

SUPPORTING INFORMATION

Biosynthesis of the (2*S*, 3*R*)-3-methyl glutamate residue of nonribosomal lipopeptides

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1. Results from feeding (2*S*, 3*S*)-MeGlu **7** to *S. coelicolor* $\Delta glmT$.
2. Results from feeding racemic 3-MeGlu diastereoisomers *rac*-**7/14** to *S. coelicolor* $\Delta glmT$.
3. Results from feeding 3-methyl-2-oxoglutarate **20** to *S. coelicolor* $\Delta glmT$.
4. Degradation and analysis of 3-MeGlu from daptomycin.
5. Deletion of *glmT* in *S. coelicolor* MT1110.
6. PCR analysis of *S. coelicolor* $\Delta glmT$.
7. Analysis of dabsylated amino acids from the CDA4a hydrosylate.
8. General experimental methods.
9. Synthesis of 3-Methyl glutamic acid (*rac*-**7/14**).

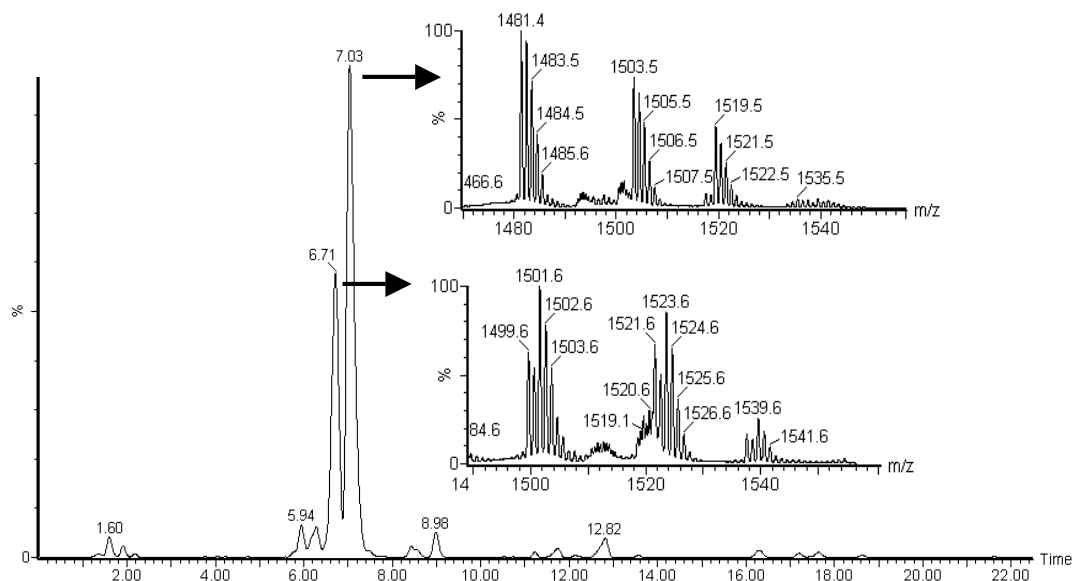


Figure 1: LC-MS chromatogram obtained after the exogenous supply of $300\mu\text{g mL}^{-1}$ (2*S*, 3*S*)-3-MeGlu to the *ΔglmT* mutant. Mass spectra of the peak at 7.03 minutes gave an m/z value of 1481.4, which corresponds to the $[\text{M}+\text{H}]^+$ ion of CDA3a. Mass spectra of the peak at 6.71 minutes gave an m/z value of 1499.6, which corresponds to the $[\text{M}+\text{H}]^+$ ion of a possible hydrolysed-linear derivative of CDA3a. Overlapping with this another product is evident which has m/z value of 1501.6 which corresponds to the $[\text{M}+\text{H}]^+$ ion of a possible hydrolysed-linear derivative of CDA3b. No MeGlu containing CDA production is evident.

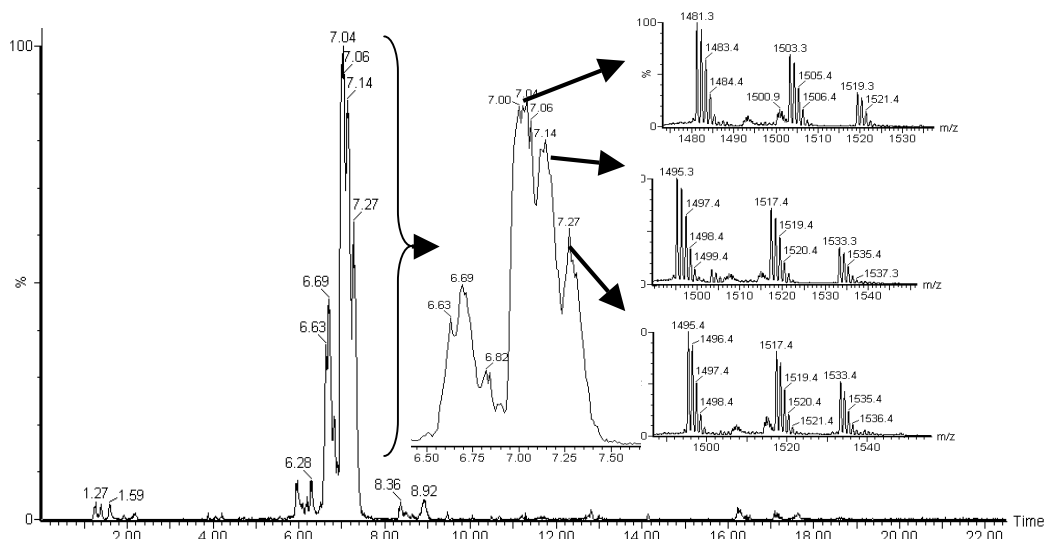


Figure 2: LC-MS chromatogram obtained after the exogenous supply of 1.2 mg mL^{-1} MeGlu *rac*-7/14 to the *ΔglmT* mutant. Mass spectra of the peak at 7.05 min gave an m/z value of 1481.3, which corresponds to the $[\text{M}+\text{H}]^+$ ion of CDA3a. Mass spectra of the peaks at 7.14 and 7.27 minutes gave m/z values of 1495.3 and 1495.4 respectively, which corresponds to the $[\text{M}+\text{H}]^+$ ion of CDA4a. Feeding and analysis were repeated on several occasions. In all cases the resulting chromatogram showed three separate peaks, two with identical mass which are tentatively assigned CDA4a and *epi*CDA4a containing a D-configured 3-MeGlu stereoisomer.

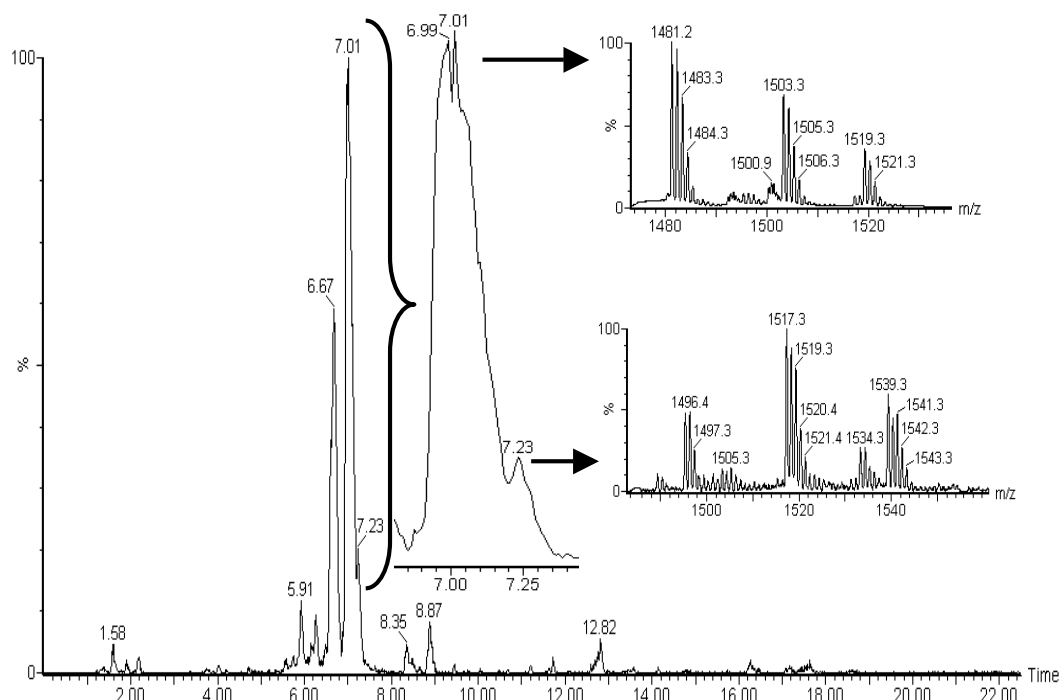


Figure 3: LC-MS chromatogram obtained after the exogenous supply of $460 \mu\text{gml}^{-1}$ 3-Methyl-2-oxoglutarate to the *ΔglmT* mutant. Mass spectra of the peak at 7.01 minutes gave an m/z value of 1481.2 which corresponds to the $[M+H]^+$ ion of CDA3a. Mass spectra of the peak at 7.23 minutes gave m/z values of 1495.4, which correspond to the $[M+H]^+$ ion of CDA4a. The low levels of MeGlu containing CDA4a produced from feeding relatively high concentrations of 3-Methyl-2-oxoglutarate may be due to alternative metabolism or poor cellular uptake.

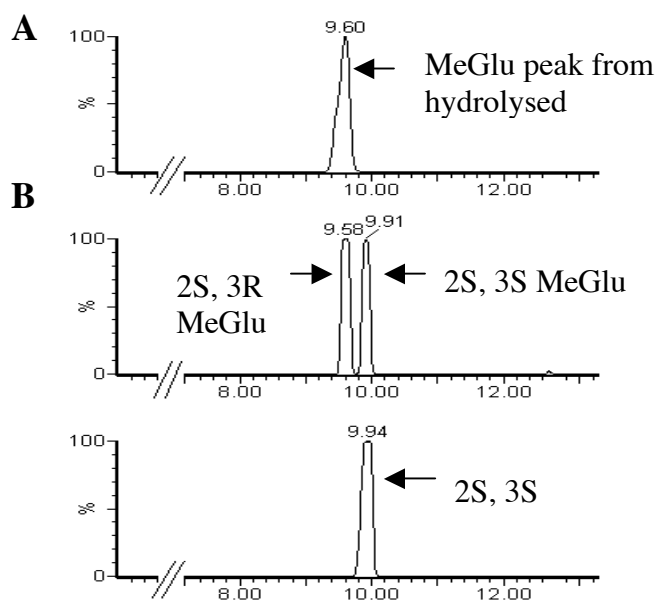


Figure 4: (A) LC-MS extracted mass chromatogram of dabsylated 3-MeGlu derived from hydrolysis of daptomycin which has a retention time 9.60 min. (B) The LC-MS chromatogram for synthetic (2*S*, 3*R*) and (2*S*, 3*S*)-3-MeGlu synthetic standards.

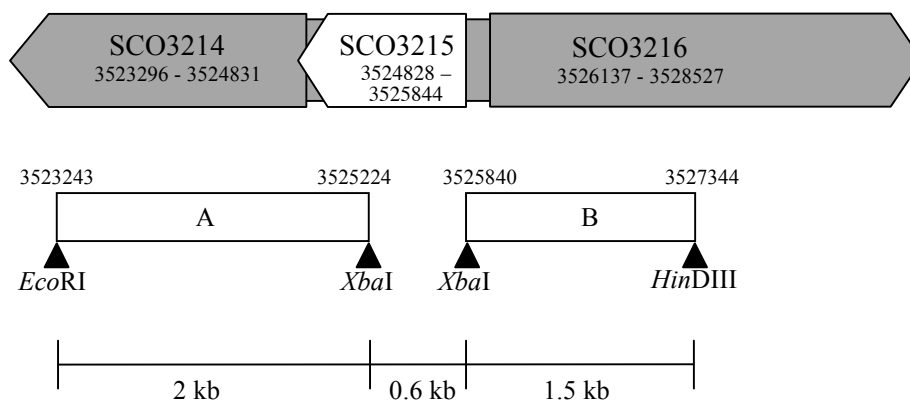


Figure 5. Deletion of SCO3215 (*glmT*). The chromosomal region to be deleted is 615 bp in size. Amplicon A, the upstream homologous region is *ca.* 2 kb in size and the B region is *ca.* 1.5 kb. Numbering according to *Streptomyces coelicolor* A3(2) genome database [<http://streptomyces.org.uk/>].

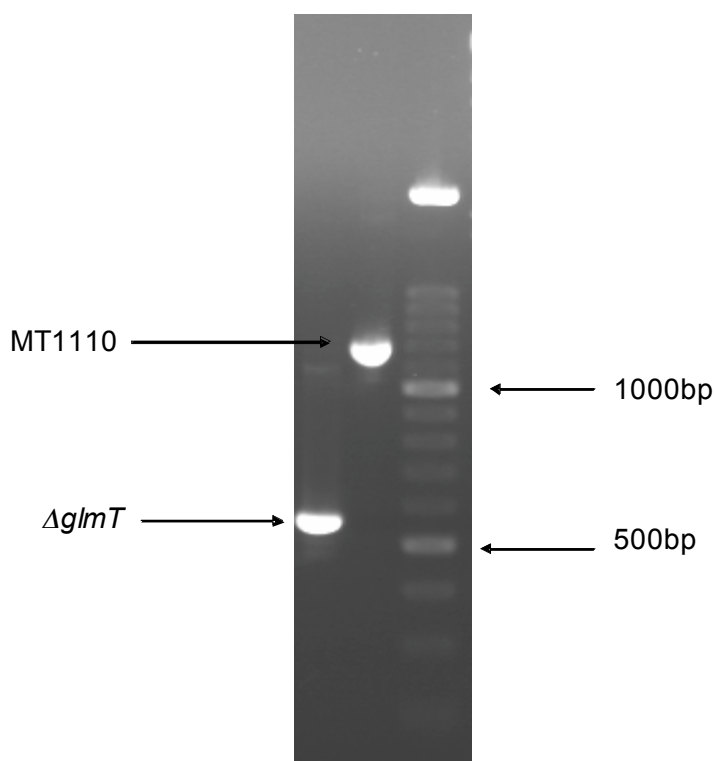


Figure 6. PCR of SCO3215 in MT1110 and the truncated SCO3215 gene in *ΔglmT*. The template DNA used was from a total DNA extraction. The forward primer (5'- TTCTGTGAGCCCGGTGCGCC) anneals between 3524755 to 3524775 and the reverse primer (5'- CATCGTCTTGCCCGCGTCCA) anneals between 3525882 to 3525902.

Lane 1: truncated SCO3215 in *ΔglmT* (PCR product size 532bp)

Lane 2: SCO3215 in MT1110 (PCR product size 1147bp)

Lane 3: 500bp DNA ladder

Dabsylated amino acids	m/z $[M+H]^+$	Retention time / min.
Ser	393	9.40
3-MeGlu	449	9.75
Thr	407	9.75
Asp	421	10.24
Gly	363	10.29
3-HydroxyAsn	437	12.82
HPG	441	13.10 (weak)
Trp	478	13.50
Z-ΔTrp	na.	na.

7. LC-MS analysis of dabsylated amino acids from the CDA4a hydrosylate (see Figure 2 of the manuscript). The table shows retention times and observed parent ions of the individual dabsylated amino acid derivatives.

8. General experimental methods. NMR spectra were recorded on either a Bruker DPX 300 (300 MHz for ^1H and 75.5 MHz for ^{13}C) or a Bruker DPX 400 (400 MHz for ^1H and 100.6 MHz for ^{13}C) unless otherwise stated. Chemical shifts in both ^1H and ^{13}C -NMR spectra are expressed in ppm downfield to tetramethylsilane and were referenced to the residual solvent peak. Coupling constants were recorded as J values in Hz and the splitting patterns were given the following abbreviations: s-singlet, d-doublet, t-triplet, m-multiplet and br-broad. Low resolution and accurate mass measurements were obtained using a Micromass LCT orthogonal acceleration time of flight mass spectrometer, equipped with an electrospray ionisation source run in positive mode. Mass spectra were also obtained from the EPSRC National Mass Spectrometry Centre (University of Wales, Swansea) using the Micromass Quattro II and Finnigan MAT 900 XLT instruments. Infra-red spectra were recorded on a Nicolet Nexus 670 FT-IR spectrometer either as a thin file on KBr discs or as a Nujoll mull. Optical rotations were carried out at *ca.* 25°C on an Optical Activity AA-IC00 polarimeter. Melting points were performed with an electrothermal capillary apparatus. Thin layer chromatography was performed on Merck silica gel (60 F254) coated on aluminium sheets and visualised under a UV (254 nm) lamp and/or developed using a potassium permanganate stain. Flash chromatography was carried out using a modest pressure of compressed air using silica gel (size 40-63 VWR international Ltd). Chemicals were obtained from Aldrich Chemical Company, Lancaster Synthesis Ltd or Novabiochem and were used without further purification unless otherwise stated. THF was distilled under argon from sodium using benzophenone

as an indicator at atmospheric pressure whilst DCM was distilled under argon from calcium hydride at atmospheric pressure. Anhydrous DMF, 1,4-dioxane, DIPEA and toluene were all purchased from Aldrich Chemical Company.

9. Synthesis of 3-Methyl glutamic acid (*rac*-7/14). Raney-Ni (0.4 g) was added to the dimethyl ester **17** (1.50 g, 6.85 mmol) in anhydrous CH₃OH (10 mL). HCO₂H (5 mL, 96%) was then added and the mixture stirred for 48 h at room temperature under N₂. The reaction mixture was then filtered through celite and the precipitate was washed with CH₃OH and EtOAc. The solvent was evaporated under reduced pressure. H₂O (20 mL) was added to the residue followed by saturated aqueous NaHCO₃ until the resulting solution was pH 8.0. The mixture was then extracted with diethyl ether (3 x 50 mL) and EtOAc (3 x 50 mL). The combined organic extracts dried over MgSO₄, filtered, and evaporated under reduced pressure. The resulting crude amine was dissolved in anhydrous CH₂Cl₂ (10 mL) under N₂ at 0 °C, di-*tert*-butyl dicarbonate (2.35 g, 10.79 mmol), triethylamine (1 mL, 7.19 mmol) and 4-DMAP (0.88 g, 7.19 mmol) were then added. The reaction mixture was allowed to warm to room temperature and stirred for 18h. H₂O (100 mL) was then added and the mixture extracted with CH₂Cl₂ (3 x 100 mL). The combined organic extracts were dried over MgSO₄, filtered, and evaporated under reduced pressure. The residue was then dissolved in THF (10 mL) at 0 °C and treated with LiOH (0.90 g, 21.5 mmol) in H₂O (2 mL) and stirred at room temperature for 18 h. H₂O (100 mL) was then added and the solution was adjusted to pH 4.0 with CH₃CO₂H and extracted with EtOAc (3 x 100 mL), dried over MgSO₄, filtered, and evaporated under reduced pressure to provide ***rac*-6/13** (1.22 g, 68 %) as a white solid. ***rac*-6/13** (1.1g, 4.21 mmol) was then transformed to 3-methyl glutamic acid.HCl salt ***rac*-7/14** as described in the main experimental section (**6** → **7**). ¹H and ¹³C NMR were consistent with a mixture of **7** and **14** as described above and as follows ¹H (400 MHz, D₂O) δ 0.89 (3H, d, *J* = 6.5 Hz, CH₃), 0.92 (3H, d, *J* = 7.5 Hz, CH₃) 2.28-2.55 (6H, m, CH₂CHCH₃), 3.97 (1H, d, *J* = 3.8 Hz, CHNH₂), 4.00 (1H, d, *J* = 3.2 Hz, CHNH₂). ¹³C NMR (75.5 MHz, D₂O) δ 14.2 (CH₃), 14.9 (CH₃), 30.8 (CHCH₃), 30.9 (CHCH₃), 37.1 (CH₂), 37.4 (CH₂), 56.4 (CHNH₂), 56.9 (CHNH₂), 170.8 (CH₂CO₂H), 171.4 (CH₂CO₂H), 176.0 (CHCO₂H), 176.1 (CHCO₂H). LRMS-ESI (*m/z*): 162.1 ([M+H]⁺, 100 %), 323 ([2M+H]⁺, 5%); HRMS-ESI (*m/z*): [M+H]⁺ calc'd for C₆H₁₂NO₄ 162.0761. Found, 162.0761.