Discovery of Cyclohexadepsipeptides with Anti-Zika Virus Activities and Biosynthesis of the Nonproteinogenic Building Block (3*S*)-Methyl-L-Proline

Bochuan Yuan,[a,+] Ziwei Wu,[a,+] Wei Ji,[b] Dong Liu,[a] Xiang Guo,[a] Donghui Yang,[a] Aili Fan,[a] Hongli Jia,[a] Ming Ma,[a,*] Wenhan Lin[a,c,*]

[a] State Key Laboratory of Natural and Biomimetic Drugs, Beijing 100191, P.R. China

[b] School of Basic Medical Sciences, Peking University, Beijing 100191, P.R. China

[c] Institute of Ocean Research, Peking University, Beijing 100191, P.R. China

[+] These authors contributed equally to this work

*Correspondence: mma@bjmu.edu.cn; whlin@bjmu.edu.cn

ORCID of Prof. Ma: 0000-0001-8311-3892

ORCID of Prof. Lin: 0000-0002-4978-4083

Table of Contents

Spectroscopic data of cyclohexadepsipeptides

Felinotoxin A (**1**): light yellowish crystal; mp 229.5-230.7 ºC, $[\alpha]_D^{25}$ -110 (*c* 0.06, MeOH). IR (KBr) ν_{max} 3385, 3292, 1670, 1632, 1446 cm-^{1}; ^{1}H and ^{13}C NMR data, see Table S1; HRESIMS *m/z* 638.3766 $[M + H]^+$ (calcd for $C_{31}H_{51}N_5O_9$, 638.3765).

Felinotoxin B (**2**): light yellowish amorphous; $[\alpha]_D^{25}$ -27 (*c* 0.06, MeOH). IR (KBr) ν_{max} 3385, 3292, 1670, 1632, 1446 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table S2; HRESIMS *m/z* 638.3761 $[M + H]^+$ (calcd for $C_{31}H_{51}N_5O_9$, 638.3765).

Felinotoxin C (**3**): white crystal; mp 185-186 ºC, $[\alpha]_D^{25}$ -56 (*c* 0.06, MeOH). IR (KBr) ν_{max} 3381, 2965, 1670, 1639, 1450 cm-^{1}; ^{1}H and ^{13}C NMR data, see Table S3; HRESIMS *m/z* 626.3757 $[M + H]^+$ (calcd for $C_{30}H_{52}N_5O_9$, 626.3765).

Felinotoxin D (**4**): white amorphous; $[\alpha]_D^{25}$ -36 (*c* 0.06, MeOH). IR (KBr) ν_{max} 3382, 2965, 1639, 1449 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table S4; HRESIMS *m/z* 626.3763 $[M + H]^+$ (calcd for $C_{30}H_{52}N_5O_9$, 626.3765).

Felinotoxin E (**5**): light yellowish amorphous; mp 235.1-235.8 ºC, $[\alpha]_D^{25}$ -18 (*c* 0.06, MeOH). IR (KBr) ν_{max} 3378, 2968, 1678, 1209, 1186 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table S5; HRESIMS *m/z* 624.3763 $[M + H]^+$ (calcd for $C_{30}H_{54}N_5O_8$, 624.3972).

Felinotoxin F (**6**): white amorphous; $[\alpha]_D^{25}$ -34 (*c* 0.06, MeOH). IR (KBr) ν_{max} 3282, 2960, 1645, 1541 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table S6; HRESIMS *m/z* 703.4182 $[M + H]^+$ (calcd for $C_{39}H_{55}N_6O_6$, 703.4183).

Felinotoxin G (**7**): white powder; $[\alpha]_D^{25}$ -13 (*c* 0.06, MeOH). IR (KBr) ν_{max} 3400, 3325, 2960, 1651, 1542 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table S7; HRESIMS *m/z* 568.3708 $[M + H]^+$ (calcd for $C_{28}H_{50}N_5O_7$, 568.3710).

Roseotoxin C (**8**): white powder; $[\alpha]_D^{25}$ -32 (*c* 0.06, MeOH). ^{1}H and ^{13}C NMR (100 MHz, DMSO-d_6) data for β-Me-Pro: δ_H 3.98 (1H, d, J = 2.0 Hz), 2.49 (1H, m), 1.03 (3H, d, J = 7.0 Hz), 1.69/1.93 (2H, m), 3.65/3.80 (2H, m); δ_C 170.3 (CO), 66.5, 36.4, 18.7, 30.3, 44.6, Ile: δ_H 6.96 (1H, d, J = 9.4 Hz, NH), 4.76 (1H, dd, J = 6.9, 9.4 Hz), 1.85 (1H, m), 0.77 (3H, d, J = 6.2 Hz), 1.25/1.39 (2H, m), 0.78 (3H, t, J = 7.0 Hz); δ_C 173.1 (CO), 52.6, 36.7, 15.1, 23.9, 11.2, NMe-Val: δ_H 4.99 (1H, d, J = 10.9

Hz), 2.19 (1H, dqq, *J* = 6.5, 6.5, 10.9 Hz), 0.84 (3H, d, *J* = 6.5 Hz), 0.86 (3H, d, *J* = 6.5 Hz), 3.13 (3H, s, NMe); δ_C 170.5 (CO), 57.1, 26.7, 19.1, 19.1, NMe-Ala: δ_H 5.17 (1H, q, *J* = 6.5 Hz), 1.17 (3H, d, *J* = 6.5 Hz), 2.54 (3H, s, NMe); δ_C 169.0 (CO), 54.8, 15.4, 27.7, β-Ala: δ_H 8.10 (1H, dd, *J* = 3.0, 10.0 Hz, NH), 2.35/2.77 (2H, m), 2.92/3.85 (2H, m); δ_C 173.3 (CO), 34.0, 32.9, 2,5-dihydroxy-4-methylpentanoic acid: δ_H 5.00 (1H, d, *J* = 11.0 Hz), 1.37/1.89 (2H, m), 1.68 (1H, m), 0.88 (3H, d, *J* = 6.6 Hz), 3.72 (2H, m); δ_C 173.1 (CO), 71.2, 33.6, 31.6, 15.9, 66.4, 23.1. $C_{30}H_{53}N_5O_8$, ESIMS *m/z* 624.4299 $[M+H]^+$, 646.4120 $[M+Na]^+$.

Roseotoxin B (**9**): white powder; $[\alpha]_D^{25}$ -50 (*c* 0.24, MeOH). ^1H and ^{13}C NMR (100 MHz, DMSO-*d*$_6$) data for β-Me-Pro: δ_H 3.97 (1H, d, *J* = 2.0 Hz), 2.47 (1H, m), 1.00 (3H, d, *J* = 7.0 Hz), 1.65/1.92 (2H, m), 3.67/3.76 (2H, m); δ_C 170.2 (CO), 66.5, 36.3, 18.6, 30.2, 44.7, Ile: δ_H 6.96 (1H, d, *J* = 9.4 Hz, NH), 4.78 (1H, dd, *J* = 6.6, 9.4 Hz), 1.83 (1H, m), 0.77 (3H, d, *J* = 6.2 Hz), 1.23/1.38 (2H, m), 0.78 (3H, t, *J* = 7.0 Hz); δ_C 173.0 (CO), 52.5, 36.7, 15.1, 23.8, 11.2, NMe-Val: δ_H 5.00 (1H, d, *J* = 10.9 Hz), 2.19 (1H, dqq, *J* = 6.5, 6.5, 10.9 Hz), 0.84 (3H, d, *J* = 6.5 Hz), 0.86 (3H, d, *J* = 6.5 Hz), 3.13 (3H, s, NMe); δ_C 170.5 (CO), 57.1, 26.7, 19.1, 19.1, 30.5 (NMe), NMe-Ala: δ_H 5.17 (1H, q, *J* = 6.6 Hz), 1.17 (3H, d, *J* = 6.6 Hz), 2.54 (3H, s, NMe); δ_C 169.0 (CO), 54.8, 15.4, 27.7, β-Ala: δ_H 8.10 (1H, dd, *J* = 3.0, 10.0 Hz, NH), 2.36/2.77 (2H, m), 2.93/3.85 (2H, m); δ_C 173.2 (CO), 34.1, 32.9, 2-methylpent-4-enoic acid: δ_H 5.04 (1H, d, *J* = 11.0 Hz), 2.50/2.51 (2H, m), 5.81 (1H, m), 5.14 (1H, brd, *J* = 11.0 Hz), 5.21 (1H, brd, *J* = 16.0 Hz); δ_C 168.6 (CO), 71.9, 34.7, 131.7, 119.3. $C_{30}H_{49}N_5O_7$, ESIMS *m/z* 592.3715 $[M+H]^+$, 614.3531 $[M+Na]^+$.

Roseotoxin A (**10**): white powder; $[\alpha]_D^{25}$ -46 (*c* 0.24, MeOH). ^1H and ^{13}C NMR (100 MHz, DMSO-*d*$_6$) data for β-Me-Pro: δ_H 3.98 (1H, d, *J* = 2.0 Hz), 2.50 (1H, m), 1.03 (3H, d, *J* = 6.9 Hz), 1.70/1.95 (2H, m), 3.61/3.81 (2H, m); δ_C 170.2 (CO), 66.5, 36.4, 18.7, 30.3, 44.6, Ile: δ_H 6.92 (1H, d, *J* = 9.3 Hz, NH), 4.76 (1H, dd, *J* = 6.9, 9.3 Hz), 1.86 (1H, m), 0.77 (3H, d, *J* = 6.2 Hz), 1.25/1.74 (2H, m), 0.78 (3H, t, *J* = 7.0 Hz); δ_C 173.0 (CO), 52.6, 36.7, 15.1, 23.9, 11.1, NMe-Val: δ_H 4.99 (1H, d, *J* = 10.9 Hz), 2.19 (1H, dqq, *J* = 6.5, 6.5, 10.9 Hz), 0.84 (3H, d, *J* = 6.5 Hz), 0.87 (3H, d, *J* =

6.5 Hz), 3.13 (3H, s, NMe); δ_C 170.5 (CO), 57.1, 26.7, 19.1, 19.1, NMe-Ala: δ_H 5.17 (1H, q, J = 6.6 Hz), 1.17 (3H, d, J = 6.6 Hz), 2.54 (3H, s, NMe); δ_C 169.2 (CO), 54.8, 15.4, 27.7, β-Ala: δ_H 8.09 (1H, dd, J = 3.0, 9.0 Hz, NH), 2.35/2.77 (2H, m), 2.93/3.86 (2H, m); δ_C 173.3 (CO), 34.0, 32.5, 2-hydroxy-4-methylpentanoic acid: δ_H 4.97 (1H, d, J = 11.3 Hz), 1.47/1.70 (2H, m), 1.74 (1H, m), 0.92 (3H, d, J = 6.5 Hz), 0.94 (3H, d, J = 6.5 Hz); δ_C 169.0 (CO), 71.5, 38.4, 24.0, 21.5, 23.1. $C_{31}H_{53}N_5O_7$, ESIMS *m/z* 608.4021 $[M+H]^+$, 630.3839 $[M+Na]^+$.

Desmethyldestruxin Ch1 (**11**): white powder; $[\alpha]_D^{25}$ -84 (*c* 0.06, MeOH). ^1H and ^{13}C NMR (100 MHz, DMSO-d_6) data for β-Me-Pro: δ_H 4.01 (1H, d, J = 2.0 Hz), 2.48 (1H, m), 1.03 (3H, d, J = 6.9 Hz), 1.68/1.94 (2H, m), 3.77/3.88 (2H, m); δ_C 170.2 (CO), 66.6, 36.5, 18.7, 30.2, 44.6, Ile: δ_H 6.92 (1H, d, J = 9.3 Hz, NH), 4.79 (1H, dd, J = 6.4, 9.3 Hz), 1.83 (1H, m), 0.78 (3H, d, J = 6.2 Hz), 1.24/1.38 (2H, m), 0.77 (3H, t, J = 7.0 Hz); δ_C 173.0 (CO), 52.5, 36.7, 15.2, 23.7, 11.2, NMe-Val: δ_H 5.02 (1H, d, J = 11.0 Hz), 2.19 (1H, dqq, J = 6.5, 6.5, 11.0 Hz), 0.86 (3H, d, J = 6.5 Hz), 0.86 (3H, d, J = 6.5 Hz), 3.13 (3H, s, NMe); δ_C 170.5 (CO), 57.1, 26.7, 19.1, 19.1, NMe-Ala: δ_H 5.17 (1H, q, J = 6.6 Hz), 1.17 (3H, d, J = 6.6 Hz), 2.54 (3H, s, NMe); δ_C 169.0 (CO), 54.8, 15.3, 27.7, β-Ala: δ_H 8.09 (1H, dd, J = 3.0, 9.6 Hz, NH), 2.35/2.78 (2H, m), 2.94/3.84 (2H, m); δ_C 173.2 (CO), 34.1, 32.9, 5-chloro-2,4-dihydroxypentanoic acid: δ_H 5.09 (1H, t, J = 7.2 Hz), 2.00 (2H, m), 3.66 (1H, m), 3.64 (2H, m); δ_C 168.7 (CO), 71.4, 35.1, 66.6, 49.4. $C_{30}H_{50}ClN_5O_8$, ESIMS *m/z* 644.3451 $[M+H]^+$, 666.3268 $[M+Na]^+$.

Destruxin A (**12**): white powder; $[\alpha]_D^{25}$ -100 (*c* 0.06, MeOH). ^1H and ^{13}C NMR (100 MHz, DMSO-d_6) data for Pro: δ_H 4.39 (1H, dd, J = 2.0, 7.5 Hz), 1.97/2.10 (2H, m), 1.74/1.98 (2H, m), 3.54/3.81 (2H, m); δ_C 170.5 (CO), 60.0, 29.0, 23.5, 46.3, Ile: δ_H 6.96 (1H, d, J = 9.4 Hz, NH), 4.80 (1H, dd, J = 6.5, 9.4 Hz), 1.83 (1H, m), 0.77 (3H, d, J = 7.0 Hz), 1.21/1.36 (2H, m), 0.78 (3H, t, J = 7.0 Hz); δ_C 173.0 (CO), 52.6, 36.8, 15.2, 23.7, 11.2, NMe-Val: δ_H 4.99 (1H, d, J = 11.0 Hz), 2.18 (1H, dqq, J = 6.5, 6.5, 11.0 Hz), 0.84 (3H, d, J = 6.5 Hz), 0.86 (3H, d, J = 6.5 Hz), 3.13 (3H, s, NMe); δ_C 170.5 (CO), 57.2, 26.7, 19.1, 19.1, 30.5 (NMe), NMe-Ala: δ_H 5.18 (1H, q, J = 6.7 Hz), 1.17 (3H, d, J = 6.7 Hz), 2.54 (3H, s, NMe); δ_C 169.0 (CO), 54.8, 15.4, 27.7, β-Ala: δ_H 8.07 (1H, dd, J = 2.0, 10.0 Hz, NH), 2.33/2.48 (2H, m), 2.93/3.84 (2H, m); δ_C 173.2 (CO), 34.0, 32.9, 2-hydroxypent-4-enoic acid: δ_H 5.02 (1H, t, J = 7.0 Hz), 2.20/2.36 (2H, m), 5.80 (1H, m), 5.12 (1H, d, J = 11.0 Hz), 5.19 (1H, d, J = 11.0 Hz); δ_C 168.2 (CO), 72.1, 34.2, 132.2, 118.8. $C_{29}H_{47}N_5O_7$, ESIMS m/z 578.3778 $[M+H]^+$, 600.3605$[M+Na]^+$.

Destruxin C (**13**): white powder; $[\alpha]_D^{25}$ -64 (c 0.06, MeOH). ^1H and ^{13}C NMR (100 MHz, DMSO-d_6) data for Pro: δ_H 4.38 (1H, dd, J = 2.0, 7.6 Hz), 2.12/2.20 (2H, m), 1.75/2.01 (2H, m), 3.49/3.86 (2H, m); δ_C 170.5 (CO), 60.0, 28.9, 23.6, 46.1, Ile: δ_H 6.96 (1H, d, J = 9.4 Hz, NH), 4.79 (1H, dd, J = 6.7, 9.4 Hz), 1.85 (1H, m), 0.78 (3H, d, J = 7.0 Hz), 1.25/1.39 (2H, m), 0.77 (3H, t, J = 7.0 Hz); δ_C 173.0 (CO), 52.6, 36.7, 15.1, 23.8, 11.2, NMe-Val: δ_H 4.97 (1H, d, J = 10.8 Hz), 2.18 (1H, dqq, J = 6.5, 6.5, 10.8 Hz), 0.84 (3H, d, J = 6.5 Hz), 0.86 (3H, d, J = 6.5 Hz), 3.13 (3H, s, NMe); δ_C 170.5 (CO), 57.2, 26.7, 19.1, 19.1, 30.5 (NMe), NMe-Ala: δ_H 5.17 (1H, q, J = 6.7 Hz), 1.17 (3H, d, J = 6.7 Hz), 2.54 (3H, s, NMe); δ_C 169.0 (CO), 54.7, 15.4, 27.7, β-Ala: δ_H 8.07 (1H, dd, J = 2.0, 10.0 Hz, NH), 2.33/2.78 (2H, m), 2.93/3.84 (2H, m); δ_C 173.3 (CO), 34.0, 32.9, 2,5-dihydroxy-methypentanedioic acid: δ_H 4.98 (1H, t, J = 7.0 Hz), 1.36/1.87 (2H, m), 1.71 (1H, m), 0.87 (3H, d, J = 7.0 Hz), 3.25 (2H, m); δ_C 169.0 (CO), 72.2, 33.4, 31.6, 15.8, 66.3. $C_{29}H_{51}N_5O_8$, ESIMS m/z 610.4145 $[M+H]^+$, 632.3973 $[M+Na]^+$.

Destruxin D (**14**): white powder; $[\alpha]_D^{25}$ -80 (c 0.06, MeOH). ^1H and ^{13}C NMR (100 MHz, DMSO-d_6) data for Pro: δ_H 4.36 (1H, dd, J = 2.0, 7.5 Hz), 2.00/2.11 (2H, m), 1.23/1.78 (2H, m), 3.54/3.83 (2H, m); δ_C 170.6 (CO), 60.1, 28.9, 23.7, 46.1, Ile: δ_H 6.96 (1H, d, J = 9.4 Hz, NH), 4.79 (1H, dd, J = 6.5, 9.4 Hz), 1.83 (1H, m), 0.78 (3H, d, J = 7.0 Hz), 1.23/1.38 (2H, m), 0.77 (3H, t, J = 7.0 Hz); δ_C 173.0 (CO), 52.6, 36.7, 15.2, 23.8, 11.2, NMe-Val: δ_H 4.97 (1H, d, J = 10.5 Hz), 2.19 (1H, dqq, J = 6.5, 6.5, 10.5 Hz), 0.84 (3H, d, J = 6.5 Hz), 0.87 (3H, d, J = 6.5 Hz), 3.13 (3H, s, NMe); δ_C 170.5 (CO), 57.2, 26.7, 19.1, 19.1, 30.5 (NMe), NMe-Ala: δ_H 5.17 (1H, q, J = 6.7 Hz), 1.17 (3H, d, J = 6.7 Hz), 2.54 (3H, s, NMe); δ_C 169.1 (CO), 54.8, 15.4, 27.7, β-Ala: δ_H 8.05 (1H, dd, J = 2.0, 10.0 Hz, NH), 2.35/2.75 (2H, m), 2.93/3.83 (2H, m);

δ_C 173.1 (CO), 34.0, 32.9, 2-hydroxy-4-methylpentanedioic acid: δ_H 4.99 (1H, t, *J* = 7.0 Hz), 1.75/2.00 (2H, m), 2.49 (1H, m), 1.13 (3H, d, *J* = 7.0 Hz); δ_C 176.8 (COOH), 168.3 (CO), 71.1, 23.6, 35.0, 16.9. $C_{30}H_{48}N_5O_9$, ESIMS *m/z* 624.3854 $[M+H]^+$, 600.3605$[M+Na]^+$.

Destruxin Ed (**15**): white powder; $[\alpha]_D^{25}$ -53 (*c* 0.06, MeOH). ^1H and ^{13}C NMR (100 MHz, DMSO-d_6) data for Pro: δ_H 4.40 (1H, dd, *J* = 2.0, 7.7 Hz), 2.00/2.09 (2H, m), 1.76/1.99 (2H, m), 3.80 (2H, m); δ_C 170.5 (CO), 60.0, 29.3, 23.5, 46.0, Ile: δ_H 6.97 (1H, d, *J* = 9.5 Hz, NH), 4.81 (1H, dd, *J* = 6.3, 9.5 Hz), 1.81 (1H, m), 0.78 (3H, d, *J* = 6.0 Hz), 1.23/1.37 (2H, m), 0.77 (3H, t, *J* = 7.0 Hz); δ_C 173.0 (CO), 52.5, 36.7, 15.2, 23.6, 11.2, NMe-Val: δ_H 5.01 (1H, d, *J* = 10.8 Hz), 2.19 (1H, dqq, *J* = 6.5, 6.5, 10.9 Hz), 0.84 (3H, d, *J* = 6.5 Hz), 0.87 (3H, d, *J* = 6.5 Hz), 3.13 (3H, s, NMe); δ_C 170.5 (CO), 57.1, 26.7, 19.1, 19.1, NMe-Ala: δ_H 5.17 (1H, q, *J* = 6.7 Hz), 1.17 (3H, d, *J* = 6.7 Hz), 2.54 (3H, s, NMe); δ_C 168.8 (CO), 54.8, 15.4, 27.7, β-Ala: δ_H 8.07 (1H, dd, *J* = 3.0, 10.0 Hz, NH), 2.35/2.77 (2H, m), 2.94/3.84 (2H, m); δ_C 173.3 (CO), 34.2, 32.9, 2,4,5-trihydroxypentanoic acid: δ_H 5.09 (1H, t, *J* = 7.0 Hz), 1.75/1.94 (2H, m), 3.41 (1H, m), 3.27/3.34 (2H, m); δ_C 169.0 (CO), 71.1, 35.0, 67.2, 65.8. $C_{29}H_{49}N_5O_9$, ESIMS *m/z* 612.3611 $[M+H]^+$.

Destruxin F (**16**): white powder; $[\alpha]_D^{25}$ -106 (*c* 0.06, MeOH). ^1H and ^{13}C NMR (100 MHz, DMSO-d_6) data for Pro: δ_H 4.40 (1H, dd, *J* = 2.0, 7.6 Hz), 1.99/2.09 (2H, m), 1.76/2.00 (2H, m), 3.76/3.80 (2H, m); δ_C 170.5 (CO), 60.0, 29.2, 23.5, 46.1, Ile: δ_H 6.96 (1H, d, *J* = 9.4 Hz, NH), 4.81 (1H, dd, *J* = 6.3, 9.4 Hz), 1.82 (1H, m), 0.77 (3H, d, *J* = 6.0 Hz), 1.23/1.36 (2H, m), 0.78 (3H, t, *J* = 7.0 Hz); δ_C 172.9 (CO), 52.6, 36.7, 15.2, 23.7, 11.2, NMe-Val: δ_H 5.00 (1H, d, *J* = 11.0 Hz), 2.18 (1H, dqq, *J* = 6.5, 6.5, 11.0 Hz), 0.84 (3H, d, *J* = 6.5 Hz), 0.87 (3H, d, *J* = 6.5 Hz), 3.13 (3H, s, NMe); δ_C 170.5 (CO), 57.1, 26.7, 19.1, 19.1, 30.5, NMe-Ala: δ_H 5.16 (1H, q, *J* = 6.6 Hz), 1.17 (3H, d, *J* = 6.6 Hz), 2.54 (3H, s, NMe); δ_C 169.0 (CO), 54.8, 15.3, 27.7, β-Ala: δ_H 8.07 (1H, dd, *J* = 2.0, 10.0 Hz, NH), 2.34/2.77 (2H, m), 2.94/3.84 (2H, m); δ_C 173.3 (CO), 34.1, 32.9, 2,4-dihydroxypentoic acid: δ_H 5.06 (1H, t, *J* = 7.0 Hz), 1.79/1.80 (2H, m), 3.62 (1H, m), 1.10 (3H, d, J = 7.0 Hz); δ_C 168.8 (CO), 71.0, 40.4, 62.2, 23.7. $C_{29}H_{49}N_5O_8$, ESIMS *m/z* 596.3654 $[M+H]^+$.

Destruxin Chl (**17**): white powder; $[\alpha]_D^{25}$ -128 (*c* 0.06, MeOH). ^1H and ^{13}C NMR (100 MHz, DMSO-d_6) data for Pro: δ_H 4.40 (1H, dd, *J* = 2.0, 7.6 Hz), 2.00/2.09 (2H, m), 1.76/2.00 (2H, m), 3.71/3.82 (2H, m); δ_C 170.5 (CO), 60.1, 29.2, 23.5, 46.2, Ile: δ_H 6.97 (1H, d, *J* = 9.4 Hz, NH), 4.81 (1H, dd, *J* = 6.4, 9.4 Hz), 1.83 (1H, m), 0.77 (3H, d, *J* = 6.0 Hz), 1.23/1.38 (2H, m), 0.78 (3H, t, *J* = 7.0 Hz); δ_C 173.0 (CO), 52.6, 36.8, 15.2, 23.7, 11.2, NMe-Val: δ_H 5.00 (1H, d, *J* = 10.9 Hz), 2.18 (1H, dqq, *J* = 6.5, 6.5, 10.9 Hz), 0.84 (3H, d, *J* = 6.5 Hz), 0.87 (3H, d, *J* = 6.5 Hz), 3.13 (3H, s, NMe); δ_C 170.5 (CO), 57.1, 26.7, 19.1, 19.2, NMe-Ala: δ_H 5.16 (1H, q, *J* = 6.7 Hz), 1.17 (3H, d, *J* = 6.7 Hz), 2.54 (3H, s, NMe); δ_C 169.0 (CO), 54.8, 15.4, 27.8, β-Ala: δ_H 8.07 (1H, dd, *J* = 2.0, 10.0 Hz, NH), 2.34/2.78 (2H, m), 2.94/3.83 (2H, m); δ_C 173.3 (CO), 34.3, 32.9, 5-chloro-2,4-dihydroxy pentanoic acid: δ_H 5.06 (1H, t, *J* = 7.0 Hz), 1.92/1.96 (2H, m), 3.72 (1H, m), 3.62/3.63 (2H, m); δ_C 168.4 (CO), 70.5, 35.0, 66.3, 46.5. $C_{29}H_{48}ClN_5O_8$, ESIMS *m/z* 630.3519 $[M+H]^+$, 652.2346 $[M+Na]^+$.

Destruxin Br1 (**18**): white crystal; mp 202.9-204.8, $[\alpha]_D^{25}$ -43 (*c* 0.06, MeOH). ^1H and ^{13}C NMR (100 MHz, DMSO-d_6) data for Pro: δ_H 4.40 (1H, dd, *J* = 2.0, 7.5 Hz), 2.00/2.09 (2H, m), 1.76/2.00 (2H, m), 3.70/3.80 (2H, m); δ_C 170.5 (CO), 60.0, 29.2, 23.5, 46.2, Ile: δ_H 6.97 (1H, d, *J* = 9.4 Hz, NH), 4.81 (1H, dd, *J* = 6.4, 9.4 Hz), 1.81 (1H, m), 0.78 (3H, d, *J* = 6.0 Hz), 1.23/1.38 (2H, m), 0.77 (3H, t, *J* = 7.0 Hz); δ_C 172.9 (CO), 52.6, 36.7, 15.2, 23.7, 11.2, NMe-Val: δ_H 5.00 (1H, d, *J* = 10.8 Hz), 2.19 (1H, dqq, *J* = 6.5, 6.5, 10.9 Hz), 0.84 (3H, d, *J* = 6.5 Hz), 0.87 (3H, d, *J* = 6.5 Hz), 3.13 (3H, s, NMe); δ_C 170.5 (CO), 57.1, 26.7, 19.1, 19.1, NMe-Ala: δ_H 5.16 (1H, q, *J* = 6.6 Hz), 1.17 (3H, d, *J* = 6.6 Hz), 2.54 (3H, s, NMe); δ_C 169.0 (CO), 54.8, 15.4, 27.7, β-Ala: δ_H 8.09 (1H, dd, *J* = 2.0, 10.0 Hz, NH), 2.36/2.78 (2H, m), 2.93/3.84 (2H, m); δ_C 173.2 (CO), 34.1, 32.9, 25-bromo-2,4-dihydroxypentanic acid: δ_H 5.06 (1H, t, *J* = 7.2 Hz), 1.95 (2H, m), 3.77 (1H, m), 3.70/3.82 (2H, m); δ_C 168.4 (CO), 71.5, 35.8, 65.8, 46.2. $C_{29}H_{48}BrN_5O_8$, ESIMS *m/z* 674.2794 $[M+H]^+$.

Isaridin C (**19**): white powder; $[\alpha]_D^{25}$ -40 (*c* 0.12, MeOH). ^1H and ^{13}C NMR (100 MHz, DMSO-d_6) data for Pro: δ_H 4.10 (1H, dd, *J* = 2.0, 8.3 Hz), 1.97, 2.08 (2H, m), 1.06, 1.69 (2H, m), 3.27 (2H, m); δ_C 171.1 (CO), 60.1, 31.5, 21.4, 46.7, Phe: δ_H 7.90 (1H, d, *J* = 7.6 Hz, NH), 4.61 (1H, ddd, *J* = 3.0, 7.6, 8.0 Hz), 2.89 (1H, dd, *J* = 3.0, 13.0 Hz), 2.96 (1H, dd, *J* = 8.0, 13.0 Hz), 7.90 (2H, *J* = 8.0 Hz), 7.28 (2H, d, *J* = 8.0 Hz), 7.26 (1H, t, *J* = 8.0 Hz); δ_C 172.5 (CO), 52.7, 34.5, 137.2, 129.0 (2C), 128.3 (2C), 126.6, NMe-Val: δ_H 5.03 (1H, d, *J* = 10.7 Hz), 2.24 (1H, dqq, *J* = 6.5, 6.5, 10.7 Hz), 0.77 (3H, d, *J* = 6.5 Hz), 0.73 (3H, d, *J* = 6.5 Hz), 2.98 (3H, s, NMe); δ_C 168.9 (CO), 56.5, 26.3, 19.2, 18.4, NMe-Leu: δ_H 4.92 (1H, d, *J* = 7.0 Hz), 1.20/2.08 (2H, m), 1.41 (1H, m), 0.92 (6H, d, *J* = 6.5 Hz), 2.86 (3H, s, NMe); δ_C 168.0 (CO), 58.0, 38.0, 24.7, 22.5, 22.1, β-Ala: δ_H 7.35 (1H, t, *J* = 5.6 Hz, NH), 2.50/2.65 (2H, m), 3.44/3.55 (2H, m); δ_C 173.1 (CO), 34.4, 35.4, 2-hydroxy-4-methylpentanoic acid: δ_H 5.22 (1H, d, *J* = 11.3 Hz), 1.47/1.64 (2H, m), 1.41 (1H, m), 0.92 (3H, d, *J* = 6.5 Hz), 0.88 (3H, d, *J* = 6.5 Hz); δ_C 168.9 (CO), 72.6, 37.7, 24.7, 20.1, 23.2. $C_{36}H_{55}N_5O_7$, ESIMS *m/z* 670.4243 $[M+H]^+$.

Isaridin A (**20**): white powder; $[\alpha]_D^{25}$ -72 (*c* 0.06, MeOH). ^1H and ^{13}C NMR (100 MHz, DMSO-d_6) data for Pro: δ_H 4.10 (1H, dd, *J* = 2.0, 7.0 Hz), 1.97/2.07 (2H, m), 1.06/1.67 (2H, m), 3.26 (1H, m), 3.30 (1H, m); δ_C 171.1 (CO), 60.1, 31.4, 21.4, 46.7, Phe: δ_H 7.90 (1H, d, *J* = 7.8 Hz, NH), 4.52 (1H, ddd, *J* = 3.0, 7.6, 7.8 Hz), 2.87 (1H, dd, *J* = 3.0, 13.0 Hz), 2.88 (1H, dd, *J* = 7.6, 13.0 Hz), 7.90 (2H, *J* = 8.0 Hz), 7.31 (2H, d, *J* = 8.0 Hz), 7.26 (1H, t, *J* = 8.0 Hz); δ_C 171.7 (CO), 53.0, 34.3, 137.5, 129.0 (2C), 128.5 (2C), 126.6, NMe-Val: δ_H 4.74 (1H, d, *J* = 10.7 Hz), 2.03 (1H, dqq, *J* = 6.5, 6.5, 10.7 Hz), 0.71 (3H, d, *J* = 6.5 Hz), 0.58 (3H, d, *J* = 6.5 Hz), 2.88 (3H, s, NMe); δ_C 169.1 (CO), 56.3, 25.7, 18.1, 18.0, NMe-Phe: δ_H 5.14 (1H, dd, *J* = 6.0, 8.2 Hz), 2.77 (1H, dd, *J* = 8.2, 12.0 Hz), 3.50 (1H, dd, *J* = 6.0, 12.0 Hz), 7.30 (2H, d, *J* = 8.0 Hz), 7.26 (2H, d, *J* = 8.0 Hz), 7.21 (1H, t, *J* = 8.0 Hz), 2.81 (3H, s, NMe); δ_C 167.7 (CO), 62.0, 34.9, 137.9, 128.8 (2C), 128.2 (2C), 126.5, 29.5 (NMe), β-Ala: δ_H 7.53 (1H, t, *J* = 5.3 Hz, NH), 2.50/2.59 (2H, m), 3.33/3.67 (2H, m); δ_C 173.3 (CO), 34.0, 35.6, 2-hydroxy-4-methylpentanoic acid: δ_H 5.30 (1H, dd, *J* = 1.2, 11.5 Hz), 1.46/1.64 (2H, m), 1.87 (1H, m), 0.92 (3H, d, *J* = 6.5 Hz), 0.98 (3H, d, *J* = 6.5 Hz); δ_C 169.0 (CO), 72.5, 37.8, 21.4, 20.0, 23.3. $C_{39}H_{53}N_5O_7$, ESIMS *m/z* 704.4279 $[M+H]^+$.

Isariin G1 (**21**): white amorphous; $[\alpha]_D^{25}$ -14 (*c* 0.06, MeOH). ^{1}H and ^{13}C NMR (100 MHz, DMSO-d_6) data for Gly: δ_H 7.90 (1H, dd, *J* = 3.1, 5.5 Hz, NH), 3.44 (1H, dd, *J* = 3.1, 12.0 Hz), 4.12 (1H, *J* = 5.5, 12.0 Hz); δ_C 169.1 (CO), 42.0, Val^1: δ_H 8.10 (1H, d, *J* = 7.1 Hz, NH), 4.00 (1H, dd, 6.5, 7.1 Hz), 1.86 (1H, m), 0.89 (3H, d, *J* = 6.5 Hz), 0.86 (3H, d, *J* = 6.5 Hz); δ_C 171.8 (CO), 58.9, 29.4, 23.0, 18.7, Leu: δ_H 8.65 (1H, d, *J* = 6.4 Hz, NH), 4.04 (1H, dt, *J* = 6.4, 6.5 Hz), 1.50 (2H, m), 1.63 (1H, m), 0.81 (3H, d, *J* = 6.5 Hz), 0.88 (3H, d, *J* = 6.5 Hz); δ_C 171.2 (CO), 51.7, 38.6, 24.1, 20.8, 19.1, Ala: 7.96 (1H, d, *J* = 6.4 Hz, NH), 4.19 (1H, dd, *J* = 6.4, 7.0 Hz), 1.22 (3H, d, *J* = 7.0 Hz); δ_C 172.0 (CO), 47.9, 17.2, Val^2: δ_H 7.47 (1H, d, *J* = 6.2 Hz, NH), 3.96 (1H, dd, *J* = 6.2, 6.5 Hz), 2.06 (1H, m), 0.88 (3H, d, *J* = 6.5 Hz), 0.84 (3H, d, *J* = 6.5 Hz); δ_C 169.3 (CO), 58.2, 29.2, 17.8, 18.9, 3-hydroxydecanoic acid: δ_H 2.34 (1H, dd, *J* = 2.5, 14.0 Hz), 2.45 (1H, dd, *J* = 6.5, 14.0 Hz), 4.93 (1H, m), 1.23-1.25 (12H, m), 0.85 (3H, t, *J* = 6.5 Hz); δ_C 170.1 (CO), 40.0, 72.0, 22.0-33.2, 13.9. $C_{31}H_{55}N_5O_7$, ESIMS *m/z* 610.4227 $[M+H]^+$.

Isariin G2 (**22**): white amorphous; $[\alpha]_D^{25}$ -5 (*c* 0.06, MeOH). ^{1}H and ^{13}C NMR (100 MHz, DMSO-d_6) data for Gly: δ_H 7.94 (1H, dd, *J* = 3.1, 5.5 Hz, NH), 3.45 (1H, dd, *J* = 3.1, 12.0 Hz, α-Ha), 4.06 (1H, dd, *J* = 5.5, 12.0 Hz, α-Hb); δ_C 169.0 (CO), 42.5, Val: δ_H 7.79 (1H, d, *J* = 7.1 Hz, NH), 4.09 (1H, dd, *J* =6.0, 7.1 Hz), 1.23 (1H, dqq, *J* = 6.0, 6.5, 6.5 Hz), 0.89 (3H, d, *J* = 6.5 Hz), 0.87 (3H, d, *J* = 6.5 Hz); δ_C 171.8 (CO), 58.2, 29.9, 22.9, 18.6, Leu: δ_H 8.64 (3H, d, *J* = 6.4 Hz, NH), 4.02 (1H, dt, *J* = 6.4, 6.5 Hz), 1.49 (2H, m), 1.64 (1H, m), 0.81 (3H, d, *J* = 6.5 Hz), 0.84 (3H, d, *J* = 6.5 Hz); δ_C 171.1 (CO), 51.9, 38.5, 24.1, 21.1, 19.0, Ala^1: δ_H 7.99 (1H, d, *J* = 6.4 Hz, NH), 4.20 (1H, dq, *J* = 6.5, 7.0 Hz), 1.20 (3H, *J* = 7.0 Hz); δ_C 171.9 (CO), 47.5, 17.2, Ala^2: δ_H 7.87 (1H, d, *J* = 6.4 Hz, NH), 4.07 (1H, dq, *J* = 6.4, 7.0 Hz), 1.30 (3H, d, *J* = 7.0 Hz); δ_C 171.7 (CO), 48.6, 16.4, 3-hydroxydodecanoic acid: δ_H 2.40 (2H, d, *J* = 6.0 Hz), 4.94 (1H, m), 0.90-1.25 (16H, m), 0.86 (3H, d, *J* = 6.0 Hz); δ_C 169.4 (CO), 39.5, 71.5, 22.9-33.4, 13.9. $C_{31}H_{55}N_5O_7$, ESIMS *m/z* 610.4227 $[M+H]^+$.

Isoisariin B (**23**): white amorphous; $[\alpha]_D^{25}$ -12 (*c* 0.06, MeOH). ^{1}H and ^{13}C NMR (100 MHz, DMSO-d_6) data for Gly: δ_H 7.91 (1H, dd, *J* = 3.8, 6.7 Hz, NH), 3.44 (1H, dd, *J* = 3.8, 14.0 Hz), 4.09 (1H, dd, *J* = 6.7, 14.0 Hz); δ_C 169.1 (CO), 41.9, Val[1]: δ_H 8.11 (1H, d, *J* = 7.6 Hz), 4.04 (1H, dd, *J* = 6.5, 7.6 Hz), 1.88 (1H, m), 0.86 (3H, d, *J* = 6.5 Hz), 0.89 (3H, d, *J* = 6.5 Hz); δ_C 171.7 (CO), 58.8, 29.5, 23.0, 18.7, Leu: δ_H 8.65 (1H, d, *J* = 6.5 Hz, NH), 4.03 (1H, m), 1.42 (2H, m), 1.61 (1H, m), 0.81 (3H, d, *J* = 6.5 Hz), 0.92 (3H, d, *J* = 6.5 Hz); δ_C 171.3 (CO), 51.9, 38.7, 24.2, 21.0, 19.0, Ala: δ_H 7.97 (1H, d, *J* = 6.5 Hz, NH), 4.19 (1H, dq, *J* = 6.5, 6.6 Hz), 1.22 (3H, d, *J* = 6.6 Hz); δ_C 171.9 (CO), 48.0, 17.3, Val[2]: δ_H 7.47 (1H, d, *J* = 7.7 Hz, NH), 4.08 (1H, dd, *J* = 6.5, 7.7 Hz), 2.07 (1H, m), 0.86 (3H, d, *J* = 6.5 Hz), 0.88 (3H, d, *J* = 6.5 Hz); δ_C 170.8 (CO), 57.7, 29.5, 17.5, 19.0, 2-hydroxy-3-methylheptanoic acid: δ_H 2.23 (1H, dd, *J* = 3.0, 14.0 Hz), 2.52 (1H, dd, *J* = 6.0, 14.0 Hz), 4.91 (1H, m), 1.67 (1H, m), 0.84 (3H, d, *J* = 6.5 Hz), 1.02, 1.37 (2H, m), 1.17, 1.25 (2H, m), 1.25 (2H, m), 0.85 (3H, t, *J* = 6.5 Hz); δ_C 169.9 (CO), 31.6, 75.5, 36.2, 14.8, 31.2, 28.9, 22.4, 13.9. $C_{30}H_{53}N_5O_7$, ESIMS *m/z* 596.4350 $[M+H]^+$.

Isariin E (**24**): white amorphous; $[\alpha]_D^{25}$ -10 (*c* 0.06, MeOH). ^{1}H and ^{13}C NMR (100 MHz, DMSO-d_6) data for Gly: δ_H 7.91 (1H, dd, *J* = 3.1, 5.5 Hz, NH), 3.44 (1H, dd, *J* = 3.1, 14.0 Hz), 4.12 (1H, dd, *J* = 5.5, 14.0 Hz); δ_C 169.2 (CO), 42.0, Val[1]: δ_H 8.11 (1H, d, *J* = 7.1 Hz), 4.00 (1H, dd, *J* = 6.5, 7.1 Hz), 1.86 (1H, m), 0.89 (3H, d, *J* = 6.5 Hz), 0.87 (3H, d, *J* = 6.5 Hz); δ_C 171.8 (CO), 59.0, 29.4, 23.1, 18.7, Leu: δ_H 8.66 (1H, d, *J* = 6.4 Hz, NH), 4.04 (1H, m), 1.50 (2H, m), 1.63 (1H, m), 0.80 (3H, d, *J* = 6.5 Hz), 0.88 (3H, d, *J* = 6.5 Hz); δ_C 171.2 (CO), 51.7, 38.6, 24.1, 20.8, 18.9, Ala: δ_H 7.97 (1H, d, *J* = 6.4 Hz, NH), 4.19 (1H, dq, *J* = 6.4, 7.0 Hz), 1.22 (1H, d, *J* = 7.0 Hz); δ_C 172.0 (CO), 47.9, 17.3, Val[2]: δ_H 7.47 (1H, d, *J* = 7.1 Hz, NH), 3.97 (1H, dd, *J* = 6.5, 7.1 Hz), 2.06 (1H, m), 0.88 (3H, d, *J* = 6.5 Hz), 0.88 (3H, d, *J* = 6.5 Hz); δ_C 170.8 (CO), 58.2, 29.2, 17.9, 19.2, 3-hydroxyhexanoic acid: δ_H 2.35 (1H, dd, *J* = 3.0, 14.0 Hz), 2.44 (1H, dd, *J* = 6.0, 14.0 Hz), 4.94 (1H, m), 1.51 (2H, m), 1.26 (2H, m), 0.85 (3H, t, *J* = 6.5 Hz); δ_C 169.3 (CO), 40.0, 71.9, 35.4, 17.8, 13.7. $C_{27}H_{47}N_5O_7$, ESIMS *m/z* 554.3866 $[M+H]^+$.

Nodupetide (**25**): white amorphous; $[\alpha]_D^{25}$ -14 (*c* 0.06, MeOH). ^{1}H and ^{13}C NMR (100 MHz, DMSO-d_6) data for Gly: δ_H 7.89 (1H, dd, *J* = 3.2, 4.8 Hz, NH), 3.45 (1H, dd, J = 3.2, 14.0 Hz), 4.08 (1H, dd, J = 4.8, 14.0 Hz); δ_C 169.1 (CO), 42.0, Val[1]: 8.08 (1H, d, J = 7.6 Hz, NH), 4.05 (1H, dd, J = 6.5, 7.6 Hz), 1.87 (1H, m), 0.89 (3H, d, J = 6.5 Hz), 0.88 (3H, d, J = 6.5 Hz); δ_C 171.7 (CO), 58.8, 29.5, 23.0, 18.7, Leu: δ_H 8.65 (1H, d, J = 6.4 Hz, NH), 4.04 (1H, m), 1.49 (2H, m), 1.64 (1H, m), 0.81 (3H, d, J = 6.5 Hz), 0.88 (3H, d, J = 6.5 Hz); δ_C 171.2 (CO), 51.8, 38.6, 24.1, 21.0, 18.9, Ala: δ_H 7.99 (1H, d, J = 7.9 Hz, NH), 4.19 (1H, dd, J = 6.5, 7.9 Hz), 1.22 (3H, d, J = 6.5 Hz); δ_C 171.9 (CO), 48.0, 17.4, Val[2]: δ_H 7.44 (1H, d, J = 6.9 Hz, NH), 4.09 (1H, dd, J = 6.5, 6.9 Hz), 2.08 (1H, m), 0.88 (3H, d, J = 6.5 Hz), 0.89 (3H, d, J = 6.5 Hz); δ_C 170.8 (CO), 57.7, 29.5, 17.6, 19.0, 3-hydroxy-4-methylhexanoic acid: 2.25 (1H, dd, J = 3.0, 14.0 Hz), 2.52 (1H, dd, J = 6.5, 14.0 Hz), 4.93 (1H, m), 1.60 (1H, m), 0.84 (3H, d, J = 6.0 Hz), m), 1.06, 1.41 (2H, m), 0.85 (3H, t, J = 6.5 Hz); δ_C 169.8 (CO), 37.7, 75.4, 38.0, 14.3, 24.5, 11.5. $C_{28}H_{49}N_5O_7$, ESIMS *m/z* 568.3743 $[M+H]^+$.

Isariin A (**26**): white amorphous; $[\alpha]_D^{25}$ -32 (*c* 0.06, MeOH). ^1H and ^{13}C NMR (100 MHz, DMSO-d_6) data for Gly: δ_H 7.90 (1H, dd, J = 3.1, 5.5 Hz, NH), 3.45 (1H, dd, J = 3.1, 14.0 Hz), 4.11 (1H, dd, J = 5.5, 14.0 Hz); δ_C 169.1 (CO), 42.0, Val[1]: δ_H 8.08 (1H, d, J = 7.1 Hz), 4.00 (1H, dd, J = 6.5, 7.1 Hz), 1.87 (1H, m), 0.89 (3H, d, J = 6.5 Hz), 0.87 (3H, d, J = 6.5 Hz); δ_C 171.7 (CO), 58.9, 29.4, 23.0, 18.7, Leu: δ_H 8.64 (1H, d, J = 6.4 Hz, NH), 4.03 (1H, m), 1.50 (2H, m), 1.23 (1H, m), 0.81 (3H, d, J = 6.5 Hz), 0.87 (3H, d, J = 6.5 Hz); δ_C 171.2 (CO), 51.7, 38.6, 24.1, 20.9, 18.9, Ala: δ_H 7.97 (1H, d, J = 6.4 Hz, NH), 4.20 (1H, dq, J = 6.4, 7.0 Hz), 1.22 (1H, d, J = 7.0 Hz); δ_C 172.0 (CO), 47.9, 17.2, Val[2]: δ_H 7.49 (1H, d, J = 6.2 Hz, NH), 3.96 (1H, dd, J = 6.2, 6.5 Hz), 2.06 (1H, m), 0.86 (3H, d, J = 6.5 Hz), 0.88 (3H, d, J = 6.5 Hz); δ_C 170.8 (CO), 58.2, 29.2, 17.9, 19.1, 3-hydroxydodecanoic acid: δ_H 2.25 (1H, dd, J = 3.0, 14.0 Hz), 2.45 (1H, dd, J = 6.0, 14.0 Hz), 4.94 (1H, m), 1.23-1.63 (16H, m), 0.85 (3H, t, J = 6.5 Hz); δ_C 169.3 (CO), 39.9, 72.0, 22.1-33.2, 13.9. $C_{33}H_{59}N_5O_7$, ESIMS *m/z* 638.4532 $[M+H]^+$.

Table S1. The ^{1}H and ^{13}C NMR data of **1** in DMSO-d_6 (δ in ppm) (600 MHz) (key ^{1}H-^{1}H COSY and HMBC correlations are shown below)

No.	δ_H (J in Hz)	δ_C	No.	δ_H (J in Hz)	δ_C
Acid		-	δ^1	0.78, t (7.0)	11.1, CH_3
CO		168.5, C	NH	6.88, d (9.4)	-
α	4.99, dd (5.0,10.0)	71.1, CH	N-MeVal		-
β	1.74, ddd (4.0, 5.0,12.0) 2.11, td (10.0,12.0)	33.3, CH_2	CO		170.5, C
γ	2.46, ddq (4.0,6.9,10.0)	34.9, CH	α	5.00, d (12.0)	57.1, CH
δ^2	1.14, d (6.9)	17.2, CH_3	β	2.18, dqq(6.4,6.4,12.0)	26.7, CH
δ^1		176.7, C	γ^1	0.84, d (6.4)	19.1, CH_3
MePro		-	γ^2	0.87, d (6.4)	19.1, CH_3
CO		170.3, C	N-CH_3	3.13, s	30.5, CH_3
α	3.96, d (2.0)	66.6, CH	N-MeAla		-
β	2.47, m	36.4, CH	CO		169.0, C
β-Me	1.05, d (7.0)	18.7, CH_3	α	5.17, q (6.6)	54.8, CH
γ	1.64, m; 1.94, m	30.5, CH_2	β	1.17, d (6.6)	15.4, CH_3
δ	3.68, m; 3.77, m	44.7, CH_2	N-CH_3	2.54, s	27.7, CH_3
Ile	-	-	Ala		-
CO	-	173.1, C	CO		173.2, C
α	4.76, dd (6.7, 9.4)	52.5, CH	α	2.35,dd (11.1, 18.2) ; 2.75, dd (4.0, 18.0)	34.0, CH_2
β	1.83, m	36.9, CH	β	2.93, ddd (2.0,11.0,11.1) 3.85, dt (4.0,11.0)	32.9, CH_2
γ^2	0.77, d (5.6)	15.1, CH	NH	8.09, dd (2.0, 11.0)	
γ^1	1.23, m; 1.39, m	23.9, CH_2			

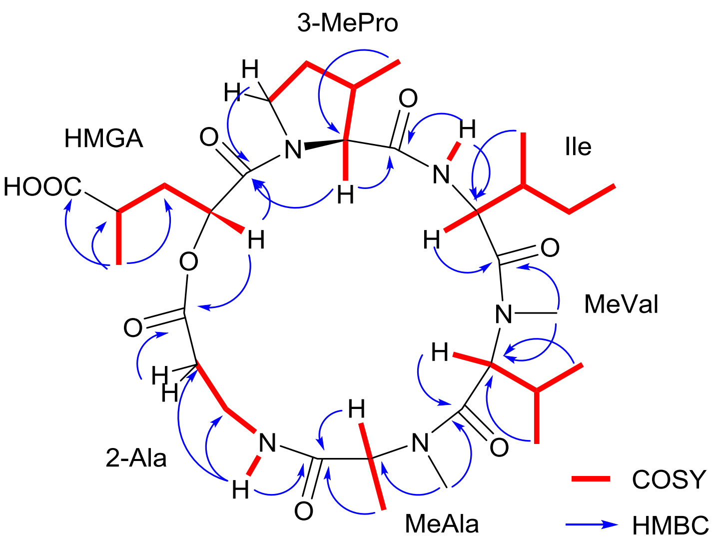


Table S2. The ^{1}H and ^{13}C NMR data of **2** in DMSO-d_6 (δ in ppm) (600 MHz) (key ^{1}H-^{1}H COSY and HMBC correlations are shown below)

No.	δ_H (J in Hz)	δ_C	No.	δ_H (J in Hz)	δ_C
Acid		-	δ^1	0.77, t (7.0)	11.2, CH_3
CO		168.7, C	NH	6.86, d (9.4)	-
α	4.96, dd (2.0, 11.0)	71.7, CH	N-MeVal	-	-
β	1.74, ddd (2.0, 11.0,12.0) 1.95, ddd (4.0,11.0,12.0)	33.4, CH_2	CO	-	170.5, C
γ	2.57, m	34.9, CH	α	4.99, m	57.1, CH
δ^2	1.15, d (6.9)	17.8, CH_3	β	2.18, m	26.6, CH
δ^1		176.6, C	γ^1	0.83, d (6.4)	19.1, CH_3
MePro		-	γ^2	0.87, d (6.4)	19.1, CH_3
CO		170.2, C	N-CH_3	3.13, brs	30.5, CH_3
α	3.97, m	66.3, CH	N-MeAla	-	-
β	2.46, m	36.6, CH	CO	-	169.0, C
β-Me	1.03, d (7.0)	18.7, CH_3	α	5.17, q (6.6)	54.7, CH
γ	1.69, m; 1.95, m	30.5, CH_2	β	1.16, d (6.6)	15.4, CH_3
δ	3.68, m; 3.75, m	44.6, CH_2	N-CH_3	2.54, s	27.7, CH_3
Ile		-	Ala	-	-
CO		173.0, C	CO	-	173.3, C
α	4.74, dd (6.7, 9.3)	52.5, CH	α	2.35, dd (11.1, 18.0) 2.78, dd (4.0, 18.0)	34.0, CH_2
β	1.84, m	36.7, CH	β	2.92, ddd (2.0,11.0,11.5) 3.84, ddd (4.0,11.0,11.5)	32.9, CH_2
γ^2	0.76, d (5.6)	15.1, CH	NH	8.07, dd (2.0, 11.0)	-
γ^1	1.22, m; 1.39, m	23.9, CH_2			

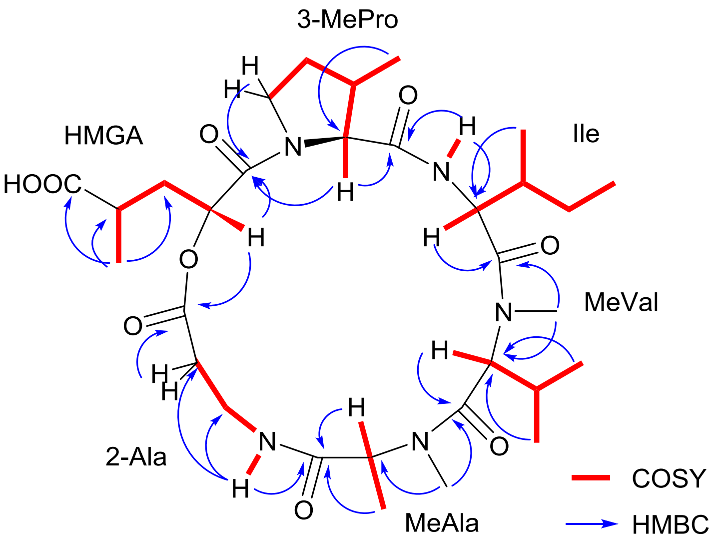


Table S3. The ^{1}H and ^{13}C NMR data of **3** in DMSO-d_6 (δ in ppm) (600 MHz) (key ^{1}H-^{1}H COSY and HMBC correlations are shown below)

No.	δ_H (J in Hz)	δ_C	No.	δ_H (J in Hz)	δ_C
Acid		-	γ^1	1.23, m; 1.37, m	23.7, CH_2
CO		169.1, C	δ^1	0.78, t (7.0)	11.2, CH_3
α	5.10, dd (5.8, 9.0)	71.0, CH	NH	6.93, d (9.5)	-
β	1.75, m; 1.97, m	33.4, CH_2	N-MeVal	-	-
γ	3.36, m	34.9, CH	CO	-	170.5, C
γ-OH	4.98, m	-	α	5.03, d (10.9)	57.1, CH
δ	3.17, m; 3.27, m	65.8, C	β	2.18, m	26.3, CH
δ-OH	4.68, m	-	γ^1	0.84, d (6.4)	19.1, CH_3
MePro	-	-	γ^2	0.87, d (6.4)	19.1, CH_3
CO	-	170.2, C	N-CH_3	3.13, brs	30.5, CH_3
α	4.00, m	66.6, CH	N-MeAla	-	-
β	2.46, m	36.6, CH	CO	-	169.0, C
β-CH_3	1.03, d (7.0)	18.8, CH_3	α	5.16, q (6.6)	54.8, CH
γ	1.65, m; 1.93, m	30.2, CH_2	β	1.17, d (6.6)	15.4, CH_3
δ	3.76, m; 3.98, m	44.5, CH_2	N-CH_3	2.54, brs	27.7, CH_3
Ile	-	-	Ala	-	-
CO	-	173.0, C	CO	-	173.4, C
α	4.81, dd (6.4, 9.4)	52.4, CH	α	2.34, m; 2.77, m	34.2, CH_2
β	1.82, m	36.7, CH	β	2.94, m; 3.84, m	32.9, CH_2
γ^2	0.76, d (5.6)	15.2, CH	NH	8.10, dd (2.3, 9.8)	-

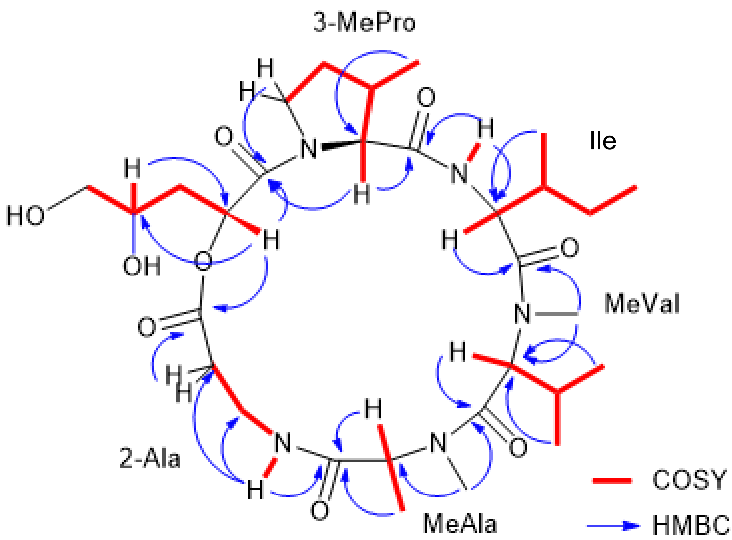


Table S4. The [1]H and [13]C NMR data of **4** in DMSO-d_6 (δ in ppm) (600 MHz) (key [1]H-[1]H COSY and HMBC correlations are shown below)

No.	δ_H (J in Hz)	δ_C	No.	δ_H (J in Hz)	δ_C
Acid	-	-	γ^1	1.25, m; 1.40, m	23.9, CH_2
CO	-	169.4, C	δ^1	0.78, m	11.2, CH_3
α	5.12, dd (3.0, 10.1)	70.4, CH	NH	6.92, d (9.4)	-
β	1.52, m; 1.93, m	34.7, CH_2	N-MeVal	-	-
γ	3.60, m	67.3, CH	CO	-	170.5, C
γ-OH	4.83, m	-	α	4.99, d (11.0)	57.1, CH
δ	3.25, m; 3.37, m	65.7, C	β	2.19, m	26.7, CH
δ-OH	4.64, m	-	γ^1	0.84, d (6.4)	19.1, CH_3
MePro	-	-	γ^2	0.87, d (6.4)	19.1, CH_3
CO	-	170.3, C	N-CH_3	3.13, brs	30.5, CH_3
α	3.98, m	66.3, CH	N-MeAla	-	-
β	2.47, m	36.5, CH	CO	-	169.0, C
β-CH_3	1.03, d (7.1)	18.7, CH_3	α	5.18, q (6.6)	54.8, CH
γ	1.68, m; 1.95, m	30.5, CH_2	β	1.17, d (6.6)	15.4, CH_3
δ	3.71, m	44.6, CH_2	N-CH_3	2.54, brs	27.7, CH_3
Ile	-	-	Ala	-	-
CO	-	173.1, C	CO	-	173.4, C
α	4.75, dd (6.9, 9.3)	52.5, CH	α	2.37, m; 2.76, m	34.0, CH_2
β	1.86, m	36.8, CH	β	2.94, t (12.3); 3.87, m	32.9, CH_2
γ^2	0.77, m	15.1, CH	NH	8.10, dd (2.2, 9.9)	-

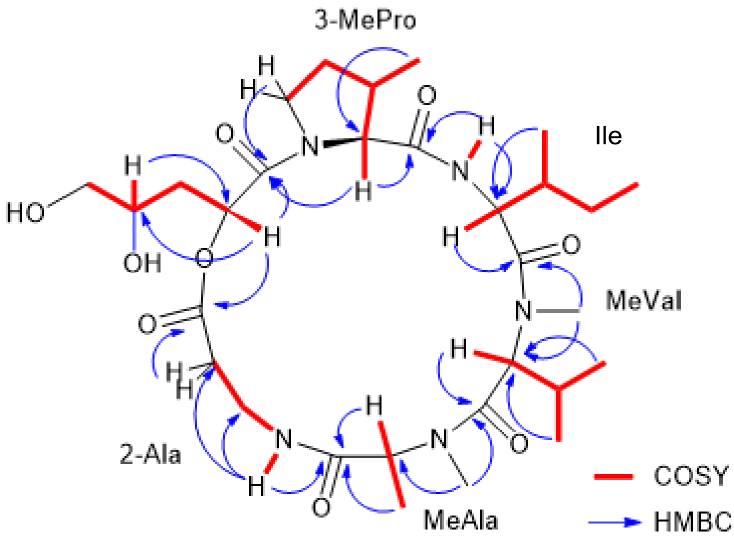


Table S5. The [1]H and [13]C NMR data of **5** in DMSO-d_6 (δ in ppm) (600 MHz) (key [1]H-[1]H COSY and HMBC correlations are shown below)

No.	δ_H (*J* in Hz)	δ_C	No.	δ_H (*J* in Hz)	δ_C
Acid	-	-	δ^1	0.78, t (7.0)	11.1, CH_3
CO	-	169.3, C	NH	6.93, d (9.3)	-
α	5.10, m	72.0, CH	N-MeVal	-	-
β	1.56, m; 1.71, m	34.0, CH_2	CO	-	170.5, C
γ	1.70, m	31.5, CH	α	4.98, m	57.1, CH
δ^2	0.91, d (6.5)	17.8, CH_3	β	2.19, m	26.6, CH
δ^1	3.28, m; 3.25, m	65.4, CH_2	γ^1	0.84, d (6.4)	19.1, CH_3
MePro	-	-	γ^2	0.87, d (6.4)	19.1, CH_3
CO	-	170.2, C	N-CH_3	3.13, brs	30.5, CH_3
α	3.97, m	66.4, CH	N-MeAla	-	-
β	2.48, m	36.4, CH	CO	-	169.0, C
β-CH_3	1.03, d (7.0)	18.6, CH_3	α	5.17, q (6.6)	54.7, CH
γ	1.66, m; 1.93, m	30.4, CH_2	β	1.17, d (6.6)	15.4, CH_3
δ	3.66, m; 3.76, m	44.6, CH_2	N-CH_3	2.54, brs	27.7, CH_3
Ile	-	-	Ala	-	-
CO	-	173.0, C	CO	-	173.0, C
α	4.75, dd (6.7, 9.2)	52.7, CH	α	2.36, m; 2.76, m	34.0, CH_2
β	1.85, m	36.7, CH	β	2.93, m; 3.86, dd	32.9, CH_2
γ^2	0.77, d (5.6)	15.1, CH	NH	8.09, dd (2.2, 9.8)	-
γ^1	1.25, m; 1.40, m	23.9, CH_2			

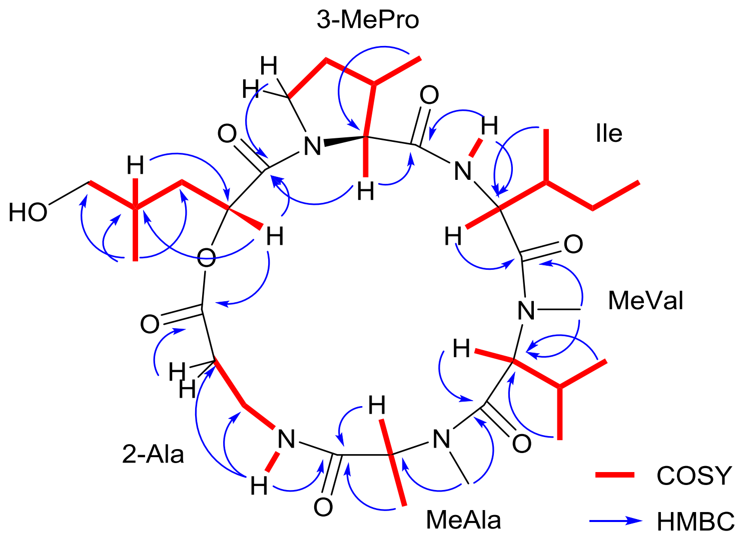


Table S6. The [1]H and [13]C NMR data of **6** in DMSO-d_6 (δ in ppm) (600 MHz) (key [1]H-[1]H COSY and HMBC correlations are shown below)

No.	δ_H (*J* in Hz)	δ_C	No.	δ_H (*J* in Hz)	δ_C
Leu	-	-	NH	8.96, d (8.0)	-
CO	-	172.5, C	N-MeVal	-	-
α	4.51, m	51.4, CH	CO	-	169.0, C
β	1.56, m; 1.29, m	37.7, CH_2	α	4.75, d (10.6)	56.5, CH
γ	1.91, m	24.2, CH	β	2.03, m	25.8, CH
δ^1	0.88, d (6.6)	19.8, CH_3	γ^1	0.56, d (6.5)	18.1, CH_3
δ^2	0.97, d (6.7)	23.6, CH_3	γ^2	0.11, d (6.4)	17.8, CH_3
NH	8.33, d (2.5)	-	N-CH_3	2.54, brs	40.4, CH_3
Pro	-	-	N-MePhe	-	-
CO	-	171.9, C	CO	-	168.0, C
α	4.08, dd (1.0, 8.4)	60.2, CH	α	5.19, dd (6.0, 8.1)	61.1, CH
β	2.01, m; 2.05, m	31.5, CH_2	β	2.83, m; 3.28, m	35.4, CH_2
γ	1.09, m; 1.67, m	21.6, CH_2	γ	-	137.8, C
δ	3.26, m; 3.30, m	46.6, CH_2	δ	7.30, m	128.6, CH
Phe	-	-	ε	7.26, m	128.1, CH
CO	-	172.5, C	ζ	7.21, m	126.4, CH
α	4.50, m	53.2, CH	N-CH_3	2.89, brs	29.1, CH_3
β	2.86, m; 2.99, m	34.2, CH_2	Ala	-	-
γ	-	137.6, C	CO	-	171.5, C
δ	7.31, m	129.1, CH	α	2.23, m; 2.41, m	34.5, CH_2
ε	7.26, m	128.5, CH	β	3.32, m; 3.54, m	35.4, CH_2
ζ	7.22, m	126.7, CH	NH	8.33, d (2.5)	-

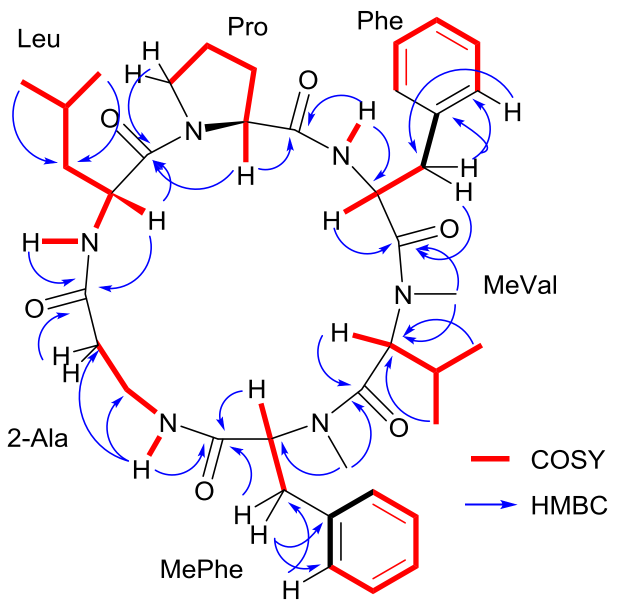


Table S7. The [1]H and [13]C NMR data of **7** in DMSO-d_6 (δ in ppm) (600 MHz) (key [1]H-[1]H COSY and HMBC correlations are shown below)

No.	δ_H (J in Hz)	δ_C	No.	δ_H (J in Hz)	δ_C
Acid	-	-	NH	7.81, m	-
1	-	169.7, C	Leu	-	-
2	2.25, m; 2.54, m	37.2, CH_2	CO	-	171.1. C
3	4.96, d (7.4)	74.5, CH	α	4.02, m	51.9, CH
4	1.64, m	36.2, CH	β	1.48, m	38.6, CH_2
5	0.81, m	14.6, CH_3	γ	1.62, m	24.1, CH
6	1.00, m; 1.33, m	31.5, CH_2	δ[1]	0.81. m	21.1, CH_3
7	1.24, m	28.7, CH_2	δ[2]	0.89, m	22.9, CH_3
8	1.23, m	22.3, CH_2	NH	8.65, d (4.9)	-
9	0.86, m	13.9, CH_3	Ala	-	-
Gly	-	-	CO	-	171.7, C
CO	-	169.0, C	α	4.18, m	47.6, CH
α	3.41, m; 4.10, m	42.3, CH_2	β	1.19, m	17.3, CH_3
NH	7.88, m	-	NH	7.96, d (7.6)	-
Val	-	-	Ala	-	-
CO	-	171.7, C	CO	-	171.7, C
α	4.10, m	58.2, CH	α	4.07, m	48.3, CH
β	1.85, m	30.0, CH	β	1.29, m	16.6, CH_3
γ[1]	0.87, m	18.9, CH_3	NH	7.79, m	-
γ[2]	0.83, m	18.7, CH_3			

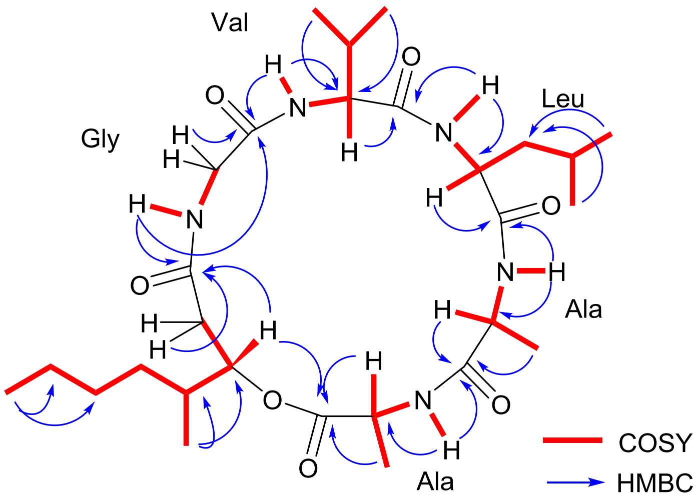


Table S8. Clusters encoding nonribosomal peptide synthases (NRPSs) (excluding PKS/NRPS hybrids) in *B. felina* SX-6-22

Clusters	NRPS modules organization	NRPSs in this study	Accession numbers of homologues	Hosts of the homologues
1	AT-C		OBT62387.1	*Pseudogymnoascus* sp.
2	CAT-C		KPM39273.1	*Neonectria ditissima*
3	AT-CATE-CAT-CAT-CA(MT)T-CA(MT)T-C	DetxA	KND86507.1	*Tolypocladium ophioglossoide*
4	AT-CTC		KFH48348.1	*Acremonium chrysogenum*
5	AT(Te)		RYP68643.1	*Monosporascus* sp.
6	AT(Te)		OTB16444.1	*Daldinia* sp.
7	T-CAT-C		KPM35145.1	*Neonectria ditissima*
8	AT(Te)		POR35295.1	*Tolypocladium paradoxum*
9	A-CAT-CT-CACT-C		KFH43976.1	*Acremonium chrysogenum*
10	T-CATE-CAT-CATE-CAT-CAT-C	IsrA	KFX41393.1	*Talaromyces marneffei*
11	AT-CATE-CAT-CAT-CA(MT)T-CA(MT)T-C	IsdA	KND86507.1	*Tolypocladium ophioglossoide*
12	ATE-CAT-CAT-C		OJJ41173.1	*Aspergillus wentii*
13	Ox-AC		KAF5494064.1	*Colletotrichum fructicola*
14	CAT-CAT-CATE-CATE-CAT-CT-C		PKS08582.1	*Lomentospora prolificans*
15	AT(Te)		RYP11740.1	*Monosporascus* sp.
16	ATR		EKG19785.1	*Macrophomina phaseolina*
17	TCA		EOD43397.1	*Neofusicoccum parvum*
18	TCA		KND92421.1	*Tolypocladium ophioglossoide*
19	CAT-CAT-C		PYI02181.1	*Aspergillus sclerotiicarbona*
20	CAT-CA(MT)T-CA(MT)T-CA(MT)T-CA(MT)T-CAT-CA(MT)T-CMT)T-CAT-CA(MT)T-CAT-C		CAA82227.1	*Tolypocladium inflatum*
21	ATR		OLN95656.1	*Colletotrichum chlorophyti*
22	CAT-CAT-CAT-C		KFH45129.1	*Acremonium chrysogenum*
23	CAT-CAT-CAT-CAT-C		XP_033604383.1	*Pseudovirgaria hyperparasitica*

Table S9. Gene annotation for *detx*, *isd* and *isr* clusters

Clusters	Name/ID	Size (bp/aa)	Accession numbers	Predicted functions	Accession numbers of homologues	Identity/ similarity (%)
detx	*BFSX_00493*	1111/346	MW271810	Zinc-type alcohol dehydrogenase-like protein	KFH42175	78/86
	BFSX_00492	2317/730	MW271809	α-1,2-Mannosidase	PNY25799	65/73
	BFSX_00491	783/261	MW271808	Putative esterase	XP_035318235	67/78
	BFSX_00490	1206/367	MW271807	Hypothetical protein	KFA76960	28/34
	BFSX_00487	1653/551	MW271806	pH signal transduction protein	POR32663	70/81
	detxA	24105/8002	MT990934	Destruxins-like nonribosomal peptide synthetase	KND86507	69/81
	detxB	2441/ 573	MT990935	Aldo/keto reductase	XP_007826234	76/87
	detxC	1608/ 513	MT990936	Cytochrome P450 monooxygenase	KAF5121804	81/90
	detxD	3945/ 1295	MT990937	ABC multidrug transporter Mdr1	KFG77710	69/83
	detxE	1229/ 377	MT990938	2-oxoglutarate-Fe(II) type oxidoreductase	TVY12977	43/58
	BFSX_00481	2759/849	MW271815	pH-response regulator protein palA	KFH40867	84/91
	BFSX_00480	1595/494	MW271814	Flotillin domain-containing protein	XP_018146344	72/80
	BFSX_00478	1037/326	MW271813	Kinase-like domain	KAF4462691	53/60
	BFSX_00477	2029/646	MW271812	UDP-glucose 4-epimerase	PNY28365	47/55
	BFSX_00476	1380/460	MW271811	3'(2'), 5'-bisphosphate nucleotidase	XP_035318213	62/71
isd	*BFSX_04284*	2241/691	MW271825	Calcium channel-like protein	KFH43535	70/78
	BFSX_04283	1463/466	MW271824	Glycoside hydrolase superfamily	KXJ88434	58/69

	BFSX_04282	1015/321	MW271823	Putative membrane protein-like protein	KFH45179	72/81
	BFSX_04281	3107/892	MW271822	SH3 domain-containing protein	XP_008098738	71/80
	BFSX_04280	1624/503	MW271821	Leucine permease transcriptional regulator	KAF4458932	73/82
	isdA	24258/8044	MT990939	Destruxins-like nonribosomal peptide synthetase	KND86507	65/78
	isdB	1413/385	MT990940	Aldo/keto reductase	XP_007826234	68/77
	BFSX_04277	2008/653	MW271820	Vacuolar protein-sorting-associated protein	TVY72203	73/82
	BFSX_04276	1140/380	MW271819	CENP-B homolog protein	GFP57757	79/90
	BFSX_04274	939/291	MW271818	Mitochondrial fission process protein-like protein	KFH45208	79/90
	BFSX_04273	927/309	MW271817	AN1-type zinc finger protein-like protein	KFH45196	85/94
	BFSX_04272	3115/1021	MW271816	DNA repair protein-like protein	KFH45197	76/84
isr	*BFSX_03676*	3189/1006	MW271830	Armadillo-like helical protein	XP_018174607	68/77
	BFSX_03674	2583/708	MW271829	High-affinity glucose transporter-like protein	KFH42623	81/87
	BFSX_03673	1689/473	MW271828	DUF89 domain-containing protein	KID97754	81/90
	BFSX_03672	1593/488	MW271827	Pisatin demethylase-like protein (P450)	KFH47378	73/87
	BFSX_03670	1380/386	MW271826	Integral membrane protein	KZL68594	49/70
	isrA	22331/7398	MT990941	Nonribosomal peptide synthetase	KFX41393	38/56
	isrB	2194/574	MT990942	AMP dependent CoA ligase	XP_006666503	56/72
	isrC	1446/482	MT990943	Acyltransferase easC	C8VPT2	50/67
	isrD	8006/2544	MT990944	Polyketide synthase	KIA75545	60/74
	BFSX_03665	1598/503	MW271835	Aminoglycoside phosphotransferase	PNY25379	56/72
	BFSX_03664	1625/410	MW271834	Integral membrane protein	KAF4342798	55/67
	BFSX_03663	9785/2981	MW271833	Polyketide synthase	PMD36174	75/85
	BFSX_03662	1138/357	MW271832	Putative zinc-binding dehydrogenase	PMD36173	80/92
	BFSX_03661	1758/542	MW271831	Cytochrome P450	PMD36172	78/88
out of cluster	*SX-p5cr1*	926/287	MT990945	Pyrroline-5-carboxylate reductase	TVY57756	50/67
out of cluster	*SX-p5cr2*	942/313	MT990946	Pyrroline-5-carboxylate reductase	KFH48959	81/89

Table S10. The substrate selectivity-conferring codes of A domains in DetxA, IsrA and IsdA

NRPSs	A domains	Substrate selectivity-conferring codes										Predicted substrates based on analysis by NRPSsp[4]	Substrates based on natural products structures
		1	2	3	4	5	6	7	8	9	10		
GrsA-A		D	A	W	T	I	A	A	I	C	K	Phe	L-Phe
DetxA	A_1	D	I	F	Y	A	I	T	T	A	K	Phe	*β*-Ala
	A_2	G	A	N	L	I	G	A	T	V	K	Trp	L-HIC
	A_3	D	M	H	D	I	G	I	H	I	K	Pro	β-Me-L-Pro
	A_4	D	G	L	F	I	G	I	P	V	K	Pro	L-Ile
	A_5	D	A	W	F	Y	G	G	T	F	K	Leu	L-Val
	A_6	D	V	W	I	Y	A	A	V	I	K	Leu	L-Ala
IsdA	A_1	D	I	F	Y	A	I	T	T	A	K	Pro	*β*-Ala
	A_2	G	A	N	L	I	G	A	T	V	K	Trp	L-HIC
	A_3	D	M	H	D	I	G	I	H	V	K	Pro	L-Pro
	A_4	D	G	F	L	I	C	Y	P	A	K	Pro	L-Phe
	A_5	D	A	W	F	V	G	G	S	F	K	Leu	L-Val
	A_6	D	M	W	T	Y	G	A	A	I	K	Leu	L-Phe
IsrA	A_2	D	I	Q	G	I	L	A	M	Q	K	Pro	Gly
	A_3	D	A	S	Q	V	G	G	I	Y	K	Phe	L-Val
	A_4	D	A	H	F	I	G	A	I	M	K	Pro	L-Leu
	A_5	D	V	M	C	G	A	S	V	L	K	Pro	L-Ala
	A_6	D	A	A	V	I	I	G	I	I	K	Val	L-Val

Table S11. Primers used in this study

Primers	Sequence (5′-3′)
Plasmids construction (CRISPR/Cas9) for gene deletion of *detxA*, *detxE*, *isdA*, *isrA*, *p5cr1* and *p5cr2* (uppercase sequences indicate protospacer of sgRNA and its complementary ribozyme region)	
detxA_sg-F	accgGGTAGCcctgatgagtccgtgaggacgaaacgagtaagctcgtcGCTACCCTGAAGTTAGCTCC
detxA_sg-R	aaacGGAGCTAACTTCAGGGTAGCgacgagcttactcgtttcgtcctcacggactcatcaggGCTACC
detxE_sg-F	accgTGACACcctgatgagtccgtgaggacgaaacgagtaagctcgtcGTGTCAACGGCGGTAAGGAG
detxE_sg-R	aaacCTCCTTACCGCCGTTGACACgacgagcttactcgtttcgtcctcacggactcatcaggGTGTCA
isdA_sg-F	accgGATGCCcctgatgagtccgtgaggacgaaacgagtaagctcgtcGGCATCTTGAATTGTCGTCG
isdA_sg-R	aaacCGACGACAATTCAAGATGCCgacgagcttactcgtttcgtcctcacggactcatcagggcatcGGCATC
isrA_sg-F	accgTATACCcctgatgagtccgtgaggacgaaacgagtaagctcgtcGGTATACCGTATACCCAACG
isrA_sg-R	aaacCGTTGGGTATACGGTATACCgacgagcttactcgtttcgtcctcacggactcatcaggggcatcGGTATA
p5cr1_sg-F	accgTGGAGAcctgatgagtccgtgaggacgaaacgagtaagctcgtcTCTCCAGGTTCTGGCTCGCC
p5cr1_sg-R	aaacGGCGAGCCAGAACCTGGAGAgacgagcttactcgtttcgtcctcacggactcatcagggcatcTCTCCA
p5cr2_sg-F	accgGGCTGCcctgatgagtccgtgaggacgaaacgagtaagctcgtcGCAGCCGAGCAGGATGATGT
p5cr2_sg-R	aaacACATCATCCTGCTCGGCTGCgacgagcttactcgtttcgtcctcacggactcatcagggcatcGCAGCC
dDNAs construction for gene deletion of *detxA*, *detxE*, *isdA*, *isrA*, *p5cr1* and *p5cr2* (lowercase sequences indicate overlap region)	
detxA_5f-F	CCGGACTCGCTGCCTGATTTAA
detxA_5f-R	AGAAGTCGGCTGCCGTTTCT
detxA_3f-F	agaaacggcagccgacttctCAGAGCGTCGCTGACTACCTGAAT
detxA_3f-R	CTGAGCTCCGGTGGTGAATGA
detxA-NF	GGATGATGTGAACCGGTGGTGG
detxA-NR	CTGCTGCGTATCTCGGTGATGAA
detxE_5f-F	ATCACAGTAGGGTACAGCGCAG
detxE_5f-R	CATGGCGCTTACACGGTGG
detxE_3f-F	caccgtgtaagcgccatgTGATGCCGGCAATTCCCTTTC
detxE_3f-R	AGAGCACCGCCATCATGCTT
detxE-NF	GTATGTATCCAGTGCACCCAGG

detxE-NR	CCACGACTACCGGTCTCACAT
isdA_5f-F	GTAGCTCGCCTCCTGGATCCAA
isdA_5f-R	ccaactttctggctcggagaCCTCTTAGATATCGGCTGCCG
isdA_3f-F	TCTCCGAGCCAGAAAGTTGGG
isdA_3f-R	CGTCATCTGTGACGACGACAT
isdA-NF	GTAAACATCTACGGACCCGCC
isdA-NR	GCTCGCCTCCACTTGTTGAAT
isrA_5f-F	CTGGAGACTCTTGTCCTTGGTG
isrA_5f-R	tcgcccacgctttgagTCTCTGTCGCCGTGACTGGTTTGA
isrA_3f-F	GAGACTCAAAGCGTGGGCGAA
isrA_3f-R	TAGATGACAAGCGCAGCGTTG
isrA-NF	AACGGATACGGGCCCTCAGA
isrA-NR	TGAGGAACCTTACCCTCAAAGG
isrA_check-F	ACTCTATCACGGTGGTTGCAT
isrA_check-R	GTTTGCCTTCAGTCTTCGGAT
p5cr1_5f-F	CTCATCATGATCGAGGCGACG
p5cr1_5f-R	GAGCAAGTTTACATAGGGAGTCACCC
p5cr1_3f-F	tgactccctatgtaaacttgctcGCGACTGTCAAGTTCTCGGAC
p5cr1_3f-R	CATTCCTGCTGAACATGTCCCAG
p5cr1-NF	CTACGAGAACGTTGGCGTCATC
p5cr1-NR	GTCACAGTGGGAGCTGTTGC
p5cr1_check-F	GGTCATTTACATTGGTGCTGGTGTC
p5cr1_check-R	ACATTGATGCCTACGAGCAAGTGTG
p5cr2_5f-F	GTTGAGGTGTTGGAGCTGGGA
p5cr2_5f-R	aggcagctttggcgtctcTAGGAGCTGACATGCTGGCTACT
p5cr2_3f-F	TAGAGACGCCAAAGCTGCCTAAG
p5cr2_3f-R	GGAATTCCTGCGGAAGATGGC
p5cr2-NF	AACCTCATGCGGACCTCGAAC
p5cr2-NR	CACCTCGACACCAGTGTCACT
p5cr2_check-F	CATATAATCTGTGACGCCGAAACACC
p5cr2_check-R	CTCAACATCTCGCAACCGATATCGTA
Plasmids construction for protein purification (lowercase sequences indicate overlap region)	
LIC_DetxE-F	tacttccaatccaatgcaATGGGTTCCACAAGCCCCAATG
LIC_DetxE-R	ttatccacttccaatgTCAGTACGCAGTGGCAACACG
LIC_P5CR1-F	tacttccaatccaatgcaATGTCCGAGAACTTGACAGTCGC
LIC_P5CR1-R	ttatccacttccaatgCTATGTAAACTTGCTCATGTTTCTCATTCTATCCG
LIC_P5CR2-F	tacttccaatccaatgcaATGGGTATTGCCATCGTCGGT
LIC_P5CR2-R	ttatccacttccaatgCTAGTTTGGCGGCTGGTTGGT

Table S12. Plasmids and strains used in this study

Name	Description	References
Plasmids		
pYBC-01a	Initial CRISPR/Cas9 plasmid (hygromycin resistance) without any sgRNA sequence for in-frame gene deletion	[1]
pMM1029	Modified pYBC-01a with sgRNA sequence for *detxA* deletion	This study
pMM1030	Modified pYBC-01a with sgRNA sequence for *isdA* deletion	This study
pMM1031	Modified pYBC-01a with sgRNA sequence for *isrA* deletion	This study
pMM1032	Modified pYBC-01a with sgRNA sequence for *detxE* deletion	This study
pMM1033	Modified pYBC-01a with sgRNA sequence for *p5cr1* deletion	This study
pMM1034	Modified pYBC-01a with sgRNA sequence for *p5cr2* deletion	This study
pMCSG7	Plasmid with His_6 tag for protein purification	[2]
pMCSG19	Plasmid with MBP tag for protein purification	[3]
pMM1035	Modified pMCSG7 with *detxE* for purification of DetxE	This study
pMM1036	Modified pMCSG19 with *p5cr1* for purification of P5CR1	This study
pMM1037	Modified pMCSG19 with *p5cr2* for purification of P5CR2	This study
***E. coli* strains**		
E. coli DH5α	For plasmid construction	Beijing TransGen Biotech Co., Ltd
E. coli BL21(DE3)	For protein overproduction	Beijing TransGen Biotech Co., Ltd
Fungi strains		
B. felina SX-6-22	Wild-type strain for destruxins, isaridins and isariins production	This study
MM10024	*B. felina* SX-6-22 with *detxA* deletion	This study
MM10025	*B. felina* SX-6-22 with *isdA* deletion	This study
MM10026	*B. felina* SX-6-22 with *isrA* deletion	This study
MM10027	*B. felina* SX-6-22 with *detxE* deletion	This study
MM10028	*B. felina* SX-6-22 with *p5cr1* deletion	This study
MM10029	*B. felina* SX-6-22 with *p5cr2* deletion	This study

Figure S1. Representative natural products that contain (3*S*/3*R*)-methyl-L-proline moieties. Neoefrapeptin F,[1] scytalidamide B,[2] paraherquamide E[3] and peniciherquamide C[4] contain (3*S*)-methyl-L-proline moieties. The (3*S*)-methyl-L-proline is used as a building block in the biosynthesis of UCS1025A.[5] Pentaminolarin[6] and bottromycin A_2[7] contain (3*R*)-methyl-L-proline moieties. The 3-methyl-L-proline moieties are labelled in red.

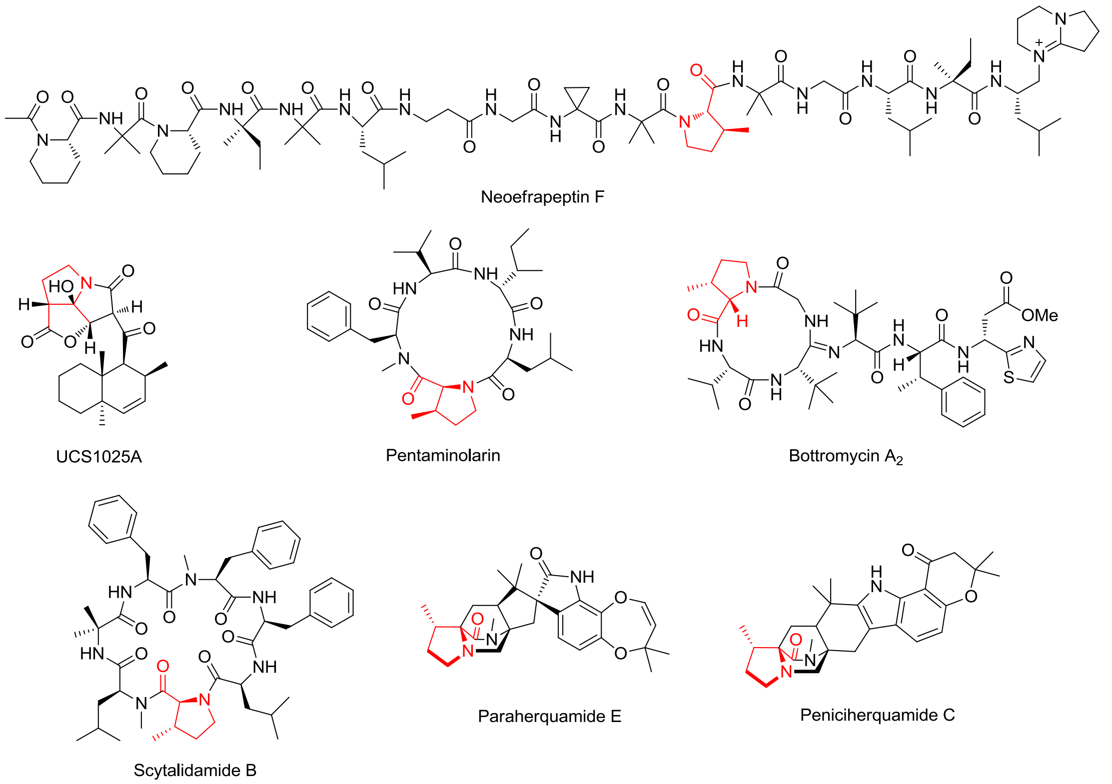
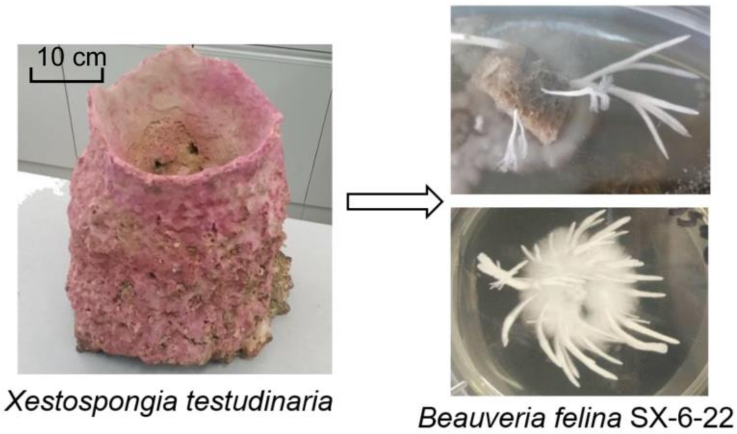


Figure S2. Morphological feature and phylogenetic analysis of *Beauveria felina* SX-6-22. (a) *B. felina* SX-6-22 isolated from marine sponge *Xestospongia testudinaria.* (b) The phylogenetic analysis of *B. felina* SX-6-22 based on ITS sequences (accession numbers were shown in parentheses).

a



b

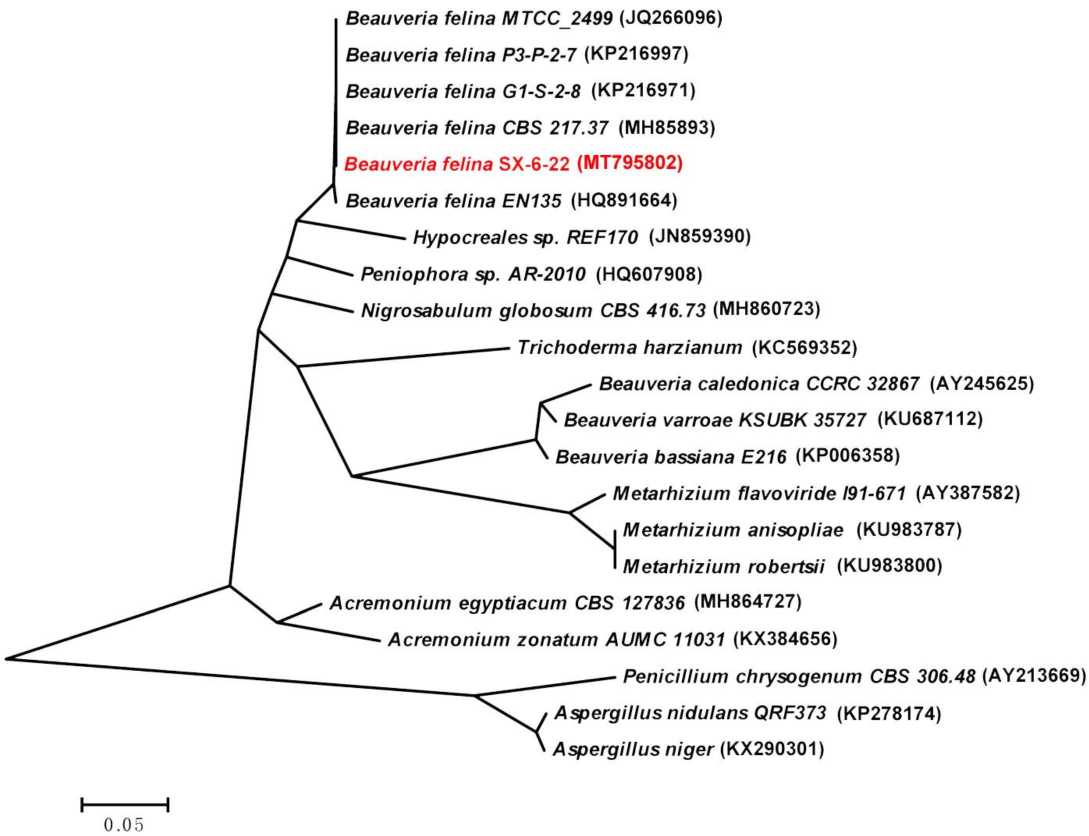


Figure S3. HPLC chromatograph and ^{1}H NMR spectrum for EtOAc extract of *B. felina* SX-6-22. (a) HPLC profile of EtOAc extract of *B. felina* SX-6-22. (b) ^{1}H NMR spectrum of the crude extract and characteristic signals related to peptides.

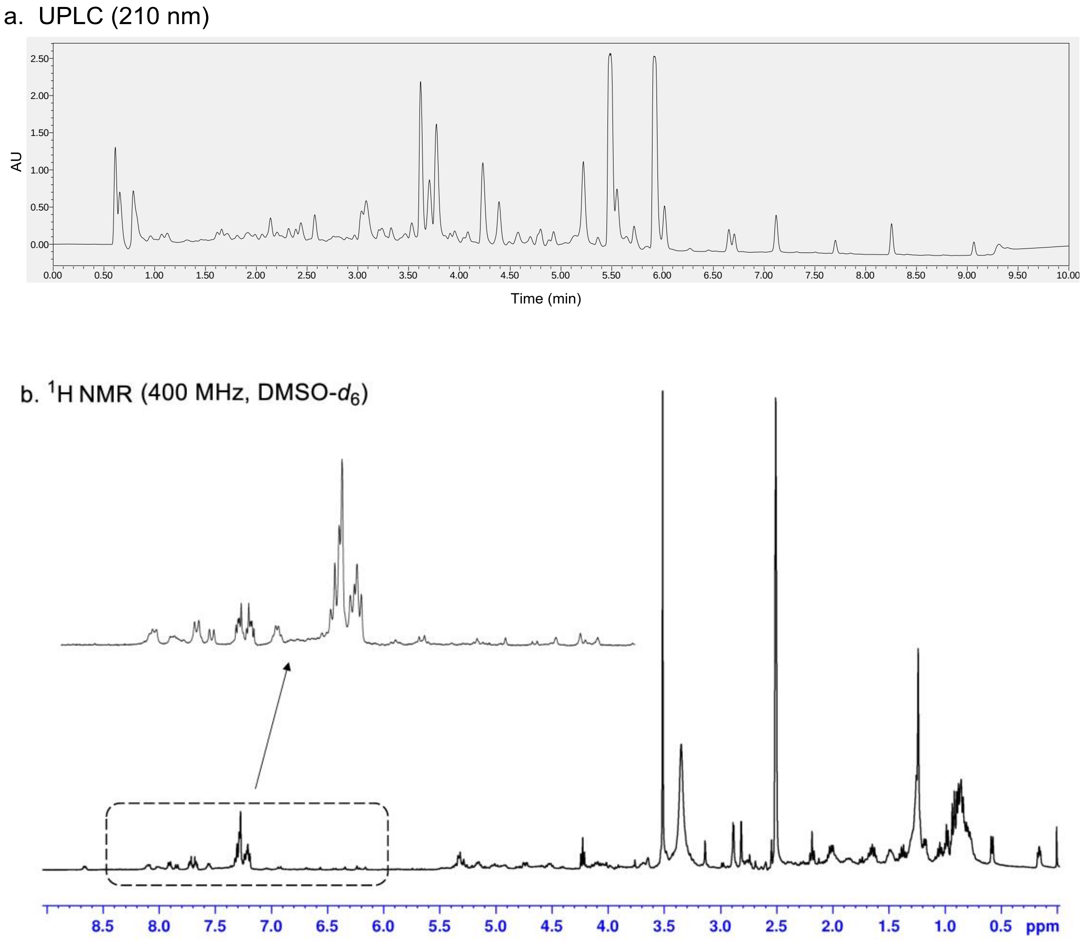


Figure S4. Global Natural Product Social Molecular Networking (GNPS) based on LC-MS data. The clusters marked in green, red and blue were related to destruxins' group, isaridins' group and isariins' group, respectively. The isolated compounds (**1-26**) were labeled with solid triangles (▲) while the compounds (**27-30**) predicted by MS^2 analysis were labeled with hollow triangles (△).

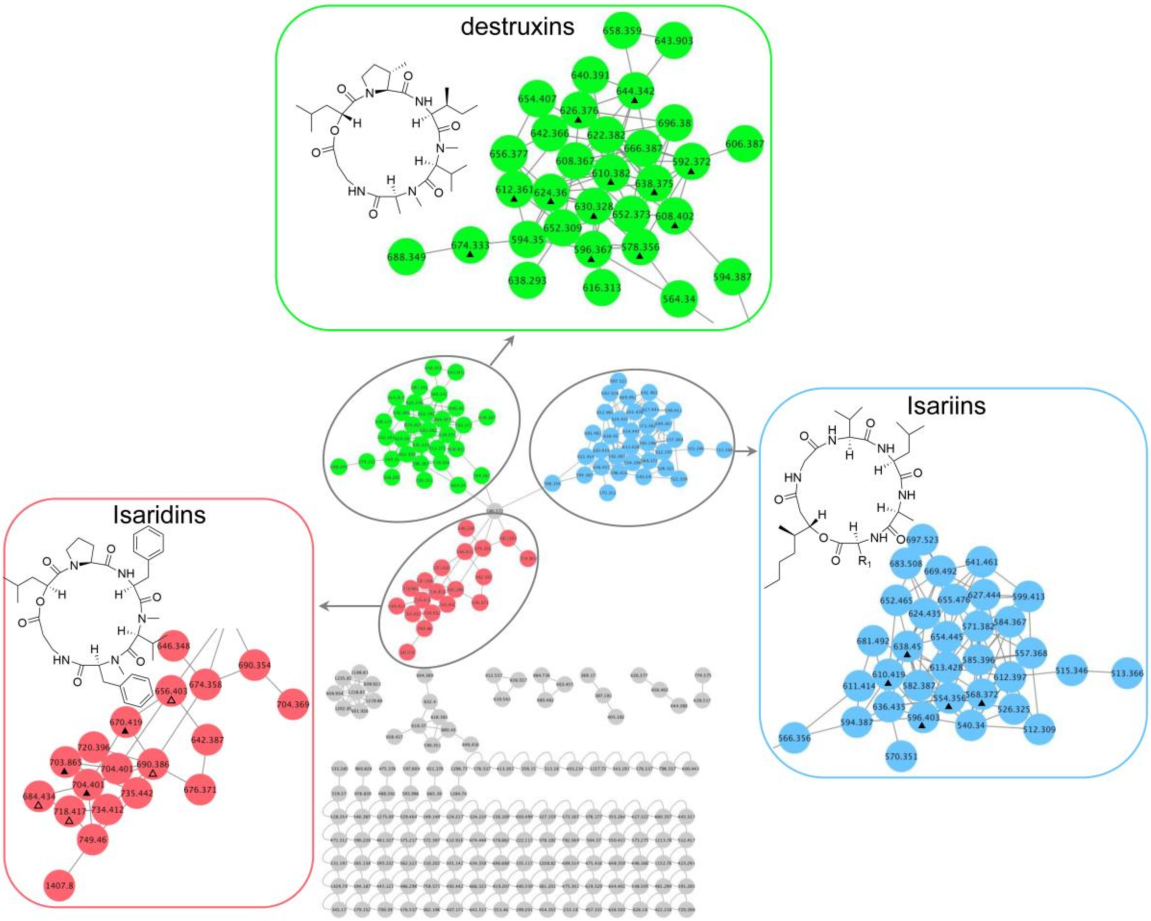
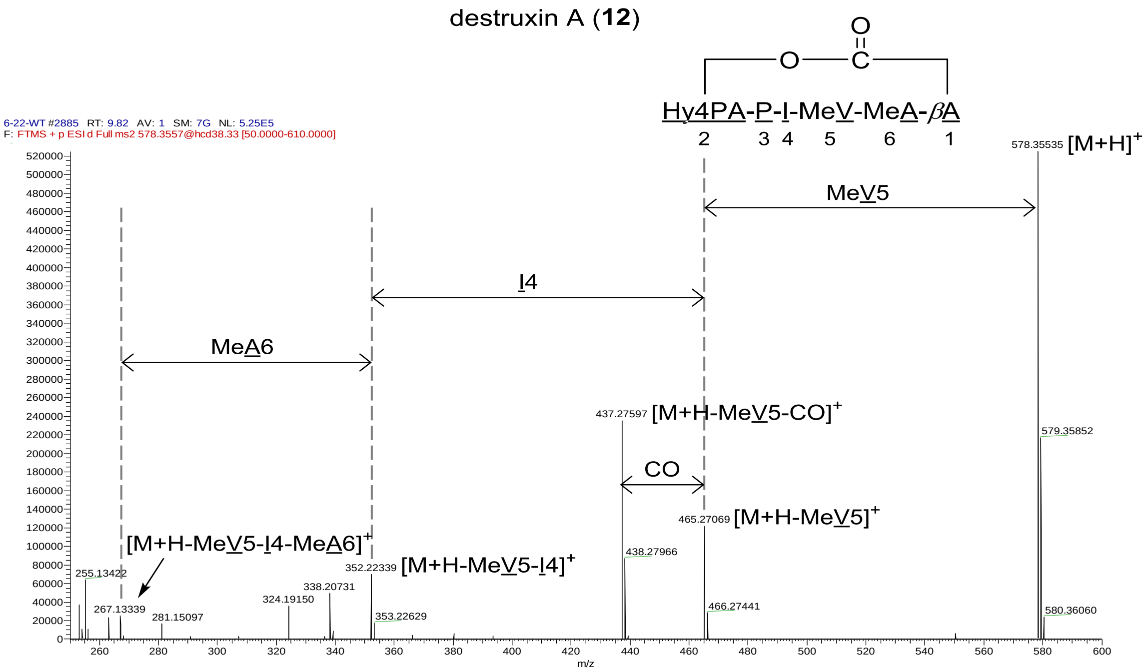
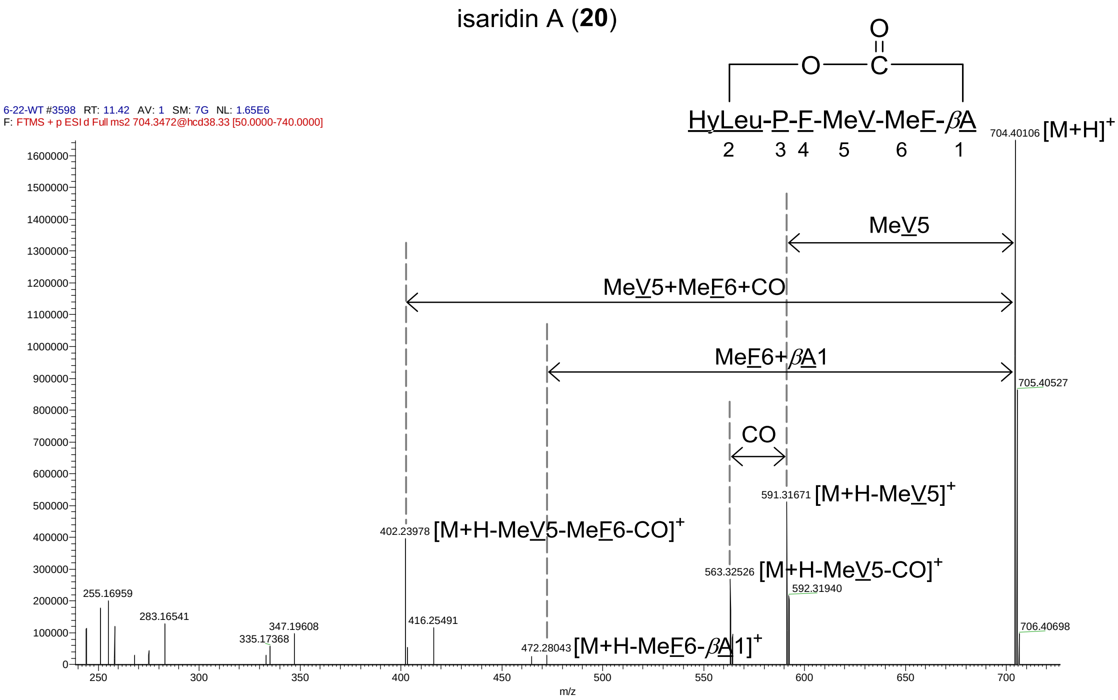


Figure S5. MS/MS spectra of known compounds (**12**, **20**, **23**, **27-30**) detected from total ion chromatogram (TIC).

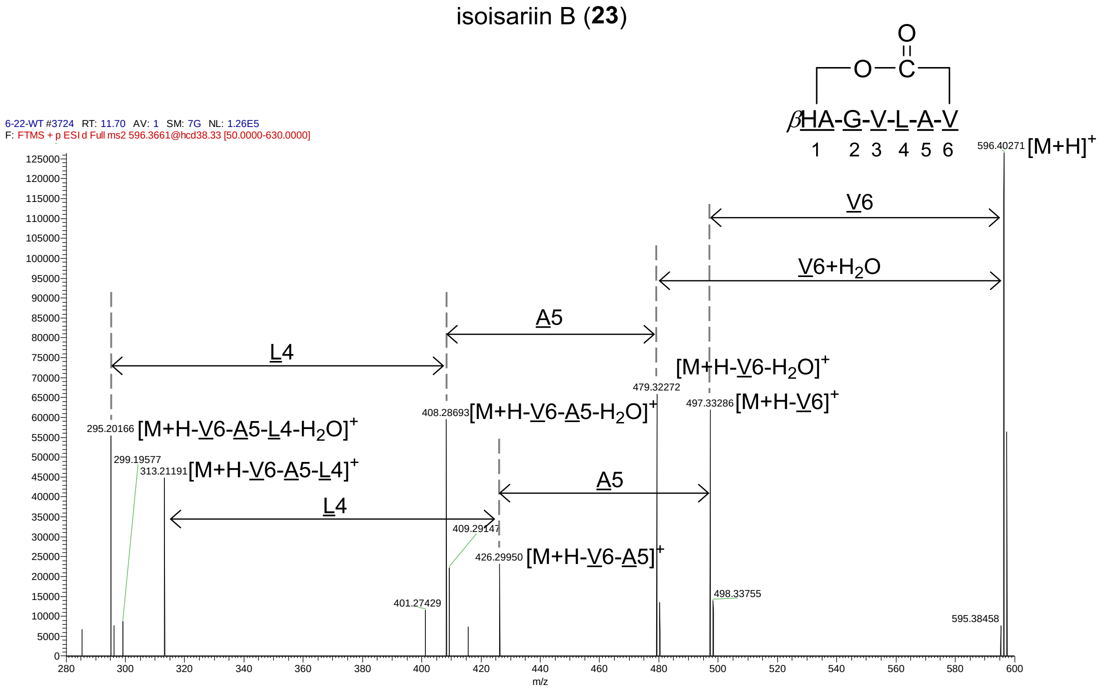
a. MS/MS spectrum of destruxin A (**12**)



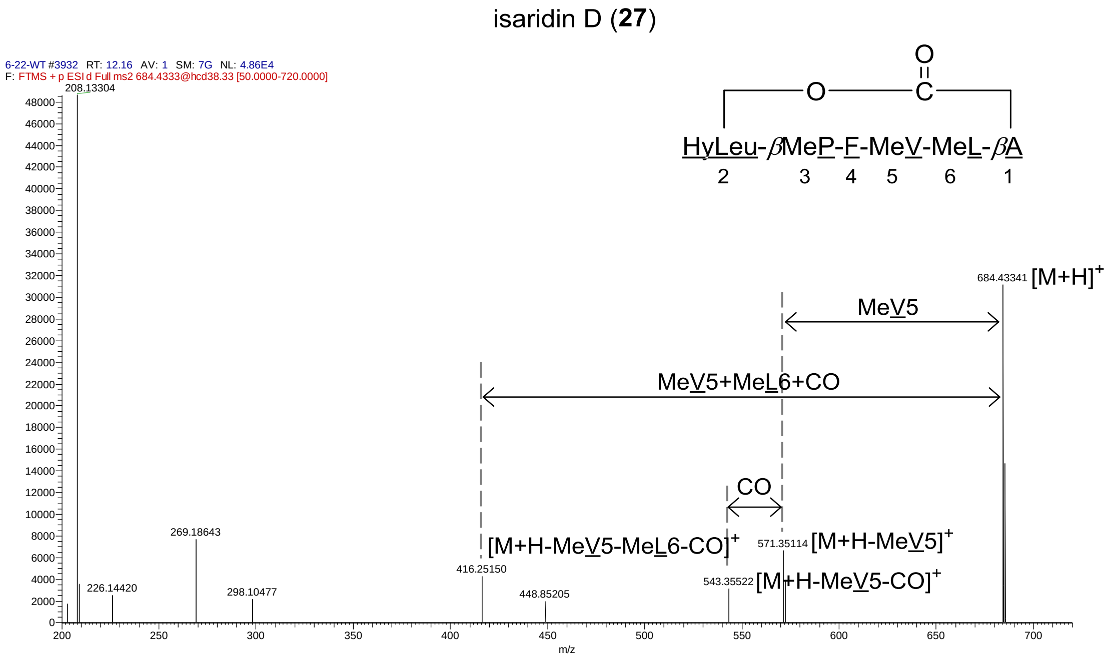
b. MS/MS spectrum of isaridin A (**20**)



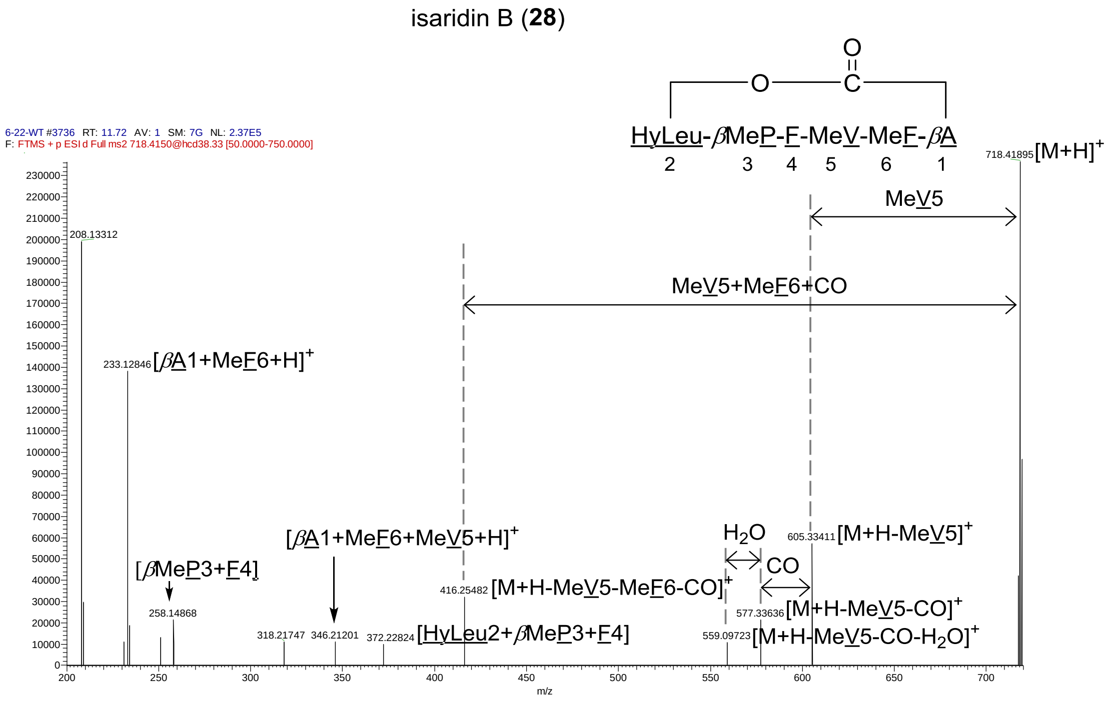
c. MS/MS spectrum of isoisariin B (**23**)



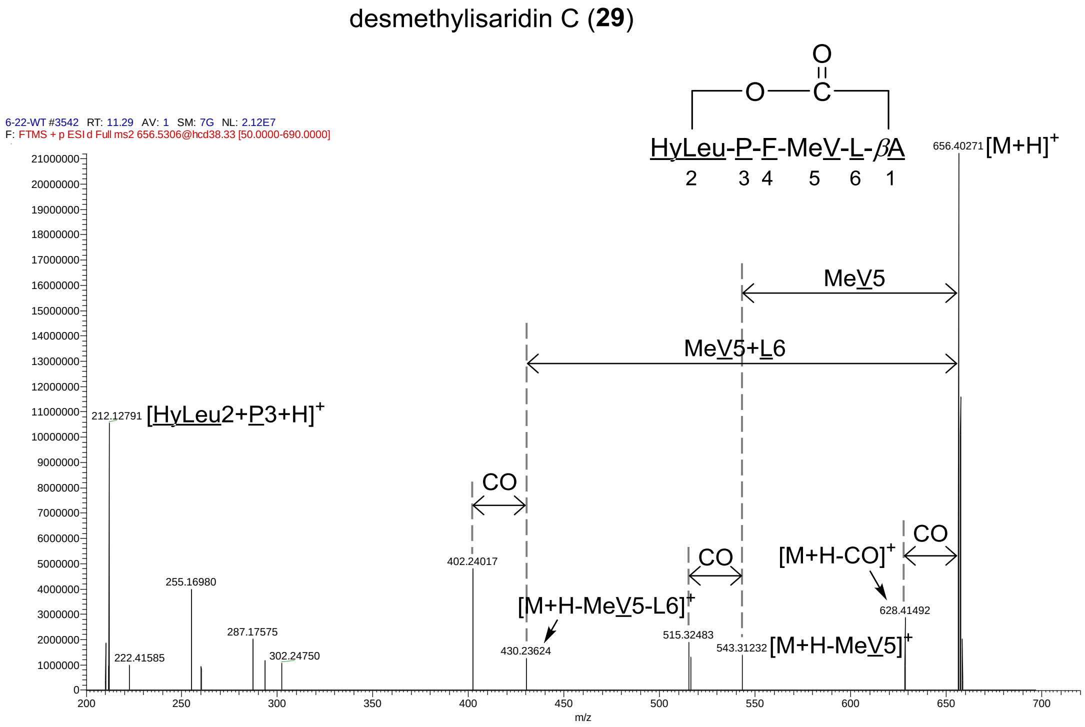
d. MS/MS spectrum of isaridin D (**27**)



e. MS/MS spectrum of isaridin B (**28**)



f. MS/MS spectrum of desmethylisaridin C (**29**)



g. MS/MS spectrum of desmethylisaridin A (**30**)

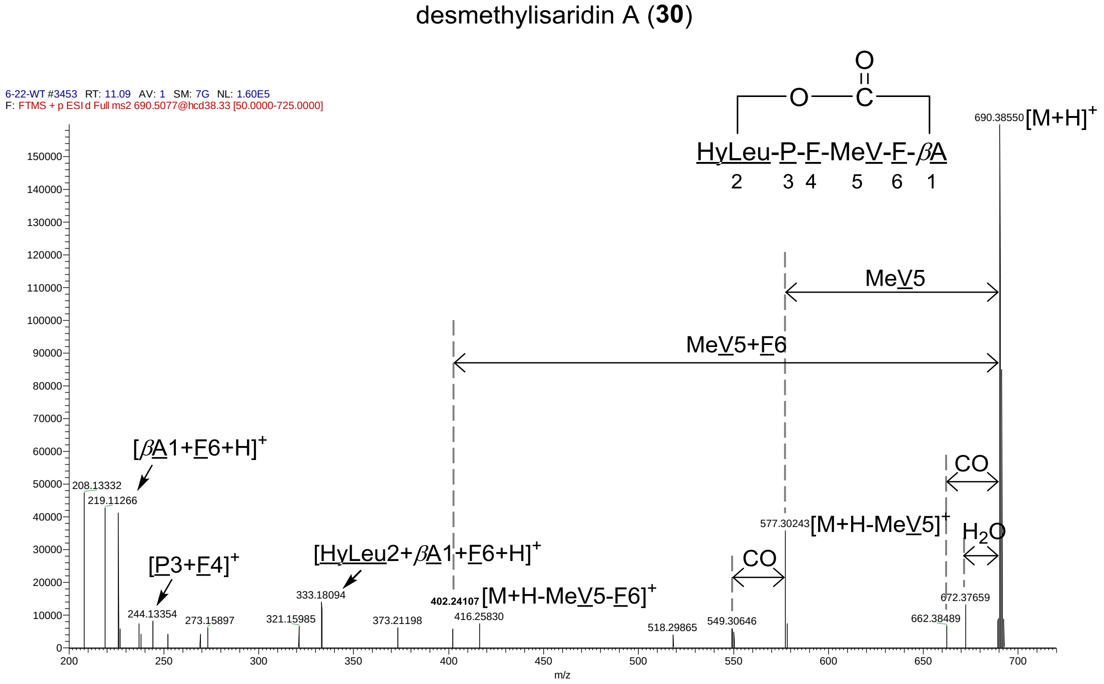


Figure S6. The Marfey's analysis for the determination of absolute configuration of **6**, based on the comparison of the HPLC retention times of the FDAA derivatives of **6**'s hydrolysates with FDAA derivatives of standard amino acids.

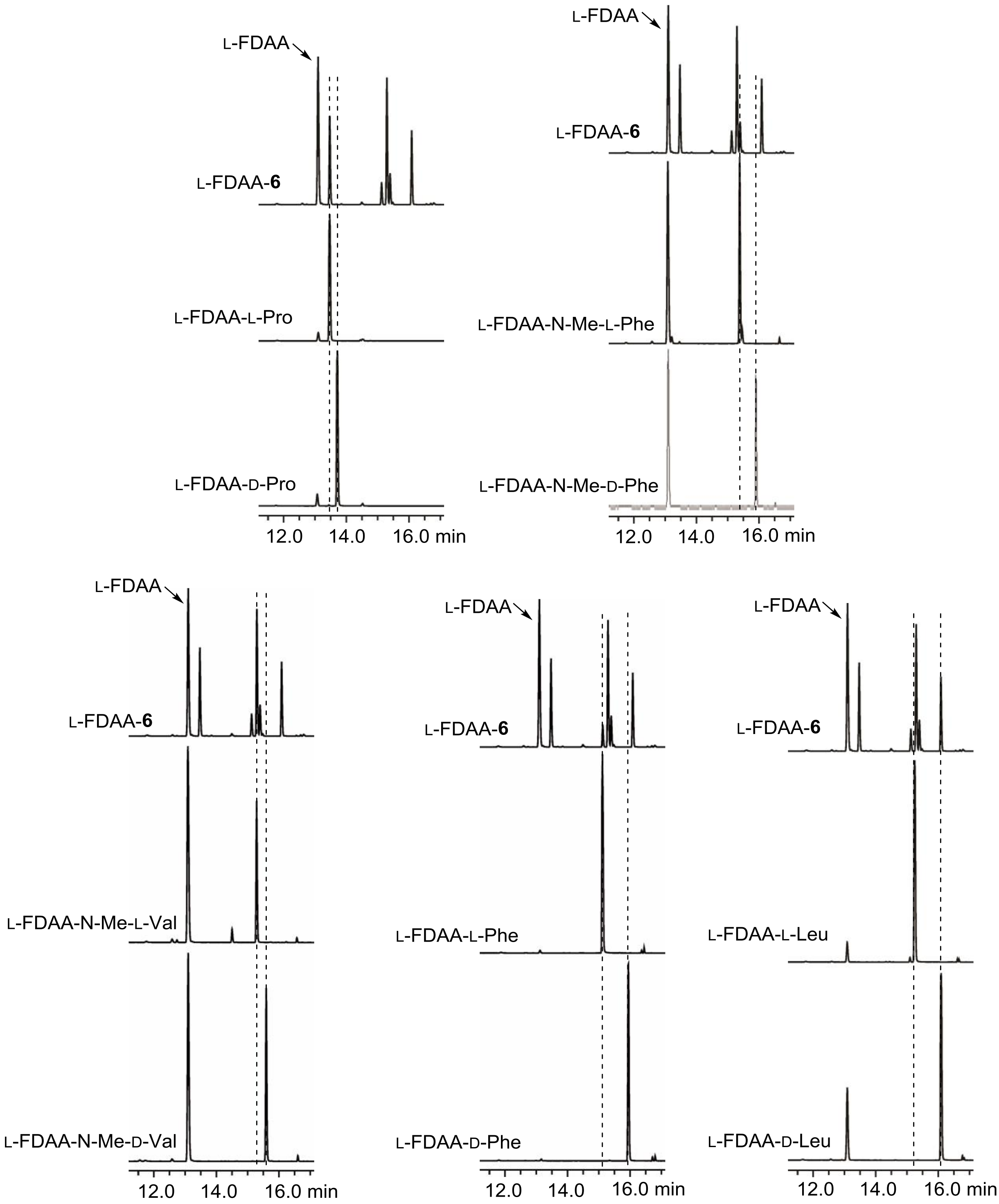
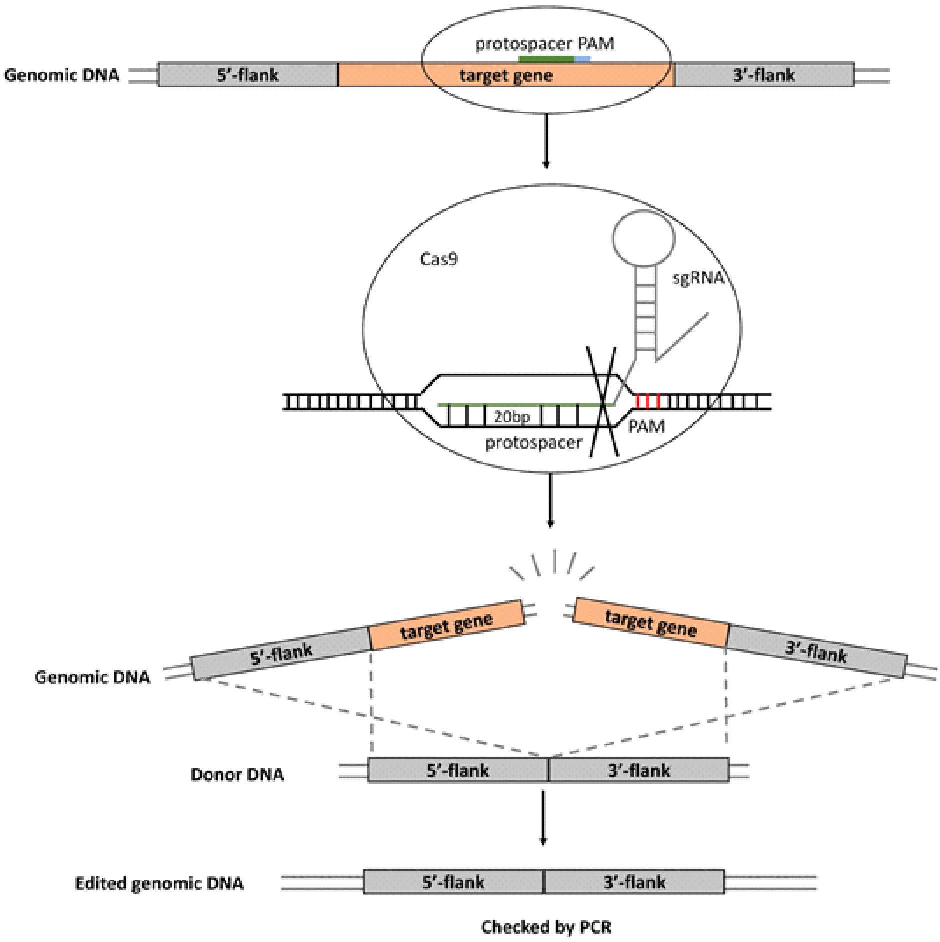


Figure S7. Schematic diagrams of gene deletion of *detxA*, *isdA*, *isrA*, *detxE*, *SX-p5cr1* and *SX-p5cr2* and PCR confirmation for mutants. (a) Schematic diagrams of gene deletion process by CRISPR/Cas9 system. (b) PCR confirmation for each of the mutants. Some gels were spliced to remove unrelated lanes among the molecular weight markers (labeled with "M"), lanes for wild-type (WT), and lanes for mutants.

a



b

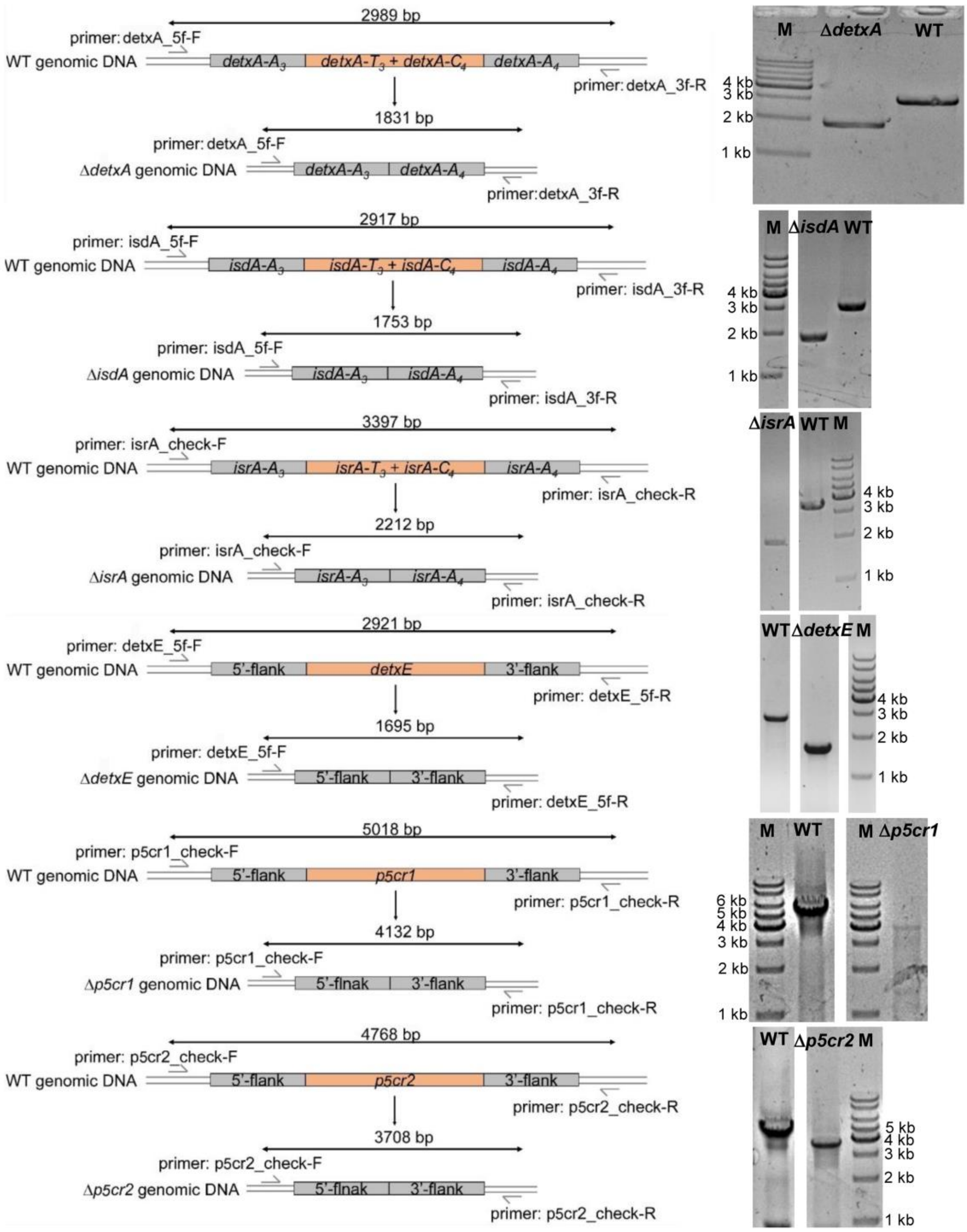

2989 bp
primer: detxA_5f-F
WT genomic DNA
detxA-A3
detxA-T3 + detxA-C4
detxA-A4
primer: detxA_3f-R
1831 bp
primer: detxA_5f-F
ΔdetxA genomic DNA
primer: detxA_3f-R
M ΔdetxA WT
4 kb
3 kb
2 kb
1 kb
2917 bp
primer: isdA_5f-F
isdA-A3
isdA-T3 + isdA-C4
isdA-A4
primer: isdA_3f-R
1753 bp
ΔisdA genomic DNA
M ΔisdA WT
3397 bp
primer: isrA_check-F
isrA-A3
isrA-T3 + isrA-C4
isrA-A4
primer: isrA_check-R
2212 bp
ΔisrA genomic DNA
ΔisrA WT M
2921 bp
primer: detxE_5f-F
5'-flank
detxE
3'-flank
primer: detxE_5f-R
1695 bp
ΔdetxE genomic DNA
WT ΔdetxE M
5018 bp
primer: p5cr1_check-F
p5cr1
primer: p5cr1_check-R
4132 bp
5'-flnak
Δp5cr1 genomic DNA
M WT M Δp5cr1
6 kb
5 kb
4768 bp
primer: p5cr2_check-F
p5cr2
primer: p5cr2_check-R
3708 bp
Δp5cr2 genomic DNA
WT Δp5cr2 M

Figure S8. LC-MS analysis of compounds accumulated in wild-type strain and mutants including Δ*detxA*, Δ*isdA* and Δ*isrA*. Compounds **1**/**2**, **3**/**4**, **5**/**8**, **21**/**22** and **7**/**25** are isomers.

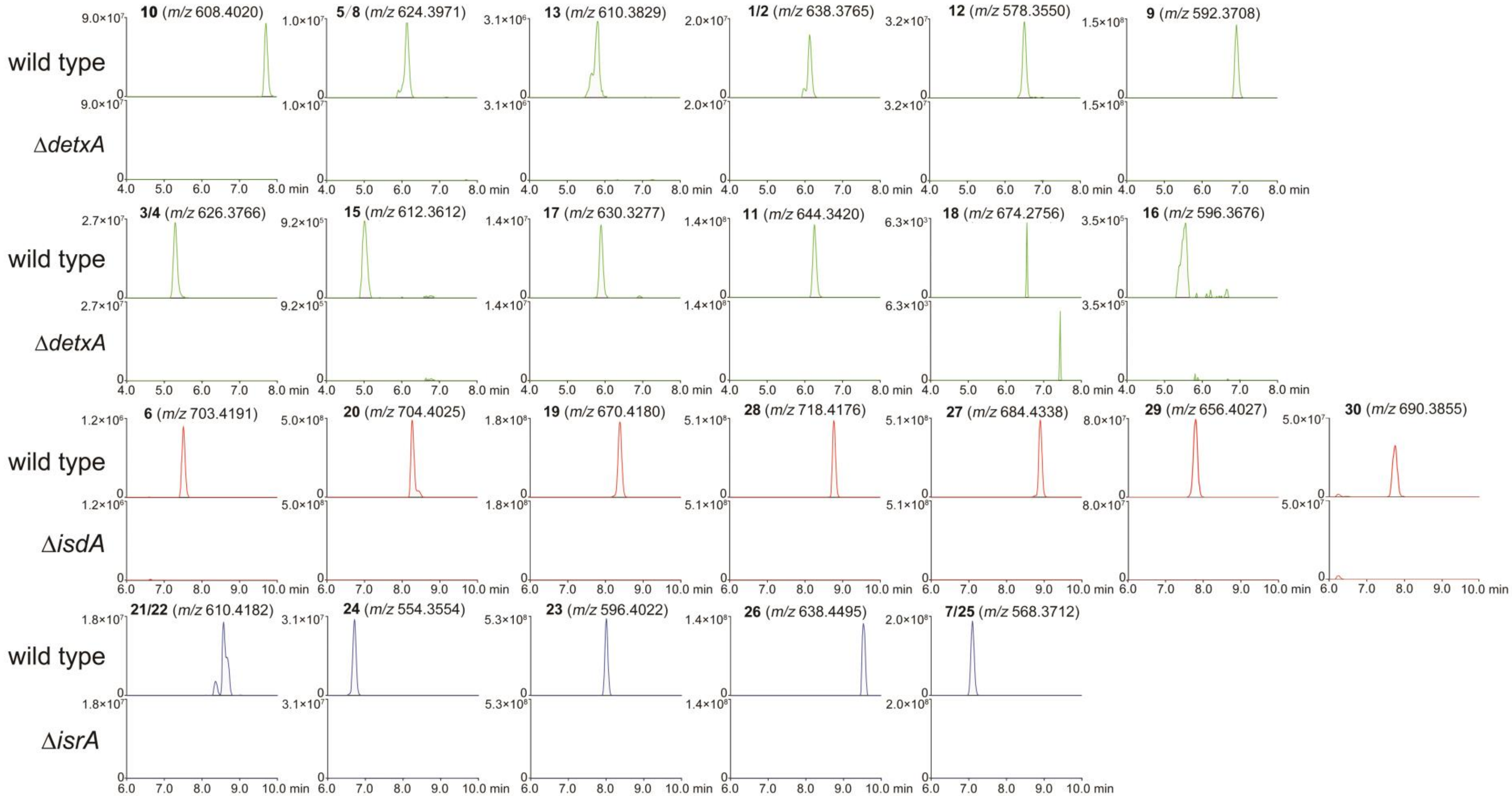


Figure S9. Proposed biogenetic oxidation of α-HIC.

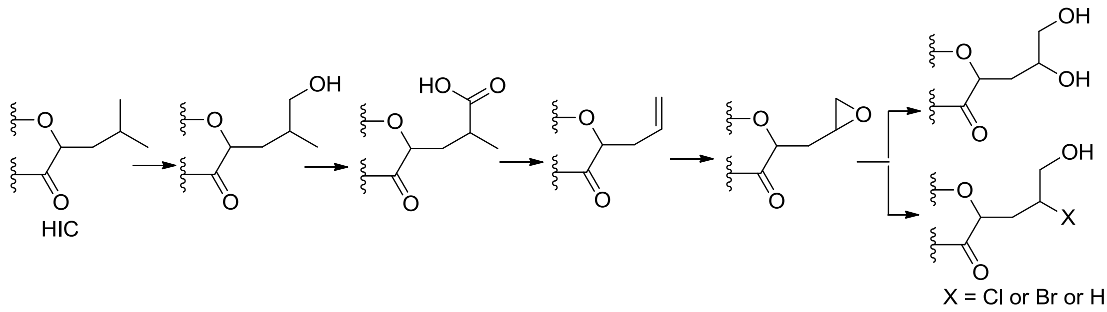


Figure S10. The extracted ion chromatogram (EIC) detection of compounds accumulated in Δ*detxE* mutant compared to wild-type strain.

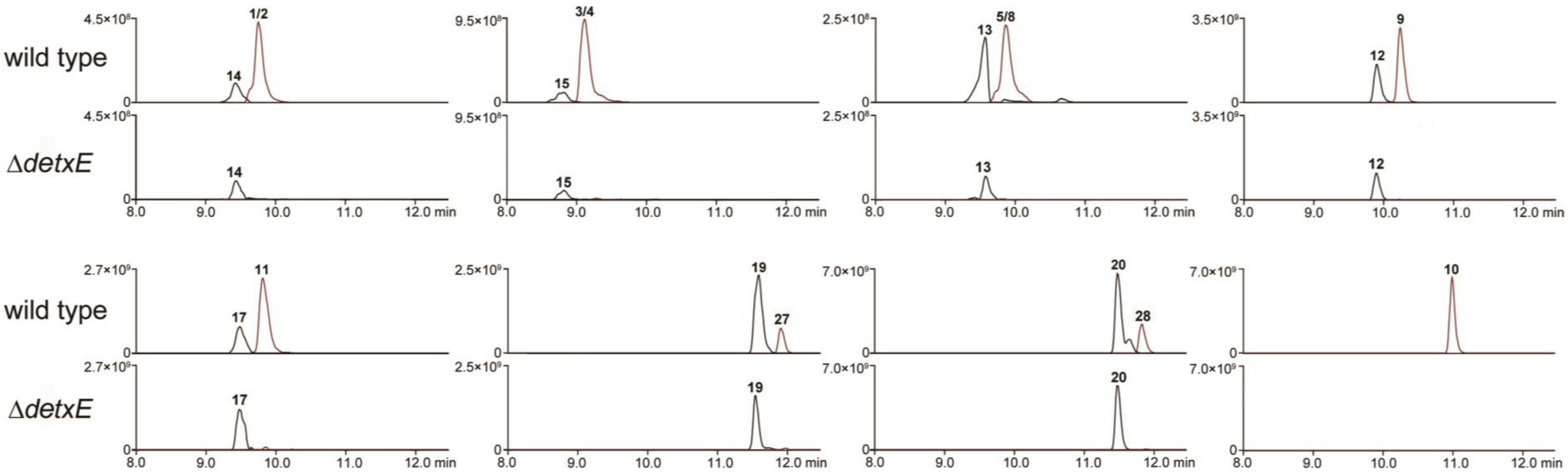


Figure S11. SDS-PAGE analysis of purified DetxE, SX-P5CR1 and SX-P5CR2.

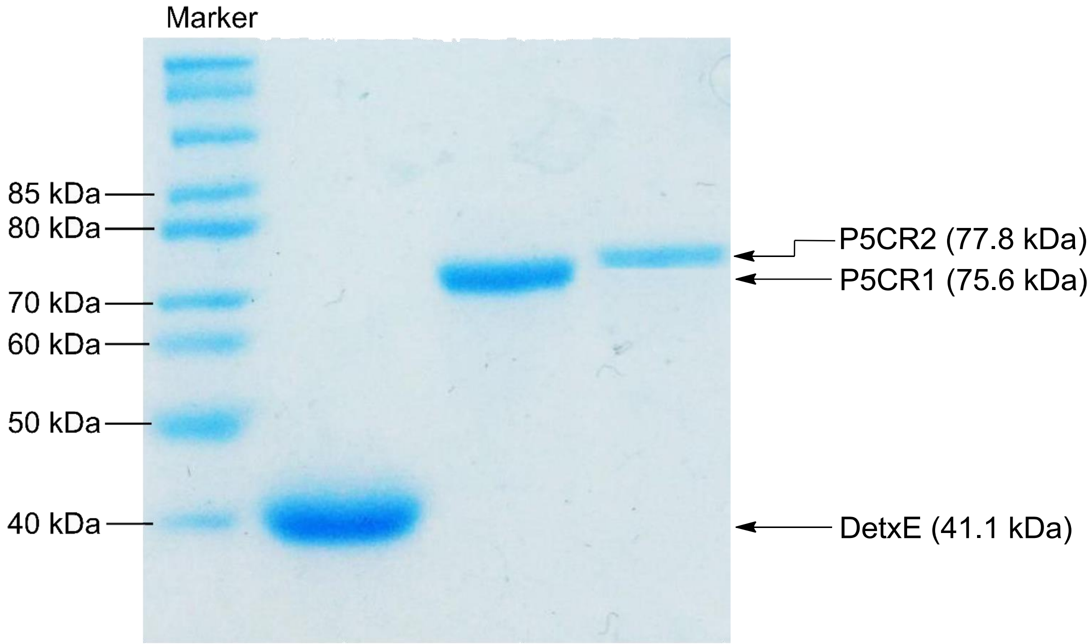


Figure S12. The UPLC profile of DetxE reaction with *o*-AB derivatization using L-isoleucine as the substrate.

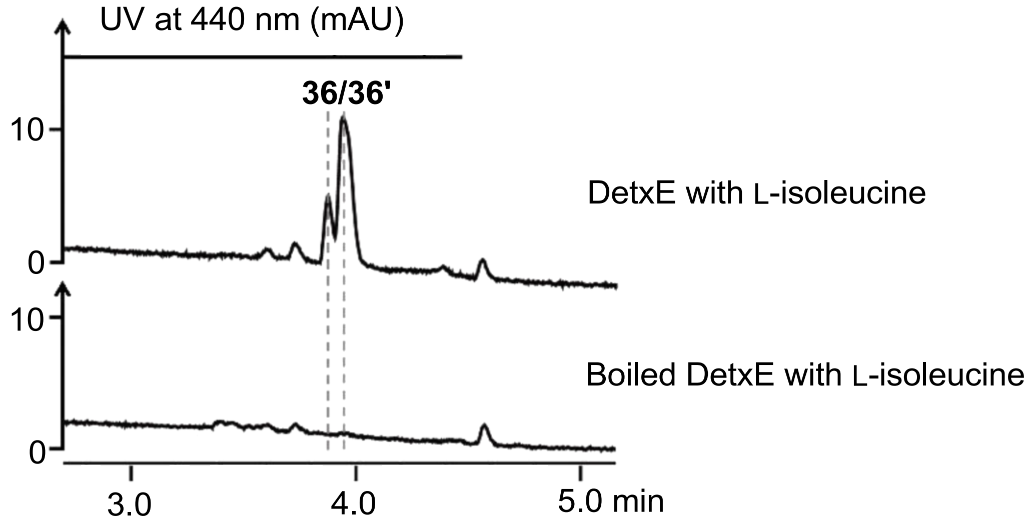


Figure S13. The HPLC profiles of DetxE reaction with Fmoc-Cl derivatization. Lane I, the reaction of DetxE with L-isoleucine as the substrate; lane II, the reaction of boiled DetxE with L-isoleucine as the substrate; lane III, the reaction of DetxE with D-isoleucine as the substrate; lane IV, the reaction of DetxE with L-*allo*-isoleucine as the substrate.

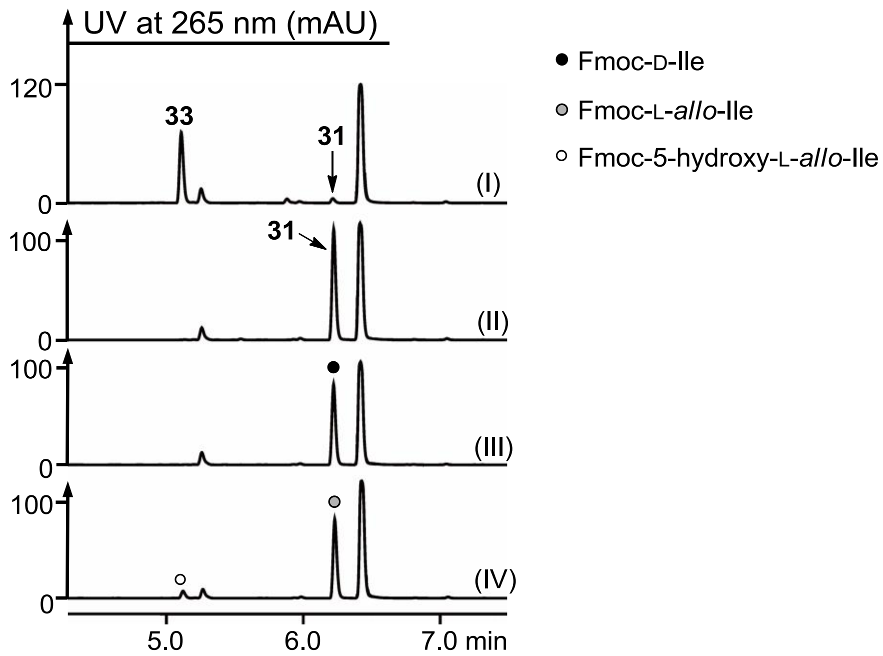


Figure S14. The EIC analysis of DetxE reactions without Fmoc-Cl derivatization. Lane I, the reaction of DetxE with L-isoleucine as the substrate; lane II, the reaction of boiled DetxE with L-isoleucine as the substrate; lane III, the reaction of DetxE with D-isoleucine as the substrate; lane IV, the reaction of DetxE with L-*allo*-isoleucine as the substrate. The *m/z* 148 ions were selected for the detection of 5-hydroxyisoleucine and *m/z* 132 ions were selected for the detection of L-isoleucine, D-isoleucine and L-*allo*-isoleucine.

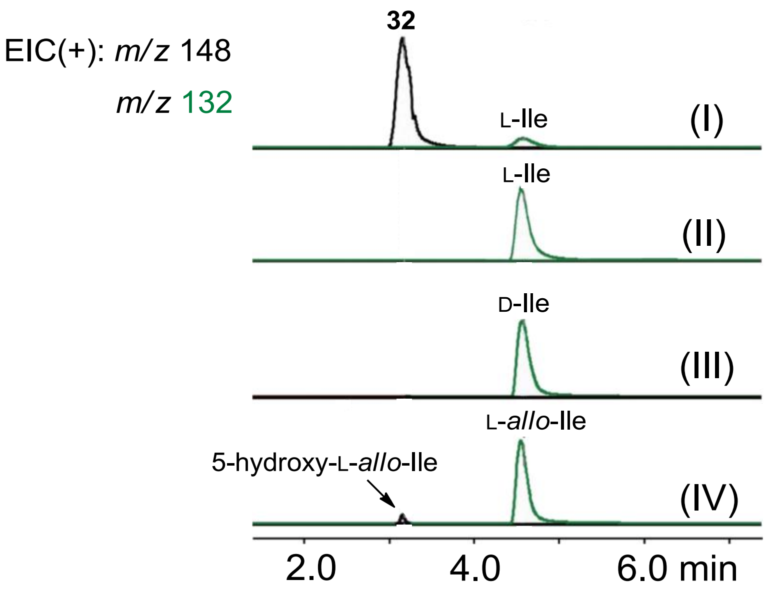


Figure S15. The biosynthetic process of (4*R*)-methyl-L-proline reported in previous literature.[8]

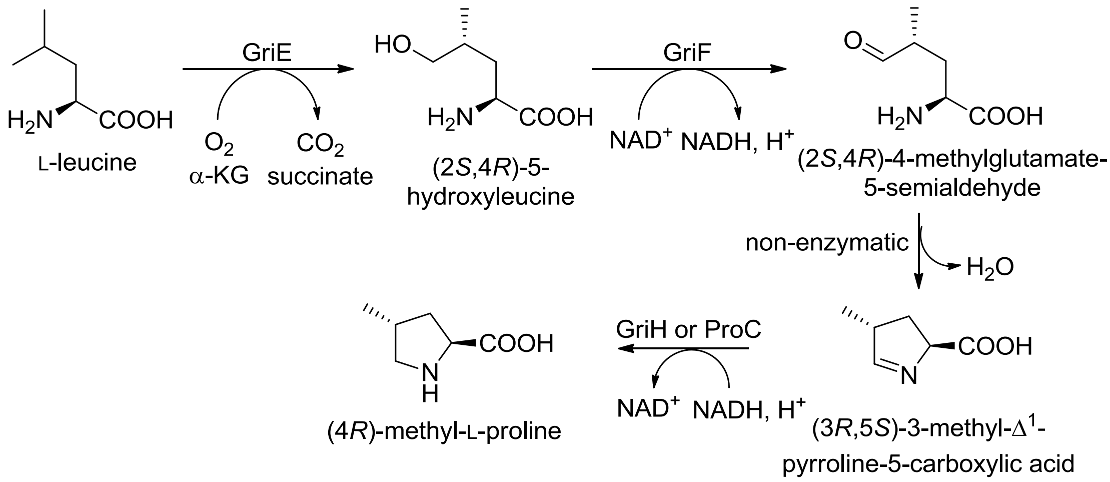


Figure S16. The EIC anaysis of DetxE combined with P5CR1/P5CR2 reactions, using L-isoleucine as the substrate and derivatized with Fmoc-Cl. Lane I, standare L-isoleucine; lane II, standard (3*S*)-methyl-L-proline; lane III, the reaction of DetxE; lane IV, the reaction of DetxE with SX-P5CR1 and NADH; lane V, the reaction of DetxE with SX-P5CR1 and NADPH; lane VI, the reaction of DetxE with SX-P5CR2 and NADH; lane VII, the reaction of DetxE with SX-P5CR2 and NADPH; lane VIII, the reaction of boiled DetxE with both SX-P5CR1 and SX-P5CR2 added. The peaks (*m/z* 352) labelled with asterisk represent the in-situ dehydration product of **33** generated in the ESIMS experiments.

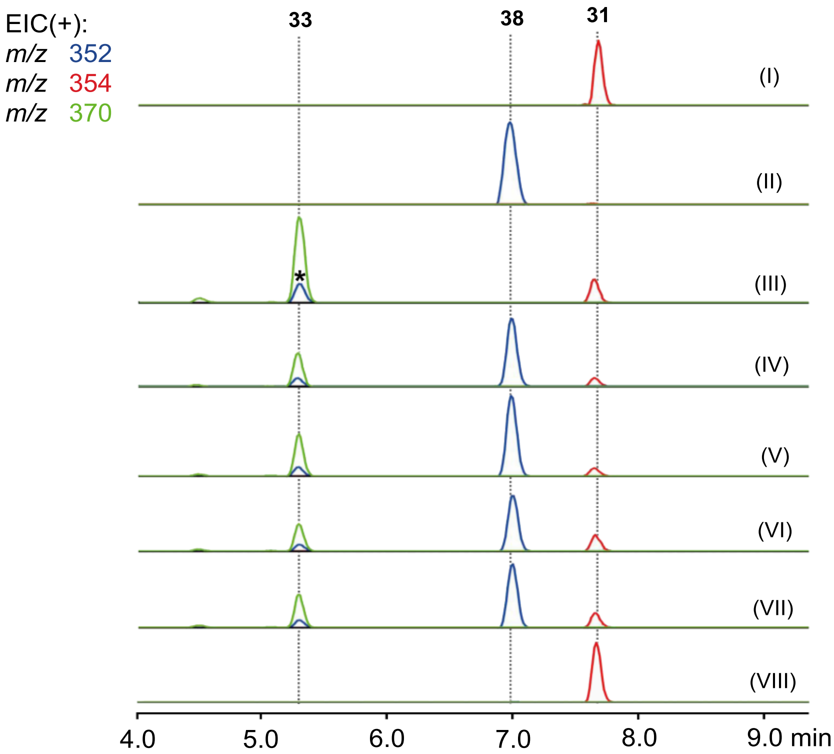
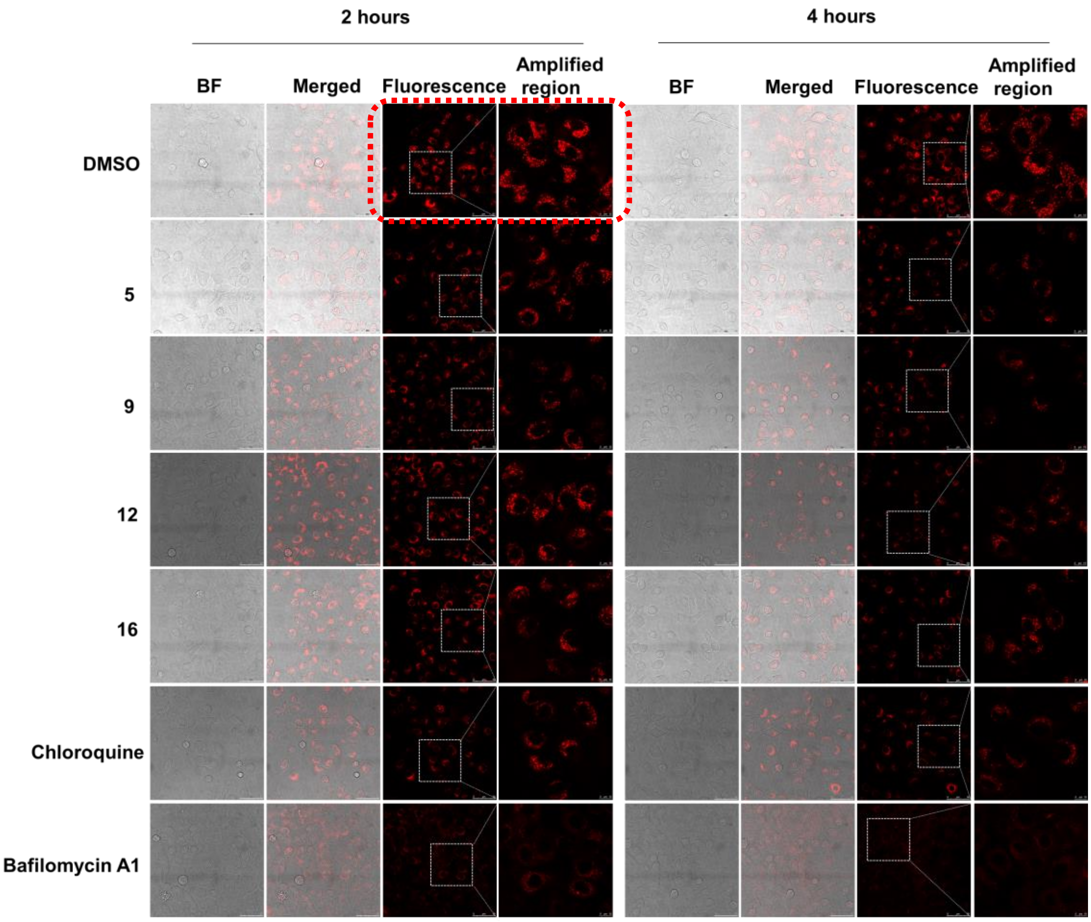
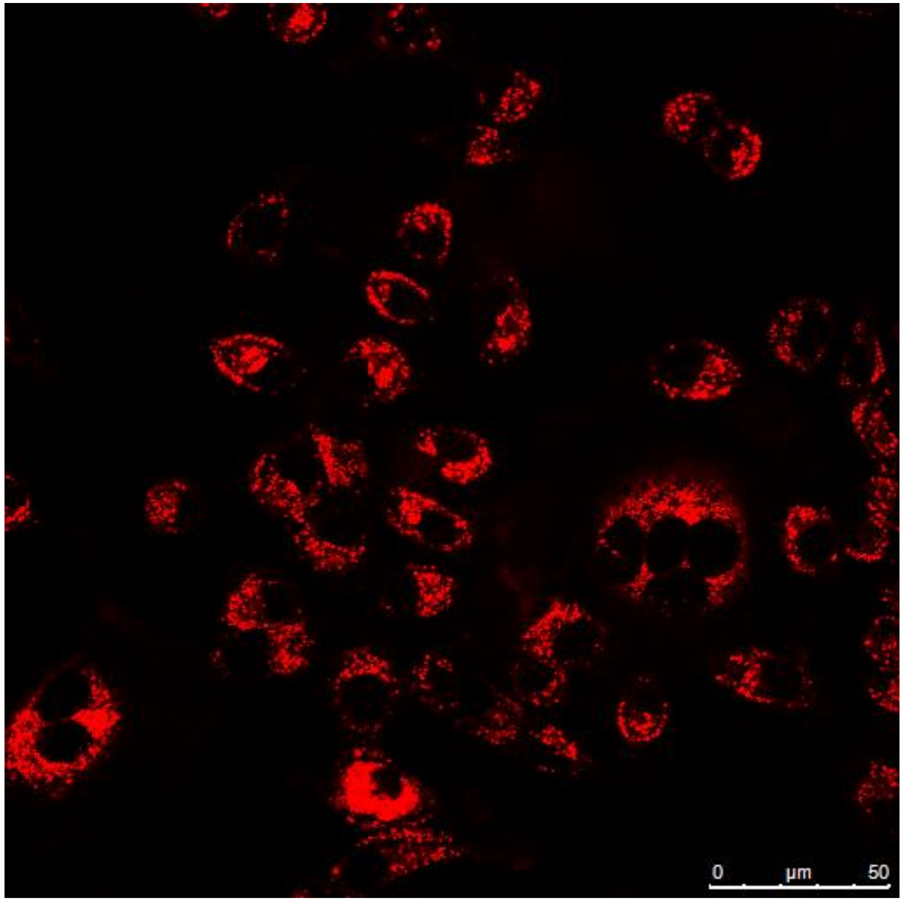


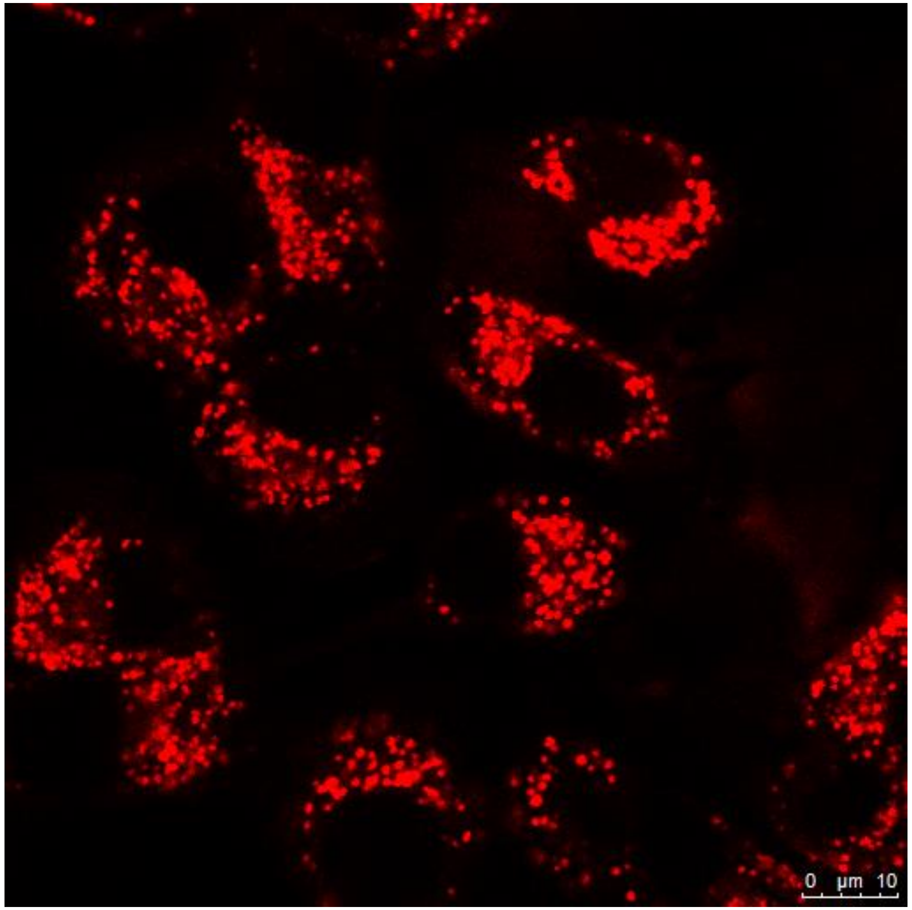
Figure S17. Detection of lysosome acidification of A549 cells and the uncropped NS5 production inhibition images. (A) Detection of lysosome acidification of A549 cells treated by compounds **5**, **9**, **10** and **16**. Two inhibitors chloroquine and bafilomycin were used as positive control and DMSO was used as negative control. Fluorescence was observed by confocal microscopy using a 60× objective lens. "BF" represents the view taken for cells in brightfield, "Fluorescence" represents the view of Lysotracker Red fluorescence, and "Merged" represents the view with BF merged with fluorescence. The images of "BF", "Merged" and "Fluorescence" have the same scale bars; and the images of "Amplified region" have the same scale bars. (B) The amplified presentation of the two images in the red dashed box in (A), showing the scale bars with measurement units. (C) and (D) The uncropped NS5 production inhibition images (corresponding to Figure 3B and 3C).

A



B





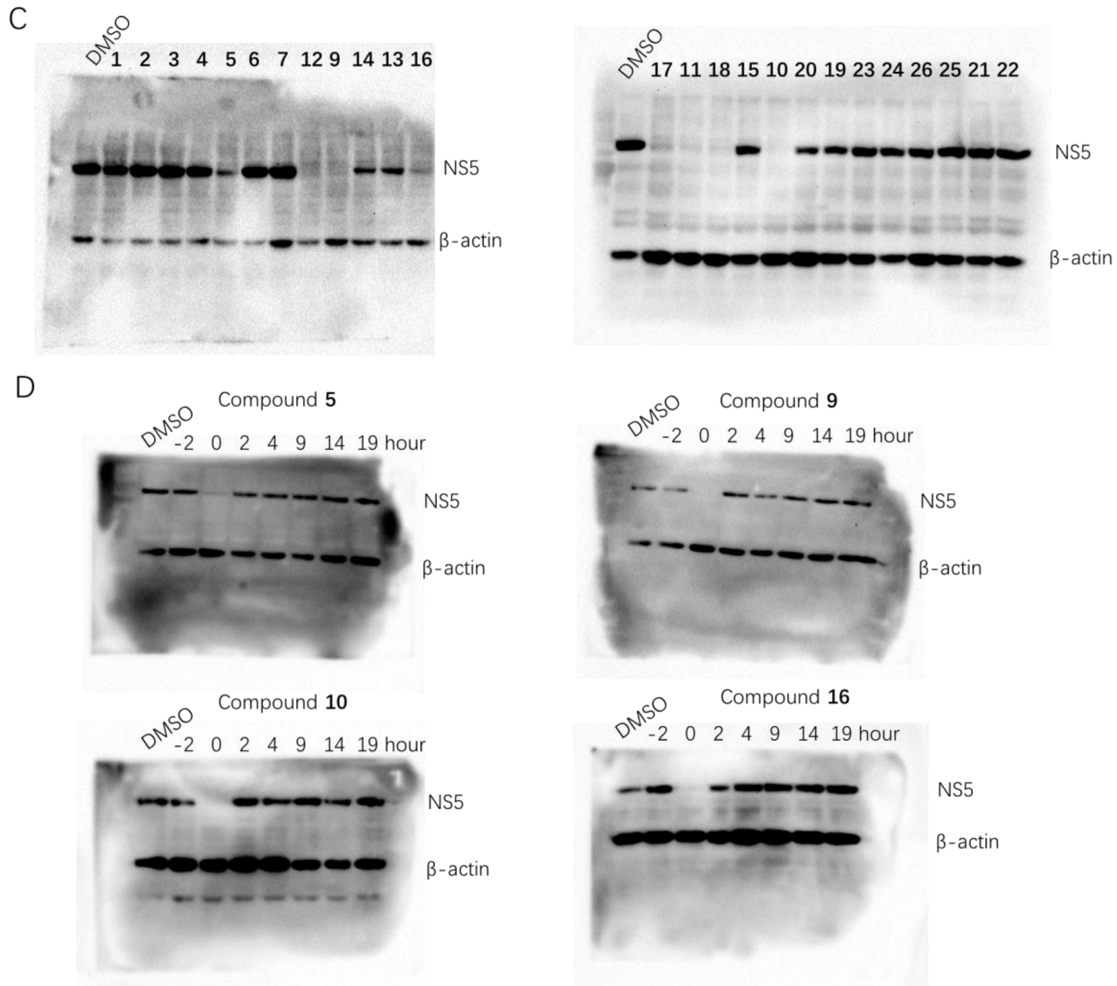

C
DMSO
1 2 3 4 5 6 7 12 9 14 13 16
NS5
β-actin
DMSO
17 11 18 15 10 20 19 23 24 26 25 21 22
NS5
β-actin
D
Compound 5
DMSO
-2 0 2 4 9 14 19 hour
NS5
β-actin
Compound 9
DMSO
-2 0 2 4 9 14 19 hour
NS5
β-actin
Compound 10
DMSO
-2 0 2 4 9 14 19 hour
NS5
β-actin
Compound 16
DMSO
-2 0 2 4 9 14 19 hour
NS5
β-actin

Figure S18. ^{1}H NMR spectrum of **1** (600 MHz, DMSO-d_6).

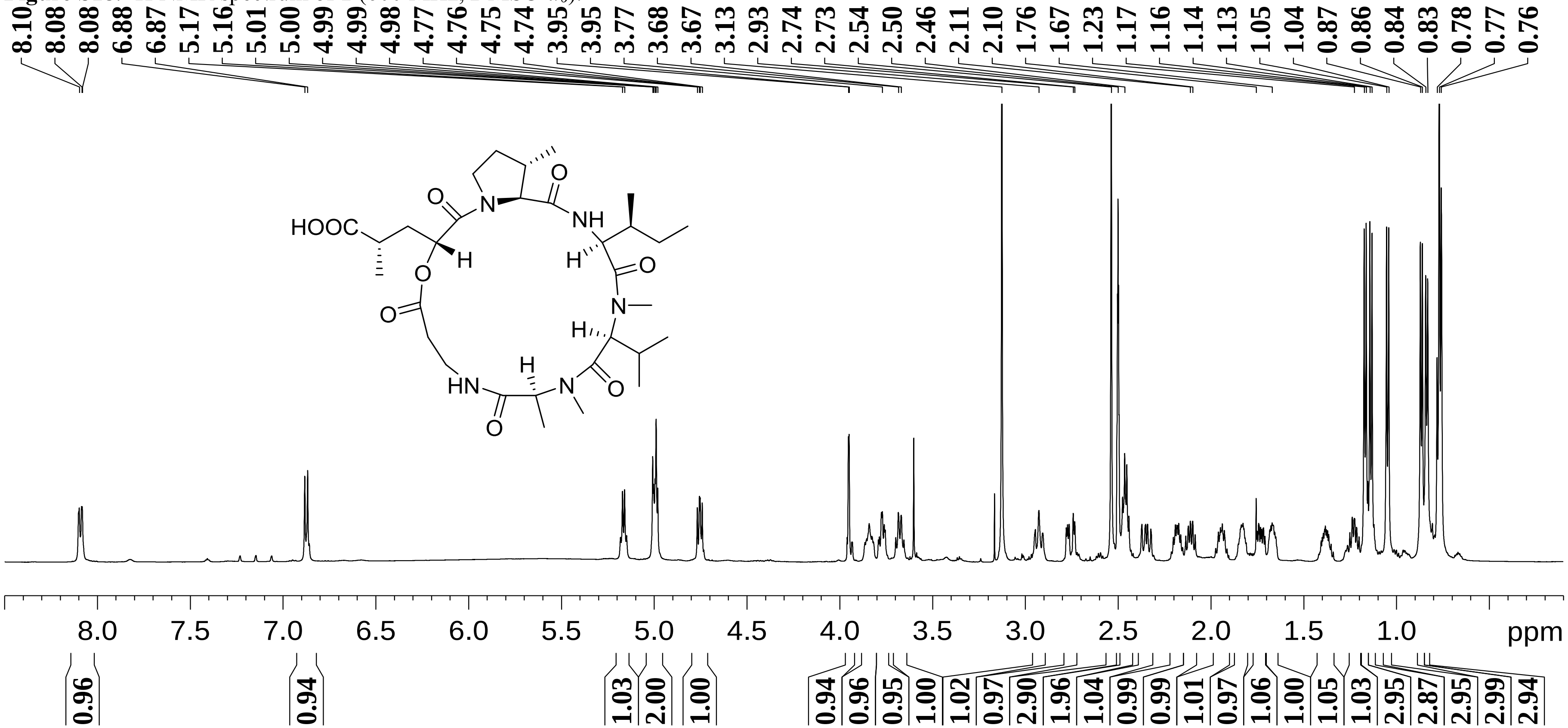


Figure S19. ^{13}C APT spectrum of compound **1** (600 MHz, DMSO-d_6) .

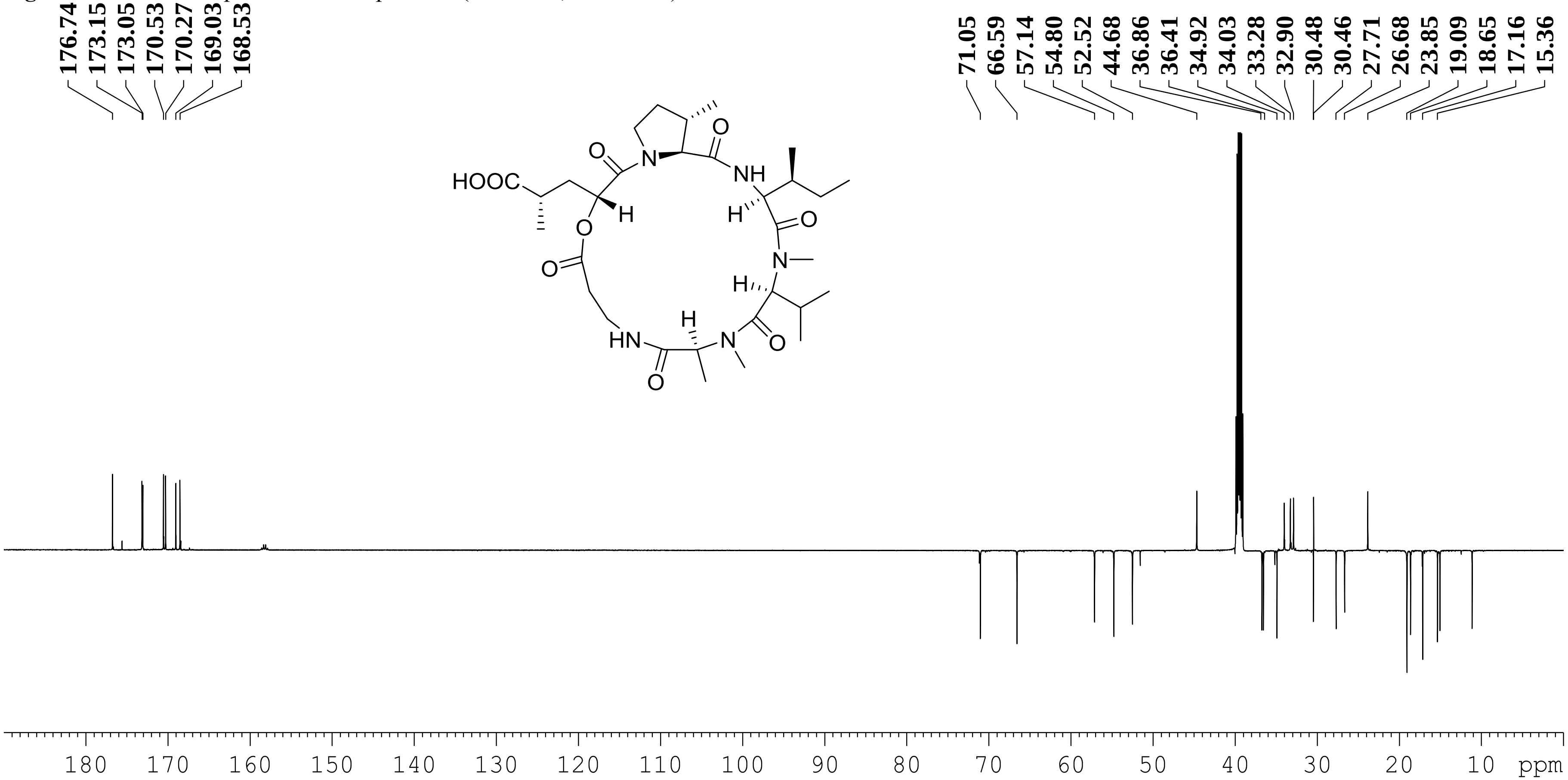


Figure S20. ^{1}H-^{1}H COSY spectrum of compound **1**.

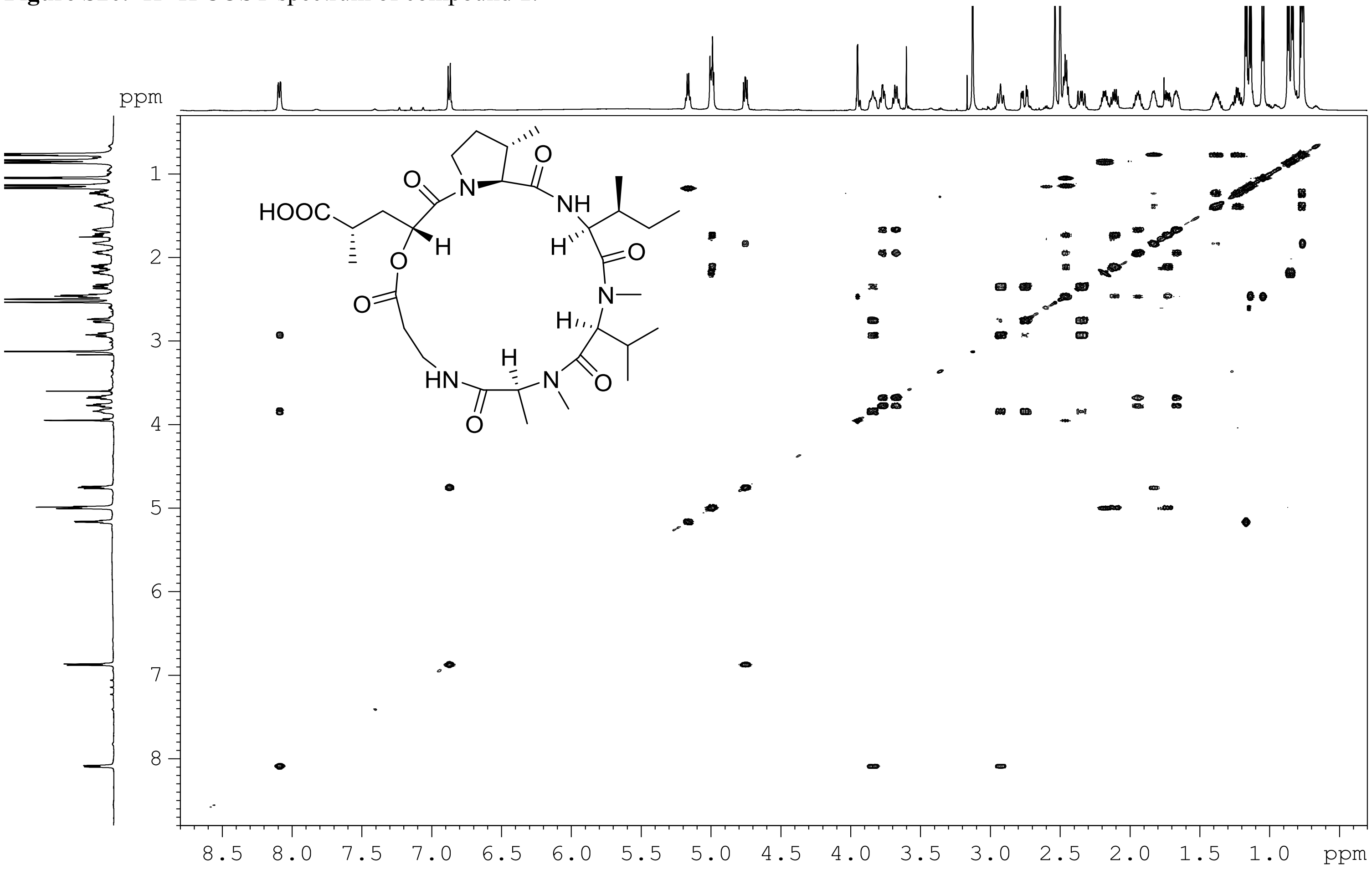


Figure S21. HSQC spectrum of compound **1**.

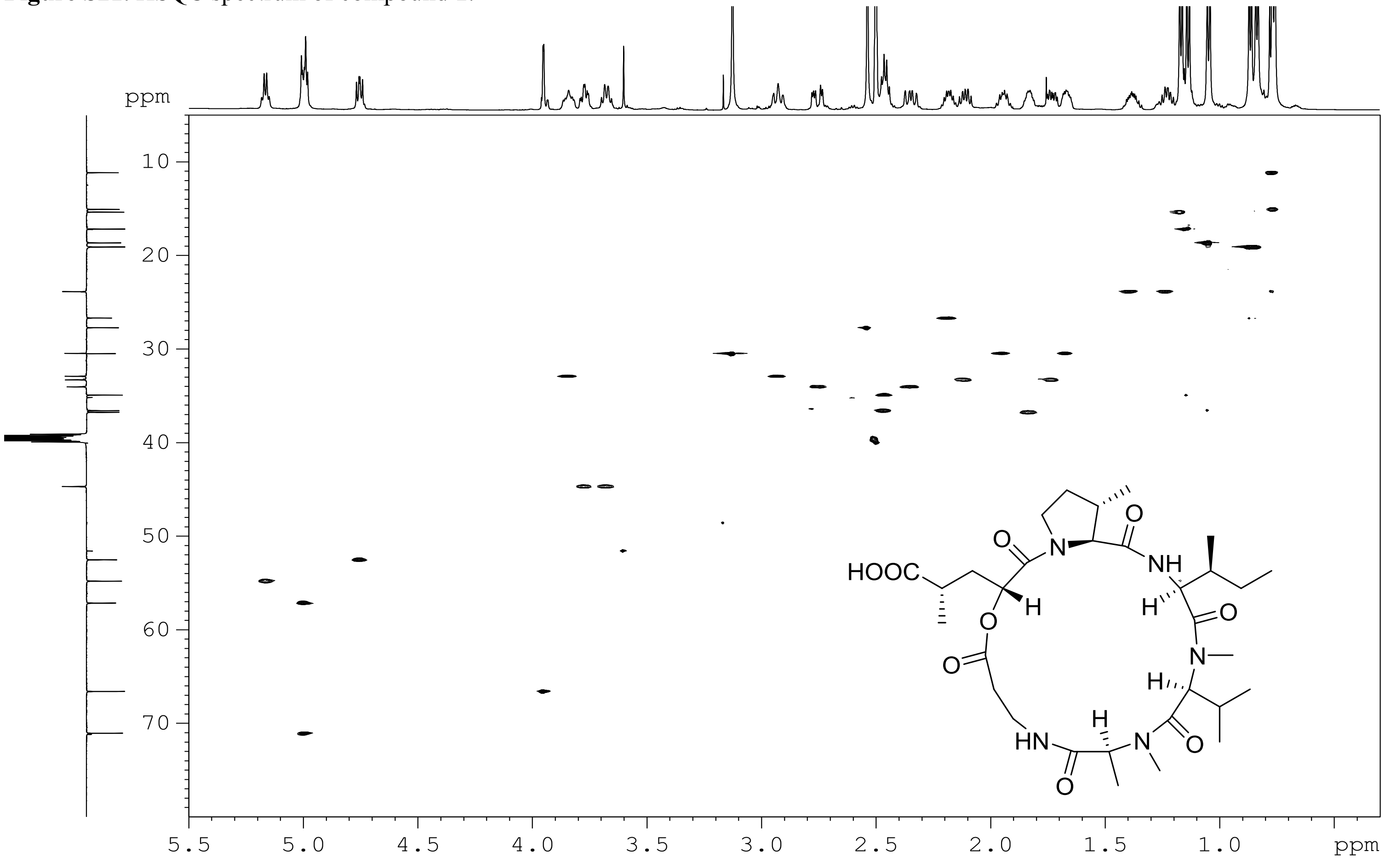


Figure S22. HMBC spectrum of compound **1**.

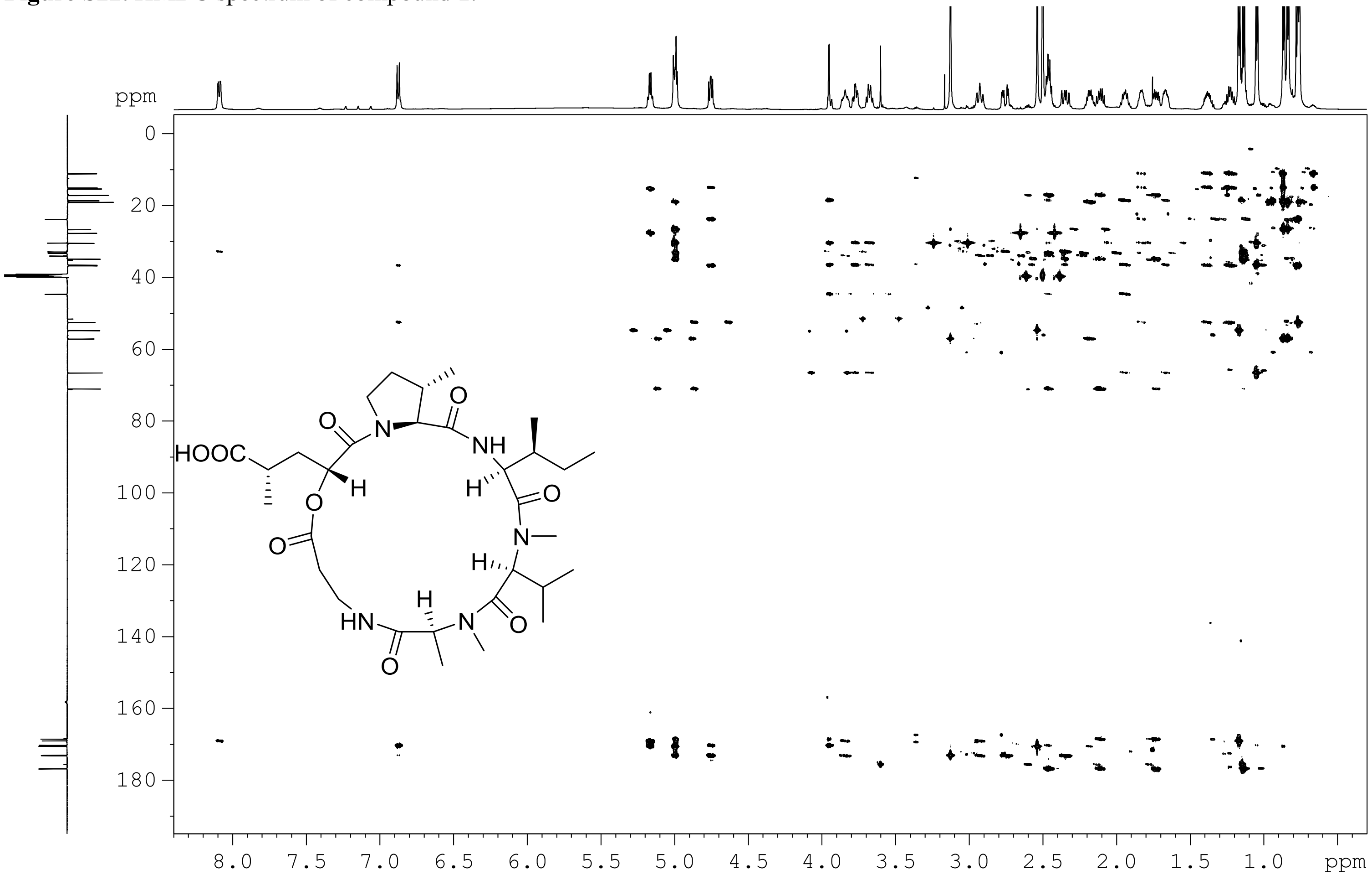


Figure S23. NOESY spectrum of compound **1**.

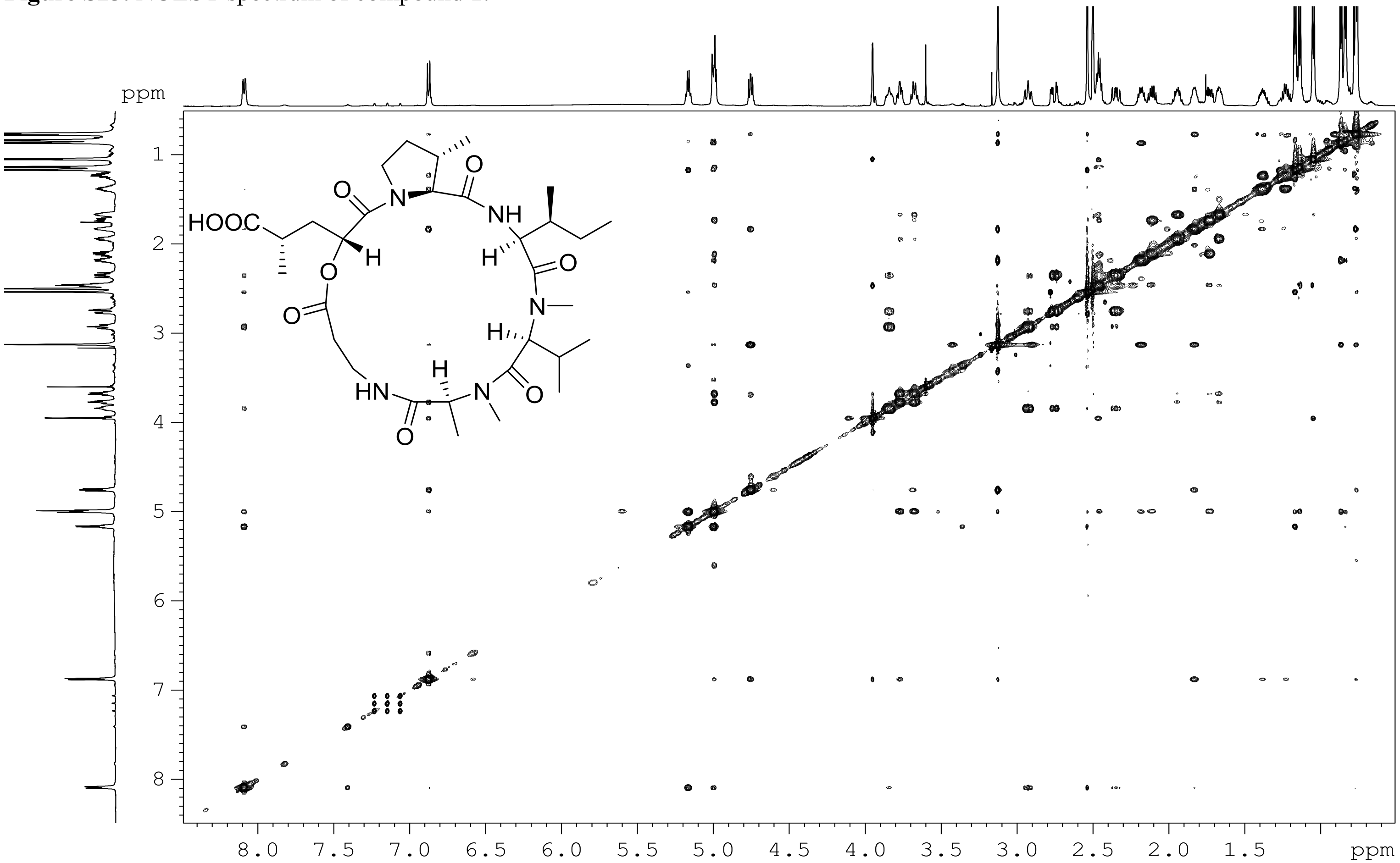


Figure S24. IR of compound **1**.

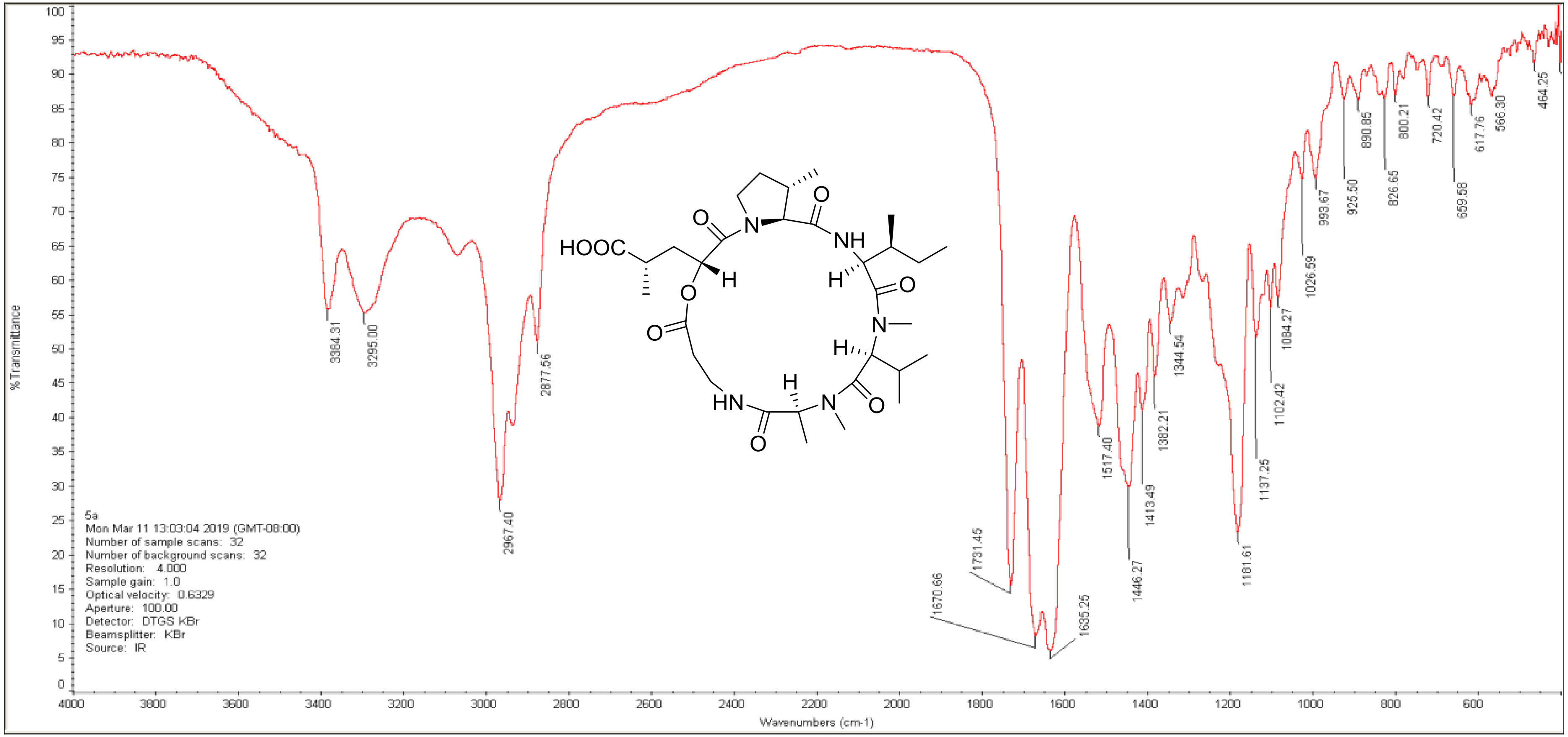
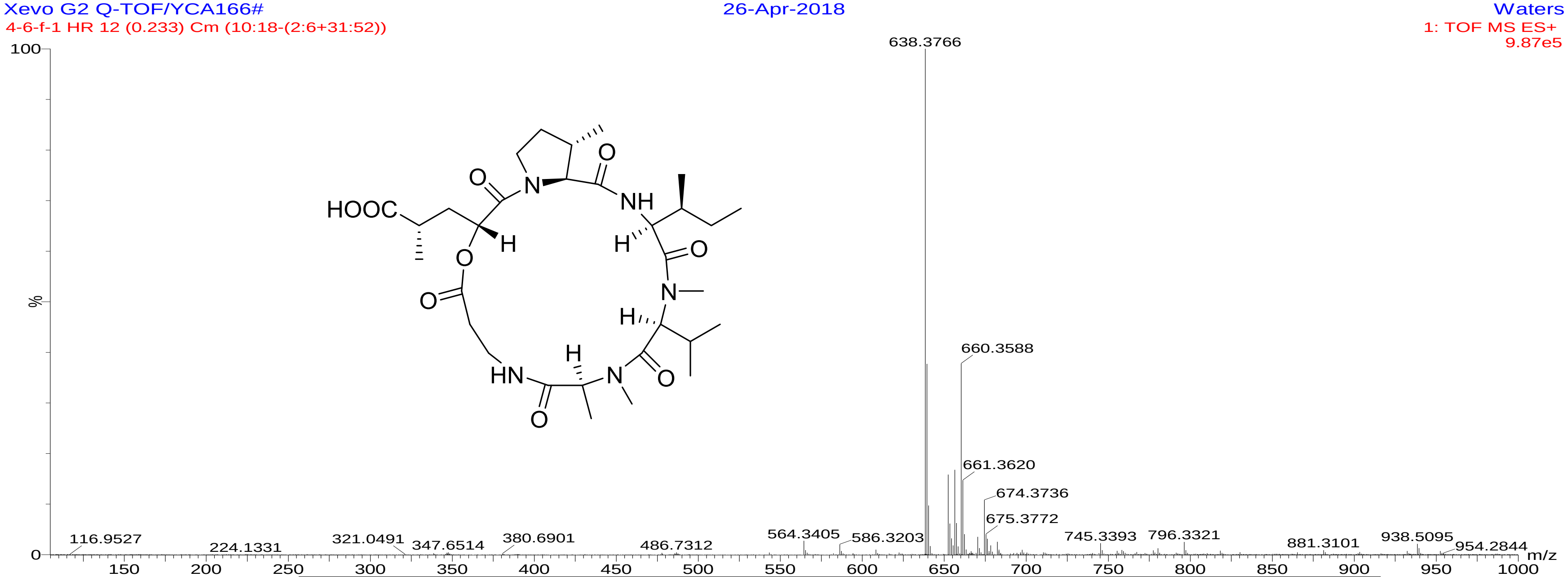


Figure S25. HRESIMS of compound **1**.



Mass	Calc.Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
638.3766	638.3765	0.1	0.2	8.5	325.1	0.484	61.61	$C_{31}H_{52}N_5O_9$

Figure S26. [1]H NMR spectrum of compound **2** (600 MHz, DMSO-d_6).

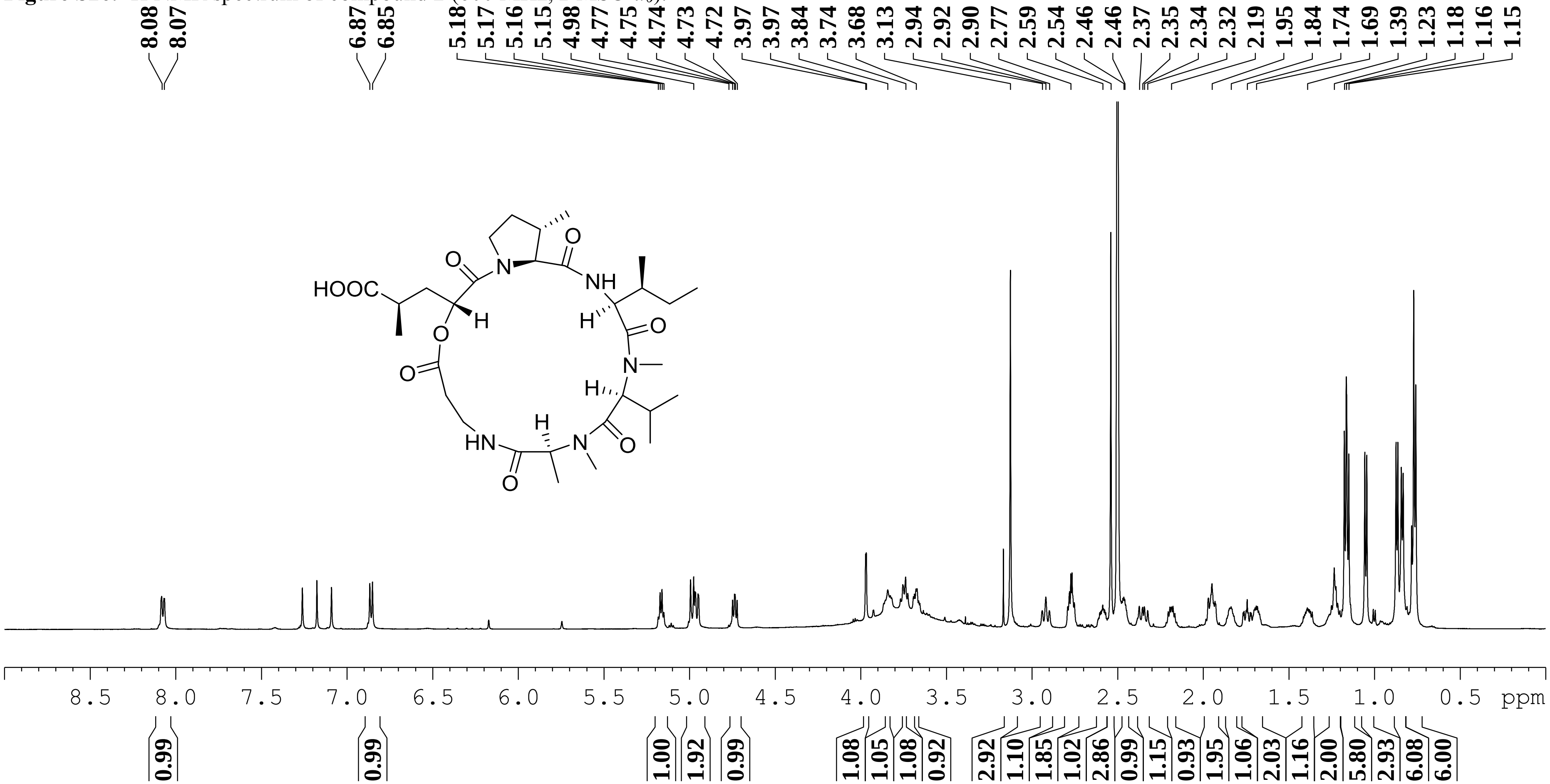


Figure S27. ^{13}C APT spectrum of compound **2** (600 MHz, DMSO-d_6) .

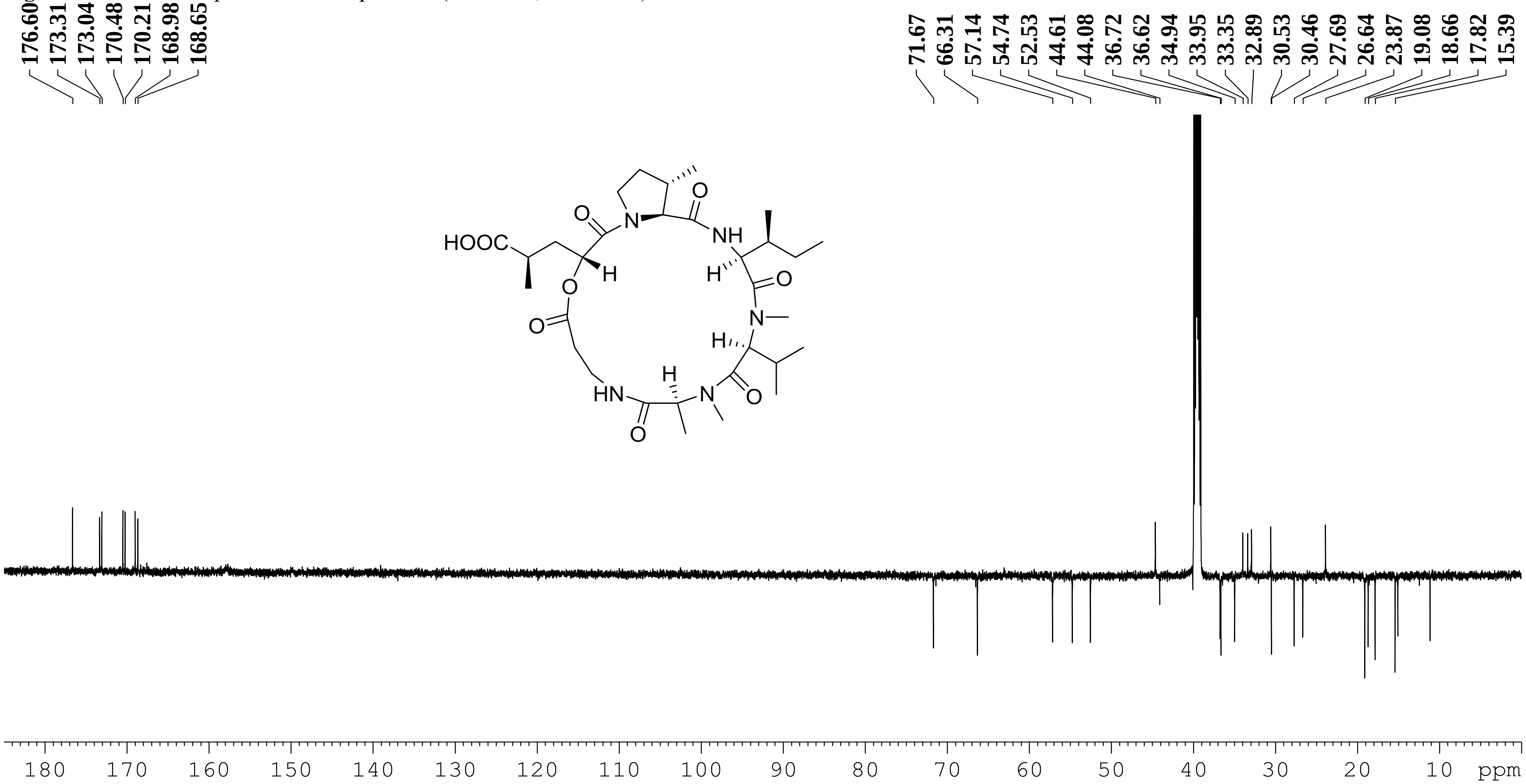


Figure S28. ¹H-¹H COSY spectrum of compound **2**.

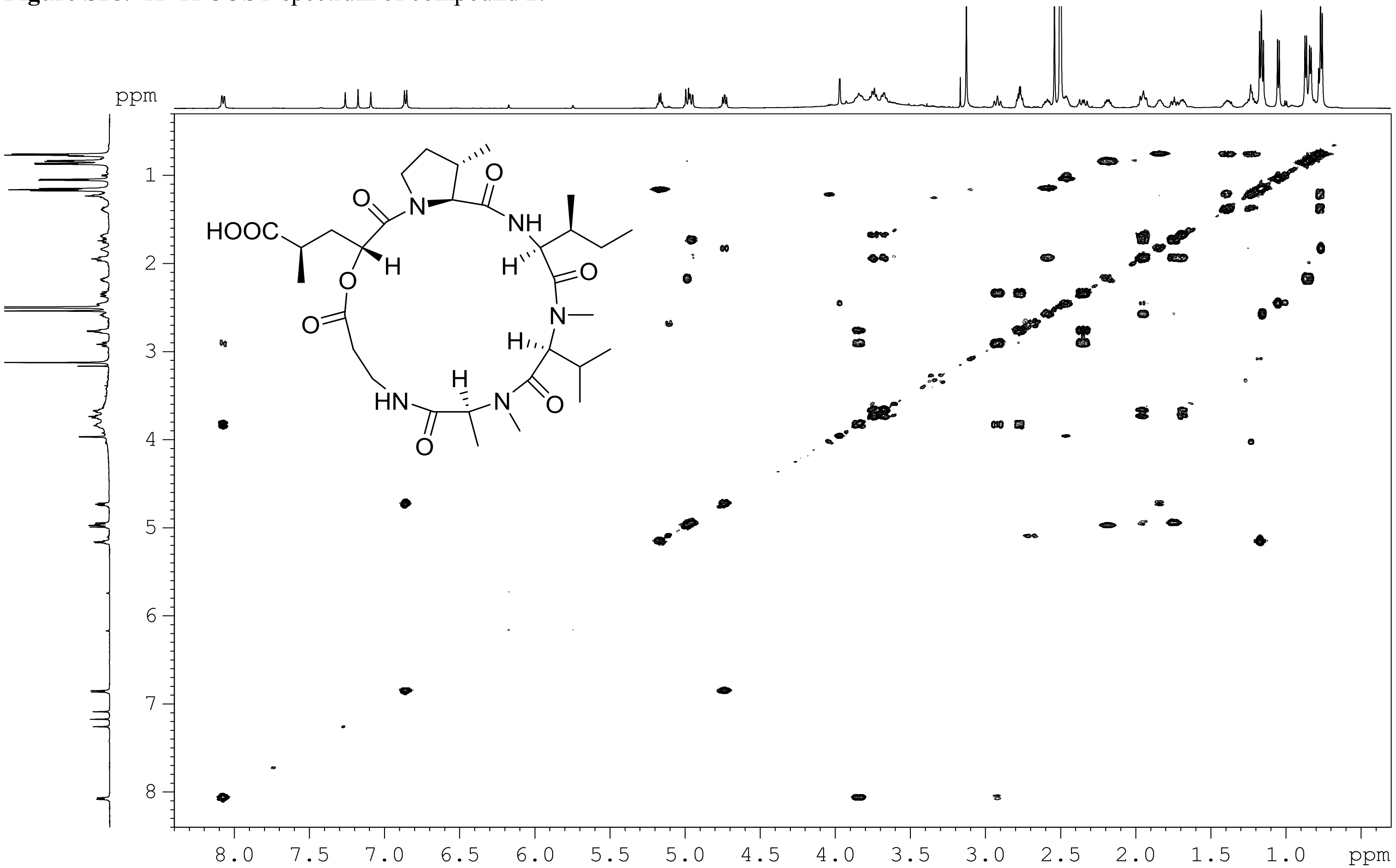


Figure S29. HSQC spectrum of compound **2**.

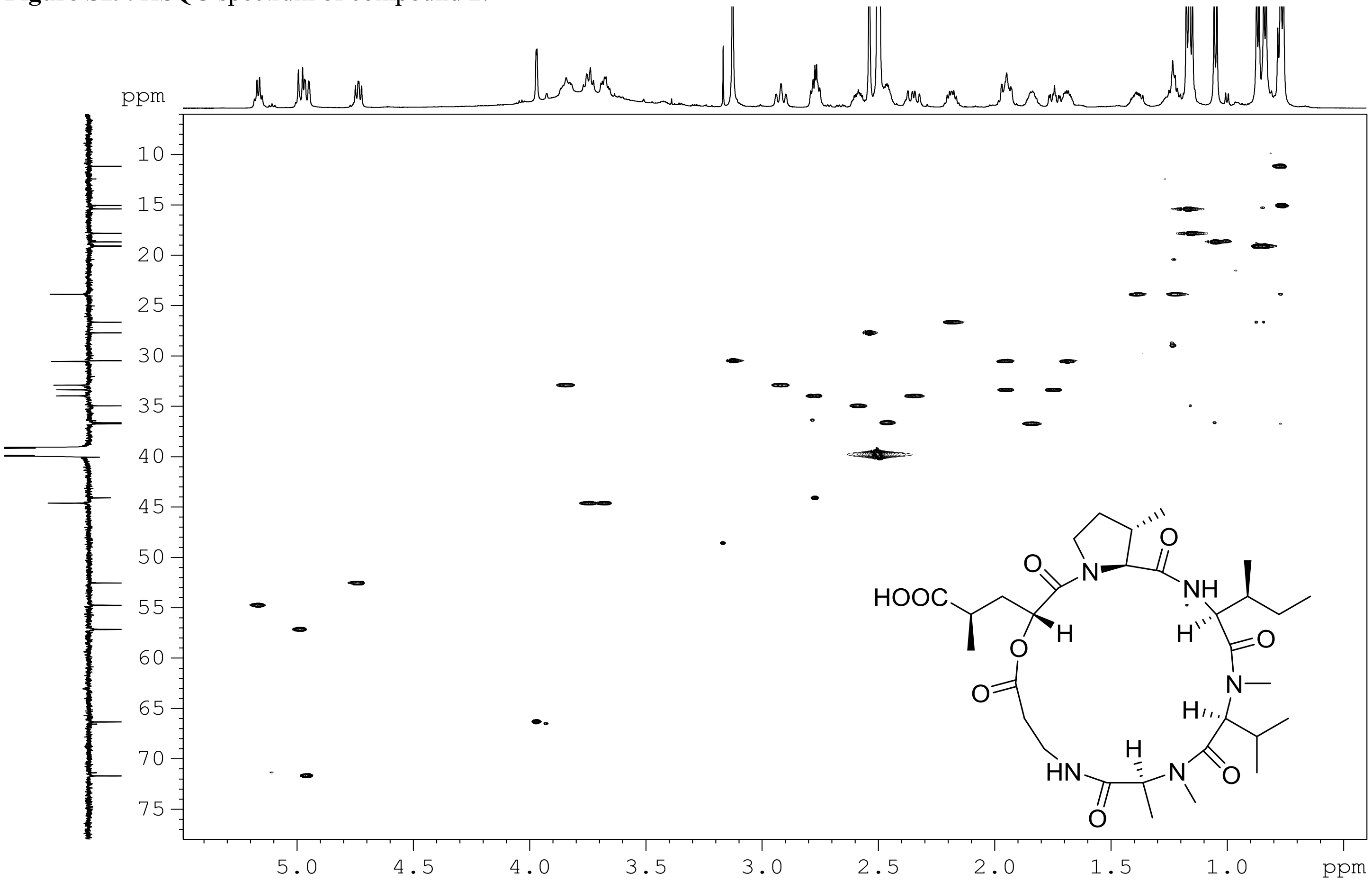


Figure S30. HMBC spectrum of compound **2**.

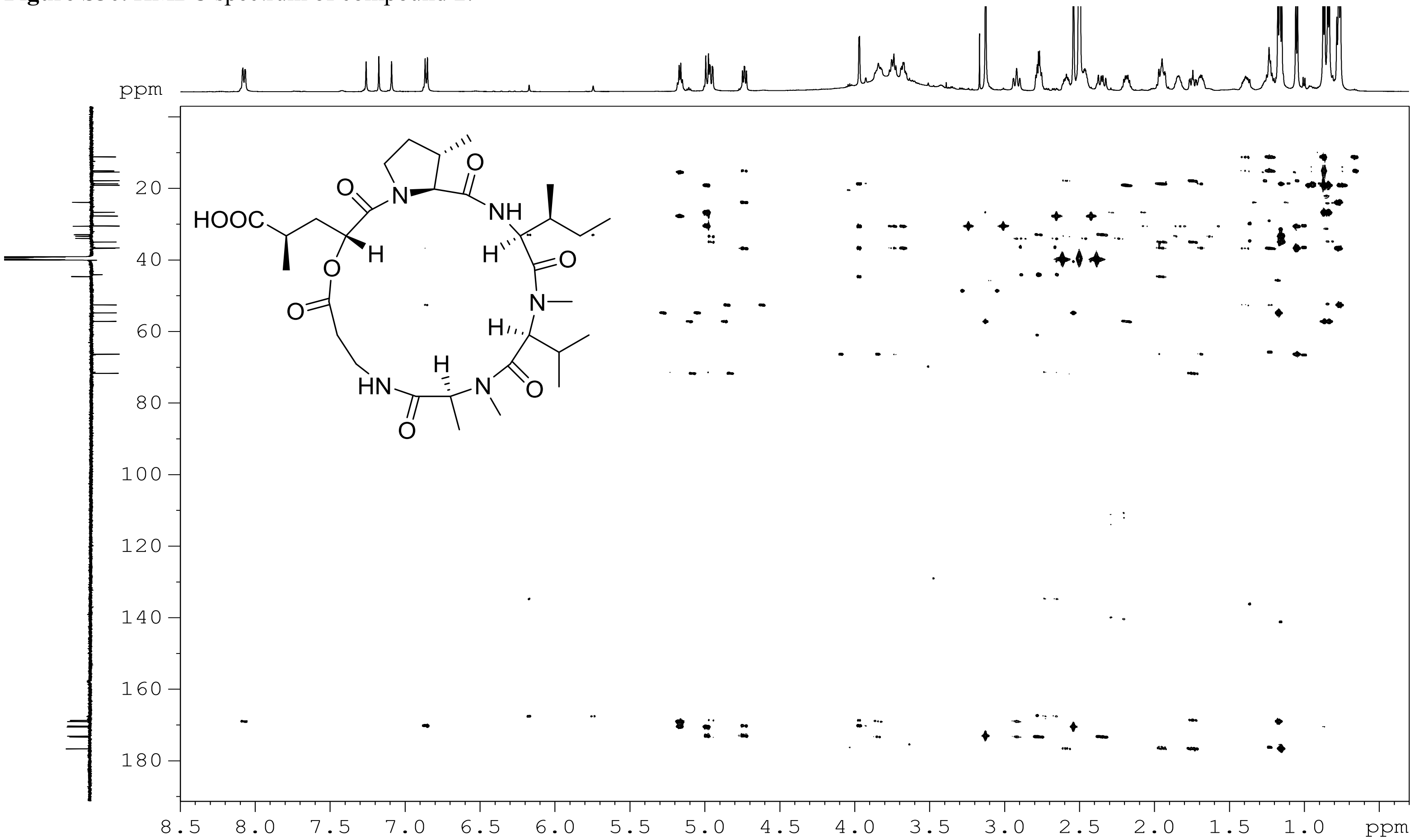


Figure S31. NOESY spectrum of compound **2**.

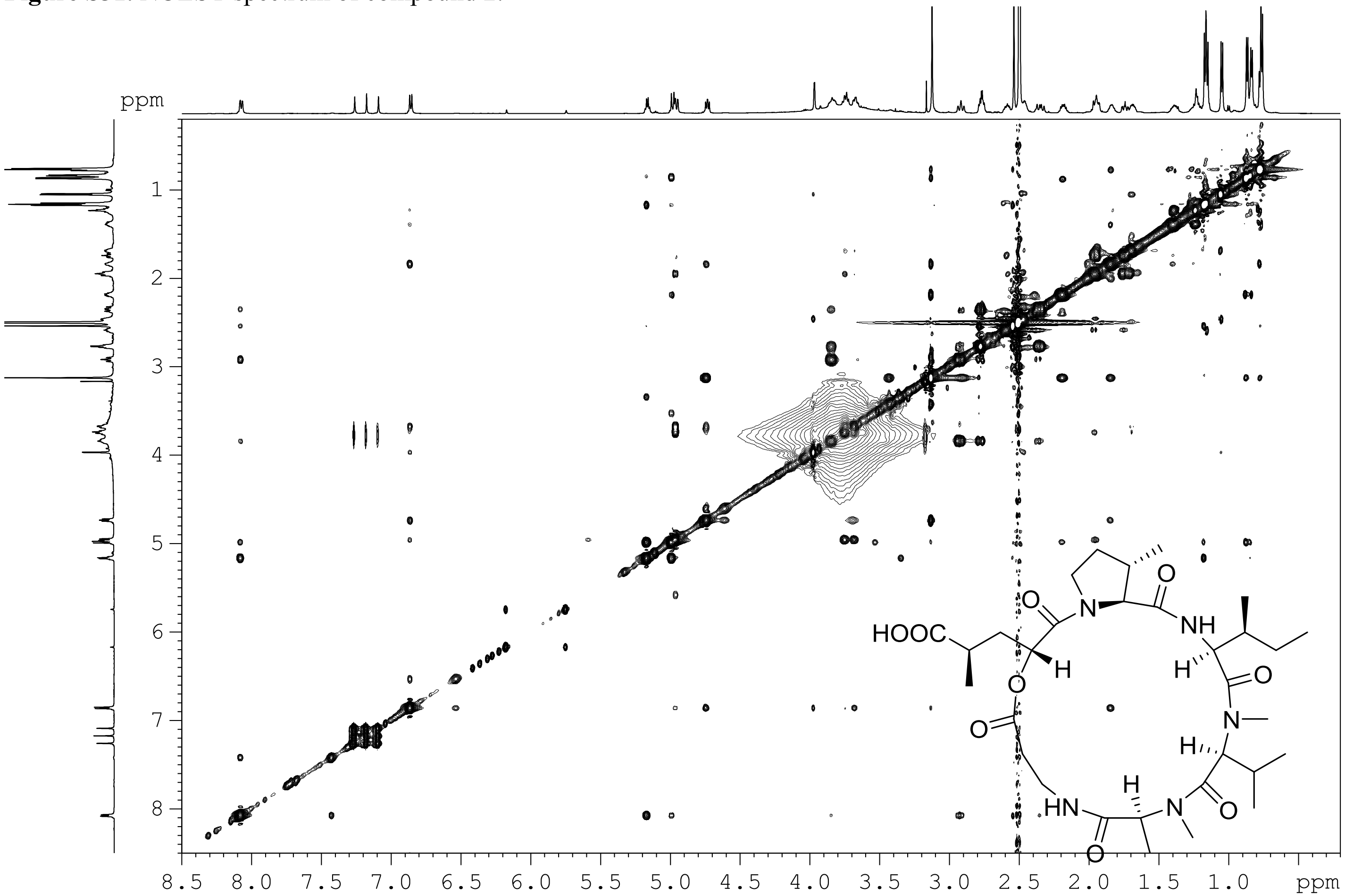


Figure S32. IR of compound **2**.

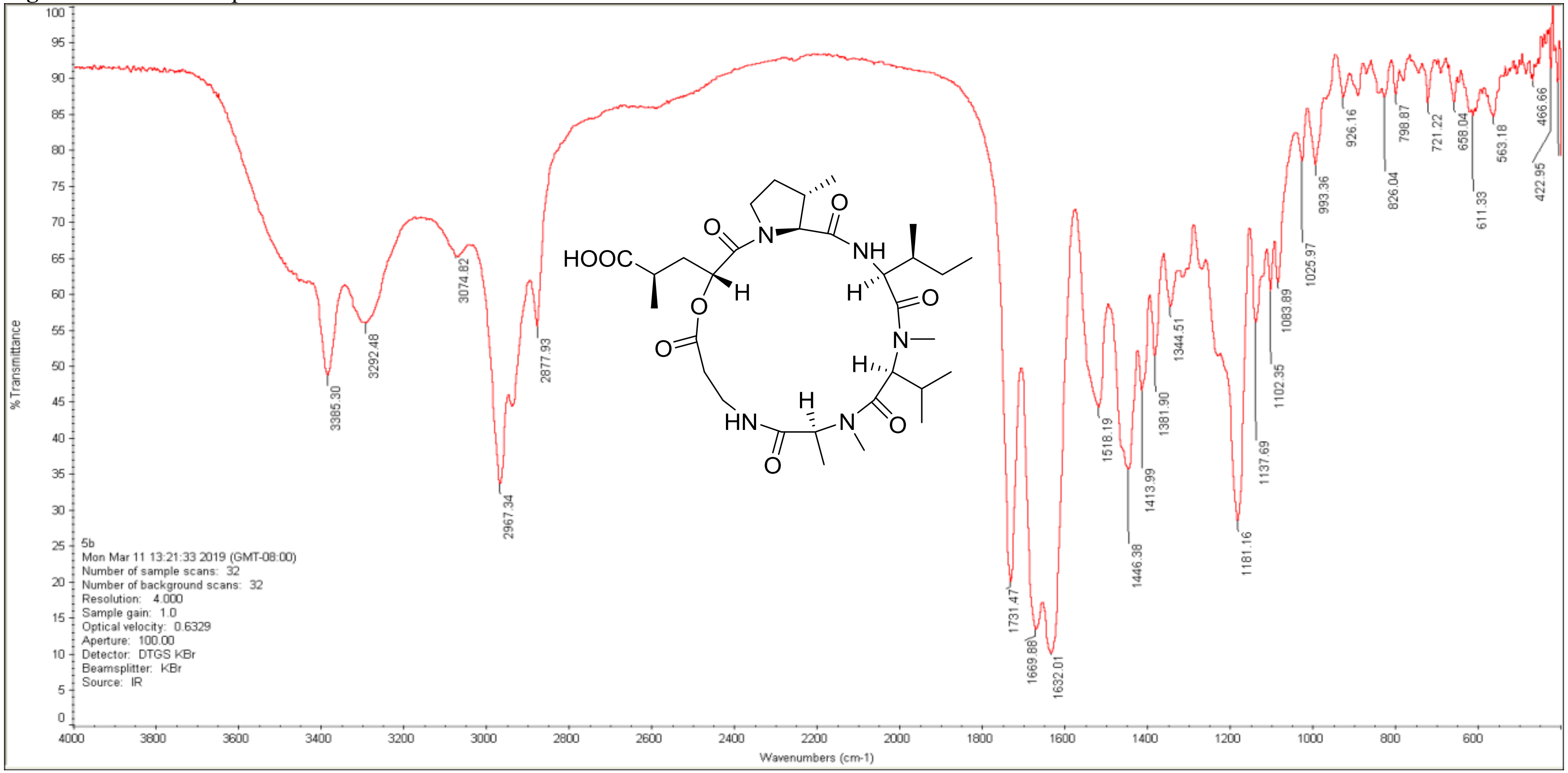
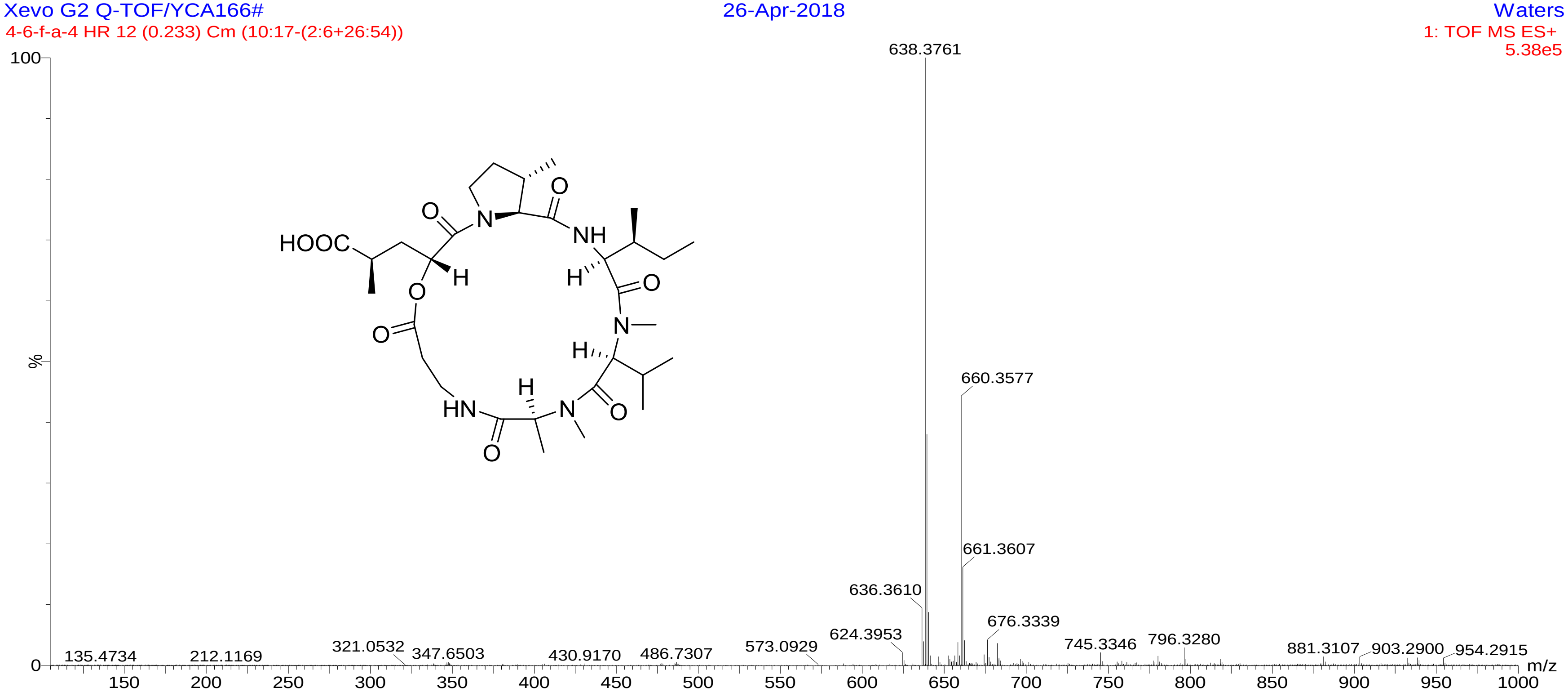


Figure S33. HRESIMS of compound **2**.



Mass	Calc.Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
638.3761	638.3765	-0.4	-0.6	8.5	193.7	1.062	64.57	$C_{31}H_{52}N_5O_9$

Figure S34. ^{1}H NMR spectrum of compound **3** (600 MHz, DMSO-d_6).

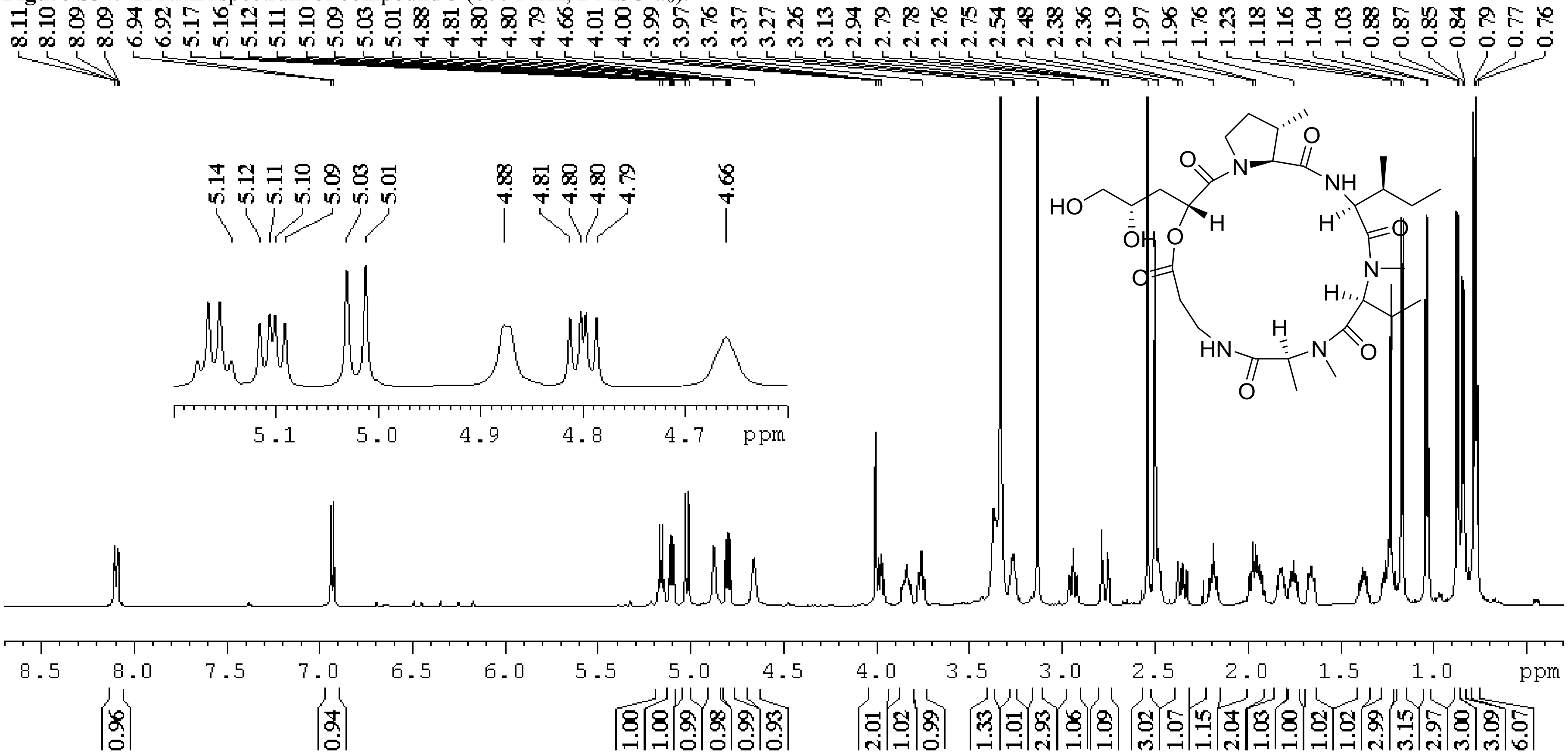


Figure S35. ^{13}C APT spectrum of compound **3** (600 MHz, DMSO-d_6).

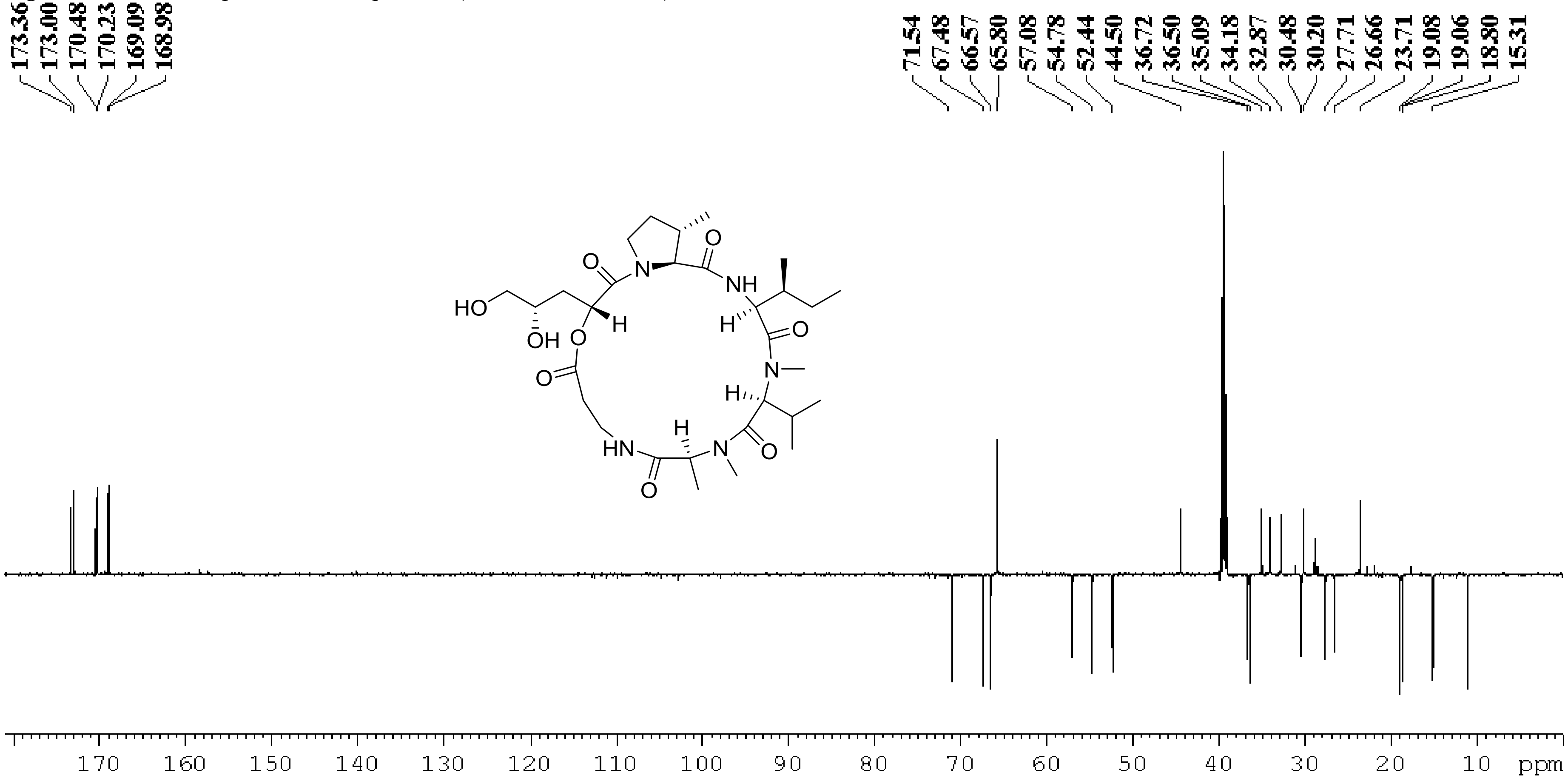


Figure S36. ^{1}H-^{1}H COSY spectrum of compound **3**.

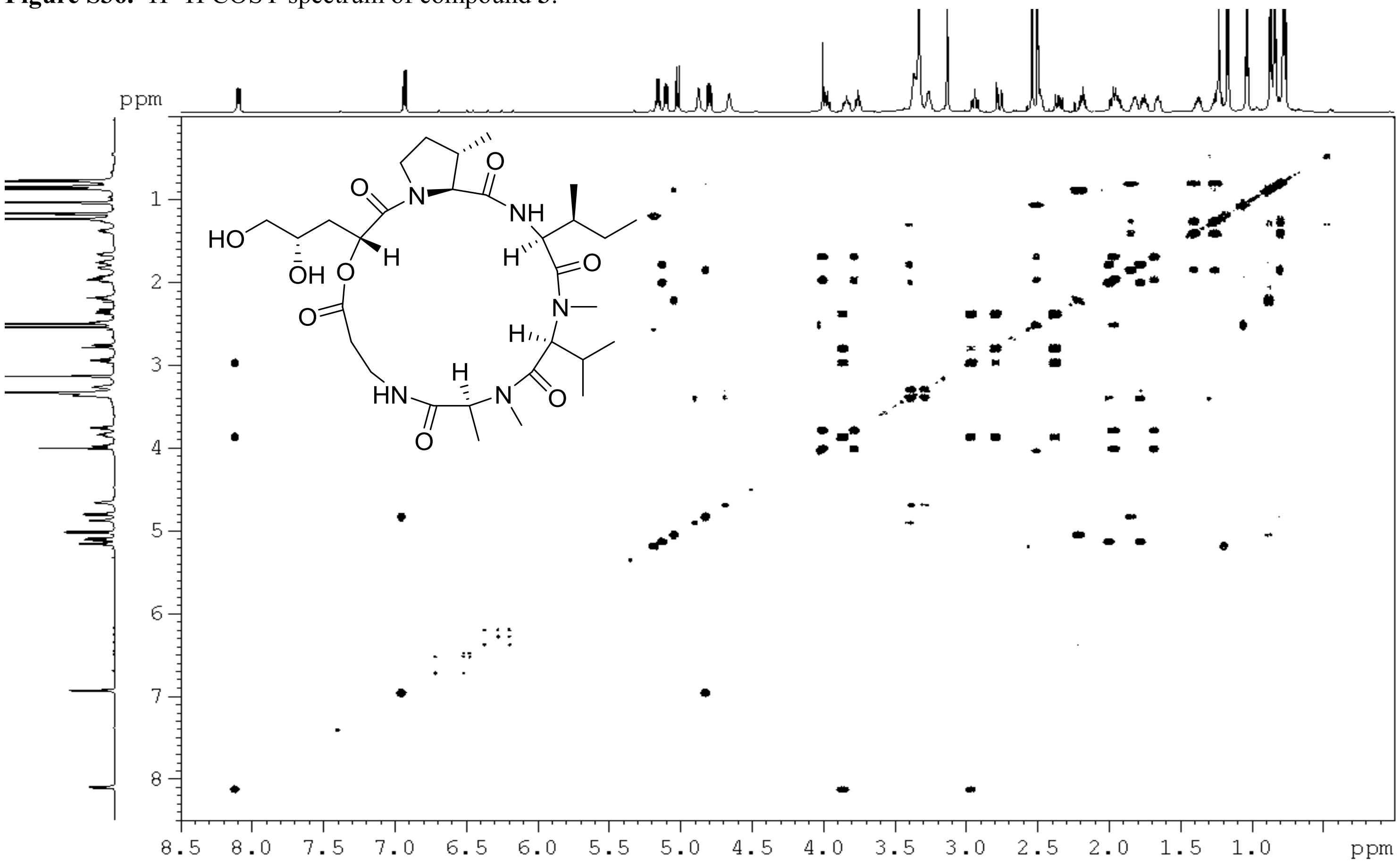


Figure S37. HSQC spectrum of compound **3**.

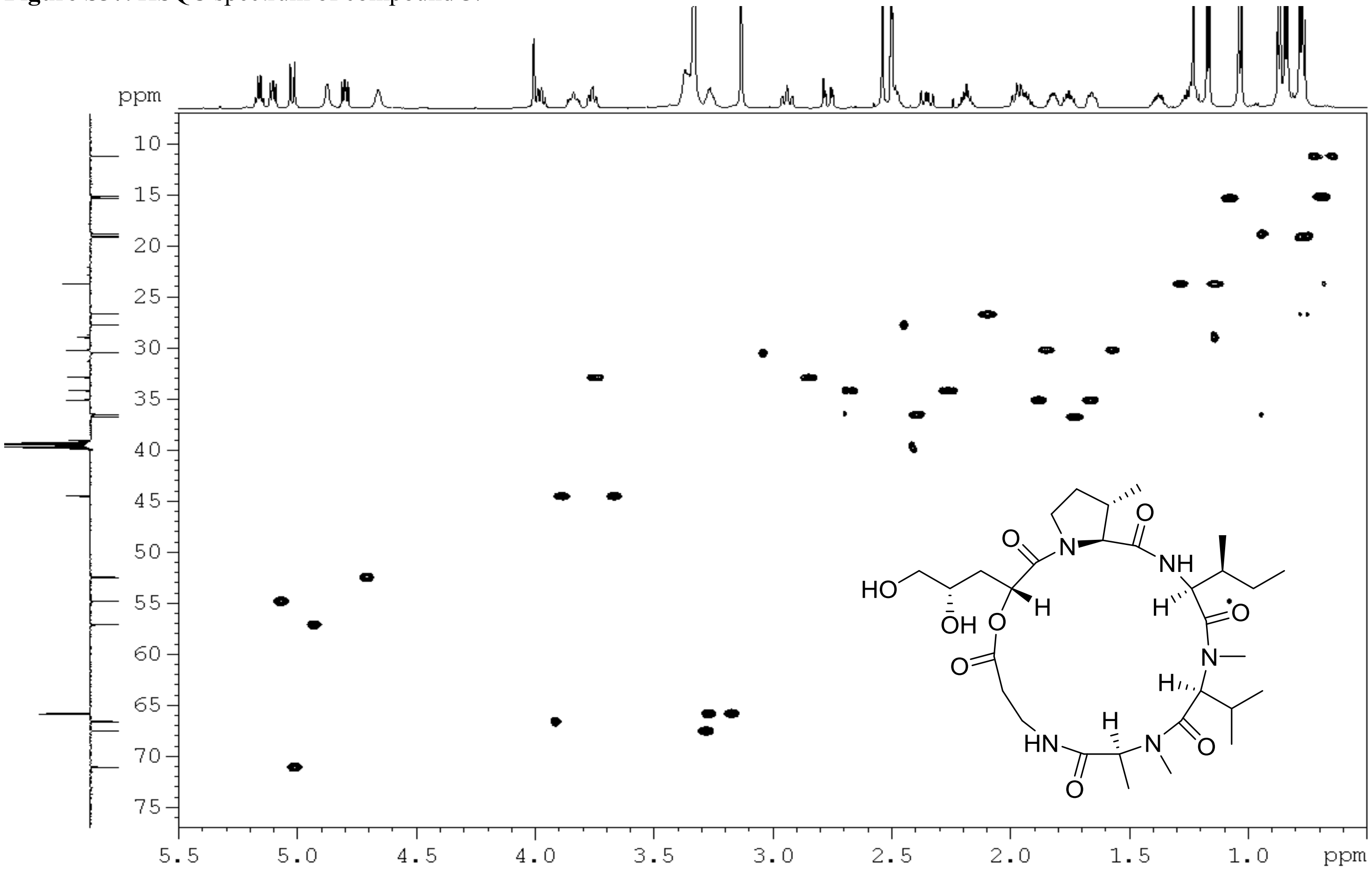


Figure S38. HMBC spectrum of compound **3**.

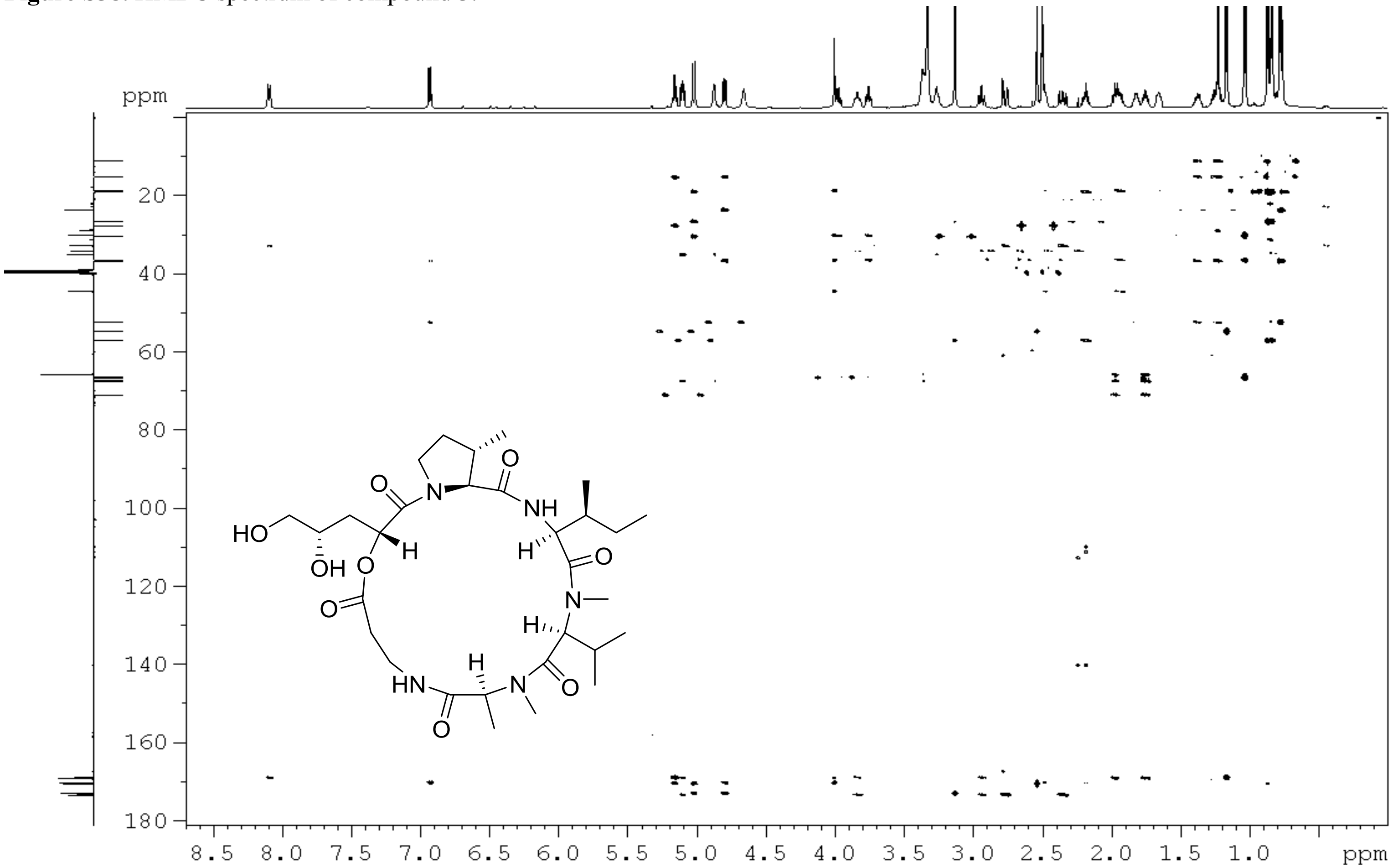


Figure S39. NOESY spectrum of compound **3**.

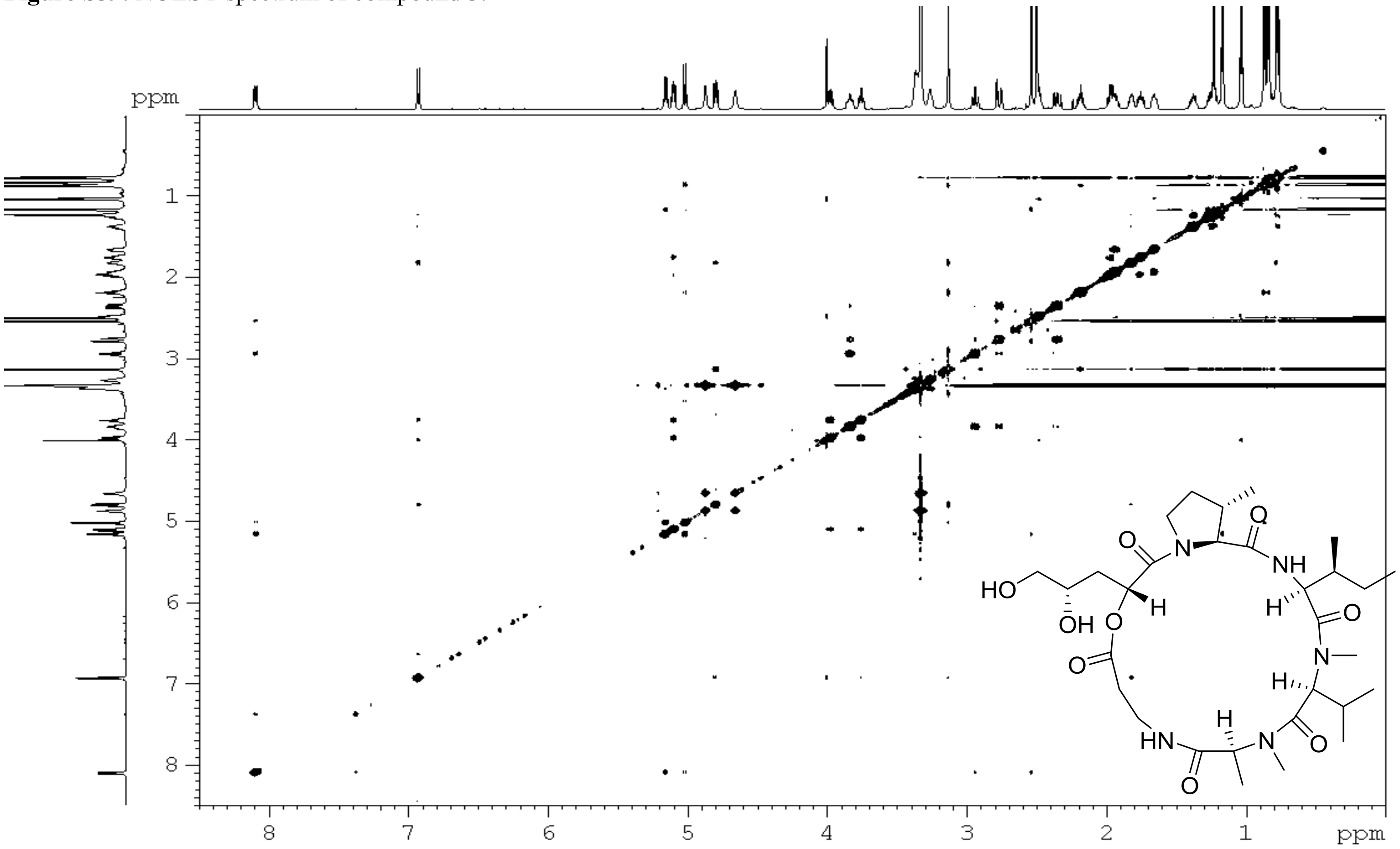


Figure S40. IR of compound **3**.

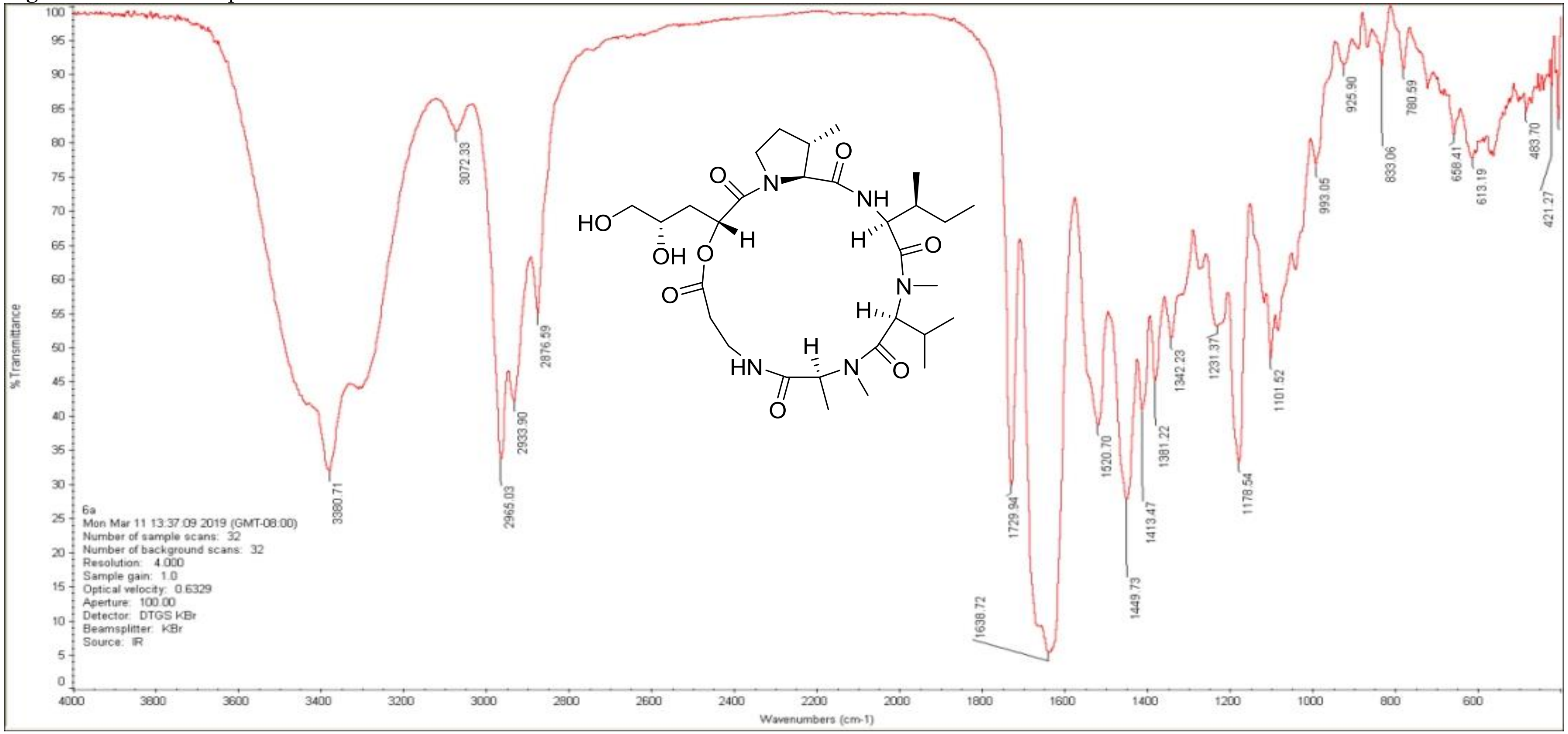
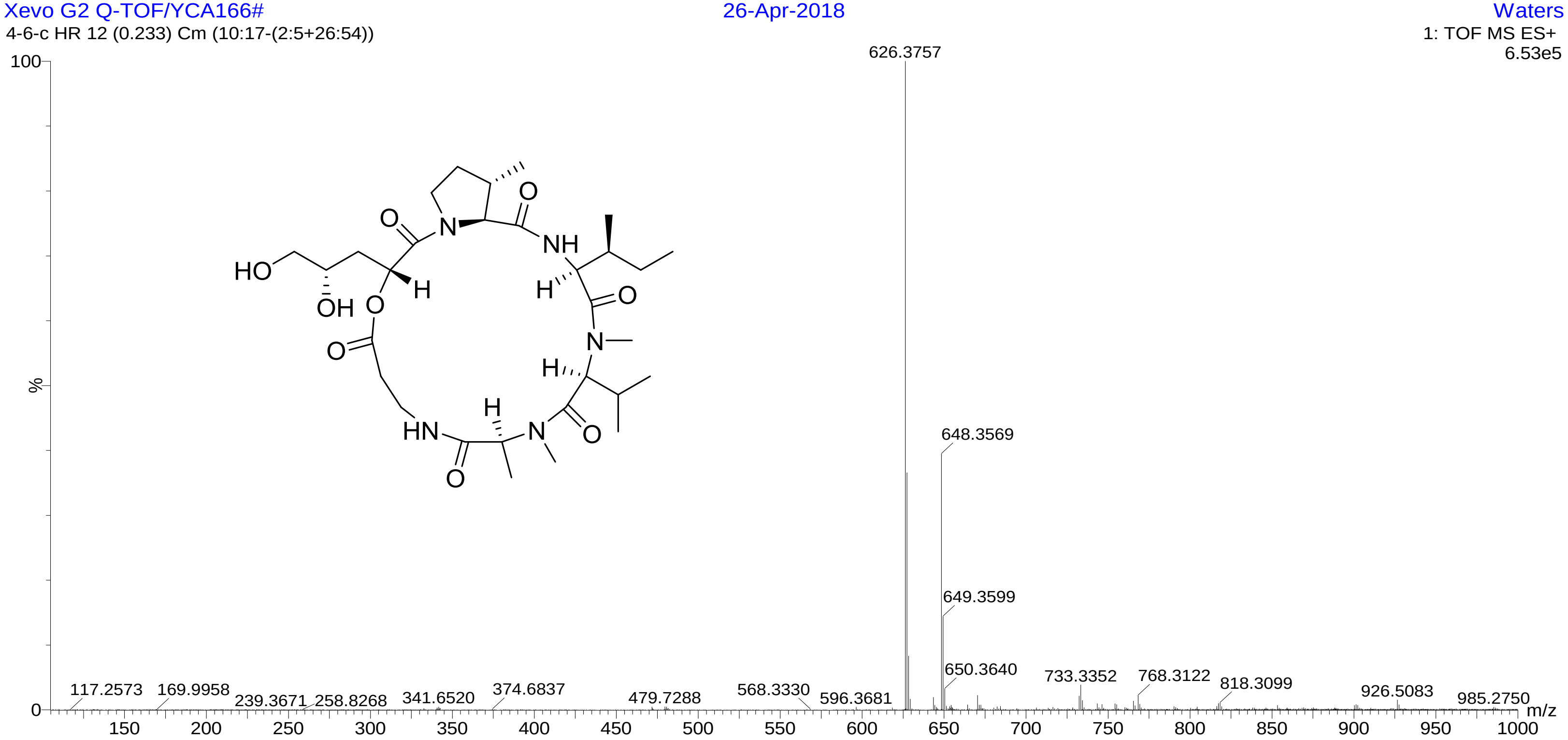


Figure S41. HRESIMS of compound **3**.



Mass	Calc.Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
626.3757	626.3765	-0.8	-1.3	7.5	188.3	0.296	74.37	$C_{30}H_{52}N_5O_9$

Figure S42. ^{1}H NMR spectrum of compound **4** (600 MHz, DMSO-d_6).

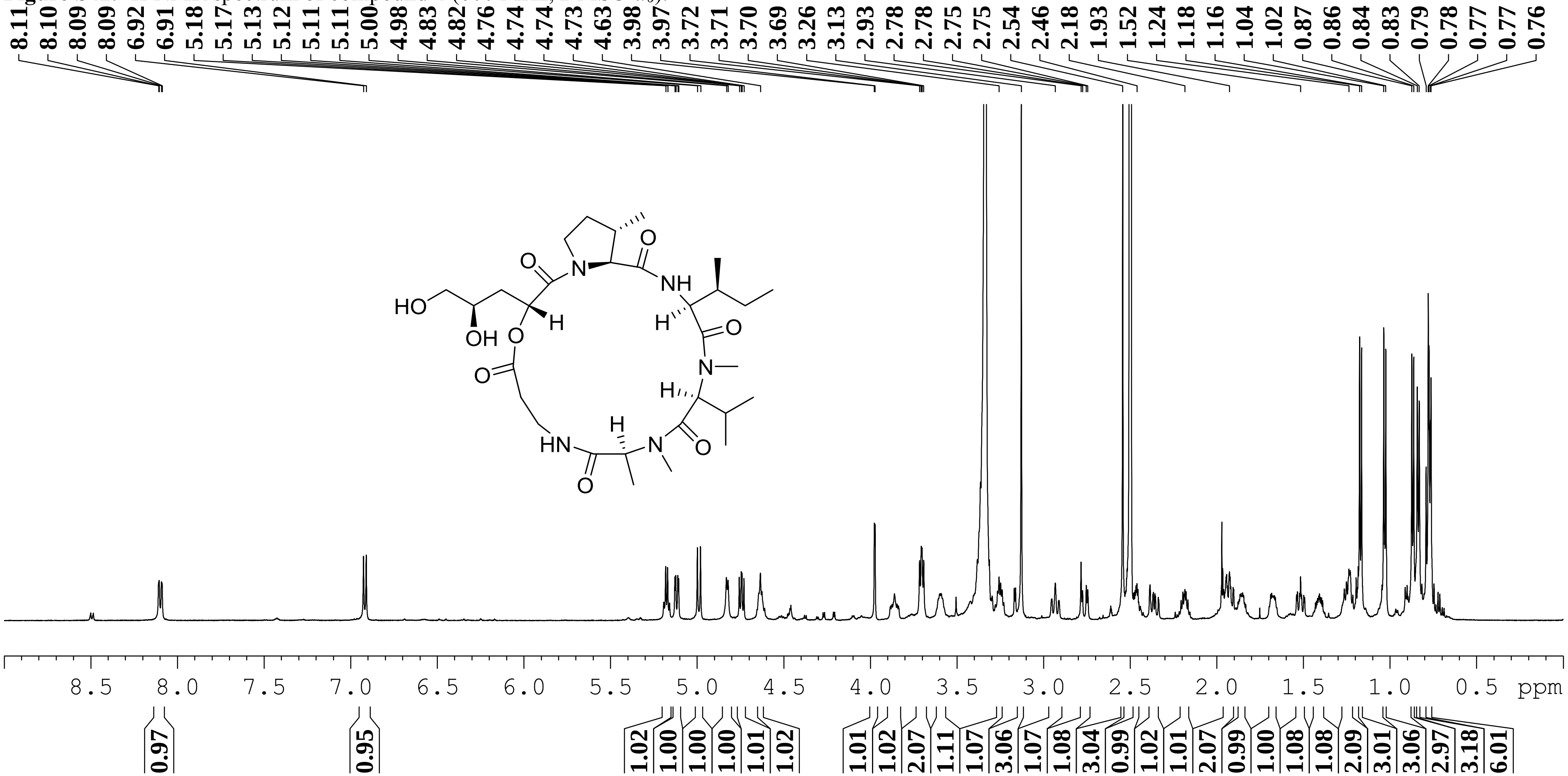


Figure S43. ^{13}C APT spectrum of compound **4** (600 MHz, DMSO-d_6) .

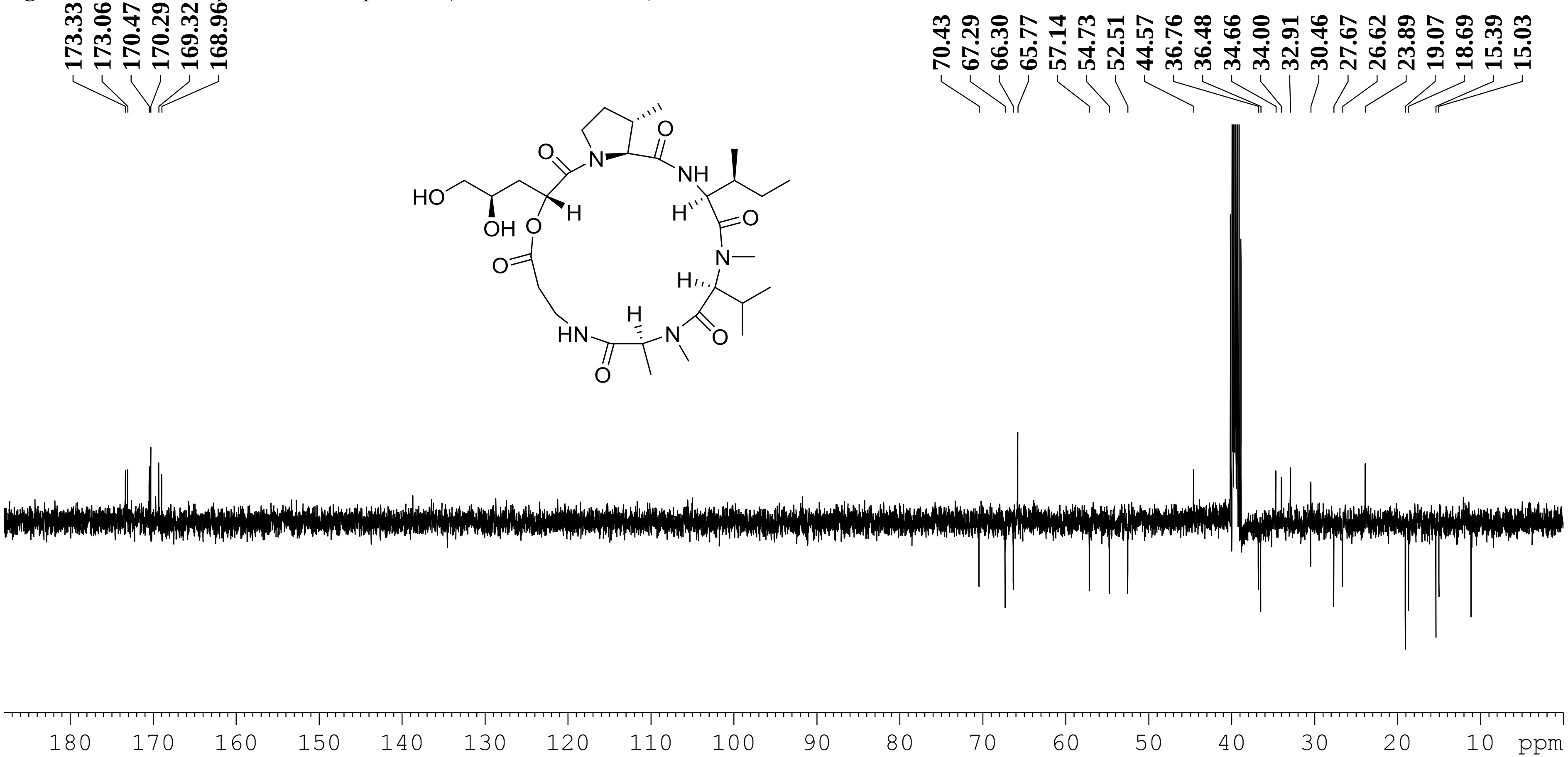


Figure S44. ^{1}H-^{1}H COSY spectrum of compound **4**.

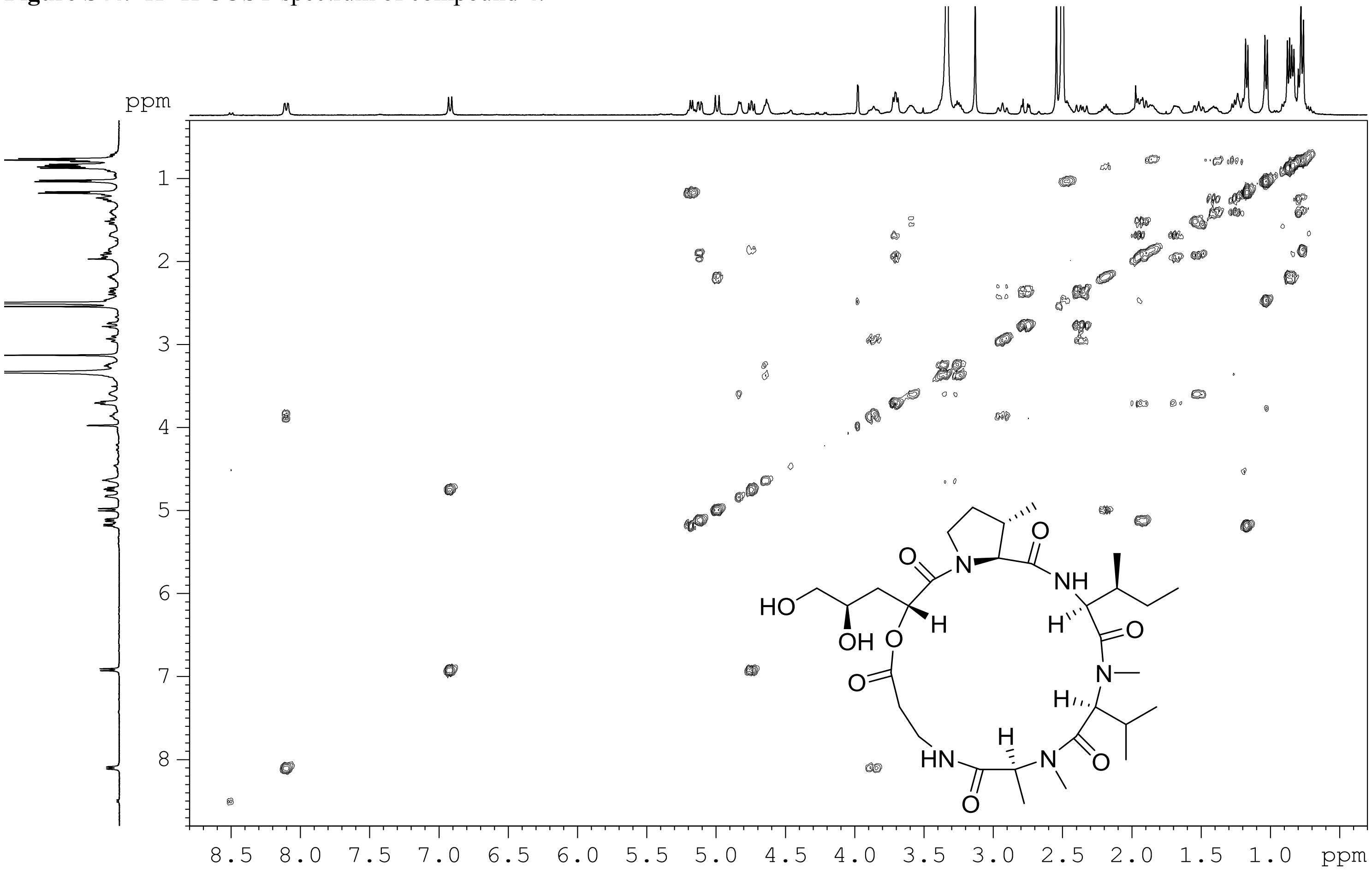


Figure S45. HSQC spectrum of compound **4**.

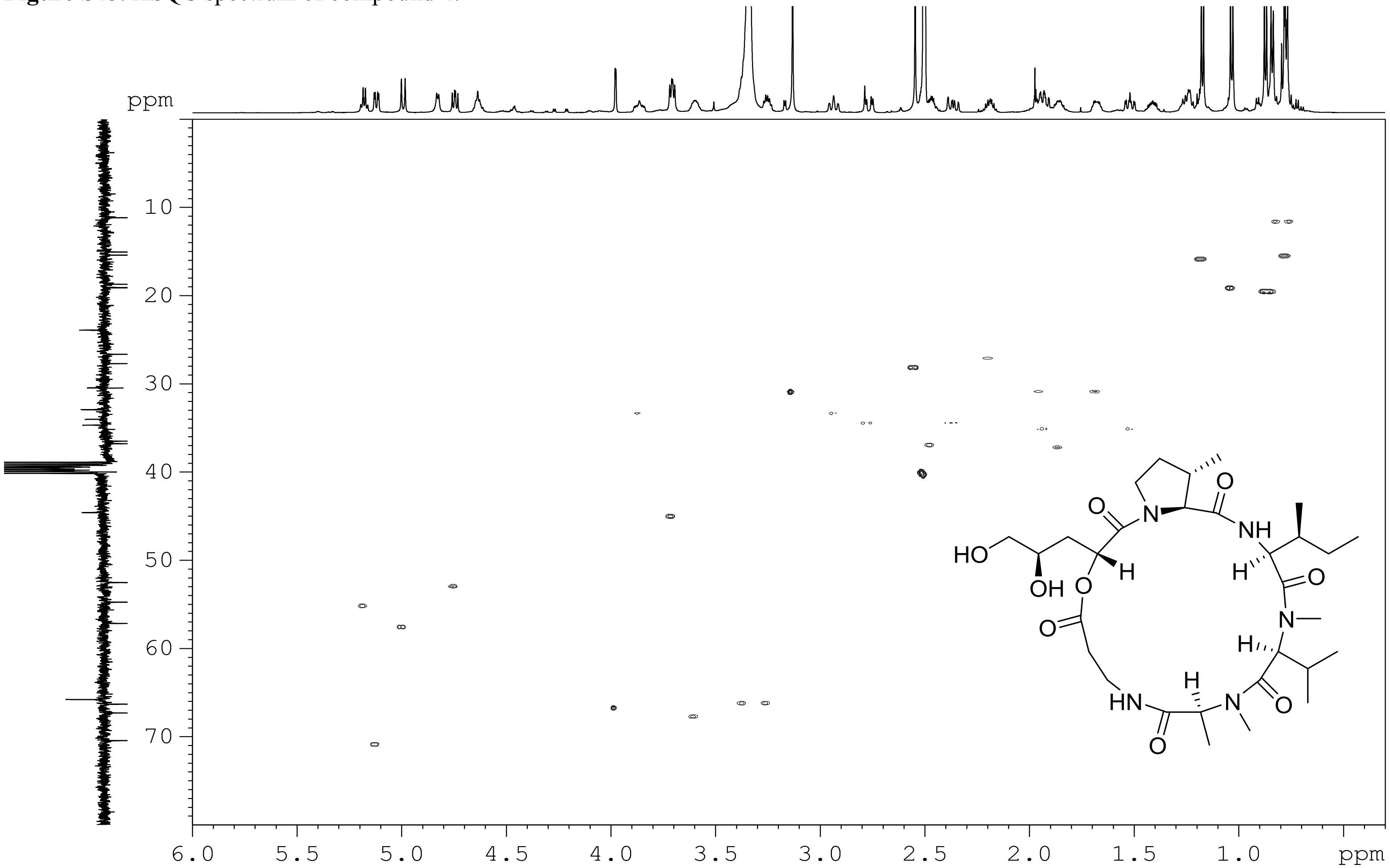


Figure S46. HMBC spectrum of compound **4**.

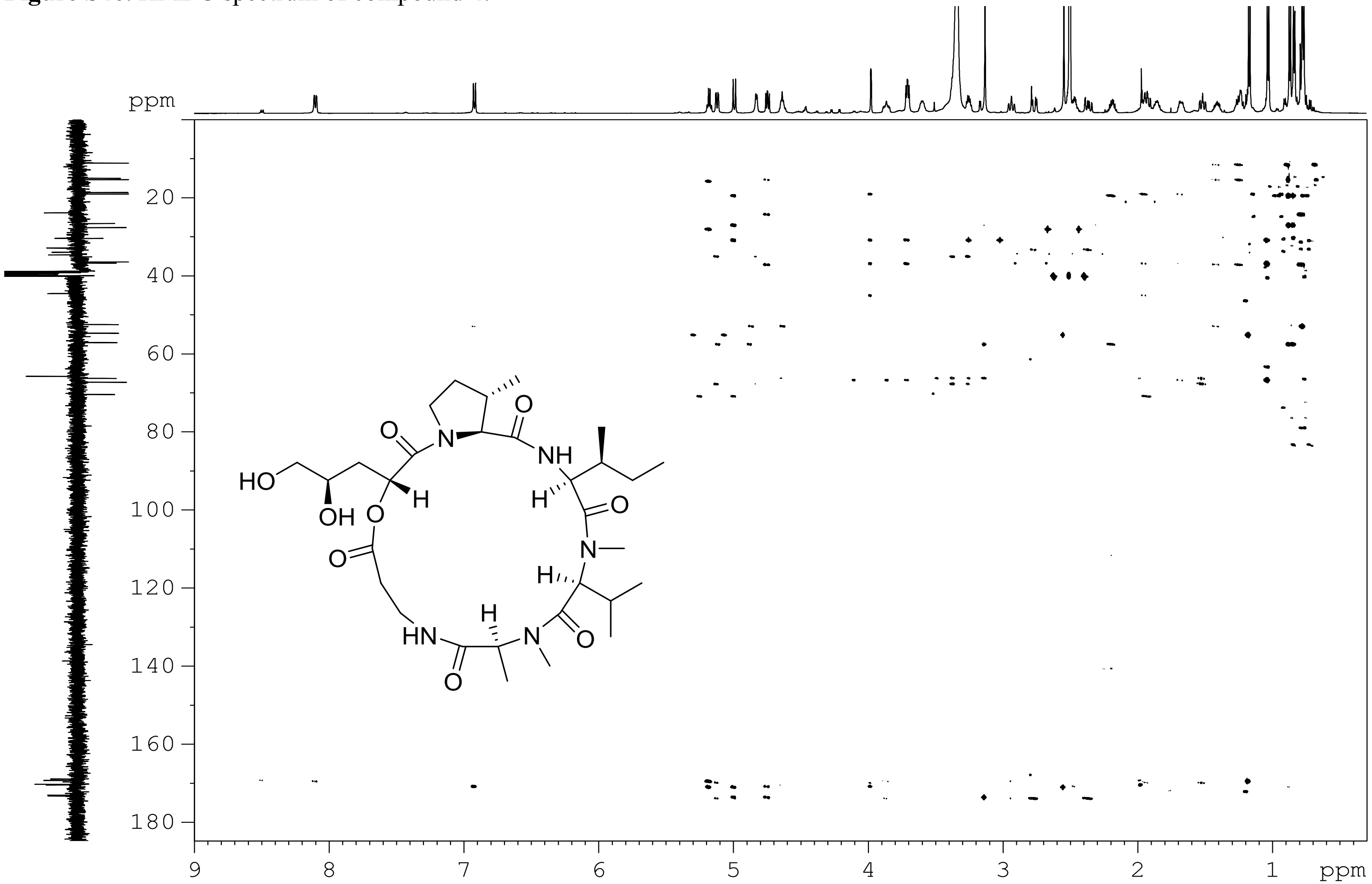


Figure S47. IR of compound **4**.

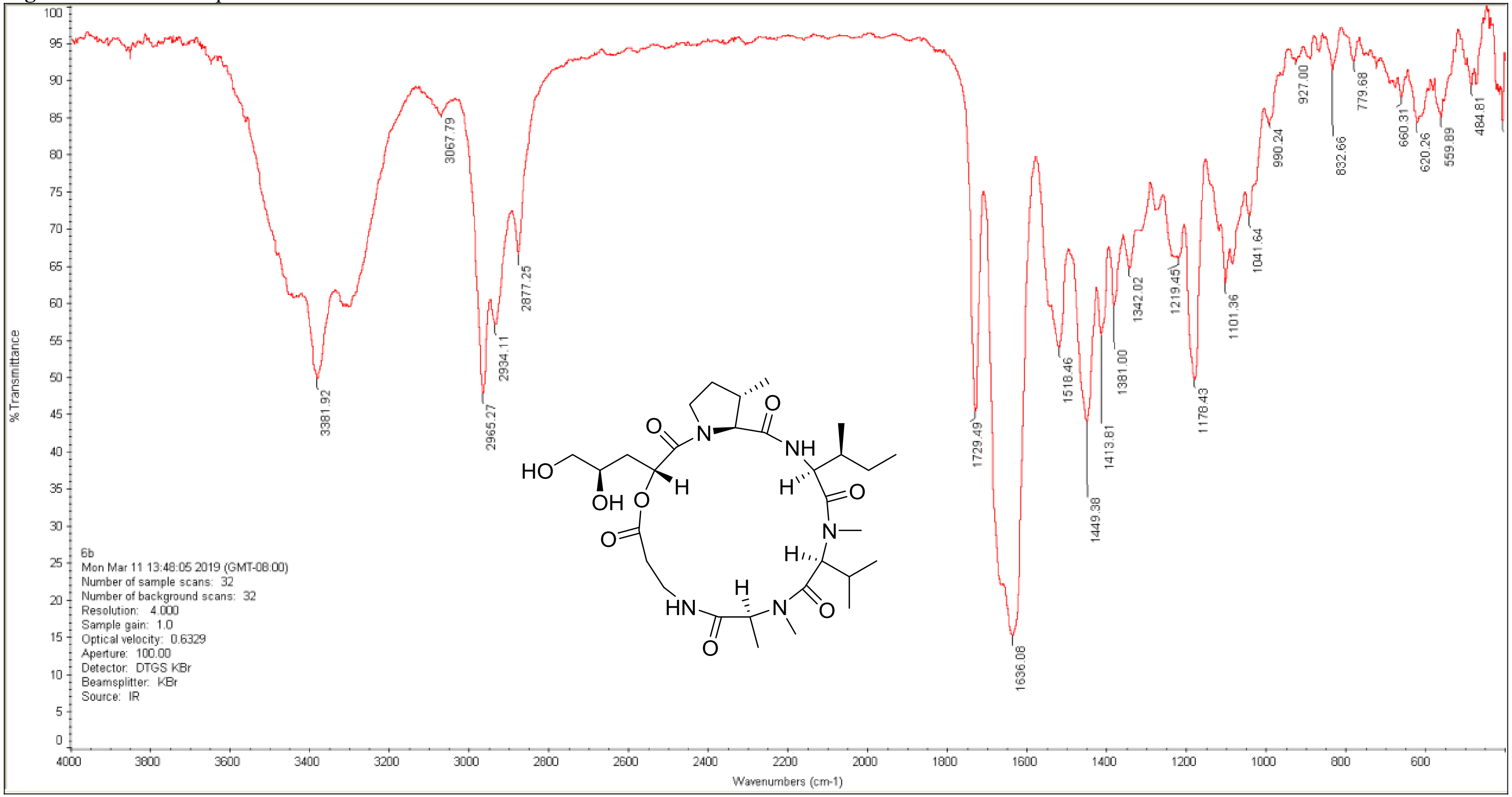
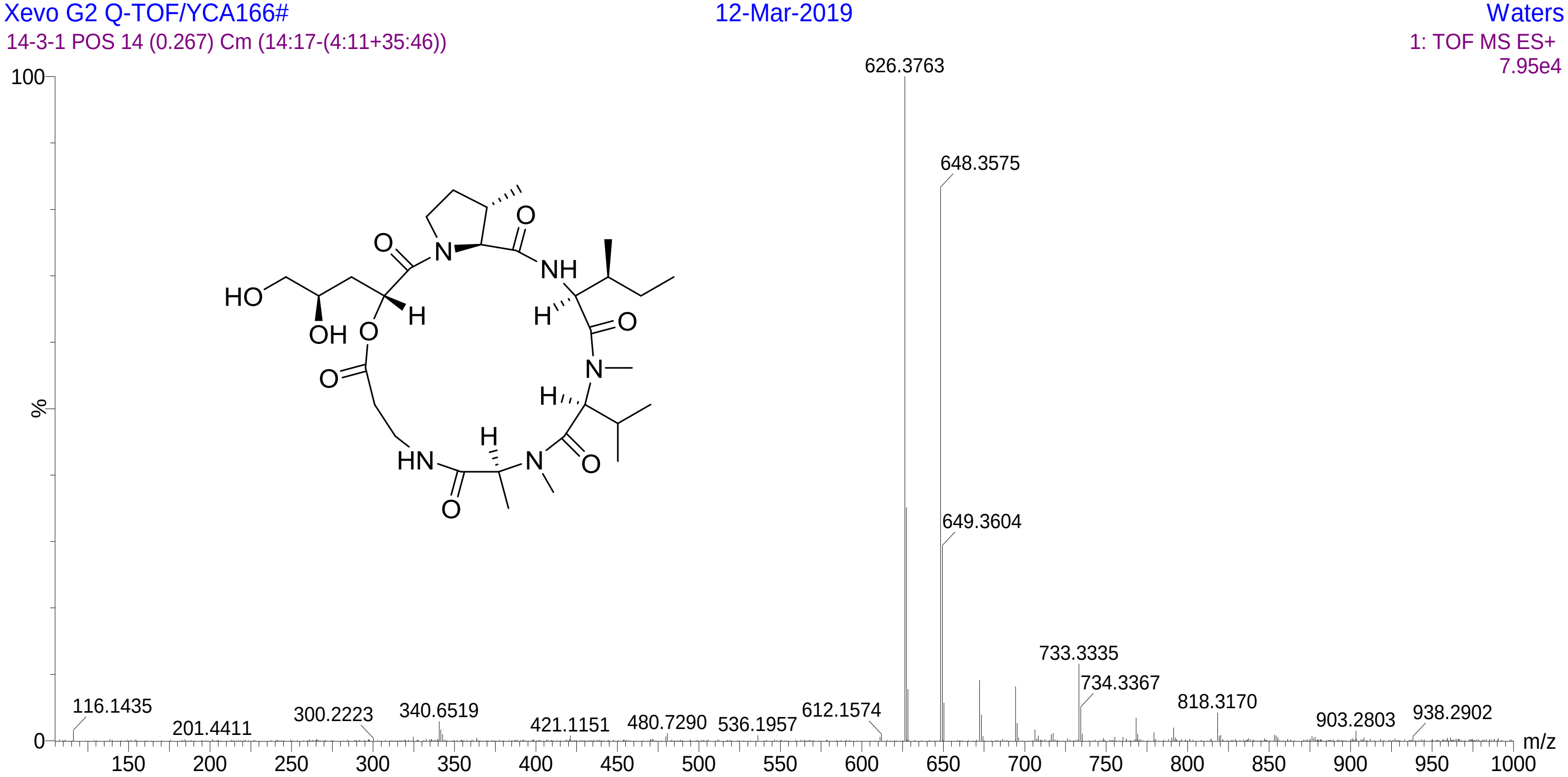


Figure S48. HRESIMS of compound **4**.



Mass	Calc.Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
626.3763	626.3765	-0.2	-0.3	7.5	37.1	0.002	99.77	$C_{30}H_{52}N_5O_9$

Figure S49. ^{1}H NMR spectrum of compound **5** (600 MHz, DMSO-d_6).

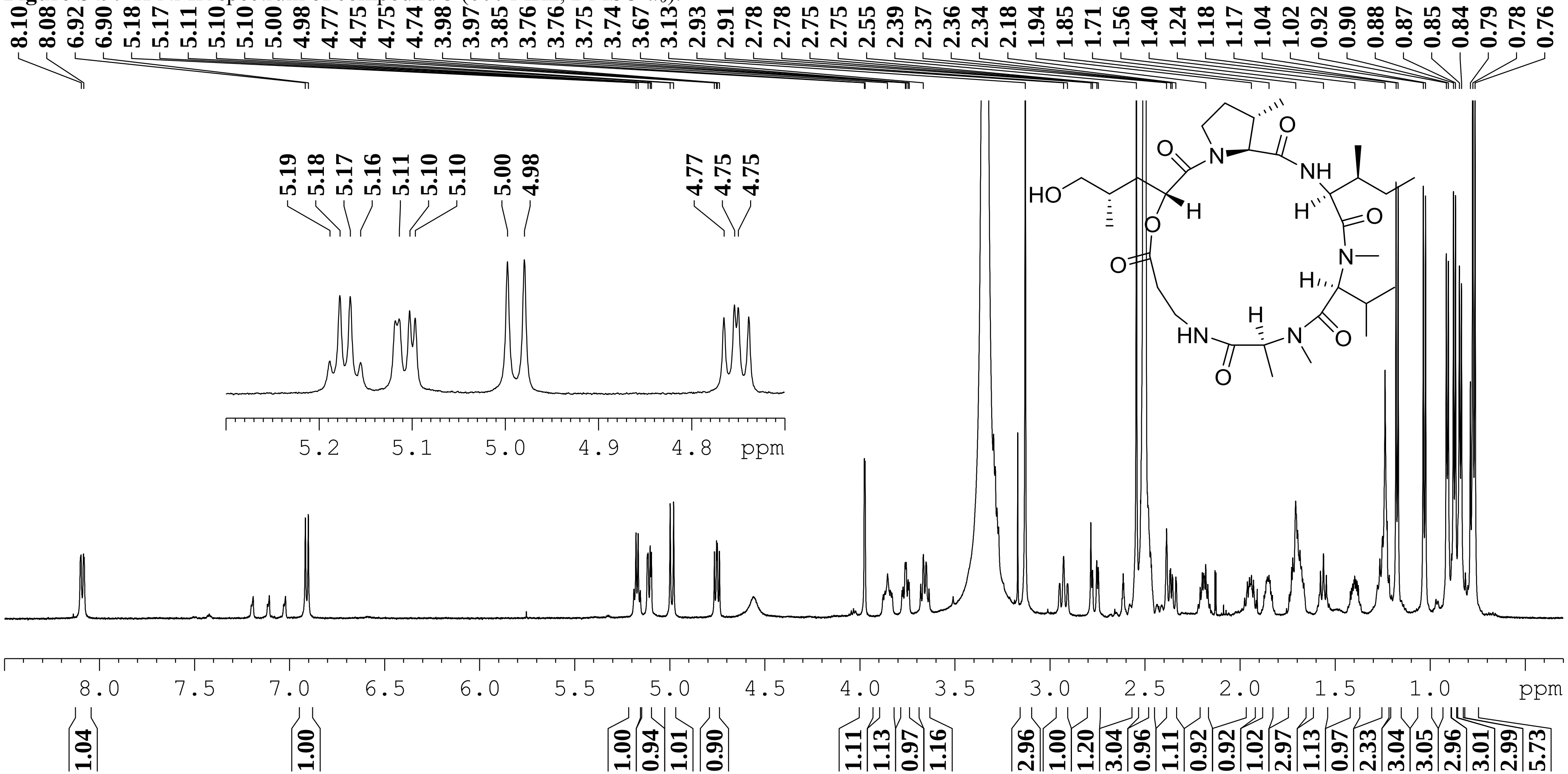


Figure S50. ^{13}C APT spectrum of compound **5** (600 MHz, DMSO-d_6) .

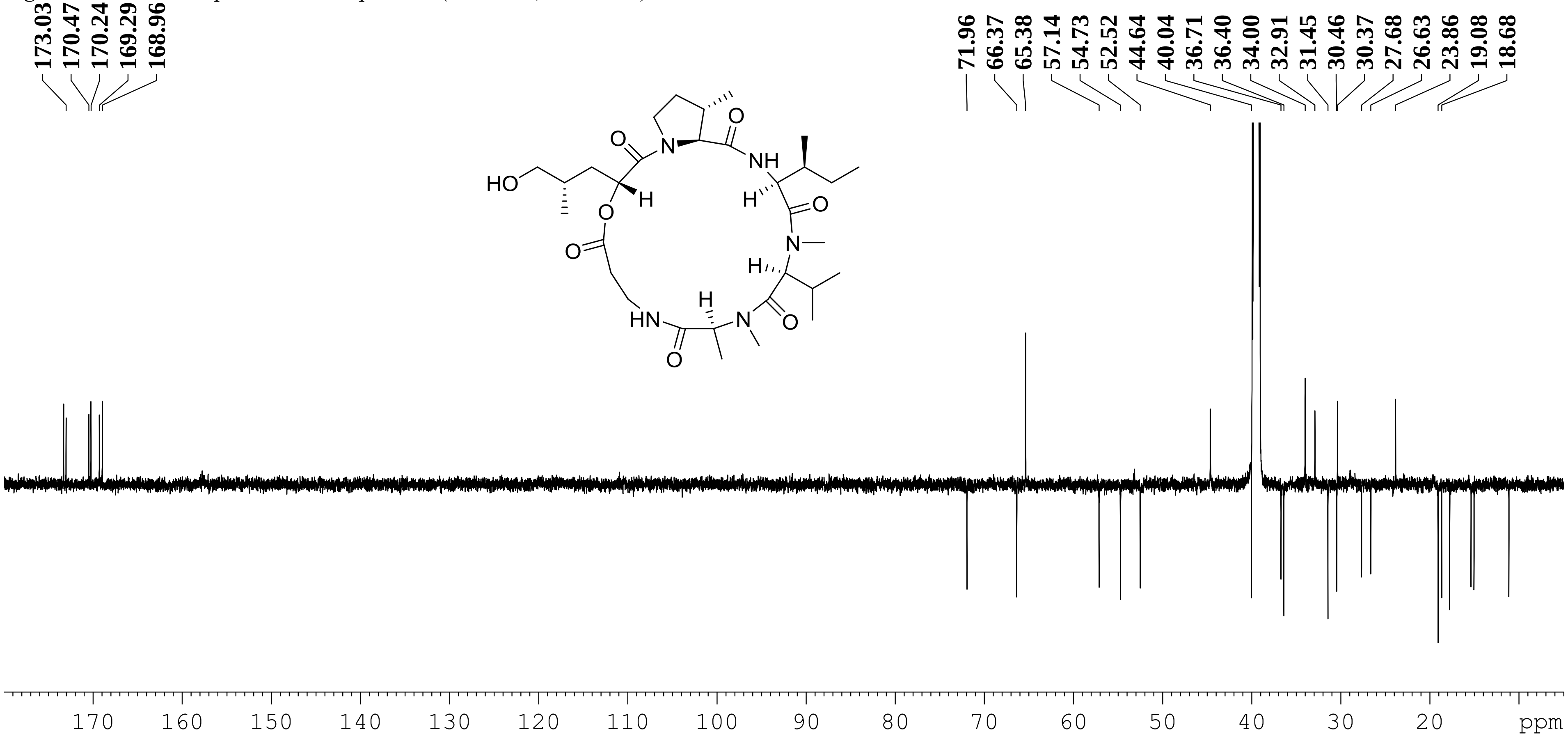


Figure S51. ^{1}H-^{1}H COSY spectrum of compound **5**.

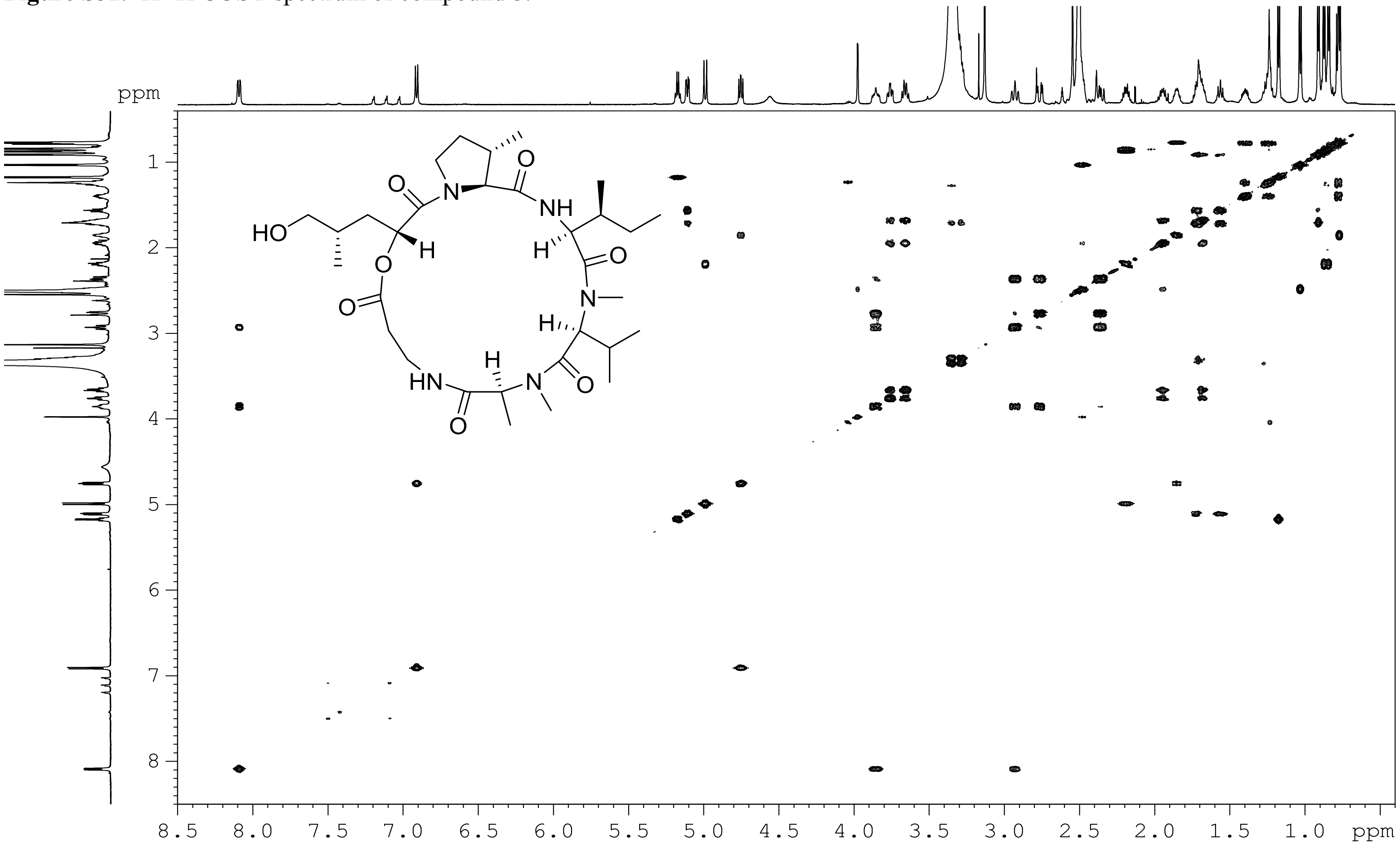


Figure S52. HSQC spectrum of compound **5**.

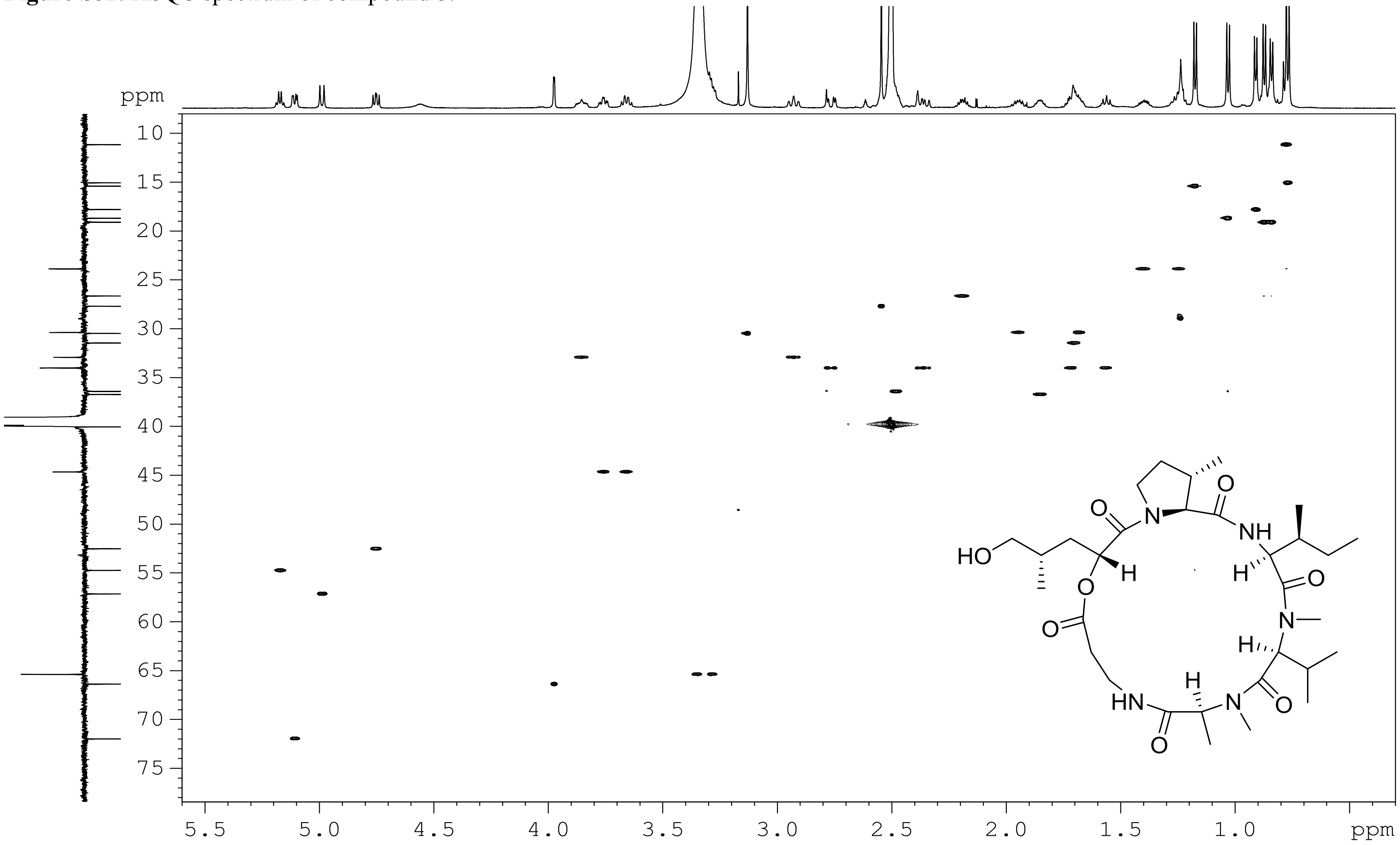


Figure S53. HMBC spectrum of compound **5**.

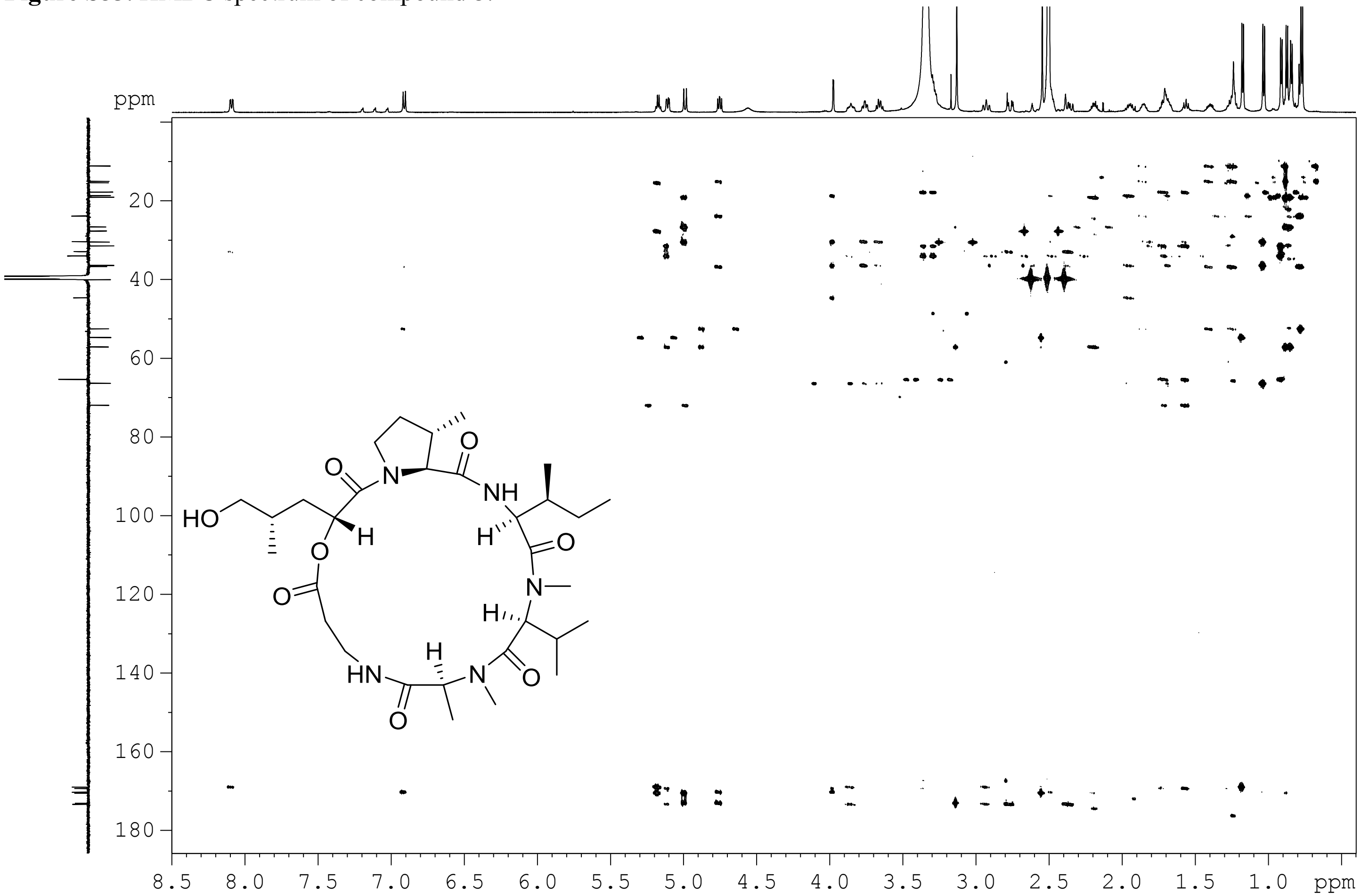


Figure S54. IR of compound **5**.

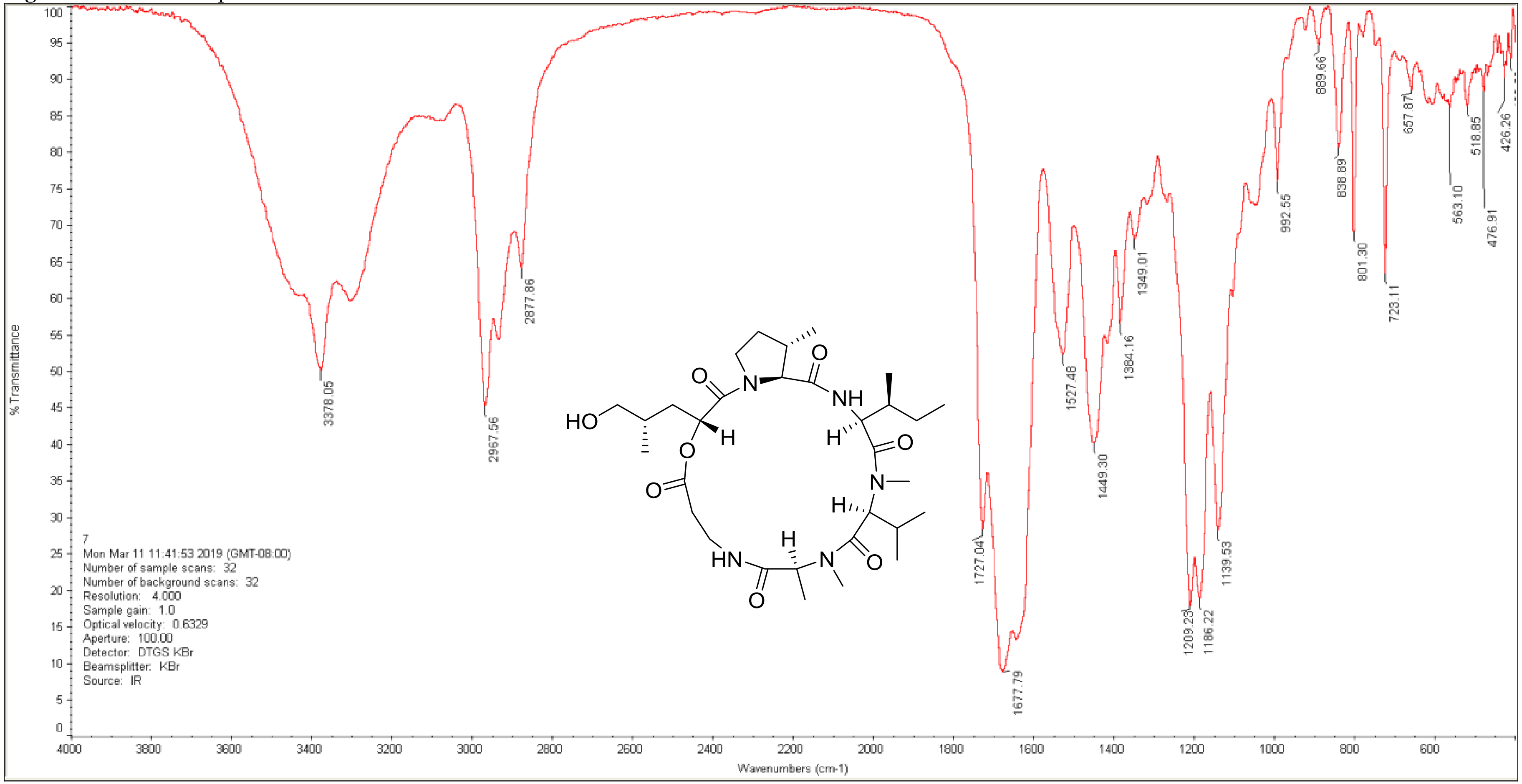
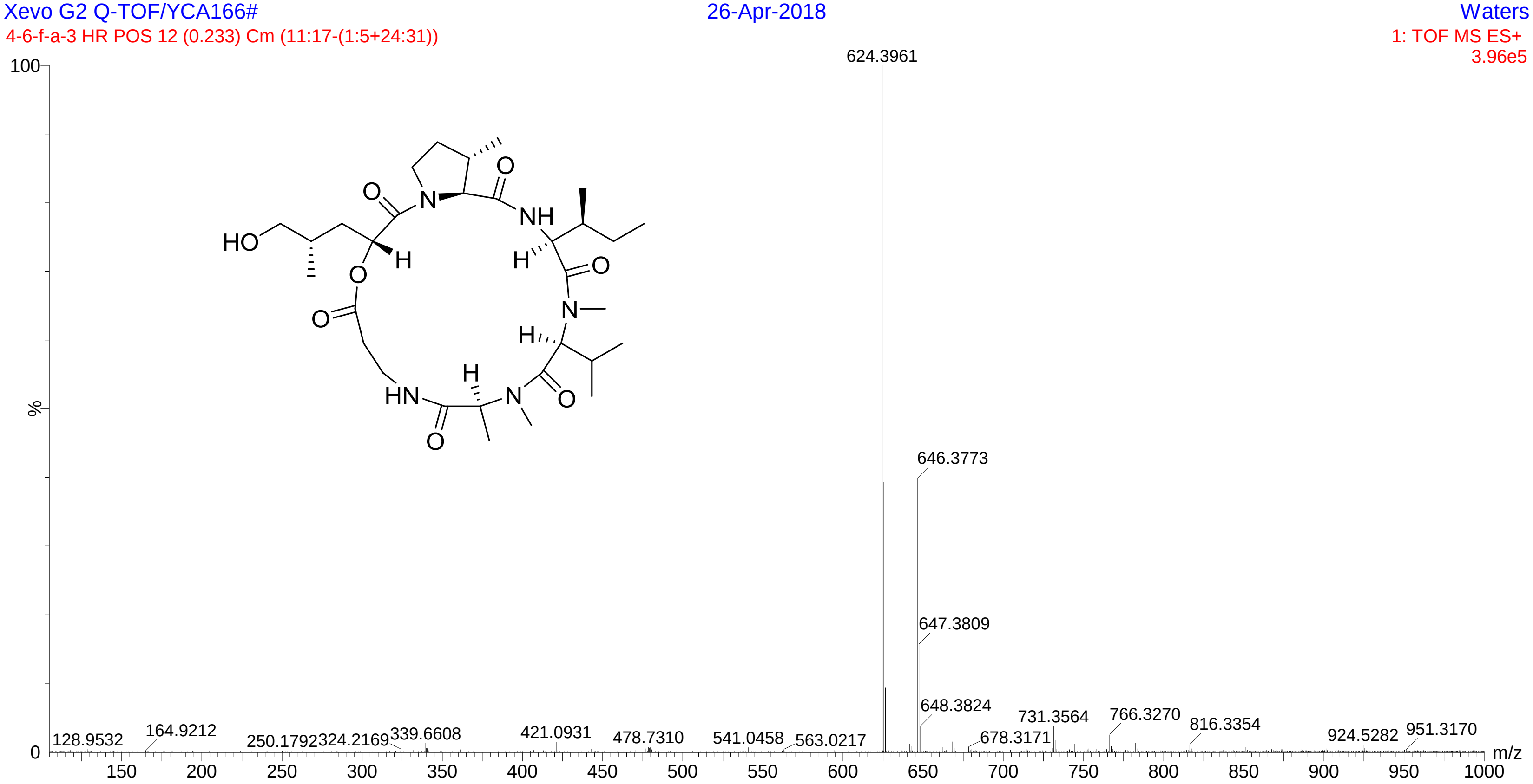


Figure S55. HRESIMS of compound **5**.



Mass	Calc.Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
626.3763	624.3972	-1.1	-1.8	7.5	239.6	1.011	46.38	$C_{31}H_{54}N_5O_8$

Figure S56. ^{1}H NMR spectrum of **6** (600 MHz, DMSO-d_6).

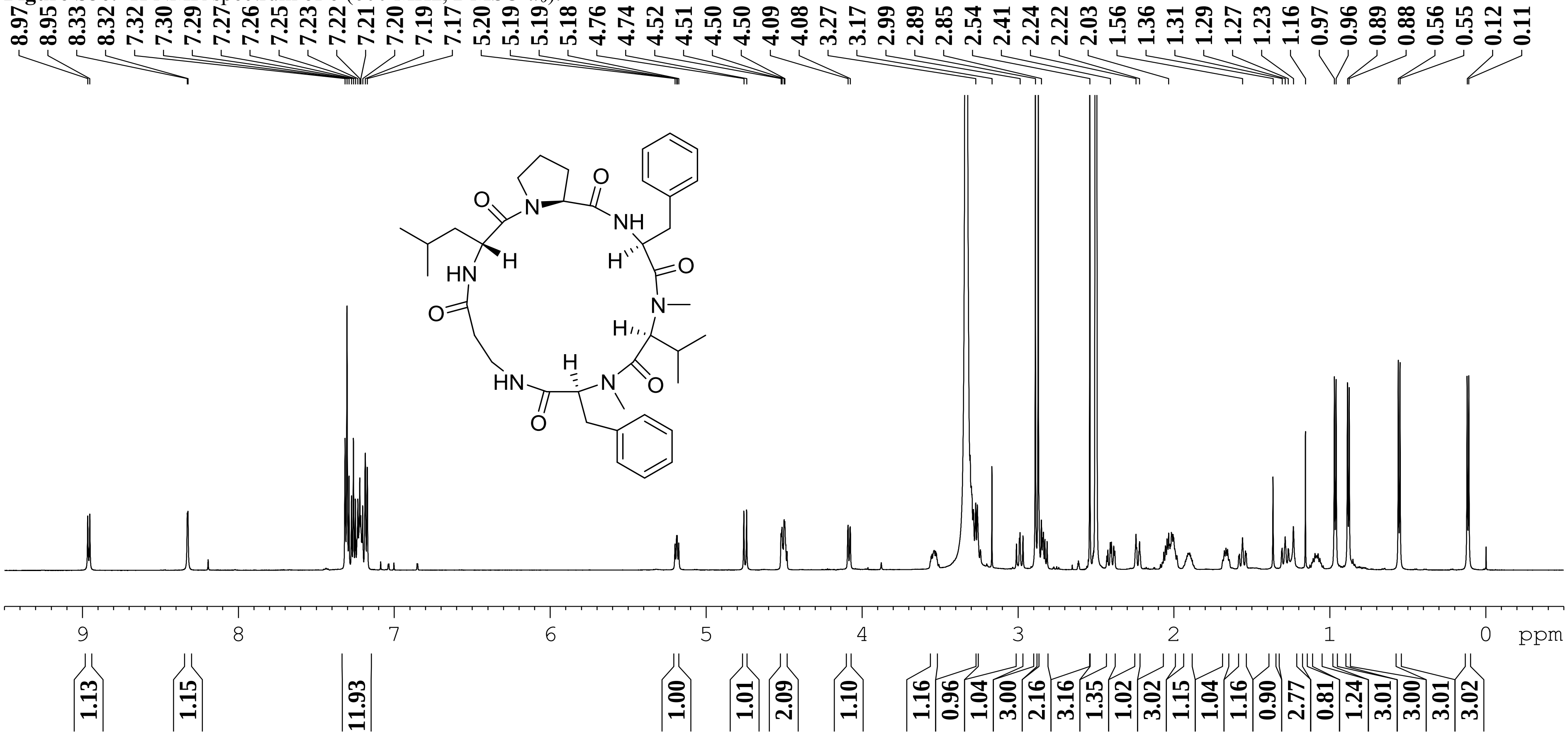


Figure S57. ^{13}C APT spectrum of **6** (600 MHz, DMSO-d_6) .

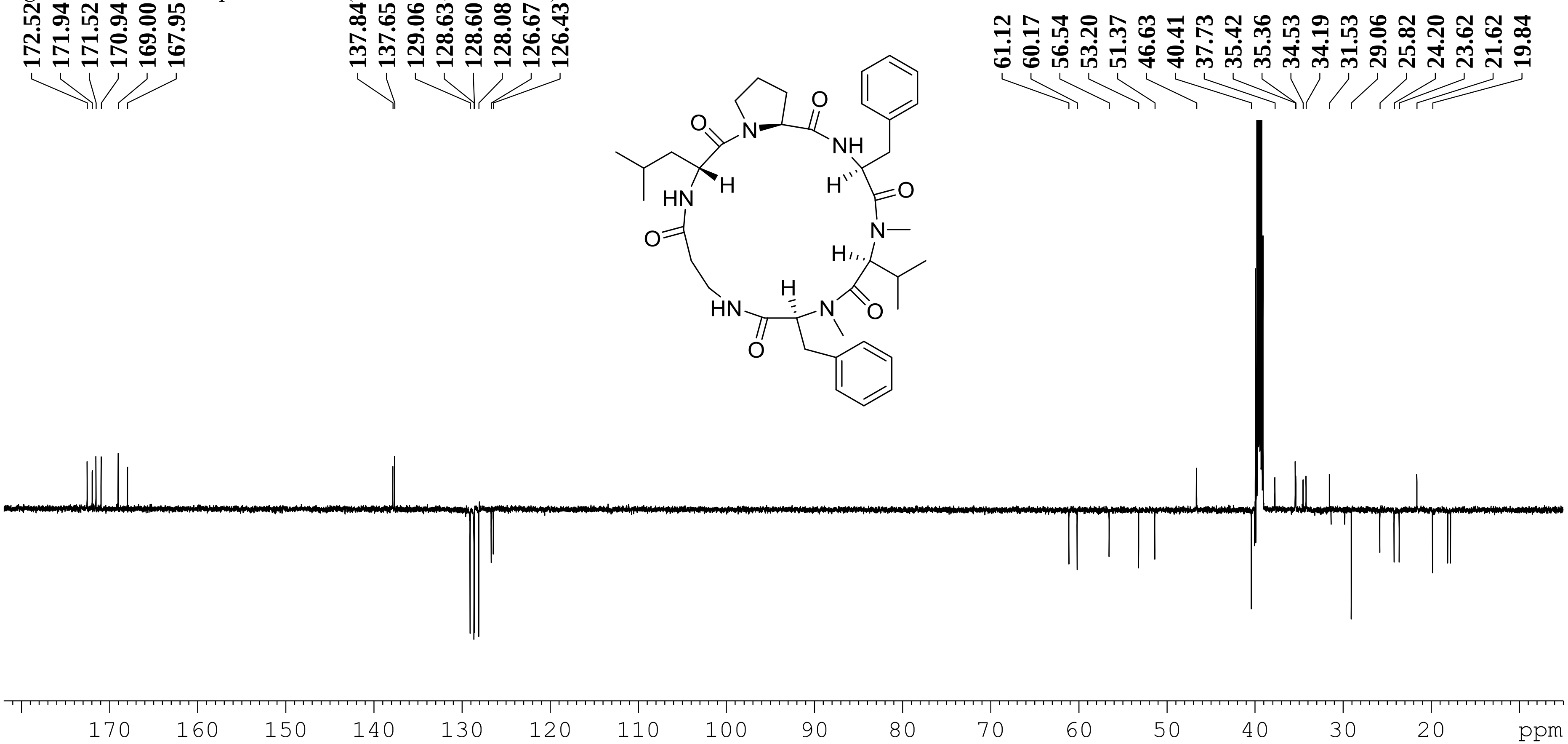


Figure S58. ^{1}H-^{1}H COSY spectrum of **6**.

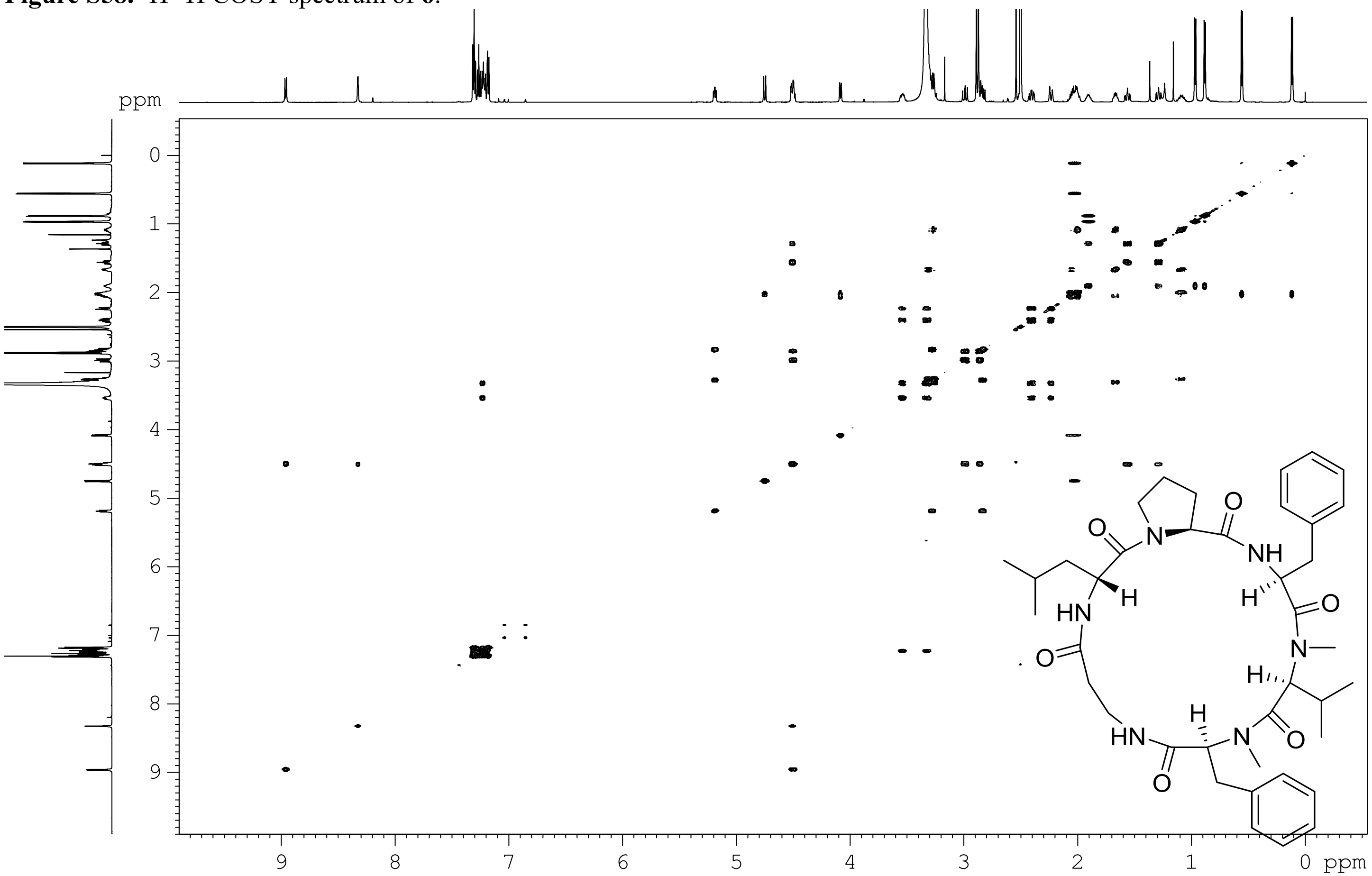


Figure S59. HSQC spectrum of **6**.

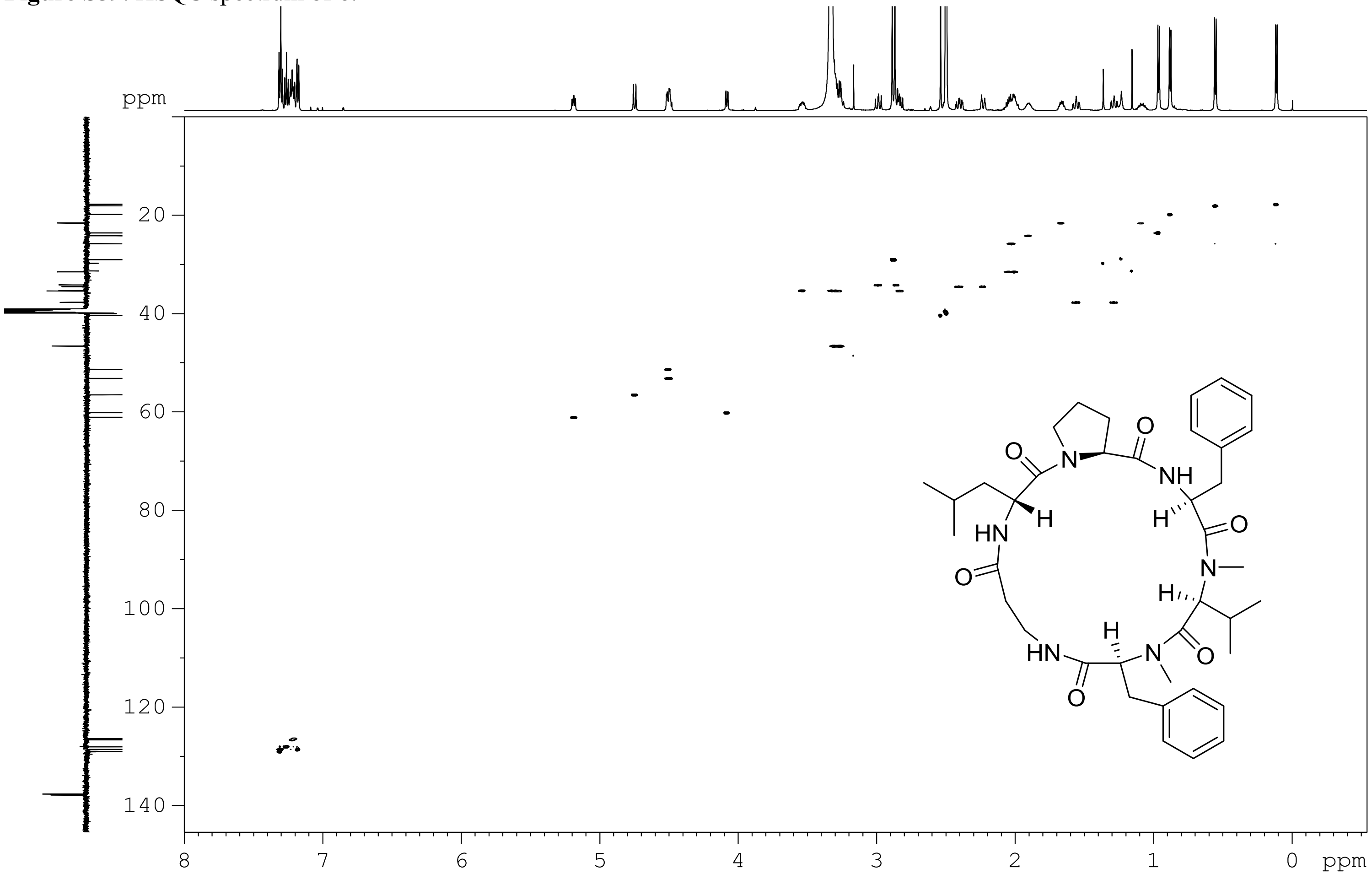


Figure S60. HMBC spectrum of **6**.

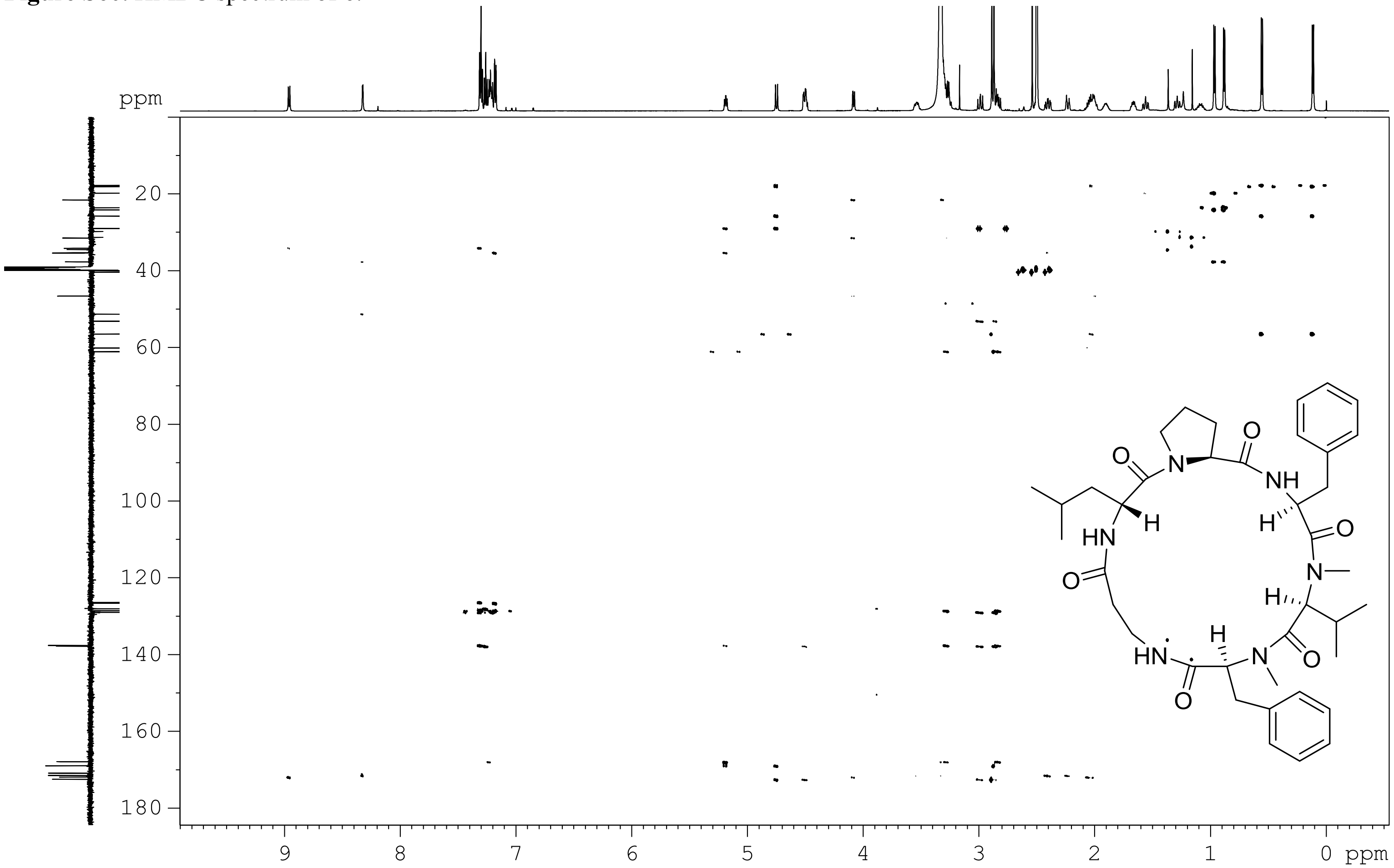


Figure S61. NOESY spectrum of **6**.

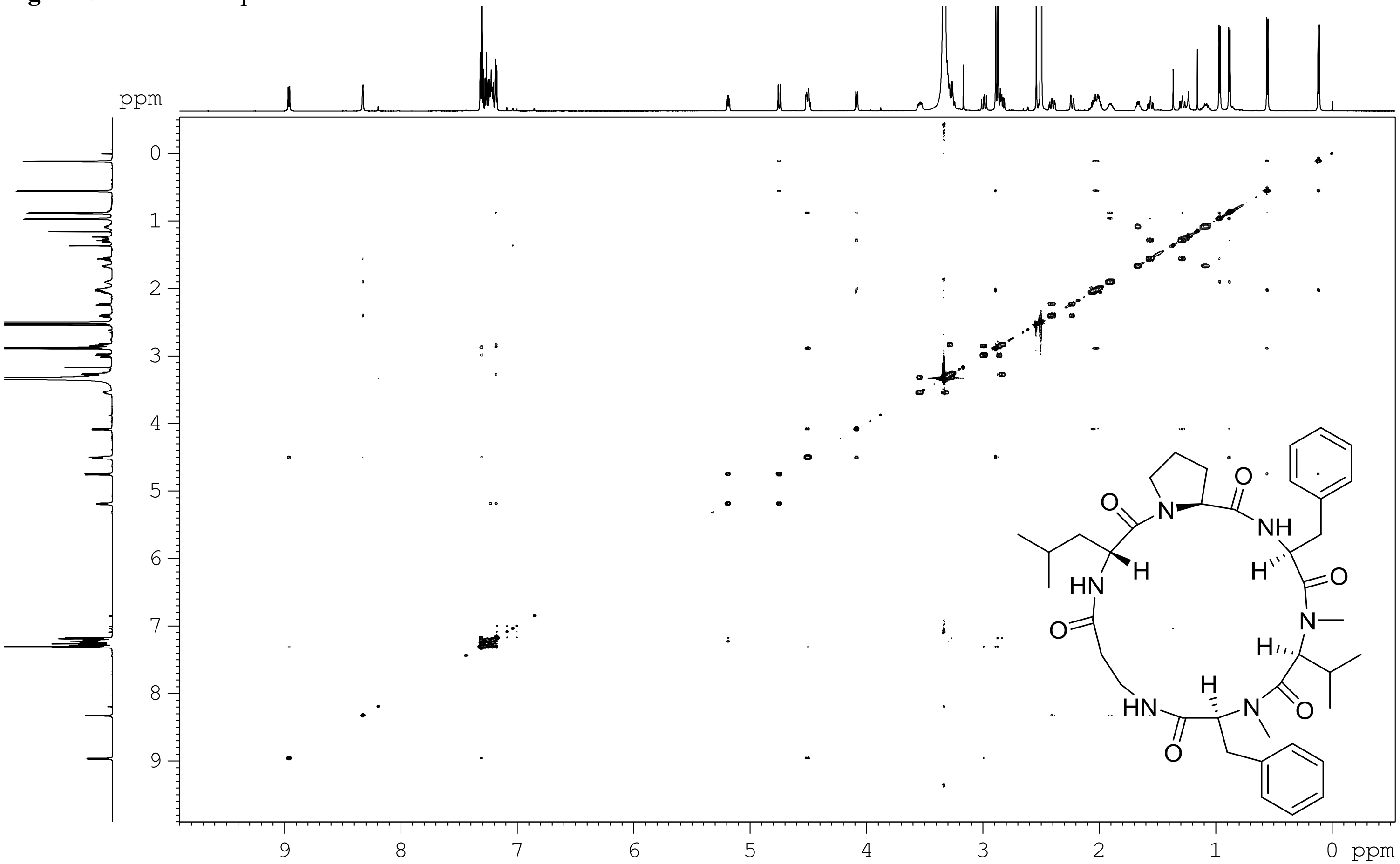


Figure S62. IR spectrum of **6**.

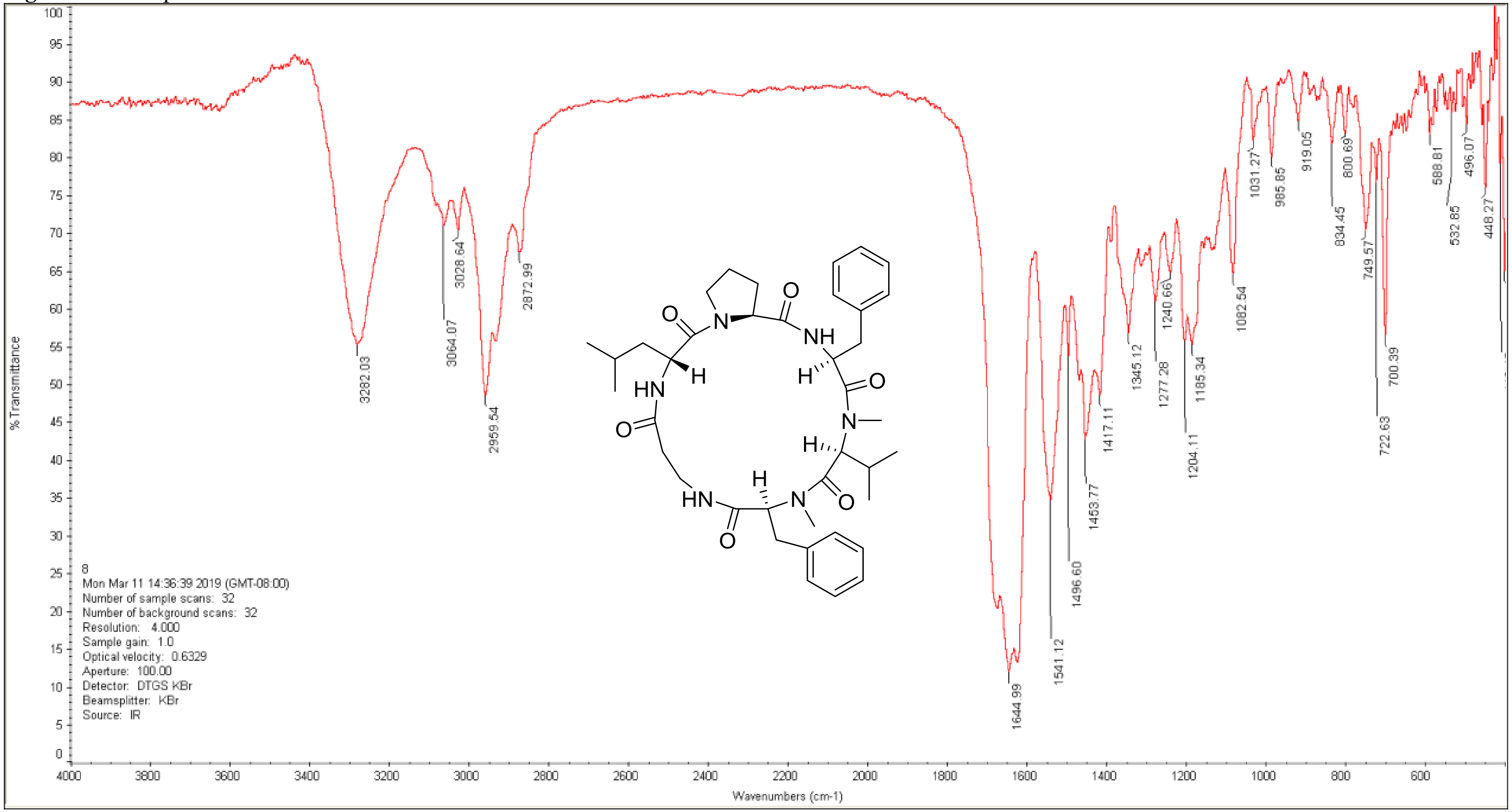
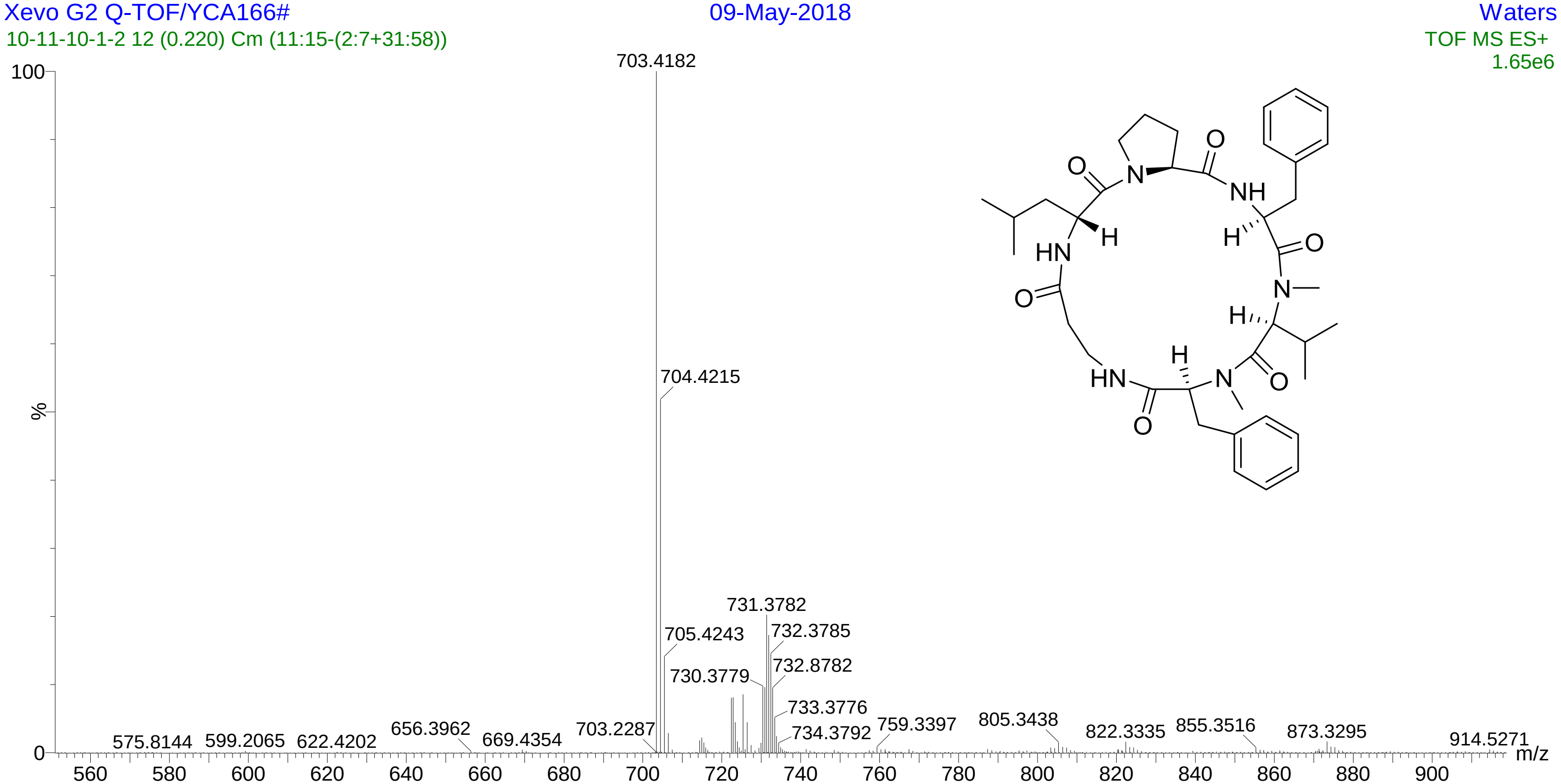


Figure S63. HRESIMS spectrum of **6**.



Mass	Calc.Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
703.4182	703.4183	-0.1	-0.1	15.5	269.7	0.089	91.47	$C_{39}H_{55}N_6O_6$

Figure S64. ^{1}H NMR spectrum of **7** (600 MHz, DMSO-d_6).

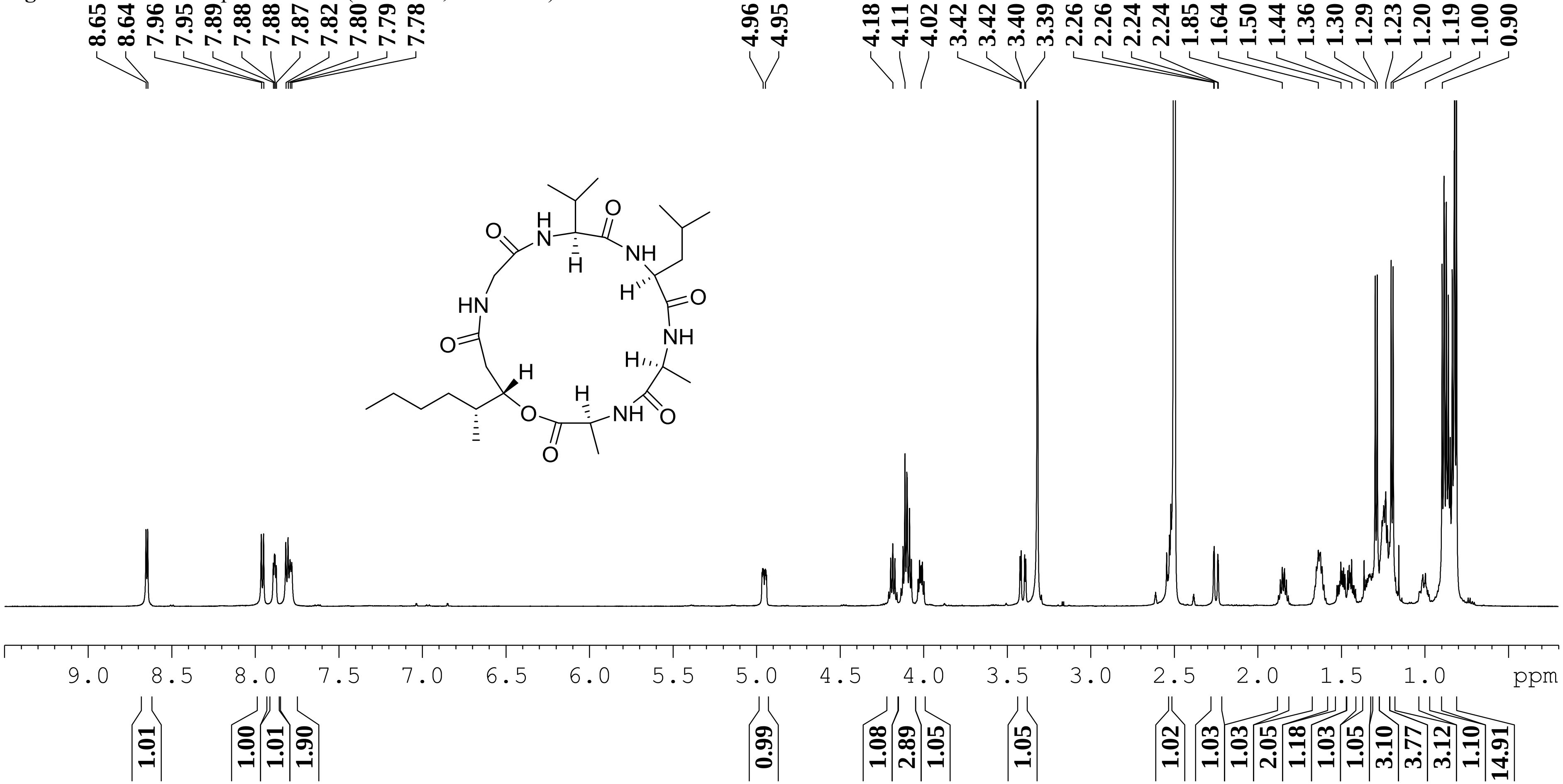


Figure S65. ^{13}C APT spectrum of **7** (600 MHz, DMSO-d_6) .

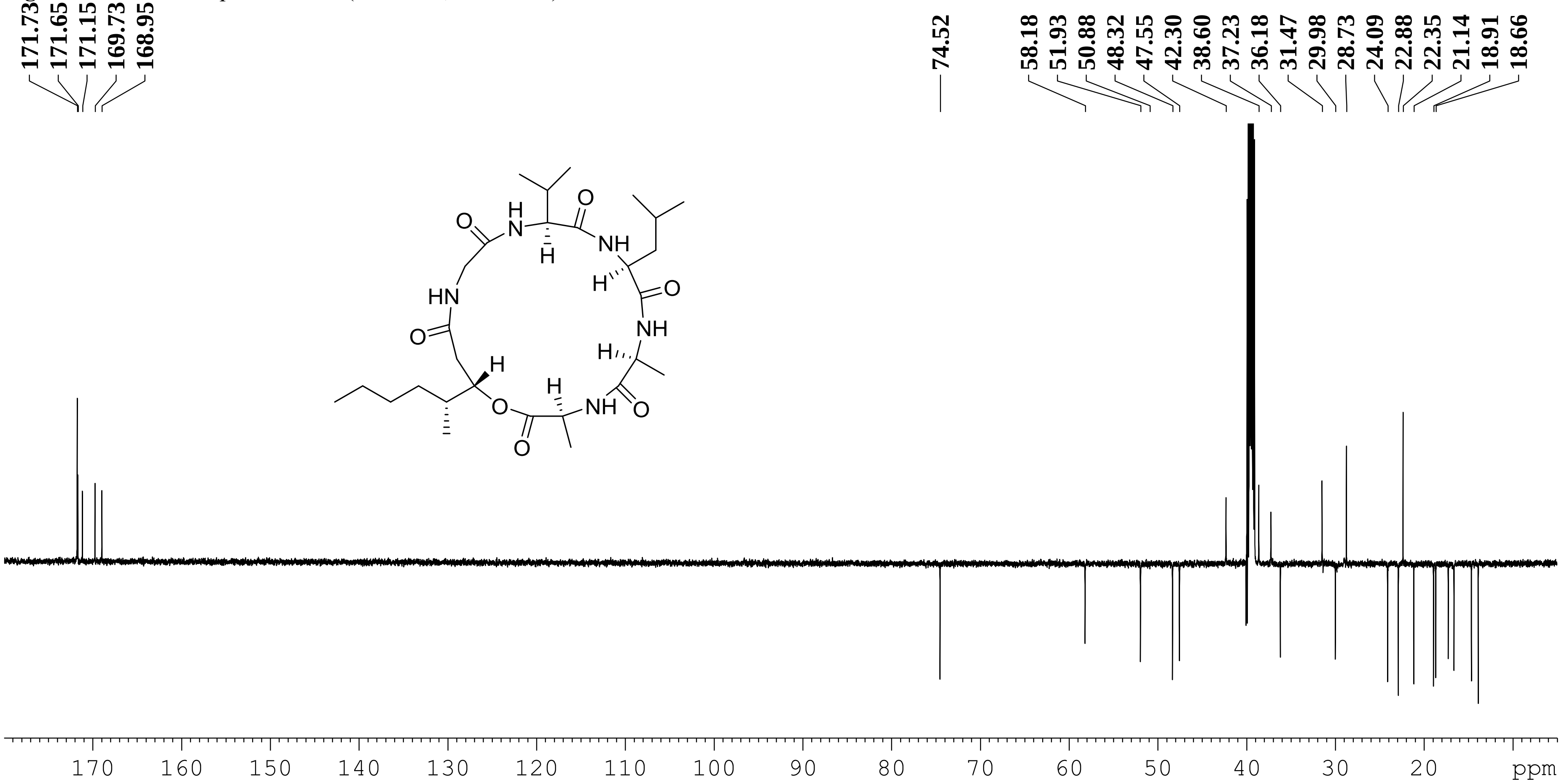


Figure S66. ^{1}H-^{1}H COSY spectrum of **7**.

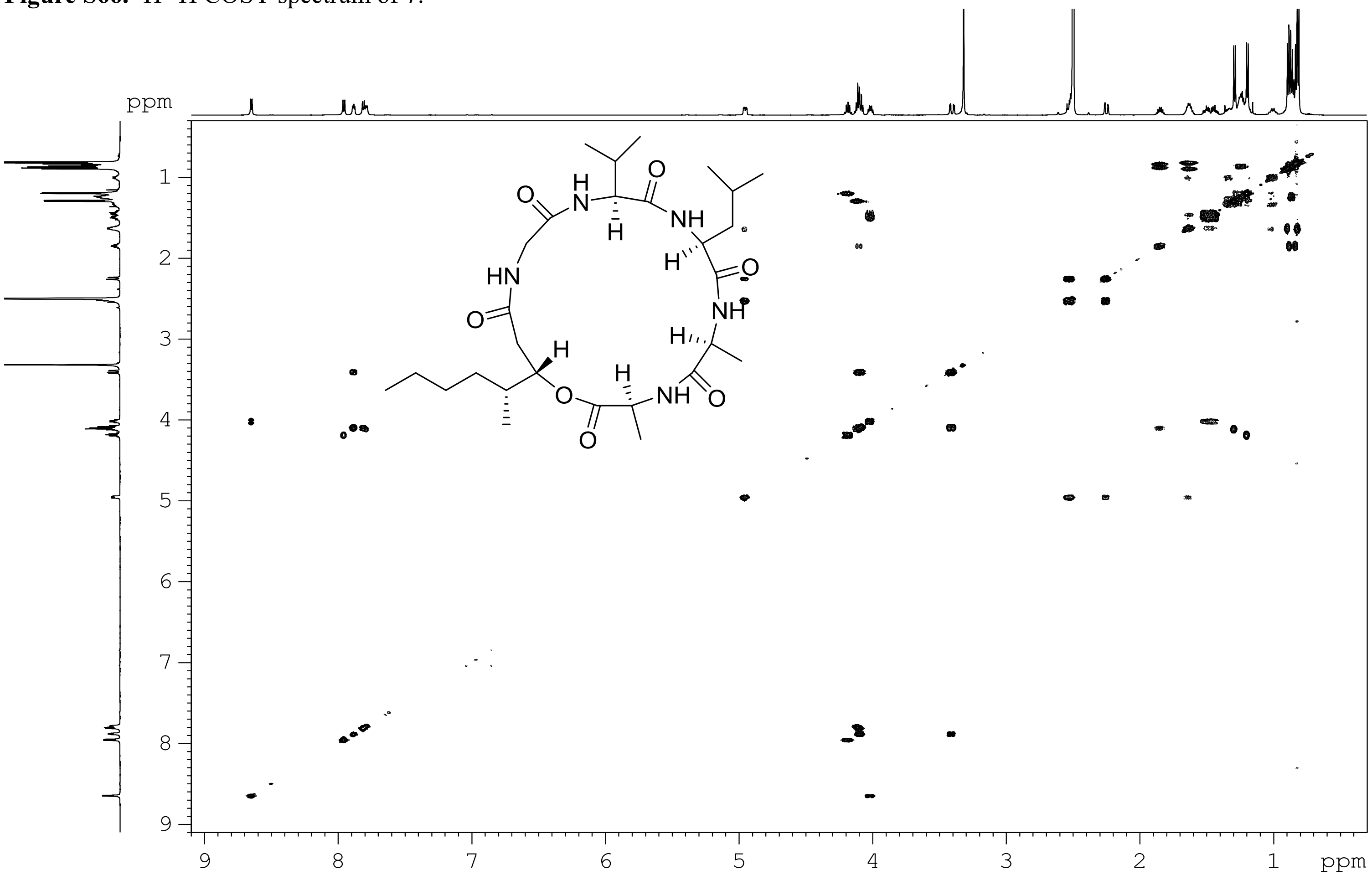


Figure S67. HSQC spectrum of **7**.

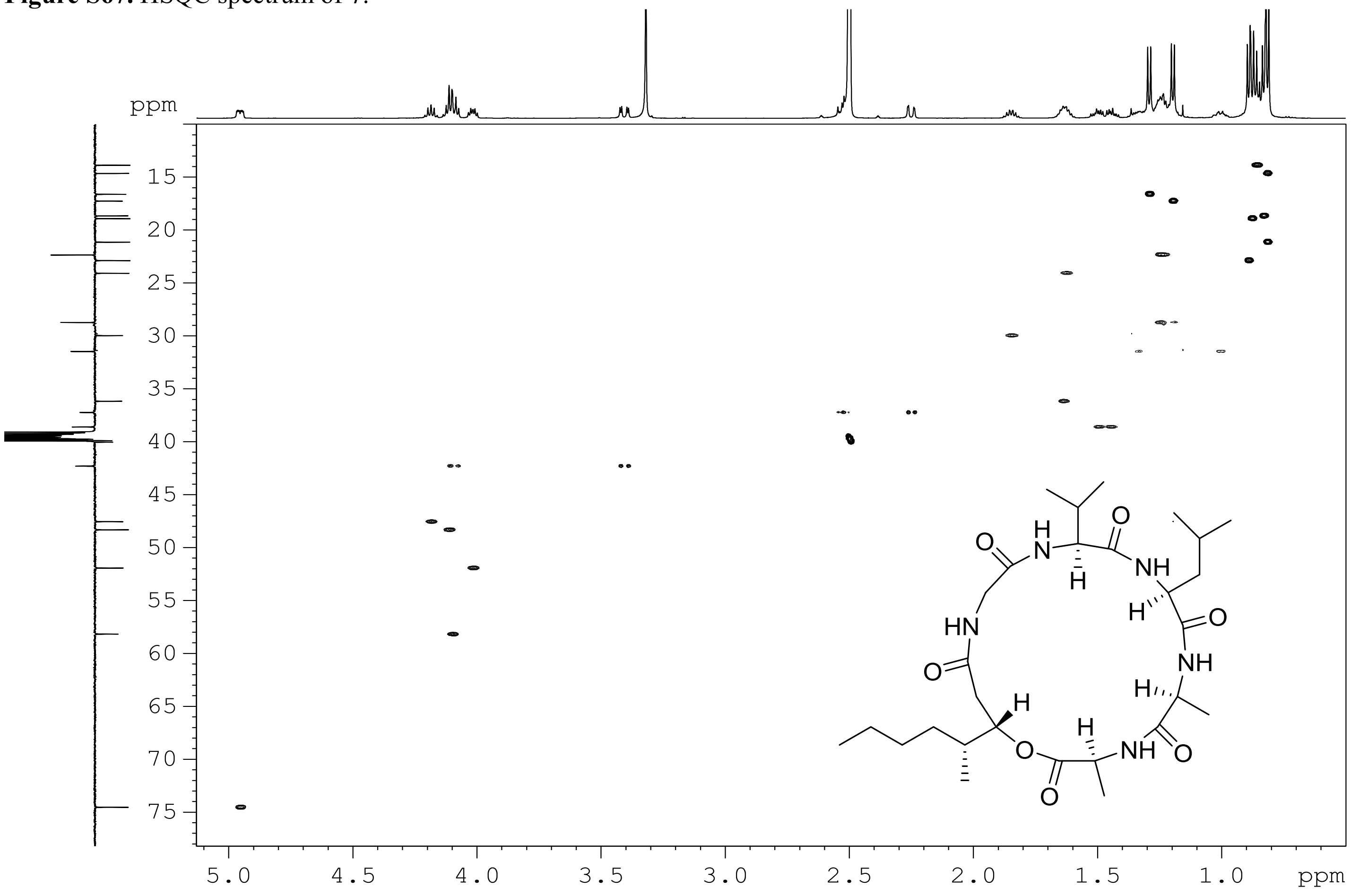


Figure S68. HMBC spectrum of **7**.

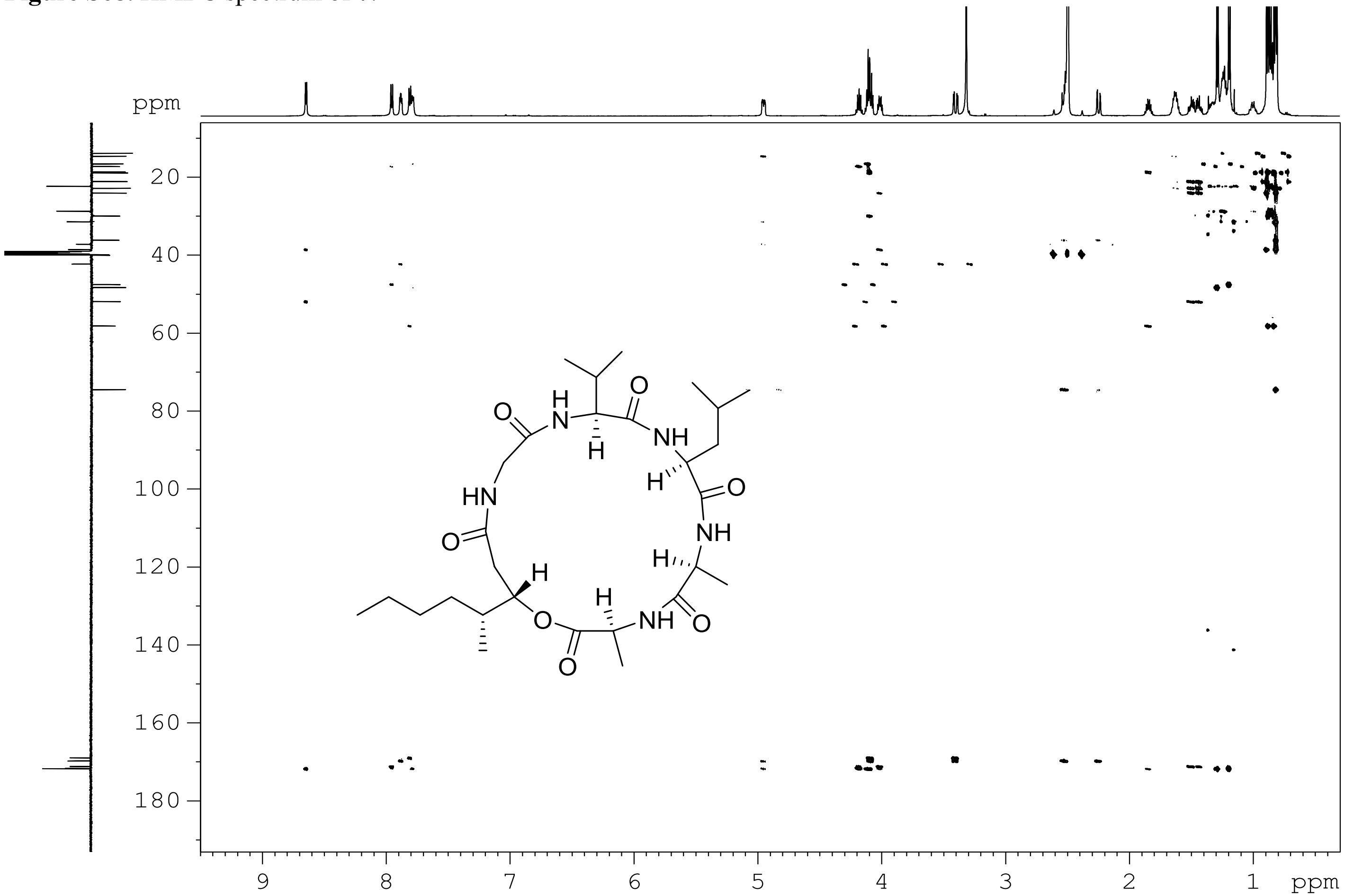


Figure S69. NOESY spectrum of **7**.

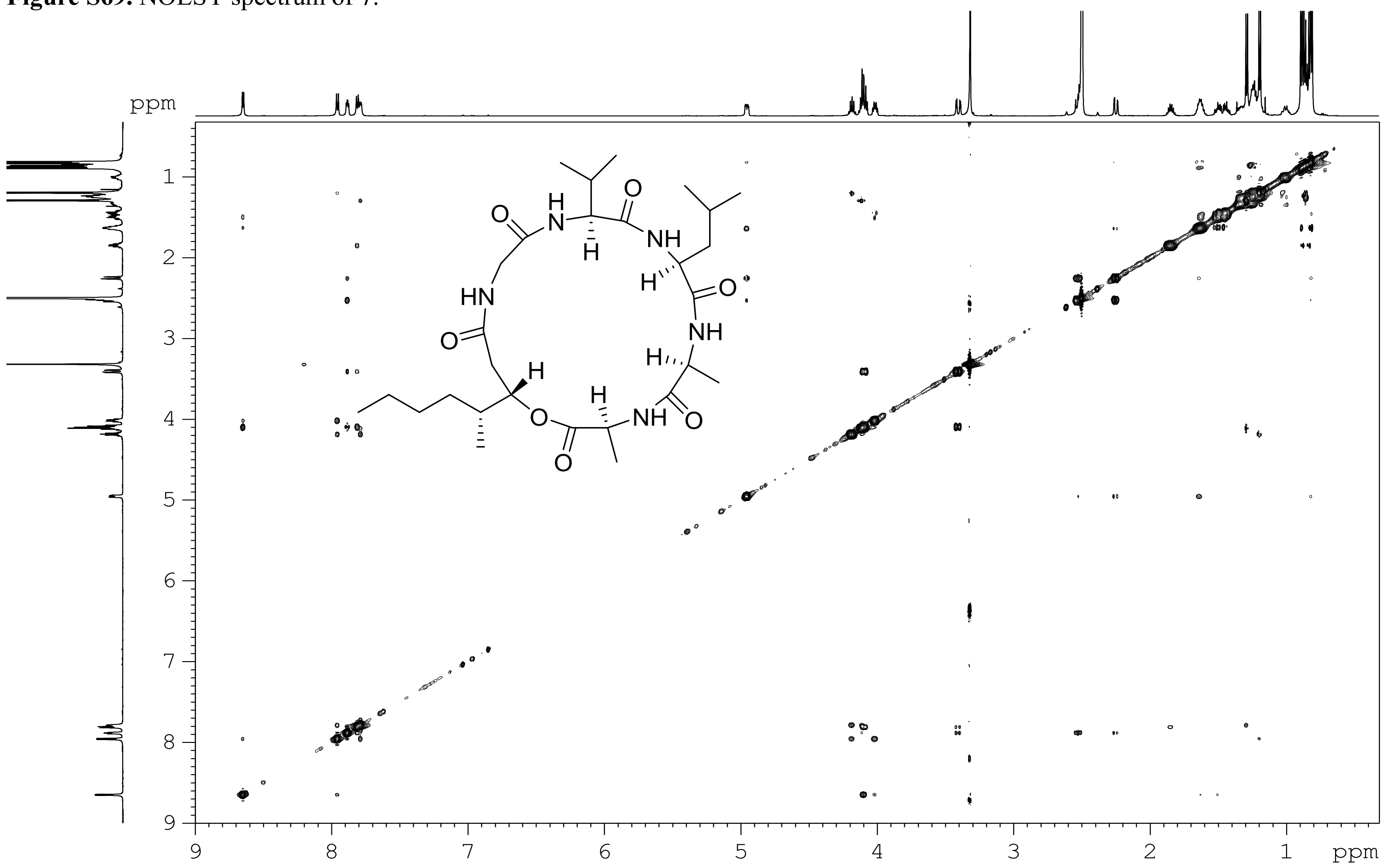


Figure S70. IR spectrum of **7**.

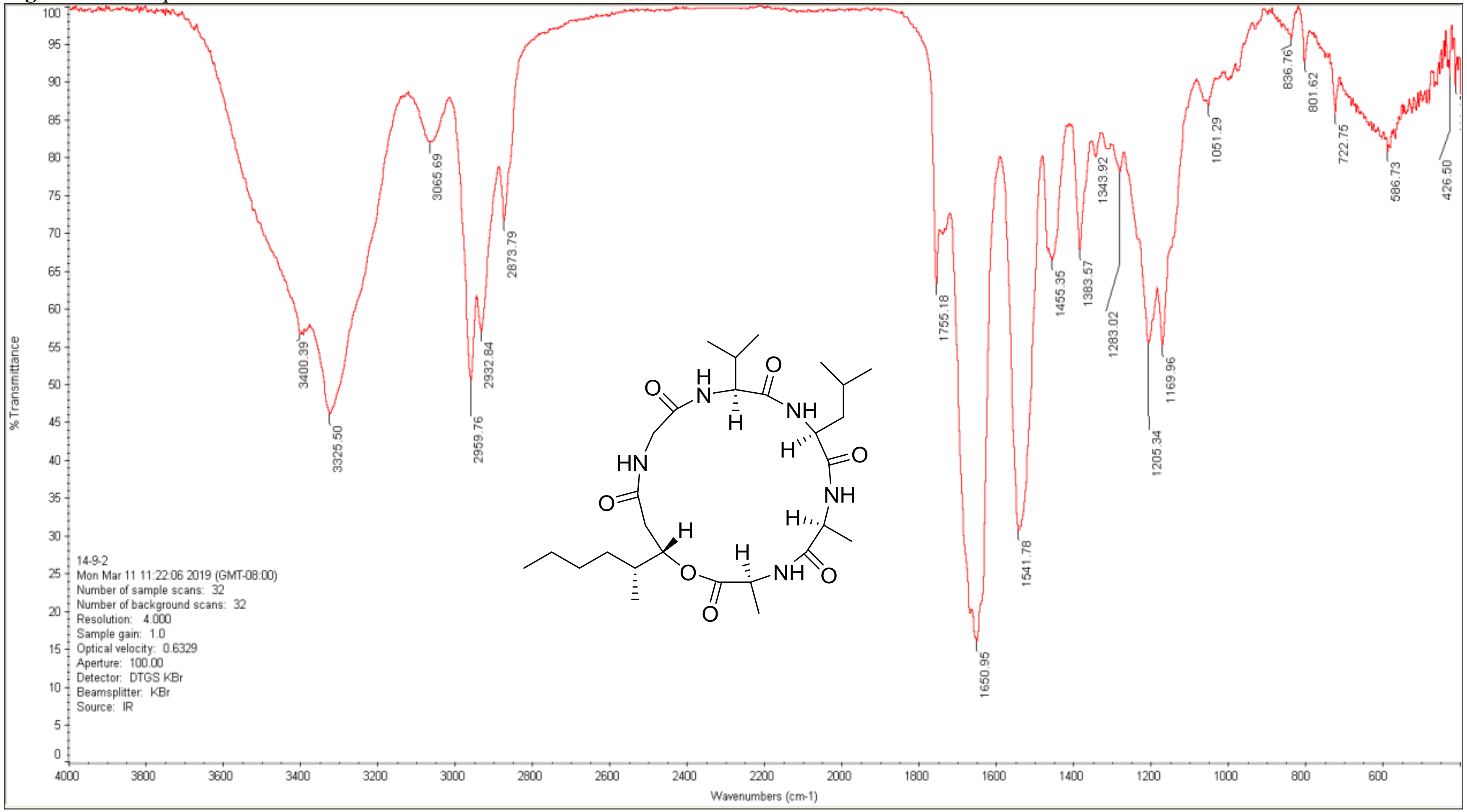
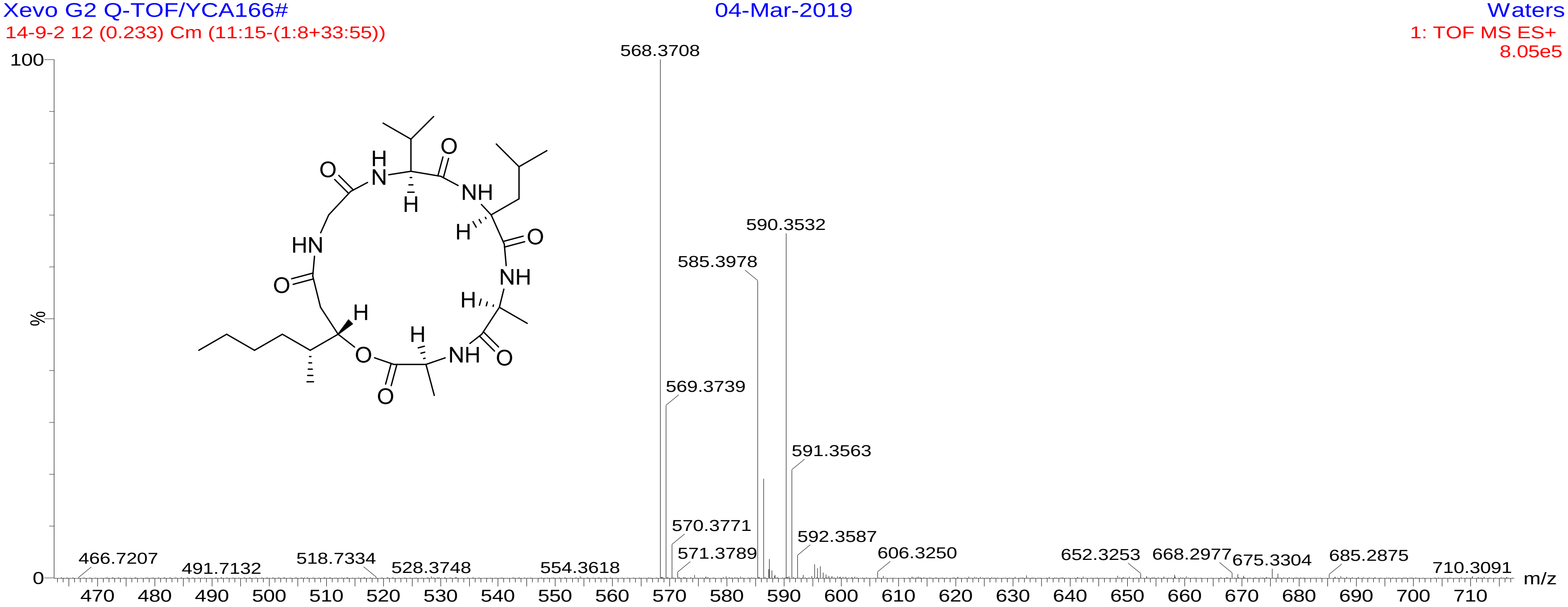


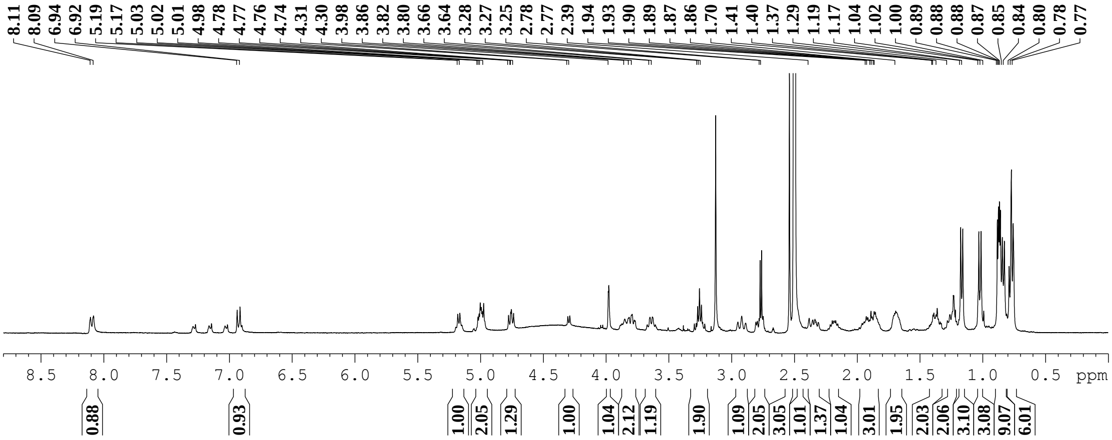
Figure S71. HRESIMS spectrum of **7**.



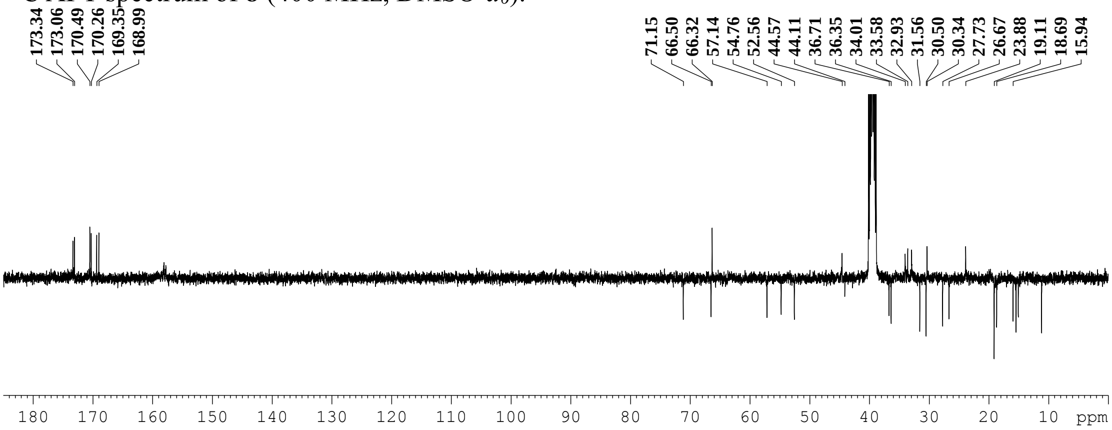
Mass	Calc.Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
568.3708	568.3710	-0.2	-0.4	6.5	250.4	0.018	98.20	$C_{28}H_{50}N_5O_7$

Figure S72. ^{1}H and ^{13}C NMR and ESIMS spectra of roseotoxin C (**8**).

^{1}H NMR spectrum of **8** (400 MHz, DMSO-d_6).



^{13}C APT spectrum of **8** (400 MHz, DMSO-d_6).



ESIMS spectrum of **8**.

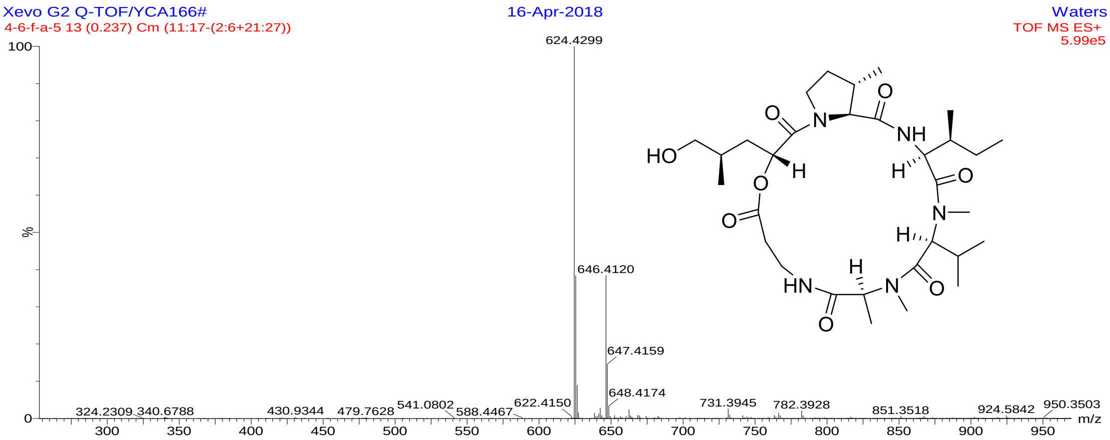
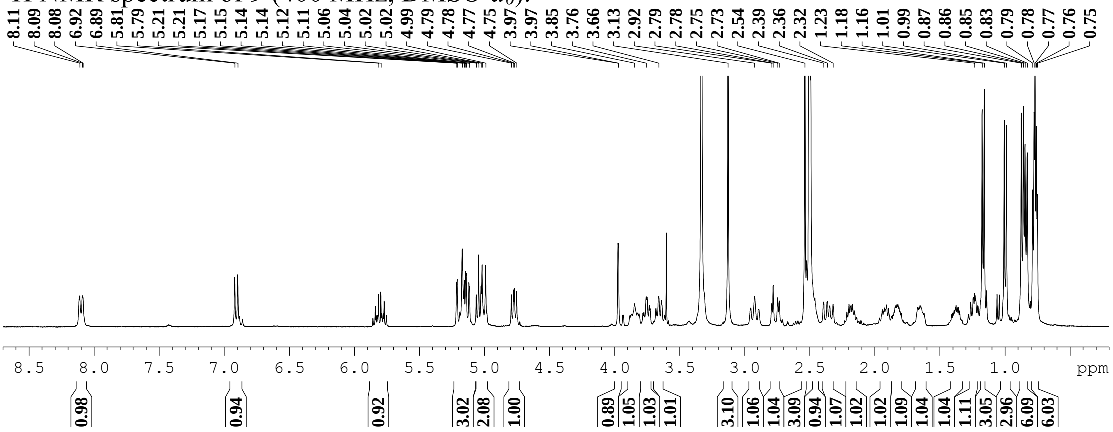
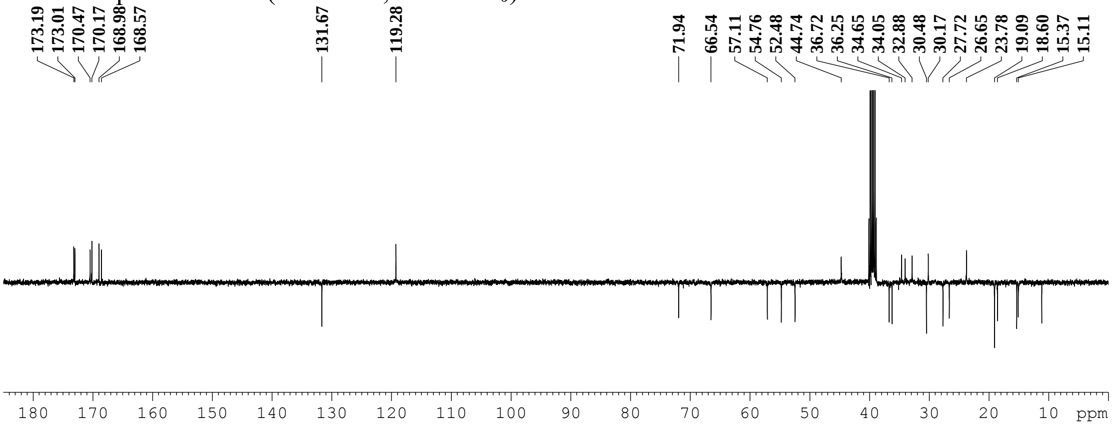


Figure S73. ^{1}H and ^{13}C NMR and ESIMS spectra of roseotoxin B (**9**).

^{1}H NMR spectrum of **9** (400 MHz, DMSO-d_6).



^{13}C APT spectrum of **9** (400 MHz, DMSO-d_6).



ESIMS spectrum of **9**.

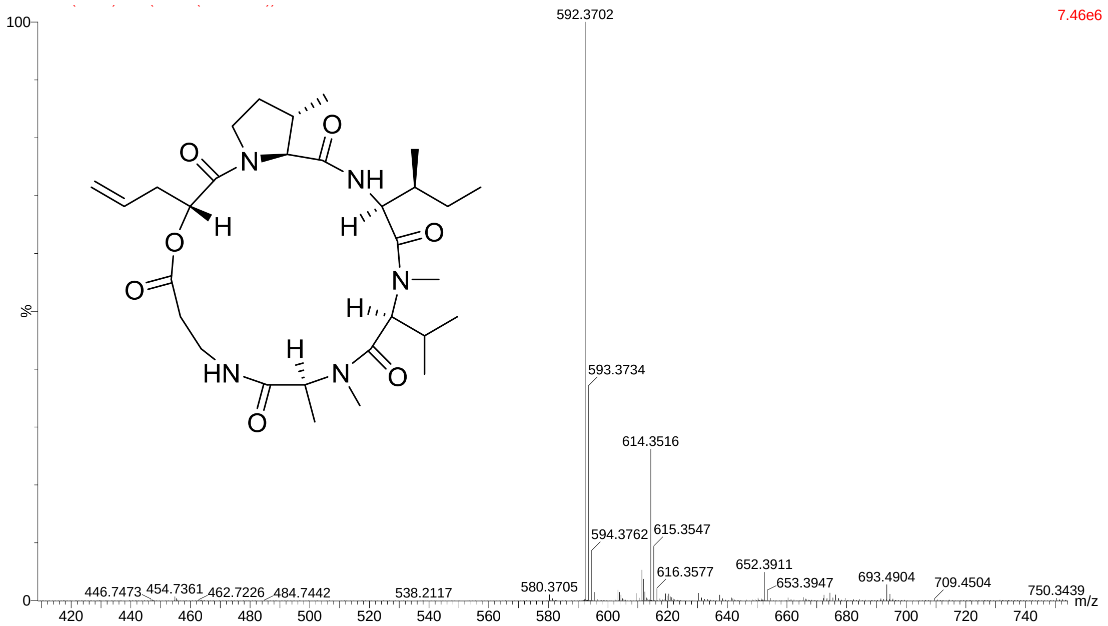
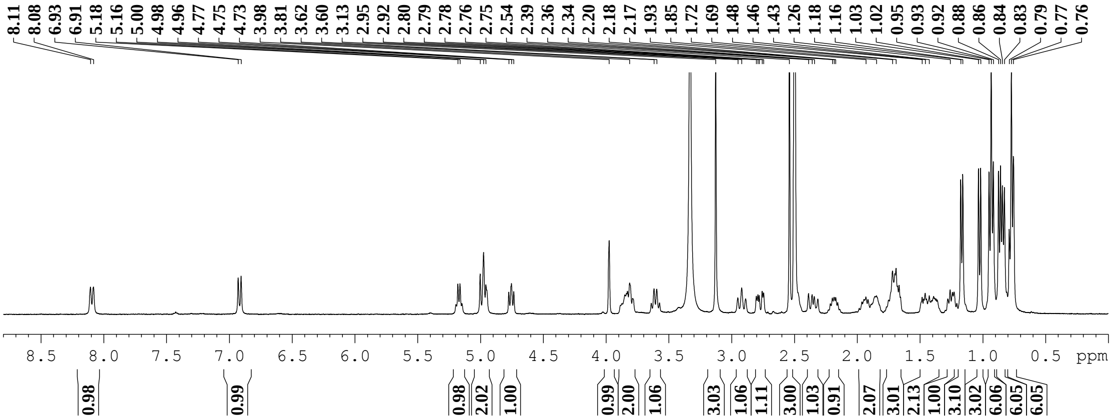
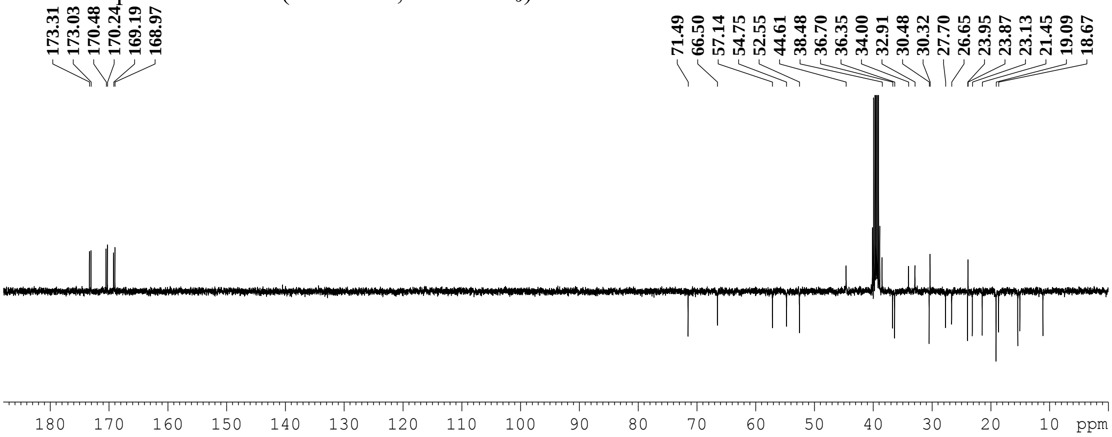


Figure S74. ^{1}H and ^{13}C NMR and ESIMS spectra of roseotoxin A (**10**)

^{1}H NMR spectrum of **10** (400 MHz, DMSO-d_6).



^{13}C APT spectrum of **10** (400 MHz, DMSO-d_6).



ESIMS spectrum of **10**.

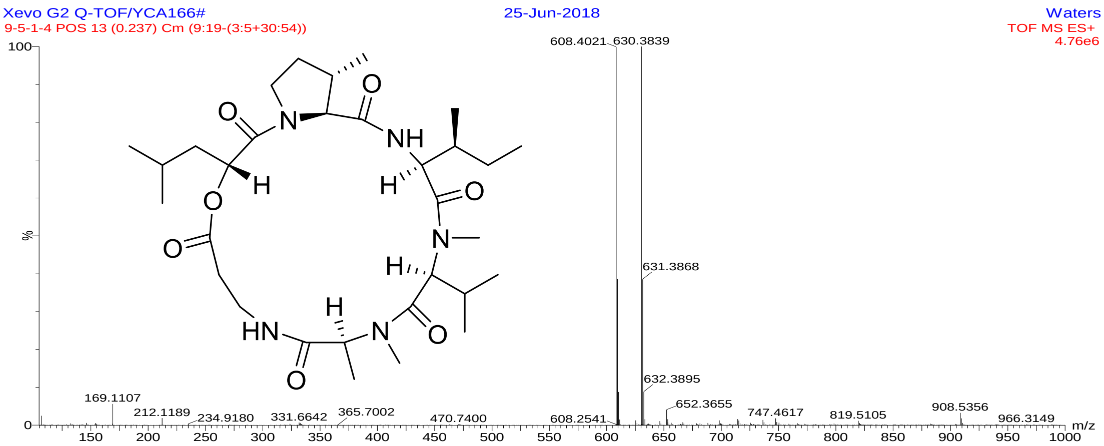
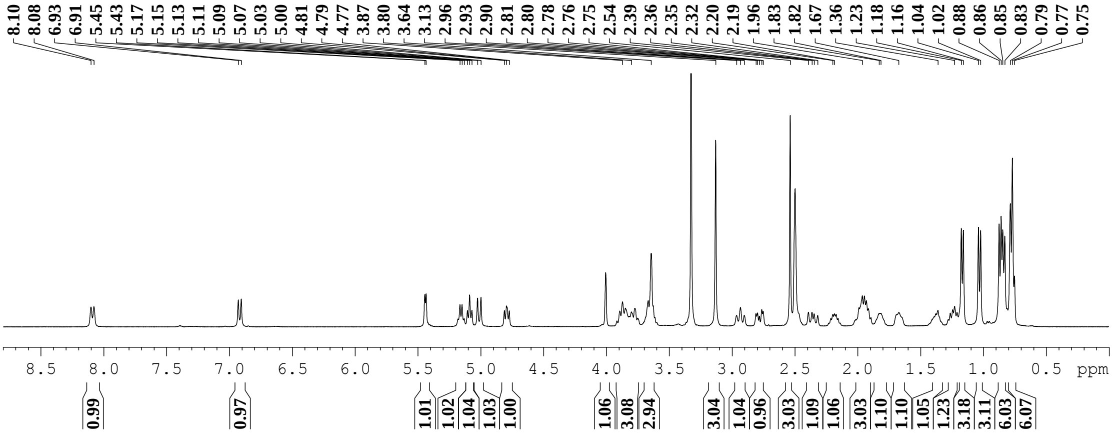
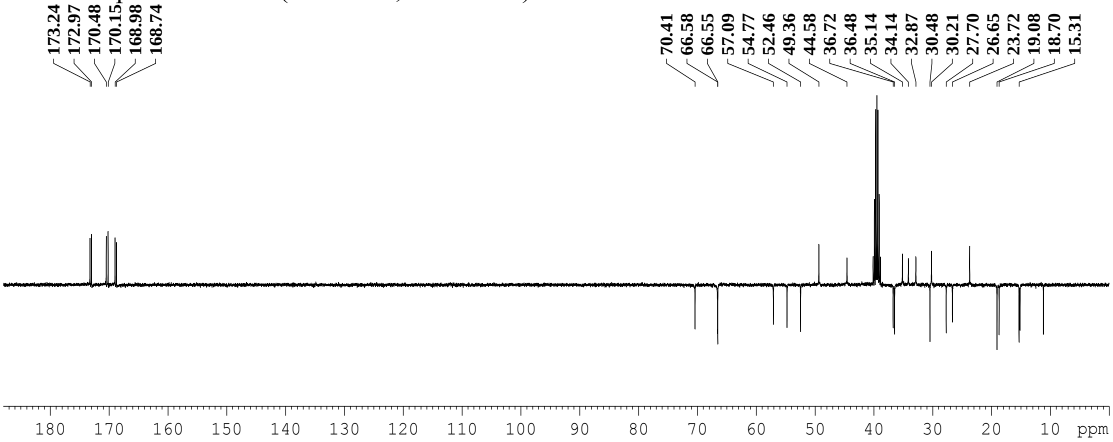


Figure S75. ^{1}H and ^{13}C NMR and ESIMS spectra of [β-MePro] destruxin E chlorohydrin (**11**).

^{1}H NMR spectrum of **11** (400 MHz, DMSO-d_6).



^{13}C APT spectrum of **11** (400 MHz, DMSO-d_6).



ESIMS spectrum of **11**.

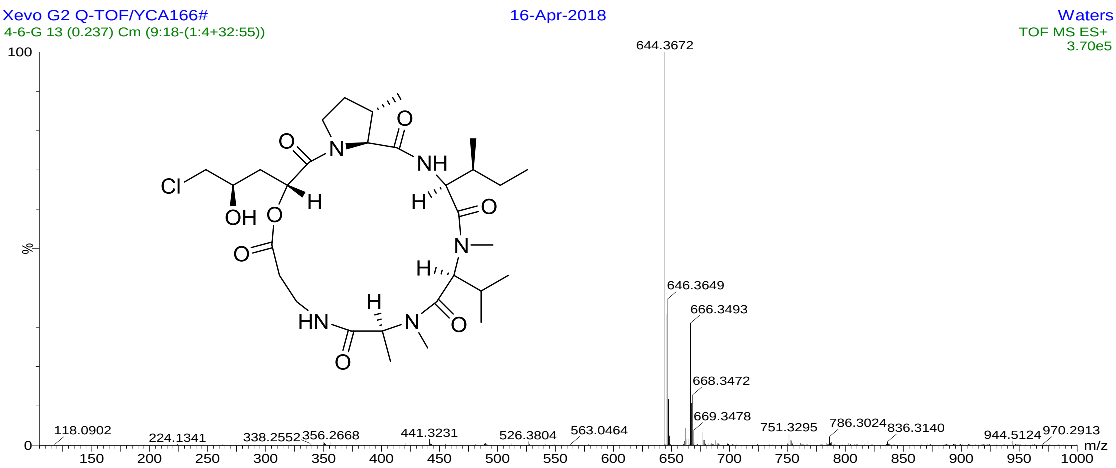
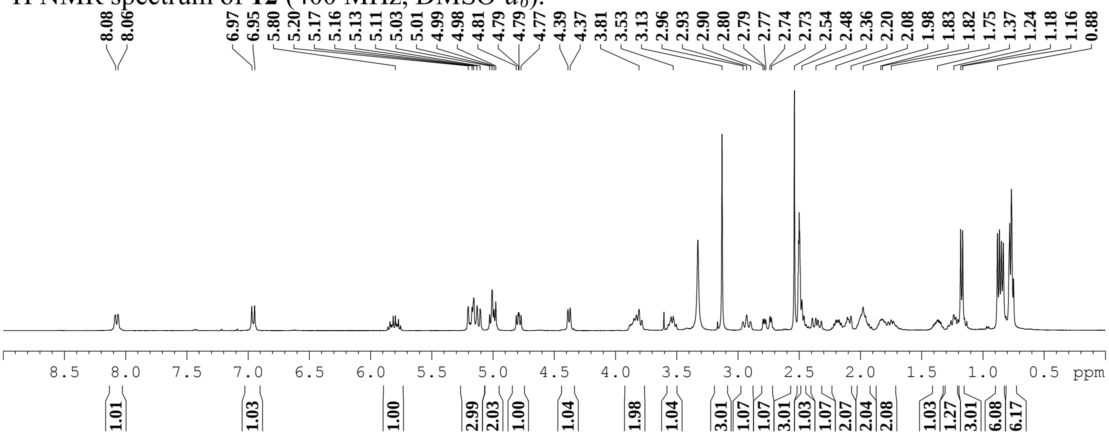
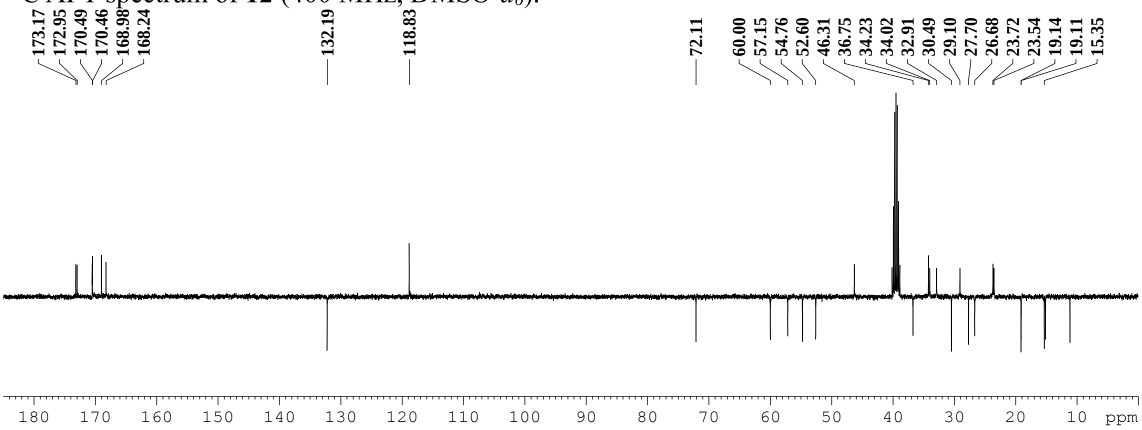


Figure S76. ^{1}H and ^{13}C NMR and ESIMS spectra of destruxin A (**12**).

^{1}H NMR spectrum of **12** (400 MHz, DMSO-d_6).



^{13}C APT spectrum of **12** (400 MHz, DMSO-d_6).



ESIMS spectrum of **12**.

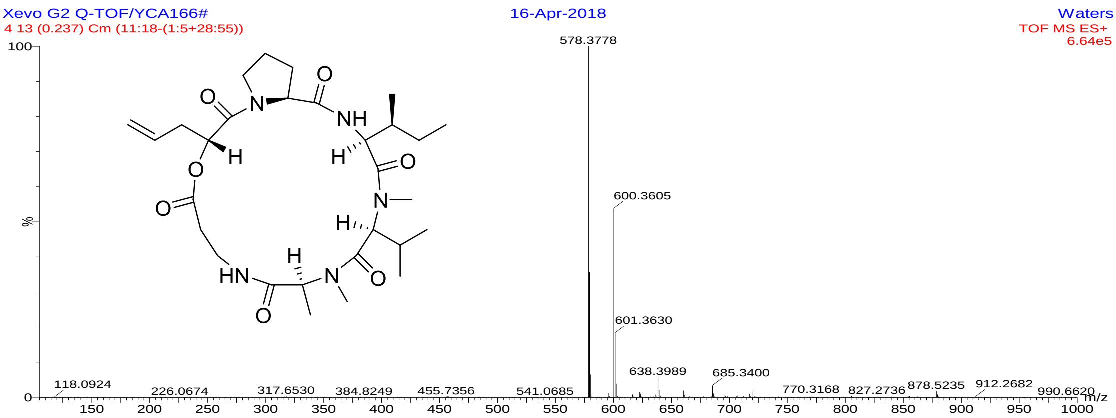
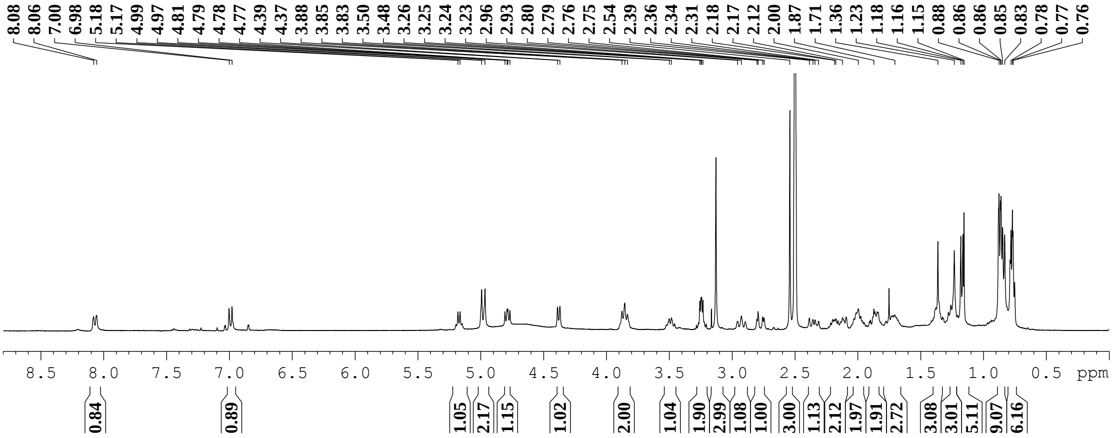
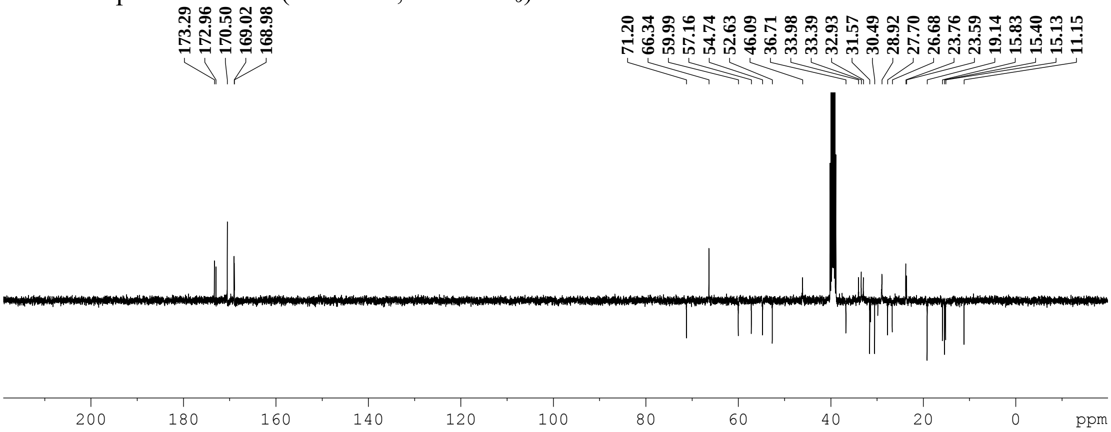


Figure S77. [1]H and [13]C NMR and ESIMS spectra of destruxin C (**13**).

[1]H NMR spectrum of **13** (400 MHz, DMSO-d_6).



[13]C APT spectrum of **13** (400 MHz, DMSO-d_6).



ESIMS spectrum of **13**.

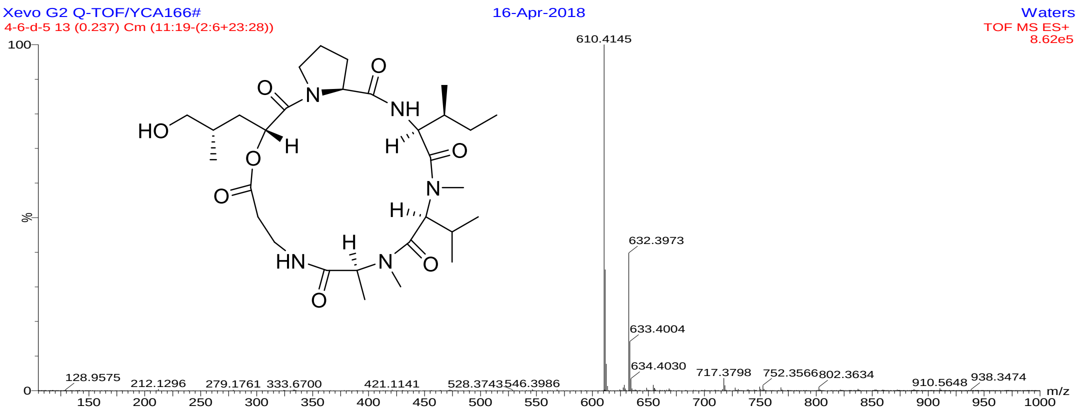
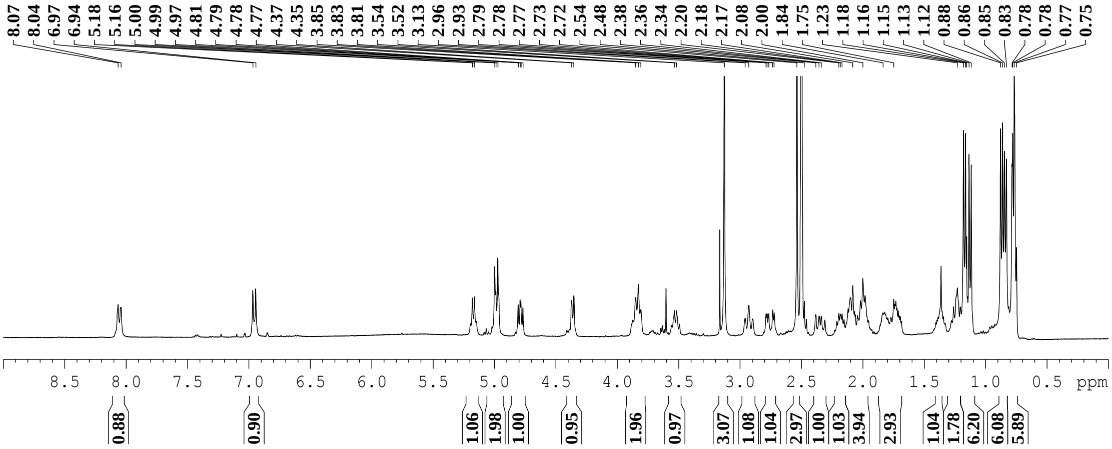
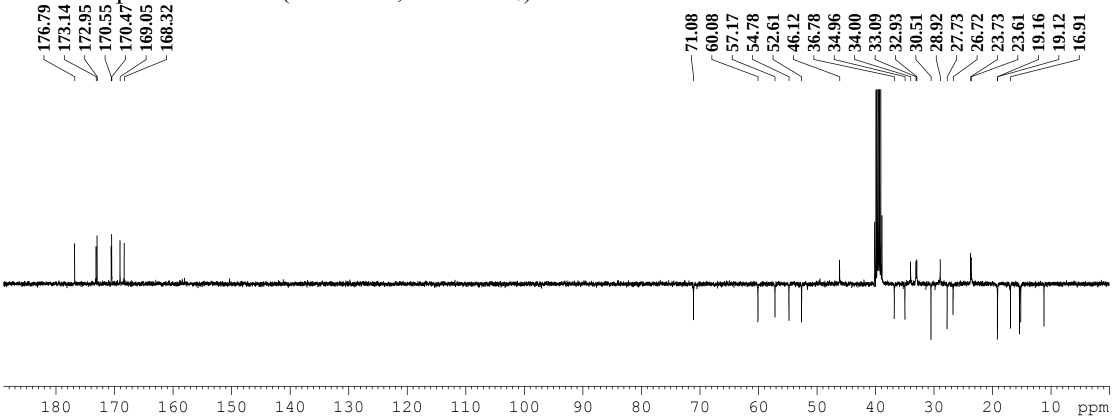


Figure S78. ^{1}H and ^{13}C NMR and ESIMS spectra of destruxin D (**14**)

^{1}H NMR spectrum of **14** (400 MHz, DMSO-d_6).



^{13}C APT spectrum of **14** (400 MHz, DMSO-d_6).



ESIMS spectrum of **14**.

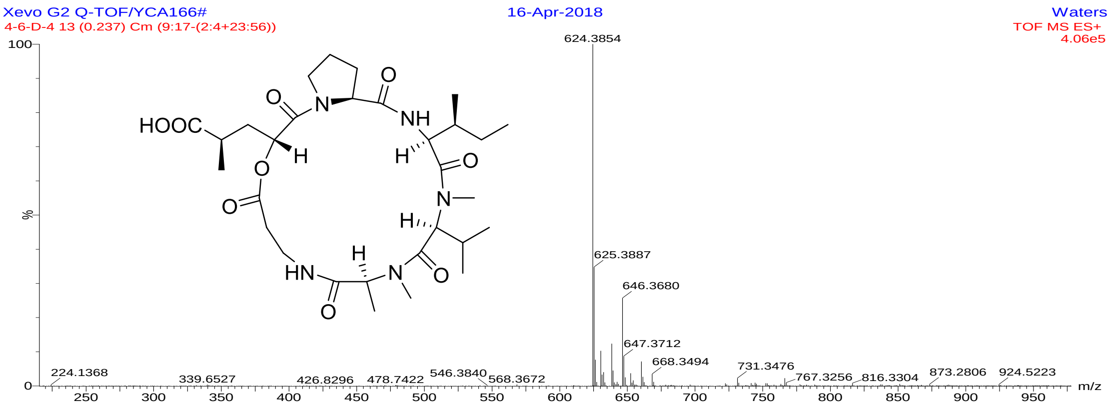
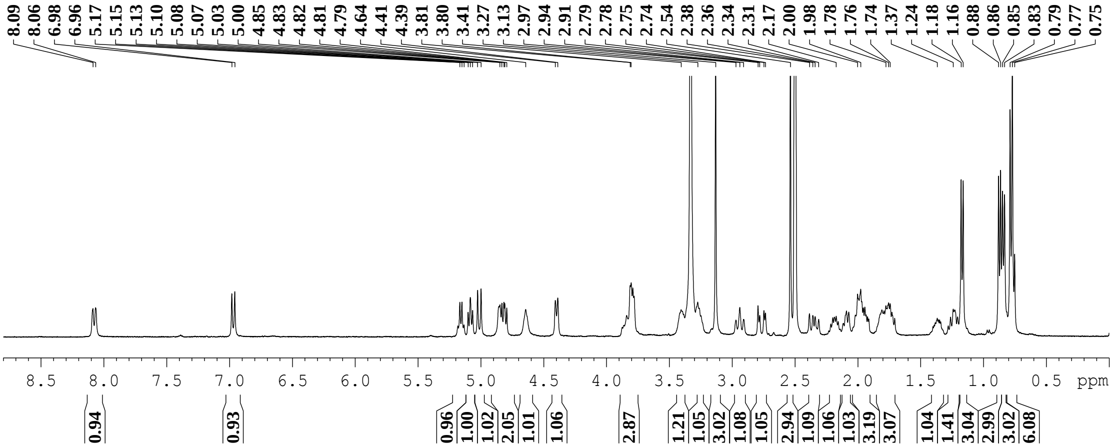
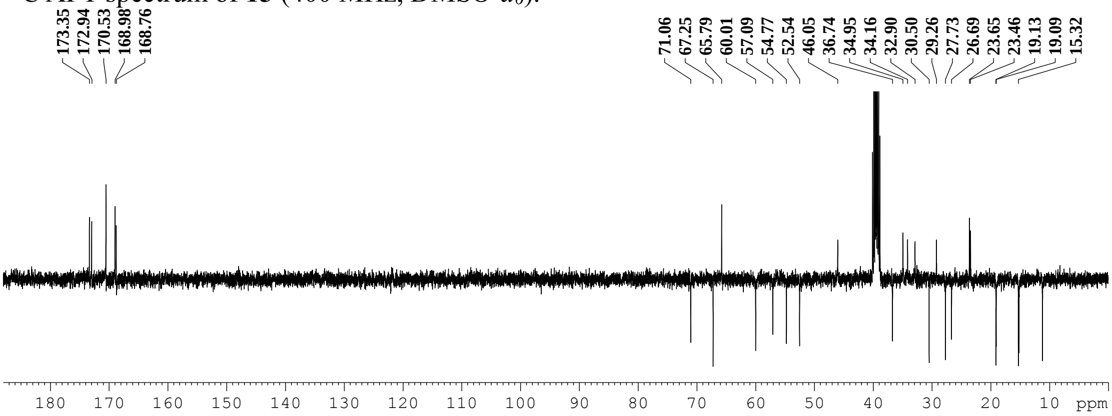


Figure S79. ^{1}H and ^{13}C NMR and ESIMS spectra of destruxin Ed (**15**).

^{1}H NMR spectrum of **15** (400 MHz, DMSO-d_6).



^{13}C APT spectrum of **15** (400 MHz, DMSO-d_6).



ESIMS spectrum of **15**.

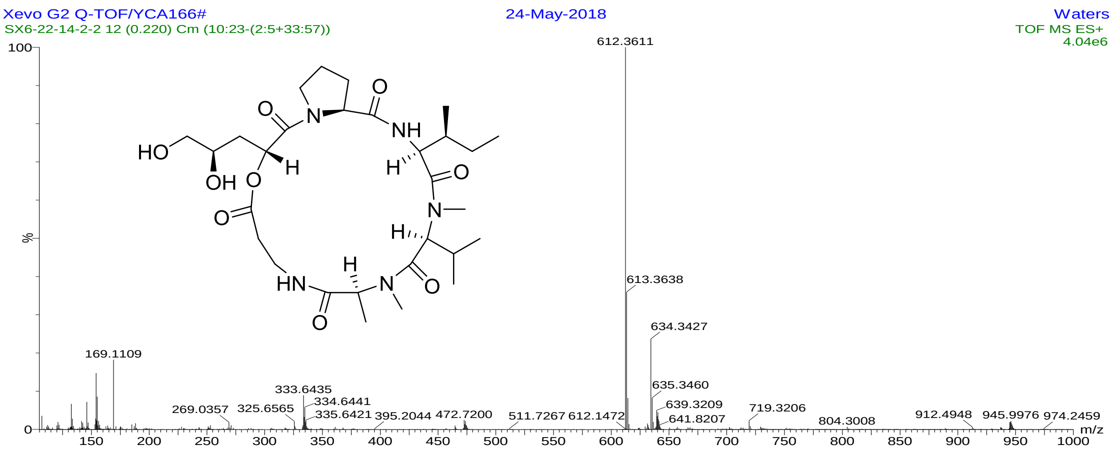
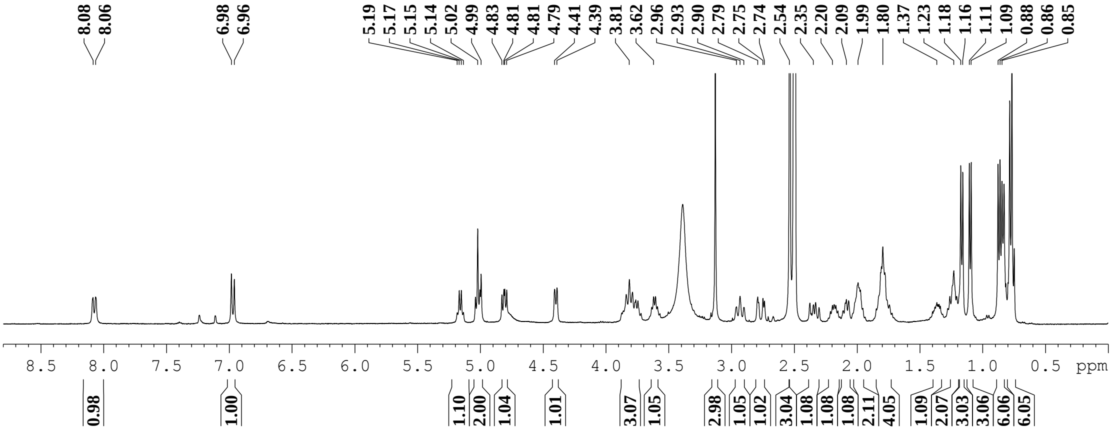
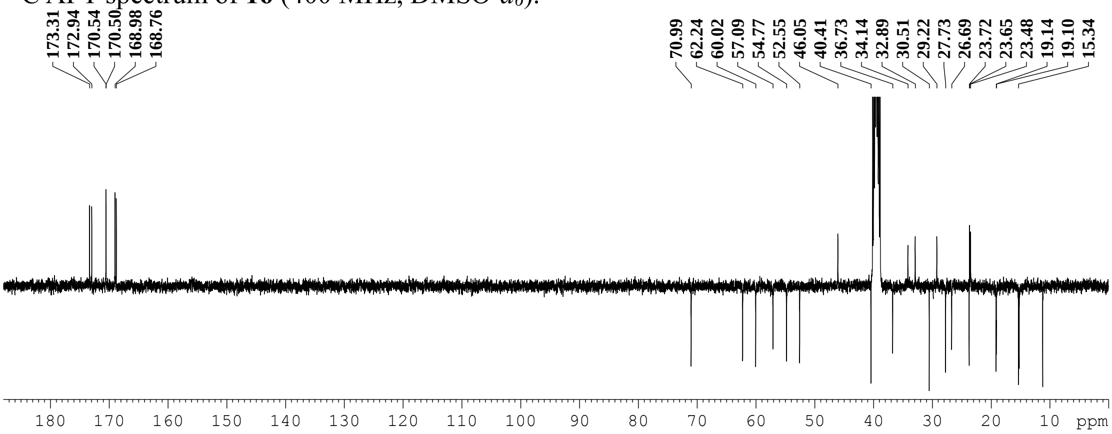


Figure S80. ^{1}H and ^{13}C NMR and ESIMS spectra of destruxin F (**16**).

^{1}H NMR spectrum of **16** (400 MHz, DMSO-d_6).



^{13}C APT spectrum of **16** (400 MHz, DMSO-d_6).



ESIMS spectrum of **16**.

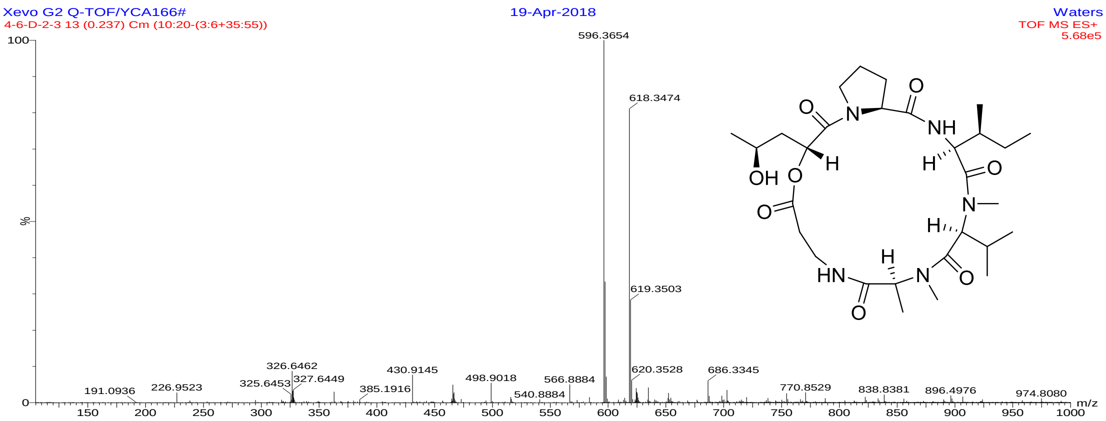
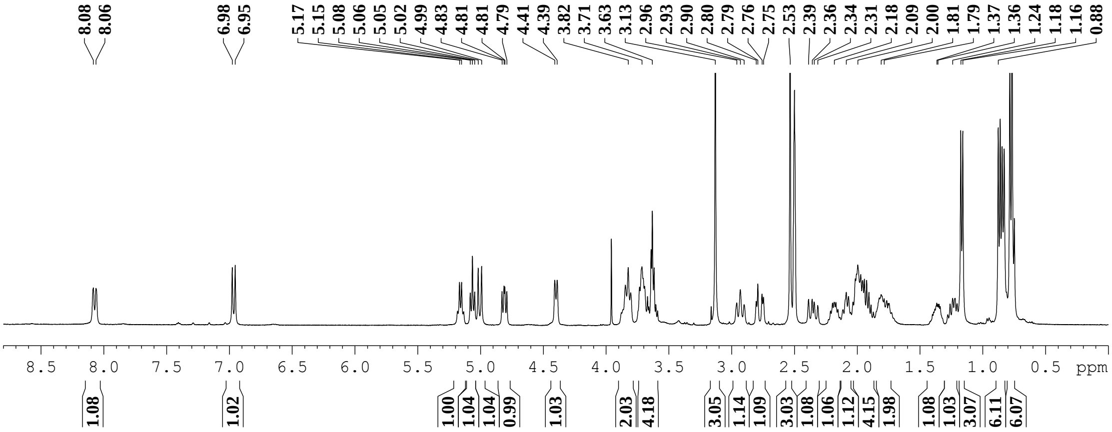
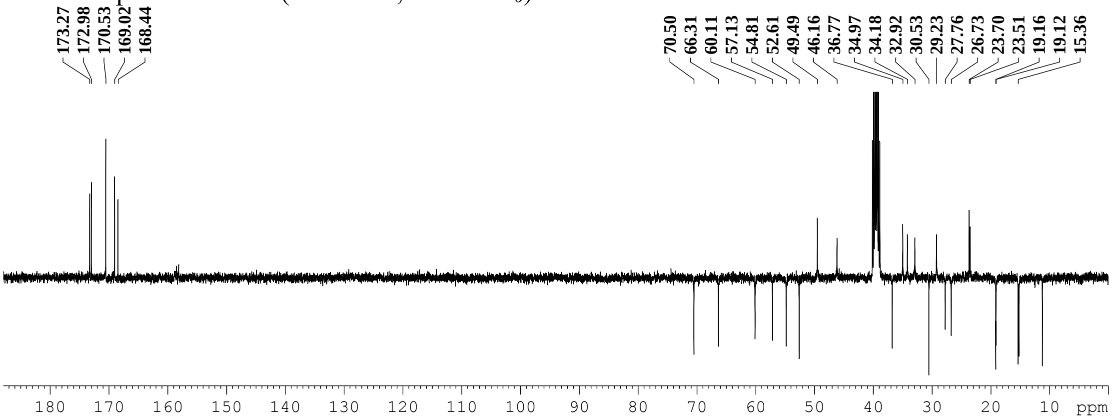


Figure S81. ^{1}H and ^{13}C NMR and ESIMS spectra of destruxin Chl (**17**).

^{1}H NMR spectrum of **17** (400 MHz, DMSO-d_6).



^{13}C APT spectrum of **17** (400 MHz, DMSO-d_6).



ESIMS spectrum of **17**.

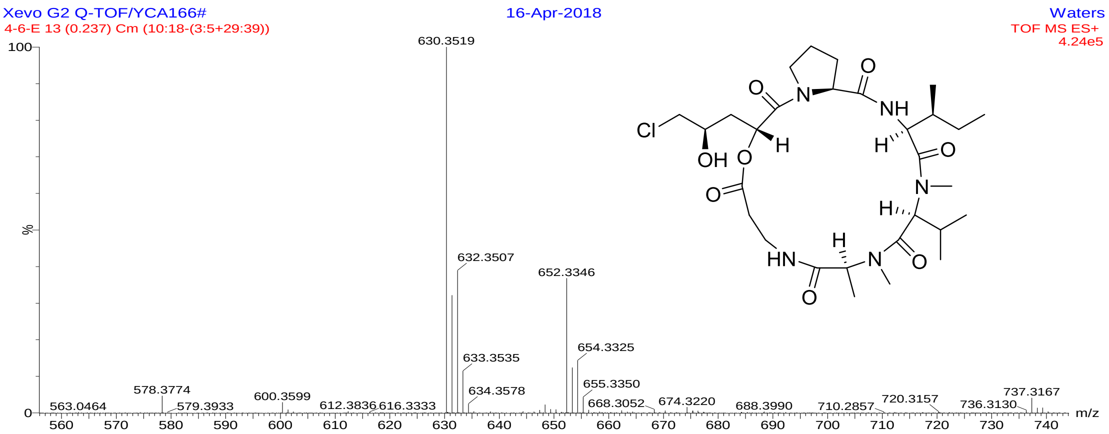
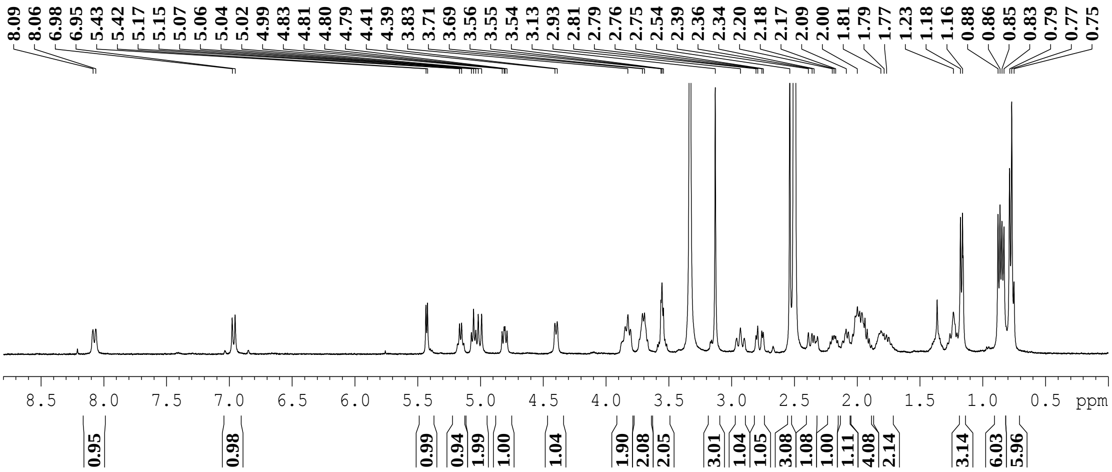
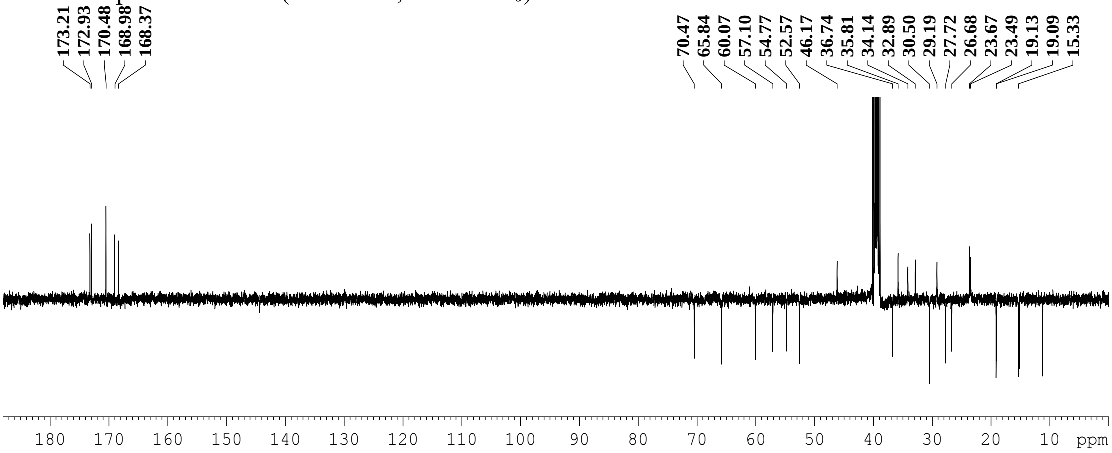


Figure S82. ^{1}H and ^{13}C NMR and ESIMS spectra of destruxin Brh (**18**).

^{1}H NMR spectrum of **18** (400 MHz, DMSO-d_6).



^{13}C APT spectrum of **18** (400 MHz, DMSO-d_6).



ESIMS spectrum of **18**.

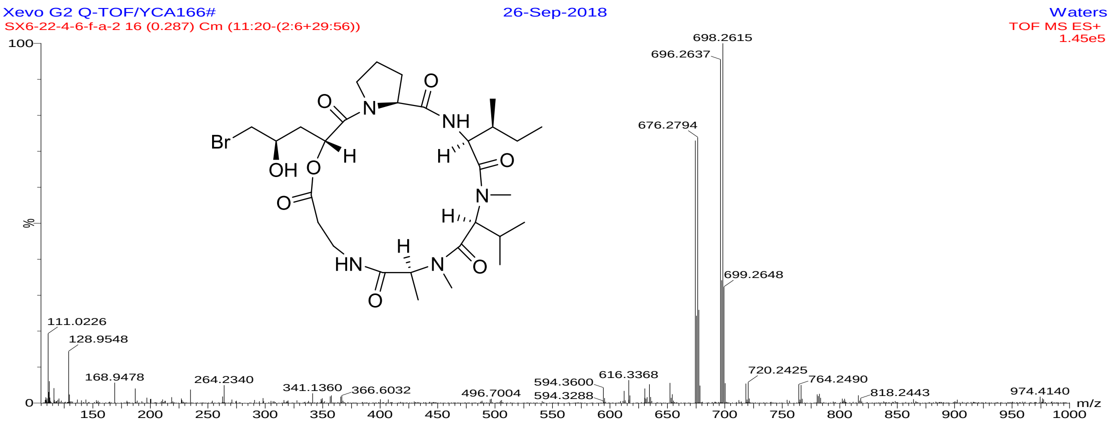
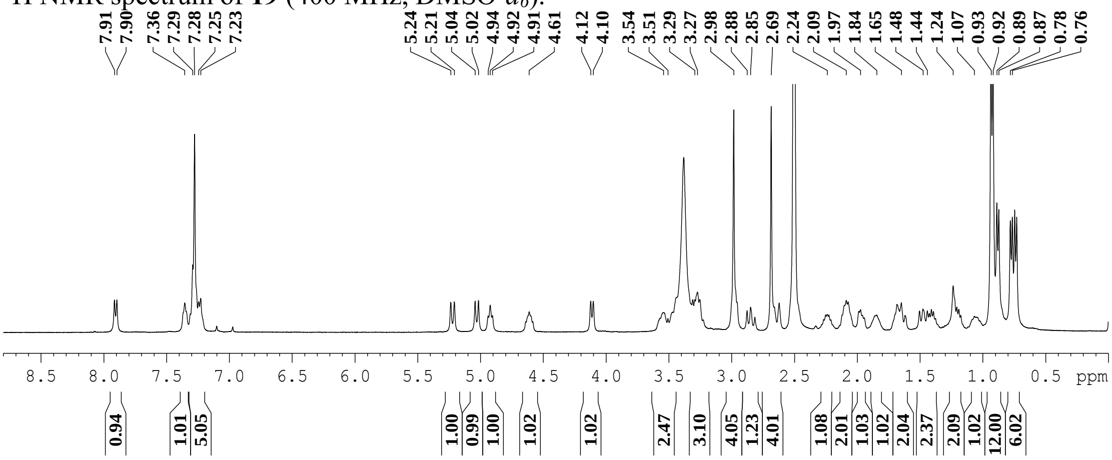
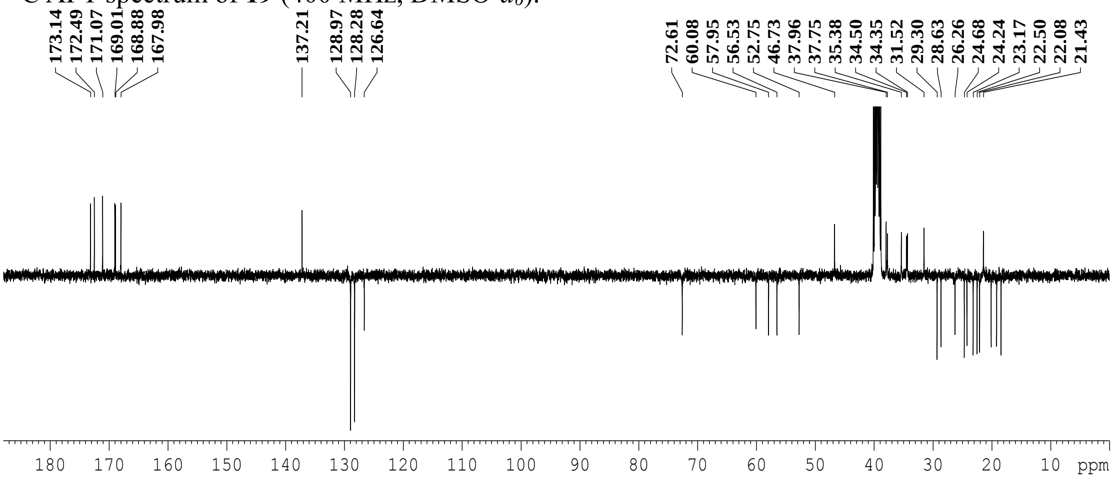


Figure S83. ^{1}H and ^{13}C NMR and ESIMS spectra of isaridin C (**19**).

^{1}H NMR spectrum of **19** (400 MHz, DMSO-d_6).



^{13}C APT spectrum of **19** (400 MHz, DMSO-d_6).



ESIMS spectrum of **19**.

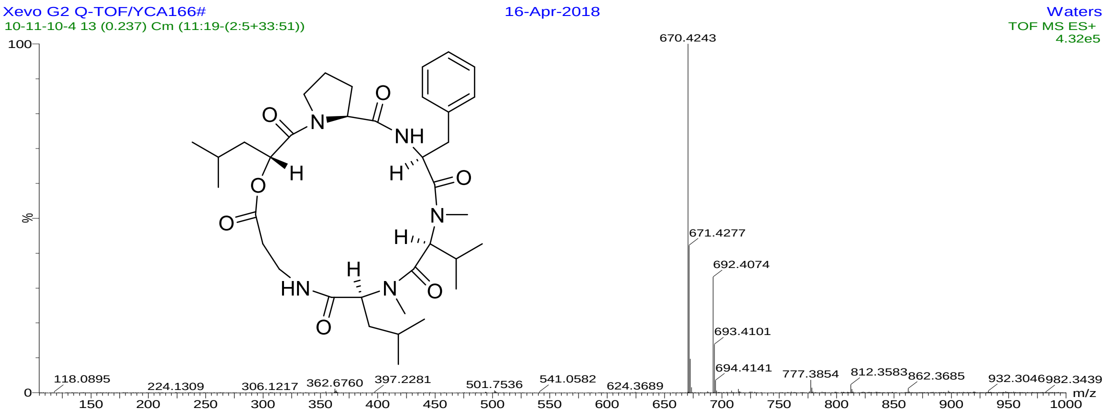
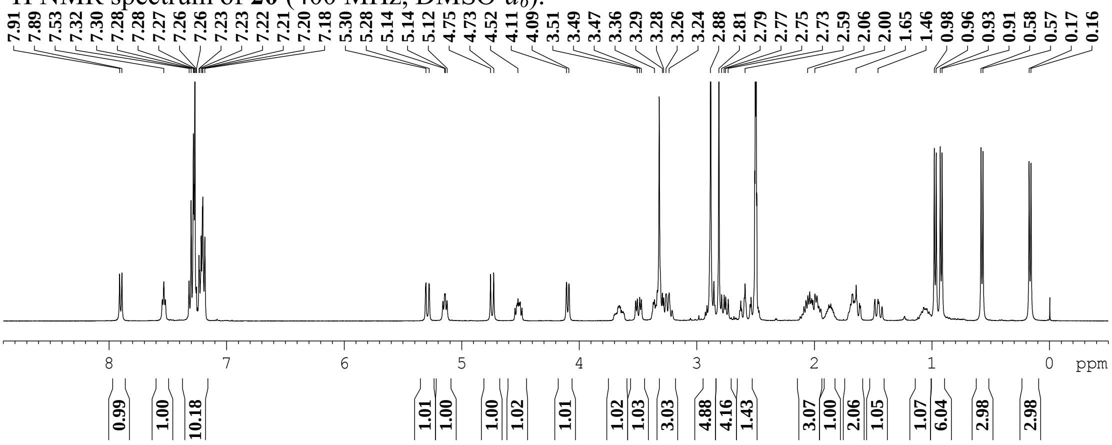
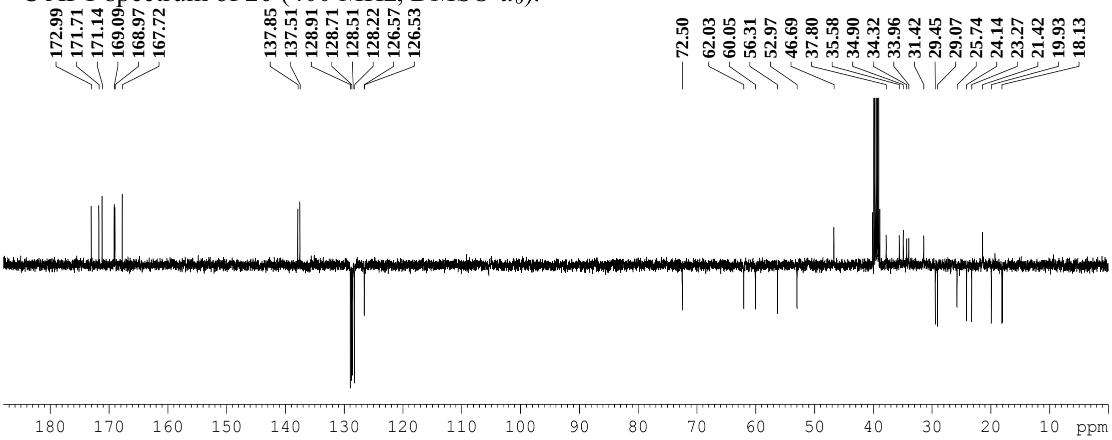


Figure S84. ^{1}H and ^{13}C NMR and ESIMS spectra of isaridin A (**20**).

^{1}H NMR spectrum of **20** (400 MHz, DMSO-d_6).



^{13}C APT spectrum of **20** (400 MHz, DMSO-d_6).



ESIMS spectrum of **20**.

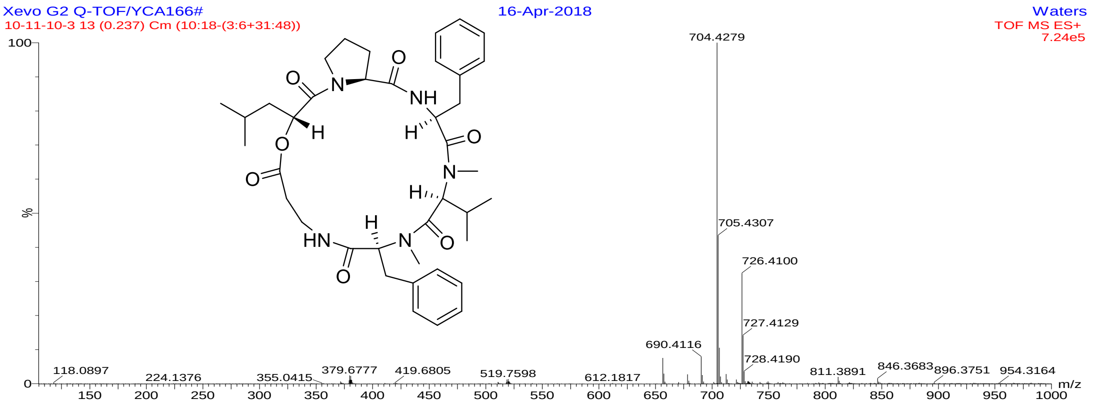
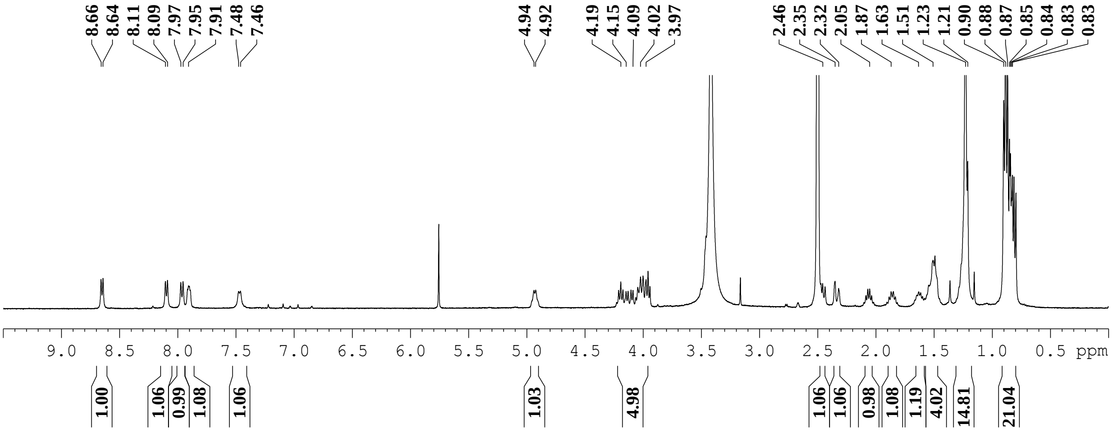
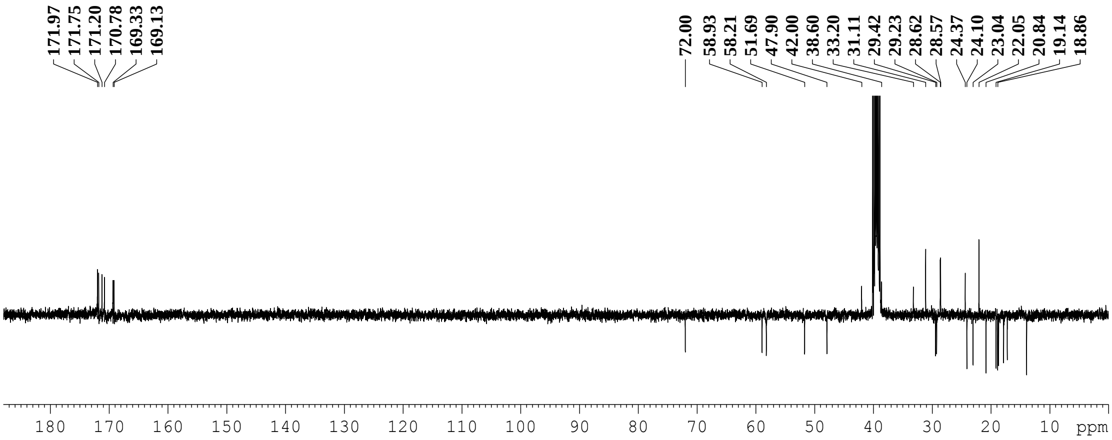


Figure S85. ^{1}H and ^{13}C NMR and ESIMS spectra of isariin G1 (**21**).

^{1}H NMR spectrum of **21** (400 MHz, DMSO-d_6).



APT spectrum of **21** (400 MHz, DMSO-d_6).



ESIMS spectrum of **21**.

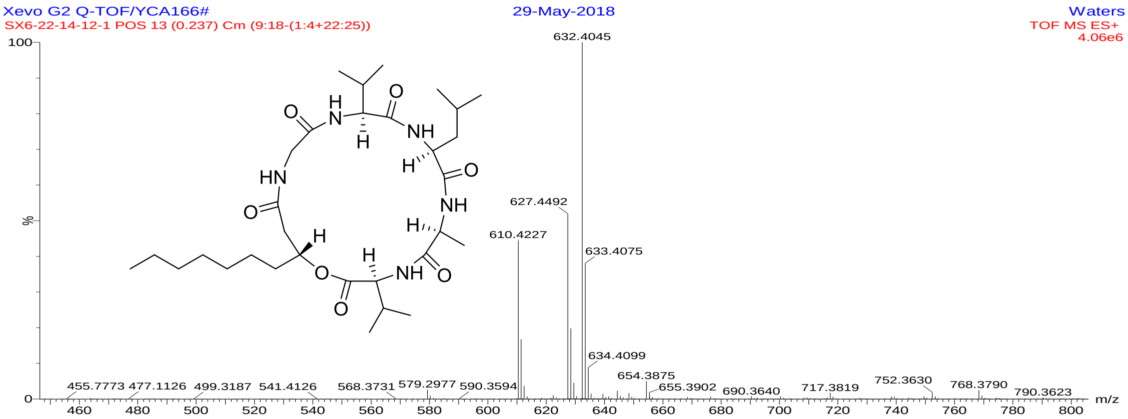
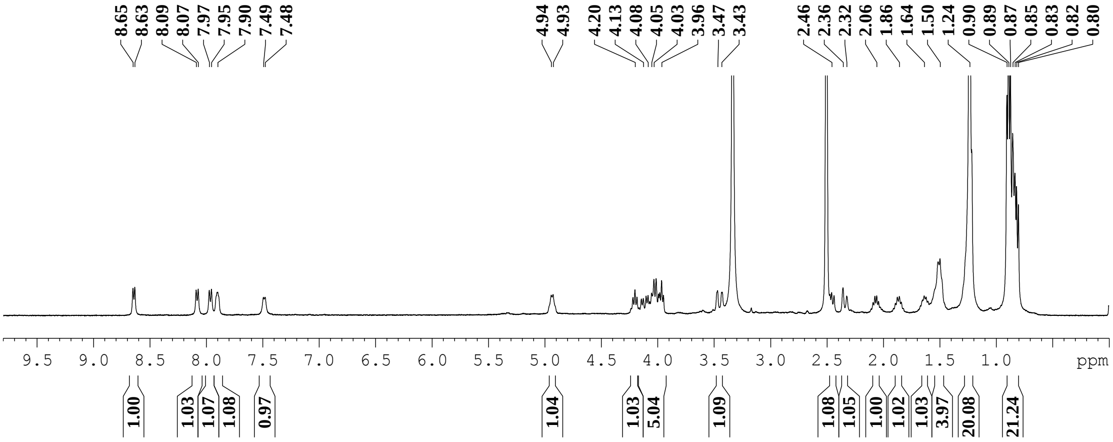
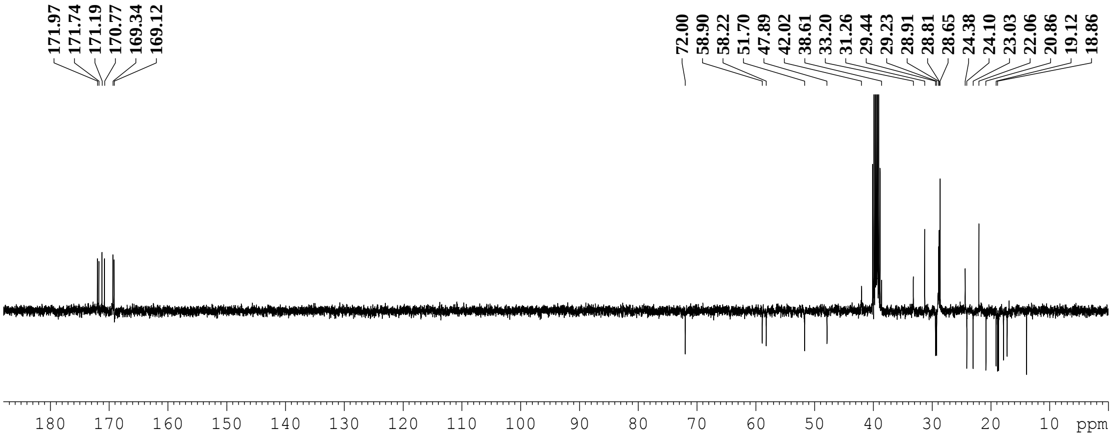


Figure S86. ^{1}H and ^{13}C NMR and ESIMS spectra of isariin G2 (**22**).

^{1}H NMR spectrum of **22** (400 MHz, DMSO-d_6).



^{13}C APT spectrum of **22** (400 MHz, DMSO-d_6).



ESIMS of **22**.

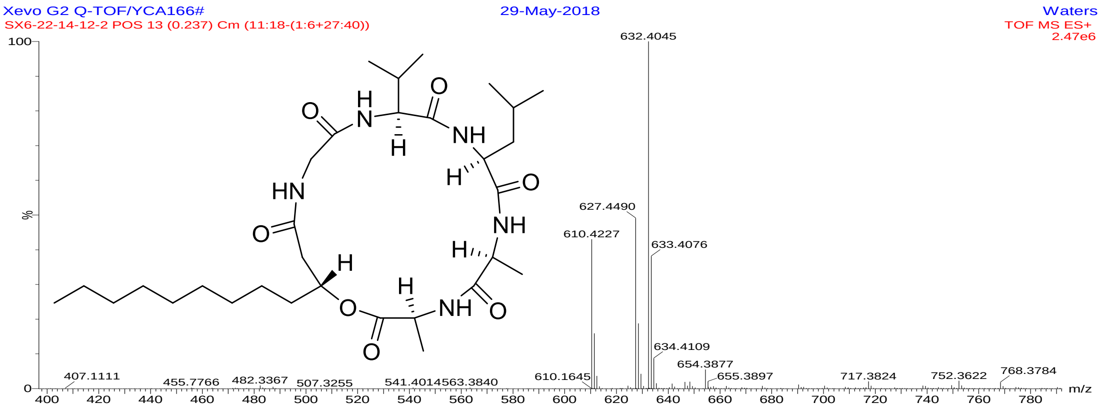
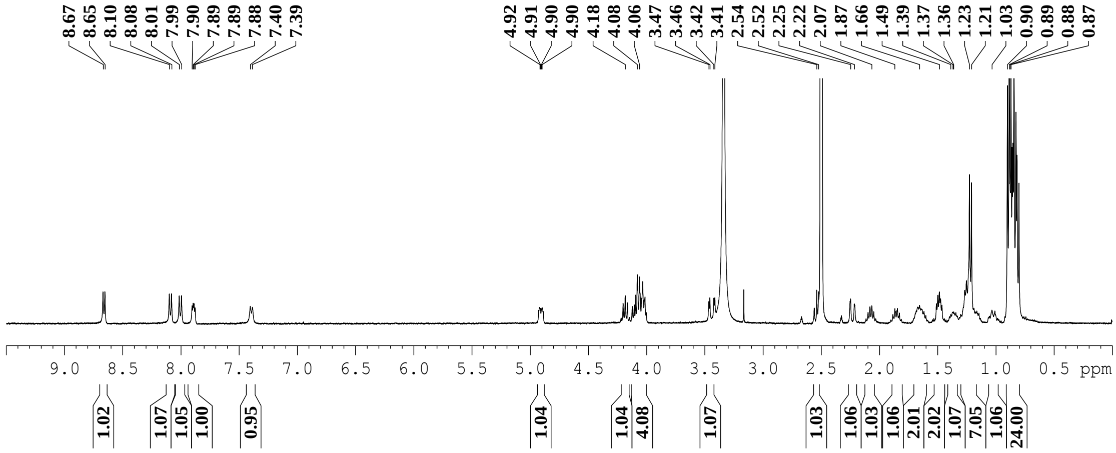
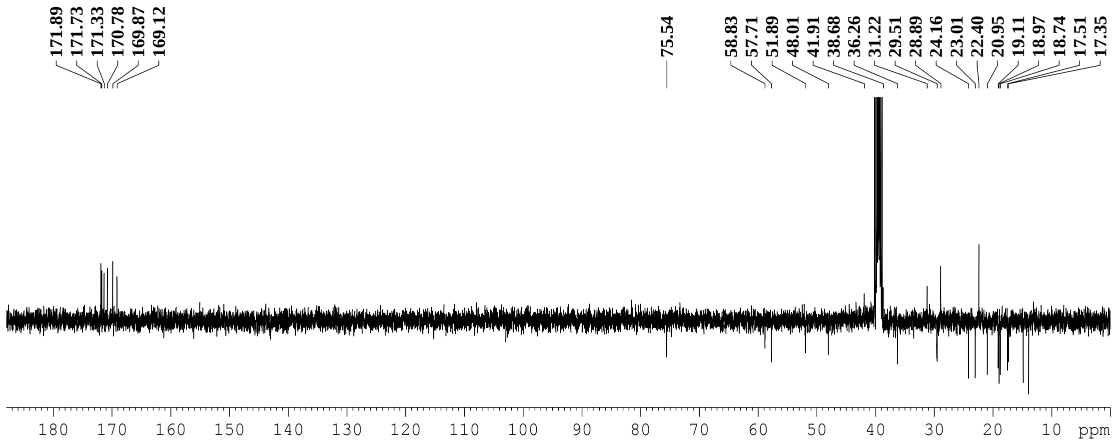


Figure S87. ^{1}H and ^{13}C NMR and ESIMS spectra of isoisariin B (**23**).

^{1}H NMR spectrum of **23** (400 MHz, DMSO-d_6).



^{13}C APT spectrum of **23** (400 MHz, DMSO-d_6).



ESIMS spectrum of **23**.

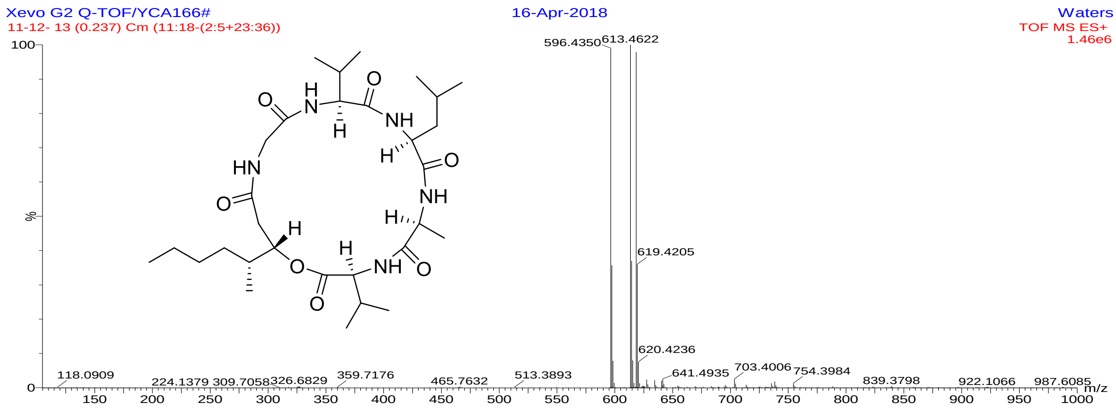
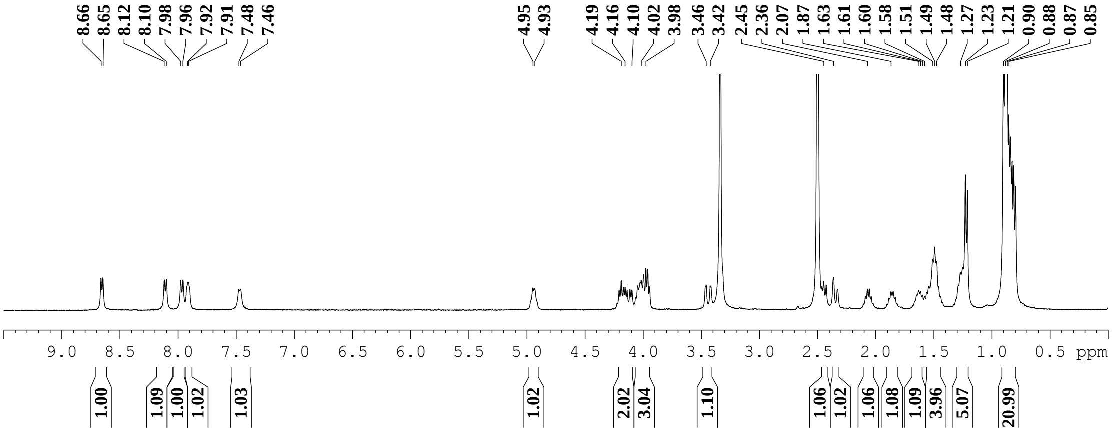
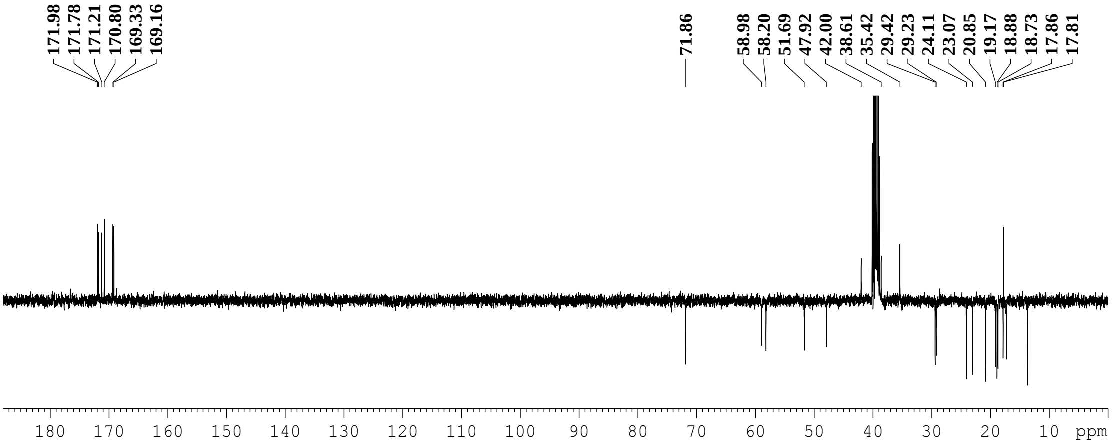


Figure S88. ^{1}H and ^{13}C NMR and ESIMS spectra of isariin E (**24**).

^{1}H NMR spectrum of **24** (400 MHz, DMSO-d_6).



^{13}C APT spectrum of **24** (400 MHz, DMSO-d_6).



ESIMS spectrum of **24**.

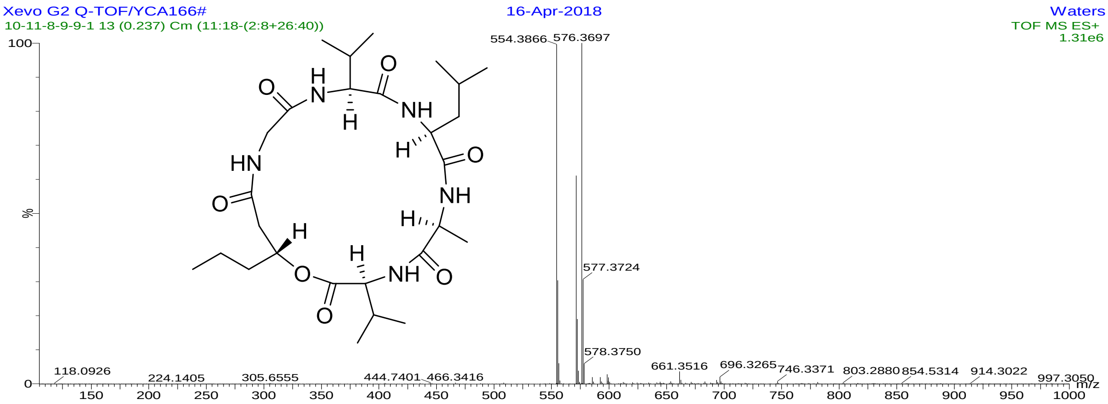
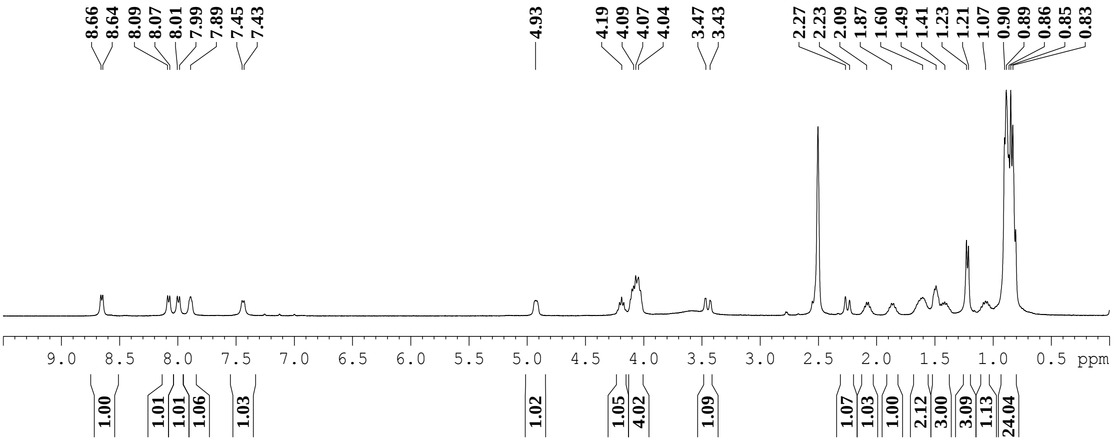
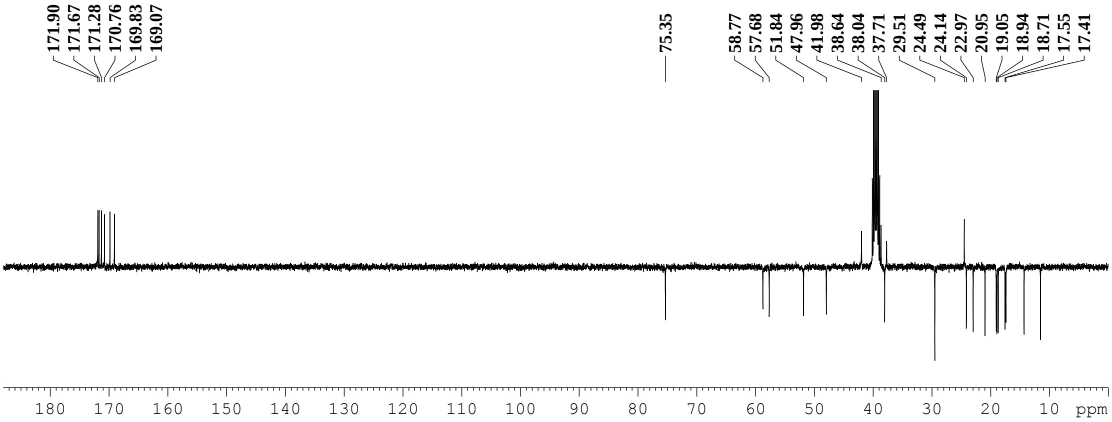


Figure S89. ^{1}H and ^{13}C NMR and ESIMS spectra of nodupetide (**25**).

^{1}H NMR spectrum of compound **25** (400 MHz, DMSO-d_6).



^{13}C APT spectrum of **25** (400 MHz, DMSO-d_6).



ESIMS spectrum of **25**.

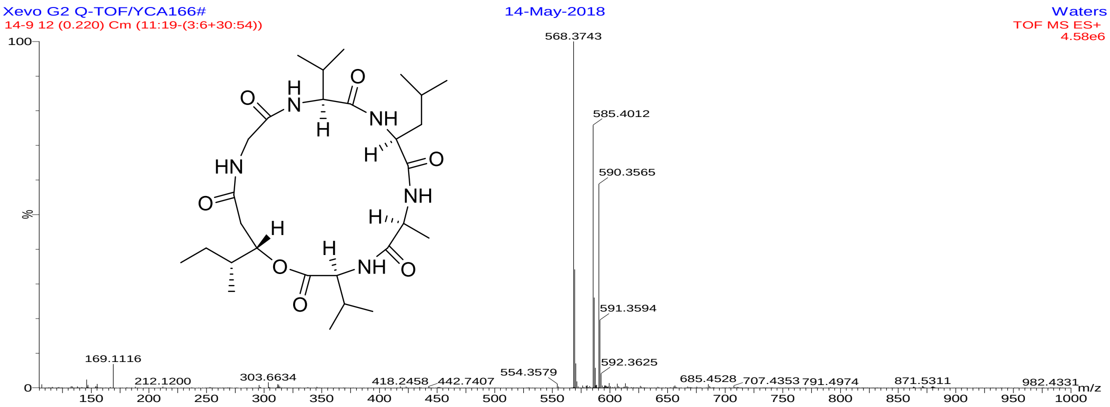
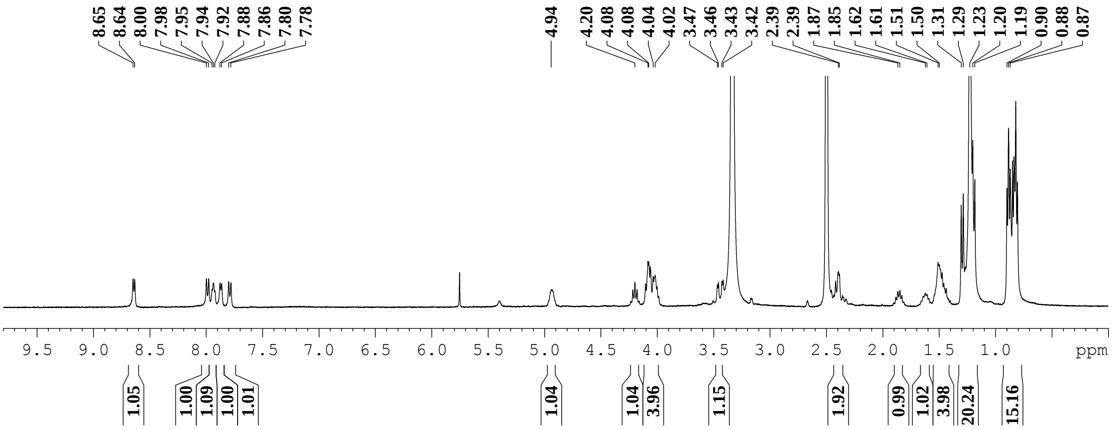
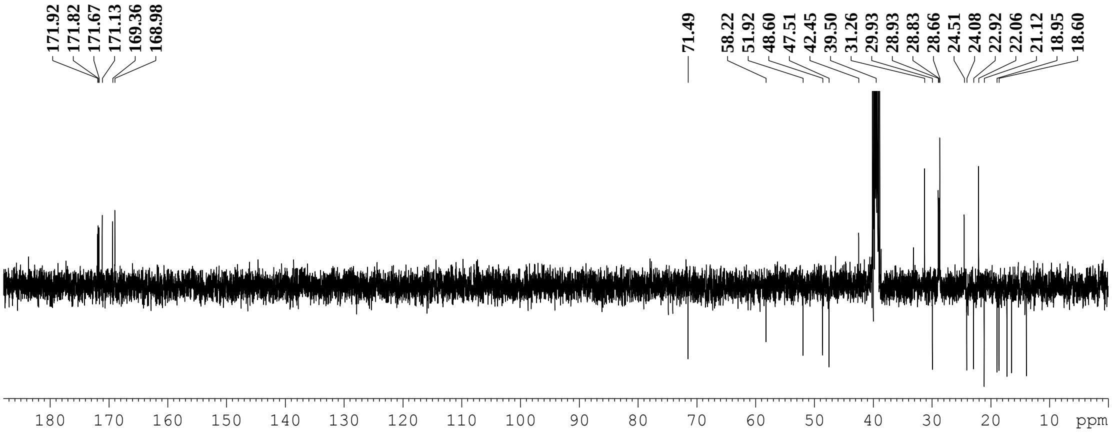


Figure S90. ^{1}H and ^{13}C NMR and ESIMS spectra of isariin A (**26**).

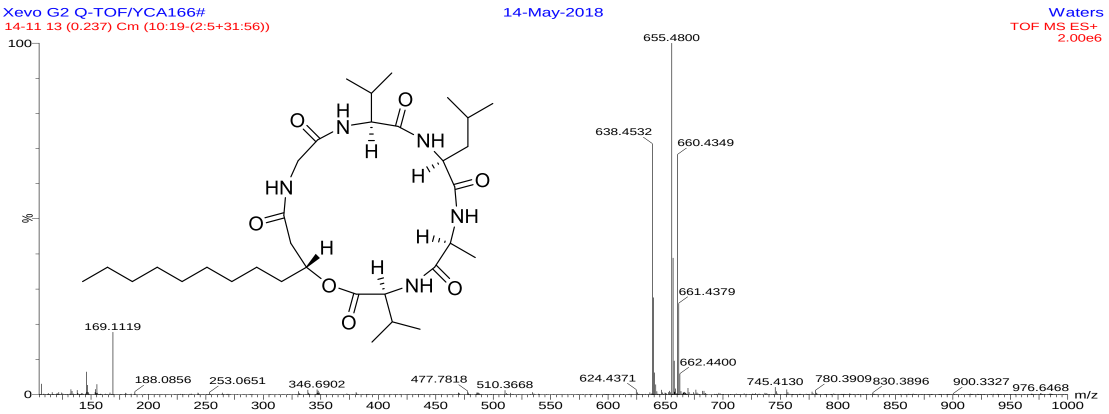
^{1}H NMR spectrum of **26** (400 MHz, DMSO-d_6).



APT spectrum of **26** (400 MHz, DMSO-d_6).



ESIMS spectrum of **26**.



References:

[1] A. Fredenhagen, L. P. Molleyres, B. Bohlendorf, G. Laue, *J Antibiot (Tokyo)* **2006**, *59*, 267.

[2] L. T. Tan, X. C. Cheng, P. R. Jensen, W. Fenical, *J Org Chem* **2003**, *68*, 8767.

[3] E. M. Stocking, J. F. Sanz-Cervera, R. M. Williams, C. J. Unkefer, *J Am Chem Soc* **1996**, *118*, 7008.

[4] S. Nishikori, K. Takemoto, S. Kamisuki, S. Nakajima, K. Kuramochi, S. Tsukuda, M. Iwamoto, Y. Katayama, T. Suzuki, S. Kobayashi, K. Watashi, F. Sugawara, *J Nat Prod* **2016**, *79*, 442.

[5] L. Li, M. C. Tang, S. Tang, S. Gao, S. Soliman, L. Hang, W. Xu, T. Ye, K. Watanabe, Y. Tang, *J Am Chem Soc* **2018**, *140*, 2067.

[6] S. Noppawana, W. Mongkolthanarukb, N. Suwannasaic, T. Senawongd, P. Moontragoone, J. Boonmakf, S. Youngmef, S. McCloskeya, *Nat Prod Res* **2018**, *34*, 464.

[7] H. Shimamura, H. Gouda, K. Nagai, T. Hirose, M. Ichioka, Y. Furuya, Y. Kobayashi, S. Hirono, T. Sunazuka, S. Omura, *Angew Chem Int Ed Engl* **2009**, *48*, 914.

[8] P. Lukat, Y. Katsuyama, S. Wenzel, T. Binz, C. Konig, W. Blankenfeldt, M. Bronstrup, R. Muller, *Chem Sci* **2017**, *8*, 7521.