Electronic Supplementary information

Role and substrate specificity of the *Streptomyces coelicolor* RedH enzyme in undecylprodiginine biosynthesis

Stuart W. Haynes, Paulina K. Sydor, Anna E. Stanley, Lijiang Song and Gregory L. Challis*

Construction of *S. coelicolor* W33 (*redH*::*aac(3)IV* mutant of *S. coelicolor* M511)

A standard PCR-targeting-based method was used.[1] PCR amplification of the *oriT-aac(3)IV* gene replacement cassette derived from pIJ773 was carried out with the forward primer 5'-TCGCGCACGCCTGACCGCCGAGGAGACACACCCGCCATGATTCCGGGGATCCGTCGACC-3' and the reverse primer 5'-GGCGGTCGTGGTCCGGTGTTGCGGCGGTGGCCGGCTTCATGTAGGCTGGAGCTGCTTC-3' using Expand high fidelity polymerase (Roche), as described.[1] The 20 and 19 nucleotides underlined at the 3' ends of the forward and reverse primers, respectively, are complementary to the 5' ends of the coding and template strands, respectively, of the knockout cassette. The remaining 39 nucleotides in the forward and reverse primers are homologous to the regions of DNA upstream and downstream, respectively, of the *redH* gene and incorporate the start (forward primer) and stop (reverse primer) codons of *redH*. PCR amplification to confirm that *redH* gene was replaced on the chromosome of *S. coelicolor* M511, as desired, was carried out with the forward primer 5'-TCTGGAAGCGCTCAACCTC-3' (1) and the reverse primer 5'-CGATGGTGTTGCCCTTCATGC-3' (2). The primers were designed to anneal outside the region of the sequence that had been replaced. Because the W33 mutant produced low levels of prodiginines, new primers were designed to prove that the mutant was not contaminated with wild type *S. coelicolor* M511. PCR amplifications were carried out using the forward primer 5'-GCGAGCAGTTCCACGCCTTCAC-3' (3) (designed to prime outside of *redH*) and with two reverse primers: 5'-GATGTCCTGCTCGCTGCCGTAC-3' (4) (designed to prime inside *redH*) and 5'-GCAGCGTCGTGTTGGCATCGTG-3' (5) (designed to prime inside the *oriT-aac(3)IV* cassette). Amplimers were generated by PCR, using combinations of the primers described above, and genomic DNA from the wild type M511 strain or the W33 mutant as template DNA. Agarose gel electrophoretic analysis of these reactions is shown in Fig.1.

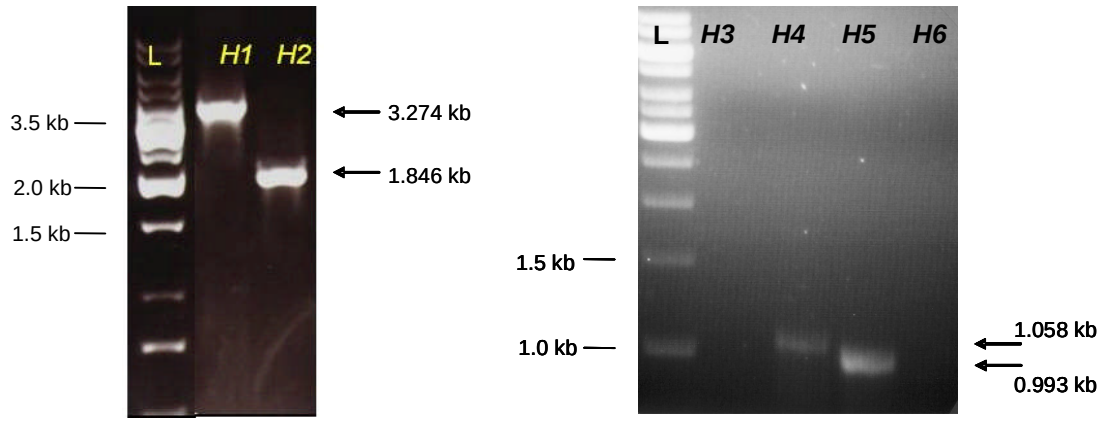


Fig.1: PCR analysis of genomic DNA from *S. coelicolor* M511 (wild type) and *S. coelicolor* W33 (*redH::aac(3)IV* mutant), L = size marker, *H1* = M511 with primers 1 and 2, *H2* = W33 with primers 1 and 2, *H3* = M511 with primers 3 and 5, *H4* = W33 with primers 3 and 5, *H5* = M511 with primers 3 and 4, *H6* = W33 with primers 3 and 4.

Complementation of the *redH*::*aac(3)IV* mutation in *S. coelicolor* W33

The forward primer 5'-AAAGGGAAGCTTCGAGGAGACACACCCGCC-3' (*Hin*dIII site underlined) and the reverse primer 5'-CCCTTTCTCGAGGGCGGTCGTGGTCCGGTG-3' (*Xho*I site underlined) were used to amplify a DNA fragment containing the *redH* gene from cosmid SC3F7 using Expand DNA polymerase (Roche). Reactions contained 1.0 μL of 100 μM solutions of each primer, 10 ng of cosmid SC3F7, 5 μL of 10X Expand polymerase buffer, 1 μL of a 10 μM dNTPs solution, 2.5 μL DMSO and 0.5 μL Roche Expand DNA polymerase made up to a total volume of 50 μL with deionised water. Reaction conditions were: initial denaturation at 94°C for 2 minutes, followed by 25 cycles of 94°C for 45 seconds, 55°C for 45 seconds and 72°C for 90 seconds, and a final elongation temperature of 72°C for 5 minutes. The products were analysed on a 1% agarose gel by electrophoresis. The 2.8 kb band was excised from the gel, purified using a gel extraction kit (Qiagen) and re-suspended in 30 μL of deionised water. 30 μL of this solution was incubated with *Hin*dIII and *Xho*I restriction enzymes (Fermentas) according to the manufacturer's instructions and separated on a 1% agarose gel by electrophoresis. The 2.8 kb band was excised from the gel, purified using a gel extraction kit (Qiagen) and re-suspended in 30 μL of deionised water. 4.5 μL of this solution was mixed with 0.5 μL of a solution of *Hin*dIII- and *Xho*I-digested pOSV556t (supplied by Prof. Jean-Luc Pernodet, Université Paris-Sud 11). The PCR product and linearized vector were ligated using the Rapid DNA Ligation Kit (Roche) following the manufacturers instructions. 2 μL of the ligation mixture was used to transform *E. coli* MC1061 competent cells following standard procedures.[2] Plasmids were purified from ampicillin and hygromycin resistant transformants and the presence of the desired insert was determined by digestion with *Hin*dIII and *Xho*I, followed by agarose gel electrophoresis. One plasmid containing

the correct insert was named pPKS1 and used to transform *E. coli* ET12567 containing pUZ8002 by electroporation. An ampicillin and hygromycin resistant colony was picked and used to transfer pPKS1 from *E. coli* to *S. coelicolor* W33 by intergenic conjugation using a standard procedure.[3] A spore stock of one hygromycin resistant transconjugant was prepared using standard procedures[3] and stored at -20ºC.

Construction of *S. venezuelae*/pPKS1

E. coli ET12567 containing pUZ8002 and pKS1 were used to transfer pPKS1 from *E. coli* to *S. venezuelae* ATCC10712 by intergenic conjugation using a standard procedure.[3] A spore stock of one hygromycin resistant transconjugant was prepared using standard procedures[3] and stored at -20ºC.

Synthesis of MBC analogues

General Procedures

Dry toluene was obtained by evaporation of the toluene/water azeotrope *in vacuo* followed by distillation from calcium hydride under argon, and stored over 4 Å molecular sieves. Dry chloroform and methanol were produced in the same way. THF was pre-dried over sodium wire and distilled from potassium prior to use. Dry diisopropylamine was obtained by distillation from sodium hydroxide pellets under argon and stored over sodium hydroxide pellets. All other reagents and solvents were used as supplied. Petroleum ether refers to the fraction of light petroleum boiling between 40°C and 60°C. Solvents were evaporated using a Buchi Rotavapor R-200 equipped with a Buchi Vacuubrand pump.

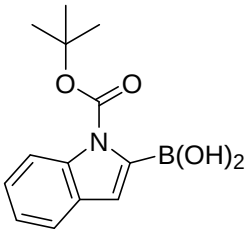
Flash column chromatography was conducted on Fluka Silica Gel (40-63 μm, 60 Å), or Basic aluminum oxide (activated basic Brockman 1 standard grade 150 mesh). TLC was performed on aluminium backed plates pre-coated with Merck silica gel 60 F_{254}. UV radiation, phosphomolybdic acid, potassium permanganate, or vanillin were used for visualisation of TLC plates, as appropriate.

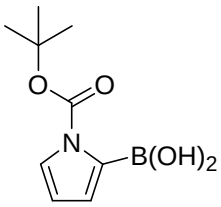
IR spectra were recorded on solid compounds using a Perkin-Elmer Avatar 320 Fourier Transformation spectrometer. Only selected absorptions are reported, in units of wavenumbers (cm^{-1}), using the following abbreviations: w, weak; m, medium; s, strong; br, broad.

^1H NMR spectra were recorded at 300, 400 or 700 MHz using Bruker DPX300, DPX400 or AV700 spectrometers respectively. Chemical shifts (δ_H) are quoted in ppm with reference to the residual solvent peak. The data in parentheses follow the order (i) multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet, (ii) number of equivalent protons, (iii) coupling constant (J): in Hz to the nearest 0.5 Hz.

^{13}C NMR spectra were recorded on Bruker DPX300, DPX400 or AV700 spectrometers at 75, 100 or 175 MHz. Chemical shifts (δ_C) are quoted in ppm with reference to the residual solvent peak.
High resolution mass spectra were recorded on a Bruker microTOF spectrometer equipped with an ESI source.
LC-MS was carried out on an Agilent 1100 HPLC instrument with the outflow connected via a splitter (10% to mass spectrometer, 90% to waste) to a Bruker Daltonics esquire HCT plus mass spectrometer equipped with an ESI source.

Synthesis of N-BOC-indole- and N-BOC-pyrrole-2-boronic acids





*n*BuLi (1.5 M in hexanes, 6.58 mmol, 1.10 equiv) was slowly added to a solution of 2,2,6,6-tetramethylpiperidine (6.58 mmol, 1.10 equiv) in THF (11 mL) at -78 °C under argon. After stirring for 10 min the mixture was allowed to warm to 0°C over 30 min and then cooled again to -78°C. A solution of N-BOC-pyrrole or N-BOC-indole (5.98 mmol, 1.0 equiv) in THF was added slowly to keep the temperature below -65°C. The reaction mixture was stirred for 2 h at -78°C, trimethyl borate (17.54 mmol, 3.0 equiv) in THF (40 mL) was added and the mixture allowed to warm to room temperature overnight.
0.3 N HCl (15 mL) was added, the volatiles were removed *in vacuo* and the residue was extracted with diethyl ether (3x20 mL). The combined organic phases were washed with water (2x6 mL) and dried over magnesium sulphate. The solution was slowly concentrated until a solid began to precipitate and then cooled to 0°C before filtering off the solid and washing with cold diethyl ether. The resulting off-white solid was dried *in vacuo*.

N-BOC-pyrrole-2-boronic acid
0.6879g (72%)
^{1}H-NMR ($CDCl_3$, 400MHz) δ: 1.55 (s, 9H), 6.15 (t, 1H, 4.0 Hz), 7.00 (d, 1H, 4.0 Hz), 7.35 (d, 1H, 4.0 Hz), 7.55 (br s, 2H, variable with concentration)
^{13}C-NMR ($CDCl_3$, 100MHz) δ: 27.95, 85.57, 112.06, 115.53, 127.04, 128.75, 152.31
IR (cm^{-1}): 3336, 3171, 2984, 1704, 1554
HRMS: *m/z* calculated for $C_9H_{14}BNO_4$: 212.1094 $[M+H]^+$; found: 212.1099.

N-BOC-indole-2-boronic acid **5b**

1.7054 g (88%)

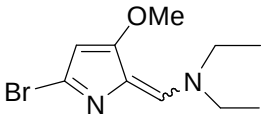
^{1}H-NMR ($CDCl_3$, 400MHz) δ: 1.65 (s, 9H), 7.15 (t, 1H, 5.5 Hz), 7.15 (s, 2H, variable with concentration), 7.25 (t, 1H, 5.5 Hz), 7.40 (s, 1H), 7.50 (d, 1H, 5.5 Hz), 7.90 (d, 1H, 5.5 Hz)

^{13}C-NMR ($CDCl_3$, 100MHz) δ: 29.50, 84.43, 112.63, 115.01, 121.12, 123.65, 124.43, 131.12, 136.63, 138.51, 150.43.

IR (cm^{-1}): 3337, 3146, 2972, 1692

HRMS: *m/z* calculated for $C_{13}H_{16}BNO_4$: 262.1251 $[M+H]^+$; found: 262.1253.

(5-Bromo-3-methoxy-pyrrol-2-ylidenemethyl)-diethyl-amine **6**[4]



To a mixture of diethylformamide (1.50 mL, 13.25 mmol, 3.0 equiv) and chloroform (5 mL) at 0°C was added a solution of phosphorous oxybromide (3.17 mL, 11.06 mmol, 2.5 equiv) in chloroform (15 mL) dropwise under argon and the resulting yellowish suspension was stirred at 0°C for 30 min under argon.

A solution of 4-methoxy-3-pyrrolin-2-one (0.500 g, 4.42 mmol, 1.0 equiv) was added dropwise to the previous solution at 0°C. The resulting mixture was warmed to room temperature then heated to 65°C for 5 hours.

After pouring the mixture onto ice, the aqueous layer was adjusted to pH 7-8 with 2 N NaOH. Ethyl acetate (20 mL) was added and the mixture was filtered over Celite to remove phosphorus salts. The aqueous layer was separated and extracted with ethyl acetate (2x 20 mL). The combined organic layers were washed with brine (2x 20 mL), dried over magnesium sulphate and evaporated *in vacuo* to obtain a brown oil. The product was purified though a pad of silica using 20% ethyl acetate/hexane as eluent, to leave a brown oil, which solidified on standing.

Yield: 0.9564 g (83%)

^{1}H-NMR ($CDCl_3$, 400MHz) δ: 1.2 (t, 3H, 7.1 Hz), 1.25 (t, 3H, 7.1 Hz), 3.30 (q, 2H, 7.1 Hz), 3.65 (s, 3H), 4.05 (q, 2H, 7.1 Hz), 5.50 (s, 1H), 6.90 (s, 1H).

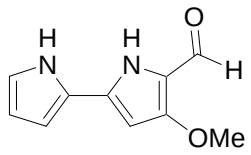
^{13}C-NMR ($CDCl_3$, 100MHz) δ: 18.36, 21.25, 44.55, 51.18, 58.01, 96.39, 120.77, 133.53, 138.67, 165.34.

IR (cm^{-1}): 2973, 2936, 1629, 1516

mp: 38-39°C

HRMS: m*/z* calculated for $C_{10}H_{15}BrN_2O$: 259.0446 $[M+H]^+$; found: 259.0448.

4-Methoxy-1H,1'H-[2,2']bipyrrolyl-5-carbaldehyde **3**[4]



To toluene (1 mL) was added $Pd(OAc)_2$ (0.30 mmol, 0.1 equiv) and triphenylphosphine (1.35 mmol, 0.45 equiv) under argon. The resulting bright yellow solution was stirred for 30 min at 70°C. A degassed solution of (5-Bromo-3-methoxy-pyrrol-2-ylidenemethyl)-diethyl-amine **6** (3.00 mmol, 1.0 equiv) and N-BOC-pyrrole-2-boronic acid (3.30 mmol, 1.1 equiv) in 10% water/dioxane (15 mL) was added, followed by sodium carbonate (9.00 mmol, 3.0 equiv) and the mixture was stirred for 3.5 h at 100°C. The solution was treated with sodium methoxide (3.00 mmol, 1.0 equiv) and stirred for 15 min at 100°C, then treated again with sodium methoxide (3.00 mmol, 1.0 equiv) and stirred for 10 min more at 100°C.

The mixture was poured into water (50 mL) and the pH was lowered to 7 with 2 N HCl and stirred for 15 min. The brown precipitate was recovered by filtration over a fritted disc funnel and washed with water (2x 15 mL), and acetone (2x 10 mL) to leave a yellow solid.

0.433 g (75%)

^{1}H-NMR (DMSO, 400MHz) δ: 3.80 (s, 3H), 6.10 (d, 1H, 4.4Hz), 6.25 (d, 1H, 4.4.Hz), 6.75 (s, 1H), 6.90 (t, 1H, 4.4Hz), 9.30 (s, 1H), 11.20 (br s, 1H), 11.40 (br s, 1H).

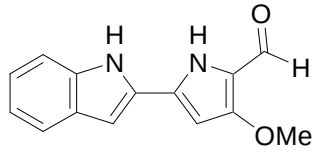
^{13}C-NMR (DMSO, 175MHz) δ: 57.70, 90.82, 108.26, 109.24, 117.87, 120.64, 123.66, 133.63, 159.05, 171.54.

IR (cm^{-1}): 3243, 3188, 3038, 2950, 2840, 1593, 1544

mp: 284°C

HRMS: *m/z* calculated for $C_{10}H_{10}N_2O_2$: 191.0821 $[M+H]^+$; found: 191.0824.

5-(1H-Indol-2-yl)-3-methoxy-1H-pyrrole-2-carbaldehyde **7b**[5]



To a solution of toluene (1 mL) was added $Pd(OAc)_2$ (0.30 mmol, 0.1 equiv) and triphenylphosphine (1.35 mmol, 0.45 equiv) under argon. The resulting bright yellow solution was stirred for 30 min at 70°C. A degassed solution of (5-Bromo-3-methoxy-pyrrol-2-ylidenemethyl)-diethyl-amine **6** (3.00 mmol, 1.0 equiv) and N-BOC-indole-2-boronic acid **5b** (3.30 mmol, 1.1 equiv) in 10% water/dioxane (15 mL) was added, followed by sodium carbonate (9.00 mmol, 3.0 equiv) and the mixture was stirred for 3.5 h at 100°C. The solution was treated with sodium

methoxide (3.00 mmol, 1.0 equiv) and stirred for 15 min at 100°C, then treated again with sodium methoxide (3.00 mmol, 1.0 equiv) and stirred for 10 min more at 100°C.

The mixture was poured into water (50 mL) and the pH was lowered to 7 with 2N HCl and stirred for 15 minutes. The resulting brown precipitate was recovered by filtration and washed with water (2x 15 mL) and then dissolved in acetone. The solvent was then removed by rotary evaporation and the resulting solid was treated with chloroform and diethyl ether (1:2, 5 mL) and the solution was left to stand for 10 minutes until a solid was obtained. The green solid was collected by filtration and washed with diethyl ether (2 x 5 mL).

0.562 g (78%)

^{1}H-NMR (DMSO, 400MHz) δ: 3.90 (s, 3H), 6.65 (s, 1H), 7.05 (t, 1H, 5.0 Hz), 7.15 (s, 2H), 7.45 (d, 1H, 5.0 Hz), 7.55 (d, 1H, 5.0 Hz), 9.50 (s, 1H), 11.50 (br s, 1H), 11.90 (br s, 1H).

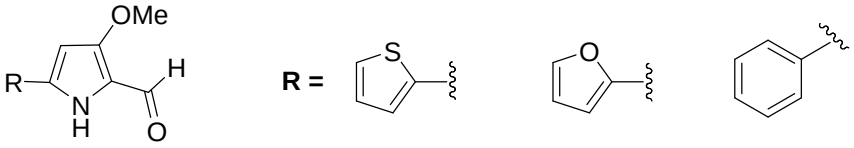
^{13}C-NMR (DMSO, 175MHz) δ: 57.92, 93.24, 100.76, 111.23, 118.67, 119.75, 120.32, 122.31, 128.12, 129.41, 131.78, 136.72, 157.91, 172.98.

IR (cm^{-1}): 3254, 3234, 3215, 2943, 1588, 1525.

mp: >300°C

HRMS: *m/z* calculated for $C_{14}H_{12}N_2O_2$: 241.0977 $[M+H]^+$; found: 241.0981.

General procedure for synthesis of other MBC analogues



As for R = 2-indolyl and 2-pyrrolyl except, the mixture was poured into water (50 mL) and the pH lowered to 7 with 2 N HCl (aq) and stirred for 15 min. The resulting mixture was extracted with ethyl acetate (3 x 75 mL). The combined organic fractions were washed with brine, dried over magnesium sulphate and evaporated to leave an oily residue which was purified by chromatography on basic alumina (20% ethyl acetate/hexane).

R = phenyl **7a**

0.1411g (70%)

^{1}H-NMR (Acetone, 400MHz) δ: 3.90 (s, 3H), 6.50 (s, 1H), 7.30 (t, 1H, 7.5 Hz), 7.40 (t, 2H, 7.5 Hz), 7.85 (d, 2H, 7.5 Hz), 9.50 (s, 1H), 10.50 (br s, 1H).

^{13}C-NMR (Acetone, 175MHz) δ: 58.29, 94.10, 120.25, 126.09, 129.13, 129.57, 131.80, 138.36, 159.06, 174.19.

IR (cm^{-1}): 3246, 2821, 1617, 1561

mp: 137-138°C

HRMS: *m/z* calculated for $C_{12}H_{11}NO_2$: 202.0868 $[M+H]^+$; found: 202.0872.

R = 2-thienyl **7d**

0.1834 g (56%)

^{1}H-NMR (Acetone, 400MHz) δ: 3.90 (s, 3H), 6.30 (s, 1H), 7.15 (dd, 1H, 4.0, 3.0 Hz), 7.50 (d, 1H, 4.0 Hz), 7.65 (d, 1H, 3.0 Hz), 9.50 (s, 1H), 10.50 (br s, 1H).

^{13}C-NMR ($CDCl_3$, 175MHz) δ: 58.27, 94.24, 119.45, 125.43, 126.67, 128.73, 133.32, 134.26, 158.52, 173.94.

IR (cm^{-1}): 3205, 3081, 2951, 1614, 1577, 1530

mp: 160-161°C

HRMS: *m/z* calculated for $C_{10}H_9NO_2S$: 208.0432 $[M+H]^+$; found: 208.0436.

R = 2-furyl **7c**

0.1861 g (60%)

^{1}H-NMR (Acetone, 400MHz) δ: 3.90 (s, 3H), 6.30 (s, 1H), 6.55 (dd, 1H, 4.0, 3.0 Hz), 6.95 (d, 1H, 4.0 Hz), 7.60 (d, 1H, 3.0 Hz), 9.50 (s, 1H), 10.50 (br s, 1H).

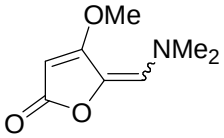
^{13}C-NMR ($CDCl_3$, 175MHz) δ: 58.38, 93.32, 108.0, 112.66, 119.88, 130.24, 144.14, 147.71, 158.95, 174.24.

IR (cm^{-1}): 3207, 2924, 1601, 1546

mp: 185-186°C

HRMS: *m/z* calculated for $C_{10}H_9NO_3$: 192.0661 $[M+H]^+$; found: 192.0665.

5-Dimethylaminomethylene-4-methoxy-5H-furan-2-one **11**



A solution of 4-methoxy-2(5H)-furanone **10** (2.00 g, 17.53 mmol, 1.0 equiv) in dimethyl formamide dimethyl acetal (20 mL) was heated in an oil bath at 110°C, resulting in slow, continuous distillation of methanol. After 3 hours the mixture was cooled to room temperature and the excess dimethyl formamide dimethyl acetal was removed *in vacuo* to leave a yellow/brown solid, which was purified by flash column chromatography (silica, ethyl acetate) to leave a yellow solid.

2.750 g (92%)

^{1}H-NMR ($CDCl_3$, 400MHz) δ: 3.05 (s, 6H), 3.85 (s, 3H), 4.90 (s, 1H), 6.05 (s, 1H).

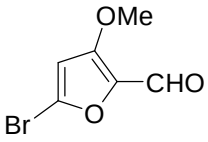
^{13}C-NMR ($CDCl_3$, 100MHz) δ: 42.45, 58.43, 80.59, 120.19, 122.78, 169.98, 171.38.

IR (cm^{-1}): 2990, 2901, 2822, 1704, 1655, 1555

m.p.: 57-58°C

HRMS: *m/z* calculated for $C_8H_{11}NO_3$: 170.0817 $[M+H]^+$; found: 170.0820.

5-Bromo-3-methoxy-furan-2-carbaldehyde **12**



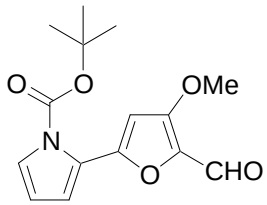
To 5-Dimethylaminomethylene-4-methoxy-5H-furan-2-one **11** (1.00 g, 5.90 mmol, 1.0 equiv) in chloroform (15 mL) at 0°C was added a solution of phosphorous oxybromide in chloroform (15 mL) dropwise. The resulting blue/green mixture was stirred and heated under reflux overnight. The reaction was quenched with water and the aqueous layer was adjusted to pH 14 (2M, NaOH). The organic layer was separated and the aqueous layer was extracted with dichloromethane (3 x 50 mL). The combined organic fractions were washed with Na_2CO_3 (20 mL, sat.) and brine (20 mL). The organic layer was dried over magnesium sulphate and evaporated to dryness, to yield an unstable white solid.

0.2854 g (24%)

^{1}H-NMR ($CDCl_3$, 400MHz) δ: 4.05 (s, 3H), 6.90 (s, 1H), 9.45 (s, 1H).

HRMS: *m/z* calculated for $C_6H_5BrO_3$: 204.9500 $[M+H]^+$; found: 204.9509.

2-(5-Formyl-4-methoxy-furan-2-yl)-pyrrole-1-carboxylic acid tert-butyl ester



To a solution of toluene (1 mL) was added $Pd(OAc)_2$ (0.10 mmol, 0.1 equiv) and triphenylphosphine (0.45 mmol, 0.45 equiv) under argon. The resulting bright yellow solution was stirred for 30 min at 70°C. A deoxygenated solution of 5-Bromo-3-methoxy-furan-2-carbaldehyde **12** (1.00 mmol, 1.0 equiv) and N-BOC-pyrrole-2-boronic acid (1.10 mmol, 1.1 equiv) in 10% water/dioxane (15 mL) was added, followed by sodium carbonate (3.00 mmol, 3.0 equiv) and the mixture was stirred for 3.5 h at 100°C. The solution was treated with sodium methoxide (1.00 mmol, 1.0 equiv) and stirred for 15 min at 100°C, then treated again with sodium methoxide (1.00 mmol, 1.0 equiv) and stirred for 10 min more at 100°C.

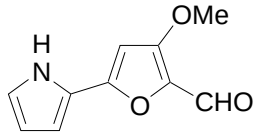
The mixture was poured into water (50 mL) and the pH was lowered to 7 with 2 N HCl and stirred for 15 min. The resulting mixture was extracted with ethyl acetate (3x 75 mL). The combined organic fractions were washed with brine, dried over magnesium sulphate and evaporated to leave an oily residue. Purification of the residue by column chromatography through basic alumina (20% ethyl acetate/hexane) yielded a viscous green oil.

0.2423 g (83%)

^{1}H-NMR (Acetone, 400MHz) δ: 1.45 (s, 9H) 4.00 (s, 3H), 6.35 (t, 1H, 3.5 Hz), 6.70 (dd, 1H, 1.5, 3.0 Hz), 6.80 (dd, 1H, 1.5, 3.0 Hz), 6.85 (s, 1H) 9.60 (s, 1H)

HRMS: *m/z* calculated for $C_{15}H_{18}NO_5$: 292.1185 $[M+H]^+$; found: 292.1192.

3-Methoxy-5-(1H-pyrrol-2-yl)-furan-2-carbaldehyde **13**



To a solution 2-(5-Formyl-4-methoxy-furan-2-yl)-pyrrole-1-carboxylic acid tert-butyl ester (0.199 g, 0.684 mmol) in THF (5 mL) was added lithium hydroxide (0.164 g, 6.84 mmol, 10 equiv) in methanol (5 mL) under argon. The resulting solution was stirred at room temperature and monitored by TLC. On completion of the reaction, the solvent was removed and the resulting yellow solid was triturated with water to yield the desired product as a yellow/green solid.

0.113 g (87%)

^{1}H-NMR (Acetone, 400MHz) δ: 4.00 (s, 3H), 6.25 (dd, 1H, 2.5, 1.0 Hz), 6.75 (d, 1H, 2.5 Hz), 6.75 (s, 1H) 7.05 (d, 1H, 2.5 Hz), 9.45 (s, 1H), 11.10 (br s, 1H).

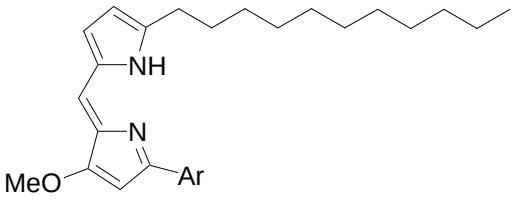
^{13}C-NMR (Acetone, 175MHz) δ: 59.72, 95.02, 109.84, 111.28, 111.38, 121.85, 123.16, 136.54, 153.31, 161.27.

mp: 154 – 155°C

IR (cm^{-1}): 3255, 3179, 3121, 2931, 2834, 1620, 1589, 1563

HRMS: *m/z* calculated for $C_{10}H_9NO_3$: 192.0661 $[M+H]^+$; found: 192.0667.

General procedure for acid catalysed coupling of MBC analogues with 2-undecylpyrrole 4[6]



To a suspension of MBC or one of the MBC analogues (0.25 mmol, 1.0 equiv) and 2-undecylpyrrole (0.30 mmol, 1.2 equiv) in methanol (5 mL) was added methanolic HCl (2 N, 100 µl). The resulting brightly coloured solution was stirred for 3 hours at room temperature. The solvent was removed *in vacuo* and the resulting solid was dissolved in ethyl acetate (20 mL) and washed with saturated aqueous sodium bicarbonate (2x 25 mL) and brine (30 mL). The organic layer was dried over magnesium sulphate and the solvent was removed *in vacuo* to leave a brightly-coloured crude solid, which was purified by flash column chromatography.

Ar = 2-Indolyl **14b**

The crude residue was purified using flash column chromatography over silica gel with gradient elution from 0-20% diethyl ether in petroleum ether.

16 mg (18%)

^{1}H-NMR ($CDCl_3$, 400MHz) δ: 0.80 (t, 3H, 7.0 Hz), 1.20 (m, 16H), 1.60 (m, 2H), 2.60 (t, 2H, 7.5 Hz), 3.80 (s, 3H), 5.75 (s, 1H), 5.95 (d, 1H, 3.5 Hz), 6.50 (d, 1H, 3.5 Hz), 6.85 (s, 1H), 6.90 (s, 1H), 7.15 (t, 1H, 6.5 Hz), 7.25 (t, 1H, 7.5 Hz), 7.50 (d, 1H, 7.5 Hz), 8.05 (d, 1H, 7.5 Hz).

IR (cm^{-1}): 3250, 3118, 2923, 2852, 1732, 1623, 1571, 1547

mp: 58-59°C

HRMS: *m/z* calculated for $C_{29}H_{37}N_3O$: 444.3015 $[M+H]^+$; found: 444.3017.

Ar = 2-Pyrrolyl **1**

The crude residue was purified using flash column chromatography over silica gel with gradient elution from 0-70% diethyl ether in petroleum ether.

51 mg (52%)

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.80 (t, 3H, 7.0 Hz), 1.20 (m, 18H), 2.10 (m, 2H), 3.90 (s, 3H), 5.75 (d, 1H, 3.5 Hz), 5.95 (s, 1H), 6.05 (t, 1H, 3.5 Hz), 6.40 (d, 1H, 3.5 Hz), 6.55 (d, 1H, 3.5 Hz), 6.60 (d, 1H, 4.0 Hz), 6.75 (s, 1H).

IR (cm^{-1}): 3267, 3102, 2919, 2849, 1613, 1575, 1561, 1547

mp: 86-87°C

HRMS: *m/z* calculated for $C_{25}H_{35}N_3O$: 394.2858 $[M+H]^+$; found: 394.2861.

Ar = 2-Thienyl **14d**

The crude residue was purified using flash column chromatography over silica gel with gradient elution from 0-30% diethyl ether in petroleum ether.

54 mg (52%)

^{1}H-NMR ($CDCl_3$, 400MHz) δ: 0.80 (t, 3H, 7.0 Hz), 1.20 (m, 16H), 1.70 (m, 2H), 2.70 (t, 2H, 7.0 Hz), 3.80 (s, 3H), 5.85 (s, 1H), 5.95 (d, 1H, 3.5 Hz), 6.50 (d, 1H, 3.5 Hz), 6.75 (s, 1H), 7.05 (dd, 1H, 4.0, 3.0 Hz), 7.30 (d, 1H, 4.0 Hz), 7.40 (d, 1H, 3.0 Hz).

IR (cm^{-1}): 3254, 2917, 2848, 1603, 1542

mp: 87-88°C

HRMS: *m/z* calculated for $C_{25}H_{34}N_2OS$: 411.2470 $[M+H]^+$; found: 411.2469.

Ar = 2-Furyl **14c**

The crude residue was purified using flash column chromatography over silica gel with gradient elution from 0-30% diethyl ether in petroleum ether.

15 mg (15%)

^{1}H-NMR ($CDCl_3$, 400MHz) δ: 0.80 (t, 3H, 7.0 Hz), 1.20 (m, 16H), 1.70 (m, 2H), 2.65 (t, 2H, 7.0 Hz), 3.80 (s, 3H), 5.90 (d, 1H, 3.5 Hz), 5.95 (d, 1H, 3.5 Hz), 6.45 (dd, 1H, 4.0, 3.0 Hz), 6.50 (d, 1H, 4.0 Hz), 6.80 (s, 1H), 6.90 (d, 1H, 3.0 Hz), 7.50 (s, 1H).

IR (cm^{-1}): 3261, 2951, 2922, 2848, 1612, 1579, 1541

mp: 70-71 °C

HRMS: *m/z* calculated for $C_{25}H_{34}N_2O_2$: 395.2699 $[M+H]^+$; found: 395.2701.

Ar = Phenyl **14a**

The crude residue was purified using flash column chromatography over silica gel with gradient elution from 0-20% ethyl acetate in hexane.

79 mg (97 %)

^{1}H-NMR ($CDCl_3$, 400MHz) δ: 0.80 (t, 3H, 6.5 Hz), 1.20 (m, 16H), 1.65 (m, 2H), 2.65 (t, 1H, 7.0 Hz), 3.80 (s, 1H), 5.90 (d, 1H, 3.5 Hz), 6.00 (s, 1H), 6.50 (d, 1H, 3.5 Hz), 6.80 (s, 1H), 7.35 (m, 3H), 7.90 (d, 2H, 7.0 Hz).

IR (cm^{-1}): 3251, 2960, 2917, 2848, 1613, 1594, 1579, 1542

mp: 100-101 °C

HRMS: *m/z* calculated for $C_{27}H_{36}N_2O$: 405.2906 $[M+H]^+$; found: 405.2902.

Feeding of MBC and analogues to the *S. coelicolor* mutants on solid R5 medium

R5 agar plates were overlaid with sterile permeable membranes (12-14000 Da molecular weight cut off, Size 20). 10 μL of a spore suspension of each mutant diluted with 100 μL of sterile water was spread on a separate plate. After 3 days incubation at 30°C, each plate was flooded with 2 mL of sterile water to which was added a solution of MBC or an analogue in DMSO (200 μl, 5 mg/mL). After a further 2 days of incubation at 30°C, the membrane was peeled off the plate and the mycelia

were scraped off into a 15 mL tube. The mycelia were extracted with 3 mL of 1:1 acetonitrile:methanol (acidified with 15 µl 2M HCl) and sonicated (15 bursts of 1 second duration). The extracts were centrifuged at 4000g for 10 min and the supernatants were analysed by LC-MS/MS.

Feeding of 2-undecylpyrrole and MBC to wild type *S. venezuelae* and *S. venezuelae*/pPKS1

R5 agar plates were overlaid with sterile permeable membranes (12-14000 Da molecular weight cut off, Size 20). 10 µL of a spore suspension of each strain diluted with 100 µL of sterile water was spread on a separate plate. After 2 days incubation at 30°C, solutions of 2-undecylpyrrole in methanol (20 x 5 µl, 50 mg/mL) and MBC in DMSO (20 x 5 µl, 5 mg/mL) were added. After a further 2 days of incubation at 30°C, the membrane was peeled off the plate and the mycelia were scraped into a 15 mL tube. The mycelia were extracted with 3 mL of 1:1 acetonitrile:methanol, (acidified with 15 µl 2M HCl) and sonicated (15 bursts of 1 second duration). The extracts were centrifuged at 4000g for 10 min and the supernatants were analysed by LC-MS/MS.

LC-MS/MS analysis of mycelial extracts

10 µL of each extract was injected onto an Agilent C8 column (150 x 4.6 mm, 5µm, column temperature 25ºC) connected to an Agilent 1100 instrument equipped with a binary pump and a diode array detector and eluted with the gradient elution profile in the table below.

Time (min)	Water (pH 3 with HCl)	Acetonitrile	Flow Rate
0	50	50	1.0
1	50	50	1.0
4	25	75	1.3
21	20	80	1.4
23	50	50	1.0

The HPLC out-flow was connected via a splitter (10% flow to MS, 90% flow to waste) to a Bruker HCT+ mass spectrometer (Bruker Daltonics, Coventry) equipped with an electrospray source operated in positive ion mode with parameters as follows: nebulizer flow 40 psi, dry gas flow 10.0 L/min, dry temperature 300ºC, capillary -4 kV, skimmer 40V, capillary exit 106 V, ion charge control target (ICC) 100,000, spectral averages 3.

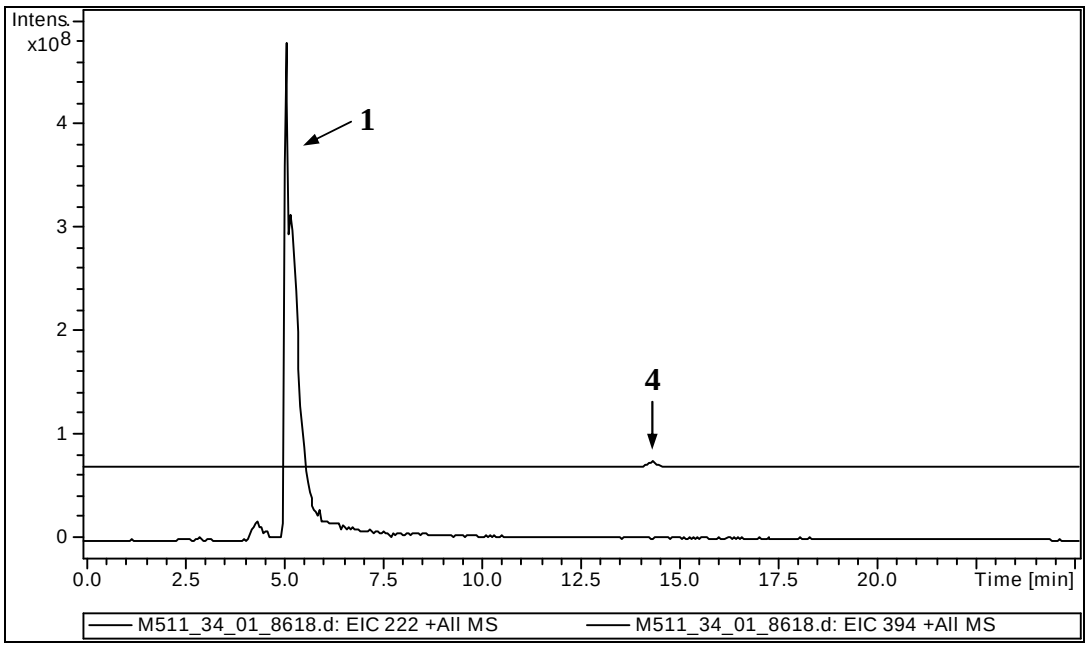


Figure 2: Extracted ion chromatograms showing levels of undecylprodiginine **1** (bottom trace), and 2-undecylpyrrole **4** (top trace) in *S. coelicolor* M511.

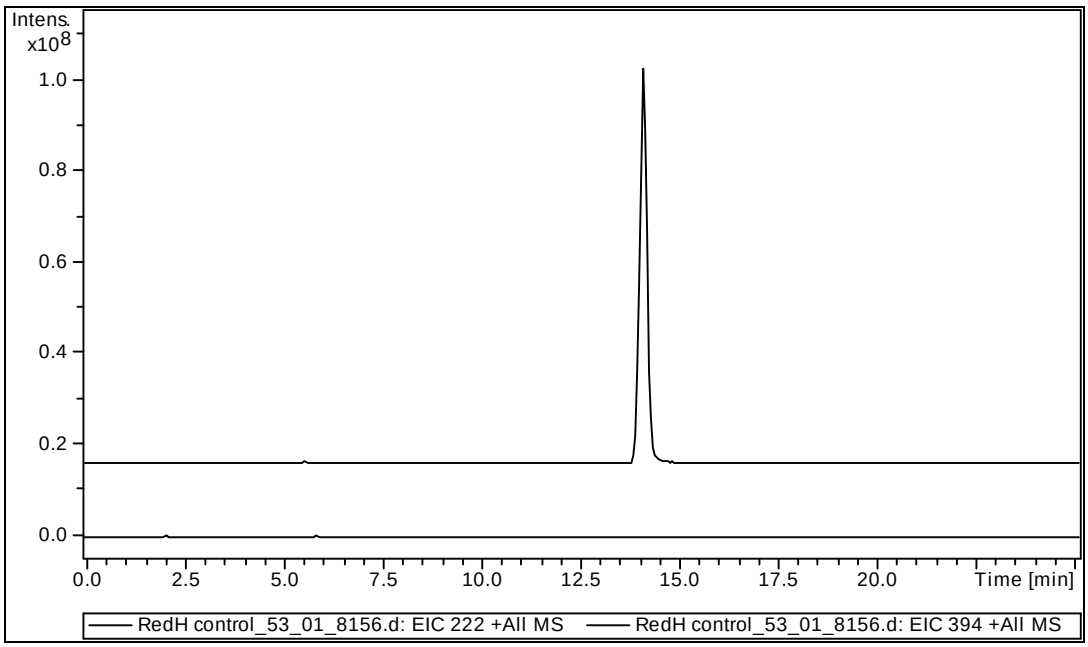


Figure 3: Extracted ion chromatograms showing levels of undecylprodiginine **1** (bottom trace), and 2-undecylpyrrole **4** (top trace) in *S. coelicolor* W33.

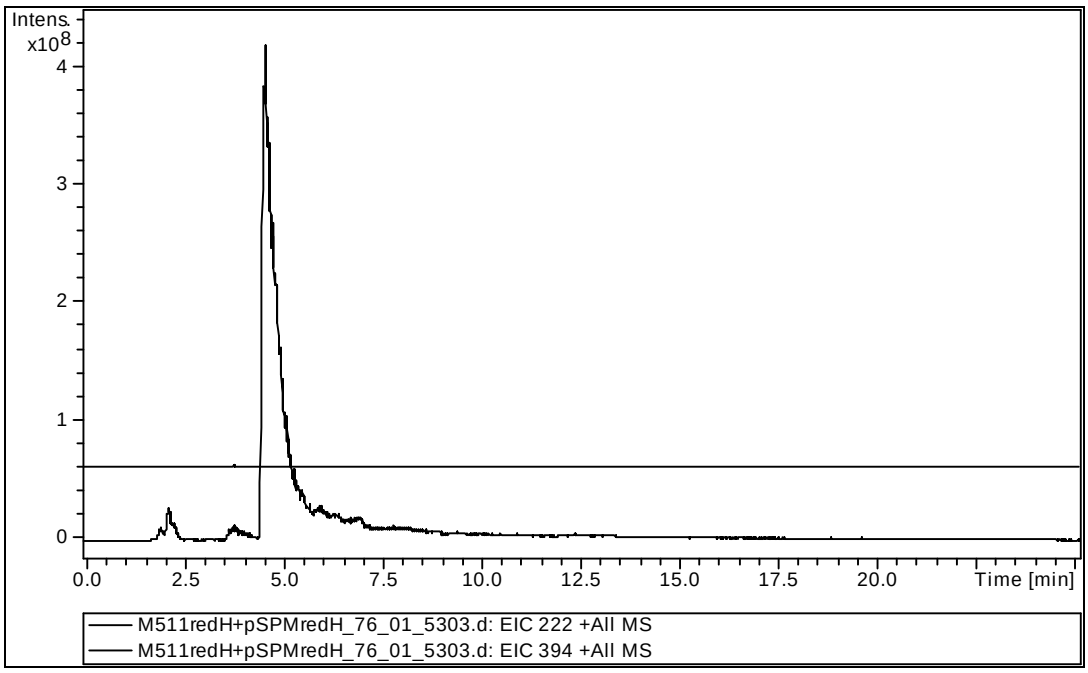


Figure 4: Extracted ion chromatograms showing levels of undecylprodiginine **1** (bottom trace), and 2-undecylpyrrole **4** (top trace) in *S. coelicolor* W33 complemented with pPSK1.

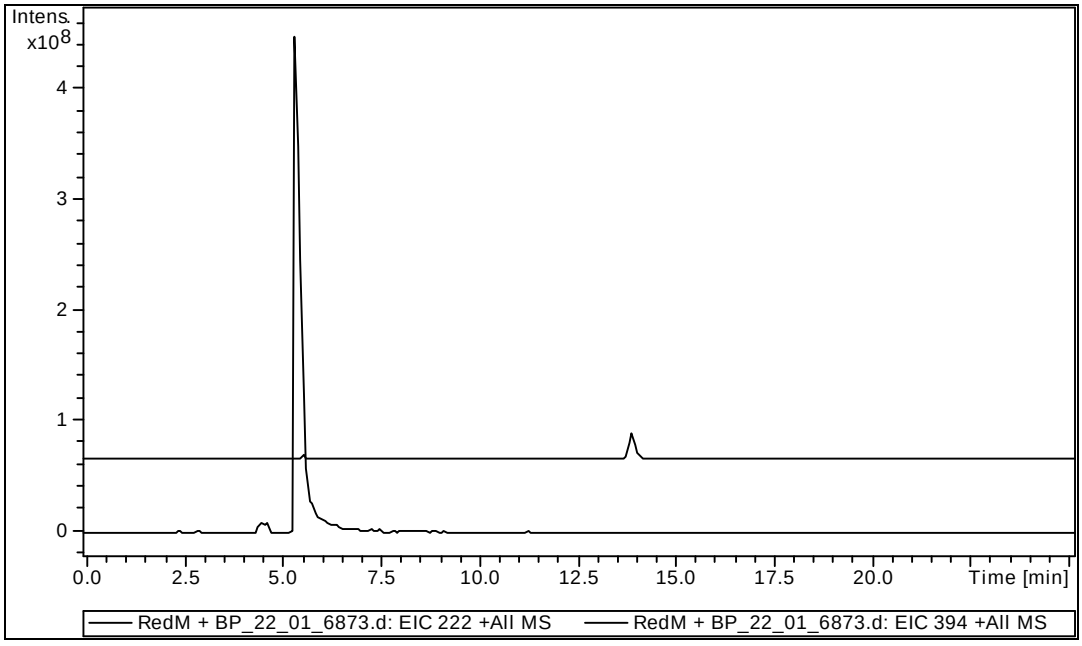


Figure 5: Extracted ion chromatograms showing levels of undecylprodiginine **1** (bottom trace), and 2-undecylpyrrole **4** (top trace) in *S. coelicolor* W39 when synthetic MBC **3** is added.

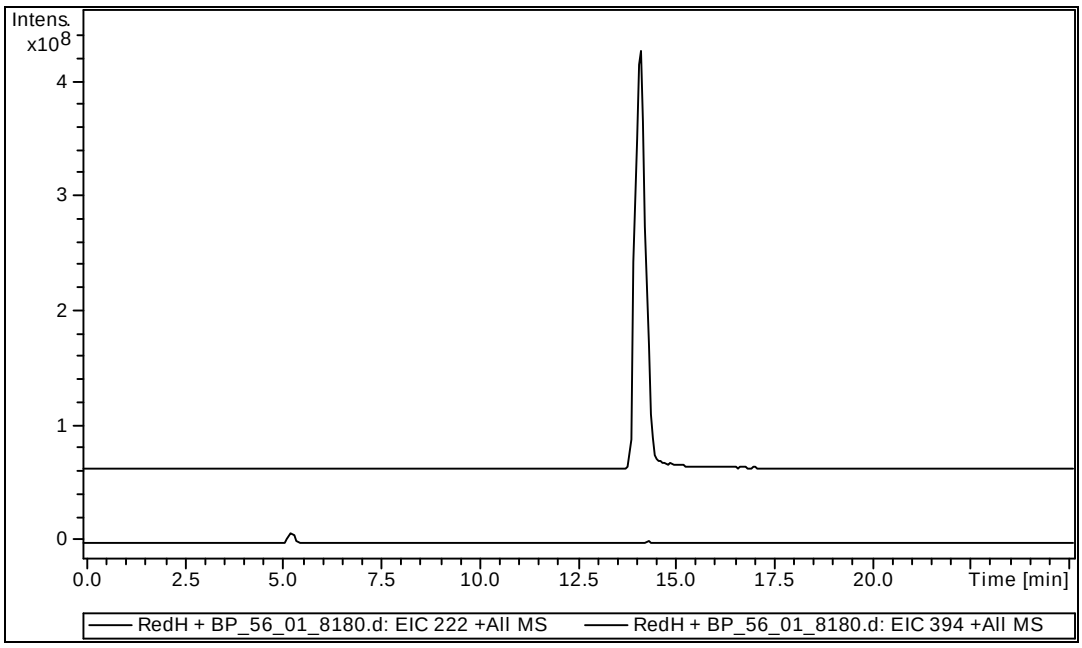


Figure 6: Extracted ion chromatograms showing levels of undecylprodiginine **1** (bottom trace), and 2-undecylpyrrole **4** (top trace) in *S. coelicolor* W33 when synthetic MBC **3** is added.

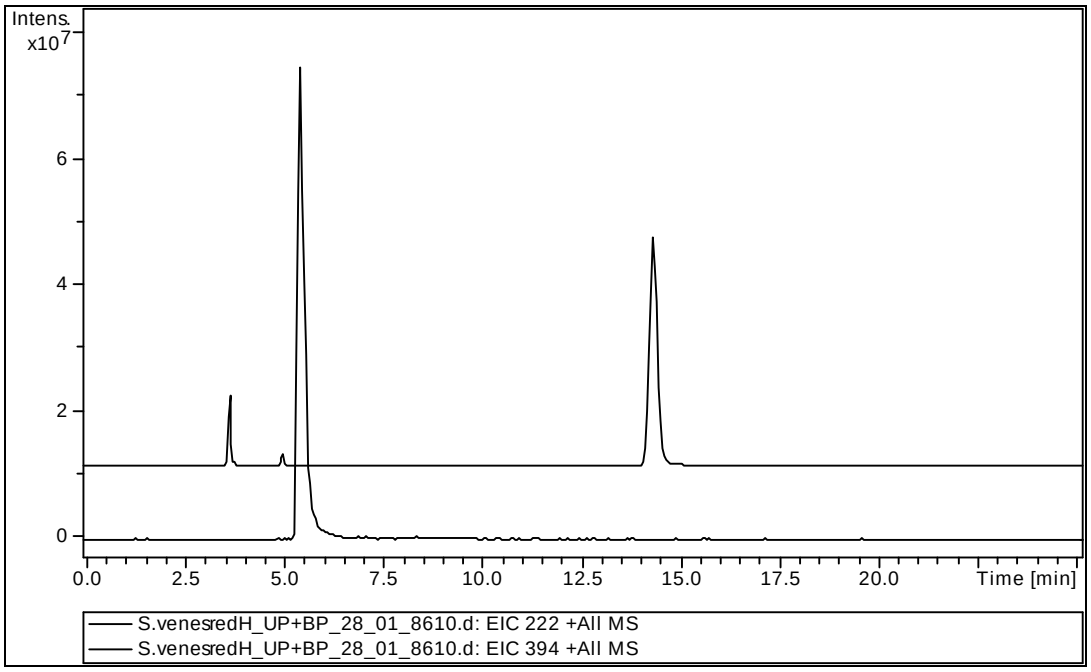


Figure 7: Extracted ion chromatograms showing levels of undecylprodiginine **1** (bottom trace) and 2-undecylpyrrole **4** (top trace) in *S. venezuelae* / pPKS1 when synthetic MBC **3** and 2-undecylpyrrole **4** were added.

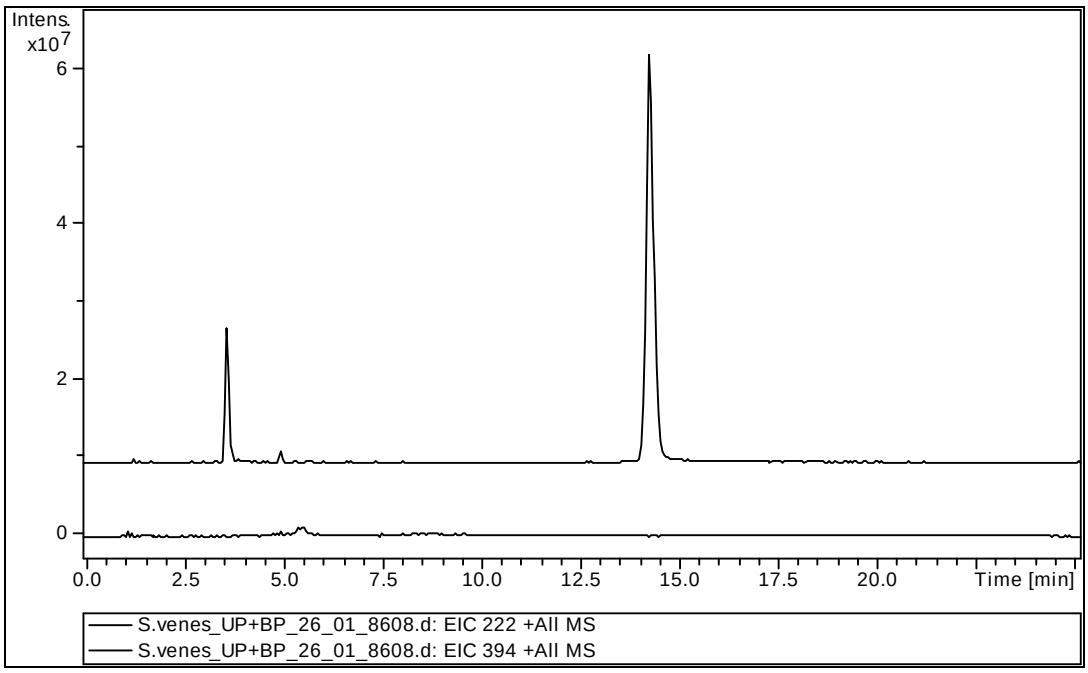


Figure 8: Extracted ion chromatograms showing levels of undecylprodiginine **1** (bottom trace) and 2-undecylpyrrole **4** (top trace) in *S. venezuelae* when synthetic MBC **3** and 2-undecylpyrrole **4** were added.

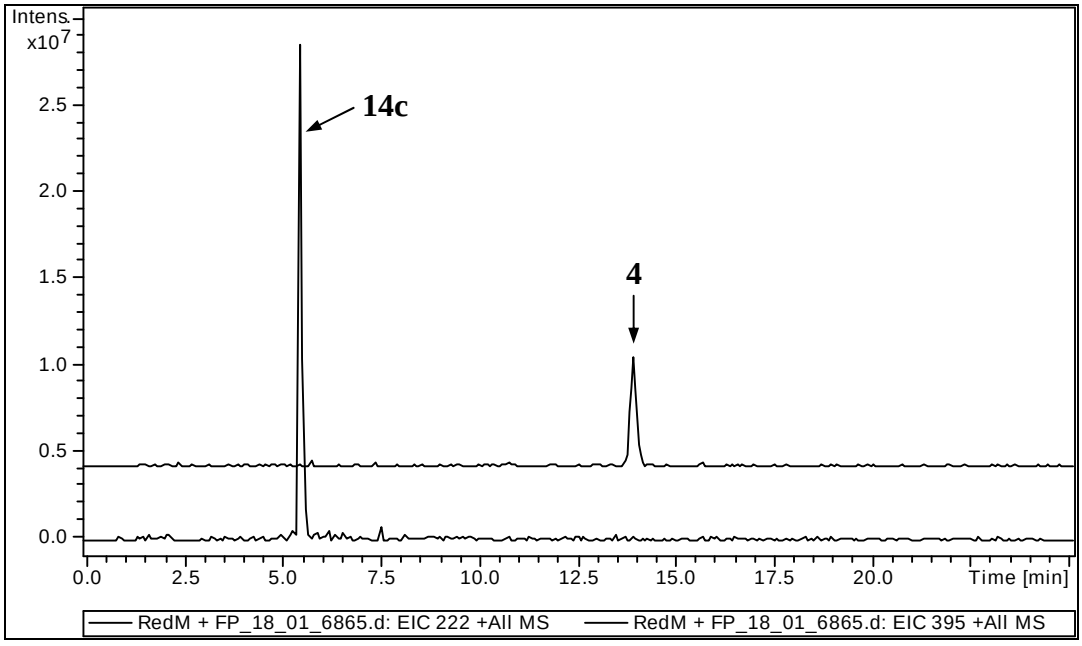


Figure 9: Extracted ion chromatograms showing levels of undecylprodiginine analogue **14c** (bottom trace) and 2-undecylpyrrole **4** (top trace) in *S. coelicolor* W39 when MBC analogue **7c** was added.

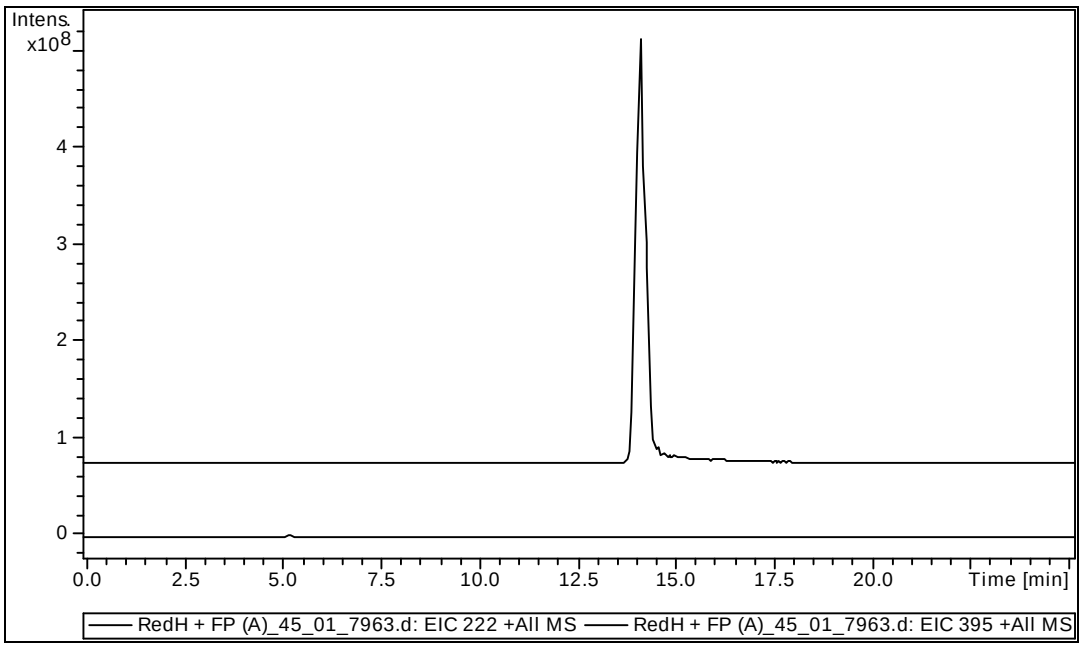


Figure 10: Extracted ion chromatograms showing levels of undecylprodiginine analogue **14c** (bottom trace) and 2-undecylpyrrole **4** (top trace) in *S. coelicolor* W33 when MBC analogue **7c** was added.

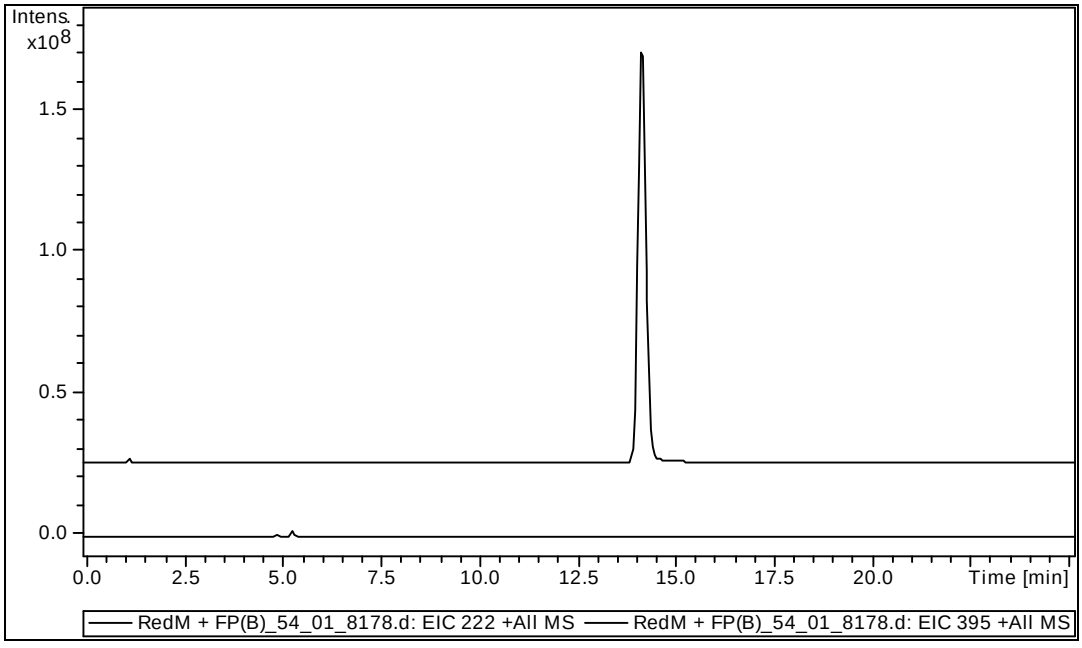


Figure 11: Extracted ion chromatograms showing levels of undecylprodiginine analogue **9** (bottom trace) and 2-undecylpyrrole **4** (top trace) in *S. coelicolor* W39 when MBC analogue **13** was added.

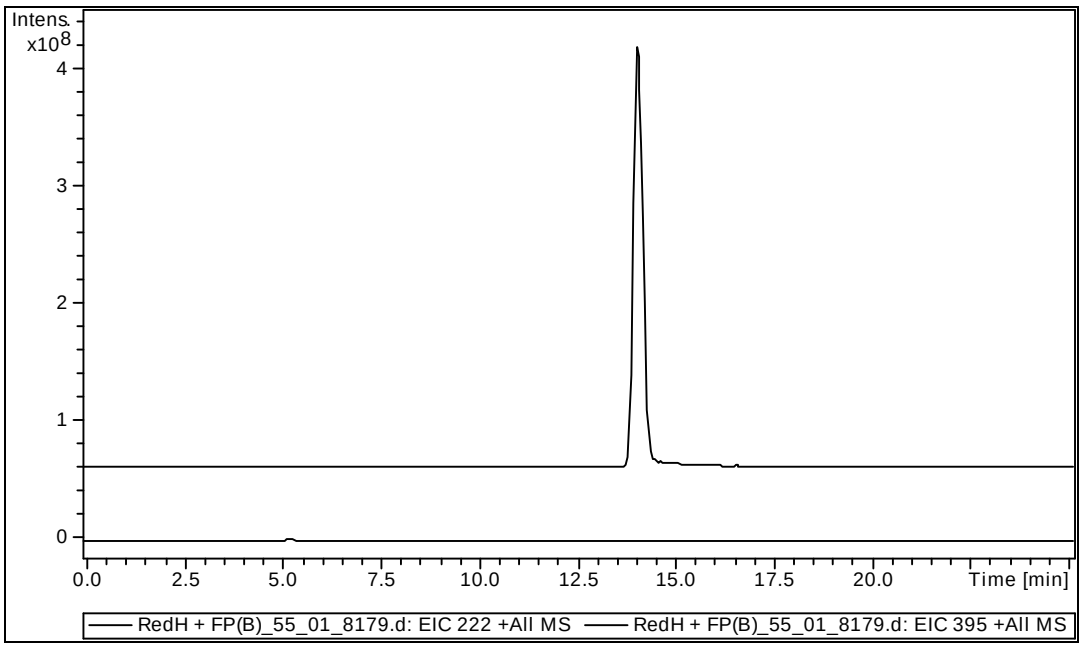


Figure 12: Extracted ion chromatograms showing levels of undecylprodiginine analogue **9** (bottom trace) and 2-undecylpyrrole **4** (top trace) in *S. coelicolor* W33 when MBC analogue **13** was added.

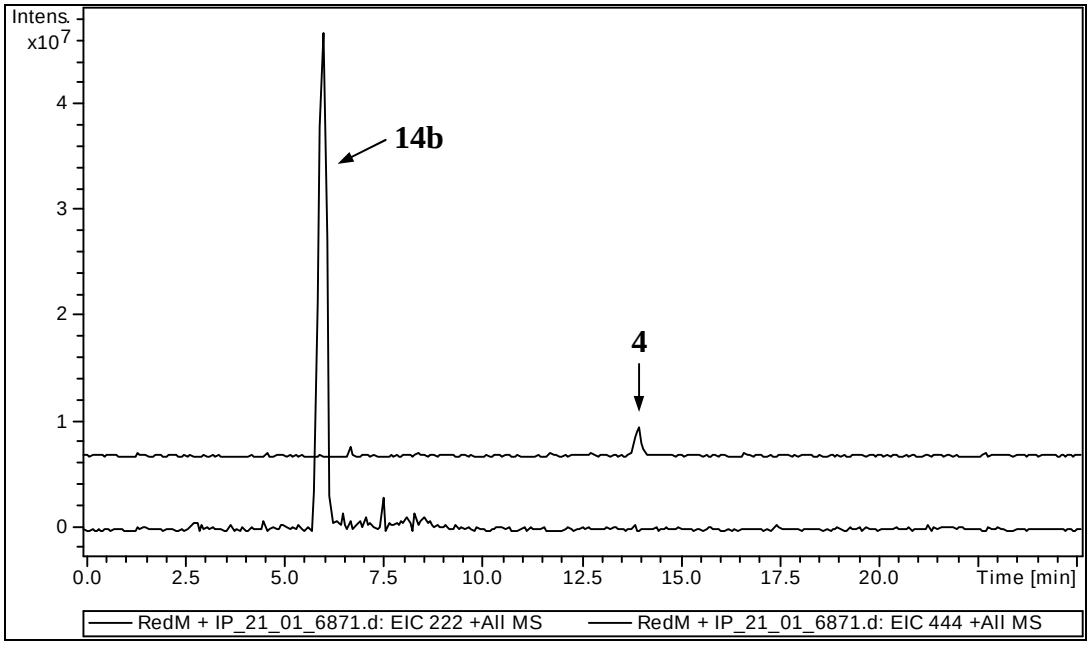


Figure 13: Extracted ion chromatograms showing levels of undecylprodiginine analogue **14b** (bottom trace) and 2-undecylpyrrole **4** (top trace) in *S. coelicolor* W39 when MBC analogue **7b** was added.

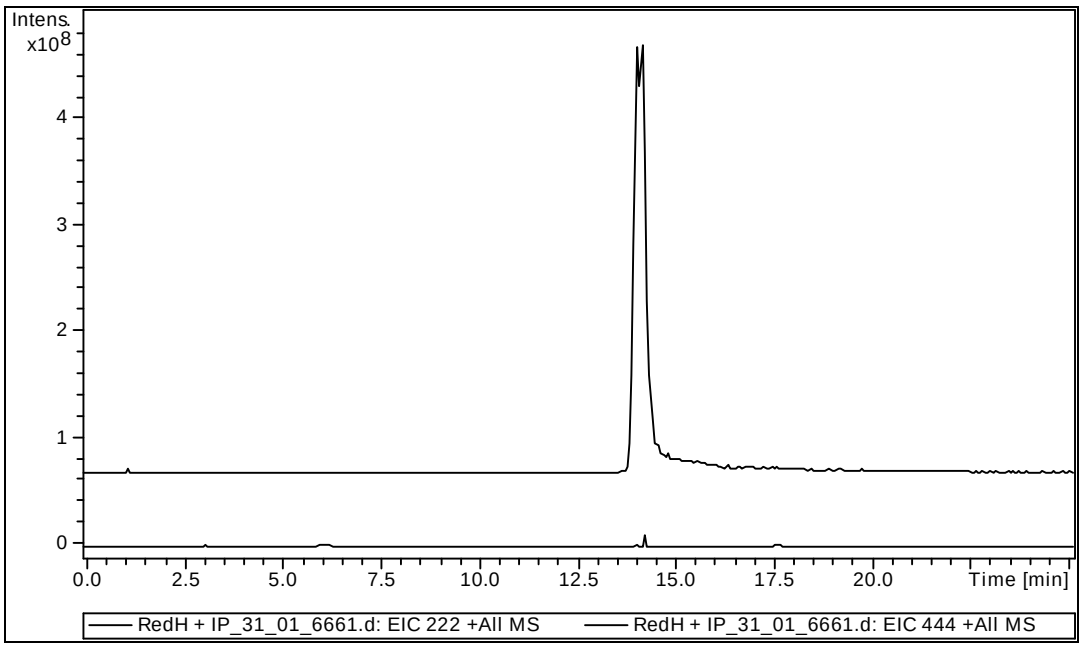


Figure 14: Extracted ion chromatograms showing levels of undecylprodiginine analogue **14b** (bottom trace) and 2-undecylpyrrole **4** (top trace) in *S. coelicolor* W33 when MBC analogue **7b** was added.

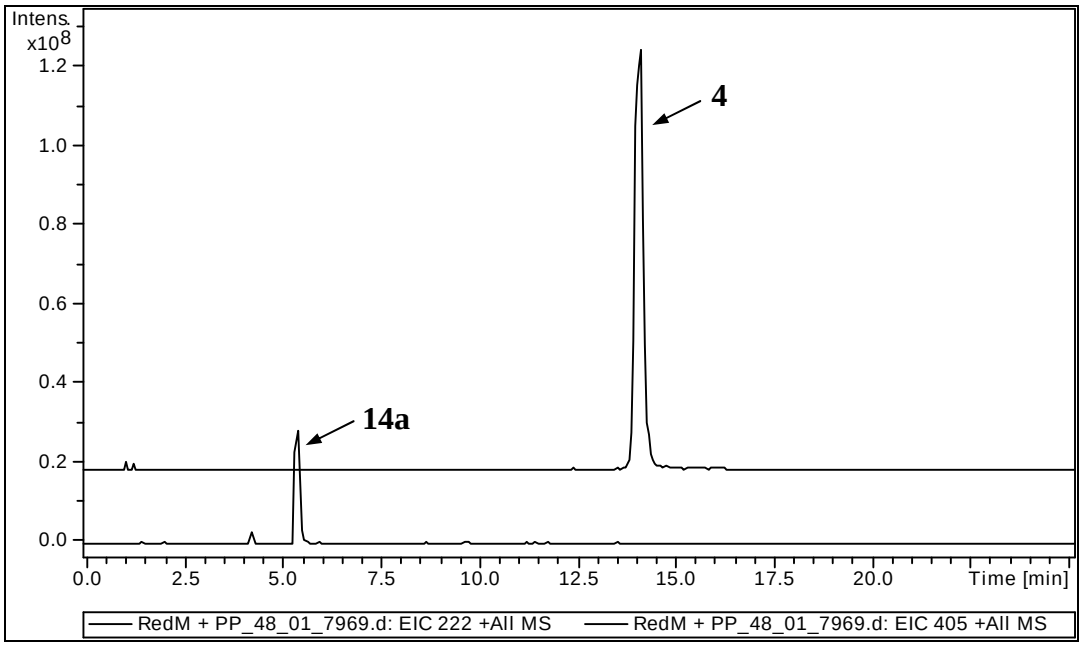


Figure 15: Extracted ion chromatograms showing levels of undecylprodiginine analogue **14a** (bottom trace) and 2-undecylpyrrole **4** (top trace) in *S. coelicolor* W39 when MBC analogue **7a** was added.

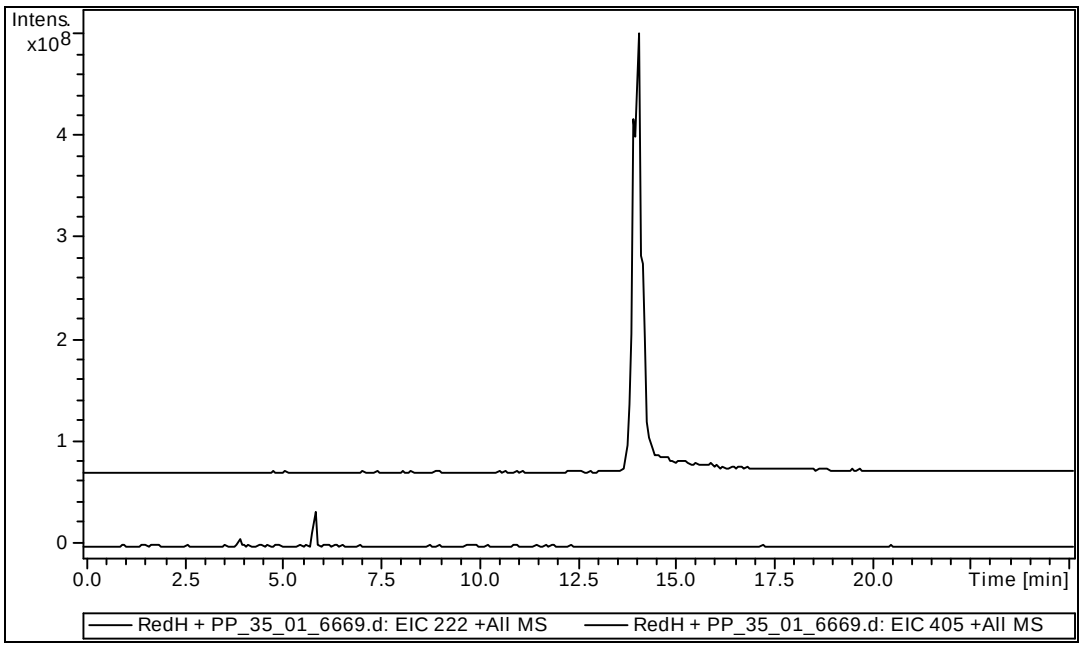


Figure 16: Extracted ion chromatograms showing levels of undecylprodiginine analogue **14a** (bottom trace) and 2-undecylpyrrole **4** (top trace) in *S. coelicolor* W33 when MBC analogue **7a** was added.

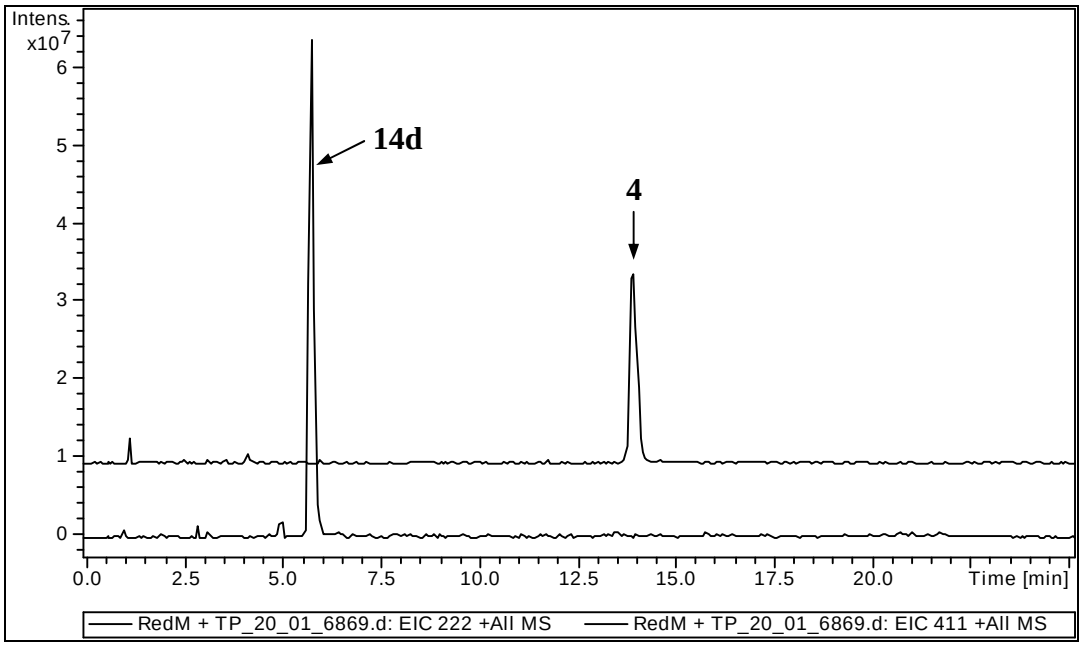


Figure 17: Extracted ion chromatograms showing levels of undecylprodiginine analogue **14d** (bottom trace) and 2-undecylpyrrole **4** (top trace) in *S. coelicolor* W39 when MBC analogue **7d** was added.

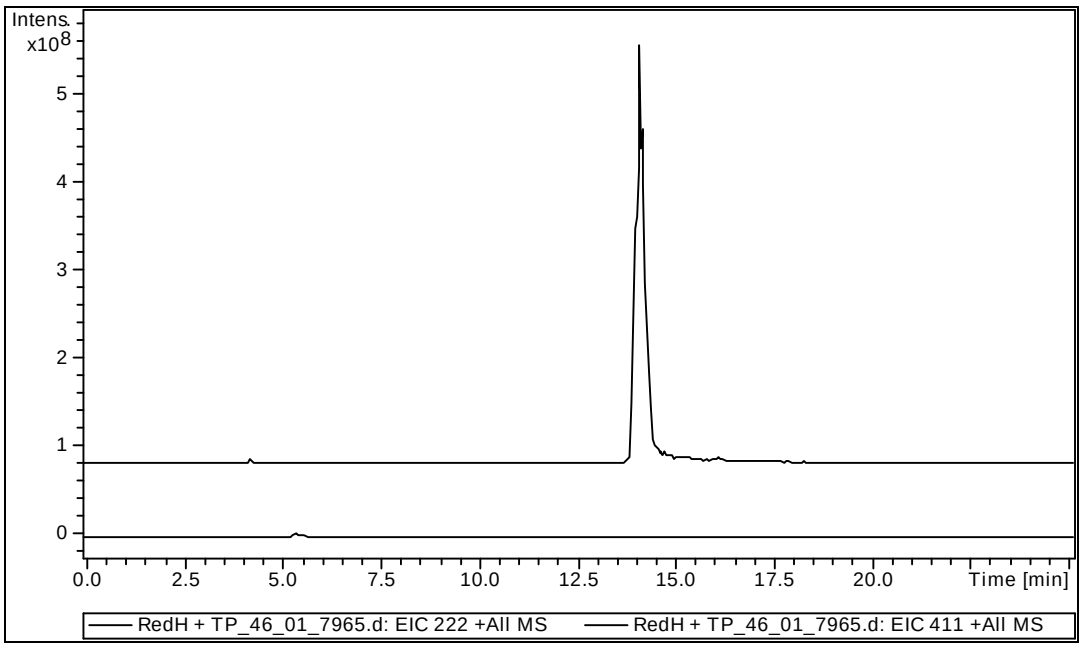
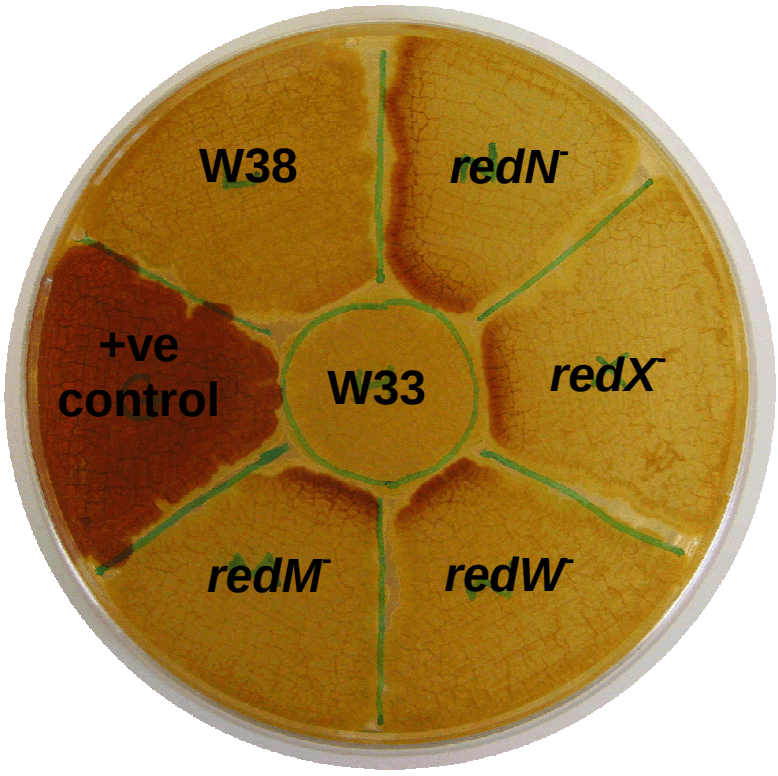


Figure 18: Extracted ion chromatograms showing levels of undecylprodiginine analogue **14d** (bottom trace) and 2-undecylpyrrole **4** (top trace) in *S. coelicolor* W33 when MBC analogue **7d** was added.

Cross feeding of MBC from *S. coelicolor* W33 (*redH*$^-$) and W38 (*redL*$^-$)[7] to *S. coelicolor redN*$^-$, *redX*$^-$, *redW*$^-$ and *redM*$^-$ mutants[8] blocked in MBC biosynthesis resulting in restoration of prodiginine production (red pigment formation)



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8. A.E. Stanley, L.J. Walton, M. Kourdi-Zerikly, C. Corre and G.L. Challis, *Chem. Commun.* 2006, 3981-3983.