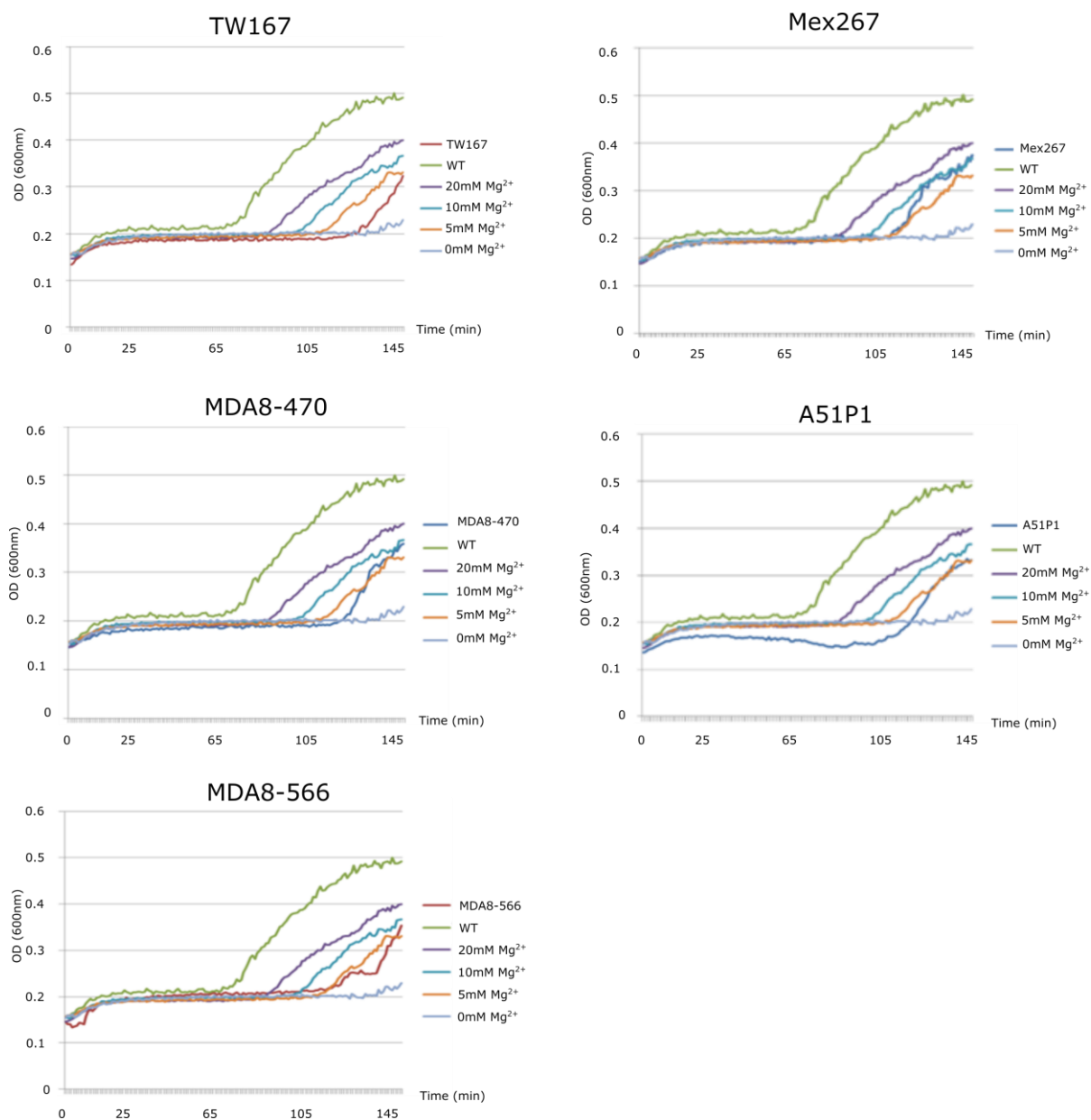


## Supplementary Information



**Supplementary Figure 1** Confirmation of the *mbl*-rescuing activity of hits from primary extract screening. The *mbl* mutant and wild type control strains were cultured in NB (96 well plates) with added Mg<sup>2+</sup> as indicated (mM). Fresh agar crush extracts from the indicated strains were added to a concentration of 6% (vol/vol). Growth was recorded as OD<sub>600</sub>.

**Supplementary Table 1** antiSMASH output of the prioritised strains

| MD8A-566  |                                     |           |           |   |   |            |
|-----------|-------------------------------------|-----------|-----------|---|---|------------|
| Region    | Type                                | From      | To        | Most similar known cluster                                    |   | Similarity |
| Region 1  | lassopeptide                        | 49,959    | 72,289    | kanamycin   | Saccharide  | 1%         |
| Region 2  | lanthipeptide-class-iv              | 159,332   | 181,995   | venezuelin  | RiPP:Lanthipeptide                                | 100%       |
| Region 3  | NRPS,T3PKS                          | 211,829   | 268,782   | violapyrone B   | Polyketide  | 28%        |
| Region 4  | NRPS                                | 414,129   | 477,202   | atratumycin   | NRP   | 7%         |
| Region 5  | terpene                             | 727,150   | 752,475   | hopene  | Terpene   | 76%        |
| Region 6  | RiPP-like                           | 1,268,156 | 1,279,454 |   |   |            |
| Region 7  | NRPS                                | 1,493,212 | 1,580,665 | friulimicin A / friulimicin B / friulimicin C / friulimicin D | NRP   | 21%        |
| Region 8  | siderophore                         | 1,708,307 | 1,722,357 | ficellomycin  | NRP   | 3%         |
| Region 9  | terpene                             | 2,087,836 | 2,108,076 | BD-12   | NRP   | 17%        |
| Region 10 | LAP,thiopeptide                     | 4,436,710 | 4,471,437 |   |   |            |
| Region 11 | butyrolactone                       | 4,984,835 | 4,994,482 |   |   |            |
| Region 12 | ectoine                             | 5,453,618 | 5,463,115 | ectoine   | Other   | 100%       |
| Region 13 | terpene                             | 5,863,622 | 5,882,692 | steffimycin D   | Polyketide:Type II + Saccharide:Hybrid /tailoring | 19%        |
| Region 14 | blactam                             | 6,137,403 | 6,159,229 | tabtoxin  | Other   | 13%        |
| Region 15 | NRPS,betalactone                    | 6,319,170 | 6,389,723 | coelichelin   | NRP   | 100%       |
| Region 16 | T2PKS,terpene,NRPS                  | 6,476,466 | 6,602,937 | spore pigment   | Polyketide  | 83%        |
| Region 17 | T2PKS,terpene                       | 6,620,932 | 6,691,617 | lugdunomycin  | Polyketide  | 55%        |
| Region 18 | T1PKS,PKS-like,NRPS                 | 6,940,306 | 7,056,880 | salinomycin   | Polyketide:Modular type I                         | 32%        |
| Region 19 | T1PKS,butyrolactone,oligosaccharide | 7,056,948 | 7,120,066 | lidamycin   | NRP + Polyketide                                  | 39%        |
| Region 20 | ectoine,T2PKS,butyrolactone         | 7,148,573 | 7,229,947 | kosinostatin  | NRP + Polyketide                                  | 22%        |

| TW167       |  |           |           |                            |  |            |
|-------------|--|-----------|-----------|----------------------------|--|------------|
| Region      | Type   | From      | To        | Most similar known cluster |  | Similarity |
| Region 1.1  | T2PKS,butyrolactone,ectoine                        | 1,882     | 83,259    | kosinostatin               | NRP + Polyketide                                 | 22%        |
| Region 1.2  | oligosaccharide,T1PKS, butyrolactone,PKS-like,NRPS | 111,705   | 291,764   | C-1027                     | Polyketide                                       | 42%        |
| tig00000012 |  |           |           |                            |  |            |
| Region 3.1  | T2PKS,terpene                                      | 252,049   | 322,751   | lugdunomycin               | Polyketide                                       | 55%        |
| Region 3.2  | NRPS,T2PKS,terpene                                 | 343,158   | 467,350   | spore pigment              | Polyketide                                       | 83%        |
| Region 3.3  | NRPS,betalactone                                   | 553,868   | 624,767   | coelichelin                |  |            |
| Region 3.4  | blactam  | 784,515   | 806,353   | tabtoxin                   | Other  | 13%        |
| Region 3.5  | terpene  | 1,060,336 | 1,081,358 | steffimycin D              | Polyketide:Type II + Saccharide:Hybrid/tailoring | 19%        |
| Region 3.6  | ectoine  | 1,480,132 | 1,490,530 | ectoine                    | Other  | 100%       |
| Region 3.7  | thiopeptide,LAP                                    | 2,471,932 | 2,506,665 |                            |  |            |

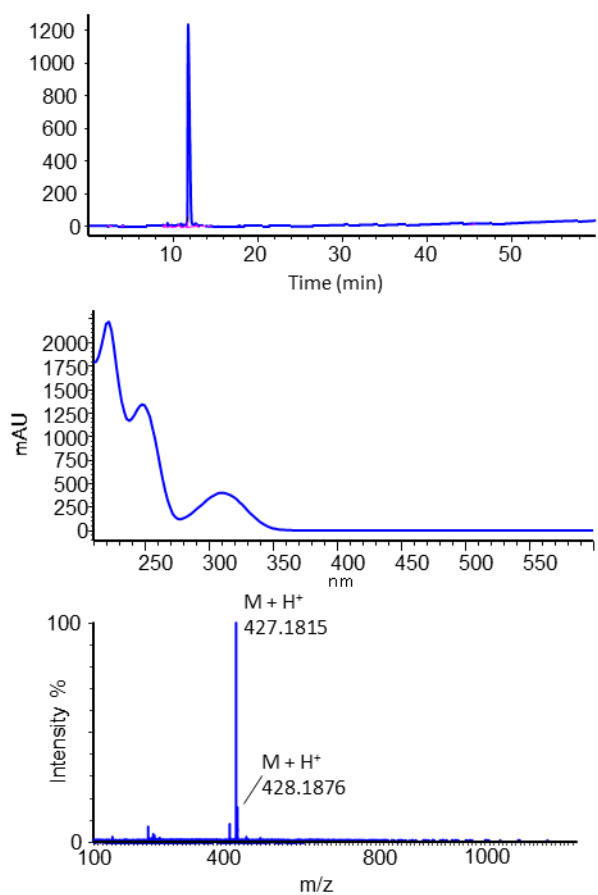
|                   |                        |           |           |  |                    |      |
|-------------------|------------------------|-----------|-----------|--|--------------------|------|
| <b>tig0000014</b> |                        |           |           |  |                    |      |
| <b>Region 5.1</b> | terpene                | 1,176,059 | 1,196,304 | BD-12  | NRP                | 17%  |
| <b>Region 5.2</b> | siderophore            | 1,561,489 | 1,575,905 | ficellomycin   | NRP                | 3%   |
| <b>Region 5.3</b> | NRPS                   | 1,703,576 | 1,791,102 | friulimicin A /<br>friulimicin B /<br>friulimicin C /<br>friulimicin D | NRP                | 24%  |
| <b>Region 5.4</b> | RiPP-like              | 2,004,590 | 2,015,888 |  |                    |      |
| <b>Region 5.5</b> | terpene                | 2,531,096 | 2,557,711 | hopene   | Terpene            | 76%  |
| <b>Region 5.6</b> | NRPS                   | 2,817,761 | 2,870,070 | cadaside A / cadaside<br>B   | NRP                | 14%  |
| <b>Region 5.7</b> | T3PKS,NRPS,NRPS-like   | 3,015,453 | 3,072,699 | violapyrone B  | Polyketide         | 28%  |
| <b>Region 5.8</b> | lanthipeptide-class-iv | 3,102,253 | 3,124,916 | venezuelin   | RiPP:Lanthipeptide | 100% |
| <b>Region 5.9</b> | lassopeptide           | 3,211,737 | 3,234,185 |  |                    |      |

|                    |                 |             |           |   |  |                   |
|--------------------|-----------------|-------------|-----------|---|--|-------------------|
| <b>Mex267</b>      |                 |             |           |   |  |                   |
| <b>Region</b>      | <b>Type</b>     | <b>From</b> | <b>To</b> | <b>Most similar known cluster</b>                                 |  | <b>Similarity</b> |
| <b>Region 1.1</b>  | NRPS,terpene    | 507,421     | 618,704   | nocobactin NA   | NRP + Polyketide                                     | 87%               |
| <b>Region 1.2</b>  | NAPAA           | 968,144     | 1,002,052 | isatropolone A / isotropolone<br>B / isotropolone C               | Polyketide   | 9%                |
| <b>Region 1.3</b>  | T1PKS           | 1,204,735   | 1,249,486 | clorobiocin   | Saccharide:Hybrid/tailoring +<br>Other:Aminocoumarin | 7%                |
| <b>Region 1.4</b>  | RiPP-like       | 1,543,949   | 1,553,780 |   |  |                   |
| <b>Region 1.5</b>  | ranthipeptide   | 2,000,320   | 2,021,825 | 2'-chloropentostatin / 2'-<br>amino-2'-deoxyadenosine             | Other  | 6%                |
| <b>Region 1.6</b>  | NRPS,RiPP-like  | 2,050,975   | 2,168,960 | Sch-47554 / Sch-47555   |  |                   |
| <b>Region 1.7</b>  | NRPS,T1PKS      | 3,843,816   | 3,925,042 | pepticcinnamin E  | NRP + Polyketide                                     | 6%                |
| <b>Region 1.8</b>  | NRPS-like       | 4,109,167   | 4,152,023 | platensimycin / platencin   | Terpene  | 5%                |
| <b>Region 1.9</b>  | NRPS            | 4,348,301   | 4,390,052 | diisonitrile antibiotic SF2768                                    | NRP  | 22%               |
| <b>Region 1.10</b> | T1PKS           | 4,441,229   | 4,487,630 |   |  |                   |
| <b>Region 1.11</b> | ectoine         | 4,508,666   | 4,519,061 | ectoine   |  |                   |
| <b>Region 1.12</b> | NRPS            | 4,595,594   | 4,651,731 |   |  |                   |
| <b>Region 1.13</b> | NRPS            | 4,955,436   | 5,019,662 | herboxidiene  | Polyketide   | 5%                |
| <b>Region 1.14</b> | NRPS            | 5,111,873   | 5,170,064 | pentalenolactone  | Terpene  | 15%               |
| <b>tig00000002</b> |                 |             |           |   |  |                   |
| <b>Region 2.1</b>  | betalactone     | 284,078     | 315,945   |   |  |                   |
| <b>Region 2.2</b>  | NRPS            | 342,406     | 387,295   | heterobactin A / heterobactin<br>S2                               | NRP  | 63%               |
| <b>Region 2.3</b>  | terpene         | 645,041     | 664,258   | carotenoid  | Terpene  | 27%               |
| <b>Region 2.4</b>  | T3PKS           | 739,330     | 780,499   |   |  |                   |
| <b>Region 2.5</b>  | arylpolyene     | 803784      | 844956    | echoside A / echoside B / echoside C /<br>echoside D / echoside E |  | 11%               |
| <b>Region 2.6</b>  | terpene         | 1,244,472   | 1,263,989 |   |  |                   |
| <b>Region 2.7</b>  | terpene         | 1,277,197   | 1,303,501 | A54145  | NRP  | 5%                |
| <b>Region 2.8</b>  | NRPS-like,NRPS, | 1,363,396   | 1,437,883 |   |  |                   |

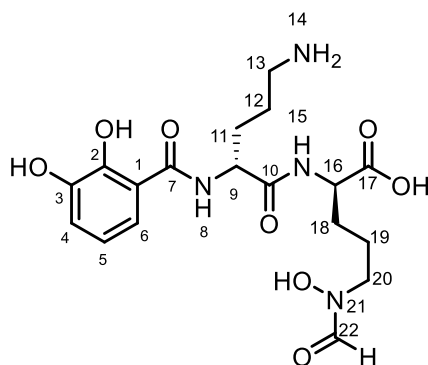
|             |             |           |           |               |                 |     |
|-------------|-------------|-----------|-----------|---------------|-----------------|-----|
|             | betalactone |           |           |               |                 |     |
| Region 2.9  | terpene     | 1,900,050 | 1,918,393 | carotenoid    | Terpene         | 18% |
| Region 2.10 | terpene     | 1,949,854 | 1,970,960 |               |                 |     |
| Region 2.11 | betalactone | 2,184,602 | 2,210,492 |               |                 |     |
| tig00000006 |             |           |           |               |                 |     |
| Region 6.1  | NAPAA       | 101,882   | 135,916   |               |                 |     |
| tig00000007 |             |           |           |               |                 |     |
| Region 7.1  | terpene     | 133,600   | 154,517   | lavendiol     | Polyketide      | 6%  |
| tig00000008 |             |           |           |               |                 |     |
| Region 8.1  | terpene     | 1         | 13,490    | carotenoid    | Terpene         | 50% |
| tig00000009 |             |           |           |               |                 |     |
| Region 9.1  | NAGGN       | 3,031     | 18,158    | botromycin A2 | RIPP:Botromycin | 6%  |

**Supplementary Table 2** BlastP analysis of the mirubactin gene cluster identified in strain MD8A-470 against the mirubactin gene cluster (BC0000392 MIBIG accession number; NCBI GenBank: CP001630.1 )

| ID mirubactin cluster | Max Score | Total Score | Query Cover        | E value   | Per. Ident | Acc. Len |
|-----------------------|-----------|-------------|--------------------|-----------|------------|----------|
| mrba                  | 244       | 244         | 96%                | 8.00E-86  | 55.89%     | 279      |
| mrbb                  | 296       | 342         | 72%                | 5.00E-99  | 57.39%     | 399      |
| mrbc                  | 588       | 588         | 98%                | 0         | 57.19%     | 553      |
| mrbd                  | 210       | 210         | 92%                | 7.00E-74  | 54.50%     | 211      |
| mrbe                  | 407       | 407         | 89%                | 7.00E-147 | 73.74%     | 297      |
| mrbf                  | 426       | 426         | 97%                | 2.00E-153 | 69.67%     | 334      |
| mrbg                  | 348       | 348         | 91%                | 1.00E-122 | 71.15%     | 342      |
| mrbh                  | 444       | 444         | 88%                | 3.00E-160 | 78.07%     | 332      |
| mrbi                  | 417       | 417         | 99%                | 6.00E-152 | 77.31%     | 262      |
| mrbj                  | 3705      | 3705        | 99%                | 0         | 62.76%     | 3328     |
| mrbk                  | 566       | 566         | 97%                | 0         | 68.05%     | 467      |
| mrbl                  |           |             | no significant hit |           |            |          |
| mrbm                  |           |             | no significant hit |           |            |          |
| mrbn                  |           |             | no significant hit |           |            |          |
| mrbo                  | 30.4      | 30.4        | 28%                | 8.00E-04  | 42.86%     | 203      |



**Supplementary Figure 2** (A) HPLC trace of purified mirubactin C. (B) UV-Vis spectrum of mirubactin C. (C) HRMS spectrum showing molecular ion  $[M+H]^+ = 427.1815$ .



**Supplementary Table 3** NMR data for mirubactin C in D<sub>2</sub>O at 298 K, 700 MHz, <sup>1</sup>H NMR collected at 700 MHz, <sup>13</sup>C{<sup>1</sup>H} NMR collected at 176 MHz, <sup>15</sup>N shifts were measured indirectly by <sup>1</sup>H-<sup>15</sup>N HMBC performed at both 8 and 12 Hz, referencing against nitromethane

| Position | $\delta_{\text{H}}$ multi, ( $J$ in Hz)                                      | $\delta_{^{13}\text{C}}$ | $\delta_{^{15}\text{N}}$ | HMBC                   |
|----------|--|--------------------------|--------------------------|------------------------|
| 1        | -  | 116.4                    |                          |                        |
| 2        | -  | 146.3 or                 |                          |                        |
| 3        | -  | 144.4                    |                          |                        |
| 4        | 6.98 (dd, $J = 7.9, 1.5$ Hz, 1H)   | 119.5                    |                          | H to C1, 2, 3, 5, 6    |
| 5        | 6.76 (t, $J = 8.0$ Hz, 1H)   | 119.6                    |                          | H to C1, 2, 3, 4, 6, 7 |
| 6        | 7.18 (dd, $J = 8.1, 1.6$ Hz, 1H)   | 119.2                    |                          | H to C1, 2, 3, 4, 5, 7 |
| 7        | -  | 169.8                    |                          |                        |
| 8        | -  | -                        | -260.5                   |                        |
| 9        | 4.53 (dd, $J = 7.8, 6.4$ Hz, 1H)   | 53.2                     |                          | H to C1, 7, 10, 11, 12 |
| 10       | -  | 172.6                    |                          | -                      |
| 11       | 1.98 – 1.91 (m, 1H)<br>1.89 – 1.82 (m, 1H)                                   | 27.8                     |                          | H to C9, 10, 12, 13    |
| 12       | 1.80 – 1.71 (m, 2H) [overlap<br>C18]   | 23.1                     |                          | *                      |
| 13       | 2.99 (ddd, $J = 8.6, 6.9, 2.1$ Hz,<br>2H)                                    | 38.8                     |                          | H to C11, 12           |
| 14       | -  | -                        | -348.8                   |                        |
| 15       | -  | -                        | -252.9                   |                        |
| 16       | 4.21 – 4.13 (m, 1H)  | 54.6                     |                          | H to C10, 17 18, 19    |
| 17       | -  | 178.1                    |                          |                        |
| 18       | 1.80 – 1.71 (m, 1H) [overlap<br>C12]<br>1.69 – 1.60 (m, 1H) [overlap<br>C19] | 28.2                     |                          | *                      |
| 19       | 1.69 – 1.60 (m, 2H) [overlap<br>C18]   | 22.7                     |                          | *                      |
| 20       | 3.56 – 3.44 (m, 2H)  | 50.0                     |                          | H to C16, 18, 19, 22   |
| 21       | -  | -                        | -199.3                   |                        |
| 22       | 7.83 and 8.18 (2 x s, 1H)  | 159.4                    |                          | H to C20               |

\* Unknown due to signal overlaps

All reagents were purchased from TCI UK Ltd (*N*<sup>α</sup>-(5-fluoro-2,4-dinitrophenyl)-L-leucinamide, *N*<sup>α</sup>-(5-fluoro-2,4-dinitrophenyl)-D-leucinamide), Alfa Aesar Ltd (L-ornithine hydrochloride, 57% hydriodic acid) and Fluorochem Ltd (D-ornithine hydrochloride). LCMS was performed on a Waters LCMS system, ACQUITY Ultra Performance LC™ (UPLC™) coupled to ACQUITY Photodiode Array (PDA) UV detector and a Xevo TQ-S mass detector.

#### **LCMS Method for Marfey's Analysis**

HPLC separation was carried out using a linear gradient of 5 – 70 % MeCN:H<sub>2</sub>O (0.1 % formic acid) over 15 minutes and a hold time at 100% of MeCN (0.1 % formic acid) for 1 minute, with an injection volume 1 μL, and a volume flow rate of 0.25 mL/min. Detection was carried out by UV (200-500 nm) and ESI-MS. Column (temp): ACQUITY UPLC® BEH C<sub>18</sub> 1.7 μm, 2.1 x 150 mm Column (40 °C)

#### **Marfey's Analysis of mirubactin C**

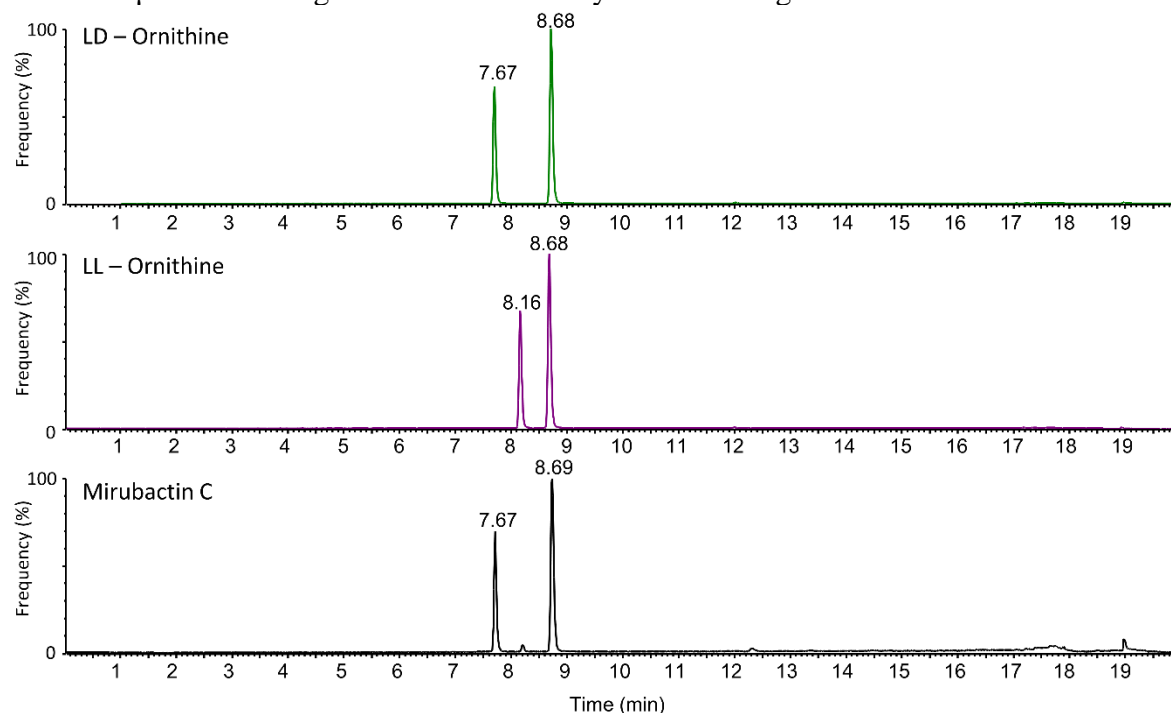
To determine the absolute stereochemistry of mirubactin C by Marfey's analysis, 500 μg of mirubactin C was dissolved in 400 μL of 57 % HI and the reaction mixture heated to 110 °C in an oil bath for 3 hours. After this time the reaction mixture was evaporated to dryness under a gentle stream of nitrogen gas overnight.

The residue material was dissolved in 240 μL of 1 M aq. NaHCO<sub>3</sub>, to which was added 170 μL 1 % *N*<sup>α</sup>-(5-fluoro-2,4-dinitrophenyl)-L-leucinamide in acetone and the reaction mixture stirred for 1 hour at 40 °C. After which the reaction was quenched with 60 μL of 2 M aq. HCl.

20 μL of this sample was diluted with 980 μL of HPLC grade water and analysed following the above LCMS method, versus standards.

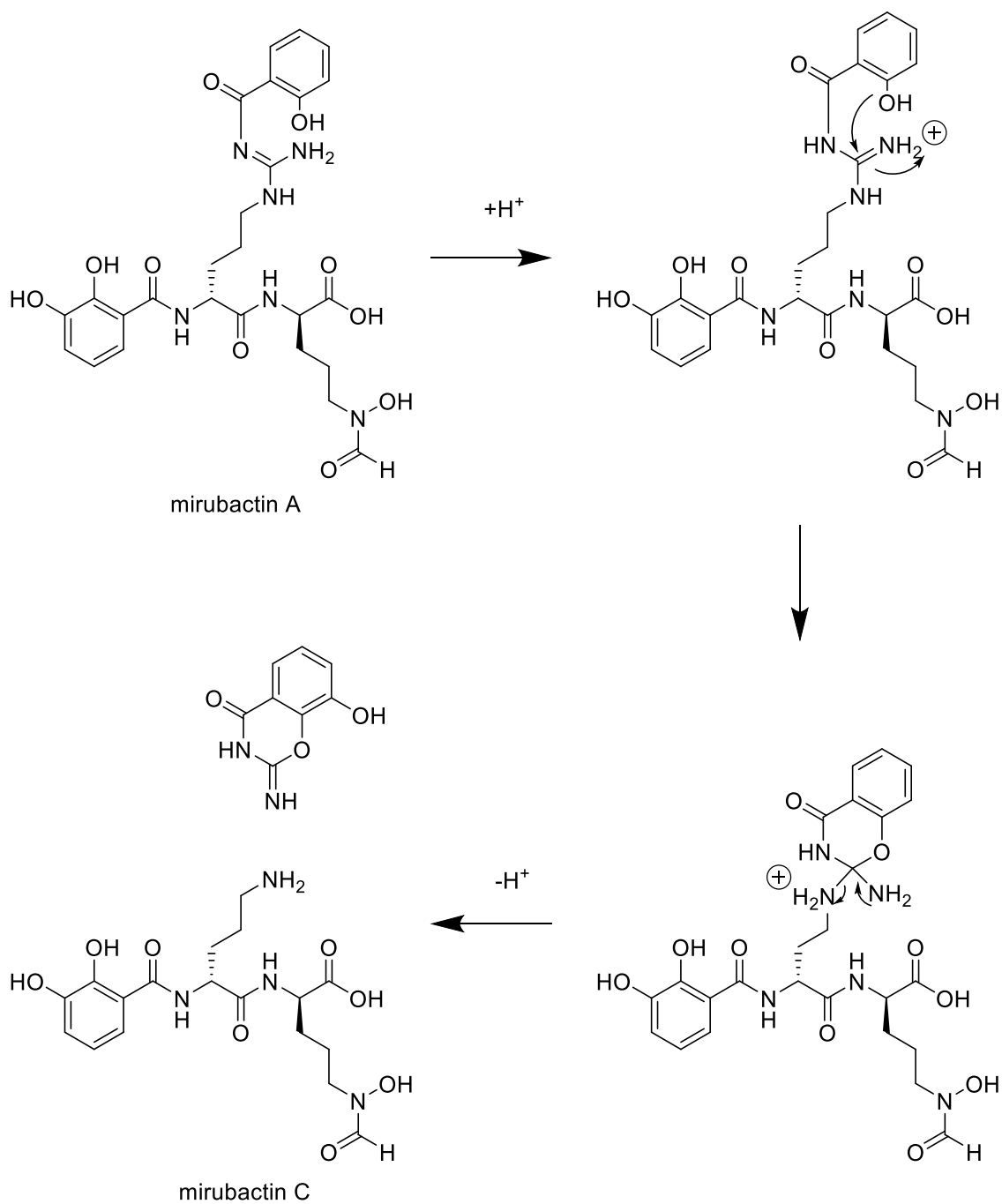
### Preparation of Standards for Marfey's Analysis

Marfey's standards for D-ornithine and L-ornithine were prepared by mixing 50  $\mu\text{L}$  of a 50 mM aq. solution of either D-ornithine or L-ornithine hydrochloride with 20  $\mu\text{L}$  of a 1 M aq. solution of  $\text{NaHCO}_3$  and 100  $\mu\text{L}$  of 1 % L-FDLA in acetone. The reaction mixtures were heated to 40  $^\circ\text{C}$  in a water bath for 1 hour to form the corresponding L-FDLA/D-ornithine and L-FDLA/L-ornithine adducts. 17  $\mu\text{L}$  of each of these samples was diluted with 983  $\mu\text{L}$  of HPLC grade water and analysed following the above LCMS method.



**Supplementary Figure 3** LCMS extracted ion chromatogram (EIC  $m/z = 427.2$ ,  $\pm 0.25$  Da) showing the samples of *N*<sup>ε</sup>-(5-fluoro-2,4-dinitrophenyl)-L-leucinamide (L-FDLA) derivatized mirubactin C (bottom) in comparison to L-FDLA/D-Ornithine (top) and L-FDLA/L-Ornithine standards (middle). LCMS Extracted ion chromatogram showing the sample of L-FDLA + mirubactin C (bottom) matched with L-FDLA + D-Ornithine standard (top)





**Supplementary Figure 4** Proposed degradation pathway of mirubactin A to mirubactin C based on Kishimoto et al (2014).<sup>34</sup>

