

# Support Information

## Diketopiperazines as cross communication *quorum-sensing* signals between *Cronobacter sakazakii* and *Bacillus cereus*

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### Characterization of *Bacillus cereus* cyclo(Pro-Leu)

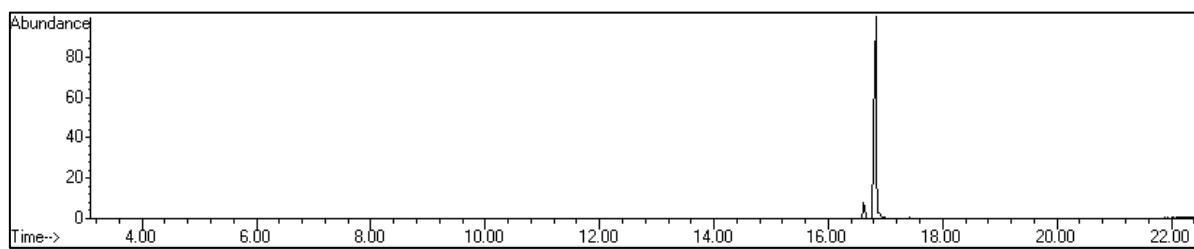


Figure S1. GC-MS chromatogram of cyclo(Pro-Leu) from *Bacillus cereus*

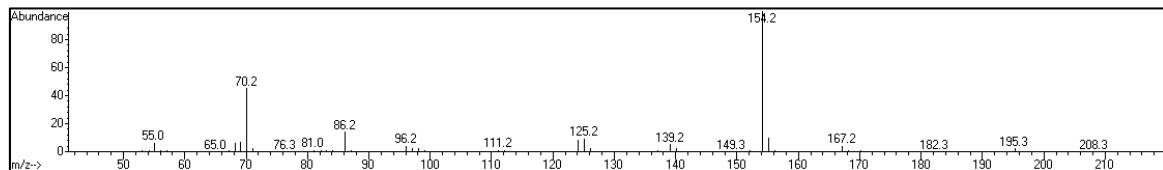
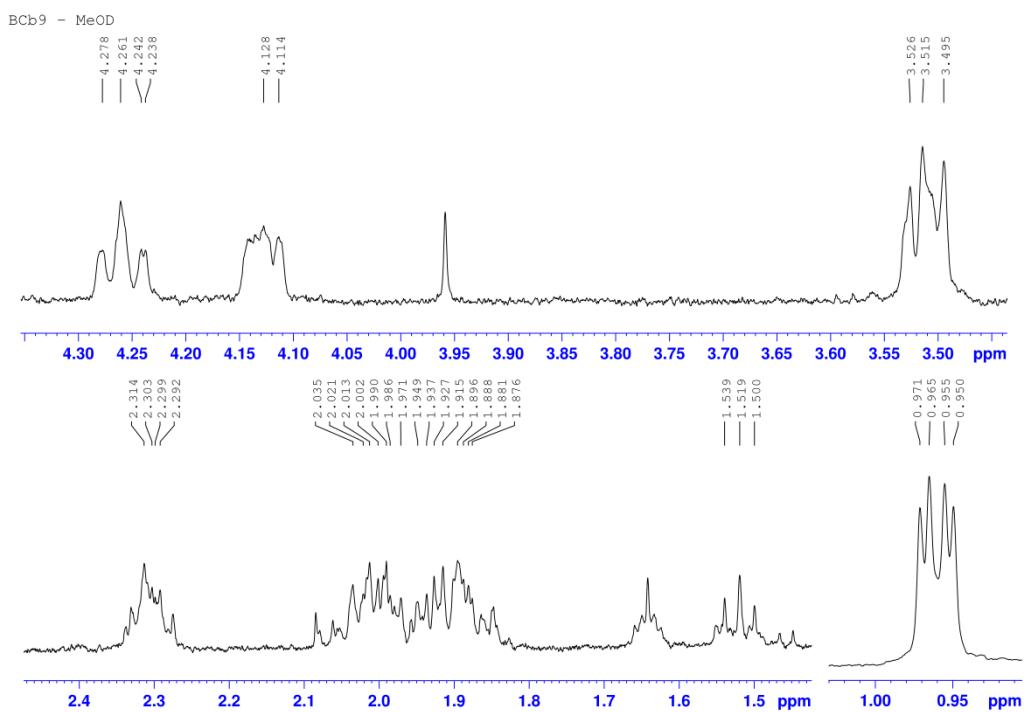
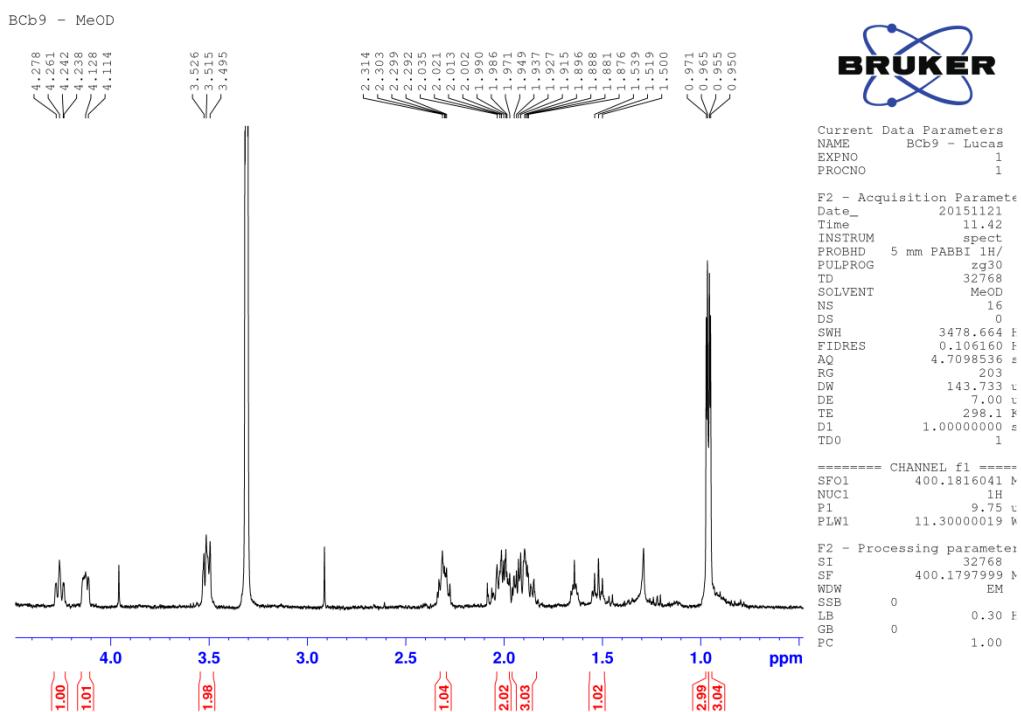
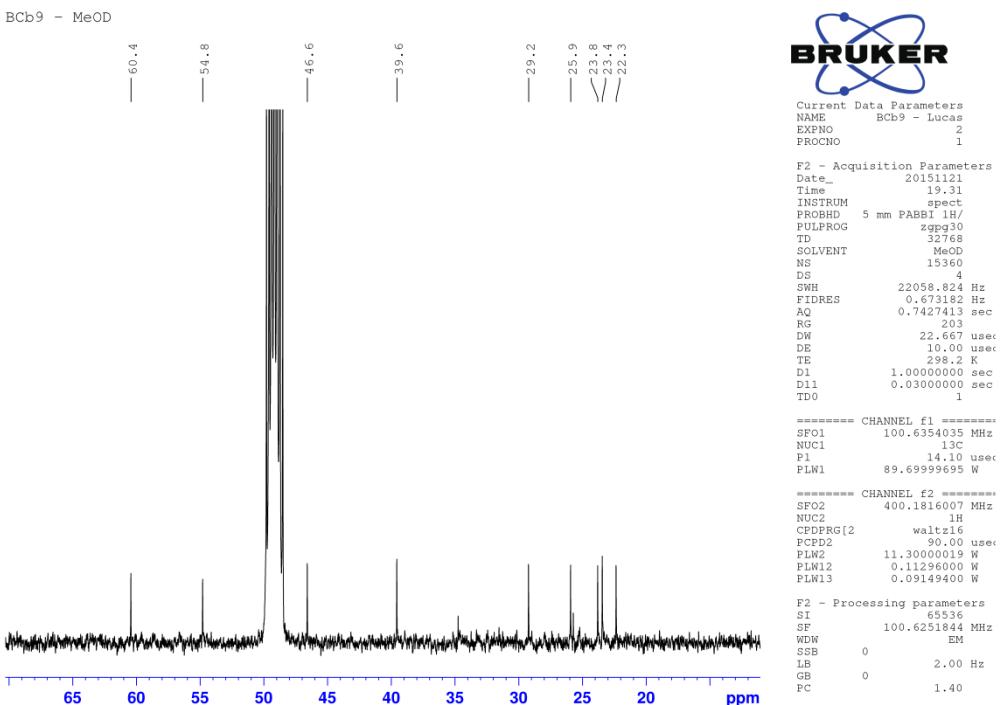


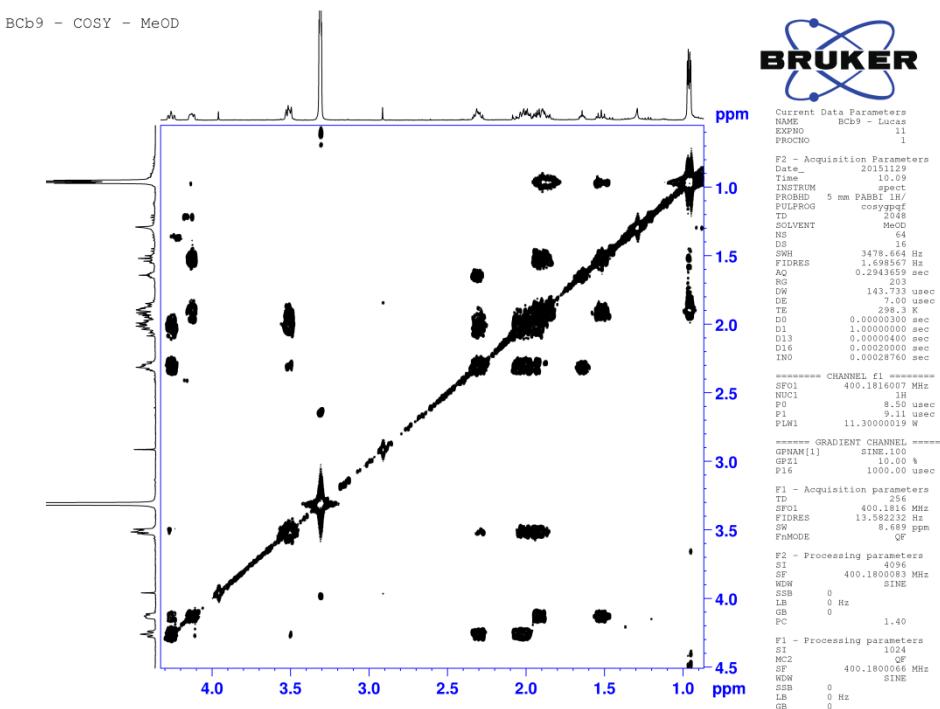
Figure S2. EI-MS spectrum of cyclo(Pro-Leu) from *Bacillus cereus*



**Figure S3.**  $^1\text{H}$  NMR spectrum of cyclo(Pro-Leu) from *Bacillus cereus* (full spectrum and expansions)

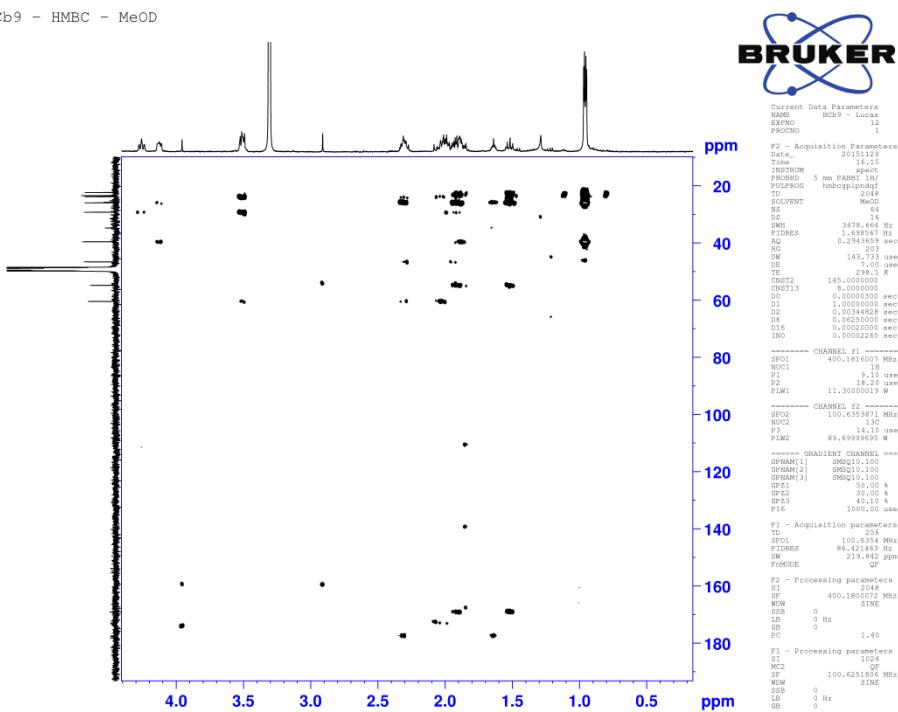


**Figure S4.**  $^{13}\text{C}$  NMR spectrum of cyclo(Pro-Leu) from *Bacillus cereus*



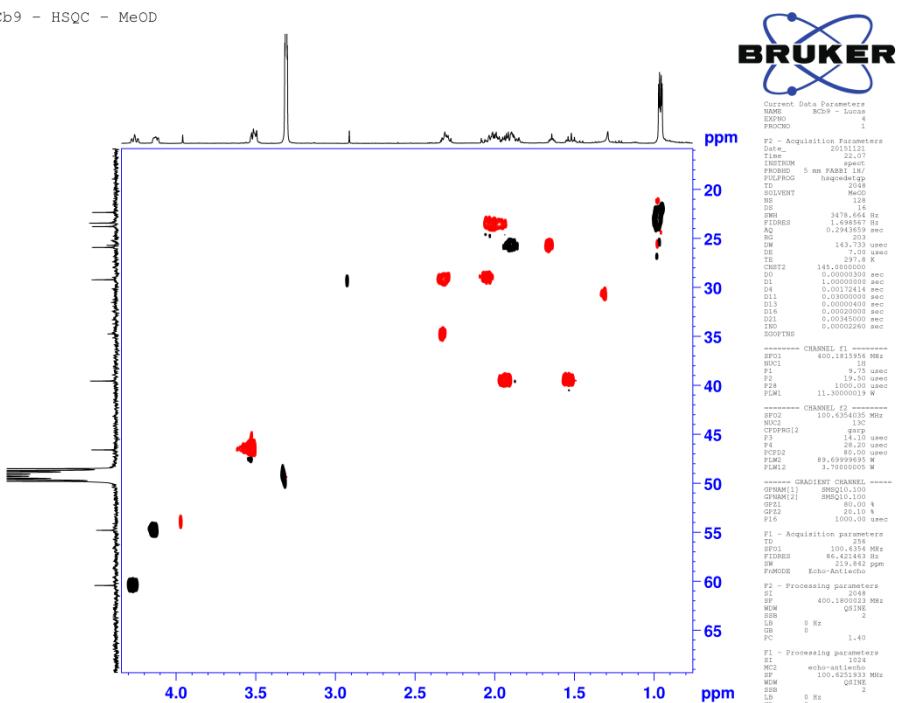
**Figure S5.** 2D NMR ( $^1\text{H}, ^1\text{H}$  - COSY) contour plot of cyclo(Pro-Leu) from *Bacillus cereus*

BCb9 - HMBC - MeOD



**Figure S6.** 2D NMR (<sup>1</sup>H, <sup>13</sup>C - HMBC) contour plot of cyclo(Pro-Leu) from *Bacillus cereus*

BCb9 - HSQC - MeOD

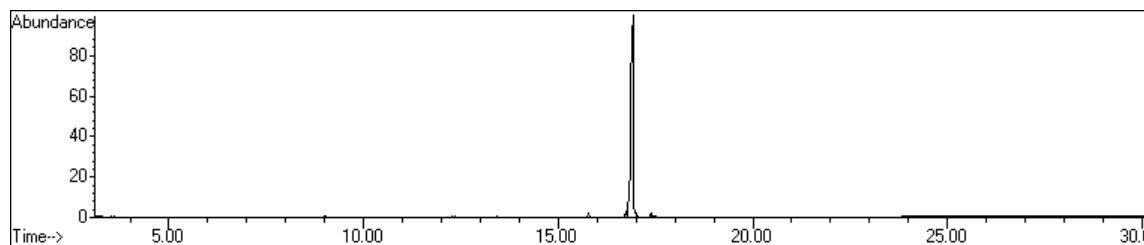


**Figure S7.** 2D NMR (<sup>1</sup>H, <sup>13</sup>C - HSQC) contour plot of cyclo(Pro-Leu) from *Bacillus cereus*

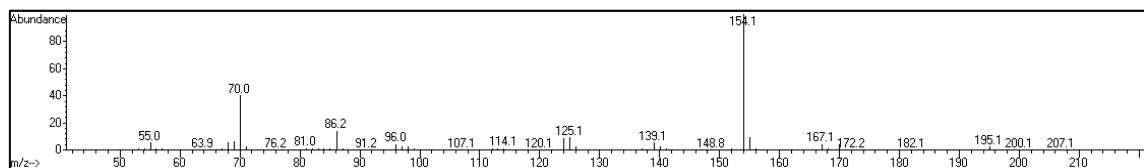
**Table S1.** <sup>1</sup>H and <sup>13</sup>C NMR data of cyclo(Pro-Leu) from natural culture (*B. cereus*) in CD<sub>3</sub>OD at 400 MHz.

Atom	<sup>13</sup> CNMR Signal (ppm)	<sup>1</sup> HNMR Signal (ppm)
<b>C1</b>	169.1	-
<b>C3</b>	46.6	3.51 (2H, m)
<b>C4</b>	23.8	(a) 1.90 (1H, m) (b) 1.99 (1H, m)
<b>C5</b>	29.2	(a) 2.30 (1H, m)  (b) 1.99 (1H, m)
<b>C6</b>	60.4	4.26 (1H, m)
<b>C7</b>	173.0	-
<b>N8</b>	-	-
<b>C9</b>	54.8	4.13 (1H, m)
<b>C10</b>	39.6	(a) 1.50 (1H, m) (b) 1.90 (1H, m)
<b>C11</b>	25.9	1.90 (1 of 3H, m)
<b>C12</b>	23.4	0.97 J 2.3 Hz (3 of 6H, d)
<b>C13</b>	22.3	0.95 J 2.3 Hz (3 of 6H, d)

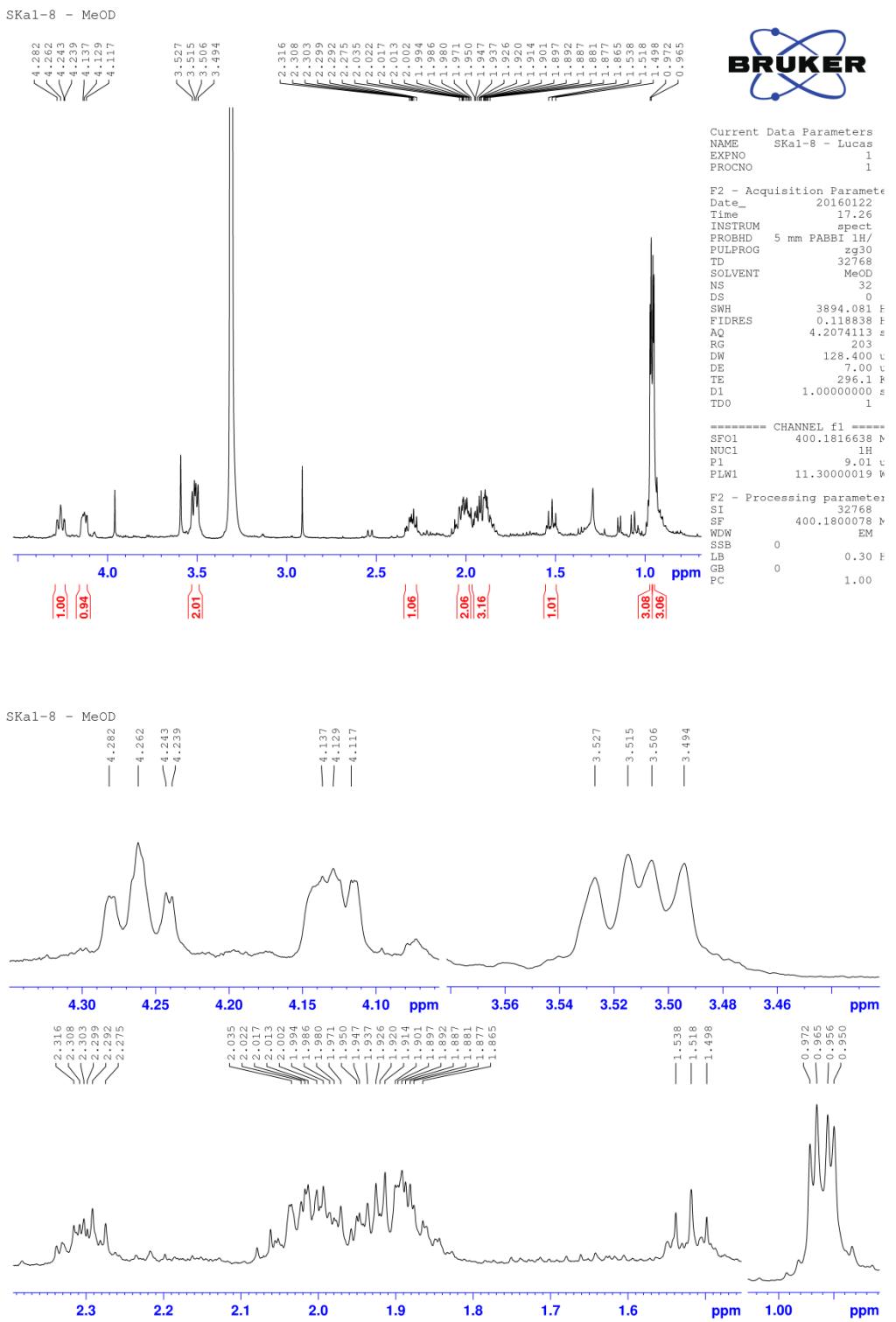
### Characterization of *Cronobacter sakazakii* cyclo(Pro-Leu)



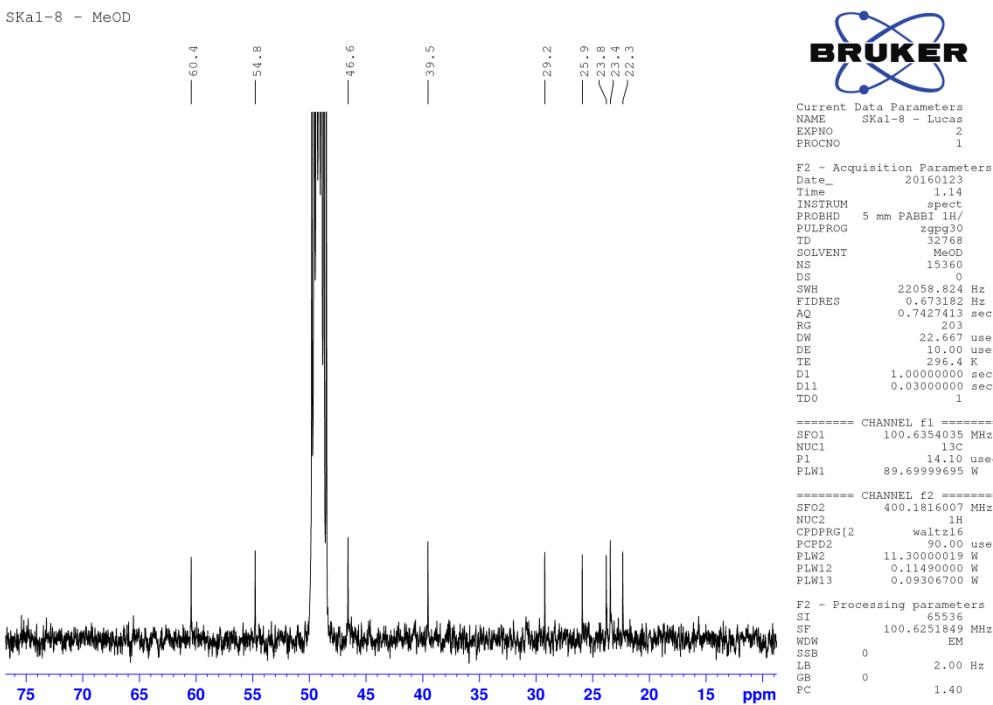
**Figure S8.** GC-MS chromatogram of cyclo(Pro-Leu) from *Cronobacter sakazakii*



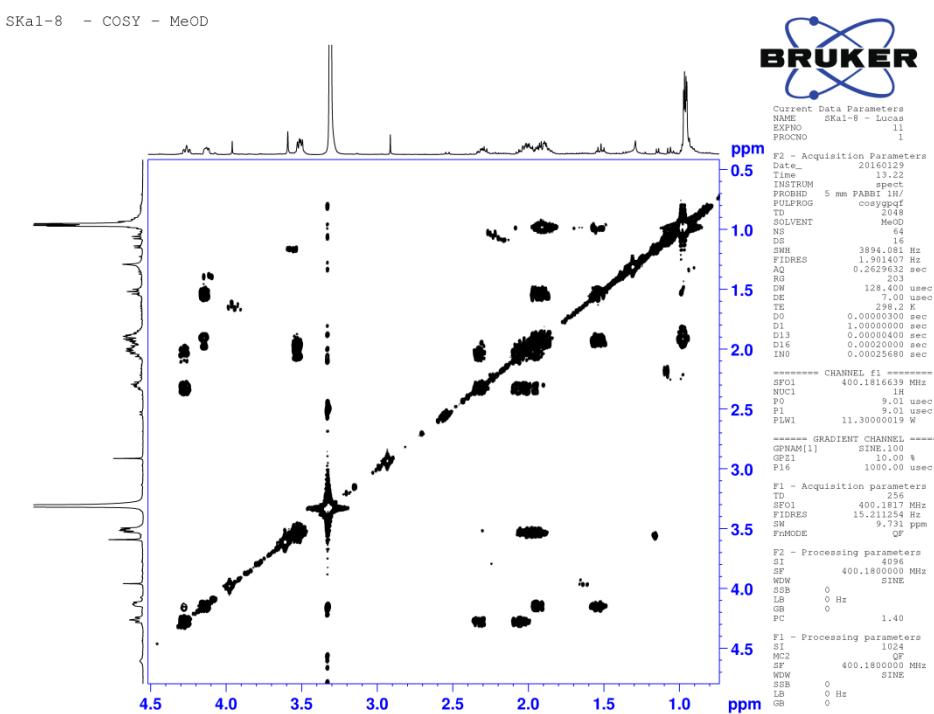
**Figure S9.** EI-MS spectrum of cyclo(Pro-Leu) from *Cronobacter sakazakii*



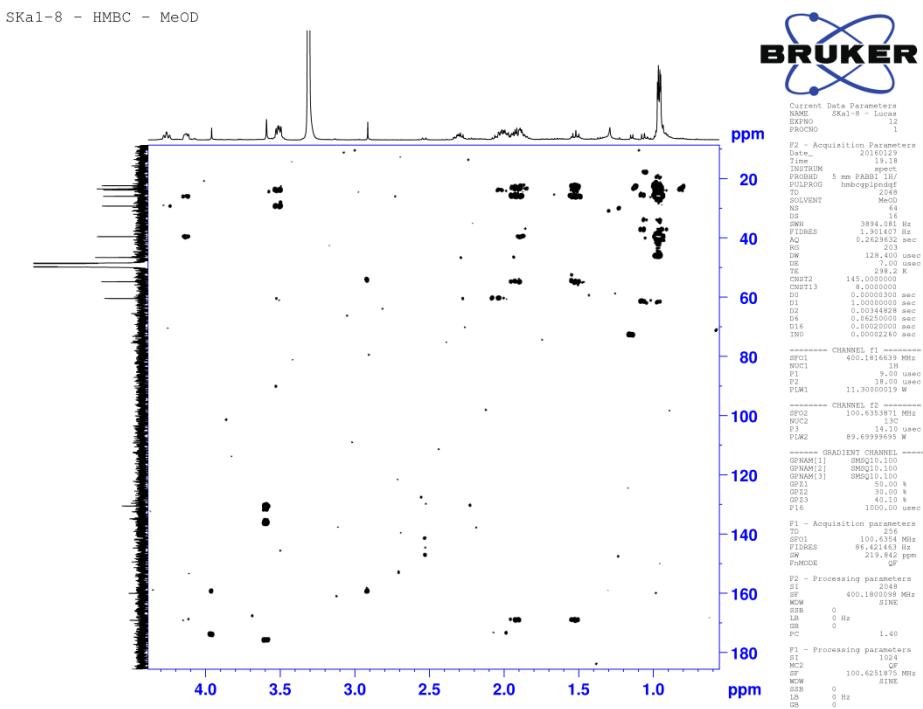
**Figure S10.**  $^1\text{H}$  NMR spectrum of cyclo(Pro-Leu) from *Cronobacter sakazakii* (full spectrum and expansions)



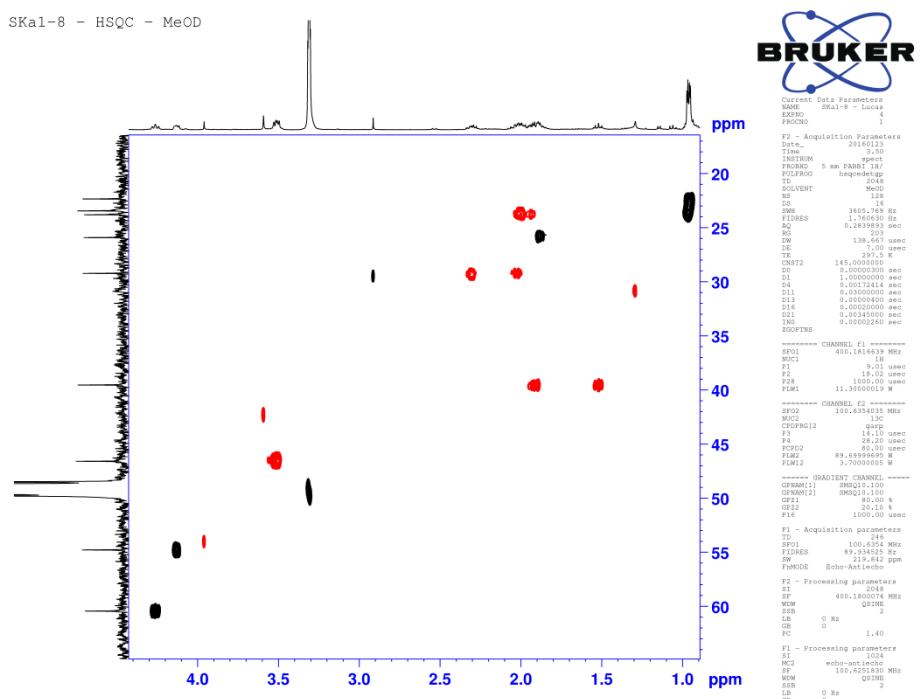
**Figure S11.**  $^{13}\text{C}$  NMR spectrum of cyclo(Pro-Leu) from *Cronobacter sakazakii*



**Figure S12.** 2D NMR ( $^1\text{H}$ ,  $^1\text{H}$  - COSY) contour plot of cyclo(Pro-Leu) from *Cronobacter sakazakii*

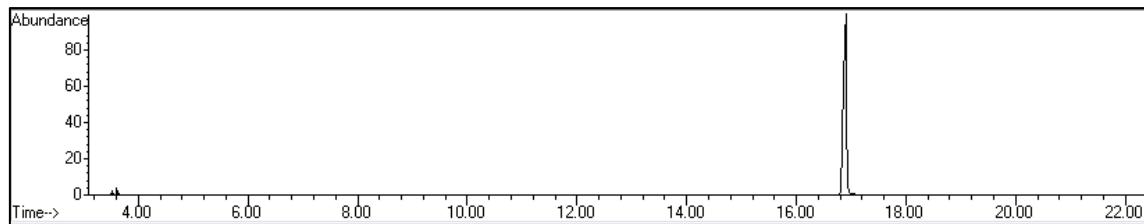


**Figure S13.** 2D NMR (<sup>1</sup>H, <sup>13</sup>C - HMBC) contour plot of cyclo(Pro-Leu) from *Cronobacter sakazakii*

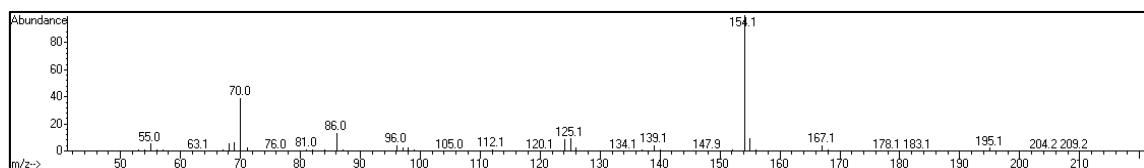


**Figure S14.** 2D NMR (<sup>1</sup>H, <sup>13</sup>C - HSQC) contour plot of cyclo(Pro-Leu) from *Cronobacter sakazakii*

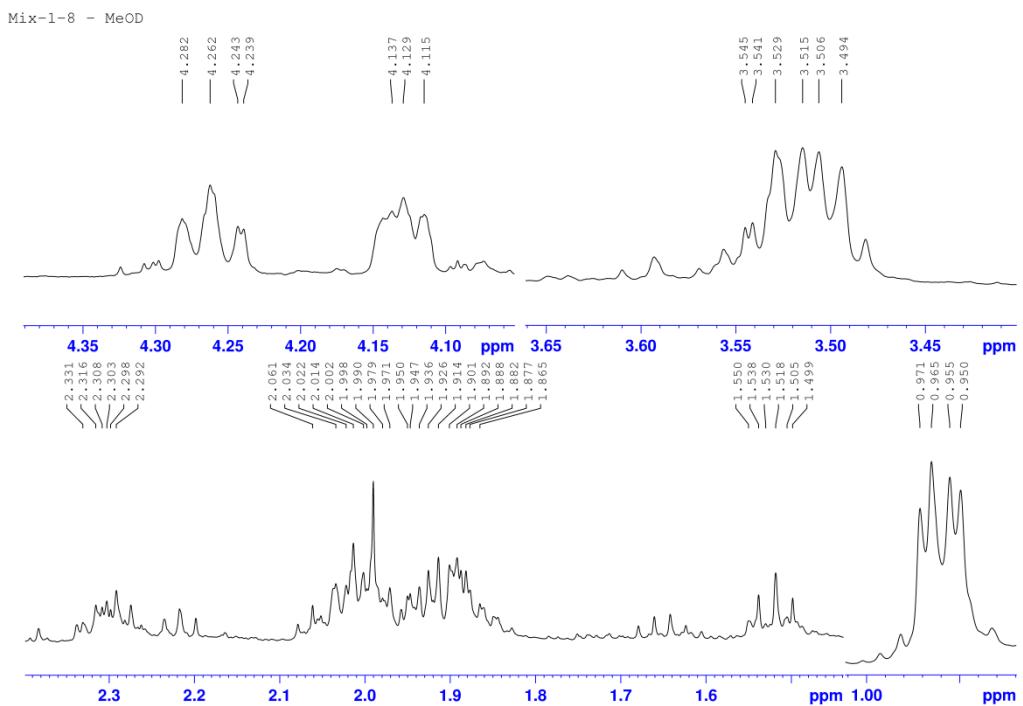
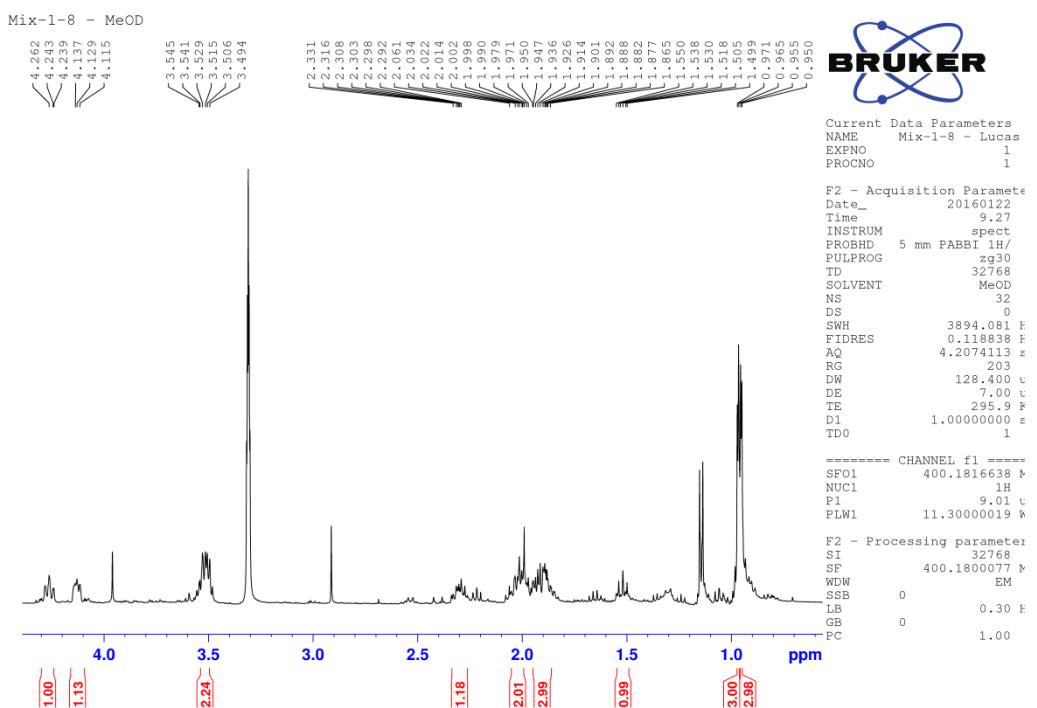
### Characterization of co-culture cyclo(Pro-Leu)



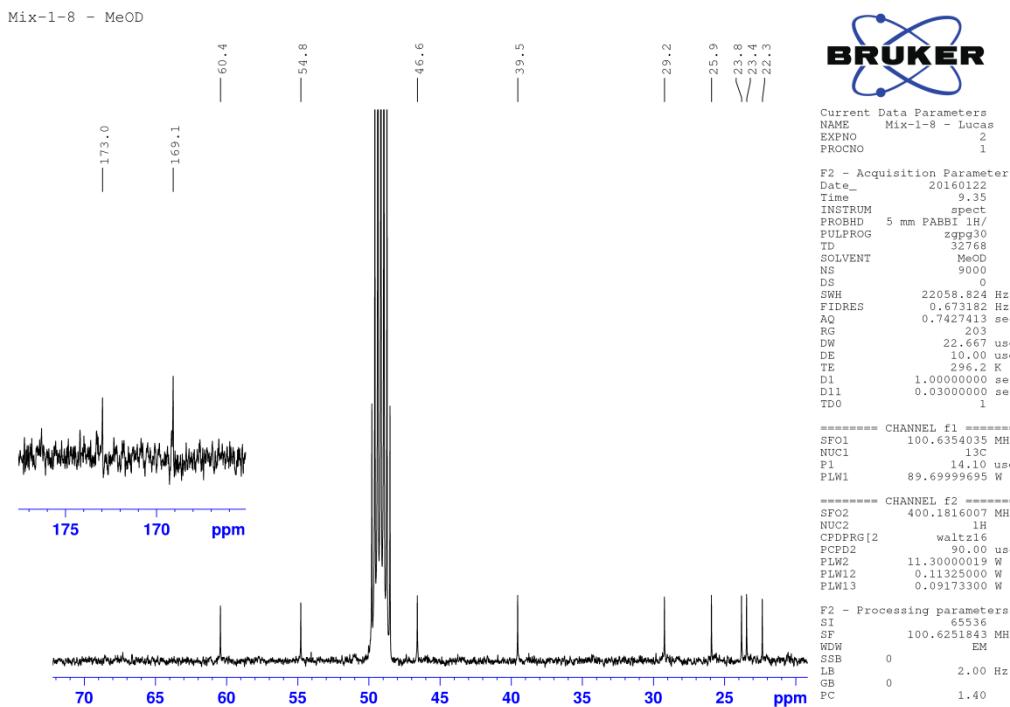
**Figure S15.** GC-MS chromatogram of cyclo(Pro-Leu) from co-culture



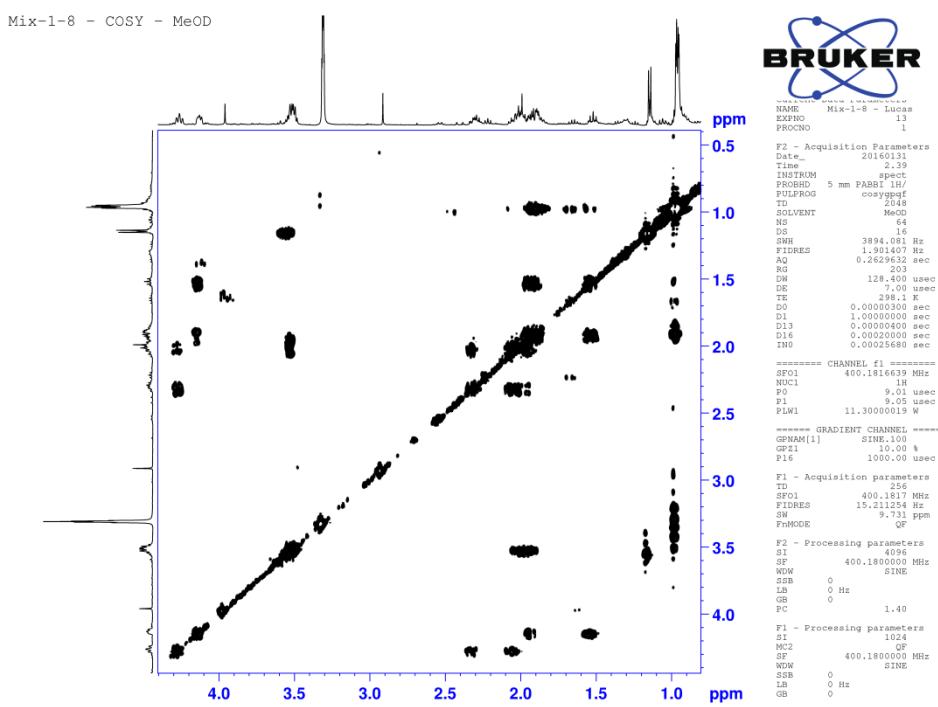
**Figure S16.** EI-MS spectrum of cyclo(Pro-Leu) from co-culture



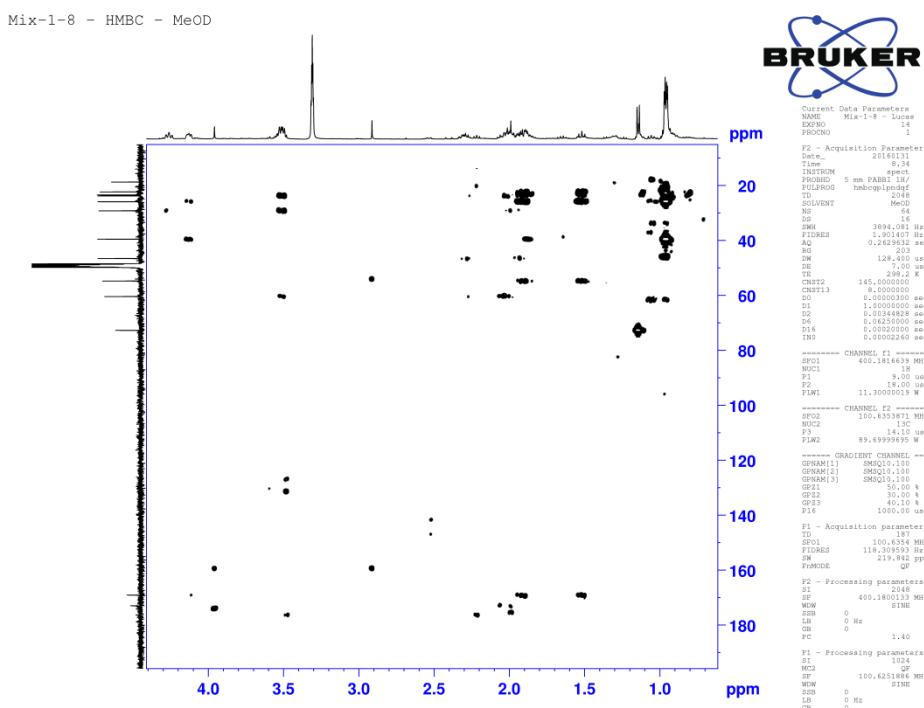
**Figure S17.**  $^1\text{H}$  NMR spectrum of cyclo(Pro-Leu) from co-culture (full spectrum and expansions)



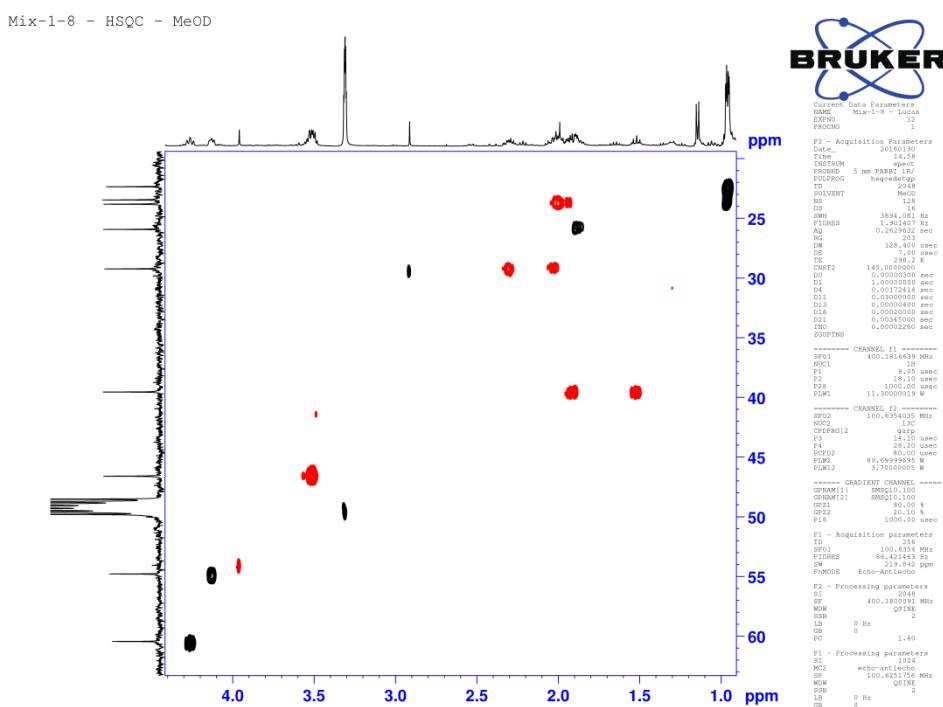
**Figure S18.**  $^{13}\text{C}$  NMR spectrum of cyclo(Pro-Leu) from co-culture



**Figure S19.** 2D NMR ( $^1\text{H}, ^1\text{H}$  - COSY) contour plot of cyclo(Pro-Leu) from co-culture

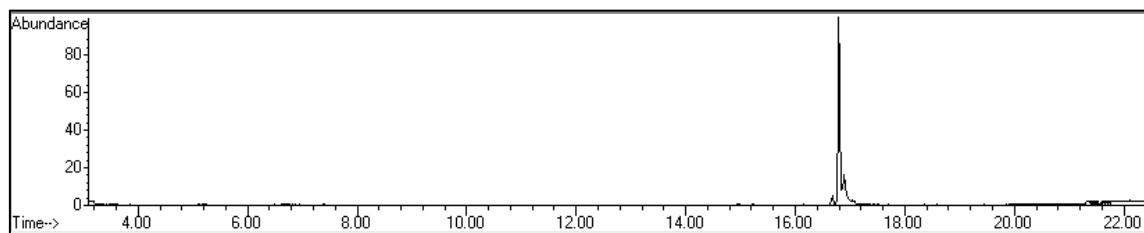


**Figure S20.** 2D NMR ( $^1\text{H}$ ,  $^{13}\text{C}$  - HMBC) contour plot of cyclo(Pro-Leu) from co-culture

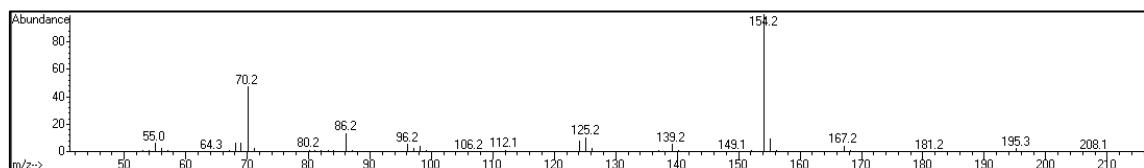


**Figure S21.** 2D NMR ( $^1\text{H}$ ,  $^{13}\text{C}$  - HSQC) contour plot of cyclo(Pro-Leu) from co-culture

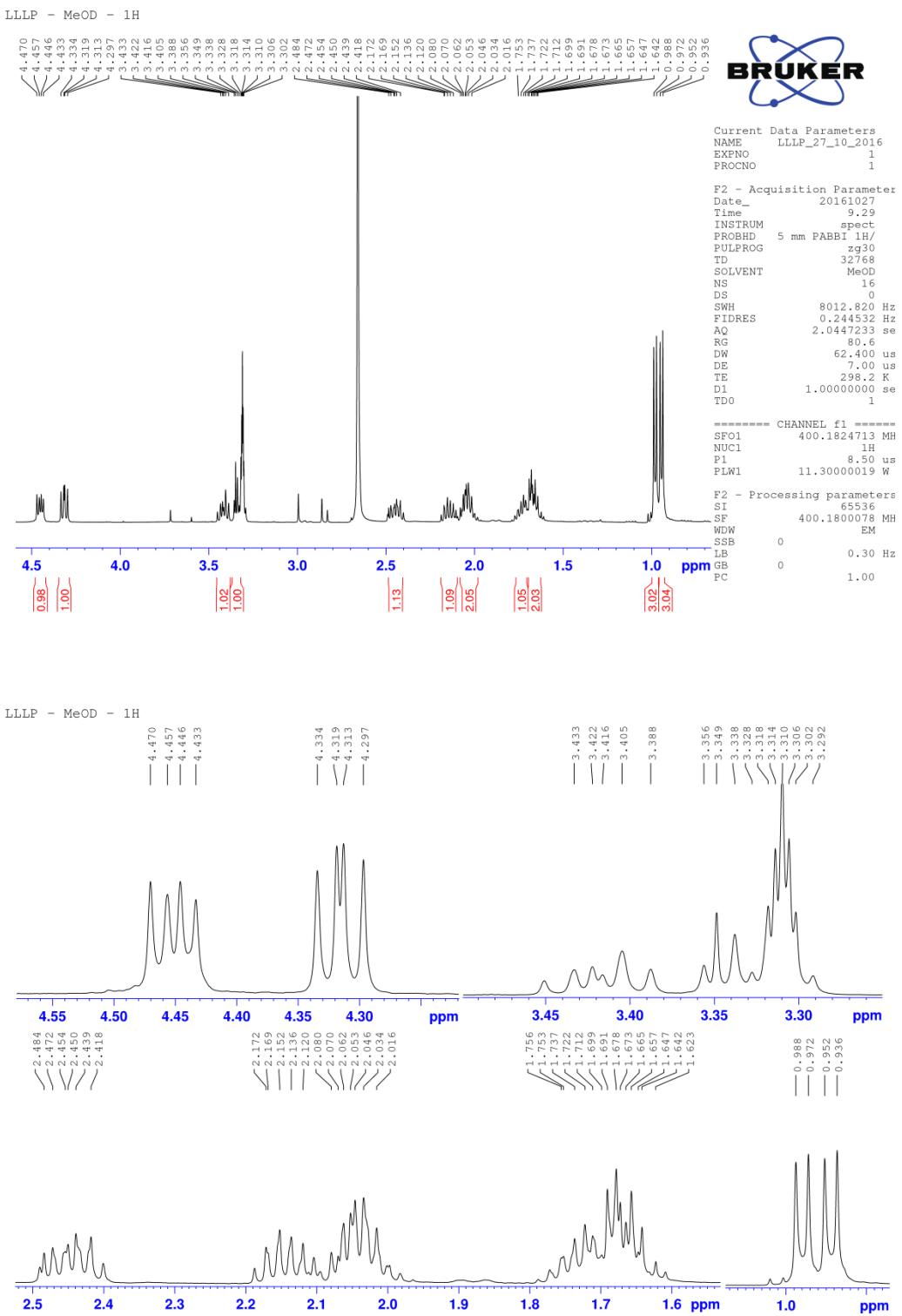
### Standard cyclo(L-Pro-L-Leu)



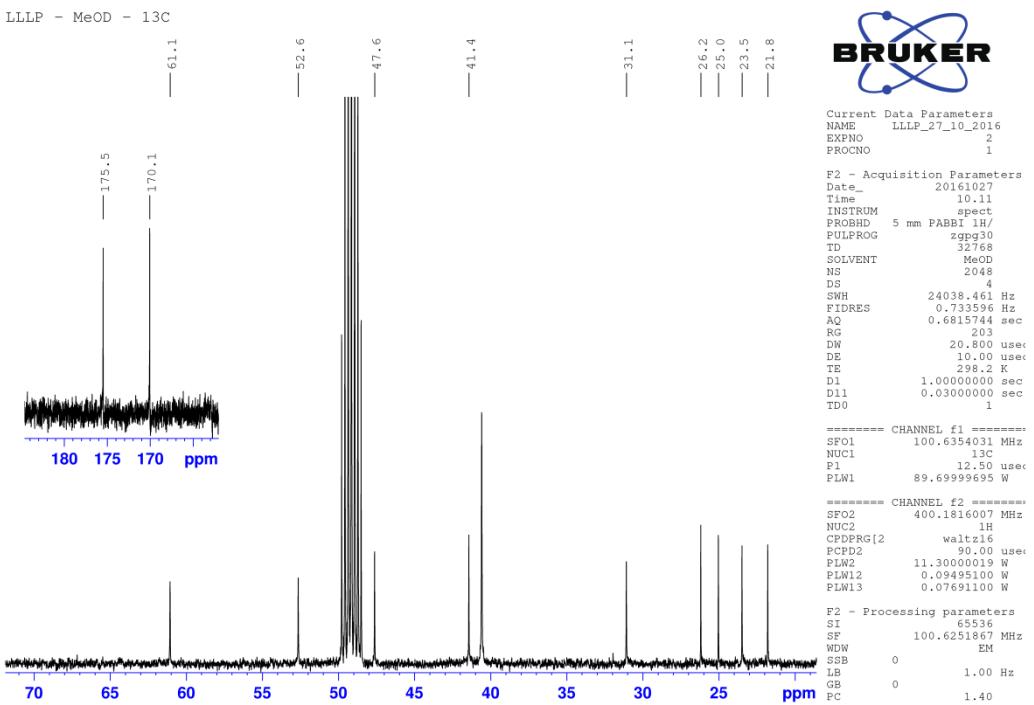
**Figure S22.** GC-MS chromatogram of standard cyclo(L-Pro-L-Leu)



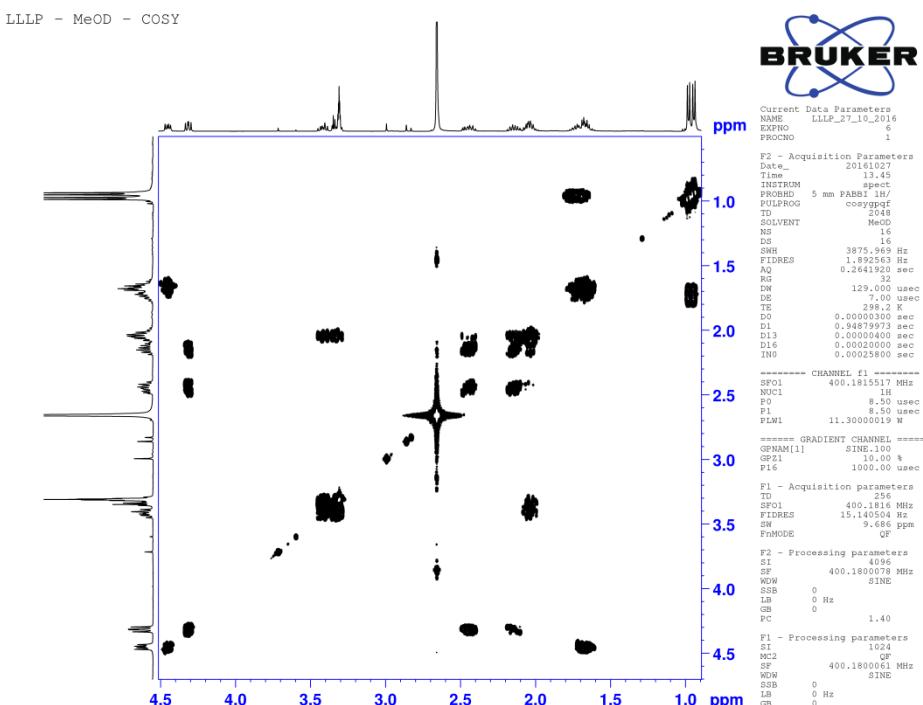
**Figure S23.** EI-MS spectrum of standard cyclo(L-Pro-L-Leu)



**Figure S24.**  $^1\text{H}$  NMR of standard cyclo(L-Pro-L-Leu) (full spectrum and expansions)



**Figure S25.**  $^{13}\text{C}$  NMR of standard cyclo(L-Pro-L-Leu)



**Figure S26.** 2D NMR ( $^1\text{H}, ^1\text{H}$  - COSY) of standard cyclo(L-Pro-L-Leu)

LLLp - MeOD - HMBC

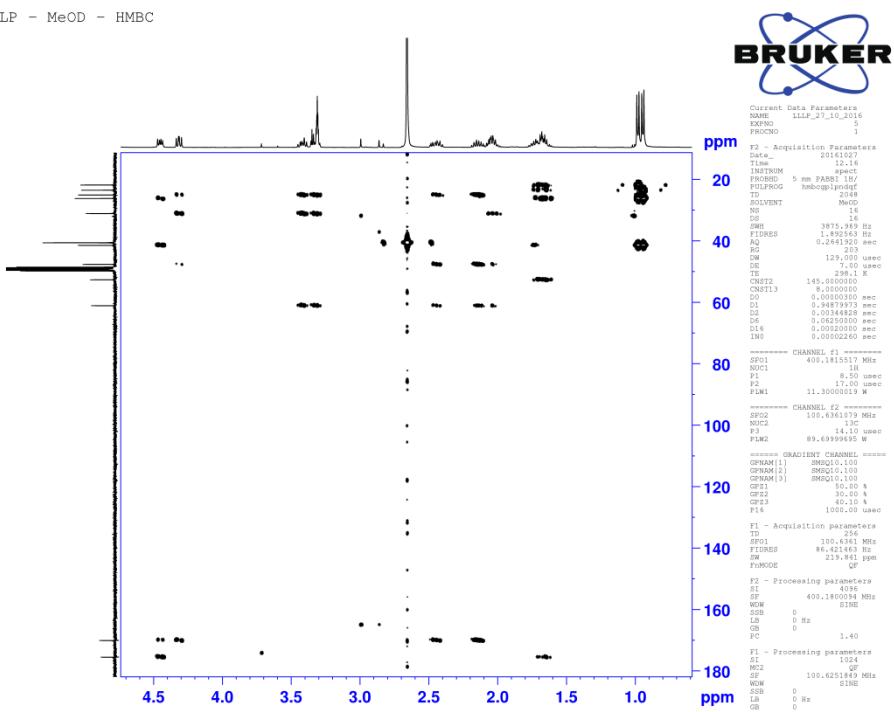


Figure S27. 2D NMR (<sup>1</sup>H, <sup>13</sup>C - HMBC) of standard cyclo(L-Pro-L-Leu)

LLLp - MeOD - HSQC

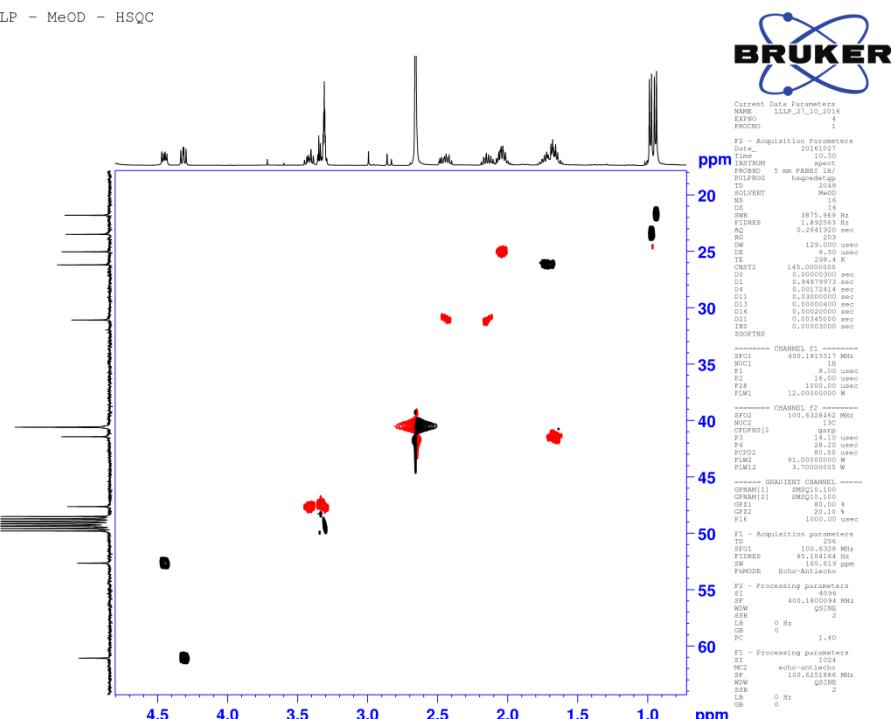
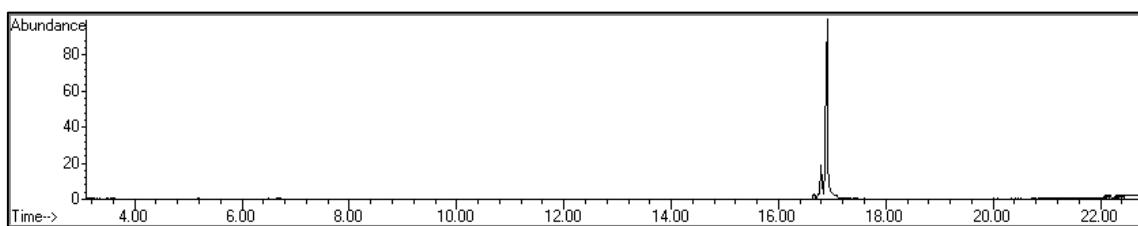
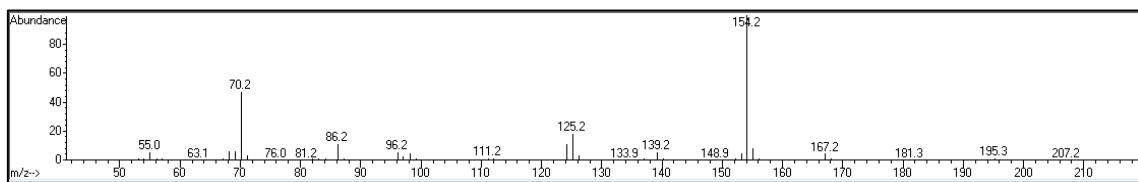


Figure S28. 2D NMR (<sup>1</sup>H, <sup>13</sup>C - HSQC) of standard cyclo(L-Pro-L-Leu)

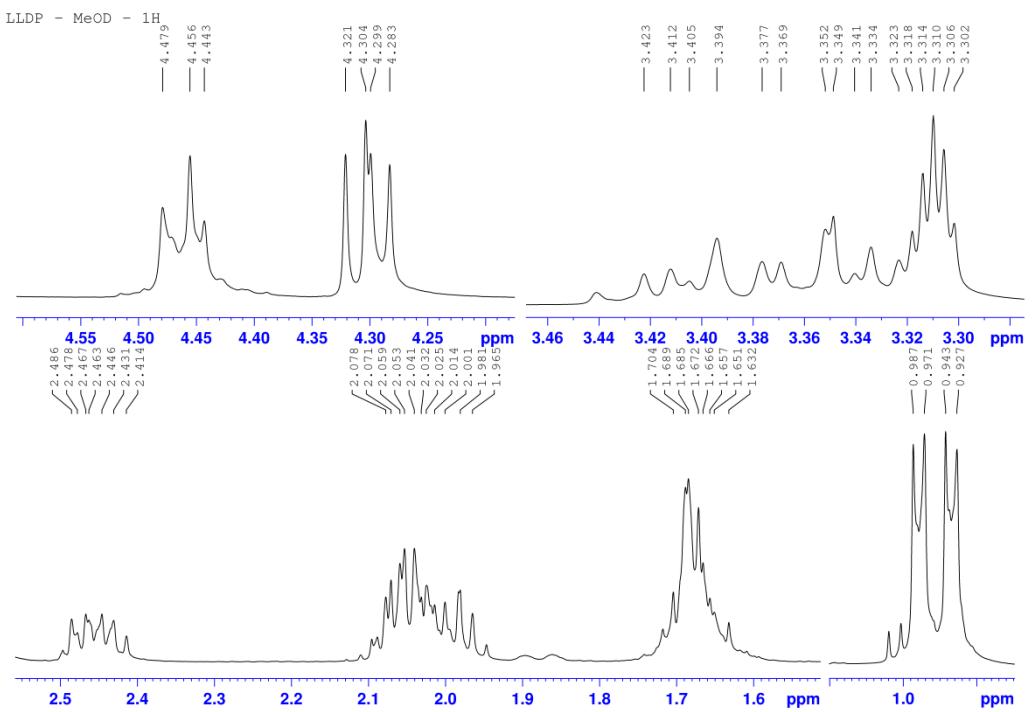
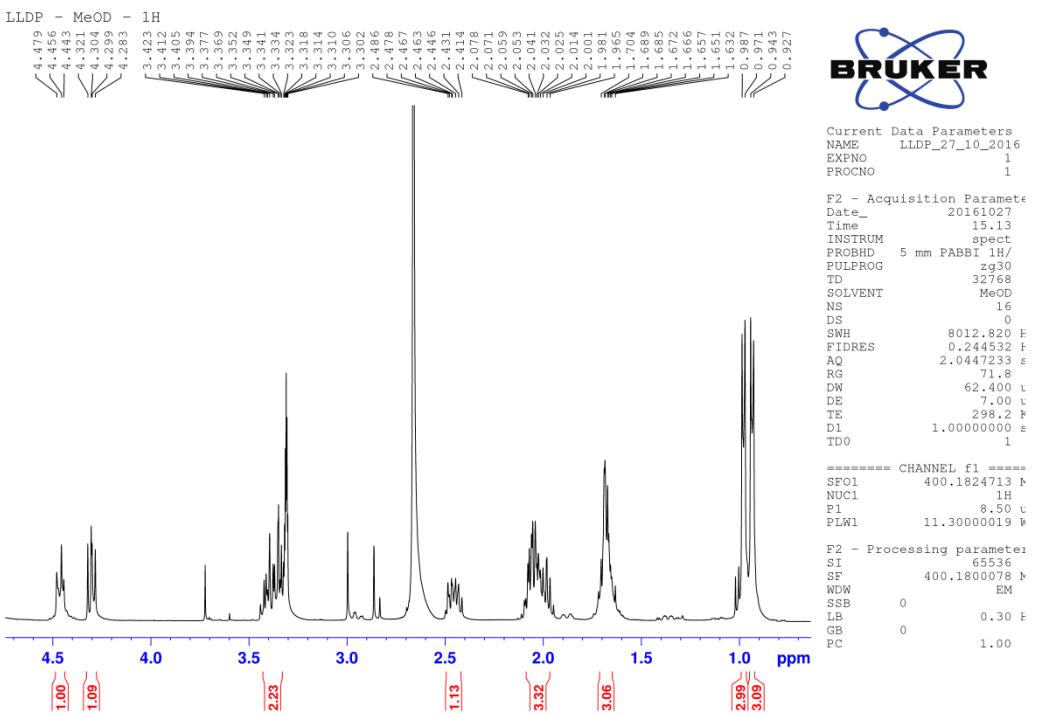
### Standard cyclo(D-Pro-L-Leu)



**Figure S29.** GC-MS chromatogram of standard cyclo(D-Pro-L-Leu)

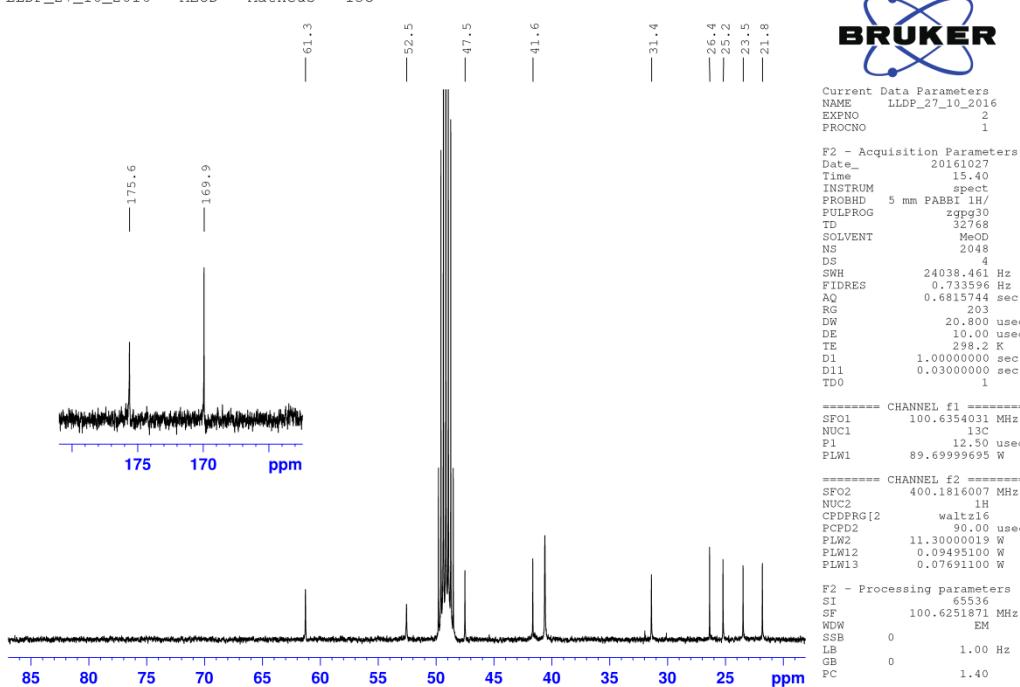


**Figure S30.** EI-MS spectrum for standard cyclo(D-Pro-L-Leu)



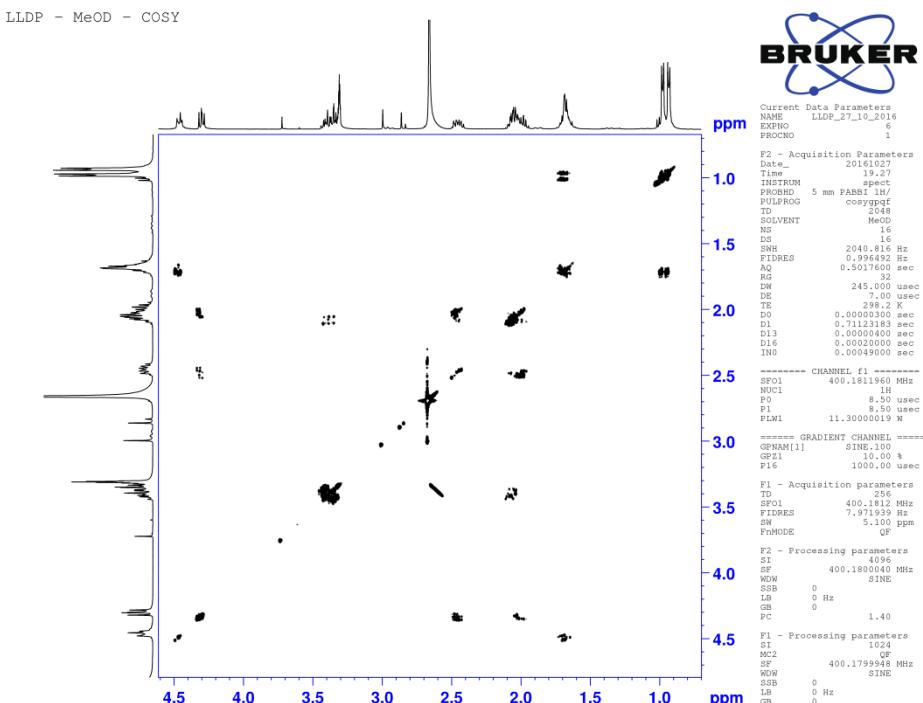
**Figure S31.**  $^1\text{H}$  NMR of standard cyclo(D-Pro-L-Leu) (full spectrum and expansions)

LLDP\_27\_10\_2016 - MeOD - Matheus - 13C



**Figure S32.** <sup>13</sup>C NMR of standard cyclo(D-Pro-L-Leu)

LLDP - MeOD - COSY



**Figure S33.** 2D NMR (<sup>1</sup>H, <sup>1</sup>H - COSY) of standard cyclo(D-Pro-L-Leu)

LLDP - MeOD - HMBC

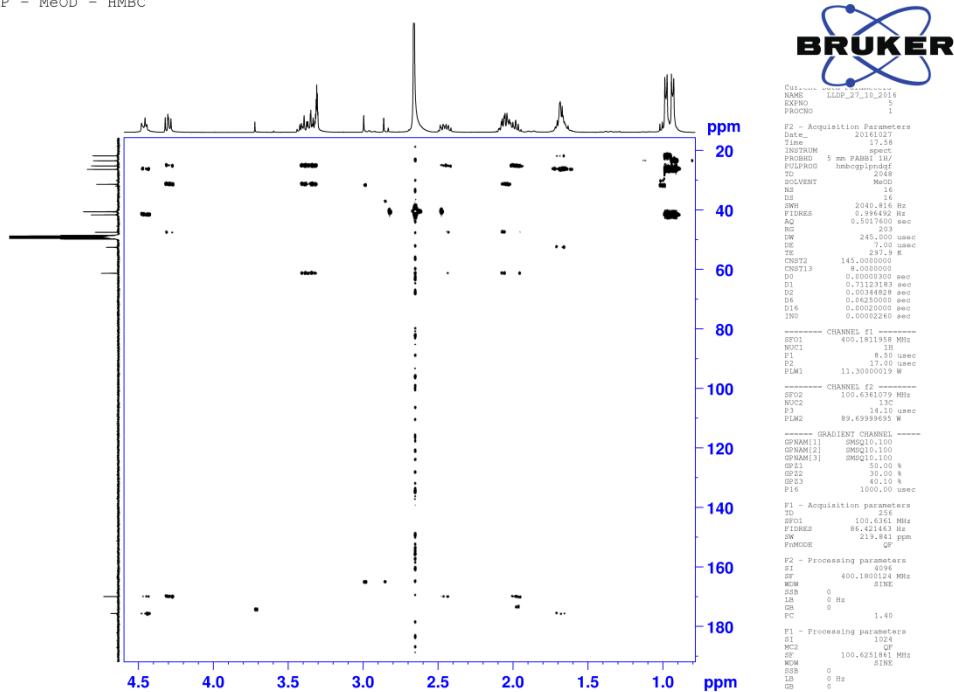


Figure S34. 2D NMR ( $^1\text{H}, ^{13}\text{C}$  - HMBC) of standard cyclo(D-Pro-L-Leu)

LLDP - MeOD - HSQC

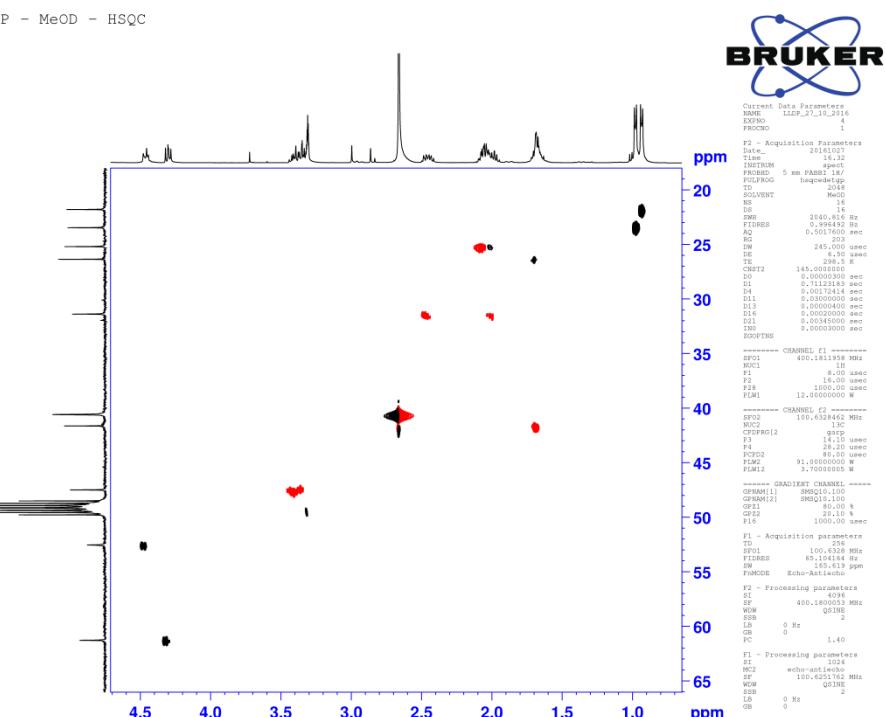
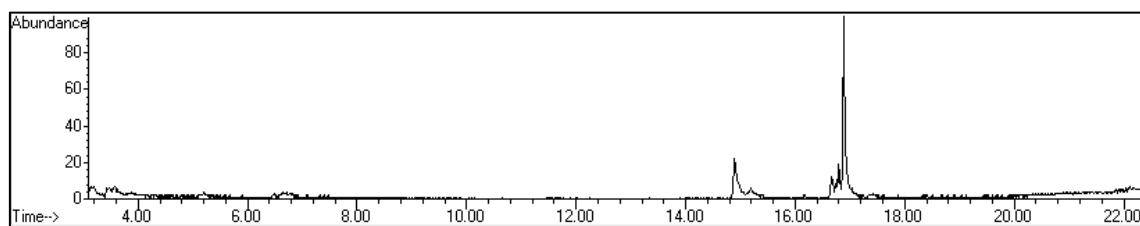
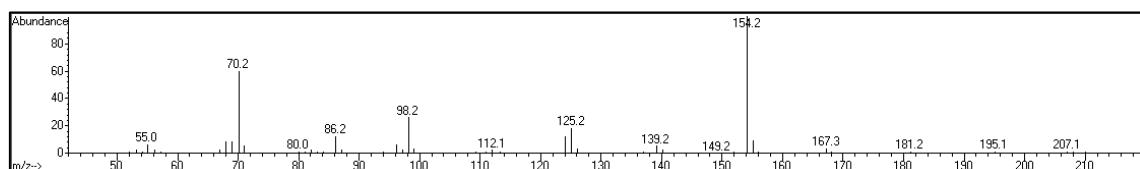


Figure S35. 2D NMR ( $^1\text{H}, ^{13}\text{C}$  - HSQC) of standard cyclo(D-Pro-L-Leu)

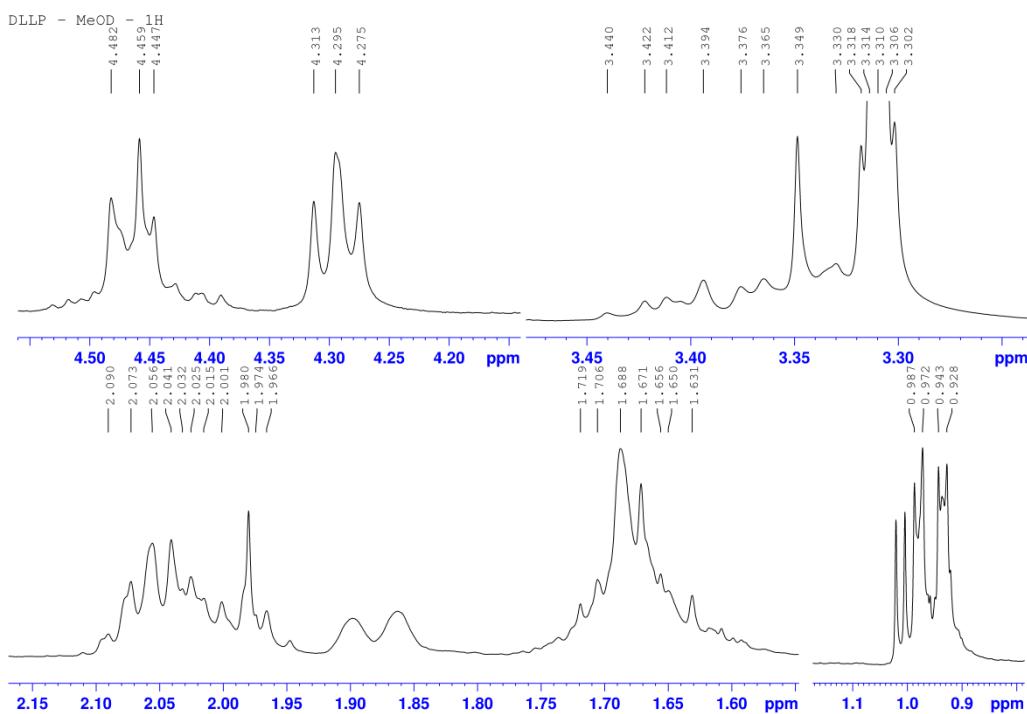
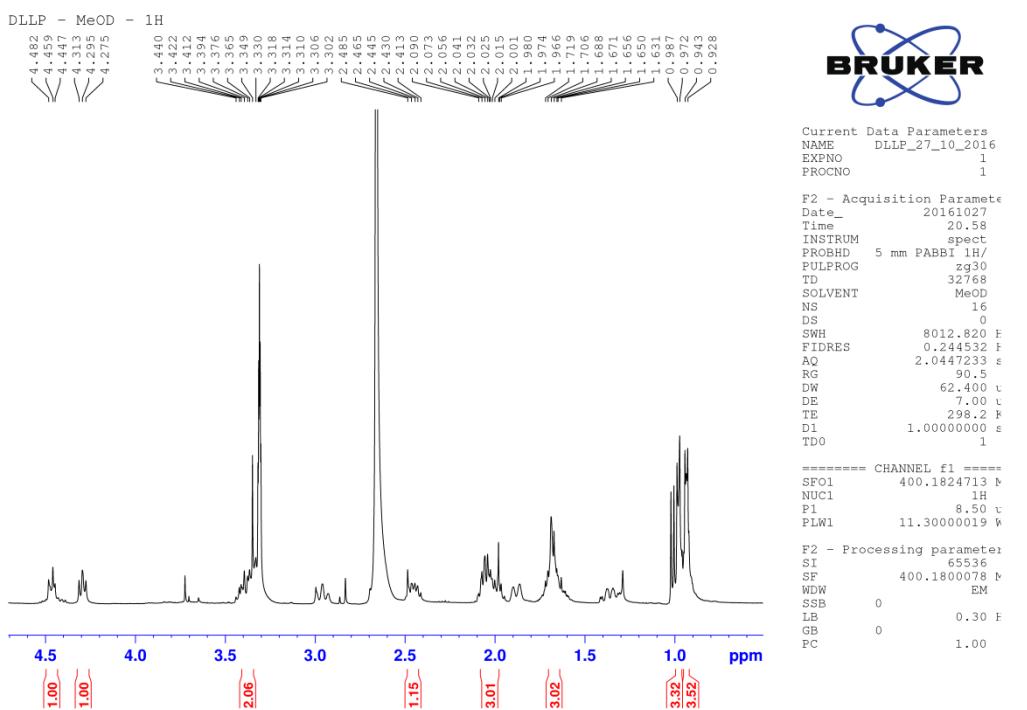
**Standard cyclo(L-Pro-D-Leu)**



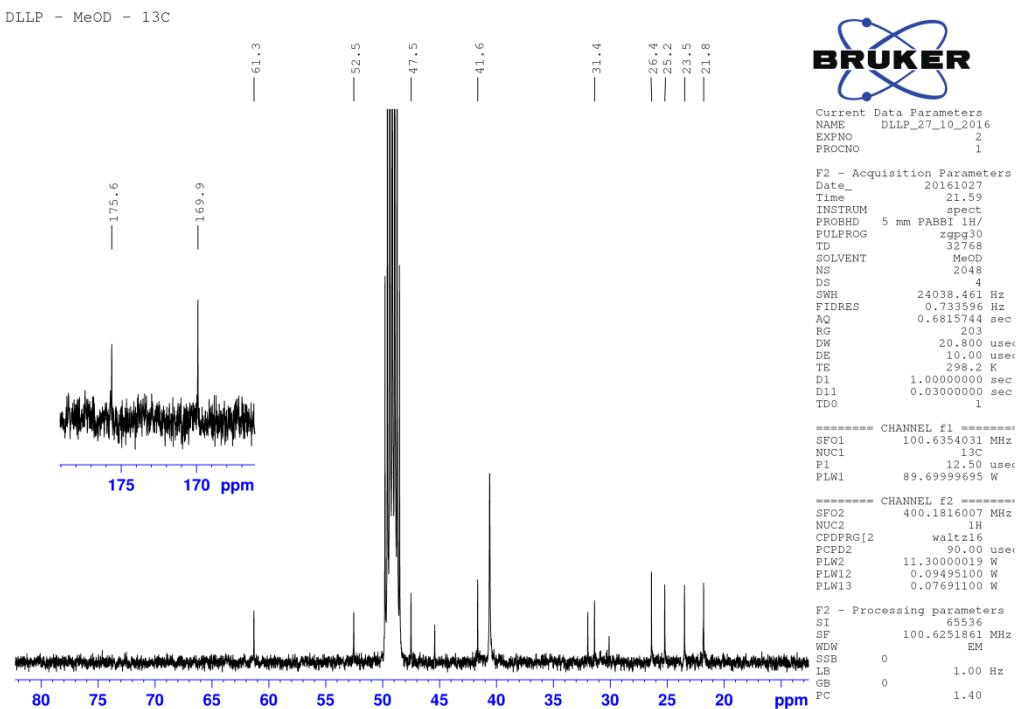
**Figure S36.** GC-MS chromatogram for cyclo(L-Pro-D-Leu)



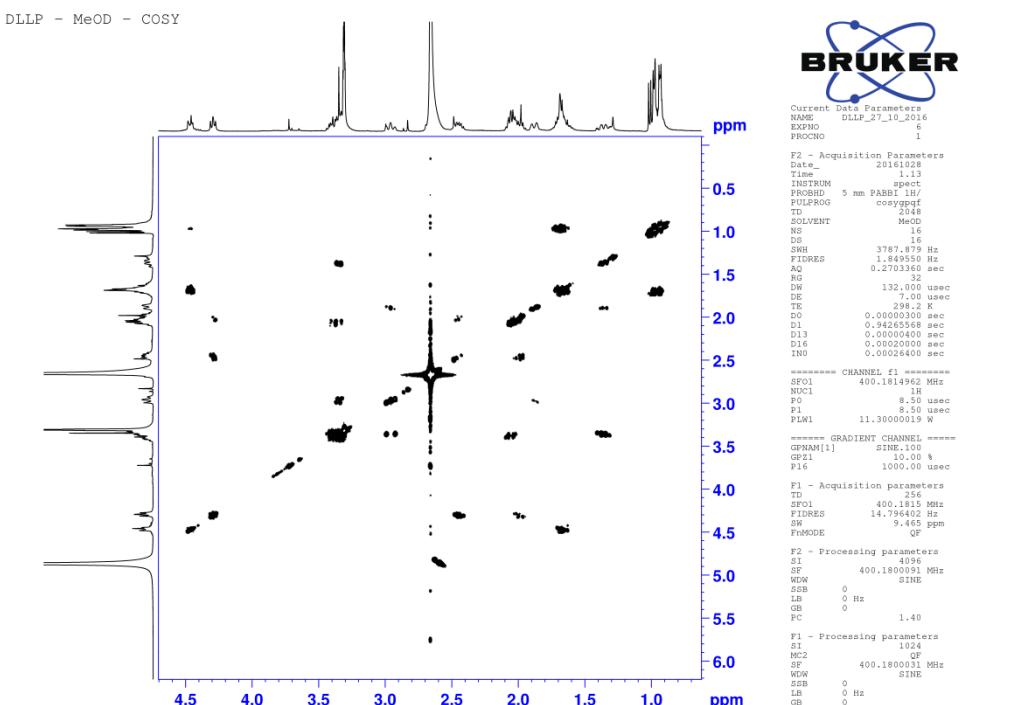
**Figure S37.** EI-MS spectrum for cyclo(L-Pro-D-Leu)



**Figure S38.**  $^1\text{H}$  NMR of standard cyclo(L-Pro-D-Leu) (full spectrum and expansions)



**Figure S39.**  $^{13}\text{C}$  NMR of standard cyclo(L-Pro-D-Leu)



**Figure S40.** 2D NMR ( $^1\text{H}, ^1\text{H}$  - COSY) of standard cyclo(L-Pro-D-Leu)

DLLP - MeOD - HMBC

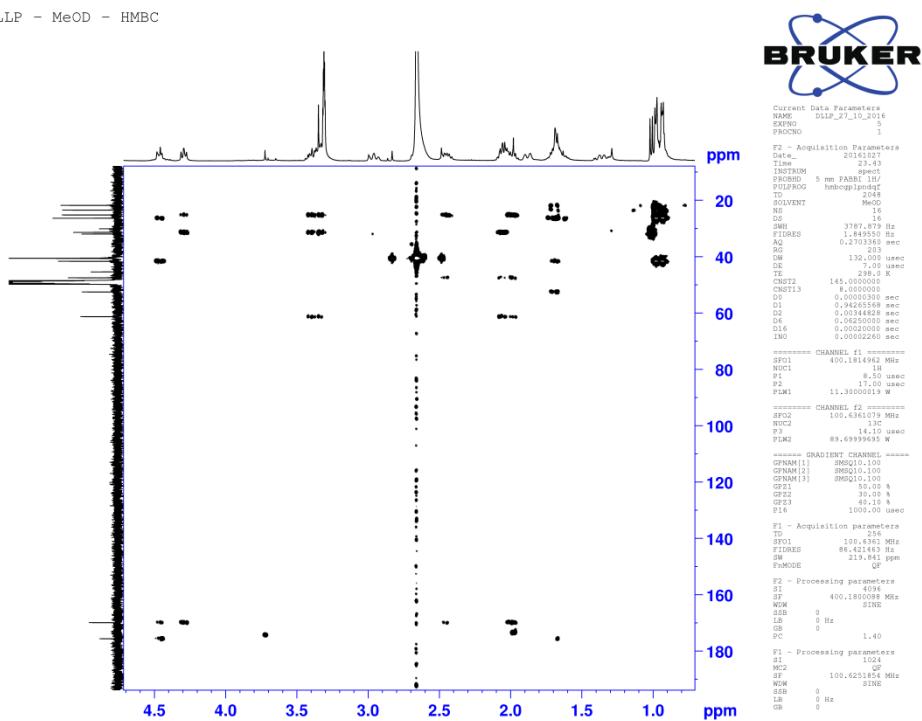
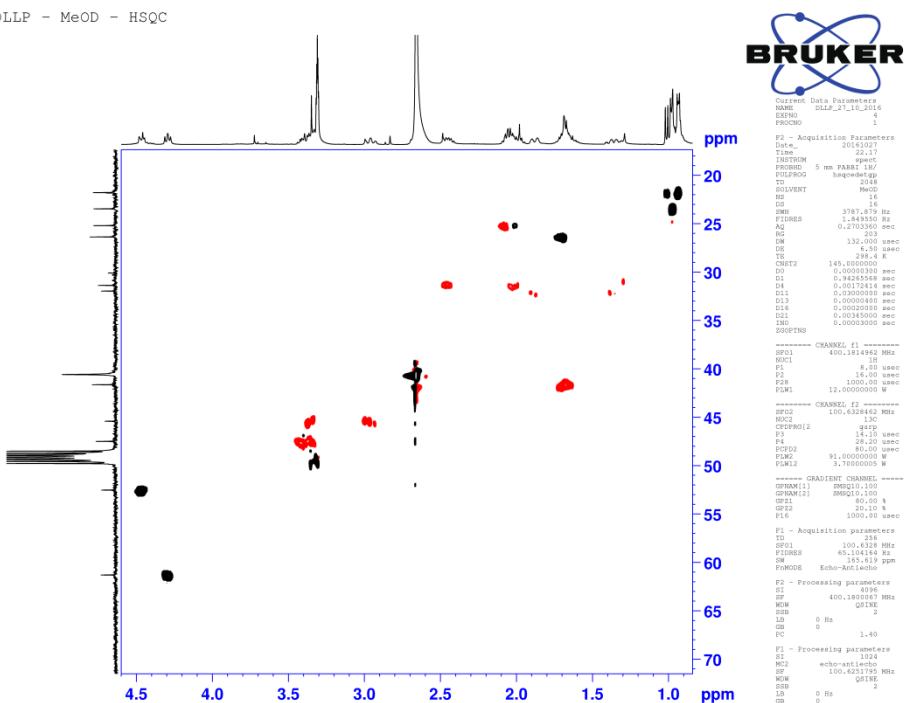
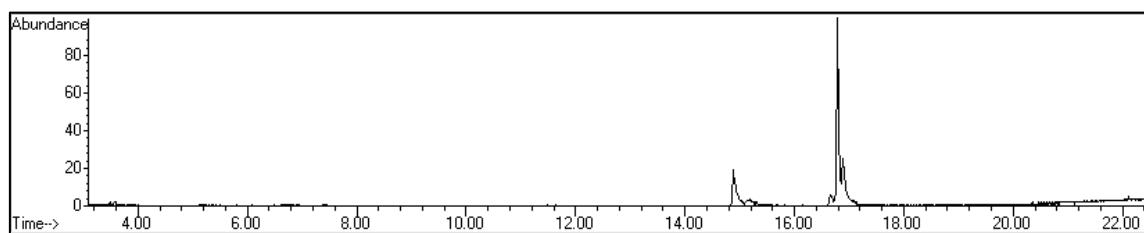


Figure S41. 2D NMR ( $^1\text{H}, ^{13}\text{C}$  - HMBC) of standard cyclo(L-Pro-D-Leu)

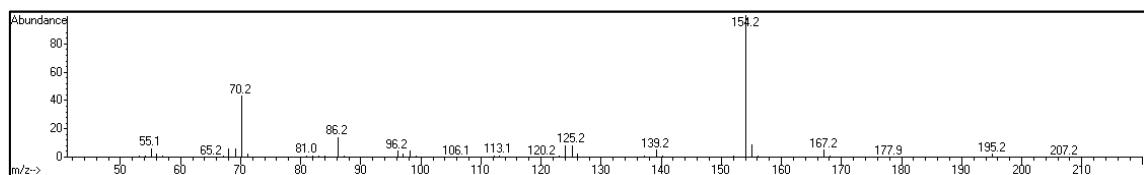
DLLP - MeOD - HSQC



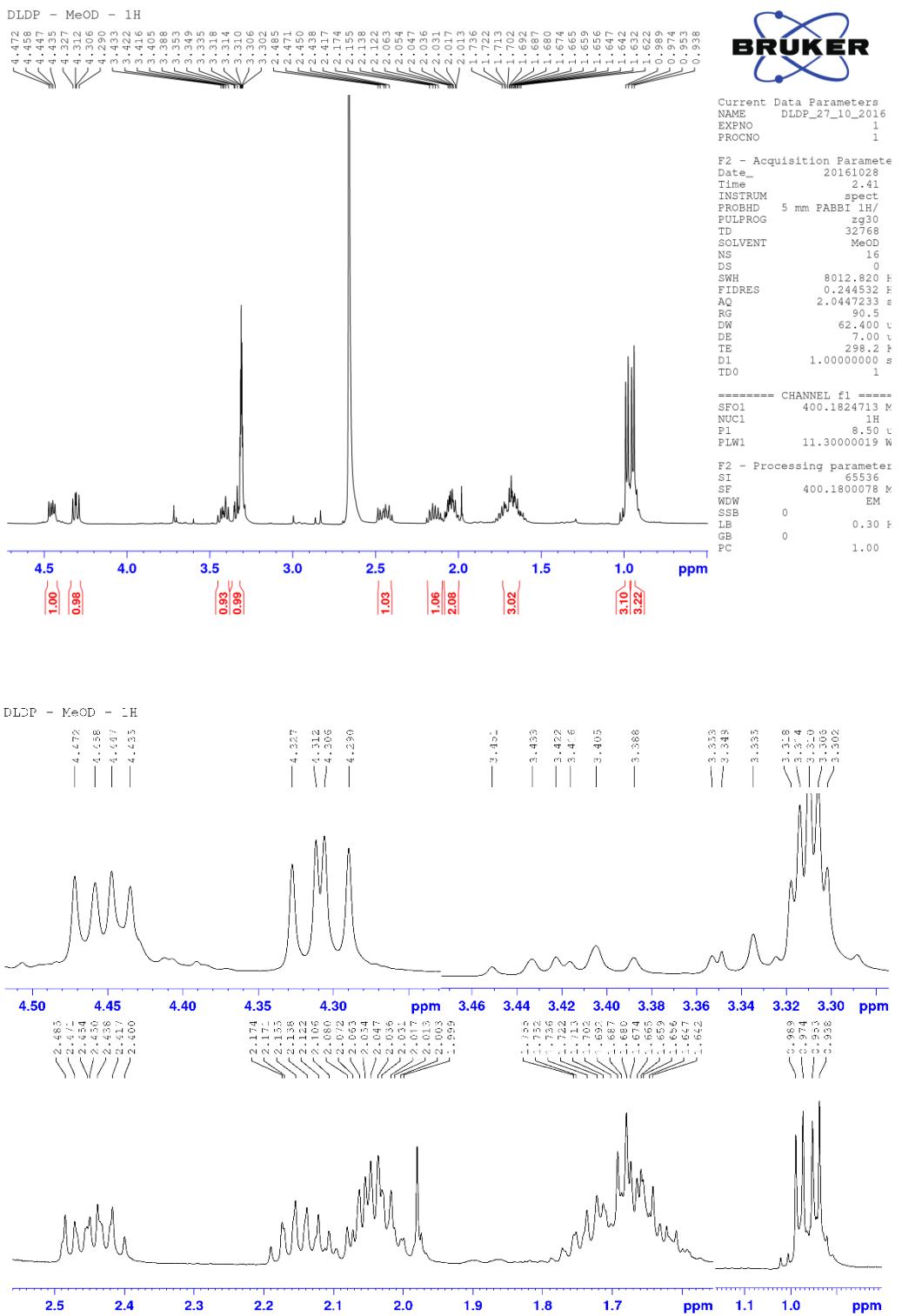
### Standard cyclo(D-Pro-D-Leu)



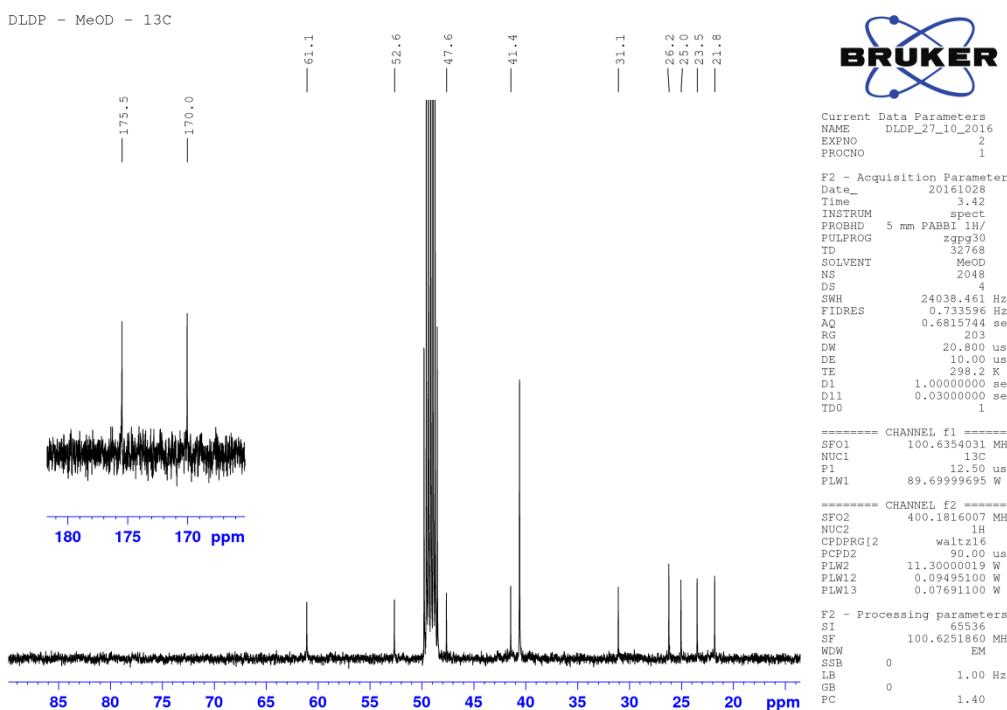
**Figure S43.** GC-MS chromatogram of standard cyclo(D-Pro-D-Leu)



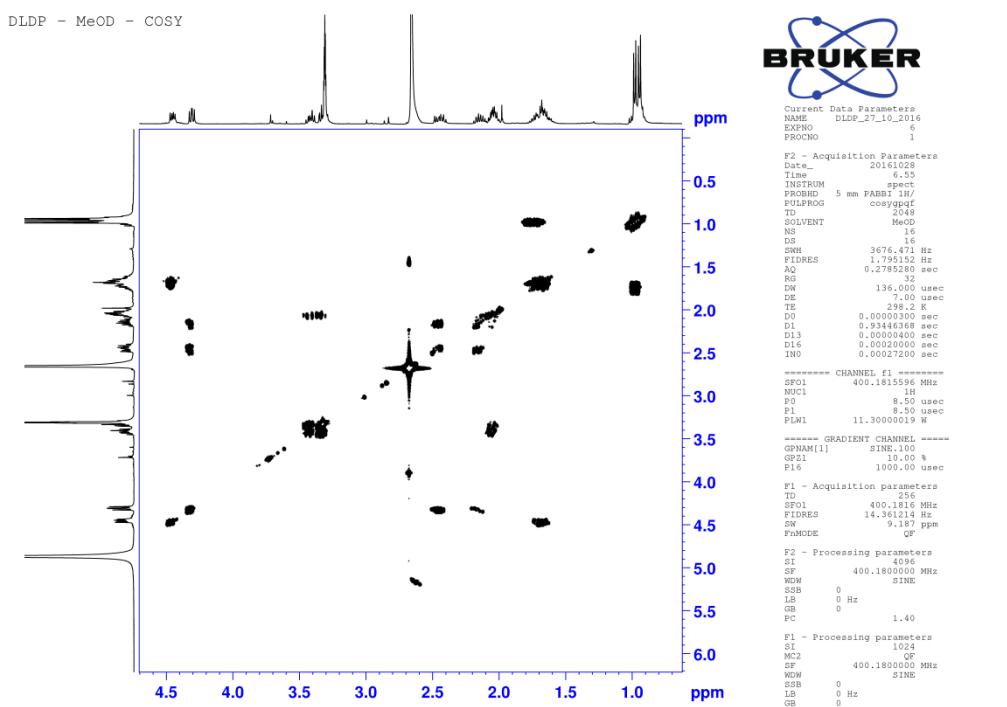
**Figure S44.** EI-MS spectrum of standard cyclo(D-Pro-D-Leu)



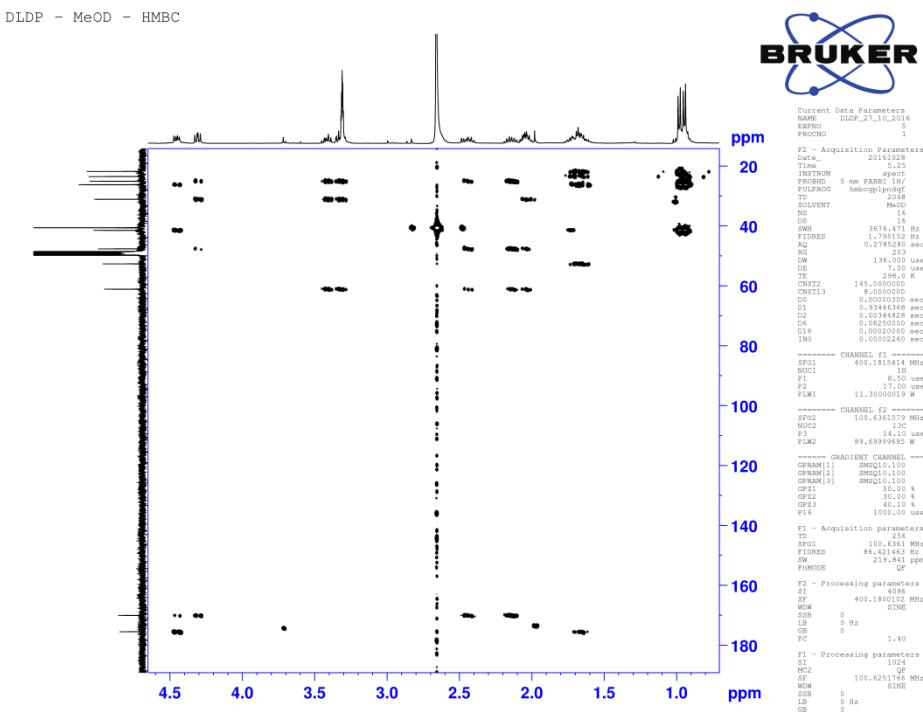
**Figure S45.**  $^1\text{H}$  NMR of standard cyclo(D-Pro-D-Leu) (full spectrum and expansions)



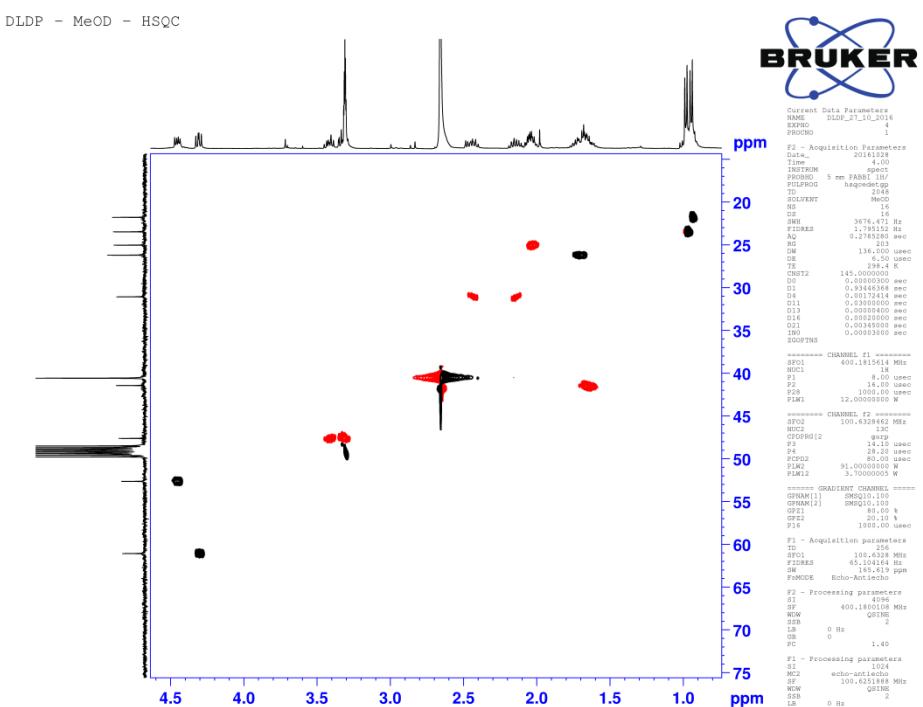
**Figure S46.**  $^{13}\text{C}$  NMR of standard cyclo(D-Pro-D-Leu)



**Figure S47.** 2D NMR ( $^1\text{H}, ^1\text{H}$  - COSY) of standard cyclo(D-Pro-D-Leu)



**Figure S48.** 2D NMR ( $^1\text{H}$ ,  $^{13}\text{C}$  - HMBC) of standard cyclo(D-Pro-D-Leu)



**Figure S49.** 2D NMR ( $^1\text{H}$ ,  $^{13}\text{C}$  - HSQC) of standard cyclo(D-Pro-D-Leu)

### Characterization of cyclo(Pro-Leu) isomers by chiral GC-FID

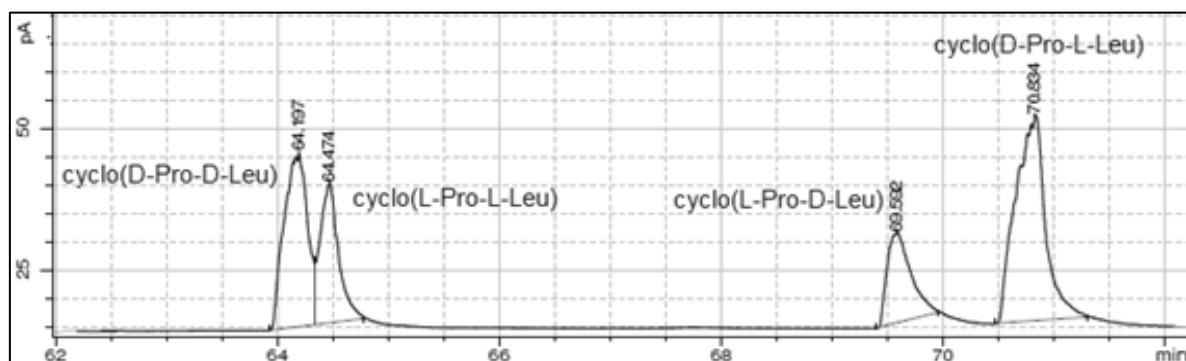


Figure S50. Chiral chromatogram for standards cyclo(Pro-Leu) isomers

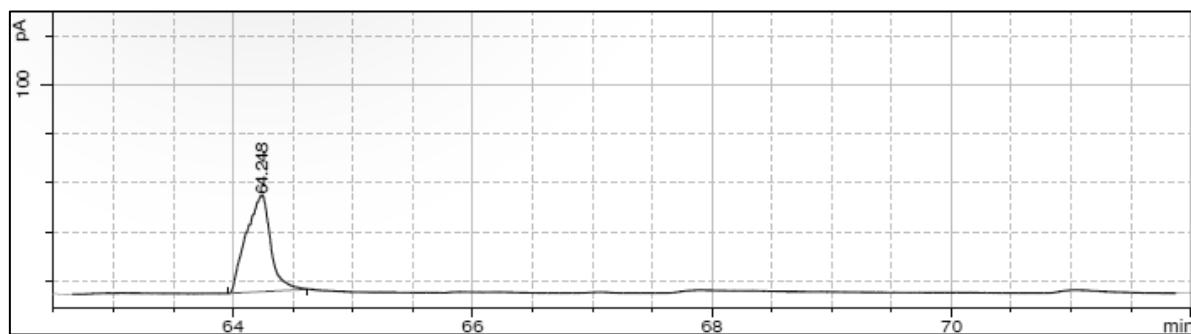


Figure S51. Chiral chromatogram for *Bacillus cereus* cyclo(Pro-Leu)

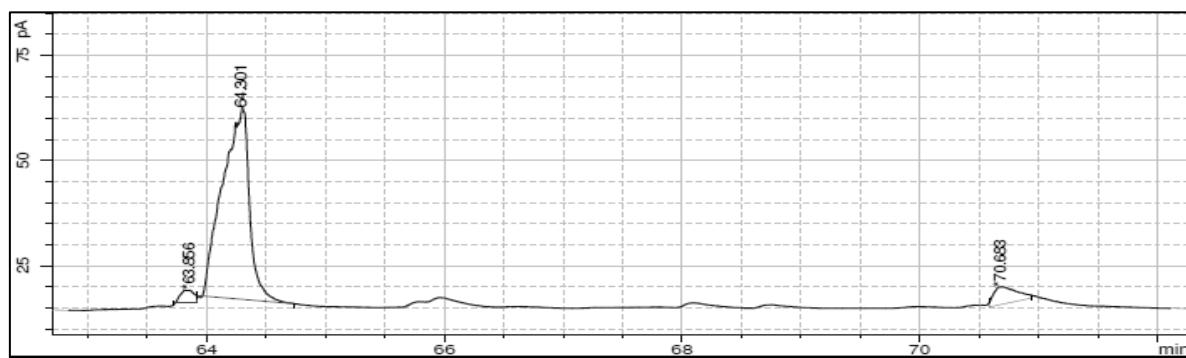


Figure S52. Chiral chromatogram for *Cronobacter sakazakii* cyclo(Pro-Leu)

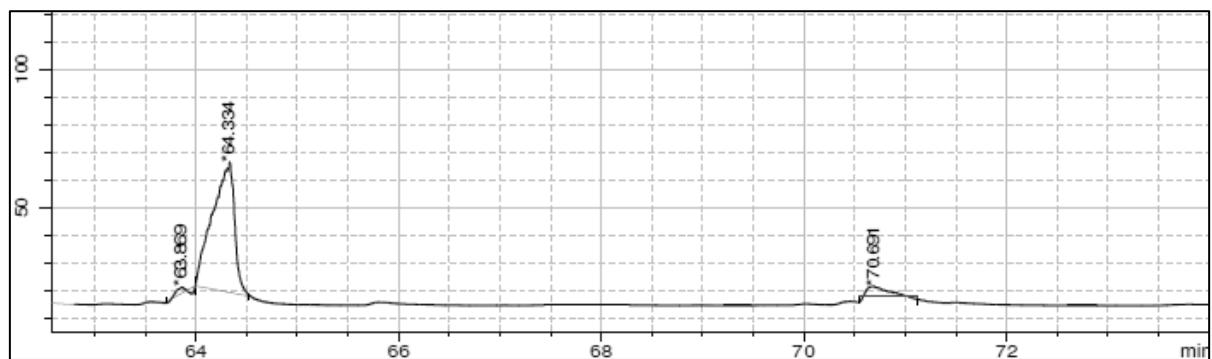
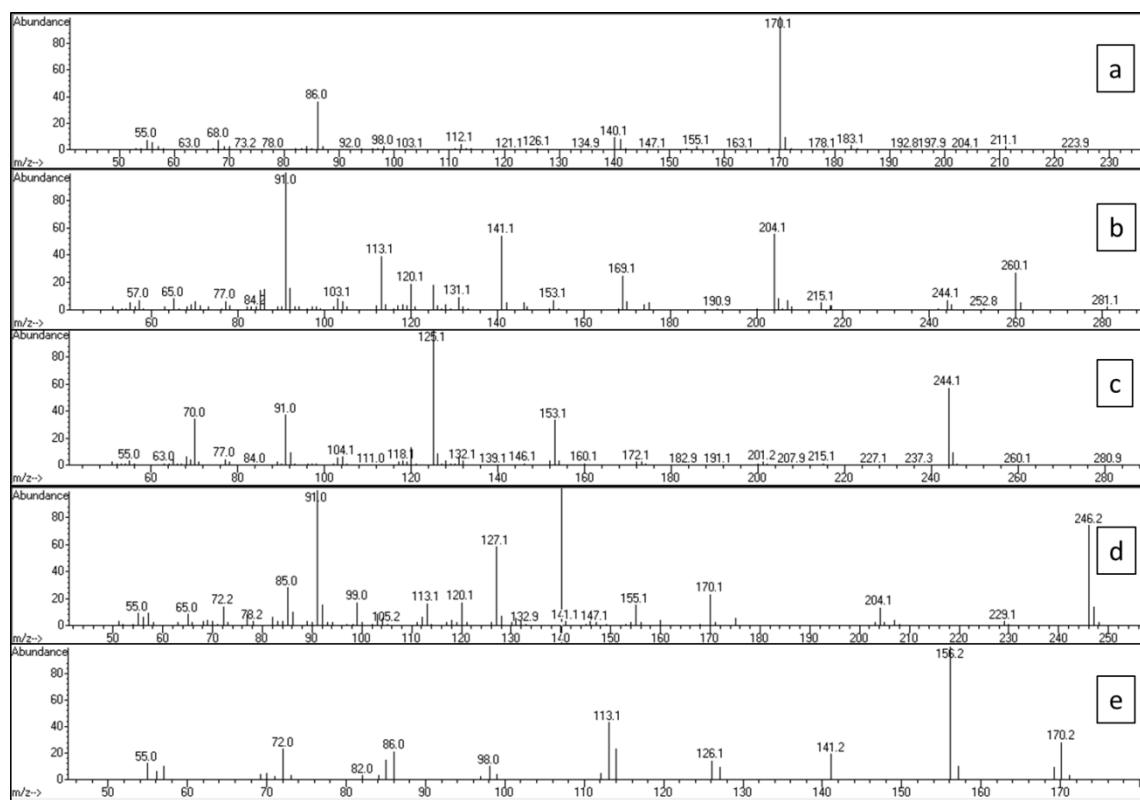


Figure S53. Chiral chromatogram for co-culture cyclo(Pro-Leu)

### Mass spectrum of others diketopiperazines



**Figure S54.** Mass spectrum for *B. cereus* and *C. sakazakii* cultures occurrence of (a) cyclo(Leu-Leu), (b) cyclo(Phe-Leu), (c) cyclo(Phe-Pro), (d) cyclo(Phe-Val) and (e) cyclo(Val-Val)

**Table S2.** EI-MS profile of DKPs found in cultures of *B. cereus*<sup>†</sup> and *C. sakazakii*\* in smaller proportions

DKPs	Molecular Weight	m/z (%)
<b>2</b> Cyclo(Val-Val)* <sup>‡</sup>	198.26	156 (99); 113 (37); 72 (26); 170 (23); 86 (22); 114 (19); 126 (17); 141 (14); 85 (12); 98 (11)
<b>3</b> Cyclo(Leu-Leu)*	226.32	170 (99); 86 (37); 171 (9); 140 (9); 141 (8); 68 (7); 55 (6); 56 (5); 112 (4); 183 (3)
<b>4</b> Cyclo(Phe-Val)*	246.30	91 (99); 246 (78); 127 (61); 170 (36); 85 (25); 113 (19); 155 (19); 120 (18); 86 (16); 92 (15)
<b>5</b> Cyclo(Phe-Leu)*	260.33	91 (99); 204 (55); 141 (54); 113 (38); 260 (27); 169 (25); 120 (19); 125 (18); 92 (16); 86 (15)
<b>6</b> Cyclo(Phe-Pro)*	244.29	125 (99); 153 (40); 244 (38); 91 (37); 70 (36); 207 (22); 170 (15); 92 (10); 68 (7); 126 (7)