# Supplementary Material

**Table S1.** Cyclic heptapeptides in the sponge *Stylissa caribica.*

| Cyclic peptide | [M+H]$^+$ | Exact Mass | Retention time RP-18[a] | Retention time PFP[b] | Citation |
|---|---|---|---|---|---|
| Stylissamide A | $C_{44}H_{61}N_8O_9$ | 845.4556 | 1.72 | 26.34 | *c,d,e* |
| Stylissamide B | $C_{44}H_{58}N_7O_8$ | 812.4341 | ND | ND | *c,d,e* |
| Stylissamide C | $C_{48}H_{60}N_7O_8$ | 862.4498 | 20.29 | 32.66 | *c,d,e* |
| Stylissamide D | $C_{45}H_{62}N_7O_8$ | 828.4654 | 19.55 | 32.48 | *c,d,e* |
| Stylissamide E | $C_{39}H_{59}N_8O_9$ | 783.4400 | 13.29 | 28.76 | *d,e* |
| Stylissamide F | $C_{43}H_{57}N_{10}O_9$ | 857.4304 | 13.94 | 32.21 | *d,e* |
| Stylissamide G | $C_{45}H_{62}N_7O_7$ | 812.4705 | ND | ND | *e* |
| Stylissamide H | $C_{44}H_{59}N_8O_8$ | 827.4450 | ND | ND | *e* |
| Stylissamide L | $C_{41}H_{53}N_8O_{10}$ | 817.3876 | 6.21 | 24.95 | this work |
| Hymenamide C | $C_{43}H_{55}N_8O_9$ | 827.4087 | 20.71 | 32.58 | *f* |
| Hymenamide F | $C_{35}H_{61}N_{10}O_8S$ | 781.4389 | 1.67 | 26.40 | *f* |
| Phakellistatin 3 | $C_{42}H_{55}N_8O_9$ | 815.4087 | 18.50 | 31.89 | *h* |
| Phakellistatin 13 | $C_{42}H_{55}N_8O_8$ | 799.4137 | 20.09 | 32.47 | *g* |
| Stylisin 1 | $C_{45}H_{62}N_7O_8$ | 828.4654 | ND | ND | *c,d,e,g* |
| Stylisin 2 | $C_{44}H_{58}N_7O_8$ | 812.4341 | ND | ND | *c,d,e,g* |

*a.* Experiments were performed with a Kinetex 5 μm, 50 mm × 2.10 mm C18 column using a flow rate of 200 μL/min and the following elution gradient: 10% MeOH for 1 min, 10%−100% MeOH over 30 min, and 100% MeOH for 10 min.

*b.* Experiments were performed with a Kinetex 5 μm, 100 mm × 2.1 mm PFP column using a flow rate of 200 μL/min and the same elution gradient of $H_2O$ and MeOH described above.

*c.* Schmidt, G.; Grube, A.; Köck, M. Stylissamides A-D - New proline-containing cyclic heptapeptides from the marine sponge Stylissa caribica. European *J. Org. Chem.* **2007**, 2, 4103–4110, doi:10.1002/ejoc.200700013.

*d.* Cychon C.; Köck,M. Stylissamides E and F, Cyclic Heptapeptides from the Caribbean Sponge Stylissa caribica. *J. Nat. Prod.* **2010,** *73*, 738–742, doi: 10.1021/np900664f.

*e.* Wang, X.; Morinaka, B. I.; Molinski, T. F. Structures and solution conformational dynamics of stylissamides G and H from the Bahamian Sponge Stylissa caribica. *J. Nat. Prod.* **2014**, *77*, 625–630, doi:10.1021/np400891s.

*f.* Grube, A.; Maier, T.; Köck, M. MS-guided Fractionation as a Fast Way to the Identification of New Natural Products – MALDI-TOF-MS Screening of the Marine Sponge Stylissa caribica. *Zeitschrift für Naturforsch. B* **2007**, *62*, 600–604, doi:10.1515/znb-2007-0420..

*g.* Mohammed, R.; Peng, J.; Kelly, M.; Hamann, M.T. Cyclic heptapeptides from the Jamaican sponge Stylissa caribica. *J. Nat. Prod.* **2006**, *69*, 1739–1744, doi:10.1021/np060006n.

*h.* This compound was not previously detected in specimens of *Stylissa caribica*. It was putatively identified by the Dereplicator tool in GNPS.

Table S2. Full NMR data of stylissamide L (1) ($^1$H 700 MHz, $^{13}$C 175 MHz, DMSO-$d_6$).

| AA | pos. | δ$_C$, type | | δ$_H$, mult (J in Hz) | NOESY | HMBC |
|---|---|---|---|---|---|---|
| Pro$^I$ | 1 | 170.3, C | | - | | |
| | 2 | 59.1, CH | | 4.34, dd (5.1, 8.6) | Ser-NH, Tyr-NH, Phe-NH | Phe-1 |
| | 3 | 28.1, CH$_2$ | a | 2.15, m | Pro$^{II}$-4b, Pro$^{II}$-3a | Pro$^I$-1 |
| | | | b | 1.75, m | Pro$^{II}$-2, Pro$^{II}$-4b | Pro$^I$-1 |
| | 4 | 24.3, CH$_2$ | | 1.87, m | | |
| | 5 | 46.7, CH$_2$ | a | 3.45, m | | |
| | | | b | 3.36, m | | Phe-1, Pro$^I$-2 |
| Pro$^{II}$ | 1 | 171.8, C | | - | | |
| | 2 | 60.1, CH | | 4.28, dd (1.5, 8.8) | Ser-NH, Pro$^I$-2, Pro$^I$-3b | Pro$^{II}$-1, Pro$^{II}$-5 |
| | 3 | 31.8, CH2 | a | 2.16, m | | Pro$^{II}$-1 |
| | | | b | 2.00, m | Ser-NH | |
| | 4 | 21.7, CH2 | a | 1.77, m | Pro$^{III}$-3a | |
| | | | b | 1.57, m | | |
| | 5 | 46.8, CH2 | a | 3.60, ddd (1.5, 8.4, 10.8) | Ser-NH | |
| | | | b | 3.33, ddd (10.8, 10.8, 7.1) | Pro$^{III}$-4b | Pro$^I$-1 |
| Ser | NH | | | 7.65, d (5.9) | Tyr-NH, Pro$^I$-2, Pro$^{II}$-2, Pro$^{II}$-5a, Pro$^{II}$-4b | Pro$^{II}$-1 |
| | 1 | 167.7, C | | - | | |
| | 2 | 60.0, CH | | 3.85, ddd (3.6, 5.9, 10.2) | Tyr-NH | Ser-1 |
| | 3 | 60.9, CH$_2$ | a | 3.46, dd (10.2, 11.9) | Tyr-NH, Tyr-5/9 | |
| | | | b | 3.14, dd (11.9, 3.6) | | |
| Tyr | NH | | | 7.34, d (9.1) | Ser-NH, Ser-3a | Ser-1 |
| | 1 | 171.5 C | | - | | |
| | 2 | 51.5 CH | | 4.88 ddd (3.2, 9.1, 10.9) | Pro$^{III}$-5a/b | Tyr-1 |
| | 3 | 37.0 CH2 | a | 3.35, dd (3.2, 13.5) | Pro$^{III}$-5a/b, Phe-NH, Gln-NH | Tyr-5/9 |
| | | | b | 2.42, dd (10.9, 13.5) | Gln-NH, Phe-NH, Pro$^{III}$-5a | Tyr-1, Tyr-4 |
| | 4 | 126.6 C | | - | | |
| | 5/9 | 130.5 CH | | 7.08, d (8.5) | Tyr-3a, Tyr-3b | Tyr-7 |
| | 6/8 | 114.9 CH | | 6.66, d (8.5) | | Tyr-7 |
| | 7 | 156.0 C | | - | | |
| | 7-OH | | | 7.42, s | | |
| Pro$^{III}$ | 1 | 171.9, C | | - | | |
| | 2 | 63.1, CH | | 4.06, t (8.7) | Phe-NH, | Pro$^{III}$-1 |
| | 3 | 28.7, CH$_2$ | a | 2.22 m | | |
| | | | b | 1.81, m | Gln-NH | Pro$^{III}$-1 |
| | 4 | 25.0, CH$_2$ | a | 2.11, m | Gln-NH, Gln-3a, Gln-3b | |
| | | | b | 1.98, m | | |
| | 5 | 46.9, CH$_2$ | a | 3.93, ddd (6.8, 9.8, 9.8) | Tyr-2, Tyr-3a | |
| | | | b | 3.82, m | Tyr-2, Gln-NH, Tyr-3a | Pro$^{III}$-3 |
| Gln | NH | | | 8.17, d (7,0) | Phe-NH, Tyr-3a, Pro$^{III}$-2, Pro$^{III}$-3b, Pro$^{III}$-4a | Pro$^{III}$-1 |
| | 1 | 170.7, C | | - | | |
| | 2 | 52.8, CH | | 4.05, ddd (4.3, 7.0, 10.0) | Phe-NH, Pro$^{III}$-4b | Gln-1 |
| | 3 | 25.9, CH$_2$ | a | 1.85, m | Pro$^{III}$-4a | Gln-1, Gln-5 |
| | | | b | 1.73, m | Pro$^{III}$-4a | Gln-5 |
| | 4 | 31.5, CH$_2$ | a | 2.13, ddd (7.2, 15.7, 7.2) | Pro$^{III}$-5b, Pro$^{III}$-3a, Pro$^{III}$-3b | Gln-5 |
| | | | b | 2.04, ddd (7.2, 15.7, 7.2) | Pro$^{III}$-4a | Gln-5 |
| | 5 | 174.5, C | | - | | |
| | 5-NH$_2$ | | | 6.92, s | Gln-4a, Gln-4b | Gln-5 |
| Phe | NH | | | 7.11, d (7.2) | Phe-2, Gln-NH, Pro$^{III}$-2, Pro$^I$-5b, Tyr-NH | Gln-1 |
| | 1 | 167.5 C | | - | | |
| | 2 | 51.5, CH | | 4.69, ddd (5.8, 7.2, 8.0) | Pro$^I$-5a, Pro$^I$-5b | Phe-1, Phe-4 |
| | 3 | 36.9 CH$_2$ | a | 3.18, dd (8.0, 14.2) | | Phe-1, Phe-4 |
| | | | b | 2.71 (5.8, 14.2) | | Phe-1, Phe-5/9 |
| | 4 | 138.0, C | | - | | |
| | 5/9 | 128.9, CH | | 7.16, d (7.5) | | |
| | 6/8 | 126.0, CH | | 7.18, t (7.3) | | |
| | 7 | 128.0, CH | | 7.22, t (7.5) | | |

Table S3. Links to LC-MS data and molecular networks.

| Description | Link |
|---|---|
| Classical Molecular Networking (Metabolomics) workflow | https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=4acb49028d7041c39fccf03dd6e8c195 |
| Feature-Based Molecular Networking workflow | https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=db7aa9e8bec64d6290cc040254f00b87 |
| Dereplicator | https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=f8b123b5ea89494481549c1a519caf5d |
| LC-MS data on Massive | https://massive.ucsd.edu/ProteoSAFe/dataset.jsp?task=f77c49cb5d73489583c08d3e66b558e1<br>FTP access: ftp://MSV000085904@massive.ucsd.edu<br>password: Sty_reviewers |

Figure S1. Molecular networks of the extract of *S. caribica* obtained with the same data and the same parameters (*m/z* tolerance 0.01 Da, cosine score > 0.55, matched peaks > 8, maximum number of neighbor nodes = 10) using (a) the program MetGem (b) the Metabolomics workflow on GNPS, and (c) the Feature-Based Molecular Network workflow on GNPS.
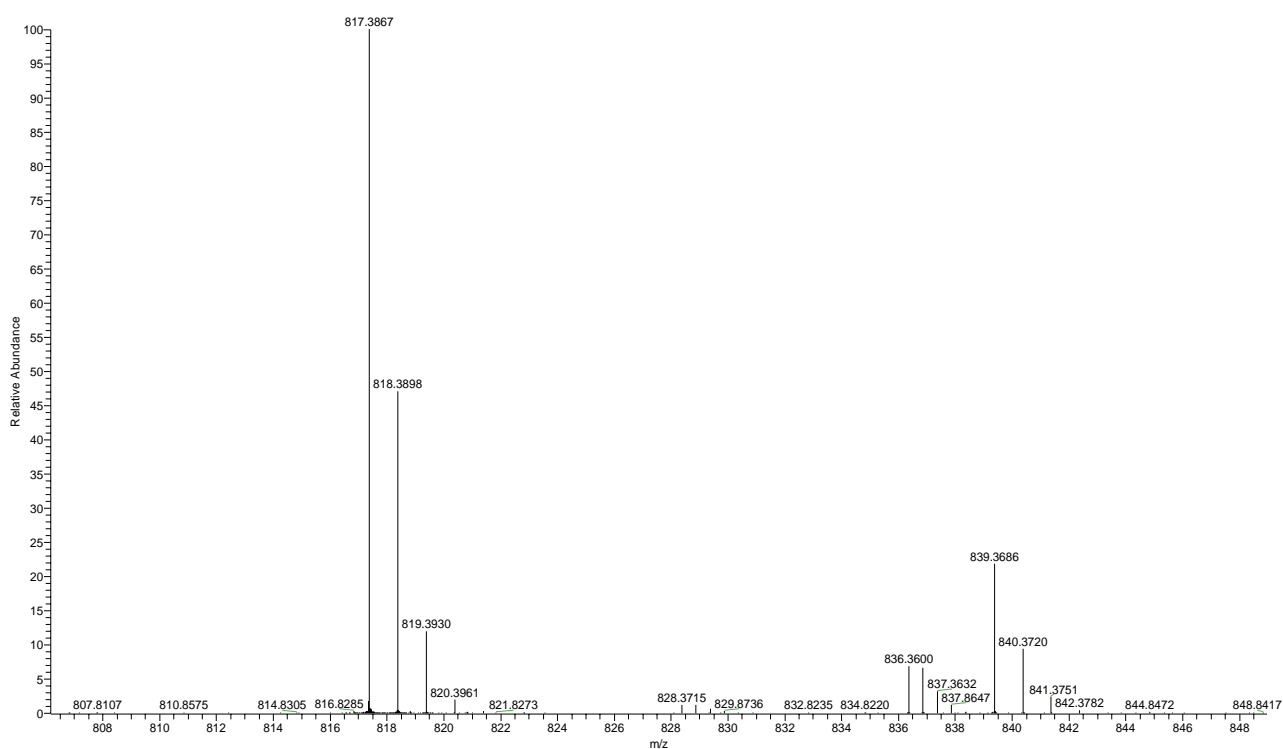
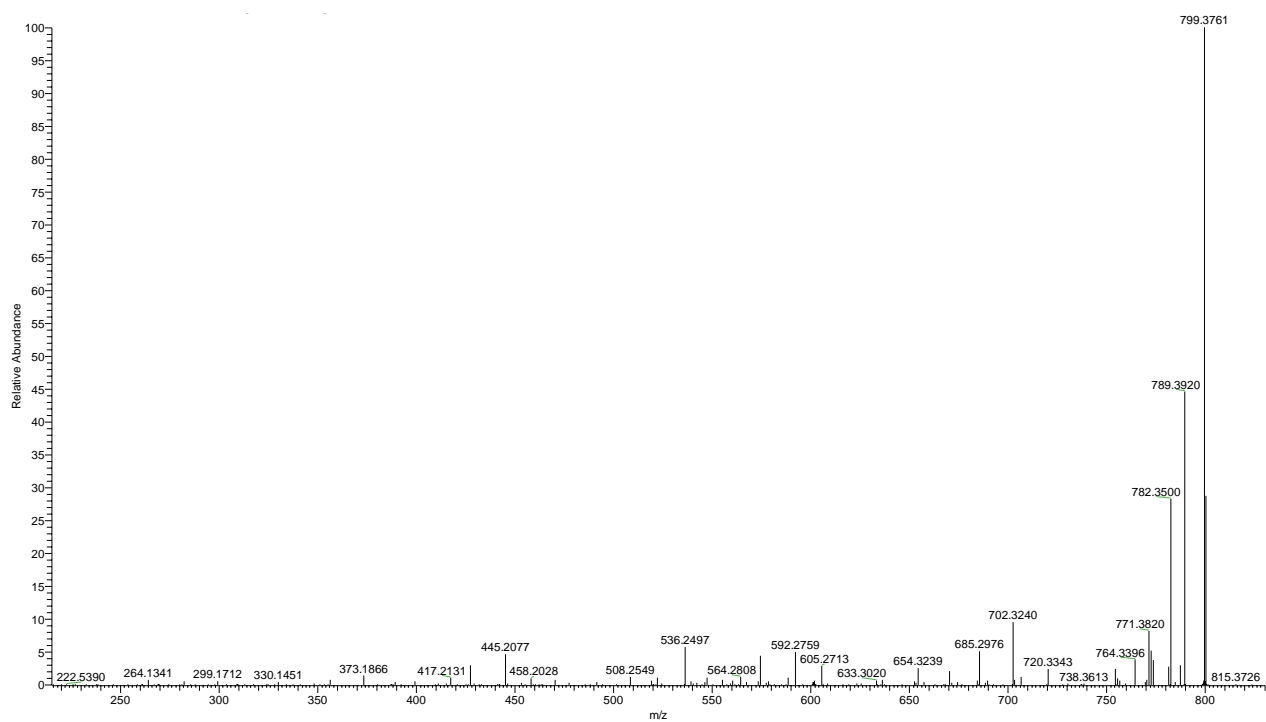Figure S2. Positive ion mode high-resolution ESI mass spectrum of stylissamide L (1).



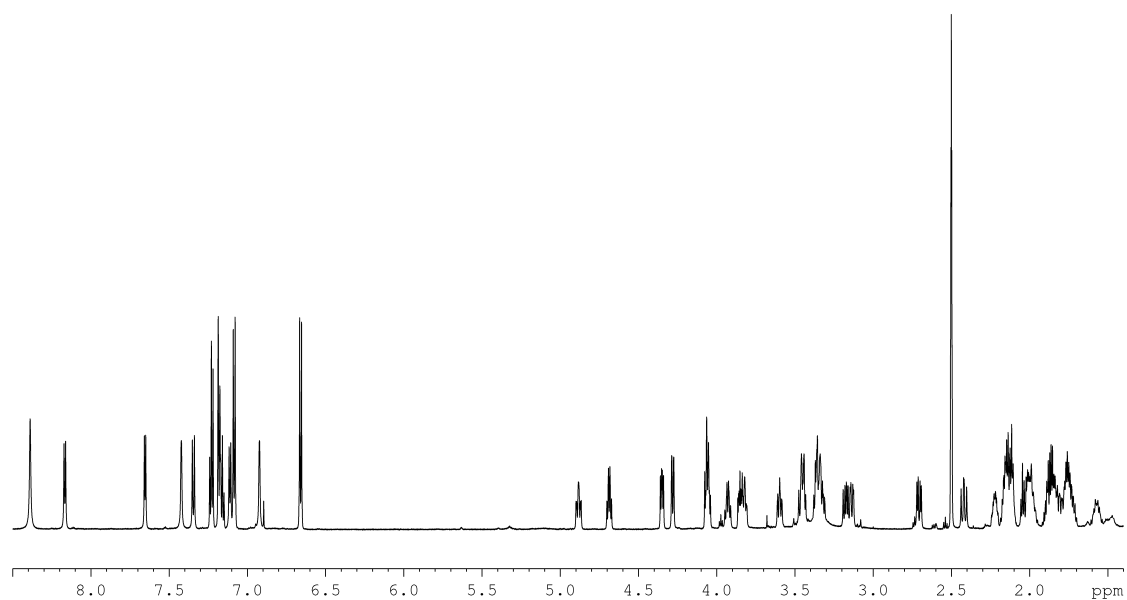Figure S3. Positive ion mode high-resolution ESI MS/MS spectrum of stylissamide L (1).

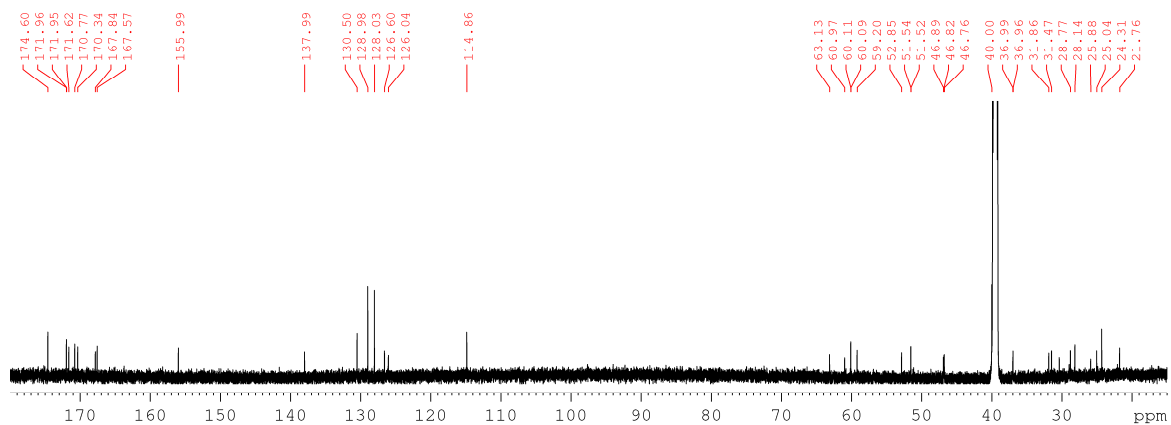Figure S4. ¹H-NMR spectrum of stylissamide L (1) (700 MHz, DMSO-*d*₆).



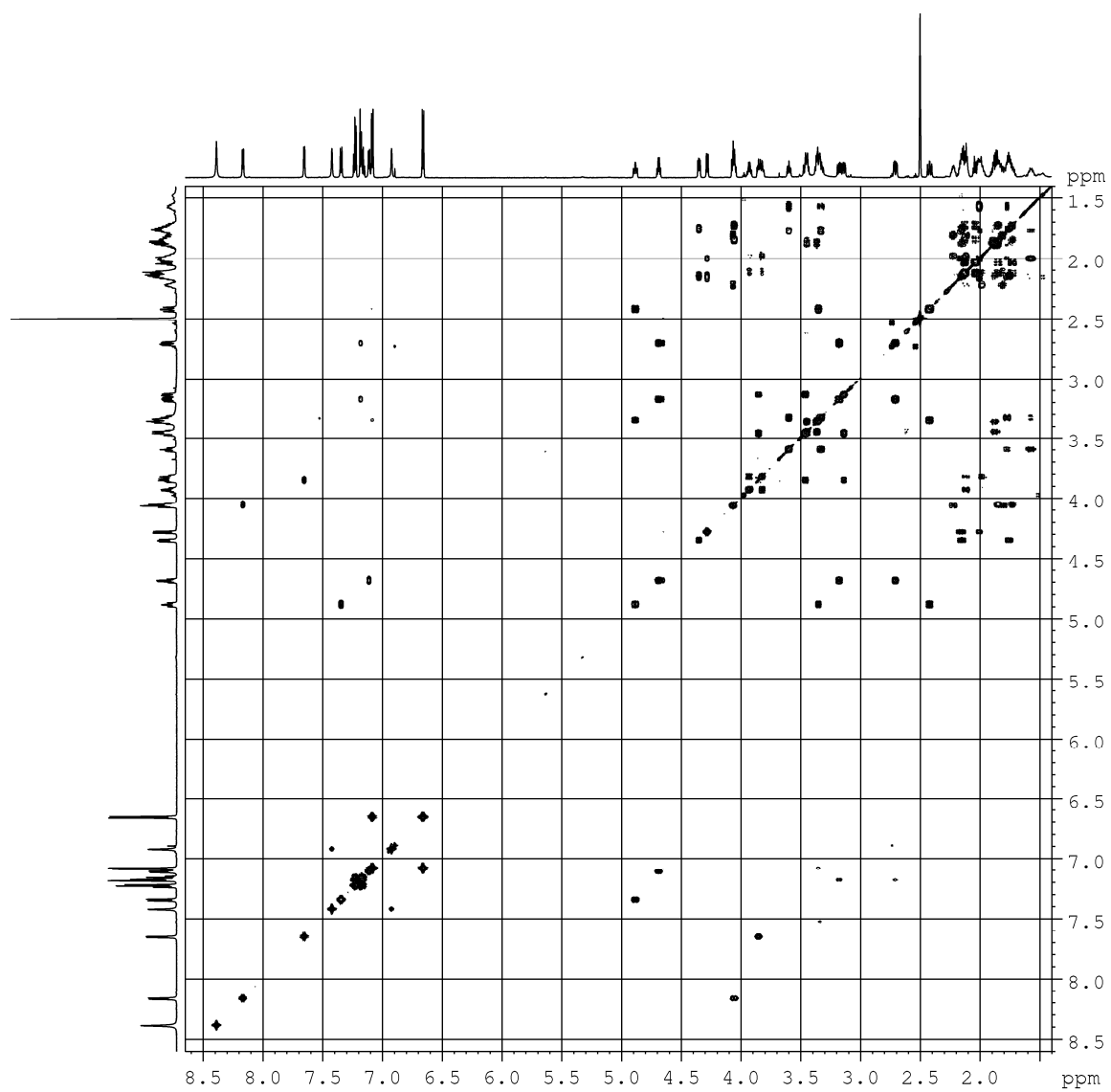Figure S5. ¹³C-NMR spectrum of stylissamide L (1) (175 MHz, DMSO-*d*₆).

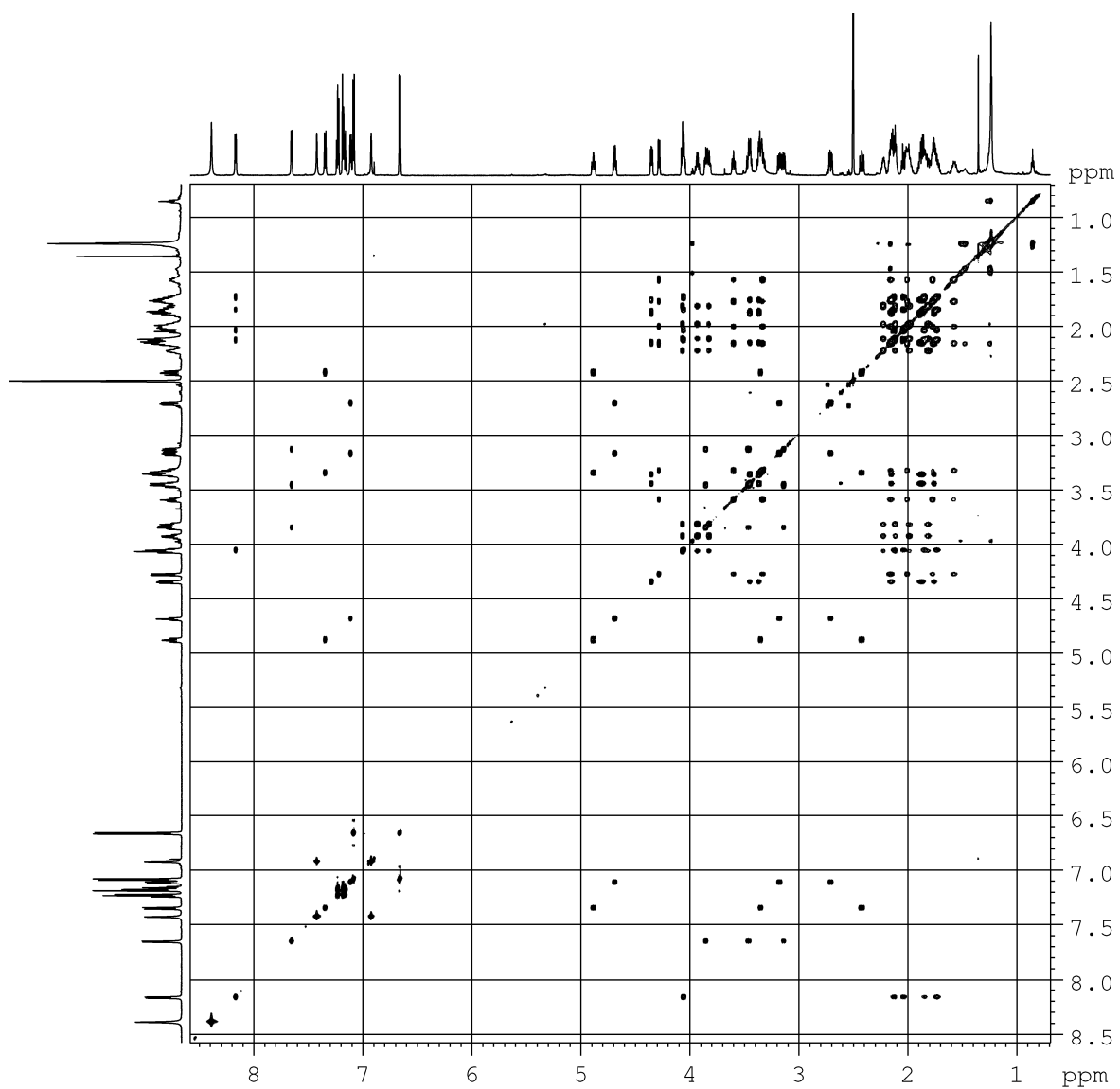Figure S6. COSY spectrum of stylissamide L (1) (700 MHz, DMSO-$d_6$).

Figure S7. TOCSY spectrum of stylissamide L (1) (700 MHz, DMSO-$d_6$).
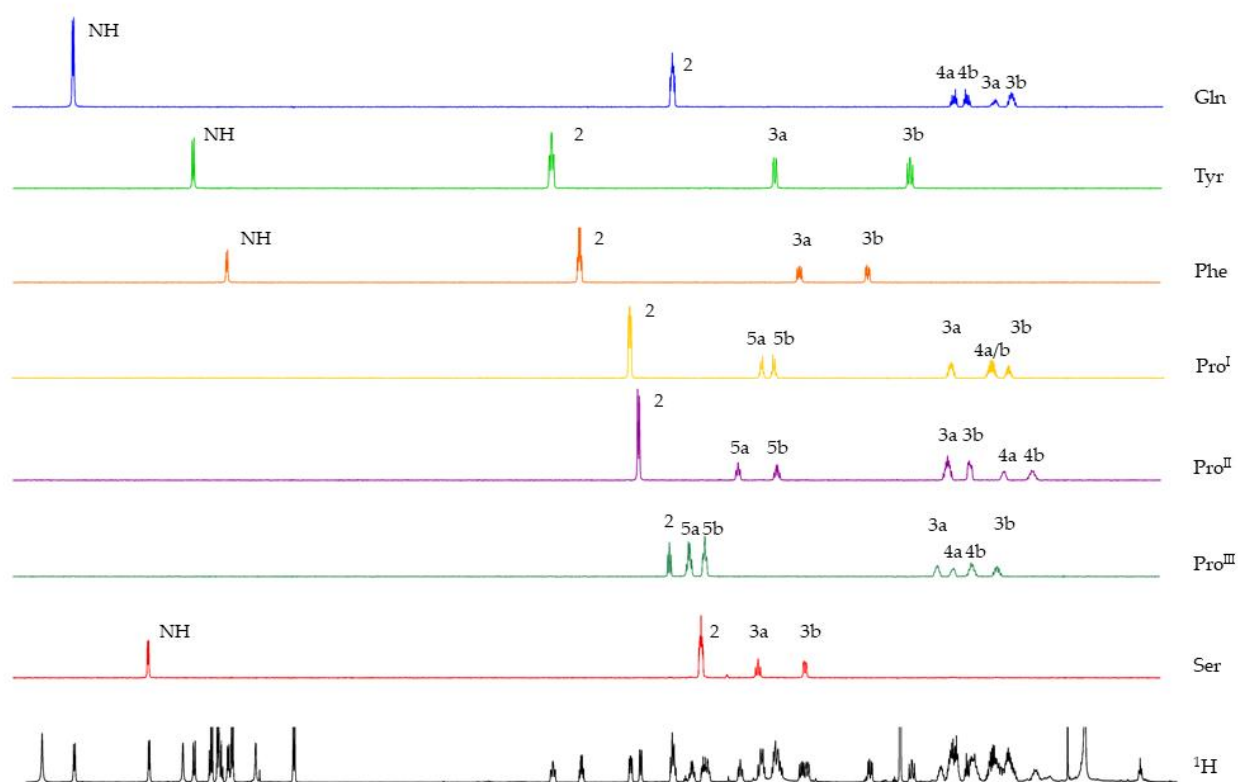
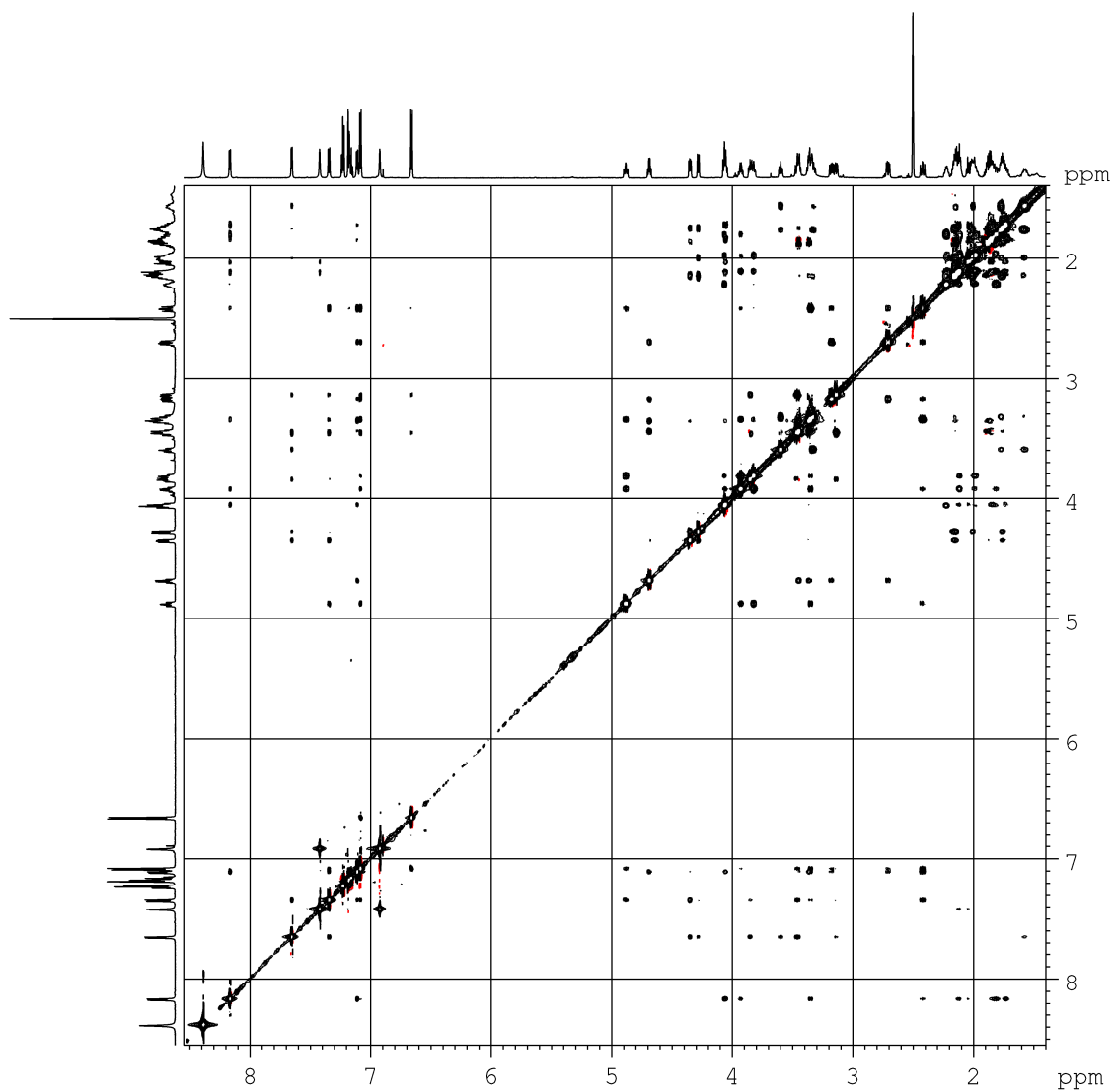Figure S8. Spin systems of stylissamide L (1) from the sections of the TOCSY spectrum.

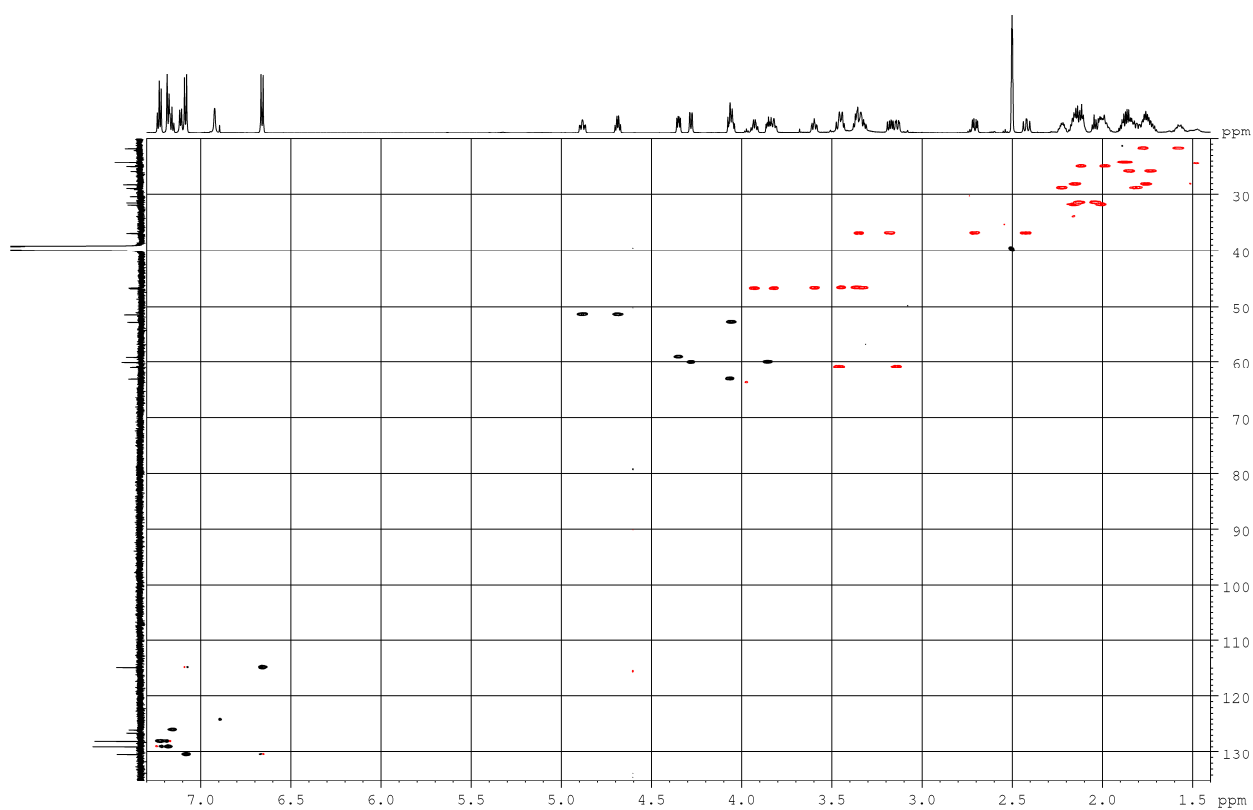Figure S9. NOESY spectrum of stylissamide L (1) (700 MHz, DMSO-$d_6$).

Figure S10. HSQC spectrum of stylissamide L (1) (700 MHz, DMSO-*d*6).

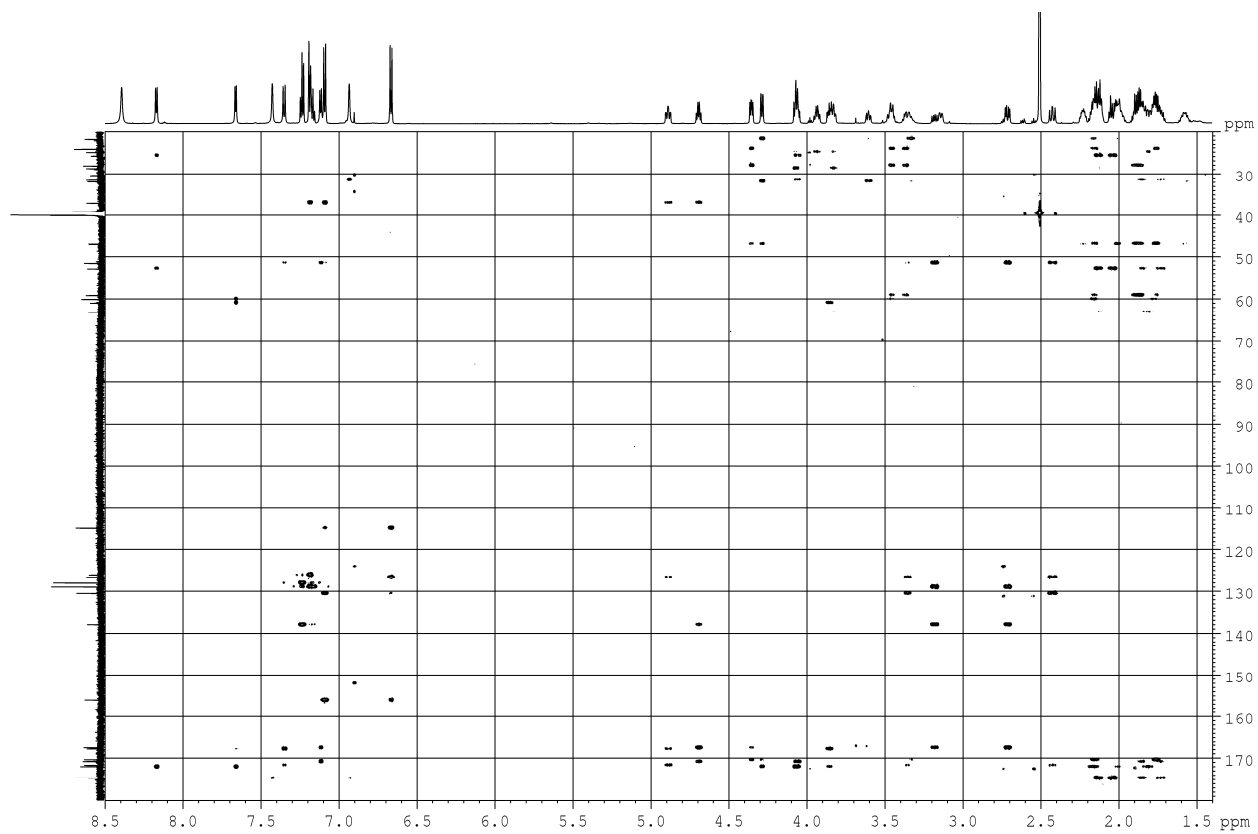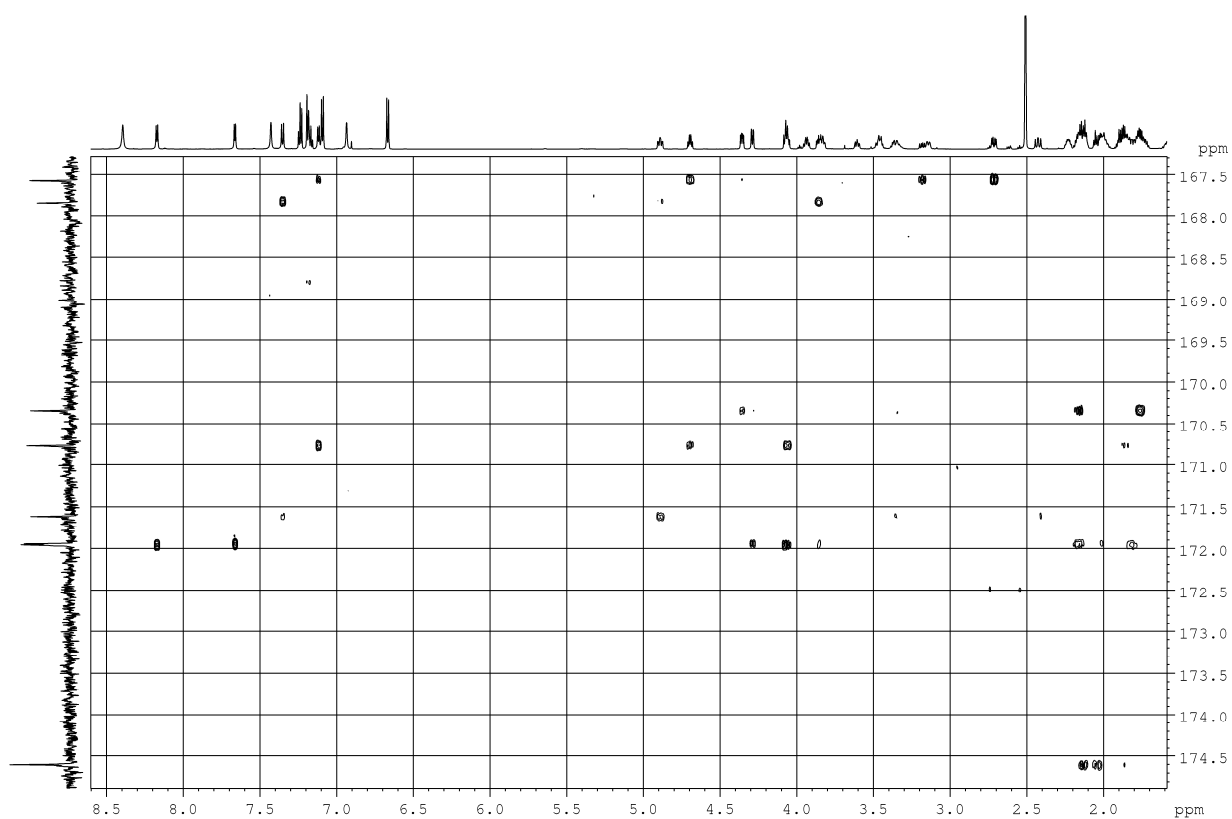Figure S11. HMBC spectrum of stylissamide L (1) (700 MHz, DMSO-$d_6$).

Figure S12. Band-selective HMBC spectrum of stylissamide L (1) (700 MHz, DMSO-$d_6$).
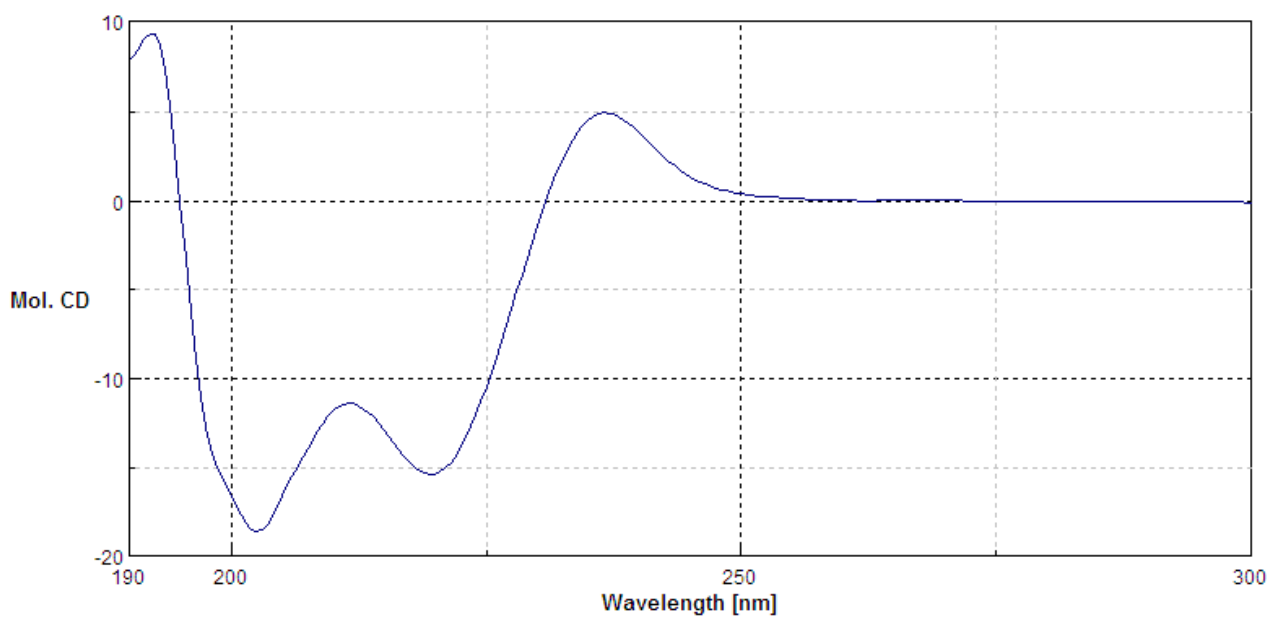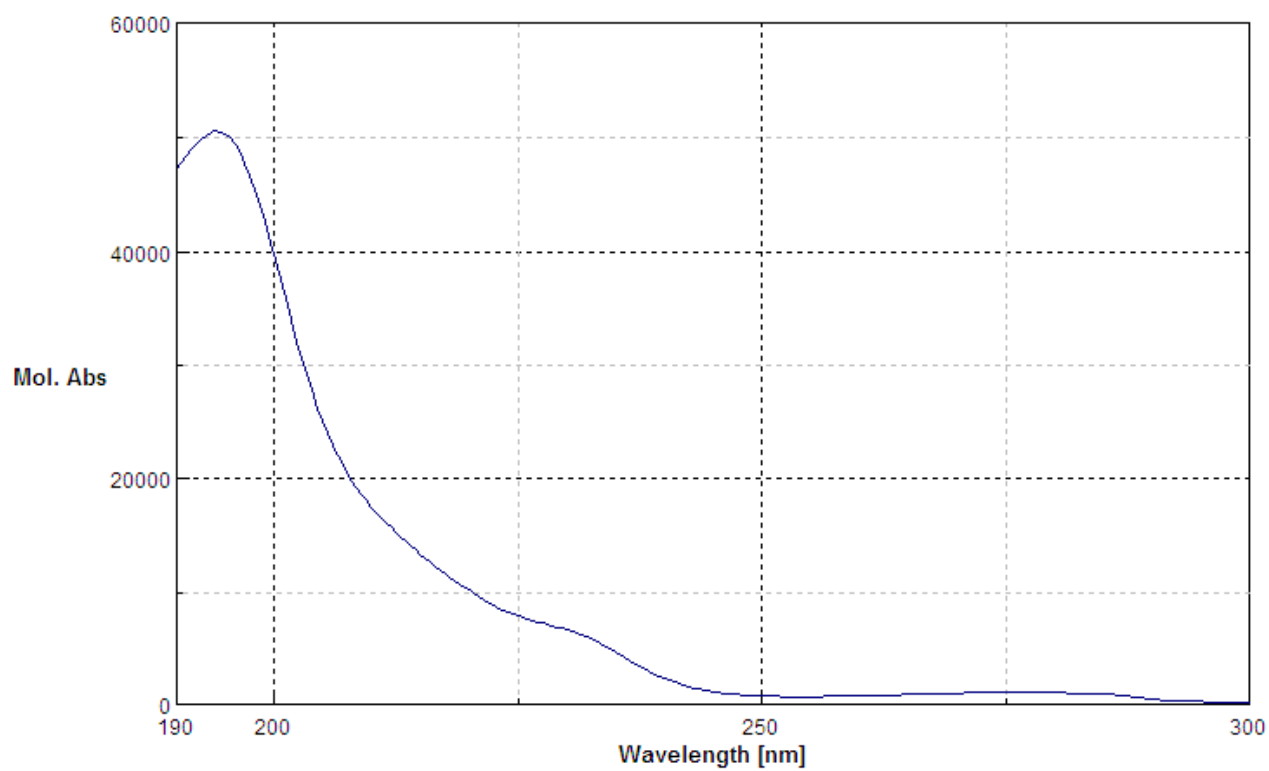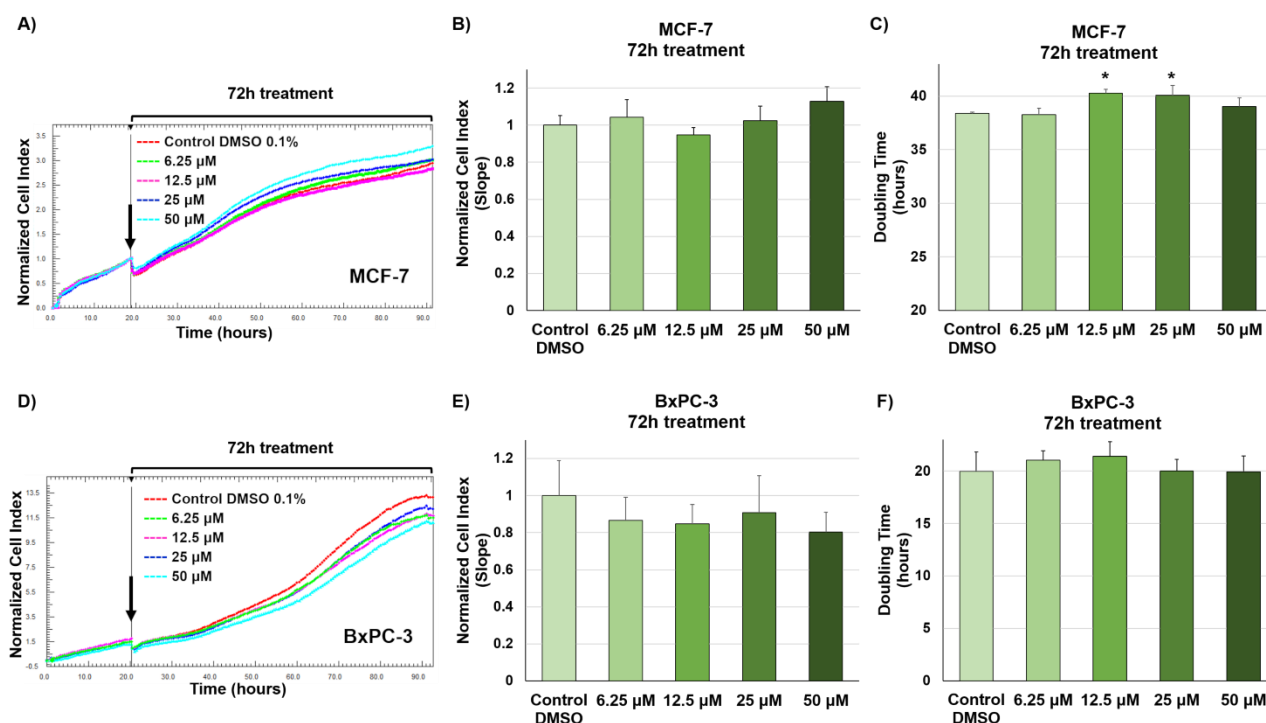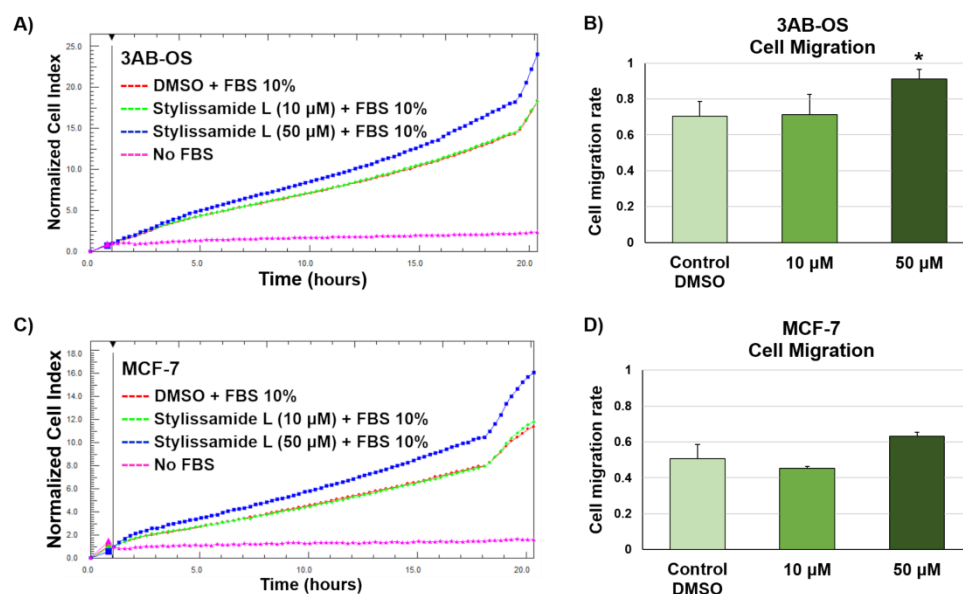
Figure S13. UV and ECD spectra of stylissamide L (1) in ACN.

**Figure S14.** Real time monitoring of cancer cell proliferation after exposure to stylissamide L (**1**) and DMSO vehicle, by using the xCELLigence System Real-Time Cell Analyzer. (**A**,**D**) Normalized cell index (NCI) traces of MCF7 (**A**) and BxPC-3 (**D**) cells treated with  different concentrations (6.25, 12.5, 25, and 50 µM) of stylissamide L (**1**) and DMSO vehicle (0.1%) for 72 hours. Black arrow shows the start of drug treatment. Each cell index value was normalized at this time. (**B**,**E**) Slope values of growth curves of MCF-7 (**B**) and BxPC-3 (**E**) cells after 72 h exposure to different concentrations (6.25, 12.5, 25, and 50 µM) of stylissamide L (**1**) and DMSO vehicle (0.1%). NCI slope values are relative to controls treated with DMSO vehicle. (**C**,**F**) Doubling times of NCI of MCF-7 (**C**) and BxPC-3 (**F**) cells after 72 h treatment with different concentrations (6.25, 12.5, 25, and 50 µM) of stylissamide L (**1**) and DMSO (0.1%). Data are presented as mean ± SD; n=3. Statistical significances are referred to the DMSO control. One-way analysis of variance (ANOVA) was applied to compare means of groups and Dunnett's method was used as a post-hoc test to compare multiple groups versus the control group. $p$-values < 0.05 were considered to be statistically significant. Statistical analysis was performed using the GraphPad Prism Software Version 5. * $p < 0.05$.

**Figure S15**. Real-time monitoring of 3AB-OS and MCF-7 cell migration after exposure to stylissamide L (**1**). (**A,C**) NCI traces of 3AB-OS (**A**) and MCF-7 (**C**) cells seeded with compound **1** or DMSO (0.1%) vehicle, in presence of 10% Fetal Bovine Serum (FBS) as the chemoattractant. Migration was monitored for 20 hours, using the xCELLigence System equipped with specially designed 16-well plates (CIM-plate 16). (**B,D**) Migration activity of 3AB-OS (**B**) and MCF-7 (**D**) cells seeded with compound **1** or DMSO (0.1%) vehicle, in presence of 10% Fetal Bovine Serum (FBS) as the chemoattractant. Cell migration rates were recorded for 20 hours and expressed as slope values of NCI curves. Data are presented as mean ± SD; n=3. Statistical significances are referred to the DMSO control. Two-group comparisons were performed using Student's t-test. P-values < 0.05 were considered to be statistically significant. Statistical analysis was performed using the GraphPad Prism Software Version 5.