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# Identification of the Biosynthetic Gene Cluster for the Anti-infective Desotamides and Production of a New Analogue in a Heterologous Host

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**Table S1.** Deduced Functions of ORFs in the DSA Gene Cluster.

| Protein | Size <sup>a</sup> | Proposed function                                                         | ID/SI <sup>b</sup> | Protein homologue and origin                                                             |
|---------|-------------------|---------------------------------------------------------------------------|--------------------|------------------------------------------------------------------------------------------|
| Orf(-3) | 474               | unknown function                                                          | 33/45              | hypothetical protein (WP_030349674.1); <i>Streptomyces scopuliridis</i>                  |
| Orf(-2) | 160               | transcriptional regulator                                                 | 97/98              | AsnC family transcriptional regulator (WP_030349675.1); <i>Streptomyces scopuliridis</i> |
| Orf(-1) | 233               | hydrolase                                                                 | 93/95              | hydrolase (WP_030349676.1); <i>Streptomyces scopuliridis</i>                             |
| DsaA    | 305               | putative transcriptional regulator                                        | 53/67              | DF17_03230 (KEF09364.1); <i>Actinoplanes</i> sp. N902-109                                |
| DsaB    | 270               | indole-3-glycerol phosphate synthase                                      | 66/78              | Z951_06575 (EXU68839.1); <i>Streptomyces</i> sp. PRh5                                    |
| DsaC    | 167               | putative YbaK/prolyl-tRNA synthetase associated domain-containing protein | 66/74              | STVIR_5719 (ELS53322.1); <i>Streptomyces viridochromogenes</i> Tue57                     |
| DsaD    | 377               | aminotransferase                                                          | 71/79              | STVIR_5718 (ELS53321.1); <i>Streptomyces viridochromogenes</i> Tue57                     |
| DsaE    | 121               | isomerase                                                                 | 74/82              | STVIR_5717 (ELS53320.1); <i>Streptomyces viridochromogenes</i> Tue57                     |
| DsaF    | 69                | MbtH domain protein                                                       | 71/79              | STVIR_5715 (ELS53318.1); <i>Streptomyces viridochromogenes</i> Tue57                     |
| DsaG    | 2642              | NRPS (C-A-PCP-C-A-PCP-TE)                                                 | 57/67              | STVIR_5714 (ELS53317.1); <i>Streptomyces viridochromogenes</i> Tue57                     |
| DsaH    | 2255              | NRPS (A-PCP-E-C- A-PCP)                                                   | 44/57              | DC60_02735 (KDR59525.1); <i>Streptomyces wadayamensis</i>                                |
| DsaI    | 2377              | NRPS (A-PCP-C-A-PCP-C)                                                    | 44/57              | XNR_0983 (AGI87382.1); <i>Streptomyces albus</i> J1074                                   |
| DsaJ    | 469               | $\beta$ -lactamase                                                        | 58/65              | STVIR_5710 (ELS53338.1); <i>Streptomyces viridochromogenes</i> Tue57                     |
| DsaK    | 919               | ABC-transporter permease                                                  | 64/77              | STVIR_5708 (ELS53336.1); <i>Streptomyces viridochromogenes</i> Tue57                     |
| DsaL    | 226               | putative ABC transporter ATP-binding protein                              | 78/87              | STVIR_5707 (ELS53335.1); <i>Streptomyces viridochromogenes</i> Tue57                     |
| DsaM    | 403               | putative Integral membrane sensor signal transduction histidine kinase    | 65/77              | STVIR_5706 (ELS53334.1); <i>Streptomyces viridochromogenes</i> Tue57                     |
| DsaN    | 219               | LuxR family two component transcriptional regulator                       | 81/89              | STVIR_5705 (ELS53333.1); <i>Streptomyces viridochromogenes</i> Tue57                     |
| DsaO    | 149               | hypothetical protein                                                      | 41/57              | STVIR_5704 (ELS53332.1); <i>Streptomyces viridochromogenes</i> Tue57                     |
| DsaP    | 228               | thioesterase                                                              | 60/73              | STVIR_5701 (ELS53329.1); <i>Streptomyces viridochromogenes</i> Tue57                     |
| DsaQ    | 379               | catabolite control protein A                                              | 85/89              | SBD_7924 (EMF50360.1); <i>Streptomyces bottropensis</i> ATCC 25435                       |
| Orf(+1) | 518               | ATP-binding protein                                                       | 85/89              | SSPG_04782 (EFD69142.1); <i>Streptomyces lividans</i> TK24                               |
| Orf(+2) | 657               | inner-membrane translocator                                               | 82/87              | SACTE_5761 (AEN13550.1); <i>Streptomyces</i> sp. SirexAA-E                               |

<sup>a</sup>in amino acids; <sup>b</sup>ID/SI: Identity/similarity.

**Table S2.** Analysis of Adenylation Domains in the DSA Cluster: Predicted Substrate Binding Pockets and Postulated Functions.

| A-domain | Substrate specific sequence | Predicted substrate | Postulated function                      |
|----------|-----------------------------|---------------------|------------------------------------------|
| DsaI-A1  | DVAMAGMV                    | No hit              | activation of L-Trp                      |
| DsaI-A2  | DALLMGAV                    | Phe/Trp             | activation of L-Leu                      |
| DsaH-A3  | DALFMGAV                    | Phe/Trp             | activation of L-Leu                      |
| DsaH-A4  | DALWMGGV                    | Val                 | activation of L-Val/ L- <i>allo</i> -Ile |
| DsaG-A5  | DLTKVGEV                    | Asn                 | activation of L-Asn/L-Asp                |
| DsaG-A6  | DLLQVGLI                    | Gly                 | activation of L-Gly                      |

**Table S3.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data for Compounds **5** and **1** in  $\text{DMSO-}d_6$ .

| Desotamide A ( <b>1</b> ) |                     |                                               | Desotamide E ( <b>5</b> ) |                                               |
|---------------------------|---------------------|-----------------------------------------------|---------------------------|-----------------------------------------------|
| Pos.                      | $\delta_{\text{C}}$ | $\delta_{\text{H}}$ , mult. ( $J$ in Hz)      | $\delta_{\text{C}}$       | $\delta_{\text{H}}$ , mult. ( $J$ in Hz)      |
|                           | Trp                 |                                               | Trp                       |                                               |
| 1                         | 170.9, C            |                                               | 170.9, C                  |                                               |
| 2                         | 55.1, CH            | 4.36, m*                                      | 54.9, CH                  | 4.30, m                                       |
| 3                         | 27.2, $\text{CH}_2$ | 3.15, dd (14.5, 4.5)<br>2.99, dd (14.5, 10.0) | 27.1, $\text{CH}_2$       | 3.21, dd (14.5, 3.5)<br>2.98, dd (14.5, 10.0) |
| 2                         | NH                  | 8.31, d (8.0)                                 | NH                        | 8.33, d (7.5)                                 |
| 1'                        | NH                  | 10.82, s                                      | NH                        | 10.84, d (2.0)                                |
| 2'                        | 123.3, CH           | 7.13, s                                       | 123.5, CH                 | 7.16, d (2.0)                                 |
| 3'                        | 109.9, C            |                                               | 110.0, C                  |                                               |
| 3'a                       | 126.9, C            |                                               | 127.0, C                  |                                               |
| 4'                        | 117.9, CH           | 7.51, d (8.0)                                 | 118.0, CH                 | 7.52, d (8.0)                                 |
| 5'                        | 118.2, CH           | 6.98, t (8.0)                                 | 118.3, CH                 | 6.98, t (7.5)                                 |
| 6'                        | 120.7, CH           | 7.06, t (8.0)                                 | 120.9, CH                 | 7.06, t (7.5)                                 |
| 7'                        | 111.2, CH           | 7.32, d (8.0)                                 | 111.3, CH                 | 7.33, d (8.0)                                 |
| 7'a                       | 136.0, C            |                                               | 136.1, C                  |                                               |
|                           | Gly                 |                                               | Gly                       |                                               |
| 1                         | 168.9, C            |                                               | 169.2, C                  |                                               |
| 2                         | 43.1, $\text{CH}_2$ | 3.90, dd (16.0, 6.5)<br>3.29, dd (16.0, 4.5)  | 43.4, $\text{CH}_2$       | 3.89, dd (15.0, 5.5)<br>3.28, dd (15.0, 5.0)  |
|                           | NH                  | 7.95, t (5.0)                                 | NH                        | 8.19, t (5.0)                                 |
|                           | Asn                 |                                               | Asp                       |                                               |
| 1                         | 170.7, C            |                                               | 170.7, C                  |                                               |
| 2                         | 49.2, CH            | 4.58, dd, (7.5, 4.0)                          | 49.3, CH                  | 4.55, dd (14.0, 6.5)                          |
| 3                         | 36.7, $\text{CH}_2$ | 2.82, dd (16.0, 5.5)<br>2.63, dd (16.0, 5.5)  | 36.5, $\text{CH}_2$       | 2.80, dd (16.0, 5.0)<br>2.64, dd (16.0, 6.5)  |
| 4                         | 171.8, C            |                                               | 171.5, C                  |                                               |
| 2                         | NH                  | 7.61, d (7.0)                                 | NH                        | 7.82, d (6.5)                                 |
|                           | NH2                 | 7.62, s<br>7.08, s                            | OH                        | 12.28, s                                      |

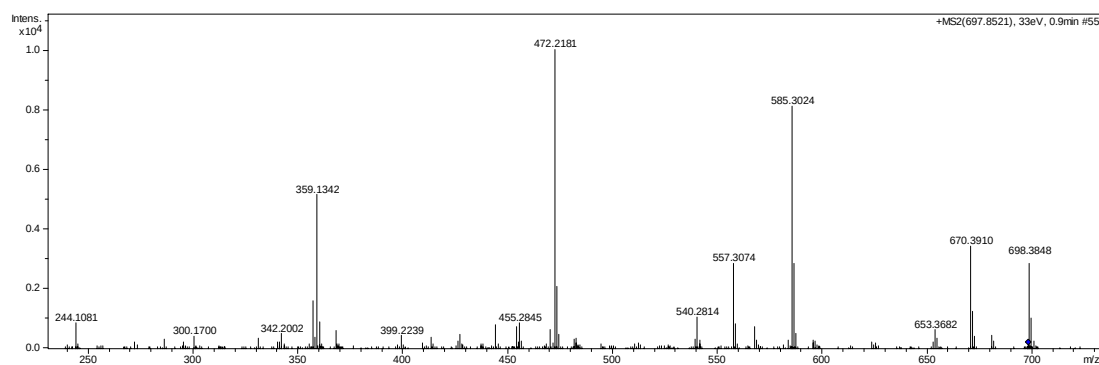
|      | Ile                   |                     |  | Ile                   |                      |  |
|------|-----------------------|---------------------|--|-----------------------|----------------------|--|
| 1    | 170.7, C              |                     |  | 170.5, C              |                      |  |
| 2    | 56.4, CH              | 4.19, dd (7.0, 4.0) |  | 56.0, CH              | 4.18, dd (8.0, 4.5)  |  |
| 3    | 35.1, CH              | 2.02, m             |  | 35.1, CH              | 1.99, m              |  |
| 4    | 25.5, CH <sub>2</sub> | 1.18, m             |  | 25.6, CH <sub>2</sub> | 1.18, m              |  |
|      |                       | 1.27, m             |  |                       |                      |  |
| 5    | 11.3, CH <sub>3</sub> | 0.83, t (7.5)       |  | 11.4, CH <sub>3</sub> | 0.81, t*             |  |
| 3-Me | 14.3, CH <sub>3</sub> | 0.84, d (6.5)       |  | 14.4, CH <sub>3</sub> | 0.82, d (6.5)        |  |
|      | NH                    | 8.25, d (7.5)       |  | NH                    | 8.22, d (6.5)        |  |
|      | Leu1                  |                     |  |                       |                      |  |
| 1    | 173.2, C              |                     |  | 172.7, C              |                      |  |
| 2    | 51.6, CH              | 4.34, m*            |  | 51.7, CH              | 4.34, m              |  |
| 3    | 39.7, CH <sub>2</sub> | 1.45, m             |  | 39.9, CH <sub>2</sub> | 1.45, m*             |  |
| 4    | 24.0, CH              | 1.53, m             |  | 24.1, CH              | 1.54, m              |  |
| 5    | 21.9, CH <sub>3</sub> | 0.84, d (6.5)       |  | 22.4, CH <sub>3</sub> | 0.83, d (6.5)        |  |
| 4-Me | 22.4, CH <sub>3</sub> | 0.84, d (6.5)       |  | 22.6, CH <sub>3</sub> | 0.84, d (6.5)        |  |
|      | NH                    | 8.31, d (5.5)       |  | NH                    |                      |  |
|      | Leu2                  |                     |  |                       |                      |  |
| 1    | 171.6, C              |                     |  | 172.0, C              |                      |  |
| 2    | 50.6, CH              | 4.37, m*            |  | 50.5, CH              | 4.40, dd (14.5, 7.5) |  |
| 3    | 41.4, CH <sub>2</sub> | 1.55, m             |  | 41.5, CH <sub>2</sub> | 1.47, m*             |  |
|      |                       | 1.45, m             |  |                       |                      |  |
| 4    | 24.3, CH              | 1.44, m             |  | 24.3, CH              | 1.46, m*             |  |
| 5    | 22.3, CH <sub>3</sub> | 0.90, d (6.0)       |  | 22.4, CH <sub>3</sub> | 0.89, d (6.5)        |  |
| 4-Me | 22.3, CH <sub>3</sub> | 0.90, d (6.0)       |  | 22.6, CH <sub>3</sub> | 0.90, d (6.5)        |  |
|      | NH                    | 7.66, d (8.0)       |  | NH                    |                      |  |

\* Resonances overlapped.

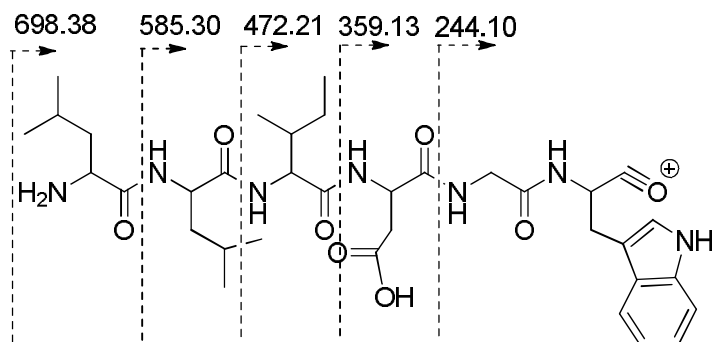
**Table S4.** Primer Pairs Used in PCR Screening of *Streptomyces scopuliridis* SCSIO ZJ46 Genomic Library.

| Locus       | Primer code | Sequences (5'-3')      | Length of deduced product |
|-------------|-------------|------------------------|---------------------------|
| <i>dsaA</i> | dsaAF       | GACTCCGTCGCTGAGCATG    | 377 bp                    |
|             | dsaAR       | TTCCGCAGGTCGATCACATA   |                           |
| <i>dsaH</i> | dsaHF       | GCTCCAGTACTCCAGCTCCGC  | 506 bp                    |
|             | dsaHR       | TTCGACATGTCGATGGCCG    |                           |
| <i>orf1</i> | orf1F       | TTGCTGCGTGTCGAAGGGAT   | 595 bp                    |
|             | orf1R       | CCTCCAGATGGTGGGTGATGAA |                           |

**Figure S1.** (+) HRESIMS/MS Spectrum of **5**.

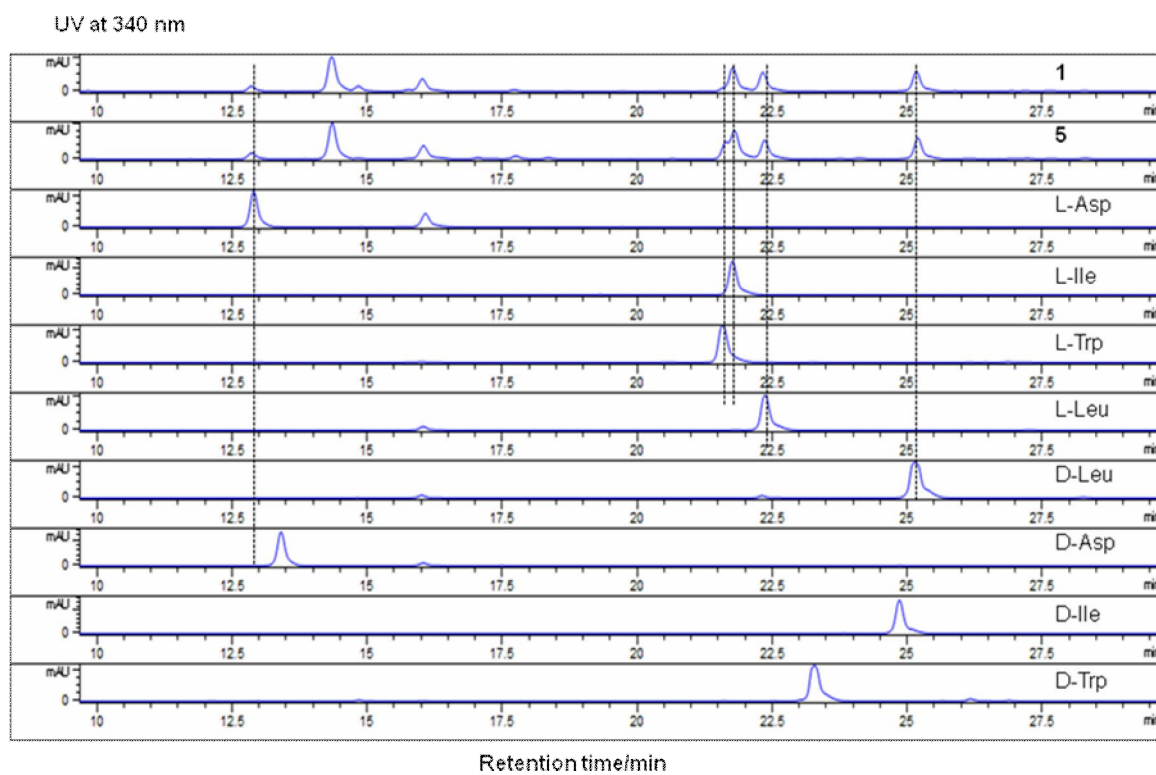


**Figure S2.** The (+) HRESIMS/MS Fragments of **5**.

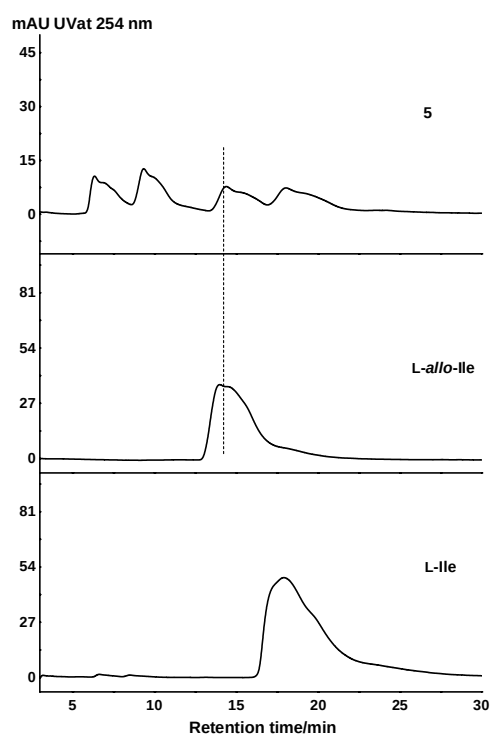




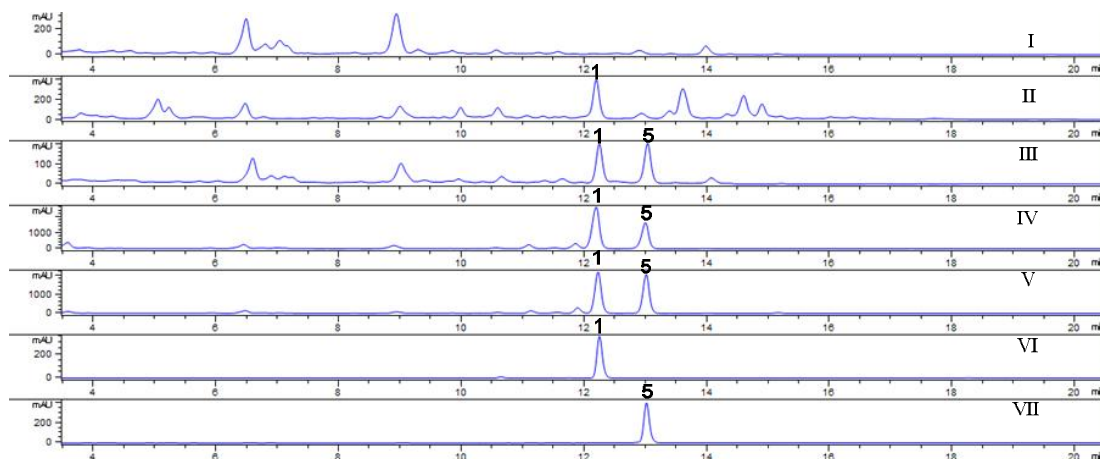
**Figure S3.** Marfey's Analysis of Acid Hydrolysates of **1** and **5** and Standard Amino Acids of L-Asp, L-Ile, L-Trp, L-Leu, D-Leu, D-Asp, D-Ile and D-Trp.



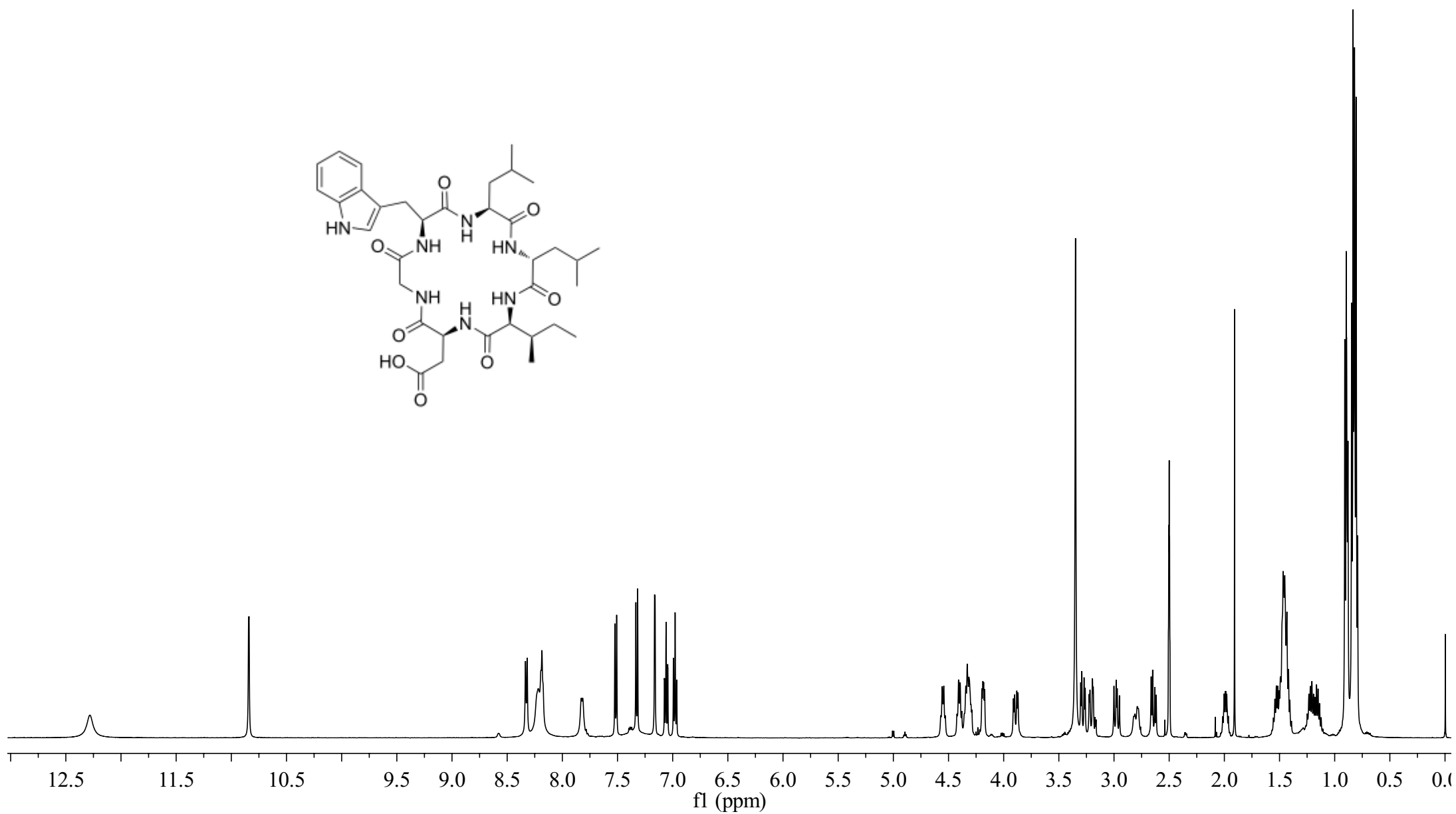
**Figure S4.** Chiral-phase HPLC Analysis of the Hydrolysate of **5** and Standard Amino Acids of L-*allo*-Ile and L-Ile.



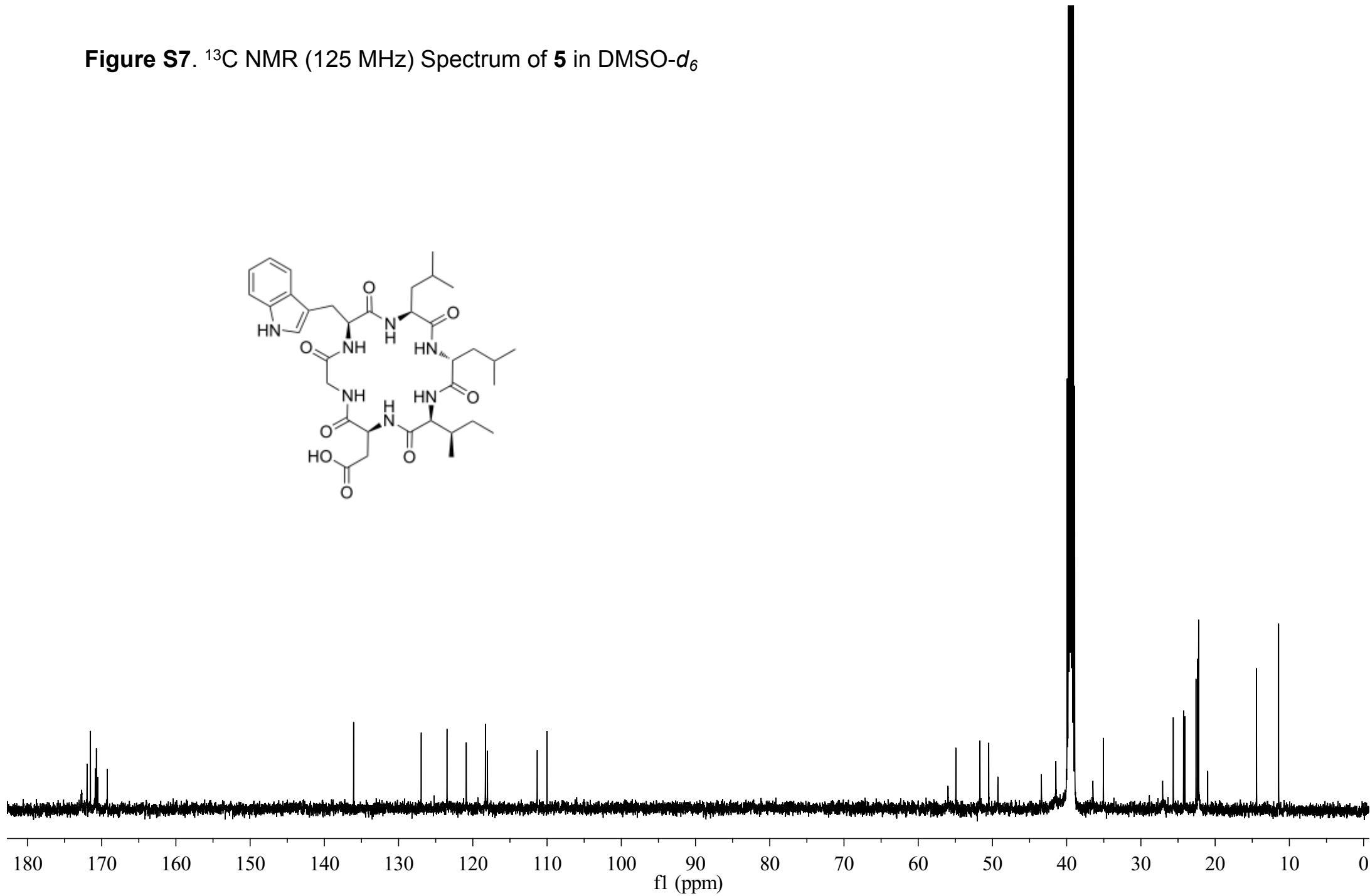
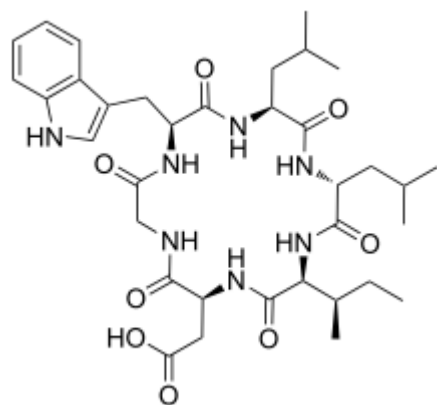
**Figure S5.** HPLC Analyses of the Fermentation Extracts of *S. coelicolor* M1152 Fed with or without Compound **1**. (I) control 1, *S. coelicolor* M1152 with supplemental 20  $\mu$ L DMSO; (II) control 2, modified Am2ab medium (50 mL) with supplemental 3.0 mg desotamide A (**1**) dissolved in 20  $\mu$ L DMSO; (III) *S. coelicolor* M1152 with supplemental 3.0 mg desotamide A (**1**) dissolved in 20  $\mu$ L DMSO; (IV) *S. coelicolor* M1152/07-6H; (V) *S. coelicolor* M1152/07-9A; (VI) purified compound **1**; (VII) purified compound **5**.



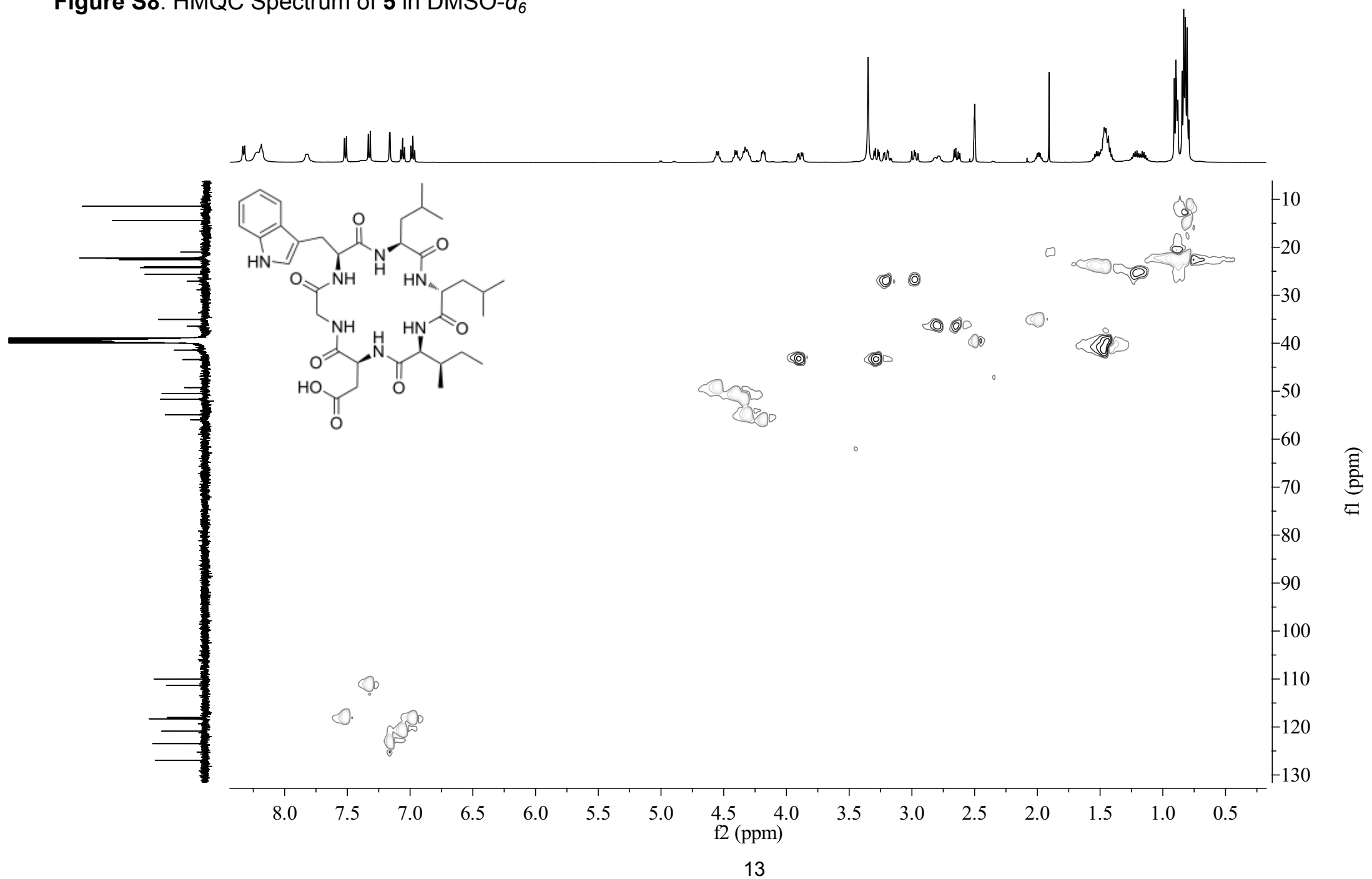
**Figure S6.**  $^1\text{H}$  NMR (500 MHz) Spectrum of **5** in  $\text{DMSO-}d_6$



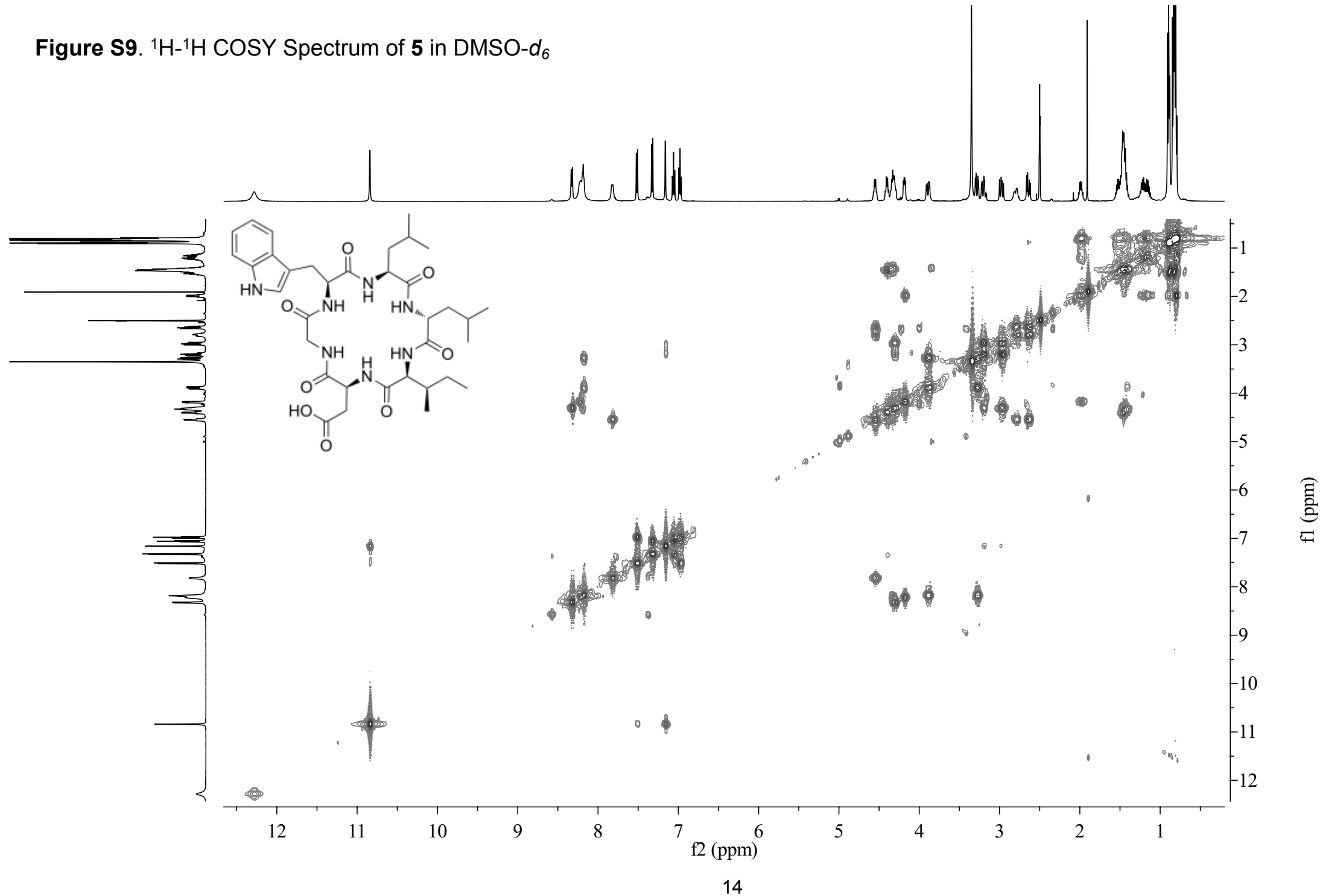
**Figure S7.**  $^{13}\text{C}$  NMR (125 MHz) Spectrum of **5** in  $\text{DMSO-}d_6$



**Figure S8.** HMQC Spectrum of **5** in DMSO-*d*<sub>6</sub>



**Figure S9.**  $^1\text{H}$ - $^1\text{H}$  COSY Spectrum of **5** in  $\text{DMSO-}d_6$



**Figure S10.** HMBC Spectrum of **5** in DMSO- $d_6$

