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Supporting Information

Functional dissection of a multimodular polypeptide of the pikromycin polyketide synthase into monomodules using a matched pair of heterologous docking domains.

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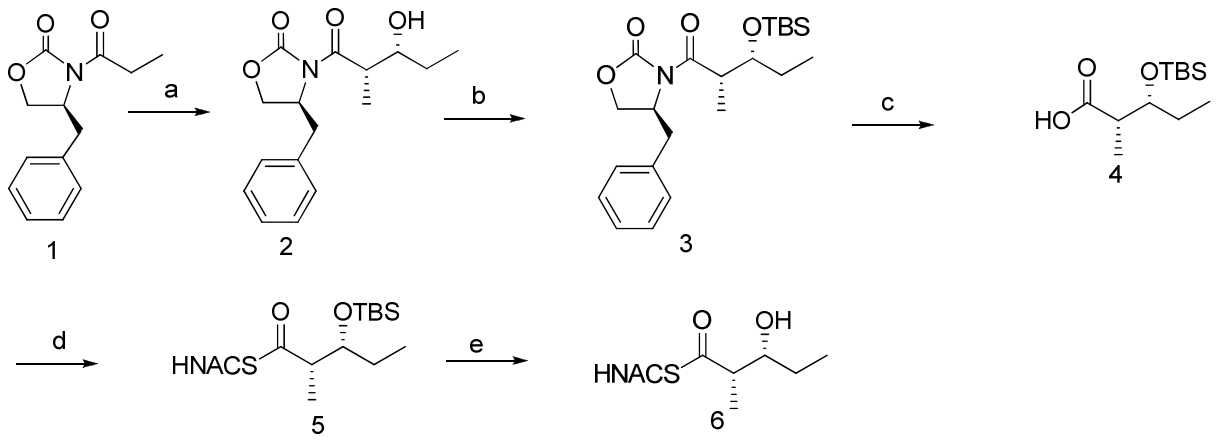
Supplementary Material

Diketide Synthesis

All reactions were carried out under nitrogen atmosphere using dry solvents under anhydrous conditions, unless otherwise noted. All solvents and reagents were purchased from Aldrich. Normal phase flash column chromatography was carried out using Davisil® silica gel (100-200 mesh, Fisher). Preparative thin-layer chromatography (PTLC) separations were carried out on 1 mm, or 2 mm E. Merck silica gel plates (60F-254). ^{1}H NMR spectra were recorded on Tecmag Libra-modified NM-500 MHz or Bruker AC-F 300 MHz spectrometers and calibrated using residual undeuterated solvent as an internal reference. ^{13}C NMR spectra were recorded on a Bruker AMX-400 MHz or Bruker AC-F 300 MHz NMR spectrometers. High-resolution mass spectra (HRMS) were recorded on a Micromass LCT Electrospray mass spectrometer performed at the Mass Spectrometry & Proteomics Facility (The Ohio State University).



Scheme 1. Synthesis of **diketide**. Reagents and conditions: a) DBBT, DIEA, CH_2Cl_2, CH_3CHO; b) TBSOTf, 2,6-Lutidine, CH_2Cl_2; c) LiOH, THF/H_2O (3:1); d) $AcHN(CH_2)_2SH$, EDCI, DMAP, CH_2Cl_2; e) 48% HF, CH_3CN, H_2O.

Compound 1 (Aldol)

To a stirred solution of Evans' acyl-oxazolidinone **1** (0.32g, 1.29 mmol) in CH_2Cl_2 (3 ml, 0.3 M) at 0^oC were added diisopropylethylamine (1.30 ml, 1.29 mmol) and dibutylboron triflate (1.0 M in CH_2Cl_2, 1.30 ml, 1.29 mmol). The resulting reaction mixture was stirred at 0^oC for 30 min and then cooled to -78^oC. Propionaldehyde (0.05 g, 0.86 mmol) in CH_2Cl_2 (1.7 ml, 0.5 M) was added and the mixture was stirred at -78^oC for 1 h and then allowed to warm to 0^oC. After stirring for 2 hrs at this temperature the reaction was quenched by addition of phosphate buffer pH 7 (1 ml). The reaction mixture was poured into a flask containing MeOH (4.3 ml) at 0^oC and treated with precooled 30% H_2O_2 (5.4 ml) and stirred at 0^oC for 1 h. MeOH was removed by rotary evaporation, saturated aqueous $NaHCO_3$ was added and the resultant aqueous layer was extracted with CH_2Cl_2 (3 x 5 ml) and purified by flash silica gel chromatography (silica gel, 10% EtOAc/hexanes) to afford the aldol, **2:** ^1H NMR (500 MHz, $CDCl_3$) δ 7.37-7.20 (m, 5H), 4.74-4.70 (m, 1H), 4.26-4.19 (m, 2H), 3.90-3.86 (m, 1H), 3.82-3.78 (m, 1H), 3.27 (dd, *J*

= 3.5, 13.5 Hz, 1H), 2.88 (m, 1H), 2.80 (dd, *J* = 10, 13.5 Hz, 1H), 1.65-1.45 (m, 2H), 1.26 (d, *J* = 7.5 Hz, 3H), 0.99 (t, *J* = 7.5 Hz, 3H).

Compound 3 (Protected Aldol)

To a solution of **2** (1.45 g, 4.97 mmol) in CH_2Cl_2 (25 ml, 0.2 M) at 0^oC was added 2,6-lutidine (1.73 ml, 14.9 mmol). After stirring for 5 min at that temperature, *tert*-butyldimethylsilyltrifluoromethane sulfonate (1.71 ml, 7.5 mmol) was added dropwise and the reaction mixture was stirred at 0°C for 20 min, after which time no starting material was detected by TLC. Saturated aqueous NH_4Cl was added. The organic phase was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 x 30 ml). The combined organic extracts were dried over anhydrous Na_2SO_4, concentrated and purified by flash silica gel chromatography (silica gel, 1% EtOAc/hexanes) to afford **3**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36-7.22 (m, 5H), 4.63-4.59 (m, 1H), 4.19-4.15 (m, 2H), 3.97 (q, *J* = 5.0 Hz, 1H), 3.91-3.87 (m, 1H), 3.31 (dd, *J* = 3.0, 13.5 Hz, 1H), 2.77 (dd, *J* = 10.0, 13.5 Hz, 1H), 1.58-1.55 (m, 2H), 1.21 (d, *J* = 7.0 Hz, 3H), 0.92-0.88 (m, 12H), 0.03 (d, *J* = 9.5 Hz, 6H).

Compound 4 (hydrolysis of chiral auxillary)

To a suspension of **3** (0.05 g, 0.123 mmol) in a mixture of THF (0.96 ml) and water (0.33 ml) at 0 ^{O}C was added $LiOH.H_2O$ (0.10 g, 0.246 mmol) and 30 % H_2O_2 (0.06 ml, 0.49 mmol). The reaction mixture was stirred at 0 ^{o}C for 1 hr after which it was quenched by addition of sodium sulfite solution (1.5 M). The mixture was concentrated and aqueous phase was washed with dichloromethane and then carefully acidified to pH 1-2 with 1N aqueous HCl. The mixture was extracted with diethyl ether and the combined organic extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. The crude

product was subjected to flash column chromatography (silica gel, 10% EtOAc/hexanes) to give **4:** ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.93 (q, *J* = 5.5 Hz, 1H), 2.65-2.60 (m, 1H), 1.60-1.47 (m, 2H), 1.14 (d, *J* = 7.5 Hz, 3H), 0.93-0.90 (m, 12H), 0.09 (d, *J* = 9.5 Hz, 6H).

Compound 5 (NAC thioester of 4)

A solution of carboxylic acid **4** (0.37 g, 1.49 mmol) in anhydrous methylene chloride (7.5 ml) was cooled to 0 ^oC for 15 min. To this solution was added N-acetyl cysteamine (47 mg, 0.394 mmol) followed by 4-(N,N-dimethylamino)pyridine (0.21 g, 1.8 mmol), and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (0.04 g, 0.30 mmol). The mixture was allowed to warm to room temperature and stirred overnight. Saturated aqueous NH_4Cl solution (7 ml) was added and the organic phase was separated. The aqueous phase was extracted with 3 x 15 ml of ether and the combined organic phases were dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, 30% EtOAc/hexanes) to afford **5:**^{1}H NMR (500 MHz, $CDCl_3$) δ 5.85 (br, 1H), 3.91 (q, *J* = 6.0 Hz, 1H), 3.48-3.40 (m, 2H), 3.01 (t, *J* = 6.0 Hz, 2H), 2.82-2.77 (m, 1H), 1.97 (s, 3H), 1.66 (s, 1H), 1.57-1.46 (m, 2H), 1.17 (d, , *J* = 6.5 Hz, 3H), 0.91-0.87 (m, 12H), 0.04 (d, *J* = 9.0 Hz, 6H).

Compound 6 (deprotection of 5)

To a solution of the protected NAC thioester (0.24 g, 0.68 mmol) in acetonitrile (6 ml, 0.1 M) and water (1.3 ml, 0.6M) was added hydrofluoric acid (1.6 ml, 48% wt in H_2O). After stirring for 2 hrs at room temperature the reaction was cool to 0^oC and pH was adjusted to 7.5 using saturated aqueous $NaHCO_3$. Acetonitrile was removed by rotary evaporation and the resultant aqueous layer was extracted with EtOAc (3 x 10 ml). The combined organic extracts dried over anhydrous Na_2SO_4, concentrated and purified by

flash silica gel chromatography (silica gel, 10% MeOH/CH_2Cl_2) to afford the final diketide NAC thioester **6:** ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.02 (br, 1H), 3.85-3.32 (m, 1H), 3.48-3.40 (m, 2H), 3.07-2.98 (m, 2H), 2.76-2.71 (m, 1H), 2.61 (s, 1H), 1.96 (s, 3H), 1.56-1.41 (m, 2H), 1.20 (d, *J* = 7.0 Hz, 3H), 0.96 (*J* = 8.0 Hz, 3H). HRMS calculated for $C_{10}H_{19}NO_3S$ + Na 256.0983; found 256.0979.

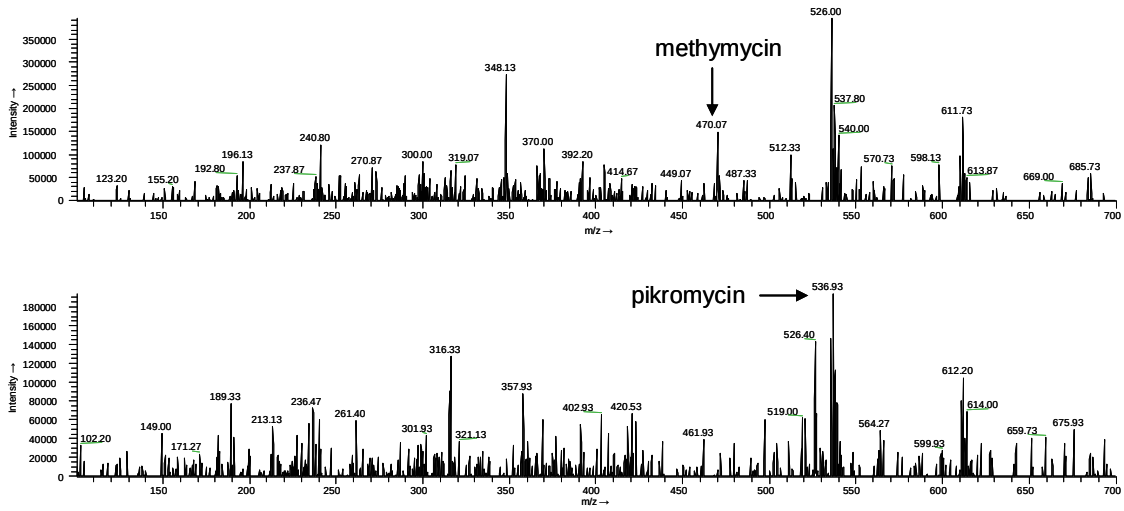


Fig. S1a: BB138/pBK51 grown in the presences of NAC thioester of 2(*S*)-methyl-3(*R*)-pentanoic acid

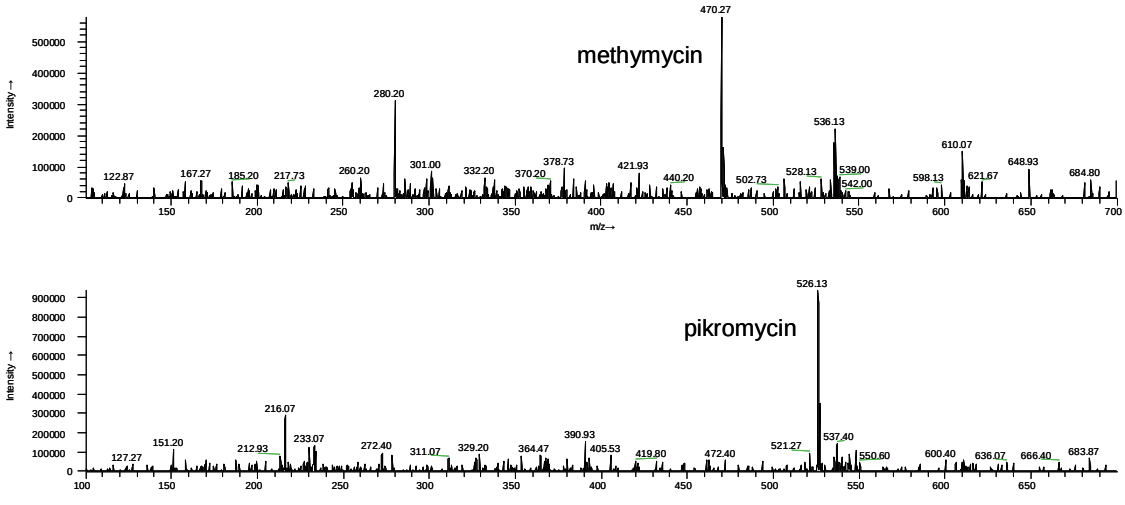


Fig. S1b: BB138/pBK51* grown in the presence of NAC thioester of 2(*S*)-methyl-3(*R*)-pentanoic acid

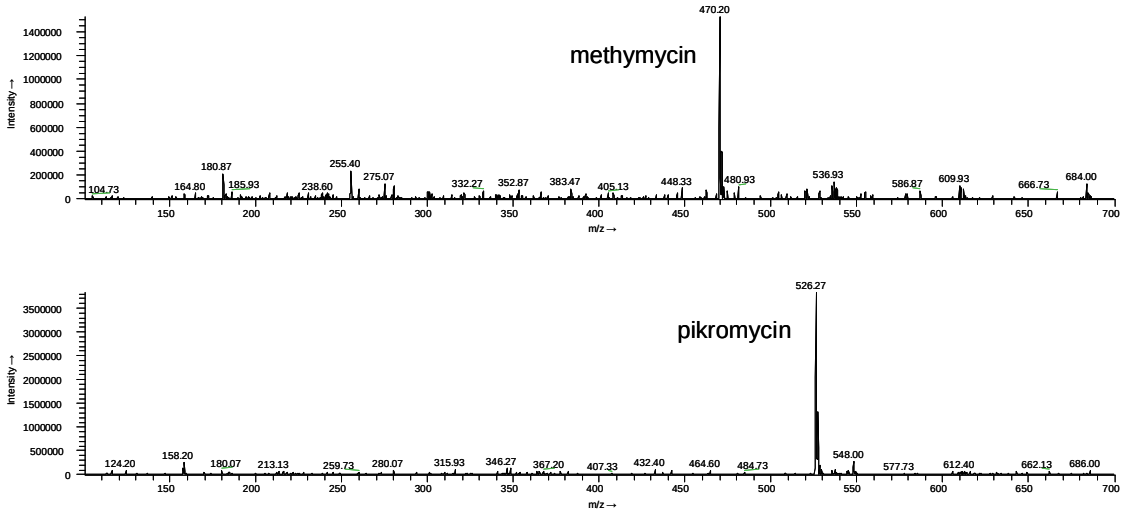


Fig. S2d: BB138/pJY7* grown in the presences of NAC thioester of 2(*S*)-methyl-3(*R*)-pentanoic acid