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# **Supporting Information**

# Second-generation probes for biosynthetic intermediate capture: towards a comprehensive profiling of polyketide assembly

Ina Wilkening,\* Silvia Gazzola,\* Elena Riva, James S. Parascandolo, Lijiang Song and Manuela Tosin\*\*

Department of Chemistry University of Warwick Library Road, Coventry CV4 7AL, UK M.Tosin@warwick.ac.uk

Ina Wilkening,\* Silvia Gazzola,\* Elena Riva, James S. Parascandolo, Lijiang Song and Manuela Tosin\*\*

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# 1. Synthesis of novel chemical probes

# 1.1. General methods

**Solvents and reagents:** Unless specified otherwise, chemicals were purchased from Sigma Aldrich, Fisher Scientific, Carbosynth or Alfa Aesar and were used without further purification. Anhydrous dichloromethane and tetrahydrofurane were purchased from VWR International (AR grade) and dried using solvent towers. Anhydrous ethyl acetate and methanol were purchased from Fisher Scientific or Sigma Aldrich. Reagent grade dichloromethane, ethyl acetate, methanol, acetonitrile, cyclohexane and chloroform were purchased from Fisher Scientific.

**Chromatography:** Analytical thin-layer chromatography (TLC) was performed on aluminium sheets precoated with silica gel 60 (F254, Merck) and visualized under ultra-violet light (short and long-wave) and using potassium permanganate (KMnO<sub>4</sub>) or vanillin stains. Silica gel was purchased from Sigma Aldrich (Tech Grade, pore size 60 Å, 230-400 mesh).

**NMR Spectroscopy:** <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded in d<sup>4</sup>-MeOD, CDCl<sub>3</sub> or  $D_2O$  on the following Bruker Avance instruments: DPX-400 400 MHz, DRX-500 500 MHz, AV III-500 HD 500 MHz or AV-600 600 MHz.

**LC-HRMS:** High-resolution mass spectra (HRMS) of newly made compounds were obtained using electrospray ionization (ESI) on a MaXis UHR-TOF (Bruker Daltonics) or on Bruker MaXis (ESI-HR-MS).

**HPLC:** Compounds were purified by semipreparative HPLC on a Phenomenex synergi<sup>™</sup> Polar RP 80 Å (250 x 10.0 mm, 4 µm) column. The mobile phase consisted of a gradient of water and acetonitrile (HPLC grade, containing 0.1% (v/v) trifluoroacetic acid) at a flow rate of 2.5 mL/min, with UV detection at 210, 254 and 280 nm.

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# 1.2. Synthesis of probes 15a-b

### Route 1



Route 2



# 1.2.1. Methyl 2-(2-(3-acetamidopropyl)-4-phenyl-1,3-dioxolan-2-yl)acetate (13a)



To a solution of the methyl ester  $1a^1$  (1.40 g, 7.00 mmol) in dry dichloromethane (40 mL), 1-phenyl-1,2ethanediol (1.92 g, 13.9 mmol) and chlorotrimethylsilane (3.51 mL, 27.9 mmol) were added dropwise under argon atmosphere. The reaction mixture was heated under reflux at 40 °C for 18 h.<sup>2</sup> The solvent was removed in vacuo, the resulting residue was dissolved in EtOAc (40 mL) and washed with 5% (w/v) NaHCO<sub>3</sub> (20 mL). The organic layer was dried over MgSO<sub>4</sub> and concentrated affording **13a** as a yellow oil (2.01 g, 90%;  $R_f = 0.60$  in 9:1 CH<sub>2</sub>Cl<sub>2</sub>:MeOH), which was used directly in the next step. A sample of this crude material was purified by semipreparative HPLC for accurate NMR characterisation ( $R_t = 23$  min, with a gradient elution from 0 to 100% MeCN over 30 minutes). A double set of signals was observed for two major isomers present in 1:1 ratio. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 1.68-1.79 (4H, m, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.95 and 1.97 (each 3H, s, CH<sub>3</sub>CONH), 1.92-2.04 (4H, m, CH<sub>2</sub>CO<sub>2</sub>), 2.80 and 2.80 (each 2H, d, J = 15.0 Hz, CH<sub>2</sub>CO), 3.27-3.33 (4H, m, CH<sub>2</sub>NH), 3.68-3.73 (2H, m, CH<sub>2</sub>O), 3.71 (6H, s, OCH<sub>3</sub>), 4.31-4.37 (2H, m, CH<sub>2</sub>O), 5.05 (1H, dd, J = 6.0, 9.3 Hz, CH), 5.15 (1H, dd, J = 6.5, 8.4 Hz, CH), 5.95 and 5.98 (each 1H, br s, NH), 7.29-7.37 (10H, m, ArH); <sup>13</sup>**C-NMR** (125 MHz, CDCl<sub>3</sub>) δ = 23.1 (CH<sub>3</sub>CO), 23.1 (CH<sub>3</sub>CO), 23.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 23.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 35.1 (CH<sub>2</sub>CO<sub>2</sub>), 35.2 (CH<sub>2</sub>CO<sub>2</sub>), 39.4 (CH<sub>2</sub>NH), 39.5 (CH<sub>2</sub>NH), 42.4 (CH<sub>2</sub>CO), 43.1 (CH<sub>2</sub>CO), 51.8 (CH<sub>3</sub>O), 51.8 (CH<sub>3</sub>O), 71.9 (CH<sub>2</sub>O), 71.9 (CH<sub>2</sub>O), 78.0 (CHO), 78.9 (CHO), 109.8 (CO<sub>2</sub>), 109.9 (CO<sub>2</sub>), 126.1 (CH arom), 126.3 (CH arom), 128.2 (CH arom), 128.3 (CH arom), 128.5 (CH arom), 128.6 (CH arom), 137.5 (C arom), 137.7 (C arom), 169.7 (CONH), 169.8 (CONH), 170.3 (CO<sub>2</sub>Me), 170.3 (CO<sub>2</sub>Me); *m/z* (HR-ESI-MS): found [M+Na]<sup>+</sup> 344.1470, C<sub>17</sub>H<sub>23</sub>NO<sub>5</sub>Na<sup>+</sup> requires 344.1468.

# 1.2.2. Methyl 2-(2-(3-d<sub>3</sub>-acetamidopropyl)-4-phenyl-1,3-dioxolan-2-yl)acetate (13b):



<sup>&</sup>lt;sup>1</sup> (a) Tosin, M.; Betancor, L.; Stephens, E.; Li, W. M. A.; Spencer, J. B.; Leadlay, P. F. *ChemBioChem*, **2010**, *11*, 539-546; (b) Tosin, M.; Demdychuk, Y.; Parascandolo, J. S.; Blasco Per, C.; Leeper, F. J.; Leadlay, P. F. *Chem. Commun.*, **2011**, *47*, 3460-3462.

<sup>&</sup>lt;sup>2</sup> Chan, T. H.; Brook, M. A.; Chaly, T. Synthesis, **1983**, *3*, 203-205.

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Compound **13b** was prepared in the same way as **13a** from methyl 6-(d<sub>3</sub>-acetamido)-3-oxohexanoate **1b**.<sup>1b</sup> <sup>1</sup>**H-NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 1.66-1.80 (4H, m, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.90-2.05 (4H, m, CH<sub>2</sub>), 2.80 and 2.81 (each 2H, d, *J* = 15.0 Hz, CH<sub>2</sub>CO), 3.27-3.34 (4H, m, CH<sub>2</sub>NH), 3.71 (6H, s, OCH<sub>3</sub>), 3.71 (2H, t, *J* =8.5 Hz, CH<sub>2</sub>O), 4.32-4.38 (2H, m, CH<sub>2</sub>O), 5.06 (1H, dd, *J* = 9.2, 5.9 Hz, CH), 5.16 (1H, dd, *J* = 8.5, 6.5 Hz, CH), 5.90 and 5.94 (each 1H, br s, NH), 7.28-7.39 (10H, m, ArH); <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  = 23.1 (CD<sub>3</sub>), 23.1 (CD<sub>3</sub>), 23.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 23.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 35.1 (CH<sub>2</sub>CO<sub>2</sub>), 35.2 (CH<sub>2</sub>CO<sub>2</sub>), 39.4 (CH<sub>2</sub>NH), 39.5 (CH<sub>2</sub>NH), 42.4 (CH<sub>2</sub>CO), 43.2 (CH<sub>2</sub>CO), 51.8 (CH<sub>3</sub>O), 51.8 (CH<sub>3</sub>O), 71.9 (CH<sub>2</sub>O), 72.0 (CH<sub>2</sub>O), 78.0 (CHO), 79.0 (CHO), 109.8 (CO<sub>2</sub>), 109.9 (CO<sub>2</sub>), 126.1 (CH arom), 126.3 (CH arom), 128.3 (CH arom), 128.4 (CH arom), 128.5 (CH arom), 128.6 (CH arom), 137.5 (C arom), 137.7 (C arom), 169.8 (CONH), 169.8 (CONH), 170.4 (COMe), 170.4 (COMe); *m/z* (HR-ESI-MS): found [M+Na]<sup>+</sup> 347.1651, C<sub>17</sub>H<sub>20</sub>D<sub>3</sub>NO<sub>5</sub>Na requires 347.1657.

# 1.2.3. Acetoxymethyl 2-(2-(3-acetamidopropyl)-4-phenyl-1,3-dioxolan-2-yl)acetate (14a)



To a solution of **13a** (2.00 g, 6.2 mmol) in dry THF (40 mL) containing 3 Å molecular sieves, potassium trimethylsilanolate (3.19 g, 24.9 mmol) was added. The reaction mixture was stirred under argon atmosphere and at room temperature for 3 h.<sup>3</sup> The reaction was then filtered and the solvent removed *in vacuo*. The residue was partitioned between EtOAc (20 mL) and water (20 mL), the aqueous layer was lyophilised affording crude potassium 2-(2-(3-acetamidopropyl)-4-phenyl-1,3-dioxolan-2-yl)acetate (**22a**, 2.14 g, 100%), which was carried forward to the next step without further purification.



For this intermediate a double set of signals was observed for two major isomers present in 1:1 ratio; <sup>1</sup>H-NMR (600 MHz, D<sub>2</sub>O)  $\delta$  = 1.65-1.78 (4H, m, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.93-2.03 (4H, m, CH<sub>2</sub>), 1.94 and 1.95 (each 3H, s, CH<sub>3</sub>CONH), 2.48-2.64 (4H, m, CH<sub>2</sub>CO), 3.22 (4H, t, *J* = 6.7 Hz, *CH*<sub>2</sub>NH), 3.78-3.86 (2H, m, CH<sub>2</sub>O), 4.34-4.45 (2H, m, CH<sub>2</sub>O), 5.15 (1H, dd, *J* = 6.2, 8.9 Hz, CH), 5.24-5.31 (1H, m, CH), 7.36-7.53 (10H, m, ArH); <sup>13</sup>C-NMR (125 MHz, D<sub>2</sub>O)  $\delta$  = 22.6 (CH<sub>3</sub>), 22.6 (CH<sub>3</sub>), 23.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 23.6 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 36.2 (CH<sub>2</sub>CO<sub>2</sub>), 36.4

<sup>&</sup>lt;sup>3</sup> Barrett, A. G. M.; Peña, M.; Willardsen, J. A. J. Org. Chem., **1996**, *61*, 1082–1100.

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(CH<sub>2</sub>CO<sub>2</sub>), 40.1 (CH<sub>2</sub>CO), 40.1 (CH<sub>2</sub>CO), 46.4 (CH<sub>2</sub>NH), 46.9 (CH<sub>2</sub>NH), 71.9 (CH<sub>2</sub>O), 71.9 (CH<sub>2</sub>O), 78.5 (CH), 79.3 (CH), 111.7 (CO<sub>2</sub>), 111.8 (CO<sub>2</sub>), 127.1 (CH arom), 127.6 (CH arom), 128.9 (CH arom), 129.4 (CH arom), 129.5 (CH arom), 129.6 (CH arom), 138.0 (C arom), 138.3 (C arom), 176.3 (CONH), 176.4 (CONH), 180.3 (COO<sup>-</sup>); *m/z* (HR-ESI-MS): found [M+Na]<sup>+</sup> 330.1311, C<sub>16</sub>H<sub>21</sub>NO<sub>5</sub>Na requires 330.1312).

To a solution of crude potassium 2-(2-(3-acetamidopropyl)-4-phenyl-1,3-dioxolan-2-yl)acetate (22a, 2.10 g, 6.1 mmol) in dry THF (40 mL), bromomethyl acetate (650 μl, 6.7 mmol) was added and the reaction mixture was stirred for 18 h at room temperature. The solvent was removed in vacuo to give 14a as a yellow oil (2.27 g, 98%), used for the next step without further purification,  $R_f = 0.25$  (EtOAc). A sample of this crude material was purified by semipreparative HPLC for accurate NMR characterisation ( $R_t = 37$  min, with a gradient elution from 0 to 50% MeCN over 30 min, then 50 to 100% MeCN over 15 min). A double set of signals was observed for two major isomers present in 1:1 ratio. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 1.65-1.79 (4H, m, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.95 and 1.97 (each 3H, s, CH<sub>3</sub>CONH), 1.92-2.04 (4H, m, CH<sub>2</sub>), 2.04 and 2.05 (each 3H, s, CH<sub>3</sub>COO) 2.83 and 2.83 (each 2H, d, J = 13.0 Hz, CH<sub>2</sub>CO), 3.26-3.33 (4H, m, CH<sub>2</sub>NH), 3.70 (2H, t, J = 8.3 Hz, CH<sub>2</sub>O), 4.29-4.37 (2H, m, CH<sub>2</sub>O), 5.04 (1H, dd, J = 9.4, 5.9 Hz, CH), 5.15 (1H, dd, J = 8.4, 6.7 Hz, CH), 5.73-5.78 (4H, m, OCH<sub>2</sub>O), 6.00 and 6.03 (each 1H, br s, NH), 7.28-7.38 (10H, m, ArH); <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  = 20.6 (CH<sub>3</sub>), 20.6 (CH<sub>3</sub>), 23.1 (CH<sub>3</sub>), 23.1 (CH<sub>3</sub>), 23.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 23.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 35.2 (CH<sub>2</sub>CO<sub>2</sub>), 35.3 (CH<sub>2</sub>CO<sub>2</sub>), 39.3 (CH<sub>2</sub>NH), 39.4 (CH<sub>2</sub>NH), 42.4 (CH<sub>2</sub>CO), 43.1 (CH<sub>2</sub>CO), 71.9 (CH<sub>2</sub>O), 72.0 (CH<sub>2</sub>O), 78.1 (CHO), 79.1 (CHO), 79.1 (OCH<sub>2</sub>O), 79.1 (OCH<sub>2</sub>O), 109.6 (CO<sub>2</sub>), 109.7 (CO<sub>2</sub>), 126.1 (CH arom), 126.2 (CH arom), 128.3 (CH arom), 128.4 (CH arom), 128.6 (CH arom), 128.6 (CH arom), 137.2 (C arom), 137.6 (C arom), 167.9 (C arom), 167.9 (C arom), 169.6 (CONH), 169.7 (CONH), 170.3 (CO), 170.3 (CO); *m/z* (HR-ESI-MS): found [M+Na]<sup>+</sup> 402.1521, C<sub>19</sub>H<sub>25</sub>NO<sub>7</sub>Na requires 402.1523.

### 1.2.4. Acetoxymethyl 2-(2-(d<sub>3</sub>-acetamidopropyl)-4-phenyl-1,3-dioxolan-2-yl)acetate (14b)



Compound **14b** was prepared in the same way as **14a** from **13b** as starting material, through potassium 2-(2-(3-d<sub>3</sub>-acetamidopropyl)-4-phenyl-1,3-dioxolan-2-yl)acetate (**22b**) as intermediate:



Intermediate characterisation of **22b**: <sup>1</sup>**H-NMR** (400 MHz, MeOD)  $\delta$  = 1.65-1.82 (4H, m, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.95-2.08 (4H, m, CH<sub>2</sub>), 2.48-2.64 (4H, m, CH<sub>2</sub>CO), 3.18-3.25 (4H, m, CH<sub>2</sub>NH), 3.54-3.65 (2H, m, CH<sub>2</sub>O), 4.31 (1H, dd, *J* = 6.0, 8.2 Hz, CH<sub>2</sub>O), 4.36 (1H, dd, *J* = 6.5, 7.7 Hz, CH<sub>2</sub>O), 5.03 (1H, dd, *J* = 7.0, 11.9 Hz, CH), 5.23 (1H, dd, *J* = 6.5, 8.4 Hz, CH), 7.36-7.53 (10H, m, ArH); <sup>13</sup>C-NMR (125 MHz, D<sub>2</sub>O)  $\delta$  = 24.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 24.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 24.9 (CD<sub>3</sub>), 24.9 (CD<sub>3</sub>), 36.4 (CH<sub>2</sub>CH), 36.7 (CH<sub>2</sub>CH), 40.9 (CH<sub>2</sub>NH), 40.9 (CH<sub>2</sub>NH), 47.4 (CH<sub>2</sub>CO<sub>2</sub>), 48.0 (CH<sub>2</sub>CO<sub>2</sub>), 73.0 (CH<sub>2</sub>O), 73.2 (CH<sub>2</sub>O), 79.1 (CH), 80.1 (CH), 112.3 (CO<sub>2</sub>), 112.5 (CO<sub>2</sub>), 127.6 (CH arom), 127.7 (CH arom), 129.2 (CH arom), 129.4 (CH arom), 129.6 (CH arom), 129.7 (CH arom), 140.0 (C arom), 140.3 (C arom), 171.8 (CONH), 176.4 (COO<sup>-</sup>), 176.4 (COO<sup>-</sup>); *m/z* (HR-ESI-MS): found [M+Na]<sup>+</sup> 333.1487, C<sub>16</sub>H<sub>18</sub>D<sub>3</sub>NO<sub>5</sub>Na<sup>+</sup> requires 333.1500.

For **14b**: <sup>1</sup>**H-NMR** (500 MHz, CDCl<sub>3</sub>)  $\delta$  = 1.65-1.80 (4H, m, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.92-2.04 (4H, m, CH<sub>2</sub>), 2.05 and 2.06 (each 3H, s, CH<sub>3</sub>COO), 2.83 and 2.84 (each 2H, d, *J* = 16.0 Hz, CH<sub>2</sub>CO), 3.28-3.35 (4H, m, CH<sub>2</sub>NH), 3.70 (2H, t, *J* = 8.3 Hz, CH<sub>2</sub>O), 4.32-4.36 (2H, m, CH<sub>2</sub>O), 5.04 (1H, dd, *J* = 6.6, 8.4 Hz, CH), 5.15 (1H, dd, *J* = 6.0, 9.3 Hz, CH), 5.73-5.78 (4H, m, OCH<sub>2</sub>O), 6.08 and 6.13 (each 1H, br s, N*H*), 7.29-7.39 (10H, m, Ar*H*); <sup>13</sup>**C-NMR** (125 MHz, CDCl<sub>3</sub>)  $\delta$  = 20.6 (CH<sub>3</sub>), 20.6 (CH<sub>3</sub>), 23.0 (CD<sub>3</sub>), 23.0 (CD<sub>3</sub>), 23.4 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 23.5 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 35.2 (CH<sub>2</sub>CO<sub>2</sub>), 35.3 (CH<sub>2</sub>CO<sub>2</sub>), 39.4 (CH<sub>2</sub>CO), 39.4 (CH<sub>2</sub>CO), 42.4 (CH<sub>2</sub>NH), 43.1 (CH<sub>2</sub>NH), 71.9 (CH<sub>2</sub>O), 72.0 (CH<sub>2</sub>O), 78.1 (CH<sub>2</sub>CH), 79.1 (OCH<sub>2</sub>O), 79.1 (OCH<sub>2</sub>O), 109.6 (CO<sub>2</sub>), 109.7 (CO<sub>2</sub>), 126.1 (CH arom), 126.3 (CH arom), 128.3 (CH arom), 128.4 (CH arom), 128.6 (CH arom), 128.6 (CH arom), 137.3 (C arom), 137.6 (C arom), 167.9 (CO), 167.9 (CO), 169.7 (CONH), 169.8 (CONH), 170.5 (CO), 170.5 (CO); *m/z* (HR-ESI-MS): found [M+Na]<sup>+</sup> 405.1701, C<sub>19</sub>H<sub>22</sub>D<sub>3</sub>NO<sub>7</sub>Na<sup>+</sup> requires 405.1682.

# 1.2.5. Methyl 2-[2-(3-acetamidopropyl)-1,3-dithiolan-2-yl]acetate (16a)



To a solution of **1a** (968 mg, 4.8 mmol) in dry  $CH_2Cl_2$  (30 mL), 1,2-ethanedithiol (2.02 mL, 24.1 mmol) was added. The reaction mixture was cooled to 0°C before dropwise addition of  $BF_3 \cdot Et_2O$  (2.97 mL, 24.1 mmol) then the reaction was heated under reflux at 40 °C for 18 h. The reaction was quenched with saturated NaHCO<sub>3</sub> solution, the organic layer was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The crude product was purified by flash chromatography (9:1 DCM:MeOH,  $R_f = 0.59$ ) to give **16a** (1.20 g, 90%). A sample of this crude material was purified by semipreparative HPLC for accurate NMR characterisation ( $R_t = 24.5$  min, with a gradient elution 0 to 40% over 15 min, then 40 to 50 over 30 min, then 50 to 100% MeCN over 5 min). <sup>1</sup>**H-NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta = 1.62-1.80$  (2H, m,  $CH_2CH_2CH_2$ ), 2.00 (3H, s,  $CH_3CONH$ ), 2.11-2.16 (2H, m,  $CH_2CS_2$ ), 3.05 (2H, s,  $CH_2$ ), 3.20-3.24 (2H, m,  $CH_2NH$ ), 3.30 (4H, s,  $SCH_2CH_2S$ ), 3.70 (3H, s,  $OCH_3$ ), 5.90 (1H, br s, NH); <sup>13</sup>**C-NMR** (125 MHz, CDCl<sub>3</sub>)  $\delta = 23.0$  (CH<sub>3</sub>), 26.8 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 39.3 (CH<sub>2</sub>), 39.5 (CH<sub>2</sub>), 39.8 (SCH<sub>2</sub>CH<sub>2</sub>S),

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48.2 (CH<sub>2</sub>CO), 51.7 (CH<sub>3</sub>O), 66.6 (CS<sub>2</sub>), 170.4 (CONH), 170.4 (COMe); *m/z* (HR-ESI-MS): found [M+Na]<sup>+</sup> 300.0701, C<sub>11</sub>H<sub>19</sub>NO<sub>3</sub>S<sub>2</sub>Na<sup>+</sup> requires 300.0699.

### 1.2.6. Acetoxymethyl 2-[2-(3-acetamidopropyl)-1,3-dithiolan-2-yl]acetate (17a)



Compound **17a** was prepared in the same way as **14a** from **16a** as starting material, proceeding through the intermediate potassium 2-[2-(3-acetamidopropyl)-1,3-dithiolan-2-yl]acetate (**23a**):



**23a**: <sup>1</sup>H-NMR (700 MHz, MeOD)  $\delta$  = 1.72-1.78 (2H, m, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.92 (3H, s, CH<sub>3</sub>CONH), 2.16-2.19 (2H, m, CH<sub>2</sub>), 2.89 (2H, s, CH<sub>2</sub>CO), 3.16 (2H, t, *J* = 7.0, CH<sub>2</sub>NH), 3.21-3.26 (4H, m, SCH<sub>2</sub>CH<sub>2</sub>S); <sup>13</sup>C-NMR (176 MHz, MeOD)  $\delta$  24.4 (CH<sub>3</sub>), 24.4 (CH<sub>2</sub>), 40.1 (CH<sub>2</sub>), 40.2 (CH<sub>2</sub>), 40.8 (CH<sub>2</sub>), 41.5 (CH<sub>2</sub>), 53.0 (CH<sub>2</sub>CO), 69.4 (CS<sub>2</sub>), 173.3 (CONH), 178.3 (COO<sup>-</sup>); *m/z* (HR-ESI-MS): found [M+H]<sup>+</sup> 286.0542, C<sub>10</sub>H<sub>17</sub>NO<sub>3</sub>S<sub>2</sub>Na<sup>+</sup> requires 286.0542.

A sample of **17a** was purified by semipreparative HPLC for accurate NMR characterisation ( $R_t = 19.5$  min, with a gradient elution 0 to 65% MeCN over 15 min, then 65 to 70 MeCN over 15 min, then 70 to 100% MeCN over 5 min). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta = 1.69-1.77$  (2H, m, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 1.98 (3H, s, CH<sub>3</sub>CONH), 2.08-2.14 (2H, m, CH<sub>2</sub>CS<sub>2</sub>), 2.11 (3H, s, CH<sub>3</sub>COO), 3.06 (2H, s, CH<sub>2</sub>CO<sub>2</sub>R), 3.26-3.31 (2H, m, CH<sub>2</sub>NH), 3.28 (4H, s, SCH<sub>2</sub>CH<sub>2</sub>S), 5.72 (2H, s, CH<sub>2</sub>), 6.01 (1H, br s, NH); <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>)  $\delta = 20.7$  (CH<sub>3</sub>), 23.1 (CH<sub>3</sub>), 26.8 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 39.3 (CH<sub>2</sub>), 39.3 (CH<sub>2</sub>), 39.3 (CH<sub>2</sub>), 48.1 (CH<sub>2</sub>CO), 66.2 (CS<sub>2</sub>), 79.1 (OCH<sub>2</sub>O), 168.5 (CONH), 169.7 (CO), 170.3 (CO); *m/z* (HR-ESI-MS): found [M+Na]<sup>+</sup> 358.0753, C<sub>13</sub>H<sub>21</sub>NO<sub>5</sub>S<sub>2</sub>Na<sup>+</sup> requires 358.0753.

### 1.2.7. Acetoxymethyl 6-acetamido-3-oxohexanoate (15a)



### Method A

To a solution of **14a** (250 mg, 0.7 mmol) in dry EtOAc (20 mL) under argon atmosphere,  $Pd(OAc)_2$  (200 mg, 0.9 mmol) and  $Pd(CF_3COO)_2$  (100 mg, 0.3 mmol) were added <sup>4</sup>.  $H_2(g)$  (1 bar) was then flushed through and

<sup>&</sup>lt;sup>4</sup> Conway, S. J.; Thuring, J. W.; Andreu, S.; Kvinlaug, B. T.; Roderick, H. L.; Bootman, M. D.; Holmes, A. B. *Aust. J. Chem.*, **2006**, *59*, 887–893.

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the mixture was stirred at room temperature for 48 h. The reaction mixture was filtered through Celite and the solvent removed *in vacuo;* following HPLC purification (see conditions below), **15a** was obtained as a yellow oil (68 mg, 40%).

### Method B

To a solution of **17a** (250 mg, 0.7 mmol) dissolved in a mixture of CH<sub>3</sub>CN (20 ml) and water (2 ml) [Bis(trifluoroacetoxy)iodo]benzene (864 mg, 2.1 mmol) was added. The reaction was stirred at room temperature for 20 minutes and then quenched with a 1:1:1 water/NaHCO<sub>3</sub>/Na<sub>2</sub>SO<sub>3</sub> mixture (20 ml in total). After an extraction with EtOAc (20 mL), the organic phase was dried over MgSO<sub>4</sub> and the solvent evaporated under reduced pressure. The crude residue was then purified by semipreparative HPLC (R<sub>t</sub> = 24 min, with a gradient elution from 0 to 50% MeCN over 30 min, then 50 to 100% MeCN over 15 min) affording **15a** as a yellow oil (125 mg, 78%). <sup>1</sup>**H**-NMR (500 MHz, (CD<sub>3</sub>)<sub>2</sub>SO)  $\delta$  = 1.57 (2H, app quin, *J* = 7.1 Hz, CH<sub>2</sub>CH<sub>2</sub>), 1.77 (3H, s, CH<sub>3</sub>CONH), 2.07 (3H, s, CH<sub>3</sub>COO), 2.52 (2H, t, *J* = 7.3 Hz, CH<sub>2</sub>), 2.97 (2H, m, CH<sub>2</sub>NH), 3.68 (2H, s, COCH<sub>2</sub>CO), 5.68 (2H, s, OCH<sub>2</sub>O), 7.80 (1H, br s, NH); <sup>13</sup>**C**-NMR (150 MHz, (CD<sub>3</sub>)<sub>2</sub>SO)  $\delta$  = 20.4 (CH<sub>3</sub>), 22.6 (CH<sub>3</sub>), 23.0 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 37.6 (CH<sub>2</sub>), 39.5 (CH<sub>2</sub>), 48.4 (CH<sub>2</sub>), 79.1 (OCH<sub>2</sub>O), 166.3 (CONH), 169.1 (CO), 169.2 (CO), 202.7 (CO); *m/z* (HR-ESI-MS): found [M+Na]<sup>+</sup> 282.0942, C<sub>11</sub>H<sub>17</sub>NO<sub>6</sub>Na<sup>+</sup> requires 282.0948.

### 1.2.8. Acetoxymethyl 6-d<sub>3</sub>-acetamido-3-oxohexanoate (15b)



Compound **15b** was prepared in the same way as **15a** (see above) from **14b** as starting material. <sup>1</sup>H-NMR (500 MHz, (CD<sub>3</sub>)<sub>2</sub>SO)  $\delta$  = 1.57 (2H, app quin, *J* = 7.1 Hz, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 2.08 (3H, s, CH<sub>3</sub>COO), 2.52 (2H, t, *J* = 7.3 Hz, CH<sub>2</sub>), 2.97 (2H, app q, *J* = 6.6, CH<sub>2</sub>NH), 3.68 (2H, s, COCH<sub>2</sub>CO), 5.68 (2H, s, OCH<sub>2</sub>O), 7.80 (1H, br s, NH); <sup>13</sup>C-NMR (150 MHz, (CD<sub>3</sub>)<sub>2</sub>SO)  $\delta$  = 20.4 (CH<sub>3</sub>), 22.6 (CH<sub>3</sub>), 23.0 (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>), 37.6 (CH<sub>2</sub>), 39.6 (CH<sub>2</sub>), 48.4 (COCH<sub>2</sub>), 79.1 (OCH<sub>2</sub>O), 166.3 (CONH), 169.1 (CO), 169.2 (CO), 202.7 (CO); *m/z* (HR-ESI-MS): found [M+Na]<sup>+</sup> 285.1132, C<sub>11</sub>H<sub>14</sub>D<sub>3</sub>NO<sub>6</sub>Na<sup>+</sup> requires 285.1136.

# 1.3. Synthesis of N-(4,6-dioxoheptyl)decanamide (20)

Compound **3** was prepared as reported previously.<sup>5</sup>

<sup>&</sup>lt;sup>5</sup> E. Riva, I. Wilkening, Ina, S. Gazzola, W. M. A. Li, L Smith, P. F. Leadlay, M. Tosin, *Angew. Chem. Int. Ed.*, **2014**, *53*, 11944—11949.

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1.3.9. Methyl-2-(2-(3-decanamidopropyl)-1,3-dithiolan-2-yl)acetate (18)



Compound **3** (800 mg, 2.55 mmol) and 1,2-ethanthiol (1.07 mL, 12.8 mmol) were dissolved in dichloromethane (16 mL) under argon atmosphere. The solution was cooled to 0 °C before  $BF_3 \cdot OEt_2$  (1.57 mL, 12.8 mmol) was slowly added. The reaction was stirred under reflux overnight. Then, a saturated solution of NaHCO<sub>3</sub> was added at 0 °C up to pH 9 and the mixture was extracted with  $CH_2Cl_2$  (3x10 mL). The crude product was purified by column chromatography (cyclohexane: EtOAc, 7:3 to 1:1)) and **18** was obtained as a white powder (600 mg, 61%).

<sup>1</sup>**H-NMR** (400 MHz, CDCl<sub>3</sub>):  $\delta = 0.87$  (3H, t, J = 6.5 Hz, CH<sub>3</sub>), 1.27 (12H, br s, (CH<sub>2</sub>)<sub>6</sub>), 1.58 (2H, m, CH<sub>2</sub>CH<sub>2</sub>CONH), 1.75 (2H, m, NHCH<sub>2</sub>CH<sub>2</sub>), 2.14 (2H, m, CH<sub>2</sub>CS<sub>2</sub>), 2.14 (2H, m, CH<sub>2</sub>CONH), 3.04 (2H, s, CH<sub>2</sub>COO), 3.29 (2H, m, NHCH<sub>2</sub>), 3.30 (4H, s, SCH<sub>2</sub>CH<sub>2</sub>S), 3.70 (3H, s, OCH<sub>3</sub>), 5.51 (1H, br s, NH); <sup>13</sup>**C-NMR** (101 MHz, CDCl<sub>3</sub>):  $\delta = 14.1$  (CH<sub>3</sub>), 22.6 (CH<sub>2</sub>CH<sub>3</sub>), 22.6 (CH<sub>2</sub>CH<sub>2</sub>CS<sub>2</sub>), 25.8 (CH<sub>2</sub>), 27.1(CH<sub>2</sub>), 29.3 (CH<sub>2</sub>), 29.3 (CH<sub>2</sub>), 29.4 (CH<sub>2</sub>), 29.4 (CH<sub>2</sub>), 36.9 (CH<sub>2</sub>CONH), 39.1 (CH<sub>2</sub>CS<sub>2</sub>), 39.4 (SCH<sub>2</sub>CH<sub>2</sub>S), 39.8 (NHCH<sub>2</sub>), 48.2 (CH<sub>2</sub>COO), 51.7

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(OCH<sub>3</sub>), 66.6 (CS<sub>2</sub>), 170.5 (C=O), 173.1 (C=O); *m/z* (HR-ESI-MS): found [M+H]<sup>+</sup> 412.1945, C<sub>19</sub>H<sub>36</sub>NO<sub>3</sub>S<sub>2</sub><sup>+</sup> requires 412.1951.

### 1.3.10. Potassium 2-(2-(3-decanamidopropyl)-1,3-dithiolan-2-yl)acetate (26)



To a solution of compound **18** (600 mg, 1.54 mmol) in dry THF (10 mL) containing 3 Å molecular sieves, was added potassium trimethilsilanolate (789 mg, 6.16 mmol). The reaction was stirred under argon atmosphere for 3 h at room temperature. Then the reaction was filtered under vacuum and concentrated. The residue was dissolved in water (5 mL), EtOAc (5 mL) was added, and the two phases were separated. The aqueous layer was freeze-dried affording **26** as a white powder (565 mg, 86%).

<sup>1</sup>**H-NMR** (400 MHz, D<sub>2</sub>O):  $\delta = 0.79$  (3H, br s, CH<sub>3</sub>), 1.22 (12H, br s, (CH<sub>2</sub>)<sub>6</sub>), 1.53 (2H, br s, CH<sub>2</sub>CH<sub>2</sub>CONH), 1.64 (2H, br s, NHCH<sub>2</sub>CH<sub>2</sub>), 1.84 (4H, s, CH<sub>2</sub>CS<sub>2</sub>), 2.01 (2H, m, CH<sub>2</sub>CO), 2.17 (2H, t, *J* = 7.2 Hz, CH<sub>2</sub>COOK), 3.13 (2H, m, CH<sub>2</sub>NH), 3.22 (4H, m, SCH<sub>2</sub>CH<sub>2</sub>S); <sup>13</sup>**C-NMR** (101 MHz, D<sub>2</sub>O):  $\delta = 13.2$  (CH<sub>3</sub>), 21.9 (CH<sub>2</sub>CH<sub>3</sub>), 25.3 (CH<sub>2</sub>CH<sub>2</sub>CS<sub>2</sub>), 26.1 (CH<sub>2</sub>), 28.2 (CH<sub>2</sub>), 28.4 (CH<sub>2</sub>), 28.5 (CH<sub>2</sub>), 28.7 (CH<sub>2</sub>), 31.1 (CH<sub>2</sub>CONH), 35.5 (CH<sub>2</sub>CONH), 38.2 (CH<sub>2</sub>CS<sub>2</sub>), 38.7 (SCH<sub>2</sub>CH<sub>2</sub>S), 39.4 (SCH<sub>2</sub>CH<sub>2</sub>S), 50.1 (CH<sub>2</sub>COO), 67.1 (CS<sub>2</sub>), 175.7 (C=O), 177.4 (C=O), 180.9 (C=O); *m/z* (ESI-MS): found [M-H]<sup>-</sup> 374.2, C<sub>18</sub>H<sub>32</sub>NO<sub>3</sub>S<sub>2</sub><sup>-</sup> requires 374.2.

### 1.3.11. Acetoxymethyl-2-(2-(3-decanamidopropyl)-1,3-dithiolan-2-yl)acetate (19)



Compound **26** (545 mg, 1.32 mmol) was suspended in dry THF (9 mL) and bromoethylacetate (140  $\mu$ L, 1.58 mmol) was added. The reaction was stirred at room temperature overnight and afterwards concentrated under vacuum. The crude product was purified by column chromatography (EtOAc/CycloHex 6:4) to yield **19** as a white powder (220 mg, 40%).

<sup>1</sup>**H-NMR** (400 MHz, CDCl<sub>3</sub>):  $\delta = 0.88$  (3H, t, J = 6.4 Hz, CH<sub>3</sub>), 1.29 (12H, br s, (CH<sub>2</sub>)<sub>6</sub>), 1.27 (2H, m, CH<sub>2</sub>CH<sub>2</sub>CONH), 1.56 (2H, m, NHCH<sub>2</sub>CH<sub>2</sub>), 2.12 (3H, s, COCH<sub>3</sub>), 2.13 (2H, m, CH<sub>2</sub>CS<sub>2</sub>), 2.13 (2H, m, CH<sub>2</sub>CONH), 3.08 (2H, s, CH<sub>2</sub>COCH<sub>3</sub>), 3.30 (2H, m, NHCH<sub>2</sub>), 3.31 (4H, s, SCH<sub>2</sub>CH<sub>2</sub>S), 5.55 (1H, br s, NH), 5.75 (1H, s, OCH<sub>2</sub>O); <sup>13</sup>C-NMR (101 MHz, CDCl<sub>3</sub>):  $\delta = 14.1$  (CH<sub>3</sub>), 20.7 (COCH<sub>3</sub>), 22.7 (CH<sub>2</sub>CH<sub>3</sub>), 22.7 (CH<sub>2</sub>CH<sub>2</sub>CS<sub>2</sub>), 25.8 (CH<sub>2</sub>), 27.1 (CH<sub>2</sub>), 29.3 (CH<sub>2</sub>), 29.4 (CH<sub>2</sub>), 29.5 (CH<sub>2</sub>), 36.9 (CH<sub>2</sub>CONH), 39.1 (CH<sub>2</sub>CS<sub>2</sub>), 39.4 (SCH<sub>2</sub>CH<sub>2</sub>S), 39.8 (NHCH<sub>2</sub>), 48.2 (CH<sub>2</sub>COO), 66.4 (CS<sub>2</sub>), 79.2 (OCH<sub>2</sub>O), 168.6 (C=O), 169.9 (C=O),173.2 (C=O); *m/z* (HR-ESI-MS): found [M+Na]<sup>+</sup> 470.2006, C<sub>21</sub>H<sub>37</sub>NNaO<sub>5</sub>S<sub>2</sub><sup>+</sup> requires 470.2005.

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### 1.3.12. Acetoxymethyl-6-decanamido-3-oxohexanoate (20)



Compound **19** (200 mg, 0.45 mmol) was dissolved in acetonitrile (12 mL) and water (1.12 mL) and [bis(trifluoroacetoxy)iodo]benzene (576 mg, 1.34 mmol) was added. The reaction was stirred at room temperature for 10 minutes, quenched with  $H_2O/NaHCO_3$  sat./ $Na_2S_2O_3$  sat. solution (1:1:1, 12 mL). The organic solvent was separated and removed under reduced pressure. The final mixture was extracted with dichloromethane (3x5 mL), the organic layer was dried, filtered, concentrated and the crude was purified by column chromatography (eluent 7:3 EtOAc/CycloHex). **20** was obtained as a white powder (80 mg, 40%). The compound was further purified by semipreparative HPLC ( $R_t = 17.7$  min, with a gradient elution from 30 to 70% MeCN over 10 min, 70% MeCN for 10 min, then 70 to 100% MeCN over 5 min).

<sup>1</sup>**H-NMR** (400 MHz, CDCl<sub>3</sub>):  $\delta = 0.86$  (3H, t, J = 6.1 Hz, CH<sub>3</sub>), 1.25 (12H, br s, (CH<sub>2</sub>)<sub>6</sub>), 1.59 (2H, m, CH<sub>2</sub>CH<sub>2</sub>CONH), 1.80 (2H, m, CH<sub>2</sub>CH<sub>2</sub>CO), 2.12 (3H, s, COCH<sub>3</sub>), 2.14 (2H, m, CH<sub>2</sub>CONH), 2.60 (2H, t, J = 6.8 Hz, CH<sub>2</sub>CO), 3.24 (2H, m, NHCH<sub>2</sub>), 3.50 (2H, s, COCH<sub>2</sub>CO), 5.73 (1H, br s, NH), 5.75 (1H, s, OCH<sub>2</sub>O); <sup>13</sup>**C-NMR** (101 MHz, CDCl<sub>3</sub>):  $\delta = 14.1$  (CH<sub>3</sub>), 20.5 (COCH<sub>3</sub>), 22.1 (CH<sub>2</sub>CH<sub>3</sub>), 23.9 (CH<sub>2</sub>), 25.8 (CH<sub>2</sub>), 29.3 (CH<sub>2</sub>), 29.3 (CH<sub>2</sub>), 29.4 (CH<sub>2</sub>), 31.8 (CH<sub>2</sub>), 31.8 (CH<sub>2</sub>), 38.5 (CH<sub>2</sub>CONH), 40.4 (NHCH<sub>2</sub>), 40.4 (CH<sub>2</sub>COCH<sub>2</sub>), 48.7 (COCH<sub>2</sub>CO), 79.5 (OCH<sub>2</sub>O), 166.1 (C=O), 169.7 (C=O), 173.4 (C=O), 200.8 (C=O); *m/z* (HR-ESI-MS): found [M+Na]<sup>+</sup> 394.2202, C<sub>19</sub>H<sub>33</sub>NNaO<sub>6</sub><sup>+</sup> requires 394.2200.

### 1.3.13. Synthesis of methyl 6-(10-azidodecanamido)-3-oxohexanoate (4)

Compound **4** was prepared as reported previously.<sup>5</sup>



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# 2. Growth of S. lasaliensis ACP12 (S970A) and mass spectrometry analysis

*S. lasaliensis* ACP12 (S970A)<sup>6</sup> was grown in M79 medium (10 mL) for 3 days at 30 °C. This seed culture was used to inoculate MYM liquid cultures (10 mL, in duplicate copy). These were incubated at 30°C and shaken at 180 rpm for a total of 5 days. After the first day of incubation, the 0.01 mmol of probes (**3**, **21**, **8**) were added daily in 50  $\mu$ L of MeOH. Control liquid cultures in absence of the probes were also prepared. After incubation all the cultures were extracted twice with ethyl acetate (10 mL x 2). The extracts were concentrated and the residues were redissolved in HPLC-grade methanol (1 mL) for mass spectrometry analysis. The Staudinger reaction on organic extracts of feeding experiments with probe **8** was performed as described preiviously.<sup>5</sup>

HPLC-HR-ESI-MS analyses of S. lasaliensis ACP12 (S970A) extracts were performed on:

1) <u>a MaXis Impact UHR-ESI-TOF</u> (Bruker Daltonics). <u>Method 1</u>: 10% B 0-2.7 min; 10-100% B 2.7-42.7 min; 100% B 42.7-62.7 min; 100-10% B 62.7-65.7 min; 10% B 65.7-77.7 min, using an Acquity UPLC HSS T3 column at a flow rate of 0.05 mL/min. <u>Method 2</u>: 5% B 0-5.3 min; 5-100% B 5.3-17.3 min; 100% B 17.3-22.3 min; 100-5% B 22.3-25.3 min; 5% B 25.3-35.3 min, using Agilent Eclipse C18 at a flow rate of 0.2 mL/min. Spectra were recorded in positive ionisation mode, scanning from *m/z* 50 to 2000, with Capillary Voltage set at 3500 V, Dry Heater set at 180°C and UV Lamp set at 210 nm. Selected ion search within 5 ppm was performed, as well as high resolution fragmentation for the putative biosynthetic intermediates.

2) <u>a Thermo Orbitrap Fusion (Q-OT-qIT, Thermo) instrument</u>. Reversed phase chromatography was used to separate the mixtures prior to MS analysis. Two columns were utilized: an Acclaim PepMap  $\mu$ -precolumn cartridge 300  $\mu$ m i.d. x 5 mm 5  $\mu$ m 100 Å and an Acclaim PepMap RSLC 75  $\mu$ m x 15 cm 2  $\mu$ m 100 Å (Thermo Scientific). The columns were installed on an Ultimate 3000 RSLCnano system (Dionex). Mobile phase buffer A was composed of 0.1% (v/v) aqueous formic acid and mobile phase B was composed of 100% acetonitrile containing 0.1% (v/v) formic acid. Samples were loaded onto the  $\mu$ -precolumn equilibrated in 2% aqueous acetonitrile containing 0.1% (v/v) trifluoroacetic acid for 8 min at 10  $\mu$ L min<sup>-1</sup> after which compounds were eluted onto the analytical column following a 75 min gradient for which the mobile phase B concentration was increased from 50% B to 99.5% over 15 min, then maintained at 99.5% B for 35 minutes, then decreased to 50% over 16 min, followed by a 9 min wash at 50% B. Eluting cations were converted to gas-phase ions by electrospray ionization and analyzed. Survey scans of precursors from 150 to 1500 *m/z* were performed at 60K resolution (at 200 *m/z*) with a 5 × 10<sup>5</sup> ion count target. Tandem MS was performed by isolation at 0.7 Th with the quadrupole, HCD fragmentation with normalized collision energy of 30, and rapid scan MS analysis in the ion trap. The MS<sup>2</sup> ion count target was set to 10<sup>4</sup> and the maximum injection time was 35 ms. A filter targeted inclusion mass list was used to select the precursor ions. The dynamic

<sup>&</sup>lt;sup>6</sup> M. Tosin, L. Smith, P. F. Leadlay *Angew. Chem. Int. Ed*, **2011**, *50*, 11930-11933.

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exclusion duration was set to 45 s with a 10 ppm tolerance around the selected precursor and its isotopes. Monoisotopic precursor selection was turned on. The instrument was run in top speed mode with 5 s cycles, meaning the instrument would continuously perform  $MS^2$  events until the list of nonexcluded precursors diminishes to zero or 5 s, whichever is shorter. Fusion runs were performed with Survey scans of precursors from 150 to 1500 *m/z* 60K resolution (at 200 *m/z*) with a 1 × 10<sup>6</sup> ion count target. Tandem MS was performed by isolation at 1.8 Th with the ion-trap, CAD fragmentation with normalized collision energy of 32, and 15K resolution scan MS analysis in the Orbitrap. The data dependent top 20 precursors were selected for  $MS^2$ .  $MS^2$  ion count target was set to 4 × 10<sup>6</sup> and the max injection time was 50 ms. The dynamic exclusion duration was set to 40 s with a 10 ppm tolerance around the selected precursor and its isotopes.

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# 2.1. Summary of captured intermediates for S. lasaliensis ACP12(S970A)



**Figure 1S**: Overview of ester probes utilized for *in vivo* intermediate capture in *S. lasaliensis* ACP12 (S970A) together with estimated *in vivo* deprotection yields (qualitative estimation by LC-MS).

**Table 1S:** Overview of intermediates captured and characterized from *S. lasaliensis* ACP12 (S970A) *via* probes **1a-b**, **3**, **4**, **15a-b** and **20** (detected on Maxis impact, MI, and Orbitrap Fusion, OF).

intermediate	putative structure	probes 1 a-b <sup>[c]</sup> R <sub>1</sub> = CH <sub>3</sub> /CD <sub>3</sub> P= CH <sub>3</sub> short chain/ Me	probe 3 <sup>(b)</sup> R <sub>1</sub> = (CH <sub>2</sub> ) <sub>8</sub> CH <sub>3</sub> P= CH <sub>3</sub> deca chain/ Me	probe 4 <sup>[c]</sup> R <sub>1</sub> = (CH <sub>2</sub> ) <sub>8</sub> N <sub>3</sub> P= CH <sub>3</sub> N <sub>3</sub> deca/ Me	probes 15 a-b <sup>[d]</sup> R <sub>1</sub> = CH <sub>3</sub> /CD <sub>3</sub> P= CH <sub>2</sub> CO <sub>2</sub> CH <sub>3</sub> short chain/ AM	probe 20 <sup>[d]</sup> R <sub>1</sub> = (CH <sub>2</sub> ) <sub>8</sub> CH <sub>3</sub> P= CH <sub>2</sub> CO <sub>2</sub> CH <sub>3</sub> deca chain/ AM
Diketides		1	✓ (MI, OF)	✓ (MI, OF)		✓ (MI, OF)
		1	✓ (OF)	(MI, OF)		✓ (MI, OF)
		1	✓ (OF)	1	1	✓ (MI, OF)
Triketides			✓ (OF)	✓ (MI, OF)		✓ (OF)
	R <sub>1</sub> H O OH		✓ (OF)			✓ (MI, OF)
			✓ (OF)			✓ (MI, OF)

		1	1	1
		(MI, OF)	(OF)	(MI, OF)
Tetraketides		1		1
		(MI, OF)		(MI, OF)
		1	1	1
	" н о он	(OF)	(MI, OF)	(MI, OF)
	RT N N	1	1	1
	H Ö	(OF)	(MI, OF)	(OF)
Pentaketides		1	1	1
	0 0	(MI, OF)	(MI, OF)	(MI, OF)
		1	1	1
	U Un	(MI, OF)	(OF)	(OF)
	RI N I STATISTICS	1	1	
	0	(MII)	(MI)	
		1	1	1
	0	(OF)	(MI, OF)	(MI, OF)
Hexaketides	Ry Ry	1		1
		(MI, OF)		(MI, OF)
	Ry Down	1		
	U Un	(MI, OF)		
Heptaketides		1		1
		(MI)		(MI)
Octaketides		1		1
		(MI, OF)		(MI, OF)
Nonaketides		1	~	1
		(MI)	(MI)	 (MI)
			1	1
			(MI)	(MI)

Undecaketides		(MI) 🗸		
				✓ (MI
Dodecaketides		<b>/</b> (MI)	(MI)	(MI)
		(((())))	((()))	(MI)
	Rr I N OH OH OH	✓ (MI)	✓ (MI)	✓ (MI)
		✓ (MI)	✓ (MI)	✓ (MI)





**Figure 25:** (A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **3** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) for probe **3** (Rt = 13.82 min) and (B) EIC for the decarboxylated probe **31** (Rt = 13.65 min) are shown. (C) The high resolution masses of probe **3** and (D) of the hydrolysed-decarboxylated probe **31** are shown.

A) Intens. EIC 298.2377 ± 0.0100 x10 5 control 2 20.2 feeding 1 0 18.5 19.0 20.0 20.5 18.0 19.5 21.0 21.5 22.0 Time [min] B) Intens 20.2 min x10<sup>5</sup> 298.2384 1.5 N ő ő  $\mathbf{H}^{\dagger}$ 1.0 C<sub>17</sub>H<sub>32</sub>NO<sub>3</sub>+ m/z [M+H]\*: 298.2377 0.5 299.2425 32 300.2441 0.0 300 299 298 301 m/z ↓ 13.01 C) NI · 1 45F7 m/z= 298.2362-298.2392 F: FTMS + p NSI Full ms 10000000 10000000 5000000 0 10 15 20 25 30 Time (min) D) 144.1020 100 RT: 13.00 HaN Full ms2 298.2362@hcd32.00 ö ö 90 C<sub>7</sub>H<sub>14</sub>NO<sub>2</sub>+ 80 m/z [M+H]\*: 144.1019 C<sub>7</sub>H<sub>11</sub>O<sub>2</sub><sup>+</sup> calc: 127.0754 70 09 of 1 127 0755 o J⊕ al multi-In qP 50 -NH<sub>3</sub> Relative C<sub>10</sub>H<sub>19</sub>O<sup>+</sup> m/z [M]<sup>+</sup>: 155.1430 40 0 || 30 . NH₃ 20 C10H22NO+ 155.1433 m/z [M+H]+: 172.1696 10 172,1697 0 300 280 120 140 240 260 160 180 200 220 m/z

**Figure 35:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **3** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **32** (Rt = 20.2 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **3** (final concentration 4 mM): EIC (Rt = 13.01 min) and (D) fragmentation of **32** with putative fragment structural assignment.



**Figure 4S**: (A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **3** (final concentration 4 mM): EIC (Rt = 12.79 min) and (B) fragmentation of putative intermediate **33** with putative fragment structural assignment.



**Figure 55:** (A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the in the presence of **3** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC), (B) high resolution mass (Rt = 14.75 min) and (C) fragmentation of putative intermediate **34** with putative fragment structural assignment are shown.

Û A) NL: 2.60E5 15.43 m/z=352.2828-352.2864 250000 -F: FTMS + p NSI Fullms 200000 Intensity 150000 100000 50000 0 5 25 10 15 20 Time (min) 0 Ъ́н C<sub>21</sub>H<sub>38</sub>NO<sub>3</sub>\* m/z [M+H]\*: 352.2846 35 B) RT: 15.35 180 Full ms2 352.2829@hcd32.00 160 Ňн ö C11H18NO\* 140 m/z [M+H]\*: 180.1383 Relative Abundance 00 00 07 180.1378 60  $\mathsf{C}_{11}\mathsf{H}_{16}\mathsf{N}^{\scriptscriptstyle +}$ m/z [M+H]+: 162.1277 40 162.1273 20 -H<sub>2</sub>O 0 150 160 170 180 190 200 210 220 230 m/z

**Figure 6S:** (A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **3** (final concentration 4 mM): EIC (Rt = 15.43 min) and (B) fragmentation of putative intermediate **35** with putative fragment structural assignment.



**Figure 7S**: A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **3** (final concentration 4 mM): EIC (Rt = 15.88 min) and (B) fragmentation of putative intermediate **36** with putative fragment structural assignment.

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**Figure 8S:** A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **3** (final concentration 4 mM): EIC (Rt = 17.05 min) and (B) fragmentation of putative intermediate **37** with putative fragment structural assignment.



**Figure 95:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **3** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **38** (Rt = 59.3 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **3** (final concentration 4 mM): EIC (Rt = 17.95min) and (D) fragmentation of **38** with putative fragment structural assignment.

A) Intens. EIC 380.3159 ± 0.0100 x10<sup>4</sup> control 1.00 59.6 feeding 0.75 0.50 0.25 0.00 B) 30 35 40 45 50 55 60 65 70 Time [min] Intens 59.6 min x10<sup>4</sup> 1.0 380.3168 0.5 C<sub>23</sub>H<sub>42</sub>NO<sub>3</sub><sup>+</sup> 381.3183 m/z [M+H]\*: 380.3159 0.0 39 380 381 382 m/z C) NL: 7.42E4 17.12 m/z= 380.3121-380.3197 60000 F: FTMS + p NSI Full ms Intensity 40000 20000 23.61 С D) 5 10 30 15 20 25 Time (min) RT: 17.14 208.1685 Full ms2 380.3143@hcd32.00 100 Luntur 90 /∥+ ∥ ∕NH O 80 C<sub>13</sub>H<sub>22</sub>NO<sup>+</sup> 70 m/z [M+H]+: 208.1696 Relative Abundance 05 09 09 30 20 10 0 200 150 250 300 350 m/z

**Figure 10S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **3** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **39** (Rt = 59.6 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **3** (final concentration 4 mM): EIC (Rt = 17.12 min) and (D) fragmentation of **39** with putative fragment structural assignment.



**Figure 11S:** (A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the in the presence of **3** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) (Rt = 17.15 min), (B) high resolution mass and (C) fragmentation of putative intermediate **40** with putative fragment structural assignment are shown.



**Figure 12S:** A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **3** (final concentration 4 mM): EIC (Rt = 18.58 min) and (B) fragmentation of putative intermediate **41** with putative fragment structural assignment.

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A)



**Figure 13S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **3** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **42** (Rt = 63.2 and 68.0 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **3** (final concentration 4 mM): EIC (Rt = 19.00 and 21.16 min) and (D) fragmentation of **42** with putative fragment structural assignment. Double peaks may arise from isomerisation (currently under investigation).

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**Figure 14S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **3** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **43** (Rt = 62.6 and 66.6 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **3** (final concentration 4 mM): EIC (Rt = 18.08 and 18.31 min) and (D) fragmentation of **43** with putative fragment structural assignment. Double peaks may arise from isomerisation (currently under investigation).



**Figure 15S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **3** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **44** (Rt = 62.4 min).



**Figure 16S:** A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **3** (final concentration 4 mM): EIC (Rt = 20.46 min) and (B) fragmentation of putative intermediate **45** with putative fragment structural assignment.



**Figure 175:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **3** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **46** (Rt = 72.8 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **3** (final concentration 4 mM): EIC (Rt = 21.85 min) and (D) fragmentation of **46** with putative fragment structural assignment.

A) Intens EIC 478.4255 ± 0.0100 x10<sup>4</sup> control 0.8 65.1 feeding 0.6 0.4 0.2 0.0 45 55 35 40 50 B) 30 60 65 70 Time [min] Intens 65.1 min x10<sup>4</sup> Ö 478.4265 ĥ 0.50 Ĥ ÓН ö 479.4279 0.25 C30H56NO3+ 480.4240 m/z [M+H]+: 478.4255 0.00 47 478 480 479 m/z C) NL: 1.81E4 m/z= 478.4231-478.4279 40000 F: FTMS + p NSI Full ms 30000 Intensity 19.59 20000 10000 С 10 25 30 5 15 20 Time (min) D) 100 RT: 19.58 Full ms2 478.4231@hcd32.00 90 80 ŇН óн 70 C<sub>20</sub>H<sub>36</sub>NO<sup>+</sup> Relative Abundance 05 09 09 m/z [M+H]+: 306.2791 30 306.2817 20 10 0 280 340 260 300 320 360 380 m/z

**Figure 18S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **3** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **47** (Rt = 65.1 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **3** (final concentration 4 mM): EIC (Rt = 19.59 min) and (D) fragmentation of **47** with putative fragment structural assignment.



**Figure 19S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **3** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **48** (Rt = 70.2 and 73.1 min). Double peaks may arise from intramolecular cyclisation or isomerisation (currently under investigation).
A) EIC 604.4936 ± 0.0100 x10 <sup>5</sup> 72.9 control 3 \_ feeding 2 1 0 35 55 65 40 45 50 60 70 Time [min] 30 B) Intens 72.8 min x10 <sup>5</sup> o 604.4941 3 `М́<sub>н</sub>+ ö ö ö ÓН 2 C<sub>37</sub>H<sub>66</sub>NO5<sup>+</sup> m/z [M+H]<sup>+</sup>: 604.4936 49 605.4974 1 606.5068 0 604 605 606 607 608 m/z NL: 6.31E5 21.40 C) m/z= 604.4906-604.4966 600000 F: FTMS + p NSI Full ms 500000 400000 Intensity 300000 200000 100000 0 35 10 5 15 20 25 30 Time (min) D) 282.2062 100 128.0706 RT: 21.40 Full ms2 604.4918@hcd32.00 90 80 ۱Œ N ö ö C<sub>16</sub>H<sub>28</sub>NO<sub>3</sub>+ 70 m/z [M]+: 282.2064 e Abundance 0 0 --Relative / 151.1481 205.1951 30 246 1234 20 163.1485 364,2861 300.2159 10 432.4531 0 600 350 400 450 500 550 150 200 250 300

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**Figure 20S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **3** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **49** (Rt = 72.9 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **3** (final concentration 4 mM): EIC (Rt = 21.40 min) and (D) fragmentation of **49** with putative fragment structural assignment. A compound of m/z [M+H]= 586.4830 (C<sub>37</sub>H<sub>64</sub>NO<sub>4</sub><sup>+</sup>) resulting from the loss of water from **49** was also detected at an identical retention time (and characterised by the same fragment highlighted in D)).

m/z



**Figure 21S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **3** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **50** (Rt = 73.0 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **3** (final concentration 4 mM): EIC (Rt = 17.83 min) and (D) fragmentation of **50** with putative fragment structural assignment.

A) Intens 72.5 EIC 732.5773 ± 0.0100 x10<sup>4</sup> control 1.00 feeding 0.75 0.50 0.25 0.00 35 40 45 50 55 60 30 70 Time [min] B) Ö Ň H H 0 ő ö óн ő óн C<sub>44</sub>H<sub>78</sub>NO7<sup>+</sup> m/z [M+H]<sup>+</sup>: 732.5773 52 Intens 72.5 min x10<sup>4</sup> 1.5 732.5777 1.0 733.5797 0.5 734.5914 0.0 734 m/z 732 733

**Figure 22S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **3** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **52** (Rt = 72.5 min).



**Figure 23S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **3** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC), (B) high resolution mass are shown for the putative intermediate **53** (Rt = 67.1 and 67.7 min) and (C) fragmentation of **53** with putative fragment structural assignment. Double peaks may arise from intramolecular cyclisation or isomerisation (currently under investigation).

A) Intens EIC 810.5754 ± 0.0100 x10 <sup>5</sup> control 70.2 feeding 2 70.6 1 0 40 45 50 55 30 35 60 65 70 Time [min] B) o `N H ö ö óн H⊥ Na Ö C<sub>47</sub>H<sub>81</sub>NNaO<sub>8</sub><sup>4</sup> m/z [M+H]+: 810.5854 юн 54 Intens. 70.2 min Intens 70.6 min x10 <sup>5</sup> 810.5856 x10 5 810.5754 2.0 1.5 1.0 1.0 811.5889 0.5 0.5 812.5917 811.5788 812,5914 0.0 0.0 812 813m/z 811 810 810 811 812 813m/z

**Figure 24S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **3** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **54** (Rt = 70.2 and 70.6 min). Double peaks may arise from intramolecular cyclisation or isomerisation (currently under investigation).





**Figure 25S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **3** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC), (B) high resolution mass are shown for the putative intermediate **55** (Rt = 70.5 and 73.4 min) and (C) fragmentation of **55** with putative fragment structural assignment. Double peaks may arise from intramolecular cyclisation or isomerisation (currently under investigation).

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# 2.3. Intermediate capture by N-(4,6-dioxoheptyl)decanamide (20)

**Figure 26S:** (A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the in the presence of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) for probe **20** (Rt = 13.44 min) and (B) EIC for the decarboxylated probe **31** (Rt = 13.00 min) are shown. (C) The high resolution masses of probe **20** and (D) of the hydrolysed- decarboxylated probe **31** are shown.

EIC 298.2377 ± 0.0100 x10 <sup>5</sup> 20.2 control 2 feeding 1 0 B) 5.0 7.5 10.0 12.5 15.0 17.5 20.0 22.5 25.0 27.5Time [min] Intens 20.2 mir ö x10 5 298.2385 N 2 ő  $\mathbf{H}^{\!+}$ ő C<sub>17</sub>H<sub>32</sub>NO<sub>3</sub>+ 1 m/z [M+H]+: 298.2377 299.2420 300.2477 32 0 299.0 300.0 298.0 m/z C) 12.99 NL: 5.87F6 m/z= 298.2362-298.2392 a hour days 5000000 F: FTMS + p NSI Full ms 4000000 Intensity 3000000 2000000 1000000 0 24 10 12 16 22 14 18 20 D) Time (min) 100 RT: 12.99 Full ms2 298.2362@hcd32.00 90 H<sub>3</sub>N ö ö 144.1019 80 C7H14NO2+ m/z [M+H]<sup>+</sup>: 144.1019 dumbruh 70 C7H11O2 calc: 127.0754 Relative Abundance 05 05 09 127.0754 0 -NH<sub>3</sub> Æ C10H19O+ m/z [M]+: 155.1430 30 0 NH<sub>3</sub> 20 155,1431 C10H22NO+ 10 m/z [M+H]+: 172.1696 172.1697 0 120 140 160 180 240 260 280 200 220 300

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A)

**Figure 27S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **32** (Rt = 20.2 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **20** (final concentration 4 mM): EIC (Rt = 12.99 min) and (D) fragmentation of **32** with putative fragment structural assignment.

m/z



**Figure 28S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **33** (Rt = 48.4 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **20** (final concentration 4 mM): EIC (Rt = 12.88 min) and (D) fragmentation of **33** with putative fragment structural assignment.

A) Intens. EIC 282.2414 ± 0.0100 x10 <sup>5</sup> control 4 15.8 feeding 2 0 7.5 15.0 17.5 B) 5.0 10.0 12.5 20.0 22.5 25.0 27.5Time [min] Intens. 15.8 min x10<sup>5</sup> 0 4 282.2427 `М́<sub>н</sub>+ 3 ő 2 C<sub>17</sub>H<sub>32</sub>NO<sub>2</sub>+ m/z[M+H]<sup>+</sup>: 282.2428 34 1 283.2468 284.2506 0 284 282 283 285 m/z C) NL: 6.18E7 15.26 m/z= 282.2414-282.2442 60000000 Lunu F: FTMS + p NSI Full ms 50000000 40000000 dan dan da 30000000 20000000 10000000 0 30 5 10 15 20 25 Time (min) D) //+ -NH RT: 15.26 100 C7H12N+ Full ms2 282.2414@hcd32.00 110.0965 m/z [M+H]+; 110.0964 120 90 80 70 e Abundance 05 0 110.5 Relative . 0 o <sup>m/z</sup> o `nH₃ 30  $C_{10}H_{19}O^{+}$ C10H22NO+ m/z [M]<sup>+</sup>: 155.1430 m/z [M+H]<sup>+</sup>: 172.1696 20 10 155,1433 172.1700 0 120 140 160 180 200 220 240 260 280

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**Figure 29S:** A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **34** (Rt = 15.8 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **20** (final concentration 4 mM): EIC (Rt = 15.26 min) and (D) fragmentation of **34** with putative fragment structural assignment.

m/z

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**Figure 30S:** (A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the in the presence of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) (Rt = 15.52 min), (B) high resolution mass and (C) fragmentation of putative intermediate **35** with putative fragment structural assignment are shown.



**Figure 31S:** A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **36** (Rt = 52.6 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **20** (final concentration 4 mM): EIC (Rt = 15.83 min) and (D) fragmentation of **36** with putative fragment structural assignment.

Intens EIC 336.2897 ± 0.0100 x104 6 control 52.6 feeding 4 2 0 45 35 40 50 55 60 65 30 Time [min] B) Intens 52.7 min x10 <sup>4</sup> 336.2890 Ň 4 C21H38NO2+ 2 m/z [M+H]+: 336.2897 337.2925 37 0 336 337 338 m/z C) NL: 1.86E5 m/z= 336.2880-336.2914 150000 F: FTMS + p NSI Full ms ntensity Û 100000 16.94 50000 0 10 25 30 35 15 20 Time (min) D) 110 // + NH -100 RT: 16.94 C<sub>11</sub>H<sub>18</sub>N⁺ Full ms2 336.2881@hcd32.00 90 m/z [M+H]+: 164.1434 80 164.1425 Relative Abundance 05 09 00 06 30 20 10 0 160 190 155 165 170 175 180 185

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A)

**Figure 32S:** A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **37** (Rt = 52.6 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **20** (final concentration 4 mM): EIC (Rt = 16.94 min) and (D) fragmentation of **37** with putative fragment structural assignment.

m/z



**Figure 33S:** A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **38** (Rt = 58.3 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **20** (final concentration 4 mM): EIC (Rt = 17.95 min) and (D) fragmentation of **38** with putative fragment structural assignment.



**Figure 34S**: A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **39** (Rt = 57.5 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **20** (final concentration 4 mM): EIC (Rt = 17.05 min) and (D) fragmentation of **39** with putative fragment structural assignment.

A) Intens EIC 382.3316 ± 0.0100 x10<sup>4</sup> 3 control feeding 24.5 2 1 0 B) 5.0 7.5 10.0 12.5 15.0 17.5 20.0 22.5 25.0 27.5 Time [min] Intens 24.5 mir x10<sup>4</sup> 382.3308 2 `М́ <sub>н</sub>і́ ő óн 1 383.2771 C23H44NO3+ 384.2739 m/z [M+H]+: 382.3316 40 0 382.5 383.0 383.5 384.0 m/z C) 17.25 NL: 1.83E5 150000 m/z= 382.3297-382.3335 F: FTMS + p NSI Full ms Intensity 100000 50000 IN. 0 15 25 5 10 зò 20 Time (min) D) RT: 17.25 110 Full ms2 382.3297@hcd32.00] 210.1853 -100 ŇĤ 90 192.1743  $C_{13}H_{22}N^{+}$ йн он 80 m/z [M+H]+: 192.1747 -H<sub>2</sub>O C<sub>13</sub>H<sub>24</sub>NO<sup>+</sup> **Relative Abundance** 70 m/z [M+H]+: 210.1852 193.1587 C<sub>13</sub>H<sub>21</sub>O<sup>+</sup> 60 Ċ m/z [M+H]<sup>+</sup>: 193.1587 50 H<sub>3</sub>N NH3 40 C<sub>10</sub>H<sub>22</sub>NO<sup>+</sup>  $C_{13}H_{24}NO^{+}$ m/z [M+H]+: 172.1696 -NH<sub>3</sub> 30 m/z [M+H]+: 210.1852 20  $\mathbf{1}$ 172.1692 10 0 170 180 190 200 210 220

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**Figure 35S:** A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **40** (Rt = 24.5 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **20** (final concentration 4 mM): EIC (Rt = 17.25 min) and (D) fragmentation of **40** with putative fragment structural assignment.

m/z

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**Figure 36S:** A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **20** (final concentration 4 mM): EIC (Rt = 18.50 min) and (B) fragmentation of putative intermediate **41** with putative fragment structural assignment.



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**Figure 375:** A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **42** (Rt = 24.1 and 25.9 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **20** (final concentration 4 mM): EIC (Rt = 19.16 and 21.12 min) and (D) fragmentation of **42** with putative fragment structural assignment. Double peaks may arise from isomerisation (currently under investigation).



**Figure 38S:** (A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the in the presence of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) (Rt = 18.89 min), (B) high resolution mass and (C) fragmentation of putative intermediate **43** with putative fragment structural assignment are shown.

A) Intens. EIC 442.3656 ± 0.0100 x10 5 2.0 control 46,5 feeding 1.5 1.0 0.5 0.0 45 55 35 40 50 60 65 Time [min] 30 B) Ö Intens 46.5 min x10 <sup>5</sup> 442.3650 `N H <sub>Na</sub>⁺ ö 1 C27H49NNaO2+ 443.3689 m/z [M+Na]+: 442.3656 444.3721 0 442.0 442.5 443.0 444.0 443.5 m/z Ън C27H50NO2+ m/z [M+H]+: 420.3836 C) 45 20.50 NL: 6.94E4 m/z= 420.3815-420.3857 60000 50000 F: FTMS + p NSI Full ms Intensity 40000 30000 20000 10000 0 5 15 25 30 10 20 Time (min) D) 248.2370 RT: 20.57 100 Full ms2 420.3820@hcd32.00] 90 -// -NH 80 C<sub>17</sub>H<sub>30</sub>N<sup>+</sup> m/z [M+H]\*: 248.2373 70 Relative Abundance 0 0 09 30 C<sub>17</sub>H<sub>29</sub>O<sup>+</sup> m/z [M+H]\*: 249.2213 20 249.2213 -10 0 248.2 248.4 248.0 248.6 248.8 249.0 249.2

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**Figure 39S:** A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **45** (Rt = 46.5 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **20** (final concentration 4 mM): EIC (Rt = 20.50 min) and (D) fragmentation of **45** with putative fragment structural assignment.

m/z



**Figure 40S:** A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **46** (Rt = 43.2 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **20** (final concentration 4 mM): EIC (Rt = 21.93 min) and (D) fragmentation of **46** with putative fragment structural assignment.



**Figure 41S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **48** (Rt = 27.1 min).



**Figure 42S:** A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **49** (Rt = 23.0 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **20** (final concentration 4 mM): EIC (Rt = 21.44 min) and (D) fragmentation of **49** with putative fragment structural assignment. A compound of m/z [M+H]= 586.4830 (C<sub>37</sub>H<sub>64</sub>NO<sub>4</sub><sup>+</sup>) resulting from the loss of water from **49** was also detected at an identical retention time (and characterised by the same fragment highlighted in D)).



**Figure 43S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **50** (Rt = 71.2 min).



**Figure 44S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **56** (Rt = 28.9 min).



**Figure 45S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **57** (Rt = 27.3 min). Double peaks may arise from intramolecular cyclisation or isomerisation (currently under investigation).



**Figure 46S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC), (B) high resolution mass are shown for the putative intermediate **53** (Rt = 26.0 min) and (C) fragmentation of **53** with putative fragment structural assignment.

A) Intens. EIC 828.5960 ± 0.0100 x10 <sup>5</sup> 25.4 control 1.0 feeding 26.7 0.5 0.0 15 20 10 25 30 Time [min] B) o Ň ő ö óн óн č Na C<sub>47</sub>H<sub>83</sub>NNaO9<sup>+</sup> m/z [M+Na]+: 828.5960 юн 58 Intens Intens 25.4 min 26.7 min x10<sup>5</sup> 1.5 x10 <sup>5</sup> 0.8 828.5974 828.5965 0.6 1.0 0.4 829.6004 829 5997 0.5 0.2 830.6042 830.6028 0.0 0.0 828 829 830 m/z 829 830 m/z C) 20.74 NL: 1.42E5 100 m/z= 806.6060-806.6222 Relative Abundance 0 0 08 00 08 F: FTMS + p NSI Full ms 19.65 0 30 5 25 10 15 20 Time (min) D) 100 RT: 19.04 = Full ms2 828.5978@hcd32.00 90 377.2661 80 70 HC Relative Abundance 05 09 09 Na C<sub>21</sub>H<sub>38</sub>NaO<sub>4</sub><sup>+</sup> m/z [M+Na]+: 377.2662 30 20 10 0 300 400 600 800 200 500 700 m/z

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**Figure 475:** LC-HRMS analysis (MaXis Impact) of the organic extracts of *S. lasaliensis ACP12 (S970A)* grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM).  $[M+H]^+$  extracted ion traces A) and high resolution mass B) are shown for a putative intermediate **58** (Rt = 25.4 and 26.7 min). Double peaks may arise from intramolecular cyclisation or isomerisation (currently under investigation).



**Figure 48S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC), (B) high resolution mass are shown for the putative intermediate **54** (Rt = 27.9 min) and (C) fragmentation of **54** with putative fragment structural assignment.



**Figure 49S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **20** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC), (B) high resolution mass are shown for the putative intermediate **55** (Rt = 26.9 and 28.5min) and (C) fragmentation of **55** with putative fragment structural assignment. Double peaks may arise isomerisation (currently under investigation).





**Figure 50S:** (A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the in the presence of **4** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) for probe **4** (Rt = 13.02 min) and (B) EIC for the decarboxylated probe **59** (Rt = 12.83 min) are shown. (C) High resolution masses of probe **4** and (D) of hydrolysed- decarboxylated probe **59** are shown.



**Figure 51S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **60** (Rt = 40.7 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **4** (final concentration 4 mM): EIC (Rt = 12.23 min) and (D) fragmentation of **60** with putative fragment structural assignment.



**Figure 52S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **61** (Rt = 15.9 min).



**Figure 53S:** (A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the in the presence of **4** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) (Rt = 13.83 min), (B) high resolution mass and (C) fragmentation of putative intermediate **62** with putative fragment structural assignment are shown.



**Figure 54S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **63** (Rt = 26.1 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **4** (final concentration 4 mM): EIC (Rt = 12.53 min) and (D) fragmentation of **63** with putative fragment structural assignment.



**Figure 55S:** (A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the in the presence of **4** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) (Rt = 16.83 min), (B) high resolution mass and (C) fragmentation of putative intermediate **64** with putative fragment structural assignment are shown.



**Figure 56S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **65** (Rt = 21.5 min).



**Figure 57S:** (A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the in the presence of **4** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) (Rt = 17.40 min) and (B) fragmentation of putative intermediate **66** with putative fragment structural assignment are shown.




**Figure 58S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **67** (Rt = 58.9 and 63.4 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **4** (final concentration 4 mM): EIC (Rt = 18.08 and 19.70 min) and (D) fragmentation of **67** with putative fragment structural assignment. Double peaks may arise from isomerisation (currently under investigation).



**Figure 59S:** (A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the in the presence of **4** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) (Rt = 17.80 min), (B) high resolution mass and (C) fragmentation of putative intermediate **68** with putative fragment structural assignment are shown.



**Figure 60S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **69** (Rt = 25.7 min).



**Figure 61S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **70** (Rt = 25.0 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **4** (final concentration 4 mM): EIC (Rt = 20.15 min) and (D) fragmentation of **70** with putative fragment structural assignment.



**Figure 62S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **71** (Rt = 21.5 min).



**Figure 63S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **72** (Rt = 20.6 min).



**Figure 64S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC), (B) high resolution mass are shown for the putative intermediate **73** (Rt = 20.0 min) and (C) fragmentation of **73** with putative fragment structural assignment.



**Figure 65S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC), (B) high resolution mass are shown for the putative intermediate **74** (Rt = 21.1 min) and (C) fragmentation of **74** with putative fragment structural assignment.



**Figure 66S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC), (B) high resolution mass are shown for the putative intermediate **75** (Rt = 20.6 and 21.2 min) and (C) fragmentation of **75** with putative fragment structural assignment. Double peaks may arise from isomerisation (currently under investigation).





**Figure 67S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** after treatment with trimethyl phosphite:  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **76** (Rt = 16.0 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **4** after treatment with trimethyl phosphite (final concentration 4 mM): EIC (Rt = 9.57 min) and (D) fragmentation of **76** with putative fragment structural assignment.



**Figure 68S:** (A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the in the presence of **4** after treatment with trimethyl phosphite:  $[M+H]^+$  extracted ion chromatogram (EIC) (Rt = 10.44 min min), (B) high resolution mass and (C) fragmentation of putative intermediate **77** with putative fragment structural assignment are shown.





**Figure 69S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** after treatment with trimethyl phosphite:  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **78** (Rt = 19.2 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **4** after treatment with trimethyl phosphite (final concentration 4 mM): EIC (Rt = 13.09 and 13.37 min) and (D) fragmentation of **78** with putative fragment structural assignment.



**Figure 70S:** (A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the in the presence of **4** after treatment with trimethyl phosphite:  $[M+H]^+$  extracted ion chromatogram (EIC) (Rt = 13.92 min min) and (B) fragmentation of putative intermediate **79** with putative fragment structural assignment are shown.





**Figure 71S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** after treatment with trimethyl phosphite:  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **80** (Rt = 20.6 and 22.2 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **4** after treatment with trimethyl phosphite (final concentration 4 mM): EIC (Rt = 14.95 and 17.02 min) and (D) fragmentation of **80** with putative fragment structural assignment. Double peaks may arise from isomerisation (currently under investigation).





**Figure 72S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** after treatment with trimethyl phosphite:  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **81** (Rt = 20.1 min). (C) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the presence of **4** after treatment with trimethyl phosphite (final concentration 4 mM): EIC (Rt = 14.50 and 15.87 min) and (D) fragmentation of **81** with putative fragment structural assignment. Double peaks may arise from isomerisation (currently under investigation).



**Figure 73S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** treated with trimethyl phosphite:  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **82** (Rt = 23.5 min).





**Figure 74S:** (A) LC-HRMS analysis (Orbitrap Fusion) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the in the presence of **4** after treatment with trimethyl phosphite:  $[M+H]^+$  extracted ion chromatogram (EIC) (Rt = 18.26 and 18.60 min), (B) high resolution mass and (C) fragmentation of putative intermediate **83** with putative fragment structural assignment are shown. Double peaks may arise from intramolecular cyclisation or isomerisation (currently under investigation).



**Figure 75S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** treated with trimethyl phosphite (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **84** (Rt = 21.8 min).



**Figure 76S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** treated with trimethyl phosphite (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC) and (B) high resolution mass are shown for the putative intermediate **85** (Rt = 21.5 and 22.4 min). Multiple peaks may arise from intramolecular cyclisation or isomerisation (currently under investigation).



**Figure 77S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** treated with trimethyl phosphite (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC), (B) high resolution mass are shown for the putative intermediate **86** (Rt = 22.8 min) and (C) fragmentation of **86** with putative fragment structural assignment. Double peaks may arise from intramolecular cyclisation or isomerisation (currently under investigation).

Ina Wilkening,\* Silvia Gazzola,\* Elena Riva, James S. Parascandolo, Lijiang Song and Manuela Tosin\*\*



**Figure 78S:** (A) LC-HRMS analysis (MaXis Impact, method 2) of the organic extracts of *S. lasaliensis* ACP12 (S970A) grown in the absence (red) and in the presence (blue) of **4** treated with trimethyl phosphite (final concentration 4 mM):  $[M+H]^+$  extracted ion chromatogram (EIC), (B) high resolution mass are shown for the putative intermediate **87** (Rt = 22.6 and 23.0 min) and (C) fragmentation of **87** with putative fragment structural assignment Double peaks may arise from isomerisation (currently under investigation).

Ina Wilkening,\* Silvia Gazzola,\* Elena Riva, James S. Parascandolo, Lijiang Song and Manuela Tosin\*\*

## 3. Spectra

# 3.1.<sup>1</sup>H- and <sup>13</sup>C-NMR of compound 13a (400 MHz, CDCl<sub>3</sub>)



# 3.2.<sup>1</sup>H- and <sup>13</sup>C-NMR of compound 13b (400 MHz, CDCl<sub>3</sub>)



## 3.3.<sup>1</sup>H- and <sup>13</sup>C-NMR of compound 14a (400 MHz, CDCl<sub>3</sub>)



# 3.4.<sup>1</sup>H- and <sup>13</sup>C-NMR of compound 14b (400 MHz, CDCl<sub>3</sub>)



# 3.5.<sup>1</sup>H- and <sup>13</sup>C-NMR of compound 16a (400 MHz, CDCl<sub>3</sub>)



# 3.6.<sup>1</sup>H- and <sup>13</sup>C-NMR of compound 17a (400 MHz, CDCl<sub>3</sub>)







# 3.8. $^{1}$ H- and $^{13}$ C-NMR of compound 15b (400 MHz, CDCl<sub>3</sub>)





# 3.10. <sup>1</sup>H- and <sup>13</sup>C-NMR of compound 24 (400 MHz, $D_2O$ )



# 3.11. <sup>1</sup>H- and <sup>13</sup>C-NMR of compound 19 (400 MHz, $CDCI_3$ )



# 3.12. <sup>1</sup>H- and <sup>13</sup>C-NMR of compound 20

