Electronic Supplementary information

Auxotrophic-precursor directed biosynthesis of nonribosomal lipopeptides with modified tryptophan residues.

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Experimental

Feeding experiments. Freshly prepared sterile SV2a liquid medium (250 mL) in a 1L conical flask equipped with a stainless steel spring was supplemented with filter-sterilized (Appleton Woods 0.2 μm syringe filters) aqueous solutions of L-His (50 $mg.L^{-1}$) and the appropriate L-tryptophan analogue (Trp, 7AW, 5HW, or 5FW) (37.5 $mg.L^{-1}$) [75 $mg.L^{-1}$ in the case of D/L-7AW] in deionised water. The flasks were then inoculated with *S. coelicolor* WH101 spore suspension (20 μL) and incubated at 28 ºC with shaking for 5 days.

Extraction and purification of CDAs. Cells were harvested by centrifugation at 4500 rpm for 15 min. The filtrates were acidified to pH 2 using 2N HCl and re-filtered. These filtrates were then passed through primed (1 volume MeOH and 2 volumes H_2O) C18 Bond-Elute cartridges and washed twice with deionised water. The elution was carried out using methanol (30%, 70% and 100%). The 70-100% collections containing CDA products were evaporated to dryness under reduced pressure and lyophilized.

Liquid Chromatography Mass Spectrometry (LC-MS). LC-MS Analysis was carried out on a Micromass LCT orthogonal acceleration time of flight (TOF) mass spectrometer, equipped with an electrospray ionization source run in positive mode, combined with a Waters 2790 separation module. Gradient elution was carried out using a reversed phase C-18 150 x 4.6 mm 3μm Phenomenex column. Spectra were processed using Waters' Masslynx V4.0 software.

Elution gradient profile:

Time (mins)	A (%)	B (%)	Flow (mL.min-1)
0.00	80.0	20.0	1.0
10.00	30.0	70.0	1.0
11.00	0.0	100.0	1.0
15.00	0.0	100.0	1.0
20.00	80.0	20.0	1.0
21.90	80.0	20.0	0.1

Solvents: A = 0.1% (v/v) Formic acid; B = Acetonitrile with 0.1% (v/v) formic acid

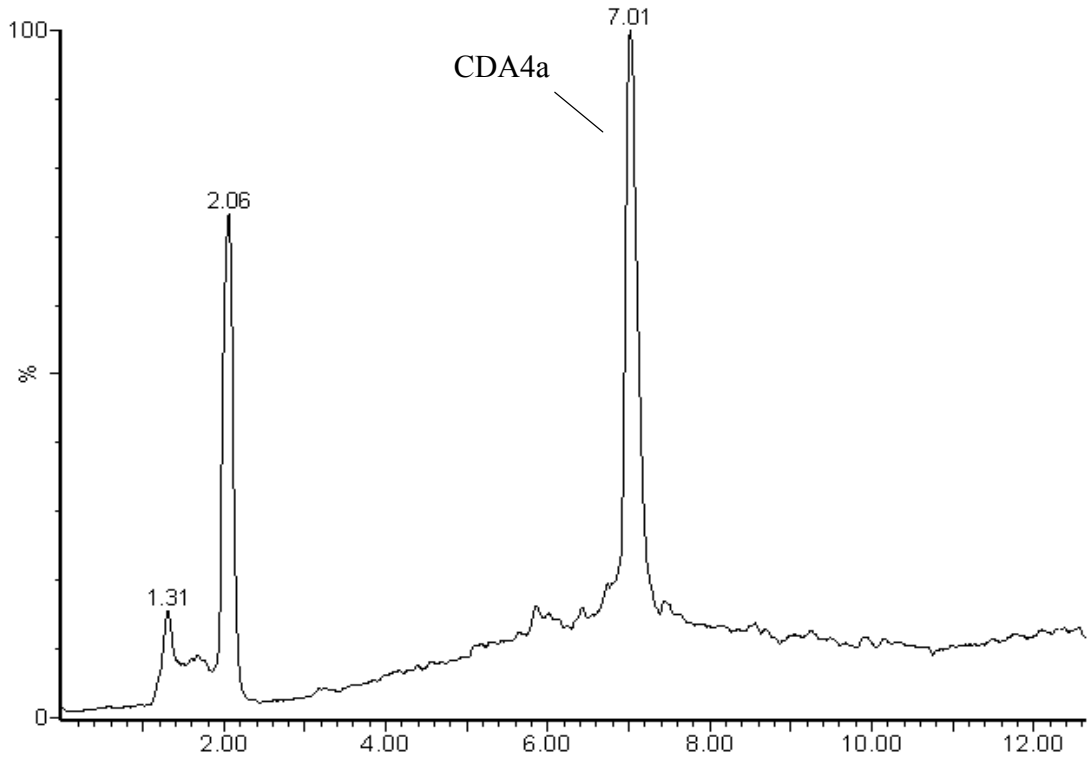


Figure S1. Total ion count (TIC) chromatogram of the WH101 strain grown in the absence of modified Trp analogs (5HW or 5FW). CDA4a is produced as the major product (R_t = 7.01 min). This control experiment was carried out under identical growth conditions as for all the feeding experiments. From analysis of the TIC chromatogram no masses corresponding to any 5HW or 5FW containing CDAs can be seen at their characteristic retention times at the threshold of detection of the ESI instrument. Mass extraction also confirms the absence of any compounds with molecular ions that match the 5HW or 5FW containing CDAs.

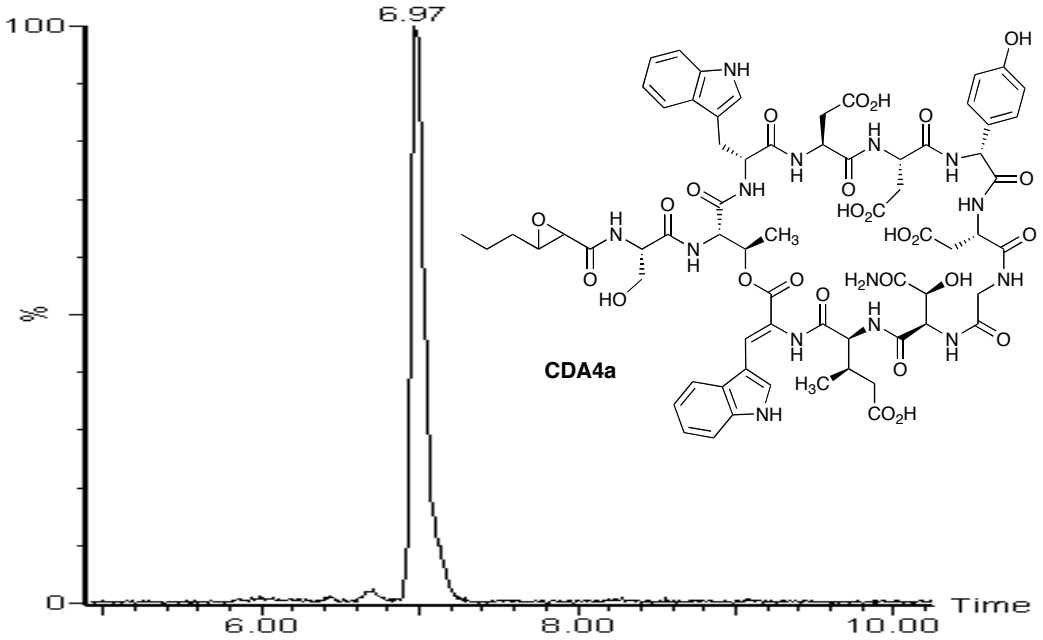


Figure S2. Mass-extraction HPLC of wild-type CDA4a produced as the major product after feeding 5HW **2** to *S. coelicolor* WH101.

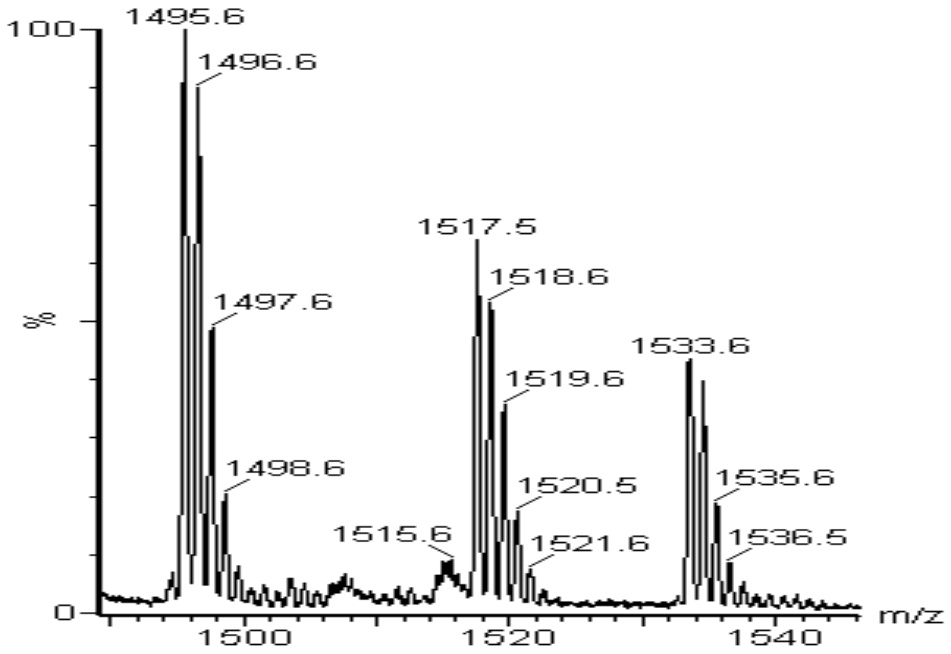


Figure S3. MS of CDA4a produced as the major product after feeding 5HW **2** to *S. coelicolor* WH101.

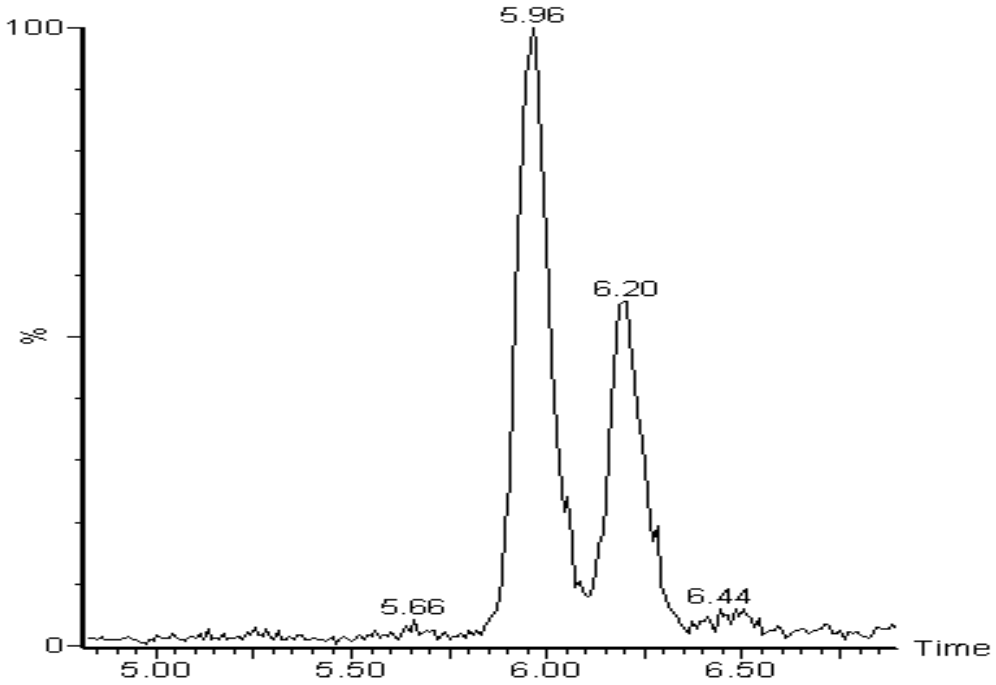


Figure S4. Mass-extraction HPLC of $(5HW)_1$CDA4a regioisomers produced after feeding 5HW **2** to *S. coelicolor* WH101.

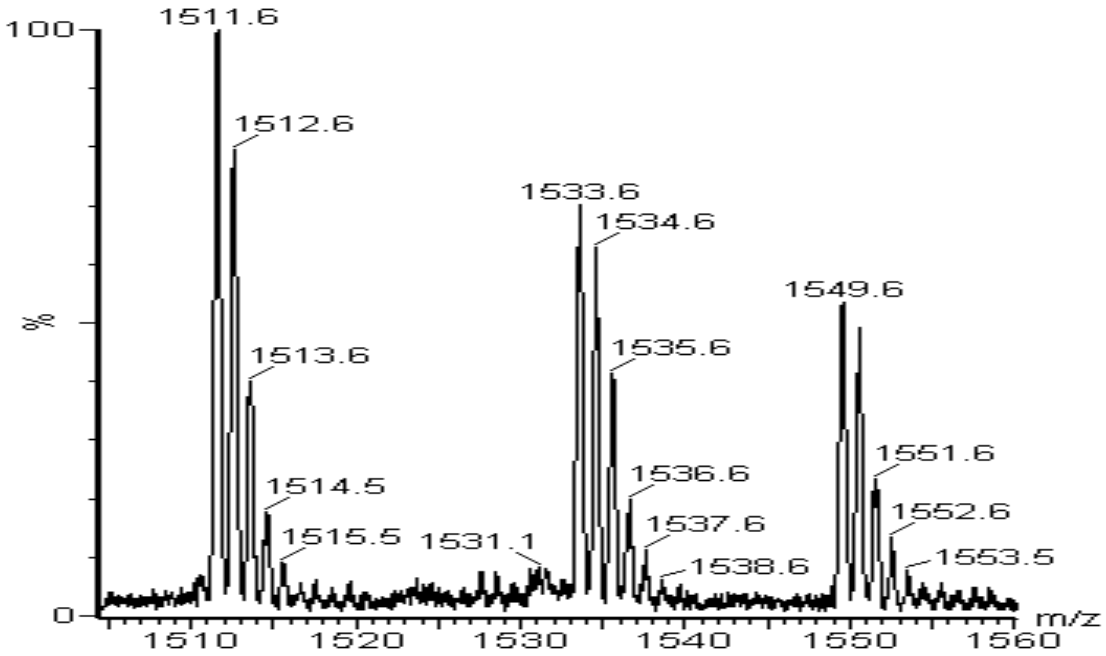


Figure S5. MS of the $(5HW)_1$CDA4a regioisomer (R_t = 5.96 min) produced after feeding 5HW **2** to *S. coelicolor* WH101.

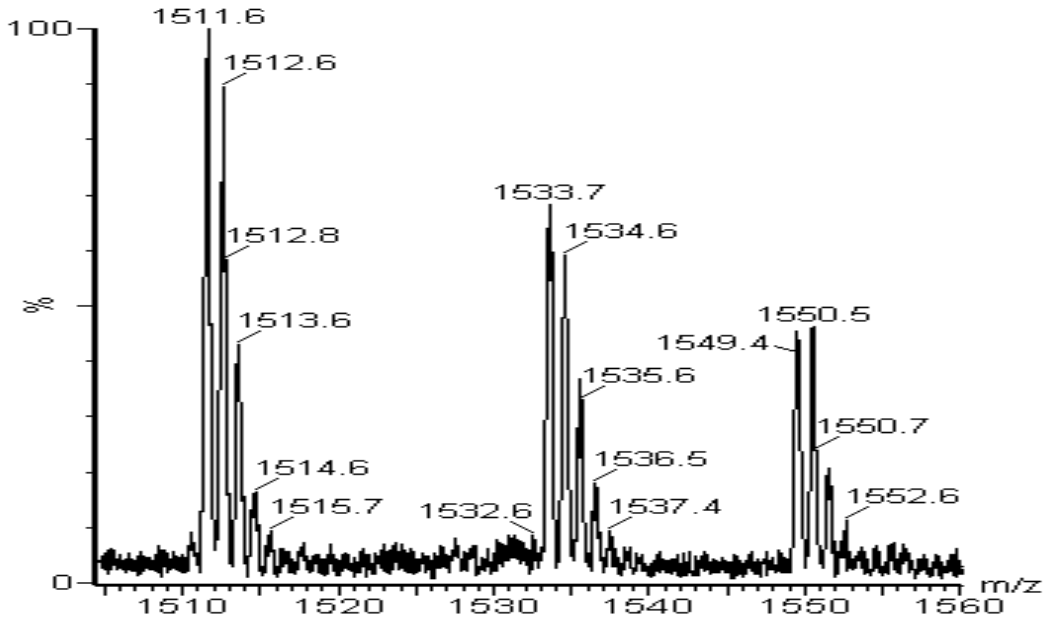
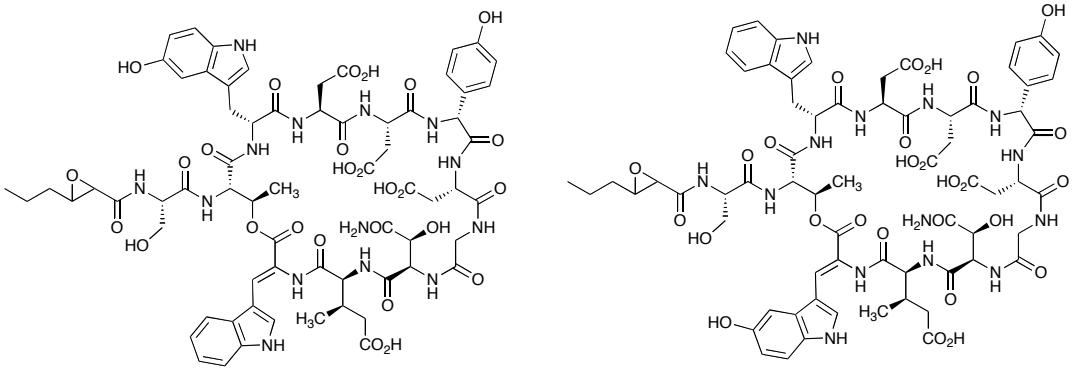


Figure S6. MS of the $(5HW)_1$CDA4a regioisomer (R_t = 6.20 min) produced after feeding 5HW **2** to *S. coelicolor* WH101.



Proposed structures of $(5HW)_1$CDA4a regioisomers

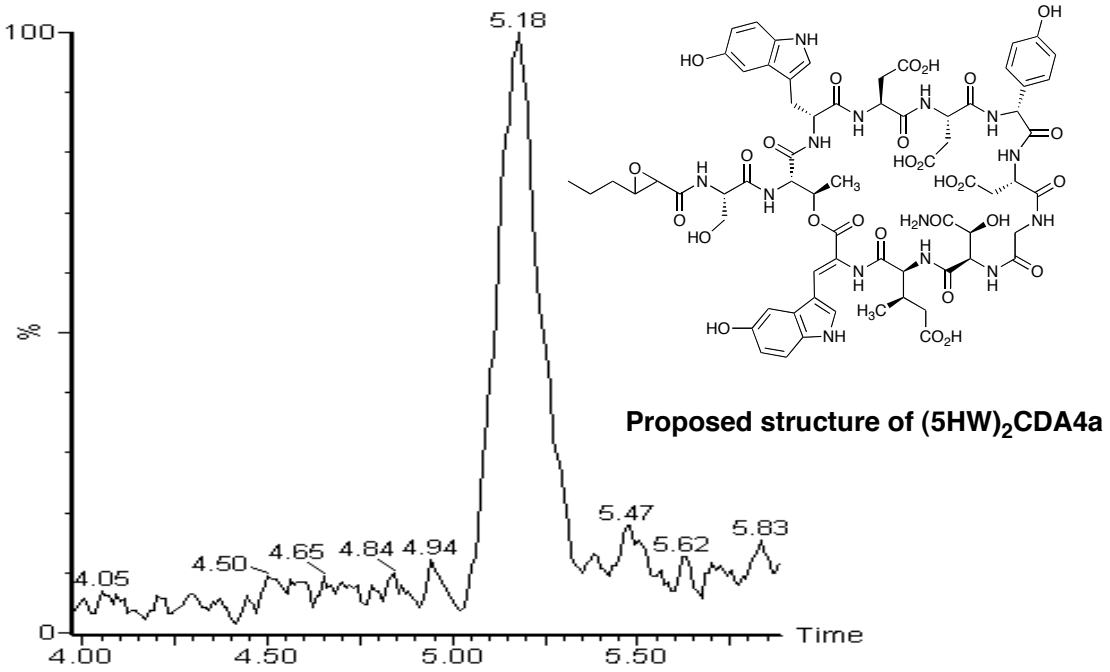


Figure S7. Mass-extraction HPLC of $(5HW)_2$CDA4a produced after feeding 5HW **2** to *S. coelicolor* WH101.

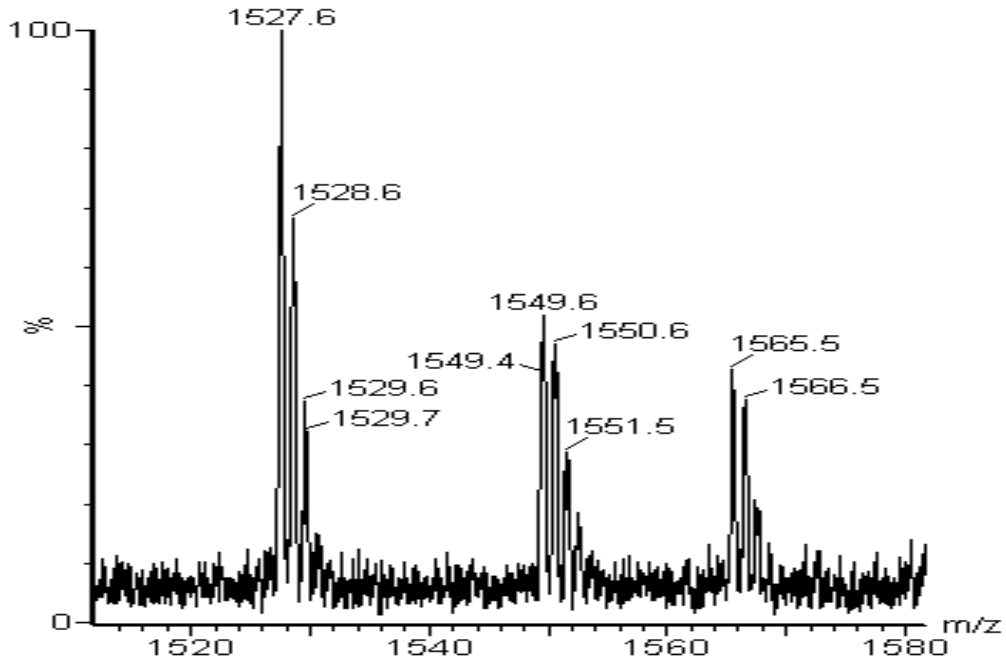


Figure S8. MS of $(5HW)_2$CDA4a produced after feeding 5HW **2** to *S. coelicolor* WH101.

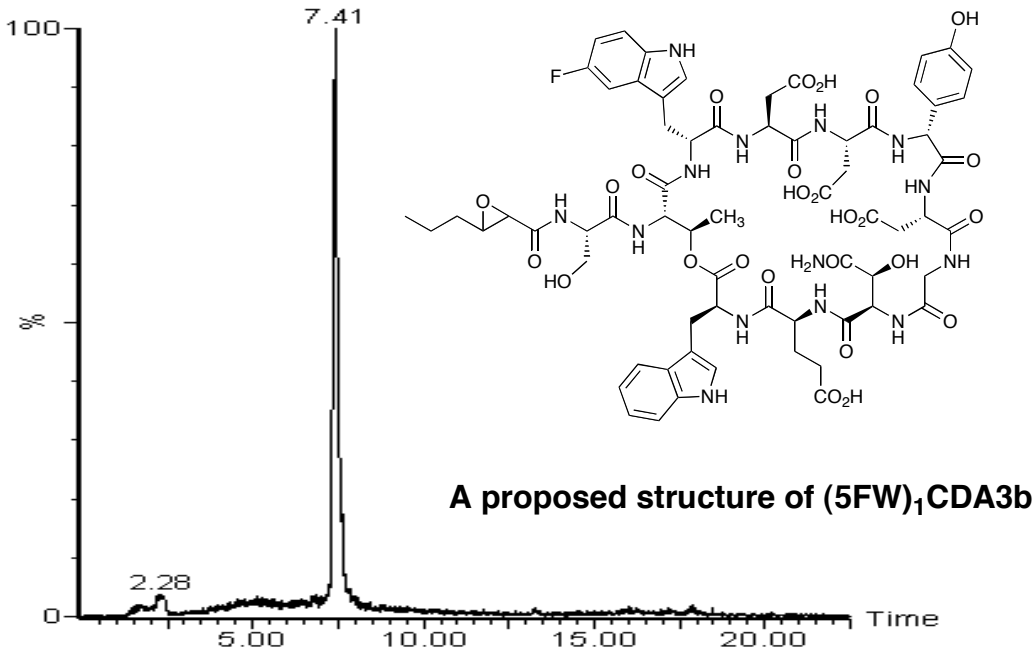


Figure S9. Mass-extraction HPLC of (5FW) $_1$CDA3b produced after feeding 5FW **3** to *S. coelicolor* WH101.

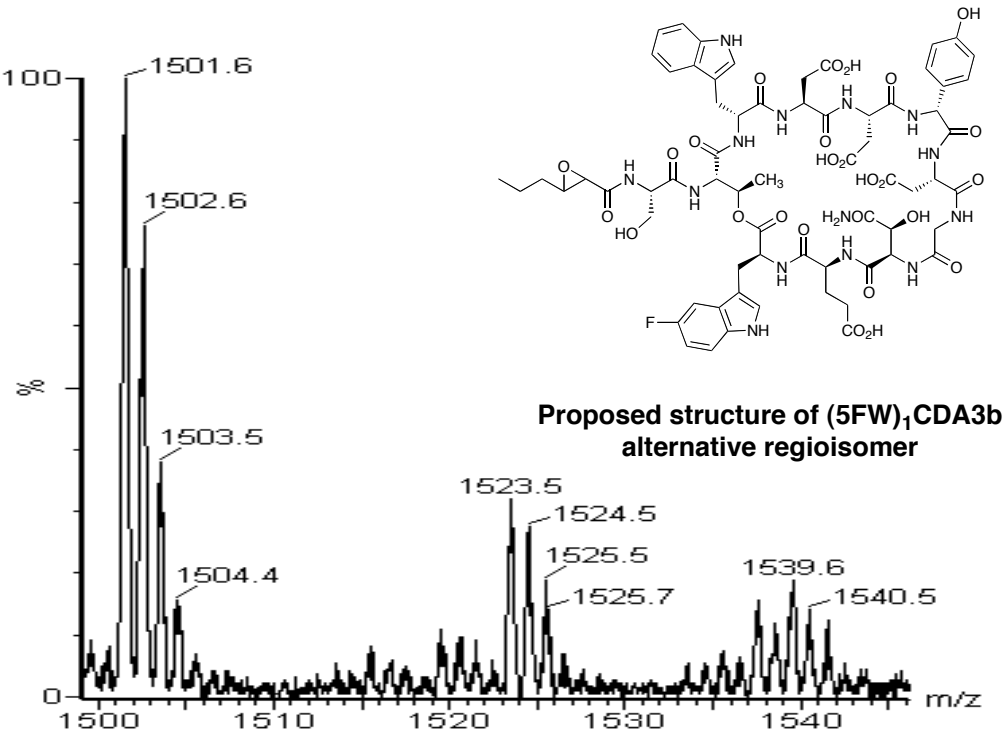


Figure S10. MS of (5FW) $_1$CDA3b produced after feeding 5FW **3** to *S. coelicolor* WH101.

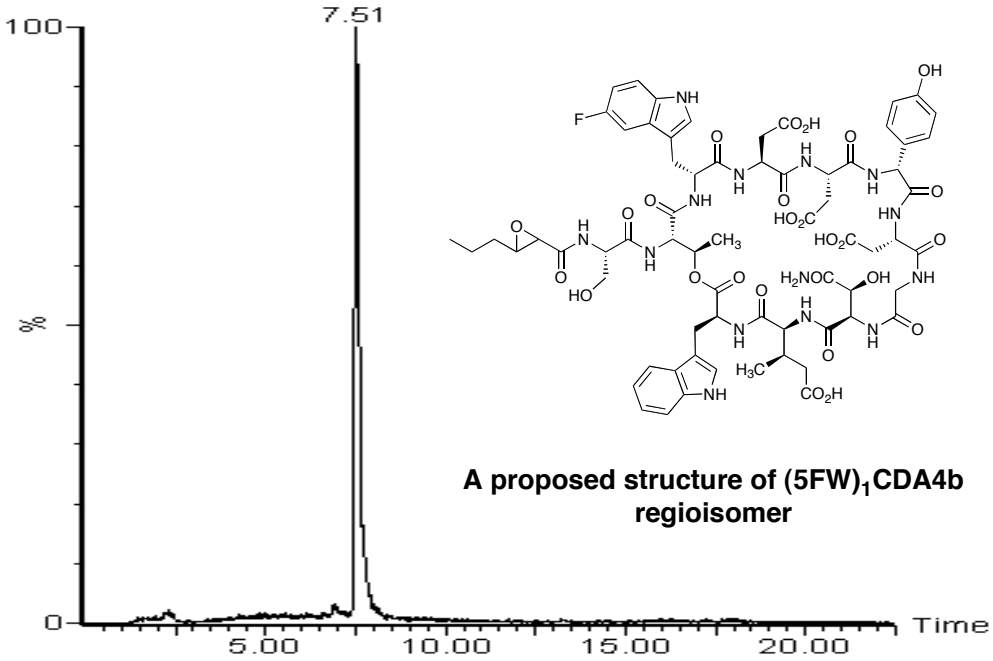


Figure S11. Mass-extraction HPLC of (5FW)$_1$CDA4b produced after feeding 5FW **3** to *S. coelicolor* WH101.

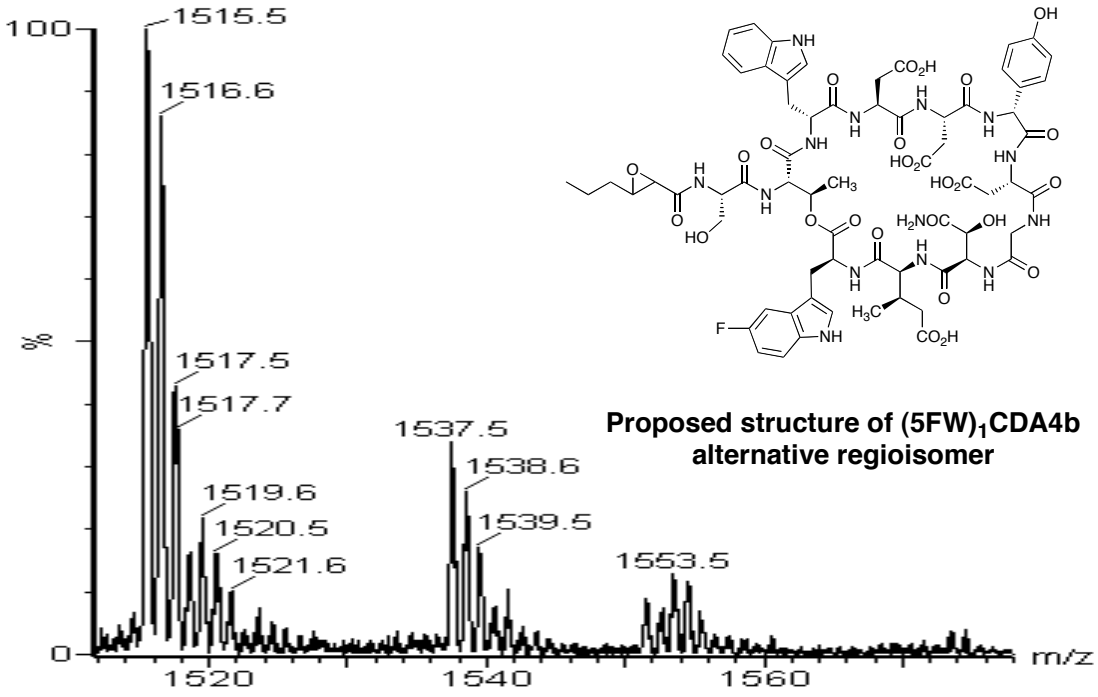


Figure S12. MS of (5FW)$_1$CDA4b produced after feeding 5FW **3** to *S. coelicolor* WH101.

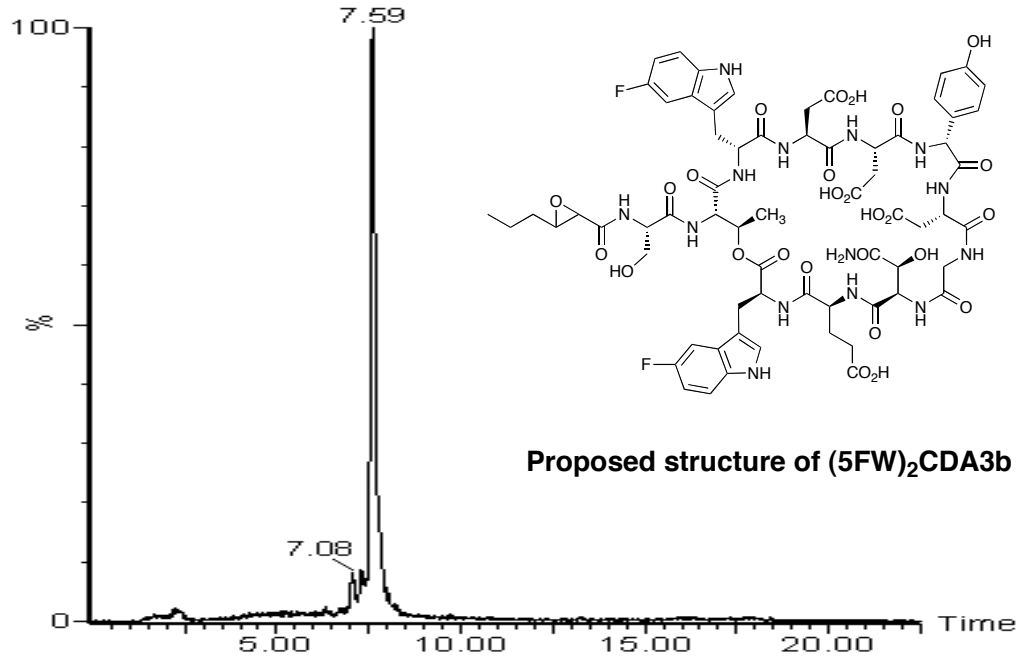


Figure S13. Mass-extraction HPLC of $(5FW)_2$CDA3b produced after feeding 5FW **3** to *S. coelicolor* WH101.

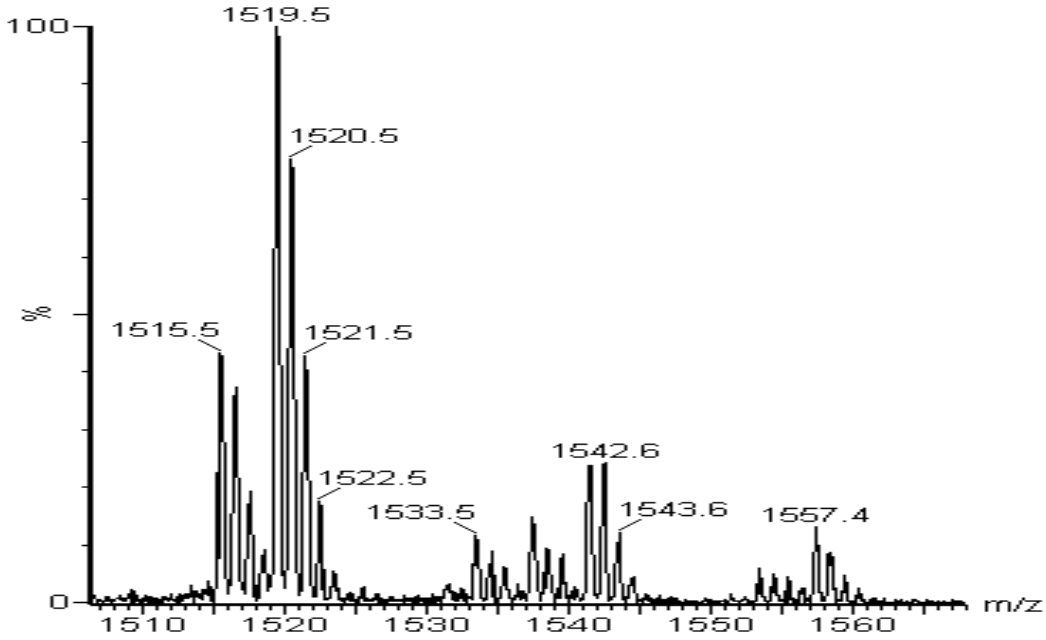


Figure S14. MS of $(5FW)_2$CDA3b produced after feeding 5FW **3** to *S. coelicolor* WH101 (plus overlap from single incorporation $(5FW)_1$CDA4b *m/z* 1515.5 $[M+H]^+$).

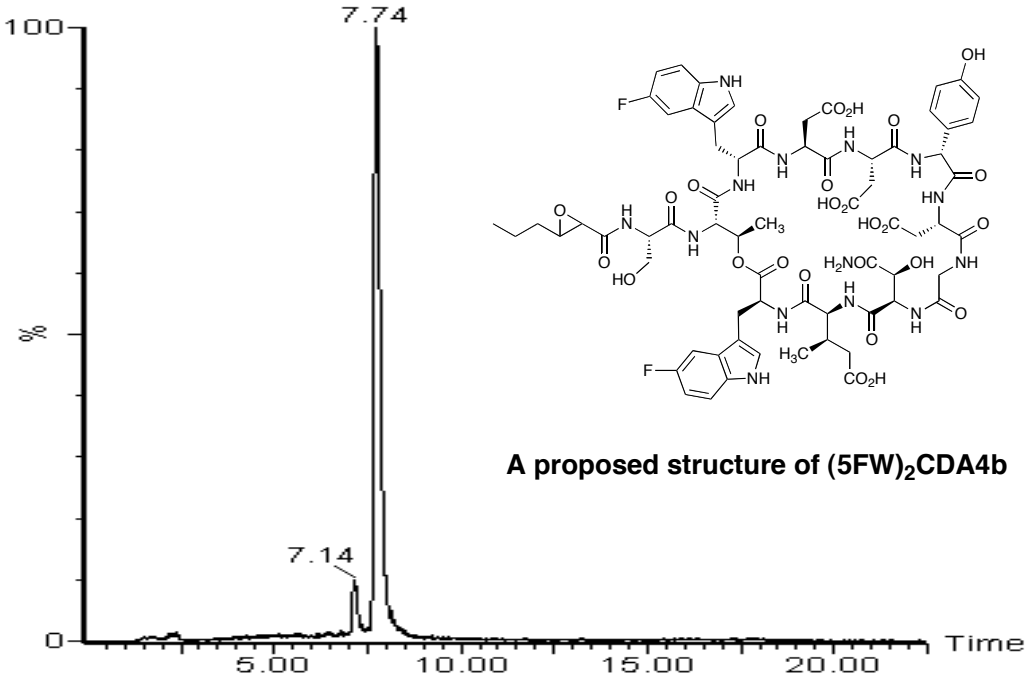


Figure S15. Mass-extraction HPLC of (5FW)$_2$CDA4b produced after feeding 5FW **3** to *S. coelicolor* WH101.

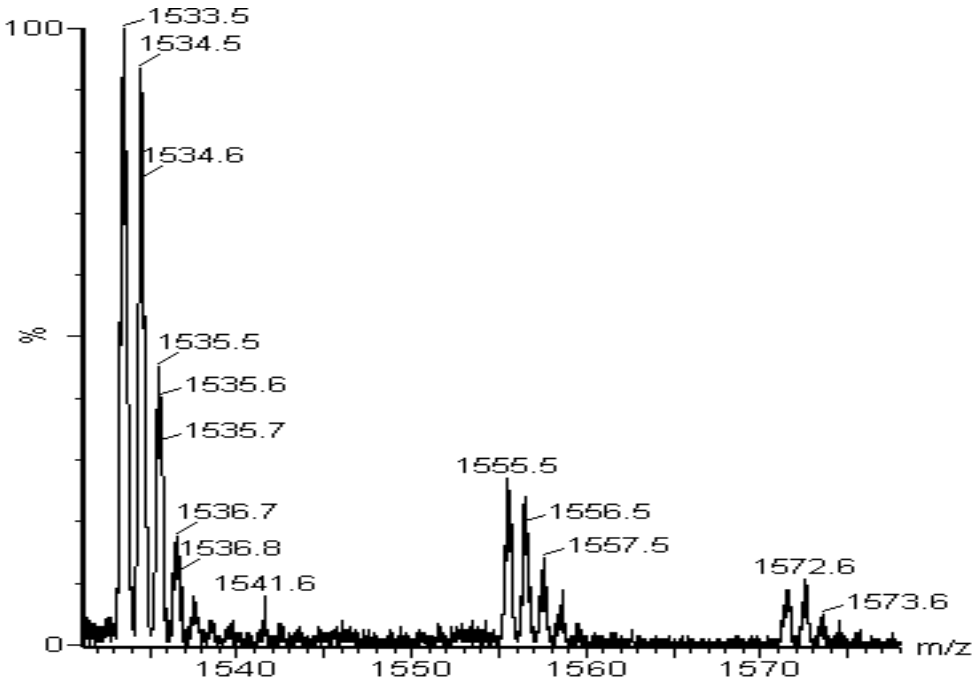


Figure S16. MS (5FW)$_2$CDA4b produced after feeding 5FW **3** to *S. coelicolor* WH101.