Biosynthesis of Thiomarinol A and Related Metabolites of *Pseudoalternomonas* sp. SANK 73390

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Supplementary information

General experimental details

All commercially available compounds were used without further purification except where stated. All moisture or air-sensitive reactions were carried out in flame-dried glassware under positive pressure of nitrogen or argon using standard syringe/septum techniques. Anhydrous solvents were obtained by passing through a modified Grubbs system of alumina columns, manufactured by Anhydrous Engineering. Petroleum ether is of the 40-60 ^{o}C boiling point range. Routine monitoring of reactions was performed using precoated Merck-Keiselgel 60 F_{254} aluminium backed TLC plates. The spots were visualised by UV_{254} light, or potassium permanganate visualising agent. Flash column chromatography was conducted as described by Still *et al.* using silica gel (obtained from Fluorochem Ltd.) as the adsorbent. [1]

Melting points were determined on an electrothermal apparatus and are uncorrected. Optical rotations were recorded using the sodium D line (λ = 589 nm) on a Bellingham and Stanley ADP220 polarimeter and the $[\alpha]_D$ values are reported in units 10^{-1} deg cm^2 g^{-1}. Infrared spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer in the solid or liquid state. ^{1}H and ^{13}C NMR spectra were recorded using either a Jeol lambda 300 MHz, Jeol Eclipse 400 MHz, Varian VNMR 400 MHz or 500 MHz spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) and the coupling constants (*J*) are in Hertz (Hz). DEPT 135, COSY and HMQC NMR spectra were routinely used to definitively assign the signals of ^{1}H and ^{13}C NMR spectra. Chemical ionisation (CI) mass spectra were recorded on a Bruker Daltonics Apex 4e 7.0T FT-MS mass spectrometer. Electrospray (ESI) mass spectra were recorded on a Micromass LCT mass spectrometer or a VG Quattro mass spectrometer. Methane was the ionisation gas used for chemical ionisation.

General fermenation procedure

*Pseudoalteromonas sp.*SANK 73390 was incubated on a marine agar plate (Becton, Dickinson and Company Difco™ marine agar 2216) at 25ºC for 24 hours. Colonies were selected and used for inoculation of a 50mL marine broth (Becton, Dickinson and Company Difco™ marine broth 2216) in a 100 mL baffled flask. This seed culture was shaken at 25 ºC and 200 rpm for 24 hours. Marine broth (100 mL per 500 mL flask) was inoculated with 1% seed culture, then incubated at 25 ºC and 200 rpm for 24 hours. The cells were separated from the media by centrifugation (7000 rpm for 15 mins). The media was extracted with ethyl acetate (3 x ~ 0.5 v/v). The cell pellet was lysed with acetone and after removal of cell debris by centrifugation and decanting, the acetone was removed *in vacuo*, and the residue reextracted with ethyl acetate. The combined ethyl acetate extracts were dried over $MgSO_4$, filtered, and the organic phase was evaporated *in vacuo* to give a crude extract. This was purified by HPLC chromatography on a Phenomenex Luna column (250 x 4.6 mm, 5µm, 40 ºC) using a 50 to 95 % methanol in water (0.005% formic acid) gradient over 45 minutes followed by a 5 minute 95 % methanol isocratic wash. Thiomarinol A (**1**) was isolated as a yellow solid (R_t 16.9 min). δ_H (CD_3OD, 500 MHz) 7. 10 (1H, s, H-5''), 6.07 (1H, br s, H-2), 5.51-5.41 (2H, m, H-10/11), 4.35 (1H, br s, H-4), 4.10 (2H, m, H-2'), 3.93 (1H, t, 3.2, H-7), 3.85 (1H, dd, 9.8, 3.0, H-6), 3.78 (1H, dd, 11.5 and 2.8, H-16), 3.70 (1H, dd, 9.6 and 1.6, H-5), 3.61 (1H, m, H-13),

[1] W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem*, **1978,** *43*, 2923

3.54 (1H, brd, 11.5, H-16), 2.40 (2H, t, 7.4, H-2'), 2.26 (1H, m, H-9), 2.21-2.11 (2H, m, H-9/H-12), 2.13 (3H, s, H-15), 1.74 (1H, m, H-8), 1.69-1.60 (4H, m, H-3'/H-7'), 1.44-1.34 (6H, m, H-4',H-5' and H-6'), 1.10 (3H, d, 6.4, H-14), 1.00 (3H, d, 6.9, H-17); δ_C (125 MHz, CD_3OD) 172.9 (C, C-1'), 169.0 (C, C-1"), 167.3 (C, C-1), 159.8 (C, C-3), 136.6 (C, C-3"), 134.3 (CH, C-11), 133.7 (CH, C-4"), 128.4 (CH, C-10), 114.8 (CH, C-2), 114.4 (C, C-2"), 112.3 (CH, C-5"), 76.1 (CH, C-5), 72.9 (CH, C-4), 70.7 (CH, C-13), 70.5 (CH, C-7), 64.6 (CH2, C-16), 64.3 (CH, C-6), 63.5 (CH2, C-8'), 43.9 (CH, C-12), 42.5 (CH, C-8), 35.2 (CH2, C-2'), 32.0 (CH2, C-9), 28.7 (CH2, C-4'), 28.6 (CH2, C-5'), 28.3 (CH2, C-7'), 25.7 (CH2, C-5'), 25.2 (CH2, C-3'), 18.9 (CH3, C-14), 15.2 (CH3, C-17), 14.9 (CH3,C-15).

Time course

Fermentation was carried out as per general procedure on 100mL scale, and 0.5 mL aliquots were removed at a series of time points. Acetone (0.5 mL) was added and the aliquots vigorously shaken, the cells separated by centrifugation and the supernatant analysed by HPLC to quantify the thiomarinol A content (calibration curve for quantification generated using authentic thiomarinol A sample at 390 nm). This indicated that production commenced after 7 hours reaching a maximum after a further 6 hours growth.

Feeding studies

Sodium [1,2-$^{13}C_2$]-acetate - Fermentation carried out as per general procedure on 1L scale, with addition of sodium [1,2-$^{13}C_2$]-acetate (25 mg/mL in water) to give a final concentration of 200 mg/L to the large scale fermentation 7 hours after inoculation. The crude extract (27 mg) was purified by HPLC to give 7.7 mg labelled thiomarinol A (Figure S5).

Sodium [2-^{13}C]-acetate. (i) Fermentation carried out as per general procedure on 1L scale, with addition of sodium [2-^{13}C]-acetate(100 mg/mL in water) to give a final concentration of 400 mg/L to the large scale fermentation 7 hours after inoculation. The crude extract (60 mg) was purified by HPLC to give 12.5 mg labelled thiomarinol A (Figure S6). The experiment was repeated and the ^{13}C NMR spectrum determined in CD_3OD (ii) Fermentation carried out as per general procedure on 0.4 L scale, with addition of sodium [2-^{13}C]-acetate(100 mg/mL in water) to give a final concentration of 200 mg/L to the large scale fermentation 7 hours after inoculation. The crude extract (125 mg) was purified by HPLC to give 8.6 mg labelled thiomarinol A (Figure S17). The purification was performed by application of HPLC chromatography on a Phenomenex Luna column (250 x 10 mm, 5µm, 40 ºC, flow: 5 mL/min) eluted isocratically for 1 minute (60% MeOH in water) followed by a gradient from 60% to 80% MeOH in water over 10 minutes to give thiomarinol A (8.6 mg, RT 8.9 mins.)

Sodium [1-^{13}C, 1-$^{18}O_2$]-acetate. Fermentation carried out as per general procedure on 0.5L scale, with addition of sodium [1-^{13}C, 1-$^{18}O_2$]-acetate (25 mg/mL in water) to give a final concentration of 200 mg/L to the large scale fermentation 7 hours after inoculation. The crude extract (35 mg) was purified by HPLC to give 5.6 mg labelled thiomarinol A (Figure S7).

[Methyl-^{13}C]-methionine. (i) Fermentation carried out as per general procedure on 0.5 L scale, with addition of [methyl-^{13}C]-methionine (20 mg/mL in water) to give a final concentration of 200 mg/L to the large scale fermentation 7 hours after inoculation. The crude extract (36 mg) was purified by HPLC to give 3.8 mg labelled thiomarinol A (Figure S8). As above the experiment was repeated and the ^{13}C NMR spectrum determined in CD_3OD (ii) Fermentation carried out as per general procedure on 0.6 L scale, with addition of [methyl-^{13}C]-methionine (100 mg/mL in water) to give a final concentration of 200 mg/L to the large scale fermentation 7 hours after inoculation. The crude extract (93 mg) was purified by HPLC to give 11.9 mg labelled thiomarinol A (Figure S18). The purification was performed by application of HPLC chromatography on a Phenomenex Luna

column (250 x 10 mm, 5µm, 40 ºC, flow: 5 mL/min) eluted isocratically for 1 minute (60% MeOH in water) followed by a gradient from 60% to 80% MeOH in water over 10 minutes to give thiomarinol A (11.9 mg, RT 8.7 mins.)

Feeding marinolic acid A to *Pseudoalteromonas* sp. SANK 73390 ΔPKS mutant. Fermentation carried out as per general procedure on 0.05L scale, with addition of marinolic acid A to give a final concentration of 100 mg/L immediately after inoculation. The crude extract was analysed by LC-MS indicating thiomarinol A is produced (Figure S19).

[2-^{13}C]-Cystine. Fermentation carried out as per general procedure on 0.3 L scale, with addition of [2-^{13}C]-cystine (10 mg/mL in water) to give a final concentration of 60 mg/L to the large scale fermentation immediately after inoculation. The crude extract (27 mg) was purified by HPLC to give 2.9 mg labelled thiomarinol A (Figure S9).

[2,3-$^{13}C_2$]-Succinic acid. Fermentation carried out as per general procedure, however supplementing with 4% glucose, on 1 L scale with addition of 7 mg [2,3-$^{13}C_2$]-succinic acid (dissolved in H_2O with a concentration of 21 mg/mL) to every flask at 6.5 hours, 8 hours and 9.5 hours after inoculation to give a final concentration 210 mg/L. The purification was performed by application of HPLC chromatography on a Phenomenex Luna column (250 x 10 mm, 5µm, 40 ºC, flow: 5 mL/min) eluted isocratically for 1 minute (60% MeOH in water) followed by a gradient from 60% to 80% MeOH in water over 10 minutes to give thiomarinol A (18.9 mg, RT 8.7 mins.) (Figure S10)

[2,3-$^{13}C_2$]-4-Hydroxybutanoic acid. Fermentation carried out as per general procedure, however supplementing with 4% glucose, on 1 L scale with addition of 7 mg [2,3-$^{13}C_2$]-4-hydroxybutanoic acid (dissolved in H_2O with a concentration of 21 mg/mL) to every flask at 6.5 hours, 8 hours and 9.5 hours after inoculation to give a final concentration 210 mg/L. The purification was performed by application of HPLC chromatography on a Phenomenex Luna column (250 x 10 mm, 5µm, 40 ºC, flow: 5 mL/min) eluted isocratically for 1 minute (60% MeOH in water) followed by a gradient from 60% to 80% MeOH in water over 10 minutes to give thiomarinol A (8.5 mg, RT 8.7 mins.) (Figures 11 and 12).

Feeding of pyrrothine to the *Pseudoalteromonas* sp. SANK 73390 ΔNPRS mutant.
Pyrrothine-HCl was dissolved in water and the pH adjusted to 7 with saturated $NaHCO_3$ solution. The aqueous solution was extracted with MeOH: $CHCl_3$ 1: 1 and the organic solvent removed to give pyrrothine as a yellow powder. This was then dissolved in water to give twelve different concentrations (8 mg/5 mL, 4 mg/5 mL, 2 mg/5 mL, 1 mg/5 mL, 0.5 mg/5 mL, 0.25 mg/5 mL, 0.13 mg/ 5 mL, 0.06 mg/ 5 mL, 0.03 mg/ 5 mL, 0.02 mg/ 5 mL, 0.01 mg/ 5 mL and 0 mg/5 mL). Each of these was added to a 50-mL scale fermentation of the **Δ**NRPS strain 6.5 hours after inoculation. Fermentation and extraction were carried out as per general procedure. The crude extracts from each fermentation was analysed by LCMS and the results are summarised in Figure S3 below. A further fermentation of the NPRS mutant was carried out on 0.2 L scale with a sterile-filtered water solution of pyrrothine (4 mg pyrrothine dissolved in 10 mL water) added 6.5 hours after inoculation (final concentration in fermentation media of 20 mg/L). Thiomarinol A (1.1 mg) was isolated and its structure confirmed by ^{1}H NMR and HR-ESI-MS.

Feeding hexanoic acid to *Pseudoalteromonas* sp. SANK 73390 ΔPKS mutant.
Fermentation of ΔPKS *Pseudoalteromonas* sp. SANK 73390 was carried out on 1.3 L scale to give 53 mg crude extract. The crude extract was purified by HPLC using a 50 to 95 % MeOH in H_2O + 0.005% formic acid over 19 mins to give xenorhabdin 1, **26**, as a yellow oily solid (< 1 mg, R_t 12.8 mins). δ_H (DMSO-d_6, 500 MHz) 10.70 (1H, br s, NH), 9.81 (1H, s, NH), 7.05 (1H, s, H5), 2.33 (2H,

t, 7.3 Hz, 2'-H_2), 1.52 (2H, q, 7.3, 3'-H_2), 1.31-1.22 (4H, m, 4'-H_2, 5'-H_2), 0.86 (3H, t, 7.1, 6'-H_3) δ_C (DMSO-d_6, 500 MHz, measured via 2D NMR experiments) 174.5 (C-1), 172.4 (C-1'), 133.9 (C-3 and C-4), 115.5 (C-2), 111.0 (C-5), 34.8 (C-2'), 25.3 (C-3'), 28.5 (C-4'), 22.1 (C-5'), 13.9 (C-6'); ν_{max}/cm^{-1} 1660 (sh), 1646, 1636 (sh), 1598, 1522; HRESIMS *m/z* = 293.0397 ± 1.0 ppm $[M]Na^+$ (293.0394 calcd for $C_{11}H_{14}O_2N_2S_2Na$).

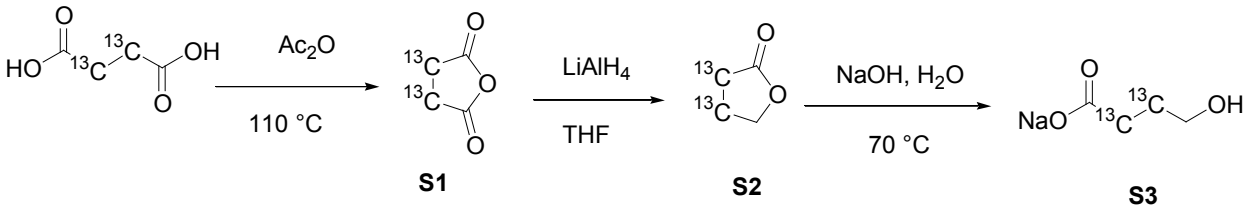
Feeding 12-hydroxydodecanoic acid to *Pseudoalteromonas* sp. SANK 73390 ΔPKS mutant.
Fermentation of ΔPKS *Pseudoalteromonas* SANK73390 was carried out on 1.2 L scale to give 58 mg crude extract. The crude extract was purified by HPLC gradient 50 to 75 % MeOH in H_2O + 0.005% formic acid over 28 mins, to yield **28** (2.1 mg, R_t 13.5 mins) and **27** (7.1 mg, R_t 21.7 mins) as yellow oily solids.

28 δ_H (DMSO-d_6, 500 MHz) 10.70 (1H, br s, NH), 9.81 (1H, s, NH), 7.05 (1H, s, 5-H), 4.31 (1H, t, 5.2, OH), 3.36 (2H, m, 12'-H_2), 2.33 (2H, t, 7.3, 2'-H_2), 1.51 (2H, q, 7.3, 3'-H_2), 1.39 (2H, q, 6.7, 11'-H_2), 1.26-1.23 (14H, m, 4'-H_2 to 10'-H_2); δ_C (DMSO-d_6, 125 MHz) 171.9 (C-1'), 167.9 (C-1), 134.0 and 133.7 (C-3 and C-4), 115.4 (C-2), 110.5 (C-5), 60.7 (C-12), 34.7 (C-2'), 32.5 (C-11'), 29.1, 28.98, 28.96, 28.90, 28.7, 28.5, 25.5, 25.1 (CH2, C3' to C10'); mp 159-160 °C; ν_{max}/cm^{-1} 2923, 1635, 1594, 1529; HRESIMS *m/z* = 393.1271 ± 3.1 ppm $[M]Na^+$ (393.1283 calcd for $C_{17}H_{26}O_3N_2S_2Na$).

27 δ_H (DMSO-d_6, 500 MHz) 10.70 (1H, br s, NH), 9.81 (1H, br s, NH), 7.05 (1H, s, 5-H), 4.31 (1H, t, 5.2, OH), 3.37 (2H, t, 6.5, 10'-H_2), 2.33 (2H, t, 7.3, 2'-H_2), 1.51 (2H, q, 6.4, 3'-H_2), 1.39 (2H, q, 6.7, 9'-H_2), 1.27-1.23 (10H, m, 4'-H_2 to 8'-H_2); δ_C (DMSO-d_6, 125 MHz) 171.8, 167.9, 134.0, 133.7, 115.4, 113.8, 110.5, 60.7, 34.7, 32.5, 28.94, 28.91, 28.7, 28.6, 25.5, 25.0; ν_{max}/cm^{-1} 2926, 1636, 1594, 1532; HRESIMS *m/z* = 365.0958 ± 3.0 ppm $[M]Na^+$ (365.0969 calcd for $C_{15}H_{22}O_3N_2S_2Na$).

Feeding phenylvaleric acid to *Pseudoalteromonas* sp.SANK 73390 ΔPKS mutant
Fermentation of ΔPKS *Pseudoalteromonas* SANK73390 carried out on 0.6 L scale to give 28 mg crude extract. The crude extract was purified by HPLC using a 50 to 95 % MeOH in H_2O + 0.005% formic acid over 19 mins to give **30** as a yellow oily solid (2.0 mg, R_t 13.3 mins). δ_H (DMSO-d_6, 500 MHz) 10.71 (1H, br s, NH), 9.84 (1H, s, NH), 7.28-7.16 (5H, m, aromatic H), 7.05 (1H, s, 5'-H), 2.58 (2H, t, 7.2, 5'-H_2), 2.37 (2H, t, 7.1 Hz, 2'-H_2), 1.58-1.51 (4H, m, 3'-H_2, 4'-H_2); δ_C (DMSO-d_6, 125 MHz) 171.7 (C-1'), 167.9 (C-1), 142.0 (C-6'), 134.0, 133.7 (C-3 and C-4), 128.2 (Ph), 125.6 (CH, Ph), 115.3 (C-2), 110.6 (C-5), 34.8, 34.5 (C2' and C5'), 30.5 (C-4'), 24.7 (C-3'); ν_{max}/cm^{-1} 2923, 1637, 1594, 1528; HRESIMS *m/z* = 355.0556 ± 1.4 ppm $[M]Na^+$ (355.0551 calcd for $C_{16}H_{16}O_2N_2S_2Na$).

Synthesis of sodium [2,3-$^{13}C_2$]-4-hydroxybutyrate



Scheme S1

[2,3-^{13}C]-Succinic anhydride (**S1**)

(Based on a procedure: Low, E.; Gawley, E. R.; *J. Am. Chem. Soc.*, **2000**, *122*, 9562).

[2,3-^{13}C]-Succinic acid (230 mg, 1.92 mmol) and acetic anhydride (0.55 mL) were added to a 2 mL round bottomed flask. The flask was sealed with a rubber septum and a nitrogen-containing balloon was attached. The reaction was heated at 110 °C for 1.5 h, after which it was concentrated under reduced pressure to give a yellow oil. The flask was then placed in an ice bath resulting in the formation of yellow crystals. The crystals were dried under vacuum to give [2,3-^{13}C]-succinic anhydride **S1** (195 mg, 99%), which was used directly in the next step without purification.

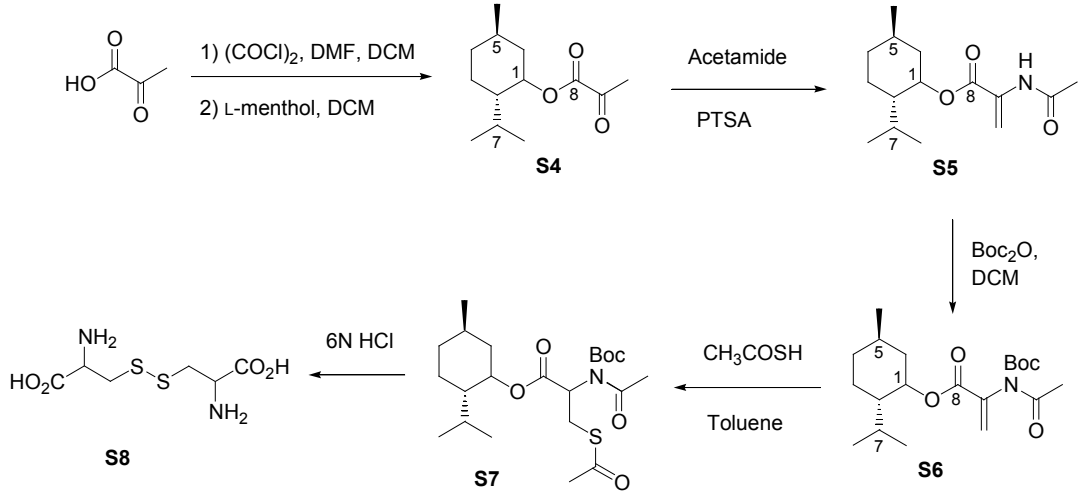
[2,3-^{13}C]-γ-Butyrolactone (**S2**)
(Based on a procedure by: Wheatley, B. M. M.; Keay, B. A. *J. Org. Chem.* **2007**, *72*, 7253).

Lithium aluminium hydride (43 mg, 1.13 mmol) was suspended in dry THF (4 mL) and refluxed for 30 min. The suspension was cooled to −78 °C and a solution of [2,3-^{13}C]-succinic anhydride **S1** (195 mg, 12.4 mmol) in dry THF (3 mL) was added dropwise. The reaction was warmed to room temperature over 1.5 h, after which it was cooled to 0 °C and 6 M aq. HCl (1.5 mL) was added slowly. The aqueous layer was separated and extracted with Et_2O (5 × 8 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give [2,3-^{13}C]-γ-butyrolactone **S2** (129 mg, 76%) as an orange oil, which was used directly in the next step without purification; δ_H (400 MHz, $CDCl_3$) 4.34 (2 H, app. tt, *J* 7.0, 2.5, 4-H_2), 2.49 (2 H, dm, *J* 136.0, 2-H_2), 2.26 (2 H, dm, *J* 136.0, 3-H_2); δ_C (100 MHz, $CDCl_3$) 27.7 (d, *J* 32.5, C-2, enhanced), 22.0 (d, *J* 32.5, C-3, enhanced).

Sodium [2,3-^{13}C]-4-hydroxybutyrate (S3)
(Based on a procedure: Weber, A. E.; Halgren, T. A.; Doyle, J. J.; Lynch, R. J.; Siegl, P. K. S.; Parsons, W. H.; Greenlee, W. J.; Patchett, A. A. *J. Med. Chem.* **1991**, *34*, 2692).

[2,3-^{13}C]-γ-Butyrolactone **S2** (80 mg, 0.91 mmol) and sodium hydroxide (36 mg, 0.91 mmol) were dissolved in H_2O (1.5 mL) and heated at 70 °C for 15 h. The reaction mixture was concentrated under reduced pressure by co-evaporation with toluene to give crude sodium [2,3-^{13}C]-4-hydroxybutyrate **S3** (116 mg, 99%) as an orange paste, which was used in feeding studies without any further purification; δ_H (400 MHz, D_2O) 3.56 (2 H, tdd, *J* 6.5, 4.0, 2.1, 4-H_2), 2.20 (2 H, dm, *J* 127.0, 2-H_2), 1.76 (2 H, dm, *J* 127.0, 3-H_2); δ_C (100 MHz, D_2O) 33.9 (d, *J* 34.0, C-2, enhanced), 28.3 (d, *J* 34.0, C-3, enhanced). (Figures 13 and 14).

Synthesis of DL-[2-^{13}C]-Cystine S8



Scheme S2

(1*R*,2*S*,5*R*)-(-)-2-Isopropyl-5-methylcyclohexyl pyruvate **S4**

Pyruvic acid (0.69 g, 7.82 mmol) was dissolved in dry DCM (20 mL) and DMF (cat.) was added under an atmosphere of nitrogen. Oxalyl chloride (0.74 mL, 8.75 mmol) was added dropwise to the solution at room temperature and the produced HCl gas was released via a needle to reduce the pressure to atmospheric pressure. The mixture was stirred under an atmosphere of nitrogen at room temperature for 16 h. (-)-Menthol (1.83 g, 11.74 mmol) was dissolved in dry DCM (3 mL) and was added dropwise to the reaction mixture and stirred for 3 h. The reaction was quenched by adding saturated $NaHCO_{3(aq)}$ (20 mL). The aqueous layer was extracted with DCM (3 × 30 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude oil was purified by flash chromatography (SiO_2, 5% EtOAc in petroleum ether 40-60 °C) giving menthyl pyruvate **S4** as a colourless oil (1.17 g, 66%). $[\alpha]_D^{24}$ -83 (*c* 1.9, EtOH), lit.[1] $[\alpha]_D$ -84.1 (*c* 1.88, absolute ethanol); δ_H (400 MHz, $CDCl_3$) 0.75 and 0.90 (each 3H, each d, *J* 7.1, 2 × 7-CH_3), 0.91 (3H, d, *J* 6.4, 5-CH_3), 1.10-2.02 (9H, m, 2-H, 3-H_2, 4-H_2, 5-H, 6-H_2 and 7-H), 2.45 (3H, s, 10-H_3), 4.81 (1H, td, *J* 11.0 and 4.5, 1-H); δc (100 MHz, $CDCl_3$) 16.2 and 20.7 (2 × 7-CH_3), 21.9 (C-5), 23.3 (CH_2), 26.2 (C-10), 26.8 (CH), 31.4 (CH), 34.0 (CH_2), 40.4 (CH_2), 46.8 (CH), 77.1 (C-1), 160.6 (C-8), 192.3 (C-9). Spectroscopic data in accord with literature.[1]
For the synthesis of [^{13}C]-**S4**, 2-[^{13}C]-sodium pyruvate (0.41 g, 3.69 mmol) was suspended in THF (5 mL) and then 1M $HCl_{(aq)}$ (2 mL) was added. The solution was stirred for 0.5 h and the aqueous phase was extracted with EtOAc (3 × 30 mL). The combined organic phase was extracted with brine (10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude [^{13}C]pyruvate **S4** was used in the next reaction without further purification.

1. K. Matsumoto and K. Harada, *J. Org. Chem.*, 1966, **31**, 1956-1958.

(1*R*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexyl 2'-acetamidacrylate **S5**
Keto ester **S4** (0.90 g, 6.06 mmol), acetamide (0.54 g, 9.09 mmol), *p*-toluenesulfonic acid (cat.) and 4-methoxyphenol (cat.) were dissolved in toluene (40 mL) and the mixture was heated to reflux for 7 h in a Dean-Stark. The solvent was evaporated *in vacuo* and the yellow oil was diluted with DCM (40 mL) and the solution washed with saturated $NaHCO_{3(aq)}$ (20 mL) and then water (20 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude oil was purified by flash chromatography (SiO_2, 20% EtOAc in petroleum ether 40-60 °C) giving acrylate **S5** as a colourless oil (0.69 g, 65%). $[\alpha]_D^{24}$ -75.0 (*c* 0.95, $CHCl_3$), lit.[2] $[\alpha]_D$ -81.2 ($CHCl_3$); δ_H(400 MHz, $CDCl_3$) 0.76 and 0.90 (each 3H, each d, *J* 7.0, 2 × 7-CH_3), 0.93 (3H, d, *J* 6.6, 5-CH_3), 0.99-2.00 (9H, m, 2-H, 3-H_2, 4-H_2 ,5-H, 6-H_2 and 7-H), 2.13 (3H, s, $NCOCH_3$), 4.80 (1H, td, *J* 11.0 and 4.5, 1-H), 5.86 (1H, d, *J* 1.5, 10-*H*H), 6.56 (1H, s, 10-H*H*), 7.78 (1H, brs, NH); δc(100 MHz, $CDCl_3$) 16.4 and 20.6 (2 × 7-CH_3), 21.9 (5-CH_3), 23.6 (CH_2), 24.7 (CH), 26.4 ($NCOCH_3$), 31.4 (CH), 34.1 (CH_2), 40.6 (CH_2), 47.0 (CH), 76.5 (C-1), 108.0 (C-10), 131.2 (C-9), 163.7 (C-8), 168.8 ($NCOCH_3$). Spectroscopic data in accord with literature.[2]
The above reaction was repeated using [^{13}C]-keto ester **S4** giving [^{13}C]-amide **S5** δ_H (400 MHz, $CDCl_3$) 0.77 and 0.91 (each 3H, each d, *J* 7.0, 2 × 7-CH_3), 0.93 (3H, d, *J* 6.6, 5-CH_3), 1.03-2.04 (9H, m, 2-H, 3-H_2, 4-H_2, 5-H, 6-H_2 and 7-H), 2.14 (3H, s, $NCOCH_3$), 4.81 (1H, td, *J* 10.9 and 4.4, 1-H), 5.86 (1H, d, *J* 1.5, 10-*H*H), 6.57 (1H, d, *J* 3.9, 10-H*H*), 7.78 (1H, brs, NH); δc (100 MHz, $CDCl_3$) 131.2 (C-9, enhanced).

2. H. Tanaka and M. Niwa, *Polymer*, 2005, **46**, 4635-4639.

(1*R*, 2*S*, 5*R*)-2-Isopropyl-5-methylcyclohexyl 2'-(*N*-(*tert*-butoxycarbonyl) acetamido)acrylate **S6**

Acrylate **S5** (0.084 g, 0.30 mmol) was dissolved in dry DCM (10 mL) and then DMAP (0.003 g, 0.03 mmol) and di-*tert*-butyl dicarbonate (0.08 g, 0.37 mmol) were added under an atmosphere of nitrogen at room temperature. The mixture was stirred for 16 h and then saturated $NaHCO_{3(aq)}$ (10 mL) was added. The aqueous layer was extracted with DCM (3×20 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude oil was purified by flash chromatography (SiO_2, 10% EtOAc in petroleum ether 40-60 °C) giving ester **S6** as a colourless oil (0.10 g, 88%). $[\alpha]_D^{21}$ -42.1 (*c* 1.04, $CHCl_3$); ν_{max}/cm^{-1} 2955 and 2870 (CH), 1744 and 1713 (C=O), 1646 (C=C) ; δ_H (400 MHz, $CDCl_3$) 0.76 and 0.88 (each 3H, each d, *J* 7.0, 2 × 7-CH_3), 0.90 (3H, d, *J* 6.2, 5-CH_3), 0.95-2.06 (9H, m, 2-H, 3-H_2, 4-H_2, 5-H, 6-H_2 and 7-H), 1.46 (9H, s, $C(CH_3)_3$), 2.54 (3H, s, $NCOCH_3$), 4.75 (1H, td, *J* 10.8 and 4.6, 1-H), 5.63 (1H, s, 10-*H*H), 6.57 (1H, s, 10-H*H*); δc(100 MHz, $CDCl_3$) 16.3 and 20.7 (2 × 7-CH_3), 21.9 (5-CH_3), 23.4 (CH_2), 25.9 (CH) 26.2 ($NCOCH_3$), 27.8 ($CO_2C(CH_3)_3$), 31.3 (CH), 34.1 (CH_2), 40.7 (CH_2), 47.1 (CH), 75.7 (C-1), 83.5 ($CO_2C(CH_3)_3$), 125.6 (C-10), 136.1 (C-9), 151.7 ($CO_2C(CH_3)_3$), 162.5 (C-8), 172.5 ($NCOCH_3$). Found (ESI) 390.2257 [MNa^+] ($C_{20}H_{33}NO_5Na$ requires 390.2251).

The above reaction was repeated using [^{13}C]-acrylate **S5** giving [^{13}C]–**S6**; δ_H (400 MHz, $CDCl_3$) 0.77 and 0.90 (each 3H, each d, *J* 7.0, 2 × 7-CH_3), 0.91 (3H, d, *J* 6.4, 5-CH_3), 0.95-2.07 (9H, m, 2-H, 3-H_2, 4-H_2, 5-H, 6-H_2 and 7-H), 1.47 (9H, s, $C(CH_3)_3$), 2.56 (3H, s, $NCOCH_3$), 4.76 (1H, td, *J* 10.8 and 4.2, 1-H), 5.63 (1H, d, *J* 5.0, 10-*H*H), 6.57 (1H, m, 10-H*H*); δc (100 MHz, $CDCl_3$) 136.1 (C-9, enhanced).

(1*R*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexyl 3'-(acetylthio)-2'-(*N*-(*tert*-butoxycarbonyl)-acetamido)propanoate **S7**

Ester **S6** (0.11 g, 0.31 mmol) was dissolved in dry toluene (5 mL) and then thioacetic acid (0.2 mL, 2.84 mmol) was added. The solution was refluxed for 16 h. The yellow solution was concentrated *in vacuo* and the crude oil was purified by flash chromatography (SiO_2, 10% ethyl acetate in petroleum ether 40-60 °C) giving ester **S7** as a 1:1 mixture of diastereomers as a colourless oil (0.12 g, 88%). For the mixture: ν_{max}/cm^{-1} 2955 and 2871 (CH), 1736 and 1697 (C=O); δ_H (400 MHz, $CDCl_3$) 0.76 and 0.87 (each 3H, each m, 2 × 7-CH_3), 0.90 (3H, m, 5-CH_3), 0.95-2.06 (9H, m, 2-H, 3-H_2, 4-H_2, 5-H, 6-H_2 and 7-H), 1.52 and 1.53 (9H, 2 × s, $CO_2C(CH_3)_3$), 2.32 (3H, s, $SCOCH_3$), 2.48 and 2.50 (3H, 2 × s, $NCOCH_3$), 3.42-3.67 (2H, m, 10-H_2), 4.67 and 4.70 (1H, 2 × td, *J* 10.8 and 4.4, 1-H), 5.28 (1H, m, 9-H); δc(100 MHz, $CDCl_3$) 16.1, 16.5, 20.7 and 20.8 (2 × 7-CH_3), 21.9 (5-CH_3), 23.2 and 23.5 (CH_2), 25.9 and 26.3 (CH), 26.5 ($NCOCH_3$), 27.9 ($CO_2C(CH_3)_3$), 28.8 and 28.9 (C-10), 30.4 ($SCOCH_3$), 31.3 (CH), 34.1 (CH_2), 40.5 and 40.6 (CH_2), 46.8 and 46.9 (CH), 55.8 and 56.0 (C-9), 75.8 and 76.1 (C-1), 84.3 and 84.4 ($CO_2C(CH_3)_3$), 152.0 ($CO_2C(CH_3)_3$), 168.9 (C-8), 172.7 ($NCOCH_3$), 194.8 and 194.9 ($SCOCH_3$). Found (ESI) 466.2249 [MNa^+] ($C_{22}H_{37}NO_6SNa$ requires 466.2239).

The above reactions was repeated with [^{13}C]–unsaturated ester **S6** giving [^{13}C]-thiol ester **S7** as a 1:1 mixture of diastereomers. Data for the mixture; δ_H (400 MHz, $CDCl_3$) 0.75 and 0.87 (each 3H, each m, 2 × 7-CH_3), 0.90 (3H, m, 5-CH_3), 0.95-2.06 (9H, m, 2-H, 3-H_2, 4-H_2, 5-H, 6-H_2 and 7-H), 1.51 and 1.52 (9H, 2 × s, $CO_2C(CH_3)_3$), 2.31 (3H, s, $SCOCH_3$), 2.48 and 2.51 (3H, 2 × s, $NCOCH_3$), 3.38-3.67 (2H, m, CH_2S), 4.62-4.73 (1H, m, 1-H), 5.07-5.45 (1H, m, 9-H); δc (100 MHz, $CDCl_3$) 55.8 and 56.0 (C-9, enhanced).

DL-Cystine S8

Ester **S7** (0.22 g, 0.50 mmol) was suspended in 6N $HCl_{(aq)}$ (5 mL) and refluxed for 16 h. The mixture was extracted with EtOAc (5 mL) and then the organic layer was extracted with water (5

mL). The aqueous layers was combined and concentrated *in vacuo* to give crude oil which was purified with ion-exchange chromatography (3% NH_3 in water) giving cystine **S8** as a white solid (0.04 g, 70%) as a mixture of diastereomers. m.p. 256-258 °C, lit.[3] m.p. 258-260 °C (dec.); δ_H (400 MHz, D_2O) 3.22 (2H, m, 3-H_2), 4.02 (1H, m, 2-H); δc (100 MHz, D_2O) 43.4 and 43.6 (C-3), 54.8 and 54.9 (C-2), 180.7 (C-1). Spectroscopic data is accord with literature.[3]
The above reaction was repeated using [^{13}C]-**S7** giving [^{13}C]-cystine **S8** as a mixture of diastereomers. δ_H (400 MHz, D_2O) 2.27-3.05 (2H, m, 3-H_2), 3.32-3.71 (1H, dm, *J*140.7, 2-H); δc (100 MHz, D_2O) 54.6 and 54.7 (C-2, enhanced); Found (MALDI) 265.00 [MNa^+] ($C_4{}^{13}C_2H_{12}N_2O_4S_2Na$ requires 265.03) (Figures 15 and 16).

3. J. L. G. Ruano, A. Parra and J. Alemán, *Green Chemistry*, 2008, **10**, 706-711.

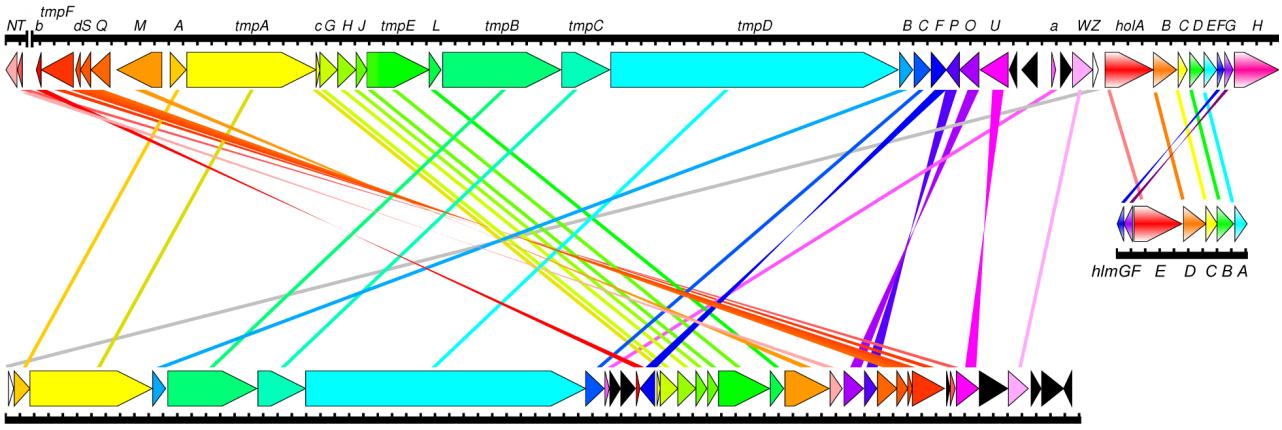


Figure S1. Thiomarinol, mupirocin and holomycin biosynthetic gene clusters.

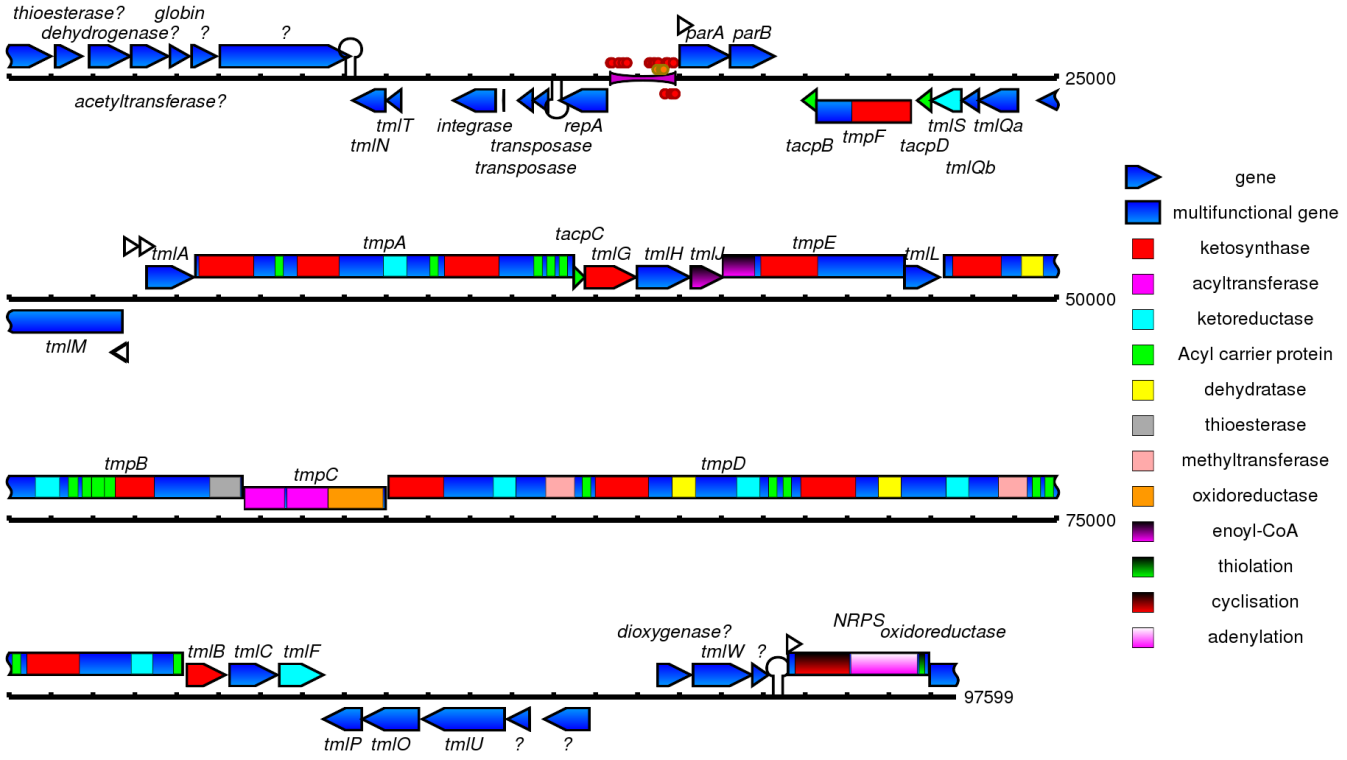
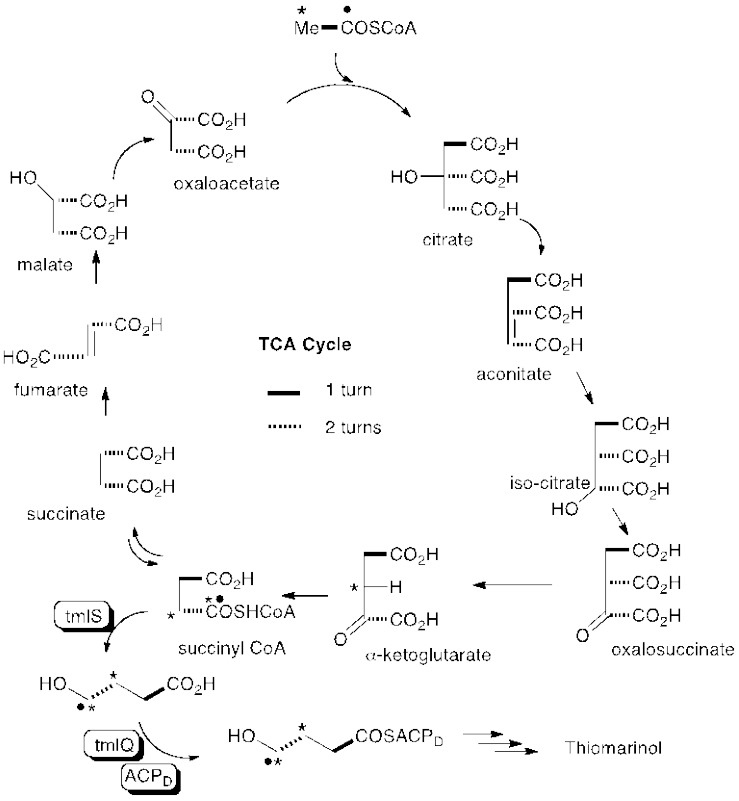


Figure S2. PKS/NRPS domain structure and gene distribution in the thiomarinol gene cluster



Scheme S3. Predicted acetate incorporation into succinyl CoA and 4-hydroxybutyrate

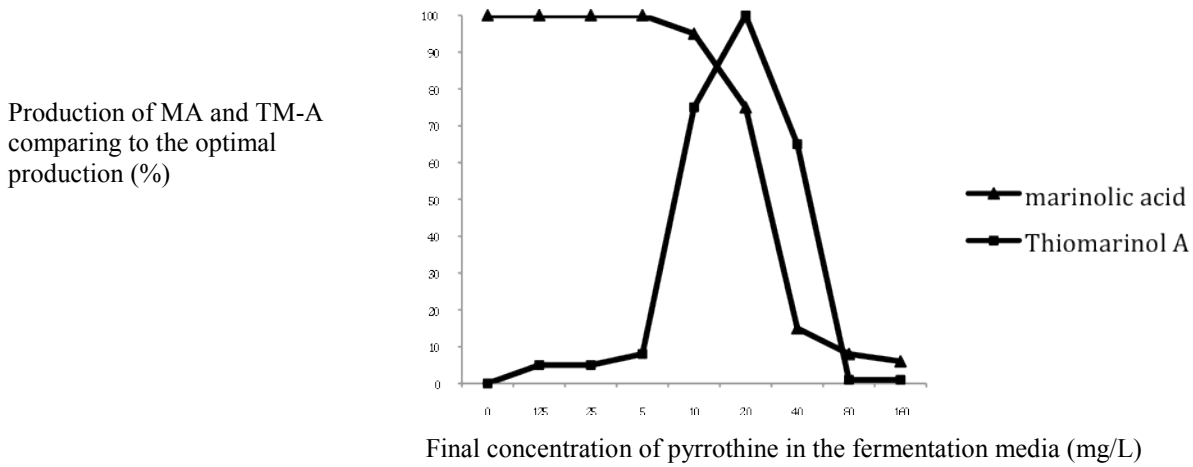


Figure S3. Restoration of thiomarinol production on addition of pyrrothine to the ΔNRPS mutant.

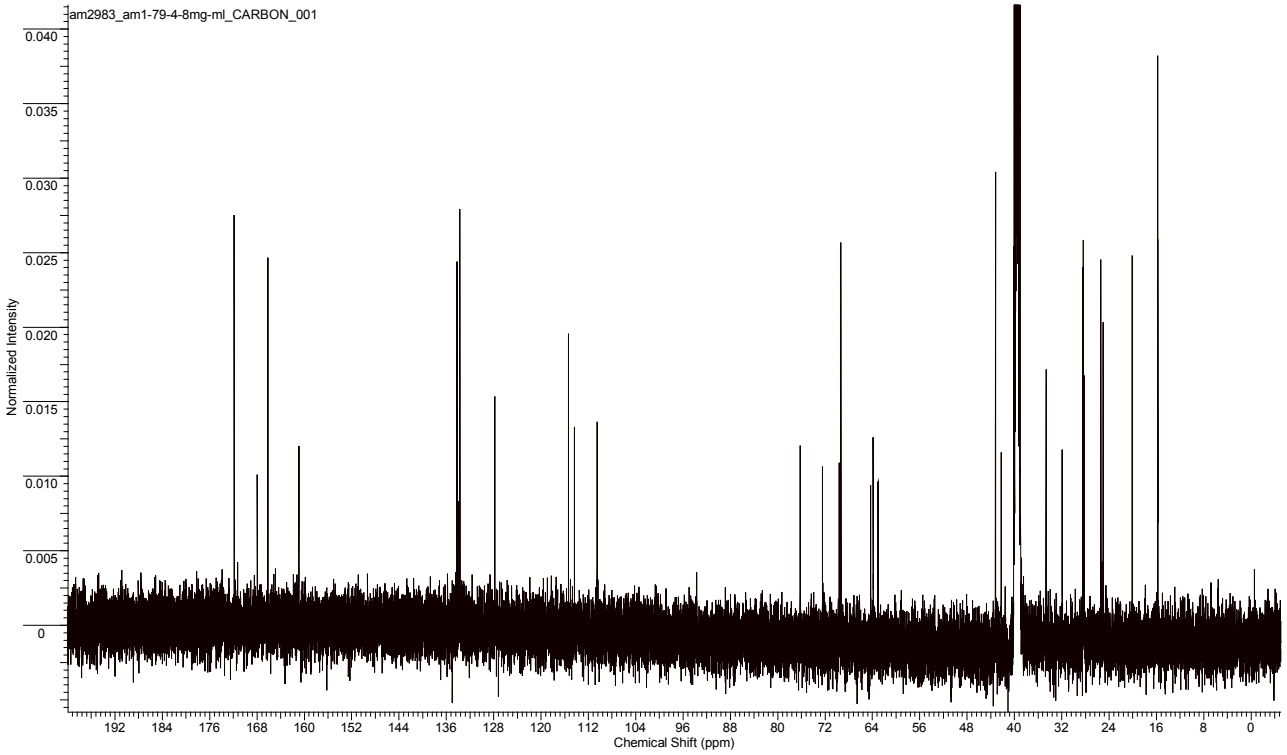


Figure S4. Thiomarinol A (DMSO, 125 MHz)

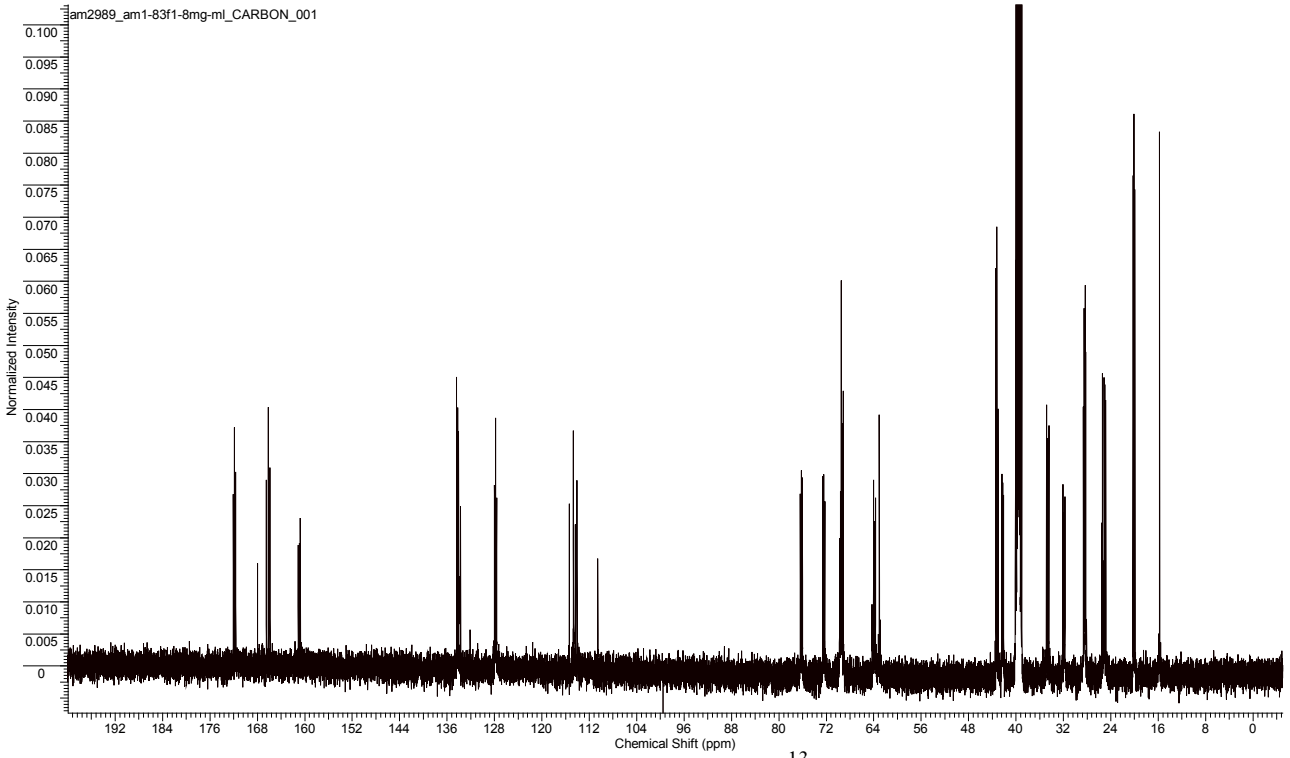


Figure S5. Thiomarinol A isolated from sodium [1,2-$^{13}C_2$]-acetate feeding experiment (DMSO, 125 MHz)

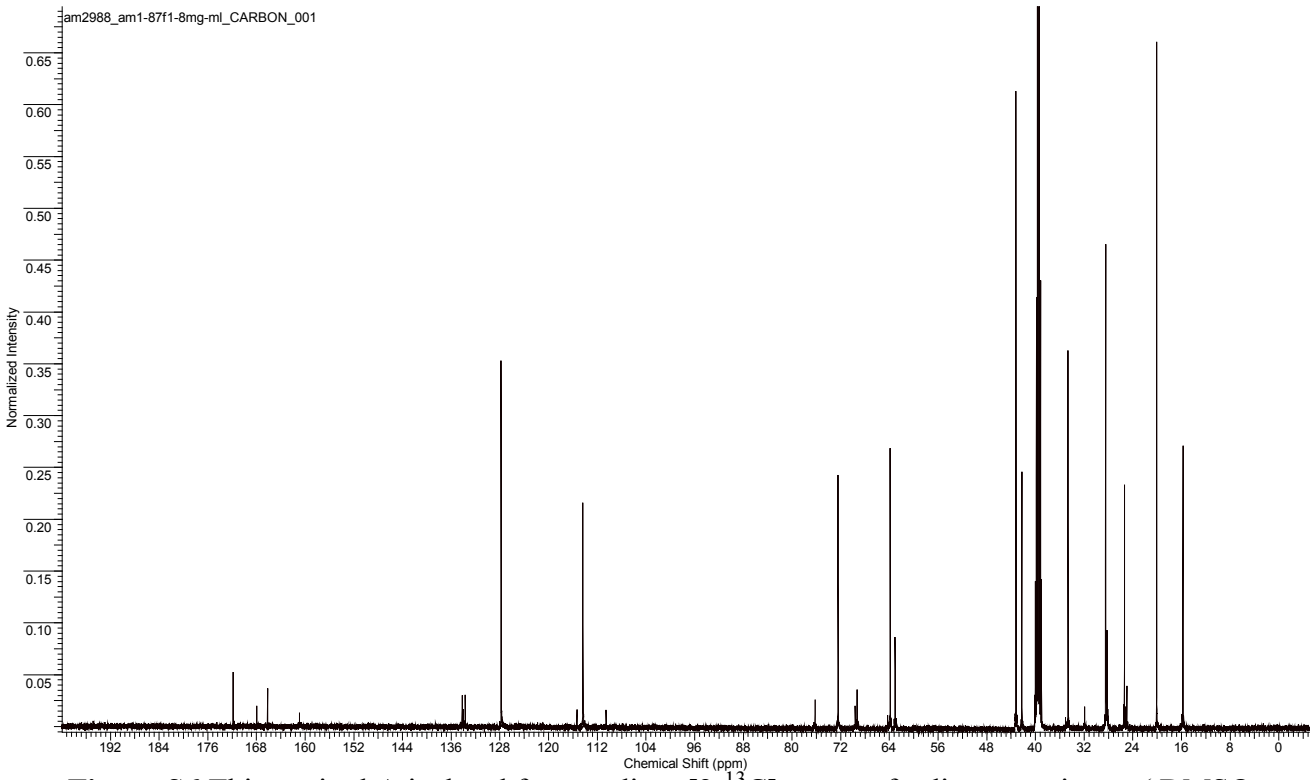


Figure S6 Thiomarinol A isolated from sodium [2-^{13}C]-acetate feeding experiment (DMSO, 125 MHz)

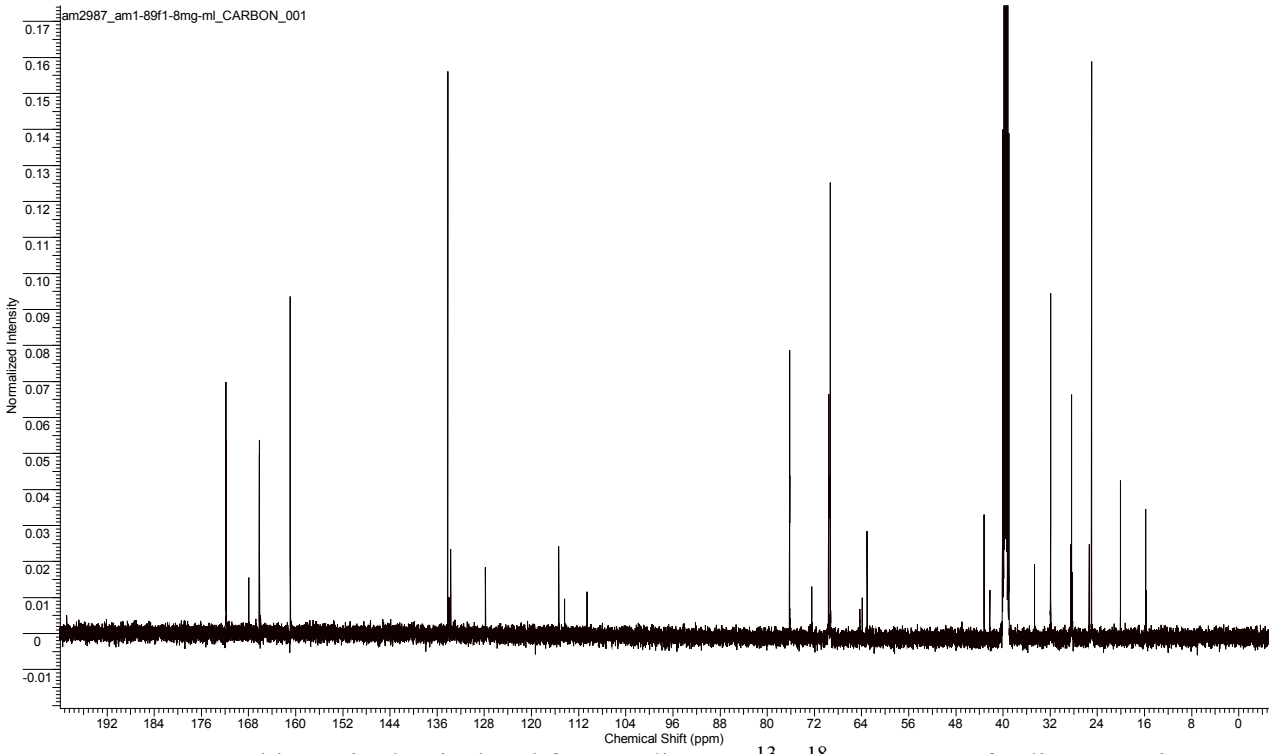


Figure S7 Thiomarinol A isolated from sodium [1-^{13}C,$^{18}O_2$]-acetate feeding experiment (DMSO, 125 MHz)

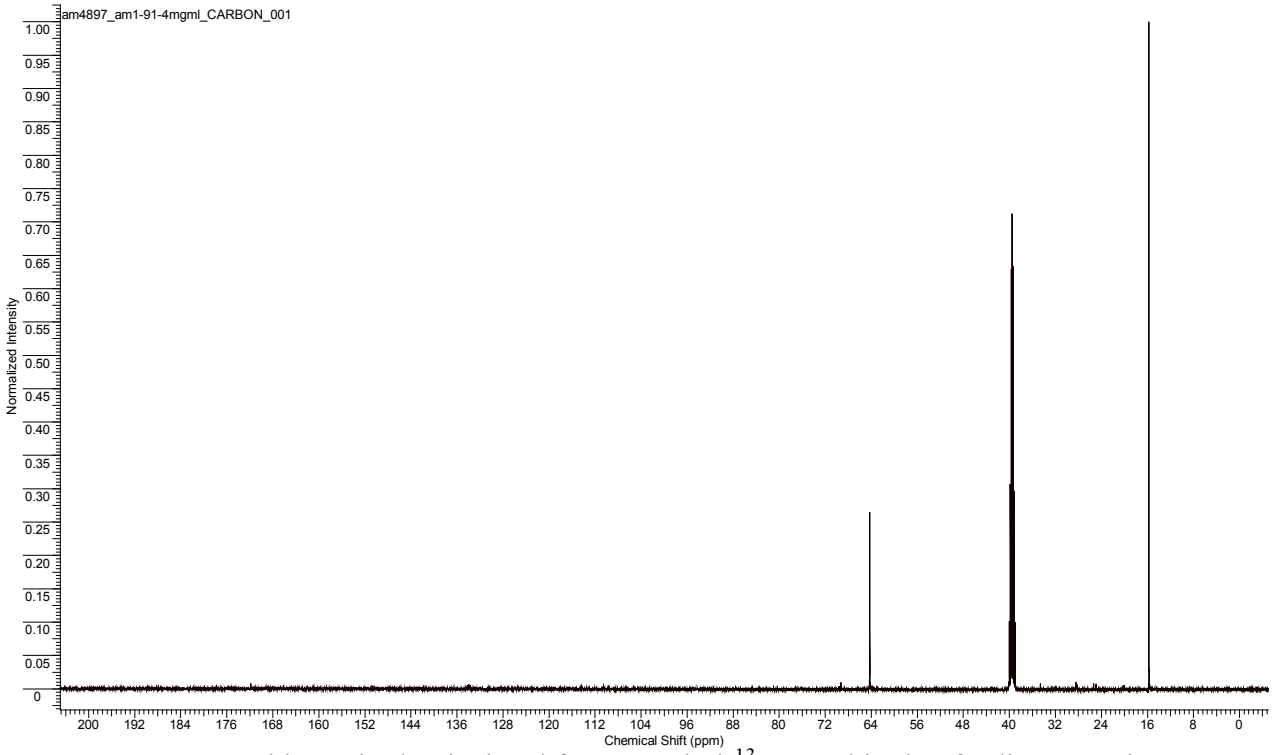


Figure S8. Thiomarinol A isolated from [methyl-^{13}C]-methionine feeding experiment (DMSO, 125 MHz)

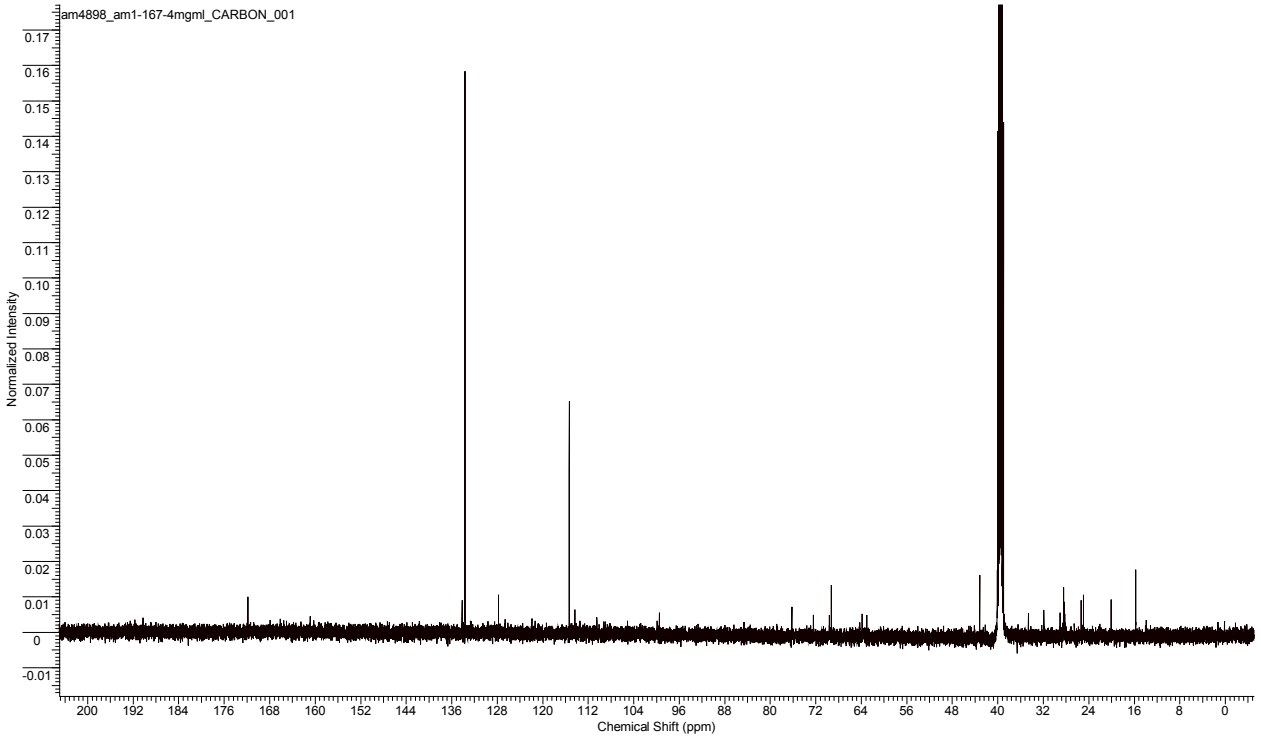


Figure S9. Thiomarinol A isolated from [2-^{13}C]-cystine feeding experiment (4 mg/mL DMSO)

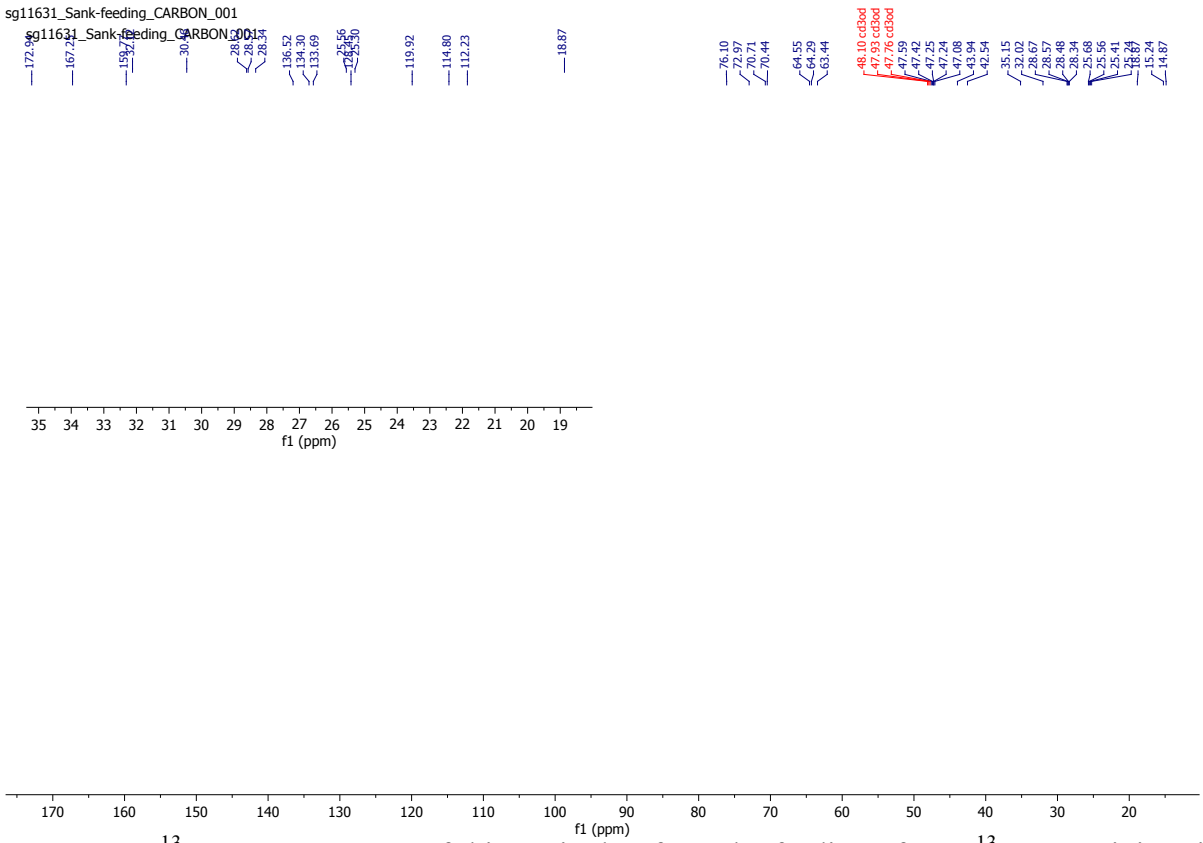


Figure S10. ^{13}C NMR spectrum of thiomarinol A from the feeding of [2,3-$^{13}C_2$]-succinic acid to *Pseudoalteromonas* SANK 73390 (CD_3OD, 125MHz).

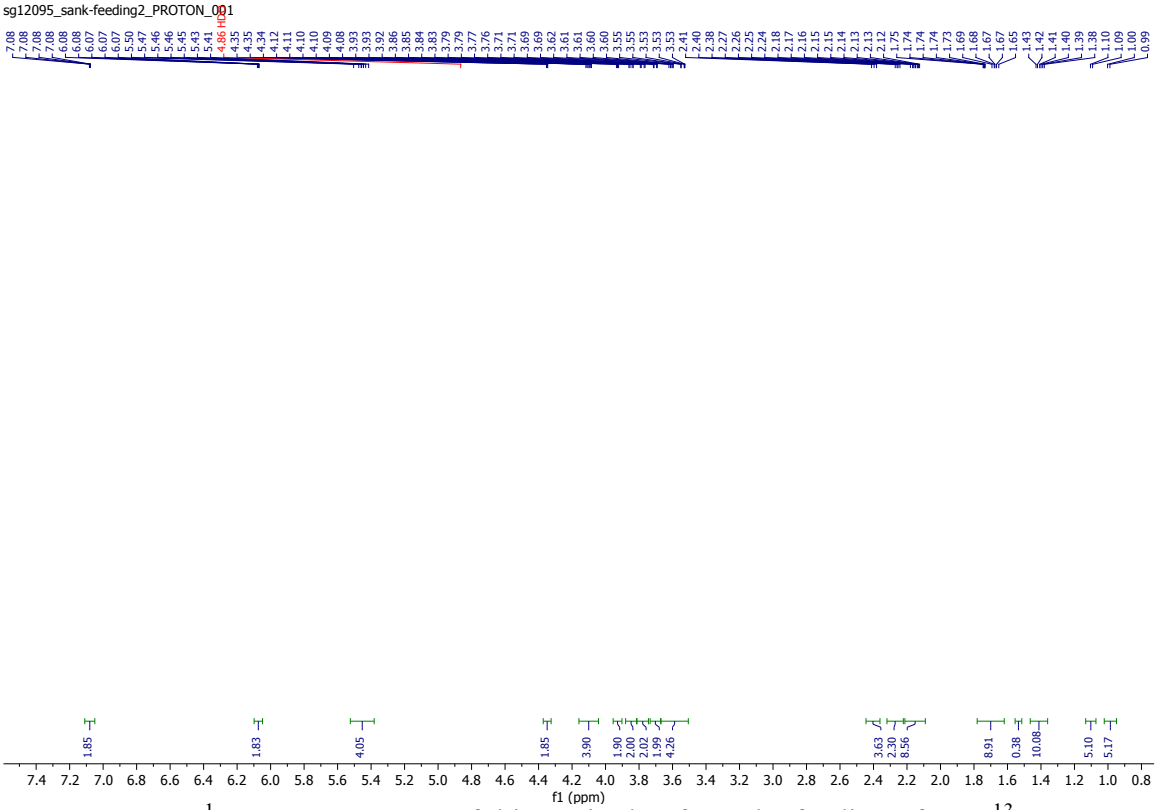


Figure S11. ^{1}H NMR spectrum of thiomarinol A from the feeding of [2,3-$^{13}C_2$]-4-hydroxybutanoic acid to *Pseudoalteromonas* SANK 73390 (CD_3OD, 600MHz)

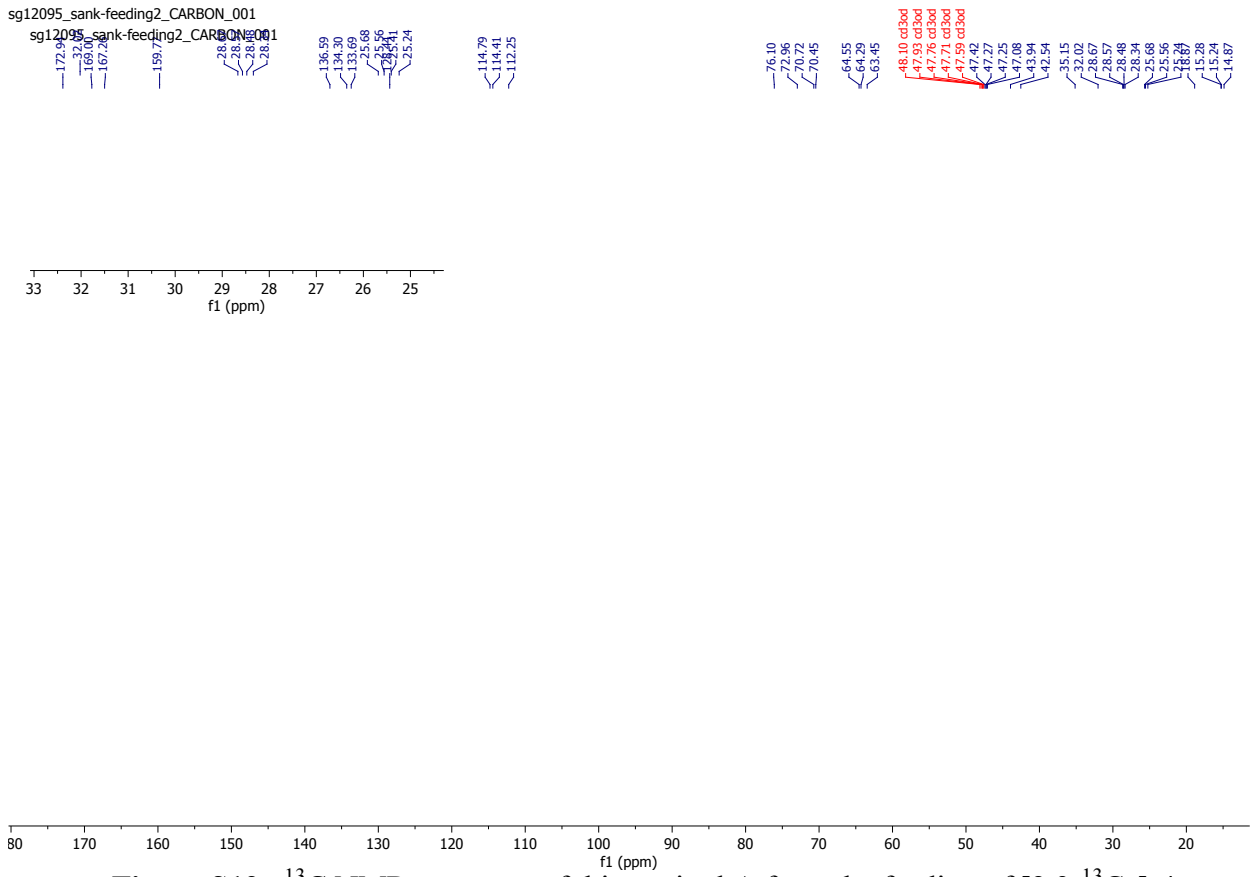


Figure S12. ^{13}C NMR spectrum of thiomarinol A from the feeding of [2,3-$^{13}C_2$]-4-hydroxybutanoic acid to *Psudoalteromonas* SANK 73390 (CD_3OD, 125MHz)

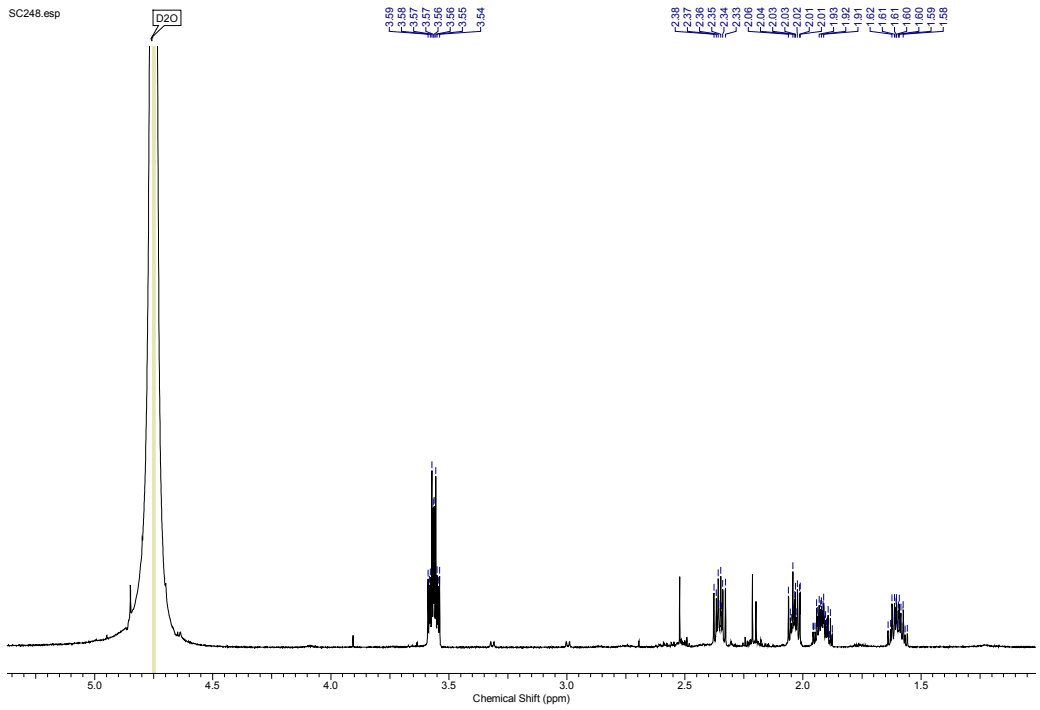


Figure S13. ^{1}H NMR spectrum (400MHz, D_2O) sodium [2,3-^{13}C]-4-hydroxybutyrate (**S3**)

.

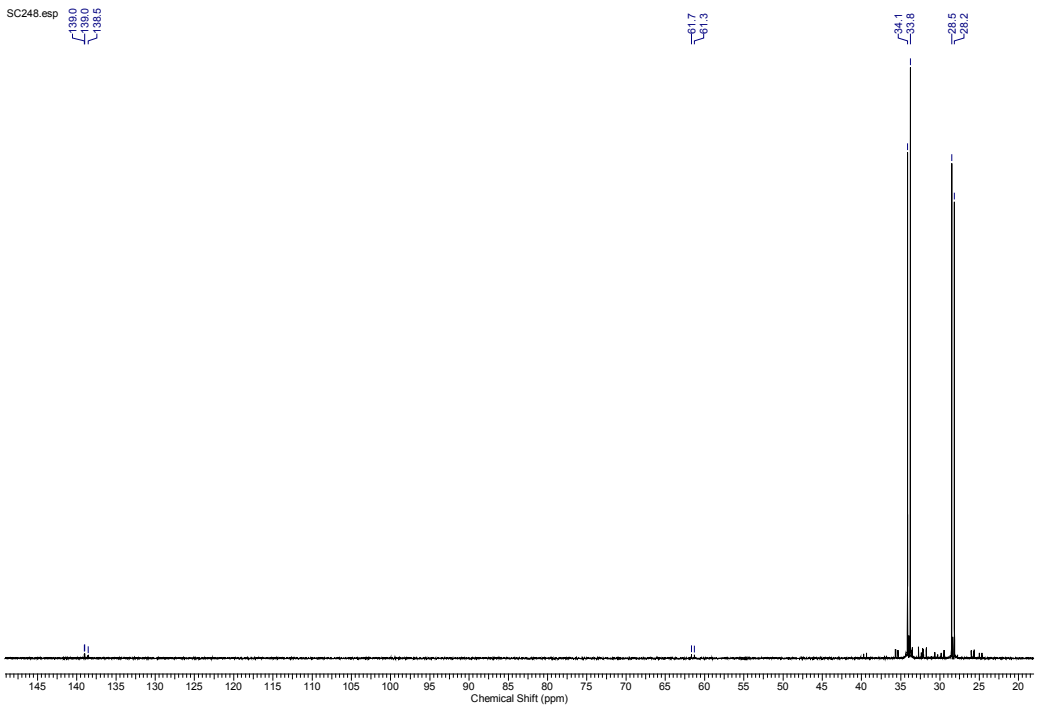


Figure S14. ^{13}C NMR spectrum (100MHz, D_2O) sodium [2,3-^{13}C]-4-hydroxybutyrate (**S3**)

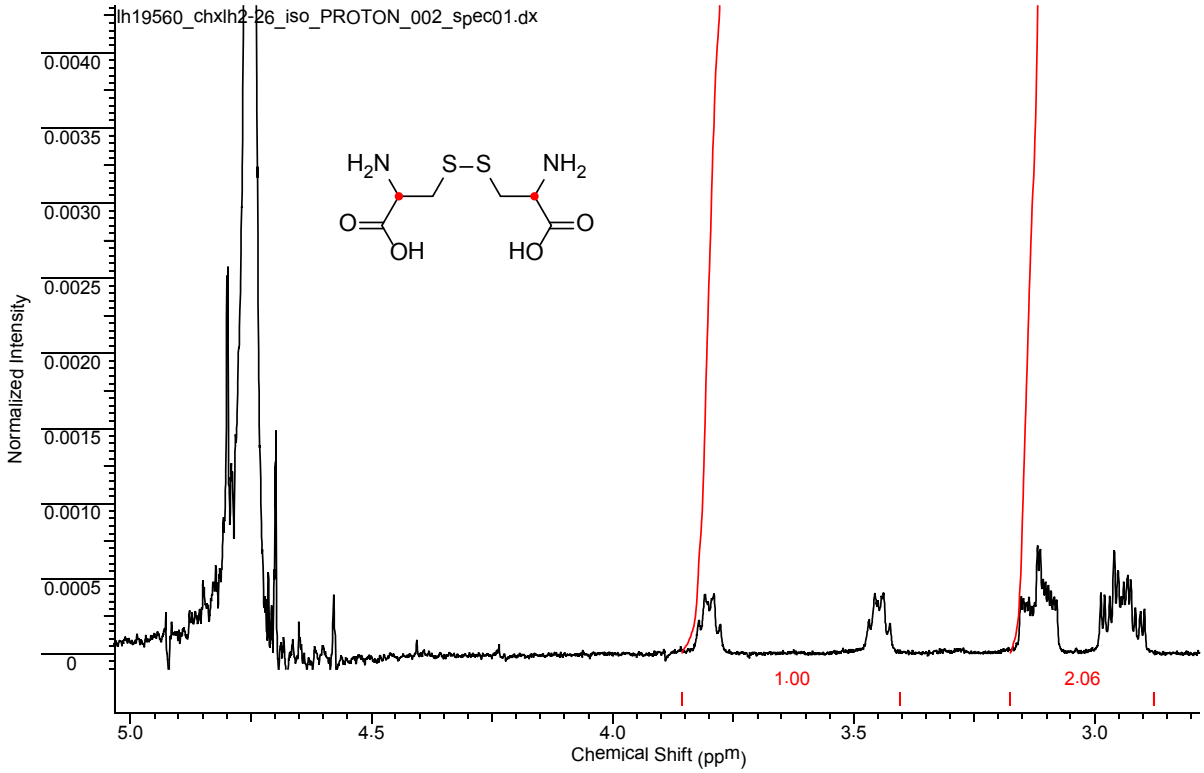


Figure S15. ^{1}H NMR spectrum (400MHz, D_2O) [2-^{13}C]-cystine (**S8**).

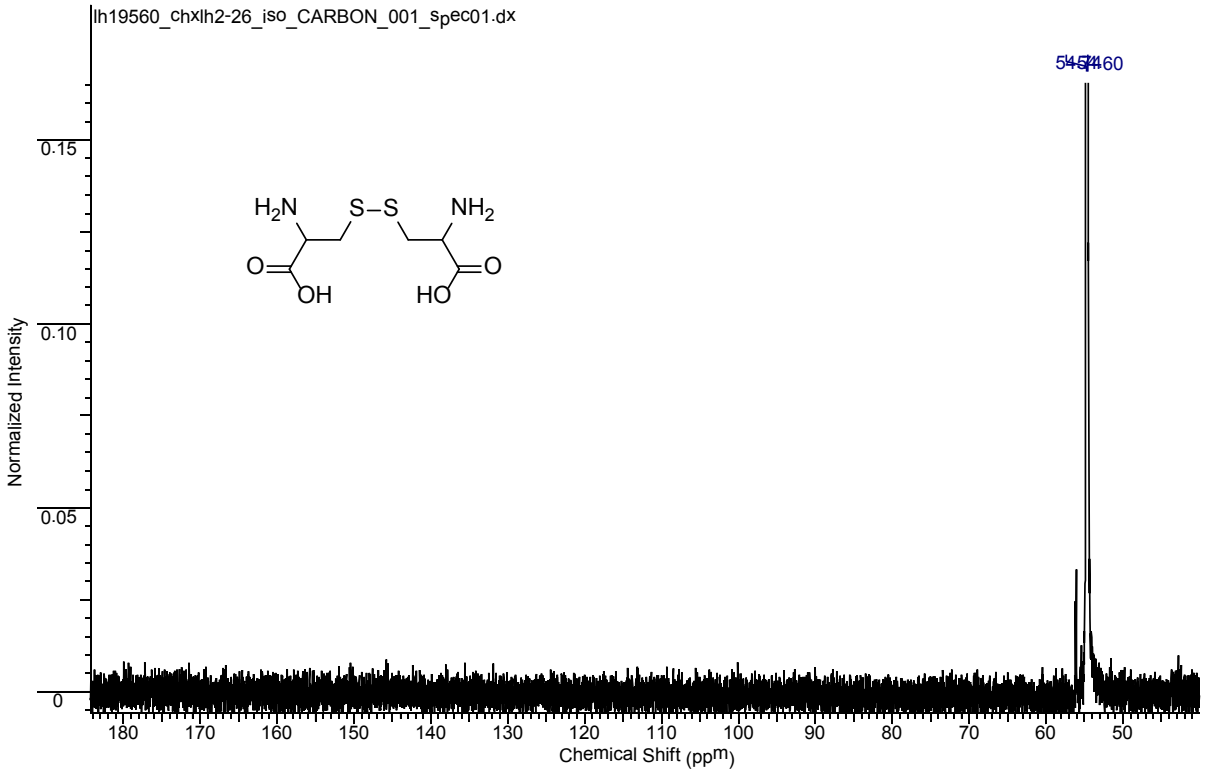


Figure S16. ^{13}C NMR spectrum (100MHz, D_2O) [2-^{13}C]-cystine (**S8**).

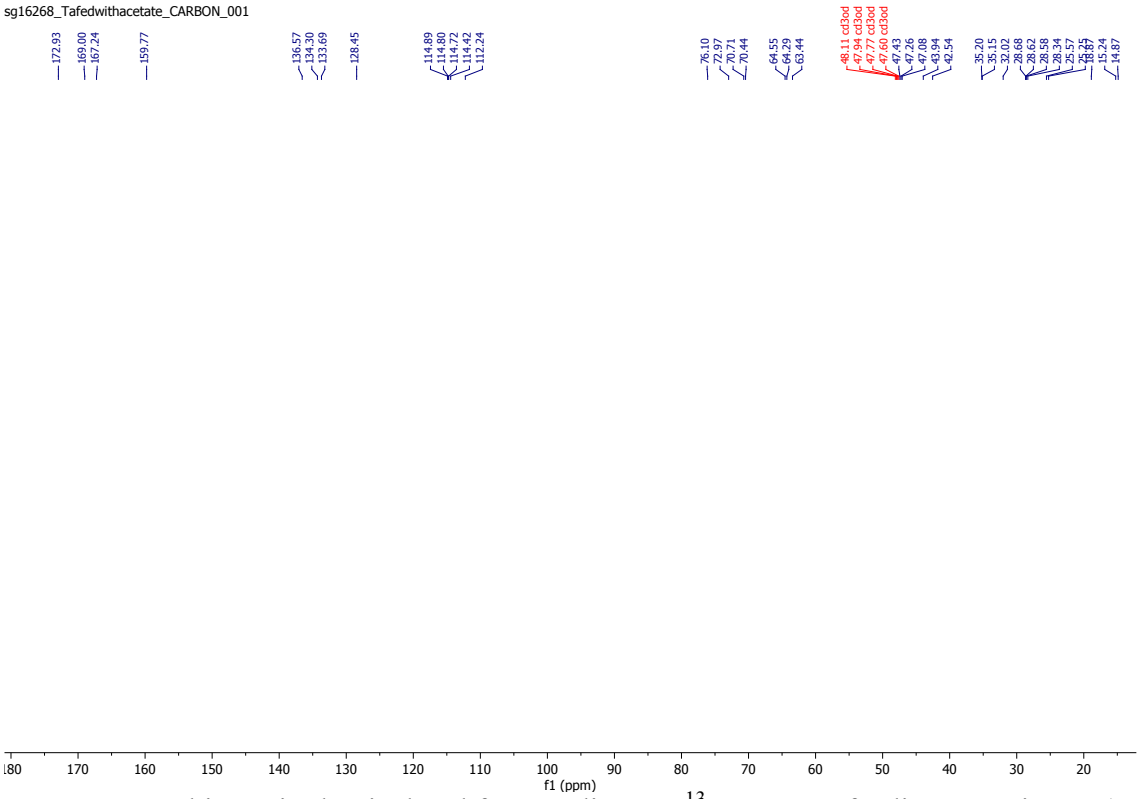


Figure S17. Thiomarinol A isolated from sodium [2-^{13}C]-acetate feeding experiment (CD_3OD, 125 MHz)

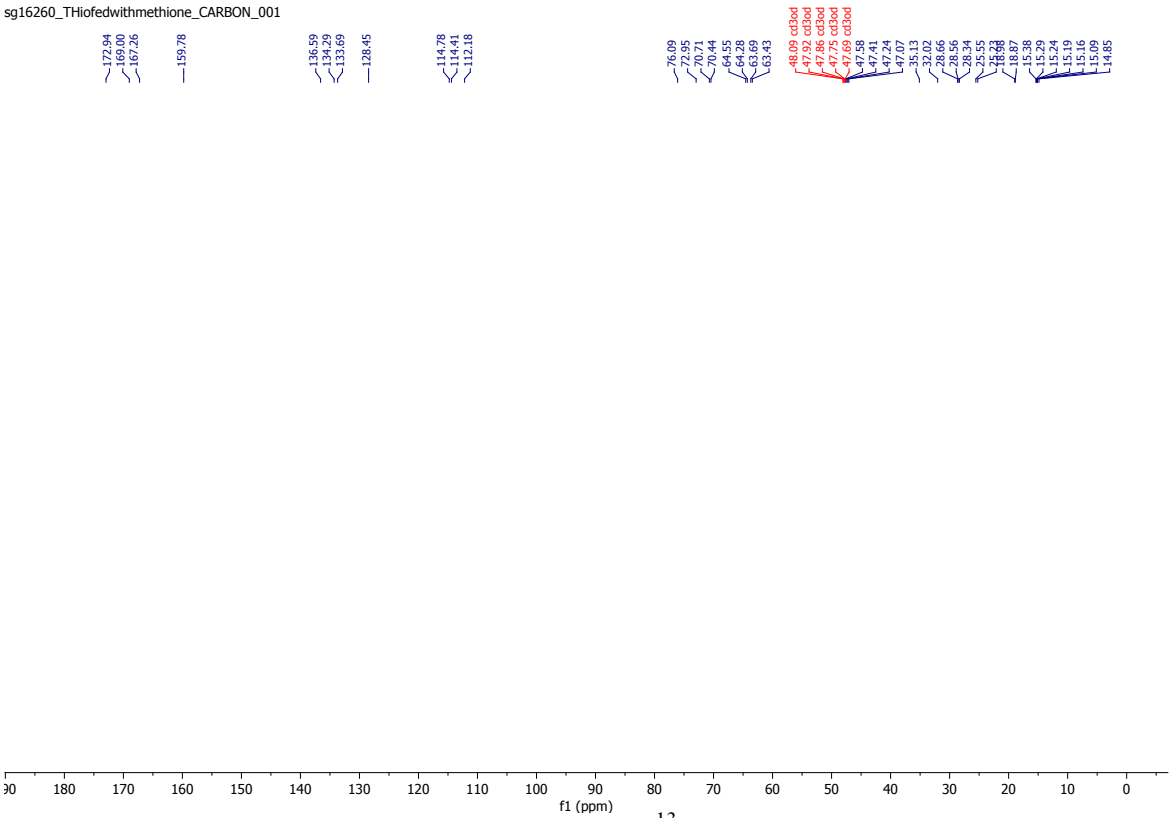


Figure S18. Thiomarinol A isolated from [methyl-^{13}C]-methionine feeding experiment (CD_3OD, 125 MHz)

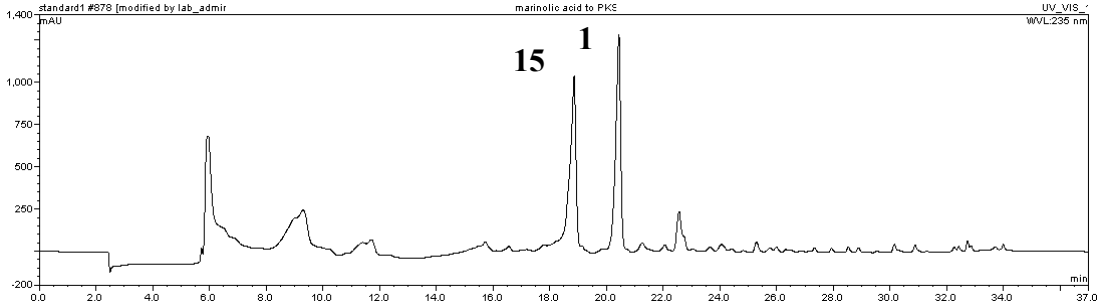


Figure S19. HPLC diode array trace of extract from feeding of marinolic acid to *ΔPKS*. **1**: Thiomarinol A; **15**: marinolic acid A.