

## Supporting information

### Overproduction and identification of butyrolactones SCB1-8 in the antibiotic production superhost *Streptomyces* M1152

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#### 1. Experimental

##### Culture conditions

AlaMM liquid medium (50 mL) was inoculated with 20  $\mu$ L spores of *Streptomyces coelicolor* M1152 and incubated (30 °C, 180 rpm). After 24 h, a solution of sterile filtered precursor ( $d_5$ -propionic acid,  $d_7$ -butyric acid,  $d_8$ -DL-valine,  $d_{10}$ -L-leucine or  $d_{10}$ -L-isoleucine) was added to give a final concentration of 1 mM. This feeding procedure was repeated at 12 h intervals until a total incubation time of 5 days, after which the metabolites were extracted from these cultures and analysed.

Metabolites were extracted with 1:1 volume of ethyl acetate, dried with  $MgSO_4$  and ethyl acetate removed by under reduced pressure. The remaining residue was resuspended in 50:50 HPLC water/methanol.

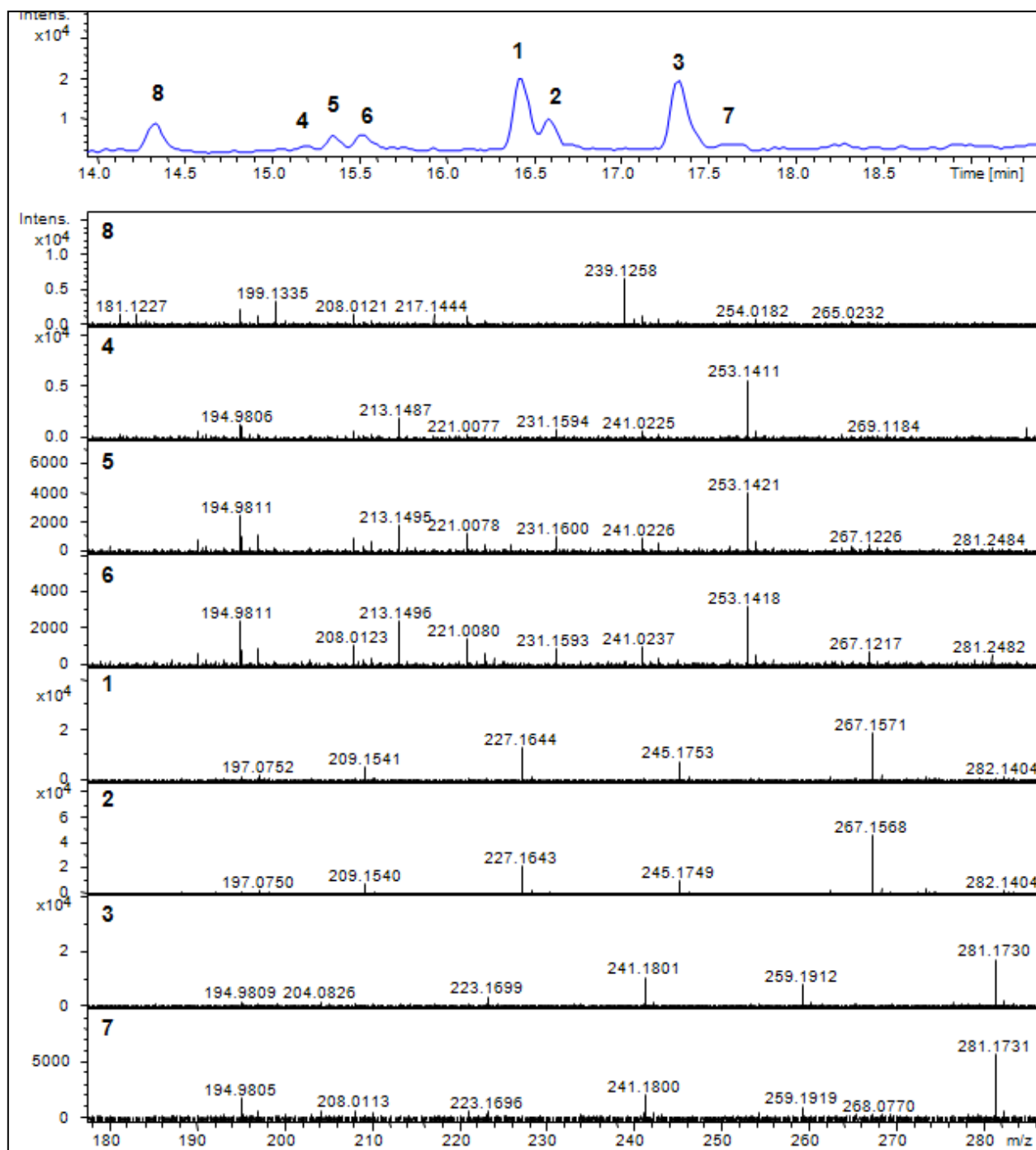
#### 2. LC-MS data

20  $\mu$ L of prepared extracts were injected through a reverse phase column (Zorbax  $C_{18}$ , size 46 x 150 mm, particle size 5  $\mu$ m) connected to an Agilent 1100 HPLC. The outflow was routed to a Bruker High Capacity Trap (HCT) + ion trap mass spectrometer with an electrospray source, operating in positive ion mode. A 5 min isocratic elution (95:5 solvent A/solvent B) was followed by gradient elution to 0:100 solvent A/solvent B over 25 min. Solvents A and B were water (0.1 % HCOOH) and methanol (0.1 % HCOOH), respectively.

The high-resolution data were obtained by performing UPLC-MS through a reverse phase column (Zorbax Eclipse Plus  $C_{18}$ , size 2.1 x 100 mm, particle size 1.8  $\mu$ m) connected to a Dionex 3000RS UHPLC coupled to Bruker Ultra High Resolution (UHR) Q-TOF MS MaXis mass spectrometer with an electrospray source. Sodium formate (10 mM) was used for internal calibration and a  $m/z = 50$  to 3000 scan range used. A 5 min isocratic elution (95:5 solvent A/solvent B) was followed by gradient elution to 0:100 solvent A/solvent B over 15.3 min. Solvents A and B were water (0.1 % HCOOH) and acetonitrile (0.1 % HCOOH), respectively.

## 2. LC-MS data

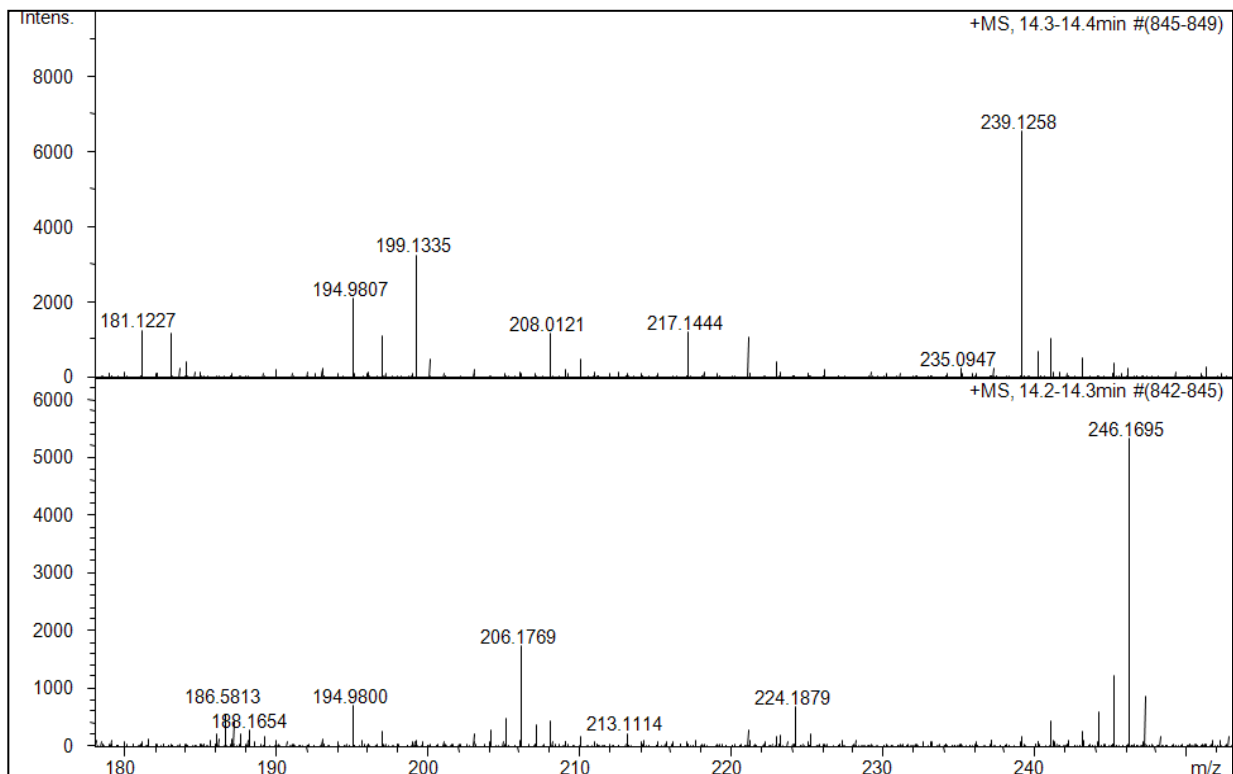
### 2.1 High resolution mass spectrometry data for SCBs 1-8



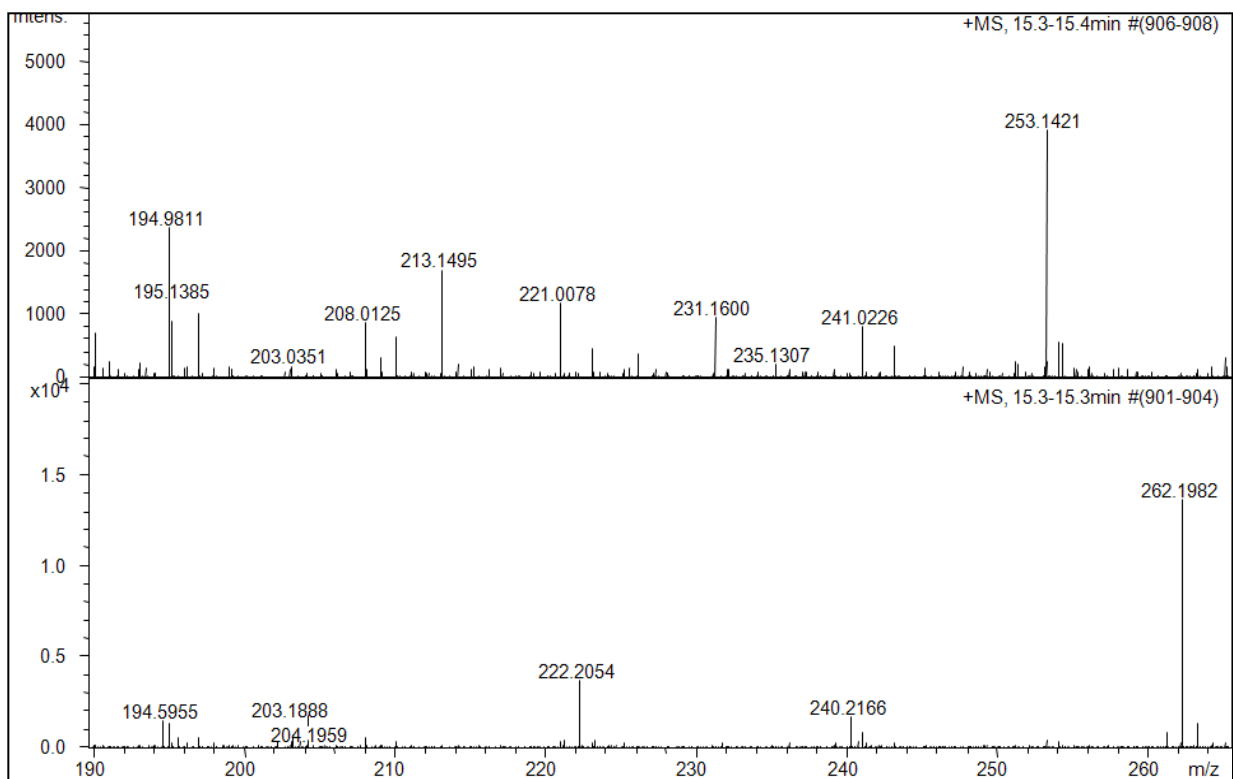
**Figure S1.** High resolution mass spectra of SCBs 1-8 present in culture extract of *S. coelicolor* M1152 grown for five days

**Table S1.** UHR-MS assignments for molecular ions and fragments observed for SCBs **1-8** extracted from *S. coelicolor* M1152 after being grown in AlaMM for five days

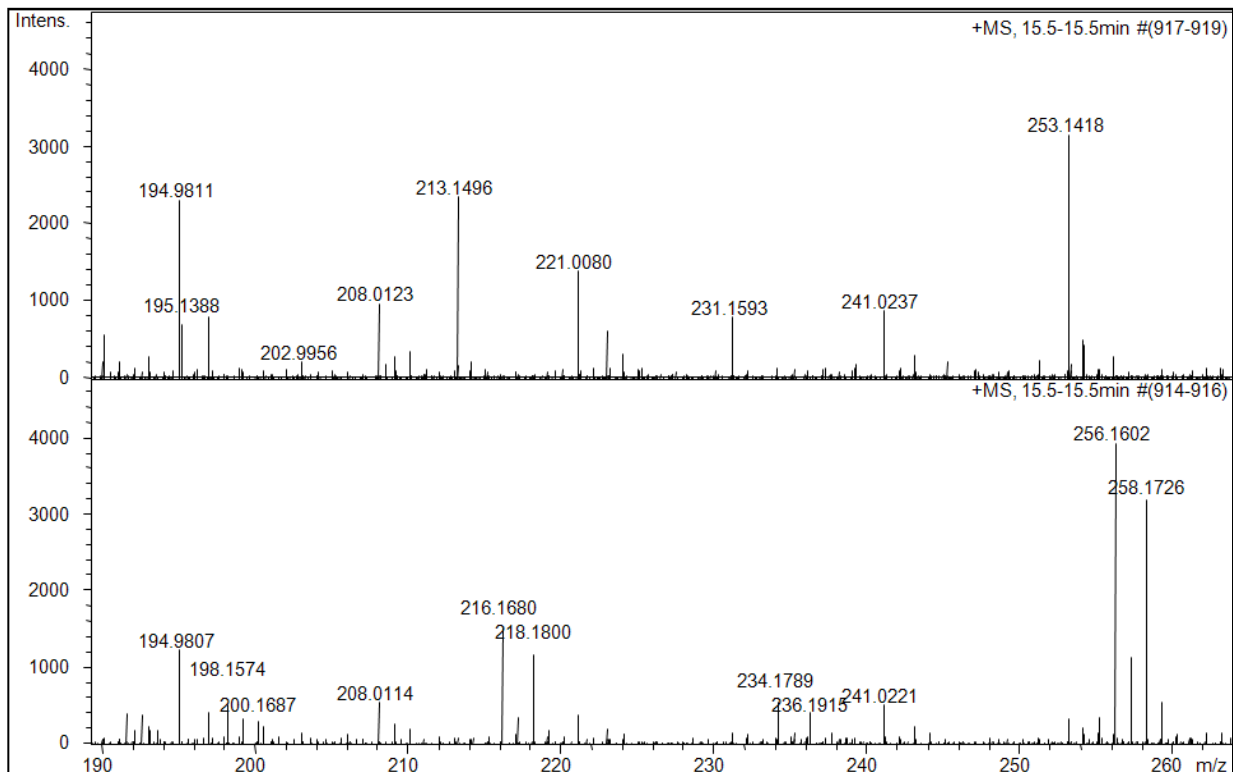
<i>retention time / min</i>	<i>compound</i>	<i>Molecular formula</i>	<i>Observed m/z</i>	<i>Calculated m/z</i>	<i>error / ppm</i>	<i>assignment</i>
14.3	SCB 8 (8)	<b>C<sub>11</sub>H<sub>20</sub>NaO<sub>4</sub></b>	239.1258	239.1254	1.9	[M+Na] <sup>+</sup>
		<b>C<sub>11</sub>H<sub>21</sub>O<sub>4</sub></b>	217.1444	217.1434	4.6	[M+H] <sup>+</sup>
		<b>C<sub>11</sub>H<sub>19</sub>O<sub>3</sub></b>	199.1335	199.1329	3.1	[M-H <sub>2</sub> O+H] <sup>+</sup>
		<b>C<sub>11</sub>H<sub>17</sub>O<sub>2</sub></b>	181.1227	181.1223	3.4	[M-2H <sub>2</sub> O+H] <sup>+</sup>
15.2	SCB 4 (4)	<b>C<sub>12</sub>H<sub>22</sub>NaO<sub>4</sub></b>	253.1411	253.1410	0.4	[M+Na] <sup>+</sup>
		<b>C<sub>12</sub>H<sub>23</sub>O<sub>4</sub></b>	231.1594	231.1591	1.5	[M+H] <sup>+</sup>
		<b>C<sub>12</sub>H<sub>21</sub>O<sub>3</sub></b>	213.1487	213.1485	1.1	[M-H <sub>2</sub> O+H] <sup>+</sup>
		<b>C<sub>12</sub>H<sub>19</sub>O<sub>2</sub></b>	195.1377	195.1380	1.2	[M-2H <sub>2</sub> O+H] <sup>+</sup>
15.3	SCB 5 (5)	<b>C<sub>12</sub>H<sub>22</sub>NaO<sub>4</sub></b>	253.1421	253.1410	4.2	[M+Na] <sup>+</sup>
		<b>C<sub>12</sub>H<sub>23</sub>O<sub>4</sub></b>	231.1600	231.1591	3.8	[M+H] <sup>+</sup>
		<b>C<sub>12</sub>H<sub>21</sub>O<sub>3</sub></b>	213.1495	213.1485	4.7	[M-H <sub>2</sub> O+H] <sup>+</sup>
		<b>C<sub>12</sub>H<sub>19</sub>O<sub>2</sub></b>	195.1385	195.1380	2.6	[M-2H <sub>2</sub> O+H] <sup>+</sup>
15.5	SCB 6 (6)	<b>C<sub>12</sub>H<sub>22</sub>NaO<sub>4</sub></b>	253.1418	253.1410	3.1	[M+Na] <sup>+</sup>
		<b>C<sub>12</sub>H<sub>23</sub>O<sub>4</sub></b>	231.1593	231.1591	1.0	[M+H] <sup>+</sup>
		<b>C<sub>12</sub>H<sub>21</sub>O<sub>3</sub></b>	213.1496	213.1485	4.9	[M-H <sub>2</sub> O+H] <sup>+</sup>
		<b>C<sub>12</sub>H<sub>19</sub>O<sub>2</sub></b>	195.1388	195.1380	4.4	[M-2H <sub>2</sub> O+H] <sup>+</sup>
16.4	SCB 1 (1)	<b>C<sub>13</sub>H<sub>24</sub>NaO<sub>4</sub></b>	267.1571	267.1567	1.7	[M+Na] <sup>+</sup>
		<b>C<sub>13</sub>H<sub>25</sub>O<sub>4</sub></b>	245.1753	245.1747	2.3	[M+H] <sup>+</sup>
		<b>C<sub>13</sub>H<sub>23</sub>O<sub>3</sub></b>	227.1644	227.1642	1.1	[M-H <sub>2</sub> O+H] <sup>+</sup>
		<b>C<sub>13</sub>H<sub>21</sub>O<sub>2</sub></b>	209.1541	209.1536	2.2	[M-2H <sub>2</sub> O+H] <sup>+</sup>
16.5	SCB 2 (2)	<b>C<sub>13</sub>H<sub>24</sub>NaO<sub>4</sub></b>	267.1568	267.1567	0.5	[M+Na] <sup>+</sup>
		<b>C<sub>13</sub>H<sub>25</sub>O<sub>4</sub></b>	245.1749	245.1747	0.6	[M+H] <sup>+</sup>
		<b>C<sub>13</sub>H<sub>23</sub>O<sub>3</sub></b>	227.1643	227.1642	0.6	[M-H <sub>2</sub> O+H] <sup>+</sup>
		<b>C<sub>13</sub>H<sub>21</sub>O<sub>2</sub></b>	209.1540	209.1536	1.7	[M-2H <sub>2</sub> O+H] <sup>+</sup>
17.3	SCB 3 (3)	<b>C<sub>14</sub>H<sub>26</sub>NaO<sub>4</sub></b>	281.1730	281.1723	2.5	[M+Na] <sup>+</sup>
		<b>C<sub>14</sub>H<sub>27</sub>O<sub>4</sub></b>	259.1912	259.1904	3.0	[M+H] <sup>+</sup>
		<b>C<sub>14</sub>H<sub>25</sub>O<sub>3</sub></b>	241.1801	241.1798	1.1	[M-H <sub>2</sub> O+H] <sup>+</sup>
		<b>C<sub>14</sub>H<sub>23</sub>O<sub>2</sub></b>	223.1699	223.1693	2.8	[M-2H <sub>2</sub> O+H] <sup>+</sup>
17.5	SCB 7 (7)	<b>C<sub>14</sub>H<sub>26</sub>NaO<sub>4</sub></b>	281.1731	281.1723	2.7	[M+Na] <sup>+</sup>
		<b>C<sub>14</sub>H<sub>27</sub>O<sub>4</sub></b>	259.1919	259.1904	5.9	[M+H] <sup>+</sup>
		<b>C<sub>14</sub>H<sub>25</sub>O<sub>3</sub></b>	241.1800	241.1798	0.9	[M-H <sub>2</sub> O+H] <sup>+</sup>
		<b>C<sub>14</sub>H<sub>23</sub>O<sub>2</sub></b>	223.1696	223.1693	1.7	[M-2H <sub>2</sub> O+H] <sup>+</sup>



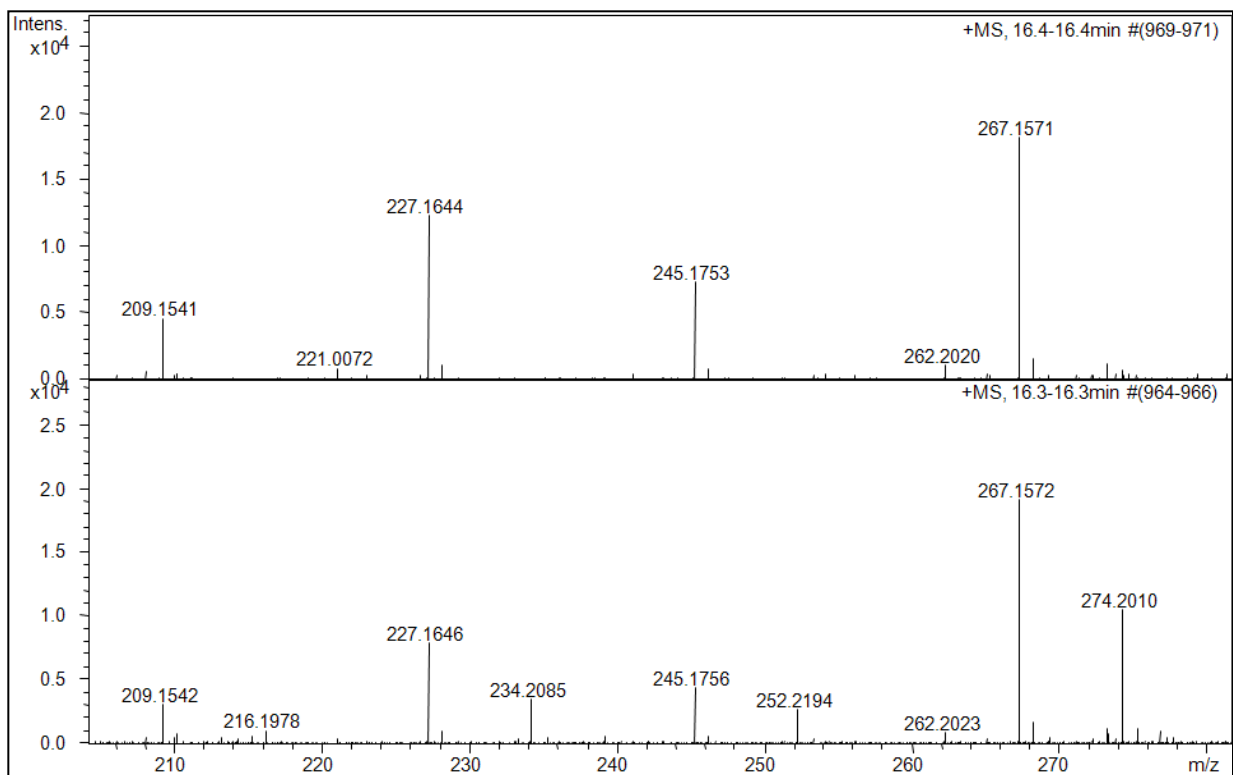
**Figure S2.** High resolution mass spectra of SCB8 (**8**) extracted from *S. coelicolor* M1152 grown on AlaMM (top panel) and AlaMM when  $d_7$ -butyric acid was added to the media (bottom panel)



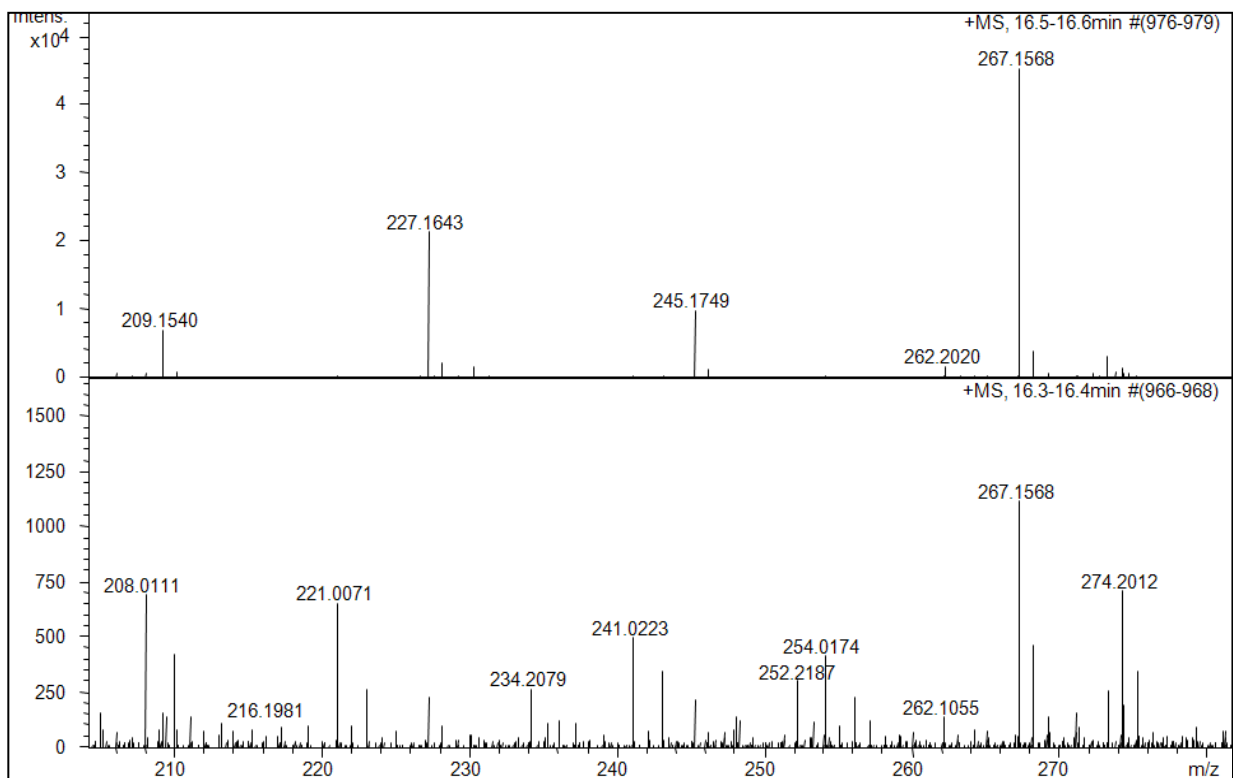
**Figure S3.** High resolution mass spectra of SCB5 (**5**) extracted from *S. coelicolor* M1152 grown on AlaMM (top panel) and AlaMM when  $d_{10}$ -leucine was added to the media (bottom panel)



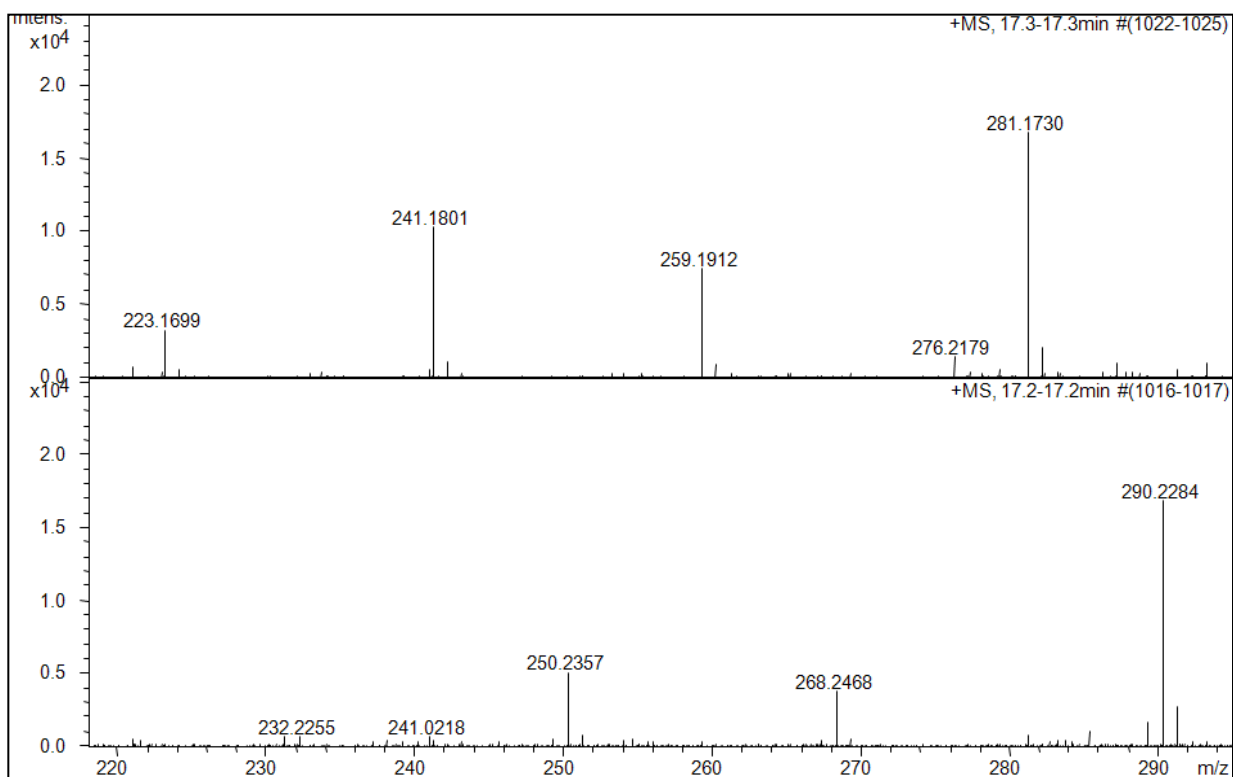
**Figure S4.** High resolution mass spectra of SCB6 (**6**) extracted from *S. coelicolor* M1152 grown on AlaMM (top panel) and AlaMM when d<sub>5</sub>-propionic acid was added to the media (bottom panel)



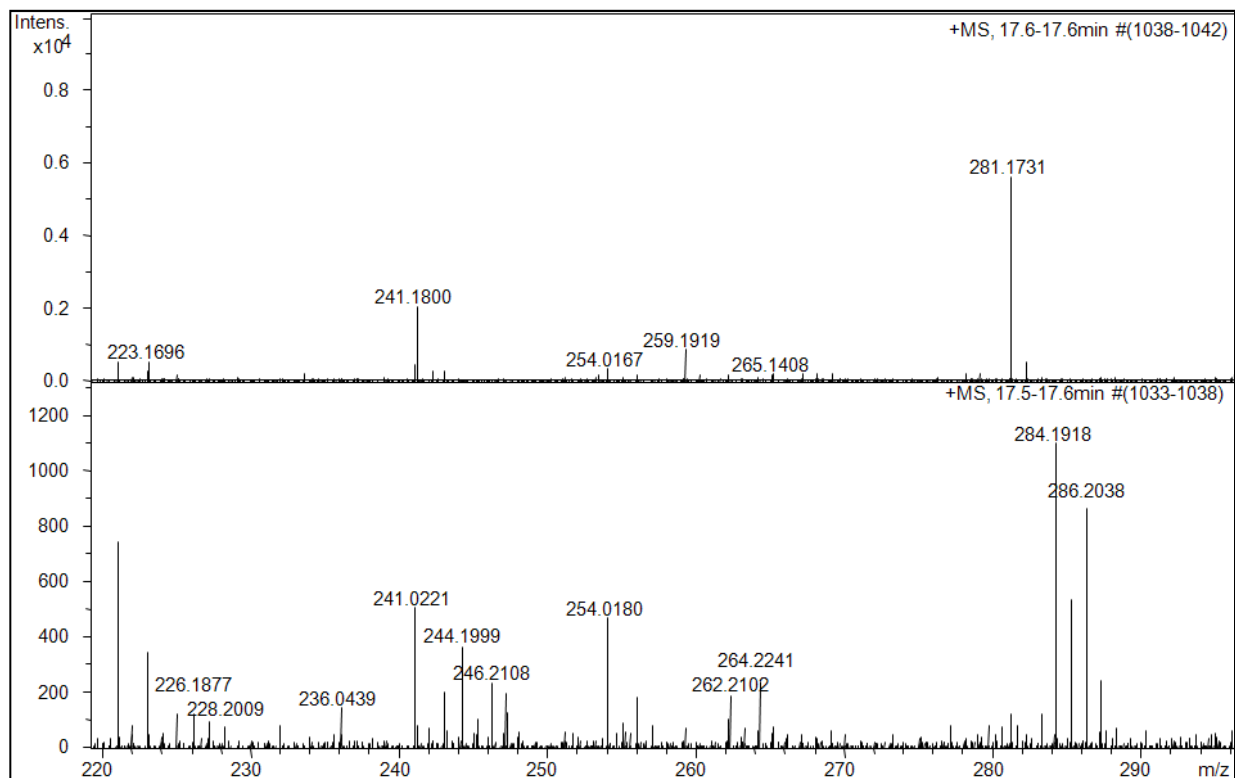
**Figure S5.** High resolution mass spectra of SCB1 (**1**) extracted from *S. coelicolor* M1152 grown on AlaMM (top panel) and AlaMM when d<sub>8</sub>-valine was added to the media (bottom panel)



**Figure S6.** High resolution mass spectra of SCB2 (**2**) extracted from *S. coelicolor* M1152 grown on AlaMM (top panel) and AlaMM when  $d_7$ -butyric acid was added to the media (bottom panel)



**Figure S7.** High resolution mass spectra of SCB3 (**3**) extracted from *S. coelicolor* M1152 grown on AlaMM (top panel) and AlaMM when  $d_{10}$ -isoleucine was added to the media (bottom panel)



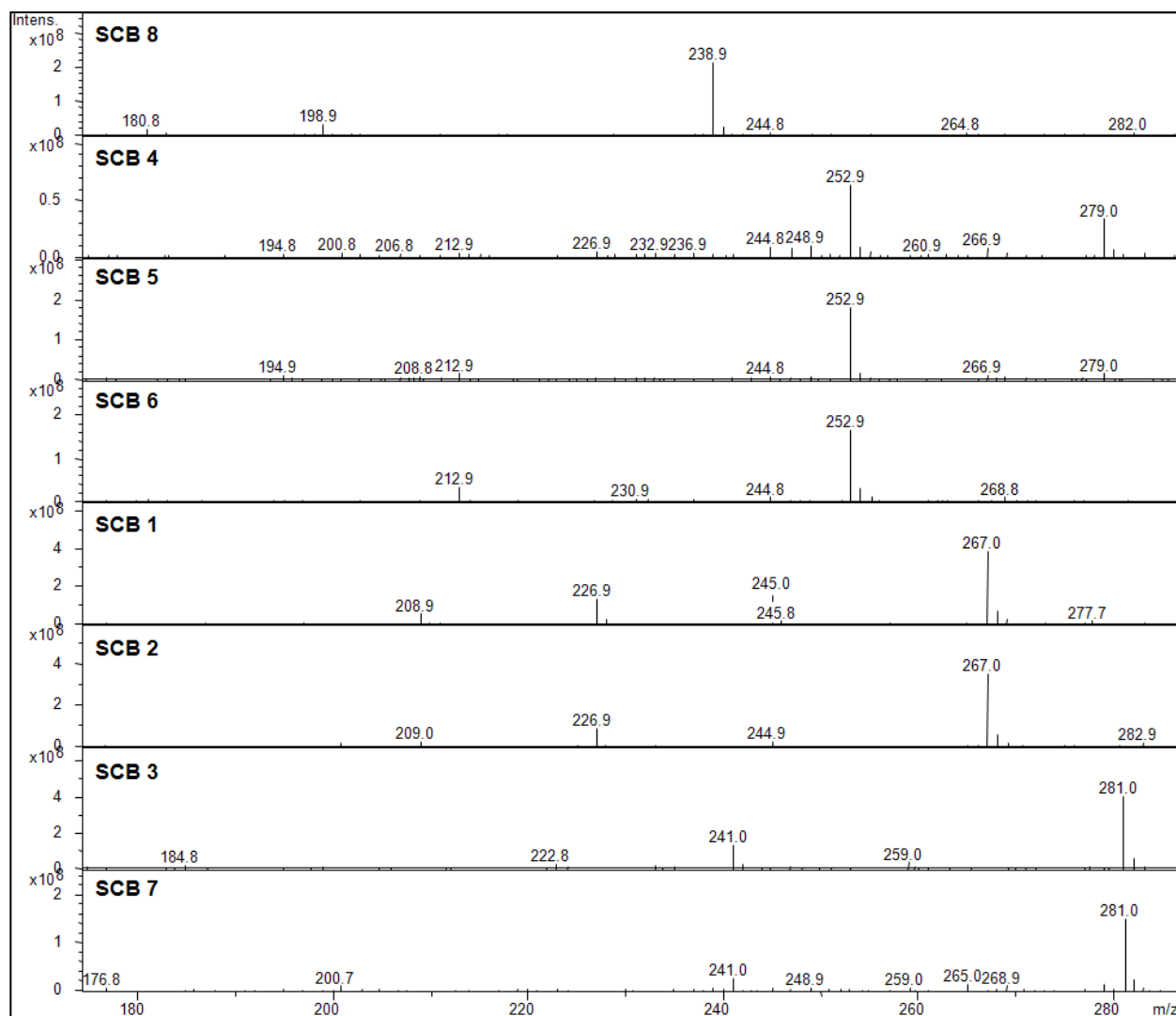
**Figure S8.** High resolution mass spectra of SCB7 (7) extracted from *S. coelicolor* M1152 grown on AlMM (top panel) and AlaMM when d<sub>5</sub>-propionic acid was added to the media (bottom panel)

**Table S2.** UHR-MS assignments for molecular ions and fragments observed for metabolites **1-8** when fed with different precursor molecules (corresponding to the spectra shown in Figures S1-S8)

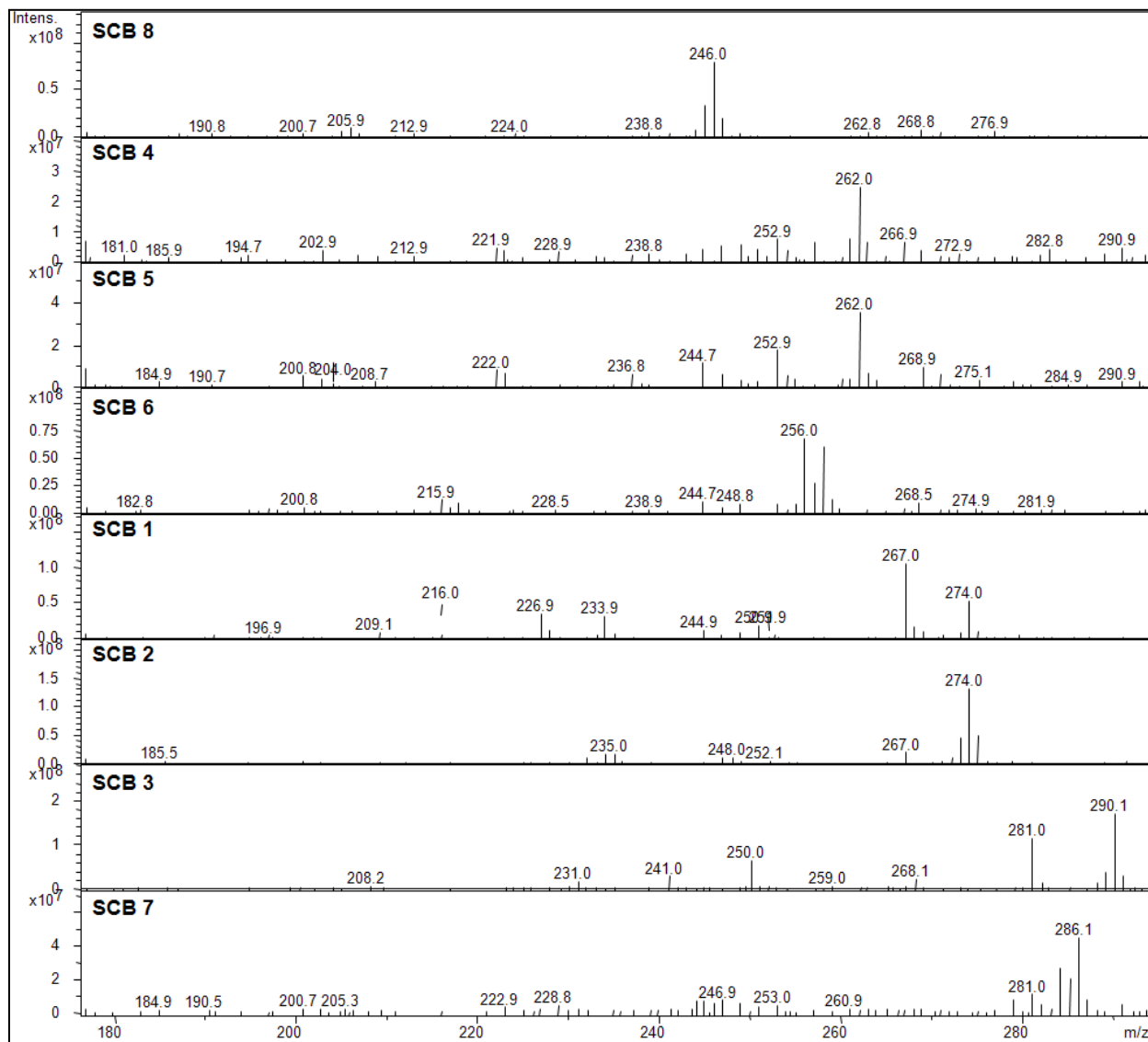
<i>retention time / min</i>	<i>compound</i>	<i>Molecular formula</i>	<i>Observed m/z</i>	<i>Calculated m/z</i>	<i>error / ppm</i>	<i>assignment</i>
14.3	d <sub>7</sub> -SCB 8 (8)	C <sub>11</sub> H <sub>13</sub> D <sub>7</sub> NaO <sub>4</sub>	246.1695	246.1693	0.6	[M+Na] <sup>+</sup>
		C <sub>11</sub> H <sub>14</sub> D <sub>7</sub> O <sub>4</sub>	224.1879	224.1874	2.5	[M+H] <sup>+</sup>
		C <sub>11</sub> H <sub>12</sub> D <sub>7</sub> O <sub>3</sub>	206.1769	206.1768	0.7	[M-H <sub>2</sub> O+H] <sup>+</sup>
		C <sub>11</sub> H <sub>10</sub> D <sub>7</sub> O <sub>2</sub>	188.1654	188.1662	4.7	[M-2H <sub>2</sub> O+H] <sup>+</sup>
15.2	SCB4 (4)	intensity of peaks too low to generate molecular formulae – see low resolution data				
15.3	d <sub>9</sub> -SCB 5 (5)	C <sub>12</sub> H <sub>13</sub> D <sub>9</sub> NaO <sub>4</sub>	262.1982	262.1975	2.5	[M+Na] <sup>+</sup>
		C <sub>12</sub> H <sub>14</sub> D <sub>9</sub> O <sub>4</sub>	240.2166	240.2156	4.3	[M+H] <sup>+</sup>
		C <sub>12</sub> H <sub>12</sub> D <sub>9</sub> O <sub>3</sub>	222.2054	222.2050	1.7	[M-H <sub>2</sub> O+H] <sup>+</sup>
		C <sub>12</sub> H <sub>10</sub> D <sub>9</sub> O <sub>2</sub>	204.0199	204.1944	7.0	[M-2H <sub>2</sub> O+H] <sup>+</sup>
15.5	d <sub>5</sub> -SCB 6 (6)	C <sub>12</sub> H <sub>17</sub> D <sub>5</sub> NaO <sub>4</sub>	258.1726	258.1724	0.7	[M+Na] <sup>+</sup>
		C <sub>12</sub> H <sub>18</sub> D <sub>5</sub> O <sub>4</sub>	236.1915	236.1905	4.3	[M+H] <sup>+</sup>
		C <sub>12</sub> H <sub>16</sub> D <sub>5</sub> O <sub>3</sub>	218.1800	218.1799	0.3	[M-H <sub>2</sub> O+H] <sup>+</sup>
		C <sub>12</sub> H <sub>14</sub> D <sub>5</sub> O <sub>2</sub>	200.1687	200.1693	3.2	[M-2H <sub>2</sub> O+H] <sup>+</sup>
15.5	d <sub>3</sub> -SCB6 (6)	C <sub>12</sub> H <sub>19</sub> D <sub>3</sub> NaO <sub>4</sub>	256.1602	256.1599	1.3	[M+Na] <sup>+</sup>
		C <sub>12</sub> H <sub>20</sub> D <sub>3</sub> O <sub>4</sub>	234.1789	234.1779	4.0	[M+H] <sup>+</sup>
		C <sub>12</sub> H <sub>18</sub> D <sub>3</sub> O <sub>3</sub>	216.1680	216.1674	3.0	[M-H <sub>2</sub> O+H] <sup>+</sup>
		C <sub>12</sub> H <sub>16</sub> D <sub>3</sub> O <sub>2</sub>	198.1574	198.1568	3.0	[M-2H <sub>2</sub> O+H] <sup>+</sup>
16.4	d <sub>7</sub> -SCB 1 (1)	C <sub>13</sub> H <sub>17</sub> D <sub>7</sub> NaO <sub>4</sub>	274.2010	274.2006	1.6	[M+Na] <sup>+</sup>
		C <sub>13</sub> H <sub>18</sub> D <sub>7</sub> O <sub>4</sub>	252.2194	252.2187	2.7	[M+H] <sup>+</sup>
		C <sub>13</sub> H <sub>16</sub> D <sub>7</sub> O <sub>3</sub>	234.2085	234.2081	1.7	[M-H <sub>2</sub> O+H] <sup>+</sup>
		C <sub>13</sub> H <sub>14</sub> D <sub>7</sub> O <sub>2</sub>	216.1978	216.1975	1.1	[M-2H <sub>2</sub> O+H] <sup>+</sup>
16.5	d <sub>7</sub> -SCB 2 (2)	C <sub>13</sub> H <sub>17</sub> D <sub>7</sub> NaO <sub>4</sub>	274.2012	274.2006	2.1	[M+Na] <sup>+</sup>
		C <sub>13</sub> H <sub>18</sub> D <sub>7</sub> O <sub>4</sub>	252.2187	252.2187	0.1	[M+H] <sup>+</sup>
		C <sub>13</sub> H <sub>16</sub> D <sub>7</sub> O <sub>3</sub>	234.2079	234.2081	0.7	[M-H <sub>2</sub> O+H] <sup>+</sup>
		C <sub>13</sub> H <sub>14</sub> D <sub>7</sub> O <sub>2</sub>	216.1981	216.1975	2.4	[M-2H <sub>2</sub> O+H] <sup>+</sup>
17.3	d <sub>9</sub> -SCB 3 (3)	C <sub>14</sub> H <sub>17</sub> D <sub>9</sub> NaO <sub>4</sub>	290.2284	290.2288	1.4	[M+Na] <sup>+</sup>
		C <sub>14</sub> H <sub>18</sub> D <sub>9</sub> O <sub>4</sub>	268.2468	268.2469	0.1	[M+H] <sup>+</sup>
		C <sub>14</sub> H <sub>16</sub> D <sub>9</sub> O <sub>3</sub>	250.2357	250.2363	2.3	[M-H <sub>2</sub> O+H] <sup>+</sup>
		C <sub>14</sub> H <sub>14</sub> D <sub>9</sub> O <sub>2</sub>	232.2255	232.2257	1.0	[M-2H <sub>2</sub> O+H] <sup>+</sup>
17.5	d <sub>5</sub> -SCB 7 (7)	C <sub>14</sub> H <sub>21</sub> D <sub>5</sub> NaO <sub>4</sub>	286.2038	286.2037	0.1	[M+Na] <sup>+</sup>
		intensity of peaks too low to generate molecular formulae				
		C <sub>14</sub> H <sub>22</sub> D <sub>5</sub> O <sub>4</sub>	246.2108	246.2112	1.8	[M-H <sub>2</sub> O+H] <sup>+</sup>
		C <sub>14</sub> H <sub>20</sub> D <sub>5</sub> O <sub>3</sub>	228.2009	228.2006	1.2	[M-2H <sub>2</sub> O+H] <sup>+</sup>
17.5	d <sub>3</sub> -SCB 7 (7)	C <sub>14</sub> H <sub>18</sub> D <sub>5</sub> O <sub>2</sub>	228.2009	228.2006	1.2	[M-2H <sub>2</sub> O+H] <sup>+</sup>
		C <sub>14</sub> H <sub>23</sub> D <sub>3</sub> NaO <sub>4</sub>	284.1918	284.1912	2.2	[M+Na] <sup>+</sup>
		C <sub>14</sub> H <sub>24</sub> D <sub>3</sub> O <sub>4</sub>	262.2102	262.2092	3.7	[M+H] <sup>+</sup>
		C <sub>14</sub> H <sub>22</sub> D <sub>3</sub> O <sub>3</sub>	244.1999	244.1987	5.0	[M-H <sub>2</sub> O+H] <sup>+</sup>
		C <sub>14</sub> H <sub>20</sub> D <sub>3</sub> O <sub>2</sub>	226.1877	226.1881	1.9	[M-2H <sub>2</sub> O+H] <sup>+</sup>



## 2.2 Low resolution mass spectrometry data for SCB1-8 (1-8)

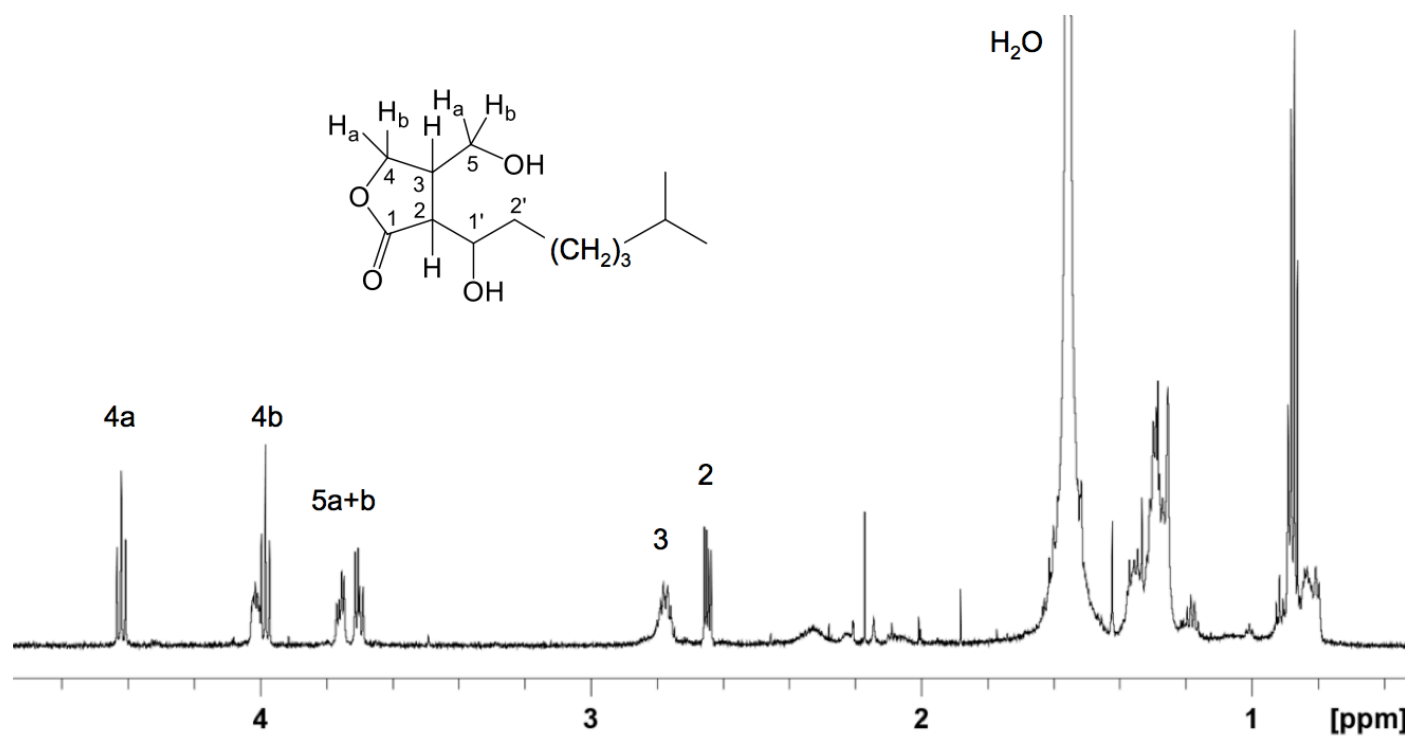


**Figure S9.** Low resolution mass spectra for metabolites **1-8** (SCB1-8) present in culture extract of *S. coelicolor* M1152 grown for five days.

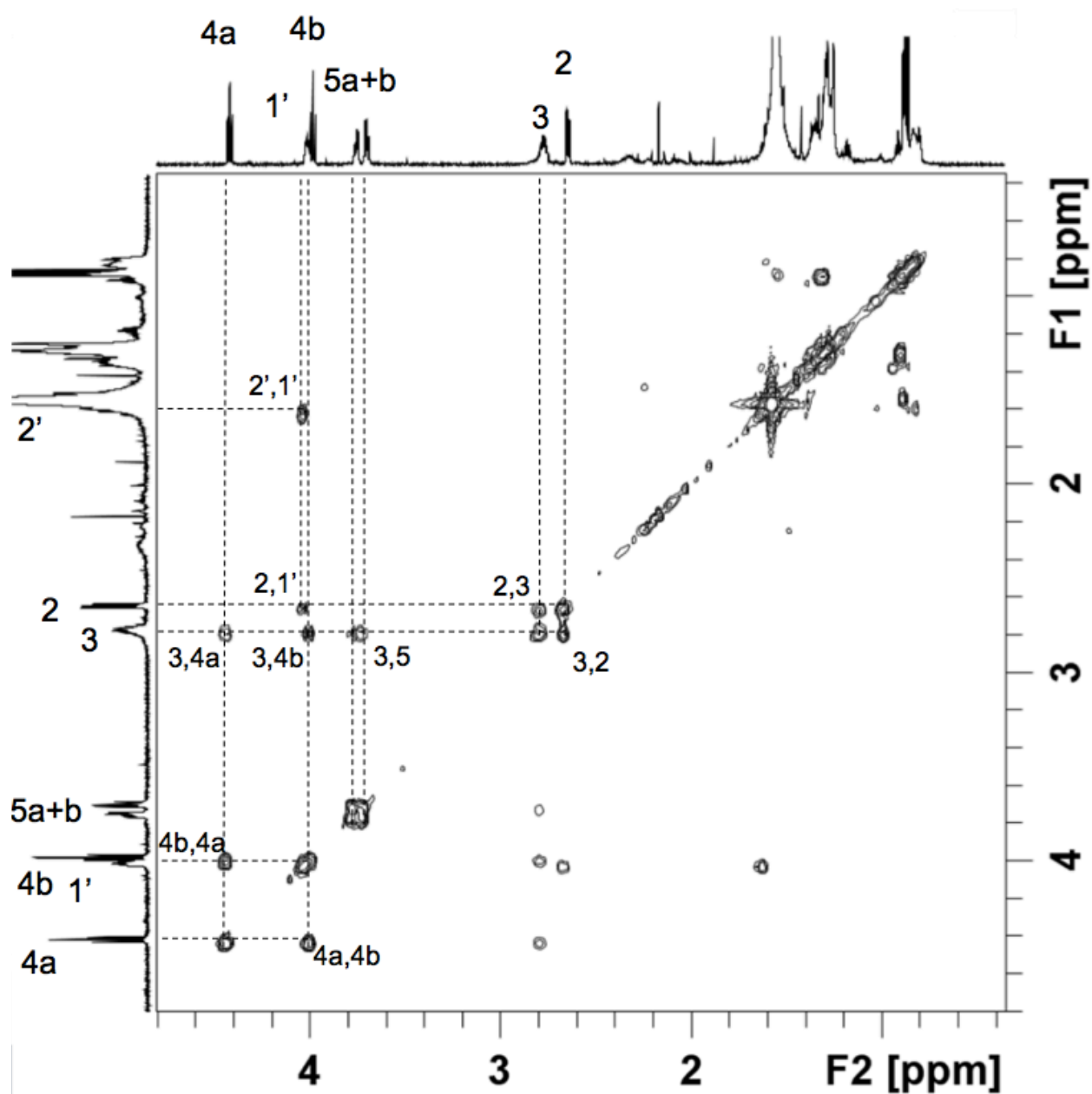


**Figure S10.** Low resolution mass spectra for metabolites **1-8** (SCBs 1-8) present in culture extract of *S. coelicolor* M1152 grown for five days in AlaMM enriched with  $d_5$ -proionic acid (SCBs 6 and 7);  $d_7$ -butyric acid (SCBs 2 and 8);  $d_8$ -valine (SCB 1);  $d_{10}$ -leucine (SCB 5) and  $d_{10}$ -isoleucine (SCBs 3 and 4)

### 3. NMR data for SCB1



**Figure S11.** <sup>1</sup>H-NMR spectrum (700 MHz, CDCl<sub>3</sub>) for SCB1 isolated from *S. coelicolor* M1152.



**Figure S12.** COSY spectrum for SCB1 isolated from *S. coelicolor* M1152; selected correlations observed in the COSY spectrum are highlighted by dashed lines.