Supplementary Materials for

Scalable Access to Arylomycins via C–H Functionalization logic

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S3
General Experimental

All reactions were carried out under an inert argon atmosphere with dry solvents under anhydrous conditions unless otherwise stated. Dry acetonitrile (MeCN), dichloromethane (DCM), diethyl ether (Et₂O), tetrahydrofuran (THF), toluene (PhMe), dimethylformamide (DMF), and triethylamine (Et₃N) were obtained by passing the previously degassed solvents through activated alumina columns. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Yields refer to chromatographically and spectroscopically (¹H NMR) homogeneous material, unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm E. Merck silica plates (60F-254), using UV light as the visualizing agent and/or KMnO₄ and heat as a developing agent. Flash silica gel chromatography was performed using E. Merck silica gel (60, particle size 0.043–0.063 mm). NMR spectra were recorded on Bruker DRX-600 and AMX-400 instruments and were calibrated using residual undeuterated solvent as an internal reference (chloroform-d: ¹H NMR δ = 7.26 ppm, ¹³C NMR δ = 77.16 ppm; methanol-d₄: ¹H NMR δ = 3.31 ppm, ¹³C NMR δ = 49.00 ppm; DMSO-d₆: ¹H NMR δ = 2.50 ppm, ¹³C NMR δ = 39.52 ppm). The following abbreviations were used to explain NMR peak multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. High-resolution mass spectra (HRMS) were recorded on an Agilent LC/MSD TOF mass spectrometer by electrospray ionization time-of-flight (ESI-TOF) reflectron experiments.

Experimental Procedures and Characterization Data for Synthesis of Arylomycin A Macrocycle

Compound S1

(S)-2-((tert-butoxycarbonyl)amino)-2-(4-hydroxyphenyl)acetic acid (S1)

Prepared from p-hydroxyphenylglycine as described in a previous literature procedure by Roberts, et al.¹ All spectral data were consistent with those previously reported.
Compound S2

\[
\text{OH} \quad \text{Boc} \quad \text{N} \quad \text{O} \\
\text{Me} \quad \text{CO}_2\text{H}
\]

\textit{tert-butyl (S)-4-(4-hydroxyphenyl)-5-oxooxazolidine-3-carboxylate (S2)}

Prepared from compound S1 as described in a previous literature procedure by Zhu, et al.\textsuperscript{2} All spectral data were consistent with those previously reported.

Compound 1

\[
\text{OH} \quad \text{Boc} \quad \text{N} \quad \text{O} \\
\text{Me} \quad \text{CO}_2\text{H}
\]

\textit{(S)-2-((tert-butoxycarbonyl)(methyl)amino)-2-(4-hydroxyphenyl)acetic acid (1)}

\textbf{Experimental:} Compound S2 (8.00 g, 28.6 mmol) was suspended in dichloromethane (40.0 mL), and the mixture was cooled to 0°C. The reaction flask was sealed and placed under a nitrogen atmosphere. Triethylsilane (18.4 mL, 13.22 g, 114 mmol, 4.0 eq.) was added to mixture via syringe while stirring. Then trifluoroacetic acid (40.0 mL) was added drop-wise via addition funnel. The resulting solution was allowed to warm to room temperature while stirring. After 12 hours, the solution was concentrated under reduced pressure at ~35°C. The resulting residue was re-suspended in water (80.0 mL) and cooled to 0°C. Solid NaHCO\textsubscript{3} was added to this solution until a pH of 7 was reached. To this solution was added 1N NaOH (aq.) (57 mL, 2 eq.), then ditertbutyldicarbonate (9.362 g, 42.9 mmol, 1.5 eq.) dissolved in THF (80 mL). The resulting solution was allowed to warm to room temperature while stirring. After stirring for 12 hours, the THF was removed under reduced pressure. The remaining aqueous layer was washed twice with hexanes. The aqueous layer was then acidified to pH 2-3 with aqueous 6N HCl, then extracted two times with dichloromethane. The organic layers were combined and rinsed with brine, dried over Na\textsubscript{2}SO\textsubscript{4}, and concentrated under reduced pressure to yield an off-white solid (5.47 g, 68%) which was used without further purification.
Compound S3

Boc-Ala-Tyr-OMe (S3)

Experimental: Boc-L-Ala-OH (4.730 g, 25.0 mmol) and L-Tyr-OMe · HCl (5.792 g, 25.0 mmol, 1 eq.) were dissolved in 80 mL of DMF. To this solution was added 1-Hydroxybenzotriazole hydrate (HOBt) (3.378 g, 25.0 mmol, 1 eq.) and Et3N (11.51 mL, 8.348 g, 82.5 mmol, 3.3 eq.). After stirring the resultant solution for 5 minutes, it was cooled to 0ºC, and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide Hydrochloride (EDC) (7.189 g, 37.5 mmol, 1.5 eq.) was added at once. The reaction mixture was allowed to warm to room temperature while stirring. The reaction was stirred for 18 hours. The resulting mixture was allowed to warm to room temperature while stirring. The reaction mixture was partitioned between EtOAc and water and extracted three times with EtOAc. The organic layers were combined and sequentially washed with sat. NaHCO₃ (aq.), sat. NH₄Cl (aq.), and brine. The organic layer was dried over Na₂SO₄, and concentrated under reduced pressure to yield a white foamy solid (8.885 g, 97% yield);

Physical State: white foam;

TLC: Rᵣ = 0.3 (60% EtOAc in hexanes)

¹H NMR (600 MHz, Chloroform-d): δ (ppm) 6.92 (d, J = 8.5 Hz, 2H), 6.70 (d, J = 8.1 Hz, 3H), 5.08 (s, 1H), 4.16 (s, 1H), 3.72 (s, 3H), 3.07 (dd, J = 14.0, 5.1 Hz, 1H), 2.99 (dd, J = 14.0, 5.9 Hz, 1H), 1.44 (s, 9H), 1.30 (d, J = 6.5 Hz, 3H);

¹³C NMR (151 MHz, Chloroform-d): δ (ppm) 172.50, 171.87, 155.61, 155.39, 130.40, 127.03, 115.58, 80.44, 53.42, 52.43, 50.12, 37.16, 37.14, 28.35, 18.31;

HRMS (m/z): calc’d for C₁₈H₂₇N₂O₆ [M+H]⁺ 367.1864, found 367.1864.

Compound S4

HCl·NH₂-Ala-Tyr-OMe (S4)
Experimental: Compound S3 (8.00 g, 21.8 mmol), was placed in an oven dried flask fixed with a magnetic stir bar, and was sealed. The flask was evacuated under reduced pressure and backfilled with N₂ three times. To this flask, was added dry MeOH (200 mL) via cannula. The resulting solution was cooled to 0°C, and acetyl chloride (7.8 mL, 8.55 g, 109 mmol, 5 eq.) was added drop-wise via syringe. The reaction was allowed to warm to room temperature while stirring. After 18 hours, the solution was concentrated under reduced pressure yielding a white foamy solid (quantitative yield).

Physical State: white foam;

^1H NMR (600 MHz, Methanol-d₄): δ (ppm) 7.04 (d, J = 8.5 Hz, 2H), 6.71 (d, J = 8.5 Hz, 2H), 4.63 (dd, J = 9.0, 5.4 Hz, 1H), 3.90 (q, J = 7.1 Hz, 1H), 3.70 (s, 3H), 3.35 (s, 1H), 3.10 (dd, J = 14.1, 5.4 Hz, 1H), 2.91 (dd, J = 14.1, 9.1 Hz, 1H), 1.49 (d, J = 7.1 Hz, 3H);

^13C NMR (151 MHz, Methanol-d₄) δ 173.19, 171.06, 157.55, 131.19, 128.60, 116.34, 55.83, 52.80, 50.06, 37.36, 17.65;

HRMS (m/z): calc’d for C₁₃H₁₈N₂O₄Na [M+Na]^+ 289.1159, found 289.1148.

Compound 2

Boc-NMe-Hpg-Ala-Tyr-OMe (2)

Experimental: Compound 1 (5.10 g, 18.1 mmol) and compound S4 (5.48 g, 18.1 mmol, 1 eq.) were dissolved in 180 mL of MeCN/DMF (4:1). To this solution was added HOBt (2.45 g, 18.1 mmol, 1 eq.) and Et₃N (8.3 mL, 6.04 g, 59.7 mmol, 3.3 eq.). After stirring the resultant solution for 5 minutes, it was cooled to 0°C, and EDC (5.19 g, 27.2 mmol, 1.5 eq.) was added at once. The reaction mixture was allowed to warm to room temperature while stirring. Reaction was stirred for 18 hours. The resulting mixture was partitioned between EtOAc and water and extracted three times with EtOAc. The organic layers were combined and sequentially washed with sat. NaHCO₃ (aq.), sat. NH₄Cl (aq.), and brine. The organic layer was dried over Na₂SO₄, and concentrated under reduced pressure. The resulting off-white solid was purified by flash column chromatography (SiO₂; 7:3 EtOAc/hexanes) yielding a white foam (6.894 g, 72%);
Physical State: white foam;

TLC: Rf = 0.30 (70% EtOAc in hexanes);

$^1$H NMR (600 MHz, Chloroform-d): δ (ppm) 8.02 (s, 1H), 7.70 (s, 1H), 7.02 (d, J = 8.0 Hz, 2H), 6.89 (d, J = 8.5 Hz, 2H), 6.70 (d, J = 8.6 Hz, 2H), 6.62 (d, J = 8.4 Hz, 2H), 5.53 (s, 1H), 4.80 – 4.74 (m, 1H), 4.49 (s, 1H), 3.69 (s, 3H), 3.08 (dd, J = 14.0, 4.6 Hz, 1H), 2.89 (dd, J = 14.1, 7.8 Hz, 1H), 2.71 (s, 3H), 2.01 (s, 2H), 1.46 (s, 9H), 1.21 (s, 3H);

$^{13}$C NMR (151 MHz, Chloroform-d) δ 172.47, 171.93, 170.77, 156.72, 156.69, 155.45, 130.50, 130.26, 127.09, 125.63, 115.98, 115.64, 81.06, 63.45, 53.85, 53.83, 52.55, 49.09, 36.93, 28.43, 28.41, 17.87;

HRMS (m/z): calc'd for C$_{27}$H$_{36}$N$_3$O$_8$ [M+H]$^+$ 530.2497, found 530.2497.

**Compound 3**

![Chemical Structure]

General Procedure:
Compound 2 was added to a flame dried flask and sealed. It was immediately evacuated and backfilled with nitrogen three times. To this was added dry acetonitrile to give a concentration of 0.0189 mmol/mL. In a separate flame dried flask, [Cu(MeCN)$_4$][PF$_6$] was added with stir bar and sealed. This was immediately evacuated and backfilled three times. To this was added dry acetonitrile via syringe to give a concentration of 0.0378 mmol/mL. To the resulting clear solution was then added an equimolar amount of freshly distilled TMEDA via syringe. This was stirred for 5 minutes under nitrogen, then purged with oxygen by bubbling it through the solution while stirring (or sonicating) for 20 minutes. This produced a deep blue solution. To another separate flame dried flask fixed with a stir bar (reaction vessel) was added 4 angstrom molecular sieves (20% weight of compound 2 being reacted), then placed under a nitrogen atmosphere. To this was added a specified volume of the Cu/TMEDA solution. While stirring under nitrogen, an equal volume of the solution of compound 6 was added to the reaction vessel over the course of 3 hours using a syringe pump or addition funnel. The reaction was allowed to stir for 3 days. After reaction completion (monitored by TLC), saturated aqueous
Na₂EDTA solution (volume equal to total reaction volume) was added at once to the reaction vessel, and allowed to stir for 1 hour. This solution as then filtered through a bed of celite and rinsed with EtOAc. Water was added to the filtrate, and extracted two times with EtOAc. The organic layers were combined, washed twice with brine and dried over Na₂SO₄. This solution was filtered and the solvent removed under reduced pressure to yield an orange solid. The crude product was purified by flash column chromatography (SiO₂; 3:2 EtOAc/toluene) yielding an off-white powder. This powder contains residual NaPF₆, and % weight of product must be obtained by qNMR (using benzoic acid as an internal standard) for proper assignment of yield.

**IMPORTANT NOTE:**
Freshly recrystallized [Cu(MeCN)₄][PF₆] must be used for optimal yields. All [Cu(MeCN)₄][PF₆] used was synthesized using the procedure reported by Kubas, and was recrystallized according to the procedure reported therein.³ Additionally, when running the reactions on small scale (<1 gram of 6), special consideration for dryness of both the solvents and glassware must be taken.

**Compound 3 (Large Scale Procedure)**
Compound 2 (5.1 g, 9.64 mmol) was dissolved in 510 mL of MeCN to give the appropriate stock concentration (see above). [Cu(MeCN)₄][PF₆] (7.186 g, 19.28 mmol) and TMEDA (2.89 ml, 2.24 g, 19.3 mmol) were mixed in 510 mL of MeCN according to the above procedure to give the appropriate stock concentration. This was solution was oxygenated by bubbling O₂ through the solution (using a balloon of O₂ attached to a 19 gauge needle, and a 20 gauge needle for gas release – occasional change of the balloon was necessary when running low) while sonicating for 20 minutes. 500 mL of this solution was transferred via syringe to a three-neck flask containing 1.0 g of molecular sieves and a stir bar, and fitted with an addition funnel, all under a N₂ balloon. 500 mL of the stock solution of compound 2 was then transferred to the addition funnel via syringe. While stirring, the stock solution was dripped into the Cu/TMEDA solution at a rate of about 5 mL/minute. After addition as complete, 5 mL of MeCN was used to rinse the addition funnel into the reaction mixture at once. This was stirred for 2.5 days, and was worked up and purified according to the procedures described above. (Dried material mass after purification = 5.861 g; weight % of purified mass = 51%; mass of 3 isolated = 2.989 g; 60% yield).

**Physical State:** off-white powder;
**TLC:** $R_f = 0.42$ (25% benzene in EtOAc) – fluoresces blue under short wave UV lamp (243 nm)

$^1$H NMR (600 MHz, Methanol-$d_4$): Mixture of isomers (see spectrum below). Determined to be a single compound via NOE exchange and variable temperature NMR;

$^{13}$C NMR (151 MHz, Methanol-$d_4$): Mixture of isomers (see spectrum below);

HRMS (m/z): calc’d for C$_{27}$H$_{34}$N$_3$O$_8$ [M+H]$^+$ 528.2340, found 528.2341.
Graphical Procedure for Cu-mediated Oxidative Macrocyclization of 2:

(Left) Recrystalized [Cu(MeCN)₄][PF₆]; (Center) Flask containing [Cu(MeCN)₄][PF₆] for reaction; (Right) Addition of MeCN to [Cu(MeCN)₄][PF₆].

(Left) [Cu(MeCN)₄][PF₆] dissolved in MeCN; (Center) Measuring out freshly distilled TMEDA; (Right) Addition of TMEDA to [Cu(MeCN)₄][PF₆] solution.

(Left) [Cu]/TMEDA solution after stirring for 5 minutes; (Center) Sparging of [Cu]/TMEDA solution with O₂; (Right) [Cu]/TMEDA solution after sparging for 20 mintues.
(Left) Pure compound 2; (Center) Flask containing compound 2; (Right) MeCN solution of compound 2.

(Left) Flame dried reaction flask containing 4 Å molecular sieves under N₂; (Center) Addition of oxygenated [Cu]/TMEDA solution to the reaction flask; (Right) Reaction flask after addition of oxygenated [Cu]/TMEDA solution.

(Left) Measuring out MeCN solution of compound 2; (Center) Addition of compound 2 to reaction flask via syringe pump; (Right) Reaction flask after complete addition of compound 2.
(Left) Saturated EDTA solution; (Center) Addition of EDTA solution to reaction flask; (Right) Reaction flask after addition of EDTA solution and stirring for 1 hour.

(Left) Filtration of reaction mixture; (Center) Extraction of aqueous layer with EtOAc; (Right) Concentration of the organic layer.

(Left) Crude product; (Right) Product after column chromatography.
Experimental Procedures and Characterization Data for Synthesis of Arylomycin Analogs

Compound S5

methyl 2-methyl-4-octylbenzoate (S5)

**Experimental:** A three neck, round bottom flask was fixed with reflux condenser and a stirbar, and placed under nitrogen. To this was Methyl 4-bromo-2-methylbenzoate (8.00 g, 34.9 mmol) and 1-octyne (4.23 g, 38.4 mmol) via syringe and trimethylamine (120 mL) was added by canula. Pd(PPh$_3$)$_2$Cl$_2$ (1.226 g, 1.746 mmol) and Cul (0.333 g, 1.746 mmol) were added quickly, and the flask was resealed. The reaction mixture was stirred at reflux for 4 hours. The mixture was cooled and extracted with dichloromethane (3 x mL). The combined organic layers were washed with brine, dried over Na$_2$SO$_4$. The solution was filtered and dried under reduced pressure. The resulting oil was purified by flash column chromatography (SiO$_2$; 100:1 to 25:1 hexanes/EtOAc). The resulting yellow oil, still containing trace methyl 4-bromo-2-methylbenzoate, was used without further purification. The oil was dissolved in dry MeOH (mL) under nitrogen in a flame-dried flask fixed with a stirbar. To this was added Pd/C (6.6 g, 6.18 mmol). The flask was purged with hydrogen gas, and stirred under a hydrogen atmosphere for 16 hours. The solution was then filtered through a celite plug and concentrated under reduced pressure. The resulting oil was placed under high vacum at 120 °C for 4 hours to remove any remaining uncoupled methyl benzoate contaminants. The resulting oil was purified by flash column chromatography (SiO$_2$; 100:1 to 25:1 hexanes/EtOAc) yielding a yellow oil (5.7 g, 62% yield over 2 steps).

**Physical State:** yellow oil;

**TLC:** $R_f = 0.31$ (10% EtOAc in hexanes);

$^1$H NMR (400 MHz, Chloroform-$d$): $\delta$ 7.86 (d, $J = 8.5$ Hz, 1H), 7.07 (s, 2H), 3.89 (s, 3H), 2.62 (d, $J = 11.0$ Hz, 5H), 1.68 – 1.59 (m, 2H), 1.37 – 1.24 (m, 10H), 0.90 (t, $J = 6.6$ Hz, 3H);

$^{13}$C NMR (151 MHz, Chloroform-$d$): $\delta$ 167.58, 147.01, 139.86, 131.39, 130.30, 126.31, 125.30, 51.18, 35.34, 31.41, 30.66, 28.97, 28.84, 28.77, 22.20, 21.38, 13.64;
**Compound S6**

2-methyl-4-octylbenzoic acid (S6)

**Experimental:** Compound S5 (5.50 g, 21.0 mmol) was dissolved in MeOH (56 mL) in a round bottom flask fixed with a stir bar and reflux condenser. To this was added 5N aqueous NaOH (56 mL, 238 mmol). The solution was stirred for 3h at 100 °C, after which it was cooled and acidified to pH 3 with 1N HCl (aq.). The mixture was then extracted with EtOAc (3x). The combined organic extracts were rinsed with brine, and dried over Na₂SO₄. The solvent was removed under reduced pressure to yield a white, analytically pure solid (5.10 g, 98% yield).

**Physical State:** white powder

**H NMR** (600 MHz, Methanol-d₄): δ 7.83 (d, J = 7.9 Hz, 1H), 7.09 – 7.05 (m, 2H), 2.63 – 2.59 (m, 2H), 2.55 (s, 3H), 1.66 – 1.59 (m, 2H), 1.31 (m, 10H), 0.89 (t, J = 7.1 Hz, 3H);

**C NMR** (151 MHz, Methanol-d₄): δ 171.31, 148.83, 141.51, 132.95, 132.22, 128.72, 126.97, 36.85, 33.17, 32.49, 30.68, 30.52, 30.49, 23.86, 22.17, 14.57;

**HRMS (m/z):** calc’d for C₁₆H₂₅O₂ [M+H]^+ 249.1849, found 249.1825.

**Compound 5**

(S)-4-((tert-butoxycarbonyl)amino)-2-(2-methyl-4-octylbenzamido)butanoic acid (5)

**Experimental:** Compound S6 (2.500 g, 10.07 mmol) and NH₂-Dab(Boc)-OMe (2.338 g, 10.07 mmol) were dissolved in DMF. To this solution was added HOBt (1.541 g, 10.07 mmol) and Et₃N (4.63 mL, 3.36 g, 33.2 mmol). After stirring the resultant solution for 5 minutes, it was cooled to 0 °C, and EDC (2.344 g, 15.10 mmol) was added at once. The reaction mixture was allowed to warm to room temperature while stirring. The reaction was then allowed to stir for 12 hours. The resulting mixture was partitioned between EtOAc and water and extracted three times with EtOAc. The organic layers were combined and sequentially washed with sat.
NaHCO₃ (aq.), sat. NH₄Cl (aq.), and brine. The organic layer was dried over Na₂SO₄, and concentrated under reduced pressure. The crude material was purified by flash column chromatography (SiO₂; 2:1 EtOAc/Hexanes) yielding a white solid. This was then dissolved in THF (85 mL) and cooled to 0 °C. To this was added 2.0 N aq. LiOH (58 mL, 116 mmol). The solution was allowed to warm to room temperature and stirred vigorously for 2 hours. The solution was then acidified to a pH of 3 with 6.0 M HCl and partitioned with EtOAc. This was extracted three times with EtOAc. The combined organic layers were rinsed with brine, dried over Na₂SO₄, and solvent removed under reduced pressure to yield a colorless, analytically pure viscous oil (3.70 g, 82.3% yield).

Physical State: extremely viscous colorless oil;

¹H NMR (600 MHz, Methanol-d₄): δ 7.40 – 7.36 (m, 1H), 7.08 – 7.04 (m, 2H), 4.59 (dd, J = 9.6, 4.4 Hz, 1H), 3.25 (dt, J = 13.0, 6.7 Hz, 1H), 3.17 (dt, J = 14.0, 7.3 Hz, 1H), 2.60 (t, J = 7.7 Hz, 2H), 2.41 (s, 3H), 2.12 (m, 1H), 1.91 (m, 1H), 1.65 – 1.58 (m, 2H), 1.44 (s, 9H), 1.36 – 1.25 (m, 11H), 0.90 (t, J = 7.1 Hz, 3H);

¹³C NMR (151 MHz, Methanol-d₄): δ 175.14, 173.45, 158.45, 146.22, 137.16, 134.84, 131.86, 128.47, 126.70, 80.18, 51.77, 38.23, 36.66, 33.04, 32.54, 30.58, 30.43, 30.27, 28.81, 23.74, 19.90, 14.47;

HRMS (m/z): calc'd for C₂₅H₄₁N₂O₅ [M+H]⁺ 449.3010, found 499.3012.

Compound 6

Experimental: Compound 3 (250 mg, 0.47 mmol) was placed in a flame dried culture tube fixed with a stir bar, then evacuated and backfilled with nitrogen three times. To this was added methanol (4.7 mL) and stirred until dissolved. This was cooled to 0 °C, and acetyl chloride (0.4 mL) was added dropwise. The solution was allowed to warm to room temperature and stirred until completion by TLC (~ 5 h). After this, the solvent was removed on a rotary evaporator, and placed under high vacuum for at least 5 hours. The resulting off white solid was then re-
suspended in DMF (2.0 mL) and Et₃N (0.20 mL, 145 mg, 1.4mmol, 3 eq.) was added to this solution and stirred. In a separate flame dried culture tube fixed with a stir bar was placed 5 (425 mg, 0.95 mmol 2 eq.) and (7-Azabenzotriazol-1-ylloxy)tripyrrolidinophosphonium hexafluorophosphate (PyAOP) (494 mg, 0.95 mmol, 2 eq.), which was then sealed, and evacuated and backfilled with nitrogen three times. To this was added DMF (2.0 mL) and stirred until all solids dissolved. N,N-diisopropylethylamine (0.20 mL, 145 mg, 1.4mmol, 3 eq.) was then added at once and the resulting solution was stirred for 15 minutes at room temperature. At this time, this solution was transferred to the vial containing crude deprotected compound 3. This new solution was then placed in an oil bath preheated to 50 ºC and stirred for 24 hours. After this time, the solution was cooled to room temperature, partitioned between EtOAc and water, and extracted two times with EtOAc. The combined organic layers were rinsed sequentially with aq. LiCl (10% w/w) and sat. NH₄Cl (aq.), and brine. The organic layer was then dried over Na₂SO₄, and the solvent was removed under reduced pressure. The crude product was purified flash column chromatography (SiO₂; 100% CH₂Cl₂ to 5% MeOH in CH₂Cl₂) yielding an off-white solid (335 mg, 82% yield).

**NOTE:** Compound 6 is isolated and carried through subsequent steps as a mixture of diastereomers at the alpha center of the diaminobutyric acid residue. Diasteromers of the final compounds tested for bioactivity were separated by HPLC and found to be diastereomERICally pure. Correct stereochemistry was assigned by comparison of chemical shifts to previously reported compounds.⁴

**Physical State:** white solid;

**TLC:** Rᵣ = 0.31 (5% MeOH in dichloromethane);

**¹H NMR** (600 MHz, Methanol-d₄): Mixture of isomers (see spectrum below);

**¹³C NMR** (151 MHz, Methanol-d₄): Mixture of isomers (see spectrum below);

**HRMS** (m/z): calc'd for [M+H]⁺ 880.4467, found 880.4462.
Compound 7

**Experimental:** Compound 6 (20.0 mg, 0.023 mmol) was placed in a vial fixed with a stirbar and dissolved in THF (0.7 mL). The solution was cooled to 0 °C, and 2M LiOH (aq.) (0.3 mL) was added slowly. The solution was allowed to warm to room temperature and stirred vigorously for 2 hours. The solution was then acidified to a pH of 3 with 1.0 M HCl and partitioned with EtOAc. This was extracted 3 times with EtOAc. The combined organic layers were rinsed with brine, dried over Na₂SO₄, and dried under reduced pressure to yield a white solid, which was used without further purification. The crude solid was placed in a vial fixed with a stirbar and placed under nitrogen. CH₂Cl₂ (0.7 mL) was added via syringe resulting in a suspension that was cooled to 0 °C. To this was added trifluoroacetic acid (0.3 mL) dropwise. The solution was allowed to warm to room temperature and stirred for 2 hours. The solvent was then removed on a rotary evaporator and the resulting oil was placed under high vacuum for at least 5 hours. The crude material was taken up in EtOH and purified by reverse phase preparatory HPLC (C₁₈; gradient of H₂O to MeCN each containing 0.1% formic acid). Isolated 6.9 mg (38% yield).

**Physical State:** white foam;

**¹H NMR** (600 MHz, Methanol-d₄): δ 8.54 (s, 1H), 7.26 (d, J = 8.6 Hz, 1H), 7.13 – 6.99 (m, 4H), 6.93 (d, J = 8.31 Hz, 1H), 6.86 – 6.79 (m, 2H), 6.52 (s, 1H), 5.14 (dd, J = 7.9, 5.6 Hz, 1H), 4.59 (s, 2H) 4.47 (d, J = 8.2 Hz, 1H), 3.37 (dd, J = 16.3, 3.0 Hz, 1H), 3.16 – 3.01 (m, 4H), 2.94 (s, 3H), 2.63 – 2.43 (m, 2H), 2.32 (s, 3H), 2.29 – 2.14 (m, 2H), 1.64 – 1.52 (m, 3H), 1.37 – 1.26 (m, 14H), 0.90 (t, J = 7.0 Hz, 3H);

**¹³C NMR** (151 MHz, Methanol-d₄): δ 178.00, 173.64, 173.34, 172.00, 170.18, 163.31, 163.08, 162.85, 153.16, 146.73, 137.06, 135.33, 133.97, 133.75, 132.10, 131.59, 130.84, 130.38, 128.28, 127.64, 127.11, 126.95, 118.63, 117.19, 62.02, 56.45, 50.56, 49.57, 37.75, 36.49, 35.80, 33.64, 33.04, 32.23, 30.78, 30.54, 30.42, 30.32, 23.73, 19.98, 19.08, 14.44;

**HRMS** (m/z): calc’d for C₄₄H₅₆N₅O₈ [M+H]+ 744.3967, found 744.3968.
Compound S7

**Experimental:** Compound 6 (110.0 mg, 0.130 mmol) and K₂CO₃ (142.0 mg, 1.02 mmol, 8 eq.) were added quickly to a flame dried culture tube fixed with a stirbar and sealed, then evacuated and backfilled with nitrogen three times. DMF (1.3 mL) was added via syringe and stirred for 5 minutes. N-Boc-2-bromoethanamine (65.0 mg, 0.29 mmol, 5 eq.) was added via syringe and the resulting solution was placed in an oil bath preheated to 50 °C and stirred for 2-3 days (monitored for complete conversion to the bis-alkylated product by LCMS as mono- and bis-alkylated products are inseparable by PTLC or flash column chromatography). After completion, the solution was cooled to room temperature and filtered over a bed of celite and rinsed with EtOAc. The filtrate was then partitioned between water and EtOAc and extracted twice. The combined organic layers were rinsed sequentially with aq. LiCl (10% w/w) and brine, dried over Na₂SO₄, and solvent removed under reduced pressure. The crude solid was purified by flash column chromatography (SiO₂; 5% MeOH in CH₂Cl₂) yielding a light yellow solid that was used directly in the next step.

**Physical State:** light yellow solid;

**TLC:** Rᵣ = 0.35 (5% MeOH in dichloromethane);

**¹H NMR** (600 MHz, Methanol-d₄): Mixture of isomers (see spectrum below);

**¹³C NMR** (151 MHz, Methanol-d₄): Mixture of isomers (see spectrum below);

**HRMS (m/z):** calc'd for C₆₁H₉₀N₇O₁₄ [M+H]⁺ 1144.6540, found 1144.6562.
Compound 8

Experimental: Compound S7 (100 mg, 0.177 mmol) was placed in a vial fixed with a stirbar and dissolved in THF (2.5 mL). The solution was cooled to 0 °C, and aq. LiOH (2M) (1 mL) was added slowly. The solution was allowed to warm to room temperature and stirred vigorously for 2 hours. The solution was then acidified to a pH of 2 with 1.0 M HCl and partitioned with EtOAc. This was extracted 3 times with EtOAc. The combined organic layers were rinsed with brine, dried over Na₂SO₄, and dried under reduced pressure to yield a white solid (100.4 mg, 69% over two steps – from 7)

Physical State: white solid;

¹H NMR (600 MHz, Methanol-d₄): Mixture of isomers (see spectrum below);

¹³C NMR (151 MHz, Methanol-d₄): Mixture of isomers (see spectrum below);

HRMS (m/z): calc’d for C₆₀H₈₆N₇O₁₄ [M–H]⁻ 1128.6238, found 1128.6231.
**Experimental:** Compound 8 (20.0 mg, 0.018 mmol) was placed in a vial fixed with a stirbar and placed under nitrogen. CH₂Cl₂ (0.7 mL) was added via syringe resulting in a suspension that was cooled to 0 °C. To this was added trifluoroacetic acid (0.3 mL) dropwise. The solution was allowed to warm to room temperature and stirred for 2 hours. The solvent was then removed on a rotary evaporator and the resulting oil was placed under high vacuum for at least 5 hours. The crude material was taken up in EtOH and purified by reverse phase preparatory HPLC (C₁₈; gradient of H₂O to MeCN each containing 0.1% TFA). Isolated 5.9 mg (30% yield).

**Physical State:** white foam;

**¹H NMR** (600 MHz, Methanol-δ₁): δ 8.39 (d, J = 9.0 Hz, 1H), 7.34 - 7.31 (m, 2H), 7.26 (dd, J = 8.6, 2.4 Hz, 1H), 7.18 (d, J = 8.6 Hz, 1H), 7.12 - 7.08 (m, 4H), 6.87 (d, J = 2.5 Hz, 1H), 6.86 (d, J = 2.4 Hz, 1H), 6.33 (s, 1H), 5.13 (dd, J = 7.9, 5.6 Hz, 1H), 4.32 - 4.21 (m, 5H), 3.49 (dd, J = 17.1, 3.3 Hz, 1H), 3.17 - 3.08 (m, 4H), 2.92 (s, 3H), 2.62 (t, J = 7.7 Hz, 2H), 2.41 (s, 3H), 2.30 - 2.23 (m, 1H), 2.16 - 2.09 (m, 1H), 1.68 - 1.60 (m, 3H), 1.37 - 1.27 (m, 14H), 0.90 (t, J = 7.0 Hz, 3H);

**¹³C NMR** (151 MHz, Methanol-δ₁): δ 174.51, 174.22, 173.32, 173.09, 172.34, 172.25, 156.60, 155.27, 146.87, 137.25, 136.62, 133.97, 133.33, 132.18, 132.13, 131.81, 131.00, 130.65, 129.91, 129.76, 128.38, 126.91, 115.77, 115.47, 66.84, 62.09, 52.24, 50.33, 50.23, 49.57, 40.34, 40.21, 37.64, 36.63, 34.49, 33.65, 33.02, 32.51, 30.57, 30.56, 30.41, 30.27, 23.72, 20.02, 19.28, 14.42;

**HRMS (m/z):** calc’d for C₄₅H₆₄N₇O₈ [M+H]⁺ 830.4811, found 830.4812.
Compound 10

![Chemical Structure]

**Experimental:** Compound 8 (30 mg, 0.027 mmol) and N-hydroxyphalmitide (4.8 mg, 0.029 mmol, 1.1 eq) was placed in an oven dried culture tube fixed with a stir bar. This was evacuated and backfilled with argon 3 times. To this was added DCM (0.5 mL) via syringe, creating a suspension. While stirring, N,N'-diisopropylcarbodiimide (4.6 μL, 3.7 mg, 0.029 mmol, 1.1 eq.) was added via syringe. After consumption of starting material as monitored by TLC (~1 h), the solvent was blown off under a stream of argon, and placed on high vacuum for 2 hours. After this, Zn powder (35.0 mg, 0.531 mmol, 20 eq.), Ni(acac)₂ (14.0 mg, 0.053 mmol, 2 eq.), and LiCl (23.0 mg, 0.531 mmol, 20 eq.) were quickly added to the tube and resealed. It was evacuated and backfilled with argon 3 times. Before stirring, acetonitrile (0.3 mL) and methyl acrylate (48 μL, 45.7 mg, 0.531 mmol, 20 eq.) were added to the vial. The resulting mixture was then stirred vigorously for 15 hours at room temperature. It was quenched with 2 mL of 1:1 water/saturated aqueous NH₄Cl. This was extracted twice with EtOAc. The combined organic layers were rinsed with brine and dried over Na₂SO₄. Solvent was removed under reduced pressure. The crude material was purified by PTLC to enrich the product (does not separate well from similar impurities). This enriched product (mass confirmed by LCMS) was then subject to the deprotection sequence described for the synthesis of compound 7. The resulting material was then purified by preparatory HPLC (C₁₈; gradient of H₂O to MeCN each containing 0.1% formic acid), yielding 6.0 mg (22% yield) of a white foam.

**Physical State:** white foam;

**¹H NMR** (600 MHz, Methanol-d₄): δ 8.50 (d, J = 8.7 Hz, 1H), 8.19 (d, J = 8.7 Hz, 1H), 7.40 – 7.32 (m, 2H), 7.28 – 7.22 (m, 1H), 7.19 (dd, J = 8.6, 1.6 Hz, 1H), 7.16 – 7.03 (m, 4H), 6.90 (d, J = 2.3 Hz, 1H), 6.82 (d, J = 2.5 Hz, 1H), 6.35 (s, 1H), 5.16 (dd, J = 7.9, 5.7 Hz, 1H), 4.78 – 4.66 (m, 1H), 4.46 – 4.35 (m, 1H), 4.34 – 4.18 (m, 5H), 3.31-3.25 (m, 3H), 3.19 – 3.09 (m, 4H), 2.93 (s, 3H), 2.64 (m, 2H), 2.45 (s, 3H), 2.41 – 2.37 (m, 2H), 2.34 – 2.25 (m, 1H), 2.19 – 2.09 (m,
1H), 2.04 – 1.93 (m, 1H), 1.80 (m, 1H), 1.63 (m, 3H), 1.41 – 1.26 (m, 16H), 0.92 (t, J = 7.0 Hz, 3H);

13C NMR (151 MHz, Methanol-d4): δ 176.80, 174.44, 173.29, 173.09, 172.29, 163.05, 162.82, 160.58, 155.30, 154.97, 146.87, 137.25, 133.96, 133.49, 132.98, 132.17, 131.80, 131.14, 130.75, 130.59, 129.73, 128.38, 126.91, 115.77, 115.42, 66.88, 62.13, 50.51, 49.57, 48.34, 40.35, 37.76, 37.64, 36.63, 33.60, 33.01, 32.50, 32.03, 31.23, 30.55, 30.40, 30.26, 23.71, 20.06, 20.02, 14.42;

HRMS (m/z): calc’d for C_{47}H_{68}N_{7}O_{8} [M+H]^+ 858.5124, found 858.5123.

**Compound 11**

![Compound 11](image)

**Experimental:** Compound 8 (50.0 mg, 0.044 mmol) and N-hydroxyphalmide (7.9 mg, 0.049 mmol, 1.1 eq) was placed in an oven dried culture tube fixed with a stirbar. This was evacuated and backfilled with argon 3 times. To this was added dichloromethane (0.5 mL) via syringe, creating a suspension. While stirring, N,N’-diisopropylcarbodiimide (7.6 µL, 6.1 mg, 0.049 mmol, 1.1 eq.) was added via syringe. After consumption of starting material by TLC (~1 h), the solvent was blown off under a stream of argon, and placed on high vacuum for 2 hours. After this, Zn powder (58.0 mg, 0.885 mmol, 20 eq.), Ni(acac)₂ (23.0 mg, 0.088 mmol, 2 eq.), and LiCl (38.0 mg, 0.885 mmol, 20 eq.) were quickly added to the tube and resealed. It was evacuated and backfilled with argon 3 times. Before stirring, acetonitrile (0.45 mL) and acrylonitrile (58.0 µL, 47.0 mg, 0.885 mmol, 20 eq.) were added to the vial. The resulting mixture was then stirred vigorously for 15 hours at room temperature. It was quenched with 2 mL of 1:1 water/saturated aqueous NH₄Cl. This was extracted twice with EtOAc. The combined organic layers were rinsed with brine and dried over Na₂SO₄. Solvent was removed under reduced pressure. The crude material was purified by PTLC to enrich the product (does not separate well from similar
impurities). This enriched product (mass confirmed by LCMS) was then subject to the deprotection sequence described for the synthesis of compound 9. The resulting material was then purified by preparatory HPLC (C_{18}; gradient of H_{2}O to MeCN each containing 0.1% TFA), yielding 9.5 mg (19% yield) of a white foam.

**Physical State:** white foam;

**{\textsuperscript{1}H NMR** (600 MHz, Methanol-d<sub>4</sub>): {\textdegree} 7.35 – 7.33 (m, 2H), 7.25 (dd, {\textit{J}} = 8.6, 2.4 Hz, 1H), 7.20 (d, {\textit{J}} = 8.5 Hz, 1H), 7.14 – 7.09 (m, 4H), 6.88 (d, {\textit{J}} = 2.4 Hz, 1H), 6.84 (d, {\textit{J}} = 2.5 Hz, 1H), 6.35 (s, 1H), 5.15 (dd, {\textit{J}} = 8.0, 5.6 Hz, 1H), 4.72 (q, {\textit{J}} = 6.8 Hz, 1H), 4.49 – 4.43 (m, 1H), 4.33 – 4.22 (m, 4H), 3.29 (q, {\textit{J}} = 6.2, 5.5 Hz, 5H), 3.17 – 3.09 (m, 4H), 2.93 (s, 3H), 2.64 (t, {\textit{J}} = 7.7 Hz, 2H), 2.52 (d, {\textit{J}} = 7.2 Hz, 2H), 2.43 (s, 3H), 2.33 – 2.25 (m, 1H), 2.17 – 2.10 (m, 1H), 2.06 – 1.99 (m, 1H), 1.89 – 1.81 (m, 1H), 1.66 – 1.59 (m, 3H), 1.40 (d, {\textit{J}} = 6.8 Hz, 3H), 1.37 – 1.28 (m, 14H), 0.92 (t, {\textit{J}} = 7.1 Hz, 3H);

**{\textsuperscript{13}C NMR** (151 MHz, Methanol-d<sub>4</sub>): 174.63, 173.29, 173.08, 172.26, 162.99, 162.75, 156.59, 155.08, 146.87, 137.25, 136.61, 133.97, 132.99, 132.91, 132.17, 131.83, 131.13, 130.67, 129.92, 129.81, 128.38, 126.91, 120.95, 115.75, 115.47, 66.88, 66.84, 62.12, 50.52, 49.57, 49.00, 48.15, 40.35, 40.20, 37.64, 37.21, 36.63, 33.61, 33.20, 33.02, 32.51, 30.58, 30.56, 30.41, 30.27, 23.72, 20.02, 19.98, 14.42, 14.36;

**HRMS** (m/z): calc’d for C_{47}H_{68}N_{8}O_{8}[M+H]^{+} 839.5178, found 839.5177.

**Compound 12**

**Experimental:** Compound 8 (20.0 mg, 0.018 mmol) and N-hydroxyphalmidine (3.2 mg, 0.019 mmol, 1.1 eq) was placed in an oven dried culture tube fixed with a stirbar. This was evacuated and backfilled with argon 3 times. To this was added dichloromethane (0.5 mL) via syringe, creating a suspension. While stirring, {\textit{N,N'-diisopropylcarbodiimide} (3.0 µL, 2.5 mg, 0.019 mmol, 1.1 eq.) was added via syringe. After consumption of starting material by TLC (~1 h), the solvent was blown off under a stream of argon, and placed on high vacuum for 2 hours. After this, Zn powder (23.0 mg, 0.35 mmol, 20 eq.), Ni(acac)<sub>2</sub> (9.0 mg, 0.040 mmol, 2.0 eq.), and LiCl (15.0
mg, 0.35 mmol, 20 eq.) were quickly added to the tube and resealed. It was evacuated and backfilled with argon 3 times. Before stirring, acetonitrile (0.5 mL) and 2-vinylpyridine (38.0 µL, 37mg, 0.35 mmol, 20 eq.) were added to the vial. The resulting mixture was then stirred vigorously for 15 hours at room temperature. It was quenched with 2 mL of 1:1 water/saturated aqueous NH₄Cl. This was extracted twice with EtOAc. The combined organic layers were rinsed with brine and dried over Na₂SO₄. Solvent was removed under reduced pressure. The crude material was purified by PTLC to enrich the product (does not separate well from similar impurities). This enriched product (mass confirmed by LCMS) was then subject to the deprotection sequence described for the synthesis of compound 9. The resulting material was then purified by preparatory HPLC (C₁₈; gradient of H₂O to MeCN each containing 0.1% formic acid), yielding 2.1 mg (10% yield) of a white foam.

**NOTE:** Compound demonstrates conformational heterogeneity as shown by exchange cross-peaks in ROESY experiment (see spectrum below). ¹H assignments below are for the most prevalent conformer.

**Physical State:** white foam;

**¹H NMR** (600 MHz, Methanol-δ₆): δ 8.45 (dd, J = 11.4, 4.8 Hz, 1H), 3.80 – 7.73 (m, 1H), 7.41 – 6.95 (m, 9H), 6.90 – 6.80 (m, 2H), 6.39 (s, 1H), 5.14 (dd, J = 7.9, 5.6 Hz, 1H), 4.79 – 4.70 (m, 1H), 4.41 – 4.32 (m, 1H), 4.19 – 4.05 (m, 5H), 3.18 – 2.96 (m, 8H), 2.92 (s, 3H), 2.64 – 2.58 (m, 2H), 2.42 (m, 3H), 2.28 – 2.16 (m, 1H), 2.14 – 1.94 (m, 2H), 1.91 – 1.83 (m, 1H), 1.65 – 1.58 (m, 3H), 1.42 – 1.36 (m, 2H), 1.35 – 1.26 (m, 14H), 0.90 (t, J = 6.9 Hz, 3H);

**¹³C NMR** (151 MHz, Methanol-δ₆): δ 174.23, 173.64, 173.14, 172.30, 170.29, 162.53, 157.07, 155.69, 155.35, 149.66, 146.72, 138.87, 137.19, 137.10, 136.42, 134.19, 133.29, 132.62, 132.11, 131.57, 130.93, 130.89, 129.86, 129.24, 128.39, 126.89, 124.70, 124.65, 122.94, 115.26, 114.74, 69.85, 62.07, 51.21, 50.59, 49.85, 49.57, 41.18, 38.01, 37.65, 37.61, 36.63, 35.94, 35.19, 33.56, 33.02, 32.52, 32.50, 30.78, 30.56, 30.55, 30.41, 30.27, 23.72, 20.04, 19.99, 14.42;

**HRMS** (m/z): calc’d for C₅₁H₇₁N₈O₆ [M+H]⁺ 891.5491, found 891.5491.
Compound 13

Experimental: Compound 8 (40.0 mg, 0.035 mmol) and N-hydroxyphalmi de (6.4 mg, 0.039 mmol, 1.1 eq) was placed in an oven dried culture tube fixed with a stirbar. This was evacuated and backfilled with argon 3 times. To this was added dichloromethane (0.5 mL) via syringe, creating a suspension. While stirring, N,N'‐diisopropylcarbodiimide (6.1 µL, 4.9 mg, 0.039 mmol, 1.1 eq.) was added via syringe. After consumption of starting material by TLC (~1 h), the solvent was blown off under a stream of argon, and placed on high vacuum for 2 hours. After this, Zn powder (5.8 mg, 0.088 mmol, 2.5 eq.), NiCl₂(H₂O)₆ (4.21 mg, 0.018 mmol, 0.5 eq.), and 4,4'-di-tert-butyl-2,2'-bipyridine (9.5 mg, 0.035 mmol, 1.0 eq.) were quickly added to the tube and resealed. It was evacuated and backfilled with argon 3 times. Before stirring, DMF (0.4 mL) and phenylsilane (32 µL, 29 mg, 0.27 mmol, 7.5 eq.) were added to the vial. The resulting mixture was then stirred vigorously for 3 hours at room temperature. It was quenched with 2 mL of 1:1 water/saturated aqueous NH₄Cl. This was extracted twice with EtOAc. The combined organic layers were rinsed with brine and dried over Na₂SO₄. Solvent was removed under reduced pressure. The crude material was purified by PTLC to enrich the product (does not separate well from similar impurities). This enriched product (mass confirmed by LCMS) was then subject to the deprotection sequence described for the synthesis of compound 7. The resulting material was then purified by preparatory HPLC (C₁₈; gradient of H₂O to MeCN each containing 0.1% TFA), yielding 7.5 mg (19% yield) of a white foam.

Physical State: white foam;

¹H NMR (600 MHz, Methanol-d₄): δ 7.37 – 7.32 (m, 2H), 7.26 (dd, J = 8.6, 2.3 Hz, 1H), 7.20 (d, J = 8.6 Hz, 1H), 7.16 – 7.08 (m, 3H), 6.93 (m, 2H), 6.36 (s, 1H), 5.15 (dd, J = 8.0, 5.6 Hz, 1H), 4.73 (q, J = 6.8 Hz, 1H), 4.27 (ddt, J = 31.8, 11.1, 4.5 Hz, 5H), 4.08 (ddd, J = 13.5, 10.0, 3.1 Hz, 1H), 3.32 – 3.27 (m, 3H), 3.23 – 3.07 (m, 5H), 2.95 (s, 3H), 2.64 (t, J = 7.7 Hz, 2H), 2.44 (s, 3H),
2.33 – 2.26 (m, 2H), 2.18 – 2.11 (m, 2H), 1.68 – 1.60 (m, 3H), 1.39 – 1.27 (m, 14H), 0.92 (t, J = 7.0 Hz, 3H);

$^{13}$C NMR (151 MHz, Methanol-$d_4$): $\delta$ 174.24, 173.35, 173.11, 171.98, 163.15, 162.92, 156.56, 155.06, 146.86, 137.25, 136.65, 134.10, 134.05, 133.99, 132.17, 131.69, 130.83, 130.73, 129.89, 129.73, 128.39, 126.91, 115.83, 115.57, 66.91, 66.86, 62.03, 50.44, 49.57, 40.35, 40.22, 39.36, 37.64, 36.63, 33.64, 33.02, 32.52, 32.11, 30.56, 30.41, 30.27, 23.72, 20.02, 19.32, 14.42;

HRMS (m/z): calc’d for C$_{44}$H$_{64}$N$_7$O$_6$ [M+H]$^+$ 786.4913, found 786.4916.
Optimization of Cu-mediated Oxidative Macrocyclization

Initial Discovery

Procedure: Compound 2 (4.0 mg, 7.56 µM) is added to a reaction vial as a stock solution in DCM, and blown down to dryness under N₂. 0.38 mL of reaction solvent is then added to the reaction vial along with a stirbar. In a separate vial, a 1:1 mixture (by moles) was made of the copper source and TMEDA in the reaction solvent to a final concentration of each reagent at 0.02 M. Then, 0.38 mL of the Cu/TMEDA solution was added to the reaction vial followed by 4 equivalents of the oxidant (if not gaseous). For reactions run under ambient air, a needle was placed in the vial septum, and for reactions run under O₂, a balloon was used. For reactions run under ambient air, all solutions were made open to air. For those reactions run with other oxidants, solutions were made under an inert atmosphere. Reactions were allowed to stir for 48 hours at room temperature after which aliquots were taken and analyzed for yield by HPLC with biphenyl as an internal standard.

| Entry | Cu Source | Oxidant | Solvent | % Yield (HPLC-DAD) |
|-------|-----------|---------|---------|--------------------|
| 1     | [Cu(OH)Cl•TMEDA]² | ambient air² | CH₂Cl₂ | 10                 |
| 2     | CuCl      | ambient air | CH₂Cl₂ | 10                 |
| 3     | CuBr      | ambient air | CH₂Cl₂ | 12                 |
| 4     | CuI       | ambient air | CH₂Cl₂ | <5                 |
| 5     | CuOAc     | ambient air | CH₂Cl₂ | <5                 |
| 6     | CuCl₂     | ambient air | CH₂Cl₂ | <5                 |
| 7     | CuBr₂     | ambient air | CH₂Cl₂ | 8                  |
| 8     | CuSO₄     | ambient air | CH₂Cl₂ | <5                 |
| 9     | Cu(OTf)₂  | ambient air | CH₂Cl₂ | <5                 |
| 10    | Cu(OAc)₃ | ambient air | CH₂Cl₂ | 8                  |
| 11    | [Cu(MeCN)₄][PF₆] | ambient air | CH₂Cl₂ | 14                 |
| 12    | CuCl      | ambient air | acetone | <5                 |
| 13    | CuCl      | ambient air | EIOAc  | <5                 |
| 14    | CuCl      | ambient air | THF    | 5                  |
| 15    | CuCl      | ambient air | MeCN   | 10                 |
| 16    | CuBr      | ambient air | acetone | 5                  |
| 17    | CuBr      | ambient air | EIOAc  | <5                 |
| 18    | CuBr      | ambient air | THF    | <5                 |
| 19    | CuOAc₂    | ambient air | acetone | <5                 |
| 20    | CuOAc₂    | ambient air | EIOAc  | <5                 |
| 21    | CuOAc₂    | ambient air | THF    | <5                 |
| 22    | [Cu(MeCN)₄][PF₆] | ambient air | CH₂Cl₂ | 20                 |
| 23    | [Cu(MeCN)₄][PF₆] | ambient air | MeCN   | 14                 |
| 24    | [Cu(MeCN)₄][PF₆] | O₂       | CH₂Cl₂ | 7                  |
| 25    | [Cu(MeCN)₄][PF₆] | O₂       | MeCN   | 20                 |
| 26    | [Cu(MeCN)₄][PF₆] | TBHP (decanes)² | MeCN | 9                  |
| 27    | [Cu(MeCN)₄][PF₆] | TBHP (decanes)² | MeCN | 5                  |
| 28    | [Cu(MeCN)₄][PF₆] | H₂O₂-Urea² | MeCN | <5                 |
| 29    | [Cu(MeCN)₄][PF₆] | oxone² | MeCN | <5                 |

²No TMEDA added to reaction mixture. ³Reactions were run open to air. ⁴Run under N₂ atmosphere.
Conditions Optimization

Procedure: See above general procedure for synthesis of compound 3. Variations from this procedure are listed in the table below (i.e. – atmosphere, temperature, addition rate). Reactions were allowed to stir for 48 hours at room temperature after which aliquots were taken and analyzed for yield by HPLC with biphenyl as an internal standard.

| Entry | Atmosphere (1 atm) | Temp. | Addn. of 6 | % Yield (HPLC-DAD) |
|-------|--------------------|-------|------------|---------------------|
| 1     | O₂                 | r.t.  | at once    | 51                  |
| 2     | O₂                 | 45 °C | at once    | 31                  |
| 3     | O₂                 | r.t.  | over 2 h   | 57                  |
| 4     | O₂                 | 45 °C | over 2 h   | 55 (1.5 h)          |
| 5     | N₂                | r.t.  | at once    | 45                  |
| 6     | N₂                | 45 °C | at once    | 50                  |
| 7     | N₂                | r.t.  | over 2 h   | 71 (4 d)            |
| 8     | N₂                | 45 °C | over 2 h   | 57                  |

*Cu/MeCN mixture sparged with O₂ for 20 minutes, then transferred to reaction vial under N₂ prior to addition of 6.

| Time Course of Entry 4 | Time Course of Entry 7 |
|------------------------|------------------------|
| **Time (h)** | **% Yield** | **Time (h)** | **% Yield** |
| 0.17 | 52 | 9 | 45 |
| 1.5 | 55 | 32 | 55 |
| 3.0 | 50 | 48 | 65 |
| 15 | 37 | 72 | 68 |
| 24 | 30 | 4 d | 71 |

*Times are after complete addition of substrate.
Copper Source and Solvent Optimization

Procedure: See above general procedure for synthesis of compound 3. Reactions were allowed to stir for 48 hours at room temperature after which aliquots were taken and analyzed for yield by HPLC with biphenyl as an internal standard.

| Entry | Cu Source    | Solvent    | % Yield (HPLC-DAD) |
|-------|--------------|------------|--------------------|
| 1     | CuI          | CH₂Cl₂     | <5                 |
| 2     | CuOAc        | CH₂Cl₂     | <5                 |
| 3     | CuCl₂        | CH₂Cl₂     | <5                 |
| 4     | CuBr₂        | CH₂Cl₂     | 9                  |
| 5     | CuSO₄        | CH₂Cl₂     | <5                 |
| 6     | Cu(OTf)₂     | CH₂Cl₂     | <5                 |
| 7     | CuCl         | CH₂Cl₂     | 21                 |
| 8     | CuCl         | acetone    | <5                 |
| 9     | CuCl         | EtOAc      | 6                  |
| 10    | CuCl         | THF        | 12                 |
| 11    | CuCl         | MeCN       | 32                 |
| 12    | CuBr         | CH₂Cl₂     | 15                 |
| 13    | CuBr         | acetone    | 5                  |
| 14    | CuBr         | EtOAc      | 6                  |
| 15    | CuBr         | THF        | 12                 |
| 16    | CuBr         | MeCN       | 37                 |
| 17    | Cu(OAc)₂     | MeCN       | 6                  |
| 18    | [Cu(MeCN)₄][PF₆] | CH₂Cl₂  | 34                 |
| 19    | [Cu(MeCN)₄][PF₆] | MeCN    | 65                 |
| 20    | [Cu(MeCN)₄][PF₆] | DMF    | <5                 |
| 21    | [Cu(MeCN)₄][PF₆] | THF    | 21                 |
| 22    | [Cu(MeCN)₄][PF₆] | MeOH   | <5                 |
| 23    | [Cu(MeCN)₄][PF₆] | HFIP   | <5                 |
| 24    | [Cu(MeCN)₄][PF₆] | MeCN w/ 10% HFIP | 12               |
Ligand Optimization

**Procedure:** See above general procedure for synthesis of compound 3. Reactions were allowed to stir for 48 hours at room temperature after which aliquots were taken and analyzed for yield by HPLC with biphenyl as an internal standard.

| Entry | Ligand (equiv.) | % Yield (HPLC-DAD) |
|-------|-----------------|-----------------|
| 1     | TMEDA (2.0)     | 65              |
| 2     | Et,N (2.0)      | no rxn          |
| 3     | L1 (2.0)        | <5              |
| 4     | L2 (2.0)        | <5              |
| 5     | L3 (2.0)        | <5              |
| 6     | L4 (2.0)        | 34              |
| 7     | L5 (2.0)        | 13              |
| 8     | L6 (2.0)        | <5              |
| 9     | L7 (2.0)        | <5              |
| 10    | L8 (2.0)        | 5               |
| 11    | L9 (2.0)        | 10              |
| 12    | L10 (2.0)       | 7               |
| 13    | L11 (2.0)       | 10              |
| 14    | L12 (2.0)       | <5              |
| 15    | TMEDA (1.0)     | 21              |
| 16    | TMEDA (0.5)     | <5              |
| 17    | TMEDA (4.0)     | 32              |
Failed Attempts of Stereo-retentive Tail Coupling

[Chemical structures and reactions]

[All conditions yield 1:1 mixture of diastereomers at designated center]
Spectral Data

Compound S3 $^1$H NMR
Compound S3 $^{13}$C NMR
Compound S4 $^1$H NMR
Compound S4 $^{13}$C NMR
Compound 2 $^1$H NMR
Compound 2 $^{13}$C NMR
Compound 3 \(^1\)H NMR
Compound 3 $^{13}$C NMR
Compound 3 COSY

(uudd) \( T_J \)
Compound 3 HSQC
Compound 3 ROESY
Compound 3 NOESY @ 55 °C
Compound 3 NOESY @ 55°C (zoom)
Compound 3 $^1$H NMR (variable temperature)

Blue = 25 °C
Red = 55 °C

Blue = 25 °C
Red = 55 °C
Compound 3 $^1$H NMR (variable temperature – zoom)

Blue = 25 °C
Red = 55 °C
Compound S6 $^1$H NMR
Compound S6 $^{13}$C NMR
Compound 5 $^1$H NMR
Compound 5 $^{13}$C NMR

![NMR Spectrum Image]
Compound 6 $^1$H NMR
Compound 6 $^{13}$C NMR
Compound 7 1H NMR

f1 (ppm)

12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1
Compound 7 $^{13}$C NMR
Compound 7 COSY
Compound 7 HSQC
Compound 8 $^1$H NMR
Compound 8 $^{13}$C NMR
Compound 9 $^{13}$C NMR
Compound 9 HMBC
Compound 10 $^{13}$C NMR
Compound 10 COSY
Compound 10 ROESY
Compound 11 $^1$H NMR
Compound 11 $^{13}$C NMR
Compound 12 $^1$H NMR
Compound 12 $^{13}$C NMR
Compound 12 COSY

\[ \text{COSY} \]
Compound 12 ROESY
Compound 13 $^1$H NMR
Compound 13 $^{13}$C NMR
Compound 13 COSY

(uudd) $\tau_J$
Compound 13 HSQC
Materials and Methods (Biological Studies)

Materials

Strains tested were MRSA strain USA300, MRSA strain COL, *S. epidermidis* RP62A, *E. coli* BAS901, *E. coli* K-12 MG1655, *Y. pestis* KIM6+, *P. aeruginosa* PAO1, *K. pneumoniae* 700721. Bacteria were routinely grown at 37 °C on Mueller-Hinton II agar (MHIIA) and cation-adjusted Mueller-Hinton II broth (MHIIB) or on trypticase soy agar (TSA) or broth (TSB).

Arylomycin A-C₁₆ Structure

![Arylomycin A-C₁₆](image)

Succeptibility Determinations

Antibiotic susceptibilities were determined using the Clinical and Laboratory Standards Institute broth microdilution method.² Briefly, antibiotics were prepared as 2-fold dilutions in 96-well plates containing cation-adjusted Mueller-Hinton II broth containing (0.01% v/v Tween-80). Stock solutions of antibiotics were made in dimethyl sulfoxide (DMSO). Wells were inoculated from a fresh plate scrape diluted to a final concentration of 5 × 10⁵ CFU/ml and incubated at 37°C. Growth observed visually at 20 h. All MICs are an average of at least three independent determinations.
Figure S1 – Arylomycin A$_2$/LepB Structure

Image generated from RCSB PDB-1T7D. Ligand-protein interactions depicted are putative hydrogen bonds as described in original report.$^5$ The lipophilic tail unresolved and therefore not included.

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