Three Siderophores from One Bacterical Assembly Line

William M. Wuest, Elizabeth S. Sattely and Christopher T. Walsh*

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115

Supporting Information

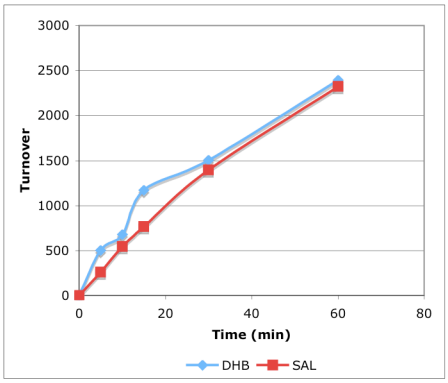
Contents:

1. Materials and General Methods. Standard recombinant DNA, molecular cloning, and microbiological procedures were performed according to the methods described by Sambrook *et al*.[1] *E. coli* competent Top10 and BL21(DE3) strains were purchased from Invitrogen. Oligonucleotide primers were synthesized by Integrated DNA Technologies (Coralville, IA). All chemicals were purchased from Sigma-Aldrich unless noted otherwise. Phusion DNA polymerase, restriction enzymes, and T4 DNA ligase were purchased from New England Biolabs. DNA sequencing was performed at the Molecular Biology Core Facilities of the Dana Farber Cancer Institute (Boston, MA). *P. entomophila* gDNA was isolated with a genomic DNA kit from Biorad. Recombinant plasmid DNA was purified with a Qiaprep kit from Qiagen. Gel extraction of DNA fragments and restriction endonuclease cleanup was done with a QIAquick PCR cleanup kit from Qiagen. Nickel-nitrilotriacetic acid-agarose (Ni-NTA) superflow resin and SDS-page gels were purchased from Qiagen. Protein samples were concentrated using 10K mwco Amicon Ultra filters from Millipore. Microfiltration devices were also purchased from Millipore (10K mwco). The concentration of purified protein solutions was determined by the method of Bradford,[2] with BSA as a standard. HPLC analysis was performed on a Beckman System Gold (Beckman Coulter) instrument on a Vydac small pore C18 column. The eluent was monitored for absorption at 237, 247, or 254 nm for all liquid chromatography analysis of salicylate derivatives and at 250 and 267 for 2,3-dihydrobenzoate derivatives. Unless otherwise noted, the mobile phase consisted of 10% acetonitrile in H_2O (each with 0.1% v/v TFA), isocratic elution. LCMS analysis was carried out on a Shimadzu LCMS-QP8000a equipped with a Luna Phenomenex C18 column. ^{1}H NMR spectra were recorded on a Varian 600 MHz spectrometer. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance resulting from incomplete deuteration as the internal standard ($CDCl_3$ δ 7.26, D_2O δ 4.79, CD_3OD δ 3.31). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants (Hz), and integration. A Cary 50 Bio scanning spectrometer was employed to record absorption spectra.

2. Cloning, Expression and Purification of Pms Proteins. The Pms proteins were prepared following the procedure previously disclosed by Sattely and Walsh.[3]

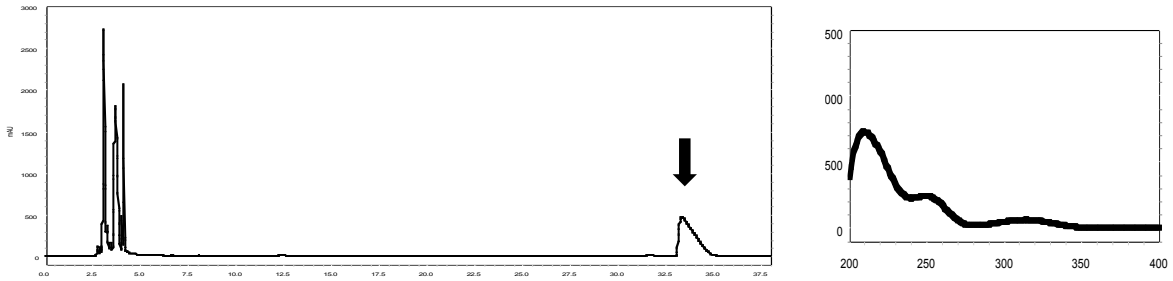
3. ATP-PPi Exchange Assay to Determine Activity of PmsE A Domain. A typical experiment (700 μL total volume) was carried as follows: $MgCl_2$ (10 mM), DTT (5 mM), ATP (5 mM), tetrasodium[^{32}P]pyrophosphate (1 mM, 1.2 μCi), BSA (100 μg/mL), and substrate acid (5 mM) were combined in reaction buffer (75 mM Tris, pH 7.75); reactions were then initiated by the addition of PmsE to a final concentration of 0.1 μM. At regular time intervals, 100 μL aliquots (90 μL for T = 0, before addition of enzyme) were quenched with a 500 μL solution of activated charcoal (1.6% w/v), 200 mM tetrasodium pyrophosphate, and 3.5% perchloric acid in water. The charcoal was pelleted by centrifugation and washed twice with a 500 μL solution of 200 mM tetrasodium pyrophosphate and 3.5% perchloric acid in water. The radioactivity of ATP bound to the charcoal was then measured by liquid scintillation counting. Turnover was calculated as (% incorporation of ^{32}P-PPi)[total PPi]/[Enz]. Results shown in SI Figure 1.

SI Figure 1. ATP-PPi Exchange Assay of the PmsE A domain with DHB and SAL.



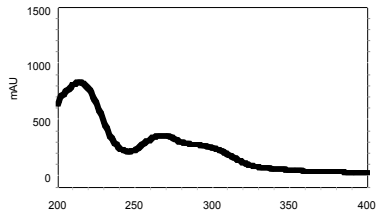
4a. Reconstitution of Acinetobactin Biosynthesis with PmsD, E, and G; Isolation of Acinetobactin (5). Holo-PmsE and holo-PmsG were prepared as indicated above by incubation with Sfp and CoA. The resulting solutions were then used directly in reconstitution experiments with PmsD. Reactions of 150 μL contained 18 μM holo-PmsE, 18 μM holo-PmsG, 3.6 μM PmsD, 360 μM dihydroxybenzoate (in 25 mM Tris, pH 8), 360 μM of N-hydroxyhistamine (prepared according to the protocol described by Sattely[3]), 360 μM L-Thr, 3 mM ATP, 50 mM Tris (pH 7), 3 mM TCEP, and 10 mM $MgCl_2$. Enzymes were combined with a homogeneous solution of substrates and reagents, with PmsD added last to initiate the first condensation event. Incubation for 4 h at ambient temperature was followed by quenching with 450 μL of cold MeOH and cooling in an ice bath for 10 min. The solution was then centrifuged at 13K for 5 min, followed by transfer of the supernatant to a one-dram vial and then concentrated in vacuo. The residue was next taken up in 150 μL of water and injected on the HPLC. The filtrate was then analyzed by reverse phase HPLC on a Vydac C18 small pore with a mobile phase of 10% MeCN in H_2O (each with 0.1% v/v TFA), isocratic elution with monitoring at 237 and 254 nm. As shown in SI Figure 3, the direct product of the reconstitution is acinetobactin. Enzymatic acinetobactin: ^{1}H NMR (D_2O): δ 8.70 (s, 1H), 7.35 (s, 1H), 7.14 (d, J = 7.9 Hz, 1H), 6.86 (d, J = 7.6 Hz, 1H), 6.64 (dd, J = 7.6, 7.9 Hz, 1H), 4.46 (m, 2H), 3.92 (dt, J = 14.1, 7.0 Hz, 1H), 3.81 (dt, J = 14.7, 6.7 Hz, 1H), 3.03 (t, J = 6.4 Hz, 2H), 1.37 (d, J = 5.3 Hz, 3H). UV analysis: λ max = 210, 248, 313 nm (literature = 210, 250, 317 nm; see SI Figure 2 for UV/vis spectrum). HRMS calcd. for $C_{16}H_{18}N_4O_5$: 347.35 [M+H]; found, 347.20.

SI Figure 2. HPLC Trace and UV/vis Spectra of Acinetobactin.



4b. Reconstitution of Anguibactin Biosynthesis with PmsD, E, and G; Isolation of Anguibactin (2). Holo-PmsE and holo-PmsG were prepared as indicated above by incubation with Sfp and CoA. The resulting solutions were then used directly in reconstitution experiments with PmsD. Reactions of 150 μL contained 18 μM holo-PmsE, 18 μM holo-PmsG, 3.6 μM PmsD, 360 μM dihydroxybenzoate (in 25 mM Tris, pH 8), 360 μM of N-hydroxyhistamine, 360 μM L-Cys, 3 mM ATP, 50 mM Tris (pH 7), 3 mM TCEP, and 10 mM $MgCl_2$. Enzymes were combined with a homogeneous solution of substrates and reagents, with PmsD added last to initiate the first condensation event. Incubation for 4 h at ambient temperature was followed by quenching with 450 μL of cold MeOH and cooling in an ice bath for 10 min. The solution was then centrifuged at 13K for 5 min, followed by transfer of the supernatant to a one-dram vial and then concentrated in vacuo. The residue was next taken up in 150 μL of water and injected on the HPLC. The filtrate was then analyzed by reverse phase HPLC on a Vydac C18 small pore with a mobile phase of 10% MeCN in H_2O (each with 0.1% v/v TFA), isocratic elution with monitoring at 237 and 254 nm. As shown in Scheme 1C (main text), the direct product of the reconstitution is anguibactin. Enzymatic anguibactin: ^{1}H NMR (D_2O): δ 8.66 (s, 1H), 7.97 (s, 1H), 7.25 (s, 1H), 6.85 (d, *J* = 7.6 Hz, 1H), 6.84 (d, *J* = 7.2 Hz, 1H), 6.66 (dd, *J* = 7.6, 7.2 Hz, 1H), 5.74 (dd, *J* = 9.1, 8.8 Hz, 1H), 4.06 (ddd, *J* = 14.1, 5.6, 2.0 Hz, 1H), 3.83 (ddd, *J* = 14.7, 6.0, 2.0 Hz, 1H), 3.50 (dt, *J* = 10.9, 2.0 Hz, 1H), 3.35 (dd, *J* = 11.2, 10.9, 1H), 3.05 (m, 2H). UV analysis: λ max = 215, 268 nm (literature = 214, 260 nm; see SI Figure 3 for UV/vis spectrum). LCMS calcd. for $C_{15}H_{16}N_4O_4S$: 349.39 [M+H]; found, 349.20.

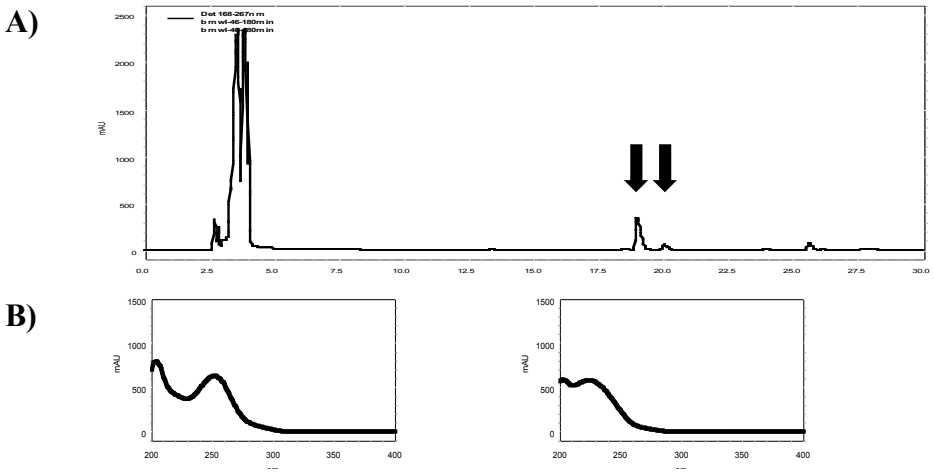
SI Figure 3. UV/vis Spectra of Anguibactin.



4c. Reconstitution of 2,3-deshydroxy-acinetobactin (10) Biosynthesis with PmsD, E, and G. Holo-PmsE and holo-PmsG were prepared as indicated above by incubation with Sfp and CoA. The resulting solutions were then used directly in reconstitution experiments with PmsD. Reactions of 150 μL contained 18 μM holo-PmsE, 18 μM holo-PmsG, 3.6 μM PmsD, 360 μM benzoic acid (in 25 mM Tris, pH 8), 360 μM of N-hydroxyhistamine, 360 μM L-Thr, 3 mM ATP, 50 mM Tris (pH 7), 3 mM TCEP, and 10 mM $MgCl_2$. Enzymes were combined with a homogeneous solution of substrates and reagents, with PmsD added last to initiate the first condensation event. Incubation for 4 h at ambient temperature was followed by quenching with 450 μL of cold MeOH and cooling in an ice bath for 10 min. The solution was then centrifuged at 13K for 5 min, followed by transfer of the supernatant to a one-dram vial and concentrated in vacuo. The residue was next taken up in 150 μL of water and injected on the HPLC. The eluent was analyzed by reverse phase HPLC according to the method used in the reconstitution of acinetobactin. As shown in SI Figure 4, the two products of the reconstitution were assigned as pre-**10** and **10** according to MS and UV absorption data. Pre-**10** (18 min): UV analysis: λ max = 204, 254 nm. LCMS for $C_{16}H_{18}N_4O_3$ [M+H]: expected, 315.35; found, 315.20. **10** (20 min): UV

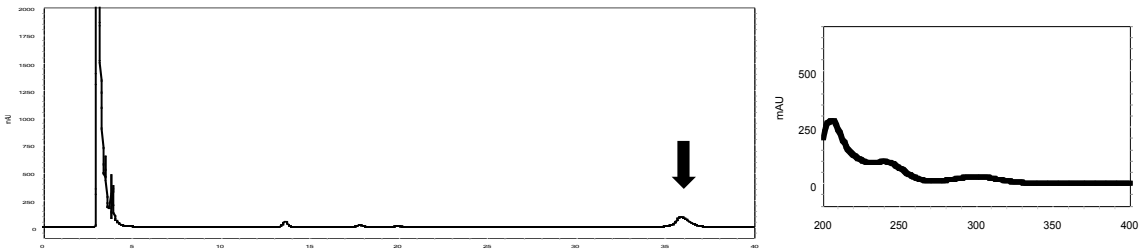
analysis: λ max = 203, 229 nm. LCMS for $C_{16}H_{18}N_4O_3$ [M+H]: expected, 315.345; found, 315.20.

SI Figure 4. HPLC Trace (A) and UV/vis Spectras (B) of 2,3-deshydroxy-pre-acinetobactin (Left, 18 min) and 2,3-deshydroxy-acinetobactin (Right, 20 min).



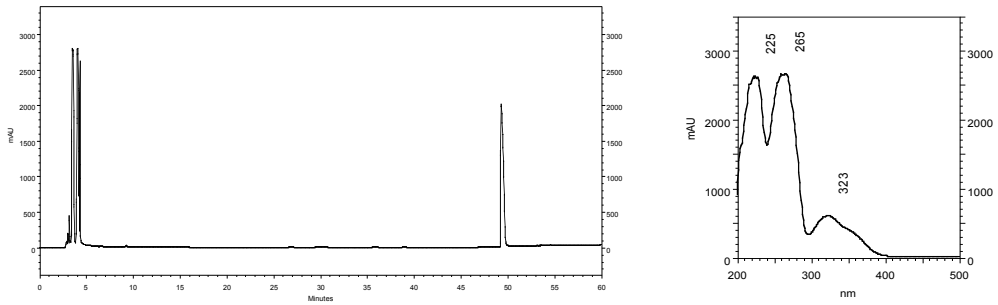
4d. Reconstitution of Desmethyl-acinetobactin (6) Biosynthesis with PmsD, E, and G. Holo-PmsE and holo-PmsG were prepared as indicated above by incubation with Sfp and CoA. The resulting solutions were then used directly in reconstitution experiments with PmsD. Reactions of 150 μL contained 18 μM holo-PmsE, 18 μM holo-PmsG, 3.6 μM PmsD, 360 μM dihydroxybenzoate (in 25 mM Tris, pH 8), 360 μM of N-hydroxyhistamine, 360 μM L-Ser, 3 mM ATP, 50 mM Tris (pH 7), 3 mM TCEP, and 10 mM $MgCl_2$. Enzymes were combined with a homogeneous solution of substrates and reagents, with PmsD added last to initiate the first condensation event. Incubation for 4 h at ambient temperature was followed by quenching with 450 μL of cold MeOH and cooling in an ice bath for 10 min. The solution was then centrifuged at 13K for 5 min, followed by transfer of the supernatant to a one-dram vial and concentrated in vacuo. The residue was next taken up in 150 μL of water and injected on the HPLC. The eluent was analyzed by reverse phase HPLC according to the method used in the reconstitution of acinetobactin. As shown in SI Figure 5, the direct product of the reconstitution was assigned as **6** according to MS and UV absorption data. UV analysis: λ max = 211, 257, 326 nm. LCMS for $C_{15}H_{16}N_4O_5$ [M+H]: expected, 333.32; found, 333.30.

SI Figure 5. HPLC Trace and UV/vis Spectra of Desmethyl-acinetobactin (36 min)



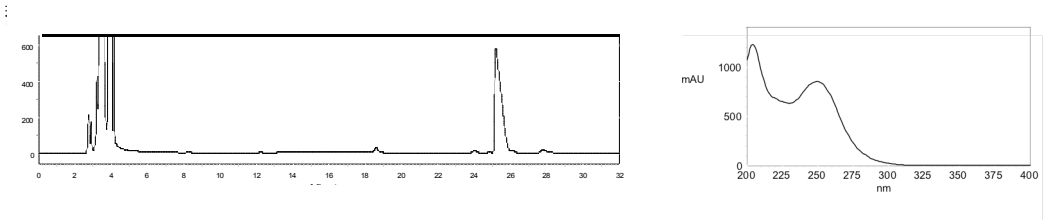
4e. Reconstitution of N-Deshydroxy-pre-acinetobactin (7) Biosynthesis with PmsD, E, and G. Holo-PmsE and holo-PmsG were prepared as indicated above by incubation with Sfp and CoA. The resulting solutions were then used directly in reconstitution experiments with PmsD. Reactions of 150 μL contained 18 μM holo-PmsE, 18 μM holo-PmsG, 3.6 μM PmsD, 360 μM 2,3-dihydroxybenzoate (in 25 mM Tris, pH 8), 360 μM of histamine, 360 μM L-Thr, 3 mM ATP, 50 mM Tris (pH 7), 3 mM TCEP, and 10 mM $MgCl_2$. Enzymes were combined with a homogeneous solution of substrates and reagents, with PmsD added last to initiate the first condensation event. Incubation for 4 h at ambient temperature was followed by quenching with 450 μL of cold MeOH and cooling in an ice bath for 10 min. The solution was then centrifuged at 13K for 5 min, followed by transfer of the supernatant to a one-dram vial and concentrated in vacuo. The residue was next taken up in 150 μL of water and injected on the HPLC. The eluent was analyzed by reverse phase HPLC according to the method used in the reconstitution of acinetobactin. As shown in SI Figure 6, the direct product of the reconstitution was assigned as **7** according to MS and UV absorption data. UV analysis: λ max = 225, 265, 323 nm. LCMS for $C_{15}H_{16}N_4O_5$ [M+H]: expected, 333.36; found, 333.20.

SI Figure 6. HPLC Trace and UV/vis Spectra of *N*-deshydroxy-pre-acinetobactin (49 min)



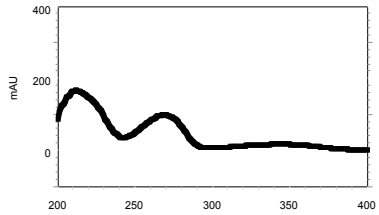
4f. Reconstitution of N,2,3-Deshydroxy-pre-acinetobactin (9) Biosynthesis with PmsD, E, and G. Holo-PmsE and holo-PmsG were prepared as indicated above by incubation with Sfp and CoA. The resulting solutions were then used directly in reconstitution experiments with PmsD. Reactions of 150 μL contained 18 μM holo-PmsE, 18 μM holo-PmsG, 3.6 μM PmsD, 360 μM benzoic acid (in 25 mM Tris, pH 8), 360 μM of histamine, 360 μM L-Thr, 3 mM ATP, 50 mM Tris (pH 7), 3 mM TCEP, and 10 mM $MgCl_2$. Enzymes were combined with a homogeneous solution of substrates and reagents, with PmsD added last to initiate the first condensation event. Incubation for 4 h at ambient temperature was followed by quenching with 450 μL of cold MeOH and cooling in an ice bath for 10 min. The solution was then centrifuged at 13K for 5 min, followed by transfer of the supernatant to a one-dram vial and concentrated in vacuo. The residue was next taken up in 150 μL of water and injected on the HPLC. The eluent was analyzed by reverse phase HPLC according to the method used in the reconstitution of acinetobactin. As shown in SI Figure 7, the direct product of the reconstitution was assigned as **9** according to MS and UV absorption data. UV analysis: λ max = 206, 254 nm. LCMS for $C_{15}H_{16}N_4O_5$ [M+H]: expected, 299.34; found, 299.30.

SI Figure 7. HPLC Trace and UV/vis Spectra of *N*,2,3-Deshydroxy-pre-acinetobactin (26 min)



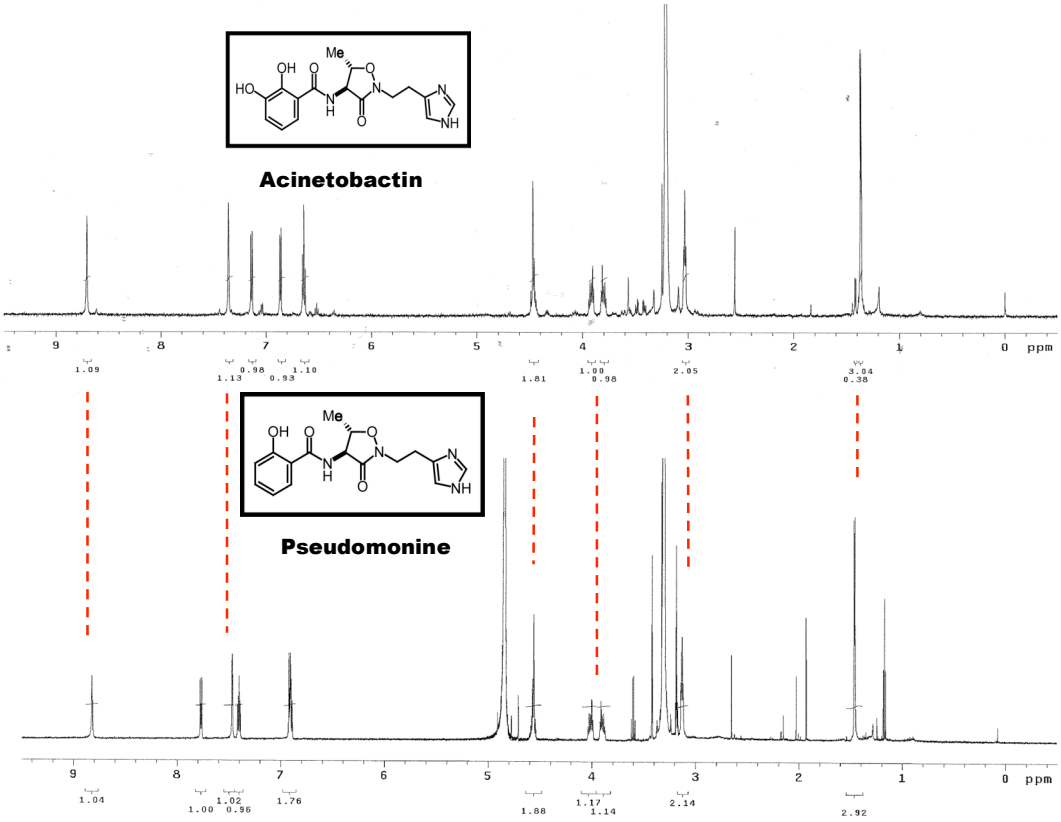
4g. Time Course of Reconstitution; Isolation of Pre-acinetobactin (1). The experiment described for the isolation of acinetobactin was carried out, but with 75 μL aliquots taken at varied time intervals to monitor the production and disappearance of **1**. Each aliquot was quenched with 225 μL of cold MeOH and cooled in an ice bath for 10 min. The solution was then centrifuged at 13K for 5 min, followed by transfer of the supernatant to a one-dram vial and concentrated in vacuo (to remove protein prior to HPLC analysis). The residue was next taken up in 150 μL of water and injected on the HPLC. Results are depicted in Scheme 1B (main text). The compound eluting at 35 minutes was analyzed further by LCMS $C_{16}H_{18}N_4O_5$ [M+H]: expected, 347.35; found 347.20. Although the mass was identical to that of acinetobactin, differences in the pre-acinetobactin UV absorption profile (see SI Figure 8 for UV/vis spectrum) supports the assignment as an acinetobactin isomer, bearing an oxazoline.

SI Figure 8. UV/vis Spectra of Pre-acinetobactin.



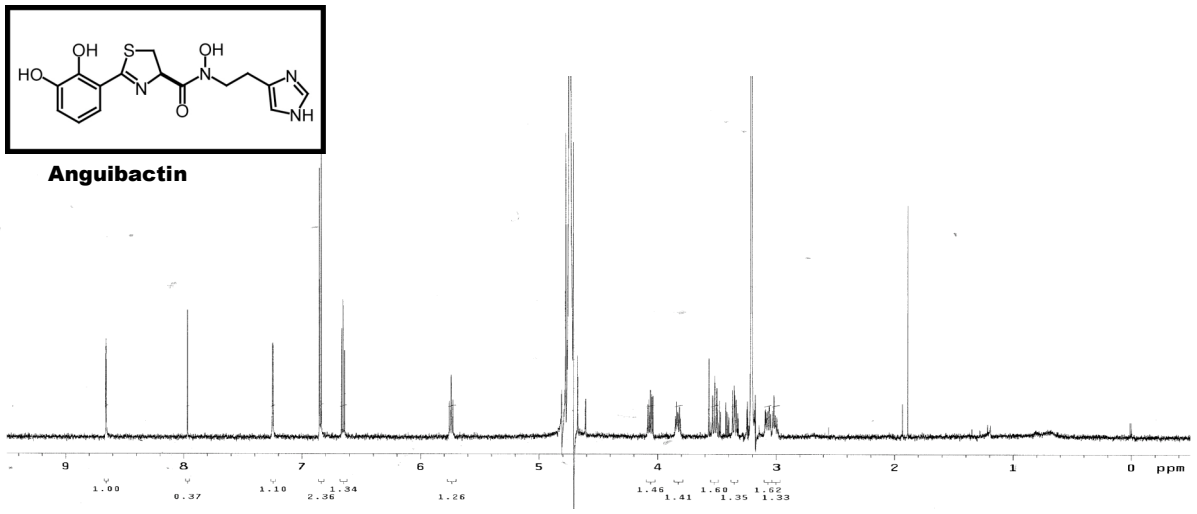
5. Large-Scale Enzymatic Preparation of Acinetobactin with PmsD, E, and G for NMR Analysis. Experiments to produce sufficient acinetobactin for characterization were carried out as described for the isolation of acinetobactin with the following exceptions: reaction volumes of 450 μL were prepared with increased substrate concentration (720 μM each of DHB, L-Thr, and *N*-hydroxyhistamine) and ATP (5 mM). Reactions were incubated at ambient temperature for 12 h, followed by quench with 450 μL cold MeOH. Precipitated protein was pelleted by centrifugation, and the supernatant was separated and concentrated in vacuo. The resulting residue was dissolved in 200 μL water and purified by reverse-phase analytical HPLC. Material (as the TFA salt) from four such experiments was combined for ^{1}H NMR (SI Figure 9).

SI Figure 9. Comparative ^{1}H NMR (D_2O) of Acinetobactin-TFA (top) and Pseudomonine-TFA (bottom).



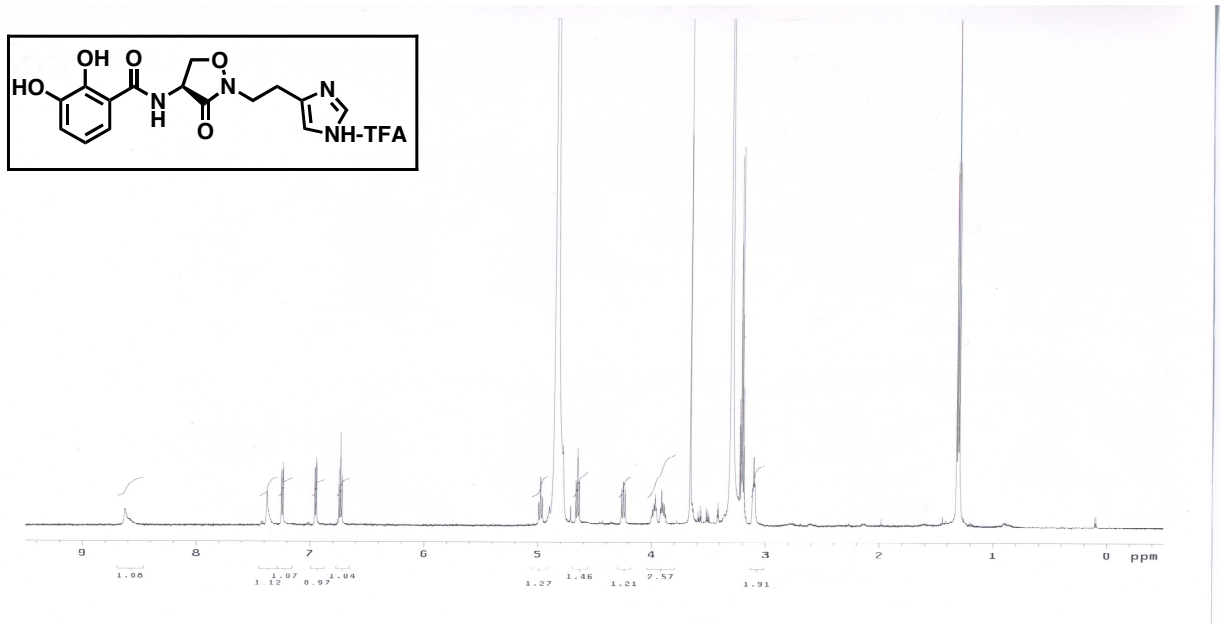
6. Large-Scale Enzymatic Preparation of Anguibactin with PmsD, E, and G for NMR Analysis. Experiments to produce sufficient anguibactin for characterization were carried out as described for the isolation of anguibactin with the following exceptions: reaction volumes of 450 μL were prepared with increased substrate concentration (720 μM each of DHB, L-Cys, and *N*-hydroxyhistamine) and ATP (5 mM). Reactions were incubated at ambient temperature for 12 h, followed by quench with 450 μL cold MeOH. Precipitated protein was pelleted by centrifugation, and the supernatant was separated and concentrated in vacuo. The resulting residue was dissolved in 200 μL water and purified by reverse-phase analytical HPLC. Material (as the TFA salt) from four such experiments was combined for ^{1}H NMR (SI Figure 10).

SI Figure 10. ^{1}H NMR (D_2O) of Anguibactin-TFA.



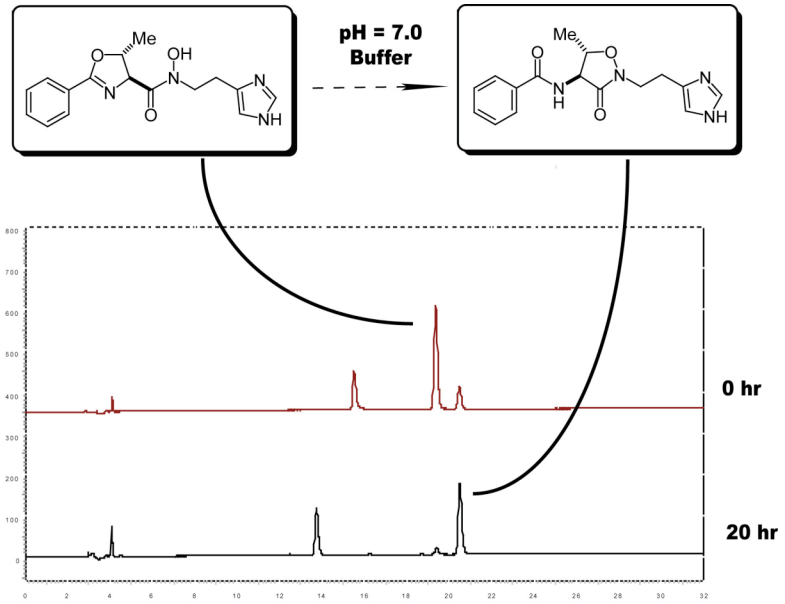
7. Large-Scale Enzymatic Preparation of Desmethyl-Acinetobactin with PmsD, E, and G for NMR Analysis. Experiments to produce sufficient desmethyl-acinetobactin for characterization were carried out as described for the isolation of desmethyl-acinetobactin with the following exceptions: reaction volumes of 450 μL were prepared with increased substrate concentration (720 μM each of DHB, L-Ser, and *N*-hydroxyhistamine) and ATP (5 mM). Reactions were incubated at ambient temperature for 12 h, followed by quench with 450 μL cold MeOH. Precipitated protein was pelleted by centrifugation, and the supernatant was separated and concentrated in vacuo. The resulting residue was dissolved in 200 μL water and purified by reverse-phase analytical HPLC. Material (as the TFA salt) from four such experiments was combined for ^{1}H NMR (SI Figure 11).

SI Figure 11. ^{1}H NMR (CD_3OD) of Enzymatic Desmethyl-acinetobactin-TFA.



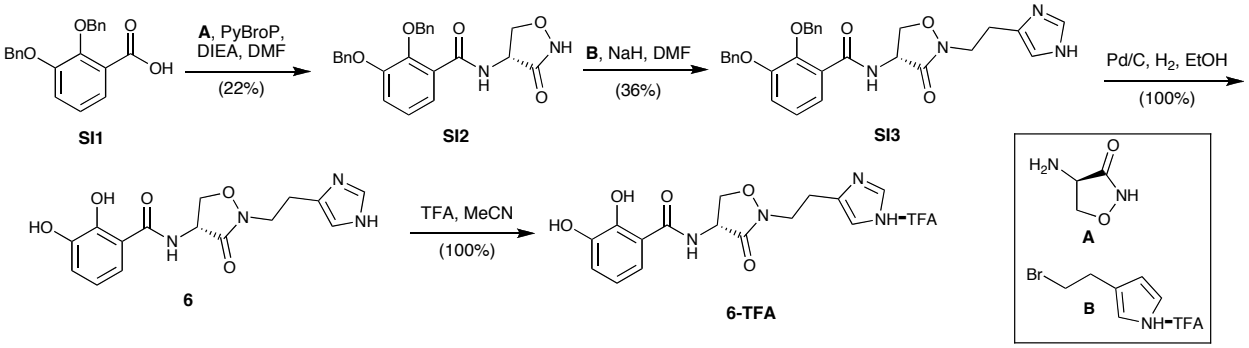
8. Non-enzymatic Rearrangement of 2,3-deshydroxy-acinetobactin (10). The acinetobactin reconstitution time course was performed as described above, however using benzoic acid in place of DHB, and each time point was analyzed by HPLC. Fractions of the eluent containing pre-**10** were collected, combined and concentrated by lyophilization. Based on apparent conversion, a 0.7 mM solution of pre-**10** was prepared in 50 mM Tris (pH 7) and was incubated (50 μL reaction volume) at ambient temperature for 20 h. The reaction was filtered through a 10K mwco membrane and analyzed by HPLC. Non-enzymatic conversion to **10** had occurred indicating that the rearrangement is likely a spontaneous process (see SI Figure 12).

SI Figure 12. Non-enzymatic Rearrangement of 2,3-deshydroxy-acinetobactin at pH 7.0 after 20 hrs (hydrolyzed product at ~14-15 min).



9. Chemical Synthesis of Desmethyl-acinetobactin (6).

SI Figure 13. Chemical Synthesis of Desmethyl-acinetobactin (**6**).



A 50 mL round bottom flask was charged with 2,3-dihydroxybenzoic acid (**SI1**) (750 mg; 2.11 mmol; 1.0 equiv. prepared according to published procedure[4]), a solution of cycloserine (**B**) in water (211 mg/1.0 mL; 2.11 mmol; 1.0 equiv.), PyBroP (1.28 g; 2.74 mmol; 1.3 equiv.),

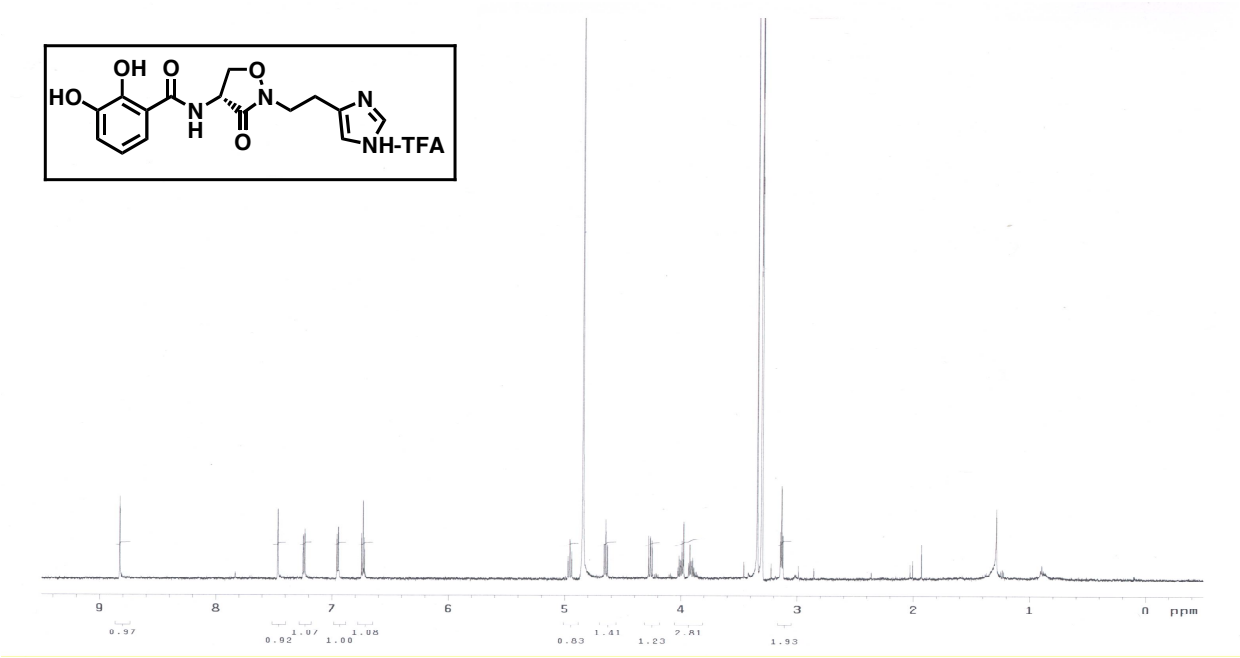
diisopropylethylamine (1.103 mL; 6.33 mmol; 3.0 equiv.) and DMF (21 mL). The resulting solution was stirred at room temperature for 12 h, and then transferred to separatory funnel. The organic layer was diluted with 5 mL ethyl acetate, washed twice with two 5 mL portions of an aqueous solution of citric acid (0.1M), followed by 5 mL of a saturated aqueous solution of sodium bicarbonate, and five 5 mL portions of water. The organic solution was then dried over sodium sulfate, filtered and concentrated in vacuo. Purification by silica gel chromatography in 1:1 CH_2Cl_2/EtOAc afforded 198 mg (0.473 mmol; 22%) of amide **SI2** as an amorphous white solid. ^{1}H NMR ($CDCl_3$): δ 8.57 (br, 1H), 7.69 (dd, *J* = 6.6, 1.5 Hz, 1H), 7.53-7.11 (m, 12H), 5.25 (d, *J* = 7.8 Hz, 1H), 5.18 (s, 2H), 5.09 (d, *J* = 7.8 Hz, 1H), 4.84-4.80 (m, 1H), 4.74 (t, *J* = 8.4 Hz, 1H), 3.81 (t, *J* = 8.4 Hz, 1H).

A solution of amide **SI2** (56.0 mg; 0.134 mmol; 1.0 equiv.) and bromide **B** (prepared according to published procedure[5]) (28.3 mg; 0.134 mmol; 1.0 equiv.) in anhydrous DMF (1.2 mL) was added to a N_2-filled, 10 mL round bottom flask at room temperature. NaH (60% in mineral oil) (10.7 mg; 0.268 mmol; 2.0 equiv.) was next added and the solution was stirred at room temperature for 3 h. The reaction was quenched with aqueous citric acid (0.1M), transferred to a separatory funnel and diluted with 5 mL of ethyl acetate. The organic layer was washed with five 5 mL portions of water, dried over sodium sulfate, filtered and concentrated in vacuo. Purification by silica gel chromatography in 10:1 EtOAc/MeOH afforded 25 mg (0.049 mmol; 36%) of amide **SI3** as an amorphous white solid. ^{1}H NMR ($CDCl_3$): δ 8.59 (br, 1H), 7.68 (dd, *J* = 6.6, 1.5 Hz, 1H), 7.57 (s, 1H), 7.48-7.42 (m, 3H), 7.42-7.35 (m, 4H), 7.33-7.29 (m, 2H), 7.20-7.12 (m, 3H), 6.84 (s, 1H), 5.22 (d, *J* = 7.8 Hz, 1H), 5.17 (s, 2H), 5.08 (d, *J* = 7.8 Hz, 1H), 4.65 (m, 1H), 4.58 (t, *J* = 9.0 Hz, 1H), 3.99-3.93 (m, 1H), 3.88 (t, *J* = 9.6 Hz, 1H), 3.79-3.72 (m, 1H), 3.00-2.95 (m, 2H).

In a sealed one-dram vessel, amide **SI3** (15.5 mg; 0.030 mmol) was dissolved in ethanol (1.0 mL). 10% Pd/C (3.0 mg) was next added and the atmosphere was then evacuated and back filled with hydrogen (1 atm). The purging process was repeated three times and the reaction was then stirred at room temperature for 90 min under a balloon of hydrogen. The solution was next filtered through a plug of celite and rinsed with ethanol (3.0 mL). The solvent was removed in vacuo providing 10 mg (0.030 mmol; 100%) of the free base of desmethyl-acinetobactin (**6**) as an amorphous white solid. ^{1}H NMR (CD_3OD): δ 7.53 (s, 1H), 7.17 (dd, *J* = 7.8, 1.5 Hz, 1H), 6.85 (dd, *J* = 7.8, 1.2 Hz, 1H), 6.84 (s, 1H), 6.31 (t, *J* = 7.8 Hz, 1H), 4.99 (t, *J* = 9.6 Hz, 1H), 4.65 (t, *J* = 8.4 Hz, 1H), 4.26 (dd, *J* = 9.6, 8.4 Hz, 1H), 3.77 (dt, *J* = 7.8, 2.4 Hz, 2H), 2.87 (dt, *J* = 7.8, 2.4 Hz, 2H).

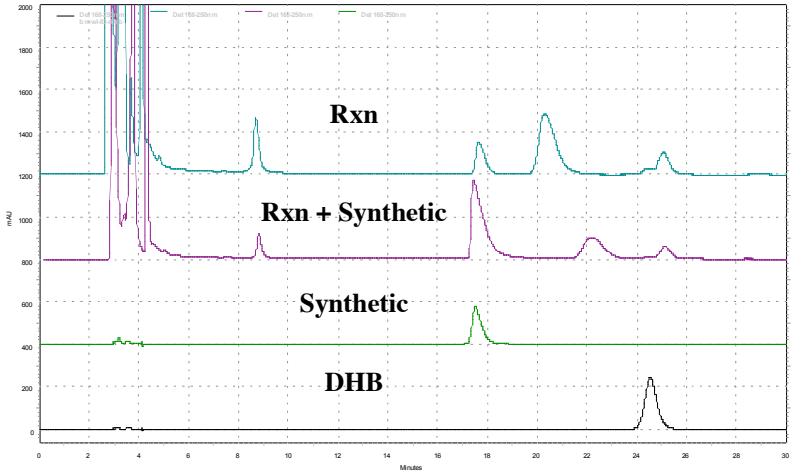
In a one-dram vessel **6** (1.4 mg; 0.0039 mmol) was dissolved in MeCN (1 mL) and TFA (3 drops) was added at room temperature. The solution was stirred for 30 min and the resulting solution was concentrated in vacuo provided 1.4 mg (0.0039; 100%) of the TFA-salt of desmethyl-acinetobactin (**6-TFA**) as an amorphous white solid. ^{1}H NMR (CD_3OD): δ 8.83 (s, 1H), 7.46 (s, 1H), 7.24 (dd, *J* = 8.4, 1.2 Hz, 1H), 6.95 (dd, *J* = 7.8, 1.2 Hz, 1H), 6.74 (dd, *J* = 8.4, 7.8 Hz, 1H), 4.96 (dd, *J* = 10.2, 9.0 Hz, 1H), 4.65 (dd, *J* = 9.0, 8.4 Hz, 1H), 4.26 (dd, *J* = 10.2, 8.4 Hz, 1H), 4.02-3.88 (m, 2H), 3.13 (t, *J* = 6.0 Hz, 2H) (SI Figure 14).

SI Figure 14. ^{1}H NMR (CD_3OD) of Synthetic Desmethyl-acinetobactin-TFA (**6-TFA**).



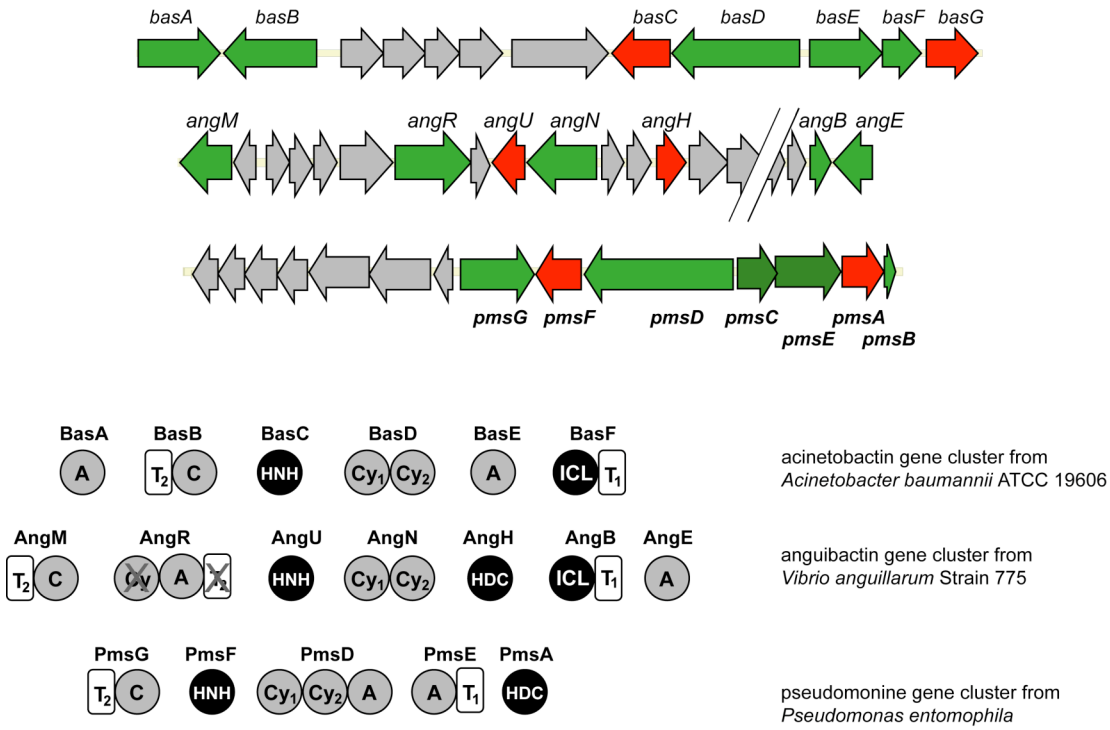
10. Desmethyl-acinetobactin Reconstitution with spiked synthetic standard. The experiment was carried out as described for the isolation of desmethyl-acinetobactin with the following exceptions. The concentrated supernatant residue was taken up in 150 µL of water and then split into two portions (75 µL each). One portion was added to a 75 µL solution of water in a HPLC vial. The second portion was added to a 75 µL solution of 0.5 µmol of synthetic desmethyl-acinetobactin in 75 µL of water in a HPLC vial. Each solution was independently analyzed by HPLC and the results are displayed in SI Figure 15.

SI Figure 15. HPLC Traces of Spiked Reconstitution Assay (non-cyclized product appears at 9 min).



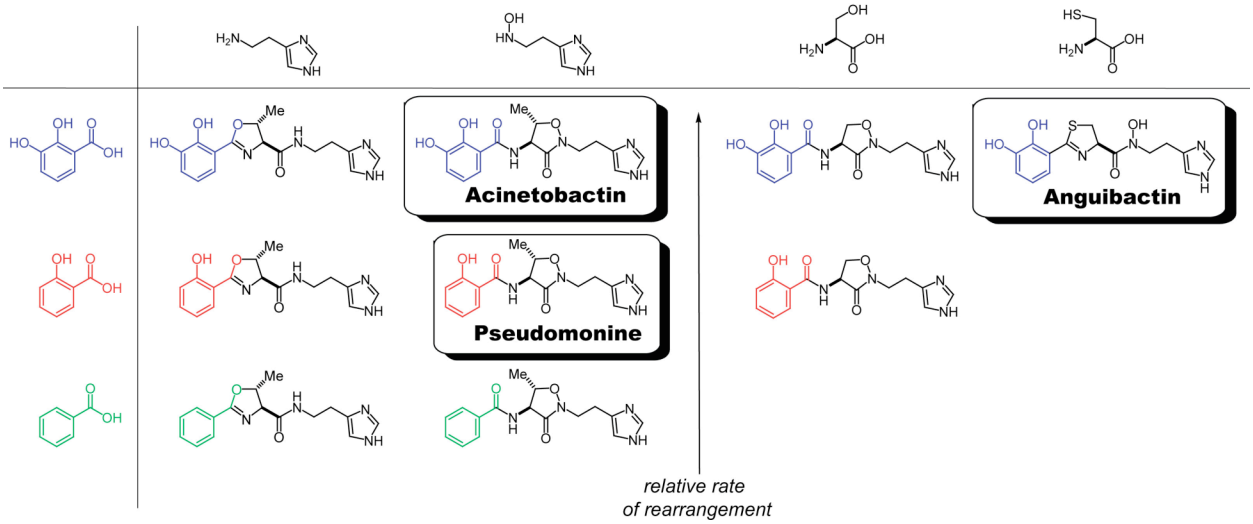
11. Siderophore Assembly Lines.

SI Figure 16. Comparison of Gene Clusters (Top) and Assignments (Bottom) of the Acinetobactin, Anguibactin and Pseudomonine Producer Organisms.



12. Siderophore and Siderophore-like Products Produced by PmsD, E, and G.

SI Figure 17. Complete List of Siderophore and Siderophore-like Compounds Biosynthetically Synthesized by PmsD, E, and G, including relative rate of rearrangement for the oxazoline family of compounds.



(1) Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual.* 3rd ed, Cold Spring Harbor Laboratory Press, Cold Spring Habor, NY, **2001**.
(2) Stoscheck, C. M. *Method. Enzymol.* **1990**, *182*, 50-68.
(3) Sattely, E. S.; Walsh, C. T. *J. Am. Chem. Soc.* **2008**, *130*, 12282-84.
(4) Gardner, R. A.; Kinkade, R.; Wang, C.; Phanstiel, O. IV. *J. Org. Chem*. **2004**. *69*, 3530-3537.
(5) Ting, R.; Lermer, L.; Perrin, D. M. *J. Am. Chem. Soc.* **2004**, *126*, 12720-12721.