 (m, 2H), 2.42 (d, J = 15.2 Hz, 1H), 2.35-2.19 (m, 4H), 2.25 (s, 6H), 2.20-2.09 (m, 3H), 1.96 (s, 1H), 1.82 (d, J = 14.3 Hz, 1H), 1.65-1.54 (m, 8H), 1.48 (s, 3H), 1.46-1.39 (m, 1H), 1.35 (d, J = 6.3 Hz, 3H), 1.32-1.21 (m, 24H), 1.17-1.12 (m, 12H), 0.98 (d, J = 7.3 Hz, 3H), 0.90-0.84 (m, 9H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 205.1, 176.9, 173.1, 172.6, 150.9, 150.9, 150.8, 150.8, 129.9, 129.8, 125.5, 125.4, 120.5, 120.4, 120.0, 119.9, 109.2, 101.1, 96.1, 86.5, 86.4, 85.4, 85.2, 82.7, 77.9, 77.7, 75.2, 73.3, 73.3, 71.7, 68.6, 63.8, 63.6, 63.6, 49.5, 47.1, 45.2, 41.3, 40.9, 39.1, 36.8, 35.8, 34.9, 34.9, 31.9, 31.8, 30.5, 29.4, 29.3, 29.2, 29.2, 25.6, 25.5, 25.2, 25.0, 23.9, 22.8, 22.8, 21.9, 21.3, 18.5, 18.3, 15.9, 14.2, 14.2, 13.8, 11.3, 10.0; **^{31}P NMR** ($CDCl_3$, 162 MHz) δ -11.7; **IR** (film, cm^{-1}) 3428, 2962, 2934, 2860, 1728, 1495, 1462, 1385, 1283, 1189, 1172, 1046, 1013, 960; **TLC** R_f = 0.43 (9/1 $CHCl_3$/MeOH v/v); **HRMS** (ES^+) *m/z* Calc'd for $C_{65}H_{105}NO_{18}P$ 1218.7069, found 1218.7028 ($M + H^+$).

Preparation of 2'-Acetyl-11-3-(Boc)-propanoylerythromycin (14)

11-3-(Boc)-propanoylerythromycin (**18**, 30.0 mg, 0.0331 mmol) was dissolved in chloroform (2.0 mL), and triethylamine (13.9 μL, 0.0994 mmol) was added. Acetic anhydride (9.4 μL, 0.0994 mmol) was added and the reaction was stirred for sixteen

hours. The reaction was purified by loading directly onto silica gel ($CHCl_3$/MeOH 95/5 v/v) to afford the colorless solid (19.6 mg, 62%).



$[\alpha]^{20.0}$: -34.7 (*c* 1.0, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 5.31 (br s, 1H), 5.04 (dd, J_1 = 10.4 Hz, J_2 = 2.4 Hz, 1H), 4.90 (s, 1H), 4.81 (d, J = 4.5 Hz, 1H), 4.77 (dd, J_1 = 10.5 Hz, J_2 = 7.5 Hz, 1H), 4.50 (d, J = 7.5 Hz, 1H), 4.36 (d, J = 7.2 Hz, 1H), 3.98 (dd, J_1 = 9.4 Hz, J_2 = 6.3 Hz, 1H), 3.56 (d, J = 8.6 Hz, 1H), 3.54-3.45 (m, 2H), 3.40-3.35 (m, 1H), 3.33 (s, 3H), 3.04 (t, J = 9.7 Hz, 1H), 2.73-2.56 (m, 3H), 2.51-2.43 (m, 1H), 2.38 (d, J = 15.2 Hz, 1H), 2.28 (d, J = 8.0 Hz, 1H), 2.26 (s, 6H), 2.18-2.10 (m, 3H), 2.06 (s, 3H), 1.79-1.71 (m, 2H), 1.64 (qd, J_1 = 7.4 Hz, J_2 = 4.9 Hz, 1H), 1.55 (dd, J_1 = 15.2 Hz, J_2 = 4.8 Hz, 1H), 1.50 (s, 3H), 1.42 (s, 9H), 1.31-1.22 (m, 18H), 1.17 (d, J = 7.1 Hz, 3H), 1.13 (d, J = 7.0 Hz, 3H), 0.95 (d, J = 7.2 Hz, 3H), 0.87 (t, J = 7.4 Hz, 3H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 176.8, 175.8, 171.7, 170.0, 155.9, 109.2, 101.9, 96.6, 85.8, 85.2, 83.7, 79.5, 78.4, 77.8, 75.3, 72.9, 71.9, 69.0, 65.6, 63.7, 49.5, 47.3, 45.4, 40.9, 40.8, 40.8, 38.9, 36.5, 36.0, 35.4, 35.1, 30.5, 28.6, 28.6, 25.8, 25.4, 23.9, 21.8, 21.6, 21.2, 18.7, 18.3, 15.9, 14.2, 11.4; **IR** (film, cm^{-1}) 3457, 2974, 2934, 2872, 2831, 2782, 1728, 1503, 1450, 1373, 1246, 1168, 1054, 1001; **TLC** R_f = 0.32 (9/1 $CHCl_3$/MeOH v/v); **HRMS** (ES^+) *m/z* Calc'd for $C_{47}H_{83}N_2O_{17}$ 947.5692, found 947.5649 ($M + H^+$).

Preparation of 11-Propionylerythromycin (16)

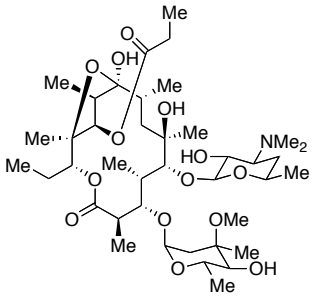
Erythromycin A (**2**, 100 mg, 0.136 mmol) was dissolved in $CHCl_3$ (1.40 mL) in a flame-dried flask. Triethylamine (5 equiv., 94.9 μL, 0.681 mmol) and the catalyst (**5**, 10 mol%, 20.0 mM soln., in CH_2Cl_2, 0.681 mL, 13.6 μmol) were then introduced

sequentially. Propionic anhydride (10 equiv., 175 μL, 1.36 mmol) was introduced and the reaction was allowed to stir at 25 °C. Reaction progress was monitored by ^{1}H NMR (400 MHz) by removing 100 μL aliquots followed by a methanol quench. The resulting solutions were passed through a silica gel plug eluting with a $CHCl_3$/MeOH (95/5 v/v) solvent system and concentrated under high vacuum. After approximately 93 hours, the reaction was deemed complete and was quenched with methanol and concentrated to dryness. The crude reaction was subjected to methanolysis for 72 hours and then concentrated to dryness. The crude reaction was loaded onto a silica gel column ($CHCl_3$/MeOH/NH_4OH, 14 M; 95/5/1 v/v/v) to afford recovered erythromycin A (**2**, 6.7 mg, 7% yield) coeluted with 11-propionoylerythromycin (**16**, 30.3 mg, 28% yield) and 4”-propionoylerythromycin (8.2 mg, 8% yield) coeluted with 4”,11-dipropionoylerythromycin (37.2 mg, 32% yield). The relative ratios of products by ^{1}H NMR was utilized along with weighed product to calculate yields. Pure 11-propionoylerythromycin (**16**) was isolated by semi-preparative HPLC and was found to be a colorless solid.

Use of *ent*-5 peptide catalyst.

Erythromycin A (**2**, 100 mg, 0.136 mmol) was dissolved in $CHCl_3$ (2.00 mL) in a flame-dried flask. Triethylamine (94.9 μL, 0.681 mmol) and the catalyst (***ent*-5**, 12.2 mg, 0.0136 mmol) were added. Propionic anhydride (174.6 μL, 1.36 mmol) was added last. The reaction was allowed to stir at room temperature for 22 hours and then quenched with methanol. The crude reaction was purified by silica gel chromatography ($CHCl_3$/MeOH 95/5 v/v) to separate the 2’+2’,11 and 2’,4”+2’,4”, 11 material. The separated mixtures were then concentrated and weighed. ^{1}H NMR analysis of the

samples allowed a ratio of products calculated with the isolated weights. The NMR analysis provided the following yields: 2'-propionoylerythromycin (30.0 mg, 28%), 2',11-dipropionoylerythromycin (**6**, 58.0 mg, 50%), 2',4"-dipropionoylerythromycin (5.0 mg, 4%) and 2',4",11-tripropionoylerythromycin (8.4 mg, 7%).



$[\alpha]^{20.0}$: -36.0 (*c* 1.0, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 5.06 (dd, J_1 = 10.8 Hz, J_2 = 2.8 Hz, 1H), 4.92 (d, *J* = 2.4 Hz, 1H), 4.82 (d, *J* = 4.8 Hz, 1H), 4.38 (d, *J* = 7.2 Hz, 1H), 4.31 (d, *J* = 8.0 Hz, 1H), 4.05 (dd, J_1 = 11.6 Hz, J_2 = 8.4 Hz, 1H), 3.58 (d, *J* = 8.0 Hz, 1H), 3.54-3.47 (m, 2H), 3.30 (s, 3H), 3.27 (dd, J_1 = 10.0 Hz, J_2 = 7.2 Hz, 1H), 3.02 (d, *J* = 9.6 Hz, 1H), 2.68 (pent, *J* = 7.6 Hz, 1H), 2.53-2.46 (m, 1H), 2.42-2.35 (m, 4H), 2.32 (s, 6H), 2.24-2.15 (m, 3H), 2.09 (br s, 1H), 1.89 (d, *J* = 12.8 Hz, 1H), 1.69-1.62 (m, 2H), 1.53 (dd, J_1 = 15.2 Hz, J_2 = 4.8 Hz, 1H), 1.50 (s, 3H), 1.42 (ddd, J_1 = 14.0 Hz, J_2 = 6.8 Hz, J_3 = 2.8 Hz, 1H), 1.33-1.17 (m, 22H), 1.12 (dd, J_1 = 7.2 Hz, J_2 = 3.2 Hz, 6H), 0.85 (t, *J* = 7.6 Hz, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 176.5, 173.8, 108.8, 104.5, 97.0, 85.5, 85.0, 79.1, 78.2, 78.1, 77.4, 74.7, 72.7, 70.9, 69.4, 65.7, 65.5, 49.6, 46.9, 46.6, 40.9, 40.6, 38.7, 37.6, 35.1, 29.9, 29.3, 28.3, 25.9, 24.2, 21.8, 21.5, 18.8, 18.4, 15.5, 14.9, 11.5, 10.5, 9.4; **IR** (film, cm^{-1}) 3471, 2874, 2942, 2892, 1727, 1463, 1381, 1281, 1174, 1111, 1061, 1017, 771; **HRMS** (EI^+) *m/z* Calc'd for $C_{40}H_{72}NO_{14}$ 790.4953, found 790.4969 ($M + H^+$).

Preparation of 11-Pent-4-enoate erythromycin (17)

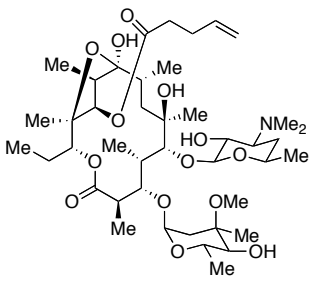
Erythromycin A (**2**, 100 mg, 0.136 mmol) was dissolved in $CHCl_3$ (1.50 mL) in a flame-dried flask. Triethylamine (5 equiv., 94.9 μL, 0.681 mmol) and the catalyst (**5**, 10 mol%, 20.0 mM soln., in CH_2Cl_2, 0.681 mL, 13.6 μmol) were then introduced

sequentially. 4-pentenoic anhydride (10 equiv., 248 mg, 1.36 mmol) was introduced and the reaction was allowed to stir at 25 °C. Reaction progress was monitored by ^{1}H NMR (400 MHz) by removing 100 μL aliquots followed by a methanol quench. The resulting solutions were passed through a silica gel plug eluting with a $CHCl_3$/MeOH (95/5 v/v) solvent system and concentrated under high vacuum. After approximately 41 hours, the reaction was deemed complete and was quenched with methanol and concentrated to dryness. The crude reaction was loaded onto a silica gel column ($CHCl_3$/MeOH, 95/5 v/v) to afford 2'-pent-4-enoate erythromycin (16.3 mg, 15% yield) coeluted with 2',11-di-pent-4-enoate erythromycin (**7**, 68.7 mg, 56% yield) and 2',4"-di-pent-4-enoate erythromycin (12.3 mg, 10% yield) coeluted with 2',4",11-tri-pent-4-enoate erythromycin (20.5 mg, 15% yield). The relative ratios of products by ^{1}H NMR was utilized along with weighed product to calculate yields. After 72 hours of methanolysis, pure 11-pent-4-enoate erythromycin (**17**) was isolated, by silica gel chromatography ($CHCl_3$/MeOH, 90/10 v/v) from the mixture of Ery A (**2**) and 11-monopent-4-enoate (**17**). The product was found to be a colorless solid.

Use of *ent*-5 peptide catalyst.

Erythromycin A (**2**, 100 mg, 0.136 mmol) was dissolved in $CHCl_3$ (2.00 mL) in a flame-dried flask. Triethylamine (94.5 μL, 0.681 mmol) and the catalyst (*ent*-**5**, 12.2 mg, 0.0136 mmol) were added. The symmetrical anhydride (248.0 mg, 1.36 mmol) was added last. The reaction was stirred for 43 hours and then quenched with methanol and concentrated. The 2' and 2',11 material was separated from the 2',4" and 2',4",11 compounds by silica gel chromatography ($CHCl_3$/MeOH, 95/5 v/v). Utilizing ^{1}H NMR analysis, the yields were: 2'-pent-4-enoate erythromycin (36.9 mg, 33%) and 2',11-di-

pent-4-enoate erythromycin (**7**, 61.5 mg, 50%), 2',4"-di-pent-4-enoate erythromycin (7.8 mg, 6%) and 2',4",11 (13.2 mg, 10%). The product was found to be a colorless solid.



$[\alpha]^{20.0}$: -27.2 (*c* 1.0, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 5.91-5.81 (m, 1H), 5.11-5.01 (m, 3H), 4.93 (d, J = 2.4 Hz, 1H), 4.82 (d, J = 4.4 Hz, 1H), 4.38 (d, J = 7.2 Hz, 1H), 4.30 (d, J = 7.2 Hz, 1H), 4.05 (dd, J_1 = 11.6 Hz, J_2 = 6.8 Hz, 1H), 3.58 (d, J = 7.6 Hz, 1H), 3.54-3.47 (m, 2H), 3.32-3.28 (m, 4H), 3.02 (d, J = 9.6 Hz, 1H), 2.70-2.59 (m, 2H), 2.52-2.42 (m, 3H), 2.38-2.32 (m, 8H), 2.25-2.16 (m, 3H), 1.88 (d, J = 13.6 Hz, 1H), 1.73 (d, J = 10.0 Hz, 1H), 1.65 (ddd, J_1 = 14.4 Hz, J_2 = 6.8 Hz, J_3 = 2.4 Hz 1H), 1.55 (dd, J_1 = 15.2 Hz, J_2 = 4.8 Hz, 1H), 1.50 (s, 3H), 1.45-1.36 (m, 2H), 1.33-1.18 (m, 19H), 1.11 (dd, J_1 = 6.8 Hz, J_2 = 4.8 Hz, 6H), 0.85 (t, J = 7.2 Hz, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 176.6, 172.5, 136.6, 115.7, 108.7, 104.5, 97.2, 85.6, 84.9, 79.3, 78.3, 78.1, 77.4, 74.6, 72.8, 70.9, 69.3, 65.8, 65.4, 49.6, 46.9, 46.8, 41.0, 40.6, 38.7, 37.7, 35.1, 34.1, 30.0, 29.6, 28.9, 25.9, 24.2, 21.9, 21.4, 18.8, 18.4, 15.5, 15.1, 11.6, 10.6; **IR** (film, cm^{-1}) 3477, 2986, 2948, 2886, 1734, 1646, 1576, 1463, 1381, 1255, 1167, 1054, 1016, 953, 758; **HRMS** (EI^+) *m/z* Calc'd for $C_{42}H_{74}NO_{14}$ 816.5109, found 816.5133 (M + H^+)

Preparation of 11-3-(Boc)-propanoylerythromycin (18)

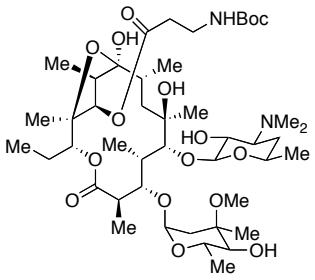
Erythromycin A (**2**, 100 mg, 0.136 mmol) was dissolved in $CHCl_3$ (1.50 mL) in a flame-dried flask. Triethylamine (5 equiv., 94.9 μL, 0.681 mmol) and peptide catalyst (**5**, 10 mol%, 20.0 mM solution in CH_2Cl_2, 0.681 mL, 13.6 μmol) were then introduced sequentially. 3-Boc-propanoic pivalic anhydride (10 equiv., 372 mg, 1.36 mmol) was

introduced and the reaction was allowed to stir at 25 °C. Reaction progress was monitored by ^{1}H NMR (400 MHz) by removing 100 μL aliquots followed by a methanol quench. The resulting solutions were passed through a silica gel plug eluting with a $CHCl_3$/MeOH (95/5 v/v) solvent system and concentrated under high vacuum. After approximately 54 hours, the reaction was deemed complete and was quenched with methanol and concentrated to dryness. The crude reaction was loaded onto a silica gel column ($CHCl_3$/MeOH, 95/5 v/v) to afford 2'-3-(Boc)-propanoylerythromycin (22.0 mg, 18% yield) coeluted with 2',11-di-3-(Boc)-propanoylerythromycin (**8**, 77.4 mg, 53% yield) and 2',4"-di-3-(Boc)-propanoylerythromycin (**3.46b**, 1.1 mg, 1% yield) coeluted with 2',4",11-tri-3-(Boc)-propanoylerythromycin (3.5 mg, 2% yield). The relative ratios of products by ^{1}H NMR was utilized along with weighed product to calculate yields. Pure 11-3-(Boc)-propanoylerythromycin (**18**) was isolated after methanolysis, along with 2'-3-(Boc)-propanoylerythromycin, by recrystallization with from acetonitrile to afford a white solid.

Use of *ent*-5 peptide catalyst.

Erythromycin A (**2**, 100 mg, 0.136 mmol) was dissolved in $CHCl_3$ (2.00 mL) in a flame-dried flask. Triethylamine (94.5 μL, 0.681 mmol) and the catalyst (***ent*-5**, 12.2 mg, 0.0136 mmol) were added. The mixed anhydride (372 mg, 1.36 mmol) was added dropwise and the reaction was allowed to stir at room temperature for 65 hours. The reaction was then quenched and concentrated. The crude mixture was purified by silica gel chromatography (hexanes/EtOAc 4/1 v/v) three times. The 2' and the 2',11 material was inseparable so the yield was calculated using total mass and NMR integration ratios. The NMR analysis provided the following yields: 2'-3-(Boc)-propanoylerythromycin

(16.0 mg, 13%), 2',11-di-3-(Boc)-propanoylerythromycin (**8**, 99.0 mg, 68%), and 2',4"-di-3-(Boc)-propanoylerythromycin (4.4 mg, 3%).



$[\alpha]^{20.0}$: -30.5 (*c* 1.0, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 5.43 (br s, 1H), 5.05 (dd, J_1 = 10.4 Hz, J_2 = 2.4 Hz, 1H), 4.93 (s, 1H), 4.81 (d, *J* = 4.8 Hz, 1H), 4.40-4.37 (m, 2H), 4.03 (m, 1H), 3.58 (d, *J* = 8.8 Hz, 1H), 3.52-3.42 (m, 3H), 3.30 (s, 3H), 3.25 (dd, J_1 = 10.4 Hz, J_2 = 7.2 Hz, 1H), 3.02 (t, *J* = 9.6 Hz, 1H), 2.71 (p, *J* = 7.2 Hz, 1H), 2.59 (dd, J_1 = 12.8 Hz, J_2 = 6.8 Hz, 1H), 2.53-2.47 (m, 1H), 2.37 (d, *J* = 15.2 Hz, 1H), 2.29 (s, 6H), 2.23-2.16 (m, 3H), 2.01 (s, 1H), 1.90 (d, *J* = 12.4 Hz, 1H), 1.69-1.61 (m, 2H), 1.53 (dd, J_1 = 15.2 Hz, J_2 = 4.4 Hz, 1H), 1.51 (s, 3H), 1.43 (s, 9H), 1.40-1.37 (m, 1H), 1.33-1.21 (m, 19H), 1.18 (d, *J* = 6.8 Hz, 3H), 1.12 (t, *J* = 7.6 Hz, 6H), 0.87 (t, *J* = 7.2 Hz, 3H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 176.7, 171.7, 156.0, 108.9, 104.4, 97.2, 85.8, 85.0, 84.7, 79.3, 79.1, 78.2, 78.1, 77.4, 74.9, 72.7, 70.9, 69.4, 65.7, 65.5, 49.5, 47.2, 46.2, 40.7, 40.5, 38.5, 37.0, 36.3, 35.4, 35.1, 29.0, 26.1, 25.5, 24.1, 21.8, 21.4, 18.8, 18.3, 15.6, 14.9, 11.5, 10.4; **IR** (film, cm^{-1}) 3458, 2974, 2936, 2879, 1734, 1514, 1469, 1381, 1255, 1174, 1061, 1016, 758; **HRMS** (EI^+) *m/z* Calc'd for $C_{45}H_{81}N_2O_{16}$ 905.5586, found 905.5582 ($M + H^+$).

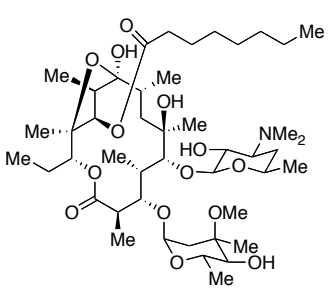
Preparation of 11-Octanoylerythromycin (19)

Erythromycin A (**2**, 100 mg, 0.136 mmol) was dissolved in $CHCl_3$ (1.50 mL) in a flame-dried flask. Triethylamine (5 equiv., 94.9 μL, 0.681 mmol) and peptide catalyst (**5**, 10 mol%, 20.0 mM solution in CH_2Cl_2, 0.681 mL, 13.6 μmol) were then introduced sequentially. Octanoyl anhydride (10 equiv., 368 mg, 1.36 mmol) was introduced and the

reaction was allowed to stir at 25 °C. Reaction progress was monitored by ^{1}H NMR (400 MHz) by removing 100 μL aliquots followed by a methanol quench. The resulting solutions were passed through a silica gel plug eluting with a $CHCl_3$/MeOH (95/5 v/v) solvent system and concentrated under high vacuum. After approximately 120 hours, the reaction was deemed complete and was quenched with methanol and concentrated to dryness. The crude reaction mixture was loaded onto a silica gel column ($CHCl_3$/MeOH, 95/5 v/v) to afford 2'-octanoylerythromycin (7.6 mg, 7% yield) that had coeluted with 2',11-dioctanoylerythromycin (**9**, 77.3 mg, 58% yield). 2',4"-Dioctanoylerythromycin (**15**) and 2',4",11 trioctanoyl erythromycin coeluted with methyloctanoate; therefore, the mixture was cleaved in methanol for 72 hours. 4"-octanoylerythromycin (7.5 mg, 6% yield) separated from 4",11-dioctanoyl erythromycin (30.7 mg, 23% yield by silica gel chromatography. The relative ratios of products by ^{1}H NMR was utilized along with weighed product to calculate yields. Pure 11-octanoylerythromycin (**19**) was isolated after 72 hours of methanolysis by silica gel chromatography ($CHCl_3$/MeOH, 90/10 v/v) from the mixture of Ery A (**2**) and 11-octanoylerythromycin (**19**). The product was found to be a colorless oil.

Use of *ent*-5 peptide catalyst. Erythromycin A (**2**, 100 mg, 0.136 mmol) was dissolved in $CHCl_3$ (2.00 mL) in a flame-dried flask. Triethylamine (94.5 μL, 0.681 mmol) and the catalyst (***ent*-5**, 12.2 mg, 0.0136 mmol) were added. The symmetrical anhydride (368.4 mg, 1.36 mmol) was added last. The reaction was stirred for 96 hours, quenched with methanol and concentrated. The 2'-monooctanoate and 2',11-dioctanoate material was separated from the 2',4"-dioctanoate and 2',4",11-trioctanoate compounds by silica gel chromatography ($CHCl_3$/MeOH, 95/5 v/v). Utilizing ^{1}H NMR analysis, the yields were:

2'-octanoylerythromycin (8.6 mg, 10%) and 2',11-dioctanoylerythromycin (**9**, 62.0 mg, 63%). The 2',4" and 2',4",11 material were coeluting with the methyl ester of the anhydride and were then subjected to methanolysis for three days. The reaction was concentrated and purified by silica gel chromatography ($CHCl_3$/MeOH 95/5 v/v) to afford 4",11-dioctanoylerythromycin (**15**, 7.8 mg, 6%) and 11-octanoylerythromycin (**19**, 9.4 mg, 8%). 4"-monooctanoate material was not observed. The total yield for the 11-octanoylerythromycin material (2',11 and 11) was 71%.



$[\alpha]^{20.0}$: -27.3 (*c* 1.0, $CHCl_3$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 5.06 (dd, J_1 = 10.0 Hz, J_2 = 2.4 Hz, 1H), 4.91 (d, J = 2.4 Hz, 1H), 4.82 (d, J = 4.8 Hz, 1H), 4.38 (d, J = 7.2 Hz, 1H), 4.31 (d, J = 6.8 Hz, 1H), 4.05 (dd, J_1 = 9.2 Hz, J_2 = 6.0 Hz, 1H), 3.59 (d, J = 8.0 Hz, 1H), 3.54-3.47 (m, 2H), 3.31 (s, 3H), 3.27 (dd, J_1 = 10.4 Hz, J_2 = 7.6 Hz, 1H), 3.02 (d, J = 8.8 Hz, 1H), 2.68 (pent, J = 7.6 Hz, 1H), 2.54-2.46 (m, 1H), 2.39-2.33 (m, 4H), 2.31 (s, 6H), 2.25-2.14 (m, 4H), 1.89 (d, J = 13.6 Hz, 1H), 1.69-1.61 (m, 4H), 1.53 (dd, J_1 = 15.2 Hz, J_2 = 4.8 Hz, 1H), 1.50 (s, 3H), 1.42 (ddd, J_1 = 14.4 Hz, J_2 = 7.2 Hz, J_3 = 3.2 Hz, 1H), 1.33-1.18 (m, 27H), 1.12 (dd, J_1 = 7.2 Hz, J_2 = 5.2 Hz, 6H), 0.89-0.83 (m, 6H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 176.7, 173.3, 108.8, 104.5, 97.0, 85.4, 85.2, 85.0, 79.1, 78.2, 78.1, 77.4, 74.8, 72.7, 70.9, 69.4, 65.7, 65.4, 49.5, 46.9, 46.5, 40.8, 40.5, 38.5, 37.4, 35.1, 34.9, 31.8, 29.3, 29.2, 25.8, 25.7, 25.0, 24.1, 22.8, 21.7, 21.4, 18.7, 18.3, 15.5, 14.8, 14.2, 11.4, 10.4; **IR** (film, cm^{-1}) 3490, 2974, 2948, 2879, 1740, 1463, 1381, 1249, 1174, 1117, 1054, 1016, 960, 752; **HRMS** (EI^+) *m/z* Calc'd for $C_{45}H_{82}NO_{14}$ 860.5735, found 860.5746 ($M + H^+$).

Assay Method for the Testing of Erythromycin A Analogs

The testing of the erythromycin A analogs utilized the MIC (minimum inhibitory concentration) method and was performed by Janie Merkel at the Center for Chemical Genomics at Yale University.

Compounds

Blinded compounds were received at 640 μg/mL concentration dissolved in 100% DMSO and stored at -20 °C until use. Compounds were taken out of the -20 °C freezer to thaw overnight at room temperature on the day prior to the experiment. The morning of the experiment, fresh compound stock plates were made. Each compound was first diluted 10-fold in Mueller Hinton II broth to a concentration of 64 μg/mL, 10% DMSO on the left most column (column 1) of a 96-well deepwell block (2 mL volume per well) to a volume of 660 μL. 330 μL of Mueller Hinton II broth was dispensed to the remaining 11 columns (columns 2-12) of the 96-well block. Two-fold serial dilutions were made starting with the solution in column 1, moving left-to-right across the plate, by transferring 330 μL into the 330 μL of broth already in the wells. Each dilution was mixed by pipetting the mixture solution into and out of pipet tips three times at a volume of 600 μL. A Matrix Impact eight-channel multi-channel pipetman use used for the dilution. No compound was added to column 12, the compound-free control column. The 8th row (row H) was a vehicle control row, containing the DMSO used to dissolve the compounds. Three source 96-well plates were created, each containing 11 dilutions of 7 test compounds, a DMSO dilution series, and no compound controls. The test compound concentrations are consistent with recommended erythromycin MIC testing concentrations for 12-point dilution series (see Table).

Compound concentrations and DMSO percentages of 96-well compound blocks		
96-well block column number	**Compound concentration of stock plate in ug/mL (2X final assay concentration)**	**DMSO concentration of stock plate (2X final assay concentration)**
1	64	10%
2	32	5%
3	16	2.5%
4	8	1.25%
5	4	0.625%
6	2	0.313%
7	1	0.156%
8	0.5	0.078%
9	0.25	0.039%
10	0.125	0.020%
11	0.0625	0.010%
12	No compound	0%

Bacterial strains

Six bacterial strains were tested in the assay (see Table). They were received in lyophilized form as Kwik-Stiks from commercial sources, and stored at 4 °C until use. Working in a biosafety hood with ventilated air handling and according to standard BL2 practices, Kwik-Stik buffers at the top of the sticks were released from sealed sterile ampules by pinching through the stick. The buffer flowed to the bottom chamber to dissolve the lyophilized bacterial pellet. Pellets were squeezed gently through the stick to help further dissolve them. The immersed Q-tip within the stick was taken out and swabbed onto the surface of Petri dishes with Trypticase soy agar supplemented with 5% sheep blood (TSA II). Plates were inverted and incubated in at 35 °C overnight. Dense

lawns of bacteria grew on each plate. Plates were sealed and stored at 4 °C for several weeks.

Two days prior to the experiment, cultures were re-streaked onto fresh TSA II plates, inverted and incubated at 35 °C overnight to generate isolated colonies.

One day prior to the experiment, 2 mL of Mueller Hinton II broth was transferred into sterile 15 mL disposable culture tubes. A sterile pipet tip transferred bacterial colonies from the restreaked plates into the media in the tubes. Tubes were incubated at 35 °C overnight to generate dense starter cultures for the day of the experiment.

Bacterial strains tested
Enterococcus faecalis ATCC 29212
Escherichia coli 25922
Escherichia coli 35218
Pseudomonas aeruginosa ATCC 27853
Staphylococcus aureus ATCC 29213
Staphylococcus aureus ATCC 700699 (TetR)
No bacterial control (control for contamination of media)

MIC Testing

Experiments presented below were modified slightly from the batch microdilution method commonly used to further reduce the experimental scale to 384-well format from 96-well format. This reduction enabled us to perform quadruplicate measurements for each compound, concentration and bacterial strain. Compounds were transferred from 96-well deepblocks into 384-well translucent plastic assay plates. Assay plates are sterile, with a tissue-culture treated surface and provided with a lid, part number Corning 3701. Transfers were performed on the Tecan Aquarius liquid handling robot, which uses a 96-tip block to aspirate 40 μL of each compound from the 96-well deepblock and to dispense 10 μL to four quadrants of each 384-well plate. Therefore, there were four

replicates for every combination of compound-compound concentration-bacterial strain (or no bacterial control). A brief centrifugation step was used to spin the compound broth solution into the bottom of the assay plate.

Bacterial cultures were first diluted in Mueller Hinton II broth to achieve visual turbidity equivalent to a 0.5 McFarland turbidity unit standard, which is a density that represents 10^5 colony forming units per microliter. These cultures were then diluted 100-fold in Mueller Hinton II broth for a volume of 20 mL in a 50 mL conical tube. Cultures were dispensed to 384-well assay plates containing compound/broth solution using a Thermo Multidrop. Cassette lines were primed with culture, and then 10 μL of culture was dispensed to each well, for a final volume of 20 μL per well. Plates were sealed with parafilm and incubated at 35 °C for 24 hours.

As an additional control, 10 μL of the culture added to assay plates was dissolved in 10 mL 1X Phosphate Buffered Saline (PBS) and 100 μL was added to a TSA II plate and incubated overnight. Colonies were observed on culture plates, but not on the culture-free media-only control plate.

Plates were read using a PerkinElmer Envision platereader using a 620 nm filter to record the optical density of the cultures. Average and standard deviations were calculated from quadruplicate measurements.

In addition to plate reading, visual assessments of culture growth were recorded. MICs are defined as the "lowest concentration of drug that completely inhibits growth of the microorganism as detected by the unaided eye", and so the lowest drug concentration where no bacteria were present was scored as the MIC in visual reads. There was an

excellent correlation of growth/non-growth among quadruplicate wells. No growth was observed on any of the bacterial-free plates suggesting sterility throughout the experiment. Growth was observed for all strains in DMSO-only rows on each assay plate, indicating viable bacteria in solution cultures. Most strains showed reduced ODs at the highest DMSO concentration tested.

The published acceptable MIC ranges for erythromycin with *Staphylococcus aureus* ATCC 29213 is 0.25-1 μg/mL and for *Enterococcus faecalis* ATCC 29212 is 1-4 μg/mL (see chapter, page 160). This experiment yielded an MIC of 1 μg/mL for *Staphylococcus aureus* ATCC 29213 and 2 μg/mL for *Enterococcus faecalis* ATCC 29212, within the acceptable ranges for both organisms when treated with erythromycin.